Research on the Mechanism of Covalent Interactions in Targeted Drug Design

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Abstract. Covalent interactions are one of the important intermolecular forces and play a significant role in the design of targeted drugs. This article provides a detailed account of the role covalent interactions play in drug-target binding and the thermodynamic and kinetic characteristics of covalent bond formation. By reviewing the relevant literature and combining with theoretical analysis, the differences in pharmacodynamics and pharmacokinetics between covalent inhibitors and traditional non-covalent inhibitors were compared. The results showed that covalent interactions could significantly enhance the binding affinity and specificity of the drug to the target protein, thereby prolonging the drug's action time and reducing the number of dosing times. And because the covalent bond is irreversible, it can avoid conformational changes of the target protein and competitive inhibition, and has great advantages in tumor treatment, etc. However, covalent drugs can also bring about problems such as off-target toxicity and immunogenicity. This article also summarizes several currently successful covalent targeted drugs, such as afatinib, osimertinib and other EGFR covalent inhibitors, and summarizes their design ideas and improvement schemes. Some design principles and development directions of covalent drugs were proposed in terms of the selection of reactive groups, the design of linkers, the properties of target amino acid residues, etc. The findings suggest that the rational use of covalent interactions can significantly increase the success rate of drug design, which is of great significance for the development of highly effective and low-toxicity targeted therapeutic drugs.

Keywords: Covalent interactions; Targeted drug design; Molecular mechanisms; Drug-target binding; Covalent inhibito.

1. Introduction

Covalent interactions are once again being given attention in today's drug development process, especially in targeted therapy drugs. Due to the development of the biopharmaceutical industry, global funding for new drug research and development has been increasing year by year. It is estimated that the average annual growth rate of global funding for new drug research and development will be 8.5% from 2019 to 2023, and targeted drugs account for a large proportion. Although traditional non-covalent drugs have achieved good results in clinical practice, they are powerless against some difficult-to-drug targets. Covalent targeted drugs can form covalent bonds with the target protein to produce more lasting and specific effects, making up for the shortcomings of traditional drugs.

In recent years, as understanding of covalent effects has deepened, more and more covalent targeted drugs have been approved for marketing by the U.S. Food and Drug Administration (FDA). For example, the launch of afatinib in 2013 was an important milestone in covalent drug design, and the subsequent successful launches of osimertinib, ibrutinib, and other drugs have also demonstrated the effectiveness of covalent drug design. According to 2023 data, more than 30 covalent targeted drugs have been approved for clinical use, covering areas[1] such as oncology, immunology, and neurology. This article mainly summarizes the role of covalent interactions in the design of targeted drugs, analyzes their principles, methods and application prospects, with the aim of providing some guidance for the design of covalent drugs.

2. The theoretical basis of covalent interactions

2.1 Chemical Principles of covalent bond formation

A covalent bond is formed by the sharing of electron pairs between two atoms, and in drug-target interactions, this covalent bond is the result of a reaction between the electrophilic group in the drug molecule and the nucleophilic amino acid residue in the protein. From a quantum chemical perspective, the overlap between molecular orbitals and the redistribution of electron clouds have significant effects on the thermodynamics and kinetics of covalent bond formation. Drug molecules typically contain reactive electrophilic groups such as acrylamide, chloroacetamide, aldehyde groups, etc. These groups are electron-deficient and can accept electron pairs provided by the nucleophilic residues of proteins. Residues such as cysteine, lysine and histidine in proteins are nucleophilic due to the presence of lone pairs of electrons and are the main sites of action for covalent reactions. In the reaction, the frontier molecular orbital theory can well describe the process of electron transfer and bonding, and the HOMO-LUMO energy level difference determines the activity and selectivity[2] of the reaction. The energy barrier formed by covalent bonds is generally large and requires suitable conditions and environments. Factors such as the pH value of the microenvironment in which the protein is located, hydrophobicity, and electrostatic field all affect the reaction.

