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Review Article

3D PRINTING IN THE PHARMACEUTICAL INDUSTRY: A SPECIAL CONSIDERATION ON MEDICAL DEVICE AND ITS APPLICATIONS.

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ABSTRACT

3 Dimensional (3D) printing has seemed to be the technology of radical development for the pharmaceutical industry, particularly in medical device manufacturing. The current review elaborates on the applications of 3D printing, challenges, and potentials in pharmaceutical medical devices. The technology allows for complicated personalized devices with accuracy and cost-effectiveness as never before, bringing in the key applications for this technology in the fields of prostheses, orthoses, surgical guides, audiology devices, and bioresorbable implants. It brings along customization, better pre-operative planning, and new drug delivery systems, but there are quality control and regulatory challenges to be faced: material selection, process validation, sterilization, and scalability. In view of this upcoming technology, the regulatory bodies are having to update their guidelines to ensure continued safety and efficacy. On the road ahead, with artificial intelligence, nanotechnology, and 4 Dimensional (4D) printing, future developments could make sophisticated medical equipment and change the management and outcome of diseases. While 3D printing opens up newer routes of innovation in the pharmaceutical industry, there are major concerns on issues of scalability and regulatory matters. This technology will thus make a significant impact on healthcare delivery through these coming decades, with changes in the global research and regulatory landscapes.

Keywords: 3D printing, Medical devices, Pharmaceutical industry, Regulatory compliance, Personalized medicine, Additive manufacturing

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INTRODUCTION

The technology of 3 Dimensional (3D) printing has been embraced by pharmaceuticals, and it is being used in the manufacture of medical devices. This new manufacturing technique has taken the market by storm since now complex, very delicate, or highly specialized medical equipment can be created at the highest possible level of precision and cost-effectiveness [1].

Additive manufacturing or 3D printing is an innovative way of building up a device in small increments from digital blueprints. When compared to traditional methods of manufacturing, this approach provides many advantages. It makes it possible to develop intricate geometries, allows customization for individual patients, and speeds up trial production, thus shortening lead time for product development cycles [2].

Since there are no rules on how 3D printers should be used in medicine, they have been employed across different categories of medical devices as well. They can make artificial limbs, personalized implants, peculiar surgical equipment, and printed organs, which surgeons use for rehearsal before operations among other uses. This can optimize inventory control while improving supply chain dynamics in the sector through the ability of the technique to produce goods as required [3].

It is opening up new avenues of innovation in drug delivery systems within the pharmaceutical field since 3D printing allows the construction of devices with precision dose control and modified release profiles. It may finally lead to individually composed medications for the patient, which perhaps may improve treatment outcomes and compliance [4].

In a few years, when the technology further improves, 3D printing will change medical equipment design, development, and uses. The change is about ushering in an age of personalized healthcare solutions to improve results overall for patients [5]. The continuing development of 3D printing within both the pharma and medical device industries at large stands as evidence of its high potential to totally revolutionize health care into efficient, personalized care options for patients worldwide [6].

Search criteria

The selections of articles for the present review were searched from specialized databases (Range of years: 2000-2024), which includes Elsevier, PubMed, and Cambridge, using the keywords 3D printing, medical devices, additive manufacturing, and Regulatory consideration. Other selections include articles from Springer, and Wiley, information from Internet sources, and online published articles from the Food and Drug Administration (FDA).

Basics of 3D printing

Additive manufacturing or 3D printing is an innovative way to create 3D objects. From a digital model, it creates 3D objects, depositing layer by layer bit by bit. It will start off reading a computer-aided design (CAD) file sliced into very thin cross-sections to let it build an object from bottom to top with the help of materials which may range from plastics and metals to ceramics, and also some biological materials. Such technology provides the ability to create intricate, customized designs with a low volume of waste and multiples the benefits that cut across all the industrial fields, including medicine, aerospace, and consumer goods. It has attracted attention because of the possibility it presents to consumers, especially because of its capability to enable rapid prototyping, on-demand production, and the creation of shapes that are inaccessible or hard to produce [7].

The whole process often involves typically three main steps as given below.

Design

The digital 3D model of medical devices starts with selecting proper CAD software, for example, SolidWorks or Autodesk Fusion 360. The first step is the creation of 2 dimensional (2D) sketches to the needs of medicine, subsequently converted into 3D geometries with a set of CAD instruments. Parametric modeling can be easily modified, while assembly modeling is intended for complex devices. Surface modeling techniques are utilized in producing organic shapes. Material properties are defined to facilitate correct simulation. The design is subjected to finite element analysis, where it has to pass structural tests and other criteria of the design. The simulation result, combined with medical

feedback will allow iteration for optimizing the design. At the last stage, the CAD model is converted into a 3D-printable file and becomes ready for production. Throughout the process, documentation has been elaborated, and often, cloud-based platforms are utilized in cooperation between engineers and medical professionals.

Printing

The most basic steps of the process to bring a digital model to an actual, 3D-printed medical device include converting a CAD model into a Stereo lithographical (slt.) file. This involved projecting the surfaces of a model into triangles by approximating the model. Then, slicing software processes that file, which splits up the entire 3D model into layers at defined, thin horizontal sections and generates machine instructions for the 3D printer in geometry code. As the designs are fed into the device, layers on top of layers of the product are created during printing. Among the most common 3D printing technologies include fused deposition modeling (FDM), where molten thermoplastics extrude the product, Stereo lithography (SLA), which makes use of Ultraviolet (UV) light to cure the liquid resin, and Selective laser sintering (SLS) which utilizes a laser in fusing the powder material. Depending on the properties of the material required, resolutions, and the final application of the product for health care, then that will be the technology to apply. Most print devices require further post-processing steps, including cleaning of the supports off the material, finishing of the surfaces, and sterilization, for them to be used medicinally.

Post-processing

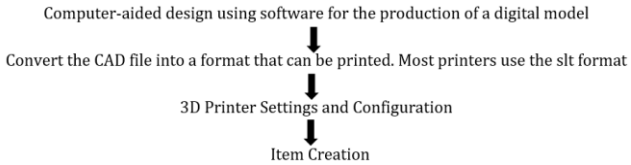
Post-processing is an important step in 3D printing medical products to ensure that the final product reaches stringent quality and safety requirements. First, support structures need to be removed. They are either manually removed or chemically dissolved based on the printing process. SLA/Digital Light Processing (DLP) resin-based prints undergo a thorough cleaning with isopropyl alcohol to remove uncured resin, followed by exposure to UV curing to attain full material strength. FDM prints can be surface finish enhanced with methods such as sanding or acetone vapor treatment. The metal prints are often heat-treated to relieve the internal stresses. All medical devices have precise dimensional checks and must undergo additional machining for critical features. Surface treatments with polishing or coating enhance both biocompatibility and functionality. Finally, the product is sterilized by a process such as ethylene oxide, gamma radiation, or autoclaving to ensure that the device is safe for medical application. All of these steps are documented for compliance with all demands of the regulatory body and for ensuring quality [2].

Need for 3D printing in the pharmaceutical industry

There are many benefits of 3D printing over traditional manufacturing methods, including a decrease in production and logistic expenses, easy production of complex and customized products, and more efficiency due to a reduction in material and energy usage [8]. 3D printing in medicine allows for faster and more affordable production of items compared to other means, such as machining. This is particularly valuable in product development, given that designs can be turned around quickly, products are easily customized, and small quantities can be produced affordably. In fact, this technology can really help slash the time taken to introduce new items into the market [9]. Compact and biocompatible electronic platforms are much needed for wearable devices and specific implants that will conduct real-time monitoring of chronic health conditions. The exploitation of 3D printing in medical devices is foreseen to go beyond anatomical models and prosthetics when electronic materials and additive manufacturing technologies with enhanced properties become available [10]. Such technology will aid in the creation of individualized or personalized electronic implants and other devices from a great variety of biocompatible materials. Success depends on getting enough feedback from health professionals and patients, but also on the opportunity to implement design improvements really fast. Fast feedback created by 3D printing accelerates the design refinement cycle [11, 12].

3D printing software

Technology for medical rapid prototyping has advanced significantly. The advancements in reconstruction techniques, image processing, and medical imaging techniques made them easier. Despite the diversity of technologies, the 3D printing process typically involves the majority of the following procedures:



Because this is a completely computer-driven process, it removes intermediary phases, hence requiring less manual labor. This technique comes with several advantages: cost savings, shortened production times, and development with any modifications if required [13].

3D printing materials and used in pharmacy

The selection of appropriate materials is crucial for the successful implementation of 3D printing in pharmacy, as these materials directly impact such as biocompatibility, mechanical properties, and degradation kinetics.

Biocompatibility

Biocompatibility is an important aspect of 3D-printed medical devices, particularly because the devices have direct contact with biological systems. The biocompatibility for a 3Dprinted medical device is therefore very necessary. For a safe and effective medical device in patients, restrictive testing is thus required by adherence to regulatory standards and continuous improvements in the field of materials and manufacturing processes [14].

Material selection

The biocompatibility of 3D-printed medical devices is very sensitive to material choice. The materials should not provoke any adverse biological responses. On the other hand, they should support tissue integration. For example, some polymers, metals, and ceramics are biocompatible [15].

Regulatory standards

In response, a few rigid standards have been set up by regulatory bodies for biocompatibility testing; this includes cytotoxicity, sensitization, irritation, and systemic toxicity studies [16].

Printing process implications

The 3D printing process itself can also affect biocompatibility due to variables such as printing resolution, layer adhesion, and other post-processing treatments associated with how materials will bind with biological systems [17].

Long-term stability

Another important factor is the biocompatibility for long periods. Materials shall not chemically degrade such that they cause harm to the patient or dysfunction of the device [18].

Customization and patient-specific devices

Although 3D printing allows customization to patient anatomy in the field of medical devices, this should not take away from considerations about biocompatibility. Materials and processes should be carefully matched both for safety and efficacy [19].

Mechanical properties

The mechanical performance of a 3Dprinted implant is expected to vary with the application involved, whether these are load-carrying implants or flexible systems, as in drug delivery. Required properties are appropriate: Strength and stiffness, Fatigue resistance, Wear resistance, and Elastic behaviour (for specific applications) [20].

Degradation kinetics

Material degradation

Depending on the nature of the application, the 3D-printed medical devices will have to bear degradation in biological environments.

Biodegradable polymers, for example, break down with time upon being resorbed or metabolized by the body. The kinetics of degradation for these materials provide the knowledge needed for the development of a device with the right lifetime [21].

Environmental factors

Many environmental parameters may be involved in degradation kinetics, such as temperature, pH, humidity, or even the presence of some enzymes or body fluids. Control and prediction of these parameters become, therefore, basic parameters for reliability and safety related to a 3D-printed medical device [22].

Long-term stability

In permanent implants, for example, orthopedic implants or dental prostheses, long-term stability and degradation resistance are very important. This means it should maintain its mechanical integrity along with biocompatibility as long as the device is intended to last [23].

Testing and validation

Run accelerated aging studies and *in vitro*/*in vivo* degradation studies to understand the degradation kinetics of a 3D-printed medical device. The results of such studies are highly valued while gaining regulatory approval and acceptance at the clinical level [24].

Table 1: Common polymers used in 3D printing

Name	Melting point	Advantages	Limitations
Acrylonitrile butadiene styrene (ABS)	105 °C	Good strength and flexibility	They are non-biodegradable and reduce in size when in contact with air.
Polylactic acid (PLA)	175 °C	Good mechanical properties; Low cost.	Long-term biocompatibility [25]
Polycaprolactone (PCL)	60 °C	Good rheological and Excellent viscoelastic properties upon heating and are minimal cost.	Long degradation time (3 y) [26]
Polycarbonate (PC)	110 °C	Tuneable mechanics and porosity.	They intake moisture from the air which can affect the performance and printing resistance [27]
High-performance polymers-PEEK (polyetheretherketone), PEKK (Polyetherketoneketone), ULTEM (polyetherimide)	350 °C	The material is highly resistant to mechanical and thermal stresses. Besides, it is an extremely strong and at the same time much lighter material than some metals.	High melting point [28]

Types of 3D printing technologies applicable in pharmacy

Stereolithography (SLA) technology

3D printing in pharmacy involves several different technologies, such as stereolithography. SLA is an additive manufacturing technique whereby a UV laser or projector hardens liquid resin into plastic. The main parts composing an SLA printer are the light, build platform and resin tank. This is a fast-prototyping method that generates fine details using an ultraviolet laser and requires only a few hours to print an object. Digital Light Processing is an older technology in 3D printing that uses lamps, increasing the printing speed by drying layers in seconds [29, 72].

Digital light processing (DLP)

DLP belongs to the additive process for producing medical devices whereby a digital projector is used to cure photopolymer resin layer by layer. It projects a 2D image onto the resin, thereby solidifying an entire layer at once. This has high precision, smooth surface finish, and faster printing speeds compared to other technologies. DLP finds a lot of applications in creating complex medical devices, like dental implants, hearing aids, and surgical guides. This is where the manufacturing of customized devices with complex geometries and fine details for the patient can be done. The ability to use biocompatible resins opens a plethora of possibilities for this technology in many medical applications, which will enhance personalization and potentially improve the quality of care delivered [72].

Continuous liquid interface production (CLIP)

The CLIP process is a fast VAT photopolymerization method that makes use of Digital Light Synthesis technology to shine a series of UV images onto a 3D printed part's cross-section, which aids in controlling the curing process with very fine resolution. The whole part is then subjected to a thermal bath or oven, raising a diversity of chemical reactions that eventually harden the part.

Material jetting

Material jetting technologies represent the newest developments in the field of 3D printing and are increasingly being explored for pharmaceutical applications. Liquid materials photopolymers are deposited as small droplets in a layer-by-layer process and subsequently cured by UV light. It presents great promise for personal medicine: from tailored dosage formats and complex geometries in controlled release to multi-drug combination products. In this respect, material jetting may be considered an attractive technique in drug delivery because of its high precision and possibilities for the elaboration of structures. The range of suitable materials is highly limited, and there are regulatory hurdles and scaling up for mass production in the way of this industry. This technology may yet hold some revolutionary techniques for the drug-manufacturing industry, such as rapid prototyping or on-demand production of tailored pharmaceutical products.

Binder jetting

Binder jetting is an additive manufacturing process wherein liquid binding agents are applied selectively to hold the layers of powdered material together. Droplets of binder come out of a print head and are dispensed onto thin layers of powder. The powder has been spread across a build platform, and then the same process occurs for each layer until a 3D object is created. Binder jetting is compatible with different materials, such as metals, ceramics, and polymers. It is particularly useful for the production of complex geometries and large parts. In medical applications, it allows for the manufacture of custom implants, surgical models, and drug-delivery devices. This technology is suitable for both prototyping and production-scale manufacturing of medical devices, with relatively fast build speeds and the ability to print multiple parts at one time [72].

Fused deposition modeling (FDM)

Fused deposition modeling works through an extrusion-based technique whereby thermoplastic filament is heated and deposited on a substrate by layer to create a 3D object. The same involves the extrusion of molten plastic via a moveable nozzle onto a build platform in a specified path. As each layer cools and solidifies, it can bond with the previous layer. It finds broad application in medical device prototyping and production due to the flexibility, cost-effectiveness, and biocompatible raw materials the technology offers. These include applications for orthotics and prosthetics, models of anatomy, and surgical guides. Although FDM may offer lower resolution compared with some other 3D printing methods, it generally displays good mechanical properties and allows the fabrication of parts that have functionality within them to be rapidly created.

Selective laser sintering (SLS)

SLS is a form of Powder Bed Fusion that produces 3D objects by the fusion of small particles of powder by the use of a high-power laser.

A high-powered laser scans each layer of the powder bed and selectively fuses the particles, and then the process is repeated as the bed is lowered.

Multi-jet fusion (MJF)

In multi-jet Fusion, the powder is laid down by a sweeping arm; binder is applied selectively on top with an inkjet-equipped arm. Precision comes from the application of a detailing agent around said area. The application of thermal energy then initiates a chemical reaction. Direct Metal Laser Sintering, on the other hand, is similar but it uses metal powder.

Directed energy deposition (DED)

Directed Energy Deposition is one of the methods mainly used in the metal industry. A 3D printing device is attached to a multi-axis robotic arm with a nozzle that applies the metal powder. It works by melting the material, applied as a powder to a surface, by use of some energy source, and immediately creating solid objects [30, 31].

What are medical device and implants?

Medical devices and implants can be summed up as those products of the pharmaceutical industry used in healthcare that are not drugs themselves, yet in most cases, help pharmaceuticals achieve the diagnosis, prevention, or cure of an ailment. These may include simple devices and tools at one end to highly complicated implants at the other. They also intersect with drug development, delivery, and administration within the context of the pharmaceutical industry.

The major points for consideration about medical devices and implants in the pharmaceutical industry include:

Drug-device combination products

3Dprinted drug-device combination products inlay pharmaceutical components into the medical device itself to improve therapeutic outcomes from that single entity. Customized, patient-need-based, and localized drug delivery systems can be designed through such a process. By 3D printing, complex geometries can be produced with precise drug incorporation to achieve increased efficacy and reduced side effects. Applications are currently being made in drug-eluting stents, antibiotic-infused implants, and targeted cancer treatment devices, providing innovative solutions in personalized medicine and improved care for patients [32].

Drug delivery systems

These 3D-printed medical devices allow for the controlled, accurate release of drugs through their delivery systems. Such structures can be tailored for targeted delivery, optimized release kinetics, and patient-dependent needs. 3D printing enables both complex geometries and multi-drug configurations; therefore, it increases therapeutic efficacy while decreasing side effects. Applications range from implantable devices to transdermal patches that can revolutionize personalized medicine and treatment strategies across a broad swath of medical fields [33].

Diagnostic devices

Medical instruments are employed to recognize illnesses or follow conditions that can impact medicines administered by the drugs. It offers customized, rapid, and cost-effective solutions for the diagnosis and monitoring of diseases through 3D-printed diagnostic devices in medical technology. These diagnostic devices can be tailored to patients' requirements or any testing needs, hence fulfilling the concept of point-of-care diagnostics. 3D printing enables the realization of complex microfluidic structures, biosensors, and lab-on-a-chip devices that increase sensitivity and accuracy in diagnostics. Their applications include portable testing kits, wearable health monitors, and individual biomarker detection systems that further precision medicine and the identification of diseases at an incipient stage [34].

Implantable drug reservoirs

3D-printed implantable drug reservoirs offer an avenue for medical devices capable of targeting sites with controlled medication delivery. Complex geometries, part of integrated structures that optimize the release kinetics, can maintain at specific sites the release of a drug. Such devices offer features that support patient-specific design, multiple compartments for different drugs, and stimuli-responsive functionalities, which possibly will help to enhance treatment efficiency while reducing systemic side effects for a range of medical applications [35].

Smart devices

These electronic gadgets can take care of a patient's health status and adjust dosages of drugs as far as possible where necessary [36].

Table 2: Comparison of traditional and 3D-printed medical device

Category	Traditional medical devices	3D-printed medical devices
Manufacturing Process	Normally mass-produced through conventional techniques such as injection molding and machining.	It is fabricated using additive manufacturing techniques wherein an object is created layer by layer [37]
Customization	Normally limited to usual sizes or configurations.	Normally limited to usual sizes or configurations [7]
Design Complexity	It is constrained by manufacturing processes' limitations.	Enables complex geometries and internal structures [3]
Materials	It is restricted to only material types that can be conventionally manufactured.	Expanding spectrum of biocompatible materials, including some that have the potential to exhibit properties, such as that of tissues [38]
Applications in Drug Delivery	It is influenced to a great extent by a lot of manufacturing-related defects.	It allows for Multiple complicating drug release profiles under one dosage form with tailored [39]

Applications of 3D printed medical devices

Prosthesis

An artificial limb, simply put, is known as a prosthesis an artificial device designed to replace the missing body part, be it in the form of a hand or a foot, or in any other shape less natural, designed to allow amputees to perform any day-to-day activity with ease.

Prosthetics can be fabricated from many materials, of which plastics like polypropylene and polyethylene constitute prosthetics and polypropylene and polyethylene are in larger use. It has been a major and primary element in the rehabilitation of amputees and has been able to function for many years of their lives. Unlike conventional prostheses in which the materials are 'molded and cast', 3D printed models are tuneable around your unique anatomy. This can result in a much softer and more aesthetically pleasing product with much less challenge in responding to their lifestyle. During the design, the orthopedic surgeon, together with the designer, employs a blend of clinical data, CAD, and software tools in making the final product. It allows for a more personal and efficient product because it can be manufactured more easily at a lower cost and is durable [41].

Orthotics

An orthoses is a device used to assist the body in performing its functions. Generally, orthoses protect the body, limit movement, support body weight, provide movement, and prevent/correct deformities. They have been used on an extensive basis in helping patients with physical dysfunction and disability due to muscular problems like fractures, sprains, arthropathy, tendinopathy, or even

neurological disorders in the brain, spinal cord, and peripheral nerves.

The conventional technique of orthoses production is rather time-consuming. Again, the shape and dimensions of the orthoses have to be pre-adjusted on a patient's body manually. Further, creating several customized, high-quality orthoses is difficult, and sometimes realization of the complex designs is also hard.

Through 3D printing, the technology becomes dimensionally accurate for orthoses by computer graphic software. This offsets the limitation of the traditional method due to the high precision of a 3D printer.

Using 3D printing, design software enables the production of orthoses with correct dimensional values and complex structures that, otherwise produced manually, would be impossible.

Unlike custom-made orthoses, which take approximately a week, a 3D printer can make an orthoses within one day, hence provoking great interest in orthoses created using 3D printing technology [42].

3D-printed surgical guides

Additive manufactured surgical guides have been at the forefront of changing the face of personalized medicine. Patient-specific tools created from advanced modalities of imaging computed tomography or magnetic resonance image allowing for accurate preoperative planning and intraoperative guidance. One of the main advantages associated with 3D-printed surgical guides is that they enhance surgical accuracy.

For instance, a study conducted by the researchers concluded that in spinal surgery, 3D-printed surgical guides can increase the accuracy of pedicle screw placement, thereby reducing the risk of neurological complications. The accuracy rate reported for 3D printed guide-assisted screw placement was 92.8%, compared to 86.6% using the freehand technique [43].

Their working applications throughout a wide surgical spectrum in orthopedic surgery, 3D printed guides have changed the landscape for total knee arthroplasty by demonstrating that the patient-specific instrumentation-including 3D printed guides-improved overall prosthetic component alignment, which likely improves the long-term results in patients [44].

3D-printed hearing aids

This additive technology enables devices with a high level of customization, improving comfort and acoustics in comparison with devices that have been conventionally manufactured. The process mostly incorporates digital scanning of the patient's ear canal, followed by making a personalized shell design and creating the same through 3D printing.

Integrating the 3D printing procedure in the manufacturing process of the hearing aid device gave several benefits. Those are:

Improved comfort and fit

Commit accurate customization provides an excellent fitting, reducing feedback and other problems to provide more comfortable wear.

Ready in no time

The manufacturing time for custom hearing aids has come down drastically with 3D printing.

Consistency

Because it is a digital process, repeatability is assured at a high level of quality control.

The 3D printing in the hearing aid industry was simply overwhelming. The custom in-the-ear hearing aids fabricated using 3D printing have moved not only the quality of products to another level but also eased manufacturing with reduced workers, time, waste of materials, and energy [45].

Bioresorbable implant

Bioresorbable implants and tissue scaffolds have taken a new dimension of advancement in the area of 3D printing of pharmaceutical and medical devices. These structures support tissue regeneration and degrade within the body; thus, no removal surgery will be required, which minimizes long-term complications that come due to permanent implants.

3D-printed bioresorbable implants are under study for many medical applications, in particular, in orthopedics and craniofacial surgery. Such devices render temporary support, undergoing degradation in some time and allowing natural body healing processes.

3D-printed polycaprolactone and hydroxyapatite composite scaffolds were frontline for bone regeneration. These scaffolds showed good biocompatibility and sufficient mechanical strength compared to natural bone [19].

Tissue scaffolds

3D printed tissue engineering scaffolds are, therefore, essential in regenerative medicine, providing structures that will very finely duplicate the extracellular matrix to support the growth of cells and the formation of tissues.

3D bioprinting techniques for tissue engineering applications: In 3D printing, there is the potential ability to control scaffold architecture, porosity, and mechanical properties so that they are conducive to cell adhesion, proliferation, and differentiation [46].

Tailored dosage forms

Tailored dosage forms have revolutionized the production in the pharmaceutical industry brought about by the emergent technology of 3D printing. This innovation gives the opportunity for making personalized medicines with corresponding exact dosing, shape, and size for the needs of individual patients. Using 3D printing, the pharmacist and healthcare provider may use a patient's age, weight, metabolism, and even genetic makeup in tailoring their medication decisions. This kind of individualized therapeutic regimens would have more pronounced therapeutic outcomes by ensuring optimal drug absorption and efficacy. Complex geometries may be prepared for modulating the rates of release of drugs, such that multi-layered tablets combine several drugs with different release profiles in one dose. This type of strategy increases patient compliance while minimizing the side effects, which are generally associated with conventional, one-size-fits-all drugs.

Controlled release mechanisms

3D printing allows for the creation of highly complex controlled-release mechanisms for drug release. Such systems could be designed to give the necessary rate of medication delivery over much more extended periods, maximizing efficacy in therapy while offering easier management for patients. However, by manipulation of the internal architecture and composition of 3D printed devices, the researchers may be able to create matrices that can uniformly and precisely control drug diffusion or erosion. As such, it allows for complex release profiles; indeed, pulsatile or chronotherapeutic delivery systems comparable to, yet respectful of, the circadian rhythm of the body can be designed. 3Dprinted implants and inserts can also be designed for the sustained release of drugs over several weeks or months, which might modify the treatment strategies of chronic diseases and the number of drug administration's [71].

Advanced inhalation devices

3D printing paved new ways in advanced inhalation device design and manufacturing for respiratory drug delivery. Devices may be designed tailored to the patient's lung volume and breathing pattern and his or her particular therapeutic need. By 3D printing on inhalers, complex internal geometries that optimize the size distribution of particles and the dynamics of flows can be achieved. The technology can also lead to multi-dose inhalers having in-built dose counting with smart features to enhance tracking for better adherence. It also supports fast prototyping and iteration of inhaler designs, accelerating the product development process and can save costs in the long term.

Transdermal and microneedle systems

The transdermal and microneedle systems are an excellent advancement in non-invasive drug delivery. 3D printing allows the

creation of specific dimensions shapes and density arrays for maximum skin penetration and drug release with 3D-printed microneedles. These systems can deliver a wide range of therapeutics, from small molecules to large proteins and vaccines, with improved bioavailability compared to traditional transdermal patches. The ability to customize microneedle geometry allows for tailoring the depth of penetration and the rate of drug release to suit different skin types and therapeutic needs. Besides, the capability to combine multiple drugs into a single microneedle patch by 3D printing can present the potential for combination therapies or staged drug delivery. The same technology may be used in order to advance the development of dissolvable microneedles that do not leave sharps waste; thus providing safety and convenience in self-administration scenarios [73].

Regulatory authorities

Regulator bodies

The Food and Drug Administration (FDA) oversees three primary categories of products: 1) drugs overseen by the Centre for Drug Evaluation and Research, 2) device-related products overseen by the FDA's Centre for Devices and Radiological Health, and 3) Blood vaccines overseen by the FDA's Centre for Biologics Evaluation and Research. Regarding medical device regulation, firms that manufacture devices distributed in the United States have several basic regulatory responsibilities. It covers registration of facilities (21Code of Federal Regulations (CFR) Part 807), listing of devices for medical application (21 CFR Part 807), premarket application notification 510 (k) (21 CFR Part 807 Subpart E), premarket approval (21 CFR Part 814), exemption for clinical investigation of a device for investigational use (21 CFR Part 812), quality system regulation (21 CFR Part 820), regulation for labeling requirements (21 CFR Part 801), and Medical Device Reporting (MDR)(21 CFR Part 803) [48].

Medical devices are ensured through the monitoring by the United States Food and Drug Administration's Centre for Devices and Radiological Health that companies manufacturing, repackaging, relabelling, or importing medical equipment for sale in the US market adhere to the medical device regulations. In terms of setting up a regulatory framework for 3D printed medical devices, this would mean that the former would be treated as any other traditional medical device regarding manufacturing processes and control requirements.

Medical devices are classified into three classes according to the FDA: Class I, Class II, and Class III, depending on the given level of device risk, with the greater class number signifying increased controls. These classes determine the controls that would be implemented for each device type. Devices under class I are mainly low-risk and normally would not require 510(k) clearance. Those under class II are generally considered of moderate risk and require a Premarket Notification, 510(k). Generally, device types that have been classified as high-risk or novel (Class III) must support a Premarket Approval (PMA) [49].

Inspection at the site of device manufacturing of higher-risk devices in Class C or D of some classification systems within 60 days of receipt of the marketing application for compliance with the requirements of quality management. The team prepares a comprehensive inspection report at the end.

Upon getting this report, the regulating agency has 45 days in which it may reach a decision. It may license the medical device for production and distribution or reject the application upon its findings [50].

Medical devices classification as per United States Food and Drug Administration (USFDA)

Medical devices are thus categorized into three classes by the USFDA regulations based upon the associated level of risk and also the regulatory measures required to ensure that they are safe and effective. What follows is a brief description of how they are classified:

Table 3: Medical device classification as per USFDA

Class I devices	Class II devices	Class III devices
The risk associated with these devices is minimal.	The devices are considered a medium-level risk-associated	These devices belong to the highest-risk device category.
These are controlled only by general controls.	These devices are, therefore, subject to both general controls and special controls.	They are associated with clinical data under the general controls and premarket approval categories for supporting claims of safety and effectiveness.
Most of the devices are exempt from the requirement for 510(k) premarket notification.	Most of these require the 510 premarket notifications.	
Examples include elastic bandages, examination gloves, and hand-held surgical instruments.	Examples include powered wheelchairs, infusion pumps, and surgical needles.	Examples include pacemakers and deep-brain stimulators.

The class of the device is determined by the FDA based on:

Intended use of the device, how it is to be used, and any potential risk to patients and users.

Devices are liable to be reclassified upon reception of new information with respect to the safety or effectiveness of a device [51].

The FDA has laid down various routes through which medical device manufacturers can get their products into the marketplace or be approved for use. All of these routes ensure that medical devices manufactured with 3D printing technology, amongst others, are safe and effective. Details for each type of submission include:

The 510(k) Premarket notification provides the common pathway to market for many medical devices, including several 3D printed ones. This, therefore means manufacturers have to bear the burden of proving that a device is essentially equivalent to another lawfully marketed predicate device. This route is commonly quicker and less burdensome than applying for Premarket Approval [52].

Probably, the most rigorous process for marketing medical devices is premarket approval (PMA). The PMA process itself has been developed to be applied for those medical devices that are associated with high risk and for which no substantially equivalent predicate device is available. This may include, concerning 3D printed devices, implantable devices, or other products for which safety and effectiveness have to be established by a large amount of data on their clinical and non-clinical performance [53].

Humanitarian device exemption, this pathway is for Devices indicated for a condition or disease that annually affects under 8,000 Americans are eligible for a humanitarian device exemption. Unlike PMA devices, although it is assumed that they will be less effective in general for all cases a built-in incentive for rare disorders is the rule [54].

De Novo classification, A new device can be placed in a low-to-moderate risk classification pathway where the FDA has the discretion to have a device that has not been previously approved and is not substantially equivalent to any other device [55].

The Investigational device exemption (IDE) process provides the opportunity for limited exemption of certain portions of the Act, allowing for an investigational device to be used in a clinical trial to gather safety and effectiveness data. This occurs before PMAs or 510(k) applications are submitted, and it involves cases where new

3D-printed devices require clinical data to back up a marketing application [56].

The type of submission required for 3D printed devices will depend on many factors, including the risk class of the device, the intended use, whether the design or material is novel, and whether suitable predicate devices are available. This regulatory pathway is influenced by several unique aspects of the 3D printing technology, including the ability to make patient-specific devices with complex internal structures.

Challenges and limitations

The challenges of 3D printing technology in the field of pharmaceutical medical devices are:

Selection and validation of raw material

Firstly, stringent selection and validation of the raw material must be ensured in the quest for quality control. It would include the active pharmaceutical ingredients and different polymers along with additives used in printing. Manufacturers are working on evolved testing protocols for proving the purity, stability, and compatibility of raw materials with the technology of 3D printing and the intended medical application [1, 71].

Material and manufacturing constraints

The futuristic domain of 3D printing in medical devices encounters significant challenges in material science and manufacturing processes. Additive manufacturing promises unparalleled geometric freedom, but it also struggles with a very limited bio-compatible palette that can withstand the rigorous demands of medical applications. Layer-by-layer deposition inherently forces the creation of anisotropic mechanical properties, which can be deleterious to the structural integrity of the final product; yet, these state-of-the-art 3D printing technologies are still challenged by multi-material integration-the crux in mimicking the complexity of biological systems' heterogeneous structures. These limitations not only limit what can be produced but also impact long-term lifetime and functionality in vivo [1, 71].

Process validation and monitoring

Another critical pillar in the area of quality maintenance is process validation. This will involve the establishment and tracking of key process parameters such as temperature, speed, and resolution at which printing is carried out. The aim of the process will be to have lot-to-lot reproducibility and to ensure that the final product possesses the desired physical and chemical characteristics. Toward this goal, the pharmaceutical industry is currently investigating state-of-the-art process analytical technologies that support in-process monitoring and adjustments in real-time [5].

Characterization and testing of products

Characterization of the finished 3D printed devices is very necessary to assure quality. Characterization typically includes a suite of analytical techniques, many of which involve spectroscopic and imaging techniques to confirm the uniformity of the drug content, structural integrity, and surface characteristics, among others. For the devices releasing medication, special attention is paid to dissolution testing and drug release profiling to prove that the medication will be released as intended [2].

Sterilization and control of contamination

Unique in nature, 3D-printed medical devices have particular challenges regarding sterilization and contamination control. Traditional methods of sterilization may not be appropriate for 3D-printed devices; therefore, innovative ways have been explored. The need to maintain a sterile printing environment and to use proper post-production sterilization techniques has grown to become paramount in the manufacturing process [57].

Regulatory compliance and adaptation

The regulatory setting for 3D-printed medical devices in pharmaceuticals will be complex. Indeed, such products often occupy the space where device and drug regulations blur. Time is of the essence as companies rush to tailor existing Good Manufacturing Practices (GMP) and set new standards unique to 3D printing technologies while staying compliant with the changing regulatory frameworks [3, 71].

Standardization efforts

The standardization efforts that would be put in will ensure that common practices for the design, manufacture, and testing of such novel devices are streamlined. It calls for the development of standard file formats for 3D designs, calibration procedures for printing equipment, and unified test methods for the finished products [58].

Personal medicine considerations

The field, moving into personalized medicine, shall also need to consider strategies for quality control with the possible customization of devices for individual patients. This brings a unique challenge in balancing consistency and safety against the flexibility that makes 3D printing so attractive for personal healthcare solutions [59].

Post-market surveillance and traceability

Post-market surveillance and traceability of 3D printed medical devices assume new significance. Tracking products effectively through an established system for the collection of real-world data on product performance for continued quality assurance and improvement of innovative products is key to quality [60].

Scalability and economic viability

3D printing indeed does well in producing customized, low-volume medical devices. However, there is a big limitation to 3D printing regarding the scaling-up requirements of mass production. From this aspect, 3D printing is economical in terms of building time and material cost, which is a condition for large-scale standardized manufacturing. Another factor is that the 3Dprinted medical devices have post-processing requirements, including removal of supports, surface finishing, and sterilization. The majority of the processes are labor-intensive and, therefore, affect scalability. A high entry threshold at the outset with great capital investment for the production of industrial-grade biomedical 3D printers presents a huge entry barrier to smaller medical device firms. These economic factors are compounded by the current limitations of technology, which severely limit applications of 3D printing to high-value, customized medical devices, which constitute a tiny fraction of the total market for medical devices [60].

Future of 3D printing in medical devices

Personalized drug delivery systems of the future

Advanced sensor technologies and responsive materials may be incorporated into next-generation, 3D-printed drug delivery systems. Such systems could have the capability of monitoring parameters of the physiological state in real-time and tailoring drug release. For example, an implant printed by 3D printing for the treatment of chronic pain could monitor inflammation markers, modulating in turn the delivery of analgesics and, therefore, optimize the relief of pain with minimal side effects. Such systems would represent a phenomenal improvement in the personalization of medicine and might offer improved treatment efficacy and quality of life [61, 71].

Bio-printed organs: simple to complex

While completely functional 3Dprinted organs are currently hoped for the future, researchers are making huge inroads in the bioprinting of less complex tissues and organ parts. Clinical use of bio-printed skin grafts for burn victims or 3Dprinted blood vessels for cardiovascular treatments may not be far off. This could be the first step that eventually leads to more biosynthetic organs, allowing us to extinguish the organ shortage and reduce rejection rates for transplants [62].

On-demand pharmaceutical manufacturing

Future pharmacies could use advanced 3D printing technologies to manufacture the drugs in stores in doses tailored to the patient's

requirements. This would be a breakthrough in the formulation of drugs that would allow more precise dosages and tailored release profiles, maybe even several drugs combined in one easy-to-swallow form. This would be especially important in regions relatively inaccessible or at times of health catastrophe due to the fast local production of critical drugs [61].

Advanced methods

Artificial intelligence (AI) aided development in 3D printed medical devices

It is very much possible that AI, integrated with 3D printing, will revolutionize the creation and production of personalized medical devices. Algorithms in artificial intelligence open up great opportunities for analyzing complex data on patients, which includes anatomical scans and measurements, for the development of exactly tailored medical devices. For instance, AI could predict gait changes for a patient's prognosis over time—then, theoretically, a change in the prosthetic fit, if applied, might result in initially poor comfort and function but better long-term comfort and function with less frequent adjustments or replacement [63].

Moreover, machine learning models applied in AI technology can detect and correct anomalies during printing in real-time, thereby improving the 3D printing process. Increased reliability and consistency of 3D-printed medical devices boost their adoption and regulatory approval for use in the future. For instance, AI-driven platforms developed by 3D Systems and Enhanced automate design and delivery for patient-specific medical devices, making them streamlined to cover growing demands for healthcare solutions personalized to a particular patient [64].

Nano technique

Applying nanotechnology to 3D-printed medical devices opens new transformative opportunities for performance and efficiency at a molecular scale. Some researchers have found ways recently in which nanoparticles can be embodied within printable biomaterials, bringing forth advanced medical devices laden with new functionalities. Good examples include the control of drug release that comes with embedding nanoparticles into 3D-printed implants. These implants could deliver drugs at an exactly controlled rate or in response to determined biological triggers, thus offering a new paradigm in localized, long-term drug delivery [65].

Another application that looks promising is 'smart' implants with the use of Nanosensors. These microscopic sensors could read continuously in the local tissue environment, detecting the first appearances of infection, inflammation, or rejection of an implant. This data can be transferred wirelessly to health providers against the background of readings by those sensors, allowing a real-time information system on the performance of an implant and the health status of a patient. This development would, therefore, greatly improve patient outcomes through timely medical interventions based on precise and real data [66].

4D medication: the upcoming frontier

4D drugs delve into 3D printing, elevating the dimensionality with time or stimuli responsiveness. How the field in its nascent form could progress is mentioned here:

Shape-shifting drug-delivery systems

Such 4Dprinted pharmaceuticals could be designed to change their form or structure depending on lost stimuli within the body. For instance, a pill may unfold in the stomach to increase the retention time in the gastric region, whereby in the long run, it would result in prolonged release of the entrapped entity. For example, another option is a drug-releasing system whose surface area changes with pH, suiting better absorption in different parts of the gastrointestinal tract. This dynamism thus adapts to a change that realizes more and better delivery of the drugs [67].

Programmable release

4D drugs can be developed from the formation of highly complex programmable release means. This implies the release of different drugs or dosages at selectively controlled times or predetermined sequences in response to different physiologic signals. This can vastly increase the effectiveness of combination therapies in cancer, infectious diseases, and other serious diseases [68].

Environment-responsive drugs

Drugs in the future will be able to adapt to the internal environment of the patient. For instance, an anti-inflammatory drug will adjust the release of the medication in response to markers that suggest the state of inflammation, thereby providing an appropriate, individualistic dose that is tuned in real-time to the patient's needs. This level of personalization will allow optimal therapeutic effects with a decrease in potential side effects [69].

Targeted activation

The 4Dprinted nanoparticles or microstructures can be designed to be activated when reaching certain targets within the body. It would increase the treatment's precision by a big margin, therefore limiting side effects, while drugs will be active at the right locations.

This will go further into the future, whereby we have self-regulating 4D medication systems capable of monitoring their effectiveness and self-modifying their behavior appropriately. They could be interlinked with wearable health monitors or implantable sensors to establish a closed-loop system of optimal drug delivery. Truly, this will make a quantum jump toward personal and adaptive healthcare [70].

CONCLUSION

It is expected that this kind of integration would further leap toward personalized healthcare in both the pharmaceutical and medical device industries. In the case of 3D printing, customized device and implant designing and drug-delivery systems are feasible according to the requirements of a patient. Such capabilities in creating complex geometries, merging independent materials, and advanced features like Nano sensors or responsive elements make this new frontier of treatment and care unprecedented.

With the evolving technology, the development of 4D printing is visible, where time or stimuli-responsiveness is added as a dimension to the printed objects. We can foresee that this will ultimately lead to shape-shifting drug delivery systems and self-regulating medications responding in real-time to the physiology of the patient.

However, their adoption is challenged by scalability, cost-effectiveness, and regulatory compliance. Guarantees of quality control and standardization during the production of customized products remain a great obstacle. Nevertheless, 3D and 4D printing will continue to remain very promising frontiers of pharmaceutical and medical device innovation because of the potential for better patient outcomes, reduced healthcare expenses, and easier drug development processes.

Further down the road, as research continues to push further forward and regulatory frameworks change, we may see these technologies diffuse into changing how we think about treatment options and disease management in the decade ahead.

ABBREVIATIONS

3D (3 dimensions), 2D (2 dimensions), 4D (4 dimensions), CAD (Computer-aided design), stl.(stereolithography format), ABS (Acrylonitrile butadiene styrene), PEEK (polyetheretherketone), PEKK (Polyetherketoneketone), ULTEM (polyetherimide), UV (Ultraviolet), Stereolithography technology (SLA), Digital light processing (DLP), Continuous liquid interface production (CLIP), Fused deposition modeling (FDM), Selective laser sintering (SLS), Multi-jet fusion (MJF), DED (Directed Energy Deposition), FDA (Food and Drug Administration), USFDA (United States Food and Drug Administration), CFR (Code of Federal Regulations), MDR (Medical Device Reporting), PMA (premarket approval), IDE (investigational device exemption), GMP (Good manufacturing practice), AI(Artificial intelligence).

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CONFLICTS OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Alhnan MA, Okwuosa TC, Sadia M, Wan KW, Ahmed W, Arafat B. Emergence of 3D printed dosage forms: opportunities and challenges. Pharm Res. 2016 Aug;33(8):1817-32. doi: 10.1007/s11095-016-1933-1, PMID 27194002.
2. Goole J, Amighi K. 3D printing in pharmaceutics: a new tool for designing customized drug delivery systems. Int J Pharm. 2016 Feb 29;499(1-2):376-94. doi: 10.1016/j.ijpharm.2015.12.071, PMID 26757150.
3. Jamroz W, Szafraniec J, Kurek M, Jachowicz R. 3D printing in pharmaceutical and medical applications recent achievements and challenges. Pharm Res. 2018 Jul 11;35(9):176. doi: 10.1007/s11095-018-2454-x, PMID 29998405, PMCID PMC6061505.
4. Khoo ZX, Teoh JE, Liu Y, Chua CK, Yang S, An J. 3D printing of smart materials: a review on recent progresses in 4D printing. Virtual Phys Prototyp. 2015 Jul 3;10(3):103-22. doi: 10.1080/17452759.2015.1097054.
5. Norman J, Madurawe RD, Moore CM, Khan MA, Khairuzzaman A. A new chapter in pharmaceutical manufacturing: 3D-printed drug products. Adv Drug Deliv Rev. 2017 Jan 1;108:39-50. doi: 10.1016/j.addr.2016.03.001, PMID 27001902.
6. Sandler N, Preis M. Printed drug delivery systems for improved patient treatment. Erratum: Trends in pharmacological sciences 37, 1070-80. Trends Pharmacol Sci. 2017 Mar;38(3):317. doi: 10.1016/j.tips.2017.01.002, PMID 28111068.
7. Chia HN, WU BM. Recent advances in 3D printing of biomaterials. J Biol Eng. 2015 Mar 1;9:4. doi: 10.1186/s13036-015-0001-4, PMID 25866560, PMCID PMC4392469.
8. Nulty J, Freeman FE, Browe DC, Burdis R, Ahern DP, Pitacco P. 3D bioprinting of pre vascularised implants for the repair of critically sized bone defects. Acta Biomater. 2021 May;126:154-69. doi: 10.1016/j.actbio.2021.03.003, PMID 33705989.
9. Feng C, Zhang M, Bhandari B. Materials properties of printable edible inks and printing parameters optimization during 3d printing: a review. Crit Rev Food Sci Nutr. 2019;59(19):3074-81. doi: 10.1080/10408398.2018.1481823, PMID 29856675.
10. Shen YW, Tsai YS, Hsu JT, Shie MY, Huang HL, Fuh LJ. Biomechanical analyses of porous designs of 3D-printed titanium implant for mandibular segmental osteotomy defects. Materials (Basel). 2022 Jan 13;15(2):576. doi: 10.3390/ma15020576, PMID 35057294, PMCID PMC8779878.
11. LI KH, Kui C, Lee EK, HO CS, Wong SH, WU W. The role of 3D printing in anatomy education and surgical training: a narrative review. Med. 2017 Jun 6;6:92. doi: 10.15694/mep.2017.000092, PMID 38406430, PMCID PMC10885259.
12. Kolesky DB, Truby RL, Gladman AS, Busbee TA, Homan KA, Lewis JA. 3D bioprinting of vascularized heterogeneous cell laden tissue constructs. Adv Mater. 2014 May 21;26(19):3124-30. doi: 10.1002/adma.201305506, PMID 24550124.
13. Madla CM, Trenfield SJ, Goyanes A, Gaisford S, Basit AW. 3D printing technologies implementation and regulation: an overview. 3D Printing of Pharmaceuticals. 2018 Aug;21-40. doi: 10.1007/978-3-319-90755-0_2.
14. Williams DF. On the mechanisms of biocompatibility. Biomaterials. 2008 Jul;29(20):2941-53. doi: 10.1016/j.biomaterials.2008.04.023, PMID 18440630.
15. Zhou LY, FU J, HE Y. A review of 3D printing technologies for soft polymer materials. Adv Funct Materials. 2020 Jul;30(28):2000187. doi: 10.1002/adfm.202000187.
16. Morrison RJ, Kashlan KN, Flanangan CL, Wright JK, Green GE, Hollister SJ. Regulatory considerations in the design and manufacturing of implantable 3D-printed medical devices. Clin Transl Sci. 2015 Oct;8(5):594-600. doi: 10.1111/cts.12315, PMID 26243449, PMCID PMC4626249.
17. Zadpoor AA, Malda J. Additive manufacturing of biomaterials tissues and organs. Ann Biomed Eng. 2017 Jan;45(1):1-11. doi: 10.1007/s10439-016-1719-y, PMID 27632024.
18. Tappa K, Jammalamadaka U. Novel biomaterials used in medical 3D printing techniques. J Funct Biomater. 2018 Feb 7;9(1):17. doi: 10.3390/jfb9010017, PMID 29414913, PMCID PMC5872103.
19. Javaid M, Haleem A. Additive manufacturing applications in medical cases: a literature-based review. Alex J Med. 2018;54(4):411-22. doi: 10.1016/j.ajme.2017.09.003.
20. WU D, Spanou A, Diez Escudero A, Persson C. 3D printed PLA/HA composite structures as synthetic trabecular bone: a feasibility study using fused deposition modeling. J Mech Behav Biomed Mater. 2020 Mar;103:103608. doi: 10.1016/j.jmbbm.2019.103608, PMID 32090935.
21. Woodruff MA, Hutmacher DW. The return of a forgotten polymer polycaprolactone in the 21st century. Prog Polym Sci. 2010 Oct 1;35(10):1217-56. doi: 10.1016/j.progpolymsci.2010.04.002.
22. Agrawal CM, Ray RB. Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. J Biomed Mater Res. 2001 May;55(2):141-50. doi: 10.1002/1097-4636(200105)55:2<141::aid-jbm1000>3.0.co;2-j, PMID 11255165.
23. Rahim TN, Abdullah AM, MD Akil H, Mohamad D, Rajion ZA. The improvement of mechanical and thermal properties of polyamide 12 3D printed parts by fused deposition modelling. Express Polym Lett. 2017 Dec 1;11(12):963-82. doi: 10.3144/expresspolymlett.2017.92.
24. Pietrzak WS, Eppley BL. Resorbable polymer fixation for craniomaxillofacial surgery: development and engineering paradigms. J Craniofac Surg. 2000 Nov;11(6):575-85. doi: 10.1097/00001665-200011060-00011, PMID 11314499.
25. Rosenzweig DH, Carelli E, Steffen T, Jarzem P, Haglund L. 3D-printed ABS and PLA scaffolds for cartilage and nucleus pulposus tissue regeneration. Int J Mol Sci. 2015 Jul 3;16(7):15118-35. doi: 10.3390/ijms160715118, PMID 26151846, PMCID PMC4519890.
26. Eshraghi S, Das S. Mechanical and microstructural properties of polycaprolactone scaffolds with one dimensional two dimensional and three dimensional orthogonally oriented porous architectures produced by selective laser sintering. Acta Biomater. 2010 Jul;6(7):2467-76. doi: 10.1016/j.actbio.2010.02.002, PMID 20144914, PMCID PMC2874084.
27. Alaboodi AS, Sivasankaran S. Experimental design and investigation on the mechanical behavior of novel 3D printed biocompatibility polycarbonate scaffolds for medical applications. J Manuf Processes. 2018 Oct 1;35:479-91. doi: 10.1016/j.jmapro.2018.08.035.
28. El Magri A, Vanaei S, Vaudreuil S. An overview on the influence of process parameters through the characteristic of 3D-printed PEEK and PEI parts. High Perform Polym. 2021 Oct;33(8):862-80. doi: 10.1177/09540083211009961.
29. Mukhtarkhanov M, Perveen A, Talamona D. Application of stereolithography based 3D printing technology in investment casting. Micromachines (Basel). 2020 Oct 19;11(10):946. doi: 10.3390/mi11100946, PMID 33086736, PMCID PMC7589843.
30. Shahrubudin N, Lee TC, Ramlan RJ. An overview on 3D printing technology: technological materials and applications. Procedia Manuf. 2019 Jan 1;35:1286-96. doi: 10.1016/j.promfg.2019.06.089.
31. Rajora A, Kumar R, Singh R, Sharma S, Kapoor S, Mishra A. 3D printing: a review on the transformation of additive manufacturing. Int J App Pharm. 2022 Jul;14(4):35-47. doi: 10.22159/ijap.2022v14i4.44597.

32. Couto DS, Perez Breva L, Cooney CL. Regenerative medicine: learning from past examples. Tissue Eng Part A. 2012 Nov;18(21-22):2386-93. doi: 10.1089/ten.TEA.2011.0639, PMID 22697402.
33. Prausnitz MR, Langer R. Transdermal drug delivery. Nat Biotechnol. 2008 Nov;26(11):1261-8. doi: 10.1038/nbt.1504, PMID 18997767, PMCID PMC2700785.
34. Califf RM. Biomarker definitions and their applications. Exp Biol Med (Maywood). 2018 Feb;243(3):213-21. doi: 10.1177/1535370217750088, PMID 29405771, PMCID PMC5813875.
35. Mitra A, Dey B. Chitosan microspheres in novel drug delivery systems. Indian J Pharm Sci. 2011 Jul;73(4):355-66. doi: 10.4103/0250-474X.95607, PMID 22707817, PMCID PMC3374549.
36. Majumder S, Mondal T, Deen MJ. Wearable sensors for remote health monitoring. Sensors (Basel). 2017 Jan 12;17(1):130. doi: 10.3390/s17010130, PMID 28085085, PMCID PMC5298703.
37. Ventola CL. Medical applications for 3D printing: current and projected uses. P T. 2014 Oct;39(10):704-11. PMID 25336867, PMCID PMC4189697.
38. Wang J, Goyanes A, Gaisford S, Basit AW. Stereolithographic (SLA) 3D printing of oral modified release dosage forms. Int J Pharm. 2016 Apr 30;503(1-2):207-12. doi: 10.1016/j.ijpharm.2016.03.016, PMID 26976500.
39. Awad A, Trenfield SJ, Goyanes A, Gaisford S, Basit AW. Reshaping drug development using 3D printing. Drug Discov Today. 2018 Aug;23(8):1547-55. doi: 10.1016/j.drudis.2018.05.025, PMID 29803932.
40. DI Prima M, Coburn J, Hwang D, Kelly J, Khairuzzaman A, Ricles L. Additively manufactured medical products the FDA perspective. 3D Print Med. 2016;2:1. doi: 10.1186/s41205-016-0005-9, PMID 29974058, PMCID PMC6027614.
41. Choo YJ, Boudier Reveret M, Chang MC. 3D printing technology applied to orthosis manufacturing: narrative review. Ann Palliat Med. 2020 Nov;9(6):4262-70. doi: 10.21037/apm-20-1185, PMID 33040564.
42. Tack P, Victor J, Gemmel P, Annemans L. 3D-printing techniques in a medical setting: a systematic literature review. Biomed Eng Online. 2016 Oct 21;15(1):115. doi: 10.1186/s12938-016-0236-4, PMID 27769304, PMCID PMC5073919.
43. Thienpont E, Schwab PE, Fennema P. A systematic review and meta-analysis of patient-specific instrumentation for improving alignment of the components in total knee replacement. Bone Joint J. 2014 Aug;96-B(8):1052-61. doi: 10.1302/0301-620X.96B8.33747, PMID 25086121.
44. Sandstrom CG. The non-disruptive emergence of an ecosystem for 3D Printing insights from the hearing aid industrys transition 1989-2008. Technol Forecasting Soc Change. 2016 Jan 1;102:160-8. doi: 10.1016/j.techfore.2015.09.006.
45. Jammalamadaka U, Tappa K. Recent advances in biomaterials for 3D printing and tissue engineering. J Funct Biomater. 2018 Mar 1;9(1):22. doi: 10.3390/jfb9010022, PMID 29494503, PMCID PMC5872108.
46. Pati F, Jang J, HA DH, Won Kim S, Rhie JW, Shim JH. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun. 2014 Jun 2;5:3935. doi: 10.1038/ncomms4935, PMID 24887553, PMCID PMC4059935.
47. 3D printing of medical devices; 2020. Available from: https://www.fda.gov/medicaldevices/products-and-medical-procedures.
48. FDA. Monkeypox response; 2022. Available from: https://www.fda.gov/emergencypreparedness-and-response/mcm-issues/fdampox-response.
49. FDA's role in 3D printing; 2017. Available from: https://www.fda.gov/medical-devices/3d-printing-medical-devices/fdas-role-3d-printing.
50. India's new medical devices rules; 2017. Available from: https://www.mpo-mag.com/issues/2018-04-01.viewfeatures/indias-new-medical-devices-rules.
51. Classify your medical device; 2020. Available from: https://www.fda.gov/medical-devices/overview-device-regulation/classify-your-medical-device.
52. FDA. Premarket notification 510(k). Available from: https://www.fda.gov/medicaldevices/premarket-submissions-selecting-and-preparing-correct-submission/premarket-notification-510k.
53. FDA. Premarket approval (PMA). Available from: https://www.fda.gov/medical-devices/premarket-submissions-selecting-and-preparing-correct-submission/premarket-approval-pma.
54. FDA. Humanitarian device exemption. Available from: https://www.fda.gov/medical-devices/premarket-submissions-selecting-and-preparing-correct-submission/humanitarian-device-exemption.
55. FDA. De novo classification request. Available from: https://www.fda.gov/medical-devices/premarket-submissions-selecting-and-preparing-correct-submission/de-novo-classification-request.
56. FDA. Investigational device exemption (IDE). Available from: https://www.fda.gov/medical-devices/premarket-submissions-selecting-and-preparing-correct-submission/investigational-device-exemption-ide.
57. Trenfield SJ, Awad A, Goyanes A, Gaisford S, Basit AW. 3D printing pharmaceuticals: drug development to frontline care. Trends Pharmacol Sci. 2018 May;39(5):440-51. doi: 10.1016/j.tips.2018.02.006, PMID 29534837.
58. Awad A, Trenfield SJ, Gaisford S, Basit AW. 3D printed medicines: a new branch of digital healthcare. Int J Pharm. 2018 Sep 5;548(1):586-96. doi: 10.1016/j.ijpharm.2018.07.024, PMID 30033380.
59. Kjar A, Huang Y. Application of micro-scale 3D printing in pharmaceutics. Pharmaceutics. 2019 Aug 3;11(8):390. doi: 10.3390/pharmaceutics11080390, PMID 31382565, PMCID PMC6723578.
60. Lamichhane S, Bashyal S, Keum T, Noh G, Seo JE, Bastola R. Complex formulations simple techniques: can 3D printing technology be the midas touch in pharmaceutical industry? Asian J Pharm Sci. 2019 Sep;14(5):465-79. doi: 10.1016/j.ajps.2018.11.008, PMID 32104475, PMCID PMC7032174.
61. Elumalai A, Nayak Y, Ganapathy AK, Chen D, Tappa K, Jammalamadaka U. Reverse engineering and 3D printing of medical devices for drug delivery and drug embedded anatomic implants. Polymers (Basel). 2023 Nov 2;15(21):4306. doi: 10.3390/polym15214306, PMID 37959986, PMCID PMC10647997.
62. Alzoubi L, Aljabali AA, Tambuwala MM. Empowering precision medicine: the impact of 3D printing on personalized therapeutic. AAPS Pharm Sci Tech. 2023 Nov 14;24(8):228. doi: 10.1208/s12249-023-02682-w, PMID 37964180.
63. Rojek I, Mikołajewski D, Dostatni E, Kopowski J. Specificity of 3D printing and AI-based optimization of medical devices using the example of a group of exoskeletons. Appl Sci. 2023 Jan 12;13(2):1060. doi: 10.3390/app13021060.
64. Park JJ, Tiefenbach J, Demetriades AK. The role of artificial intelligence in surgical simulation. Front Med Technol. 2022 Dec 14;4:1076755. doi: 10.3389/fmedt.2022.1076755, PMID 36590155, PMCID PMC9794840.
65. Das M, Ambekar RS, Panda SK, Chakraborty S, Tiwary CS. 2D nanomaterials in 3D/4D-printed biomedical devices. J Mater Res. 2021 Oct 14;36(19):4024-50. doi: 10.1557/s43578-021-00287-2.
66. Dos Santos J, DE Oliveira RS, DE Oliveira TV, Velho MC, Konrad MV, DA Silva GS. 3D printing and nanotechnology: a multiscale alliance in personalized medicine. Adv Funct Materials. 2021 Apr;31(16):2009691. doi: 10.1002/adfm.202009691.
67. Pingale P, Dawre S, Dhapte Pawar V, Dhas N, Rajput A. Advances in 4D printing: from stimulation to simulation. Drug Deliv Transl Res. 2023 Jan;13(1):164-88. doi: 10.1007/s13346-022-01200-y, PMID 35751000.
68. Ahmed A, Arya S, Gupta V, Furukawa H, Khosla A. 4D printing: fundamentals materials applications and challenges. Polymer. 2021 Jul 16;228:123926. doi: 10.1016/j.polymer.2021.123926.
69. Shirazi SF, Gharehkhani S, Mehrali M, Yarmand H, Metselaar HS, Adib Kadri N. A review on powder-based additive manufacturing for tissue engineering: selective laser sintering and inkjet 3D printing. Sci Technol Adv Mater. 2015 May

5;16(3):033502. doi: 10.1088/1468-6996/16/3/033502, PMID 27877783, PMCID PMC5099820.

70. An J, Chua CK, Mironov V. A perspective on 4D bioprinting. Int J Bioprinting. 2016;2(1). doi: 10.18063/IJB.2016.01.003.
71. Yadav A, Yadav M, Yadav AK, Mishra S, Jena J, Rai JK. Printing technique: a review on the applications in pharmaceutical manufacturing. Int J Pharm Pharm Sci. 2024 Apr;16(4):11-7. doi: 10.22159/ijpps.2024v16i4.50139.
72. Pund A, Magar M, Ahirrao Y, Chaudhari A, Amritkar A. 3D printing technology: a customized advanced drug delivery. Asian J Pharm Clin Res. 2022 Aug;15(8):23-33. doi: 10.22159/ajpcr.2022.v15i8.45136.
73. Economidou SN, Lamprou DA, Douroumis D. 3D printing applications for transdermal drug delivery. Int J Pharm. 2018 Jun 15;544(2):415-24. doi: 10.1016/j.ijpharm.2018.01.031, PMID 29355656.

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Review Article

UNDERSTANDING NANO-BIO-INTERACTIONS WITH CORRESPONDING BIOLOGICAL RESPONSES: INSIGHTS AND IMPACT ON NANO ASSEMBLY AND DISASSEMBLY

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ABSTRACT

Using stimuli-responsive Bio Interactions with controlled nano-assembly is proving a potent method for generating theranostic nanosystems that satisfy the needs of modern medicine for example, targeted delivery which is very helpful for cancer treatment with minimum side effects. However, because of the limitations in our knowledge, this promising topic is still in the proof-of-concept stage. This study provides an overview of the most recent theoretical and experimental advancements in biological fate, functional activity of nano-assemblies, and nano-bio interactions with exogenous stimulus-triggered systems (Light-responsive systems, Ultrasound-responsive systems, Magnetic field-responsive systems, and Thermal-responsive systems)endogenous stimulus-triggered systems (Ph-Responsive Systems, Redox-responsive systems, Enzyme-responsive systems) and multi stimuli system**.** Related biological consequences reactions. Firstly, we intend to thoroughly explain these relationships in this review. The relationship between interaction studies and nano-based stimuli; the important physicochemical characteristics of *in vivo* stimuli, such as responsive assembly and disassembly; biological applications; and pharmacokinetic (pk) parameters based on nano-bio interaction.

Keywords: Nano-bio interaction, Nanotechnology, 2-Dimensional, Assembly, Disassembly

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INTRODUCTION

The Introduction and all sections of this review article were searched from specialized databases such as Elsevier, PubMed, Science Direct, Springer, and Google Scholar, and online published articles from the International Journal of Applied Pharmaceutics, Nature, JDDST, Nanomedicine, Nano Today, ACS Omega, etc. with searching keywords nano-bio interaction, Assembly, Disassembly, stimuli responsive. The range of the year of literature review article 1989-2023.

The range for nanoscale is 1 to 100 nanometres. Nanotechnology plays a vital role in pharmaceutical sciences, disease treatment, identification, etc. Nanotechnology gives facility to the targeted delivery, which is a very interesting topic for understanding how Nanomaterials (NMs) interact with biological molecules, to understand that concept, we discuss some important topics in this review. First, Nanomedicine entering into biological fluids, engineered nanomaterial can rapidly interact with various biomolecules, which mainly contain the three following aspects;

(1) Absorption of a biomolecule on the surface of nanomaterials (2) reconstruction and change of functional proteins and (3) redox reaction between nanomaterial and biomolecule.

Then, NMsenter the cell with different uptake inhibitors and state-of-the-art techniques such as transmission electron microscopy or confocal microscopy are used to study the cellular trafficking of NMs. The NMs react with the cells with uptake pathways, including the clathrin-mediated, caveolae-mediated, and lipid raft-mediated endocytosis and phagocytosis, as well as pinocytosis and micropinocytosis. Phagocytosis is normally for specialized cells such as monocytes and macrophages. Small size and protein adsorption in cell culture media, NMs are mostly consumed by cells through endocytosis, trapped into endosomes, entered into lysosomes, and then excluded from the cells. However, some NMs can get out of endosomes and enter other organelles such as cytosol, mitochondrion, and nucleus [1, 2].

How nano-based stimuli connect with interaction studies

Nano-based stimuli

The influence of nanotechnology in medicine is substantial, particularly in utilizing nanomaterials like metallic Nanoparticles (NPs), which offer numerous advantages. These intelligent nanomaterials are highly sought after due to their responsiveness to a range of extrinsic (e. g., optical, ultrasound, magnetic fields, and thermal state) and intrinsic (e. g., pH, redox potential, and enzymes) stimuli. Leveraging their interaction with the biological system, they hold promise for the evolution of highly effective therapeutic administration systems [3, 4].

Exogenous stimulus-triggered systems

The extrinsic stimulus-triggered system engages drug delivery through external factors like light, ultrasound, magnetic fields, and temperature. This system aims to reduce inter-patient variability by directly applying physical stimuli to the specific tissue for triggering drug release. It offers numerous advantages, enabling controlled and targeted drug release while minimizing side effects on surrounding healthy tissues [5, 6] All the factors are discussed below.

