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Better understanding of biomolecules as drug target: a literature review

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ABSTRACT

Drugs are small biological therapeutic agents that play a crucial role in enhancing human health. They have been widely used in the treatment of various diseases, with conventional medicine being one of the most common approaches. However, conventional medicine has several limitations, including non-specific drug targeting, limited efficacy, low stability, poor absorption rates, and degradation due to enzymatic activity and low pH in the digestive system. Efforts to improve drug targeting have led to the development of molecular-based therapies, particularly through the use of biomolecules as drug targets. Drug targeting aims to optimize therapeutic effects by focusing on specific targets while minimizing side effects. This review article explores research findings from the PubMed database, applying specific inclusion and exclusion criteria. It discusses the types of drugs and biomolecular targets, including G protein-coupled receptors (GPCRs), enzymes, ion channel receptors, and DNA, as well as the mechanisms through which drugs interact with these targets in the treatment of various diseases. Additionally, the advantages and disadvantages of these biomolecular targets are examined. In conclusion, the four biomolecules discussed each present unique characteristics as drug targets, highlighting the importance of carefully selecting appropriate molecules based on an understanding of their functions, properties, and interactions with drugs in the body.

ABSTRACT

Obat merupakan agen terapeutik berukuran kecil dan bersifat biologis yang berperan dalam meningkatkan kesehatan manusia. Obat telah digunakan dalam berbagai macam pengobatan, salah satunya dalam pengobatan konvensional. Namun, pengobatan konvensional memiliki beberapa kekurangan seperti target obat yang tidak spesifik, efektivitas obat yang terbatas, stabilitas dan tingkat absorpsi obat yang rendah, serta degradasi obat karena pengaruh enzim dan pH yang rendah pada sistem gastrointestinal. Pengobatan konvensional memberikan solusi penargetan obat menjadi lebih berkembang ke arah molekuler, yaitu dengan menggunakan biomolekul sebagai target obat. Penargetan obat ini merupakan upaya untuk mengoptimalkan efektivitas obat terhadap target secara spesifik dan meminimalisir efek samping yang dihasilkan dari pengobatan konvensional. Metode yang digunakan yaitu eksplorasi hasil penelitian pada situs PubMed sebagai sumber utama dengan kriteria ekslusi dan inklusi. Ulasan artikel ini akan membahas tentang jenis obat dan target biomolekul yang terdiri dari reseptor terkait protein G (GPCR), enzim, reseptor kanal ion dan DNA, mekanisme kerja obat terhadap target obat pada beberapa penyakit, serta kelebihan dan kekurangan dari keempat biomolekul sebagai target obat. Kesimpulan yang diperoleh yaitu keempat biomolekul memiliki spesifitas masing-masing sebagai target obat sehingga perlu diperhatikan pemilihan molekul yang tepat melalui pemahaman fungsi, karakteristik dan interaksi obat terhadap target di dalam tubuh.

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INTRODUCTION

Drugs are chemical compounds that affect the structure or function in the body and useful in the treatment, cure, mitigation, prevention, or diagnosis of a disease. Drugs can be used to cure or alleviate the symptoms of a disease or certain mental medical conditions.[1] The type of treatment often used in the medical world is conventional medicine, which is chemically isolated from natural resources such as plants, fungi, bacteria or animals or synthesized from organics compounds. The drug treatment aims to alleviate the symptoms of a disease by increasing the desired clinical effect or improving the negative side effects of the main drug.[2] Some conventional treatments such as pharmaceutical drugs,[3] surgery, chemotherapy, radiation (cancer treatment).[4] and psychotherapy[4] have been developed since many years ago.

The use of conventional treatment often causes some unwanted side effects. The use of pharmaceutical drugs in high doses (in order to penetrate the plasma membrane of infected cells,[9] causing drug resistance (both treatment of bacterial infections and cancer).[10] In addition to the occurrence of drug resistance, there are also obstacles such as the difficulty of access to conventional treatment in several countries.[11] The use of conventional treatment in practice often causes some unwanted side effects, such as the use of pharmaceutical drugs in high doses in order to penetrate the plasma membrane of infected cells,[5] drug resistance in the treatment of bacterial infections and cancer.[6] Conventional drug delivery systems have several shortcomings, such as limited effectiveness, non-specific drug targets, short drug residence time, weak drug biodistribution.[7] Disadvantages in terms of less effective bioavailability such as oral drug distribution can cause drugs to be degraded by enzymes and low pH in the gastrointestinal system,[8] biocompatibility, biodegradability, and drug distribution into the body.[9,10]

The use of biomolecules as drug targets also enables the development of therapies for complex diseases. Advances in knowledge of the human genome and molecular biology accelerate the process of developing current data to provide a variety of information through bioinformatics on drug-target interactions that support the development of drug targeting.[11] Drug target discovery utilizes two current approaches combining molecular and systemic approaches coupled with in vivo and in vitro validation to assess drug efficacy.[12]

