Advantages and Disadvantages of Covalent Inhibitors

Subjects: Chemistry, Medicinal

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The formation of covalent bonds that target proteins can offer drugs diverse advantages in terms of target selectivity, drug resistance, and administration concentration. The most important factor for covalent inhibitors is the electrophile (warhead), which dictates selectivity, reactivity, and the type of protein binding (i.e., reversible or irreversible) and can be modified/optimized through rational designs. Furthermore, covalent inhibitors are becoming more and more common in proteolysis, targeting chimeras (PROTACs) for degrading proteins, including those that are currently considered to be ‘undruggable’.

Keywords: covalent inhibitors ; PROTACs ; drug discovery ; covalent drugs

1. Introduction

Medical research has progressed exponentially over the last century [1]. In a lot of cases, diseases that were considered to be death sentences 100 years ago can now be cured using drugs [2]. For instance, the discovery of antibiotics has drastically increased human life expectancy and reduced the progress and severity of symptoms [1][2]. Drug discovery is centered around the development of active substances [3]. Depending on the desired result, different active substances have been designed to have distinct pharmacodynamic properties (e.g., pain relief or blood pressure reduction) [3]. Many small molecule drugs can inhibit and, therefore, prevent the biological activity of a protein of interest (POI), while a few drugs can stimulate POI activity. Regardless of the functions of small molecule drugs, the effects generally depend on the interaction between the active substance (e.g., an inhibitor, effector, or activator) and the POI (e.g., an enzyme, protein, ion channel, or receptor) [3]. These interactions can be divided into two general categories: non-covalent interactions and covalent interactions (**Figure 1**) [4].

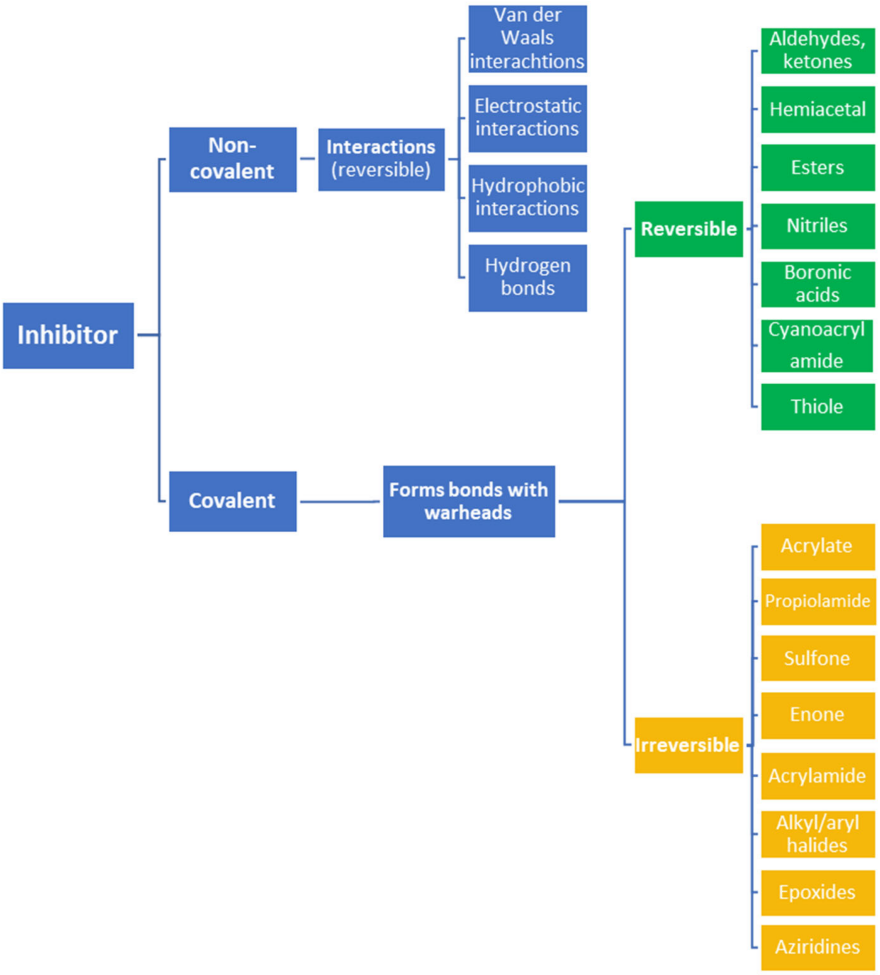


Figure 1. Different types of inhibitors and their potential interactions and bindings, as well as the corresponding functional groups for the formation of reversible and irreversible covalent bonds.

Due to negative experiences with covalent-reactive compounds (especially with highly reactive drug metabolites, which can trigger immunogenicity and idiosyncratic drug reactions), covalent inhibitors did not enjoy widespread popularity until 1990 [5][6]. At that time, most research groups and companies developed active compounds with non-covalent binding properties [6]. **Figure 2** displays a timeline of the development or commercialization of covalent binding agents over the years [6][7][8][9][10][11].

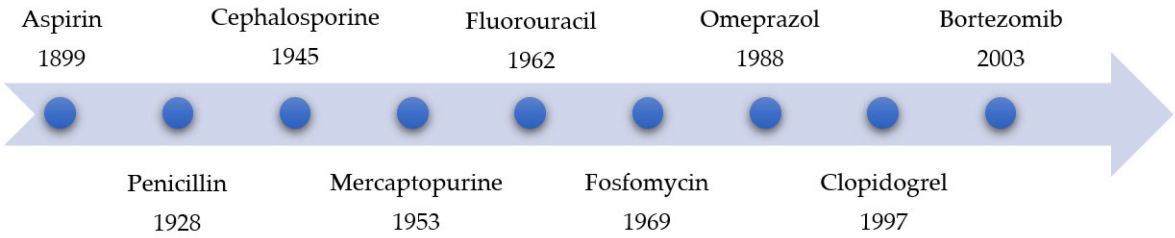


Figure 2. The timeline of the development of various covalent inhibitors with the associated years of discovery.

More recent studies have shown that chemical optimization can enhance the activity and target specificity of covalent inhibitors in clinical use, which has greatly encouraged scientists to develop more covalent inhibitors [6]. **Figure 3** illustrates the number of publications in the SciFinder portal containing the terms ‘covalent drug’ and ‘inhibitor covalent’ over time.