Light-responsive systems

Light-responsive systems represent an extensively explored exogenous system in drug delivery. Electromagnetic radiation with diverse wavelengths, including ultraviolet, visible, and near-infrared light, can modify the structure of light-responsive nanocarriers, thereby enabling controlled drug release at specific locations. The versatility of light as a stimulant from its adaptable nature and high precision, makes it highly applicable for targeted drug delivery and control. Photothermal effects of light, converting it into heat, have been extensively investigated. This concept involves heat generated from light activating heat-sensitive nanocarriers, disrupting their nanostructure by breaking hydrophobic and hydrophilic linkages, thereby facilitating drug release at desired sites. For instance, Li *et al.* developed nanocarriers equipped with the hydrated moieties AMD3100 and the lipophilic NIR light-to-heat converters IR780 [7]. Guardado-Alvarez *et al.* capitalized on the high spatial resolution, subcutaneous delivery, and attenuated diffusion properties of NIR radiation to achieve drug release from developed siliceous mesostructured NPs jointly with a disulfide-attached β-cyclodextrin cap. Optical-responsive bio-inert micellar therapeutic delivery systems further underscore the significance of light as a stimulus. The creation of coumarin-modified block copolymers in micelle-drug conjugates demonstrated controlled drug emit of the oncolytic

mediator 5-FU under UV irradiation (254 nm) [8]. Peng and colleagues engineered a photo-responsive hydrogel by linking trans-configurated nitrophenyl groups to dextran and pairing it with a cyclodextrin-decorated dextran. Upon exposure to light, the azobenzene is isomerized, causing the release of encapsulated entities. Utilizing Upconverting Nanoparticles (UCNPs), dynamic optically triggered substances can be charged. Xiang *et al.* developed UCNPs containing an amphipolar di-block copolymer with a UV-responsive lipophilic sphere, releasing drugs under the influence of NIR radiation (wavelength 908 nm), altering the micelle structure due to UV light absorption by the copolymer and inducing disproportion in amphiphilic balance [9-11].

Ultrasound-responsive systems

Ultrasound waves possess thermal, mechanical, and radiation force properties, which contribute to targeted drug release. The medical field widely explores ultrasound technology for imaging-guided drug delivery because of its reliability, parenchyma-penetrating ability, non-intrusion, and precise space-time domination, enabling focused treatment on specific areas [12]. In the exploration of ultrasound potential, an innovative ultrasound-reactive system loaded with doxorubicin was devised employing poly (D, L-lactide-co-glycoside)-methoxy-poly (ethylene glycol) to complex doxorubicin. This system was transformed into stable nanobubbles through boiling. Minimal drug release was observed in the absence of ultrasound exposure [13-15]. Another study focused on Microbubbles (MBs) as ultrasound-enhancing agents carrying mRNA-lipoplexesNPs. Numerous studies have utilized MBs for ultrasound-facilitated gene delivery. For instance, Hossack *et al.* developed a method utilizing ultrasound-facilitated plasmid DNA delivery from cationic MBs for vascular myocytes. Microbubbles carrying reporter plasmid DNA were sonicated near smooth muscle cells in an *in vitro* setting using varying acoustic pressures (ranging from 0 to 950 kilopascals) and pulse durations (ranging from 0 to 100 cycles) [16-18].

Magnetic field-responsive system

Recently, magnetically sensitive systems attained considerable cognitive engagement medical field account of advancements in Magnetic Nanoparticles (MNPs) and their application in biomedical and clinical domains. These systems incorporate fabricated metal and superparamagnetic oxide MNPs that exhibit responsiveness to both internal and external stimuli [19, 20]. MNPs play a pivotal role in magnetic-responsive nano-systems due to their biocompatibility and facile synthesis via various techniques such as hydrothermal methods, ignition, thermal decomposition, chemical vapor deposition, and carbon arc, among others. Studies indicate that MNPs, being small with a substantial specific surface area, facilitate cellular activities and signaling for ex vivo and in situ remote control cellular, enabling a promising potential for drug delivery systems [21, 22].

Previously, magnetoactive nanomembrane-based PNIPAAM nanogels and NPs were developed. Results indicated that these membranes were cell-bio-friendly and maintained their modifiable flow characteristics post-45 days of subcutaneous embedding [23]. Various mechanisms, including magneto-driven hyperthermia and directed therapeutic delivery, have been investigated to comprehend magnetic-reactive systems [24]. For further exploration of these mechanisms, Thirunavukkarasu *et al.* fabricated Superparamagnetic Iron Oxide NPs (SIONPs) for therapeutic applications. In this study, SIONPs and Doxorubicin (DOX) were loaded into a poly (α-hydroxy acids) (PHA) medium, reacting to the thermal effects induced by SIONPs under magnetic field influence, consequently facilitating the release of DOX. Ex-vivo assay demonstrated thermotolerance of the PHA medium, showing approximately 37 °C, drug release reached 39%, while at 45 °C, it increased to 57% [25]. In separate work, Wang *et al.* developed an implantable chitosan-based hydrogel integrated with magnetic properties, incorporating hydrophobic (rifampicin) and hydrophilic (Adriamycin) medications, exhibiting controlled drug release at targeted sites [26]. Reports indicate that alternative magnetic fields can control the timing and dosage of drug release from nano-formulations. The formulation of Pluronic/poly (ethylene imine) polymeric nanospheres stands as an illustration of an alternative magneto-sensitive system, enabling the triggered delivery of siRNA at targeted sites. Stable nano-sized polyelectrolyte complexes containing anionic siRNA-PEG conjugates facilitated the release of drugs through cleavable disulfide linkages [27]. A study introduced the utilization of triblock copolymer, poly [(acrylic acid)-block-(N-isopropyl acrylamide)-block-(acrylic acid)], in magnetic field-responsive systems. These copolymers, self-assembled into magnetic nanocarriers (SAMNs) by immobilizing amine groups on iron oxides (Fe3O4-NH2), were further grafted with folic acid for additional targeting effects. Encapsulation of the hydrophobic anticancer drug curcumin, into SAMNs resulted in enhanced curcumin release upon exposure to the magnetic field because of the paramagnetic behaviour of SAMNs [29].

Thermal responsive systems

Thermal-responsive systems represent an area of exploration in extrinsic triggered systems within medicinal fields for both intervention and assessment techniques. Tumour sites typically exhibit higher temperatures (40–42 °C) compared to healthy tissues (37 °C). Thus, heat-sensitive nanospheres sustain their load at ambient temperatures and liberate upon exposure to hyperthermia in infected sites [30]. Two primary strategies dominate the exploration of thermo-responsive systems. In the first, drug loaders are synthesized to emit the drug when exposed to hyperthermia. For instance, polycaprolactone (N-isopropyl acrylamide) (PNIPAM) conjugated with nanostructured carbon effectively released drug molecules in response to higher temperatures (40 °C). This resulted in a substantial decrease in impaired cellular functionality was observed at 40 °C, showing a 20% reduction in viability relative to the baseline viability observed at 37 °C following nanotherapeutic intervention. The secondary approach revolves around utilizing pharmaceutical carriers engineered for an immediate or rapid release triggered by elevated temperatures induced via an external stimulus. This trigger initiated a thermo-responsive agent in the vector to generate thermal energy, prompting rapid drug liberation at the intended area [31, 32]. The integration of block polymers in thermo-responsive systems has provided a new area in the pharmaceutical field. Block polymers with thermo-responsive monomers enable the manipulation of the Lower Critical Solution Temperature (LCST) to a desired point. PEG-b-PNIPAAM copolymers formed micelles via Atom Transfer Radical Polymerization (ATRP) when exceeding the thermal threshold. These micelles, comprising PNIPAM-b-PMMA, exhibited enhanced drug liberation upon reaching temperatures beyond the LCST point of 38 °C [33, 34]. The therapeutic efficacy of hydrophobic anti-neoplastic agent Paclitaxel (PTX) was notably enhanced when loaded into nanoparticles, given the formulation's prolonged vascular transit time conditions, with the LCST surpassing normal body temperature [35, 36]. An innovative method using a temperature-responsive macromolecular drug carrier, Elastin-like polypeptide (ELP), effectively localized neoplasms. ELP clusters attached to tumor blood vessels only expose tumors to 41.5 °C heat. Upon returning to febrile temperature, these particles solubilized within the plasma, augmenting vascular density and facilitating increased recanalization of ELPs penetrating neoplastic vascular networks, leading to substantial extravascular accumulation [37-39]. Another approach to achieve thermal responsiveness in drug formulation is through cryotherapy. Zhang *et al.* fabricated nanospheres with a core-shell loaded with pluronic F127 and chitosan, leveraging their inherent heat-triggered swelling and shrinking characteristic. These nanospheres, when combined with cryotherapy, demonstrated a high permeable barrier and effective encapsulation of small therapeutic agents, showing promise in disease treatment [40-42].

Endogenous stimuli-responsive systems

Biological responses can be elicited through the utilization of endogenous stimuli, including fluctuations in pH levels, variations in tissue-specific enzyme concentrations, and gradients in reduction-oxidation (redox) potentials [43].

Ph-responsive systems

The biological system encompasses distinct pH gradients across various organs, offering an advantage for pH-responsive nanocarriers. These carriers undergo conformational changes or cleavage of pH-responsive bonds at particular pH values, releasing

their cargo drugs precisely at the intended site. Hemodynamic and healthy tissues typically maintain a pH of approximately 7.4; conversely, tumor interstitial spaces and inflamed areas have an approximate pH of 6.5. Intracellular organelles like endosomes exhibit a pH of 5–6, while late lysosomes have a pH range of 4–5 [44-47]. Numerous materials exhibit responsiveness to pH stimuli, encompassing both organic and inorganic substances. Recently, dendritic polymers have gained prominence in pH stimuli-responsive systems due to their manipulative properties, including solubility, volume, and conformation [48]. Their biological effectiveness is enhanced when combined with Polyethylene glycol, altering structure, size, and biocompatibility. Moreover, coupling dendritic polymers with antitumor drugs through hydrazine bonds has shown increased effectiveness in cancer treatment [49]. pH-responsive nano-systems have been employed to deliver hydrophobic anticancer drugs to specific sites. For instance, the fabrication of Curcumin (CUR) DOX-loaded polyethylene glycol nanoparticles combined with transferrin (Tf) demonstrated accelerated release of both CUR and DOX in mildly acidic environments, highlighting the pH-dependent drug release in this setup [50]. Another innovative approach engages emergent-organization hyaluronic acid NPs using calcium phosphate to create hydroxyapatite NPs incorporated DOX. These minerals dissolve upon exposure to low pH, thereby releasing the drug at specific tumor sites [51]. Moreover, polymers comprising ionizable clusters, like amines and carbonic acids, show promise in developing pH-responsive nanospheres. Utilizing a PMAA–PMA copolymer significantly enhanced the bioequivalence of cyclosporine A, exhibiting the drug emitted at pH>6, thereby preserving the drug from acid decomposition after passing through the stomach [52]. Ulbrich *et al.* developed an HPMA (N-(2-hydroxypropyl) methacrylamide) polymer linked utilizing hydrazone groups as pH-triggered linkers for attaching the antineoplastic agent DOX. These conjugates remained stable at pH 7.4 and effectively emit the drug at pH 5. Additionally, they coupled an antibody to the polymer backbone to attain efficient directing of T cell lymphoma EL 4 cells [53-55].

Redox-responsive systems

In our biological system, the extracellular and intracellular spaces exhibit a redox potential difference of approximately ~100–1000 fold, along intercellular environment being oxidative and intracellular being reductive. An emerging paradigm shift in therapeutics involves this redox potential gradient in redox-responsive systems for targeted drug delivery [56]. Redox-sensitive nanocarriers, particularly in gene delivery, offer promising avenues for protecting plasmid DNA or siRNA outside the cell and releasing them upon cellular entry [57]. Recent investigations by Xiao *et al.* highlighted an oxidation-reduction signaling system using silica nanoparticles conjugated alongside DOX via an amphiphilic peptide, including a disulfide bond. These nanoparticles securely retained the drug DOX, exhibiting minimal escape in blood circulation and normal cells. Intracellularly, rapid and substantial drug release occurred only upon cleavage of the disulfide bond interjacent the nanospheres and the drug due to the redox potential gradient. This system capitalizes on substantial variances in GSH levels amidst tumor cells, the extracellular matrix, and normal cells, resulting in mitigated toxicity and increased tumor selectivity [58]. Similarly, Zhang *et al.* employed DOX with a PFG polymer, exploring redox-responsive systems extensively by employing a preclinical breast carcinoma prototype. They integrated thioketal, a Reactive Oxygen Species (ROS) potential stimulator for tumor cells, resulting in enhanced intracellular drug delivery via Glutathione (GSH) activation. This redox-responsive nanocarrier exhibited significantly higher drug-loading efficiency, improved stability, and enhanced cellular uptake [59]. Studies have observed that GSH can upset disulfide coupling within NPs in redox-triggered systems [60]. Additionally, Tirelli *et al.* demonstrated that polysulfide-containing nanocarriers can respond to oxidants present in their surroundings [61].

Enzyme-responsive systems

Enzyme-triggered systems primarily depend on ester hydrolysis by various enzymes within the biological system [64]. Cathepsin B (CTSB) finds extensive use in enzyme-responsive systems for site-specific drug delivery due to its amplification of carcinoma [65]. Tarassoli *et al.* investigated CTSB for the emission of indocyanine green (ICG) from polyglutamate (PGA) NPs. They developed biodegradable and self-assembled PGA-NPs incorporating ICG, reporting tumour-targeted drug emission and low toxic profile due to CTSB overexpression [66]. Mao and Gan formulated hydrophilic-phobic poly(glycidol-block-ε-caprolactone) (PG-b-PCL) encapsulating a model compound. In addition to lipase, blockage of PCL decreased, suggesting that the enzyme could still split the ester bonds owing to the kinetics of amalgamation and segregation [67, 68]. Moreover, Minko and colleagues linked paclitaxel to PAMAM G4 dendrimers and then succinate. This system released the drug when ester bonds were broken by esterase, exhibiting superior cell toxicity than the unbound moiety [69]. Aimetti *et al.* investigated a 4-arm PEG norbornene hydrogel using a peptide cross-linker with terminus thiol moiety. Drug release from this system occurred upon contact with human neutrophil elastase [70]. Additionally, Ghavami *et al.* fabricated Phospholipase-Sensitive Liposomes (PSL) as a drug-delivery system, where liposome degradation was triggered by tumor cell-derived phospholipase A2 (sPLA2). The activation of PNA release by>80% of phospholipase indicated its potential as an agent to release drugs from enzyme-responsive systems [72]. An enzyme-responsive nanomaterial based on an HPMA triblock copolymer was developed, self-assembling into NPs approximately 85 nm in diameter. This system precisely emits the antineoplastic agent paclitaxel in the cancer microenvironment [73].

Multi stimuli-responsive systems

Multi-stimuli triggered systems, capable of responding to two or more distinct stimuli, are garnering significant interest in therapeutic applications. These systems combine stimuli like pH, temperature, redox potential, magnetic fields, and more within the same matrix [74]. They function as intelligent carriers, precisely releasing their payload at targeted sites in controlled amounts [75]. Examples of multi-stimuli responsive systems include pH-redox, photo-magnetic, and thermo-redox combinations [76]. Qian *et al.* investigated a multi-stimuli system where the outcomes of the principal stimulus served as an auxiliary stimulus to enhance specificity and synergistic efficacy. They developed a conjugated polymeric nanoparticle exposed to light irradiation, resulting in O_2 generation and inducing cellular apoptosis [77]. In a related study, Lu and associates explored a tri-stimuli delivery system (redox/pH/photo-responsive) comprising organo-silica and copper sulfide nanoparticles (DOX-CuS@PMO) cross-linked by thiol bonds. Their biological evaluation using the U87MG human glioblastoma cell line and a glioblastoma mouse model demonstrated enhanced cellular internalization upon mild laser irradiation of DOX-CuS@PMO [78]. Researchers have also employed bi-stimuli systems, where pH and NIR stimulus are used to fabricate hollow mesoporous copper sulfide NPs (HMCuS NPs) loaded with DOX and coated with hyaluronic acid (HA). This system efficiently delivers the payload to tumor sites as the outer layer of DOX-loaded nanoparticles degrades due to hyaluronidase, influenced by pH and NIR. Similarly, the sensitivity of multi-responsive systems to temperature and redox potential has been explored, enabling specific drug release in the tumor microenvironment [79-81].

Section snippets

The key physicochemical parameters of *in vivo* stimuli-responsive assembly-disassembly

Nanoparticle assemblies and disassemblies play an integral role in nanoparticle functionality, ensuring stability before reaching the target site and subsequently activating their function through assembly or disassembly in situ [82]. This stimuli-responsive assembly-disassembly can lead to unexpected biological outcomes [83]. Recent studies have shown the emerging selective "turn-on" performance of *in vivo* stimulus-responsive nano-assemblies and disassemblies, demonstrating their potency in various therapeutic conditions, such as tumor treatment. Comprising eco-friendly polymers, triggered groups, and pharmaceutically bio-active molecules, these *in vivo* stimulus-responsive systems are designed for targeted drug delivery. They energetically react to the intrinsic microenvironment, enhancing therapy efficacy and enabling control over degradation speed and

clearance from the body. The strategies for delectable assembly/disassembly mechanisms revolve around corrupting the delicate equilibrium between the entropy and enthalpy of nano-systems upon cellular/extracellular stimuli in target tissues. These stimuli include the acidity of the microenvironment, overexpressed proteins/enzymes, and high levels of reduced GSH and ROS among others. Dynamic nano assembly/disassembly-based drug delivery systems are adaptable structures that change in response to biological microenvironments [85-87]. Researchers are increasingly interested in exploring stimuli-responsive controllable assembly/disassembly strategies to enhance the efficiency of drug-associated nanosystems. Typically, small units are assembled into nanoscale assemblies to achieve tumor accumulation via the Enhanced Permeability and Retention (EPR) effect, minimizing rapid excretion. Upon reaching the complex tumor microenvironment, these triggered nano assemblies undergo disassembly, releasing their inner active substances, thereby enhancing their therapeutic efficacy. Stimulus-responsive modification can also alter the characteristics of nanoparticles, particularly their size and shape, to achieve desired effects in imaging, therapy, and bioelimination. Therefore, a detailed discussion on pH-stimuli-responsive assembly-disassembly, redox-responsive assembly-disassembly, and enzyme-responsive assembly-disassembly is needed [88, 89].

Ph stimuli-responsive assembly-disassembly

The pH conditions within various human body organs, including the blood (pH 7.2–7.4), endosomes (pH 5.0–6.2), and even the tumor interstitial environment (pH 6.5–6.8), can trigger pH-stimuli-triggered assembly-disassembly processes within cells. The design of pH-responsive diagnostic nanosystems primarily relies on the protonation of specific moieties (e. g., amines). These triggers disrupt the hydrophilic-lipophilic balance, resulting in direct drug release at the target site. The wide usage of pH values at the target site serves as a broad-spectrum stimulus, ultimately enhancing pH-stimuli-responsive assembly-disassembly [90, 91]. In tumor therapy, pH-responsive disassembly has shown promise. Molecules of interest encapsulated in pH-triggered amphiphilic polymers aggregate in the hydrophobic core and undergo self-quenching. They are triggered to disassemble into highly fluorescent molecular units within the acidic tumor microenvironment [92]. Distinguishing neoplastic tissues from surrounding healthy tissues can be achieved with high specificity through pH-responsive assembly-disassembly. For instance, glycyrrhizic acid-modified gold nanoparticles assemble at normal tissue pH (pH 7.4) and disassemble at the tumor extracellular pH (pH 6.8), facilitating cellular uptake of the nanoparticles. This reversible pH-stimuli-responsive assembly-disassembly process can enhance Computed Tomography (CT) imaging for tumor therapy [93]. Redox-responsive assembly-disassembly techniques have gained attention due to the differing redox potential between normal and abnormal tissues. Research focuses on redox-sensitive linkers, such as disulfide bonds, which play a crucial role in this strategy [94]. In redox-responsive nanosystems, payload drug release occurs through the reduction of disulfide bonds, breaking the cross-linkers used for assembly, and degrading hydrophilic bonds, leading to disassembly. Advancements in redox-responsive assembly-disassembly involve utilizing Nile red-based amphiphiles bearing redox-cleavable disulfide bonds that exhibit enhanced binding to the system [95]. Disulfide bonds respond differently in the tumor microenvironment, promoting the self-assembly of small units. For instance, Liang and colleagues developed a Cys(StBu)-Lys(Ru(bpy)3 2+)-CBT probe for tumor imaging that self-assembles into Ru(bpy)3 2+NPs within cells under the influence of the redox potential [96]. Enzyme-responsive assembly-disassembly strategies leverage the catalytic activity of endogenous enzymes. Diseases often involve the overexpression of specific enzymes like Alkaline Phosphatase (ALP), matrix metalloproteinases (MMPs), and furin, paving the way for enzyme-responsive assembly-disassembly. Nanoparticles carrying drug payloads reach the target site and release drugs upon catalysis by these overexpressed enzymes, so many stimuli actions with assembly mechanisms and responses are discussed below in table 1 [97]. Drugs combined with enzyme-sensitive linkers, incorporated into amphiphilic polymers, aggregate in the hydrophobic core. Upon contact with specific enzymes overexpressed at the target site, these assemblies disassemble and release the drug [98]. Furthermore, endogenous enzymes are explored to enhance the accumulation of nanoparticles in biological systems, particularly in tumor imaging. Combining tumor probes with in situ enzyme-responsive assembly-disassembly mechanisms can "turn on" tumor-associated enzyme activity, enabling imaging in living cells [99].

Table 1: List of stimuli-responsive assembly and disassembly

Stimulus		Preparation	Responses	Assembly mechanism	Ref
pH	3 amines	2-(Dipropylamino)ethyl methacrylate (DPAMA)	Disassembly	Lipophilic effect	[100]
	Imidazole	Octadecylamine-p(API-Asp)10, Pt NPs	Disassembly	Lipophilic effect	[101]
	2-Pyridylamine	Au-DNA-αCDs	Assembly	Complementary nucleotide bonding	[102]
	Hydrazone	AuNP(Au-AK), azide	Assembly	Click chemistry	[103]
	Cytosine rich i-motif array	DNA with G-quadruplex and cytosine rich i-module array, DOX, Au NPs	Assembly	i-motif shift	[104]
Redox	Disulfide bond	Paclitaxel-loaded poly(ethylene glycol)-disulfide-paclitaxel conjugate NPs	Disassembly	Lipophilic effect	[105]
	Disulfide bond	Polyethylene glycol-polylactic acid with disulfide	Disassembly	Hydrogen bond	[106]
	Disulfide bond	Poly(ethylene glycol)-b-poly(l-lysine)-bpoly(l-phenylalanine)	Disassembly	Disulfide cross-links	[107]
	Disulfide bond	5-[4-(Prop-2-yn-1-yloxy)benzyl]-1,3-dioxolane-2,4-dione Tyr(alkynyl)-O-carboxyanhydrides, bis-(azidoethyl) disulfide as a cross-linker	Assembly	Click chemistry	[108]
Enzyme	Gelatin	Gelatin, IONPs@Au	Disassembly	Lipophilic effect	[109]
	GCNSGGRMSMPVSNGG– HYD	Maleimide-functionalized HA	Disassembly	Ionic forces	[110]
	GPLGLAGGERDG	Carboxylic acid-functionalized norbornene with GPLGLAGGERDG	Assembly	Lipophilic effect	[111]
	GPLGLAGGWGERDGS	Alex647-PPA-l (l-amino acid peptide)	Assembly	Lipophilic effect	[112]
	Pro-Leu-Gly-Val-Arg-Gelatinase	Purpurin 18-Pro-Leu-Gly-Val-Arg-Gly (P18-PLGVRG)	Assembly	π–π stacking	[113]
	Phosphate bond	Indocyanine green	Assembly	NapFFKYp Head-to-tail arrangement	[114]

ADME (Absorption distribution metabolism excretion) in nano-interaction

The pharmacokinetics of nanoscale formulations differ significantly from those of conventional formulations. Understanding the relationship between drug pharmacokinetics, encompassing absorption, distribution, metabolism, and elimination, and nanoscale preparation is crucial. The impact of characteristics (such as size, surface polarity, charge, and bioadhesive properties) of NPs on ADME profiles is paramount [115].

Size

The size of NPs plays a pivotal role in the formulation of ADME as it influences uptake by enterocytes and M cells within the biological system. Additionally, cellular uptake through the paracellular route

depends on NPs size, particularly when the NPs substance inherently enhances transmissivity by opening a permeability barrier [116]. Studies have demonstrated size-dependent absorption mechanisms; for instance, carboxylated chitosan-grafted poly (methyl methacrylate) NPs of varying sizes (300, 600, and 1000 nm) were evaluated *in vitro* using caco-2 mono-cultures and co-cultures with M cells. Smaller particles exhibited greater transportation through all routes compared to larger NPs. Similar size-dependent ADME trends have been observed in studies with decomposable PLGA NPs using the Caco-2 model [117].

Surface polarity

Surface polarity significantly affects nano-bio interactions and ADME. Enhancing NP stability through surface polarity helps prevent NP aggregation in the gut lumen and may reduce enzymatic degradation [118]. However, increased surface polarity might decrease intestinal permeability [119]. This property is often utilized to reduce protein adsorption on NP surfaces, potentially leading to reduced hepatic clearance [120].

Charge

The charge on NPs affects formulation stability, influencing NPs clustering in the gut lumen and the absorption process. Upon contact with the biological system, the charge density of NPs can undergo alterations [121]. Assimilated into the peripheral circulation, charged NPs, specifically ones, interact with plasma proteins, leading to aggregation. Superiorly charged NPs are likely to exhibit increased accumulation in target tissues [122].

Bioadhesive properties

Bioadhesive NPs impact the ADME process by prolonging the dwell span in the gut and maintaining extended interaction with gut exterior barriers, potentially improving ADME [123-124]. Recent studies have explored the impact of bioadhesive NPs not only on absorption but also on NP distribution within the biological system. For example, attaching a biocompatible layer (poly(butadiene-maleic anhydride-co-L dopa to non-biocompatible Polystyrene (PS) beads resulted in exceptional enhanced cellular intake [125, 126].

Pharmacokinetic (pk) parameter based on nano-bio interaction

The comprehensive analysis of ADME parameters concerning drug-loaded NPs can be challenging due to variations in NP formulations and their interactions within biological systems. ADME data is often confined to Cmax, Tmax, and AUC, with limited research detailed parameters like $t_{1/2}$, C, V_{dss}, or MRT [127]. Sonaje *et al.* conducted a study investigating the pharmacokinetics, pharmacodynamics (blood glucose), and biodistribution of pH-triggered NPs comprising chitosan and poly(gamma-glutamic acid). The comparison was made between oral and subcutaneous administration in rats using Single-photon Emission Computed Tomography (SPECT). The investigation results revealed that orally given aspart-insulin was incorporated into the peripheral circulation, whereas the nanocarrier was mostly held in the gut following hypodermal administration. Peak aspart-insulin concentration in peripheral tissue/plasma occurred at 20 min post-injection. A comparison of PD/PK profiles between orally administered as part-insulin and SC infusion of NPH-insulin, moderate-acting insulin preparation, suggested the potential of this nano-bio system as a non-invasive alternative for basal insulin regimen [128]. Another study, inspired by this work, focused on hepato-selective agent delivery carriers comprising chitosan/poly (ethylene glycol)–glycyrrhetinic acid NPs. The PK analysis of these NPs was conducted using single-photon emission computed tomography (SPECT), while cellular uptake was assessed using human hepatic carcinoma cells (QGY-7703 cells). Results indicated the remarkable hepato-selective ability of CTS/PEG–GA NPs, maintaining high levels during the experiment, with liver accumulation reaching 51.3% at 3 h post-injection [129].

Two-dimensional (2D) nanomaterials interactions with biological moieties

2D materials refer to substances alongside a thickness of meagernanometres, typically existing as laminate materials with intense in-plane bonds and weak van der Waals-like linkage amidst laminae. These nanomaterials have gained attention in pharmaceuticals derived their unique structural and physiochemical characteristics [130]. Their sheet-like planar morphology, held together by weak van der Waals forces, endows 2D nanomaterials with exceptional optical, electrical, and mechanical properties, elevating their significance [131]. Moreover, their large surface area-to-volume ratio allows the loading of various pharmaceutically active agents onto their surfaces through non-covalent interactions [132, 133]. This high surface tunability enables the design of biocompatible nanomaterials for applications spanning drug delivery, bioimaging, biosensors, stimulus-responsive theranostic agents, and regenerative medicine [134-136]. The interaction of 2D nanomaterials with biomedical systems, encompassing cells, cytoplasmic organelles, and biomolecules, is remarkable due to their large surface areas providing high surface energy and numerous active centers. This enhanced interaction holds potential for numerous healthcare utilization, comprising tissue engineering, additive manufacturing, neoplastic treatment, biosensing, and more [137-139]. Research into new 2D materials and their interactions with biological moieties remains a focal area. Graphene, the ancient identified 2D nanomaterial, finds applications across diverse research fields, comprising therapeutics, sensing, and energy. For instance, graphene oxide (GO), derived from graphene modified with carboxylic acid, epoxide, and hydroxyl groups, exhibits amphiphilic properties and is highly useful in pharmaceutical applications for stabilizing hydrophobic drugs in solution [140-142]. Additionally, reduced graphene oxide (rGO) synthesized via spray drying forms iron oxide-decorated rGO microspheres, displaying synergistic neoplastic treatment. The photothermal properties of rGO enable NIR stimulus, accelerating doxorubicin release and increasing temperature, responding to NIR intensity. Contrasted to 1D or 3D nanomaterials, 2D nanomaterials have the supreme therapeutic application due to their particular surface area, resulting in an abundant quantity of surface atoms compared to volume atoms [143].

Biological applications

In recent times, nanotechnology has emerged as a ground-breaking field impacting pharmaceuticals, materials science, and electronics. Nano-bio interactions in pharmaceuticals have notably advanced with the availability of various nano-based formulations exhibiting superior responses compared to conventional ones. Among these, liposomes stand out as a pivotal carrier system, demonstrating excellent responsiveness to endogenous stimuli like temperature and pH conditions, significantly enhancing therapeutic agent delivery [144-146]. In the field of nanomedicine, exploring nano-bio interfaces remains pivotal for designing safe and effective drug delivery systems, targeting pathological sites, understanding metabolism, and ensuring biocompatibility [147-149]. While the application of nano-bio interactions in pharmaceuticals, especially in drug delivery systems and oral routes, presents numerous advantages, detailed mechanistic studies are imperative to understand the influence of nanoparticles on ADME profiles, thereby enhancing formulation safety and efficacy [150]. Studies by Pascal Ickrath *et al.* highlighted the impact of nano-bio systems on dermal zinc oxide NPs formulations in human mesenchymal cells, revealing cytotoxicity at elevated doses and genomic instability at average to minimal doses, with prolonged exposure exacerbating cell necrosis impact [151]. Additionally, the work of Luisana Di Cristo *et al.* delved into nano-bio systems' role in inhalation therapies delivered via aerosol [152, 153]. Moreover, nano-biosystems play pivotal roles not only in pharmacy but also in biosensing, electronics, and imaging—the vastness of their applications beckons further exploration [154, 155]. This is achieved by controlling drug release within the tumoral vascular and interstitial space, improving liposomal clusters in tumoral tissue through increased blood flow and tumor vasculature penetrability [156]. Responsive drug delivery systems targeting the tumor microenvironment are extensively employed to enhance selectivity in tumor imaging and therapy while minimizing normal cytotoxicity, ultimately elevating therapeutic effectiveness [157, 158]. The development of pH-sensitive dynamic nano assemblies, leveraging upconversion NPs (UCNPs), offers significant therapeutic potential in cancer therapy by capitalizing on the acidic tumor microenvironment the implementation of pH-responsive drug delivery systems facilitates the precise diagnosis of minor orthotopic

Hepatocellular Carcinoma (HCC) by functioning as a Magnetic Resonance Imaging (MRI) contrast agent, pivotal for monitoring the progression of cancer [159]. Neurological diseases like Alzheimer's, Parkinson's, ALS, Huntington's disease, epilepsy, and ischemic stroke possess complex, ambiguous pathogenic mechanisms. Current diagnostic methods fall short of meeting clinical demands. Hence, there's a pressing need for sensitive, specific probes for early diagnosis and therapy. Designing DNDDS offers a new ray of hope in initial assessment, evaluation, and rational treatment of neurological diseases [160, 161]. Tissue injuries and infections often accompany inflammation, recruiting various inflammatory cells and generating ROS. This oxidative stress exacerbates injuries. Additionally, the infected site exhibits a slightly acidic microenvironment. DNDDS designed based on these micro-environmental features alleviates oxidative stress at injured/infected tissues, thereby improving therapeutic efficiency in treating injuries and infections; nano-bio interactions studies play a pivotal role in the pharma industry; some approved products are also enlisted in table 2 [162].

Table 2: Nano-bio responsive approved formulation

Approved product	Drug release	Disease	Company	Ref
Depocyt	Cytarabine	Oncogenic	Pacira Pharma	[163]
Onivyde	Irinotecan	Pancreat cancer	Merrimack pharmaceuls	[164]
Arikace	Amikacin	Lung infections	Transave Inc.	[165]
T4N5 liposlotin	T4 endonucle V	Xeroderma pigmentosum	AGI DermaticInc.	[166]
DaunoXome	Daunorubicin	Kaposi's Sarcoma	NeXstar	[167]
Marqibo	Vincristine	Acute lymphoblastic leukemia	Talon Therapeutics	[168]
OSI-211	Lurtotecan	Ovarian cancer	OSI pharma	[169]
Thermedox	Doxorubicin	Metastatic liver cancer	Celsion	[170]

Future approach

For future study, so many research problems are still in the way of understanding: The first point is a lack of complete knowledge of the biological mechanisms of nanomaterials. The second point is to effectively use nanomaterials as more intelligent therapeutic and diagnostic modalities, a regulation approach for their catalytic activity needs to be developed. The third point is that the complex environment *in vivo* must be considered in research on nano-bio interactions. The fourth and last point is more attention should be paid to theoretical simulation to accurately and deeply investigate the nano-bio interactions. Thus, more efforts should be made in the research of nano-bio interactions.

CONCLUSION

If we are heading off to advance nanomedicine, research on the nano-bio interactions of stimuli-based nanomaterials is crucial. This is because drug delivery, metabolism, pathological site targeting, safe and effective nanomedicine, and biocompatibility with minimal side effects all are impacted by nano-bio interactions. We outlined recent developments in nano-bio interactions of nanomaterials in this review. With these developments, nanomaterials will likely play a significant role in biomedicine in the future, particularly in the treatment of cancer-related illnesses.

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ABBREVIATIONS

Nanomaterials: NMs, Nanoparticles: NPs, Utilizing Upconverting Nanoparticles: UCNPs, Superparamagnetic Iron Oxide Nano Particles: SIONPs, Doxorubicin: DOX, Self-Assembled into Magnetic Nanocarriers: SAMNs, Lower Critical Solution Temperature: LCST, Atom Transfer Radical Polymerization: ATRP, Paclitaxel: PTX, Elastin-Like Polypeptide: ELP, Curcumin: CUR, Glutathione: GSH, Reactive Oxygen Species: ROS, Cathepsin B: CTSB, Phospholipase-Sensitive Liposomes: PSL, Enhanced Permeability and Retention: EPR, Computed Tomography: CT, Alkaline Phosphatase: ALP, Polystyrene: PS, Single-Photon Emission Computed Tomography: SPECT, Hepatocellular Carcinoma: HCC, Magnetic Resonance Imaging: MRI

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CONFLICT OF INTERESTS

The authors declare no competing interest

REFERENCES

1. Zhang Z, Zhang D, Wei L, Wang X, Xu Y, Li HW. Temperature responsive fluorescent polymer nanoparticles (TRFNPs) for cellular imaging and controlled releasing of drug to living cells. Colloids Surf B Biointerfaces. 2017 Nov;159:905-12. doi: 10.1016/j.colsurfb.2017.08.060, PMID 28898952.
2. Raza A, Hayat U, Rasheed T, Bilal M, Iqbal HM. Smart materials based near-infrared light responsive drug delivery systems for cancer treatment: a review. J Mater Res Technol. 2019;8(1):1497-509. doi: 10.1016/j.jmrt.2018.03.007.
3. Xiang J, Tong X, Shi F, Yan Q, Yu B, Zhao Y. Near-infrared light triggered drug release from UV-responsive diblock copolymer coated up conversion nanoparticles with high monodispersity. J Mater Chem B. 2018;6(21):3531-40. doi: 10.1039/c8tb00651b, PMID 32254448.
4. Mavuso S, Choonara YE, Marimuthu T, Kumar P, Du Toit LC, Kondiah PP. A dual pH/Redox responsive copper ligand nanoliposome bioactive complex for the treatment of chronic inflammation. Int J Pharm. 2016;509(1-2):348-59. doi: 10.1016/j.ijpharm.2016.05.069, PMID 27269194.
5. Li H, Yang X, Zhou Z, Wang K, Li C, Qiao H. Near infrared light triggered drug release from a multiple lipid carrier complex using an all-in-one strategy. J Control Release. 2017 Sep 10;261:126-37. doi: 10.1016/j.jconrel.2017.06.029, PMID 28666728.
6. Jin Q, Mitschang F, Agarwal S. Biocompatible drug delivery system for photo-triggered controlled release of 5-fluorouracil. Biomacromolecules. 2011;12(10):3684-91. doi: 10.1021/bm2009125, PMID 21863834.
7. Peng K, Tomatsu I, Kros A. Light controlled protein release from a supramolecular hydrogel. Chem Commun (Camb). 2010;46(23):4094-6. doi: 10.1039/c002565h, PMID 20464018.
8. Gwon K, Jo EJ, Sahu A, Lee JY, Kim MG, Tae G. Improved near infrared mediated hydrogel formation using diacrylated pluronic F127-coated upconversion nanoparticles. Mater Sci Eng C Mater Biol Appl. 2018 Sep 1;90:77-84. doi: 10.1016/j.msec.2018.04.029, PMID 29853148.
9. Yi C, Yu Z, Ren Q, Liu X, Wang Y, Sun X. Nanoscale ZnO based photosensitizers for photodynamic therapy. Photodiagnosis Photodyn Ther. 2020 Jun;30:101694. doi: 10.1016/j.pdpdt.2020.101694, PMID 32109615.
10. Li Q, Li W, Di H, Luo L, Zhu C, Yang J. A photosensitive liposome with NIR light triggered doxorubicin release as a combined photodynamic chemo therapy system. J Control Release. 2018 May;277:114-25. doi: 10.1016/j.jconrel.2018.02.001, PMID 29408424.
11. Luo Z, Jin K, Pang Q, Shen S, Yan Z, Jiang T. On-demand drug release from dual-targeting small nanoparticles triggered by high intensity focused ultrasound enhanced glioblastoma targeting therapy. ACS Appl Mater Interfaces. 2017;9(37):31612-25. doi: 10.1021/acsami.7b10866, PMID 28861994.

12. De Cock I, Lajoinie G, Versluis M, De Smedt SC, Lentacker I. Sonoprinting and the importance of microbubble loading for the ultrasound mediated cellular delivery of nanoparticles. Biomaterials. 2016 Mar;83:294-307. doi: 10.1016/j.biomaterials.2016.01.022, PMID 26796042.
13. Dewitte H, Vanderperren K, Haers H, Stock E, Duchateau L, Hesta M. Theranostic mRNA loaded microbubbles in the lymphatics of dogs: implications for drug delivery. Theranostics. 2015;5(1):97-109. doi: 10.7150/thno.10298, PMID 25553101.
14. Paris JL, Manzano M, Cabanas MV, Vallet Regi M. Mesoporous silica nanoparticles engineered for ultrasound-induced uptake by cancer cells. Nanoscale. 2018;10(14):6402-8. doi: 10.1039/C8NR00693H, PMID 29561558.
15. Papa AL, Korin N, Kanapathipillai M, Mammoto A, Mammoto T, Jiang A. Ultrasound sensitive nanoparticle aggregates for targeted drug delivery. Biomaterials. 2017 Sep;139:187-94. doi: 10.1016/j.biomaterials.2017.06.003, PMID 28618348.
16. Hosseini Nassab N, Samanta D, Abdolazimi Y, Annes JP, Zare RN. Electrically controlled release of insulin using polypyrrole nanoparticles. Nanoscale. 2017;9(1):143-9. doi: 10.1039/c6nr08288b, PMID 27929180.
17. Phillips LC, Klibanov AL, Wamhoff BR, Hossack JA. Targeted gene transfection from microbubbles into vascular smooth muscle cells using focused ultrasound-mediated delivery. Ultrasound Med Biol. 2010;36(9):1470-80. doi: 10.1016/j.ultrasmedbio.2010.06.010, PMID 20800174.
18. Yang HY, Li Y, Lee DS. Multifunctional and stimuli-responsive magnetic nanoparticle-based delivery systems for biomedical applications. Advanced Therapeutics. 2018;1(2):1800011. doi: 10.1002/adtp.201800011.
19. Su FY, Chen J, Son HN, Kelly AM, Convertine AJ, West TE. Polymer-augmented liposomes enhancing antibiotic delivery against intracellular infections. Biomater Sci. 2018;6(7):1976-85. doi: 10.1039/c8bm00282g, PMID 29850694.
20. Hoare T, Santamaria J, Goya Gf V. Iron oxide loaded nanotheranostics: major obstacles to *in vivo* studies and clinical translation. J Control Release. 2009;198(10):35-54.
21. Zhou X, Wang L, Xu Y, Du W, Cai X, Wang F. A pH and magnetic dual response hydrogel for synergistic chemo magnetic hyperthermia tumor therapy. RSC Adv. 2018;8(18):9812-21. doi: 10.1039/c8ra00215k, PMID 35540837.
22. Wang Y, Li B, Xu F, Han Z, Wei D, Jia D. Tough magnetic chitosan hydrogel nanocomposites for remotely stimulated drug release. Biomacromolecules. 2018;19(8):3351-60. doi: 10.1021/acs.biomac.8b00636, PMID 29995388.
23. Lee K, Bae KH, Lee Y, Lee SH, Ahn CH, Park TG. Pluronic/polyethylenimine shell crosslinked nanocapsules with embedded magnetite nanocrystals for magnetically triggered delivery of siRNA. Macromol Biosci. 2010;10(3):239-45. doi: 10.1002/mabi.200900291, PMID 19924685.
24. Zhu Y, Tao C, Sanchez Moreno P. DNA capped Fe_3O_4/SiO_2 magnetic mesoporous silica nanoparticles for potential controlled drug release and hyperthermia. Recent Adv Synth Biomed Appl Nanomater. 2015;5:29. doi: 10.1039/C5RA00701A.
25. Khoee S, Karimi MR. Dual drug-loaded Janus graphene oxide based thermoresponsive nanoparticles for targeted therapy. Polymer. 2018 Apr 25;142:80-98. doi: 10.1016/j.polymer.2018.03.022.
26. Yang J, Zhai S, Qin H, Yan H, Xing D, Hu X. NIR controlled morphology transformation and pulsatile drug delivery based on multifunctional phototheranostic nanoparticles for photoacoustic imaging guided photothermal-chemotherapy. Biomaterials. 2018 Sep;176:1-12. doi: 10.1016/j.biomaterials.2018.05.033, PMID 29842986.
27. Aathimanikandan SV, Savariar EN, Thayumanavan S. Temperature sensitive dendritic micelles. J Am Chem Soc. 2005;127(42):14922-9. doi: 10.1021/ja054542y, PMID 16231948.
28. Li Y, Pan S, Zhang W, Du Z. Novel thermo sensitive core-shell nanoparticles for targeted paclitaxel delivery. Nanotechnology. 2009;20(6):065104. doi: 10.1088/0957-4484/20/6/065104, PMID 19417372.
29. Haba Y, Kojima C, Harada A, Kono K. Comparison of thermosensitive properties of poly(amidoamine) dendrimers with peripheral N-isopropylamide groups and linear polymers with the same groups. Angew Chem Int Ed Engl. 2007;46(1-2):234-7. doi: 10.1002/anie.200603346, PMID 17124702.
30. Liu YY, Shao YH, Lu J. Preparation properties and controlled release behaviors of pH-induced thermosensitive amphiphilic gels. Biomaterials. 2006;27(21):4016-24. doi: 10.1016/j.biomaterials.2006.02.042, PMID 16563494.
31. Dreher MR, Liu W, Michelich CR, Dewhirst MW, Chilkoti A. Data from thermal cycling enhances the accumulation of a temperature-sensitive biopolymer in solid tumors. Cancer Res. 2007 May 1;67(9):4418-24. doi: 10.1158/0008-5472.CAN-06-4444.
32. Carmona Moran CA, Zavgorodnya O, Penman AD, Kharlampieva E, Bridges SL JR, Hergenrother RW. Development of gellan gum containing formulations for transdermal drug delivery: component evaluation and controlled drug release using temperature-responsive nanogels. Int J Pharm. 2016;509(1-2):465-76. doi: 10.1016/j.ijpharm.2016.05.062, PMID 27260133.
33. Gao Y, Chan Cu, Gu Q, Lin X, Zhang W, Yeo Dc. Controlled nanoparticle release from stable magnetic microbubble oscillations. NPG Asia Mater. 2016;8(4):e260, doi: 10.1038/am.2016.37.
34. Said SS, Campbell S, Hoare T. Externally addressable smart drug delivery vehicles: current technologies and future directions. Chem Mater. 2019;31(14):4971-89. doi: 10.1021/acs.chemmater.9b01798.
35. Tannock IF, Rotin D. Acid pH in tumors and its potential for therapeutic exploitation. Cancer Res. 1989;49(16):4373-84. PMID 2545340.
36. Kato Y, Ozawa S, Miyamoto C, Maehata Y, Suzuki A, Maeda T. Acidic extracellular microenvironment and cancer. Cancer Cell Int. 2013;13(1):89. doi: 10.1186/1475-2867-13-89, PMID 24004445.
37. Gerweck LE, Seetharaman K. Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. Cancer Res. 1996;56(6):1194-8. PMID 8640796.
38. Ohkuma S, Poole B. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. Proc Natl Acad Sci USA. 1978;75(7):3327-31. doi: 10.1073/pnas.75.7.3327, PMID 28524.
39. Pan D, She W, Guo C, Luo K, Yi Q, Gu Z. Pegylated dendritic diaminocyclohexyl-platinum (II) conjugates as pH-responsive drug delivery vehicles with enhanced tumor accumulation and antitumor efficacy. Biomaterials. 2014;35(38):10080-92. doi: 10.1016/j.biomaterials.2014.09.006, PMID 25263685.
40. Cui T, Zhang S, Sun H. Co-delivery of doxorubicin and pH-sensitive curcumin prodrug by transferring targeted nanoparticles for breast cancer treatment. Oncol Rep. 2017;37(2):1253-60. doi: 10.3892/or.2017.5345, PMID 28075466.
41. Han HS, Lee J, Kim HR, Chae SY, Kim M, Saravanakumar G. Robust PEGylated hyaluronic acid nanoparticles as the carrier of doxorubicin: mineralization and its effect on tumor targetability *in vivo.* J Control Release. 2013;168(2):105-14. doi: 10.1016/j.jconrel.2013.02.022, PMID 23474029.
42. Dai J, Nagai T, Wang X, Zhang T, Meng M, Zhang Q. pH-sensitive nanoparticles for improving the oral bioavailability of cyclosporine a. Int J Pharm. 2004 Aug;280(1-2):229-40. doi: 10.1016/j.ijpharm.2004.05.006, PMID 15265562.
43. Ulbrich K, Etrych T, Chytil P, Jelinkova M, Rihova B. Antibody targeted polymer-doxorubicin conjugates with pH-controlled activation. J Drug Target. 2004;12(8):477-89. doi: 10.1080/10611860400011869, PMID 15621674.
44. Bae Y, Fukushima S, Harada A, Kataoka K. Design of environment-sensitive supramolecular assemblies for intracellular drug delivery: polymeric micelles that are responsive to intracellular pH change. Angew Chem Int Ed Engl. 2003;42(38):4640-3. doi: 10.1002/anie.200250653, PMID 14533151.
45. Bae Y, Kataoka K. Intelligent polymeric micelles from functional poly(ethylene glycol) poly(amino acid) block copolymers. Adv Drug Deliv Rev. 2009;61(10):768-84. doi: 10.1016/j.addr.2009.04.016, PMID 19422866.
46. Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med.

2001;30(11):1191-212. doi: 10.1016/s0891-5849(01)00480-4, PMID 11368918.
47. Mintzer MA, Simanek EE. Nonviral vectors for gene delivery. Chem Rev. 2009;109(2):259-302. doi: 10.1021/cr800409e, PMID 19053809.
48. Xiao D, Jia HZ, Ma N, Zhuo RX, Zhang XZ. A redox-responsive mesoporous silica nanoparticle capped with amphiphilic peptides by self-assembly for cancer-targeting drug delivery. Nanoscale. 2015;7(22):10071-7. doi: 10.1039/c5nr02247a, PMID 25978679.
49. Zhang Y, Guo Q, An S, Lu Y, Li J, He X. ROS switchable polymeric nanoplatform with stimuli-responsive release for active targeted drug delivery to breast cancer. ACS Appl Mater Interfaces. 2017;9(14):12227-40. doi: 10.1021/acsami.6b16815, PMID 28350451.
50. Jhaveri AM, Torchilin VP. Multifunctional polymeric micelles for delivery of drugs and siRNA. Front Pharmacol. 2014 Apr 25;5:77. doi: 10.3389/fphar.2014.00077, PMID 24795633.
51. Ren H, Wu Y, Ma N, Xu H, Zhang X. Side chain selenium-containing amphiphilic block copolymers: redox controlled self assembly and disassembly. Soft Matter. 2012;8(5):1460-6. doi: 10.1039/C1SM06673K.
52. Oba M, Vachutinsky Y, Miyata K, Kano MR, Ikeda S, Nishiyama N. Antiangiogenic gene therapy of solid tumor by systemic injection of polyplex micelles loading plasmid DNA encoding soluble flt-1. Mol Pharm. 2010;7(2):501-9. doi: 10.1021/mp9002317, PMID 20178335.
53. Liberti MV, Locasale JW. Correction to: the warburg effect: how does it benefit cancer cells. Trends Biochem Sci. 2016;41(3):287. doi: 10.1016/j.tibs.2016.01.004, PMID 29482833.
54. Calderon M, Welker P, Licha K, Graeser R, Kratz F, Haag R. Development of efficient macromolecular prodrugs derived from dendritic polyglycerol. J Control Release. 2010;148(1):e24-5. doi: 10.1016/j.jconrel.2010.07.036, PMID 21529602.
55. Gong F, Peng X, Luo C, Shen G, Zhao C, Zou L. Cathepsin B as a potential prognostic and therapeutic marker for human lung squamous cell carcinoma. Mol Cancer. 2013;12(1):125. doi: 10.1186/1476-4598-12-125, PMID 24139065.
56. Tarassoli SP, De Pinillos Bayona AM, Pye H, Mosse CA, Callan JF, Mac Robert A. Cathepsin B-degradable NIR responsive nanoparticulate platform for target specific cancer therapy. Nanotechnology. 2017;28(5):055101. doi: 10.1088/1361-6528/28/5/055101, PMID 28029105.
57. Mao J, Gan Z. The influence of pendant hydroxyl groups on enzymatic degradation and drug delivery of amphiphilic poly [glycidol-block-(epsilon-caprolactone)] copolymers. Macromol Biosci. 2009;9(11):1080-9. doi: 10.1002/mabi.200900104, PMID 19634151.
58. Calderon M, Graeser R, Kratz F, Haag R. Development of enzymatically cleavable prodrugs derived from dendritic polyglycerol. Bioorg Med Chem Lett. 2009;19(14):3725-8. doi: 10.1016/j.bmcl.2009.05.058, PMID 19553109.
59. Khandare Jj, Jayant S, Singh A, Chandna P, Wang Y, Vorsa N. Dendrimer versus linear conjugate: influence of polymeric architecture on the delivery and anticancer effect of paclitaxel. Bioconjug Chem. 2006;17(6):1464-72. doi: 10.1021/bc060240p, PMID 17105225.
60. Aimetti AA, Machen AJ, Anseth KS. Poly(ethylene glycol) hydrogels formed by thiol-ene photopolymerization for enzyme responsive protein delivery. Biomaterials. 2009;30(30):6048-54. doi: 10.1016/j.biomaterials.2009.07.043, PMID 19674784.
61. Zhao C, Zhuang X, He P, Xiao C, He C, Sun J. Synthesis of biodegradable thermo- and pH-responsive hydrogels for controlled drug release. Polymer. 2009;50(18):4308-16. doi: 10.1016/j.polymer.2009.07.010.
62. Thamphiwatana S, Gao W, Pornpattananangkul D, Zhang Q, Fu V, Li J. Phospholipase A2-responsive antibiotic delivery via nanoparticle stabilized liposomes for the treatment of bacterial infection. J Mater Chem B. 2014;2(46):8201-7. doi: 10.1039/C4TB01110D, PMID 25544886.
63. Cai H, Wang X, Zhang H, Sun L, Pan D, Gong Q. Enzyme sensitive biodegradable and multifunctional polymeric conjugate as theranostic nanomedicine. Appl Mater Today. 2018 Jun;11:207-18. doi: 10.1016/j.apmt.2018.02.003.
64. Lei B, Chen M, Wang Y, Zhang J, Xu S, Liu H. Double security drug delivery system DDS constructed by multi-responsive (pH/redox/US) microgel. Colloids Surf B Biointerfaces. 2020;193:111022. doi: 10.1016/j.colsurfb.2020.111022, PMID 32416517.
65. Guragain S, Bastakoti BP, Malgras V, Nakashima K, Yamauchi Y. Multi stimuli-responsive polymeric materials. Chemistry. 2015;21(38):13164-74. doi: 10.1002/chem.201501101, PMID 26219746.
66. Wang L, Liu L, Dong B, Zhao H, Zhang M, Chen W. Multi stimuli responsive biohybrid nanoparticles with cross-linked albumin coronae self-assembled by a polymer protein biodynamer. Acta Biomater. 2017;54:259-70. doi: 10.1016/j.actbio.2017.03.009, PMID 28286038.
67. Qian C, Yu J, Chen Y, Hu Q, Xiao X, Sun W. Light-activated hypoxia responsive nanocarriers for enhanced anticancer therapy. Adv Mater. 2016;28(17):3313-20. doi: 10.1002/adma.201505869, PMID 26948067.
68. Lu N, Huang P, Fan W, Wang Z, Liu Y, Wang S. Tri-stimuli responsive biodegradable theranostics for mild hyperthermia enhanced chemotherapy. Biomaterials. 2017;126:39-48. doi: 10.1016/j.biomaterials.2017.02.025, PMID 28254692.
69. Hegazy M, Zhou P, Wu G, Wang L, Rahoui N, Taloub N. Construction of polymer coated core-shell magnetic mesoporous silica nanoparticles with triple responsive drug delivery. Polym Chem. 2017;8(38):5852-64. doi: 10.1039/C7PY01179B.
70. Feng Q, Zhang Y, Zhang W, Shan X, Yuan Y, Zhang H. Tumor targeted and multi-stimuli responsive drug delivery system for near-infrared light-induced chemo-phototherapy and photoacoustic tomography. Acta Biomater. 2016;38:129-42. doi: 10.1016/j.actbio.2016.04.024, PMID 27090593.
71. Li F, Lu J, Kong X, Hyeon T, Ling D. Dynamic nanoparticle assemblies for biomedical applications. Adv Mater. 2017 Apr;29:14. doi: 10.1002/adma.201605897, PMID 28224677.
72. Rabiee N, Yaraki MT, Garakani SM, Garakani SM, Ahmadi S, Lajevardi A. Recent advances in porphyrin based nanocomposites for effective targeted imaging and therapy. Biomaterials. 2020 Feb;232:119707. doi: 10.1016/j.biomaterials.2019.119707, PMID 31874428.
73. Li F, Qin Y, Lee J, Liao H, Wang N, Davis TP. Stimuli-responsive nano assemblies for remotely controlled drug delivery. J Control Release. 2020 Jun 10;322:566-92. doi: 10.1016/j.jconrel.2020.03.051, PMID 32276006.
74. Low LE, Wu J, Lee J, Tey BT, Goh BH, Gao J. Tumor responsive dynamic nanoassemblies for targeted imaging therapy and microenvironment manipulation. J Control Release. 2020 Aug 10;324:69-103. doi: 10.1016/j.jconrel.2020.05.014, PMID 32423874.
75. Hirsjarvi S, Sancey L, Dufort S, Belloche C, Vanpouille Box C, Garcion E. Effect of particle size on the biodistribution of lipid nanocapsules: comparison between nuclear and fluorescence imaging and counting. Int J Pharm. 2013;453(2):594-600. doi: 10.1016/j.ijpharm.2013.05.057, PMID 23747436.
76. Yuan Y, Zhang CJ, Kwok RT, Mao D, Tang BZ, Liu B. Light up probe based on AIE gens: dual signal turn on for caspase cascade activation monitoring. Chem Sci. 2017;8(4):2723-8. doi: 10.1039/c6sc04322d, PMID 28553507.
77. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. Nat Rev Cancer. 2011;11(9):671-7. doi: 10.1038/nrc3110, PMID 21833026.
78. Harris RJ, Cloughesy TF, Liau LM, Prins RM, Antonios JP, Li D. pH-weighted molecular imaging of gliomas using amine chemical exchange saturation transfer MRI. Neuro Oncol. 2015;17(11):1514-24. doi: 10.1093/neuonc/nov106, PMID 26113557.
79. Darwich Z, Klymchenko AS, Dujardin D, Mely Y. Imaging lipid order changes in endosome membranes of live cells by using a nile red-based membrane probe. RSC Adv. 2014;4(17):8481-8. doi: 10.1039/C3RA47181K.
80. Dou Y, Guo Y, Li X, Li X, Wang S, Wang L. Size tuning ionization to optimize gold nanoparticles for simultaneous enhanced CT imaging and radiotherapy. ACS Nano. 2016;10(2):2536-48. doi: 10.1021/acsnano.5b07473, PMID 26815933.
81. Karimi M, Ghasemi A, Sahandi Zangabad P, Rahighi R, Moosavi Basri SM, Mirshekari H. Smart micro/nanoparticles in stimulus

responsive drug/gene delivery systems. Chem Soc Rev. 2016;45(5):1457-501. doi: 10.1039/c5cs00798d, PMID 26776487.
82. Niko Y, Arntz Y, Mely Y, Konishi GI, Klymchenko AS. Disassembly driven fluorescence turn on of polymerized micelles by reductive stimuli in living cells. Chemistry. 2014;20(50):16473-7. doi: 10.1002/chem.201405040, PMID 25347980.
83. Li J, Hai Z, Xiao H, Yi X, Liang G. Intracellular self assembly of $Ru(bpy)^{32+}$ nanoparticles enables persistent phosphorescence imaging of tumors. Chem Commun (Camb). 2018;54(28):3460-3. doi: 10.1039/C8CC01759J, PMID 29560995.
84. Li H, Wang P, Deng Y, Zeng M, Tang Y, Zhu WH. Combination of active targeting enzyme triggered release and fluorescent dye into gold nanoclusters for endomicroscopy guided photothermal/photodynamic therapy to pancreatic ductal adenocarcinoma. Biomaterials. 2017 Sep;139:30-8. doi: 10.1016/j.biomaterials.2017.05.030, PMID 28582716.
85. Chien MP, Carlini AS, Hu D, Barback CV, Rush AM, Hall DJ. Enzyme-directed assembly of nanoparticles in tumors monitored by *in vivo* whole animal imaging and ex vivo super-resolution fluorescence imaging. J Am Chem Soc. 2013;135(50):18710-3. doi: 10.1021/ja408182p, PMID 24308273.
86. Ma X, Wang Y, Zhao T, Li Y, Su LC, Wang Z. Ultra pH sensitive nanoprobe library with broad pH tunability and fluorescence emissions. J Am Chem Soc. 2014;136(31):11085-92. doi: 10.1021/ja5053158, PMID 25020134.
87. Yu J, He X, Wang Z, Liu S, Hao D, Li X. Combination of starvation therapy and Pt-NP based chemotherapy for synergistic cancer treatment. J Mater Chem B. 2021;9(32):6406-11. doi: 10.1039/d1tb01222c, PMID 34318860.
88. Gao X, Yue Q, Liu Z, Ke M, Zhou X, Li S. Guiding brain tumor surgery via blood-brain barrier permeable gold nanoprobes with acid triggered MRI/SERRS signals. Adv Mater. 2017 Jun;29:21. doi: 10.1002/adma.201603917, PMID 28295679.
89. Park H, Kim J, Jung S, Kim WJ. DNA AU nanomachine equipped with i-motif and G-quadruplex for triple combinatorial anti-tumor therapy. Adv Funct Materials. 2018;28(5):1705416. doi: 10.1002/adfm.201705416.
90. Chuan X, Song Q, Lin J, Chen X, Zhang H, Dai W. Novel free paclitaxel loaded redox responsive nanoparticles based on a disulfide-linked poly (ethylene glycol)-drug conjugate for intracellular drug delivery: synthesis characterization and antitumor activity *in vitro* and *in vivo*. Mol Pharm. 2014;11(10):3656-70. doi: 10.1021/mp500399j, PMID 25208098.
91. Koo AN, Min KH, Lee HJ, Lee SU, Kim K, Kwon IC. Tumor accumulation and antitumor efficacy of docetaxel loaded core shell corona micelles with shell-specific redox responsive cross links. Biomaterials. 2012;33(5):1489-99. doi: 10.1016/j.biomaterials.2011.11.013, PMID 22130564.
92. Zhang Z, Yin L, Tu C, Song Z, Zhang Y, Xu Y. Redox responsive core cross-linked polyester micelles. ACS Macro Lett. 2013;2(1):40-4. doi: 10.1021/mz300522n, PMID 23536920.
93. Li L, Fu S, Chen C, Wang X, Fu C, Wang S. Microenvironment driven bioelimination of magnetoplasmonic nanoassemblies and their multimodal imaging guided tumor photothermal therapy. ACS Nano. 2016;10(7):7094-105. doi: 10.1021/acsnano.6b03238, PMID 27309678.
94. Purcell BP, Lobb D, Charati MB, Dorsey SM, Wade RJ, Zellars KN. Injectable and bioresponsive hydrogels for on-demand matrix metalloproteinase inhibition. Nat Mater. 2014;13(6):653-61. doi: 10.1038/nmat3922, PMID 24681647.
95. Gallo J, Kamaly N, Lavdas I, Stevens E, Nguyen QD, Wylezinska Arridge M. CXCR4-targeted and MMP-responsive iron oxide nanoparticles for enhanced magnetic resonance imaging. Angew Chem Int Ed Engl. 2014;53(36):9550-4. doi: 10.1002/anie.201405442, PMID 25045009.
96. Huang P, Gao Y, Lin J, Hu H, Liao HS, Yan X. Tumor-specific formation of enzyme instructed supramolecular self-assemblies as cancer theranostics. ACS Nano. 2015;9(10):9517-27. doi: 10.1021/acsnano.5b03874, PMID 26301492.
97. He C, Yin L, Tang C, Yin C. Size-dependent absorption mechanism of polymeric nanoparticles for oral delivery of protein drugs. Biomaterials. 2012;33(33):8569-78. doi: 10.1016/j.biomaterials.2012.07.063, PMID 22906606.
98. Verma MS, Liu S, Chen YY, Meerasa A, Gu FX. Size tunable nanoparticles composed of dextran-b-poly(D, L-lactide) for drug delivery applications. Nano Res. 2012;5(1):49-61. doi: 10.1007/s12274-011-0184-z.
99. Gaumet M, Gurny R, Delie F. Localization and quantification of biodegradable particles in an intestinal cell model: the influence of particle size. Eur J Pharm Sci. 2009;36(4-5):465-73. doi: 10.1016/j.ejps.2008.11.015, PMID 19124077.
100. Pawar VK, Meher JG, Singh Y, Chaurasia M, Surendar Reddy B, Chourasia MK. Targeting of gastrointestinal tract for amended delivery of protein/peptide therapeutics: strategies and industrial perspectives. J Control Release. 2014;196:168-83. doi: 10.1016/j.jconrel.2014.09.031, PMID 25305562.
101. Xu Q, Ensign LM, Boylan NJ, Schon A, Gong X, Yang JC. Impact of surface polyethylene glycol (PEG) density on biodegradable nanoparticle transport in mucus ex vivo and distribution *in vivo*. ACS Nano. 2015;9(9):9217-27. doi: 10.1021/acsnano.5b03876, PMID 26301576.
102. Guo J, Bourre L, Soden DM, O Sullivan GC, O Driscoll C. Can non viral technologies knockdown the barriers to siRNA delivery and achieve the next generation of cancer therapeutics? Biotechnol Adv. 2011;29(4):402-17. doi: 10.1016/j.biotechadv.2011.03.003, PMID 21435387.
103. Bourganis V, Karamanidou T, Samaridou E, Karidi K, Kammona O, Kiparissides C. On the synthesis of mucus permeating nanocarriers. Eur J Pharm Biopharm. 2015;97(A):239-49. doi: 10.1016/j.ejpb.2015.01.021, PMID 25661586.
104. Guo J, Fisher KA, Darcy R, Cryan JF, O Driscoll C. Therapeutic targeting in the silent era: advances in non-viral siRNA delivery. Mol Biosyst. 2010;6(7):1143-61. doi: 10.1039/c001050m, PMID 20431817.
105. Prego C, Fabre M, Torres D, Alonso MJ. Efficacy and mechanism of action of chitosan nanocapsules for oral peptide delivery. Pharm Res. 2006;23(3):549-56. doi: 10.1007/s11095-006-9570-8, PMID 16525861.
106. Huang Y, Leobandung W, Foss A, Peppas NA. Molecular aspects of muco and bioadhesion. J Control Release. 2000;65(1-2):63-71. doi: 10.1016/S0168-3659(99)00233-3.
107. Reineke J, Cho DY, Dingle YL, Cheifetz P, Laulicht B, Lavin D. Can bioadhesive nanoparticles allow for more effective particle uptake from the small intestine? J Control Release. 2013;170(3):477-84. doi: 10.1016/j.jconrel.2013.05.043, PMID 23796432.
108. Fan T, Chen C, Guo H, Xu J, Zhang J, Zhu X. Design and evaluation of solid lipid nanoparticles modified with peptide ligand for oral delivery of protein drugs. Eur J Pharm Biopharm. 2014;88(2):518-28. doi: 10.1016/j.ejpb.2014.06.011, PMID 24968819.
109. Longmire M, Choyke PL, Kobayashi H. Clearance properties of nano sized particles and molecules as imaging agents: considerations and caveats. Nanomedicine (Lond). 2008;3(5):703-17. doi: 10.2217/17435889.3.5.703, PMID 18817471.
110. Sonaje K, Lin KJ, Wey SP, Lin CK, Yeh TH, Nguyen HN. Biodistribution pharmacodynamics and pharmacokinetics of insulin analogues in a rat model: oral delivery using pH-responsive nanoparticles vs. subcutaneous injection. Biomaterials. 2010;31(26):6849-58. doi: 10.1016/j.biomaterials.2010.05.042, PMID 20619787.
111. Tian Q, Zhang CN, Wang XH, Wang W, Huang W, Cha RT. Glycyrrhetinic acid-modified chitosan/poly(ethylene glycol) nanoparticles for liver targeted delivery. Biomaterials. 2010;31(17):4748-56. doi: 10.1016/j.biomaterials.2010.02.042, PMID 20303163.
112. Konwarh R, Karak N, Rai SK, Mukherjee AK. Polymer-assisted iron oxide magnetic nanoparticle immobilized keratinase. Nanotechnology. 2009;20(22):225107. doi: 10.1088/0957-4484/20/22/225107, PMID 19433867.
113. Yang Z, Lu Y, Yang Z. ChemInform abstract: mesoporous materials: tunable structure morphology and composition. ChemInform. 2009;40:27. doi: 10.1002/chin.200927203.
114. Aghili Z, Taheri S, Zeinabad HA, Pishkar L, Saboury AA, Rahimi A. Investigating the interaction of Fe nanoparticles with lysozyme by biophysical and molecular docking studies. Plos