Identification of drug targets will help determine the relationship between drug activity and phenotype, determine the mechanism of drug action, determine effective targets for therapeutic drugs, resistance mechanisms to possible toxicity in more detail.[13] Currently, small molecular drugs mostly target various molecular components such as enzymes, DNA, receptors, ion channels through the mechanism of action of enzyme inhibitors, ion channel blockers, and inhibitors of the replication process.[14] The advantages of drug targeting include increasing drug efficacy, reducing side effects, increasing drug concentration at the desired site, and allowing the use of lower doses but still providing a significant effect in treating.[15]

MATERIAL AND METHODS

This review uses primary literature retrieved from PubMed with specific keywords, such as GPCR, enzyme, ion channel receptors, and DNA. All articles were selected based on inclusion and exclusion criteria. The inclusion articles criteria were full free access and have been published within the last 10 yr. We also have consideration to exclude the selected articles based on the type of drug target, FDA approval, drug mechanism of action, and clinical trials-

based experiment. Drug efficacy rates above 50% have been used as additional requirements for quality assessment. After a continuous screening process, 40 articles which then represent 10 articles for each drug target were selected.

RESULTS

A total of 1982 articles were collected from PubMed, after screening only 40 articles were included in this review (TABLE 1).

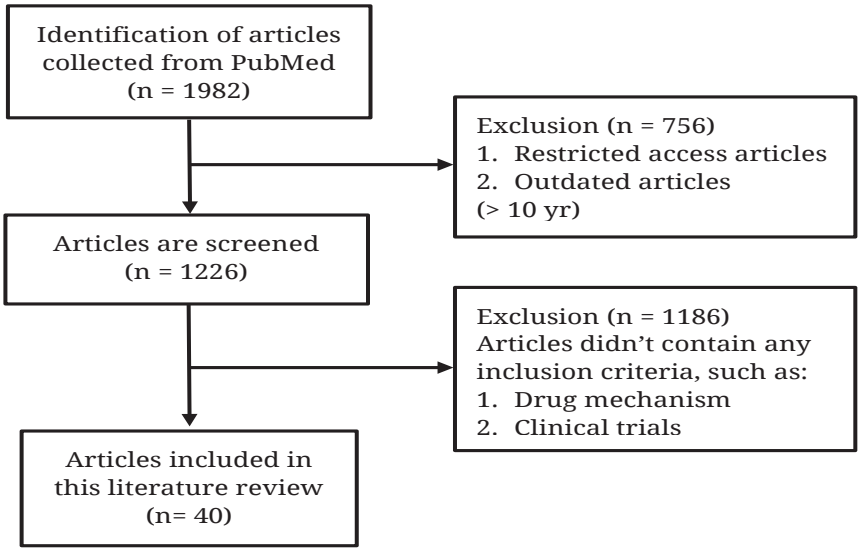

FIGURE 1. PRISMA flow chart of biomolecules as a drug target

TABLE 1. List of drugs targeting biomolecules and their mechanism of action

Specific target	Drug	Mechanism	Indications	Ref
GPCR				
• *β*2AR (*β*2-adrenergic receptors)	LABAs (salmeterol, formoterol, vilanterol, and olodaterol)	Relaxation of bronchial smooth muscle.	Severe asthma	16
• *β*2AR (*β*2-adrenergic receptors)	SABAs (Albuterol, levalbuterol)	Relaxation of bronchial smooth muscle.	Severe asthma	17
• PTH1 (parathyroid hormone receptors)	Tymlos (abaloparatide)	Parathyroid type 1 receptor conformation binding selectivity that favors anabolic activity.	Osteoporosis	18
• GLP1 (glucagon-like peptide 1)	Semaglutide	Patients with type 2 diabetes reported a dose-dependent reduction in weight and HbA1c levels after using semaglutide.	Diabetes mellitus type 2	19
• OX_2R (OX_2 receptor)	Suvorexant	Maintaining the stability of an extracellular salt bridge network and preventing the transmembrane helix movements required for activation.	Insomnia	20

Table 1 cont.

