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’.

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].

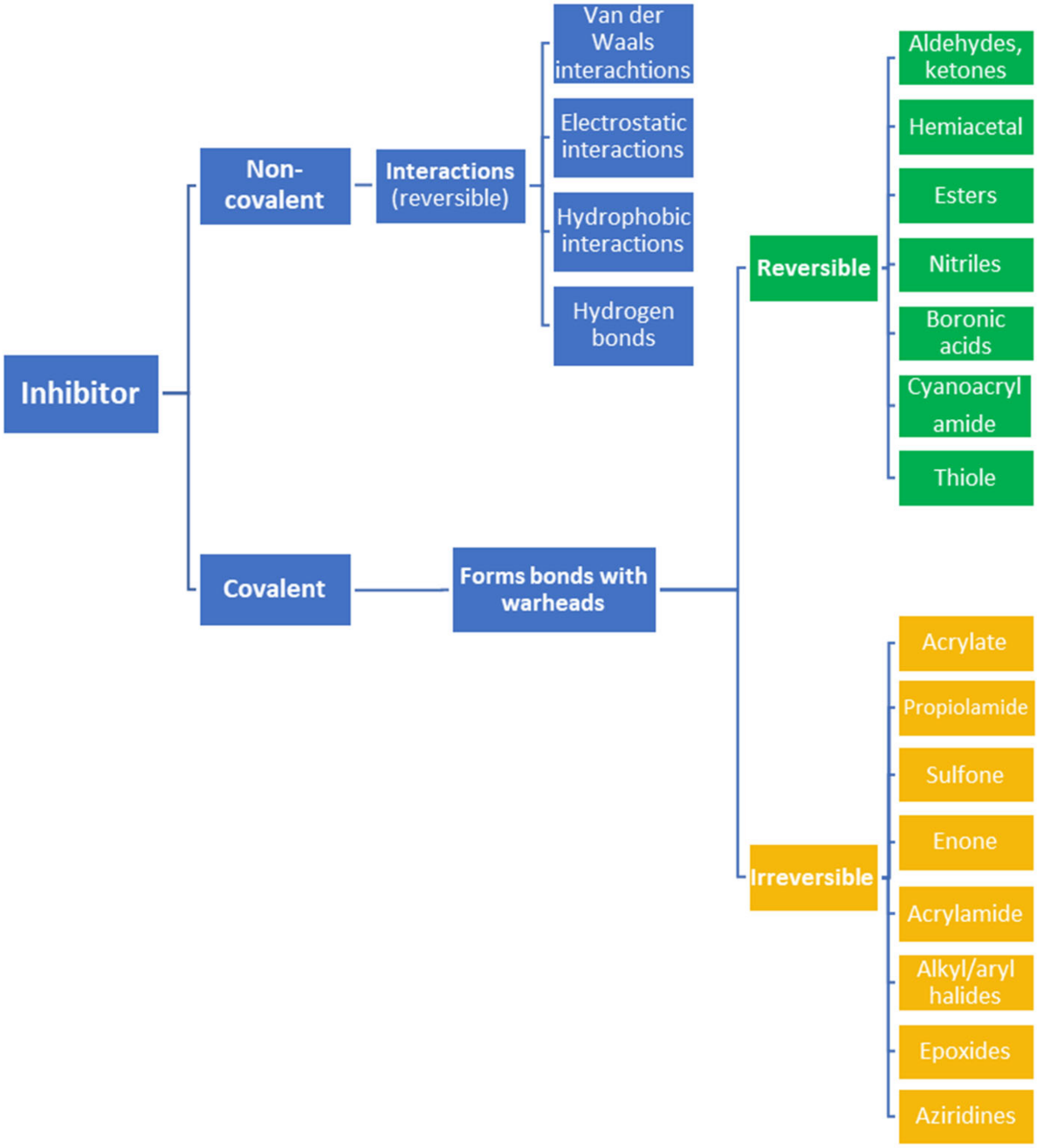


Figure 1. Different types of inhibitors and their potential interactions and bindings, as well as the corresponding functional groups for the formation of reversible and irreversible covalent bonds.

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].