One. 2016;11(10):e0164878. doi: 10.1371/journal.pone.0164878, PMID 27776180.
115. You CC, Agasti SS, De M, Knapp MJ, Rotello VM. Modulation of the catalytic behavior of α-chymotrypsin at monolayer-protected nanoparticle surfaces. 2006 Nov 15;128:14612-8. doi: 10.1021/ja064433z.
116. Bera S, Dhar J, Dasgupta R, Basu G, Chakraborti S, Chakrabarti P. Molecular features of interaction involving hen egg white lysozyme immobilized on graphene oxide and the effect on activity. Int J Biol Macromol. 2018;120(B):2390-8. doi: 10.1016/j.ijbiomac.2018.09.007, PMID 30218729.
117. Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV. Electric field effect in atomically thin carbon films. Science. 2004;306(5696):666-9. doi: 10.1126/science.1102896, PMID 15499015.
118. Xu M, Liang T, Shi M, Chen H. Graphene like two-dimensional materials. Chem Rev. 2013;113(5):3766-98. doi: 10.1021/cr300263a, PMID 23286380.
119. Georgakilas V, Tiwari JN, Kemp KC, Perman JA, Bourlinos AB, Kim KS. Noncovalent functionalization of graphene and graphene oxide for energy materials biosensing catalytic and biomedical applications. Chem Rev. 2016;116(9):5464-519. doi: 10.1021/acs.chemrev.5b00620, PMID 27033639.
120. Ghosal K, Sarkar K. Biomedical applications of graphene nanomaterials and beyond. ACS Biomater Sci Eng. 2018;4(8):2653-703. doi: 10.1021/acsbiomaterials.8b00376, PMID 33434995.
121. Manzeli S, Ovchinnikov D, Pasquier D, Yazyev OV, Kis A. 2D transition metal dichalcogenides. Nat Rev Mater. 2017;2(8):17033. doi: 10.1038/natrevmats.2017.33.
122. McCallion C, Burthem J, Rees Unwin K, Golovanov A, Pluen A. Graphene in therapeutics delivery: problems solutions and future opportunities. Eur J Pharm Biopharm. 2016 Jul;104:235-50. doi: 10.1016/j.ejpb.2016.04.015, PMID 27113141.
123. Wu T, Jiang Q, Wu D, Hu Y, Chen S, Ding T. What is new in lysozyme research and its application in food industry? a review. Food Chem. 2019 Feb 15;274:698-709. doi: 10.1016/j.foodchem.2018.09.017, PMID 30372997.
124. Fu X, Lu Y, Guo J, Liu H, Deng A, Kuang C. K237-modified thermosensitive liposome enhanced the delivery efficiency and cytotoxicity of paclitaxel *in vitro.* J Liposome Res. 2019;29(1):86-93. doi: 10.1080/08982104.2018.1458863, PMID 29671386.
125. Monteiro LO, Lopes SC, Barros AL, Magalhaes Paniago R, Malachias A, Oliveira MC. Phase behavior of dioleyphosphatidylethanolamine molecules in the presence of components of pH-sensitive liposomes and paclitaxel. Colloids Surf B Biointerfaces. 2016 Aug 1;144:276-83. doi: 10.1016/j.colsurfb.2016.04.011, PMID 27100854.
126. Khosravi Z, Sharma S, Farnoud AM. Submicron polymeric particles accelerate insulin fibrillation by surface adsorption. Biointerphases. 2019;14(2):021001. doi: 10.1116/1.5083821, PMID 30841701.
127. Rocha S, Thunemann AF, Pereira Mdo C, Coelho M, Mohwald H, Brezesinski G. Influence of fluorinated and hydrogenated nanoparticles on the structure and fibrillogenesis of amyloid beta-peptide. Biophys Chem. 2008;137(1):35-42. doi: 10.1016/j.bpc.2008.06.010, PMID 18625543.
128. Linse S, Cabaleiro Lago C, Xue WF, Lynch I, Lindman S, Thulin E. Nucleation of protein fibrillation by nanoparticles. Proc Natl Acad Sci USA. 2007;104(21):8691-6. doi: 10.1073/pnas.0701250104, PMID 17485668.
129. Tian X, Chong Y, Ge C. Understanding the nano bio interactions and the corresponding biological responses. Front Chem. 2020 Jun 10;8:446. doi: 10.3389/fchem.2020.00446, PMID 32587847.
130. Griffin BT, Guo J, Presas E, Donovan MD, Alonso MJ, O Driscoll CM. Pharmacokinetic pharmacodynamic and biodistribution following oral administration of nanocarriers containing peptide and protein drugs. Adv Drug Deliv Rev. 2016;106(B):367-80. doi: 10.1016/j.addr.2016.06.006, PMID 27320644.
131. Ickrath P, Wagner M, Scherzad A, Gehrke T, Burghartz M, Hagen R. Time-dependent toxic and genotoxic effects of zinc oxide nanoparticles after long term and repetitive exposure to human mesenchymal stem cells. Int J Environ Res Public Health. 2017;14(12):1590. doi: 10.3390/ijerph14121590, PMID 29258234.
132. Di Cristo L, Maguire Cm, Mc Quillan K, Aleardi M, Volkov Y, Movia D. Towards the identification of an *in vitro* tool for assessing the biological behavior of aerosol supplied nanomaterials. Int J Environ Res Public Health. 2018 Mar 21;15(4):563. doi: 10.3390/ijerph15040563, PMID 29561767.
133. Roy S, Deo KA, Singh KA, Lee HP, Jaiswal A, Gaharwar AK. Nano-bio interactions of 2D molybdenum disulfide. Adv Drug Deliv Rev. 2022 Aug;187:114361. doi: 10.1016/j.addr.2022.114361, PMID 35636569.
134. Chaudhary K, Kumar K, Venkatesu P, Masram DT. In-depth understanding of a nano-bio interface between lysozyme and Au NP-immobilized N-doped reduced graphene oxide 2-D scaffolds. Nanoscale Adv. 2020;2(5):2146-59. doi: 10.1039/d0na00155d, PMID 36132509.
135. Tang Z, Xiao Y, Kong N, Liu C, Chen W, Huang X. Nano bio interfaces effect of two-dimensional nanomaterials and their applications in cancer immunotherapy. Acta Pharm Sin B. 2021;11(11):3447-64. doi: 10.1016/j.apsb.2021.05.004, PMID 34900529.
136. Ta T, Porter TM. Thermosensitive liposomes for localized delivery and triggered release of chemotherapy. J Control Release. 2013;169(1-2):112-25. doi: 10.1016/j.jconrel.2013.03.036, PMID 23583706.
137. Li F, Du Y, Liu J, Sun H, Wang J, Li R. Responsive assembly of upconversion nanoparticles for pH-activated and near-infrared triggered photodynamic therapy of deep tumors. Adv Mater. 2018;30(35):e1802808. doi: 10.1002/adma.201802808, PMID 29999559.
138. Lee YJ, Lee JM, Lee JS, Lee HY, Park BH, Kim YH. Hepatocellular carcinoma: diagnostic performance of multidetector CT and MR imaging a systematic review and meta-analysis. Radiology. 2015;275(1):97-109. doi: 10.1148/radiol.14140690, PMID 25559230.
139. Frost B, Diamond Mi. Prion-like mechanisms in neurodegenerative diseases. Nat Rev Neurosci. 2010;11(3):155-9. doi: 10.1038/nrn2786, PMID 20029438.
140. Viles JH. Metal ions and amyloid fiber formation in neurodegenerative diseases copper zinc and iron in alzheimers parkinsons and prion diseases. Coord Chem Rev. 2012;256(19-20):2271-84. doi: 10.1016/j.ccr.2012.05.003.
141. Wu H, Li F, Wang S, Lu J, Li J, Du Y. Ceria nanocrystals decorated mesoporous silica nanoparticle-based ROS scavenging tissue adhesive for highly efficient regenerative wound healing. Biomaterials. 2018;151:66-77. doi: 10.1016/j.biomaterials.2017.10.018, PMID 29078200.
142. Glantz MJ, La Follette S, Jaeckle KA, Shapiro W, Swinnen L, Rozental JR. Randomized trial of a slow release versus a standard formulation of cytarabine for the intrathecal treatment of lymphomatous meningitis. J Clin Oncol. 1999;17(10):3110-6. doi: 10.1200/JCO.1999.17.10.3110, PMID 10506606.
143. Hong K, Drummond DC, Kirpotin D. Liposomes useful for drug delivery. United States Patent US 8 Hermes Biosciences Inc Inventor Assignee; 2012. p. 147.
144. Li Z, Zhang Y, Wurtz W, Lee JK, Malinin VS, Durwas Krishnan S. Characterization of nebulized liposomal amikacin (Arikace) as a function of droplet size. J Aerosol Med Pulm Drug Deliv. 2008;21(3):245-54. doi: 10.1089/jamp.2008.0686, PMID 18759656.
145. Yarosh DB, Kibitel JT, Green LA, Spinowitz A. Enhanced unscheduled DNA synthesis in UV-irradiated human skin explants treated with T4N5 liposomes. J Invest Dermatol. 1991;97(1):147-50. doi: 10.1111/1523-1747.ep12479314, PMID 2056185.
146. Petre CE, Dittmer DP. Liposomal daunorubicin as a treatment for kaposis sarcoma. Int J Nanomedicine. 2007;2(3):277-88. PMID 18019828.
147. Sarris AH, Hagemeister F, Romaguera J, Rodriguez MA, McLaughlin P, Tsimberidou AM. Liposomal vincristine in relapsed non-hodgkins lymphomas: early results of an ongoing phase II trial. Ann Oncol. 2000;11(1):69-72. doi: 10.1023/a:1008348010437, PMID 10690390.
148. Chang HI, Yeh MK. Clinical development of liposome-based drugs: formulation characterization and therapeutic efficacy. Int

J Nanomedicine. 2012;7:49-60. doi: 10.2147/IJN.S26766, PMID 22275822.

149. Chen J, He CQ, Lin AH, Gu W, Chen ZP, Li W. Thermosensitive liposomes with higher phase transition temperature for targeted drug delivery to tumor. Int J Pharm. 2014;475(1-2):408-15. doi: 10.1016/j.ijpharm.2014.09.009, PMID 25218394.
150. Wang Y, Cai R, Chen C. The nano-bio interactions of nanomedicines: understanding the biochemical driving forces and redox reactions. Acc Chem Res. 2019;52(6):1507-18. doi: 10.1021/acs.accounts.9b00126, PMID 31149804.
151. Bhatt S, Punetha VD, Pathak R, Punetha M. Graphene in nanomedicine: a review on nano-bio factors and antibacterial activity. Colloids Surf B Biointerfaces. 2023 Jun;226:113323. doi: 10.1016/j.colsurfb.2023.113323, PMID 37116377.
152. Mahmoudi M, Landry MP, Moore A, Coreas R. The protein corona from nanomedicine to environmental science. Nat Rev Mater. 2023;8(7):422-38. doi: 10.1038/s41578-023-00552-2, PMID 37361608.
153. Saeed S, Ud Din SR, Khan SU, Gul R, Kiani FA, Wahab A. Nanoparticle: a promising player in nanomedicine and its theranostic applications for the treatment of cardiovascular diseases. Curr Probl Cardiol. 2023;48(5):101599. doi: 10.1016/j.cpcardiol.2023.101599, PMID 36681209.
154. Han X, Gong C, Yang Q, Zheng K, Wang Z, Zhang W. Biomimetic nano drug delivery system: an emerging platform for promoting tumor treatment. Int J Nanomedicine. 2024 Jan 18;19:571-608. doi: 10.2147/IJN.S442877, PMID 38260239.
155. Gong Z, Peng S, Cao J, Tan H, Zhao H, Bai J. Advances in the variations and biomedical applications of stimuli-responsive nano drug delivery systems. Nanotechnology. 2024 Jan 10;35(13). doi: 10.1088/1361-6528/ad170b, PMID 38198449.
156. Nanotechnology-based delivery systems to overcome drug resistance in cancer. Medical Review. 2024 Feb 20;4(1):5–30. doi: 10.1515/mr-2023-0058.
157. Moradi Kashkooli F, Hornsby TK, Kolios MC, Tavakkoli J. Ultrasound mediated nano-sized drug delivery systems for cancer treatment: multi-scale and multi-physics computational modeling. WIREs Nanomed Nanobiotechnol. 2024;16(1):e1913. doi: 10.1002/wnan.1913.
158. Hahn J, Ding S, IM J, Harimoto T, Leong KW, Danino T. Bacterial therapies at the interface of synthetic biology and nanomedicine. Nat Rev Bioeng. 2024;2(2):120-35. doi: 10.1038/s44222-023-00119-4, PMID 38962719.
159. Zhang P, Xiao Y, Sun X, Lin X, Koo S, Yaremenko AV. Cancer nanomedicine toward clinical translation: obstacles opportunities and future prospects. Med. 2023;4(3):147-67. doi: 10.1016/j.medj.2022.12.001, PMID 36549297.
160. Yellanki SK, Manoj AS, TM. Preparation and *in vitro* evaluation of metoprolol-loaded bovine serum albumin nanoparticles. Asian J Pharm Clin Res. 2021;14(1):213-7. doi: 10.22159/ajpcr.2021.v14i1.39738.
161. Sriramcharan P, Natarajan J, Raman R, Nagaraju G, Justin A, Senthil V. A review on green synthesis of cerium oxide nanoparticles: focus on central nervous system disorders. Int J App Pharm. 2022;14(4):94-102. doi: 10.22159/ijap.2022v14i4.44487.
162. Abdellatif MM, Ahmed SM, El-Nabarawi MA, Teaima M. Nano delivery systems for enhancing oral bioavailability of drugs. Int J App Pharm. 2023;15(1):13-9. doi: 10.22159/ijap.2023v15i1.46758.
163. Narayana S, Ahmed Mg, Nasrine A. Development of nano in situ gels of bevacizumab for the treatment of ocular angiogenesis: *in vitro* assessment of anti-angiogenesis activity and molecular docking analysis. Int J App Pharm. 2023;15(4):201-13. doi: 10.22159/ijap.2023v15i4.47860.
164. Zhang M, Lu H, Xie L, Liu X, Cun D, Yang M. Inhaled RNA drugs to treat lung diseases: disease-related cells and nano-bio interactions. Adv Drug Deliv Rev. 2023 Dec;203:115-44. doi: 10.1016/j.addr.2023.115144, PMID 37995899.
165. Saiding Q, Zhang Z, Chen S, Xiao F, Chen Y, Li Y. Nano-bio interactions in mRNA nanomedicine: challenges and opportunities for targeted mRNA delivery. Adv Drug Deliv Rev. 2023 Dec;203:115-6. doi: 10.1016/j.addr.2023.115116, PMID 37871748.
166. Liu J, Guo M, Chen C. Nano-bio interactions: a major principle in the dynamic biological processes of nano assemblies. Adv Drug Deliv Rev. 2022 Jul;186:114318. doi: 10.1016/j.addr.2022.114318, PMID 35533787.
167. Lin JH. Editor. Research article nano-bio interfacial interactions determined the contact toxicity of $nTiO_2$ to nematodes in various soils science of the total; 2022.
168. Ayushi Priyam. Multi-endpoint assessments for *in vitro* nano-bio interactions and uptake of biogenic phosphorus nanomaterials using HEK293 cells. Environ Sci: Adv. 2023:2:749-66. doi: 10.1039/D2VA00318J.
169. Shounak Roy, Kaivalya A Deo, Kanwar Abhay Singh. Nano-bio interactions of 2d molybdenum disulphide. Advanced Drug Delivery. 2022 Aug:187:114361. doi: 10.1016/j.addr.2022.114361.
170. Jagriti Gupta, Pradeep Kumar Vaid, Eepsita Priyadarshini, Paulraj Rajamani. Nano-bio convergence unveiled: systematic review on quantum dots protein interaction their implications and applications. Biophysical Chemistry. 2024 Jul;310:107238. doi: 10.1016/j.bpc.2024.107238.

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Review Article

EXPLORING POTENTIAL OF NOVEL HETEROCYCLIC COMPOUNDS AND THEIR STRUCTURE-ACTIVITY RELATIONSHIP IN PROSTATE CANCER TREATMENT

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ABSTRACT

Prostate cancer is one of the leading causes of male death globally, and its overall incidence flaunts a rising trend over the years. Currently available treatment modalities for prostate cancer suffer from severe toxicity, unpredictable efficacy, high costs, and the emergence of resistance towards anti-cancer compounds. This substantiates the need to develop novel and potent anti-proliferative agents against prostate cancer. Multiple cellular mechanisms underlie the development of prostate cancer and, thus, multiple drug gable targets. In recent years, researchers have been conducting a myriad of investigations in this direction. This work recapitulates the synthesis of 78 such molecules based on recent references. These compounds are classified and tabulated according to the moiety that they possess. Further, the review study highlights the potent member of each chemical class. In addition, the review provides fundamental insights into the design and development of such compounds through the structure-activity relationship of each series of compounds, thereby unlocking new doors for future exploration.

Keywords: Anti-cancer agents, Anti-proliferative activity, Druggable targets, Novel, Potent, Prostate cancer, Synthetic compounds

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INTRODUCTION

Cancer is a leading cause of mortality worldwide, with over 10 million deaths in 2020 [1]. According to the estimates of GLOBOCAN 2020, the predicted number of new cancer cases could reach 28.4 million in the next 20 y [2]. Prostate Cancer (PCa) is the second most common cancer in men worldwide, with an incidence rate that can go as high as 83 in 100,000 people [2, 3]. PCa being a heterogeneous disease, the diversity in clinical, spatial, morphology and molecular genetics adds to the complexity of the disease [4-6]. PCa has multifactorial etiology with a wide range of modifiable and non-modifiable risk factors [7]. Certain established factors that pose risks are elderly age, positive family history, and African ancestry [8-10]. In addition, environmental factors, dietary habits and lifestyle can have effects on risk of developing PCa and its advancement [11, 12]. Widely used treatment modalities for PCa are surgery, radiotherapy, and/or Androgen Deprivation Therapy (ADT) [13, 14]. ADT, the mainstay of treatment of metastatic hormone-sensitive PCa treatment, has high rates of relapse where PCa cells develop castration resistance and grow aggressively [15-17]. Advent of immunotherapy has tremendously revolutionized cancer treatment [18-20]. However, these novel agents suffer from drawbacks such as unpredictable efficacy, immunotoxicity and high cost [21-23]. Moreover, identifying the dominant cancer immune drivers, pose a major challenge in selecting the type of immunotherapeutic agent [24]. Hence, the quest for new drug candidates against PCa is imperative to circumvent the problems posed by currently available therapies and address unmet needs such as enhanced survival rates, minimal toxicity, improved effectiveness and lower cost. In the recent times, a wide array of molecules derived from natural sources and synthetic approaches have been tested for their potent anti-proliferative actions that can be used to treat PCa. This review study presents the preparation of significant anti-PCa compounds that are categorized based on the basic nucleus that they contain, along with the insights into their synthesis and Structure-Activity Relationship (SAR). In addition, this work identifies the most consistent and promising molecule and their targets. The reviewed compounds depict an interesting possibility to tackle PCa.

MATERIALS AND METHODS

This work reviews research articles published between 2000 to 2020 and are accessed from ScienceDirect, Scopus, Elsevier, Springer, Pubmed, web of science. The summary of preparation of potential anti-PCa compounds according to the functional group they possess as follows:

Thiazolidines and thiadiazolines

Gududuru and others reported on the synthesis of an array of 2-aryl-4-oxo-thiazolidin-3-yl amide analogues of which three 4-thiazolidinone derivatives exhibited maximum potency with an IC50 (Half-maximal inhibitory concentration) value of 39.6, 11.5 and 22.1 µM against RH 7777a cells [25].

Of the 1,3,4-thiadiazolines that were synthesized by De Monte *et al.*, N-(4-Acetyl-5-ethyl-4,5-dihydro-5-phenyl-1,3,4-thiadiazol-2-yl)acetamide was identified as the most potent agent against PC3, SKMEL-5 and SK-MEL-28 cell lines (table 1) [26].

Trifluoromethyl substituted anilide

A study carried out by Basset to *et al.* described the synthesis of trifluoromethyl substituted anilides and demonstrated three perfluorinated derivatives to exhibit the highest potency with IC50 value of 37.51, 16.28 and 7.57 µM against DU-145 cells (table 2)[28].

Dibromotyrosine analogues

Of the dibromotyrosine analogues synthesized by Sallam *et al.*, 2,6-Dibromo-1-(trans, trans-farnesyl oxy)benzene-4-acetic acid ethyl ester was identified to exhibit the highest inhibitory potency with an IC50 value of 16.5 µM (table 3)[29].

Steroid analogues

Bruno *et al.* investigated the synthesis of VN/124-1 analogs (5, 3-β-hydroxy-17-(1H-benzimidazole) androsta-5,16-diene derivatives of which 3-β-Hydroxy-17-(1H-benzimidazol-1-yl)androsta-5,16-dien was identified as the most potent agent against CYP7 with an IC50 value of 47 nM [30].

A series of Platinum(II) complexes conjugated at 7αposition of 17β-acetyl-testosterone were synthesized by Fortin *et al.*, among which 17β-acetyl testosterone-7α-platinum(II) complex exhibited the highest inhibitory potency with an IC50 value of 5.2 µM against PCacell lines [31].

Bastien *et al.* synthesized 7α-testosterone chlorambucil hybrid that binds to and inhibits androgen receptor and is employed against hormone-dependent PCa [32].

Heng *et al.* described the synthesis of Nickel (II) complex with testosterone thiosemicarbazone with IC50 of 14.1±1.2, 6.6±1.7,13.8±2.2 against PC3, LNCaP, HCT 116 cell line respectively [33].

A study carried out by Shi*et al.* described the synthesis of poly substituted steroidal pyridines and identified 2-Ethoxyl-4-(pyridin-3-yl)-6-[(3'β, 17'β)-3' (hydroxyl) androst-5'-en-17'-yl] pyridine to be the most potent cytotoxic molecule with an IC50 of 1.55 μM against PC-3 cells [34].

Sethi*et al.* developed diosgenin-indomethacin pro-drugs among which 3β, 25R-Spirost-5-ene-3yl (2, 3-dimethylphenyl) aminobenzoate was reported to be the pro-drug with highest potency [35].

Preparation of azole derivatives of[17(20)E]-21-norpregnene was reported by Dalidovich *et al.* of which 3β-hydroxy-5-ene (IC50= 10μM and 42μMagainst LNCaP and PC-3 cell lines respectively) and isoxazole moieties (IC50=72μM and 67μMagainst LNCaP and PC-3 cell lines respectively) showed the highest inhibitory potency [36].

Jorda *et al.* described the synthesis of an array of galeterone derivatives such as steroid-fused azacycles of which 3b-Acetoxy-40-methylandrost-5-eno[16,17:4,5]pyrido[2,3-b] indole was a promising lead compound with IC50 value of 0.315 μM [37].

A study carried out by Komendantova *et al.* described the synthesis of steroidal 1,3,4-thiadiazines analogues and demonstrated fbruno(N-arylcarbamoyl)17 [1′,3′,4′]thiadiazine-substituted androstenesto exhibit the highest potency with IC50 value of2.1–6.6 μM [38].

Of the new steroidal imidazoles series prepared by Hou *et al.*, 20-(1′-methylimidazol-2-yl)-20-hydroxy-pregnan-4-alkene-3-oxime was identified to possess the highest potency with IC50 of 0.5 μM for AR inhibition(table 4.) [39].

1,3-diaryl-2-propen-1-ones (Chalcones)

1,3-diaryl-2-propen-1-ones (Chalcones) analogues were synthesized by Nagaraju *et al.* and identified 1,3-disubstituted-2-propen-1-ones as potent molecules with IC50 = 8.4μM (table 5) [42].

Table 1: Synthesis of thiazolidines and thiadiazolines

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Thiazolidines and thiadiazolines				
a) 2-aryl-4-oxo-thiazolidin-3-yl-amides	Scheme 1: Mercaptoacetic acid, aldehydes of aromatic kind and glycine methyl ester are condensed so as to form ester intermediate which is further subjected to base catalyzed hydrolysis and subsequent reaction with suitable amines in the presence of 1-hydroxybenzotriazole monohydrate or ethylene dichloride. Scheme 2: In the presence of 4-dimethylaminopyridine as a catalyst, acid reacts with various isocyanates. Scheme 3: Product so formed is subjected to exhaustive reduction with tetrahydrofuran or borane under conditions of reflux or oxidation reaction using oxidizing agents such as potassium permanganate and hydrogen peroxide[25].	Lysophosphatidic Acid Receptor [27]	[25]	4-thiazolidinones
b) 1,3,4-thiadiazolines	Reaction between carbonyl compounds and thiosemicarbazide bathed in ethanol in the presence of acetic acid as catalyst produces thiosemicarbazone intermediates which when treated with symmetrical anhydrides (as solvent) forms 1,3,4-thiadiazolines. Subsequently, oxidation reaction is carried out using potassium permanganate in acetic acid in the presence of water and hydrogen peroxide [26].	Kinesin Eg5 ATPase	[26]	N-(4-Acetyl-5-ethyl-4,5-dihydro-5-phenyl-1,3,4-thiadiazol-2-yl) acetamide

Table 2: Synthesis of trifluoromethyl substituted anilides

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Trifluoromethyl substituted anilides:				
a) Bicalutamide derivatives	Suitable aniline when treated with methacryloyl chloride indimethyl acetamide generates phenylacrylamide intermediate when treated with hydrogen peroxide in excess and trifluoroacetic anhydride in dichloromethane yields suitable epoxides. Further, using commercial thiophenols and phenolsepoxides ring is cleaved to furnish an array of thioethers and ethers. Thioethers are oxidized by treatment with meta-chloroperoxybenzoic acid to produce suitable sulfones.	Androgen Receptor	[28]	Perfluorinated derivatives
b) Enzalutamide derivatives	Substituted anilines with acetone and trimethylsilyl cyanide follow Strecker reaction to furnish suitable cyanomines, which when treated with isothiocyanates in dimethylformamide and subsequent addition of hydrogen chloride and methanol yields desired product [28].			

Table 3: Synthesis of dibromo tyrosine analogues

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Dibromotyrosine analogues	Scheme 1: Phenolic hydroxyl group is esterified with different alkyl and aryl acid chlorides, employing N, N-dimethylaminopyridine catalyst. Scheme 2: In the presence of sodium hydride, the same phenolic hydroxyl group is esterified with different of alkyl and aryl bromides [29].	ATP binding site of VEGFR2	[29]	2,6-Dibromo-1-(trans, trans-Farnesyl oxy)benzene-4-acetic acid ethyl ester

Table 4: Synthesis of steroids analogues

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Steroid analogues				
a) VN/124-1 (TOK-001) derivatives				
i) 3-ξ-Fluoro-17-(1H-benzimidazol-1-yl)androsta-5,16-dien	Reaction of 3-β-Hydroxy-17-(1H-benzimidazol-1-yl)androsta-5,16-dien bathed in dichloromethane with diethyl aminosulfur trifluoride yields product.	Androgen Receptor	[30]	3-β-Hydroxy-17-(1H-benzimidazol -1-yl)androsta-5,16-dien
ii) 3-β-O-Mesyl-17-(1H-benzimidazol-1-yl)androsta-5,16-dien	Reaction between 3-β-hydroxy-17-(1H-benzimidazol-1-yl) androsta-5,16-diene in pyridine and methane sulfonyl chloride at a temperature of 0° C. The resulting solution is subsequently flooded over ice-water mixture so as to form precipitate of product.			
iii) 3-α-Azido-17-(1H-benzimidazol-1-yl)androsta-5,16-dien	Reaction between 3-β-O-Mesyl-17-(1H-benzimidazol-1-yl) androsta-5,16-dien in dimethylformamide and sodium azide, heated and poured into ice-water.			
iv) 3-β-O-Sulfamoyl-17-(1H-benzimidazol-1-yl)androsta-5,16-dien	Reaction between 3 β-hydroxy-17-(1H-benzimidazol-1-yl) androsta-5,16-diene in dimethylformamide and potassium tertiary-butoxide under cold conditions followed by treatment with sulfamoyl chloride in toluene. Unreacted reagents are inactivated by using water saturated with ammonium chloride. Further, the product formed is extracted with ethyl acetate.			
v) 3-β-Hydroxy-17-(1H-benzimidazol-1-yl)androsta-5-ene	Reaction of 3b-Hydroxy-17-(1H-benzimidazol-1-yl) androsta-5,16-dien in ethanol with hydrazine hydrate and acetic acid followed by heating, cooling, concentrating under vacuum and subsequent treatment with water saturated with sodium bicarbonate.			
vi) 3-β-Acetoxy-17-chloro-16-formyl-5-α-androstan-16-ene	Reaction of trans-androstane in pyridine at 0° C with acetic anhydride. A solution of 3-β-acetoxy-5 α-androst-17-one in dry chloroform is poured into cold and a uniform solution containing phosphorus oxychloride and dimethyl formamide. The resulting blend is further subjected to reflux under argon. Further, the concentrated mixture was poured onto ice, extracted using a cocktail of ethyl acetate and ether and subsequently washed with brine.			
vii) 3-β-Acetoxy-17-(1H-benzimidazol-1-yl)-16-formyl-5α-androstan 16-ene	Addition of 3-β-acetoxy-17-chloro-16-formyl-5-α-androstan-16-ene to a mixture of benzimidazole and potassium carbonate in dry dimethylformamide so as to form an admixture which is subsequently allowed to attain room temperature and subsequently flooded onto ice-cold water so as to obtain product precipitate.			
viii) 3-β-Acetoxy-17-(1H-benzimidazol-1-yl)-5 α-androsta-16-ene	3-β-Acetoxy-17-(1H-benzimidazol-1-yl)-16-formyl-5-α-androsta-16-ene is dissolved in dry benzonitrile to form a solution which is further refluxed using Palladium on activated charcoal as a catalyst, cooled to room temperature, filtrate is evaporated, and product obtained.			
ix) 3-β-Hydroxy-17-(1H-benzimidazol-1-yl)-5-α-androsta-16-ene.	Acetate of 3-β-Acetoxy-17-(1H-benzimidazol-1-yl) androstaneis dissolved in methanol in an atmosphere of inert argon gas, followed by reaction with 10% methanolic potassium hydroxide. The solution thus obtained is concentrated under diminished pressure poured onto ice water. White precipitate of the product obtained by filtration is washed and dried.			
x) 3-β-Acetoxy-17-(1H benzimidazol-1 yl)androstane	3-β-Acetoxy-17-(1H-benzimidazol-1-yl)-5-α-androsta-16-ene was dissolved in ethanol and treated with hydrazine hydrate, acetic acid and heat while the solution was continuously bubbled with air. The reaction mixture further cooled, concentrated, and poured onto ice cold water. Subsequently, water saturated with sodium bicarbonate was used to obtain precipitate of product.			
xi) 3-β-Hydroxy-17-(1H-benzimidazol-1-yl)-5-α androstane	Reaction of acetate of 3-β-Acetoxy-17-(1H-benzimidazol-1-yl)-5-α-androsta-16-ene in methanol with10% methanolic potassium hydroxide. The resulting mixture was stirred, concentrated and when poured onto ice water to obtain precipitate of product.			
xii) 17-(1H-Benzimidazol-1-yl)androsta-4,16-dien-3-one	An admixture of compounds N-methylmorpholine-N-oxide, 3-β-Hydroxy-17-(1H-benzimidazol-1-yl)-5-α-androstane and dichloromethane was prepared which was further subjected to treatment with tetrapropylammoniumperruthenate. To resulting solution, ethyl acetate was added to dilute and washed using aqueous solution of sodium chloride and sodium bicarbonate [30].			
b) Platinum(II) complexes conjugated at position 7a of 17 β-acetyl-testosterone				
Compounds	Reaction between 7 α-(E)-4-chlorobut-2-enyl-4-androsten-17b-ol-3-one acetate and specific amino acid using cesium carbonate bathed in methyl ethyl ketone	Androgen Receptor	[31]	17 β-acetyl testosterone-7 α-platinum(II) complex
i) 17β acetyl testosterone-7 α-tert-butyloxycarbonyl amino acids				
ii) 17 β-acetyl-testosterone-7 αamino acids	Reaction between 17 β acetyl testosterone-7 α-tert-butyloxycarbonyl amino acids and trifluoroacetic acid bathed in methylene chloride yields 17 β-acetyl-testosterone-7 αamino acids			
iii) 17 β-acetyl-testosterone-7 α-platinum(II) complexes	Reaction of 17 β-acetyl-testosterone-7 αamino acids with potassium tetrachloroplatinate bathed in a cocktail of water and dimethylformamide [31].			

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
c) 7 α-testosteronechlorambucil hybrid	Scheme 1: Synthesis involves Nucleophilic Bimolecular type of substitution reaction. First step of synthesis involves Olefin Crossmetathesis reaction of 7 α–allyltestosterone derivative using allyl chloride and Hoveyda-Grubbs catalyst in the presence of dichloromethane to yield an intermediate 7 α-(4 chloro-but-2-enyl) testosterone. This is followed by hydrolysis reaction of acetate with hydrogen chloride in methanol and substitution reaction in the presence of chlorambucil, allyl chloride and sodium bicarbonate in a cocktail of water and dimethylformamide. Scheme 2: Hydrolysis of 7α-allyltestosterone derivative in the presence of hydrogen chloride yielded 7 α–allyl testosterone. Treatment of chlorambucil with oxalyl chloride, allyl alcohol and pyridine bathed in dichloromethane produced acid allyl ester derivative. The ester and 7-αallyl testosterone in the presence of Hoveyda-Grubbs catalyst (2nd generation) in dichloromethane yields product [32].	Androgen Receptor	[32]	----
d) Nickel (II) complex with testosterone thiosemicarbazone	Reaction between estosterone in ethanol and ethanolic thiosemicarbazide and subsequent treatment with ethanol at 78° C results in formation of a Schiff base ligand composed of testosterone, thiosemicarbazide and its nickel (II) complex [33].	Androgen Receptor and DNA binding	[33]	----
e) Poly-substituted steroidal pyridines	Scheme 1: Pregnenolone and aldehydes of aromatic kind bathed in ethanol undergoes Aldol Condensation in the presence of aluminum oxide/potassium fluoride catalyst. Resulting steroidalα, β-unsaturated ketone is treated with malononitrile and sodium ethoxide followed by acetylation reaction using acetyl chloride, triethylamine, 4-dimethylaminopyridine, methylene chloride. Scheme 2: Malononitrile in the presence of sodium ethoxide undergoes 1, 4-Michael addition reaction to form an intermediate which isomerizes to produce an enamine which, when subjected to dehydration of intramolecular kind and ambient oxidation in the presence of air yields product [34].	Androgen Receptor [40]	[34]	2-Ethoxyl-4-(pyridin-3-yl) -6-[(3'β, 17'β)-3'-(hydroxyl) androst-5'-en-17'-yl]pyridine
f) Diosgenin-indomethacin pro-drugs	Mefenamic acid and indomethacin are coupled with diosgenin in the presence of an ionic liquid N-methyl-2 pyrrolidone hydrogen sulfate to generate pro-dugs [35].	Matrix metalloproteinase-2(MMP-2) and MMP-9 [41]	[35]	3β, 25R-Spirost-5-ene -3yl (2, 3-dimethyl phenyl) aminobenzoate
g) Azole analogues of [17(20)*E*]-21-norpregnene				
i) isoxazole, 1,2,3-triazole, tetrazole derivatives of [17(20)E]-21-norpregnene	1,3-dipolar cycloaddition reaction of azides or nitrile oxides to produce nitriles or acetylenes and subsequent dehydration reaction of 17 beta-hydroxy-17beta-methylene-azoles to derivatives of norpregnene	CYP17A1	[36]	3β-hydroxy-5-ene-and isoxazole moieties
ii) 1,2,4-oxadiazole derivatives of [17(20)E]-21-norpregnene	The synthesis 1,2, 4-oxadiazoles is through the generation of acetimidamides[36].			
h) Galeterone analogues including steroid-fused azacycles				
i) Steroid-fused azacycles (benzimidazolopyrimidines ii) 17-(benzimidazol-1-ylimino) steroid derivatives iii) 16-α-(benzimidazol-2-ylamino) steroid derivatives iv) 16 α-(benzothiazol-2-ylamino) steroid derivatives	Scheme 1: Condensation reaction of 2-aminobenzimidazoles and 16-dehydropregnenolone acetate yields Azacycle fused steroids. Scheme 2: Enone undergoes Aza-Michael reaction and a subsequent electrophilic substitution employing acid as a catalyst, resulting in cyclization, spontaneously occurring dehydration, and aromatization [36].	Androgen receptor	[36]	3-β-Acetoxy-40-methylandrost-5-eno[16,17:4,5]pyrido[2, 3-b] indole
i) Steroidal 1,3,4-thiadiazines				
i) Spiro[1,3,4] thiadiazine ii) 16β-hydroxyspiro-androsteno-17,6′[1,3,4]thiadiazines iii) 17-(6′*H*-1′,3′,4′-thiadiazine-2′-carboxamide)androst-5,17-dienes	Scheme 1: The traditional nucleophilic oxirane ring opening reaction of 16β,17β-epoxypregnenolone with NH-nucleophile and subsequent aromatization-driven dehydration. Scheme 2: In the presence of a catalytic quantity of sulphuric acid, reaction between thiohydrazides with both electron-withdrawing and donating groups on the aryl moiety and16β, 17β-epoxypregnenolone yields desired product. Scheme 3: Reaction of 21-bromopregna-5,16-dien-20-one with oxamic acid thiohydrazides under mild basic circumstances to yield product [38].	Androgen receptor	[38]	(N-arylcarbamoyl) 17 [1′,3′,4′]thiadiazine -substituted androstenes
j) Steroidal imidazoles	The essential intermediates are produced by the hydrolysis of 3β-hydroxy-pregnane-5-alkene-20-one-3 acetate in the presence of potassium carbonate and its hydroxy group is protected. This intermediate undergoes nucleophilic substitution reaction with N-methylimidazole in the presence of n-butyl lithium to yield product [39].	Androgen Receptor/ CYP17	[39]	20-(1′-methylimidazol-2-yl)-20-hydroxy-pregnan-4-alkene-3-oxime

Table 5: Synthesis of chalcones

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
5. 1,3-diaryl-2-propen-1-ones (Chalcones)	Scheme 1: With 3-chloro-2-methylpropene in acetone, res acetophenone undergoes Mono-allylation reaction in the presence of anhydrous potassium carbonate and catalytic amount of sodium iodide. Subsequently, Claisen rearrangement reaction occurs with allyl–aryl ether in N, N-diethyl aniline to form rearranged product, which upon treatment with catalytic amount of p-toluenesulfonic acid in chloroform results in generation of benzofuran. Phenol undergoes benzylation with treated with benzyl bromide and subsequent cyclization upon treatment with p-toluenesulfonic acid in refluxing toluene as a catalyst. Scheme 2: Claisen–Schmidt condensation reaction of ethanone with several benzaldehydes and pyrazolaldehydesin ethanolic solution of sodium hydroxide to form 1,3-disubstituted-2-propen-1-ones [42].	Androgen Receptor [43]	[42]	1,3-disubstituted-2-propen-1-ones

Table 6: Synthesis of flavonols and flavones

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Flavonols and flavones				
a) Methoxyflavonols	Scheme 1: 20-hydroxy acetophenone and benzaldehyde undergoes Claisen-Schmidt condensation reaction to yield 20-hydroxy chalcones, which under AlgareFlynneOyamada conditions (hydrogen peroxide, sodium hydroxide) generates desirable methoxy flavonols	Androgen receptor	[44]	3,3',4',5'-Tetramethoxyflavone, 6-Fluoro-3',4',5'-trimethoxyflavonol,
b) Hydroxyflavonols	Demethylation of methoxy flavonols with boron tribromide to generate corresponding hydroxy flavonols			7-Fluoro-3',4',5'-trimethoxyflavonol
c) 4',6'-difluoro-2'-hydroxyacetophenone	Scheme 2: O-acetylation of 3,5-difluorophenol and subsequent Fries rearrangement catalysed by aluminum chloride.			
d) Tetramethoxyflavone	Scheme 3: Methylation of 3',4',5'-trimethoxy-flavonol with iodomethane generates tetramethoxyflavone [44].			
a) 3-O-substituted-3',4',5'-trimethoxyflavonols	Scheme 1: Trimethoxyflavonolsare synthesized through a four-day one-pot process employing hydroxylacetophenone and trimethoxyl benzaldehyde as the precursors. Scheme 2: Trimethoxyflavonol undergoes O-alkylation reaction with suitable alkyl halide in aprotic solvent dimethylformamide and potassium carbonate as the base to generate 3-O-alkyltrimethoxyflavonols. Scheme 3: 3', 4', 5'-trimethoxyflavonol were transformed into twelve novel 3-O-aminoalkyl-3', 4', 5'-trimethoxyflavonols in 2 steps [45].	5-alpha reductase enzyme	[45]	3',4',5'-trimethoxyflavonols and 3-O-dialkylaminoalkyl-3',4',5'-trimethoxyflavonols

Table 7: Synthesis of gold (III) alkane-diamine complexes

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Gold(III) alkanediamine complexes	Trihydrate auric acid reacts with diamine ligand equivalents to produce complexes of Gold(III) alkane-diamine [46].	thioredoxin reductase	[46]	[Au(diamine)2]3+(in PC3 cells), [Au(diamine)Cl2]+(in SGC7901 and A2780/A2780 cis cells)

Table 8: Synthesis of5-substituted-3, 4-diphenyl furan-2-ones

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
5-substituted-3,4-diphenyl furan-2-ones: a) 5-alkylidene substituted-3-phenylfuran-4-(4 methanesulfonyl phenyl)-2 ones b) Rofecoxib and its analogues	Reaction of rofecoxib or itsanalogs and anhydrous sodium carbonate in methanol andrelevant aldehyde or ketone to form precipitate of product Reaction of sodium hydroxide with phenyl acetic acid or 2-chlorophenylacetic acid or 4-fluorophenylacetic acid in dimethylformamide, followed by reaction of 2-bromo-1-(4 (methylsulfonyl) phenyl) ethanone so as to form intermediate which further reacts with diisopropylamine, acidification with hydrochloric acid, forming precipitate of product [47].	Cyclooxygenases (COXs)	[47]	3-(2-chloro-phenyl)-4-(4-methanesulfonyl-phenyl)-5-(1-methoxy-ethyl)-5Hfuran-2-one

Flavonols and flavones

Britton *et al.* reported on the synthesis of flavonols of which 3,3',4',5'-Tetramethoxyflavone, 6-Fluoro-3',4',5'-trimethoxyflavonol and 7-Fluoro-3',4',5'-trimethoxyflavonol were identified as the molecules with an excellent inhibitory potency with IC50 of 2.6, 3.3 and 4.0μM respectively [44].

Xiang Li*et al.* developed a series of 3-O-substituted-3',4',5'-trimethoxyflavonols of which 3',4',5'-trimethoxyflavonols exhibited potent inhibitory action with IC50 values of 32.1 μM and 3-O-dialkylaminoalkyl-30,40,50-trimethoxyflavonols demonstrated amarginal improvement in inhibitory potency against proliferation of LNCaP cell lines (table 6) [45].

Gold (III) alkanediamine complexes

Mehboob *et al.* developed gold (III) alkanediamine complexes and demonstrated [Au(diamine)2]3+complex to exhibit highest potency with IC50 value of 1–6μMagainst PC3 cells and [Au(diamine)Cl2]+complex to exhibit highest efficacy in SGC7901 and A2780/A2780 cis cells (table 7) [46].

Diphenyl furanone analogues

Liu and others reported on the development of 5-substituted-3, 4-diphenyl furan-2-ones, of which 3-(2-chloro-phenyl)-4-(4-methanesulfonyl-phenyl)-5-(1-methoxy-ethyl)-5Hfuran-2-one possessed the most potent inhibitory action against PC3 (IC50 = 20μM), PC3 PCDNA (IC50 = 5 μM), PC3 SKP2 (IC50 = 5 μM) and DU145 cell lines (IC50 = 25 μM)(table 8) [47].

Aryl –pyrazol derivatives

Nakao *et al.* developed an array of 1-aryl-3,4-substituted-1H-pyrazol-5-ol derivatives of which 1-(5-methyl-1H-benzimidazol-2-yl)-4-benzyl-3-methyl-1H-pyrazol-5-ol demonstrated a potent inhibitory action against PCA-1/ALKBH3 both *in vitro* and *in vivo* (table 9) [48].

Table 9: Synthesis of1-aryl-3,4-substituted-1H-pyrazol-5-ol derivatives

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
1-aryl-3,4-substituted-1H-pyrazol-5-ol derivatives: a) 1,3,4-substituted-5-hydroxy-pyrazoles b) 1-(substituted benzimidazol-2-yl)-5-hydroxy-3-methyl-4-(subsituted benzyl) pyrazoles	Condensation reaction between N-substituted hydrazines and derivatives of ethyl acetoacetate [48].	PCA-1/ALKBH3	[48]	1-(5-methyl-1H-benzimidazol-2-yl)-4-benzyl-3-methyl-1H-pyrazol-5-ol

Emetine dithiocarbamate ester derivatives

Of the emetine dithiocarbamate ester derivatives synthesized by Akinboye *et al.*, 1-(3-Ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2Hpyrido[2,1-a]isoquinolin-2-yl-methyl)-6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-carbodithioic acid (4-bromophenyl carbamoyl)-methyl ester possessed potent inhibitory action against LNCaP (IC50 = 1.698 μM), PC3(IC50 = 1.507μM), and DU145 (IC50 = 1.603 μM) cell lines (table 10) [49].

Arylpiperazine derivatives

A study conducted by Chen *et al.* described the synthesis of arylpiperazine derivatives and demonstratedN-(4-(2-(4-Phenylpiperazin-1-yl)ethyl)benzyl)-1H-indole-2-carboxamide dihydrochloride (IC50 = 5.50μmol/l), N-(4-(2-(4-Phenylpiperazin-1-yl)ethyl)benzyl)benzofuran-2-carboxamide (IC50 = 5.17μmol/l) and N-(4-(2-(4-Phenylpiperazin-1-yl)ethyl)benzyl)benzo[b]thio-phene-2-carboxamide dihydrochloride (IC50 = 8.21 μmol/l) against DU145 cells (table 11) [50].

Silibinins

Vue *et al.* developed a series of silibinins of which 7-O-Methylsilibinin (IC50 of 10 2.76±0.18, 7.92±0.55, 2.39±0.97 against LNCaP, DU 145, PC-3 respectively) and 7-O-ethylsilibinin (IC50 of 2.58±0.07, 7.59±0.66, 3.25±0.31 against LNCaP, DU 145, PC-3 respectively) exhibited potent inhibitory action [51].

Vue *et al.* investigated the synthesis of 20-O-alkyl-2,3-dehydrosilybins and 5,20-O-dialkyl-2,3-dehydrosilybins. The study further demonstrated 5-O-heptyl-2,3-dehydrosilybin to be the most potent member of them all, having an IC50 value of less than 8 μM and potency 7 to 29 times more than silybin (table 12) [52].

Table 10: Synthesis of emetinedithiocarbamate ester derivatives

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Emetine dithiocarbamate ester derivatives	Ethanolic solution of sodium hydroxide containing salt of emetine dihydrochloride is treated with carbon disulfide and subsequently with different alkylating agents in acetonitrile to form dithiocarbamate ester derivatives of emetine [49].	Androgen Receptor	[49]	1-(3-Ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2Hpyrido[2,1-a]isoquinolin-2-yl-methyl)-6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-carbodithioic acid (4-bromophenylcarbamoyl)-methyl ester

Table 11: Synthesis of aryl piperazine derivatives

Type of derivative	Type of reaction involved in synthesis	Target	Ref	Potent compound
Arylpiperazine derivatives	Scheme 1: (4-(2-(4-phenylpiperazin-1-yl)ethyl)phenyl) methanamine) in toluene reacts with corresponding acid anhydride to generate an intermediate. The solution of it is prepared to which ethyl acetate is added in a dropwise manner followed by hydrochloric acid in ethyl acetate. Scheme 2: Reaction between (4-(2-(4-phenylpiperazin-1-yl)ethyl)phenyl) methanamine bathed in dichloromethane, appropriate acid,2-(7-aza-1H benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate and N, N-diisopro-pylethylamine. The solution of the intermediate is prepared to which ethyl acetate is added in a drop wise manner followed by hydrochloric acid in ethyl acetate. Scheme 3: Reduction reaction between 2-(4-(bromomethyl) phenyl)acetic acid and borane–methyl sulfide complex, followed by nucleophilic substitution reaction occurring between intermediate so obtained and 1-phenylpiperazine with methyl cyanide as solvent in the presence of potassium carbonate, followed by reaction with 4-toluene-sulfonyl chloride along with trimethylamine using dichloromethane as solvent and 4-dimethylaminopyridine as a catalyst. Finally, treatment with various phenols in the presence of potassium carbonate to obtain product [50].	Alpha 1-adrenergic receptor	[50]	N-(4-(2-(4-Phenylpiperazin-1-yl)ethyl)benzyl)-1H-indole-2-carboxamide dihydrochloride and N-(4-(2-(4-henylpiperazin-1-yl)ethyl)benzyl)benzofuran-2-carboxamide and N-(4-(2-(4-Phenylpiperazin-1-yl)ethyl)benzyl)benzo[b]thio-phene-2-carboxamide dihydr-ochloride

Table 12: Synthesis of silibinins

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Silibinins				
a) Eight 7-O-alkylsilibinins, b) Eight 7-O-alkyl-2,3-dehydrosilibinins, c) Eight 3,7-O-dialkyl-2,3 dehydrosilibinins	Selective methylation and benzylation at phenolic hydroxyl group at 7th carbon atom of silibinin in the presence of potassium carbonate and anhydrous acetone using methyl iodide and benzyl bromide [51].	Epidermal Growth Factor Receptor (EGRF) [53]	[51]	7-O-Methylsilibinin and 7-O-ethylsilibinin
i) 20-O-alkyl-2,3-dehydrosilybins and 5,20-O-dialkyl-2,3-dehydrosilybins	Under completely anaerobic conditions, silybin is converted to 7-O-benzylsilybin employing acetone solvent and equivalents of benzyl bromide and potassium carbonate. Solvent is switched from acetone to dimethylformamide and addition of benzyl bromide and potassium carbonate oxidation yields an intermediate which undergoes selective benzylation at 3-hydroxide and subsequent reaction with suitable alkyl halide using dimethylformamide as the solvent and potassium carbonate as the base to produce dibenzylsilybins that further upon treatment with ammonium formate undergoes debenzylation reaction using Palladium on activated charcoal as a catalyst.	Epidermal Growth Factor Receptor (EGRF) [53]	[52]	5-O-heptyl-2,3-dehydrosilybin
ii)5-O-alkyl-2,3-dehydrosilybins	Silybin undergoes benzylation reaction at 7thhydroxide group under complete anaerobic conditions, followed by a subsequent aerobic oxidation generates intermediate that further undergoes dibenzylation reaction occurring at 3rd OH and 20thhydroxide group to generate 3,7,20-O tribenzyl-2,3-dehydrosilybin. The intermediate thus formed undergoes alkylation reaction at of 5thhydroxide group and a subsequent global debenzylation reaction with ammonium formate using palladium carbon as catalyst to produce desired product [52].			

Table 13: Synthesis of indole derivatives

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Indole derivatives:				
i) Indeno[1,2-b]indole derivatives	Scheme 1: Indanone undergoes Fischer Indolization reaction through the formation of respective phenylhydrazones. Scheme 2: 2-nitrobenzylidenephtalide is produced either by reaction between phthalide-phosphonium bromide and 2-nitrobenzaldehyde followed by cyclization reaction or by cyclization of intramolecular type of 2-(2-nitrophenylethyl)benzoic acid. The analogues of nitrobenzylidenephtalide so formed are subjected to transformation and reduction reaction to yield desired products. Scheme 3: Reaction between ninhydrine, amines of aliphatic and aromatic kind, or enaminones of alicyclic and cyclic type producesvic-dihydroxy-indenoindolones [54].	Matrix metallo proteinases	[54]	7,7-dimethyl-5-[(3,4-dichlorophenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9 bhexahydroindeno [1,2-b]indole-9,10-dione
ii) Thiosemicarbazone-indole derivatives	Scheme 1: 2-(1H-indol-3-yl)ethan-1-amine reacts with carbon disulfide in the company of triethylamine. Further the compound so formed undergoes addition reaction with 4-dimethylaminopyridine and di-tert-butyl-dicarbonate to generate an intermediate, which upon treatment with hydrazine hydrate and a subsequent Schiff base condensation reaction with 5-methylpicolinaldehyde driven by acid-catalysis yields desired thiosemicarbazone-indole analogues Scheme 2: Reaction of 4-aminocyclohexane-1-carboxylic acid with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, hydroxybenzotriazole and 2-(1H-indol-3-yl) ethan-1-amine to yield amide derivatives. Scheme 3: Reaction of substituted amines with 6-nitronicotinic acid or 4-nitrobenzoic acid in the company of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide and 1-Hydroxybenzotriazolein dichloromethane, and the subsequent reaction with hydrogen using palladium as a catalyst to generate corresponding byproducts, which are subjected to reaction similar to that of Scheme 1 [55].	Ribonucleotide reductase	[55]	(E)-N-(2-(2-methyl-1H-indol-3-yl)ethyl)-4-(2-((5-methylpyridin-2-yl)methylene)hydrazine-1-carbothioamido)benzamide

Indole derivatives

Lobo *et al.* developed derivatives of indeno [1,2-b]indole of which 7,7-dimethyl-5-[(3,4-dichlorophenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9bhexahydroindeno[1,2-b]indole-9,10-dione possessed strong anti-proliferative potency against PC-3 (IC50 value of 10.70±0.07μM), LNCaP (9.57±0.55μM) and MatLyLu cell line (5.96±0.28μM) [54].

Of the thiosemicarbazone indole derivatives synthesized by Xu He *et al.*, (E)-N-(2-(2-methyl-1H-indol-3-yl)ethyl)-4-(2-((5-methylpyridin-2-yl)methylene)hydrazine-1-carbothioamido)benzamide demonstrated potent inhibitory action with the IC50 value of 0.054 μM (table 13) [55].

Triazole derivatives

Mandalapu *et al.* synthesized triazole hybrids of curcumin of which (3*E*,5*E*)–1–((1–(substitutedbenzyl)–1*H*–1,2,3–triazol–4–yl)methyl)–3,5–bis(substituted benzylidene) piperidin–4–ones exhibited potent action against PC-3 (IC50 = 8.8μM) and DU-145 cell lines (IC50 = 9.5μM)[56].

Madasu*et al.* reported on the synthesis of1,2,3-triazole hybrids of myrrhanone B of which meta-hydroxy phenyl 1,2,3–triazole (IC50: 6.57±0.62 μM) and deoxyuridine 1,2,3–triazole (IC50: 10.85±0.90 μM) were found to be the most potent antiproliferative agents against PC-3 cell line (table 14) [57].

Table 14: Synthesis of triazole derivatives

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Triazole derivatives				
a) Triazole hybrids of curcumin	Reaction between substituted benzaldehydes and 4–piperidinone hydrochloride hydrate with concentrated hydrochloric acid in glacial acetic acid and subsequent alkalinization with potassium carbonate in a blend of acetone and water generates substituted benzylidenepiperidin–4–onederivatives, which further reacts with propargyl bromide in acetone. Reaction of carbon disulfide with aq. potassium hydroxide in dichloromethane yields dithiocarbamate potassium salts which in turn react with propargyl bromide in a blend of water and acetone. Further substituted benzylidenepiperidin–4–one analogues or substituted benzylidene–4–oxopiperidine–1–carbodithioate undergoes reaction with substituted benzyl azides using copper sulphate pentahydrate as catalyst and sodium ascorbate [56].	Cell survival protein Akt	[56]	(3*E*,5*E*)–1–((1–(substitutedbenz-yl)–1*H*–1,2,3–triazol–4–yl)methyl)–3,5–bis(substitutedbenzylidene) piperidin–4–ones
b) myrrhanone B-1,2,3-triazole hybrids	Propargylation followed by Huisgen's 1,3-dipolar cycloaddition reaction. Reaction of (5S,8R,9R,10S)-3-oxo-8-hydroxy-30-carboxypolypoda 13E,17E,21E-triene with propargyl bromide in company of potassium carbonate in dry acetone yields an intermediate. Parallel to this, substituted aromatic azides, deoxy uridine and protected uridine azides are prepared. Intermediate formed reacts with substituted azides in sodium ascorbate and copper sulphate pentahydrate in the water-tetrahydrofuran solution's presence [57].	Epidermal Growth Factor Receptor (EGFR) [58]	[57]	Meta-hydroxy phenyl 1,2,3–triazole and deoxyuridine 1,2,3 – triazole

Table 15: Synthesis of quinoline derivatives

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Quinoline derivatives	Scheme 1: Quinolines were oxidized using m-chloroperoxybenzoic acid in chloroform at room temperature, yielding the critical intermediate quinoline N-oxide, which was then condensed with different substituted 2-aminopyridines to generate product. Scheme 2: Under basic circumstances, methyl iodide is used to oxidise substituted 8-hydroxyquinoline that is protected by a methyl group, followed by coupling reaction with suitable pyridines and subsequent demethylation using tribromoborane in a nitrogen environment to produce thioethers. Intermediates generated through coupling, on the other hand, are hydrolyzed by strong hydrochloric acid and deprotected to yield products. Scheme 3: In the presence of triethylamine, 5-substituted 8-hydroxy quinoline reacts with methanesulfonyl chloride, and subsequent N-oxidation reaction. The intermediate formed were condensed with 2-hydroxypyridine and are further hydrolyzed with sodium hydroxide to yield the appropriate ethers [59].	PIM-1 kinase (Proviral Insertion site in Moloney murine leukemia virus)	[59]	2-(pyridine-2-amino)quinolin-8-ol

Quinoline derivatives

Of the quinoline derivatives developed by Li *et al.*, 2-(pyridine-2-amino) quinolin-8-olpossessed potent antitumor activity with IC50 value of 0.75 μM (table 15) [59].

Diheteroarylnona-tetraen-ones

Zhang *et al.* synthesized 1,9-diheteroarylnona-1,3,6,8-tetraen-5-ones, of which (1*E*,3*E*,6*E*,8*E*)-1,9-Bis(3-fluoropyridin-4-yl)nona-1,3,6,8-tetraen-5-one (IC50 value 2.36±0.56 μM, 1.21±0.43 μM, 2.43±1.31 μM against PC-3, DU145, LNCaPcell lines respectively) and (1E,3E,6E,8E)-1,9-Bis(1-(pentan-3-yl)-1H-imidazol-2-yl)nona-1,3,6,8-tetraen-5-one (IC50 value 1.14±0.12 μM, 1.78±0.13 μM, 2.17±0.2 μM against PC-3, DU145, LNCaPcell lines respectively) were the two most potent members (table 16) [60].

Boswellic acid derivatives

Li *et al.* described the synthesis of acetyl-11-keto-β-boswellic acid derivatives of which N-(2-cyano-3,11-dioxo-ursan-1,12-dien-24-oyl)-piperazine exhibited a potent inhibitory action and demonstrated IC50 value of 0.04 and 0.27 μM against PC-3 and LNCaP cell lines respectively [62].

Huang *et al.* investigated on the synthesis of ring-A modified 11-keto-boswellic acid derivatives of which 3-oxo-2-carboxylmethylene derivative of 11-keto-boswellic acid derivatives showed potent action with 0.46 μMIC50 value(table 17) [63].

Table 16: Synthesis of1,9-diheteroarylnona-1,3,6,8-tetraen-5-ones

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
1,9-Diheteroarylnona-1,3,6,8-tetraen-5-ones	Using potassium carbonate as base and water and ethanol as co-solvents,1,3 bis(diethyl phosphonate) acetone and (E)-3-aryl-2-propenal undergoes Horner-Wadsworth-Emmons reaction. The (E)-3-aryl-2-propenals are generated through a one to four-day Wittig reaction of the suitable carbaldedyde with (triphenylphosphoranylidene) aldehyde in dimethylformamide. Potassium carbonate was used to generate 1-alkyl-1H-imidazole-2-carbaldehydes from 1H-imidazole-2-carbaldehydes [60].	NF-κb-regulated gene products [61]	[60].	1,9-Bis(3-fluoropyridin-4-yl) 1,3,6,8-tetraen-5-one nona derivative1,9-Bis(1-(pentan-3-yl)imidazol-2-yl) 1,3,6,8-tetraen-5-onenona derivative

Table 17: Synthesis of boswellic acid derivatives

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Boswellic acid derivatives				
a) Acetyl-11-keto-β-boswellic acid derivatives	Following esterification and hydrolysis of free acid, thionyl chloride is added to chloroform to produce the suitable acyl chloride, which is subsequently treated with sodium methoxide/methanol. The protecting group was chosen to be benzyl because of the efficiency with which it could be removed using titanium tetrachloride bathed in dichloromethane. The acetate group is hydrolyzed by potassium hydroxide in methanol. Other derivatives are synthesized by reaction with 2-iodoxybenzoic acid in dimethyl sulfoxide and subsequent treatment with iodine in the presence of pyridine. Starting material for generating 2-cyano and 2-trifluoromethyl derivatives is 2-iodo analogues. Substitution reaction in the presence of cuprous cyanide bathed in N-methyl pyrrolidinone generates 2-cyano derivatives. Substituted analogues, when treated with methyl 2,2-difluoro-2-(fluorosulfonyl) acetate bathed in HMPT/DMF and cuprous iodine generates 2-trifluoromethyl analogues. Removal of benzyl group by titanium tetrachloride/methylene chloride produces free acid, which subsequently with thionyl chloride produces suitable acyl chloride. Further, product so formed undergoes condensation with several nitrogen containing heterocycles to yield amides [62].	PIN 1 (Protein Interaction with Never in mitosis A1)	[62]	N-(2-cyano-3,11-dioxo-ursan-1,12-dien-24-oyl)-piperazine
b) Ring A modified 11-keto-boswellic acid derivatives	Scheme 1: In the presence of potassium carbonate, Acetyl-11-Keto-b-Boswellic Acid derivatives (AKBA) are benzylated by treating them with benzyl bromide; subsequently, benzylated products are hydrolyzed with sodium ethoxide and further treated with 2-chloroacetyl chloride. The intermediate formed in turn is treated with amines or substituted phenols and further debenzylation with titanium tetrachloride is carried out. Scheme 2: AKBA is treated with various alcohols or amines and are subsequently subjected to series of reactions, such as hydrolysis and oxidisation using Jones' reagent, sequentially. Further, Aldol reaction of intermediate is carried out and subjected to treatment with glyoxylic acid monohydrate under basic circumstances and further substituted with benzaldehydes in the presence of potassium hydroxide, ethanol. Scheme 3: Intermediates obtained from Aldol Reaction are reacted with diethyl oxalate followed by cyclization. The resultant intermediate is then treated with hydroxylamine hydrochloride or hydrazine hydrate and subsequently subjected to ester hydrolysis [63].	PIN 1 (Protein Interaction with Never in mitosis A1)	[63]	3-oxo-2-carboxyl methylene derivative of 11-keto-boswellic acid derivatives

Indoline and Isoindoline derivatives

Among the 2-(4-phenylthiazol-2-yl) isoindoline-1,3-dione) isoindoline derivatives synthesized by Saravanan *et al.*, derivatives with 4-aromatic substituent on the 1,3-thiazole core presenting IC50 value of 5.96±1.6 μM were potent of all [64].