Specific target	Drug	Mechanism	Indications	Ref
• CASR	Parsabiv (etelcalcetide)	Reducing serum PTH concentrations.	Hyperparathyroidism	21
• 5HT2A, 5HT1A	Rexulti (brexpiprazole)	Neurite outgrowth elicited by benzpiprazole is mediated by 5-HT1A and 5-HT2A receptors.	Depression	22
• P2Y12	Kengreal (cangrelor)	Reduce percutaneous coronary intervention-related ischemic complications without increasing major bleeding.	Percutaneous coronary intervention	23
• PAR1 (protease-activated receptor-1)	Zontivity (vorapaxar)	Reduce the expression of HSP90**β**, the interaction between HSP90**β** and transforming growth factor-**β** (TGF**β**) receptor II, and the TGF**β**/Smad signaling pathway. These mechanisms also inhibit the stimulation of fibroblasts induced by thrombin in a dose-dependent manner and the signal transduction of JAK2/STAT1/3.	Pulmonary fibrosis	24
• NK1R (neurokinin 1 receptor)	Varubi (rolapitant)	Rolapitant quickly entered the amygdala, hypothalamus, and neocortex, three brain areas related to reward and anxiety regulation.	Alcohol self-administration in action, alcohol use on a voluntary basis, and the recurrence of alcohol-seeking behavior due to stress.	25
Enzymes				
• Cyclooxygenase (COX) 1 & 2	Aspirin	Inhibiting synthesis of the procoagulant Thromboxane A2	Ischemic stroke	26
• Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase	Statin drugs (lovastatin, simvastatin, atorvastatin)	Competitively inhibit HMG-CoA reductase in mevalonate synthesis pathway thereby reducing cholesterol synthesis in the liver	Dyslipidemia and cardiovascular disease	27
• Angiotensin converting enzyme (ACE)	Captopril and lisinopril	ACE competitive inhibitor, leads to blood pressure reduction by inhibiting conversion of angiotensin I to angiotensin II	Hypertension	28
• Guanylate cyclase type C	Linaclotide	Guanylate cyclase type C activator, results in an elevation of cGMP levels	Chronic idiopathic constipation	29
• Xanthine oxidase (XO)	Allopurinol and febuxostat	Competitive inhibitor of xanthine oxidase which decreases the formation of uric acid by inhibiting hydroxylation of hypoxanthine to xanthine and xanthine to uric acid	Hyperuricemia and gout	30

Table 1 cont.

Specific target	Drug	Mechanism	Indications	Ref
• Dihydropteroate synthase	Sulfa drugs	Dihydropteroate synthase inhibitor by directly competing with the substrate *p*ABA results in disruption of folate biosynthesis	Antibacterial	31
• Dihydrofolate reductase (DHFR)	Methotrexate	Inhibits Dihydrofolate Reductase, reducing the amount of tetrahydrofolate required for the synthesis of nucleotide bases	Lymphoma, acute lymphoblastic leukemia, and osteosarcoma	32
• Thyroid peroxidase (TPO)	Methimazole and Propylthiouracil	Thyroid Peroxidase inhibitor by interfering oxidation and organification of iodine	Hyperthyroidism	33
• 5'-Lipoxygenase	Zileuton	Inhibitor of 5'-Lipoxygenase, leads to synthesis disruption of leukotriene B4 (LTB4) and cysteinyl leukotrienes (CysLT)	Asthma	34
• **γ**-Aminobutyric acid aminotransferase (GABA-AT)	Vigabatrin (Sabril)	Active-site directed inhibitor of GABA-AT by blocking the degradation of brain GABA	Epilepsy and drug addiction	35
Ion channel				
• Calcium channel	Nimodipine	Lowering the CaV1.2 mRNA expression and block the calcium uptake	Diabetic encephalopathy	36
• Calcium channel	Nifedipine	Activating PPAR**γ** to suppresses MCP-1 and ABCA1	Atherosclerosis	37
• Sodium channel	Lamotrigine	Inhibit Na voltage-gated ion channel through a dual-pocket mechanism	Epilepsy	38
• Sodium-glucose cotransporter 2 (SGLT2)	Empagliflozin	Direct inhibition of SGLT2 channel to reduce glycated hemoglobin	Diabetes mellitus type 2	39
• Potassium channel	Dalfampridine	Blocking the potassium channel exposed through MS-related demyelination.	Multiple sclerosis	40
• ATP-sensitive potassium (K ATP)	Carbamazepine	Inhibiting KATP channel activity primarily by abolishing the stimulatory effect of MgADP	Congenital hyperinsulinism, neonatal diabetes, DEND syndrome	41
Ion channel				
• Kv1.3 potassium channel	Dalazatide	Blocking the Kv1.3 potassium channel and inhibit the T cell migration	Plaque psoriasis	42
• Epithelial sodium channel	Amiloride	Blockade renin-angiotensin-aldosterone system (RAAS)	Proteinuric kidney	43

TABLE 1. Cont.