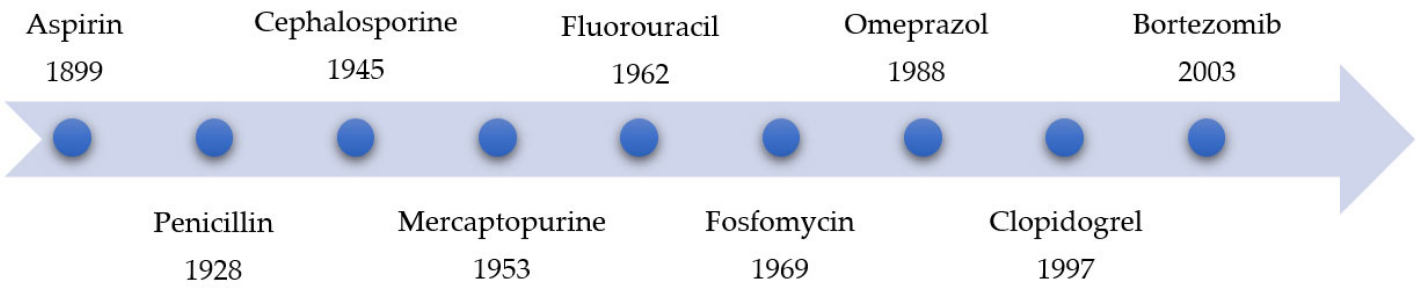
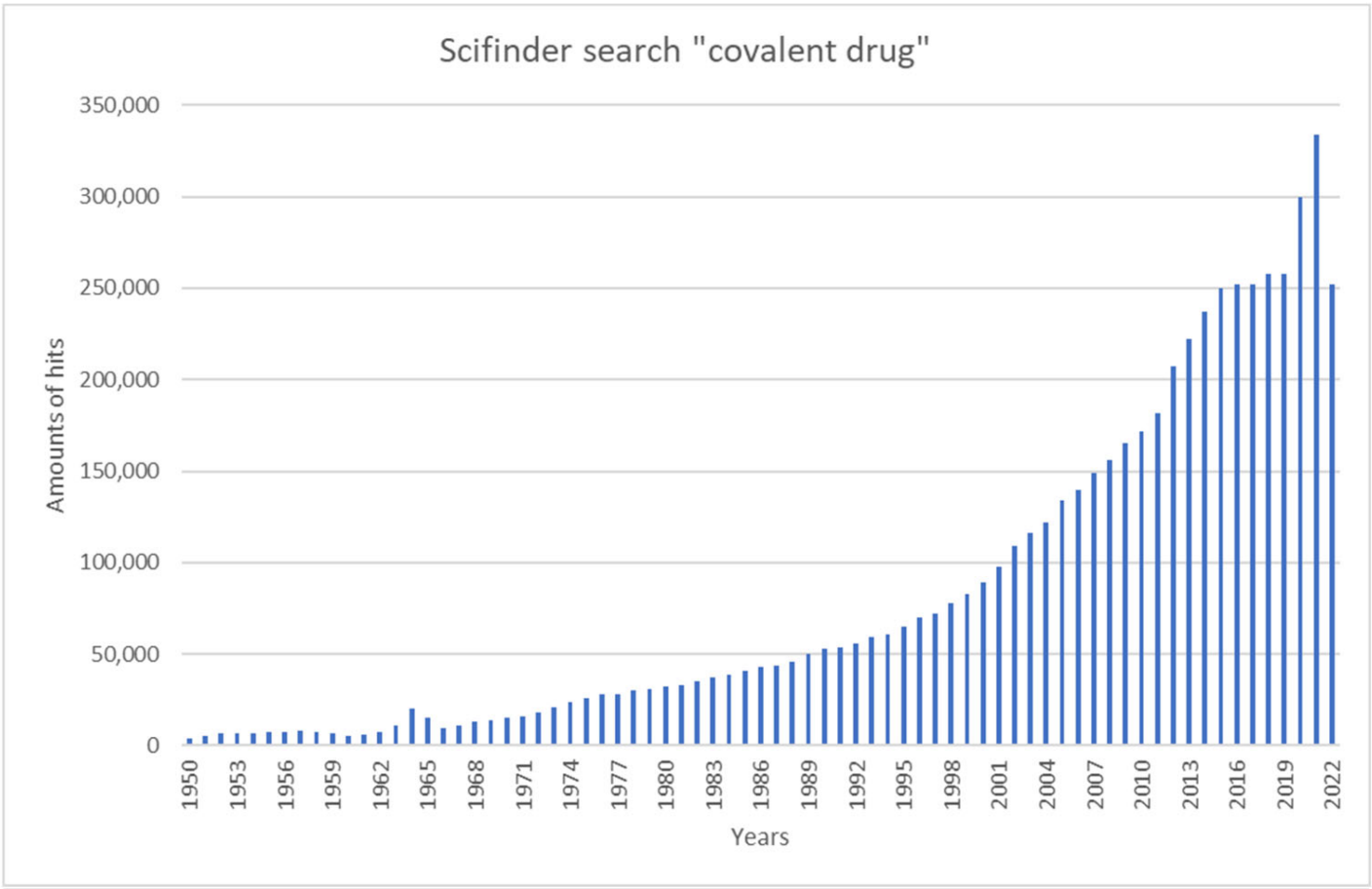


Figure 2. The timeline of the development of various covalent inhibitors with the associated years of discovery.

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.