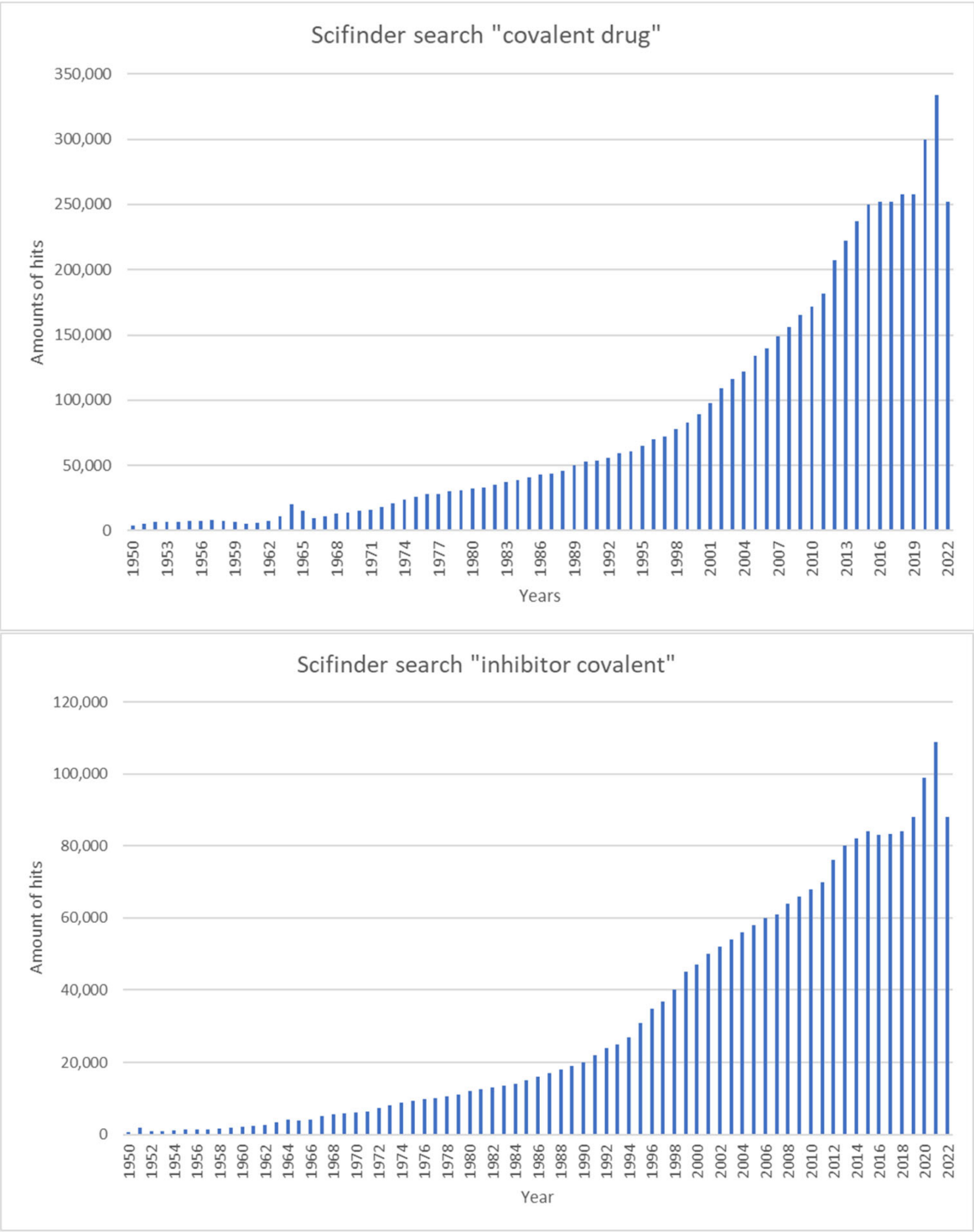
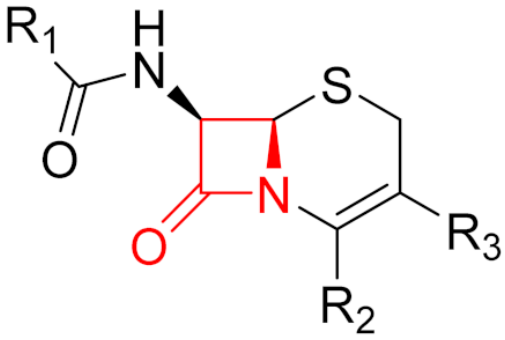
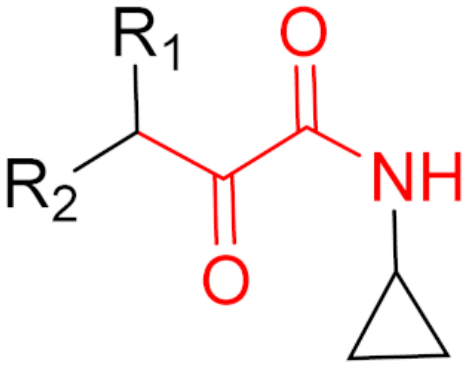
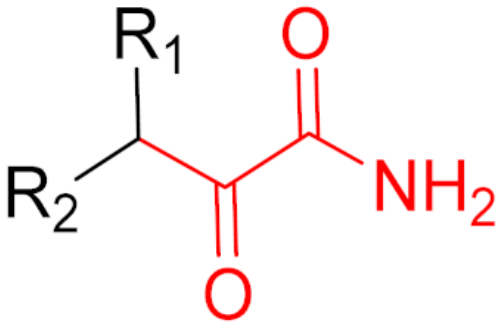
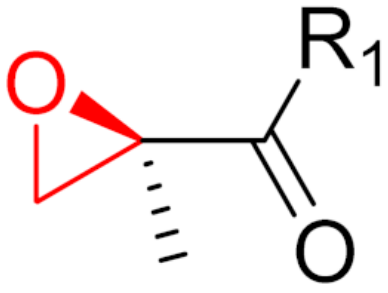
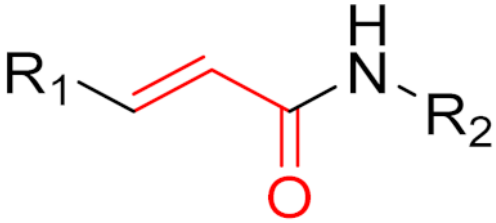
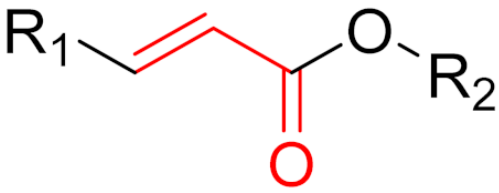
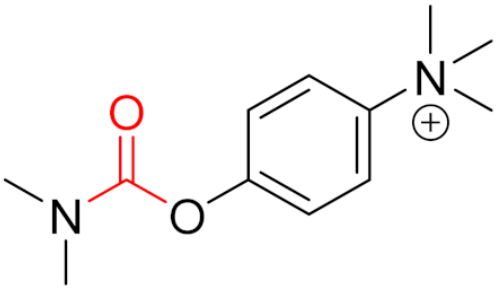
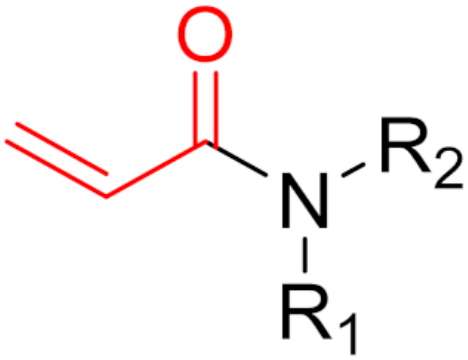
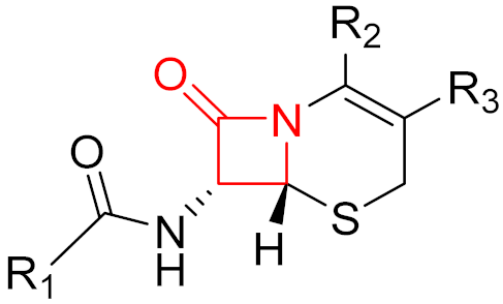
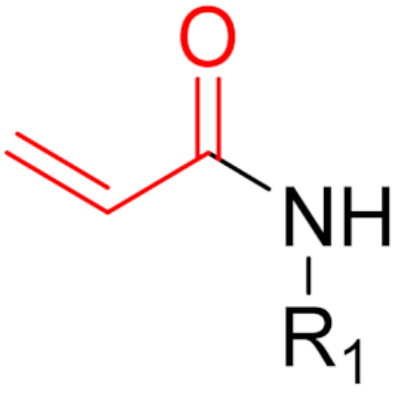