Kumar *et al.* developed a series of spiro-chrome no indoline-triones of which the two compounds identified to possess highest potency were 5' bromospiro[indeno[2',1':5,6]pyrano[3,2–c]chromene-7,3'-indoline]-2',6,8(7aH,12bH)-trione (IC50=0.025±0.002 μM) and 1'-allylspiro[indeno[2',1':5,6]pyrano[3',2–c]chromene-7,3-indoline]-2',6,8(7aH,12bH)-trione (IC50=0.081±0.002 μM) (table 18) [65].

Table 18: Synthesis of indoline and isoindoline derivatives

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Indoline and isoindoline derivatives				
a) 2-(4-phenylthiazol-2-yl) isoindoline-1,3-dione)isoindolinederivatives	In the presence of triethylamine, 2-bromo-1-(4-substituted phenyl)ethanone reacts with thiourea bathed in ethanol. Resulting product is further refluxed with phthalic anhydride in acetic acid [64].	Androgen Receptor	[64]	Derivatives with 4-aromatic moiety on 1,3-thiazole core
b) 10,10-dimethyl-9,10,11,11a-tetrahydro-6H spiro[chromeno [4,3–b]chromene-7,3-indoline]-2',6,8 (7aH)-triones	Reaction involving various Lewis acids and bifunctional organocatalysts. This reaction is carried out under green conditions, employing an admixture of cyclic diketone, isatins, 4-hydroxycoumarin, and β-diketone [65].	Alkaline Phosphatase	[65]	5'-bromospiro -trione and 1'-allylspiro-trione analogues

Amino-aroylnaphthalenes

Rai *et al.* developed a series of 1-Amino-2-Aroyl naphthalenes of which potent molecule 4-amino-3-aroyl/heteroaroyl-2-methylsulfanylnapthalene-1-carbonitriles presented IC50 values of 14μM (table 19) [66].

4-azaandrostenes analogues

Of the series of 4-azaandrostenes analogues synthesized by Brito *et al.*, 16E-[(4-methylphenyl) methylidene]-4-azaandrost-5-ene-3,17-dione (IC50 =28.28 μM) was identified to possess the highest inhibitory potency (table 20) [67].

Table 19: Synthesis of1-Amino-2-Aroyl naphthalenes

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
1-Amino-2-Aroyl naphthalenes	Reaction between 2-(1-cyano-2,2-bis-methylsulfanyl-vinyl)-benzonitrile and acetophenone in the presence of base generates appropriate functionalized naphthalenes [66].	cytochrome P450 receptor	[66]	4-amino-3-aroyl/heteroaroyl-2-methylsulfanylnapthalene-1-carbonitriles

Table 20: Synthesis of4-azaandrostenes analogues

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
4-azaandrostenes	Androstenedione undergoes oxidative cleavage at the enone system. Oxidative cleavage is followed by Azacyclization reaction. The intermediate thus formed undergoes Aldol Condensation with several aldehydes to yield the desired product [67].	5-alpha reductase enzyme.	[67]	16E-[(4-methylphenyl) methylidene]-4-azaandrost -5-ene-3,17-dione (in androgen-independent PC-3 cells)

Pyrazine analogues

Seo *et al.* described the preparation of a series of3,4-dihydropyrrolo[1,2-a]pyrazine of which potent molecule was identified to be (3R*,4S*)-3-(4-bromophenyl)-4-(4-fluorobenzoyl)-2-(2-oxo-2-phenylethyl)-3,4-dihydropyrrolo[1,2-a]pyrazin-2-ium bromide with an IC50 value of 1.18±0.05 μM (table 21) [68].

Table 21: Synthesis of 3,4-dihydropyrrolo[1,2-a] –pyrazine

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
3,4-dihydropyrrolo[1,2-a]-pyrazine	In-situ imine generation from the reaction of aldehyde and ammonium acetate reacts with N-substituted pyrrole-2-carboxaldehyde. Alkylation of basic nitrogen of product so obtained forming 4-acyl-3,4 dihydropyrrolo [1,2-a] pyrazines from N-substituted pyrrole-2-carboxaldehyde with (hetero) arylaldehydes and ammonium acetate in the presence of potassium carbonate in ethanol [68].	Caspase-3	[68]	(3R*,4S*)-3-(4-bromophenyl) -4-(4-fluorobenzoyl)-2-(2-oxo-2-phenylethyl)-3,4-dihydro Pyrrolo [1,2-a]pyrazin-2-ium bromide

Table 22: Synthesis of nitrogen-containing derivatives of O-tetramethyl quercetin

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Nitrogen-containing derivatives of O-tetramethyl quercetin:				
a) 3′,4′,5,7-*O*-tetramethylquercetin b) 3,3′,4′,7-*O*-Tetramethylquercetin c) four 3-*O*-aminoalkyl-3′,4′,5,7-*O*-tetramethylquercetins	Scheme 1: Global Methylation of Rutin followed by glycoside hydrolysis Scheme 2: Manthey and Guthrie's technique for selective tetramethylation of quercetin was used. Scheme 3: Firstly, 3,4,5,7-O-tetramethylquercetin is O-alkylated with suitable dibromoalkanes. Subsequently, resulting 3-O-bromoalkyl-3,4,5,7-O –tetramethylquercetinsis N-alkylated with appropriate amines. Base and polar aprotic solvent employed in the synthesis is potassium carbonate and N, N-dimethylformamide respectively.	Androgen Receptor	[69]	5-*O*-(*N*,*N*-dibutylamino)propyl -3,3′,4′,7-*O*-tetramethyl quercetin
d) 5-*O*-aminoalkyl-3,3,4,7-*O*-tetramethylquercetins	Scheme 4: From 3,3,4,7-O-tetramethylquercetin, twenty-four different 5-O-aminoalkyl-3,3,4,7-O-tetramethylquercetins are generated [69].			

Nitrogen-containing derivatives of O-tetramethyl quercetin

Rajaram *et al.* synthesized nitrogen-containing derivatives of O-tetramethyl quercetin among which5-O-(*N*,*N*dibutylamino)propyl-3,3′,4′,7-*O*-tetramethylquercetin demonstrated potent anti-proliferative action(IC50 =0.55–2.82 μM) (table 22) [69].

Ruthenium (II) complexes

Grandis *et al.* developed Ruthenium (II) complexes bearing Lawsone among which[Ru(lawsone)(bis [diphenylphosphino] methane)(2,2′-bipyridine)]PF6(IC50 value of 1.9 to 4.8 μM against DU-145 and A549 cell lines respectively) and [Ru(lawsone)(bis [diphenylphosphino] methane)(1,10-phenanthroline)]PF6(IC50 values ranging from 1.3-3.0 μM against DU-145 and MDA-MB-231cell lines, respectively) exhibited the highest potency [70].

Olmo*et al.* synthesized heterofunctional ruthenium (II) carbosilane dendrons of which {[PTA(η^5-C_5H_5)RuNCPh(o-N)]-G_2-[$(CH_2)_3N(CH_3)_2]_4$}Cl (IC50 =1.8 μM in HeLa; 1.4 μM in PC-3) was identified as potent complex among all [71].

Olmo*et al.* described the synthesis of cyclopentadienyl ruthenium (II) carbosilanemetallodendrimers of which G1-{[NCPh(o-N)Ru(η^5-C_5H_5)(PTA)]Cl}$_4$ and G2-{[NCPh(o-N)Ru(η^5-C_5H_5)(PTA)]Cl}$_8$possesed potent inhibitory action with IC50 of 8.3 μM and 6.6 μMrespectively (table 23) [72].

Table 23: Synthesis of ruthenium (II) complexes

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Ruthenium (II) containing complexes:				
a) Lawsone-containing ruthenium (II) complexes:				
i) [Ru(lawsone)(bipy or phen)$_2$]PF$_6$	The precursor complex [RuCl$_2$(bipy or phen)$_2$] of cis form reacts with lawsone (law) ligand under argon atmosphere.	Death receptor-upregulates BAX and downregulates BCL-2 expression.	[71]	[Ru(lawsone)(bis[diphenyl phosphino] methane)(2,2′-bipyridine)]PF$_6$ and [Ru(lawsone)(bis [diphenylphosphino] methane)(1,10-phenanthroline)]
ii)[Ru(lawsone)([diphenylphosphino] methane)(bipy or phen)]PF$_6$	The initial generation of precursor complexes. These are generated using cis and trans forms of [RuCl$_2$(PPh$_3$)$_2$(bipy or phen)] and (diphenylphosphino)methane of toluene. The reaction mixture is a refluxedinan atmosphere provided by argon gas [71].			
a) Heterofunctionalruthenium(II) carbosilane dendrons [Ru(η^5-C$_5$H$_5$)(PTA)Cl](PTA 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane)	Coordination of metallic centre to the focal point of dendritic wedges of different generations. Secondly, replacement of acetonitrile ligand by 1,3,5-triaza-7-Phosphatricyclo-Decane (PTA) is carried out. In a second step, without isolating the previous products a reaction of ligand exchange by PTA forms product [72].	Vasoactive Intestinal Peptide (VIP) and Growth Hormone Releasing Hormone (GHRH) receptors [73]	[72]	{[PTA(η^5-C$_5$H$_5$)RuNCPh(o-N)] -G2-[(CH$_2$)$_3$N(CH$_3$) $_2$]$_4$}Cl
b) Cyclopentadienyl ruthenium (II) carbosilanemetallodendrimers:				
i) The first generation metallodendrimer[G0{NCPh(o-N)Ru(η^5-C$_5$H$_5$)(CH$_3$CN)}]PF$_6$	Dendrimers which are functionalized with groups such as iminopyridine, G0-[NCPh(o-N)], G1-[NCPh(o-N)]$_4$ and G2-[NCPh(o-N)]$_8$ are used. Coordinating metallic centre, ligands and counterion exchange in an inert atmosphere produces suitable metallo dendrimer.	Vasoactive Intestinal Peptide (VIP) and Growth Hormone Releasing Hormone (GHRH) receptors [73]	[72]	G1-{[NCPh(o-N)Ru(η^5-C$_5$H$_5$) (PTA)]Cl}$_4$ and G2-{[NCPh(o-N)Ru(η^5-C$_5$H$_5$) (PTA)Cl}$_8$
ii) The second generation metallodendrimer[G1{NCPh(o-N)Ru(η^5-C$_5$H$_5$)(CH$_3$CN)}$_4$]PF$_6$	Synthesis following scheme similar as described above using the following reagents-dendritic ligand (II) of first generation and ruthenium precursor.			
iii) [G2{NCPh(o-N)Ru(η^5-C$_5$H$_5$)(CH$_3$CN)}$_8$]PF$_6$	Synthesis following scheme similar as described above using the following reagents-ruthenium precursor and dendritic ligand (II) of first generation.			
iv) G0-{[NCPh(o-N)Ru(η^5-C$_5$H$_5$)(PTA)]PF$_6$}$_4$	Reaction of complex[G0{NCPh(o-N)Ru(η^5-C$_5$H$_5$)(CH$_3$CN)}]PF$_6$ with 1,3,5-triaza-7 phosphoadamantane PTA ligand.			
v) G1-{[NCPh(o-N)Ru(η^5-C$_5$H$_5$) (PTA)]PF$_6$}$_4$	Reaction of complex [G1{NCPh(o-N)Ru(η^5-C$_5$H$_5$)(CH$_3$CN)}$_4$]PF$_6$ with PTA			
vi)G2-{[NCPh(o-N)Ru(η^5-C$_5$H$_5$)(PTA)]PF$_6$}$_8$	Reaction of [G2{NCPh(o-N)Ru(η^5-C$_5$H$_5$)(CH$_3$CN)}$_8$]PF6with PTA			
vii) G0-{[NCPh(o-N)Ru(η^5-C$_5$H$_5$)(PTA)]Cl}$_4$	To the complex G0-{[NCPh(o-N) Ru((η^5-C$_5$H$_5$)(PTA)]PF$_6$}$_4$ in a mixture of acetone/water, ion exchange resin is used to carry out exchange of counter ion.			
viii)G1-{[NCPh(o-N)Ru(η^5-C$_5$H$_5$)(PTA)]Cl}$_4$	To the complex G1{[NCPh(oN)Ru(η^5 C$_5$H$_5$)(PTA)]PF$_6$}$_4$ in a mixture of acetone/water, ion exchange resin is used to carry out an exchange of counterion.			
xi) G2-{[NCPh(o-N)Ru(η^5-C$_5$H$_5$)(PTA)]Cl}$_8$	To the complex G2{[NCPh(oN)Ru(η^5 C$_5$H$_5$) (PTA)]PF$_6$}$_8$ in an acetone/water, ion exchange resin is used to carry out exchange of counter ion [50].			

Table 24: Synthesis of N-heterocyclic nitro prodrugs

Type of derivative	Type of reaction involved in synthesis	Target	Ref	Potent compound
24. N-heterocyclic nitro pro-drugs:				
a) Nitro-containing triazine derivatives	A nucleophilic substitution reaction of cyanuric chloride and amines of aromatic kind such as 2,4-dinitrophenylhydrazine and 2-nitro, 3-nitro, 4-nitro, 2,4-dinitro, 3,5-dinitroaniline and is further refluxed with acetic acid.	Ssap-Ntr Bnitroreductase enzyme	[74]	Pyrimidine derivative
a) Urea derivatives of nitrophenyls and piperazine	Urea analogs of nitrophenyls and piperazine are generated by Curtius rearrangement reaction. Nitrobenzoyl chlorides react with sodium azide to produce derivatives of nitrobenzoylazide. Unstable nitrophenyl isocyanate formed via nitrogen output at temperature of reflux of toluene reacts with piperazine to yield product.			
b) Carbamate derivatives of nitrophenyls and piperazine	Rivett and Wilshire's method employes 1,4-bis (chlorocarbonyl) piperazine, which is generated through the reaction of piperazine with phosgene in pyridine and subsequent reactions at room temperature with bis(chlorocarbonyl) piperazine and nitrophenols (2-nitro, 3-nitro and 4-nitro) in dimethylformamide in the presence of sodium hydride.			
c) Pyrimidine derivative	At reflux temperature 2,4 dichloropyrimidine reacts with 4-nitroaniline in diluted hydrochloric acid to yield pyrimidine containing nitro pro-drugs [74].			

N-heterocyclic nitro pro-drugs

Güngor*e t al.* reported on the generation of N-heterocyclic nitro pro-drugs and demonstrated pyrimidine derivatives to possess highest inhibitory potency against PC3 cells (IC50 = 1.75 nMto 1.79 nM) (table 24) [74].

Dinitrobenzamide mustards

Basiri *et al.* described the synthesis of dinitrobenzamide mustards and identified mustards containing alcohol side chain counterparts as compound with maximum potency with IC50 value of 26±2 μM (table 25) [75].

Table 25: Synthesis of dinitrobenzamide mustards

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Dinitrobenzamide Mustards	Scheme 1: 2-chloro-3,5-dinitrobenzoic acid bathed in methanol is esterified by an acid catalyst. Further, the protected dinitrobenzoate is treated with bis(chloroethyl)amine and subsequently with aqueous potassium hydroxide in dioxane so as to cleave the methyl group, yielding benzoic acid which in turn reacts with oxalyl chloride under mild circumstances to form acid chloride. The acid chloride upon treatment with β-Alanine t-butyl ester or ethanolamine affords desirable product in situ [75].	Activation by nitroreductase enzyme followed by DNA alkylation	[75]	Dinitrobenzamide Mustards Containing alcohol side chain

Thiohydantoin derivatives

Wang *et al.* developed an array of thiohydantoin analogues of which4-(4,4-dimethyl-5-oxo-3-(1-oxoisochroman-6-yl)-2-thioxoimidazolidin-1-yl)-2(trifluoromethyl)benzonitrile demonstrated to posses potent inhibitory action with IC50 value of 1.936μM against LNCaP and 0.730μM againstLNCaP/AR(table 26) [76].

Table 26: Synthesis of thiohydantoin analogues

Type of derivative	Type of reaction involved in synthesis	Target	Reference	Potent compound
Thiohydantoin Derivatives	Suzuki coupling reaction of several boric acid or borate esters, followed by oxidation reaction using selenium dioxide at methyl group and a subsequent Pinnick oxidation to furnish appropriate carboxylic acids. Further, reduction of quinoline core so as to afford tetrahydroquinoline and formation of esters from relevant carboxylic acids is carried out. At the end, cyclization reactions yielded products [76].	Androgen Receptor	[76]	4-(4,4-dimethyl-5-oxo -3-(1-oxoisochroman-6-yl) -2-thioxoimidazolidin-1 yl)-2(trifluoromethyl) benzonitrile

Structure-activity relationship studies

Among thiazolidines, electron-donating substituent, sulfoxide or sulfone moiety conferred potent cytotoxicity to these analogues, whereas electron-withdrawing groups at ortho-position decreased their anticancer activity [25].

Incorporation of a C5-ethyl group of the thiadiazoline ring resulted in an enhanced inhibitory effects on mitosis and kinesin activity in cell lines of PC3, SKMEL-5 and SK-MEL-28 [26].

Among anilides, replacing central methyl moiety of bicalutamide with a trifluoromethyl moiety yielded derivatives with potent anti-proliferative action and superior stability with enzymes of phase 1 biotransformation while maintaining appreciable membrane permeability [28].

In dibromotyrosine derivatives, incorporation of C-1acetyl ester group conferred higher anti-migratory activity, whereas incorporation of ether group conferred higher anti-proliferative activity to these analogues [29].

In vitro SAR studies of β-hydroxy-androstadiene derivatives indicated that 3β-OH group is crucial to exert androgen receptor down-regulation. Isosteric substitution of 3β-OH moiety with fluoro group at C-3 resulted in significant reduction in this activity, whereas substitution with azido moiety completely terminated the action. Chemical alterations such as incorporation of 16-methyl alcohol or the substitution of 17-benzimidazole with purines that can be either substituted or unsubstituted led to a significant decrease in the down-regulation of androgen receptor [30].

The SAR results of poly-substituted steroidal pyridines revealed that analogues with heterocyclic rings at 4-position of the pyridine ring demonstrated superior anti-proliferative action against various tumor cell lines than phenyl-substituted counterparts. Moreover, it was deduced that the presence of an additional 4-pyridine moiety conferred appreciable growth inhibition activity to the derivatives [34].

Among norpregnene azole derivatives, compounds with 3β-hydroxy-5-ene-and isoxazole groups had the most potent inhibitory action against LNCaP and PC-3 cells. Derivatives with 3-oxo-4-ene-isoxazole groups demonstrated moderate anti-proliferative potency. Of oxadiazoles, 3β-hydroxy-5-ene-derivative was the only potent inhibitor among the series [36].

Of the chalcones synthesized by Nagaraju *et al.*, benzylated derivatives showed greater cytotoxicity than the corresponding debenzylated derivatives owing to higher lipophilicity of benzylated counterparts [42].

Findings of a study conducted on synthesis of flavonols imply that 3',4',5'-arrangement of either hydroxy or methoxy groups imparts potency to flavonols and that the methoxy derivatives have superior growth arrest activity as compared to their hydroxy counterparts [44].

The results of an investigation that involved synthesis of 3-O-substituted-trimethoxyflavonols indicates that incorporation of dipentylaminopropyl moiety increases the anti-proliferative potency as well as the ability to induce apoptosis in PC-3 cell lines. The study also concluded that potency of these derivatives could be enhanced by modifications at 3-OH group [45].

In diphenyl furanone derivatives, incorporation of fluoro group at para-position and/or chloro group at ortho-position on C-3 phenyl ring along with suitable modifications at 5 position of central furanone yields analogues with potent anti-proliferative effects [47].

An investigation on aryl-substituted-pyrazol-ol derivatives suggested that incorporating phenyl or naphthyl ring on 4 position-methyl moiety of the pyrazole ring generated potent anti-proliferative agents. Derivatives containing carboxyl moiety or those lacking the methyl group exhibited equivalent activities. Compounds with a phenyl ring

attached at the 3-position demonstrated lower activities. The removal or substitution of aromatic rings on the benzimidazole moiety resulted in weaker PCA-1/ALKBH3 inhibitors [48].

The arylpiperazine derivatives with an o-methylsulphonyl moiety on the phenyl ring possessed potent inhibitory action against LNCaP cell lines [50].

The SAR results of silibinins indicated that anti-proliferative potency of the derivatives can be enhanced by making chemical modifications on the C-7 phenolic hydroxyl moiety. Bioavalability of these analogues could be improved by incorporating suitable functional group through a linker to hydroxyl group at 7 position of silibinin and 2,3-dehydrosilibinin [51].

O-alkyldehydrosilibinins with modified hydroxyl group at either 3, 5, or 7 position have potent anti-proliferative action against androgen-sensitive PCa cell lines. However, derivatives with an enhanced ability to induce PC-3 cell apoptosis can be obtained by incorporating an alkyl group at hydroxyl group at either 5, or 7 position [52].

Results of the study on indeno [1,2-b]indole suggested that analogues with dimethyl group at 7 positions and dichloro phenyl group at 5 positions are essential features that confer anti-proliferative potency to these derivatives [54].

Among thiosemicarbazone indole derivatives, it was observed that benzamide moiety in the linker was responsible for selective cytotoxicity of these compounds. Moreover, a reduction in the potency of these analogues was seen when NNS donor was replaced with other donor chelators, indicating that NNS donor conferred significant anti-proliferative potency towards tumor cell lines [55].

The SAR results of triazole hybrids of curcumin indicated that anti-proliferative potency of the derivatives can be enhanced by incorporating 4-methyl groups at R1 position on the 1,2,3-triazole scaffold [56].

The results of the study on diheteroaryl nona-tetra enones, suggested that analogues with pyridine-4-yls and quinolin-4-yl heteroaromatic rings confer anti-proliferative potency to these derivatives [60].

The SAR results of acetyl-keto boswellic acid derivatives indicated that anti-proliferative potency of the derivatives can be enhanced by incorporating electron-withdrawing moiety on ring A and a nitrogen atom containing heterocycle at C-24 [62].

Among spiro-chromeno indoline-triones derivatives, it was observed that groups like bromo at R2 position, carbonyl available in isatin, 4-hydroxycoumarin and 1H-indene-1,3(2H)-dione support electron withdrawal thus enhance therapeutic effects of these analogues. Presence of abundance of functional groups ensures a higher bioavailability and anti-proliferative effects [65].

Among derivatives of O-tetramethyl quercetin 5-O-Aminoalkyl-3,3′,4′,7-O-tetramethylquercetins are considered to be a superior scaffold for further design and development quercetin anti-prostate drugs [69].

The SAR results of lawsone-containing ruthenium(II) complexes indicated that those containing phosphine ligand displayed potent anti-proliferative action by enhancing lipophilicity of the complex, thereby increasing its cytotoxicity against various tumour cell lines [70].

Among N-heterocyclic nitro pro-drugs, it was observed that para-nitrosubstituted piperazine-urea moiety and ortho-nitrosubstituted piperazine-carbamate moiety conferred significant anti-proliferative potency to these pro-drugs [74].

The SAR results of dinitrobenzamide mustards indicated that insertion of a carboxylic acid group into the construct of dinitrobenzamide mustard yields agents with superior hypoxia-selectivity than their alcohol counterparts [75].

The development and spread of particular cancer cells can be prevented by targeted treatment [77]. Fewer efficacies in the present cancer therapy, patient non-compliance, drug resistance and uncertainty of current candidates in a clinical trial have led to the need for the development of potential anticancer agents [78-80].

CONCLUSION

This present review summarizes the synthesis of significant anti-PCa agents their SAR studies and reflects current advancements and attempts in the field of cancer research. This review work intends to provide a basic insight into the design and development of novel molecules against PCa; thereby paving the way for future exploration.

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The authors confirm that there is no conflict of interest related to the manuscript.

AUTHORS CONTRIBUTIONS

Kavana Krishna Nayak was involved in writing the manuscript, conducting the literature search, and interpreting the results. Lalit Kumar was involved in supervision, critical review, and literature search. Ruchi Verma contributed by providing the idea, designing the study, supervising, performing critical reviews, writing and editing the manuscript, conducting the literature search.

REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Pineros M, Znaor A. Cancer statistics for the year 2020: an overview. Int J Cancer. 2021;149(4):778-89. doi: 10.1002/ijc.33588, PMID 33818764.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021 May;71(3):209-49. doi: 10.3322/caac.21660, PMID 33538338.
3. Giona S. Chapter 1. The epidemiology of prostate cancer. In: Bott SR, Ng KL, editors. Prostate cancer. Brisbane (AU). Exon Publications; 2021 May 27. doi: 10.36255/exonpublications.prostatecancer.epidemiology.2021, PMID 34181376.
4. Haffner MC, Zwart W, Roudier MP, True LD, Nelson WG, Epstein JI. Genomic and phenotypic heterogeneity in prostate cancer. Nat Rev Urol. 2021;18(2):79-92. doi: 10.1038/s41585-020-00400-w, PMID 33328650, PMCID PMC7969494.
5. Tolkach Y, Kristiansen G. The heterogeneity of prostate cancer: a practical approach. Pathobiology. 2018;85(1-2):108-16. doi: 10.1159/000477852, PMID 29393241.
6. Fisher R, Pusztai L, Swanton C. Cancer heterogeneity: implications for targeted therapeutics. Br J Cancer. 2013 Feb 19;108(3):479-85. doi: 10.1038/bjc.2012.581, PMID 23299535.
7. NG KL. Chapter 2. The etiology of prostate cancer. In: Bott SR, Ng KL, editors. Prostate cancer. Brisbane (AU). Exon Publications; 2021 May 27. PMID 34181375.
8. Pienta KJ, Esper PS. Risk factors for prostate cancer. Ann Intern Med. 1993 May 15;118(10):793-803. doi: 10.7326/0003-4819-118-10-199305150-00007, PMID 8470854.
9. Stangelberger A, Waldert M, Djavan B. Prostate cancer in elderly men. Rev Urol. 2008;10(2):111-9. PMID 18660852, PMCID PMC2483315.
10. McHugh J, Saunders EJ, Dadaev T, McGrowder E, Bancroft E, Kote Jarai Z. Prostate cancer risk in men of differing genetic ancestry and approaches to disease screening and management in these groups. Br J Cancer. 2022 Jun;126(10):1366-73. doi: 10.1038/s41416-021-01669-3, PMID 34923574, PMCID PMC9090767.
11. Wilson KM, Mucci LA. Diet and lifestyle in prostate cancer. Adv Exp Med Biol. 2019;1210:1-27. doi: 10.1007/978-3-030-32656-2_1, PMID 31900902.
12. Pouresmaeili F, Hosseini SJ, Farzaneh F, Karimpour A, Azargashb E, Yaghoobi M. Evaluation of environmental risk factors for prostate cancer in a population of Iranian patients. Asian Pac J

Cancer Prev. 2014;15(24):10603-5. doi: 10.7314/apjcp.2014.15.24.10603, PMID 25605146.
13. Chen FZ, Zhao XK. Prostate cancer: current treatment and prevention strategies. Iran Red Crescent Med J. 2013 Apr;15(4):279-84. doi: 10.5812/ircmj.6499, PMID 24082997, PMCID PMC3785898.
14. Bracarda S, Logothetis C, Sternberg CN, Oudard S. Current and emerging treatment modalities for metastatic castration-resistant prostate cancer. BJU Int. 2011 Apr;107 Suppl 2:13-20. doi: 10.1111/j.1464-410X.2010.10036.x, PMID 21382150.
15. Melloni C, Roe MT. Androgen deprivation therapy and cardiovascular disease. Urol Oncol. 2020 Feb;38(2):45-52. doi: 10.1016/j.urolonc.2019.02.010, PMID 30879969.
16. LI JR, Wang SS, Chen CS, Cheng CL, Hung SC, Lin CH. Conventional androgen deprivation therapy is associated with an increased risk of cardiovascular disease in advanced prostate cancer a nationwide population-based study. Plos One. 2022 Jun 28;17(6):e0270292. doi: 10.1371/journal.pone.0270292, PMID 35763533.
17. Ritch C, Cookson M. Recent trends in the management of advanced prostate cancer. F1000Res. 2018 Sep 21;7. doi: 10.12688/f1000research.15382.1, PMID 30345007.
18. Sahu M, Suryawanshi H. Immunotherapy: the future of cancer treatment. J Oral Maxillofac Pathol. 2021 May-Aug;25(2):371. doi: 10.4103/0973-029X.325257, PMID 34703141.
19. Kelly PN. The cancer immunotherapy revolution. Science. 2018 Mar 23;359(6382):1344-5. doi: 10.1126/science.359.6382.1344, PMID 29567702.
20. Patnaik A. Revolutionizing cancer treatment: the challenges of immunotherapy. Immunol Disord Immunother. 2023;8(1):137. doi: 10.35248/2593-8509.23.8.137.
21. Couchoud C, Fagnoni P, Aubin F, Westeel V, Maurina T, Thiery Vuillemin A. Economic evaluations of cancer immunotherapy: a systematic review and quality evaluation. Cancer Immunol Immunother. 2020 Oct;69(10):1947-58. doi: 10.1007/s00262-020-02646-0, PMID 32676716.
22. Cha HR, Lee JH, Ponnazhagan S. Revisiting immunotherapy: a focus on prostate cancer. Cancer Res. 2020 Apr 15;80(8):1615-23. doi: 10.1158/0008-5472.CAN-19-2948, PMID 32066566.
23. Gupta S, Shukla S. Limitations of immunotherapy in cancer. Cureus. 2022 Oct 29;14(10):e30856. doi: 10.7759/cureus.30856, PMID 36465776.
24. Porta Pardo E, Godzik A. Mutation drivers of immunological responses to cancer. Cancer Immunol Res. 2016 Sep 2;4(9):789-98. doi: 10.1158/2326-6066.CIR-15-0233, PMID 27401919.
25. Gududuru V, Hurh E, Dalton JT, Miller DD. Synthesis and antiproliferative activity of 2-aryl-4-oxo-thiazolidin-3-yl-amides for prostate cancer. Bioorg Med Chem Lett. 2004;14(21):5289-93. doi: 10.1016/j.bmcl.2004.08.029, PMID 15454213.
26. DE Monte C, Carradori S, Secci D, D Ascenzio M, Guglielmi P, Mollica A. Synthesis and pharmacological screening of a large library of 1,3,4-thiadiazolines as innovative therapeutic tools for the treatment of prostate cancer and melanoma. Eur J Med Chem. 2015 Nov 13;105:245-62. doi: 10.1016/j.ejmech.2015.10.023, PMID 26498571.
27. Gududuru V, Hurh E, Sullivan J, Dalton JT, Miller DD. SAR studies of 2-arylthiazolidine-4-carboxylic acid amides: a novel class of cytotoxic agents for prostate cancer. Bioorg Med Chem Lett. 2005;15(18):4010-3. doi: 10.1016/j.bmcl.2005.06.032, PMID 16005217.
28. Bassetto M, Ferla S, Pertusati F, Kandil S, Westwell AD, Brancale A. Design and synthesis of novel bicalutamide and enzalutamide derivatives as antiproliferative agents for the treatment of prostate cancer. Eur J Med Chem. 2016 Aug 8;118:230-43. doi: 10.1016/j.ejmech.2016.04.052, PMID 27131065.
29. Sallam AA, Ramasahayam S, Meyer SA, El Sayed KA. Design synthesis and biological evaluation of dibromotyrosine analogues inspired by marine natural products as inhibitors of human prostate cancer proliferation invasion and migration. Bioorg Med Chem. 2010;18(21):7446-57. doi: 10.1016/j.bmc.2010.08.057, PMID 20884214.
30. Bruno RD, Vasaitis TS, Gediya LK, Purushottamachar P, Godbole AM, Ates Alagoz Z. Synthesis and biological evaluations of putative metabolically stable analogs of VN/124-1 (TOK-001): head to head anti-tumor efficacy evaluation of VN/124-1 (TOK-001) and abiraterone in LAPC-4 human prostate cancer xenograft model. Steroids. 2011;76(12):1268-79. doi: 10.1016/j.steroids.2011.06.002, PMID 21729712.
31. Fortin S, Brasseur K, Morin N, Asselin E, Berube G. New platinum(II) complexes conjugated at position 7α of 17β-acetyl testosterone as new combi-molecules against prostate cancer: design synthesis structure-activity relationships and biological evaluation. Eur J Med Chem. 2013 Oct;68:433-43. doi: 10.1016/j.ejmech.2013.08.011, PMID 23994871.
32. Bastien D, Hanna R, Leblanc V, Asselin E, Berube G. Synthesis and preliminary *in vitro* biological evaluation of 7α-testosterone chlorambucil hybrid designed for the treatment of prostate cancer. Eur J Med Chem. 2013;64:442-7. doi: 10.1016/j.ejmech.2013.04.027, PMID 23665800.
33. Heng MP, Sinniah SK, Teoh WY, Sim KS, Ng SW, Cheah YK. Synthesis of a DNA-targeting nickel (II) complex with testosterone thiosemicarbazone, which exhibits selective cytotoxicity towards human prostate cancer cells (LNCaP). Spectrochim Acta A Mol Biomol Spectrosc. 2015;150(1):360-72. doi: 10.1016/j.saa.2015.05.095, PMID 26057090.
34. Shi YK, Wang B, Shi XL, DI Zhao YD, YU B, Liu HM. Synthesis and biological evaluation of new steroidal pyridines as potential anti-prostate cancer agents. Eur J Med Chem. 2018 Feb 10;145:11-22. doi: 10.1016/j.ejmech.2017.12.094, PMID 29310026.
35. Sethi A, Singh P, Yadav N, Yadav P, Banerjee M, Singh RP. Greener approach for synthesis of novel steroidal prodrugs using ionic liquid their DFT study and apoptosis activity in prostate cancer cell line. J Mol Struct. 2019;1180:733-40. doi: 10.1016/j.molstruc.2018.12.009.
36. Dalidovich TS, Hurski AL, Morozevich GE, Latysheva AS, Sushko TA, Strushkevich NV. New azole derivatives of [17(20)E]-21-norpregnene: synthesis and inhibition of prostate carcinoma cell growth. Steroids. 2019;147(20):10-8. doi: 10.1016/j.steroids.2018.08.004, PMID 30149075.
37. Jorda R, Reznickova E, Kielczewska U, Maj J, Morzycki JW, Siergiejczyk L. Synthesis of novel galeterone derivatives and evaluation of their *in vitro* activity against prostate cancer cell lines. Eur J Med Chem. 2019;179:483-92. doi: 10.1016/j.ejmech.2019.06.040, PMID 31271960.
38. Komendantova AS, Scherbakov AM, Komkov AV, Chertkova VV, Gudovanniy AO, Chernoburova EI. Novel steroidal 1,3,4-thiadiazines: synthesis and biological evaluation in androgen receptor-positive prostate cancer 22Rv1 cells. Bioorg Chem. 2019 Oct;91:103142. doi: 10.1016/j.bioorg.2019.103142, PMID 31400555.
39. Hou Q, HE C, Lao K, Luo G, You Q, Xiang H. Design and synthesis of novel steroidal imidazoles as dual inhibitors of AR/CYP17 for the treatment of prostate cancer. Steroids. 2019;150:108384. doi: 10.1016/j.steroids.2019.03.003, PMID 30885648.
40. Scherbakov AM, Komkov AV, Komendantova AS, Yastrebova MA, Andreeva OE, Shirinian VZ. Steroidal pyrimidines and dihydrotriazines as novel classes of anticancer agents against hormone-dependent breast cancer cells. Front Pharmacol. 2017;8:979. doi: 10.3389/fphar.2017.00979, PMID 29375380.
41. Chen PS, Shih YW, Huang HC, Cheng HW. Diosgenin a steroidal saponin inhibits migration and invasion of human prostate cancer PC-3 cells by reducing matrix metalloproteinases expression. Plos One. 2011;6(5):e20164. doi: 10.1371/journal.pone.0020164, PMID 21629786.
42. Nagaraju M, Gnana Deepthi E, Ashwini C, Vishnuvardhan MV, Lakshma Nayak V, Chandra R. Synthesis and selective cytotoxic activity of novel hybrid chalcones against prostate cancer cells. Bioorg Med Chem Lett. 2012;22(13):4314-7. doi: 10.1016/j.bmcl.2012.05.016, PMID 22668451.
43. Zhou J, Geng G, Batist G, WU JH. Syntheses and potential anti-prostate cancer activities of ionone-based chalcones. Bioorg Med Chem Lett. 2009;19(4):1183-6. doi: 10.1016/j.bmcl.2008.12.089, PMID 19138519.
44. Britton RG, Horner Glister E, Pomenya OA, Smith EE, Denton R, Jenkins PR. Synthesis and biological evaluation of novel flavonols as potential anti-prostate cancer agents. Eur J Med Chem. 2012 Aug;54:952-8. doi: 10.1016/j.ejmech.2012.06.031, PMID 22789812.

45. LI X, Lee M, Chen G, Zhang Q, Zheng S, Wang G. 3-O-Substituted-3′,4′,5′-trimethoxyflavonols: synthesis and cell based evaluation as anti-prostate cancer agents. Bioorg Med Chem. 2017;25(17):4768-77. doi: 10.1016/j.bmc.2017.07.022, PMID 28760528.
46. Monim Ul Mehboob M, Altaf M, Fettouhi M, Isab AA, Wazeer MI, Shaikh MN. Synthesis spectroscopic characterization and anti-cancer properties of new gold(III) alkanediamine complexes against gastric prostate and ovarian cancer cells; crystal structure of [Au2(pn)2(Cl)2]Cl2·H2O. Polyhedron. 2013;61:225-34. doi: 10.1016/j.poly.2013.05.054.
47. Liu GZ, XU HW, Wang P, Lin ZT, Duan YC, Zheng JX. Stereoselective synthesis and anti-proliferative effects on prostate cancer evaluation of 5-substituted-3,4-diphenylfuran-2-ones. Eur J Med Chem. 2013 Jul;65:323-36. doi: 10.1016/j.ejmech.2013.04.062, PMID 23735281.
48. Nakao S, Mabuchi M, Shimizu T, Itoh Y, Takeuchi Y, Ueda M. Design and synthesis of prostate cancer antigen-1 (PCA-1/ALKBH3) inhibitors as anti-prostate cancer drugs. Bioorg Med Chem Lett. 2014;24(4):1071-4. doi: 10.1016/j.bmcl.2014.01.008, PMID 24461353.
49. Akinboye ES, Bamji ZD, Kwabi Addo B, Ejeh D, Copeland RL, Denmeade SR. Design synthesis and cytotoxicity studies of dithiocarbamate ester derivatives of emetine in prostate cancer cell lines. Bioorg Med Chem. 2015;23(17):5839-45. doi: 10.1016/j.bmc.2015.06.072, PMID 26187015.
50. Chen H, XU F, Liang X, XU BB, Yang ZL, HE XL. Design synthesis and biological evaluation of novel arylpiperazine derivatives on human prostate cancer cell lines. Bioorg Med Chem Lett. 2015;25(2):285-7. doi: 10.1016/j.bmcl.2014.11.049, PMID 25488843.
51. Vue B, Zhang S, Zhang X, Parisis K, Zhang Q, Zheng S. Silibinin derivatives as anti-prostate cancer agents: synthesis and cell-based evaluations. Eur J Med Chem. 2016;109:36-46. doi: 10.1016/j.ejmech.2015.12.041, PMID 26748997.
52. Vue B, Zhang X, Lee T, Nair N, Zhang S, Chen G. 5-or/and 20-O-alkyl-2,3-dehydrosilybins: synthesis and biological profiles on prostate cancer cell models. Bioorg Med Chem. 2017;25(17):4845-54. doi: 10.1016/j.bmc.2017.07.035, PMID 28756013.
53. Ramasamy K, Agarwal R. Multitargeted therapy of cancer by silymarin. Cancer Lett. 2008 Oct 8;269(2):352-62. doi: 10.1016/j.canlet.2008.03.053, PMID 18472213.
54. Lobo G, Monasterios M, Rodrigues J, Gamboa N, Capparelli MV, Martinez Cuevas J. Synthesis crystal structure and effect of indeno[1,2-b]indole derivatives on prostate cancer *in vitro*. Potential effect against MMP-9. Eur J Med Chem. 2015;96:281-95. doi: 10.1016/j.ejmech.2015.04.023, PMID 25899333.
55. HE ZX, Huo JL, Gong YP, An Q, Zhang X, Qiao H. Design synthesis and biological evaluation of novel thiosemicarbazone indole derivatives targeting prostate cancer cells. Eur J Med Chem. 2021 Jan 15;210:112970. doi: 10.1016/j.ejmech.2020.112970, PMID 33153765.
56. Mandalapu D, Saini KS, Gupta S, Sharma V, Yaseen Malik M, Chaturvedi S. Synthesis and biological evaluation of some novel triazole hybrids of curcumin mimics and their selective anticancer activity against breast and prostate cancer cell lines. Bioorg Med Chem Lett. 2016;26(17):4223-32. doi: 10.1016/j.bmcl.2016.07.053, PMID 27496212.
57. Madasu C, Karri S, Sangaraju R, Sistla R, Uppuluri MV. Synthesis and biological evaluation of some novel 1,2,3-triazole hybrids of myrrhanone B isolated from commiphora mukul gum resin: identification of potent antiproliferative leads active against prostate cancer cells (PC-3). Eur J Med Chem. 2020;188:111974. doi: 10.1016/j.ejmech.2019.111974, PMID 31883489.
58. XU X, Zhang J, Zhang Z, Wang M, Liu Y, LI X. Systems pharmacology in combination with proteomics reveals underlying mechanisms of xihuang pill against triple-negative breast cancer. Bioengineered. 2020;11(1):1170-88. doi: 10.1080/21655979.2020.1834726, PMID 33092442.
59. LI K, LI Y, Zhou D, Fan Y, Guo H, MA T. Synthesis and biological evaluation of quinoline derivatives as potential anti-prostate cancer agents and Pim-1 kinase inhibitors. Bioorg Med Chem. 2016;24(8):1889-97. doi: 10.1016/j.bmc.2016.03.016, PMID 26979485.
60. Zhang X, Wang R, Perez GR, Chen G, Zhang Q, Zheng S. Design synthesis and biological evaluation of 1,9-diheteroarylnona-1,3,6,8-tetraen-5-ones as a new class of anti-prostate cancer agents. Bioorg Med Chem. 2016;24(19):4692-700. doi: 10.1016/j.bmc.2016.08.006, PMID 27543391.
61. Mbese Z, Khwaza V, Aderibigbe BA. Curcumin and its derivatives as potential therapeutic agents in prostate colon and breast cancers. Molecules. 2019 Nov 30;24(23):4386. doi: 10.3390/molecules24234386, PMID 31801262.
62. LI K, LI L, Wang S, LI X, MA T, Liu D. Design and synthesis of novel 2-substituted 11-keto-boswellic acid heterocyclic derivatives as anti-prostate cancer agents with Pin1 inhibition ability. Eur J Med Chem. 2017;126:910-9. doi: 10.1016/j.ejmech.2016.09.089, PMID 27997878.
63. Huang M, LI A, Zhao F, Xie X, LI K, Jing Y. Design synthesis and biological evaluation of ring modified 11-keto-boswellic acid derivatives as pin1 inhibitors with remarkable anti-prostate cancer activity. Bioorg Med Chem Lett. 2018;28(19):3187-93. doi: 10.1016/j.bmcl.2018.08.021, PMID 30153964.
64. Saravanan K, Elancheran R, Divakar S, Anand SA, Ramanathan M, Kotoky J. Design synthesis and biological evaluation of 2-(4-phenylthiazol-2-yl) isoindoline-1,3-dione derivatives as anti-prostate cancer agents. Bioorg Med Chem Lett. 2017;27(5):1199-204. doi: 10.1016/j.bmcl.2017.01.065, PMID 28162857.
65. Kumar MR, Manikandan A, Sivakumar A, Dhayabaran VV. An eco-friendly catalytic system for multicomponent one-pot synthesis of novel spiro chromeno indoline triones and their anti-prostate cancer potentials evaluated via alkaline phosphatase inhibition mechanism. Bioorg Chem. 2018 Dec;81:44-54. doi: 10.1016/j.bioorg.2018.07.037, PMID 30118985.
66. Rai R, Dutta RK, Singh S, Yadav DK, Kumari S, Singh H. Synthesis biological evaluation and molecular docking study of 1-amino-2-aroylnaphthalenes against prostate cancer. Bioorg Med Chem Lett. 2018;28(9):1574-80. doi: 10.1016/j.bmcl.2018.03.057, PMID 29606573.
67. Brito V, Santos AO, Alves G, Almeida P, Silvestre S. Novel 4-azapregnene derivatives as potential anticancer agents: synthesis antiproliferative activity and molecular docking studies. Molecules. 2022;27(18). doi: 10.3390/molecules27186126, PMID 36144856.
68. Seo Y, Lee JH, Park SH, Namkung W, Kim I. Expansion of chemical space based on a pyrrolo[1,2-a]pyrazine core: synthesis and its anticancer activity in prostate cancer and breast cancer cells. Eur J Med Chem. 2020 Feb 15;188:111988. doi: 10.1016/j.ejmech.2019.111988, PMID 31901746.
69. Rajaram P, Jiang Z, Chen G, Rivera A, Phasakda A, Zhang Q. Nitrogen containing derivatives of O-tetramethylquercetin: synthesis and biological profiles in prostate cancer cell models. Bioorg Chem. 2019 Jun;87:227-39. doi: 10.1016/j.bioorg.2019.03.047, PMID 30904813.
70. DE Grandis RA, Santos PW, Oliveira KM, Machado AR, Aissa AF, Batista AA. Novel lawsone containing ruthenium(II) complexes: synthesis characterization and anticancer activity on 2D and 3D spheroid models of prostate cancer cells. Bioorg Chem. 2019 Apr;85:455-68. doi: 10.1016/j.bioorg.2019.02.010, PMID 30776556.
71. Sanz Del Olmo N, Maroto Diaz M, Quintana S, Gomez R, Holota M, Ionov M. Heterofunctional ruthenium(II) carbosilane dendrons a new class of dendritic molecules to fight against prostate cancer. Eur J Med Chem. 2020 Dec 1;207:112695. doi: 10.1016/j.ejmech.2020.112695, PMID 32882608.
72. Sanz Del Olmo N, Bajo AM, Ionov M, Garcia Gallego S, Bryszewska M, Gomez R. Cyclopentadienyl ruthenium(II) carbosilane metallodendrimers as a promising treatment against advanced prostate cancer. Eur J Med Chem. 2020;199:112414. doi: 10.1016/j.ejmech.2020.112414, PMID 32438200.
73. Sanchez Milla M, Munoz Moreno L, Sanchez Nieves J, Maly M, Gomez R, Carmena MJ. Anticancer activity of dendriplexes against advanced prostate cancer from protumoral peptides and cationic carbosilane dendrimers. Biomacromolecules. 2019;20(3):1224-34. doi: 10.1021/acs.biomac.8b01632, PMID 30669830.
74. Gungor T, Tokay E, Guven Gulhan U, Hacıoglu N, Celik A, Koçkar F. Prodrugs for nitroreductase based cancer therapy-4: towards prostate cancer targeting: synthesis of N-heterocyclic nitro prodrugs Ssap NtrB enzymatic activation and anticancer

evaluation. Bioorg Chem. 2020 Dec;105:104450. doi: 10.1016/j.bioorg.2020.104450, PMID 33189994.

75. Basiri A, Zhang W, Garrison J. Synthesis and evaluation of new dinitrobenzamide mustards in human prostate cancer. Bioorg Med Chem Lett. 2021;31:127697. doi: 10.1016/j.bmcl.2020.127697, PMID 33220402.
76. Wang A, Wang Y, Meng X, Yang Y. Design synthesis and biological evaluation of novel thiohydantoin derivatives as potent androgen receptor antagonists for the treatment of prostate cancer. Bioorg Med Chem. 2021;31:115953. doi: 10.1016/j.bmc.2020.115953, PMID 33388655.
77. Mehdi S, Chauhan A, Dhutty A. Cancer and new prospective to treat cancer. Int J Curr Pharm Sci. 2023;15(6):16-22. doi: 10.22159/ijcpr.2023v15i6.3078.
78. Mathew C, Lal N, Lakshmi S, Aswathy TR, Varkey J. Antioxidant anticancer and molecular docking studies of novel 5-benzylidene substituted rhodanine derivatives. Int J Pharm Pharm Sci. 2023;15(7):7-19. doi: 10.22159/ijpps.2023v15i7.47421.
79. Jahan SM, Kabir S, Jinatrahana, Sazianowshin SS, Salam S, Islam J. Study of the physiological role of Streblus asper as a chemopreventive agent on human prostate cancer (DU-145) cell line. Asian J Pharm Clin Res. 2024;17(7):126-30. doi: 10.22159/ajpcr.2024v17i7.50977.
80. Bamaniya BB, Mavuduru RS, Bora GS, Sharma AP, SK, Rastogi A. Serum testosterone as a marker of response to androgen deprivation therapy in metastatic prostate cancer. Asian J Pharm Clin Res. 2023;16(8):176-80. doi: 10.22159/ajpcr.2023.v16i8.48075.

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Review Article

A REVIEW ON RECENT ADVANCES IN HYDROGELS AS DRUG DELIVERY SYSTEM

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ABSTRACT

Hydrogels are hydrophilic three-dimensional polymeric networks which has the capability to absorb water or biological fluids. These polymeric network is formulated through chemical crosslinking or physical crosslinking mechanisms. Several polymers of synthetic and natural origin can be used to form hydrogels. Mechanical properties, swelling and biological properties are about the most significant hydrogels properties that can affect their morphology and structure. Hydrogels are promising biomaterials due to their significant properties as hydrophilicity, biodegradability, biocompatibility and non-toxicity. These characteristics make hydrogels appropriate for medical and pharmaceutical application. This review discusses the types of hydrogels, their properties, mechanism of preparation and applications of hydrogels as drug delivery system.

Keywords: Hydrogels, Networks, Hydrophilic, Drug targeting, Drug delivery, Crosslinking

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INTRODUCTION

Hydrogels are three-dimensional networks of polymers that have the capability of absorbing high amounts of water or any biological fluids in the body, which utilized in various biomedical applications. Hydrogels have the ability to swell and preserve these fluids within its structure without being dissolved [1]. In general, hydrogels properties depend on their chemical composition that response to various stimuli for example, heating, light, pH, and chemicals. Moreover, due to the large amount of water that they can retain in their structure, they have a degree of flexibility that make them identical and analogue to natural tissue [2]. The absorption capacity of hydrogel rises from the hydrophilic functional groups that are linked to the main polymeric backbone. Among these hydrophilic functional groups are the hydroxyl (OH-), amine (NH2), carboxyl (COOH-) and sulfate (SO3H-) groups [3]. Hydrogel polymer hydrated to different degrees depending on the type of the aqueous surrounding fluids and polymer structure; the hydrophilic groups and the backbone. However, hydrogels display a swelling performance in the aqueous surroundings rather than being dissolved as a result of the critical crosslink's found between network chains [3]. There are two significant types of hydrogel crosslinks. The first type is the physical crosslink, such as, entanglements or crystallites and the second one is the chemical type that includes tie-points and junctions. These crosslinks within the polymer network arises from the presence of Vander Waals interactions, hydrogen bonds, covalent bonds or physical entanglements [4]. The first appearance of hydrogel was in 1960 when Wichterle and Lim suggested the usage of poly (2-hydroxyethyl methacrylate) hydrophilic networks in contact lenses. Subsequently, hydrogels use has spread to several pharmaceutical and biomedical applications [5]. A number of terms have been used for hydrogels, for instance intelligent gels and smart gels. Hydrogels take these terms because they can behave in intelligent and smart way in observing the predominant stimuli and responding to it by forming changes in their physical or chemical properties, causing the release of the drug entrapped inside it in a controlled mode [6].

In contrast to other artificial biomaterial technologies, hydrogels are closely similar to the biological tissues in their physical properties due to their somewhat high content of water and elastic consistency. Actually, Hydrogels are polymers which can preserve water many times their individual weight. They are carboxylic acids polymers. In water the acid groups ionize, so a several negative charge appears along the surface of the polymer. And this affects the hydrogel in two ways. First, the polymer is enforced to enlarge due to the repulsion forces of the negative charges. Second, the negative charges attracted the polar molecules of water, so the viscosity of the formed mixture will increase because the polymer chains now use more space and resist the solvent molecules flow around them. An equilibrium between the polymer and the water around it in now formed, but the distortion of this equilibrium can be happened in a different of ways. Increasing the ionic concentration of the solution by for example, the addition of salt, the ions of positive charge attached to the negative charged sites on the polymer surface, so charges neutralizing effectively occurs. As a result, the polymer collapse on itself again. As well, adding an alkali material eradicates the acid ions and causes the equilibrium position to move to the right; addition of acid has an opposite effect. There are numerous hydrogels that can expand or contract by varying the pH values, ionic concentrations and temperatures [7].

Furthermore, Hydrogels can be formed from natural polymers as chitosan, hyaluronic acid, cellulose, sodium alginate, albumin, dextran and gelatin. These hydrogels are biodegradable, biocompatible and support the activities of the cells. Through the past two decades, synthetic hydrogels replaced the natural hydrogels. Because synthetic hydrogels have long life stability, high water absorption capability, and strong gel texture. In addition to that synthetic hydrogels have well-defined structure which could be adjusted to enhance their properties and function [8]. Synthetic hydrogel polymers include polyvinyl pyrrolidone, polyurethane, polyvinyl alcohol, polyacrylate, poly hydroxyethyl methaacrylate, polyethylene glycol, polymidine and derivatives [9].

Recently, as a result of hydrogel properties as water absorption, biocompatibility, soft structure, low adsorption of protein because of low surface tension and similarity to biological structure, researchers have paid more attention for using hydrogels in various medical applications including release of therapeutic agents (drugs, proteins orgenes), contact lenses, tissue engineering and wound coverings.

Various available methods for *in vivo* administration of drugs using hydrogel which are based on the pathological condition and localization, such as topical subcutaneous, oral, orthotopic, intraperitoneal, rectal and ocular [10].

For this review paper, the key phrases employed in the literature search were 'Hydrogels', 'Hydrophilic polymers', 'Hydrophobic polymers', 'Crosslinking', 'Hydrogels preparation', 'Hydrogels properties and characteristics' and 'Drug targeting using hydrogels', using 'Pubmed', 'Google search engine', 'Cross references', 'Science direct', 'Scopus' and 'Google Scholar'. Since 1998 and by initial peer-review of the obtained articles some of them which contained the specified keywords have been involved in this review article.

The primary objective of this review article is to discuss one of the main subjects of biomaterials research, which is hydrogels. This article will focus in hydrogels definition, properties, methods of preparation and their application in drug delivery and various medical fields.

Types of hydrogels

Based on the mechanism of cross-linking

Hydrogels can be categorized into two groups based on the nature of cross-linking reaction. The first type is the permanent hydrogel as it formed from covalent bonds among its polymeric chains. The second type formed as a result of physical interactions among the polymeric chains of the hydrogel, these interaction includes ionic interaction, molecular entanglement and hydrogen bonding. This type called physical hydrogels. However, this type of crosslinking may not be permeant junction, its only sufficient to preserve the hydrogel from being dissolved in an aqueous media [10].

Based on polymer type

Furthermore, based on polymer type, hydrogels are distributed into two categories: synthetic and natural hydrogels. Hydrogels formulated using natural or synthetic polymers. Natural polymers like chitosan, proteins as gelatin, lysozyme, collagen, fibrin and fibrin, or polysaccharides as alginate and hyaluronic acid. However synthetic hydrogels are formulated through polymerization of monomers chemically. They have a wide-ranging of chemical and mechanical properties. Synthetic polymers include poly vinyl alcohol, poly acrylamide, poly ethylene glycol and poly N-isopropyl acrylamide. These polymers should be biodegradable and biocompatible [11].

Based on stimuli-response method

Moreover, Hydrogels may be classified into conventional or stimuli-responsive hydrogels. Conventional hydrogels may not be affected by any change in the temperature, pH or electric field of the environment surrounding them because they are chains of cross-linked polymer which absorb water and swollen and reversibly release water solutions when placed in an aqueous media [12].

On the other hand, the stimuli responsive hydrogels also called smart hydrogels are sensitive to various stimuli as physical, chemical or biological and have the advantage of controlling drug release from hydrogel system in response to external or internal selected triggers [13].

These hydrogels undergo notable alterations in their permeability, network structure and swelling behavior. Any external stimuli as electric field and light have been applied using stimuli producing devices, whereas internal stimuli arise from the intrinsic body environment. Features such as hydrophobic and hydrophilic balance, monomers type, confirmation of chemical groups, cross-link density or osmotic pressure, also affect the way gels response to the stimuli [14].

Numerous chemical, physical and biological stimuli have been used to prompt several responses to the hydrogel delivery systems as shown in fig. 1.

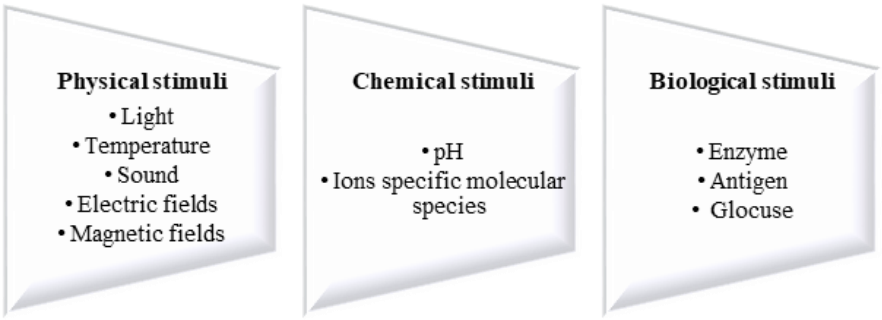

Fig. 1: Different factors that can stimulate and affect hydrogels

Hydrogel properties

Porosity

Hydrogels pore size and extent control drug loading and release through diffusion-controlled mechanism [15] which obeys the first law of Fickian diffusion [16, 17]. Hydrogel porosity also affects the tissue engineering process and is related inversely to the stiffness of the scaffold decreasing [18].

According to pore size, hydrogels are classified into four different classes; nano-porous, micro-porous, macro-porous and super-porous hydrogels [19]. Nano-porous hydrogels have small-sized pores between 1 to 10 nm. This tiny pore size is due to the dense polymer chains of these hydrogels and leads low loading and diffusion capacities, which restrict their use in effective drug delivery [19, 20]. In contrast, The pore size of micro, macro, and super porous hydrogels allows solute diffusion and water convection in the pores to diverse extents [19, 21, 22].

Porosity can be measured by different techniques some of them are traditional as unit cube analysis, Archimedes method, mass technique, liquid displacement method, and other modern and more accurate technique such as: using electronic microscopy, micro-CT, also it is called X-ray micro-tomography [23].

Many studies (table 1) tend to increase pore size for different purposes, it was reported that gas foaming, freeze drying, solvent casting, and phase separation can be used to made controlled macroporous hydrogels [24–27].

Although increasing hydrogel pore size leads usually to increase the extent and rate of drug loading and release, it decreases the mechanical strength of hydrogels which is a serious challenge especially for super-porous hydrogels [22, 28]. Researchers (table 1) reported that this problem can be solved by controlling many factors especially the amount and type of crosslinking agent as increasing the crosslinking agent amount enhances the mechanical strength of hydrogels and super-porous hydrogels [20, 28].

Hydrogel swelling capacity

Hydrogels have an amazing ability for swelling. In water, hydrogels are able to absorb from 10-20% up to thousands of times of their dry weight [23]. when hydrogels contact with a thermodynamically compatible solvent (water and water-soluble materials)the solvent penetrates into the polymeric network through the pores by the capillary and convection force and lead to hydrogel swelling [19, 22, 29]. Particularly, this swelling is responsible for the rubbery and soft properties of hydrogel which is similar to the soft tissue and also have a role in dug loading and release [30].

Drug loading volume can be enhanced by increasing swelling ability of hydrogels. Though, it is unfavorable in many circumstances,

mainly in biomedical applications as soft actuating, internal wound closure, tissue engineering, bioelectronics, and others. This is because of hydrogel swelling frequently leads to a volume expansion, which resulted in deteriorate of the mechanical characteristics of the hydrogel and when applied *in vivo* it will resulted in undesirable oppression on the adjacent tissues. In contrast, hydrogels with anti-swelling property are almost not adjust their volume when subjected to an aqueous environment, consequently preserving its original mechanical performance, size-stability and facilitating their potential application [31].

However, physical and chemical crosslinks restricted the control on hydrogels swelling [32].

Swelling is controlled mainly with two forces: osmotic force and elasticity force, when a balance between these two forces is achieved, the equilibrium is reached and no additional swelling occur and the network structure is maintained against any deformities [19]. The understanding of hydrogel swelling behavior is important especially for controlling drug loading and release. Swelling-controlled drug release occur when the rate of drug diffusion is faster than the rate of hydrogel swelling [33, 34], in such model of release the higher the rate of hydrogel swelling leads higher rate of drug release [17].

The swelling rate and nature are affected by internal and external factors.

The internal factors include:

- The pores number and size [19, 23], super porous hydrogels are capable of rapid swelling and shrinking [29, 35].
- The crosslinking extent [23, 32, 36], increasing crosslinks increases the hydrogel stiffness and rigidity and restrict the movement of polymer chain leading to a limit swelling and shrinking ability [32].
- Polymer content and type [36], swelling can be decreased by reducing the average molecular weight of the polymer segments among crosslinks [37], also increasing hydrophobic polymer limits the swelling extent [23, 32].
- The repulsive and attractive interactions which exist between the networks will highly influence hydrogel volume, intra and inter molecular-interactions as Van der Waals attractions and hydrogen bonding, as well as the hydrophobic interactions also affect the degree of swelling in the hydrogel [38].

While the external factors that affect swelling rate and extent are:

- Medium pH [37, 38], pH effect is dependent on hydrogel composition, for example, in a study on natural polymers hydrogels, the swelling and release were higher in acidic medium (pH 3.9) compared to that once in (pH 7.1) [39], while other study reported that The water uptake increased with increasing pH in their designed hydrogel [40].
- Medium temperature [32], swelling weight and volume may decreased by decreasing the medium temperature of temperature-sensitive hydrogels [36].
- Photo irradiation and pressure showed marked ability to affect hydrogel swelling [36].

Flexibility, stretching and mechanical strength

Swelling of hydrogels requires a degree of flexibility which is often inversely proportion to the hydrogel mechanical strength, hydrogels low mechanical strength and rupturing ability is a strong cause to limit hydrogels use, hydrogels have a few energy dissipation mechanisms to slow crack propagation and have irregularly distributed crosslinking points and polymer chains of varying lengths between those points. This uneven distribution of stress among the polymer chains makes it easy for cracks to form. Researchers (table 1) have made numerous attempts to enhance the mechanical strength of hydrogels [41]. A number of methods have been developed in order to enhance hydrogel mechanical strength and thermal stability.

Below is a summary for two promising modern methods for enhancing mechanical strength:

Double network (DN) hydrogels, DN hydrogels have been proven to exhibit remarkable mechanical strength. These gels can withstand strain of 92% and fracture compressive stress of 17.2 MPa, as well as strain of 1000-2000% and fracture tensile stress of 1-10 MPa. Additionally, they also demonstrate great mechanical toughness with tearing fracture energy of 102-103 J m^2 [42].

