International Journal of Pharmaceutical Research and Development

ISSN Print: 2664-6862
ISSN Online: 2664-6870
Impact Factor: RJIF 8.55
IJPRD 2025; 7(2): 209-215
www.pharmaceuticaljournal.net
Received: 20-06-2025
Accepted: 24-07-2025

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Modern perspectives on covalent and reversible covalent inhibitors: From selectivity to clinical translation

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DOI: https://www.doi.org/10.33545/26646862.2025.v7.i2c.181

Abstract
Covalent inhibitors have re-emerged as powerful agents in drug discovery, offering the advantages of high potency, sustained target engagement, and the potential to overcome resistance mechanisms. Traditionally viewed with caution due to concerns over off-target reactivity and toxicity, covalent drugs are now recognized as transformative therapeutic options, particularly in oncology, virology, and immunology. The discovery of selective electrophilic warheads and advances in computational chemistry have enabled precise targeting of nucleophilic amino acid residues such as cysteine, lysine, and serine. In parallel, the development of reversible covalent inhibitors has introduced a new dimension to drug design by combining durable binding with controllable pharmacodynamics, exemplified by recent breakthroughs in KRAS G12C inhibitors. This review summarizes the mechanistic principles, chemical strategies, and clinical progress of both irreversible and reversible covalent inhibitors. Special emphasis is placed on warhead innovations, resistance management, and the integration of structural and chemo proteomic tools in guiding selectivity. With several FDA-approved covalent drugs and an expanding pipeline of clinical candidates, covalent and reversible covalent inhibitors are poised to redefine the landscape of targeted therapeutics.

Keywords: Allosteric sites, covalent inhibitors, electrophilic warheads, kinase inhibitors, reversible covalent inhibition, targeted drug discovery

1. Introduction
Covalent inhibitors have long been a part of medicinal chemistry, with early examples such as aspirin and penicillin demonstrating the therapeutic value of irreversible enzyme modification [1, 2]. However, for several decades, covalent drugs were met with skepticism due to concerns about off-target reactivity, immunogenicity, and toxicity [3]. In recent years, advances in chemical biology and structural characterization have led to a paradigm shift, establishing covalent inhibition as a rational and controllable strategy in modern drug discovery [4]. The core principle of covalent inhibition lies in the design of electrophilic warheads that react with nucleophilic amino acid residues, most commonly cysteine, within the active or allosteric sites of target proteins [5]. This irreversible interaction often translates into enhanced potency, prolonged pharmacodynamic effects, and the ability to overcome resistance mutations that weaken non-covalent binding [6]. Beyond cysteine, recent innovations have expanded the repertoire to lysine, tyrosine, serine, and threonine residues, thereby broadening the scope of ligandable proteins [7]. A notable advancement is the emergence of reversible covalent inhibitors, which combine the benefits of durable target engagement with improved safety by allowing dissociation over time [8]. Such inhibitors are exemplified by KRAS G12C modulators (sotorasib and adagrasib) that achieve selective and reversible targeting of a historically "undruggable" oncogene [9, 10]. This approach has gained traction in oncology, virology, and immunology, where resistance to traditional therapies remains a major challenge [11]. The resurgence of covalent drug design has been supported by powerful tools such as chemo proteomics, which enables mapping of reactive amino acids across the proteome, and computational modelling, which predicts covalent binding kinetics and selectivity [12, 13].

Moreover, the integration of structural biology methods, particularly cryo-electron microscopy and high-resolution X-ray crystallography, has allowed precise visualization of covalent interactions at the atomic level [14]. Currently, several covalent inhibitors have received FDA approval, including ibrutinib (BTK inhibitor), osimertinib (EGFR inhibitor), and sotorasib (KRAS G12C inhibitor), while many others are progressing through clinical development [15-17]. This review provides a comprehensive overview of covalent and reversible covalent inhibitors in drug discovery.