Specific target	Drug	Mechanism	Indications	Ref
Ion channel				
• Transient receptor potential cation channel member A1 (TRPA1)	GDC-0034 (Proline sulfonamide)	Bind to closed state of TRPA1 and restrict the conformational change related with gating mechanism	Asthma, airway inflammation	44
• Calcium released-related calcium (CRAC) channel	Auroxa	Inhibit calcium channel and the release of proinflammatory cytokine	Pneumonia in COVID-19	45
DNA				
• Breast cancer susceptibility gene 1 and 2 (BRCA1 and 2)	Olaparib, rucaparib, niraparib	Combat homologous recombination-deficient (HRD) cancers with Poly (ADP-ribose) polymerase inhibitor (PARPi)	Ovarian cancer	46
• DNA damage response (DDR) genes	Camonsertib	Synthetically lethal by loss-of-function (LOF) ataxia telangiectasia-mutated (ATM)	Ovarian cancer	47
• DNA polymerase theta (Polθ)	RP-6685 Novobiocin	Against breast and ovarian cancers harboring BRCA-mutant alleles. Blocks the activity of a protein called DNA polymerase theta, which helps repair DNA that has become damaged as cells grow and divide.	Breast and Ovarian cancer Tumors that have Alterations in DNA Repair Genes	48
• BCL-2 (B-cell lymphoma 2) protein (BCL2 gene)	Venetoclax	Inhibits BCL-2 (B-cell lymphoma 2)	Small lymphocytic lymphoma (SLL)	49
• Single strand DNA breaks	Talazoparib	Inhibits the polyadenosine 5′-diphosphoribose polymerase (PARP) enzymes, which play a critical role in repairing DNA single-strand breaks	Early triple negative breast cancer	50
• *BDNF* gene Val66Met polymorphism	Antipsychotic drugs	BDNF modulates the major neurotransmitter systems including the dopaminergic, glutamatergic, and serotonergic systems	Schizophrenia	51
• Trophoblast cell surface antigen 2 (TROP2)	Datopotamab deruxtecan (Dato-DXd, DS-1062a)	Internalized into tumor cells followed by intracellular trafficking to lysosome and DXd release, which induced DNA damage and apoptosis in TROP2	Negative breast cancer and urothelial carcinoma	52

TABLE 1. Cont.

Specific target	Drug	Mechanism	Indications	Ref
• DNA damage response (DDR)-related gene	Toripalimab, lenvatinib, and gemcitabine plus oxaliplatin (GEMOX)	Inhibits PD-1 (programmed cell death protein 1) protein on T cells, inhibits a number of enzymes involved and inhibits DNA synthesis	Intrahepatic cholangiocarcinoma (ICC)	53
• EGFR (epidermal growth factor receptor) protein	Osimertinib	Osimertinib inhibits EGFR, which is essential for the growth and proliferation of lung cancer cells	Non-small cell lung cancer (NSCLC)	54
• Cancer-associated of proliferating cell nuclear antigen (caPCNA)	Dacarbazine, cyclophosphamides, and Busulfan	Exploit this genetic instability by inducing additional DNA damage to overwhelm the repair system in cancer cells	Cancer	55

DISCUSSION

Target identification during the early stage of the drug discovery process plays an important role in successfully developing novel therapeutic agents. A 'drug target' refers to biomolecules that directly bind to drugs through specific binding sites and alter signaling pathways, leading to physiological response to achieve therapeutic benefits.[56] Most drug targets are protein including receptor, enzyme, ion channel, and transporters. Yet, some drugs bind directly with DNA rather than protein.[57] According to database curation conducted by Santos *et al.*,[58] 893 human and pathogen-derived biomolecules are primarily involved as targets of 1,578 FDA-approved drugs. G-protein coupled receptor (GPCR) make up the largest group of drug targets (33%), accompanied by enzyme and other biomolecules (30%), ion channel includes both voltage-gated and ligand-gated (18%), nuclear receptor (16%), and kinase (3%). FDA-approved small molecule and biologic drugs are most widely used for nervous system therapeutic area, involving 249 drugs commonly act as antiepileptics (N03A), antipsychotic (N05A), and antidepressants (N06B). Followed by antineoplastic and immunomodulating agents (209 drugs), cardiovascular (205), antiinfections (204), alimentary tract and metabolism system (190), sensory organs (154), and many more.

An extensive knowledge of a drug's mechanism of action is fundamental to completely understanding its biological effects on the organism. Despite that, mechanisms of several drugs remain unclear even if the targets have been recognized. Therefore, molecular biology approaches are needed to obtain comprehensive insight of the interaction between drug and drug targets for achieving desired pharmacological effect.