DN hydrogels have significantly enhanced mechanical properties, which are likely due to their distinctive contrasting network structure and tough network entanglement, the principles for preparing tough chemically linked DN hydrogels can be summarized as follows [42]: first, use a hard and brittle polyelectrolyte as the first network, and the second network is a soft and ductile neutral polymer. Second, ensure that the molar concentration of the second network is 20-30 times that of the first network. Finally, tightly crosslink the first network while the second network is loosely cross-linked to achieve a strong asymmetric gel structure [43–45]. With these design principles in mind, the two-step polymerization method has been shown to be feasible for producing different tough DN hydrogels [46].

Although the high mechanical strength of DN hydrogels they also can hold a high water content (~90wt%) [42], so DN hydrogels can work as a stable carriers to release and deliver therapeutic drugs or biomolecules in a precise manner [46]. These advances have also led to new designs of biocompatible DN hydrogels for tissues regeneration as cartilage [47].

Nano composite hydrogels, these systems are distinguished by their unique network structure. They can be defined as hydrated polymeric networks, covalently or physically cross-linked with one another and/or with nanoparticles [48]. nanoparticles incorporation in hydrogels structure is a hopeful alternative to overcome the weak mechanical strength of traditional hydrogels, some results displayed that the nanocomposite hydrogel strength in distilled water and 0.9 wt% NaCl solution can reach 198.85 and 204.23 mJ/g, respectively, which were 13 times larger compared with the gel strength of matrix so it can withstand deformation such as elongation and torsion, it also behave free in swelling and deswelling [49, 50]. In addition, nanocomposite hydrogels are shown to be multi-responsive with improved physical, mechanical, and biological properties that may be obtained.

These nanoparticles that can be incorporated with hydrogels include inorganic nanoparticles as clay, graphene, hydroxyapatite, and metallic nanoparticles and organic/polymeric nanoparticles, which could be used as fillers to strengthen the hydrogel matrix and bring the hydrogel new functionalities as well [51].

Mechanism of hydrogel formation

Hydrogels are networks of polymers having hydrophilic nature. Generally, hydrogels are formed using hydrophilic monomers. However, hydrophobic monomers are sometimes used in preparation of hydrogel. Either natural or synthetic polymers could be used in preparation of hydrogels [10]. The synthetic polymers have hydrophobic properties and chemically stronger compared to natural polymers. Because of their mechanical strength the degradation rate of synthetic polymers is slow, but this mechanical strength enhances their stability as well. A balance between these two opposed properties should be achieved to prepare an optimum hydrogel design. Moreover, Natural polymers could be used in the preparation of hydrogels provided that these polymers have appropriate functional groups [66].

Hydrogels can be formulated by choosing the monomer or polymer type and the kind of hydrogel formation techniques. Hydrogels are designed by two methods either chemical crosslinking or physical crosslinking.

Table 1: Classification of studies that aimed to improve hydrogel properties according to the improved property and the improvement method

Property	First author	Property improvement method	References
Porosity	K. Kabiri	Control the addition time and sequence, and shorten the gelation time of the porosity generators (Acetone and sodium bicarbonate).	[52]
	Maya Ovadia	Synthesis of polys high internal phase emulsions which are extremely porous polymers and control their compositions.	[40]
	HV Chavda	Addition of low crosslinker concentrations led to a better porous structure.	[53]
	Kourosh Kabiri	Foaming conducted in the course of polymerization and dewatering of the as-synthesized gels.	[54]
	Nasim Annabi	Utilizing high-pressure CO2 to prepareα-elastin porous hydrogels.	[55]
	Manisha Pandey	Using solubilized bacterial cellulose in hydrogel synthesis.	[56]
	N. Vishal Gupta	Polymerization of crosslinking agent and using gas blowing method, Ac-Di-Sol as a stabilizer and bicarbonate as a foaming agent to produce the porous structure.	[57]
Mechanical strength	Jian Ping Gong	Synthesis of double-network gels to have both great water content and large toughness and mechanical strength.	[42]
	Haque Md Anamul		[58]
	Xuefeng Li		[59]
	Rakesh K. Mishra	Increasing the PVP content in the hydrogel membranes.	[60]
	Swati Sharma	Using cuminaldehyde and chitosan for gel preparation by covalent bonding between free carbonyl and amino group of cuminaldehyde and chitosan, respectively.	[61]
Absorption	Ali Pourjavadi	Optimizing a number of variables of the implant copolymerization (i. e. the initiator, the monomer, and the crosslinker concentration) to attain a hydrogel with improved swelling capacity.	[62]
	Qandeel Zahra	Adding Acrylamide-2-methyl propane sulfonic acid to enhance the swelling of hydrogels because of its polyelectrolyte nature.	[63]
	Maria Lazaridou	Synthesis chitosan copolymers (CS-g-SBMA) grafted with [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide in different molar ratios.	[64]
	Manisha Pandey	Graft polymerization of acrylamide on bacterial cellulose solubilized in an NaOH/urea solvent system and crosslinked by *N*,*N*′-methylenebisacrylamide under microwave irradiation.	[56]
	Swati Sharma	Using cuminaldehyde and chitosan for gel preparation by covalent bonding between free carbonyl and amino group of cuminaldehyde and chitosan, respectively.	[61]
Release	Baoqi Cai	Synthesis\hydrogel based on dynamic covalent bond, composed of 3-acrylamidophenyl boronic acid copolymerized with 2-lactobionamidoethyl methacrylate (p(APBA-*b*-LAMA)) by means of the association of boronic acid with diols.	[65]

Chemical cross-linking

Chemical crosslinking is a method that is often employed to create hydrogels. This process entails the use of polymer chains possessing functional groups that can form covalent bonds, which are cross-linked using multifunctional molecules or ions [67]. One such example is the creation of alginate hydrogels through ionic interactions between alginate polymer chains and divalent cations [68]. This interaction helps to stabilize the hydrogel structure, preventing it from dissolving in water and physiological fluids. Compared to physical hydrogels, chemically crosslinked hydrogel networks offer greater control, as their synthesis and applications are not solely reliant on pH. Chemical crosslinking provides the ability to modify the physical characteristics of the hydrogel [69]. The cross-linking process involved the use of small cross-linker molecules, photosensitive agents, polymer-polymer conjugation or enzyme-catalyzed reactions.

Small molecule crosslinking, the easiest method for producing hydrogels using small-molecule cross-linking is to mix a polymer, a small-molecule cross-linker, and an appropriate solvent. These substances, known as cross-linkers, are made up of at least two active functional groups that facilitate the formation of bonds between polymer chains [70]. Examples of these small molecules include formaldehyde, genipin, glutaraldehyde, diethyl squarate, blocked di-isocyanates and ethylene glycol diglycidyl ether [71].

Enzymatic cross-linking, it is a gentle and practical approach for in-situ hydrogel formation as it is a cytocompatible process, because no exogenous substances are used. Most of the enzymes used in hydrogel cross-linking are like those found in our bodies that catalyze biological processes. Several enzymes have been involved in this process, including lysyl oxidase, plasma amine oxidase, Transglutaminases TG, peroxidases, tyrosinase, phosphopantetheinyl transferase, Horseradish peroxidase, Laccase and phosphatases [72]. This process can take place under minor conditions as physiological temperature, an aqueous medium and neutral pH, which are compatible with body cells. The main advantage of this method is enzyme specificity as toxicity could be avoided, and no cytotoxicity may be generated. Moreover, the on-site creation of covalently bound hydrogels without the need of cross-linker molecules is another advantage of Enzymatic-based hydrogels. However, this method is expensive and difficult to produce [73].

Photo cross-linking, formation of hydrogels through photo-crosslinking relies on the presence of photo-sensitive functional groups. These groups are attached to a polymer, allowing it to form cross-links when exposed to specific wavelengths of light as UV light. This method offers spatiotemporal control over the reaction, fast cross-linking, preservation of the shape of hydrogels and room temperature environments. However, the light intensity, duration of exposure and concentration of photo-initiator are critical parameters which can cause cell damage. UV light (290–320 nm) could be replaced by visible light such as green (505 nm) and blue (405 nm) lights, as their intensity is relatively similar to that of UV, and they do not harm the cells. Chitosan, PEG and gelatin have been extensively studied for this purpose [67]. This technique can be applied to encapsulate bioactive molecules, like growth factors, for a variety of purposes, including wound healing [74].

Polymer-Polymer cross-linking (hybrid polymer networks), this process involves the interaction between a building block of one polymer chain and a building block of a different polymer chain with distinct characteristics. Consequently, before the cross-linking process, the polymers need to be modified by incorporating specific reactive functional groups. The choice of covalent connections can be tailored to control the cross-linking speed, select the desired reactive functional groups, and determine the biodegradability of the resultant compound [75].

For more details, (table 2) summarizes the advantages and disadvantages of each of the above methods.

Physical cross-linking

Hydrogels can be formulated using reversible or physical cross-linked networks. Molecular entanglements, supramolecular chemistry or physicochemical interactions such as hydrophobic interactions, hydrogen bonds, charge condensation, are responsible for holding physically cross-linked hydrogels together. The interest in physical or reversible gels has grown due to their simple production process and the absence of cross-linking agents so the primary benefit of physical crosslinking methods is their biomedical safety, as they do not involve the use of chemical crosslinking agents that may be cytotoxic [70]. The choice of hydrocolloid type, concentration, and pH is vital in achieving various gel textures. Many techniques have been

documented for the synthesis of physically cross-linked hydrogel [67].

Several methods have been documented for creating physically cross-linked hydrogels (table 3) shows some of them.

Table 2: Advantages and this advantage of chemical crosslinking method

Methods	Advantages	Disadvantages	References
Small molecule crosslinking	Mild conditions Fast gelation Good mechanical properties Spontaneous reaction	The possible toxicity of unreacted residual cross-linker agents *in vivo*.	[71]
Enzymatic crosslinking	No exogenous reagents. Specificity Spontaneous reaction. Fast gelation Control over the reaction. Mild conditions	The change of activity during stock solution storage. The enzyme costs are additional costs.	[72]
Photo-crosslinking	Fast gelation. Stabilization of weak cross-linked hydrogels Room temperature conditions. Spatiotemporal control of the reaction.	Light irradiation may affect cells. Needs precise regulation of photo-initiator, light intensity and duration of exposure.	[74]
Polymer-Polymer crosslinking	Biological inert gel Rapid formation of gel. Flexibility in multiple bonds types formation.	Multi-step purification and preparation	[75]

Table 3: Summary of physically cross linking method for hydrogel preparation

Method	Description	Method of hydrogel formation	Example	References
Crystallization	Involves creating a strong and extremely elastic hydrogel by freezing-thawing process.	The highly elastic gel is produced by subjecting the aqueous polymer solution to a repeated freeze–thaw process.	Freeze-thawing of xanthan and polyvinyl alcohol.	[70]
Hydrophobic interaction	Polymers undergo hydrophobic interaction must have both hydrophobic and hydrophilic domain, which is called amphiphilic.	This method involves increasing the temperature of the copolymers to the critical micelle temperature then they aggregate to form spherical micelles. These micelles composed of an outer layer of hydrated swollen hydrophilic chains with dehydrated hydrophobic blocks in the core.	Hydrogel made from polysaccharides such as dextran, chitosan and pullulan.	[76]
Ionic interactions	Hydrogels cross-linked using specific ions.	Cross-linking of Hydrogel is done using suitable ions under gentle conditions at physiological pH and at room temperature.	Alginate could cross-linked through calcium ions.	[69]
Polyelectrolyte complexes (PECs)	PECs are prepared by electrostatic interactions in an aqueous solution, between two polyelectrolytes with opposite charged.	Oppositely charged polymers will stick together forming soluble and insoluble complexes dependent on the pH and concentration of the particular solutions.	Polyatomic chitosan with polyanionic xanthan.	[76]
Hydrogen bonding interaction	Hydrogen bonded hydrogel of polymers carrying carboxyl groups.	Hydrogen bonded hydrogel could be obtained by dropping the pH of aqueous solution of polymers carrying carboxyl groups by dispersing the polymer into 0.1M HCl.	Hydrogen-binding of carboxy methyl cellulose polymer.	[69]
Stereo complexation	A synergistic interaction among polymer chains or small molecules with same chemical composition, but different stereochemistry.	Stereo complexation does not require harsh organic solvents or chemical cross-linkers.	Hydrogel prepared via crosslinking of lactic acid oligomers with opposite chirality.	[72]

Applications of hydrogels as a drug delivery system

Drug delivery systems play significant role in improving drugs therapeutic efficacy by overcoming the limitations of the traditional drug preparations. Some of these limitations are poor solubility, low bioavailability and short half-life, which considerably can influence the efficacy of the drug and require a frequent dosing [77]. Conversely, controlled drug delivery systems, like polymer-based hydrogels, display a hopeful solution for these limitations by permitting continued drug release for a long time period. This sustained and controlled release property assists in the maintenance of drug concentrations in the target site within the required range as well as avoiding any unexpected decrease or increase in blood-drug concentration that can result in suboptimal treatment results. As a result of extending the duration of drug release, the drug bioavailability will be enhanced using the polymer-based hydrogel systems as well as the frequency of drug dosing will be reduced which will support patient convenience and compliance [78]. Furthermore, hydrogels drug delivery systems have the advantage of delivering the drug to the target tissues or organs. Targeting drug delivery could be achieved by incorporating directing ligands or by altering the properties of hydrogel's. This approach will minimize the drug systemic exposure and diminishes the possible side effects, as well as enhancing the therapeutic efficiency at the intended site.

Furthermore, hydrogel drug delivery systems could be designed to accommodate various active ingredients with diverse physicochemical properties. In particular, these hydrogel systems offer flexibility in drug release and loading mechanisms, which allows for the delivery of numerous drug types as peptides, proteins and nucleic acids [79].

Hydrogels have been generally used for the preparation of controlled drug delivery systems for long time period. Once the hydrogel system that bears a drug comes in contact with an aqueous medium, the water will penetrate into the hydrogel system and the drug will be dissolved. Mainly, Diffusion phenomena occurs when the drug that is dissolved diffuses to the surrounding aqueous medium and gets out of the hydrogel delivery system [80].

Particularly, all of these distinctive properties makes the polymer-based hydrogels an attractive and promising technology for drug delivery and the current research in this area is predicted to result in preparation of novel and advanced drug delivery systems.

The following are some of the hydrogels applications as drug delivery system:

Controlled drug release

Polymer-based hydrogels can be utilized to control and adjust the release of drugs by altering the behavior of hydrogel swelling. This can be achieved by modifying the chemical structure or by adjustment of hydrogel crosslinking density [81].

Targeted drug delivery

Polymer-based hydrogels could be formulated to precisely target definite cell, tissue or organ. By combining targeting moieties for instance peptides or antibodies into the hydrogel, the drug could be targeted to a precise site inside the body [82].

Oral drug delivery

The oral bioavailability of drugs could be improved using polymer-based hydrogels. The drug will be protected from degradation and released in a controlled manner in the gastrointestinal tract [83].

Transdermal drug delivery

Transdermal delivery is a painless way of administration drugs systemically by applying a drug preparation onto healthy skin [84, 85]. Polymer-based hydrogels could be also used for drug delivery trough transdermal route. The drug is incorporated inside the hydrogel matrix and the hydrogel system then applied topically to the skin. The drug release will be in an organized manner over time [85].

Implantable drug delivery systems

Polymer-based hydrogels can be employed as implantable drug delivery systems, in which the hydrogel is applied inside the body and the drug is released over long period of time. In particular, these systems are utilized for chronic diseases therapy or in delivering long-acting drugs [86].

Gene delivery

The genetic material as DNA or RNA could be delivered to the intended site using polymer-based hydrogels. The genetic material is incorporated into the hydrogel matrix. Mainly, this will protect the genetic material from degradation and enforce its delivery to the target site [87].

Hydrogels success as drug delivery systems can be judged by a number of marketed formulations (table 4).

Table 4: Some of hydrogel-based products on the market

Active ingredient	Product	Hydrogel based product	Polymer system	Remarks of hydrogel based product	References
Diltiazem	Cardizem®	Diltiazem SQZ Gel™	Chitosan and polyethylene glycol	pH-Sensitive, provides less-frequent administrations than a traditional tablet product thus enhances patient compliance as it once a-day tablet of diltiazem hydrochloride.	[88]
Metronidazole	MetroGel Vaginal ®	Hycore-V™ and Hycore-R™	Not disclosed	Localized delivery of metronidazole for vaginal and rectal infections, respectively. Thus minimize its side effects.	[89]
Dexamethasone	Maxidex®	DEXTENZA™	PEG	Post-operative inflammation, allergic conjunctivitis providing coverage up to 30 d of sustained steroid release.	[90]
Morphine sulfate	Oramorph®	Moraxen ®	PU (PEG-diol)	Improved bioavailability of morphine.	[91]
Dinoprostone	Dinoglandin®	Propess®	PU (PEG-diol)	Induction of labor,the vagina pessary stays for 24 h and slowly releasing the hormone.	[92]
Histrelin acetate	-	Supprelin® LA	2-Hydroxyethyl/propyl methacrylate, trimethylolpropane trimethacrylate	Treatment of children with central precocious puberty, provides sustained release for 12 mo	[93]

CONCLUSION

Hydrogels became a core of many studies due to their flexible structure, the capacity to hold a large amount of active ingredients, and their ability to respond to different stimuli. Studies and research about hydrogels started in 1960, and many trials to include hydrogels in dosage forms have been performed. However, hydrogel use in pharmaceutical technology is limited due to many obstacles that affect its quality. Many studies have attempted to overcome different limitations and make the better hydrogel formulation, which shows that hydrogels are on their way to becoming a milestone in many dosage forms.

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AUTHORS CONTRIBUTIONS

Dr. Aya M. Ghanem (1st author, Corresponding author): Study the concept, design of the manuscript, review of literature and paper writing. Dr. Sondos Ahmad Ashour (2nd author): Data collection, review of literature, data interpretation and paper writing. Dr. Ruaa M Hussien (3rd author): Review of literature, planning and conceptualization.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Ahmed EM. Hydrogel: preparation characterization and applications: a review. J Adv Res. 2015 Mar;6(2):105-21. doi: 10.1016/j.jare.2013.07.006, PMID 25750745.
2. Chamkouri H. A review of hydrogels their properties and applications in medicine. AJBSR. 2021 Feb 3;11(6):485-93. doi: 10.34297/AJBSR.2021.11.001682.
3. Sanchez Cid P, Jimenez Rosado M, Romero A, Perez Puyana V. Novel trends in hydrogel development for biomedical applications: a review. Polymers. 2022 Jul 26;14(15):3023. doi: 10.3390/polym14153023, PMID 35893984.
4. Giordano S, Gallo E, Diaferia C, Rosa E, Carrese B, Borbone N. Multicomponent peptide based hydrogels containing chemical functional groups as innovative platforms for biotechnological applications. Gels. 2023 Nov 15;9(11):903. doi: 10.3390/gels9110903, PMID 37998993.
5. Karoyo AH, Wilson LD. A review on the design and hydration properties of natural polymer based hydrogels. Materials (Basel). 2021 Feb 26;14(5):1095. doi: 10.3390/ma14051095, PMID 33652859.
6. Cao H, Duan L, Zhang Y, Cao J, Zhang K. Current hydrogel advances in physicochemical and biological response driven biomedical application diversity. Signal Transduct Target Ther. 2021 Dec 16;6(1):426. doi: 10.1038/s41392-021-00830-x, PMID 34916490.
7. HO TC, Chang CC, Chan HP, Chung TW, Shu CW, Chuang KP. Hydrogels: properties and applications in biomedicine. Molecules. 2022 May 2;27(9):2902. doi: 10.3390/molecules27092902.
8. Cai MH, Chen XY, FU LQ, DU WL, Yang X, Mou XZ. Design and development of hybrid hydrogels for biomedical applications: recent trends in anticancer drug delivery and tissue engineering. Front Bioeng Biotechnol. 2021 Feb 17;9:630943. doi: 10.3389/fbioe.2021.630943, PMID 33681168.
9. Catoira MC, Fusaro L, DI Francesco D, Ramella M, Boccafoschi F. Overview of natural hydrogels for regenerative medicine

applications. J Mater Sci Mater Med. 2019 Oct 10;30(10):115. doi: 10.1007/s10856-019-6318-7, PMID 31599365.
10. LU L, Yuan S, Wang J, Shen Y, Deng S, Xie L. The formation mechanism of hydrogels. Curr Stem Cell Res Ther. 2018;13(7):490-6. doi: 10.2174/1574888X12666170612102706, PMID 28606044.
11. Peng W, LI D, Dai K, Wang Y, Song P, LI H. Recent progress of collagen chitosan alginate and other hydrogels in skin repair and wound dressing applications. Int J Biol Macromol. 2022 May 31;208:400-8. doi: 10.1016/j.ijbiomac.2022.03.002, PMID 35248609.
12. Chen M, Wang Y, Zhang J, Peng Y, LI S, Han D. Stimuli responsive DNA based hydrogels for biosensing applications. J Nanobiotechnology. 2022 Jan 21;20(1):40. doi: 10.1186/s12951-022-01242-x, PMID 35062945.
13. Jiang Y, Wang Y, LI Q, YU C, Chu W. Natural polymer based stimuli responsive hydrogels. Curr Med Chem. 2020;27(16):2631-57. doi: 10.2174/0929867326666191122144916, PMID 31755377.
14. Wang C, Zhang J. Recent advances in stimuli responsive DNA based hydrogels. ACS Appl Bio Mater. 2022 May 16;5(5):1934-53. doi: 10.1021/acsabm.1c01197, PMID 35138079.
15. Das S, Kumar V, Tiwari R, Singh L, Singh S. Recent advances in hydrogels for biomedical applications. Asian J Pharm Clin Res. 2018 Nov;11(11):62-8. doi: 10.22159/ajpcr.2018.v11i11.27921.
16. Amsden B. Solute diffusion within hydrogels. Mechanisms and models. Macromolecules. 1998 Nov 1;31(23):8382-95. doi: 10.1021/ma980765f.
17. Ghasemiyeh P, Mohammadi Samani S. Hydrogels as drug delivery systems; pros and cons. Trends Pharmacol Sci. 2019 Mar 1;5(1):7-24.
18. Gerecht S, Townsend SA, Pressler H, Zhu H, Nijst CL, Bruggeman JP. A porous photocurable elastomer for cell encapsulation and culture. Biomaterials. 2007;28(32):4826-35. doi: 10.1016/j.biomaterials.2007.07.039, PMID 17692371.
19. Ganji F, Vasheghani FS, Vasheghani FE. Theoretical description of hydrogel swelling: a review. Iranian Polymer Journal. 2010;19(5):375-98.
20. Yin L, Fei L, Cui F, Tang C, Yin C. Superporous hydrogels containing poly(acrylic acid-co-acrylamide)/O-carboxymethyl chitosan interpenetrating polymer networks. Biomaterials. 2007 Feb 1;28(6):1258-66. doi: 10.1016/j.biomaterials.2006.11.008, PMID 17118443.
21. Lima CS, Balogh TS, Varca JP, Varca GH, Lugao AB, A Camacho Cruz L. An updated review of macro micro and nanostructured hydrogels for biomedical and pharmaceutical applications. Pharmaceutics. 2020 Oct;12(10):970. doi: 10.3390/pharmaceutics12100970, PMID 33076231.
22. Hafezi Moghaddam R, Dadfarnia S, Shabani AM, Moghaddam ZH, Tavakol M. Electron beam irradiation synthesis of porous and non porous pectin based hydrogels for a tetracycline drug delivery system. Mater Sci Eng C Mater Biol Appl. 2019 Sep 1;102:391-404. doi: 10.1016/j.msec.2019.04.071, PMID 31147010.
23. Garg S, Garg A. Hydrogel: classification properties preparation and technical features. Asian J Biomater Res. 2016 Sep 4;2:163-70.
24. Holback H, Yeo Y, Park K. 1-Hydrogel swelling behavior and its biomedical applications. Rimmer S, editor. Woodhead Publishing Series in Biomaterials Biomedical Hydrogels; 2011. p. 3-24.
25. Sornkamnerd S, Okajima MK, Kaneko T, Kaneko T. Tough and porous hydrogels prepared by simple lyophilization of LC gels. ACS Omega. 2017 Aug 31;2(8):5304-14. doi: 10.1021/acsomega.7b00602, PMID 31457799.
26. Luo R, WU J, Dinh ND, Chen CH. Gradient porous elastic hydrogels with shape-memory property and anisotropic responses for programmable locomotion. Adv Funct Materials. 2015;25(47):7272-9. doi: 10.1002/adfm.201503434.
27. DE France KJ, XU F, Hoare T. Structured macroporous hydrogels: progress challenges and opportunities. Adv Healthc Mater. 2018;7(1):1700927. doi: 10.1002/adhm.201700927, PMID 29195022.
28. Am R, Prabhu SS, JD JR, Sundaram SK, RM, TK V. Hydrogel formulation from calotropis gigantea plant extract against foot ulcer causing bacteria in diabetes. Asian J Pharm Clin Res. 2021 Nov 7;14(11):96-9.
29. Gemeinhart RA, Park H, Park K. Pore structure of superporous hydrogels. Polym Adv Technol. 2000;11(8-12):617-25. doi: 10.1002/1099-1581(200008/12)11:8/12<617::AID-PAT12>3.0.CO;2-L.
30. Thakur S, Thakur VK, Arotiba OA. History classification properties and application of hydrogels: an overview. In: Hydrogels recent advances. Thakur VK, Thakur MK, editors. Gels Horizons: From Science to Smart Materials: 2018. p. 29-50. doi: 10.1007/978-981-10-6077-9_2.
31. Zhan Y, FU W, Xing Y, MA X, Chen C. Advances in versatile anti-swelling polymer hydrogels. Mater Sci Eng C Mater Biol Appl. 2021 Aug 1;127:112208. doi: 10.1016/j.msec.2021.112208, PMID 34225860.
32. Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm. 2000 Jul 3;50(1):27-46. doi: 10.1016/s0939-6411(00)00090-4, PMID 10840191.
33. Lin CC, Metters AT. Hydrogels in controlled release formulations: network design and mathematical modeling. Adv Drug Deliv Rev. 2006 Nov 30;58(12-13):1379-408. doi: 10.1016/j.addr.2006.09.004, PMID 17081649.
34. Kim D, Park K. Swelling and mechanical properties of superporous hydrogels of poly (acrylamide-co-acrylic acid)/polyethylenimine interpenetrating polymer networks. Polymer. 2004 Jan 1;45(1):189-96. doi: 10.1016/j.polymer.2003.10.047.
35. Gemeinhart RA, Chen J, Park H, Park K. pH-sensitivity of fast responsive superporous hydrogels. J Biomater Sci Polym Ed. 2000 Jan 1;11(12):1371-80. doi: 10.1163/156856200744390, PMID 11261878.
36. Huglin MB, Liu Y, Velada J. Thermoreversible swelling behaviour of hydrogels based on N-isopropylacrylamide with acidic comonomers. Polymer. 1997 Nov 1;38(23):5785-91. doi: 10.1016/S0032-3861(97)00135-3.
37. Brannon Peppas L, Peppas NA. Equilibrium swelling behavior of pH-sensitive hydrogels. Chem Eng Sci. 1991 Jan 1;46(3):715-22. doi: 10.1016/0009-2509(91)80177-Z.
38. Kasai RD, Radhika D, Archana S, Shanavaz H, Koutavarapu R, Lee DY. A review on hydrogels classification and recent developments in biomedical applications. Int J Polym Mater Polym Biomater. 2023;72(13):1059-69. doi: 10.1080/00914037.2022.2075872.
39. Younis MK, Tareq AZ, Kamal IM. Optimization of swelling drug loading and release from natural polymer hydrogels. IOP Conf Ser Mater Sci Eng. 2018 Dec;454(1):012017. doi: 10.1088/1757-899X/454/1/012017.
40. Ovadia M, Silverstein MS. High porosity responsive hydrogel copolymers from emulsion templating. Polym Int. 2016;65(3):280-9. doi: 10.1002/pi.5052.
41. Huang T, XU HG, Jiao K, Zhu LP, Brown H, Wang H. A novel hydrogel with high mechanical strength: a macromolecular microsphere composite hydrogel. Adv Mater. 2007 May 15;19(12):1622-6. doi: 10.1002/adma.200602533.
42. Gong JP. Why are double network hydrogels so tough? Soft Matter. 2010 Jun 8;6(12):2583-90. doi: 10.1039/b924290b.
43. Haque MA, Kurokawa T, Kamita G, Gong JP. Lamellar bilayers as reversible sacrificial bonds to toughen hydrogel: hysteresis self-recovery fatigue resistance and crack blunting. Macromolecules. 2011 Nov 22;44(22):8916-24. doi: 10.1021/ma201653t.
44. HU J, Hiwatashi K, Kurokawa T, Liang SM, WU ZL, Gong JP. Microgel reinforced hydrogel films with high mechanical strength and their visible mesoscale fracture structure. Macromolecules. 2011 Oct 11;44(19):7775-81. doi: 10.1021/ma2016248.
45. Suekama TC, HU J, Kurokawa T, Gong JP, Gehrke SH. Double network strategy improves fracture properties of chondroitin sulfate networks. ACS Macro Lett. 2013 Feb 19;2(2):137-40. doi: 10.1021/mz3006318, PMID 35581775.
46. Chen Q, Chen H, Zhu L, Zheng J. Fundamentals of double network hydrogels. J Mater Chem B. 2015 Mar 31;3(18):3654-76. doi: 10.1039/c5tb00123d, PMID 32262840.
47. Chen Y, Dong K, Liu Z, XU F. Double network hydrogel with high mechanical strength: performance progress and future perspective. Sci China Technol Sci. 2012 Aug 1;55(8):2241-54. doi: 10.1007/s11431-012-4857-y.

48. Argenta DF, Dos Santos TC, Campos AM, Caon T. Chapter 3-hydrogel nanocomposite systems: physic chemical characterization and application for drug delivery systems. Mohapatra SS, Ranjan S, Dasgupta N, Mishra RK, Thomas S, editors. Elsevier: Nanocarriers for drug delivery; 2019. p. 81-131. doi:10.1016/B978-0-12-814033-8.00003-5.
49. Haraguchi K, Takehisa T. Nanocomposite hydrogels: a unique organic–inorganic network structure with extraordinary mechanical optical and swelling/de-swelling properties. Adv Mater. 2002 Aug 16;14(16). doi: 10.1002/1521-4095(20020816)14:16<1120::AID-ADMA1120>3.0.CO;2-9.
50. XU K, Wang J, Xiang S, Chen Q, Yue Y, SU X. Polyampholytes superabsorbent nanocomposites with excellent gel strength. Compos Sci Technol. 2007 Dec 1;67(15-16):3480-6. doi: 10.1016/j.compscitech.2007.02.009.
51. Das D, Kar T, Das PK. Gel nanocomposites: materials with promising applications. Soft Matter. 2012 Feb 2;8(8):2348-65. doi: 10.1039/C1SM06639K.
52. Kabiri K, Omidian H, Hashemi SA, Zohuriaan Mehr MJ. Synthesis of fast-swelling superabsorbent hydrogels: effect of crosslinker type and concentration on porosity and absorption rate. Eur Polym J. 2003 Jul 1;39(7):1341-8. doi: 10.1016/S0014-3057(02)00391-9.
53. Chavda H, Patel C. Effect of crosslinker concentration on characteristics of superporous hydrogel. Int J Pharm Investig. 2011;1(1):17-21. doi: 10.4103/2230-973X.76724, PMID 23071915.
54. Kabiri K, Zohuriaan Mehr MJ. Porous superabsorbent hydrogel composites: synthesis morphology and swelling rate. Macro Materials & Eng. 2004;289(7):653-61. doi: 10.1002/mame.200400010.
55. Annabi N, Mithieux SM, Boughton EA, Ruys AJ, Weiss AS, Dehghani F. Synthesis of highly porous crosslinked elastin hydrogels and their interaction with fibroblasts *in vitro*. Biomaterials. 2009 Sep 1;30(27):4550-7. doi: 10.1016/j.biomaterials.2009.05.014, PMID 19500832.
56. Pandey M, Mohd Amin MC, Ahmad N, Abeer MM. Rapid synthesis of superabsorbent smart swelling bacterial cellulose/acrylamide based hydrogels for drug delivery. Int J Polym Sci. 2013 Aug 28;2013:1-10. doi: 10.1155/2013/905471.
57. Gupta NV, Shivakumar HG. Preparation and characterization of superporous hydrogels as pH-sensitive drug delivery system for pantoprazole sodium. Curr Drug Deliv. 2009 Oct 1;6(5):505-10. doi: 10.2174/156720109789941722, PMID 19863492.
58. Haque MD A, Kurokawa T, Gong JP. Super tough double network hydrogels and their application as biomaterials. Polymer. 2012 Apr 17;53(9):1805-22. doi: 10.1016/j.polymer.2012.03.013.
59. LI X, Wang H, LI D, Long S, Zhang G, WU Z. Dual ionically cross linked double-network hydrogels with high strength toughness swelling resistance and improved 3D printing process ability. ACS Appl Mater Interfaces. 2018 Sep 19;10(37):31198-207. doi: 10.1021/acsami.8b13038, PMID 30148345.
60. Mishra RK, Datt M, Banthia AK. Synthesis and characterization of pectin/PVP hydrogel membranes for drug delivery system. AAPS Pharm Sci Tech. 2008 Jun 1;9(2):395-403. doi: 10.1208/s12249-008-9048-6, PMID 18431673.
61. Sharma S, Jain P, Tiwari S. Dynamic imine bond based chitosan smart hydrogel with magnified mechanical strength for controlled drug delivery. Int J Biol Macromol. 2020 Oct 1;160:489-95. doi: 10.1016/j.ijbiomac.2020.05.221, PMID 32479933.
62. Pourjavadi A, Kurdtabar M, Mahdavinia GR, Hosseinzadeh H. Synthesis and super swelling behavior of a novel protein based superabsorbent hydrogel. Polym Bull. 2006 Oct 1;57(6):813-24. doi: 10.1007/s00289-006-0649-5.
63. Zahra Q, Minhas MU, Khan S, WU PC, Suhail M, Iqbal R. Fabrication of polyethylene glycol hydrogels with enhanced swelling; loading capacity and release kinetics. Polym Bull. 2022 Jul 1;79(7):5389-415. doi: 10.1007/s00289-021-03740-8.
64. Lazaridou M, Nanaki S, Zamboulis A, Papoulia C, Chrissafis K, Klonos PA. Super absorbent chitosan based hydrogel sponges as carriers for caspofungin antifungal drug. Int J Pharm. 2021 Sep 5;606:120925. doi: 10.1016/j.ijpharm.2021.120925, PMID 34303816.
65. Cai B, Luo Y, Guo Q, Zhang X, WU Z. A glucose sensitive block glycopolymer hydrogel based on dynamic boronic ester bonds for insulin delivery. Carbohydr Res. 2017 Jun 5;445:32-9. doi: 10.1016/j.carres.2017.04.006, PMID 28395252.
66. Zhang J, Zhao D, LU K. Mechanisms and influencing factors of peptide hydrogel formation and biomedicine applications of hydrogels. Soft Matter. 2023 Oct 11;19(39):7479-93. doi: 10.1039/d3sm01057k, PMID 37756117.
67. Parhi R. Cross linked hydrogel for pharmaceutical applications: a review. Adv Pharm Bull. 2017 Dec;7(4):515-30. doi: 10.15171/apb.2017.064, PMID 29399542.
68. Pragya A, Mutalik S, Younas MW, Pang SK, SO PK, Wang F. Dynamic cross-linking of an alginate acrylamide tough hydrogel system: time resolved in situ mapping of gel self assembly. RSC Adv. 2021 Mar 10;11(18):10710-26. doi: 10.1039/d0ra09210j, PMID 35423570.
69. Bashir S, Hina M, Iqbal J, Rajpar AH, Mujtaba MA, Alghamdi NA. Fundamental concepts of hydrogels: synthesis properties and their applications. Polymers. 2020 Nov 16;12(11):2702. doi: 10.3390/polym12112702, PMID 33207715.
70. Berger J, Reist M, Mayer JM, Felt O, Gurny R. Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. Eur J Pharm Biopharm. 2004 Jan;57(1):35-52. doi: 10.1016/s0939-6411(03)00160-7, PMID 14729079.
71. Xue X, HU Y, Wang S, Chen X, Jiang Y, SU J. Fabrication of physical and chemical crosslinked hydrogels for bone tissue engineering. Bioact Mater. 2022;12:327-39. doi: 10.1016/j.bioactmat.2021.10.029, PMID 35128180.
72. Naranjo Alcazar R, Bendix S, Groth T, Gallego Ferrer G. Research progress in enzymatically cross linked hydrogels as injectable systems for bioprinting and tissue engineering. Gels. 2023 Mar 15;9(3):230. doi: 10.3390/gels9030230, PMID 36975679.
73. Badali E, Hosseini M, Mohajer M, Hassanzadeh S, Saghati S, Hilborn J. Enzymatic crosslinked hydrogels for biomedical application. Polym Sci A. 2022 Feb;63:S1-S22.
74. MA H, Peng Y, Zhang S, Zhang Y, Min P. Effects and progress of photo-crosslinking hydrogels in wound healing improvement. Gels. 2022 Sep 23;8(10):609. doi: 10.3390/gels8100609, PMID 36286110.
75. Czarnecki S, Rossow T, Seiffert S. Hybrid polymer network hydrogels with tunable mechanical response. Polymers. 2016;8(3):82. doi: 10.3390/polym8030082, PMID 30979176.
76. XU J, Zhu X, Zhao J, Ling G, Zhang P. Biomedical applications of supramolecular hydrogels with enhanced mechanical properties. Adv Colloid Interface Sci. 2023 Nov;321:103000. doi: 10.1016/j.cis.2023.103000, PMID 37839280.
77. Palmese LL, Thapa RK, Sullivan MO, Kiick KL. Hybrid hydrogels for biomedical applications. Curr Opin Chem Eng. 2019 Jun;24:143-57. doi: 10.1016/j.coche.2019.02.010, PMID 31844607.
78. Villalba Rodriguez AM, Martinez Gonzalez S, Sosa Hernandez JE, Parra-Saldivar R, Bilal M, Iqbal HM. Nanoclay/polymer based hydrogels and enzyme-loaded nanostructures for wound healing applications. Gels. 2021 May 14;7(2):59. doi: 10.3390/gels7020059, PMID 34068868.
79. Zhang Y, Huang Y. Rational design of smart hydrogels for biomedical applications. Front Chem. 2020;8:615665. doi: 10.3389/fchem.2020.615665, PMID 33614595.
80. Golmohamadi M, Davis TA, Wilkinson KJ. Diffusion and partitioning of cations in an agarose hydrogel. J Phys Chem A. 2012 Jun 28;116(25):6505-10. doi: 10.1021/jp212343g, PMID 22394244.
81. Lacroce E, Rossi F. Polymer based thermoresponsive hydrogels for controlled drug delivery. Expert Opin Drug Deliv. 2022 Oct;19(10):1203-15. doi: 10.1080/17425247.2022.2078806, PMID 35575265.
82. Moriya G, Mazumder R, Padhi S, Mishra R. Gastrorententive hydrogels responsive to external stimuli for novel drug delivery. Int J App Pharm. 2024 Jul 7;16(4):1-14. doi: 10.22159/ijap.2024v16i4.51051.
83. Kakkar V, Narula P. Role of molecularly imprinted hydrogels in drug delivery a current perspective. Int J Pharm. 2022 Sep 25;625:121883. doi: 10.1016/j.ijpharm.2022.121883, PMID 35870667.

84. Ghanem AM. A review on recent advances in transdermal drug delivery systems of tamsulosin. Int J App Pharm. 2024 Mar;16(2):28-33. doi: 10.22159/ijap.2024v16i2.49950.
85. Khan F, Atif M, Haseen M, Kamal S, Khan MS, Shahid S. Synthesis classification and properties of hydrogels: their applications in drug delivery and agriculture. J Mater Chem B. 2022 Jan 5;10(2):170-203. doi: 10.1039/d1tb01345a, PMID 34889937.
86. Dimatteo R, Darling NJ, Segura T. In situ forming injectable hydrogels for drug delivery and wound repair. Adv Drug Deliv Rev. 2018 Mar 1;127:167-84. doi: 10.1016/j.addr.2018.03.007, PMID 29567395.
87. MO F, Jiang K, Zhao D, Wang Y, Song J, Tan W. DNA hydrogel based gene editing and drug delivery systems. Adv Drug Deliv Rev. 2021 Jan;168:79-98. doi: 10.1016/j.addr.2020.07.018, PMID 32712197.
88. Andriano K. Controlled release of highly water soluble drugs from the SQZ gel oral drug delivery system. Drug Deliv Technol. 2010 Jan;10(1):42.
89. https://www.rxlist.htm#description.Com/metrogel-vaginal-drug. [Last accessed on 20 Feb 2024].
90. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/125277s000PharR_P1.pdf. [Last accessed on 20 Aug 2024].
91. https://www.drugs.com/pro/maxidex.html. [Last accessed on 23 Apr 2023].
92. https://www.medicines.org.uk/emc/product/135/smpc#gref. [Last accessed on 15 Mar 2024].
93. https://www.supprelinla.com. [Last accessed on 15 Mar 2024].

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Review Article

AN EMERGING ERA IN DRUG DELIVERY SYSTEM FOR TREATMENT OF MALARIA: WAVE FROM CONVENTIONAL TO ADVANCED TECHNOLOGY

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ABSTRACT

Colonization of the erythrocytic stages of Plasmodium falciparum has become a challenging aspect in every drug delivery system because it is responsible for each clinical manifestation and life-threatening complication in malaria. With the emergence of resistance in malarial parasites in the recent past, developing a vaccine against malaria is still a long-drawn-out affair. However, recent reports of the recombinant protein-based vaccine against malaria vaccine from Glaxo Smith Kline have initiated a new ray of hope. In such a scenario, the onus of developing a reliable drug against the disease remains the mainstay in fighting against malaria. This review delves into the various attempts carried out by researchers in the past to develop a drug against the erythrocytic stages of the malaria parasite and throws light on a very recent outcome that provides targeted delivery of the drug to the infected erythrocyte using a nanotechnology-based approach. Considering the eventful journey in the beginning, it was the discovery of chloroquine that created an epoch in the treatment of malaria. Due to its low cost and high efficacy, it became the most widely used antimalarial. Until the 1960s, Chloroquine (CQ) was the best solution against malaria, but the scenario changed in the 1970s due to widespread clinical resistance in Plasmodium falciparum and Plasmodium vivax in various parts of the world. This, in turn, led to the development of novel drug delivery systems using liposomes and Solid Lipid Nanoparticles (SLN) for more effective and site-specific delivery of chloroquine to the infected erythrocytes. Such attempts led to a later use of the nanotechnology-based approach which included the use of nanospheres and nanoparticulate drug carriers.

Keywords: Malaria, Novel drug approaches, Nanotechnology, Artificial intelligence

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INTRODUCTION

Malaria is a serious and sometimes fatal disease caused by a parasite that commonly infects a certain type of mosquito that feeds on humans. People who get malaria are typically very sick with high fevers, shaking chills, and flu-like illness [1]. Although a variety of antimalarial drugs are available for treatment, public health emergencies regarding malaria are increasing due to the spread of specific types of malaria parasites that are resistant to these drugs. In part because of the moderate to high costs of these drugs and the often uncontrolled counterfeit antimalarial market, most people in malaria-endemic countries have no immediate access to affordable, effective antimalarial therapy [2]. Also, the prevention of malaria with chemoprophylactic drugs is often not successful and is accompanied by many problems. Lastly, large-scale efforts for eradicating malaria through vector control strategies have met with little success and are not feasible against the persistence of the disease in many parts of the world today. Malaria is a disease that is both preventable and curable. However, it remains a serious public health problem in many countries. Malaria presents a risk for 3.2 billion people globally and caused 576,000 deaths in 2015 [3]. The following malaria data for 2022 were highlighted in the World Malaria Report 2023. In 2022, there were around 249 million malaria cases worldwide, an increase of 5 million over 2021. An anticipated 608,000 people will die from malaria worldwide in 2022, an almost 6% rise from 2019. In 2022, the African continent bore the brunt of the malaria load, accounting for 94% of global cases and 95% of malaria-related deaths, with children under the age of five accounting for approximately 78% of these deaths [4]. Around 1.27 billion people on the African continent were susceptible to malaria infection, with 186 cases and 47 fatalities per 100,000 people. Africa has seen a 7.6% decrease in malaria incidence and mortality since 2015 [5]. The disease is caused by parasites of the Plasmodium species and is transmitted to humans through the bites of infected mosquitoes. Most deaths are caused by Plasmodium Falciparum, which is the most prevalent and the most fatal malaria parasite in Africa. 90% of all malaria deaths occur in sub-Saharan Africa. Other high-risk groups include pregnant women and children less than 5 years of age [6]. In non-endemic countries, imported cases of malaria occur frequently due to human migration and travel. Malaria can be fatal if not treated promptly with an effective antimalarial medicine. However, an accurate diagnosis and appropriate treatment of malaria, particularly Plasmodium falciparum infection, is complex [7]. This is because the clinical symptoms of malaria are nonspecific and can be mistaken for other febrile illnesses and access to healthcare and effective antimalarial treatment is poor in many endemic regions. This all leads to increased drug pressure and resistance of the parasites to antimalarial medicines, making malaria control significantly more difficult and furthering the burden of the disease [8]. Due to these factors, malaria is a continual threat to the developing world and can have a significant impact on economic development. Malaria viruses are spread by Anopheles mosquitoes. The mosquito bites are used as host to mature the parasites in the mosquito's stomach [9]. These parasites then travel to the salivary glands of mosquitoes, and the cycle is repeated during the next mosquito bite. Various research have been conducted previously that undoubtedly attests to numerous therapeutics used in treating malaria. From 1990 to 2024 there have been more than 988 reviews and research articles in the PubMed database indicating their significant significance. The systemic review inclusion of 222 studies indicated the therapeutic advantages in the treatment of malaria.

Epidemiology

The epidemiology of malaria is tightly related to transmission intensity, acquired immunity, and clinical symptoms. Malaria is caused by Plasmodium parasites, which are spread through the bite of infected female Anopheles mosquitoes [10]. Immunity is highest in locations with intense transmission, with children under the age of five being the most vulnerable group, particularly in Africa, where the majority of malaria-related deaths occur. Displaced populations from low-transmission areas are more vulnerable due to a lack of acquired immunity, needing extensive intervention measures to prevent morbidity and mortality. Plasmodium parasites, which are spread by Anopheles mosquitoes carrying the infection, cause malaria. Plasmodium falciparum is the deadliest of the four primary species that infect people, along with Plasmodium vivax,

Plasmodium ovale, and Plasmodium malaria [11]. Around the equator, malaria is endemic throughout a large area, mostly in tropical and subtropical parts of Africa, Asia, and Latin America. Globally, there were predicted to be 247 million cases of malaria in 2021, with 619,000 fatalities from the disease; 94% of cases and deaths were in the African region as per the reports from the World Health Organization (WHO). Each region has a different level of malaria transmission; some have high, moderate, or low transmission [12]. The spleen rate, yearly parasite incidence, and entomologic inoculation rate are examples of epidemiologic metrics. Since malaria immunity is developed via repeated exposure, young children under the age of five in high-transmission regions have the greatest fatality rates. As the rate of transmission declines, more people of all ages fall sick, and cerebral malaria becomes more prevalent than severe anemia [13]. Due to a lack of immunity, displaced persons migrating from low-to-high-transmission zones are most vulnerable to serious illness. Chronic impacts of malaria can include anemia and unfavorable pregnancy outcomes [14]. The genetic variety of Plasmodium species, climatic change, and interruptions to prevention and control efforts, as shown during the coronavirus disease 2019 pandemic are the factors that affect the epidemiology of malaria. To address the changing global malaria load, ongoing surveillance, research into parasite resistance, and coordinated control techniques are crucial.

Etiology

Malaria is caused by the complex lifecycle of Plasmodium parasites, which results in typical cyclical fevers and varied incubation times among species. Malaria can cause symptoms ranging from moderate to severe, including paroxysmal fever, anemia, and potentially fatal consequences such as cerebral malaria and multi-organ failure [15]. The female Anopheles mosquito, which requires a blood meal to produce eggs, is the vector of human malaria, with distinct species preferences and behaviors determining transmission dynamics. The illness's etiology also includes aspects such as the genetic variety of Plasmodium species, the evolution of drug-resistant strains, and the effects of climate change on disease distribution, emphasizing the need for complete understanding and effective control techniques [16]. Plasmodium is the genus of single-celled parasites that cause malaria. Human infections are most frequently caused by plasmodium falciparum, plasmodium vivax, plasmodium ovale, and plasmodium malariae. The way these parasites infect people is by biting by a female Anopheles mosquito carrying the infection. The parasites enter the circulation from an infected mosquito bite and go to the liver where they develop. The adult parasites re-enter the systemic circulation and infect Red Blood Cells (RBC) after a few days [17]. The parasites grow quickly inside the RBC, rupturing the infected cells. Depending on the species, this cycle continues with the parasites infecting new RBC every 48 to 72 h. Certain Plasmodium species, including Plasmodium vivax and Plasmodium ovale can lie dormant in the liver for months or even years before springing back to life and inducing another illness relapse [18]. Additionally, organ transplants, blood transfusions, and mother-to-child transmission during pregnancy and childbirth can all result in malaria transmission. Depending on the variety of Plasmodium involved, malaria can vary in severity. The deadliest strain Plasmodium falciparum, can result in serious side effects such as organ failure, respiratory distress, and brain malaria. Generally, the sickness is caused by other species such as Plasmodium vivax and Plasmodium ovale in lesser forms [19].

Life-cycle of malaria

Malaria's life cycle (fig. 1) begins when a female Anopheles mosquito carrying Plasmodium parasites bites a human host. During its blood meal, the mosquito injects sporozoites into the bloodstream. These sporozoites travel to the liver, where they infect hepatocytes and undergo replication, resulting in thousands of merozoites. Once mature, the merozoites enter the bloodstream and begin the symptomatic phase of malaria. Merozoites multiply rapidly within RBC, causing the cells to rupture and release additional parasites [20].

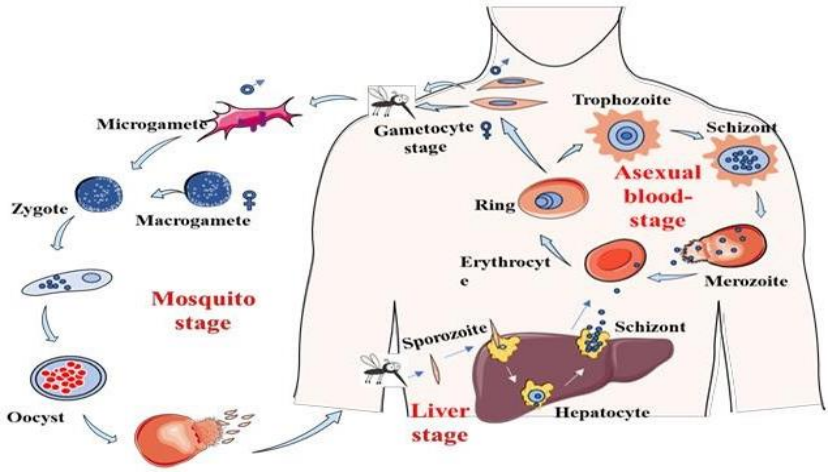

Fig. 1: Life cycle of malaria [20]

This cycle of invasion, replication, and rupture causes malaria's characteristic symptoms, such as fever, chills, and anemia. Some merozoites mature into sexual forms known as gametocytes, which can be consumed by another mosquito during a blood meal, completing the cycle [21].

Challenges in malaria treatment

Cluster and shady environments such as pools, lagoons, slow-moving streams, or rice fields are the most common places where mosquitoes live. These types of mosquitoes are very vicious because they tend to bite humans during the night. Humans are attracted to these places because they are looking for places to perform various activities, like setting up a new village or moving to open a new estate for their livelihood. During the opening activities of the new village or estate, the shelter is a temporary wooden house that can be used for a certain period before they decide to make it permanent [22]. A lot of people's density is one of the reasons this layout phase is too inviting for mosquitoes. Malaria itself is a disease that thrives among the poor. The general pattern is that poor livelihood usually means a conducive environment for malaria. This is because of the development of the area where they live and the malaria parasites share the same characteristic of temporary residence [23]. In today's situation, the movement of humans and the rising increase of global tourist employees, apart from the various levels of societies that live or work in undeveloped areas, are potential vectors for malaria transmission. The protective immunity to symptomatic malaria is acquired slowly over several years and may be lost after leaving an endemic area [24]. This also applies to people residing in

undeveloped areas. Lack of immunity to malaria symptoms in general non-immune persons may increase the chance of getting severe disease and can lead to death. This is due to a mistaken perception that malaria is a disease that only affects people at a certain level of society and that protection from severe diseases and death has never been a main priority of malaria control and treatment, especially for the poor. Nowadays, antimalarial drugs are not only taken for malaria treatment but also for prevention from getting malaria during their journey, especially for global tourist employees, and may be taken for a long time for people in the high-risk group to avoid severe diseases. This situation indicates that the drug is not only used under malaria diagnosis and the cost may be expensive for some prevention methods [25].

Traditional drug delivery methods for the treatment of malaria

The traditional methods for drug delivery include oral administration, intramuscular injection, and intravenous injection (table 1). For intramuscular injection, the surface area of the injection and the state of the blood circulation are too varied to provide a clear and predictable pathway for the drug to follow [26].

Table 1: Drugs traditionally available for the treatment of malaria

Drug	Route of administration	Type of dosage form	Applications	References
Arteether/Lumefantrine	Oral	Tablet	Uncomplicated plasmodium falciparum malaria	[27-29]
Artesunate	Parenteral	Injection	Severe malaria	[30, 31]
Atovaquone	Oral	Tablet	Malaria prophylaxis	[32, 33]
Atovaquone/proguanil	Oral	Tablet	Malaria prophylaxis, uncomplicated malaria	[34]
Chloroquine	Oral	Tablet	Plasmodium vivax, Plasmodium malaria, Plasmodium ovale malaria	[35]
Hydroxy chloroquine sulfate	Oral	Tablet	Malaria prophylaxis, treatment of malaria	[36]
Primaquine	Oral	Tablet	Radical cure of Plasmodium vivax, Plasmodium ovale malaria.	[37]
Pyrimethamine sulfadoxine	Oral	Tablet	Radical cure of Plasmodium vivax, Plasmodium ovale malaria	[38]
Quinine	Oral, parenteral	Tablet	Severe malaria, uncomplicated malaria	[39, 40]
Pyrimethamine	Oral	Tablet	Malaria prophylaxis, treatment of malaria	[41]
Tafenoquine	Oral	Tablet	Radical cure of Plasmodium vivax.	[42]

This results in inconsistent drug absorption and can lead to ineffective treatment. Intravenous injection has a more reliable drug administration mechanism. Despite increased access to blood circulation, however, where the malaria parasite resides, the drugs need to pass through numerous metabolic and physical barriers which still leave the efficacy of treatment questionable. In both cases, the traditional delivery methods offer no targeting of the drug to the infected cells and as a result, show partially effective methods of treatment. This often results in the need for large doses of drug, which can lead to toxicity, especially in the case of intravenous injection, where access to the bloodstream can result in high drug levels in the blood. Oral administration is the simplest method of drug delivery and is still the most commonly used today. Usually, tablets or capsules are administered incorporating the drug into a binding agent which will dissolve when it reaches the stomach [43].

Oral administration

Oral administration is the most used route for the delivery of drugs. Its popularity stems from its ease, convenience, and patient compliance, and its capacity for controlled dosing and good distribution characteristics make it an attractive route for drug administration. The digestive system and liver can act as a site for drug metabolism [44]. Though this may render some drugs inactive, others are altered into more therapeutically active forms. Thus, oral administration is a favorable option for drug delivery to the liver using antimalarial agents to treat liver-stage malaria. Oral drug delivery aims to release the drug at a specific site in the body and release the drug in a controlled manner to ensure maximum efficacy in treatment with minimum dosage. This can be accomplished using specific targeting and timed release of drugs. Based on drug properties and form, drugs can be targeted to release in the stomach, lymph, or liver. The high biological availability of drugs in the stomach may be ideal for toxic drugs to kill parasites in erythrocytes; however, it may cause stomach irritation and inflammation. Drugs targeted to release in Kupffer cells can target parasites in Kupffer cells and prevent hepatic schizogony [45]. Malaria infection is caused by the inoculation of sporozoites into the dermis, while female Anopheles mosquitoes ingest blood from human beings. The sporozoites are carried by the blood to the liver, where they invade hepatocytes. After undergoing one or more multiplication cycles, each resulting in the release of hundreds or thousands of merozoites, the infected hepatocytes rupture and release the merozoites into the blood. The merozoites then invade RBC where they develop and eventually multiply, resulting in generating more merozoites, which cause malaria-associated morbidity. The blood-stage parasites are responsible for the clinical manifestations of the disease. The objective of treatment is to prevent and cure the disease and stop transmission. Current shortcomings of drugs include drug resistance, limited effectiveness in gametocyte and liver stages, high toxicity, low patient compliance, and prevention of post-treatment mosquitoes. Many antimalarial drugs have been developed; however, only a minority have been developed specifically for treating malaria. This minority of drugs, specifically antimalarial agents, can be delivered using targeted drug delivery systems, unwrapping the potential of these drugs [46].

Intravenous injection

Traditional methods of drug delivery are currently the most prevalent form of treatment for malaria. These methods are dangerous and difficult to use but can also carry substantial risks to the patient's health and recovery. In severe cases of malaria, intravenous administration has been the preferred option for treatment [47]. This method increases the bioavailability of the drug and is effective when treating severe cases. It can be difficult to use in field situations or for widespread treatment. Intravenous treatment requires healthcare personnel to be available for multiple doses over 24-48 h, making it difficult for patients in remote or rural areas to access treatment. This form of treatment is also risky as the wrong administration of a drug can cause severe systemic toxicity or even death to the patient. These risks and difficulties associated with traditional drug delivery for malaria are the driving factors behind the development of novel drug delivery systems [48].

Intramuscular injection

The second most common mode of drug administration is via intramuscular injection. It is often used for drugs that cannot be digested as in oral administration or when a more localized effect is desired that cannot be obtained through intravenous administration. The injection is a bolus dose that slowly gets absorbed at the injection site. Since blood flow in muscle tissue is lower than that in veins, absorption of the drug may be delayed. However, the bioavailability of intramuscular administration is complete and there is no risk of immediate alteration of the drug by the body [49]. The slow absorption and sustained release of the drug from muscle tissue can be useful in treating malaria. Drugs like quinacrine, which is no longer used in the Western world, have a high solubility in lipids and would remain in fatty tissue for several weeks. Other drugs that can crystallize and cause irritation at the injection site are

less suited for this method of delivery. High levels of blood flow are also required as in the case of artesunate and artemether which are less suited for intramuscular injection. Overall, this mode of delivery is not specially tailored for treating malaria and has not been studied extensively for this disease [50].

Combination therapies and drug delivery

Antimalarial combination therapy has a potential role in fulfilling the postulated requirement. Its successful application and subsequent resistance management can provide a superior solution to the current situation and change the expected course of antimalarial drug resistance. In theory, antimalarial drug resistance can be managed by changing the drug pressure equation in favor of host immunity. Resistance is an inevitable outcome of the repeated use of any antimalarial monotherapy. Its emergence and rate of subsequent spread are determined by the duration of post-treatment prophylaxis and the force of infection [51]. It has a devastating impact on malaria morbidity and mortality, increasing both outcomes more than the original disease burden. The last half-century has seen the rapid spread of antimalarial drug resistance. This process was initiated with CQ resistance on the Thailand-Cambodia border in the late 1950s. CQ use had a massive impact on reducing malaria burdens in many parts of the world, but resistance substantially increased disease burdens compared to pre-CQ levels. Subsequent uses of antifolate drugs (sulphadoxine-pyrimethamine), 4-aminoquinolines (amodiaquine, chloroquine, and mefloquine), and more latterly atovaquone have seen similar substantial increases in malaria morbidity and mortality in areas of their use. Between 1989 and 2003, resistance to insider reduced child survival in sub-Saharan Africa by 1-6% in areas of drug use. The overall multiplicative effect of antimalarial drug resistance is an increase in all-cause childhood mortality [52].

Novel drug delivery approaches

Targeted drug delivery (fig. 2) of the anti-malarial drugs using site-specific drug delivery systems is one of the major advantages of the drug delivery system-based approaches to improving the efficacy of prophylaxis and the treatment of malaria. The most ambitious aim of treatment or prevention of the disease is complete eradication of the parasite in the body and prevention of re-infection. To achieve this goal, effective killing of the parasite without damage to the host should be carried out [53]. Antimalarial drugs act mainly on the infected RBC; free or hemozoin-bound drugs are at best only partially effective and at worst, toxic to the host. Thus, the drug must be actively or passively targeted to infect RBC. Christoph and his colleagues developed a novel *in vivo* targeting system based on the high affinity of infected RBC for endothelial receptors. This was achieved by administration of drug-loaded carrier erythrocytes which bound to the site of infection and released the drug, resulting in specific and highly effective therapy in animal models. Advance in this type of strategy was the development of carrier RBC which were infected *in vitro* with Plasmodium and thus acquired high affinity for infected RBC. Although this approach had outstanding potential it was not pursued, presumably due to safety concerns and the fact that it would not be relevant to human infections. An alternative method of RBC drug targeting is binding of the drug to RBC ghosts, which are then re-infused into the patient.

Fig. 2: Novel drug delivery approaches in malaria [54, 55]

This would also be an effective system albeit costly. A summary and appraisal of the various methods of RBC drug targeting are available, including an exhaustive review of the first-generation targeted antimalarial drugs. Carriers for targeted drug delivery for treating severe disease are another attractive option, although the potential for adverse effects on the parasite and not the host in this case, will necessitate special precautions [56].

Targeted drug delivery

Targeted drug delivery is a cutting-edge strategy in innovative medication delivery that seeks to deliver pharmacologically active substances to a particular target place within the body. Reducing medications adverse effects and improving treatment are the main goals of targeted delivery systems. Passive targeting and active targeting are the two basic approaches for targeted medication delivery. Drug-loaded nanoparticles are passively accumulated in the tumor via increased permeability, retention, and other special physiological properties of the drug delivery system. As a result, concentration of the medicine at the tumor location is better than in healthy tissues. Targeting ligands, such as antibodies, peptides, or small molecules can attach to over-expressed receptors on target and are used to functionalize the drug carrier in active targeting. This makes it possible for the medication payload to be delivered to the targeted target more precisely. The medication's effectiveness and selectivity may be further improved by active targeting. The creation of several nano-carrier systems, such as liposomes, polymeric nanoparticles, and inorganic nanoparticles for targeted medication administration, has been made possible by advancements in nanotechnology. It is possible to design these nanocarriers to enhance the pharmacokinetics, biodistribution, and intracellular absorption of medications [57]. By carefully delivering lethal medications to cancerous growths with the least amount of damage to healthy tissues targeted drug delivery has demonstrated the potential to enhance therapy. Overcoming biological obstacles, increasing penetration, and optimizing carrier design are ongoing difficulties. The combined use of passive and active targeting techniques can improve treatment efficacy in a complementary manner. Tailored drug delivery is an effective strategy for new medication delivery, which makes use of the distinct biology of disease and nanotechnology to enhance the efficacy and selectivity of pharmacotherapy [58].

Nanoparticles in malaria treatment

The nanoparticles have become a viable new medication delivery method in the treatment of malaria. Plasmodium parasites produce malaria a difficult illness with limited treatment options due to issues with medication resistance, low bioavailability, short half-lives, and non-specific targeting. By strengthening targeted delivery, lowering side effects, and boosting pharmacokinetic profile, nanostructured drug delivery devices can assist in overcoming these constraints [59]. Using both passive and active targeting techniques, nanoparticles may be designed to specifically target the malaria parasite's home, the infected RBC. The increased permeability and retention effect allows nanoparticles to collect preferentially in the tumor-like vasculature of infected tissues and provides the basis for passive targeting. To facilitate more targeted distribution of the antimalarial drug payload, active targeting entails functionalizing the nanoparticle surface with ligands that can bind to receptors

over-expressed on infected RBC. Many nano-carrier systems have been studied for the treatment of malaria, including liposomes, polymeric nanoparticles, and inorganic nanoparticles. By encapsulating antimalarial medications, these nanocarriers can increase the medicine's solubility, stability, and control in improving therapeutic efficacy and lowering toxicity. The promise of nanotechnology to overcome the drawbacks of traditional malaria treatments has been shown by ongoing research in this area. To reach their full potential in treating this worldwide health burden, these nano-based drug delivery technologies still require research and clinical translation [60].