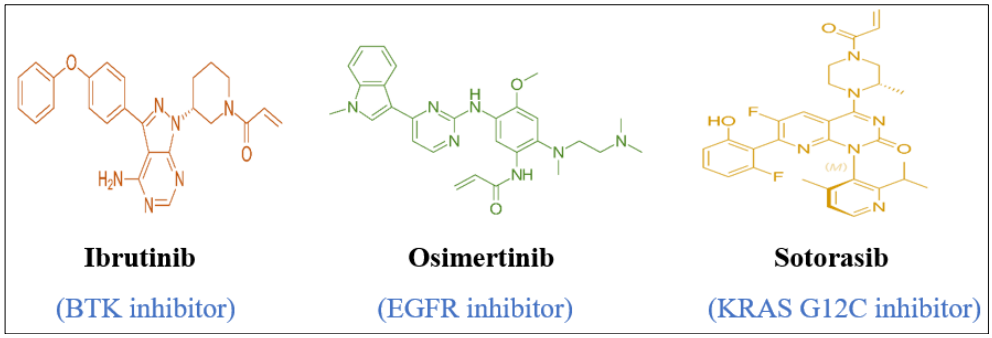

Fig 1: Structures of FDA-approved covalent inhibitors [15-17]

2. Fundamentals of Covalent Inhibition

Covalent inhibition relies on the ability of a small molecule to form a stable, often irreversible, bond with a target protein through a chemically reactive functional group. This strategy distinguishes covalent inhibitors from classical non-covalent drugs, which rely solely on reversible interactions such as hydrogen bonding, hydrophobic contacts, and electrostatics [18]. The covalent interaction not only enhances binding affinity but also prolongs pharmacological activity by ensuring sustained target occupancy, even after the free drug has been cleared from systemic circulation [19].

2.1 Mechanism of Covalent Bond Formation

Most covalent inhibitors are designed to undergo a two-step binding process: an initial non-covalent recognition event positions the electrophilic warhead in proximity to a nucleophilic amino acid side chain, followed by a chemical reaction that generates the covalent bond [20]. This two-step mechanism contributes to high selectivity, since productive covalent modification typically requires precise alignment within the protein active site [21].

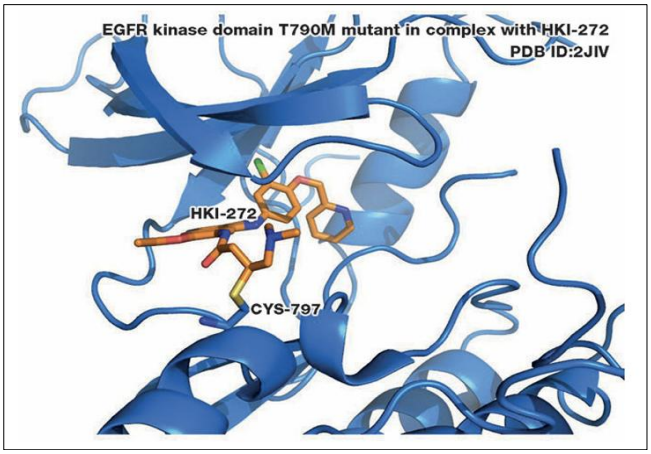

Fig 2: Targeted covalent inhibitors (TCIs), such as HKI-272 (shown in the picture in complex with a protein), first form a reversible association and then a covalent bond between an electrophile on the ligand and a nucleophilic center in the protein. This can offer benefits such as high potency and extended duration of action, and new approaches can limit the risk of serious adverse reactions [21]

2.2 Electrophilic Warheads in Drug Design

The choice of electrophilic warhead is central to covalent inhibitor design. Traditional warheads such as acrylamides have been widely employed for cysteine targeting in kinase inhibitors, while aldehydes and boronic acids are preferred in protease and proteasome inhibitors [22]. More recently, innovative chemotypes including sulfonyl fluorides, nitriles, and fluorosulfates have been introduced to expand residue selectivity and fine-tune reactivity [23, 24]. These warheads allow medicinal chemists to modulate covalent binding kinetics, minimizing off-target reactivity while retaining potency.

2.3 Target Amino Acid Residues

Covalent drugs have historically focused on cysteine residues, given their high nucleophilicity and relative rarity in the proteome, which improves selectivity [25]. However, advances in chemo proteomics and warhead chemistry have enabled targeting of other nucleophilic residues such as lysine, tyrosine, serine, and threonine [26].