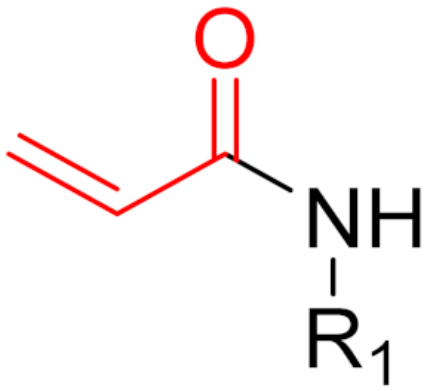
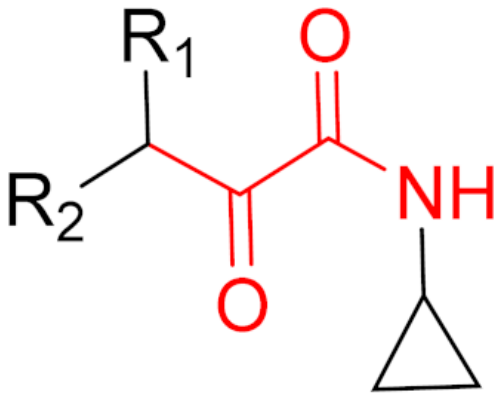
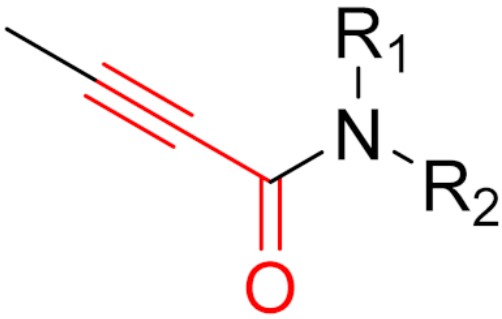
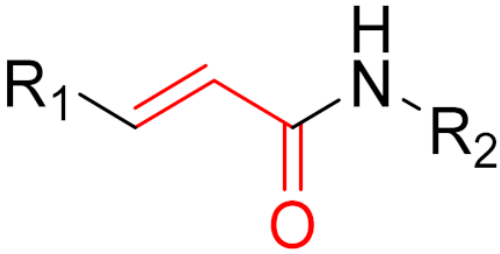
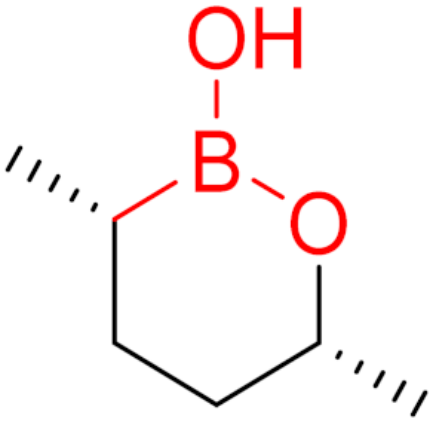
Figure 3. The significant increase in publications associated with covalent inhibitor development in the years from 1950 to 2022. The graphs show the number of publications with the keywords of 'covalent drug' (**top**) or 'inhibitor covalent' (**bottom**) in the SciFinder portal over the last 72 years.

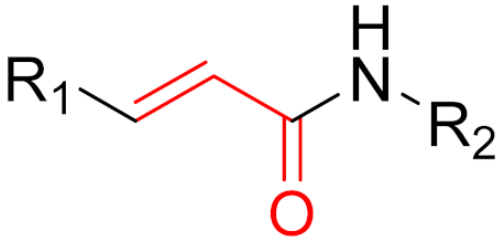
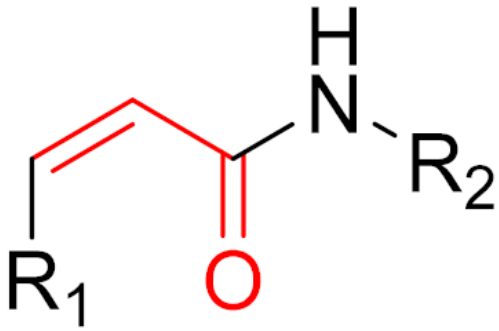
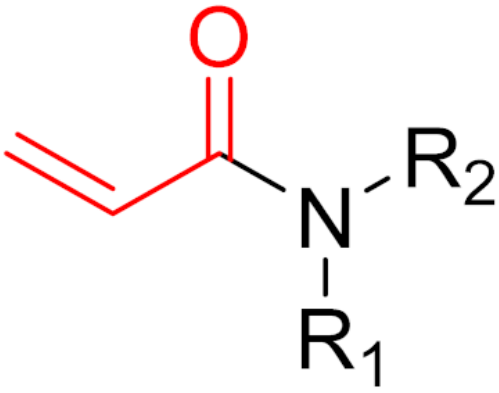
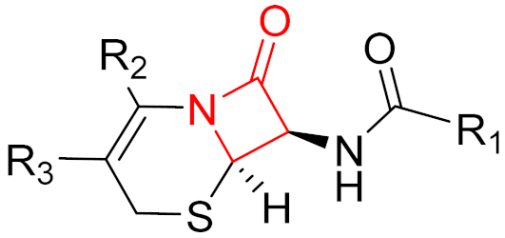
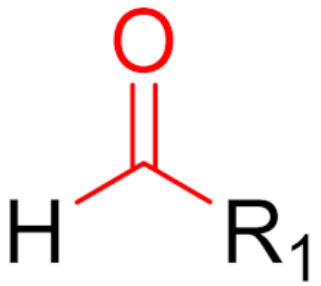
Although covalent binders can be toxic due to the undesired modification of off-target proteins or haptenization [8][12], compounds that rely on covalent mechanisms are among the major classes of small molecules, representing about 30% of all active substances on the market. **Table 1** shows the covalent inhibitors that have been approved by the FDA since 2010.

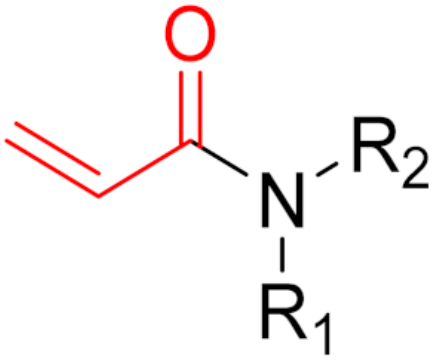
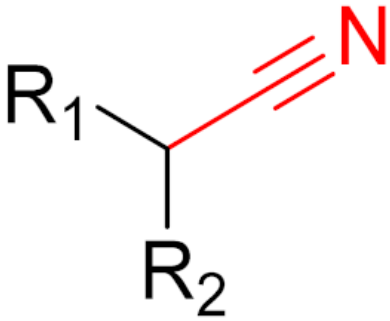
Table 1. The FDA-approved covalent inhibitors since 2010, with a structural representation of the warhead and a description of the inhibitor's function.