Microneedles for transdermal delivery

A potential method for new medication delivery intended for transdermal administration is use of microneedles. Microneedles are tiny needles that can pierce the stratum corneum, the skin's outermost layer, to allow for less invasive transdermal medication administration. The transdermal medication delivery method with microneedles has several significant benefits. First off, in contrast to conventional transdermal patches, microneedles can temporarily generate microchannels in the skin, which facilitates medication penetration through the skin barrier. Because microneedles may penetrate via the stratum corneum, the primary barrier to skin penetration results in better medication administration and increased bioavailability. Furthermore, compared to hypodermic needles, microneedles are tiny enough to be inserted into the skin without generating a great deal of discomfort, which improves patient compliance. Additionally, microneedles are a versatile platform for innovative drug delivery techniques because they may be constructed in various shapes, such as solid, coated, dissolving, and hollow to fit diverse drug compositions and delivery needs. Transdermal administration using microneedles has been investigated for a variety of medications, including vaccines, small compounds, peptides, proteins, and even nanoparticles. The goal of ongoing research is to further improve drug delivery efficiency, stability, and patient acceptance by refining microneedles design, materials, and production. Research on microneedles-based transdermal drug delivery systems is ongoing and several candidates have advanced through clinical trials. Microneedles are a clever and extremely promising new drug delivery method that might enhance transdermal administration [61].

Nanocarriers for the treatment of malaria

The pharmacokinetic profile of beneficial medications that have not been widely used in pharmacotherapy because of their high toxicity, low bioavailability, and poor water solubility can be improved using nanocarriers. Nanocarriers have been suggested for the diagnosis and treatment of malaria as well as the creation of vaccines. It's also possible that using inefficient pharmaceutical doses of antimalarials drug could contributes resistance in malaria parasites. Because nanotechnology systems can precisely target medications to their site of action, they may provide a better therapeutic effect. Due to the administration of low medication concentrations in the presence of a high parasitic load, malaria parasites frequently acquire treatment resistance. Furthermore, by altering their biodistribution and lowering toxicity, nanotechnology may revive the usage of outdated and harmful medications. This benefit is especially significant for the treatment of malaria because, especially for the antimalarial used in clinical settings, there is a need to find novel dosage forms that can effectively deliver medications to parasite-infected cells [62]. In addition to enabling the use of harmful antimalarial drugs, nanocarriers may improve vaccine formulation ability to elicit an immunological response. The goal of this review is to clarify some biological elements of malaria and connect them to nanotechnology as a potentially effective therapy approach. Taking into consideration the unique characteristics of malaria parasites, several methods for delivering antimalarial drugs as well as the processes that enable their targeted administration to Plasmodium-infected cells will be highlighted. In particular, the focus will be on polymeric-based nanosystems (fig. 3), like nanocapsules and nanospheres for the treatment of malaria, as well as lipid-based nanocarriers, like liposomes, SLN, nanoemulsions, and microemulsions. These nanocarriers are spherical vesicles made of phospholipid bilayers and are viable carriers for innovative drug delivery strategies since they can successfully encapsulate hydrophilic and hydrophobic medicines. This nanocarrier shape enables them to dissolve in water in their aqueous core and dissolve in fat in their phospholipid bilayer. As medication delivery systems, nanocarriers have several benefits. By altering drug absorption, lowering metabolism, and directing the medication to the site of action, they can raise the therapeutic index of pharmaceuticals. Because of their different biodistribution, liposomal formulations of pharmaceuticals are more effective in treating patients in preclinical models and people than traditional formulations [63]. These nanocarriers have less toxicity and adverse consequences since their composition is made up of lipids that are non-immunogenic, biodegradable, and inert to biology. Targeting ligands may be easily included on the liposome surface to enable active targeting to certain locations. Nanocarriers have less toxicity and adverse consequences since their composition is made up of lipids that are non-immunogenic, biodegradable, and inert to biology. Targeting ligands may be easily included on the carrier's surface to enable active targeting to certain locations. The preparation process affects the entrapment efficiency of medicines in nanocarriers. For the transport of a broad variety of physiologically active substances, including tiny molecules, proteins, peptides, and nucleic acids, liposomes have been thoroughly studied. Their potential has been demonstrated in several therapeutic applications, including administration of antibiotics and antifungal drugs, gene therapy, and cancer treatment.

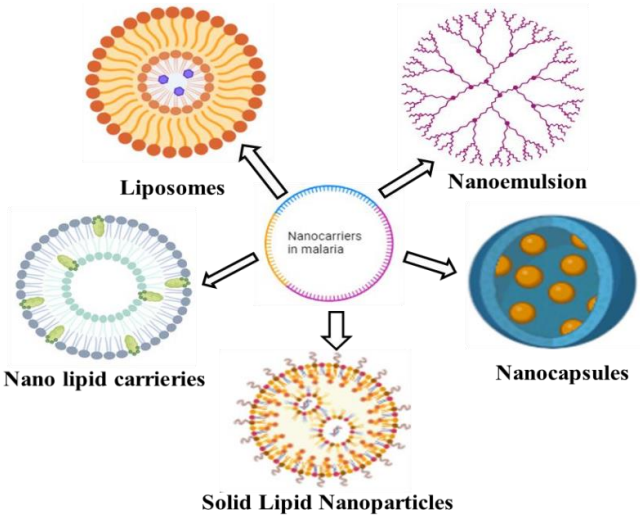

Fig. 3: Nanocarriers for the treatment of malaria [64, 65]

Despite the benefits, there are still some obstacles to be overcome in the development of nano-carrier drug delivery systems, including enhancing stability, raising drug loading, and getting through biological barriers to enable targeted delivery. Research is still being done to overcome these obstacles and maximize liposomes' potential as sophisticated and adaptable nanocarriers for cutting-edge drug delivery strategies. Recent studies on nanotechnology in malaria treatment. Over time, there have been more and more papers on nanoparticles to cure malaria. One search on the National Library of Medicine databases is only one article between 1990 and 2000 and eight between 2001 and 2010. This article analyzes 103 papers in total that discuss the use of nanoparticles in the treatment of malaria. Each year, they are broken into the following numbers: 5 articles in 2017, 9 articles in 2018, 13 articles in 2019, 24 articles in 2020, and 21 articles in 2021. There will be a substantial increase in the number of papers in 2022 that discuss research on the use of nanoparticles to treat malaria. 31 publications on this topic may be consulted in 2022. Numerous researches focused on the use of nanotechnology in treating malaria from 2019 to 2022 confirmed encouraging findings indicating the potential of nanosystems. On the other hand, there are surprisingly few documented active medication delivery-based clinical studies for the treatment of malaria. Over the past ten years, dendrimers have attracted attention for a variety of biological uses including the transport of drugs, genes as well as agents for diagnostic imaging [66]. Preclinical research in treating malaria with nanotechnology in the intra-erythrocytic stage of Plasmodium falciparum in both phases of parasite growth (asexual and sexual) for example, small gold nanoparticles based on glucose or nano gold clusters were produced without nonspecific connections or destruction of RBC. The antibacterial impact of ciprofloxacin loaded into glucose or nano gold clusters were 50% more than that of the drug alone, indicating its potential for use in medicine. In Plasmodium falciparum cultures, silver nanoparticles formed from Artemisia leaf extract showed strong antimalarial efficacy. In an experimental malaria model, silver nanoparticles from salvia officinalis leaf extract showed hepatoprotective and antiplasma actions, lowering parasitemia and liver oxidative stress indicators. To overcome issues in the treatment of malaria, such as the severity of the disease, the critical studies based on nanotechnology are being conducted. These studies are primarily concerned with reducing drug toxicity, stopping Plasmodium sp. transmission, increasing drug efficacy, and preventing multidrug resistance [67].

New drugs in clinical and preclinical development

Antimalarial medicines in clinical and preclinical development under development globally are analyzed in the WHO study from 2021. For the first time, antimalarial medication candidates in clinical development including biological agents and unconventional therapies-are evaluated in the clinical pipeline review. The preclinical development of vaccines, biological agents, direct-acting small compounds, and non-traditional medications is the main emphasis of the preclinical pipeline section. However, the preclinical pipeline reveals an unstable environment with significant turnover, protracted timelines, and difficult benchmarks before possibly entering the market [68, 69].

Table 2: Drugs in clinical and pre-clinical development

Name of drug	Mechanism of action	Safety	Efficacy	Development stage	References
Artemisinin and derivatives	Inhibition of parasite calcium ATPase	Generally well-tolerated; concerns about resistance	Highly effective against Plasmodium falciparum; rapid reduction in parasitemia	Approved	[70, 71]
Lumefantrine	Inhibition of beta-haematin formation	Generally safe; common side effects include headache	Highly effective when combined with Artemether	Approved	[72]
Atovaquone/ Proguanil	Disruption of mitochondrial electron transport (Atovaquone) and folate synthesis (Proguanil)	Mild side effects like nausea; liver toxicity rare	High efficacy against Plasmodium falciparum	Approved	[73]
Fosmidomycin	Inhibits isoprenoid biosynthesis	Generally well tolerated; few side effects	Shows promise in multi-drug resistant malaria	Clinical Phase II	[74]
KAF156 (Lumefantrine analog)	Acts on the apicoplast, inhibiting protein synthesis	Not fully established; ongoing studies on safety	Promising efficacy in early trials	Clinical Phase II	[75]
P218	Targets the Plasmodium falciparum 4-quinolones resistance transporter	Safety profile under investigation	Promising early results against resistant strains	Preclinical	[76]
Tafenoquine	Inhibition of parasite development and replication	Risk of hemolysis in G6PD-deficient patients	Effective in preventing relapse of Plasmodium vivax	Approved	[77]
Dihydroartemisinin-Piperaquine (DHP)	Similar to Artemisinin, with prolonged action of Piperaquine	Generally well tolerated; some GI disturbances	High cure rates; effective against multi-drug resistant strains	Approved	[78]
NITD609	Inhibits Plasmodium falciparum Plasmepsin IV	Safety data pending from ongoing trials	Effective against asexual stages of Plasmodium. falciparum	Preclinical	[79]
Nioxin	Inhibits various stages of the *Plasmodium* life cycle	Safety profile under investigation	Preliminary efficacy observed	Preclinical	[80]
Pyramax (artesunate/ amodiaquine)	Combination therapy enhancing efficacy	Generally well tolerated; some side effects noted	Effective against uncomplicated malaria	Approved	[81]
Methylene blue	Inhibition of the heme detoxification pathway e	Concerns regarding potential toxicity	Potential activity against malaria	Preclinical	[82]

Challenges in treatment of malaria

Finally, it is difficult to predict the long-term safety and effectiveness of a new drug formulation. New adverse effects could emerge and in some cases, the newer drugs might not be as well tolerated as the older drugs. All these issues are relevant to the global public sector and malaria control programs but are especially pertinent to developing private-sector aims to develop new antimalarial drugs. An understanding of how the characteristics of new drugs will impact their use and how the drugs will fit into the larger global antimalarial landscape is critical to the private sector's success in bringing new therapies to market. High rates of compliance and proper use of the antimalarial drugs are critical. The best drug in the world will not have a large impact on public health if it is not used

correctly. Malaria patients in many contexts are difficult to reach and difficult to treat. This makes drug administration and patient monitoring quite difficult [83]. Another issue from the standpoint of the communities being treated is the perception of new drug formulations as compared to the traditional drugs that they are accustomed to using. The newer drugs may not be accepted immediately, and in some cases, the new drugs may not be as effective in all of the same contexts as the traditional drugs. This might create a temptation to continue using the older drugs in some situations, especially if the newer drugs are not cost-prohibitive. Although major advances have been made in the realm of antimalarial drug delivery systems but not all facts of these systems have been fully explored. The caveat to new drug formulations is perhaps their implementation. Even if a new effective and inexpensive drug therapy becomes available, it may be of limited value if it cannot be deployed easily and inexpensively. It is important that these new therapies reach the target populations and in many cases, this has not yet been accomplished with existing therapies [84].

Regulatory considerations

The main objective of regulatory authorities is to ensure the drugs that are available to patients are safe, effective, and of good quality. In general, pre-clinical studies (toxicology, pharmacokinetics/pharmacodynamics, and efficacy studies) including clinical trials in humans are required to show that the drug is safe and effective. Data from the clinical studies are used as a basis for the regulatory authority to decide whether the drug should be approved and granted marketing authorization/distribution in the specific country. Comprehensive studies to show that the drug is effective and safe in a field setting are not required, but it is often difficult for a new antimalarial drug to be accepted based on results from studies conducted outside of areas where malaria is endemic. Clinical trials provide evidence that the drug benefits outweigh the risks. There is a consensus that drugs should not be harmful, but for the treatment of malaria, a drug should have a high benefit-to-risk ratio because the disease is potentially fatal. High standards for quality and manufacture are also set by regulatory authorities to ensure that drugs are consistently and properly made. The drug regulatory authorities, such as the Food and Drug Administration in the United States of America the medicines and Healthcare Products Regulatory Agency in the United Kingdom, and similar agencies in other countries have set requirements for approving a drug that is intended to be used in humans to diagnose, prevent, or treat a disease. The requirements for regulatory approval of antimalarial drugs are not different from other drugs. However, there could be some variations in the requirements depending on the endemicity of malaria in the region and the perceived public health value of the drug in that specific region [85].

Drug resistance

Anti-malarial drugs have been the basis for treatment and control of the disease for over 50 years. The increase of drug resistance by the parasite species to the available drugs has results in increased morbidity and mortality of the disease. The main thrust of drug research up to this period was the identification of cheap, safe, and effective treatment that would alleviate symptoms and cure the infection in a single dose. This would be of most benefit to the patient and negate the need for complicated and costly drug administration involving a cocktail of different drugs to circumvent resistance and achieve a radical cure for the different parasite species. A single-dose cure would also be the ideal tool for the large-scale elimination of malaria from a region. Currently, the most advanced anti-malarial drug in trials is the novel arteether-mefloquine combination (artemisone) developed with the specific goal of reducing the time for resistance to develop compared to existing drugs in the artemisinin group [86].

List of recent antimalarial drugs with patent granted

Antimalarial drugs with patent granted are highlighting those medications in the table that are currently protected by patents indicating that their developers or manufacturers have secured legal monopolies on their production and distribution. This distinction is important in understanding the landscape of antimalarial medications as it reflects the ongoing commercial and legal status of these drugs in the market [87].

Table 3: Patent on antimalarial drugs

S. No.	Patent number	Application number	Title of Invention	Patent grant date	References
1	281417	1507/KOLNP/2010	Antimalarial compounds with flexible side chains	17/03/2017	88
2	288805	131/KOLNP/2009	Vaccines for malaria	27/10/2017	89
3	289775	2074/DEL/2004	The antimalarial compound from ghomphostema given	21/11/2017	90
4	289823	1181/MUM/2009	Combined measles malaria vaccine	22/11/2017	91
5	293596	32/MUM/2013	Intranasal microemulsion of an antimalarial drug artemether	28/02/2018	92
6	298988	2798/MUM/2011	Bioactive composition for the prophylaxis and treatment of malaria, method of manufacturing and using the same	19/07/2018	93
7	310142	1773/MUM/2011	Novel plasmodium protein as malarial vaccine and drug target	27/03/2019	94
8	310371	7489/CHENP/2014	Green chemistry synthesis of the malarial drug amodiaquine and analogs thereof	29/03/2019	95

Integration of technology and drug delivery

An entirely different technology with the potential to revolutionize malaria vaccine delivery is the use of particle-based delivery systems. These systems can be designed to deliver the vaccine to specific cells such as macrophages or dendritic cells by selection of optimal particle and surface properties. For example, a recent study using virus-like particles showed that a vaccine targeting liver stages of malaria can provide unprecedented protection (>95%) using only a single dose. Additional benefits of particle-based vaccines are needle-free administration and the potential for thermal stability removing the need for cold storage and distribution, a major issue for vaccines in the developing world [96]. A multidisciplinary approach to drug and vaccine delivery technologies for malaria spanning the fields of material science, protein engineering, and biomechanics is essential for optimal prophylaxis and treatment. For example, targeting anti-malarial drugs to infected erythrocytes, the disease-causing cell, is a promising strategy being explored by several research labs. Successful targeting would increase drug localized drug concentration several-fold while avoiding healthy erythrocytes and reducing drug concentrations and side effects. Profiling the mechanical and shape-changing properties of these infected erythrocytes promises new methods for targeting drugs and vaccines. Simulation and measurement of the forces exerted by infected cells as they attempt to pass through inter endothelial slits in the spleen, as well as the altered cell and membrane stiffness, are providing valuable information for the design of effective drug targeting strategies prohibitive [97].

Role of artificial intelligence (AI) and machine learning (ML) in malaria

AI and ML are playing a significant role in improving malaria diagnosis and treatment in several ways. AI-powered microscopes

can accurately detect malaria parasites in blood samples, meeting WHO standards. These systems can scan blood films and use detection algorithms to identify parasites, reducing the burden on microscopists and increasing patient capacity. Studies show the AI system can identify malaria parasites with 88% accuracy compared to expert microscopists [98, 99].

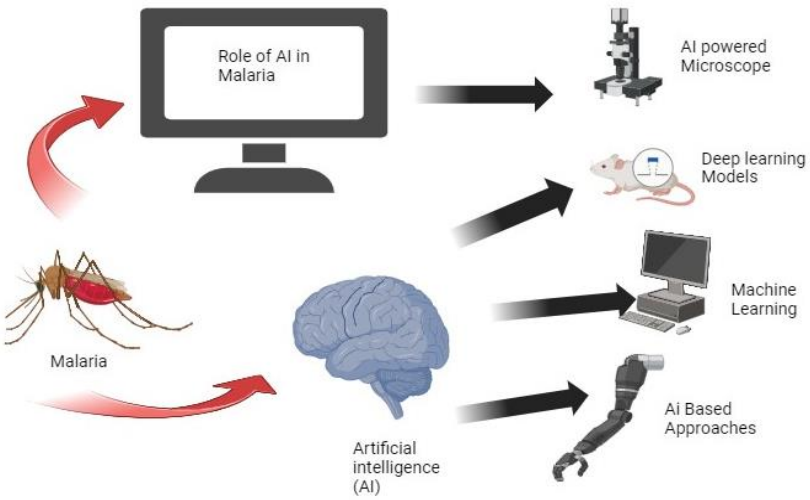

Fig. 4: AI-ML in treatment of malaria [100, 101]

Deep learning models are being used to analyze microscope images and determine the type and stage of malaria infection. This is particularly useful in remote areas with limited resources as the AI can interpret results even if a trained provider is not available. The models identify key characteristics in the images that indicate malaria. Machine learning algorithms are being developed to predict malaria outbreaks by analyzing atmospheric, epidemiological, geographic, and other data from remote sensors. This allows health officials to proactively notify at-risk populations, implement mosquito control measures, and allocate resources to areas likely to see outbreaks. Factors like temperature, humidity, and rainfall patterns are used to predict hotspots [102, 103]. AI-based approaches are being integrated with current malaria microscopy methods to strengthen surveillance and diagnostic capabilities, which is crucial for malaria elimination efforts. Investing in AI microscopy can improve sensitivity and accuracy, which are prerequisites for elimination. In summary, AI is bridging gaps in malaria diagnosis, treatment, and prevention by automating microscopy, predicting outbreaks and enhancing current methods. As AI continues to advance, it will play an increasingly important role in reducing the global malaria burden [104, 105].

CONCLUSION

In conclusion, the emergence of novel drug delivery mechanisms for the treatment of malaria has presented a promising prospect for resource-poor countries by improving patient compliance and reducing the likelihood of developing drug-resistant malarial parasites. The newer drug delivery systems provide a potentially useful means to addressing the problem of under-treatment of the most vulnerable malaria patient populations-pregnant women and young children. The advent of microfabrication technologies and the understanding of malarial pathogenesis have enabled researchers to engineer highly sophisticated and more targeted drug delivery systems to combat the disease. However, most of the technologies are still in the developmental phase and it's estimated that it will take 10-15 years before putting them into practical use. Thus, implementation of these new drugs and technologies will require a long-term sustained commitment from governments and private sectors to ensure that people afflicted with malaria will benefit from these new drugs shortly. It is also important for the current and future generations of scientists and researchers in this field to maintain a high level of enthusiasm in hopes of eventually eradicating the disease that has plagued the world for centuries. Considering the unrelenting pressures of poverty, the return on investment for the effort to cure malaria is arguably higher than for virtually any other disease. For these reasons, new tools and drugs must continue to be developed to combat malaria.

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AUTHORS CONTRIBUTIONS

Tamnna Sharma: performed the literature search, conceptualized the review, and wrote the original draft preparation. Dr. Abhishek Sharma: Contributed to manuscript writing, provided critical revisions, and editing the final version of the paper.

CONFLICT OF INTERESTS

The authors declared no conflict of interest, financial or otherwise.

REFERENCES

1. Fikadu M, Ashenafi E. Malaria: an overview. Infect Drug Resist. 2023 May 29;16:3339-47. doi: 10.2147/IDR.S405668, PMID 37274361.
2. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA. Malaria DAM. Lancet. 2014 Mar;9918:723-35.
3. Phillips MA, Burrows JN, Manyando C, Van Huijsduijnen RH, Van Voorhis WC, Wells TN. Malaria. Nat Rev Dis Primers. 2017 Mar 3;3:17050. doi: 10.1038/nrdp.2017.50, PMID 28770814.
4. Ashley EA, Pyae Phyo A. Malaria. Lancet. 2018 Mar 21;391(10130):1608-21. doi: 10.1016/S0140-6736(18)30324-6. PMID 29631781.
5. Menard D, Dondorp A. Antimalarial drug resistance: a threat to malaria elimination. Cold Spring Harb Perspect Med. 2017 Jul 5;7(7):a025619. doi: 10.1101/cshperspect.a025619, PMID 28289248.
6. Olliaro P, Wells TN. The global portfolio of new antimalarial medicines under development. Clin Pharmacol Ther. 2009 Jun;85(6):584-95. doi: 10.1038/clpt.2009.51, PMID 19404247.
7. Delves MJ, Angrisano F, Blagborough AM. Antimalarial transmission-blocking interventions: past present and future. Trends Parasitol. 2018 Sep;34(9):735-46. doi: 10.1016/j.pt.2018.07.001, PMID 30082147.
8. Greenwood BM, Targett GA. Malaria vaccines and the new malaria agenda. Clin Microbiol Infect. 2011 Nov 17;17(11):1600-7. doi: 10.1111/j.1469-0691.2011.03612.x, PMID 21883665.
9. Doolan DL, Dobano C, Baird JK. Acquired immunity to malaria. Clin Microbiol Rev. 2009 Jan 22;22(1):13-36. doi: 10.1128/CMR.00025-08, PMID 19136431.

10. Tilley L, Dixon MW, Kirk K. The plasmodium falciparum infected red blood cell. Int J Biochem Cell Biol. 2011 Jun;43(6):839-42. doi: 10.1016/j.biocel.2011.03.012, PMID 21458590.
11. Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C. Dihydroartemisinin piperaquine resistance in plasmodium falciparum malaria in Cambodia: a multisite prospective cohort study. Lancet Infect Dis. 2016 Mar 16;16(3):357-65. doi: 10.1016/S1473-3099(15)00487-9, PMID 26774243.
12. Fairhurst RM, Dondorp AM. Artemisinin resistant plasmodium falciparum malaria. Microbiol Spectr. 2016 Jun;4(3). doi: 10.1128/microbiolspec.EI10-0013-2016, PMID 27337450.
13. Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax malaria: neglected and not benign. Am J Trop Med Hyg. 2007;77(6)Suppl:79-87. doi: 10.4269/ajtmh.2007.77.79, PMID 18165478.
14. Miller LH, Ackerman HC, SU XZ, Wellems TE. Malaria biology and disease pathogenesis: insights for new treatments. Nat Med. 2013 Feb 19;19(2):156-67. doi: 10.1038/nm.3073, PMID 23389616.
15. Baird JK. Resistance to therapies for infection by plasmodium vivax. Clin Microbiol Rev. 2009 Jul 22;22(3):508-34. doi: 10.1128/CMR.00008-09.
16. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. Nat Rev Drug Discov. 2004 Jun 3;3(6):509-20. doi: 10.1038/nrd1416, PMID 15173840.
17. Hoffman SL, Vekemans J, Richie TL, Duffy PE. The march toward malaria vaccines. Vaccine. 2015;49 Suppl 4:S319-33.
18. White NJ. Malaria parasite clearance. Malar J. 2017 May 16;16(1):88. doi: 10.1186/s12936-017-1731-1.
19. Rieckmann KH, Davis DR, Hutton DC. Plasmodium vivax resistance to chloroquine. Lancet. 1989 Jun 2;8673:1183-4. doi: 10.1016/s0140-6736(89)91792-3.
20. Bousema T, Drakeley C. Epidemiology and infectivity of plasmodium falciparum and plasmodium vivax gametocytes in relation to malaria control and elimination. Clin Microbiol Rev. 2011 Apr 24;24(2):377-410. doi: 10.1128/CMR.00051-10, PMID 21482730.
21. Arama C, Troye Blomberg M. The path of malaria vaccine development: challenges and perspectives. J Intern Med. 2014 May;275(5):456-66. doi: 10.1111/joim.12223, PMID 24635625.
22. Tatem AJ, Smith DL, Gething PW, Kabaria CW, Snow RW, Hay SI. Ranking of elimination feasibility between malaria-endemic countries. Lancet. 2010 Oct;376(9752):1579-91. doi: 10.1016/S0140-6736(10)61301-3, PMID 21035838.
23. Trape JF, Tall A, Diagne N, Ndiath O, Ly AB, Faye J. Malaria morbidity and pyrethroid resistance after the introduction of insecticide treated bednets and artemisinin based combination therapies: a longitudinal study. Lancet Infect Dis. 2011 Dec 11;11(12):925-32. doi: 10.1016/S1473-3099(11)70194-3, PMID 21856232.
24. Cowman AF, Healer J, Marapana D, Marsh K. Malaria: biology and disease. Cell. 2016 Oct;167(3):610-24. doi: 10.1016/j.cell.2016.07.055, PMID 27768886.
25. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of plasmodium falciparum malaria. Nature. 2005 Feb;434(7030):214-7. doi: 10.1038/nature03342, PMID 15759000.
26. Sharma A, Patel T. Overview of antimalarial drugs: clinical and traditional perspectives. J Trop Med. 2024 Oct 15;4:123-30.
27. Pongvongsa T, Phommasone K, Phongmany P, Paboriboune P, Van Leth F, White NJ. Therapeutic efficacy of chloroquine for treatment of plasmodium vivax in Southern Laos. Am J Trop Med Hyg. 2019 Sep;101(3):553-7.
28. Rathore D, McCutchan TF, Sullivan M, Kumar S. Antimalarial drugs: current status and new developments. Expert Opin Investig Drugs. 2005 Jul 14;14(7):871-83. doi: 10.1517/13543784.14.7.871, PMID 16022576.
29. Meshnick SR. Artemisinin antimalarials: mechanisms of action and resistance. Med Trop (Mars). 1998;58(3) Suppl :13-7. PMID 10212891.
30. Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, Chhaganlal KD. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open label randomised trial. Lancet. 2010 Nov;376(9753):1647-57. doi: 10.1016/S0140-6736(10)61924-1, PMID 21062666.
31. Krishna S, Planche T, Agbenyega T, Woodrow C, Agranoff D, Bedu Addo G. Bioavailability and preliminary clinical efficacy of intrarectal artesunate in Ghanaian children with moderate malaria. Antimicrob Agents Chemother. 2001 Feb;45(2):509-16. doi: 10.1128/AAC.45.2.509-516.2001, PMID 11158748.
32. Pefanis A, Giamarellou H, Karayiannakos P, Donta I. Efficacy of ceftazidime and aztreonam alone or in combination with amikacin in experimental left sided pseudomonas aeruginosa endocarditis. Antimicrob Agents Chemother. 1993;37(2):308-13. doi: 10.1128/AAC.37.2.308, PMID 8452362.
33. Looareesuwan S, Wilairatana P, Glanarongran R, Indravijit KA, Supeeranontha L, Chinnapha S. Atovaquone and proguanil hydrochloride followed by primaquine for treatment of plasmodium vivax malaria in Thailand. Trans R Soc Trop Med Hyg. 1999 Nov;93(6):637-40. doi: 10.1016/s0035-9203(99)90079-2, PMID 10717754.
34. Blanshard A, Hine P. Atovaquone proguanil for treating uncomplicated plasmodium falciparum malaria. Cochrane Database Syst Rev. 2021 Jan 15;1(1):CD004529. doi: 10.1002/14651858.CD004529.pub3, PMID 33459345.
35. White N. Antimalarial drug resistance and combination chemotherapy. Philos Trans R Soc Lond B Biol Sci. 1999 Apr 29;354(1384):739-49. doi: 10.1098/rstb.1999.0426, PMID 10365399.
36. Schrezenmeier E, Dorner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol. 2020;16(3):155-66. doi: 10.1038/s41584-020-0372-x, PMID 32034323.
37. Baird JK, Hoffman SL. Primaquine therapy for malaria. Clin Infect Dis. 2004 Nov;39(9):1336-45. doi: 10.1086/424663, PMID 15494911.
38. Gutman J, Kachur SP, Slutsker L, Nzila A, Mutabingwa T. Combination of probenecid sulphadoxine-pyrimethamine for intermittent preventive treatment in pregnancy. Malar J. 2012 Feb 11;11:39. doi: 10.1186/1475-2875-11-39, PMID 22321288.
39. Achan J, Tibenderana JK, Kyabayinze D, Wabwire Mangen F, Kamya MR, Dorsey G. Effectiveness of quinine versus artemether-lumefantrine for treating uncomplicated falciparum malaria in Ugandan children: randomised trial. BMJ. 2009 Jul 21;339:b2763. doi: 10.1136/bmj.b2763, PMID 19622553.
40. Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN. Quinine an old anti-malarial drug in a modern world: role in the treatment of malaria. Malar J. 2011 May 24;10:144. doi: 10.1186/1475-2875-10-144, PMID 21609473.
41. Plowe CV, Kublin JG, Dzinjalamala FK, Kamwendo DS, Mukadam RA, Chimpeni P. Sustained clinical efficacy of sulfadoxine pyrimethamine for uncomplicated falciparum malaria in Malawi after 10 y as first-line treatment: five-year prospective study. BMJ. 2004 Mar 6;328(7439):545. doi: 10.1136/bmj.37977.653750.EE, PMID 14757706.
42. Markus MB. Safety and efficacy of tafenoquine for *plasmodium vivax* malaria prophylaxis and radical cure: overview and perspectives. Ther Clin Risk Manag. 2021 Sep 8;17:989-99. doi: 10.2147/TCRM.S269336, PMID 34526770.
43. Das S, Saha B, Hati AK, Roy S. Evidence of artemisinin resistant plasmodium falciparum malaria in eastern India. N Engl J Med. 2018 Nov;379(20):1962-4. doi: 10.1056/NEJMc1713777, PMID 30428283.
44. LE Minh G, Peshkova AD, Andrianova IA, Sibgatullin TB, Maksudova AN, Weisel JW. Impaired contraction of blood clots as a novel prothrombotic mechanism in systemic lupus erythematosus. Clin Sci (Lond). 2018 Feb;132(2):243-54. doi: 10.1042/CS20171510, PMID 29295895.
45. Qiu F, Liu J, MO X, Liu H, Chen Y, Dai Z. Immunoregulation by artemisinin and its derivatives: a new role for old antimalarial drugs. Front Immunol. 2021 Sep 12;12:751772. doi: 10.3389/fimmu.2021.751772, PMID 34567013.
46. Tiwari MK, Chaudhary S. Artemisinin derived antimalarial endoperoxides from bench side to bedside: chronological

advancements and future challenges. Med Res Rev. 2020 Jul;40(4):1220-75. doi: 10.1002/med.21657, PMID 31930540.
47. Akpa PA, Ugwuoke JA, Attama AA, Ugwu CN, Ezeibe EN, Momoh MA. Improved antimalarial activity of caprol-based nanostructured lipid carriers encapsulating artemether-lumefantrine for oral administration. Afr Health Sci. 2020 Dec 20;20(4):1679-97. doi: 10.4314/ahs.v20i4.20, PMID 34394228.
48. Guo W, LI N, Ren G, Wang RR, Chai L, LI Y. Murine pharmacokinetics and antimalarial pharmacodynamics of dihydroartemisinin trimer self-assembled nanoparticles. Parasitol Res. 2021 Jul;120(8):2827-37. doi: 10.1007/s00436-021-07208-6, PMID 34272998.
49. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of plasmodium falciparum malaria. In: Mendis KN, editor. Malaria: biology and disease. 2nd ed. Cambridge: Academic Press; 2009. p. 201-22.
50. Mandai SS, Francis F, Challe DP, Seth MD, Madebe RA, Petro DA. High prevalence and risk of malaria among asymptomatic individuals from villages with high rates of artemisinin partial resistance in Kyerwa District North Western Tanzania. J Trop Med. 2024 Jun 26;23(1):197. doi: 10.1186/s12936-024-05019-5.
51. Damme W. Malaria control during mass population movements and natural disasters. J Refugee Stud. 2004 Jun 17:145-6.
52. Al Awadhi M, Ahmad S, Iqbal J. Current status and the epidemiology of malaria in the middle east region and beyond. Microorganisms. 2021 Feb 9;9(2):1-20. doi: 10.3390/microorganisms9020338, PMID 33572053.
53. Elyazar IR, Hay SI, Baird JK. Malaria distribution prevalence drug resistance and control in Indonesia. Adv Parasitol. 2011;74:41-175. doi: 10.1016/B978-0-12-385897-9.00002-1, PMID 21295677.
54. Woodbury DJ, Miller C. Nystatin induced liposome fusion a versatile approach to ion channel reconstitution into planar bilayers. Biophys J. 1990 Apr;58(4):833-9. doi: 10.1016/S0006-3495(90)82429-2, PMID 1701101.
55. Venugopal K, Hentzschel F, Valkiunas G, Marti M. Plasmodium asexual growth and sexual development in the haematopoietic niche of the host. Nat Rev Microbiol. 2020 Mar 18;18(3):177-89. doi: 10.1038/s41579-019-0306-2, PMID 31919479.
56. Breman JG, Alilio MS, White NJ. Defeating malaria in Asia the pacific Americas middle east and Europe. 1st ed. Baltimore: Johns Hopkins University Press; 2011. p. 203-20.
57. Warrell DA, Gilles HM, editors. Essential malariology. 4th ed. Oxford: Oxford University Press; 2002. p. 122-43.
58. Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria. 1st ed. Bethesda: National Institutes of Health; 2002. p. 78-102.
59. Wernsdorfer WH, McGregor I, Editors. Malaria: principles and practice of malariology. 2nd ed. New York: Churchill Livingstone; 1988. p. 45-67.
60. Ashley EA, White NJ, Krishna S. The pathophysiology of malaria. 3rd ed. Berlin: Springer; 2014. p. 101-34.
61. Snow RW, Omumbo JA. Malaria. In: Warrell DA, Cox TM, Firth JD, editors. Oxford textbook of medicine. 5th ed. Oxford: Oxford University Press; 2010. p. 960-70.
62. White NJ, Nosten F. Malaria treatment and control: principles and practice. In: Mansons tropical diseases. 23rd ed. London: Elsevier; 2014. p. 178-90.
63. Van Den Berg H, Kyaw A, Huxley R. Global burden of disease due to malaria. In: Allen D, editor. Global health and diseases: current trends. 1st ed. New York: Wiley; 2018. p. 100-30.
64. Tiwari MK, Chaudhary S. Artemisinin derived antimalarial endoperoxides from bench side to bed side: chronological advancements and future challenges. In: Sharma V, editor. Antimalarial drug development: challenges and opportunities. 1st ed. Berlin: Springer; 2020. p. 112-45.
65. Baird JK, Sudimac W. Malaria in Indonesia: epidemiology and control strategies. In: Kurniawan M, Editor. Tropical infectious diseases: principles pathogens and practice. 1st ed. Jakarta: University of Indonesia Press; 2015. p. 305-20.
66. Gething PW, Smith DL, Patil AP. A new approach to malaria mapping and model selection. In: Smith DL, Editor. Malaria: progress and prospects. 1st ed. New York: Springer; 2011. p. 155-80.
67. Cotter C, Sturrock HJ, Hsiang MS, Liu J, Phillips AA, Hwang J. The changing epidemiology of malaria elimination: new strategies for new challenges. In: Hay SI, Guerra CA, Tatem AJ. editors. Malaria: a global perspective. 1st ed. London: Imperial College Press; 2016. p. 79-97.
68. Kahn K, Collinson MA, Sayed R. Health and demographic surveillance in the agincourt health and socio-demographic surveillance system. In: Dorrington R, Bradshaw D, editors. The epidemiology of malaria in South Africa: a review. 1st ed. Cape Town: South African Medical Research Council; 2014. p. 45-60.
69. Sharma A, Patel T. Agents in clinical and pre-clinical for treatment of malaria. Advances in antimalarial research. 3rd ed. Boston: Global Health Press; 2024. p. 102-4.
70. McIntosh HM, Olliaro P. Artemisinin derivatives for treating uncomplicated malaria. Cochrane Database Syst Rev. 2000 Apr;1999(2):CD000256. doi: 10.1002/14651858.CD000256, PMID 10796519.
71. Elizabeth A Ashley, Aung Pyae Phyo, Charles J Woodrow. Malaria WNJ. Lancet. 2018 Apr;391(10130):299-310.
72. Siqueira Neto JL, Wicht KJ, Chibale K, Burrows JN, Fidock DA, Winzeler EA. Antimalarial drug discovery: progress and approaches. Nat Rev Drug Discov. 2023 Oct 22;22(10):807-26. doi: 10.1038/s41573-023-00772-9, PMID 37652975.
73. Tse EG, Korsik M, Todd MH. The past present and future of anti-malarial medicines. Malar J. 2019 Mar 18;18(1):93. doi: 10.1186/s12936-019-2724-z, PMID 30902052.
74. Dondorp A, Nosten F, Stepniewska K, Day N, White N, South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) Group. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. Lancet. 2005;366(9487):717-25. doi: 10.1016/S0140-6736(05)67176-0, PMID 16125588.
75. Gamo FJ, Sanz LM, Vidal J, DE Cozar C, Alvarez E, Lavandera JL. Thousands of chemical starting points for antimalarial lead identification. Nature. 2010;465(7296):305-10. doi: 10.1038/nature09107, PMID 20485427.
76. Alaithan H, Kumar N, Islam MZ, Liappis AP, Nava VE. Novel therapeutics for malaria. Pharmaceutics. 2023 Jun 15;15(7):1800. doi: 10.3390/pharmaceutics15071800, PMID 37513987.
77. Chu CS, Hwang J. Tafenoquine: a toxicity overview. Expert Opin Drug Saf. 2021;20(3):349-62. doi: 10.1080/14740338.2021.1859476, PMID 33306921.
78. Maiga FO, Wele M, Toure SM, Keita M, Tangara CO, Refeld RR. Artemisinin based combination therapy for uncomplicated plasmodium falciparum malaria in mali: a systematic review and meta-analysis. Malar J. 2021 Aug 20;20(1):356. doi: 10.1186/s12936-021-03890-0, PMID 34461901.
79. Gamo FJ, Sanz LM, Vidal J, DE Cozar C, Alvarez E, Lavandera JL. Thousands of chemical starting points for antimalarial lead identification. Nature. 2010 May 20;465(7296):305-10. doi: 10.1038/nature09107, PMID 20485427.
80. Charman SA, Andreu A, Barker H, Blundell S, Campbell A, Campbell M. An *in vitro* toolbox to accelerate anti-malarial drug discovery and development. Malar J. 2020 Aug;19(1):1. doi: 10.1186/s12936-019-3075-5, PMID 31898492.
81. Akinbo OF. Safety and efficacy of pyramax in uncomplicated Malaria. J Infect Dis. 2022 Mar;225(6):1061-8.
82. Schmitt M. Methylene blue and its derivatives in the treatment of malaria. Curr Pharmacol Rep. 2017 Jan 3;1:20-5.
83. White NJ. Antimalarial drug resistance. In: Denny P, editor. Malaria: global status and challenges. 1st ed. Geneva: World Health Organization; 2009. p. 15-34.
84. Rieckmann KH, Davis DR, Hutton DC. Drug resistance in plasmodium falciparum: a new challenge for malaria control. In: Wernsdorfer WH, McGregor I, editors. Malaria: principles and practice of malariology. 2nd ed. New York: Churchill Livingstone; 1988. p. 775-93.
85. Chethan Kumar HB, Hiremath J, Yogisharadhya R, Balamurugan V, Jacob SS, Manjunatha Reddy GB. Animal disease surveillance: its importance & present status in India. Indian J Med Res. 2021;153(3):299-310. doi: 10.4103/ijmr.IJMR_740_21, PMID 33906992.
86. Okech B, Muga H, Nyabera J. Factors influencing the effectiveness of malaria treatment in rural Kenya. Malar J. 2022 Mar 21;1:123.

87. Dondorp AM, Nosten F, YI P, Das D, Phyo AP, Tarning J. Artemisinin resistance in plasmodium falciparum malaria. N Engl J Med. 2009 Jul;361(5):455-67. doi: 10.1056/NEJMoa0808859, PMID 19641202.
88. Njaria PM, Okombo J, Njuguna NM, Chibale K. Chloroquine containing compounds: a patent review (2010-2014). Expert Opin Ther Pat. 2015 Jun 25;25(9):1003-24. doi: 10.1517/13543776.2015.1050791, PMID 26013494.
89. Mariano RM, Goncalves AA, Oliveira DS, Ribeiro HS, Pereira DF, Santos IS. A review of major patents on potential malaria vaccine targets. Pathogens. 2023 Feb 3;12(2):247. doi: 10.3390/pathogens12020247, PMID 36839519.
90. Costantini C, Aisien MS, Bogh C. Malaria vector control in West Africa: current status and future perspectives. Malar J. 2015 Sep 14;218.
91. Nguetse CN, Tchoua R, Djouaka R. The role of insecticide resistance in malaria transmission dynamics: a systematic review. PLOS Negl Trop Dis. 2020 May 14;5.
92. Tayade NG, Nagarsenker MS. Development and evaluation of artemether parenteral microemulsion. Indian J Pharm Sci. 2010 Sep;72(5):637-40. doi: 10.4103/0250-474X.78536, PMID 21694999.
93. Lin J, LI T, Yang Y. Advances in the research of malaria vaccines. Infect Dis Pover. 2020 Mar 9;1:19.
94. Arendse LB, Wyllie S, Chibale K, Gilbert IH. *Plasmodium* kinases as potential drug targets for malaria: challenges and opportunities. ACS Infect Dis. 2021 Mar 12;7(3):518-34. doi: 10.1021/acsinfecdis.0c00724, PMID 33590753.
95. Gohain M, Malefo MS, Kunyane P, Scholtz C, Burah S, Zitha A. Process development for the manufacture of the antimalarial amodiaquine dihydrochloride dehydrate. Org Pros Res Develop. 2024 Dec 28;1:124-31.
96. Kokwaro G. Ongoing challenges in the management of malaria. Malar J. 2009 Aug 8;8 Suppl 1(S1):1-6.
97. Rosenthal PJ. Malaria in 2022: challenges and progress. Am J Trop Med Hyg. 2022 Jun;106(6):1565-7. doi: 10.4269/ajtmh.22-0128, PMID 35413687.
98. Umeyor C, Kenechukwu F, Uronnachi E, Chime S, Reginald Opara J, Attama A. Recent advances in particulate antimalarial drug delivery systems: a review. Int J Drug Deliv. 2013 Jan;5(1):1-14.
99. Mendis KN, Sina BJ, Marchesini P, Carter R. The neglected burden of plasmodium vivax malaria. In: Snow RW, Guerra CA, Tatem AJ. editors. Malaria: a global perspective. 1st ed. London: Imperial College Press; 2016. p. 37-54.
100. Baird JK. Malaria: overview and impact on public health. In: Warrell DA, Gilles HM, Editors. Essential malariology. 4th ed. Oxford: Oxford University Press; 2002. p. 1-20.
101. Kotepui M, Kirdmanee C, Koonboon S. Advances in malaria diagnosis and treatment: a review. Malar J. 2020 Jan 19;1:335.
102. Sturrock HJ, Ntshalintshali N, Mthembu DJ. The effect of environmental change on malaria transmission in South Africa: a systematic review. Malar J. 2021 Jan 20;1:201.
103. Patel DM, Shah HR, Doshi H, Shastri DD. Herbal plants with antimalarial potential: a review. Asian J Pharm Clin Res. 2018 Jan 11;1:45-50.
104. Singh MP, Kalita B. Plant based antimalarial agents: an overview. Int J Pharm Pharm Sci. 2020 Mar 12;3:67-72.
105. Roy R, Biswas P, Mehta S. Antimalarial drug resistance: a growing challenge in India. Int J Curr Pharm Res. 2020 Jan 12;1:33-9.

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Original Article

FORMULATION AND OPTIMIZATION OF BUDESONIDE COLON-TARGETED TABLETS USING CONTROLLED POROSITY OSMOTIC PUMP TECHNOLOGY

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ABSTRACT

Objective: Formulation and optimization of Budesonide (BDU) controlled porosity osmotic pump tablets (CPOP) to treat Nocturnal Asthma (NA) by adopting the Quality by design approach was set as objective of this research work.

Methods: Solubility of Budesonide was enhanced by converting in to the form of BUD Solid dispersions, using poloxamer 188. Controlled Porosity Osmotic pump (CPOP) tablets of budesonide were formulated by wet granulation technique. Quality by design approach using Box-Behnken design was adopted to optimize the selected critical factors. The optimized formulation was compared with the marketed extended-release formulation.

Results: The percentage of drug released at 4 h (D4), 7 h (D7), and 10 h (D10) were identified as response factors during the optimization phase. Statistical analysis showed that a combination of 200 mg of the SPM coat, 19.72 mg of Eudragit S 100 for the enteric coating, and 69.74 mg of guar gum in the core could achieve drug release rates of 9.4% after 4 h, 55.9% after 7 h, and 96.6% after 10 h of administration for the CPOP tablets.

Conclusion: The results indicated that the CPOP tablets were successfully formulated for colon-targeted drug release.

Keywords: Design of experiments, CPOP tablets, Box-behnken design, ANOVA test, Enteric coating, Semipermeable membrane, Polysaccharides

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INTRODUCTION

Asthma is a chronic disorder in many people across the globe. There have been numerous studies on asthma concluding the symptoms got worsen in the night during sleep. This kind of asthma is called as '*nocturnal asthma (NA)*'. According to National Heart, Lung and Blood Institute (NHLBI, A branch of National Institute of Health, USA), the lung function because of NA becomes worsen in the night with increased symptoms and airway resistance, thus requiring medication at that time [1]. Asthmatic attacks are less common in the first half of night. The air-way resistance increases progressively through the night and much greater during the sleep [2, 3]. Hence, this chronopathology of NA suggests that administration of anti-asthmatic drugs that are developed based on Chrono pharmaceutical technology is most desirable in consideration of the patient convenience. This can be achieved only through formulating Colon Targeting Drug Delivery Systems (CTDDS). These systems are formulated such that they prevent the drug release in the upper GIT and allow the drug to release only after crossing the ileocecal valve that connects the ileum to colon. After administration, passage of intact solid dosage form to the colon generally requires around 6-8 h (around 2-3 h to cross stomach and around 4-5 h to cross small intestine). Administration of these solid CTDDS after night meal at around 9 pm can produce the dosage form in the colon at around 4 am in the next day morning. So, the immediate release of any anti-asthmatic drug loaded can produce the desired plasma concentration at the most needed time to prevent the early morning attacks of asthma.

Budesonide (BUD) is one of the treatment options to treat patients with mild to moderate asthma symptoms. Budesonide belongs to the class of corticosteroids acts as bronchodilator to relieve the pain and inflammation associated with asthma attacks [4, 5]. Budesonide has high first-pass metabolism (around 90% of the dose) form the upper GIT resulting absorption of only around 10% of the administered dose. In contrast, this drug exhibited significantly high absorption around 60-80% from the ileum and colon [6, 7]. Further, it is well evidenced that budesonide can cause bleeding in the upper GIT [8] which is asymptomatic and can be discovered only when serious hemorrhage occurs. These facts suggest that it is of great necessity for Budesonide to be released only in the lower GIT i. e. in the ileum and colon for better absorption as well as preventing GI bleeding. Hence, this drug is also best-suitable for developing into CTDDS.

As per literature BUD belongs to the class-II of Biopharmaceutical classification system (BCS), which is characterized by poor solubility and high permeability [9]. Such type of APIs exhibits dissolution limited bioavailability. As the current study focusing on the targeted drug release site is colon [10, 11], and by considering the less availability of fluids in colon, it is necessary to improve the solubility of BUD. Different approaches are available in the literature to improve the solubility of the drugs; among all the approaches, solid dispersion was found to have various advantages like ease of preparation, reduced cost for preparation, improve wettability. Osmotic drug delivery systems are the most promising drug delivery systems with controlled drug release manner with aid of osmotic pressure [12].

Controlled Porosity Osmotic Pumps (CPOP) is one of the most dependable osmotic drug delivery systems to have desired drug release for the diseases associated with circadian rhythms like asthma [13] CPOP systems have unique advantage over conventional osmotic systems that they don't need mechanical drilling of orifice on the semipermeable membrane (SPM) for drug release. Instead, the SPM contains a substance that is dissolved/eroded/degraded in the favorable conditions upon administration and provide numerous micropores to allow the drug release [14, 15]. Use of natural polysaccharides like chitosan and guar gum gained an advantage because of its gelling property. These polysaccharides are degraded by the enzymes of colonic microflora once reaches to the colonic region.

Present research consisted of enhancing solubility of the BUD by solid dispersions, developing CPOP tablets with polysaccharides and coating them with enteric polymers to so as to minimize the drug release in the upper GIT and achieving the targeted release in the lower GIT. Quality by design (QbD) [16] was applied to study the influence of several factors on the drug release form the CPOP tablets also to optimize the formulation towards achieving the desired drug release profile.

MATERIALS AND METHODS

Materials

Budesonide received as gift sample from Hetero Drugs Pvt. Ltd, Hyderabad, Poloxamer-188, PEG-6000, guar gum, mannitol, Cellulose Acetate (CA) 320S, CA 398-10 was purchased from Sigma Aldrich Chemicals Co., USA, Eudragit S 100 were received as gift sample from Evonik industries, Povidone K-30 was received as gift sample from JRS Pharma. All other solvents and reagents used were of analytical grade.

Preparation of BUD-solid dispersion

BUD solid dispersions (BSDs) were prepared using solvent evaporation method [17, 18]. Briefly drug and carrier (poloxamer-188) were dissolved at 1:1, 1:5 and 1:2 ratio in round-bottomed flask containing isopropyl alcohol (IPA) and were named as BSD1, BSD2 and BSD3, respectively. Further these mixtures were subjected for evaporation of solvent using rotavapor. The dried BSDs were collected and stored until further usage.

Characterization of the BSDs

Solubility was performed using shake flask method. Briefly 10 ml of water was taken in a conical flask and excess amount of BSD was added to the media subjected for shaking until 24 h. After 24 h. the mixture was filtered and the filtrate was estimated for drug content using UV spectrophotometer [19]. X-ray diffraction studies were performed for the BUD API and formulated BSDs to detect changes in crystallinity using Thermo Fisher Scientific X-Ray Diffractometer [20, 21].

Formulation development of CPOP tablets

Colon-targeted CPOP tablets were formulated with core containing the solid dispersion of BDU, osmogenic agent and rate-controlling polymer. CPOP tablets were prepared at various combinations of the factors according to the design and were characterized. Design of experiments (DoE) analysis was carried out to identify the most significant factors based on their influence on the drug release from the tablets. To design the Budesonide CPOP Tablets, three factors were optimized by Box-Behnken design (BBD) [22, 23]. Factor A: The weight of the SPM coating, composed of cellulose acetate and the pore-forming agent PEG-6000, was optimized to support the osmotic drug release mechanism for budesonide. Factor B: The amount of guar gum (a polysaccharide) in the CPOP tablets controls the release of budesonide, specifically in colonic regions where the galactosamine enzyme is present. Factor C: The concentration of Eudragit S 100 was optimized to create an effective enteric coating for CPOP tablets. For optimization studies, three response factors % Drug released after 4 h (D4) as R1, % Drug released after 7 h (D7) as R2, % Drug released after 10 h (D10) as R3. The combinations of the above factors at their levels according to the Box-Behnken design were shown in table 1.

Table 1: List of dependent and independent variables in box-behnken design for budesonide colon-targeted tablets

Factor	Name	Units	Level used	
			LOW (-1)	HIGH (+1)
A	Semipermeable Membrane	(mg)	200	400
B	Guar gum	(mg)	40	160
C	Eudragit S100	(mg)	10	20
Response	**Name**	**Units**	**Goal**	
R_1	Drug released after 4 h (D_4)	%	≤10%	
R_2	Drug released after 7 h (D_7)	%	≤60%	
R_3	Drug released after 10 h (D_{10}) (D10)	%	≥95%	

Core tablet preparation

Core CPOP tablets were prepared using conventional wet granulation technology using Povidone K-30 as binder and water as granulating aid [24]. Core composition of CPOP contains Solid dispersion of BSD and osmogen (Mannitol). The wet granules were dried, lubricated and compressed with 8 mm round punches to get 1.8 mm thickness tablets.

Application of semipermeable coating to CPOP

The prepared SPM mix was applied on core tablets using a sandwich compression approach. Half quantity of SPM mix per unit was placed in 12 mm die cavity, followed by the core tablet was placed in the cavity and compressed with minimal force and the remaining half quantity of SPM mix was also added to the die cavity and compressed to form the CPOP tablets [25]. Enteric coating was applied on the formulated CPOP tablets using Eudragit S 100 polymer [26].

Evaluation of CPOP tablets

Physical characterization of CPOP tablets

The manufactured CPOP tablets were evaluated for thickness, tensile strength, packing fraction, friability and % drug content as per the commonly used procedures.

Drug release study

The drug release study for the CPOP tablets was conducted using a USP type-2 apparatus at 100 RPM for 2 h in 500 ml of 0.1N HCl, followed by a pH 7.4 phosphate buffer up to 10 h [27]. To the dissolution media galactomannanse from *Aspergillus niger* was added after 3 h of the dissolution in buffer stage to make the dissolution medium simulated to colonic medium for the digestion of guar gum [28, 29]. The sample was collected after 2 h in acid stage, after 2 h in buffer stage (cumulative time point of 4 h to acid stage-D4), after 5 h in buffer stage (cumulative time point of 7 h to-D7), after 8 h in buffer stage (cumulative time point of 10 h to-D10).

Optimization of critical factors

Factors having a significant impact on the response factors were optimized using Box-Behnken design [30]. The model-suggested formulations were made and further optimization was also done to get the best suitable formulation with desired responses (R1-% drug release after 4 h (D4), R2-% drug release after 7 h (D7), R3-% drug release after 10 h (D10). The general model corresponds to the following equation:

$$Y_0 = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 \quad (1)$$

Where Y is the measured response associated with each factor level combination; b0 is an intercept; b1 to b23 are the regression coefficients; and X1, X2, and X3 are the independent variables. The formulation compositions of the Budesonide CPOP tablet are presented in table 2.

RESULTS AND DISCUSSION

Characterization of the BSDs

The solubility of the BSDs was observed in the range of 0.439 mg/ml to 0.845 mg/ml, which was about 10–20 folds increment in comparison with API solubility of 0.041 mg/ml [31]. Among all the formulations the BSD3 was found to have highest solubility of 0.845 mg/ml, followed by BSD2 with 0.706 mg/ml solubility, followed by BSD1 with 0.439 mg/ml solubility. The results are displayed in fig. 1.

Table 2: Formulation compositions of the budesonide CPOP tablets

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A	B	C	R1	R2	R3
	mg	mg	mg	%	%	%
1	300	40	20	9.7	49.6	92.7
2	200	40	15	12.5	61.8	100.5
3	300	40	10	9.4	56.5	91.1
4	200	160	15	14.2	62.3	96.4
5	400	40	15	9.5	50.9	90.8
6	*300*	*100*	*15*	*7.2*	*42.7*	*81.6*
7	200	100	20	8.4	55.6	91.5
8	300	160	10	10.9	60.5	95.7
9	*300*	*100*	*15*	*7.2*	*42.7*	*81.6*
10	400	100	10	7.9	49.1	84.3
11	300	160	20	5.8	37.9	79.4
12	200	100	10	8.1	48.2	92.3
13	*300*	*100*	*15*	*7.2*	*42.7*	*81.6*
14	400	160	15	6.3	41.4	83.1
15	*300*	*100*	*15*	*7.2*	*42.7*	*81.6*
16	400	100	20	6.3	56.9	90.9
17	*300*	*100*	*15*	*6.3*	*42.7*	*81.6*

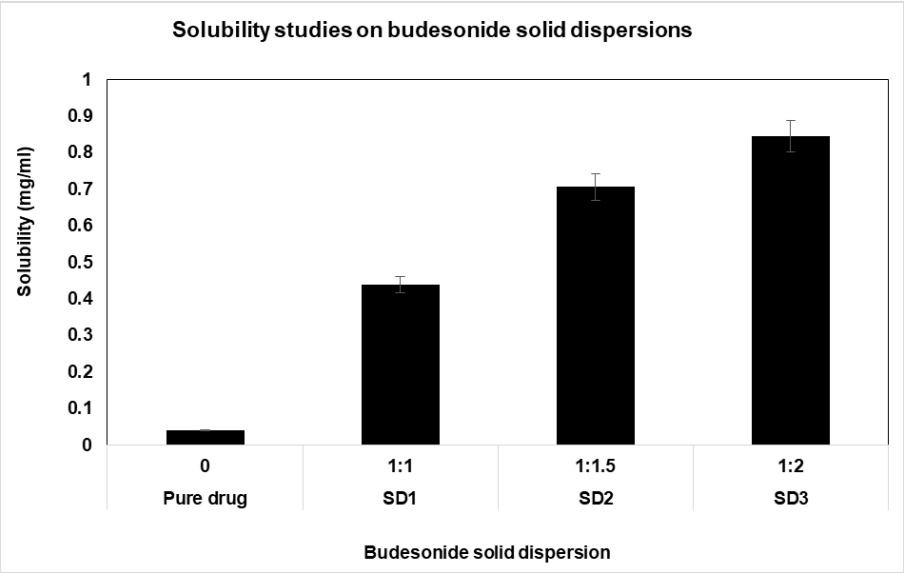

Fig. 1: Comparison of budesonide solubility profiles in pure form and solid dispersion forms (1:1, 1:1.5 and 1:2), *All the results are presented as mean±standard deviation for *n* = 3

The Solid dispersions dissolution rate depends on the proportion of the poloxamer 188 in the solid dispersions. An enhancement of dissolution rate of budesonide is because of its amorphous state, which increases the as increase of the weight fraction of the poloxamer 188 up to its saturation solubility. From the solubility data it was found that there is drastic improvement in solubility with an increment in the carrier concentration i. e. poloxamer 188. As the desired solubility achieving with 1:2 drug and carrier ratio, the same will be used for further preparation of CPOP tablets.

XRD studies were also performed for the formulated SDs to know the crystallinity of the formulation. XRD was performed for both the API and the prepared BSDs. The XRD spectrum of Budesonide API was found to have the share, high intense peaks, which indicating the crystal nature of API, whereas the XRD spectrum formulated BSDs was found to have the broad, less intense peaks, which is confirming the form conversion of API from crystalline to amorphous after solid dispersion formation [32]. The XRD spectrum is displayed in fig. 2.

Physicochemical properties of the CPOP tablets

The optimized formulations were evaluated for physical property evaluation. The results were shown in table 3. From the physical property evaluation, it was clear that the formulated CPOP tablets were rigid enough to maintain their integrity throughout lifecycle [33]. Friability data of the optimized formulations were found to be within limit as per USP (<1.0%).

Drug release study

Dissolution was performed for optimized formulations as mentioned above. Dissolution results were displayed in fig. 3. All the 13 batches were shown less than 10% drug release as per USP enteric coating criteria [34]. The D4 was found to be in the range of 5.8 to 14.2%, D7 was found to be in the range of 37.9 to 62.3%, as per the objective of the study, almost 100% drug release was observed after 10 h (D10). From the dissolution data it is evident that there is control over the drug release as per the required pattern for colon-targeted drug delivery. After 5 h, with addition of the galactomannanse from *Aspergillus niger* to the dissolution medium, there is an increase in % drug release rate, which might be due to the soluble nature of polysaccharide matter present in the SPM thereby forming pores on the surface of the CPOP tablets to facilitate the drug release [35, 36]. The compartitive dissolution studies of optimized formulation and the marketed formulation displayed in fig. 4.

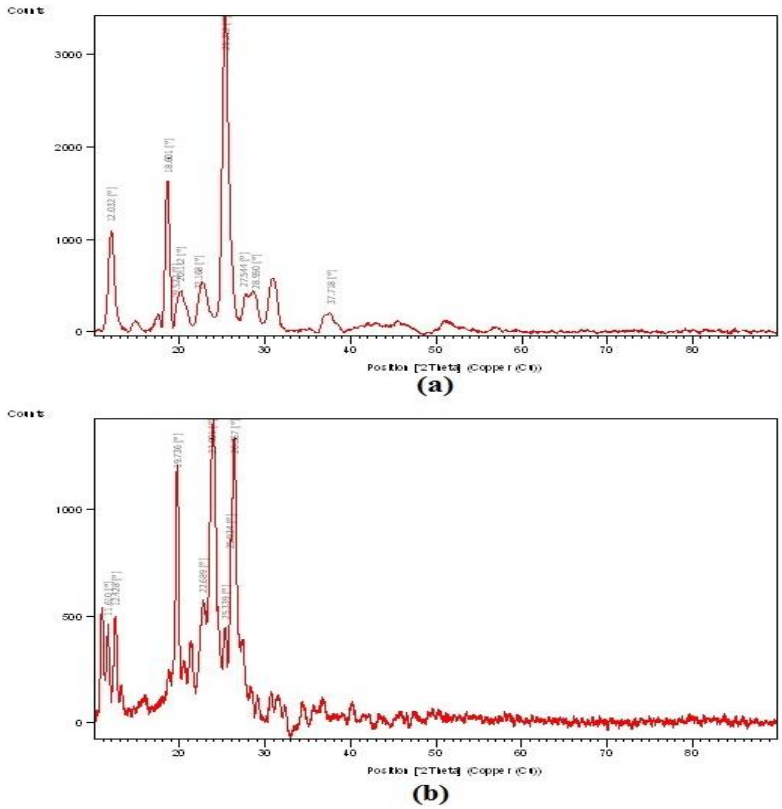

Fig. 2: XRD spectrum of a) Pure budesonide, b) Budesonide solid dispersion

Table 3: Post-compression physical properties of the screening formulations OF1 – OF13

S. No.	Formulation	Thickness (mm)	Tensile strength (N/mm²)	Packing fraction (P_f)	Friability (%)	Drug content (%)
1	OF1	2.08±0.04	0.63±0.06	0.93±0.04	0.18±0.06	98.7±2.6
2	OF2	2.12±0.06	0.61±0.07	0.94±0.03	0.21±0.05	98.2±1.2
3	OF3	2.11±0.03	0.65±0.04	0.91±0.07	0.24±0.02	101.9±0.8
4	OF4	2.09±0.08	0.66±0.08	0.95±0.08	0.13±0.06	100.5±1.6
5	OF5	2.52±0.06	0.62±0.05	0.92±0.05	0.16±0.03	99.4±2.1
6	OF6	2.58±0.12	0.59±0.04	0.93±0.03	0.21±0.06	98.5±2.4
7	OF7	2.56±0.05	0.63±0.02	0.90±0.06	0.14±0.05	101.7±1.5
8	OF8	2.61±0.03	0.60±0.05	0.95±0.02	0.18±0.03	100.2±1.8
9	OF9	2.63±0.07	0.64±0.03	0.93±0.03	0.12±0.02	98.9±2.2
10	OF10	3.13±0.11	0.58±0.09	0.91±0.04	0.25±0.03	101.3±1.9
11	OF11	3.10±0.06	0.63±0.05	0.94±0.03	0.27±0.04	99.8±2.7
12	OF12	3.08±0.09	0.60±0.07	0.95±0.08	0.15±0.03	98.4±3.1
13	OF13	3.12±0.06	0.62±0.03	0.93±0.05	0.16±0.06	100.6±1.3

*All the results are presented as mean±standard deviation for n = 3

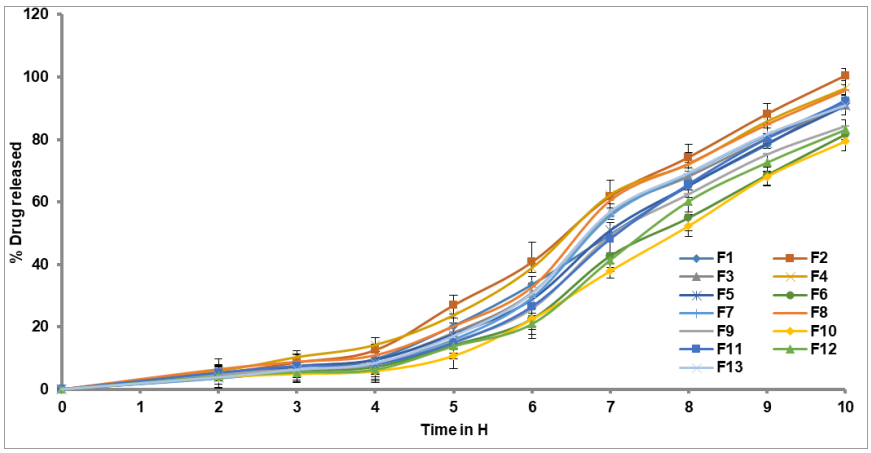

Fig.3: *in vitro* drug release profile of optimized BDU CPOP tablets,
***All the results are presented as Mean±Standard deviation for n = 3**

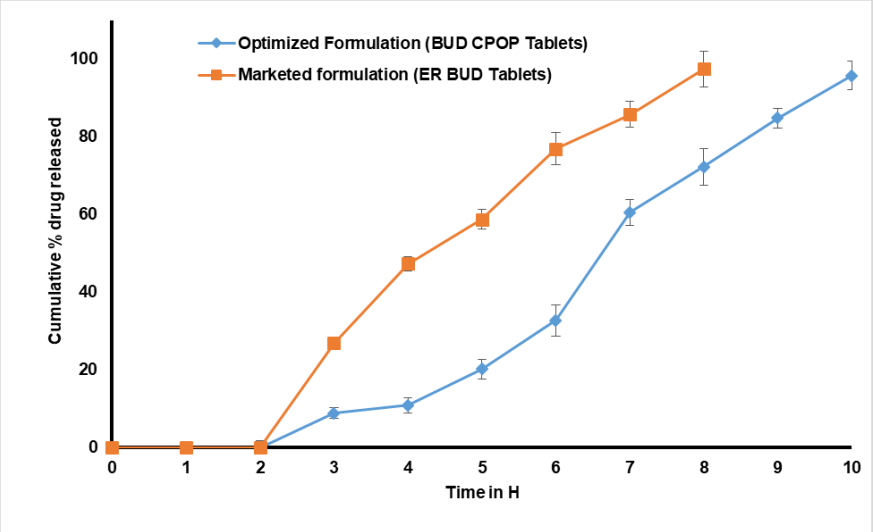

Fig. 4: *In vitro* dissolution profile of BUD CPOP colon target tablets and marketed BUD extend release tablets, *All the results are presented as mean±standard deviation for *n* = 3

Design of experimental analysis of the responses

To find the model fitness, sequential model sum of square analysis was applied to find suitable regression model for every response with the selected factors by the Design Expert software [37]. From the results, as given by the software, it was observed that the linear model was the best fit to explain the impact of factors for all the three responses. This suggested linear model was subjected to diagnosis tests to confirm its suitability and significance by ANOVA test, normal plot of residuals as well as predicted vs actual plots illustrated in fig. 4.