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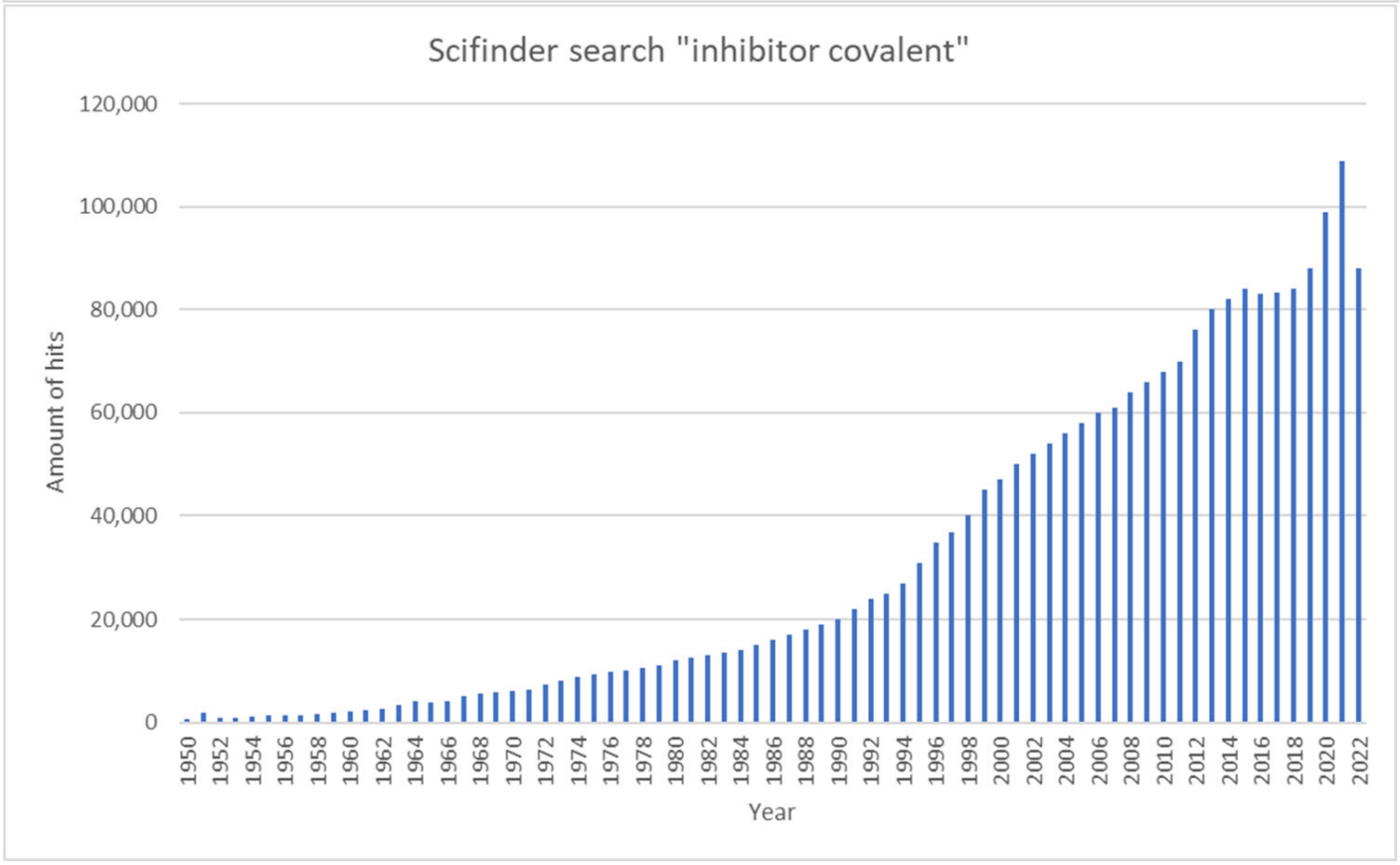
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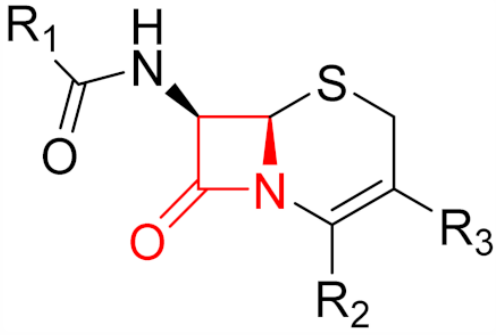
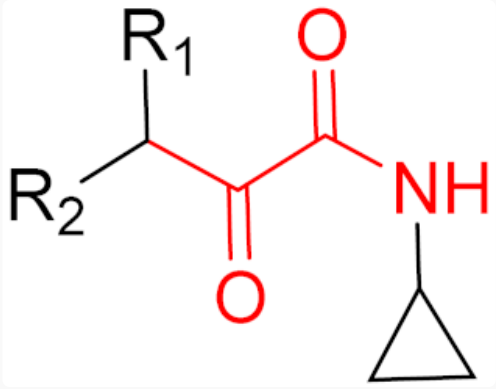
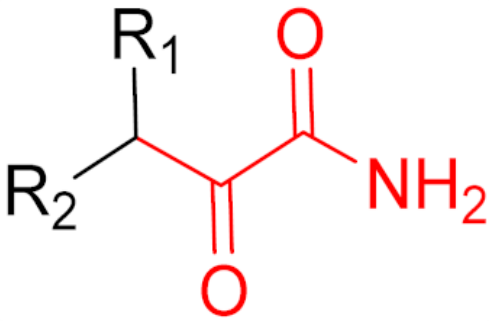
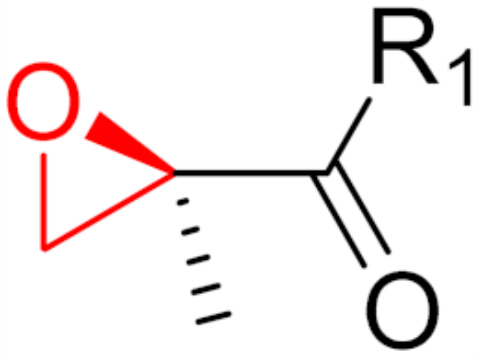
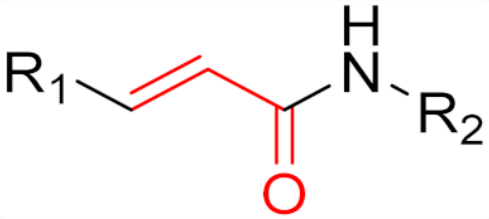
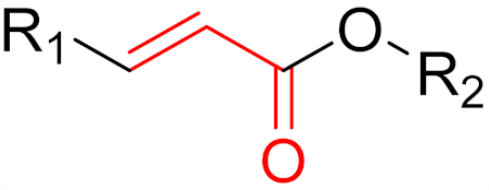
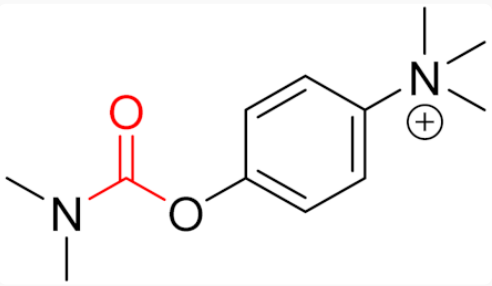