Year	Name of Drug	Warhead	Function
2010	Ceftaroline (β-lactam)		β-lactam antibiotic
2011	Telaprevir (α-ketoamide)		HCV protease inhibitor
2011	Boceprevir (α-ketoamide)		HCV protease inhibitor
2011	Abiraterone (-)	-	Prostate cancer treatment
2012	Carfilzomib (epoxide)		Proteasome inhibitor (cancer)
2013	Afatinib (α,β-unsaturated carbonyl)		EGFR tyrosine kinase inhibitor

Year	Name of Drug	Warhead	Function
2013	Dimethyl fumarate (α,β-unsaturated carbonyl)		Immunomodulatory drug
2013	Neostigmine (carbonyl group)		Acetylcholinesterase inhibitor
2013	Ibrutinib (α,β-unsaturated carbonyl)		EGFR tyrosine kinase inhibitor
2014	Ceftolozane (β-lactam)		β-lactam antibiotic
2015	Osimertinib (α,β-unsaturated carbonyl)		EGFR tyrosine kinase inhibitor

Year	Name of Drug	Warhead	Function
2015	Olmutinib (α,β-unsaturated carbonyl)		EGFR tyrosine kinase inhibitor
2016	Narlaprevir (α-ketoamide)		HCV protease inhibitor
2017	Acalabrutinib (α,β-unsaturated propargylamide)		Bruton's tyrosine kinase inhibitor
2017	Neratinib (α,β-unsaturated carbonyl)		EGFR tyrosine kinase inhibitor
2017	Vaborbactam (boronic acid)		Non-β-lactam β-lactamase inhibitor

Year	Name of Drug	Warhead	Function
2018	Dacomitinib (α,β-unsaturated carbonyl)		EGFR tyrosine kinase inhibitor
2019	Selinexor (α,β-unsaturated carbonyl)		Nuclear export inhibitor
2019	Zanubrutinib (α,β-unsaturated carbonyl)		Bruton's tyrosine kinase inhibitor
2019	Cerfiderocol (β-lactam)		β-lactam antibiotic
2019	Voxelotor (aldehyde)		Hemoglobin oxygen-affinity modulator

Year	Name of Drug	Warhead	Function
2021	Sotorasib (α,β-unsaturated carbonyl)		KRAS G12C inhibitor
2021	Nirmatrevir (nitrile)		SARS-CoV-2 main protease inhibitor

Aspirin is an essential medicine that is recommended by the WHO for anti-inflammatory, fever reduction, and pain relief functions [10][12]. Aspirin can irreversibly acetylate cyclooxygenase (COX-1 and COX-2) enzymes, which are the key catalysts in response to the formation of pro-inflammatory prostaglandins [10]. Upon transferring its acetyl group to the hydroxy group of the side chain of Ser_{530}, aspirin blocks the binding of arachidonic acid (a substrate to cyclooxygenase), thereby inhibiting its activity [13]. Moreover, due to the irreversible binding, oxygenase remains inhibited until the cell is degraded [13]. The mechanism of action of aspirin is illustrated in **Figure 4**.

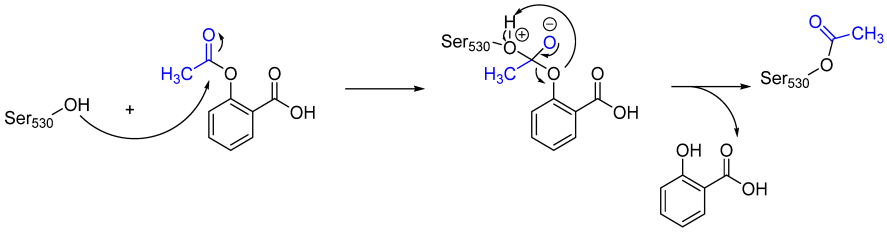


Figure 4. The mechanism of action of aspirin via the irreversible inhibition of COX-1 and COX-2. The reactive acetyl group of aspirin reacts with the hydroxy group of the Ser_{530} side chain, which is shown in blue.

Saxagliptin [14] is an active substance that is used to treat diabetes mellitus type 2. In contrast to aspirin, which is an irreversible covalent inhibitor, saxagliptin reversibly inhibits the enzyme dipeptidyl peptidase 4 (DPP-4) via the amino acid residue Ser_{630} and is a reversible covalent inhibitor [14]. DPP-4 is a serine exopeptidase that contains a catalytic triad comprising Ser_{630}, His_{740}, and Asp_{708} in its binding pocket, which degrades the hormone glucagon-like peptide 1 (GLP-1) and, in turn, prevents the release of insulin [14]. Higher insulin concentrations reduce the glucagon concentration and, thus, blood glucose levels [14]. Because saxagliptin is a reversible covalent inhibitor, the duration of inhibition depends on the reverse reaction or hydrolysis of the covalent complex [14]. The mechanisms of inhibition and the release of DPP-4 are shown in **Figure 5**.

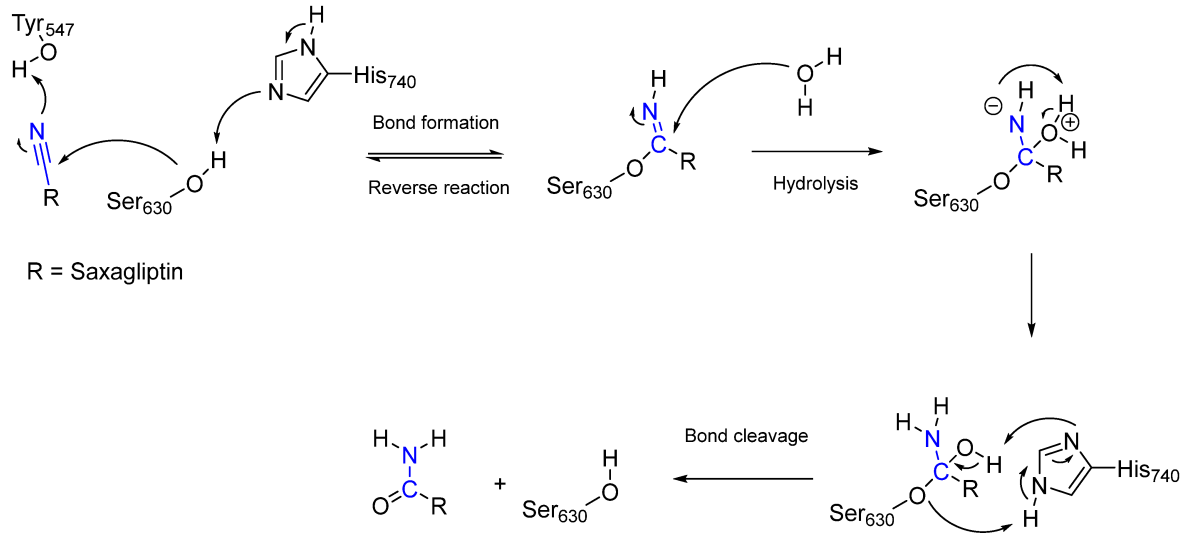


Figure 5. A graphical representation of the covalent inactivation of DPP-4 via the reaction of the nitrile group (highlighted in blue) with the catalytic Ser_{630} using the example of saxagliptin. The reverse reaction and the cleavage of the covalent bond between saxagliptin and DPP-4 complex by water are also shown.

2. Advantages and Disadvantages of Covalent Inhibitors

The ability of covalent inhibitors to form chemical bonds with target proteins can have several advantages [15]. For instance, in many cases, the enzymatic activity of a protein is related to a non-covalent or only transiently covalent interaction with its substrate [15]. Thus, the displacement of irreversible covalent inhibitors using a natural substrate is nearly impossible [15]. In general, the dose of a drug is positively correlated to its toxicity [16]. In comparison to non-covalent inhibition, the covalent bond formation can enable full target occupancy even at relatively low concentrations [16]. In addition, covalently binding drugs are usually less susceptible to drug resistance that is caused by mutations in chemotherapy, as long as the covalent binding modes remain unaffected by the mutations [17]. However, changes that affect the formation of covalent binding often lead to drug resistance, such as mutations of the nucleophile, blockages of binding sites, or reductions in nucleophilic characteristics [6][16]. The advantages and disadvantages of covalent and non-covalent inhibitors are shown in **Table 2**.

Table 2. The advantages and disadvantages of covalent and non-covalent inhibitors.