ANOVA test results are shown in table 4 and it's confirmed the suitability of the selected design for all the selected factors and the occurred responses [38]. The *p*-value was found to be less than 0.05, which is confirming the significance of model terms. The normal plot of residuals and the predicted versus actual plot are displayed in fig. 4. All the data points in the normal plot of residuals were aligned linearly without any sigmoid shape alignment. The predicted vs actual plots illustrated that the points were uniformly aligned around the 45 line. These observations are concluding that the selected models for the three responses are suitable and the same can be moved for optimization stage [37].

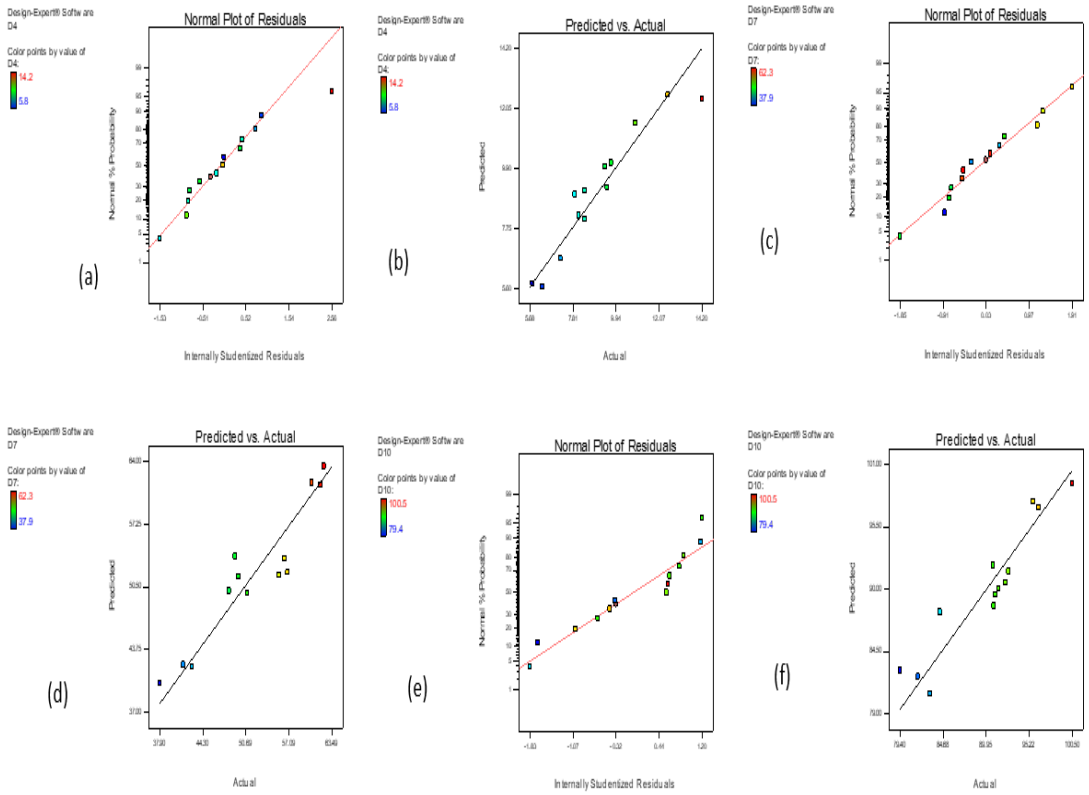

Fig. 4: a) Normal plot of residuals for the Response D_4, b) Predicted versus actual plot for the Response D_4, c) Normal plot of residuals for the response D_7 d) Predicted versus actual plot for the Response D_7, e) Normal plot of residuals for the response D_{10} d) Predicted versus actual plot for the response D_{10}

Table 4: Results of ANOVA test for response surface linear model for the response D4

Response	Source	SS[a]	Df[b]	MSS[c]	F value	p-Value	Inference[d]
D4	Model	60.56	3	20.19	27.47	<0.0001	Significant
	A-SPM coat weight	36.98	1	36.98	50.31	<0.0001	Significant
	B-PS Conc.	10.58	1	10.58	14.39	0.0043	Significant
	C-Amount of RCP	13.01	1	13.01	17.69	0.0023	Significant
	Residual	6.61	9	0.73			
	Core Total	67.18	12				
D7	Model	672.23	3	224.08	23.36	0.0001	Significant
	A-SPM coat weight	262.21	1	262.21	27.33	0.0005	Significant
	B-PS Conc.	284.41	1	284.41	29.65	0.0004	Significant
	C-Amount of RCP	125.61	1	125.61	13.09	0.0056	Significant
	Residual	86.33	9	9.59			
	Core Total	758.56	12				
D10	Model	410.19	3	136.73	22.78	0.0002	Significant
	A-SPM coat weight	153.13	1	153.13	25.51	0.0007	Significant
	B-PS Conc.	64.98	1	64.98	10.83	0.0094	Significant
	C-Amount of RCP	192.08	1	192.08	32.00	0.0003	Significant
	Residual	54.02	9	6.00			
	Core Total	464.20	12				

Note: [a]-Sum of Squares; [b]-Degrees of Freedom; [c]-mean Sum of Squares; [d]-p-Value less than 0.05 indicates model terms are significant

The effects of the selected factors on the responses are illustrated in fig. 5. The factors A and C were found to be negative on drug release whereas the factor B has positive impact on drug release. With increase in SPM coating weight, the thickness of the coating will also increase thereby decrease in drug release rate. This could be due to the increased resistance for water permeation; thereby, limited pressure development would result in decreased drug release at thicker coats [39, 40]. The drug release was found to be sustained after 5 h of dissolution, with increased concentration of polysaccharide i. e guargum. The impact of polysaccharide concentration on drug release was found to be more in case of D7 and D10 rather D4. This could be attributed to the addition of galactomannanse from *Aspergillus niger* to dissolution medium after 5 h. Galactomannanse is an enzyme that is secreted in the intestinal microflora and is responsible for the digestion of the polysaccharides in the intestine [28, 29].

The goal of drug release of prepared BUD CPOP tablets was to be pulsatile drug release pattern. The effect of the formulation factors on drug release was more complex. To study the effect of formulation factors (A, B and C) on the drug release of prepared tablet responses (R1: after 4h (D4), R2: after 7 h (D7), and R3: after 10h (D10), multiple linear regression analysis was done using polynomial equation (1,2 and 3).

$$R1 = +7.02 - 1.65A - 0.4875B - 0.7625C - 1.23AB - 0.4750AC - 1.35BC + 1.16A^2 + 2.44B^2 - 0.5100C^2 - \ldots\ldots\ldots\ldots (1)$$

$$R2 = +42.70 - 3.70A - 2.09B - 1.79C - 2.50AB + 0.1000AC - 3.93BC + 6.36A^2 + 5.04B^2 - + 3.39C^2 - \ldots\ldots\ldots\ldots (2)$$

$$R3 = +81.60 - 3.95A - 2.56B - 1.11C - 0.9000AB + 1.85AC - 4.48BC + 5.56A^2 + 5.54B^2 + 2.59C^2 - \ldots\ldots\ldots\ldots (3)$$

The equations revealed that all three factors A: SPM coat weight, B: Amount of PS and C: amount of Eudragit S 100 impact on the BUD release from the CPOP tablets. To simulate the intestinal conditions in the *in vitro* drug release studies, this enzyme is added to the dissolution medium after 5 h of the test [41]. So that, the drug release observed here can be correlated to the *in vivo* conditions. Might be the presence of this enzyme in dissolution media (mimicking the colon fluid conditions) accelerating the digestion of the polysaccharide, followed by the formation of pores on the surface of the tablet to facilitate the drug release [42]. These results signified that controlling the levels of the SPM coat weight, Amount of Eudragit S 100 and the amount of PS, the drug release from the CPOP tablets can be controlled and can achieve the desired colon-targeted drug delivery [43].

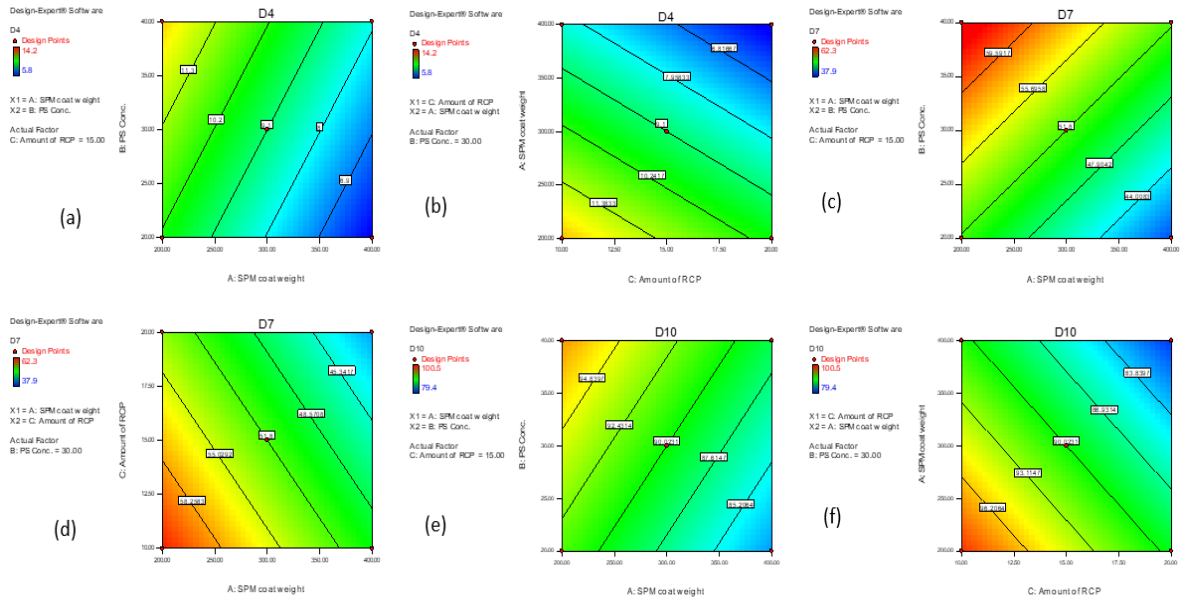

Fig. 5: Contour plot showing the effects of a) the factors A and B on the D4; b) the factors A and C on the D4; c) the factors A and B on the D7, d) the factors A and C on the D7, e) the factors A and B on the D10, f) factors A and C on the D10

Optimization of formulation

Formula optimization was performed to find the best suitable combination of the factors to achieve the desired responses. The desirability of responses is to have the minimum drug release (below 10%) after 4 h, half amount of drug release at 7 h (less than 60%) and the maximum amount of drug release at 10 h (above 90%) after dissolution to meet the objective of the formulation development of CPOP tablets of attaining maximum drug release in colon region that is after 6 h of administration.

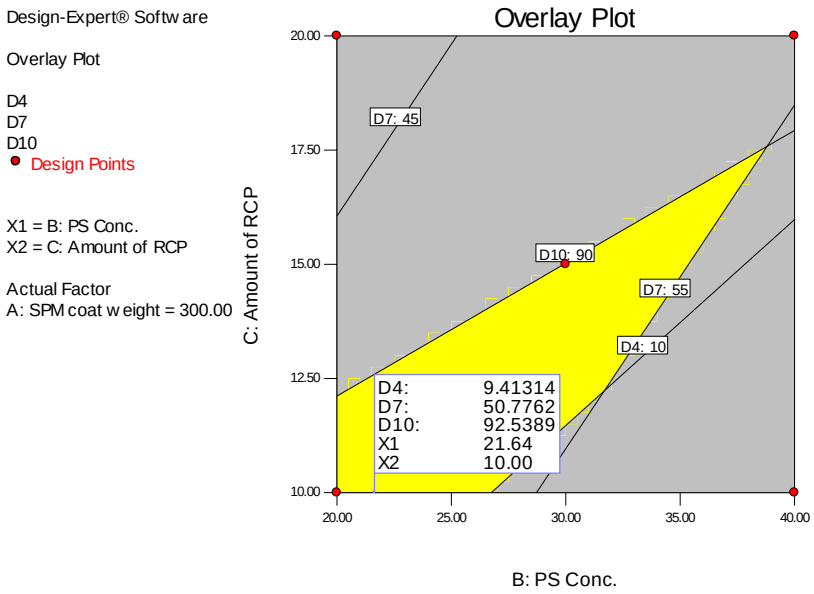

Fig. 6: Overlay plot showing the Design space (the yellow region: the yellow color part of the plot suggests the best possible combinations of factors B and C to get desired responses)

The overlay plot of graphical optimization with set of desirability function is displayed in fig. 6. The yellow color part of the plot suggests the best possible combinations of factors B and C to get desired responses. The plot indicates the combination of factor A at 200 mg SPM coat weight can produce CPOP tablets with desired drug profile. The combination of factors and the predicted drug release profiles are displayed in table 5.

Table 5: Comparison of the predicted and observed values of the responses for the optimized budesonide CPOP tablets

Factors combination	Responses	Predicted values	Observed values	% Error
A: SPM coat weight (200 mg)	R1: D4 (%)	9.9	9.4	5.05
B: PS conc. (69.74 mg)	R1: D7 (%)	56.94	55.9	-1.86047
C: Amount of Eudragit S 100 (19.72 mg)	R1: D10 (%)	95.0	96.6	1.656315

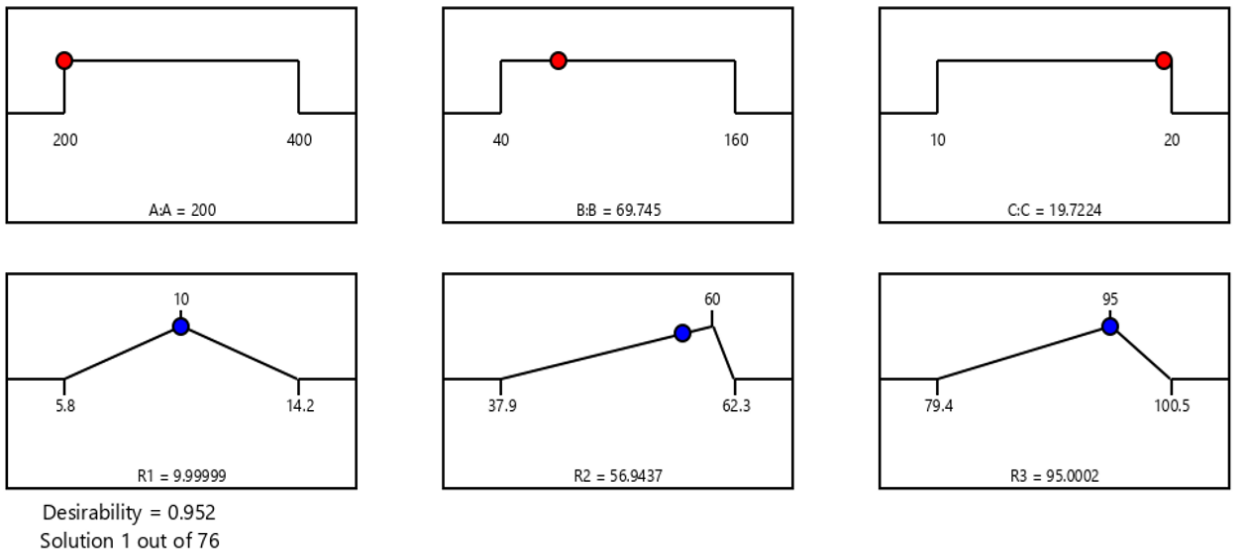

Fig. 7: Numerical optimization of BUD loaded CPOP tablets using box-bhenken design

A new batch of CPOP tablets was formulated with the design-suggested combination and evaluated for dissolution profile (fig. 7). The observed values were found to be correlating with predicted values by design space. So, the combination was selected as optimum formulation of Budesonide CPOP tablets for colon-targeted drug delivery. The difference between observed and predicted values was calculated using the following equation (4).

$$\text{Error (\%)} = (\text{difference between observed and predicted values})/\text{predicted value} \times 100 \quad \ldots\ldots (4)$$

The optimized formulation containing BUD showed with small error values (R1: 5.05, R2:-1.860, and R3: 1.65. This reveals that mathematical models obtained from the Box-Behnken design were well-fitted [44]. Comparison of in vitro dissolution profile of BUD CPOP colon target tablets and marketed BUD extended-release tablet was shown in fig. 8. The drug release was entirely inhibited at stomach pH, indicating that the concentration of the enteric-coated polymer was effective [45]. This demonstrates the reliability of the optimized procedure in predicting the operating parameters for the preparation of BUD CPOP tablets for colon targeting.

CONCLUSION

Budesonide SD was formulated to improve the dissolution rate with the help of poloxamer 188 as a carrier by solvent evaporation method. The formulated solid dispersions were found to have improved solubility in comparison with BUD plain drug. The solid dispersions were further evaluated to know the changes in crystallinity using X-ray diffractometer and from the XRD studies, it was found that the crystal form of the BUD was changed into amorphous form. The CPOP tablet was manufactured using QbD as a tool to optimize by the Box-Behnken design. Further, the significance of the model for each response was analysed by the ANOVA. From the optimization study it was concluded that increment in rate controlling polymer in core and the increment of semipermeable coat weight are controlling the drug release, whereas the increased polysaccharide content in semipermeable coat mixture leads to the higher drug release after 6 h and the model is validated using the correlation between observed and predicted values. The current study concluded that CPOP tablets effectively controlled drug release during their transit through the gastrointestinal tract (GIT) and provided targeted drug delivery for treating conditions related to circadian rhythms.

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Nil

ABBREVIATION

BDU: Budesonide, CPOP: Controlled Porosity Osmotic Pump Tablets, NA: Nocturnal Asthma, QbD: Quality By Design, ANOVA: Analysis of Variance, NHLBI: National Heart, Lung and Blood Institute, CTDDS: Colon Targeting Drug Delivery Systems, GIT: Gastro-Intestinal Tract, GI: Gastro Intestine, API: Active Pharmaceutical Ingredient, SPM: Semipermeable Membrane, PEG: Poly Ethylene Glycol, CA: Cellulose Acetate, BSD: BUD solid dispersions, IPA: Isopropyl Alcohol, BBD: Box-Behnken Design, DoE: Design of experiments, USP: United States of Pharmacopoeia, HCl: Hydrochloric acid, XRD: X-Ray Diffraction.

AUTHORS CONTRIBUTIONS

Ismail. Y conceptualized the review, planned it, and examined the study; and Ismail. Y edited, reviewed, and oversaw the document; Vijaya Kumar Voleti produced the majority of the manuscript, conducted a literature search, created the tables, figures, and references; After reading the published version of the manuscript, all writers have given their approval.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest

REFERENCES

1. Paudel KR, Jha SK, Allam VS, Prasher P, Gupta PK, Bhattacharjee R. Recent advances in chronotherapy targeting respiratory diseases. Pharmaceutics. 2021;13(12):2008. doi: 10.3390/pharmaceutics13122008, PMID 34959290.
2. Venkataswamy NL. Review article on pulsatile drug delivery system. Asian J Pharm Clin Res. 2021 Jun 6;14(6):48-59. doi: 10.22159/ajpcr.2021.v14i6.41476.
3. Vimal Raj E, Ubaidulla Uthumansha. A current update and future prospects of chronomodulated drug delivery approaches. Afr J Biogr. 2024;6(15):276-86. doi: 10.48047/AFJBS.6.15.2024.275-291.
4. Richard B, Tim H, Stefan P, Per G, Angus H, Thomas B. Evaluation of budesonide formoterol for maintenance and reliever therapy among patients with poorly controlled asthma systematic review and meta-analysis. JAMA Netw Open. 2022 Mar 1;5(3):e220615.
5. Mogal RT, Galgatte UC, Chaudhari PD. Floating pulsatile drug delivery of ranitidine hydrochloride for nocturnal acid breakthrough: design optimization *in vitro* and *in vivo* evaluation. Int J Pharm Pharm Sci. 2013;5(3):722-7.
6. O Donnell S, O Morain CA. Therapeutic benefits of budesonide in gastroenterology. Ther Adv Chronic Dis. 2010;1(4):177-86. doi: 10.1177/2040622310379293, PMID 23251737.
7. Szefler SJ. Pharmacodynamics and pharmacokinetics of budesonide: a new nebulized corticosteroid. J Allergy Clin Immunol. 1999;104(4):S175-83. doi: 10.1016/S0091-6749(99)70059-X.
8. Behera R, Sethi S. Association of upper GI bleed in patients with budesonide and SSRI therapy in chrons disease: a comparative clinical study. J Med Sci Clin Res. 2019;7(6):139-47. doi: 10.18535/jmscr/v7i6.26.
9. Radke R, Jain K. Enhancement of solubility and bioavailability of bcs class-II ambrisentan: *in vitro, in vivo* and ex vivo analysis. Int J App Pharm. 2022;14(1):67-74. doi: 10.22159/ijap.2022v14i1.43347.
10. Campos JC, Ferreira DC, Lima S, Reis S, Costa PJ. Swellable polymeric particles for the local delivery of budesonide in oral mucositis. Int J Pharm. 2019 Jul 20;566:126-40. doi: 10.1016/j.ijpharm.2019.05.057, PMID 31129342.
11. Jain SK, Jain A. Target specific drug release to the colon. Expert Opin Drug Deliv. 2008;5(5):483-98. doi: 10.1517/17425247.5.5.483, PMID 18491977.
12. Jayendrakumar P, Shalin P, Shwetaben P. Comprehensive review on osmotic drug delivery system. WJPR. 2021 Apr;10(5):523-50. doi: 10.20959/wjpr20215-20303.
13. Keraliya RA, Patel C, Patel P, Keraliya V, Soni TG, Patel RC. Osmotic drug delivery system as a part of modified release dosage form. ISRN Pharm. 2012;2012:528079. doi: 10.5402/2012/528079, PMID 22852100.
14. Shahi SR, Zadbuke NS, Gulecha B, Shivanikar SS, Shinde SB. Design and development of controlled porosity osmotic tablet of diltiazem hydrochloride. J Adv Pharm Technol Res. 2012;3(4):229-36. doi: 10.4103/2231-4040.104714, PMID 23378944.
15. Sanap LS, Savkare AD. Controlled porosity osmotic pump a review. Int J Pharm Res Dev. 2014;5(12):71-80.
16. Kurapati P, Chinni S. Development and optimization of rabeprazole chrono-modulated drug delivery systems. Int J App Pharm. 2024;16(3):332-41. doi: 10.22159/ijap.2024v16i3.50189.
17. Tran P, Pyo YC, Kim DH, Lee SE, Kim JK, Park JS. Overview of the manufacturing methods of solid dispersion technology for improving the solubility of poorly water-soluble drugs and application to anticancer drugs. Pharmaceutics. 2019;11(3):132. doi: 10.3390/pharmaceutics11030132, PMID 30893899.
18. Ubaidulla U, Senthilkumar B, Ahmad FJ, Khar RK. Studies on suspension of nimesulide solid dispersion: development characterization and *in vivo* evaluation. Indian J Pharm Sci. 2005;67(4):422-6.
19. Ravindra KK, Chintan VP, Aditee CP. Validation of novel analytical RP-HPLC method for determination of formoterol fumarate and budesonide in inhalation suspension pharmaceutical dosage form. Res J Pharm Technol. 2021;14(8). doi: 10.52711/0974-360X.2021.00761.

20. Liu H, Zhou LL, Wei LL, Hong Guo G, Nie SF, Yang XG. Preparation of budesonide poly (ethylene oxide) solid dispersions using supercritical fluid technology. Drug Dev Ind Pharm. 2007;33(9):959-66. doi: 10.1080/03639040601134181, PMID 17891582.
21. Bharathi Priya K, Kulathuran Pillai K, Nalini CN, Udhumansha U. Formulation and characterization of 5-fluorouracil and metformin biodegradable nanospheres for treating colon cancer. Futur J Pharm Sci. 2024;10(1):145. doi: 10.1186/s43094-024-00713-2.
22. Huang Y, YU H, Xiao C. pH-sensitive cationic guar gum/poly (acrylic acid) polyelectrolyte hydrogels: swelling and *in vitro* drug release. Carbohydrate Polymers. 2007;69(4):774-83. doi: 10.1016/j.carbpol.2007.02.016.
23. Ubaidulla U, Sinha P, Sangavi T, Rathnam G. Development of silymarin entrapped chitosan phthalate nanoparticles for targeting colon cancer. J Nat Rem. 2022;22(4):659-71. doi: 10.18311/jnr/2022/29816.
24. Prashant LP, Sunil VA, Darshan RT. Formulation characterization and *in vitro* dissolution studies of metadoxine tablets prepared by various granulation methods. J Pharm Allied Sci. 2021;10(2).2712-19. doi: 10.22270/jmpas.v10i2.1066.
25. Tripathi P, Ubaidulla U, Roop Kishan Khar V. Floating drug delivery system. IJRDPL. 2012;1(4):1-10.
26. Jeong YI, Ohno T, HU Z, Yoshikawa Y, Shibata N, Nagata S. Evaluation of an intestinal pressure controlled colon delivery capsules prepared by a dipping method. J Control Release. 2001;71(2):175-82. doi: 10.1016/s0168-3659(01)00211-5, PMID 11274749.
27. Vaishnavi BP, Raosaheb SS, Pooja SS, Shubham RP, Aniket JP. Formulation and *in vitro* evaluation of oral colon targeted sustained released tablet of budesonide. Turk J Physiother Rehabil. 2022;32:3.
28. Sawada T, Tsuruoka T, Ueda N, Marubayashi H, Nojima S, Morikawa J. Thermally conductive molecular assembly composed of an oligo(ethylene glycol)-modified filamentous virus with improved solubility and resistance to organic solvents. Polym J. 2020;52(7):803-11. doi: 10.1038/s41428-020-0328-7.
29. Pandey M, Choudhury H, Sahleni K, Chetty Annan N, Bhattamisra SK, Gorain B. Budesonide loaded pectin/polyacrylamide hydrogel for sustained delivery: fabrication characterization and *in vitro* release kinetics. Molecules. 2021;26(9):2704. doi: 10.3390/molecules26092704, PMID 34062995.
30. Mahapatra AP, Saraswat R, Botre M, Paul B, Prasad N. Application of response surface methodology (RSM) in statistical optimization and pharmaceutical characterization of a patient compliance effervescent tablet formulation of an antiepileptic drug levetiracetam. Futur J Pharm Sci. 2020;6(1):82. doi: 10.1186/s43094-020-00096-0.
31. Michailidou G, Papageorgiou GZ, Bikiaris DN. β-cyclodextrin inclusion complexes of budesonide with enhanced bioavailability for COPD treatment. Appl Sci. 2021;11(24):12085. doi: 10.3390/app112412085.
32. Jara MO, Warnken ZN, Williams RO. Amorphous solid dispersions and the contribution of nanoparticles to *in vitro* dissolution and *in vivo* testing: niclosamide as a case study. Pharmaceutics. 2021;13(1):97. doi: 10.3390/pharmaceutics13010097, PMID 33466598.
33. Chaudhary A, Tiwari N, Jain V, Singh R. Microporous bilayer osmotic tablet for colon-specific delivery. Eur J Pharm Biopharm. 2011;78(1):134-40. doi: 10.1016/j.ejpb.2011.01.004.
34. Uthumansha U, Prabahar K, Gajapathy DB, El-Sherbiny M, Elsherbiny N, Qushawy M. Optimization and *in vitro* characterization of telmisartan loaded sodium alginate beads and its *in vivo* efficacy investigation in hypertensive induced animal model. Pharmaceutics. 2023;15(2):709. doi: 10.3390/pharmaceutics15020709, PMID 36840031.
35. Ibrahim IM. Advances in polysaccharide-based oral colon targeted delivery systems: the journey so far and the road ahead. Cureus. 2023 Jan 11;15(1):e33636. doi: 10.7759/cureus.33636, PMID 36788847.
36. Garcia MA, Varum F, Al-Gousous J, Hofmann M, Page S, Langguth P. *In vitro* methodologies for evaluating colon-targeted pharmaceutical products and industry perspectives for their applications. Pharmaceutics. 2022 Jan 26;14(2):291. doi: 10.3390/pharmaceutics14020291, PMID 35214024.
37. Srikar G, Rani AP. Tenofovir loaded poly (lactide-co-glycolide) nanocapsules: formulation optimization by desirability functions approach. Indian J Pharm Educ Res. 2020;54(2S):s230-40. doi: 10.5530/ijper.54.2s.79.
38. Grandhi S, Rani AP, Pathuri R. Voriconazole solid lipid nanoparticles: optimization of formulation and process parameters. Res J Pharm Technol. 2018;11(7):2829-35. doi: 10.5958/0974-360X.2018.00522.X.
39. Soliman L, Ibrahim W. Effect of coating thickness and polyethylene glycols molecular weight on diltiazem hydrochloride release from controlled porosity osmotic pump tablets. Res J Pharm Technol. 2022;15(9):4043-7. doi: 10.52711/0974-360X.2022.00678.
40. Gundu R, Pekamwar S, Shelke S, Kulkarni D, Shep S. Development optimization and pharmacokinetic evaluation of biphasic extended release osmotic drug delivery system of trospium chloride for promising application in the treatment of overactive bladder. Future J Pharm Sci. 2021;7(1):1-20.
41. Niranjan GK, Sima S, Balaji M, Omprakash S, Thomas JW. Anovel dissolution media for testing drug release from a nanostructured polysaccharide based colon-specific drug delivery system: an approach to alternative colon media. Int J Nanomedicine. 2016 Mar 17;11:1089-95. doi: 10.2147/IJN.S97177.
42. Jain D, Raturi R, Jain V, Bansal P, Singh R. Recent technologies in pulsatile drug delivery systems. Biomatter. 2011;1(1):57-65. doi: 10.4161/biom.1.1.17717, PMID 23507727.
43. Sahoo CK, Sahoo NK, Rao SR, Sudhakar M, Satyanarayana K. A review on controlled porosity osmotic pump tablets and its evaluation. Bull Fac Pharm Cairo Univ. 2015;53(2):195-205. doi: 10.1016/j.bfopcu.2015.10.004.
44. Bharathi Priya K, Kulathuran Pillai K, Nalini CN, Udhumansha U. Design and optimizing of metformin and 5-fluorouracil co-loaded nanospheres by box behnken experimental design for the treatment of colorectal cancer. Int J Curr Sci Res Rev. 2024;07(07):4717-26. doi: 10.47191/ijcsrr/V7-i7-16.
45. Pandey S, Vijayendra Swamy SM, Ubaid Ulla UM, Gupta A, Patel H, Yadav JS. Cell line and augument cellular uptake study of statistically optimized sustained release capecitabine loaded eudragit S100/PLGA(poly(lacticco- glycolic acid)) nanoparticles for colon targeting. Curr Drug Deliv. 2017;14(6):887-99. doi: 10.2174/1567201813666160817150621, PMID 27538461.

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Original Article

PREVENTING DIABETIC KIDNEY DISEASE: A SYSTEMATIC REVIEW OF CURRENT PHARMACOLOGICAL APPROACHES

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ABSTRACT

Objective: This review examines the growing global burden of Diabetic Nephropathy (DN), a major complication of Diabetes Mellitus (DM) and a leading cause of Chronic Kidney Disease (CKD) and End-Stage Renal Disease (ESRD). With diabetes rates increasing, DN presents a significant health challenge. Current treatments manage established DN, but preventive strategies targeting high-risk individuals are urgently needed. This review evaluates current and emerging therapies for DN prevention.

Methods: A comprehensive literature search was conducted across multiple databases (PubMed, Web of Science, SCOPUS and others) to identify studies on the treatment and prevention of DN in DM patients. Eligible studies included Randomized Controlled Trials (RCT), cohort studies and meta-analyses published upto 2024, focusing on outcomes like albuminuria, Glomerular Filtration Rate (GFR) and ESRD incidence.

Results: Current treatments, including Sodium Glucose Co-transporter 2 (SGLT2) inhibitors, Angiotensin-Converting Enzyme (ACE) inhibitors and Angiotensin Receptor Blocker (ARB), effectively reduce albuminuria and slow progression. Emerging therapies, such as antioxidants (*Alpha-Lipoic Acid* (*ALA*), *Resveratrol*), Mineralocorticoid Receptor Antagonists (MRA) and Endothelin Receptor Antagonists (ERA), show promise in improving kidney function and reducing inflammation. Other potential therapies targeting Oxidative Stress (OS), inflammation and fibrosis, such as Advanced Glycation End products(AGE) inhibitors and Tumor Necrosis Factor-α (TNF-α) inhibitors, have demonstrated preclinical efficacy but require further validation.

Conclusion: While current therapies slow DN progression, they do not offer definitive prevention. Emerging treatments targeting oxidative stress, inflammation and fibrosis show promise in reducing kidney damage. However, challenges like side effects and long-term safety remain. Further research is needed to establish the efficacy of these therapies and develop personalized strategies for preventing DN in high-risk populations.

Keywords: Diabetic kidney disease, Diabetic nephropathy, DN, DKD, Preventive therapy, Preventing diabetic nephropathy, Preventing DKD, Diabetes complications

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INTRODUCTION

Diabetes mellitus (DM) is a global metabolic disorder with a rapidly increasing incidence, rising from 108 million cases in 1980 to 451 million in 2017 and projected to affect 693 million people by 2045 [1, 2]. This alarming trend presents a major healthcare challenge worldwide. Among the chronic complications of DM, Diabetic Nephropathy (DN) is one of the most serious and feared. Affecting approximately 40% of individuals with diabetes, DN significantly contributes to the burden of Chronic Kidney Disease (CKD) and End-Stage Renal Disease (ESRD), making diabetes the leading cause of ESRD globally [3]. Cardiovascular complications also present a major concern for individuals with DN, with cardiovascular disease being the primary competing risk before patients reach stage 4 CKD [4].

DN is a long-term, progressive kidney condition that typically manifests after 10 to 20 years of diabetes, initially characterized by microalbuminuria and progressing to macroalbuminuria and eventual renal impairment [5, 6]. Early intervention is crucial, as strict control of blood glucose and Blood Pressure (BP) has been shown to slow disease progression [7]. However, despite advances in treatment, including the use of ACE Inhibitors (ACEI), Angiotensin II Receptor Blockers (ARB) and newer therapies like Sodium Glucose co-Transporter (SGLT2) inhibitors, the majority of current interventions focus on managing the condition after it has already developed rather than preventing its onset.

This presents a critical gap in diabetes care. While existing therapies help control blood glucose and mitigate renal and cardiovascular complications, there is a pressing need for preventive strategies that can specifically target high-risk diabetic individuals before DN develops. Identifying and developing preventive therapies is essential to reduce the incidence of DN, ESRD and the need for Renal Replacement Therapy (RRT). By shifting focus toward early prevention, we can significantly improve patient outcomes, reduce healthcare costs and alleviate the growing burden of diabetes-related kidney disease. This review will explore the current landscape of drug treatments for DN and examine emerging preventive approaches that could transform the management of Diabetic Kidney Disease (DKD).

MATERIALS AND METHODS

Literature search strategy

A comprehensive literature search was performed to identify relevant studies examining treatment and preventive strategies for DN and DKD in individuals with DM. The search was conducted in multiple electronic databases, including Springer, Wiley, Web of Science, PubMed, Google Scholar, SCOPUS, Embase and Cochrane Library, with no restriction on publication date up to 2024. To further enhance the breadth of the review, references cited within included articles were also manually searched.

The following keywords and Boolean operators were used in the search: "Diabetic Nephropathy" OR "Diabetic Kidney Disease", "Diabetes Mellitus" AND "Treatment" OR "Prevention", "SGLT2 inhibitors" OR "ACE inhibitors" OR "Angiotensin Receptor Blockers", "Investigational drugs to prevent Diabetic Kidney disease", "Investigational drugs to prevent Nephropathy". The search strategy was refined using these terms in combination to ensure inclusion of studies relevant to both the treatment and prevention of DN/DKD.

Inclusion and exclusion criteria

Articles were considered for inclusion if they met the following criteria: Published in peer-reviewed journals, investigated the treatment or prevention of DN/DKD in patients with Type 1 Diabetes Mellitus (T1DM) or Type 2 Diabetes Mellitus (T2DM), reported clinical outcomes related to albuminuria, Glomerular Filtration Rate (GFR) or the incidence of ESRD, involved Randomized

Controlled Trials (RCT), cohort studies or meta-analyses and published between 2000 and 2024.

Studies were excluded if: They focused on non-diabetic kidney disease, the population consisted exclusively of patients without diabetes, they did not report relevant outcomes for DN/DKD or lacked sufficient clinical data, they were not published in peer-reviewed journals (e. g., conference abstracts) and the full text was unavailable or did not provide usable data for analysis.

Data extraction and evaluation

Data from the included studies were extracted and evaluated based on the quality of the evidence and the relevance of the findings. Key variables extracted included study design, patient population, type of intervention or preventive measure, outcomes related to renal function (e.g., albuminuria progression, GFR) and any reported adverse events.

Risk of bias assessment

The risk of bias for each included study was assessed using the Cochrane Risk of Bias Tool for RCT and the Newcastle-Ottawa Scale for observational studies. Bias was evaluated across several domains: Selection bias (e.g., random sequence generation, allocation concealment), Performance bias (e.g., blinding of participants and personnel), Detection bias (e.g., blinding of outcome assessment), Reporting bias (e.g., selective reporting of outcomes).

Studies were categorized as having low, moderate or high risk of bias in each domain. Any disagreements regarding bias assessments were resolved through discussion among the authors. Sensitivity analyses were conducted to assess the robustness of the review findings, especially in studies with a high risk of bias.

RESULTS AND DISCUSSION

Pathophysiology of DKD

The mechanisms underlying DKD arise from the interplay of three primary processes: hemodynamic, metabolic, and inflammatory factors. Each process contributes differently depending on an individual's genetic background, which explains variability in disease progression.

Hemodynamic factors

A crucial element of the hemodynamic aspect of DKD is the Renin-Angiotensin-Aldosterone System (RAAS). Renin, secreted by juxtaglomerular cells near the afferent arterioles, is pivotal for RAAS activation. Angiotensin II, produced through this pathway, binds to AT1 and AT2 receptors: AT1 receptor activation leads to increased resistance in efferent arterioles and elevated intraglomerular pressure maintaining renal filtration rates and AT2 receptor activation promotes vasodilatory Prostaglandin (PG) release, which offers a protective counterbalance [8].

Elevated angiotensin II levels contribute to renal injury through non-hemodynamic mechanisms: Stimulates aldosterone secretion and Promotes release of inflammatory chemokines, such as MCP-1 and TGF-β [9, 10].

Metabolic factors

Hyperglycemia, Insulin Resistance (IR) and dyslipidemia contribute to the progression of DKD. Excess glucose load in the proximal tubule upregulates SGLT-1 and SGLT-2, enhancing glucose and sodium reabsorption. This leads to decreased sodium delivery to the distal nephron and impaired tubuloglomerular feedback, disrupting normal glomerular hemodynamics [11-13].

Local factors

Factors like Endothelin-1, Reactive Oxygen Species (ROS) and Thromboxane A2 (TXA2) increase the tone of efferent arterioles contributing to glomerular hypertension. IR increases the production of Cyclooxygenase-2 (COX-2), prostanoids and the kallikrein-kinin system, resulting in the dilation of the afferent arterioles.

Activation of the Renin-Angiotensin System (RAS) can damage Glomerular Endothelial Cells (GEC), increasing fenestrations and triggering apoptosis. Hyperglycemia promotes the formation of Advanced Glycation End products (AGE), which attach to their RAGE receptors that reduces Nitric Oxide (NO) availability and increase activity of Transforming Growth Factor-Beta (TGF-β), a fibrotic factor. Diabetes accelerates the aging of Endothelial Progenitor Cells (EPC), diminishing their reparative capabilities.

Podocyte dysfunction

Podocytes exhibit dysregulated production of Vascular Endothelial Growth Factor (VEGF). Damage to podocytes results in foot process effacement and podocyte loss, which is a key mechanism in the development of albuminuria in diabetic patients [14-17].

Inflammation and fibrosis

Inflammation and fibrosis play crucial roles in the development of DKD. Infiltration of renal tissue by macrophage is a significant characteristic of DKD. Hyperglycemia and angiotensin II contribute to the recruitment of macrophages, which amplify inflammation through cytokine release. Activation of Mineralocorticoid Receptors (MR) intensifies the inflammatory response and contributes to glomerular damage by promoting sodium reabsorption and potassium excretion [18-20].

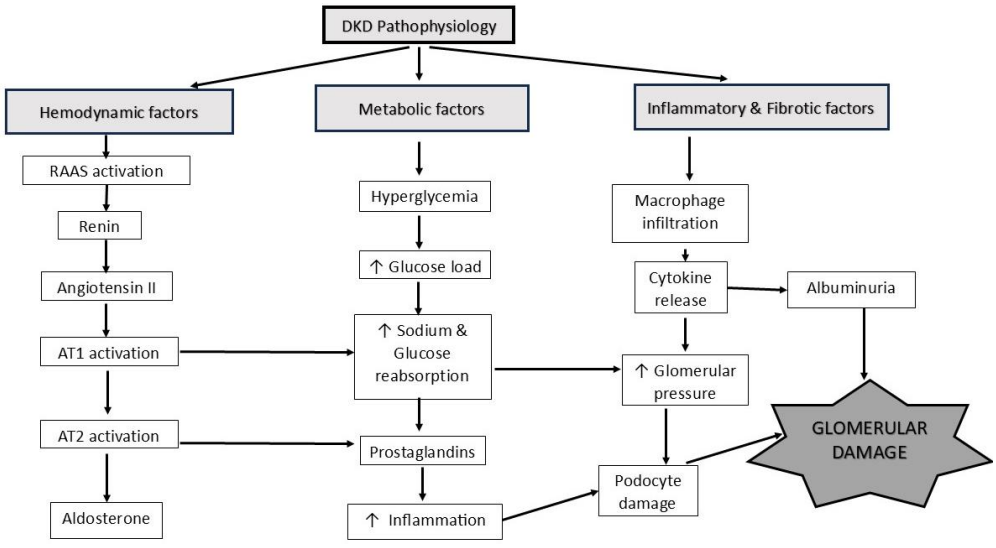

Fig. 1: Pathophysiology of DKD

Drugs to prevent DKD

Preventing DKD involves a multifaceted approach aimed at managing the underlying risk factors associated with diabetes. First and foremost, maintaining tight blood glucose control through diet, exercise and medications is essential to prevent damage to the kidneys. Monitoring and managing BP is equally critical, as high BP can accelerate kidney damage. Keeping BP within target levels (typically<130/80 mm Hg) through lifestyle changes and medications like ACE inhibitors or ARB is recommended. Reducing excess weight, avoiding smoking and limiting alcohol intake are also key lifestyle modifications that improve overall kidney health. Additionally, controlling cholesterol levels with statins can help reduce the cardiovascular risk that often accompanies diabetes and contributes to kidney dysfunction. Regular screening for early signs of kidney damage, such as albuminuria (protein in the urine) allows for early intervention and monitoring. In some cases, medications like SGLT2 inhibitors may be prescribed to further protect the kidneys. By addressing these modifiable risk factors along with a focus on diet, exercise and regular medical check-ups, individuals with diabetes can significantly reduce their risk of developing diabetic kidney disease.

Current treatment options

Glycemic control

Glycemic control is fundamental in managing DKD. The American Diabetes Association (ADA) recommends an A1C target of<7% for most adults with diabetes, while the American College of Physicians (ACP) suggests a target range of 7-8% for patients with long-standing diabetes or limited life expectancy [21, 22]. Studies show that aggressive glycemic control (e.g., A1C<6%) can reduce DKD incidence, but it may increase the risk of hypoglycemia, especially in older adults or those with cardiovascular disease [23]. Therefore, treatment should be individualized based on the patient's risk profile.

DPP-4 Inhibitors (e.g., *Sitagliptin, Saxagliptin*) reduce albuminuria independently of glucose control and are generally well tolerated. However, they may increase the risk of Heart Failure (HF) and have uncertain long-term benefits in preventing ESRD [24-26].

GLP-1 Receptor Agonists (e.g., *Semaglutide*, *Liraglutide*) protect renal endothelial cells and reduce Oxidative Stress (OS), improving kidney function and albuminuria. However, gastrointestinal side effects and risks of pancreatitis may limit their use [27-29].

SGLT2 Inhibitors (e.g., *Empagliflozin*, *Canagliflozin*) are a breakthrough in DKD management, significantly reducing renal disease progression and the need for RRT, as shown in the EMPA-REG OUTCOME trial [30, 31]. They can cause urinary and genital infections and dehydration and their long-term renal benefits in broader populations are still under study.

Thiazolidinediones (e.g., *Pioglitazone*) can reduce albuminuria but are limited by side effects such as weight gain and edema, with unclear benefits in preventing ESRD [32].

BP control

Control of BP is crucial in preventing the progression of DKD. While a target BP of<140/90 mm Hg is generally recommended, achieving this may not be appropriate for all patients [33, 34].

ACE Inhibitors (e.g., *Enalapril*, *Ramipril*) are effective in reducing albuminuria and mortality in DKD but can cause hyperkalemia, hypotension and renal impairment, especially in patients with existing kidney dysfunction [35, 36].

Aldosterone Antagonists (e.g., *Spironolactone*) lower proteinuria and BP, particularly in patients already on ACE inhibitors or ARB. However, they carry risks of hyperkalemia and gynecomastia and their benefit in advanced DKD remains unclear [37, 38].

ARB (e. g., *Losartan*, *Valsartan*) also reduce albuminuria and slow DKD progression. Like ACE inhibitors, they can cause hyperkalemia and hypotension and their long-term efficacy in advanced CKD is still under investigation [39].

MRA such as *Spironolactone*, *Eplerenone* and nonsteroidal MRAs like *Finerenone* have shown promise in reducing albuminuria and improving renal outcomes in DKD. Nonsteroidal MRAs, including *Finerenone* have a lower incidence of hyperkalemia and may offer a safer alternative to traditional therapies. Similarly, *esaxerenone* and *KBP-5074*, other nonsteroidal MRAs have also been found to significantly reduce Urine Albumin to Creatinine Ratio (UACR) in patients with diabetes and CKD [40-53]. These agents, especially when combined with ACE inhibitors or ARB, provide cardiorenal protection, though potassium monitoring is essential.

Table 1: Current treatment options to prevent DKD

Drug category	Drug	Mechanism of action	Dose	Side effects	Cost	Reference number
Glycemic control	DPP-4 Inhibitors	Inhibit DPP-4, increasing incretin hormones, improving insulin secretion, and reducing albuminuria	*Sitagliptin* (100 mg daily), *Saxagliptin* (5 mg daily)	Nasopharyngitis, heart failure risk, hypoglycemia (rare)	Moderate	[24-26]
	GLP-1 Receptor Agonists	Increase insulin secretion, decrease glucagon secretion, and reduce renal oxidative stress, improving albuminuria	Semaglutide (0.25 mg weekly), Liraglutide (0.6 mg daily)	Nausea, vomiting, pancreatitis risk, Hypoglycemia	High	[27-29]
	SGLT2 Inhibitors	Block sodium-glucose cotransporter 2 (SGLT2), reducing glucose reabsorption in the kidney, improving albuminuria, and slowing renal disease progression	*Empagliflozin* (10 mg daily), *Canagliflozin* (100 mg daily)	UTIs,Mycotic genital infections, dehydration, DKA (rare)	High	[30, 31]
	Thiazolidinediones	Activate PPAR-γ receptors, improving insulin sensitivity and reducing albuminuria	*Pioglitazone* (15-45 mg daily)	Weight gain, edema, heart failure risk	Moderate	[32]
BP control	ACE Inhibitors	Inhibit angiotensin-converting enzyme, reducing aldosterone, promoting vasodilation, and lowering proteinuria	*Enalapril* (5-40 mg daily), *Ramipril* (2.5-10 mg daily)	Hyperkalemia, hypotension, Cough, Increase serum creatinine level, Teratogenicity	Low	[35, 36]
	Aldosterone Antagonists	Block aldosterone receptors, reducing sodium retention, proteinuria, and BP	*Spironolactone* (25-100 mg daily)	Hyperkalemia, gynecomastia	Low	[37, 38]
	ARB	Block angiotensin II receptors, reducing vasoconstriction and aldosterone release, improving albuminuria	*Losartan* (25-100 mg daily), *Valsartan* (40-160 mg daily)	Hyperkalemia, hypotension, Increase in serum creatinine	Moderate	[39]
	MRA	Block mineralocorticoid receptors, reducing proteinuria, blood pressure, and kidney damage	*Spironolactone* (25-100 mg daily), *Finerenone* (10-20 mg daily)	Hyperkalemia, hyponatremina, gynecomastia, hypovolemia (*Spironolactone*)	High	[40-53]

Critical analysis

Despite the availability of various pharmacological agents to control glycemia and blood pressure in DKD, several key limitations remain. First, while ACE inhibitors, ARB and SGLT2 inhibitors have demonstrated efficacy in slowing the progression of renal disease, none of these therapies are curative. They predominantly serve as disease-modifying agents, with benefits primarily in reducing albuminuria and delaying the progression to more severe stages of DKD. However, in many patients, particularly those with advanced disease these agents provide only limited protection.

Furthermore, side effects are a significant concern. ACE inhibitors and ARBs may cause hyperkalemia and renal dysfunction, while SGLT2 inhibitors increase the risk of urinary and genital infections. Thiazolidinediones, while effective in some cases, can exacerbate heart failure, weight gain and edema, limiting their use in certain patient populations.

As a result, there is an urgent need for individualized treatment approaches in managing DKD. Treatment regimens should be tailored to the patient's specific stage of disease, comorbid conditions and risk factors. A more personalized approach incorporating factors such as age, underlying cardiovascular risk and co-morbidities may optimize therapeutic outcomes and minimize adverse effects.

Experimental treatment

Antioxidants

Alpha-lipoic acid (ALA)

A potent antioxidant that neutralizes ROS and reduces OS, *ALA* protects against DKD. It improves renal function, reduces fibrosis and decreases inflammatory cytokines (IL-6, TNF-α) by modulating pathways like p38 MAPK and NF-κB [54-56]. Clinical trials show *ALA* reduces urinary albumin excretion, a key marker of kidney dysfunction in diabetes. Typical dosages range from 600–1,200 mg/day [57-59].

Resveratrol

This polyphenol regulates oxidative stress, inflammation and autophagy in DN. It reduces ROS, enhances antioxidant defenses and improves kidney function through the AMPK/SIRT1/Nrf2 and Keap1/Nrf2 pathways [60, 61]. Studies show it also reduces proteinuria and improves renal structure [62, 63]. Combined with other treatments, *Resveratrol* may enhance DN management [64]. Network pharmacology highlights therapeutic targets for *Resveratrol* in DKD [65].

Curcumin

Known for anti-inflammatory, antioxidant and anti-apoptotic effects, curcumin protects the kidneys by activating Nrf2 and inhibiting NF-κB. It reduces OS, inflammation and fibrosis, particularly in DN [66-70]. *Curcumin* nanoparticles (nCUR) have shown promise in delaying DKD progression, even without controlling hyperglycemia [71]. It also modulates inflammation in CKD patients [72-75].

Sulbutiamine

It is a synthetic vitamin B1 derivative. *Sulbutiamine* reduces OS, improves kidney function and suppresses inflammatory markers in DN models [76].

Schisandrin B (Sch B)

It isa plant-derived lignan that targets mitochondrial dysfunction and Epithelial-Mesenchymal Transition (EMT) in renal tubular cells, reducing fibrosis and improving mitochondrial function in DKD. *Sch B* acts through the TGF-β1, PI3K/Akt, and AMPK pathways [77].

AGE formation inhibitors

Diphlorethohydroxycarmalol (*DPHC*) is found in brown seaweed. *DPHC* inhibits the AGE-RAGE interaction, preventing MGO-induced renal damage and regulating apoptosis [78]. Other AGE inhibitors like *Aminoguanidine* show promise, although clinical trials have been limited due to side effects [79-81].

Aldose reductase inhibitors (ARIs)

*WJ-39*and*Epalrestat*are ARI that inhibit the polyol pathway to protect against diabetic kidney damage. *WJ-39* improves mitochondrial function and reduces fibrosis in preclinical models [82]. *Epalrestat* has beneficial effects on renal function and interacts with key pathways (AGE-RAGE, TNF, HIF-1) [83], though gastrointestinal side effects limit its clinical use.

MRA

Esaxerenone is an MR blocker. *Esaxerenone* reduces albuminuria in DN independent of BP-lowering effects [84].

Endothelin-1 receptor A (ETA) antagonists

Atrasentan and *Zibotentan* are ETA-selective antagonists that reduce glomerular permeability and proteinuria, showing potential for DN and CKD. *Atrasentan* has shown promise in reducing renal events and albuminuria [85-91]. However, side effects like fluid retention and hepatotoxicity are concerns with long-term use.

mTOR inhibitors

mTOR signaling, activated by high glucose and cytokines in diabetes, promotes cell proliferation and fibrosis in kidney cells, contributing to DKD. mTOR activation impairs autophagy and promotes OS, inflammation and podocyte damage. *Rapamycin*, an mTOR inhibitor, shows promise in preclinical models but has side effects, including proteinuria and IR [92-105]. Other mTOR-targeting agents, like thiazolidinediones (*Rosiglitazone*), aldosterone antagonists (*Spironolactone*) and plant compounds (e.g., *Tripterygium glycoside*), show protective effects in DKD [106-114]. Vitamin D Receptor (VDR) activation, through DDIT4 upregulation, inhibits mTOR, mitigating kidney injury and fibrosis. These findings support mTOR inhibition as a potential DKD therapy, though further research is needed [115-121].

TNF-α inhibitors

TNF-α and its receptors, TNFR1 and TNFR2, play a role in the progression of DKD. Inhibiting TNF activity in diabetic models reduces proteinuria, sodium retention and kidney hypertrophy. Soluble TNF receptors like TNFR: Fc and *Etanercept* show promise in mitigating renal damage, suggesting TNFR as a key therapeutic target in DKD [122-126].

Pentoxifylline (PTX)

PTX has shown benefits in DKD by reducing proteinuria, improving kidney function (creatinine clearance), controlling inflammation and OS. *PTX* also improves lipid profiles, lowering LDL-C and Triglycerides (TGL) and reduces TNF-α levels. These multifactorial effects underscore its potential in DKD management [127-134]. Large-scale studies are needed to confirm *PTX*'s therapeutic potential.

Protein kinase C inhibitors (PKCI)

PKC β overactivity contributes to DN via collagen production and fibrosis. *Ruboxistaurin*, a selective PKC β inhibitor, reduces glomerular hyperfiltration and proteinuria in diabetic rats [135, 136]. *Echinochrome A* (*EchA*), derived from sea urchins, also inhibits PKC and improves renal function in diabetic models by reducing OS and fibrosis [137].

Nox1/4 inhibitors

NOX1 and NOX4 enzymes generate ROS, promoting inflammation and fibrosis in DKD. *GKT137831*, a dual NOX1/4 inhibitor, shows protective effects in preclinical DN models. The NOX-E36 inhibitor reduced albuminuria in DN patients, suggesting its potential for preventing kidney damage [138-140].

Nrf2 activators

Nrf2 activation enhances antioxidant capacity, reduces inflammation and prevents fibrosis, critical in DN. *Bardoxolone Methyl*, an Nrf2 activator, improved GFR in diabetic patients, though cardiovascular concerns led to trial termination [141, 142].