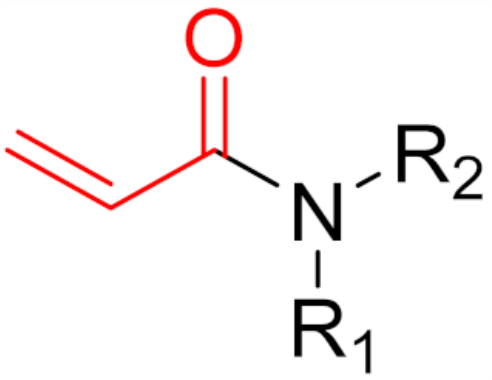
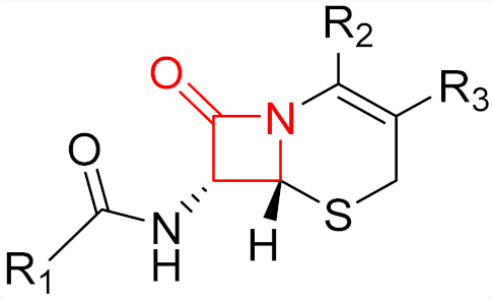
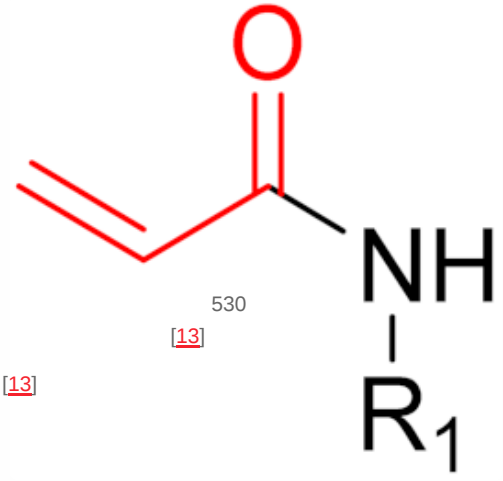
Figure 3. The significant increase in publications associated with covalent inhibitor development in the years from 1950 to 2022. The graphs show the number of publications with the keywords of 'covalent drug' (**top**) or 'inhibitor covalent' (**bottom**) in the SciFinder portal over the last 72 years.