2.2 Classification and Characteristics of Covalent interactions

Covalent interactions can be classified into different types based on their reaction mechanisms and product properties, and each type has its own characteristics and biological effects. Nucleophilic substitution is a common type of covalent reaction, with SN1 and SN2 forms, and SN2 is more frequently used in drug development due to its stereochemical properties. Michael addition is a process in which an unsaturated compound binds to a nucleophile to form a covalent bond. This reaction has good selectivity and moderate reactivity and is widely used in the design of covalent inhibitors. Aldehyde-ketone chemistry is the reaction of a carbonyl compound with an amine or thiol group to form a Schiff base or thioacetal, which is generally reversible. Click chemistry reactions, such as copper-catalyzed azide-alkyne cycloaddition, have the advantages of high efficiency and high selectivity and have great application value[3] in medicinal chemistry. Different covalent reactions vary greatly in terms of reaction rate, product stability and biocompatibility, and it is necessary to select the appropriate covalent reaction based on the properties of different targets and therapeutic purposes. Technologies.

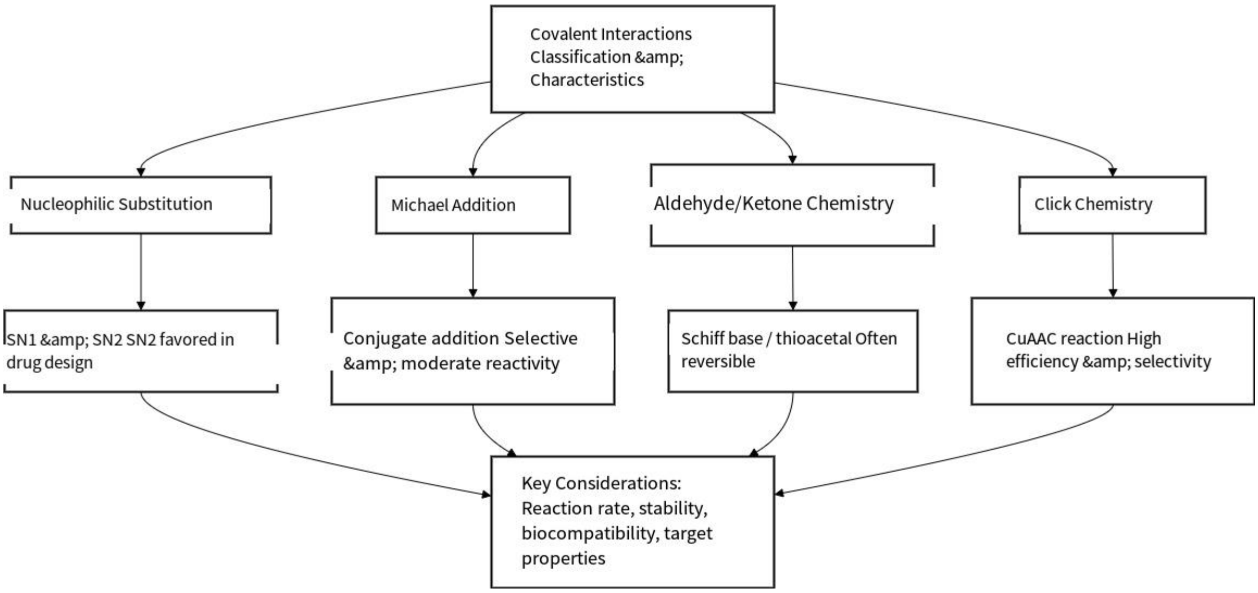

Figure 1. Classification and characteristics of covalent interactions

2.3 Reversibility and irreversibility of covalent modifications

The reversibility of covalent modification determines the characteristics of drug action and safety. Different covalent bonds have different stabilities and dissociation properties. Chemical bonds formed by irreversible covalent modifications are generally stronger, such as carbon-sulfur bonds, carbon-nitrogen bonds, etc., which are difficult to break naturally in the body and can have a longer duration of action. Reversible covalent modifications use weaker covalent bonds or dynamic equilibrium to bind drugs to their targets. For example, the borate ester bonds formed by boric acid drugs with serine residues have PH-dependent reversibility. Semi-reversible covalent modifications fall between the two, releasing drugs slowly through hydrolysis or other biochemical reactions to act sustained-release. The reversibility of the modification affects the pharmacokinetic properties of the drug, its toxic side effects, and the way it is administered, while irreversible modifications can produce long-lasting effects, they are also prone to off-target toxicity[4].