2.4 Advantages and Limitations

Covalent inhibition offers several advantages:

- High potency through irreversible target engagement.
- Extended pharmacodynamic duration, often allowing less frequent dosing.
- Potential to overcome resistance mutations, especially in kinases [27].

Nonetheless, challenges remain, particularly regarding off-target reactivity, immunogenicity from hapten formation, and difficulty in predicting covalent reactivity across biological contexts [28].

3. Reversible vs. Irreversible Covalent Inhibitors

Covalent inhibitors can be broadly categorized into irreversible and reversible types, depending on whether the covalent bond formed with the protein target is permanent or can dissociate under physiological conditions.

3.1 Irreversible Covalent Inhibitors

Irreversible inhibitors form a stable covalent bond that permanently inactivates the target protein. Their efficacy is driven by high potency and sustained pharmacological activity, often resulting in prolonged therapeutic effects even after the drug is cleared from plasma [29]. This feature allows

less frequent dosing and effective suppression of rapidly regenerating targets. A landmark example is ibrutinib, an irreversible inhibitor of Bruton's tyrosine kinase (BTK), approved for B-cell malignancies. Ibrutinib contains an acrylamide warhead that forms a covalent bond with Cys481 in BTK, leading to durable kinase inactivation [30]. Similarly, osimertinib, a third-generation epidermal growth factor receptor (EGFR) inhibitor, irreversibly targets the cysteine residue at position 797, enabling treatment of T790M-mutant non-small cell lung cancer (NSCLC) [31]. These drugs illustrate how irreversible covalent inhibition can overcome resistance mutations that limit the efficacy of first-generation non-covalent inhibitors. Despite these advantages, irreversible inhibitors are associated with safety concerns, such as off-target modification, hapten-driven immune reactions, and potential long-term toxicity due to permanent protein inactivation [32]. Therefore, their design requires careful optimization of electrophilic reactivity to achieve selectivity.

3.2 Reversible Covalent Inhibitors

Reversible covalent inhibitors represent a more recent innovation, designed to form a covalent bond that is kinetically stable yet thermodynamically reversible under physiological conditions [33]. This balance allows strong and durable binding without permanent inactivation, reducing the risk of cumulative toxicity. A breakthrough in this class is the development of KRAS G12C inhibitors such as sotorasib (AMG 510) and adagrasib (MRTX849). These drugs selectively target the mutant cysteine at codon 12 of KRAS, forming a reversible covalent bond with the cysteine thiol group, thereby locking KRAS in its inactive GDP-bound state [34, 35]. Importantly, these inhibitors spare wild-type KRAS and other isoforms, achieving high selectivity that was historically unattainable for KRAS-targeted therapies. Reversible covalent inhibition has also been applied in kinase drug design, with warheads such as cyan acrylamides enabling tunable residence times on cysteine-containing kinases [36]. This approach has opened opportunities for targeting proteins previously considered "undruggable" while maintaining a favourable therapeutic index.

4. Chemical Strategies and Warhead Innovations

Over the past decade, medicinal chemistry has shifted from "highly reactive, non-specific modifiers" to tuned electrophiles that achieve controlled reactivity, broadening the therapeutic applications of covalent drugs.

4.1 Traditional Electrophilic Warheads

The most widely used electrophile in approved covalent drugs is the acrylamide group, which has been successfully employed in kinase inhibitors such as ibrutinib and osimertinib [37]. Acrylamides provide a balance between stability under physiological conditions and sufficient reactivity toward thiols, making them well-suited for targeting cysteine residues.

Other established warheads include:

- Aldehydes, used in protease inhibitors such as boceprevir (HCV NS3 protease) and nirmatrelvir (SARS-CoV-2 main protease), forming reversible covalent hemiacetal adducts [38].
- Boronic acids, exemplified by bortezomib, a proteasome inhibitor that covalently binds the threonine hydroxyl group in the catalytic site [39].

These traditional warheads established the clinical feasibility of covalent inhibition and inspired subsequent innovations.