Type of Inhibitor	Advantages	Disadvantages
Non-Covalent	• Large non-covalent compound library • Easier to evade toxicity in comparison to irreversible covalent inhibitors (long-term inhibition) • No need for strong or activated nucleophiles	• Comparatively low selectivity • Not very potent • Limited to non-covalent binding affinity • Mostly poor reactivity

Type of Inhibitor	Advantages	Disadvantages
Covalent	• Can be administered at lower doses • Higher potency • Longer duration of time/inhibition • Less sensitive to pharmacokinetic parameters • Can provide higher selectivity • Higher biochemical efficiency • Lower risk of drug resistance • Able to target undruggable proteins • Binding properties can be influenced by the choice of warhead (i.e., reversible or irreversible)	• May cause unexpected toxicity or hypersensitivity • May cause drug-induced toxicity • The potential immunogenicity of the resulting target adducts • The need for strong or activated nucleophiles • The need for accessible nucleophile • May not be suitable for targets with fast enzyme turnover or fast degradation

For instance, the epidermal growth factor receptor (EGFR) is a surface receptor that belongs to the tyrosine kinase family [18]. Upon binding to EGF, EGFR transduces external signals to cells for proliferation. Gefitinib [18][19] is an adenosine triphosphate (ATP)-competitive protein kinase inhibitor that has a significant impact on EGFR-related signaling pathways by blocking the adenosine triphosphate (ATP)-binding sites of enzymes, resulting in the inhibition of the enzymes. However, drug resistance has often been reported due to the occurrence of various mutations during long-term treatments [20]. The most common mutation is the replacement of threonine at position 790 with methionine (T790M), which alters the binding pocket and prevents the binding [20]. T790 is known as the gatekeeper residue because the amino acid residue is critical for access to and the size of the binding pocket. The exchange of the polar amino acid threonine for the bulky nonpolar amino acid methionine leads to increased resistance to first- and second-generation EGFR inhibitors. After treatment, the receptors remain active, and tumor cells continue to proliferate [20]. In contrast, afatinib is a covalent EFGR inhibitor that can irreversibly bind to mutated and WT EGFR; however, it can lead to dose-dependent toxicity and has a stronger affinity for the wild-type EGFR [21][22]. Osimertinib is a third-generation EGFR inhibitor that shows improved selectivity and less toxicity than afatinib and has a stronger affinity to mutant EGFR than wild-type EGFR. Therefore, osimertinib can be used to circumvent the dose-limiting toxicity of second-generation inhibitors [22]. Clinical studies have reported less drug resistance to afatinib and Osimertinib [23] by tumors with $EGFR^{T790M}$ [20][22]. The structures of gefitinib, afatinib, and osimertinib are shown in **Figure 6**.

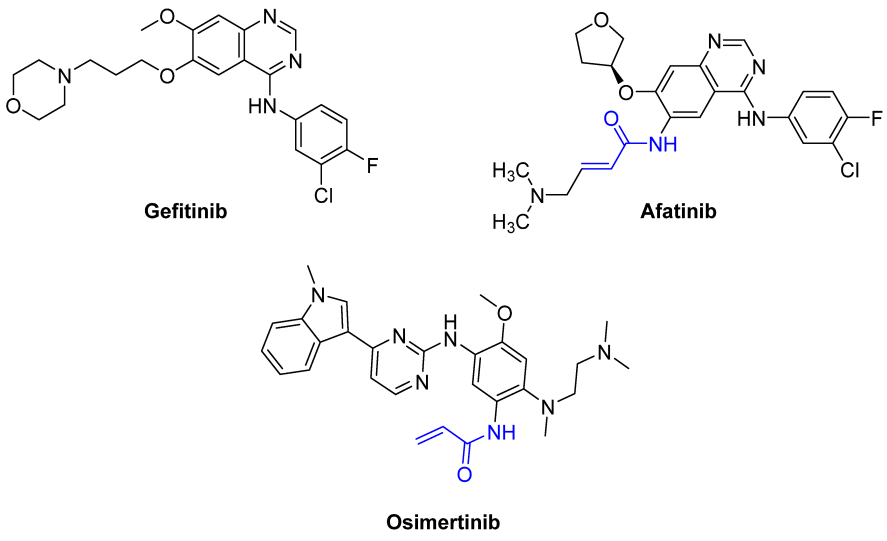


Figure 6. The structures of the selected first, second and third-generation EGFR inhibitors. Gefitinib (**left**) interacts non-covalently with receptors, while afatinib (**right**) and osimertinib (**bottom**) form covalent bonds with the thiol group of the cysteine side chain of the receptors. The warheads of afatinib and osimertinib (marked in blue) are responsible for the binding to the receptors.

However, as mentioned above, certain mutations that prevent the formation of covalent bonds, such as $EGFR^{C797S}$, confer resistance to Osimertinib [24]. This highlights that in contrast to non-covalent drugs, clinical applications of covalent drugs still need to overcome several drawbacks [6][25]. Rapid, irreversible inhibition can be advantageous for covalent inhibitors; however, this feature can also lead to undesirable long-term effects (e.g., toxicity) when proteins are inhibited over a long period of time and are not metabolized due to the long turnover of the proteins. Nevertheless, in an impressive discussion, Juswinder Singh was able to illustrate that covalent protein kinase inhibitors do not appear to exhibit higher toxicity than non-covalent inhibitors [22]. However, it can be assumed that as long as proteins are in the system, undesired interactions can occur, as shown by the following example [25][26][27]. Clopidogrel is a prodrug for thrombosis prevention, which can inhibit the adenosine diphosphate receptor P2Y12 [26][27]. P2Y12 is a member of the inhibitory G-protein-coupled purine receptor family and promotes platelet aggregation [27][28]. In the human body, clopidogrel is metabolized through oxidation and subsequent hydrolysis [27]. During this conversion, various diastereomers are formed, among which the only active metabolite is that with (S)/(R) configuration (**Figure 7**) [29]. This active metabolite irreversibly inhibits P2Y12 through a reaction between the thiol group of the active metabolite (**Figure 7**; highlighted in blue) and the Cys_{97} side chain within the first extracellular loop of P2Y12, which forms a disulfide bridge. As a result, platelet aggregation is prevented [28].