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

Year	Name of Drug	Warhead	Function
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
2013	Dimethyl fumarate (α,β-unsaturated carbonyl)		Immunomodulatory drug

Year	Name of Drug	Warhead	Function
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) [10][12]	 [13]	EGFR tyrosine kinase inhibitor [10]

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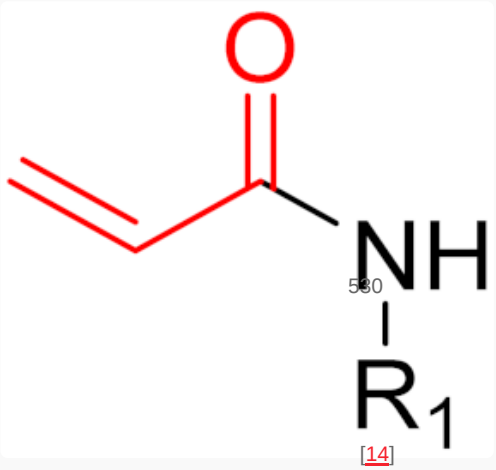
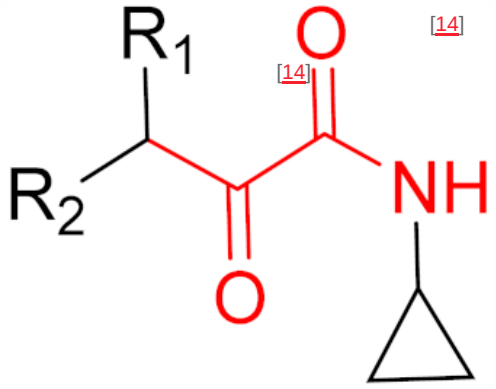
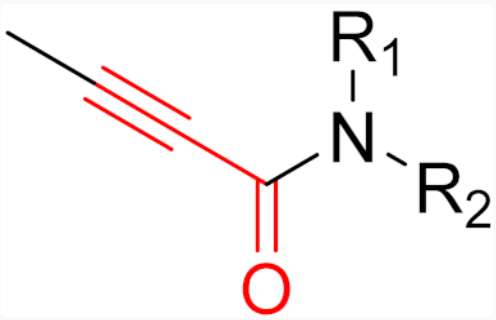
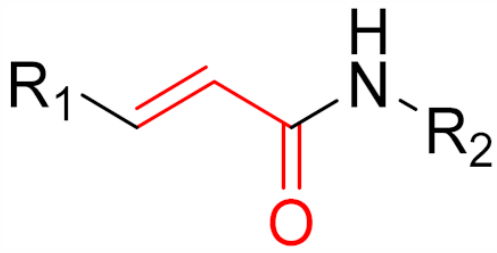
Year	Name of Drug	Warhead	Function
2015	Olmutinib (α,β-unsaturated carbonyl) [14]	 [14]	EGFR tyrosine kinase inhibitor
2016	Narlaprevir (α-ketoamide)	 [14]	HCV protease inhibitor
2017	Acalabrutinib (α,β-unsaturated propargylamide)		Bruton's tyrosine kinase inhibitor

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

Year	Name of Drug	Warhead	Function
2017	Neratinib [15][16]		EGFR tyrosine kinase inhibitor [16]
	(α,β-unsaturated carbonyl) [16]		

[17]

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
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	• 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

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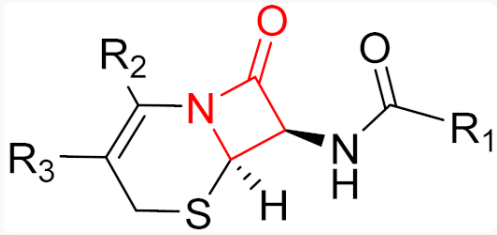
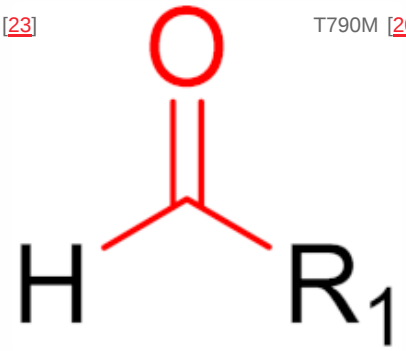
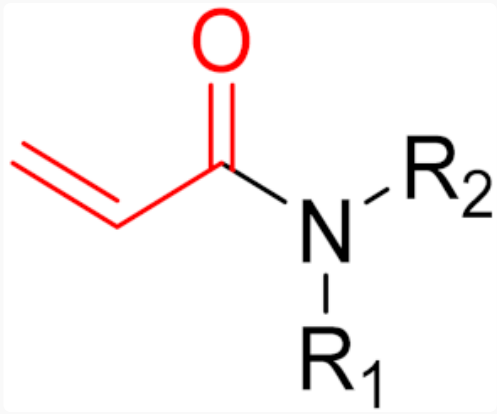
Type of Inhibitor	Advantages	Disadvantages
[18]	• Binding properties can be influenced by the choice of warhead (i.e., reversible or irreversible)	[18][19]
2019 (α,β-unsaturated carbonyl)	 [20]	inhibitor [20]
2019 [21][22]	Cerfiderocol [20] (β-lactam)	 [22]	β-lactam antibiotic
2019	Voxelotor (aldehyde)	 [23]	Hemoglobin oxygen-affinity modulator
2021	Sotorasib (α,β-unsaturated carbonyl)		KRAS G12C inhibitor

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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

Year	Name of Drug	Warhead	Function
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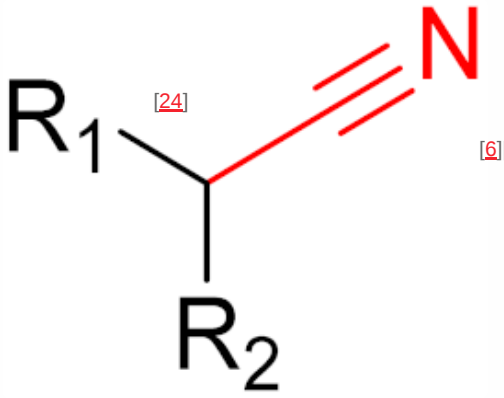
2021	Nirmatrevir	 [24]	SARS-CoV-2 main protease inhibitor [6][25]
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receptor P2Y12 [26][27]. P2Y12 is a member of the inhibitory G-protein-coupled purine receptor family and promotes platelet aggregation [27][28]. In the human body, clopidogrel is metabolized 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].