3. Design Strategies for Covalent Targeted Drugs

3.1 Selection and identification of target proteins

Target protein selection is one of the fundamental tasks in covalent drug design, based on the structural characteristics of the protein, its functional importance, and whether it is suitable for drug action. A good covalent target should have easily accessible and reactive amino acid residues, especially nucleophilic amino acid residues such as cysteine and lysine that are located in the active center or allosteric center. Bioinformatics methods and structural biology studies can be used to identify suitable reactive residues on the surface or in the pocket of proteins and to evaluate their accessibility and chemical microenvironment. The association of the target to the disease and its expression levels in different tissues are also important considerations. Selecting targets that are highly expressed in diseased tissues and lowly expressed in normal tissues is beneficial for improving the therapeutic index. The structural stability and conformational flexibility of the protein also affect the effect and selectivity of covalent reactions. A rigid structure is conducive to accurate molecular recognition, while a certain degree of flexibility is conducive to inducing fit. In addition, the biological function of the target and its position in the signaling pathway determine the efficacy of the drug, and key node proteins generally produce greater biological effects [5].

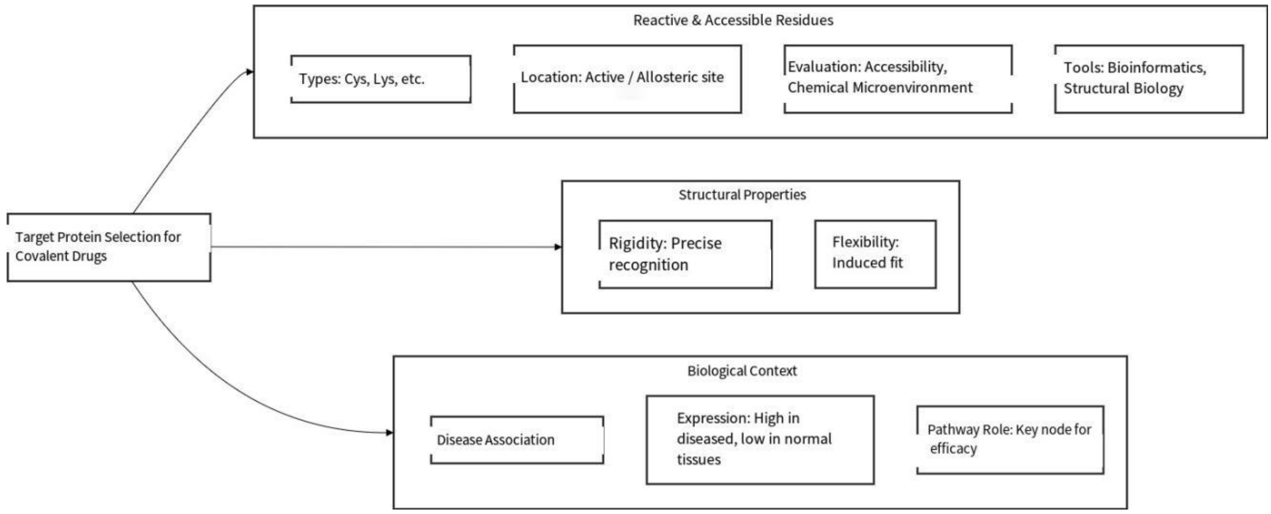

Figure 2. Framework for Target protein selection and recognition strategies in the design of covalent targeted drugs