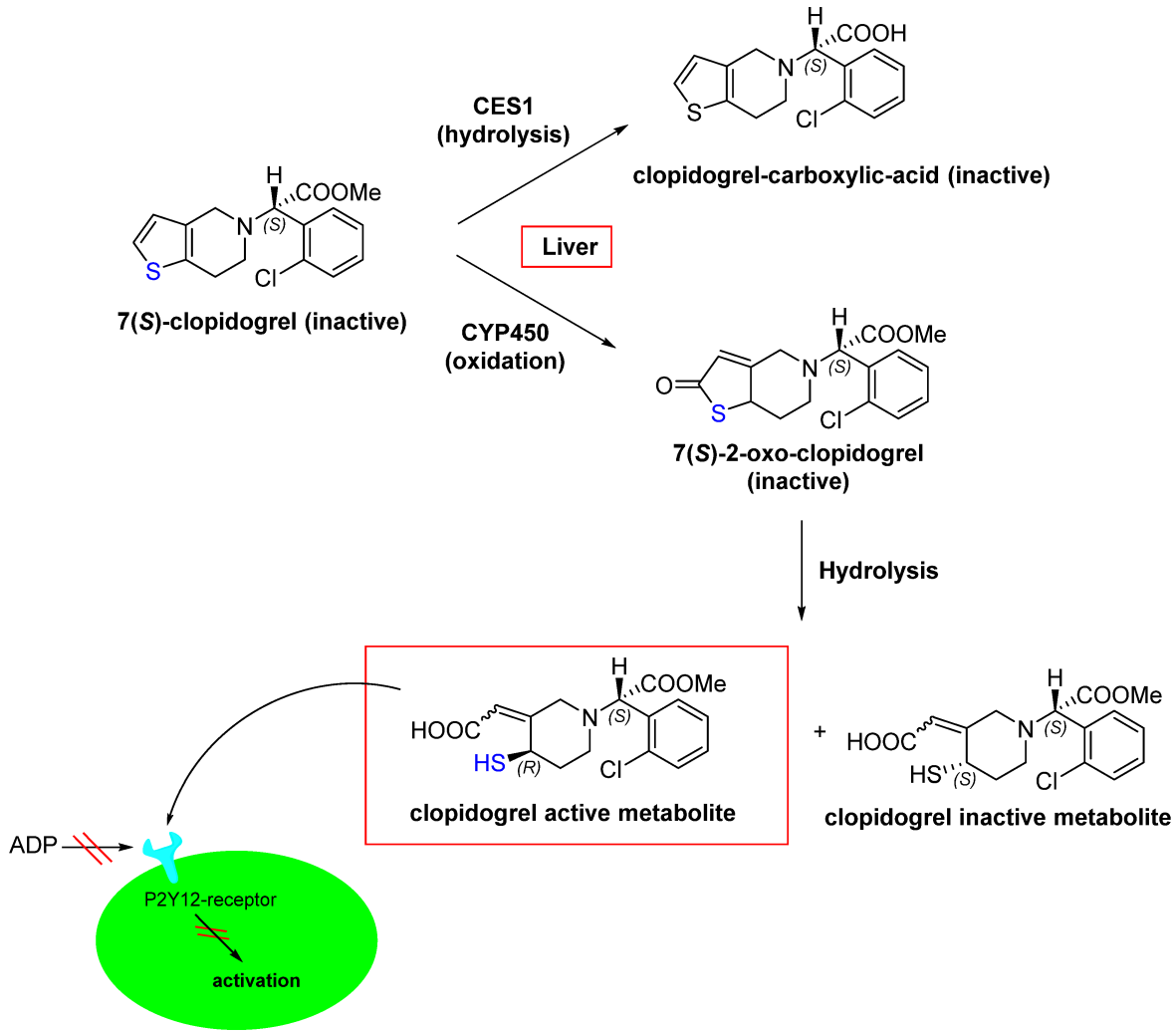


Figure 7. The metabolic pathway of clopidogrel (in)activation, considering the configuration required for the covalent inhibition of the P2Y12-receptor. CES1 (carboxylesterase 1) catalyzes the hydrolysis of 7(S)-clopidogrel into clopidogrel carboxylic acid, which is inactive. Cytochromes P450 (CYP450) are oxidoreductases and enable the oxidation of 7(S)-clopidogrel in the first step. CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4/5 are implicated as cytochrome P450 enzymes involved in the metabolism of clopidogrel. The warhead is highlighted in blue.

Because this action slows down blood clotting, long-term irreversible inhibition can lead to prolonged bleeding times, resulting in certain critical consequences, especially in the case of accidents or emergency operations after taking clopidogrel [28]. Additionally, the inhibition of platelet aggregation is sometimes also related to unusual bleeding from vessels in the eyes or lungs [28].

Another factor that negatively impacts the development of covalent agents is the rapid compensation to inhibition by newly synthesized target proteins because of the homeostasis of the body upon inhibition [16]. This is particularly problematic for

proteins with very high protein turnover or in cases where protein turnover is increased by disease, treatment, or other circumstances, as shown in the following examples.

In a review by Shringarpure et al., they described that oxidative stress could increase the intracellular degradation of short-lived and long-lived proteins and that progressive oxidation further increases the degradation of proteins via proteasomes [30]. In another case, Davies et al. described that Crohn's disease leads to abnormal protein turnover [31]. It has also been shown that children with active disease have increased protein turnover [31]. With conventional treatments, protein breakdown and synthesis are reduced, resulting in no changes in net protein balance in remission [31]. In the case of rapid protein turnover, re-administration is required to reach the critical concentration for the inhibition of the target protein. However, excessive drug intake may induce severe side effects (e.g., toxicity) [16].

3. Mechanisms of Action

The entire process involving the interaction between a target and a covalent inhibitor up to the formation of a covalent bond takes place in two steps [6]. The first step is the reversible association between the inhibitor and the target protein [6]. In the second step, a reaction takes place that forms a covalent bond [6]. This is exemplified by telaprevir, which reversibly inhibits the viral NS3.4A protease of the hepatitis C virus (HCV; Figure 8) [32].

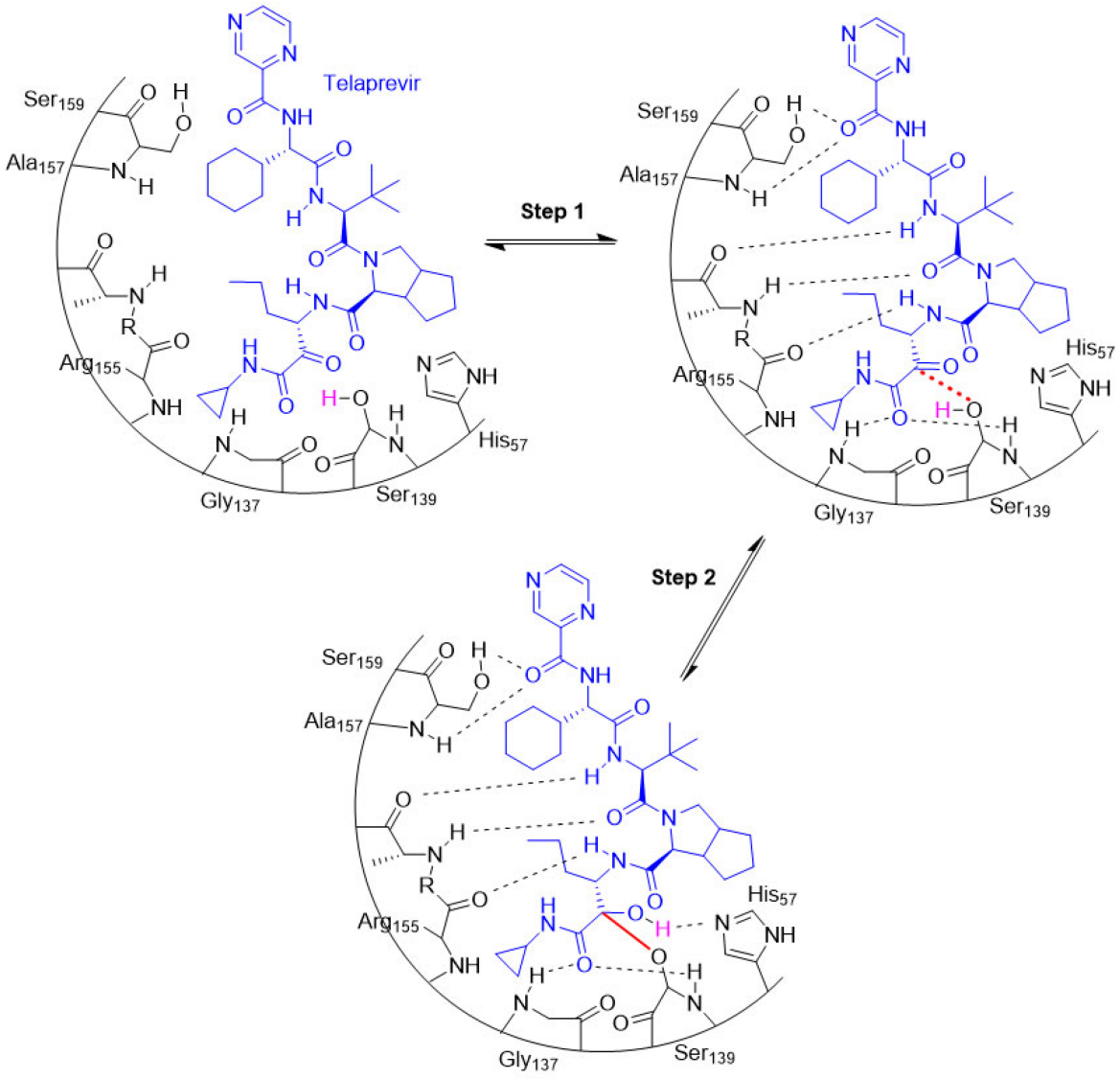


Figure 8. An illustration of the entire two-step process (i.e., association and bond formation) using telaprevir, the example HCV protease inhibitor. Telaprevir inhibits the viral NS3.4A protease of the hepatitis C virus.