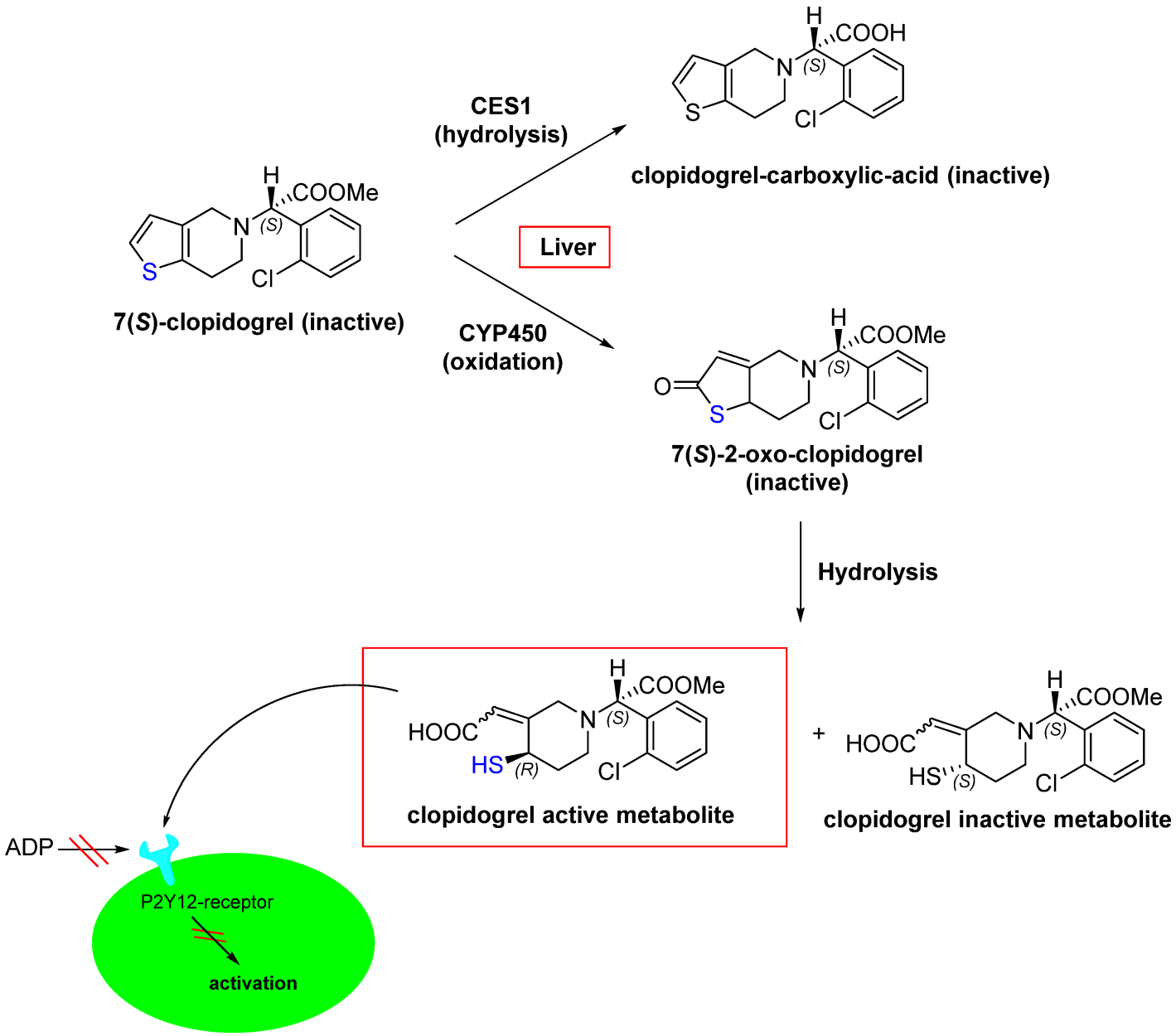

Figure 7. The metabolic pathway of clopidogrel (in)activation, considering the configuration required for the covalent inhibition of the P2Y12-receptor. CES1 (carboxylesterase 1) catalyzes the hydrolysis of 7(S)-clopidogrel into clopidogrel carboxylic acid, which is inactive. Cytochromes P450 (CYP450) 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].