3.2 Design and optimization of reactive groups

The selection and optimization of reactive groups is one of the key steps in the design of covalent drugs and directly affects the drug's reactivity, selectivity, and safety. Acrylamide groups are widely used as covalent warheads due to their appropriate reactivity and good selectivity, and can undergo Michael addition reactions with cysteine residues to form stable covalent bonds. Chloroacetamide groups have higher reactivity but poorer selectivity and are prone to off-target effects when reacting with many nucleophilic residues. Aldehyde and ketone groups can bind to lysine or arginine by forming imine bonds. The reaction is reversible but not stable enough. Boric acid and its derivatives can form reversible borate ester bonds with serine and threonine, which are widely used in the design of protease inhibitors. The electronic effect and steric hindrance of the reactive group can greatly affect its reactivity. The reactivity can be changed by introducing electron-withdrawing or electron-donating groups, and the appropriate steric hindrance is beneficial for improving selectivity[6]. Modern computational chemistry methods, such as density functional theory calculations, can predict the reactivity and selectivity of different reactive groups and provide guidance for the design of reactive groups.

3.3 Structural design of the connecting arms

The linker arm serves as a bridge between the pharmacological active group and the covalent reactive group, and its design has a significant impact on the overall properties of the drug. The length of the linker arm should be appropriate so that the reactive group can reach the target residue smoothly. If it is too short, it may cause steric hindrance; if it is too long, it will reduce reaction efficiency and selectivity. Flexible linker arms, such as alkyl chains, can give drugs more conformational freedom to better accommodate protein changes, but they may also reduce binding specificity. Rigid linker arms such as aromatic rings or heterocyclic structures can provide more definite spatial constraints for drugs, thereby enhancing binding selectivity and affinity. The chemical properties of the linker arms, such as polarity, hydrophobicity, and the ability of hydrogen bond donor and acceptor, affect the membrane permeability and bioavailability [7] of the drug. Adding appropriate functional groups to the linker arm can alter the physicochemical properties and pharmacokinetic characteristics of the drug.

3.4 Considerations of selectivity and specificity

The design of selectivity and specificity for covalent drugs is a crucial factor in ensuring treatment safety and requires consideration from multiple aspects to achieve effective targeting. Sequence selectivity is achieved based on the specific amino acid sequence of the target protein. During protein evolution, some amino acid sequences are conserved while others are variable, which can be utilized to design specific recognition elements. Structural selectivity is based on the unique three-dimensional structure of the target protein, and through molecular docking and structural optimization design, drug molecules can be well matched to the protein binding site. Reaction selectivity is achieved by altering the kinetic parameters of the covalent reaction, such as the reaction rate constant, activation energy, and the stability of the reaction intermediates. Tissue selectivity involves designing prodrug molecules[8] with tissue-specific activation based on the different levels of expression of the target protein in different tissues and the different microenvironments they are in. Time selectivity is achieved by controlling the kinetic processes of covalent bond formation and cleavage to precisely control the duration of drug action. Combining multiple selectivity strategies can significantly increase the safety range of covalent drugs, reduce the occurrence of adverse reactions, and facilitate their clinical application.

4. Examples of Covalent interactions in Drug design

4.1 Covalent design of tyrosine kinase inhibitors

Tyrosine kinase inhibitors are an important class of anti-tumor drugs, and covalent design is currently a hot direction in medicinal chemistry research. Epidermal growth factor receptor tyrosine kinase inhibitors are one of the most successful examples of covalent design, with both afatinib and osimertinib showing good efficacy. Afatinib uses its acrylamide reactive group to irreversibly covalently bind to cysteine residues in the EGFR kinase domain, effectively blocking wild-type EGFR and common activating mutations such as L858R and exon 19 deletion. Because covalent binding can avoid the problem of ATP competitive inhibition, it works[9] well even at high ATP concentrations. Osimertinib is a third-generation EGFR covalent inhibitor that, while retaining the advantages of covalent binding, has good selectivity for the T790M resistance mutation through the rational design of the molecule. The acrylamide group of the drug forms a covalent bond with Cys797 while the molecular structure is adjusted to accommodate the changes in the binding pocket caused by the T790M mutation, significantly enhancing the therapeutic effect on drug-resistant tumors. Clinically, osimertinib achieved an objective response rate of 61% in patients with T790M-positive non-small cell lung cancer and a median progression-free survival of 10.1 months, fully demonstrating the advantages of the covalent design.