Often, covalent inhibitors carry electrophilic groups, which react with nucleophilic residue on the target enzymes [6][33]. The warheads can be epoxides, aziridines, esters, ketones, nitriles, or another similar group [33]. For example, penicillin is a covalent inhibitor with beta-lactam as the warhead, which reacts with the active serine residue in the D-alanine transpeptidase [11]. Transpeptidases are essential for cross-linking in the biosynthesis of bacterial cell walls [11]. Irreversible bonds inhibit transpeptidase, resulting in the lysis of bacterial cells due to their instability [11]. The mechanism of action of this irreversible inhibition is shown in Figure 9.

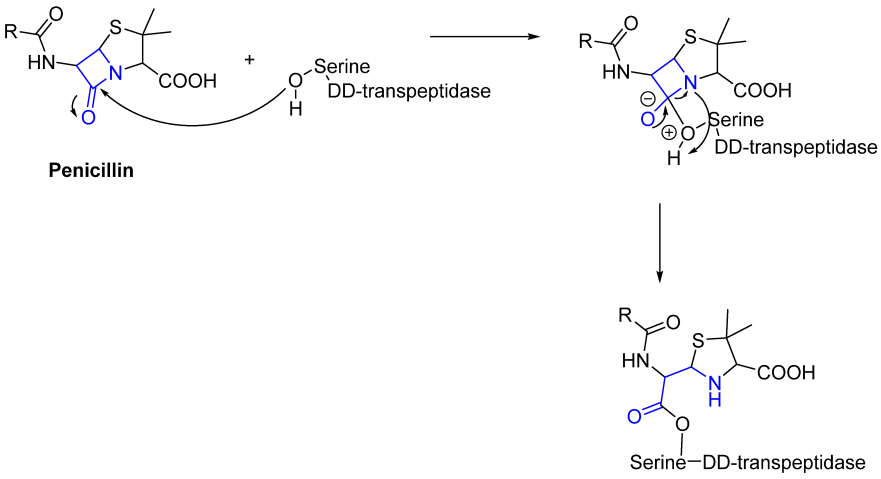


Figure 9. The mechanism of action of the irreversible inhibition of DD-transpeptidase by penicillin. The warhead of penicillin (β-lactam; highlighted in blue) reacts with the serine side chain of DD-transpeptidase.

To date, many new functional groups have been found that can form covalent bonds with sulfur-containing functional groups, as shown in Figure 10 [34]. The advantage of these groups is that they can directly react with the cysteine in target proteins at its active site without a prior metabolic activation [34]. Figure 10 displays various warheads that are involved in the formation of irreversible and reversible bonds [34]. In most cases, inhibitors occupy the binding pockets, which prevents substrates from forming bonds (i.e., competitive inhibition) [34]. Nevertheless, occupation in the active sites of target proteins is not always necessary [34]. For instance, a few inhibitors can bind to the remote sides of target enzymes, resulting in the alternation of the binding pocket [34]. These inhibitors are called allosteric inhibitors [34]. In rare cases, uncompetitive inhibition can occur, in which inhibitors bind exclusively to enzyme–substrate complexes. This results in the formation of enzyme–substrate–inhibitor complexes, which ensures that the enzymes do not convert the substrates; therefore, no products are formed [35]. Irreversible inhibitors are divided into two types: affinity label inhibitors and mechanism-based inhibitors (suicide inhibitors) [36]. Affinity label inhibitors resemble enzyme substrates and enter the active sites of enzymes, where irreversible covalent bonds are formed, and the active sites are modified without enzymatic conversion [36]. Suicide inhibitors bind to active sites in the same way as substrates, triggering the enzymatic properties of the enzymes [36]. During the enzymatic process, intermediaries are formed that cannot be further converted or split off. As a result, no further substrates can be converted. Aspirin and penicillin are examples of suicide inhibitors [36].

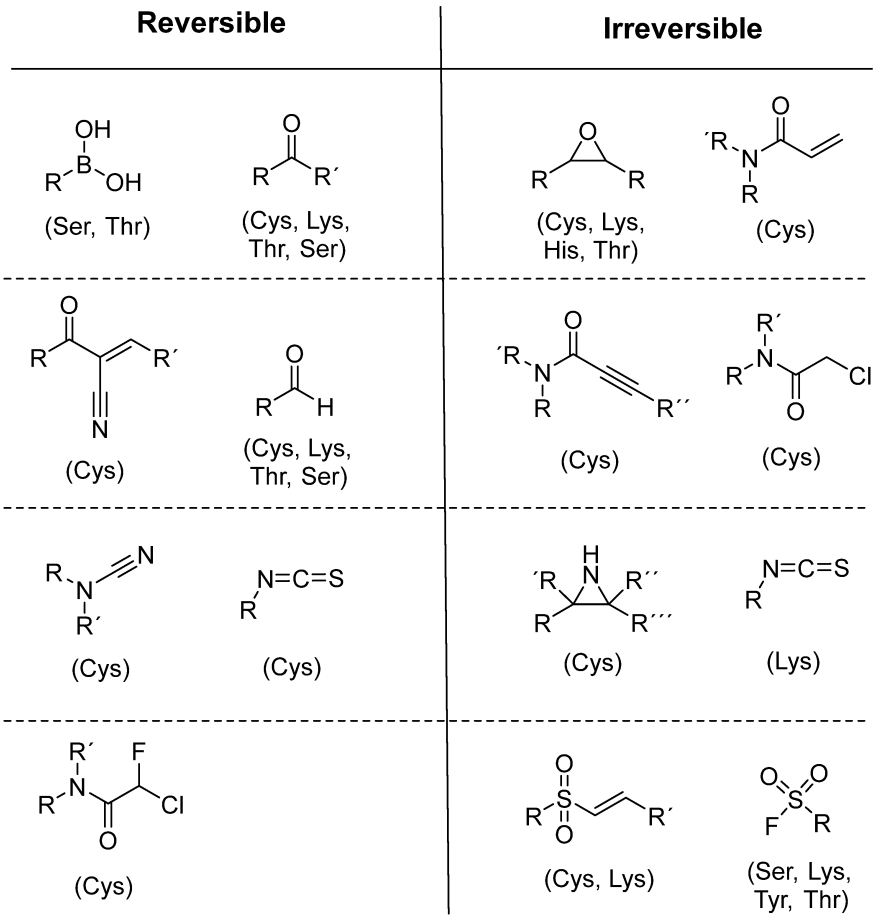


Figure 10. Warheads form irreversible and reversible bonds. The primary targeting amino acid residues are shown in brackets below the respective structures [37][38].

The formation of a bond can have different mechanisms. Many electrophilic warheads can react with a nucleophile via Michael addition, for example, [37]. The attacking nucleophile (e.g., carbanion, amine or thiol) serves as a Michael donor and the electrophile (e.g., α,β-unsaturated carbonyl compound) as a Michael acceptor [37]. A more detailed description of the different types of covalent reactions in drug development and the associated groups has been presented in great detail by Gehringer et al. [37] and Shindo et al. [38] and is not exclusively discussed here.