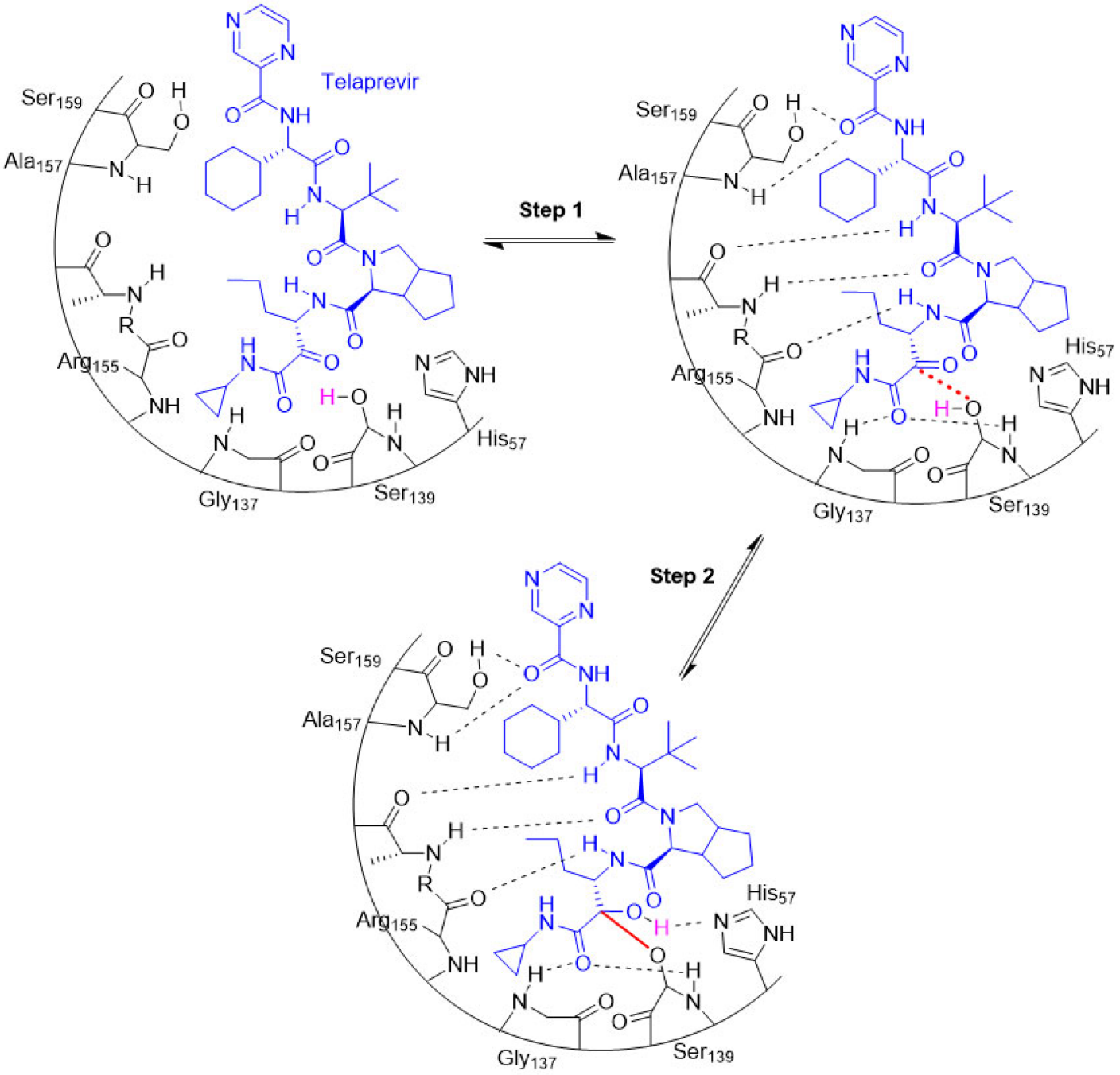

Figure 8. An illustration of the entire two-step process (i.e., association and bond formation) using telaprevir, the example HCV protease inhibitor. Telaprevir inhibits the viral NS3.4A protease of the hepatitis C virus.

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.

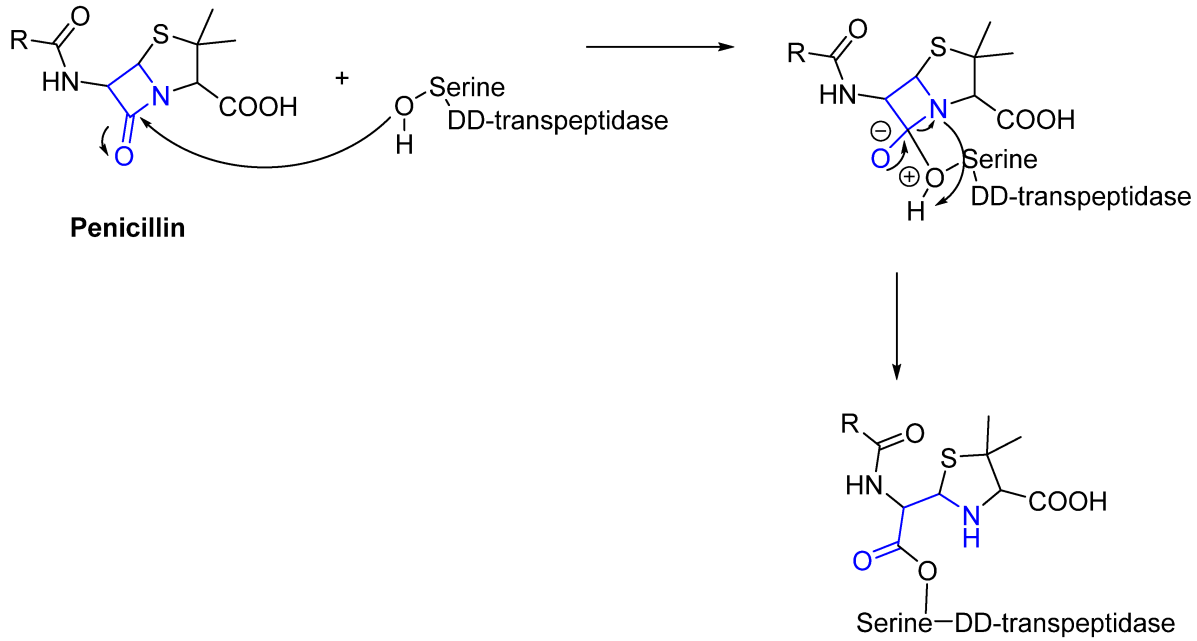

Figure 9. The mechanism of action of the irreversible inhibition of DD-transpeptidase by penicillin. The warhead of penicillin (β-lactam; highlighted in blue) reacts with the serine side chain of DD-transpeptidase.