4.2 Covalent mechanism of protease inhibitors

The covalent design of protease inhibitors takes advantage of the nucleophilic attack nature of the protease catalytic process, mimics the way natural substrates bind to proteases for effective inhibition. Serine protease inhibitors are a good example of the application of covalent mechanisms, and their design ideas are derived from the covalent acyl-enzyme intermediate produced during the catalysis of serine proteases. BTK inhibitors such as bosutinib form a covalent bond with Cys481 through their pyridine-pyrimidine core structure, which irreversibly inhibits Bruton's tyrosine kinase and plays a significant[10] role in the treatment of B-cell malignancies. Cysteine protease inhibitors also use covalent binding methods, such as cathepsin inhibitors forming covalent bonds with the active site of cysteine through electron affinity groups like vinyl sulfone or fluoromethyl ketone. These inhibitors are designed to adjust the electron affinity of the reactive group so that they can selectively bind to the target protein in a physiological environment without a non-specific reaction. Bortezomib, a proteasome inhibitor, is another form of covalent inhibition in which the boric acid group forms a reversible covalent bond with the threonine residue in the proteasome catalytic subunit, thereby preventing protein degradation and achieving anti-cancer effects. The new generation of proteasome inhibitor carfazomib, which is developed in recent years, contains epoxone structures that can form irreversible covalent bonds and has better anti-cancer effects and less peripheral neurotoxicity in the treatment of multiple myeloma.

4.3 Cases of covalent drug development targeting other targets

Covalent drug design methods have been applied to a variety of important therapeutic targets and have made significant progress in the treatment of many diseases. JAK kinase inhibitors are examples of covalent design for the treatment of autoimmune diseases, using covalent bonds formed with conserved cysteine residues to achieve long-lasting inhibition [11]. E3 ligases in the ubiquitin-proteasome system represent a new direction in covalent drug research. PROTAC technology combined with covalent binding selectively degrades target proteins, bringing new hope to previously considered "undruppable" targets. The successful development of KRAS G12C inhibitors AMG510 and MRTX849 represents significant progress in the design of covalent drugs for "undrupable" targets, which form covalent bonds with cysteine in KRAS G12C mutants, putting them inactive. Clinical data show that AMG510 has an objective response rate of 37.1% in patients with KRAS G12C-positive non-small cell lung cancer. Histone deacetylase inhibitors such as vorinota play a significant role in the treatment of hematological malignancies by coordinating their hydroxamic acid groups with the zinc ion at the enzyme's active site. In addition, covalent antiviral drugs such as remdesivir, which enter the body in its prodrug form, are converted into active nucleoside analogues and covalently bind to viral RNA polymerase to exert antiviral effects, have shown good results in the treatment of COVID-19. These successful cases all demonstrate that covalent interactions have great application prospects and good clinical effects [12] in various disease areas.

5. Conclusions

This paper elaborates on the role of covalent interactions in targeted drug design and their application prospects, and explores the mechanism of action of covalent drugs and their clinical advantages at the molecular level. Through the study of examples of tyrosine kinase inhibitors, protease inhibitors, etc., it was found that covalent interactions can significantly enhance the affinity of drugs for target proteins and the duration of action, thereby addressing issues such as drug resistance and competitive inhibition that exist in traditional non-covalent drugs. In tumor treatment, Covalent targeted drugs such as osimertinib and AMG510 can well overcome drug resistance mutations and provide new ideas for the development of "undruggable" target drugs.

Covalent drug design needs to ensure high activity while avoiding adverse side effects and immunogenicity issues. Future research should focus on the design of reactive groups, the improvement of target selectivity, and the study of new covalent binding methods. With the development of computational chemistry and structural biology, covalent drug design will become increasingly accurate and effective, thus better serving personalized treatment. In conclusion, covalent interactions are an important means of drug design today and will play an increasingly important role in future drug research for the benefit of humanity [13].

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