GPCRs as drug target

G-protein-coupled receptors are a superfamily of seven transmembrane-spanning proteins that generate in a majority of organs and are activated by a diverse array of extracellular ligands. Several biological processes, such as neurotransmission, chemotaxis, inflammation, cell proliferation, cardiac and smooth muscle contraction, as well as visual and chemosensory perception, are influenced by signaling through these receptors. A particular kind of

membrane protein known as GPCR is necessary for cell signaling. It is an important focal point for therapeutic research and is involved in a number of physiological processes.[59]

The mechanism of GPCR as a drug target involves the binding of a drug to the GPCR, which causes a conformational change in the receptor and triggers downstream signaling cascades. This activation or inhibition of GPCR signaling can lead to therapeutic effects in various diseases. Throughout signal transduction, agonists and ligands bind to the extracellular area of GPCRs, inducing the protein to first modify shape and become activated. By separating Gα from Gβγ, this activated GPCR further converts the inactive G protein to active G protein complex.[60] Additionally, helix-helix interaction ensures the GPCRs maintain their functional tertiary structure, which is essential for ligand binding, receptor folding and stability, and ligand-induced conformational changes that lead to G protein coupling.[61]

According to their amino acid sequences, the human GPCR family is classified into classes A (rhodopsin), B (secretin and adhesion), C (glutamate), and F (Frizzled) subfamilies.[62] Class A GPCRs such as 5HTR2A and P2Y12 (TABLE 1), also known as rhodopsin-like receptors, play a significant role in human disease. They are involved in a wide range of physiological processes such as sensory perception, neurotransmission, and immune response. These GPCRs have been implicated in numerous diseases including cardiovascular disorders, metabolic disorders, neurological disorders, and cancer.[63] 5HT2A is a specific subtype of serotonin receptor that has been implicated in the pathophysiology of depression. It is believed that alterations in the signaling and activity of 5HT2A receptors may contribute to the development and maintenance of depressive symptoms. Targeting 5HT2A receptors with drugs that modulate their activity has shown promise in the treatment of depression.[64,65] Meanwhile, adenosine 5' diphosphate (ADP)-binding platelet P2Y12 receptor (P2Y12R) is necessary for hemostasis, thrombosis, and platelet function. Mild-to-moderate bleeding diathesis appears in patients with hereditary P2Y12R abnormalities.[66] Particularly in the case of cardiovascular disorders, these receptors are crucial in the development of numerous human diseases. In the biomechanically induced aggregation mechanism, P2Y12 can directly contribute to thrombus formation and can also activate PI3K kinase and Syk kinase to drive thrombus formation.[67]

Receptors for peptide hormones, such as PTH1 and GLP1, belong in the class B GPCRs of the secretin family (TABLE 1).[68] These types of receptors are significant drug targets in a variety of human diseases, such as diabetes, osteoporosis, cancer, neurodegeneration, cardiovascular disease, headaches, and psychiatric disorders.[69] PTH1, also referred to as the parathyroid hormone receptors, is involved in bone structure maintenance.[70] By encouraging osteoblast growth and reducing osteoblast cell death or apoptosis, PTH can increase the number of bone-forming cells.[71] While glucagon works to maintain blood glucose levels through the conversion of glycogen to glucose, the incretins glucagon-like peptide 1 (GLP-1) and glucagon itself are members of the glucagon subfamily. The primary way that the incretins lower blood glucose is through the release of insulin.[72]

Moreover, class C GPCRs are a distinct family of receptors characterized by their unique structural features and functional roles in various physiological processes. This class includes important receptors such as metabotropic glutamate receptors (mGluRs), γ-aminobutyric acid B receptors (GABA-B), calcium-sensing receptors (CaSR), and taste receptors. Class C GPCRs play critical roles in various physiological processes, including calcium homeostasis, neurotransmission, taste sensation, and signal modulation. The calcium-sensing

receptor (CaSR) regulates calcium levels in the bloodstream by detecting extracellular calcium concentrations and modulating parathyroid hormone (PTH) secretion accordingly. Modulating CaSR (TABLE 1) activity presents potential therapeutic avenues for managing disorders related to calcium imbalance, such as osteoporosis or hyperparathyroidism.[26]

Last but not least, class F GPCRs, consisting of ten Frizzled (FZD) paralogs and smoothened (SMO), play crucial roles in embryonic development, stem cell regulation, and tumorigenesis. Specifically, FZDs mediate WNT signaling, whereas SMO mediates Hedgehog signaling. Despite lacking conserved motifs like those in class A GPCRs, such as the E/DRY motif, Class F receptors exhibit ligand-induced and constitutive activities involving the opening of the transmembrane bundle by a TM6 swing-out motion, akin to Class A and B GPCRs' activation mechanisms.[73,74] Drug mechanisms targeting Class F receptors aim to disrupt aberrant signaling pathways contributing to diseases like cancer. For instance, inhibitors designed to interfere with the GLI transcription factors downstream of SMO activation show promise in treating basal-cell carcinomas caused by aberrant Hedgehog signaling. Similarly, modulators of Wnt/Fzd interactions could offer therapeutic options for conditions influenced by dysregulation of this pathway. Further research will continue to uncover specific druggable sites within these receptors, enabling more effective treatments tailored towards disrupting pathologic signaling while preserving physiologic functions.[74]