Table 2: Experimental treatment to prevent DKD

Drug category	Drug	Mechanism of action	Study model	Study outcome	Challenges in development	Reference number	Why it can be used in preventing DKD?
Antioxidants	*ALA*	Neutralizes ROS, reduces OS, modulates inflammatory and fibrotic pathways	Animal (Preclinical), Human (Clinical)	Preclinical studies showed protective effects on kidney function, reduced hyperglycemia, prevented glomerulosclerosis and fibrosis. Clinical trials showed decreased albumin excretion.	Mild side effects (GI discomfort, hypoglycemia), optimal dosage and long-term safety need further study	[54-59]	OS is a key driver of DKD and *ALA* targets this pathway, offering potential for kidney protection.
	Resveratrol	Modulates AMPK/SIRT1/Nrf2 and Keap1/Nrf2 pathways, reduces ROS, enhances antioxidant enzymes, improves kidney function.	Animal (Preclinical), Human (Clinical)	Reduced proteinuria, improved kidney structure, decreased inflammatory markers. Subgroup analyses showed beneficial effects with or without co-treatment with other medications.	Long-term efficacy and optimal dosage still under investigation.	[60-65]	Given the role of inflammation and oxidative stress in DKD, resveratrol could help reduce these factors, slowing kidney damage.
	Curcumin	Reduces OS, prevents renal damage, enhances mitochondrial function, modulates Nrf2 and NF-κB.	Animal (Preclinical), Human (Clinical)	Reduced renal inflammation and fibrosis, improved kidney function in diabetic rat models. In human trials, reduced inflammation in CKD patients.	More research needed for precise mechanisms and clinical application.	[62-66, 103-106]	*Curcumin* offers antioxidant, anti-inflammatory, and antifibrotic properties, essential for combating DKD progression.
	Sulbutiamine	Reduces OS, suppresses inflammatory markers, improves kidney function.	Animal (Preclinical)	Reduced fasting blood glucose, improved kidney function (decreased urea, creatinine), reduced inflammation, and improved histopathological changes in kidneys of diabetic rats.	Limited human data on long-term effects.	[76]	Targets OS and inflammation, core contributors to DKD, with promising effects in early studies.
	Sch B	Inhibits EMT, improves mitochondrial function, reduces ROS, enhances ATP production.	Animal (Preclinical)	Prevented EMT in renal tubular cells, improved mitochondrial function, reduced fibrosis and OS.	Limited human studies and clinical validation.	[77]	Inhibiting EMT and improving mitochondrial function may help prevent fibrosis and functional decline in DKD.
AGE formation inhibitors	*DPHC*	Inhibits AGE-RAGE interaction, regulates apoptosis, enhances Nrf2 pathway.	Animal (Preclinical)	Prevented AGE-related kidney damage, suppressed RAGE protein expression, reduced renal damage in diabetic rats.	Limited clinical data, need for larger trials.	[78]	AGE accumulation accelerates DKD progression; targeting AGE-RAGE interactions may slow down this process.
	Aminoguanidine	Inhibits AGE formation by trapping reactive carbonyl compounds and preventing glycoxidation.	Animal (Preclinical), Human (Clinical)	Reduced renal AGE accumulation and mesangial expansion in diabetic rats, but minimal benefits in human trials for overt nephropathy	Discontinued due to toxicity and adverse effects in humans.	[79-81]	AGEs contribute to fibrosis and inflammation in DKD, making their inhibition crucial for slowing disease progression.
Aldose Reductase Inhibitors (ARIs)	*WJ-39*	Inhibits aldose reductase, reducing polyol pathway activation, improves mitochondrial function, reduces fibrosis.	Animal (Preclinical)	Protected against renal tubular damage in diabetic rats, improved mitochondrial function and reduced fibrosis.	Long-term safety and efficacy in humans remain to be confirmed.	[82]	The polyol pathway is linked to DKD progression, and inhibition could reduce kidney damage and fibrosis.
	Epalrestat	Inhibits aldose reductase, reducing renal metabolic disturbances and inflammatory pathways.	Human (Clinical), Animal (Preclinical)	Reduced renal dysfunction and metabolic disturbances in DN patients, decreased inflammation.	Gastrointestinal side effects and liver enzyme abnormalities limit use.	[83]	Inhibition of aldose reductase could provide a direct therapeutic benefit in addressing metabolic disturbances in DKD.
MRA	*Esaxerenone*	Blocks MR, reduces albuminuria independent of BP reduction.	Human (Clinical)	Reduced Urine Albumin-to-Creatinine Ratio (UACR) in patients with DN, independent of BP effects.	Further research needed to clarify mechanisms and efficacy across different populations.	[84]	Blocking MR can reduce albuminuria, a key marker of kidney damage in DKD.
ETA	*Ambrisentan*, *Macitentan*, *Sitaxentan*, *BQ-123*,	Selective antagonism of ETA receptors, reducing vasoconstriction,	Preclinical, Clinical	Reduced glomerular permeability, lower BP, potential for treating resistant hypertension, DN	Side effects: hepatotoxicity, fluid retention, anemia, particularly with long-term	[85-91]	ETA can mitigate vasoconstriction and inflammation, both of which

Drug category	Drug	Mechanism of action	Study model	Study outcome	Challenges in development	Reference number	Why it can be used in preventing DKD?
	Darusentan, *Avosentan*, *Atrasentan*, *Zibotentan*	inflammation and renal damage			use of *Sitaxentan, Avosentan*		contribute to DKD progression.
	Bosentan, Tezosentan, Aprocitentan	Dual antagonism of ETA and ETB receptors, vasodilation, renal protection	Preclinical, Clinical	*Bosentan* approved for PAH; *Tezosentan* does not reduce cardiovascular events; *Aprocitentan* lowers BP in resistant hypertension	Liver dysfunction, fluid retention, lack of cardiovascular event reduction in clinical trials	[163-169]	Dual antagonism of endothelin receptors can help combat renal vasoconstriction and improve kidney outcomes in DKD.
mTOR Inhibitor	*Rapamycin*, Thiazolidinediones (e. g., *Rosiglitazone*), Aldosterone antagonists (e. g., *Spironolactone*)	Inhibition of mTOR signaling pathway, preventing podocyte damage and glomerular hypertrophy	Preclinical, Clinical	Reduced kidney injury, improved glomerular function, reduced fibrosis and inflammation	Side effects: proteinuria, renal tubular necrosis, insulin resistance, immune suppression	[92-105]	mTOR inhibitors can protect glomerular integrity and prevent kidney injury, crucial for preventing DKD.
	Tripterygium Glycoside, Triptolide, Radix Astragali, Paecilomyces Cicadidae, Dihydromyricetin, Ginsenoside Rg1, Kaempferol	Modulation of mTOR signaling, enhancing autophagy, reducing epithelial-mesenchymal transition and apoptosis	Preclinical, *In vitro*	Protection of renal function, delayed DKD progression, improved autophagic processes	Limited clinical data, potential safety concerns, unclear mechanisms of action	[106-114]	Modulating mTOR signaling could enhance kidney function and delay DKD progression by promoting autophagy and reducing fibrosis.
TNF-α Inhibitors	*Infliximab, Etanercept*	Inhibition of TNF signaling, reduction of inflammation, sodium retention and renal hypertrophy	Preclinical (STZ rats), Clinical (human)	Reduced urinary TNF excretion, attenuated kidney damage, decreased albuminuria	Limited understanding of TNFR1 vs TNFR2 contributions, variable outcomes across models	[122-126]	TNF-α is a major pro-inflammatory mediator in DKD; its inhibition could help reduce kidney inflammation and damage.
PTX	*PTX*	Anti-inflammatory, reduces oxidative stress, improves lipid profile and enhances kidney function	Clinical (human), Preclinical	Reduced UACR, improved creatinine clearance, reduced inflammation and OS	Need for large-scale, longitudinal studies to confirm findings, safety profile concerns	[127-134]	PTX's anti-inflammatory and antioxidant effects make it a potential therapy to reduce kidney damage in DKD patients.
PKCI	*Ruboxistaurin, EchA*	Inhibition of PKC β and downstream pathways, reducing fibrosis and oxidative stress	Preclinical (rat, mouse), Clinical	Reduced glomerular hyperfiltration, proteinuria, improved renal function in DN models	Limited human trials, efficacy and safety concerns, need for long-term data	[135-137]	PKC activation plays a role in DKD progression; inhibiting it could help reduce fibrosis and renal damage in diabetes.
Nox1/4 Inhibitor	*GKT137831, NOX-E36*	Inhibition of NOX1 and NOX4, reducing ROS production and kidney damage	Preclinical (mouse), Clinical (human)	Significant reduction in albuminuria, potential efficacy in preventing kidney damage	Lack of long-term clinical data, variable response across patient populations	[138-140]	NOX enzymes contribute to OS in DKD; inhibiting them may prevent kidney injury and slow disease progression.
Nrf2 Activator	*Bardoxolone Methyl*	Activation of Nrf2 pathway, enhancing antioxidant capacity and reducing inflammation	Clinical (human)	Increased glomerular filtration rate (GFR), but trial terminated early due to cardiovascular events	Safety concerns (cardiovascular risks), need for further trials to confirm long-term benefits	[141, 142]	Activating Nrf2 enhances kidney antioxidant defenses, which is critical in managing OS in DKD.
JAK-STAT Inhibitor	*Baricitinib*	Inhibits JAK1 and JAK2, suppresses inflammation and reduces albuminuria.	Phase II clinical trial in type 2 diabetic patients with DKD.	Significant reduction in albuminuria (40%), reduction in pro-inflammatory biomarkers (e. g., CXCL10, CCL2). Common AE: anemia.	Potential safety concerns with long-term use (e. g., anemia, infection). Further large-scale trials needed.	[143-154]	Shows promising results in reducing inflammation, albuminuria, and fibrosis in DKD patients.
	Ruxolitinib	It blocks JAK1 and JAK2, leading to a decrease in inflammation and fibrosis while also regulating podocyte autophagy.	Preclinical animal models (STZ-induced Wistar rats, HG-induced MPC-5 cell model).	Reduced proteinuria, decreased levels of inflammatory markers (TNF-α, TGF-β1, NF-κB), and fibrosis markers (vimentin).	No clinical trials yet for DKD, limited full animal studies.		Potential for reducing kidney fibrosis and inflammation in DKD, though further research needed.
	Nifuroxazide	Inhibits JAK2 and Tyk2, suppresses STAT3	Preclinical studies in STZ-induced SD rats,	Reduced oxidative stress, inflammation, and renal fibrosis.	Lack of clinical trials for DKD, but long-term safety		High oral safety promising anti-inflammatory and

Drug category	Drug	Mechanism of action	Study model	Study outcome	Challenges in development	Reference number	Why it can be used in preventing DKD?
		phosphorylation, reduces oxidative stress and inflammation.	UUO rats.	Improved glucose metabolism.	in clinical use suggests promise.		antioxidative effects could be useful in DKD treatment.
	Sinomenine	Inhibits JAK2/STAT3/SOCS1 pathway, reduces inflammation, fibrosis, and apoptosis.	Preclinical studies in STZ-induced SD rats.	Reduced apoptosis of renal cells, along with decreased inflammation and fibrosis. Skin lesions and gastrointestinal discomfort noted.	Potential side effects such as skin lesions and gastrointestinal issues.		Potential therapeutic option for inflammation and fibrosis in DKD through JAK/STAT modulation.
	Silymarin	It blocks the JAK2/STAT3/SOCS1 and TGF-β/Smad signaling pathways, leading to a reduction in inflammation, oxidative stress, and fibrosis.	Preclinical studies in STZ-induced SD rats.	Improved podocyte injury, reduced oxidative stress, and renal fibrosis. Gastrointestinal discomfort reported as AE.	Teratogenicity concerns in animal studies, limited clinical data.		May help reduce oxidative stress and renal fibrosis in DKD patients with favorable safety profile in clinical use.
	Total Glucosides of Paeony (TGP)	Inhibits JAK2/STAT3 pathway, suppresses macrophage activation, reduces renal inflammation and fibrosis.	Preclinical studies in Wistar rats with STZ-induced DKD.	Inhibited macrophage infiltration and fibrosis, reduced progression of DKD.	No clinical data yet for DKD.		Potential anti-inflammatory and fibrosis-reducing effects make it promising for DKD management.
	Paeoniflorin	Inhibits JAK2/STAT3 pathway, reduces macrophage infiltration and inflammatory responses.	Preclinical studies in STZ-induced C57BL/6J mice.	Alleviated kidney inflammation and fibrosis, improved kidney protection.	No significant adverse reactions in clinical trials.		May provide effective anti-inflammatory and protective effects against DKD.
	Isoliquiritigenin	Inhibits JAK2/STAT3 pathway, reduces inflammation and oxidative stress, protects against renal fibrosis.	Preclinical studies in HFD/STZ-induced SD rats.	Reduced renal fibrosis and inflammation, decreased IL-6 and ICAM-1 levels.	Minimal side effects reported.		Promising in alleviating oxidative stress and fibrosis, potential for DKD prevention.
	Momordica Charantia	Inhibits JAK2/STAT3/STAT5/SOCS3/4 pathways, reduces renal inflammation and fibrosis.	Preclinical studies in STZ-induced Wistar rats.	Reduced renal inflammatory response, modulated JAK/STAT pathways, reduced kidney damage.	No major adverse effects, but further studies on long-term use are needed.		Potential for modulating inflammatory pathways, a promising candidate for DKD prevention.
	Danzhi Jiangtang Capsule	Inhibits JAK/STAT pathway, reduces oxidative stress and inflammation in DKD.	Preclinical studies in HFD/STZ-induced SD rats and AGE-induced GMC model.	Reduced renal dysfunction, alleviated inflammatory injury in rats, associated with JAK/STAT inhibition.	Limited clinical data, but *in vitro* and animal studies suggest efficacy.		Potential as a complementary treatment for DKD by targeting oxidative stress and inflammation.
	ErHuang Formula	Inhibits CXCL6/JAK/STAT3 pathway, reduces inflammation fibrosis, and improves kidney function.	Preclinical studies in HFD/STZ-induced SD rats and HG-induced NRK-49F cells.	Reduced fibrosis, decreased inflammation and renal dysfunction.	Need for more extensive clinical trials.		Potential to reduce renal fibrosis and inflammation, useful in DKD management.
Adhesion and chemokine molecule inhibitors	*ASP8232* (VAP-1 inhibitor), *Emapticap Pegol* (CCL2-CCR2 inhibitor), *NOX-A12* (CXCL12 inhibitor)	Blockade of adhesion molecules and chemokines, reducing immune cell migration and kidney inflammation	Clinical (Phase II), Preclinical	Reduced proteinuria, renal protection, slowed progression of kidney injury	Limited options for targeting adhesion molecules need for additional clinical research	[155-161,162]	Targeting adhesion molecules can block immune cell migration to the kidneys, reducing inflammation and fibrosis in DKD.

JAK-STAT inhibitors

JAK inhibitors, such as *tofacitinib* (JAK1/3) and *baricitinib* (JAK1/2), have shown effectiveness in treating inflammatory and autoimmune diseases, including rheumatoid arthritis [143]. *Ruxolitinib*, another JAK1/2 inhibitor, is approved for myelofibrosis [144] and has also been studied in autoimmune diseases like Crohn's disease and psoriasis [145, 146]. In early clinical trials, JAK inhibitors have demonstrated potential in treating DKD by improving renal function and reducing inflammatory markers [147, 148]. *Baricitinib*, for instance, has reduced albuminuria and inflammation in type 2 diabetic patients with DKD (NCT01683409). However, the long-term safety of these treatments, especially concerning anemia and infection risks, requires further investigation. Additionally, *Ruxolitinib* and *Nifuroxazide*, which inhibit the JAK/STAT pathway, have shown promise in experimental models for DKD treatment by reducing fibrosis and inflammation [149, 150]. Natural products like *Sinomenine*, *Silymarin* and *Paeoniflorin* have also been studied for their ability to modulate the JAK/STAT pathway and offer potential therapeutic benefits for DKD [151, 152]. Other medications, such as liraglutide and vitamin D, have been found to inhibit the JAK/STAT pathway and alleviate DKD-related inflammation and fibrosis, although further clinical trials are needed to confirm their long-term efficacy and safety [153, 154].

Adhesion and chemokine molecule inhibition in DKD

Adhesion molecules (ICAM-1, VCAM-1, VAP-1) and chemokines (e. g., CCL2) contribute to kidney inflammation and damage in DKD. Targeting adhesion molecules with VAP-1 inhibitors like *ASP8232* and blocking the CCL2-CCR2 pathway with *Emapticap Pegol* reduced proteinuria in Phase II trials [155-161]. CXCL12 inhibition also alleviated kidney damage in diabetic mice [162].

Critical analysis

The development of experimental drugs for DKD has been notably slow despite promising preclinical results and the urgent need for effective therapies. One significant reason for this lag is the high attrition rate during clinical trials, often due to issues related to safety, efficacy and side effects. Many drugs that show potential in animal models fail to replicate these outcomes in human trials. For example, while antioxidants like ALA and curcumin show positive effects in preclinical studies, they often have limited bioavailability or cause mild side effects in humans which hampers their clinical adoption. Similarly, AGE formation inhibitors like *Aminoguanidine*, despite showing promise in animal models, were discontinued in human trials due to toxicity concerns. Additionally, the complexity of DKD's pathophysiology, involving multiple pathways such as OS, inflammation, fibrosis and metabolic dysregulation, makes it difficult to pinpoint a single therapeutic target. As a result, clinical trials frequently fail to achieve the desired outcomes, slowing the development of effective treatments.

Moreover, regulatory and financial hurdles further delay the introduction of new DKD therapies. Clinical trials, particularly those for chronic conditions like DKD, require long follow-up periods to assess long-term safety and effectiveness, which increases both time and cost. This is particularly challenging for drugs targeting multiple pathways, such as mTOR inhibitors or ETA, where potential side effects like fluid retention or cardiovascular risks must be carefully managed. Limited funding, especially for phase III trials and a lack of consensus on optimal biomarkers for disease progression, also contribute to the slow pace of drug development. As a result, while the number of experimental drugs in the DKD pipeline is growing, many faces significant obstacles before they can be approved for widespread clinical use, further delaying advances in treatment for this progressive and debilitating disease.

Table 3: Comparison of current drugs Vs experimental drugs to prevent DKD

Criteria	Current treatment options	Experimental treatment options
Therapeutic efficacy	-DPP-4 Inhibitors: Improve insulin secretion and reduce albuminuria. Proven efficacy in DKD management. -SGLT2 Inhibitors: Reduce glucose reabsorption and albuminuria, slow progression of kidney disease. -ACE Inhibitors/ARBs: Reduce proteinuria, control BP and protect kidney function.	-Antioxidants (*ALA, Resveratrol, Curcumin, Sulbutiamine, Schisandrin B*): Show promising effects on reducing OS, improving kidney function and preventing fibrosis in preclinical and early clinical trials. -AGE Formation Inhibitors (*DPHC, Aminoguanidine*): Show potential in reducing kidney damage by preventing AGE-RAGE interaction. -ARI: Potential to reduce kidney damage by inhibiting the polyol pathway. -MRA (*Esaxerenone*): Show efficacy in reducing albuminuria independent of BP. -Endothelin-1 Receptor Antagonist (*Ambrisentan, Macitentan, Bosentan*): Potential to reduce glomerular permeability and inflammation. -mTOR Inhibitors (*Rapamycin, Tripterygium Glycoside*): Demonstrate efficacy in reducing kidney injury, improving glomerular function and decreasing inflammation and fibrosis. -TNF-α Inhibitors (*Infliximab, Etanercept*): Show potential in reducing kidney inflammation and albuminuria. -*PTX*: Demonstrates potential in improving kidney function and reducing inflammation. -Nox1/4 Inhibitors (*GKT137831, NOX-E36*): Show potential to reduce ROS production and kidney damage. -Nrf2 Activators (*Bardoxolone Methyl*): Demonstrated increased glomerular filtration rate, though concerns over cardiovascular safety exist. -JAK-STAT Inhibitors (*Baricitinib*): Reduced proteinuria in early-phase trials, suggesting efficacy in DKD management. -Adhesion and Chemokine Molecule Inhibitors: Target adhesion molecules to reduce inflammation and kidney damage.
Cost	-Moderate to High: SGLT2 inhibitors, GLP-1 agonists, and other medications like ACE inhibitors or ARBs can be expensive, especially newer drugs like SGLT2 inhibitors.	-Varies: Many experimental treatments are still in clinical trials and are not yet commercially available, making the cost difficult to determine. Some therapies might be cost-effective if they enter the market but others, especially novel biologics or small molecules, may be expensive.

CONCLUSION

In conclusion, while significant progress has been made in identifying and implementing strategies to prevent DKD, many of the current interventions primarily focus on slowing disease progression rather than offering definitive prevention. Lifestyle modifications such as weight loss, physical activity and dietary changes remain foundational in reducing the risk of DKD and pharmacological treatments such as ACE inhibitors, ARB and SGLT2 inhibitors have demonstrated efficacy in mitigating the onset of kidney complications. However, these interventions are not without limitations and while they can delay the progression of kidney damage, they do not fully prevent the disease in all patients.

Given the promising yet early-stage nature of many emerging therapies such as antioxidants (e. g., *ALA, Resveratrol, Curcumin*), MRA and novel agents like JAK-STAT inhibitors, the urgency for more extensive and rigorous clinical trials becomes evident. Early clinical data may suggest potential benefits, but they are not sufficient to justify the optimistic outlook that some of these therapies might prevent DKD in the long term. Therefore, a key priority is the acceleration of clinical studies to validate these promising treatments, particularly for high-risk populations who may benefit most from early intervention.

Additionally, as DKD is a complex and multifactorial disease, a more personalized approach to prevention by incorporating individual risk factors such as age, comorbid conditions and underlying cardiovascular risk will be essential in optimizing prevention strategies. Tailoring interventions to the specific needs of each patient has the potential to prevent the onset of DKD while minimizing adverse effects. Ultimately, a concerted effort to advance clinical trials and refine prevention strategies will be crucial in reducing the burden of DKD and improving outcomes for those at risk.

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CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest.

REFERENCES

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet. 2016;387(10027):1513-30. doi: 10.1016/S0140-6736(16)00618-8, PMID 27061677.
2. Federation ID. IDF diabetes atlas brussels. Belgium: international diabetes federation; 2021.
3. Garg AX, Kiberd BA, Clark WF, Haynes RB, Clase CM. Albuminuria and renal insufficiency prevalence guides population screening: results from the NHANES III. Kidney Int. 2002;61(6):2165-75. doi: 10.1046/j.1523-1755.2002.00356.x, PMID 12028457.
4. Naaman SC, Bakris GL. Diabetic nephropathy: update on pillars of therapy slowing progression. Diabetes Care. 2023;46(9):1574-86. doi: 10.2337/dci23-0030, PMID 37625003.
5. Sagoo MK, Gnudi L. Diabetic nephropathy: an overview. Methods Mol Biol. 2020;2067:3-7. doi: 10.1007/978-1-4939-9841-8_1, PMID 31701441.
6. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit Dahm KA, Zoungas S. Diabetic kidney disease. Nat Rev Dis Primers. 2015 Jul 30;1:15018. doi: 10.1038/nrdp.2015.18, PMID 27188921.
7. Papadopoulou Marketou N, Kanaka Gantenbein C, Marketos N, Chrousos GP, Papassotiriou I. Biomarkers of diabetic nephropathy: a 2017 update. Crit Rev Clin Lab Sci. 2017;54(5):326-42. doi: 10.1080/10408363.2017.1377682, PMID 28956668.
8. Carey RM, Wang ZQ, Siragy HM. Role of the angiotensin type 2 receptor in the regulation of blood pressure and renal function. Hypertension. 2000;35(1 Pt 2):155-63. doi: 10.1161/01.hyp.35.1.155, PMID 10642292.
9. Ames MK, Atkins CE, Pitt B. The rennin angiotensin aldosterone system and its suppression. J Vet Intern Med. 2019;33(2):363-82. doi: 10.1111/jvim.15454, PMID 30806496.
10. Ruiz Ortega M, Lorenzo O, Suzuki Y, Ruperez M, Egido J. Proinflammatory actions of angiotensins. Curr Opin Nephrol Hypertens. 2001;10(3):321-9. doi: 10.1097/00041552-200105000-00005, PMID 11342793.
11. Poursharif S, Hamza S, Braam B. Changes in proximal tubular reabsorption modulate microvascular regulation via the TGF system. Int J Mol Sci. 2022;23(19)11203. doi: 10.3390/ijms231911203.
12. Tuttle KR. Back to the future: glomerular hyperfiltration and the diabetic kidney. Diabetes. 2017;66(1):14-6. doi: 10.2337/dbi16-0056, PMID 27999101.
13. Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium-glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects potential mechanisms and clinical applications. Circulation. 2016;134(10):752-72. doi: 10.1161/circulationaha.116.021887, PMID 27470878.
14. Tonneijck L, Muskiet MH, Smits MM, Van Bommel EJ, Heerspink HJ, Van Raalte DH. Glomerular hyperfiltration in diabetes: mechanisms clinical significance and treatment. J Am Soc Nephrol. 2017;28(4):1023-39. doi: 10.1681/ASN.2016060666, PMID 28143897.
15. Astor BC, Hallan SI, Miller ER 3rd, Yeung E, Coresh J. Glomerular filtration rate albuminuria and risk of cardiovascular and all-cause mortality in the US population. Am J Epidemiol. 2008;167(10):1226-34. doi: 10.1093/aje/kwn033, PMID 18385206.
16. Bello AK, Hemmelgarn B, Lloyd A, James MT, Manns BJ, Klarenbach S. Associations among estimated glomerular filtration rate proteinuria and adverse cardiovascular outcomes. Clin J Am Soc Nephrol. 2011;6(6):1418-26. doi: 10.2215/CJN.09741110, PMID 21527648.
17. El Sayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D. Chronic kidney disease and risk management: standards of care in diabetes-2023. Diabetes Care. 2023;46 Suppl 1:S191-202. doi: 10.2337/dc23-S011, PMID 36507634.
18. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on kidney in cardiovascular disease high blood pressure research clinical cardiology and epidemiology and prevention. Circulation. 2003;108(17):2154-69. doi: 10.1161/01.CIR.0000095676.90936.80, PMID 14581387.
19. Cantero Navarro E, Rayego Mateos S, Orejudo M, Tejedor Santamaria L, Tejera Munoz A, Sanz AB. Role of macrophages and related cytokines in kidney disease. Front Med (Lausanne). 2021 Jul 8;8:688060. doi: 10.3389/fmed.2021.688060, PMID 34307414.
20. Tesch GH. Macrophages and diabetic nephropathy. Semin Nephrol. 2010;30(3):290-301. doi: 10.1016/j.semnephrol.2010.03.007, PMID 20620673.
21. Usher MG, Duan SZ, Ivaschenko CY, Frieler RA, Berger S, Schutz G. Myeloid mineralocorticoid receptor controls macrophage polarization and cardiovascular hypertrophy and remodeling in mice. J Clin Invest. 2010;120(9):3350-64. doi: 10.1172/JCI41080, PMID 20697155.
22. American Diabetes Association. 6. Glycemic targets: standards of Medical Care in diabetes-2018. Diabetes Care. 2018 Jan;41 Suppl 1:S55-64. doi: 10.2337/dc18-S006, PMID 29222377.
23. Qaseem A, Wilt TJ, Kansagara D, Horwitch C, Barry MJ, Forciea MA. Hemoglobin A1c targets for glycemic control with pharmacologic therapy for nonpregnant adults with type 2 diabetes mellitus: a guidance statement update from the American College of Physicians. Ann Intern Med. 2018;168(8):569-76. doi: 10.7326/M17-0939, PMID 29507945.
24. Groop PH, Cooper ME, Perkovic V, Emser A, Woerle HJ, Von Eynatten M. Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. Diabetes Care. 2013;36(11):3460-8. doi: 10.2337/dc13-0323, PMID 24026560.
25. Groop PH, Cooper ME, Perkovic V, Hocher B, Kanasaki K, Haneda M. Linagliptin and its effects on hyperglycaemia and albuminuria in patients with type 2 diabetes and renal dysfunction: the randomized MARLINA-T2D trial. Diabetes Obes Metab. 2017;19(11):1610-9. doi: 10.1111/dom.13041, PMID 28636754.
26. Scirica BM, Braunwald E, SAVOR-TIMI RI. 53 steering committee and investigators. Heart failure saxagliptin and diabetes mellitus: observations from the SAVOR-TIMI 53 randomized trial. Circulation. 2014;132(15):1579-88.
27. Marso SP, Daniels GH, Brown Frandsen K, Kristensen P, Mann JF, Nauck MA. Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2016;375(4):311-22. doi: 10.1056/NEJMoa1603827, PMID 27295427.
28. Fujita H, Morii T, Fujishima H, Sato T, Shimizu T, Hosoba M. The protective roles of GLP-1R signaling in diabetic nephropathy: possible mechanism and therapeutic potential. Kidney Int. 2014;85(3):579-89. doi: 10.1038/ki.2013.427, PMID 24152968.

29. Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jodar E, Leiter LA. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. N Engl J Med. 2016;375(19):1834-44. doi: 10.1056/NEJMoa1607141, PMID 27633186.
30. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, Von Eynatten M, Mattheus M. Empagliflozin and progression of kidney disease in type 2 diabetes. N Engl J Med. 2016;375(4):323-34. doi: 10.1056/NEJMoa1515920, PMID 27299675.
31. Barnett AH, Mithal A, Manassie J, Jones R, Rattunde H, Woerle HJ. Efficacy and safety of empagliflozin added to existing antidiabetes treatment in patients with type-2 diabetes and chronic kidney disease: a randomised double blind placebo controlled trial. Lancet Diabetes Endocrinol. 2014;2(5):369-84. doi: 10.1016/S2213-8587(13)70208-0, PMID 24795251.
32. Sarafidis PA, Bakris GL. Protection of the kidney by thiazolidinediones: an assessment from bench to bedside. Kidney Int. 2006;70(7):1223-33. doi: 10.1038/sj.ki.5001620, PMID 16883325.
33. Whelton PK, Carey RM, Aronow WS. PCNA guideline for the prevention detection evaluation and management of high blood pressure in adults: a report of the American college of cardiology/American heart association task force on clinical practice guidelines. J Am Coll Cardiol. 2017;71(19):e127-248. doi: 10.1016/j.jacc.2017.11.005, PMID 29146533.
34. ACCORD Study Group, Cushman WC, Evans GW, Byington RP, Goff DC JR, Grimm RH JR. Effects of intensive blood pressure control in type 2 diabetes mellitus. N Engl J Med. 2010;362(17):1575-85. doi: 10.1056/NEJMoa1001286, PMID 20228401.
35. LV J, Perkovic V, Foote CV, Craig ME, Craig JC, Strippoli GF. Antihypertensive agents for preventing diabetic kidney disease. Cochrane Database Syst Rev. 2012;12(12):CD004136. doi: 10.1002/14651858.CD004136.pub3, PMID 23235603.
36. Haller H, Ito S, Izzo JL, Januszewicz A, Katayama S, Menne J. Olmesartan for the delay or prevention of microalbuminuria in type 2 diabetes. N Engl J Med. 2011;364(10):907-17. doi: 10.1056/NEJMoa1007994, PMID 21388309.
37. Currie G, Taylor AH, Fujita T, Ohtsu H, Lindhardt M, Rossing P. Effect of mineralocorticoid receptor antagonists on proteinuria and progression of chronic kidney disease: a systematic review and meta-analysis. BMC Nephrol. 2016;17(1):127. doi: 10.1186/s12882-016-0337-0, PMID 27609359.
38. Bolignano D, Palmer SC, Navaneethan SD, Strippoli GF. Aldosterone antagonists for preventing the progression of chronic kidney disease. Cochrane Database Syst Rev. 2014;(4):CD007004. doi: 10.1002/14651858.CD007004.pub3, PMID 24782282.
39. Menne J, Ritz E, Ruilope LM, Chatzikyrkou C, Viberti G, Haller H. The randomized olmesartan and diabetes microalbuminuria prevention (roadmap) observational follow up study: benefits of ras blockade with *olmesartan* treatment are sustained after study discontinuation. J Am Heart Assoc. 2014;3(2):e000810. doi: 10.1161/jaha.114.000810, PMID 24772521.
40. Berbenetz NM, Mrkobrada M. Mineralocorticoid receptor antagonists for heart failure: systematic review and meta-analysis. BMC Cardiovasc Disord. 2016;16(1):246. doi: 10.1186/s12872-016-0425-x, PMID 27905877.
41. Abdelhakim AM, Abd El Gawad M. Impact of mineralocorticoid receptor antagonist in renal transplant patients: a systematic review and meta-analysis of randomized controlled trials. J Nephrol. 2020;33(3):529-38. doi: 10.1007/s40620-019-00681-4, PMID 31828668.
42. Tromp J, Ouwerkerk W, Van Veldhuisen DJ, Hillege HL, Richards AM, Van Der Meer P. A systematic review and network metaanalysis of pharmacological treatment of heart failure with reduced ejection fraction. JACC Heart Fail. 2020;33(3):73-84.
43. Chung EY, Ruospo M, Natale P, Bolignano D, Navaneethan SD, Palmer SC. Aldosterone antagonists in addition to renin angiotensin system antagonists for preventing the progression of chronic kidney disease. Cochrane Database Syst Rev. 2020;10(10):CD007004. doi: 10.1002/14651858.CD007004.pub4, PMID 33107592.
44. Rossing K, Schjoedt KJ, Smidt UM, Boomsma F, Parving HH. Beneficial effects of adding spironolactone to recommended antihypertensive treatment in diabetic nephropathy: a randomized double masked cross over study. Diabetes Care. 2005;28(9):2106-12. doi: 10.2337/diacare.28.9.2106, PMID 16123474.
45. Esteghamati A, Noshad S, Jarrah S, Mousavizadeh M, Khoee SH, Nakhjavani M. Long term effects of addition of mineralocorticoid receptor antagonist to angiotensin II receptor blocker in patients with diabetic nephropathy: a randomized clinical trial. Nephrol Dial Transplant. 2013;28(11):2823-33. doi: 10.1093/ndt/gft281, PMID 24009294.
46. Epstein M, Williams GH, Weinberger M, Lewin A, Krause S, Mukherjee R. Selective aldosterone blockade with eplerenone reduces albuminuria in patients with type 2 diabetes. Clin J Am Soc Nephrol. 2006;1(5):940-51. doi: 10.2215/CJN.00240106, PMID 17699311.
47. El Mokadem M, Abd El Hady Y, Aziz A. A prospective single blind randomized trial of ramipril eplerenone and their combination in type 2 diabetic nephropathy. Cardiorenal Med. 2020;10(6):392-401. doi: 10.1159/000508670, PMID 32998143.
48. Bertocchio JP, Barbe C, Lavaud S, Toupance O, Nazeyrollas P, Jaisser F. Safety of eplerenone for kidney transplant recipients with impaired renal function and receiving cyclosporine A. Plos One. 2016;11(4):e0153635. doi: 10.1371/journal.pone.0153635, PMID 27088859.
49. Bakris GL, Agarwal R, Chan JC, Cooper ME, Gansevoort RT, Haller H. Effect of finerenone on albuminuria in patients with diabetic nephropathy: a randomized clinical trial. JAMA. 2015;314(9):884-94. doi: 10.1001/jama.2015.10081, PMID 26325557.
50. Bakris GL, Agarwal R, Anker SD, Pitt B, Ruilope LM, Rossing P. Effect of finerenone on chronic kidney disease outcomes in type 2 diabetes. N Engl J Med. 2020;383(23):2219-29. doi: 10.1056/NEJMoa2025845, PMID 33264825.
51. Ito S, Shikata K, Nangaku M, Okuda Y, Sawanobori T. Efficacy and safety of esaxerenone (CS− 3150) for the treatment of type-2 diabetes with microalbuminuria: a randomized double blind placebo controlled phase II trial. Clin J Am Soc Nephrol. 2019;14(8):1161-72. doi: 10.2215/CJN.14751218, PMID 31248950.
52. Ito S, Kashihara N, Shikata K, Nangaku M, Wada T, Okuda Y. Esaxerenone (CS-3150) in patients with type 2 diabetes and microalbuminuria (esax-dn): phase 3 randomized controlled clinical trial. Clin J Am Soc Nephrol. 2020;15(12):1715-27. doi: 10.2215/CJN.06870520, PMID 33239409.
53. Bakris G, Pergola PE, Delgado B, Genov D, Doliashvili T, VO N. Effect of KBP-5074 on blood pressure in advanced chronic kidney disease: results of the block CKD study. Hypertension. 2021;78(1):74-81. doi: 10.1161/hypertensionaha.121.17073, PMID 33966452.
54. Grdovic N, Rajic J, Arambasic Jovanovic J, Dinic S, Tolic A, Dordevic M. α-Lipoic acid increases collagen synthesis and deposition in nondiabetic and diabetic rat kidneys. Oxid Med Cell Longev. 2021;2021:6669352. doi: 10.1155/2021/6669352, PMID 33777319.
55. Charlton A, Garzarella J, Jandeleit Dahm KA, Jha JC. Oxidative stress and inflammation in renal and cardiovascular complications of diabetes. Biology (Basel). 2020;10(1):18. doi: 10.3390/biology10010018, PMID 33396868.
56. Zhang H, MU J, DU J, Feng Y, XU W, Bai M. *Alpha lipoic acid* could attenuate the effect of chemerin induced diabetic nephropathy progression. Iran J Basic Med Sci. 2021;24(8):1107-16. doi: 10.22038/ijbms.2021.50792.11570, PMID 34804428.
57. Vakali E, Rigopoulos D, Carrillo AE, Flouris AD, Dinas PC. Effects of *alpha* lipoic acid supplementation on human diabetic nephropathy: a systematic review and meta-analysis. Curr Diabetes Rev. 2022;18(6):e140921196457. doi: 10.2174/1573399817666210914103329, PMID 34521329.
58. Jiang M, Sun H, Zhang H, Cheng Y, Zhai C. ALA/*LA* inhibited renal tubulointerstitial fibrosis of DKD db/db mice induced by oxidative stress. Research square; 2024. doi: 10.21203/rs.3.rs-3956527/v1.
59. Jeffrey S, Samraj PI, Raj BS. The role of *alpha lipoic acid* supplementation in the prevention of diabetes complications: a comprehensive review of clinical trials. Curr Diabetes Rev. 2021;17(9):e011821190404. doi: 10.2174/1573399817666210118145550, PMID 33461470.

60. Gowd V, Kang Q, Wang Q, Wang Q, Chen F, Cheng KW. Resveratrol: evidence for its nephroprotective effect in diabetic nephropathy. Adv Nutr. 2020;11(6):1555-68. doi: 10.1093/advances/nmaa075, PMID 32577714.
61. Xian Y, Gao Y, LV W, MA X, HU J, Chi J. Resveratrol prevents diabetic nephropathy by reducing chronic inflammation and improving the blood glucose memory effect in non obese diabetic mice. Naunyn Schmiedebergs Arch Pharmacol. 2020;393(10):2009-17. doi: 10.1007/s00210-019-01777-1, PMID 31970441.
62. Salami M, Salami R, Mafi A, Aarabi MH, Vakili O, Asemi Z. Therapeutic potential of resveratrol in diabetic nephropathy according to molecular signaling. Curr Mol Pharmacol. 2022;15(5):716-35. doi: 10.2174/1874467215666211217122523, PMID 34923951.
63. LI KX, JI MJ, Sun HJ. An updated pharmacological insight of resveratrol in the treatment of diabetic nephropathy. Gene. 2021;780:145532. doi: 10.1016/j.gene.2021.145532, PMID 33631244.
64. Sattarinezhad A, Roozbeh J, Shirazi Yeganeh B, Omrani GR, Shams M. Resveratrol reduces albuminuria in diabetic nephropathy: a randomized double-blind placebo-controlled clinical trial. Diabetes Metab. 2019;45(1):53-9. doi: 10.1016/j.diabet.2018.05.010, PMID 29983230.
65. Chen S, LI B, Chen L, Jiang H. Uncovering the mechanism of resveratrol in the treatment of diabetic kidney disease based on network pharmacology molecular docking and experimental validation. J Transl Med. 2023;21(1):380. doi: 10.1186/s12967-023-04233-0, PMID 37308949.
66. Zhu X, XU X, DU C, SU Y, Yin L, Tan X. An examination of the protective effects and molecular mechanisms of curcumin a polyphenol curcuminoid in diabetic nephropathy. Biomed Pharmacother. 2022;153:113438. doi: 10.1016/j.biopha.2022.113438, PMID 36076553.
67. Pricci M, Girardi B, Giorgio F, Losurdo G, Ierardi E, DI Leo A. Curcumin and colorectal cancer: from basic to clinical evidences. Int J Mol Sci. 2020;21(7):2364. doi: 10.3390/ijms21072364, PMID 32235371.
68. Asadi S, Goodarzi MT, Karimi J, Hashemnia M, Khodadadi I. Does curcumin or metformin attenuate oxidative stress and diabetic nephropathy in rats? J Nephropathol. 2018;8(1):8. doi: 10.15171/jnp.2019.08.
69. Al Tamimi JZ, Al Farga NA, Alshammari AM, Mowyna B, Yahya MN. Curcumin reverses diabetic nephropathy in streptozotocin-induced diabetes in rats by inhibition of PKC/p66Shc axis and activation of FOXO-3a. J Nutr Biochem. 2021 Jan;87:108515. doi: 10.1016/j.jnutbio.2020.108515.
70. Gao L, LV Q, Wang Y, Zhang D, Ding W, Cao L. Research on mechanism of curcumin with chitosan nanoparticles in regulating the activity of podocytes in diabetic nephropathy through alleviating oxidative stress and inflammation. Sci Adv Mater. 2022;14(4):752-9. doi: 10.1166/sam.2022.4249.
71. Ganugula R, Nuthalapati NK, Dwivedi S, Zou D, Arora M, Friend R. Nanocurcumin combined with insulin alleviates diabetic kidney disease through P38/P53 signaling axis. J Control Release. 2023;353:621-33. doi: 10.1016/j.jconrel.2022.12.012, PMID 36503070.
72. Shing CM, Adams MJ, Fassett RG, Coombes JS. Nutritional compounds influence tissue factor expression and inflammation of chronic kidney disease patients *in vitro*. Nutrition. 2011;27(9):967-72. doi: 10.1016/j.nut.2010.10.014, PMID 21295946.
73. Alvarenga L, Salarolli R, Cardozo LF, Santos RS, DE Brito JS, Kemp JA. Impact of curcumin supplementation on expression of inflammatory transcription factors in hemodialysis patients: a pilot randomized double-blind controlled study. Clin Nutr. 2020;39(12):3594-600. doi: 10.1016/j.clnu.2020.03.007, PMID 32204978.
74. Alvarenga L, Cardozo LF, DA Cruz BO, Paiva BR, Fouque D, Mafra D. Curcumin supplementation improves oxidative stress and inflammation biomarkers in patients undergoing hemodialysis: a secondary analysis of a randomized controlled trial. Int Urol Nephrol. 2022;54(10):2645-52. doi: 10.1007/s11255-022-03182-9, PMID 35347555.
75. Salarolli RT, Alvarenga L, Cardozo LF, Teixeira KT, DE SG Moreira L, Lima JD. Can curcumin supplementation reduce plasma levels of gut-derived uremic toxins in hemodialysis patients? A pilot randomized double-blind controlled study. Int Urol Nephrol. 2021;53(6):1231-8. doi: 10.1007/s11255-020-02760-z, PMID 33438085.
76. Ghaiad HR, Ali SO, Al Mokaddem AK, Abdelmonem M. Regulation of PKC/TLR-4/NF-kB signaling by sulbutiamine improves diabetic nephropathy in rats. Chem Biol Interact. 2023 Aug 25;381:110544. doi: 10.1016/j.cbi.2023.110544, PMID 37224990.
77. Liu W, LI F, Guo D, DU C, Zhao S, LI J. Schisandrin B alleviates renal tubular cell epithelial-mesenchymal transition and mitochondrial dysfunction by kielin/chordin-like protein upregulation via akt pathway inactivation and adenosine 5 monophosphate (amp) activated protein kinase pathway activation in diabetic kidney disease. Molecules. 2023 Nov 29;28(23):7851. doi: 10.3390/molecules28237851, PMID 38067580.
78. Cho CH, Yoo G, Kim M, Lee CJ, Choi IW, Ryu B. Diphlorethohydroxycarmalol a phlorotannin contained in brown edible seaweed Ishige okamurae, prevents AGE-related diabetic nephropathy by suppression of AGE-RAGE interaction. Food Biosci. 2023 Jun;53:102659. doi: 10.1016/j.fbio.2023.102659.
79. Coughlan MT, Cooper ME, Forbes JM. Can advanced glycation end-product inhibitors modulate more than one pathway to enhance renoprotection in diabetes? Ann N Y Acad Sci. 2005;1043(1):750-8. doi: 10.1196/annals.1333.087, PMID 16037302.
80. Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. Am J Nephrol. 2004;24(1):32-40. doi: 10.1159/000075627, PMID 14685005.
81. Forbes JM, Thorpe SR, Thallas Bonke V, Pete J, Thomas MC, Deemer ER. Modulation of soluble receptor for advanced glycation end products by angiotensin-converting enzyme-1 inhibition in diabetic nephropathy. J Am Soc Nephrol. 2005;16(8):2363-72. doi: 10.1681/ASN.2005010062, PMID 15930093.
82. Yang L, XU L, Hao X, Song Z, Zhang X, Liu P. An aldose reductase inhibitor WJ-*39* ameliorates renal tubular injury in diabetic nephropathy by activating PINK1/Parkin signaling. Eur J Pharmacol. 2024;967:176376. doi: 10.1016/j.ejphar.2024.176376, PMID 38336014.
83. Song T, Wang R, Zhou X, Chen W, Chen Y, Liu Z. Metabolomics and molecular dynamics unveil the therapeutic potential of epalrestat in diabetic nephropathy. Int Immunopharmacol. 2024;140:112812. doi: 10.1016/j.intimp.2024.112812, PMID 39094360.
84. Okuda Y, Ito S, Kashihara N, Shikata K, Nangaku M, Wada T. The renoprotective effect of esaxerenone independent of blood pressure lowering: a post hoc mediation analysis of the ESAX-DN trial. Hypertens Res. 2023;46(2):437-44. doi: 10.1038/s41440-022-01008-w, PMID 36100672.
85. Liang F, Glascock CB, Schafer DL, Sandoval J, Cable LA, Melvin L. Darusentan is a potent inhibitor of endothelin signaling and function in both large and small arteries. Can J Physiol Pharmacol. 2010;88(8):840-9. doi: 10.1139/Y10-061, PMID 20725142.
86. Heerspink HJ, Parving HH, Andress DL, Bakris G, Correa Rotter R, Hou FF. Atrasentan and renal events in patients with type 2 diabetes and chronic kidney disease (SONAR): a double-blind, randomised placebo-controlled trial. Lancet. 2019;393(10184):1937-47. doi: 10.1016/S0140-6736(19)30772-X, PMID 30995972.
87. Scott LJ. Sitaxentan: in pulmonary arterial hypertension. Drugs. 2007;67(5):761-70. doi: 10.2165/00003495-200767050-00007, PMID 17385944.
88. Enseleit F, Luscher TF, Ruschitzka F. Darusentan a selective endothelin a receptor antagonist for the oral treatment of resistant hypertension. Ther Adv Cardiovasc Dis. 2010;4(4):231-40. doi: 10.1177/1753944710373785, PMID 20660536.

89. Mann JF, Green D, Jamerson K, Ruilope LM, Kuranoff SJ, Littke T. Avosentan for overt diabetic nephropathy. J Am Soc Nephrol. 2010;21(3):527-35. doi: 10.1681/ASN.2009060593, PMID 20167702.
90. Anguiano L, Riera M, Pascual J, Soler MJ. Endothelin blockade in diabetic kidney disease. J Clin Med. 2015;4(6):1171-92. doi: 10.3390/jcm4061171, PMID 26239552.
91. Zhou Y, Chi J, Huang Y, Dong B, LV W, Wang YG. Efficacy and safety of endothelin receptor antagonists in type 2 diabetic kidney disease: a systematic review and meta-analysis of randomized controlled trials. Diabet Med. 2021;38(1):e14411. doi: 10.1111/dme.14411, PMID 33000477.
92. Thomas MC. Targeting the pathobiology of diabetic kidney disease. Adv Chronic Kidney Dis. 2021;28(4):282-9. doi: 10.1053/j.ackd.2021.07.001, PMID 34922684.
93. Sharov AV, Burkhanova TM, Taskın Tok T, Babashkina MG, Safin DA. Computational analysis of molnupiravir. Int J Mol Sci. 2022;23(21):13026. doi: 10.3390/ijms232113026, PMID 36362453.
94. Zang N, Cui C, Guo X, Song J, HU H, Yang M. Cgas-sting activation contributes to podocyte injury in diabetic kidney disease. Iscience. 2022;25(10):105145. doi: 10.1016/j.isci.2022.105145, PMID 36176590.
95. Yang Z, Liu F, QU H, Wang H, Xiao X, Deng H. 1, 25(OH)2D3 protects β cell against high glucose-induced apoptosis through mTOR suppressing. Mol Cell Endocrinol. 2015 Oct 15;414:111-9. doi: 10.1016/j.mce.2015.07.023, PMID 26213322.
96. Shi L, Xiao C, Zhang Y, Xia Y, Zha H, Zhu J. Vitamin D/vitamin D receptor/Atg16L1 axis maintains podocyte autophagy and survival in diabetic kidney disease. Ren Fail. 2022;44(1):694-705. doi: 10.1080/0886022X.2022.2063744, PMID 35469547.
97. Chen DP, MA YP, Zhuo L, Zhang Z, Zou GM, Yang Y. 1,25-dihydroxyvitamin D3 inhibits the proliferation of rat mesangial cells induced by high glucose via DDIT4. Oncotarget. 2018;9(1):418-27. doi: 10.18632/oncotarget.23063, PMID 29416624.
98. Wang B, Peng L, Ouyang H, Wang L, HE D, Zhong J. Induction of DDIT4 impairs autophagy through oxidative stress in dry eye. Invest Ophthalmol Vis Sci. 2019;60(8):2836-47. doi: 10.1167/iovs.19-27072, PMID 31266058.
99. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010;21(4):556-63. doi: 10.1681/ASN.2010010010, PMID 20167701.
100. Herb M, Schramm M. Functions of ROS in macrophages and antimicrobial immunity. Antioxidants (Basel). 2021;10(2):313. doi: 10.3390/antiox10020313, PMID 33669824.
101. Kogot Levin A, Hinden L, Riahi Y, Israeli T, Tirosh B, Cerasi E. Proximal tubule mTORC1 is a central player in the pathophysiology of diabetic nephropathy and its correction by SGLT2 inhibitors. Cell Rep. 2020;32(4):107954. doi: 10.1016/j.celrep.2020.107954, PMID 32726619.
102. Reifsnyder PC, Flurkey K, TE A, Harrison DE. Rapamycin treatment benefits glucose metabolism in mouse models of type 2 diabetes. Aging (Albany NY). 2016;8(11):3120-30. doi: 10.18632/aging.101117, PMID 27922820.
103. Mori H, Inoki K, Masutani K, Wakabayashi Y, Komai K, Nakagawa R. The mTOR pathway is highly activated in diabetic nephropathy and rapamycin has a strong therapeutic potential. Biochem Biophys Res Commun. 2009;384(4):471-5. doi: 10.1016/j.bbrc.2009.04.136, PMID 19422788.
104. Murakami N, Riella LV, Funakoshi T. Risk of metabolic complications in kidney transplantation after conversion to mTOR inhibitor: a systematic review and meta-analysis. Am J Transplant. 2014;14(10):2317-27. doi: 10.1111/ajt.12852, PMID 25146383.
105. Paluri RK, Sonpavde G, Morgan C, Rojymon J, Mar AH, Gangaraju R. Renal toxicity with mammalian target of rapamycin inhibitors: a meta-analysis of randomized clinical trials. Oncol Rev. 2019;13(2):455. doi: 10.4081/oncol.2019.455, PMID 31857859.
106. Flaquer M, Lloberas N, Franquesa M, Torras J, Vidal A, Rosa JL. The combination of sirolimus and rosiglitazone produces a renoprotective effect on diabetic kidney disease in rats. Life Sci. 2010;87(5-6):147-53. doi: 10.1016/j.lfs.2010.06.004, PMID 20600147.
107. LI D, LU Z, XU Z, JI J, Zheng Z, Lin S. Spironolactone promotes autophagy via inhibiting PI3K/AKT/mTOR signalling pathway and reduce adhesive capacity damage in podocytes under mechanical stress. Biosci Rep. 2016;36(4):e00355. doi: 10.1042/BSR20160086, PMID 27129295.
108. Wang MZ, Wang J, Cao DW, TU Y, Liu BH, Yuan CC. Fucoidan alleviates renal fibrosis in diabetic kidney disease via inhibition of NLRP3 inflammasome-mediated podocyte pyroptosis. Front Pharmacol. 2022;13:790937. doi: 10.3389/fphar.2022.790937, PMID 35370636.
109. Liu H, Wang Q, Shi G, Yang W, Zhang Y, Chen W. Emodin ameliorates renal damage and podocyte injury in a rat model of diabetic nephropathy via regulating AMPK/Mtor mediated autophagy signaling pathway. Diabetes Metab Syndr Obes. 2021 Mar 18;14:1253-66. doi: 10.2147/DMSO.S299375, PMID 33776462.
110. LV L, Zhang J, Tian F, LI X, LI D, YU X. Arbutin protects HK-2 cells against high glucose-induced apoptosis and autophagy by up-regulating microRNA-27a. Artif Cells Nanomed Biotechnol. 2019;47(1):2940-7. doi: 10.1080/21691401.2019.1640231, PMID 31319730.
111. Wei L, Jian P, Erjiong H, Qihan Z. Ginkgetin alleviates high glucose evoked mesangial cell oxidative stress injury inflammation and extracellular matrix (ECM) deposition in an AMPK/mTOR mediated autophagy axis. Chem Biol Drug Des. 2021;98(4):620-30. doi: 10.1111/cbdd.13915, PMID 34148304.
112. Dong R, Zhang X, Liu Y, Zhao T, Sun Z, Liu P. Rutin alleviates end MT by restoring autophagy through inhibiting HDAC1 via PI3K/AKT/mTOR pathway in diabetic kidney disease. Phytomedicine. 2023 Apr;112:154700. doi: 10.1016/j.phymed.2023.154700, PMID 36774842.
113. Tao M, Zheng D, Liang X, WU D, HU K, Jin J. Tripterygium glycoside suppresses epithelial to mesenchymal transition of diabetic kidney disease podocytes by targeting autophagy through the mTOR/Twist1 pathway. Mol Med Rep. 2021;24(2):592. doi: 10.3892/mmr.2021.12231, PMID 34165172.
114. LI XY, Wang SS, Han Z, Han F, Chang YP, Yang Y. Triptolide restores autophagy to alleviate diabetic renal fibrosis through the miR-141-3P/PTEN/Akt/mTOR pathway. Mol Ther Nucleic Acids. 2017 Aug 25;9:48-56. doi: 10.1016/j.omtn.2017.08.011, PMID 29246323.
115. Yang F, QU Q, Zhao C, Liu X, Yang P, LI Z. Paecilomyces cicadae fermented *Radix astragali* activates podocyte autophagy by attenuating PI3K/AKT/mTOR pathways to protect against diabetic nephropathy in mice. Biomed Pharmacother. 2020 Sep;129:110479. doi: 10.1016/j.biopha.2020.110479, PMID 32768963.
116. Guo L, Tan K, Luo Q, Bai X. Dihydromyricetin promotes autophagy and attenuates renal interstitial fibrosis by regulating miR-155-5p/PTEN signaling in diabetic nephropathy. Bosn J Basic Med Sci. 2020;20(3):372-80. doi: 10.17305/bjbms.2019.4410, PMID 31668144.
117. Wang T, Gao Y, Yue R, Wang X, Shi Y, XU J. Ginsenoside Rg1 alleviates podocyte injury induced by hyperlipidemia via targeting the mTOR/NF-κB/NLRP3 axis. Evid Based Complement Alternat Med. 2020;2020(1):2735714. doi: 10.1155/2020/2735714, PMID 33133213.
118. Sheng H, Zhang D, Zhang J, Zhang Y, LU Z, Mao W. Kaempferol attenuated diabetic nephropathy by reducing apoptosis and promoting autophagy through AMPK/mTOR pathways. Front Med (Lausanne). 2022;9:986825. doi: 10.3389/fmed.2022.986825, PMID 36530875.
119. Wang WJ, Jiang X, Gao CC, Chen ZW. Salusin-α mitigates diabetic nephropathy via inhibition of the Akt/mTORC1/p70S6K signaling pathway in diabetic rats. Drug Chem Toxicol. 2022;45(1):283-90. doi: 10.1080/01480545.2019.1683572, PMID 31665937.
120. Zhang Y, Wang Y, Luo M, XU F, LU Y, Zhou X. Elabela protects against podocyte injury in mice with streptozocin-induced diabetes by associating with the PI3K/Akt/mTOR pathway.

Peptides. 2019 Apr;114:29-37. doi: 10.1016/j.peptides.2019.04.005, PMID 30959144.
121. WU C, MA X, Zhou Y, Liu Y, Shao Y, Wang Q. Klotho restraining Egr1/TLR4/mTOR axis to reducing the expression of fibrosis and inflammatory cytokines in high glucose cultured rat mesangial cells. Exp Clin Endocrinol Diabetes. 2019;127(9):630-40. doi: 10.1055/s-0044-101601, PMID 29890551.
122. DI Petrillo K, Coutermarsh B, Gesek FA. Urinary tumor necrosis factor contributes to sodium retention and renal hypertrophy during diabetes. Am J Physiol Renal Physiol. 2003;284(1):F113-21. doi: 10.1152/ajprenal.00026.2002, PMID 12388406.
123. Moriwaki Y, Inokuchi T, Yamamoto A, KA T, Tsutsumi Z, Takahashi S. Effect of TNF-alpha inhibition on urinary albumin excretion in experimental diabetic rats. Acta Diabetol. 2007;44(4):215-8. doi: 10.1007/s00592-007-0007-6, PMID 17767370.
124. Omote K, Gohda T, Murakoshi M, Sasaki Y, Kazuno S, Fujimura T. Role of the TNF pathway in the progression of diabetic nephropathy in KK-A(y) mice. Am J Physiol Renal Physiol. 2014;306(11):F1335-47. doi: 10.1152/ajprenal.00509.2013, PMID 24647715.
125. Karkar AM, Smith J, Pusey CD. Prevention and treatment of experimental crescentic glomerulonephritis by blocking tumour necrosis factor alpha. Nephrol Dial Transplant. 2001;16(3):518-24. doi: 10.1093/ndt/16.3.518, PMID 11239025.
126. Ramesh G, Reeves WB. TNFR2-mediated apoptosis and necrosis in cisplatin induced acute renal failure. Am J Physiol Renal Physiol. 2003;285(4):F610-8. doi: 10.1152/ajprenal.00101.2003, PMID 12865254.
127. Zandi Nejad K, Eddy AA, Glassock RJ, Brenner BM. Why is proteinuria an ominous biomarker of progressive kidney disease? Kidney Int Suppl. 2004;66(92):S76-89. doi: 10.1111/j.1523-1755.2004.09220.x, PMID 15485426.
128. Lin SL, Chiang WC, Chen YM, Lai CF, Tsai TJ, Hsieh BS. The renoprotective potential of pentoxifylline in chronic kidney disease. J Chin Med Assoc. 2005;68(3):99-105. doi: 10.1016/S1726-4901(09)70228-X, PMID 15813241.
129. Garcia FA, Pinto SF, Cavalcante AF, Lucetti LT, Menezes SM, Felipe CF. Pentoxifylline decreases glycemia levels and TNF-alpha, iNOS and COX-2 expressions in diabetic rat pancreas. Springerplus. 2014;3(1):283. doi: 10.1186/2193-1801-3-283, PMID 24991532.
130. Goicoechea M, Garcia DE Vinuesa S, Quiroga B, Verdalles U, Barraca D, Yuste C. Effects of pentoxifylline on inflammatory parameters in chronic kidney disease patients: a randomized trial. J Nephrol. 2012;25(6):969-75. doi: 10.5301/jn.5000077, PMID 22241639.
131. Sahın S, Altok K, Pasaoglu H, Omeroglu S, Derıcı B, Erten U. The protective effects of pentoxifylline on contrast induced nephropathy in rats. Akdeniz Tıp Derg. 2019;5(3):429-38.
132. Gonzalez Espinoza L, Rojas Campos E, Medina Perez M, Pena Quintero P, Gomez Navarro B, Cueto Manzano AM. Pentoxifylline decreases serum levels of tumor necrosis factor alpha interleukin 6 and C-reactive protein in hemodialysis patients: results of a randomized double-blind controlled clinical trial. Nephrol Dial Transplant. 2012;27(5):2023-8. doi: 10.1093/ndt/gfr579, PMID 21968012.
133. Varma A, Das A, Hoke NN, Durrant DE, Salloum FN, Kukreja RC. Anti-inflammatory and cardioprotective effects of tadalafil in diabetic mice. Plos One. 2012;7(9):e45243. doi: 10.1371/journal.pone.0045243, PMID 23028874.
134. Kuwabara T, Mori K, Mukoyama M, Kasahara M, Yokoi H, Saito Y. Urinary neutrophil gelatinase-associated lipocalin levels reflect damage to glomeruli proximal tubules and distal nephrons. Kidney Int. 2009;75(3):285-94. doi: 10.1038/ki.2008.499, PMID 19148153.
135. Joy SV, Scates AC, Bearelly S, Dar M, Taulien CA, Goebel JA. Ruboxistaurin a protein kinase C β inhibitor as an emerging treatment for diabetes microvascular complications. Ann Pharmacother. 2005;39(10):1693-9. doi: 10.1345/aph.1E572, PMID 16160002.
136. Dash A, Maiti R, Bandakkanavar TK, Pandey BL. Novel drug treatment for diabetic nephropathy. HK J Nephrol. 2011;13(1):19-26. doi: 10.1016/S1561-5413(11)60003-3.
137. Pham TK, Nguyen TH, Yun HR, Vasileva EA, Mishchenko NP, Fedoreyev SA. Echinochrome prevents diabetic nephropathy by inhibiting the PKC-Iota pathway and enhancing renal mitochondrial function in DB/DB mice. Mar Drugs. 2023;21(4):222. doi: 10.3390/md21040222, PMID 37103361.
138. Gorin Y, Cavaglieri RC, Khazim K, Lee DY, Bruno F, Thakur S. Targeting NADPH oxidase with a novel dual Nox1/Nox4 inhibitor attenuates renal pathology in type 1 diabetes. Am J Physiol Renal Physiol. 2015;308(11):F1276-87. doi: 10.1152/ajprenal.00396.2014, PMID 25656366.
139. Menne J, Eulberg D, Beyer D, Baumann M, Saudek F, Valkusz Z. C-C motif ligand 2 inhibition with emapticap pegol *(*NOX-E36*)* in type 2 diabetic patients with albuminuria. Nephrol Dial Transplant. 2017;32(2):307-15. doi: 10.1093/ndt/gfv459.
140. Boels MG, Koudijs A, Avramut MC, Sol WM, Wang G, Van Oeveren Rietdijk AM. Systemic monocyte chemotactic protein-1 inhibition modifies renal macrophages and restores glomerular endothelial glycocalyx and barrier function in diabetic nephropathy. Am J Pathol. 2017;187(11):2430-40. doi: 10.1016/j.ajpath.2017.07.020, PMID 28837800.
141. DE Zeeuw D, Akizawa T, Agarwal R, Audhya P, Bakris GL, Chin M. Rationale and trial design of bardoxolone methyl evaluation in patients with chronic kidney disease and type 2 diabetes: the occurrence of renal events (beacon). Am J Nephrol. 2013;37(3):212-22. doi: 10.1159/000346948, PMID 23467003.
142. DE Zeeuw D, Akizawa T, Audhya P, Bakris GL, Chin M, Christ Schmidt H. Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. N Engl J Med. 2013;369(26):2492-503. doi: 10.1056/NEJMoa1306033, PMID 24206459.
143. Taylor PC. Clinical efficacy of launched JAK inhibitors in rheumatoid arthritis. Rheumatol (Oxf Engl). 2019;58 Suppl 1:17-26. doi: 10.1093/rheumatology/key225, PMID 30806707.
144. Plosker GL. *Ruxolitinib*: a review of its use in patients with myelofibrosis. Drugs. 2015;75(3):297-308. doi: 10.1007/s40265-015-0351-8, PMID 25601187.
145. Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O Shea JJ. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. Nat Rev Drug Discov. 2017;16(12):843-62. doi: 10.1038/nrd.2017.201, PMID 29104284.
146. Kim HO. Development of JAK inhibitors for the treatment of immune mediated diseases: kinase targeted inhibitors and pseudokinase targeted inhibitors. Arch Pharm Res. 2020;43(11):1173-86. doi: 10.1007/s12272-020-01282-7, PMID 33161563.
147. Brosius FC, Tuttle KR, Kretzler M. JAK inhibition in the treatment of diabetic kidney disease. Diabetologia. 2016;59(8):1624-7. doi: 10.1007/s00125-016-4021-5, PMID 27333885.
148. Tuttle KR, Brosius FC, Adler SG, Kretzler M, Mehta RL, Tumlin JA. JAK1/JAK2 inhibition by baricitinib in diabetic kidney disease: results from a phase 2 randomized controlled clinical trial. Nephrol Dial Transplant. 2018;33(11):1950-9. doi: 10.1093/ndt/gfx377, PMID 29481660.
149. El Kady MM, Naggar RA, Guimei M, Talaat IM, Shaker OG, Saber Ayad M. Early renoprotective effect of ruxolitinib in a rat model of diabetic nephropathy. Pharmaceuticals (Basel). 2021;14(7):608. doi: 10.3390/ph14070608, PMID 34202668.
150. Elsherbiny NM, Zaitone SA, Mohammad HM, El Sherbiny M. Renoprotective effect of nifuroxazide in diabetes-induced nephropathy: impact on NFκB oxidative stress and apoptosis. Toxicol Mech Methods. 2018;28(6):467-73. doi: 10.1080/15376516.2018.1459995, PMID 29606028.
151. Zhu M, Wang H, Chen J, Zhu H. Sinomenine improve diabetic nephropathy by inhibiting fibrosis and regulating the JAK2/STAT3/SOCS1 pathway in streptozotocin-induced diabetic rats. Life Sci. 2021 Jan 15;265:118855. doi: 10.1016/j.lfs.2020.118855, PMID 33278392.
152. Gholami M, Moallem SA, Afshar M, Amoueian S, Etemad L, Karimi G. Teratogenic effects of silymarin on mouse fetuses. Avicenna J Phytomed. 2016;6(5):542-9. PMID 27761424.
153. Winiarska A, Knysak M, Nabrdalik K, Gumprecht J, Stompor T. Inflammation and oxidative stress in diabetic kidney disease: the targets for SGLT2 inhibitors and GLP-1 receptor agonists. Int J Mol Sci. 2021;22(19):10822. doi: 10.3390/ijms221910822, PMID 34639160.

154. Yang Y, Lei Y, Liang Y, FU S, Yang C, Liu K. Vitamin D protects glomerular mesangial cells from high glucose-induced injury by repressing JAK/STAT signaling. Int Urol Nephrol. 2021;53(6):1247-54. doi: 10.1007/s11255-020-02728-z, PMID 33942213.
155. Navarro Gonzalez JF, Mora Fernandez C, Muros DE Fuentes M, Garcia Perez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. Nat Rev Nephrol. 2011;7(6):327-40. doi: 10.1038/nrneph.2011.51, PMID 21537349.
156. LI HY, Lin HA, Nien FJ, WU VC, Jiang YD, Chang TJ. Serum vascular adhesion protein-1 predicts end-stage renal disease in patients with type 2 diabetes. Plos One. 2016;11(2):e0147981. doi: 10.1371/journal.pone.0147981, PMID 26845338.
157. Qian Y, LI S, YE S, Chen Y, Zhai Z, Chen K. Renoprotective effect of rosiglitazone through the suppression of renal intercellular adhesion molecule-1 expression in streptozotocin-induced diabetic rats. J Endocrinol Invest. 2008;31(12):1069-74. doi: 10.1007/BF03345654, PMID 19246972.
158. Rubio Guerra AF, Vargas Robles H, Lozano Nuevo JJ, Escalante Acosta BA. Correlation between circulating adhesion molecule levels and albuminuria in type-2 diabetic hypertensive patients. Kidney Blood Press Res. 2009;32(2):106-9. doi: 10.1159/000210554, PMID 19342863.
159. DE Zeeuw D, Renfurm RW, Bakris G, Rossing P, Perkovic V, Hou FF. Efficacy of a novel inhibitor of vascular adhesion protein1 in reducing albuminuria in patients with diabetic kidney disease (ALBUM): a randomised placebo-controlled phase 2 trial. Lancet Diabetes Endocrinol. 2018;6(12):925-33. doi: 10.1016/S2213-8587(18)30289-4, PMID 30413396.
160. Wada J, Makino H. Inflammation and the pathogenesis of diabetic nephropathy. Clin Sci (Lond). 2013;124(3):139-52. doi: 10.1042/CS20120198, PMID 23075333.
161. Giunti S, Barutta F, Perin PC, Gruden G. Targeting the MCP-1/CCR2 system in diabetic kidney disease. Curr Vasc Pharmacol. 2010;8(6):849-60. doi: 10.2174/157016110793563816, PMID 20180766.
162. Sayyed SG, Hagele H, Kulkarni OP, Endlich K, Segerer S, Eulberg D. Podocytes produce homeostatic chemokine stromal cell-derived factor-1/CXCL12 which contributes to glomerulosclerosis podocyte loss and albuminuria in a mouse model of type 2 diabetes. Diabetologia. 2009;52(11):2445-54. doi: 10.1007/s00125-009-1493-6, PMID 19707743.
163. Enevoldsen FC, Sahana J, Wehland M, Grimm D, Infanger M, Kruger M. Endothelin receptor antagonists: status quo and future perspectives for targeted therapy. J Clin Med. 2020;9(3):824. doi: 10.3390/jcm9030824, PMID 32197449.
164. Wang Y, Chen S, DU J. Bosentan for treatment of pediatric idiopathic pulmonary arterial hypertension: state of the art. Front Pediatr. 2019 Jul 23;7:302. doi: 10.3389/fped.2019.00302, PMID 31396496.
165. Schlaich MP, Bellet M, Weber MA, Danaietash P, Bakris GL, Flack JM. Dual endothelin antagonist aprocitentan for resistant hypertension (Precision): a multicentre blinded randomised parallel-group phase 3 trial. Lancet. 2022;400(10367):1927-37. doi: 10.1016/S0140-6736(22)02034-7, PMID 36356632.
166. Murugesan N, GU Z, Fadnis L, Tellew JE, Baska RA, Yang Y. Dual angiotensin II and endothelin a receptor antagonists: synthesis of 2 substituted N-3-isoxazolyl biphenylsulfonamides with improved potency and pharmacokinetics. J Med Chem. 2005;48(1):171-9. doi: 10.1021/jm049548x, PMID 15634011.
167. Zhao Q, Guo N, Chen J, Parks D, Tian Z. Comparative assessment of efficacy and safety of ambrisentan and bosentan in patients with pulmonary arterial hypertension: a meta analysis. J Clin Pharm Ther. 2022;47(2):146-56. doi: 10.1111/jcpt.13481, PMID 34319626.
168. Persson BP, Rossi P, Weitzberg E, Oldner A. Inhaled tezosentan reduces pulmonary hypertension in endotoxin-induced lung injury. Shock. 2009;32(4):427-34. doi: 10.1097/SHK.0b013e31819e2cbb, PMID 19197226.
169. Iglarz M, Binkert C, Morrison K, Fischli W, Gatfield J, Treiber A. Pharmacology of macitentan an orally active tissue targeting dual endothelin receptor antagonist. J Pharmacol Exp Ther. 2008;327(3):736-45. doi: 10.1124/jpet.108.142976, PMID 18780830.