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].

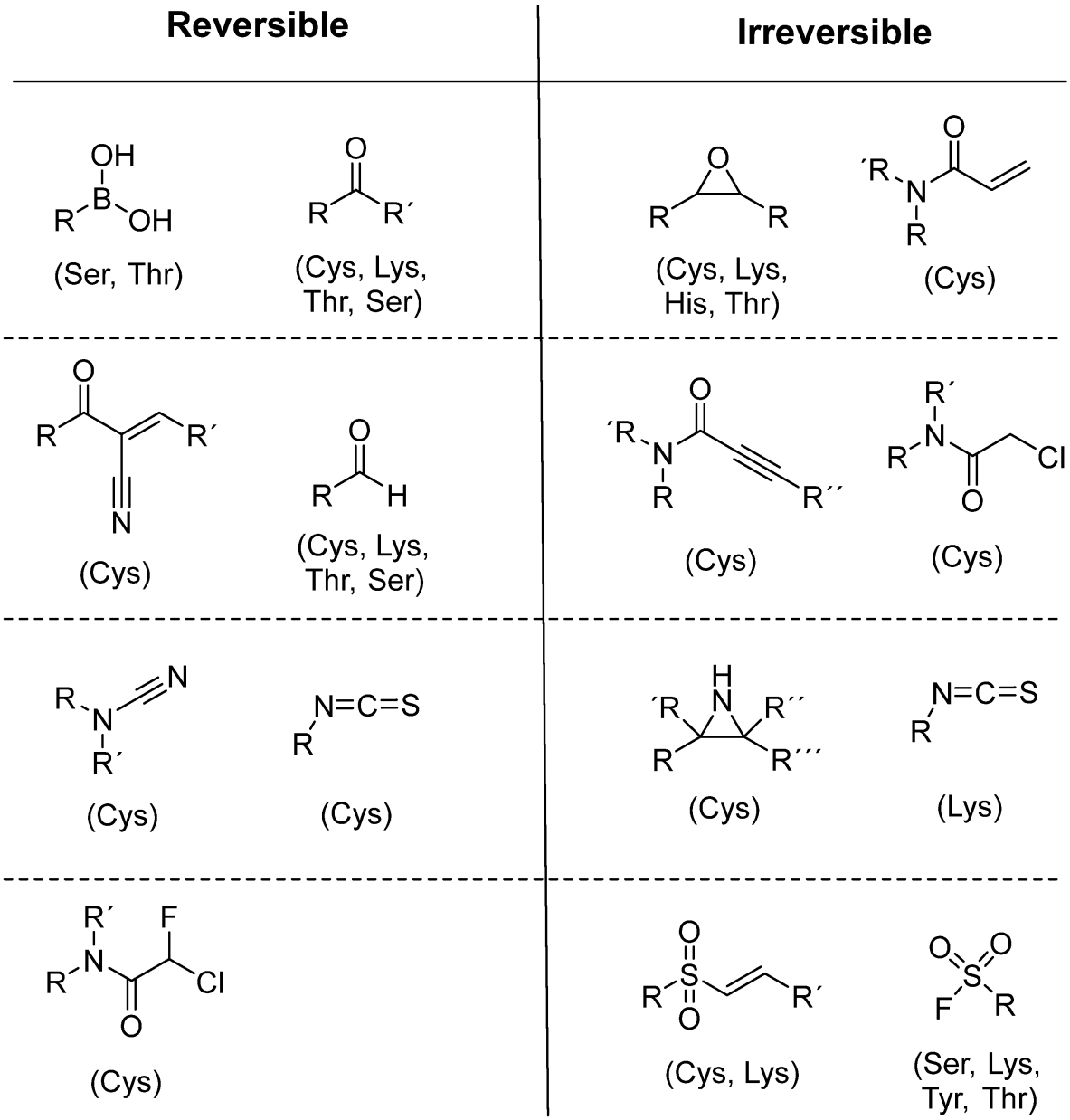

Figure 10. Warheads form irreversible and reversible bonds. The primary targeting amino acid residues are shown in brackets below the respective structures [37][38].

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 oncology 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, USA, 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, 137, 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 pitfalls. 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 Saxagliptin. 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 covalent 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, 889–906.

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, 786–792.
19. Nakamura, Y.; Oka, M.; Soda, H.; Shiozawa, K.; Yoshikawa, M.; Itoh, A.; Ikegami, Y.; Tsurutani, J.; Nakatomi, K.; Kitazaki, 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 lung 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.