G-protein coupled receptors are a widely studied class of cell surface receptors that play a crucial role in various physiological processes. Although GPCRs have been successful targets for drug development, there are several limitations and challenges associated with targeting them. Firstly, limitation is the potential for off-target effects, this means that drugs targeting a specific GPCR may also interact with other receptors, leading to unintended side effects.[75] Secondly, the complexity of GPCR signaling pathways. The signaling pathways involving GPCRs are highly intricate and interconnected, making it difficult to selectively modulate the desired pathway without affecting others. Thirdly, the limited structural information available for GPCRs, which hinders the rational design of drugs.[76] Moreover, GPCRs exhibit a high degree of structural flexibility, undergoing conformational changes upon ligand binding, which further complicates the rational design of drugs targeting GPCRs.[77,78]

Understanding the characteristics and functioning of GPCRs as a drug target is crucial in developing effective medications for several reasons. Firstly, GPCRs are one of the largest and most important protein families involved in cellular signaling and regulation. Secondly, they are involved in a wide range of physiological processes and pathways, making them attractive targets for drug intervention. Thirdly, GPCRs have been successfully targeted by numerous drugs and account for a significant portion of pharmaceuticals on the market. By knowing the characteristics and functioning of GPCRs as a drug target, researchers can better understand how to manipulate these receptors with medications. This knowledge can lead to the development of more targeted and effective drugs with fewer side effects. Additionally, understanding GPCRs allows researchers to identify the endogenous ligands that naturally bind to these receptors. This can provide insights into the normal physiological roles of GPCRs and potential therapeutic targets. Moreover, by understanding the structure and functioning of GPCRs, researchers can design drugs that specifically target these receptors and modulate their activity. Ultimately, this knowledge can drive the discovery of novel drugs that have better

efficacy, improved safety profiles, and reduced side effects.[79,80]

Enzyme as drug target

Enzymes are exceptionally powerful biocatalysts capable of promoting chemical transformations that take place in any living cell of an organism including metabolism, cell growth, division, and motility, as well as cellular signaling with high efficiency and selectivity. The existence of substrate binding sites which can be exploited and modified are one of the considerations of targeting enzymes for drug development.[81] According to Agarwal *et al.*,[82] enzymes comprise 27% of FDA-approved oral drugs. Hydrolase are the most common class of enzymatic drug targets followed by oxidoreductases, transferase, isomerase, lyase, and ligase.[83] Drugs acting on enzymes can either inhibit or activate them. Inhibition of enzymes has become a recurrent strategy for drug design because of upregulating mechanisms being prevalent in numerous diseases.

Enzyme inhibitors can either decrease or totally inhibit enzyme catalytic activity through three common mechanisms called competitive, non-competitive, and uncompetitive inhibition.[84] Competitive inhibitor drugs serve as substrate analog to occupy the active site of enzymes and prevent substrate attachment[85] (FIGURE 2b). A great number of therapeutic agents are developed through this regulation. The most prominent atherosclerotic cardiovascular disease drug named Statins has been declared as 3-hydroxy-3-methylglutarate CoA reductase competitive inhibitor. Randomized trials conducted by Hong *et al.*,[86] showed the reduction of low-density lipoprotein cholesterol (LDL-C) levels around 50-70 mg/dL after high-intensity statin therapy for around 3 yr. While some drugs act as competitive inhibitors, non-competitive inhibitors perform another binding property. In non-competitive inhibition, the inhibitor binds to the allosteric site of enzymes, leading to configurational change and disrupting enzyme's catalytic activity[85] (FIGURE 2c). Aspirin, a cardiovascular drug, establishes a non-competitive mechanism by inhibiting synthesis of Thromboxane A2. Randomized trials elucidated that aspirin treatment reduced the 6 weeks risk of recurrent ischemic stroke by about 60% and disabling fatal ischemic stroke by about 70%.[87] A third category, uncompetitive inhibitor, also considered as an inhibition type which disturbs enzyme-substrate complexes, leading to dead end complex formation[84] (FIGURE 2d). Unfortunately, this phenomenon is unusual in the drug development process and excluded in this current review.