References

1. Johnson, J.R.; Williams, G.; Pazdur, R. End points and United States Food and Drug Administration approval of oncolo gy drugs. J. Clin. Oncol. 2003, 21, 1404–1411.
2. Dumit, J. Drugs for Life: How Pharmaceutical Companies Define Our Health; Duke University Press: Durham, NC, US A, 2012.
3. Rowland, M.; Tozer, T.N. Clinical Pharmacokinetics/Pharmacodynamics; Lippincott Williams and Wilkins: Philadelphia, PA, USA, 2005.
4. Aljoundi, A.; Bjij, I.; El Rashedy, A.; Soliman, M.E. Covalent versus non-covalent enzyme inhibition: Which route should we take? A justification of the good and bad from molecular modelling perspective. Protein J. 2020, 39, 97–105.
5. Jollow, D.; Mitchell, J.; Potter, W.; Davis, D.; Gillette, J.; Brodie, B. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. J. Pharmacol. Exp. Ther. 1973, 187, 195–202.
6. Baillie, T.A. Targeted covalent inhibitors for drug design. Angew. Chem. Int. Ed. 2016, 55, 13408–13421.
7. Jack, D.B. One hundred years of aspirin. Lancet 1997, 350, 437–439.
8. Singh, J.; Petter, R.C.; Baillie, T.A.; Whitty, A. The resurgence of covalent drugs. Nat. Rev. Drug Discov. 2011, 10, 307–317.
9. Baillie, T.A. The contributions of Sidney D. Nelson to drug metabolism research. Drug Metab. Rev. 2015, 47, 4–11.
10. Lei, J.; Zhou, Y.; Xie, D.; Zhang, Y. Mechanistic Insights into a Classic Wonder Drug Aspirin. J. Am. Chem. Soc. 2015, 1 37, 70–73.
11. Fleming, A. Penicillin. Br. Med. J. 1941, 2, 386.
12. Kumalo, H.M.; Bhakat, S.; Soliman, M.E. Theory and applications of covalent docking in drug discovery: Merits and pitf alls. Molecules 2015, 20, 1984–2000.
13. Vane, J.; Botting, R. The mechanism of action of aspirin. Thromb. Res. 2003, 110, 255–258.
14. Wang, Y.-H.; Zhang, F.; Diao, H.; Wu, R. Covalent Inhibition Mechanism of Antidiabetic Drugs—Vildagliptin vs Saxaglipt in. ACS Catal. 2019, 9, 2292–2302.
15. Smith, A.J.; Zhang, X.; Leach, A.G.; Houk, K. Beyond picomolar affinities: Quantitative aspects of noncovalent and cov alent binding of drugs to proteins. J. Med. Chem. 2009, 52, 225–233.
16. Adeniyi, A.A.; Muthusamy, R.; Soliman, M.E. New drug design with covalent modifiers. Expert Opin. Drug Discov. 2016, 11, 79–90.
17. Ghosh, A.K.; Samanta, I.; Mondal, A.; Liu, W.R. Covalent inhibition in drug discovery. ChemMedChem 2019, 14, 889–9 06.
18. Kobayashi, S.; Boggon, T.J.; Dayaram, T.; Jänne, P.A.; Kocher, O.; Meyerson, M.; Johnson, B.E.; Eck, M.J.; Tenen, D. G.; Halmos, B. EGFR mutation and resistance of non–small-cell lung cancer to gefitinib. N. Engl. J. Med. 2005, 352, 78 6–792.
19. Nakamura, Y.; Oka, M.; Soda, H.; Shiozawa, K.; Yoshikawa, M.; Itoh, A.; Ikegami, Y.; Tsurutani, J.; Nakatomi, K.; Kitaza ki, T. Gefitinib (“Iressa”, ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, reverses breast cancer resistance protein/ABCG2–mediated drug resistance. Cancer Res. 2005, 65, 1541–1546.
20. Koehler, J.; Schuler, M. Treatment, Afatinib, erlotinib and gefitinib in the first-line therapy of EGFR mutation-positive lun g adenocarcinoma: A review. Oncol. Res. 2013, 36, 510–518.
21. Dungo, R.T.; Keating, G.M. Afatinib: First global approval. Drugs 2013, 73, 1503–1515.
22. Singh, J. The Ascension of Targeted Covalent Inhibitors. J. Med. Chem. 2022, 65, 5886–5901.

23. Greig, S.L. Osimertinib: First Global Approval. Drugs 2016, 76, 263–273.
24. Shaikh, M.; Shinde, Y.; Pawara, R.; Noolvi, M.; Surana, S.; Ahmad, I.; Patel, H. Emerging Approaches to Overcome Acquired Drug Resistance Obstacles to Osimertinib in Non-Small-Cell Lung Cancer. J. Med. Chem. 2022, 65, 1008–1046.
25. Cohen, S.D.; Pumford, N.R.; Khairallah, E.A.; Boekelheide, K.; Pohl, L.R.; Amouzadeh, H.; Hinson, J.A. Selective protein covalent binding and target organ toxicity. Toxicol. Appl. Pharmacol. 1997, 143, 1–12.
26. Coukell, A.J.; Markham, A. Clopidogrel. Drugs 1997, 54, 745–750.
27. Jarvis, B.; Simpson, K. Clopidogrel. Drugs 2000, 60, 347–377.
28. Bryant, J.; Post, J.M.; Alexander, S.; Wang, Y.-X.; Kent, L.; Schirm, S.; Tseng, J.-L.; Subramanyam, B.; Buckman, B.; Islam, I. Novel P2Y12 adenosine diphosphate receptor antagonists for inhibition of platelet aggregation (I): In vitro effects on platelets. Thromb. Res. 2008, 122, 523–532.
29. Bluet, G.; Blankenstein, J.; Brohan, E.; Prévost, C.; Chevé, M.; Schofield, J.; Roy, S. Synthesis of the stabilized active metabolite of clopidogrel. Tetrahedron 2014, 70, 3893–3900.
30. Shringarpure, R.; Davies, K.J.A. Protein turnover by the proteasome in aging and disease. Free Radic. Biol. Med. 2002, 32, 1084–1089.
31. Davies, A.; Nixon, A.; Muhammed, R.; Tsintzas, K.; Kirkham, S.; Stephens, F.B.; Moran, G.W. Reduced skeletal muscle protein balance in paediatric Crohn's disease. Clin. Nutr. 2020, 39, 1250–1257.
32. Perry, C.M. Telaprevir. Drugs 2012, 72, 619–641.
33. Bauer, R.A. Covalent inhibitors in drug discovery: From accidental discoveries to avoided liabilities and designed therapies. Drug Discov. Today 2015, 20, 1061–1073.
34. Johnson, L.N.; Noble, M.E.; Owen, D.J. Active and inactive protein kinases: Structural basis for regulation. Cell 1996, 85, 149–158.
35. Ouertani, A.; Neifar, M.; Ouertani, R.; Masmoudi, A.S.; Mosbah, A.; Cherif, A. Effectiveness of enzyme inhibitors in biomedicine and pharmacotherapy. Adv. Tissue Eng. Regen. Med. Open Access 2019, 5, 85–90.
36. Hajizadeh, M.; Moosavi-Movahedi, Z.; Sheibani, N.; Moosavi-Movahedi, A.A. An outlook on suicide enzyme inhibition and drug design. J. Iran. Chem. Soc. 2022, 19, 1575–1592.
37. Gehringer, M.; Laufer, S.A. Emerging and Re-Emerging Warheads for Targeted Covalent Inhibitors: Applications in Medicinal Chemistry and Chemical Biology. J. Med. Chem. 2019, 62, 5673–5724.
38. Shindo, N.; Ojida, A. Recent progress in covalent warheads for in vivo targeting of endogenous proteins. Bioorg. Med. Chem. 2021, 47, 116386.