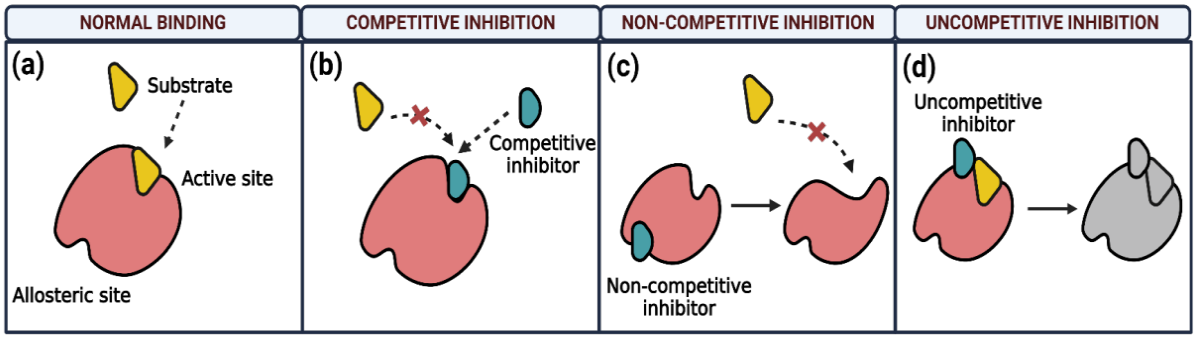

FIGURE 2. Three kind of enzyme inhibition mechanism; (a) Common binding of enzyme; (b) Competitive inhibitor act as substrate analog; (c) Non-competitive inhibitor bind to allosteric site leads to conformational change; and (d) Uncompetitive inhibitor bind to enzyme-substrate complex (https://BioRender.com)

Drugs that act as an enzyme activator can accelerate biochemical reaction through enzyme modification (ie. phosphorylation) or low molecular weight positive modulators.[88] There are particular conditions where enzyme activators comprise therapeutic benefits. For instance, activator of guanylyl cyclase C (linaclotide), increases phosphorylation rate resulting in an elevation of cGMP levels and induces several cellular responses related to chronic idiopathic constipation improvement.[34] The effectiveness and efficacy of linaclotide treatment against gastrointestinal disease have been studied by Peng *et al*.,[89] Group of irritable bowel syndrome (IBS) patients treated with linaclotide (290 μg/d) showed a significantly greater improvement than placebo in all secondary endpoints from the first 2 wk.

Several pharmaceutical agents targeting enzymes have been evolved for a wide range of applications, including cancer, gastrointestinal, respiratory, cardiovascular, and infectious diseases. However, research related to enzymatic drug targets faces various challenges and limitations. Certain drugs are known to interact with others through drug-drug interactions, leading to unwanted side effects. Besides, developing and manufacturing enzymatic drug targets can be very expensive, making them cost-prohibitive under certain circumstances. Other challenges lie in the similarity of enzyme structure resulting in lack of drug specificity and increasing off-target probability. Enzymes are also known to have short *in vivo* half-life and in particular, several studies found that the immune system exhibits enzyme clearance. The future of enzyme-based drug expansion lies in the development of selective and potent drugs as well as the ability to target enzymes involved in multiple pathways. Combination of enzyme targeting drugs with other therapeutic strategies such as gene therapy also give promising approaches.[90,91]

Ion channel as drug target

Ion channel-targeting drugs are novel but essential in modern pharmacology, significantly influencing numerous physiological processes.[92,93] It offers a precise and potent way to alter cellular signaling and function. Multiple sclerosis secondary-progressive multi-arm randomization trial (MS-SMART) has evaluated the neuroprotective effect of amiloride among other agents in patients with secondary progressive multiple sclerosis. The study included 90 patients per treatment arm and assessed the percentage of brain volume change (PBVC) as the primary outcome. Results indicated a reduction in PBVC of 0.5% in the amiloride group compared to placebo over 96 wk.[94] A consensus report from experts in China highlighted the safe and effective use of ion channel drugs for managing chronic pain, emphasizing the need for standardized practices based on clinical evidence.[95] That therapeutic approach spans a broad potential spectrum of medical conditions based on the 2 prominent families of ion channel-targeting drugs.

Voltage-gated ion channels are transmembrane proteins that open and close in response to changes in membrane potential.[96] They control cellular excitability and signal transmission by regulating the flow of several ions, such as sodium, potassium, and calcium.[97] Representing a primary target for pharmacological intervention due to its fundamental role in orchestrating cellular excitability and signaling. As mentioned in TABLE 1, the use of nimodipine, a voltage-gated ion channel-targeting drug, has also successfully normalized the activity of intracellular free calcium levels in diabetic animal models. In a study with randomized controlled trial involving 1,000 patients with trigeminal

neuralgia, carbamazepine was found to reduce pain episodes by approximately 70% compared to placebo over 12 wk.[98] A meta-analysis involving 38 studies with over 8,000 patients showed that gabapentin significantly reduced pain scores compared to placebo, with a mean difference of -1.42 (95% CI: -1.73 to -1.10) on a 0-10 scale, indicating substantial efficacy in managing neuropathic pain.[99] Compared to ligand-gated, voltage-gated channels offer unique opportunities for precise modulation of neuronal activity, making them particularly attractive targets for therapeutic intervention in various neurological and cardiovascular disorders. This emphasis on voltage-gated ion channels underscores their significance in drug discovery and development efforts to address complex physiological and pathological conditions.

On the other hand, ligand-gated ion channels are responsible for opening or closing in response to the binding of ligand-specific molecules, such as neurotransmitters or hormones.[100] Ligand-gated ion channels are crucial for neurotransmission and involve several processes, such as synaptic plasticity and sensory perception.[101] A study involving 404 patients demonstrated that memantine reduced cognitive decline as measured by the Alzheimer's disease assessment scale-cognitive subscale (ADAS-Cog) by an average of 2.2 points compared to placebo over six months.[102] The evidence shows that ion channel-targeted drugs are not only responsible for synaptic diseases in particular but are also involved in other health problems.

However, the experiment regarding ion channel-targeting drugs faces significant challenges in present and future development. One of the current challenges lies in specificity and safety, such as off-target effects and potential adverse reactions that could happen. For example, while targeting sodium channels can relieve pain, it may also disrupt regular neuronal activity, causing side effects like dizziness or sedation.[103] This necessitates extensive safety profiling in clinical trials. Furthermore, the ion channel structure complexity and their regulatory mechanism pose a pressing challenge in drug development and optimization, such as individual genetic differences that can affect patients' response to ion channel drugs, complicating treatment strategies. For instance, polymorphisms in P2X receptor genes have been associated with variations in blood pressure responses to treatments targeting these channels.[104] The present and future of this field will likely depend on overcoming these hurdles to enhance the efficacy and safety of new therapeutic agents.

DNA as drug target

Nucleic acids are important for transferring the genetic code from DNA and translating it into specific proteins. Hybridization can be used to target the activity of specific genes and genes have potential use in drug development. For example, if a gene carried by a virus is silenced by a drug, the virus may not be able to survive inside the cells it has infected and will die. Another example is if cancer cells grow uncontrollably because a gene is permanently stuck in the active position, it can be stopped by suppressing its expression by administering drugs that target DNA.[105] RNA has also been shown to be closely involved in gene regulation, namely the process of turning genes on or off at specified times and locations in living cells. Drugs that target DNA work in different ways in gene therapy. Intercalation is the process of changing the shape of DNA so that it cannot be used by ribosomes, for example chloramphenicol changes the shape of DNA. Alkylation is the process by which a drug can change the shape of DNA so that it cannot be used by ribosomes. An

example of a drug that uses an alkylation mechanism is neomycin. Apart from that, there is also a mechanism by which this drug can damage DNA replication.[106] This has implications for diseases such as cancer, where many genes are deregulated, leading to uncontrolled cell growth. For example, combination of toripalimab, lenvatinib, and gemcitabine plus oxaliplatin (GEMOX) as the first therapy for advanced stage ICC, which is done by inhibiting the PD-1 (programmed cell death protein 1) protein on T cells, which is a mechanism used by cancer cells to avoids detection and destruction by the immune system, inhibits a number of enzymes involved and inhibits DNA synthesis.[53] According to Okajima *et al*.,[52] drugs that target DNA datopotamab deruxtecan can internalized into tumor cells followed by intracellular trafficking to lysosome and DXd release, which induced DNA damage and apoptosis in TROP2.

The drugs shown in TABLE 1 are intended to bind to and stop the expression of specific genes, either at the RNA level or directly on DNA. The mechanism by which drugs target DNA can have an effect on the properties of the nucleic acid molecule, as well as the way it hybridizes or binds to its target sequence meaning that it sometimes connects to other areas, causing off-target effects such as immunogenicity. Immunogenicity refers to the ability of a molecule to enhance the immune system, which is an undesirable characteristic of drugs administered to humans. The main problem with nucleic acid-based drugs is their delivery to target tissues.[107] To target DNA as a target for a drug can be optimized by knowing that the selected target DNA is directly involved in treating the disease being targeted, ensuring that the target DNA can be accessed by the drug, the DNA to be targeted has high specificity thereby reducing the potential for mistargeting or targeting DNA. It is also important to test the right dose to achieve a therapeutic effect without excessive side effects, considering the possibility of resistance to the drug so that the drug remains effective in treating a disease. The DNA that will be used as a drug target should be stable so that there are no significant changes in the DNA structure that could reduce the action of the drug used.

CONCLUSION

G-protein coupled receptors, one of the biomolecules involved in modulating receptor activity and various physiological processes, can be targeted by affecting relevant signaling pathways to provide therapeutic effects against certain diseases, such as osteoporosis and carcinoma cancer. Targeting biomolecules, like enzymes, has the potential to overcome diseases such as cancer and infections through the understanding of the enzyme inhibition or activation mechanism as well as the mechanism between enzymes and substrates. Targeting drugs at ion channels that regulate ion transmission has the potential to treat various neurological and cardiovascular disorders. A DNA and RNA can be targeted especially in the treatment of diseases such as cancer by altering gene expression or damaging DNA involved in disease progression. Further research needs to be done that focuses on understanding selective and specific drug delivery mechanisms, the structure and the complexity of each biomolecule, and the interactions between drugs and biomolecules to improve treatment effectiveness.

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