4.2 Next-Generation Warheads

Recent progress has expanded the chemical toolbox to include novel electrophiles with tunable reactivity:

- **Sulfonyl fluorides (SuFEx chemistry):** React with tyrosine, lysine, and serine residues with high selectivity [40]. Their moderate reactivity and stability in aqueous media make them promising for site-selective modification.
- **Nitriles and cyanacrylamides:** Exhibit reversible covalent binding, especially to cysteine residues in kinases [41].
- **Fluorosulfates and sulfonyl triazoles:** Emerging "privileged" warheads with broader residue selectivity, enabling modulation of less reactive nucleophiles [42].
- **Electrophilic fragments (fragment-based covalent drug discovery):** Weak warheads designed for proteome-wide screening of ligandable residues [43].

These innovations allow medicinal chemists to target a wider spectrum of amino acids beyond cysteine and tune covalent binding kinetics for safety.

4.3 Target Expansion beyond Cysteine

While cysteine remains the most exploited residue due to its high nucleophilicity, emerging warheads are enabling covalent modification of other amino acids:

- Lysine-targeting electrophiles (e.g., sulfonyl triazoles, fluorosulfates) have been applied in bromodomain inhibitors [44].
- Tyrosine-directed warheads (e.g., aryl fluorosulfates) exploit the unique positioning of phenolic hydroxyls in protein binding sites [45].
- Serine/threonine electrophiles (e.g., carbamates, β-lactams) continue to play central roles in protease inhibitors and antibiotics [46].

Such diversification expands the "ligandable proteome," allowing access to previously intractable drug targets.

4.4 Reversible Covalent Warheads

The introduction of reversible covalent warheads represents a significant innovation, enabling tunable residence times and improved safety profiles. For example:

- Cyanoacrylamides enable reversible cysteine targeting in kinases, with controlled dissociation under physiological conditions [47].
- Ketoamides and nitriles form reversible covalent interactions in viral protease inhibitors such as nirmatrelvir [48].
- Boronic esters and aldehydes have shown reversible engagement of catalytic residues in proteasomes and proteases [49].

These designs combine the potency of covalent binding with the safety of reversibility, driving a new generation of clinical candidates.

4.5 Design Considerations for Warhead Selection

When designing warheads, several factors must be balanced:

1. **Reactivity:** Must be sufficient to engage the target residue but low enough to avoid nonspecific reactions.

2. **Selectivity:** Enhanced by positioning within a pre-organized binding pocket.
3. **Reversibility:** Increasingly favoured to reduce toxicity risk.
4. **Stability:** Should withstand metabolic degradation until reaching the target.

5. Therapeutic Applications

The clinical success of covalent inhibitors demonstrates their broad utility across multiple therapeutic areas. Recent advances have expanded their application beyond traditional oncology targets into viral, autoimmune, and metabolic diseases, while reversible covalent inhibitors are opening safer, tunable modalities.

5.1 Oncology

Cancer remains the largest therapeutic domain for covalent inhibitors, particularly due to the prevalence of oncogenic kinases with nucleophilic cysteine residues near the ATP-binding pocket.

- **BTK inhibitors:** *Ibrutinib* was the first covalent BTK inhibitor approved for B-cell malignancies, followed by next-generation agents such as *acalabrutinib* and *zanubrutinib*, designed with improved selectivity [50, 51].
- **EGFR inhibitors:** *Osimertinib* is a third-generation EGFR inhibitor approved for NSCLC patients harboring the T790M resistance mutation, exploiting covalent binding to Cys797 [52].
- **KRAS G12C inhibitors:** *Sotorasib* and *adagrasib* represent breakthrough covalent inhibitors targeting the previously "undruggable" KRAS G12C mutant, validated in lung and colorectal cancers [53].

These examples underscore the ability of covalent inhibitors to address resistance mutations and intractable oncogenes.

5.2 Viral Infections

Covalent inhibitors have proven indispensable in antiviral therapy, particularly for viral proteases:

- HCV NS3/4A protease inhibitors such as *boceprevir* and *telaprevir* exploit reversible covalent ketoamide warheads [54].
- HIV protease inhibitors with covalent modifications were among early proofs-of-concept, though limited by toxicity [55].
- Most recently, SARS-CoV-2 Mpro inhibitors, including *nirmatrelvir (Paxlovid)*, employ reversible covalent nitrile warheads, demonstrating rapid adaptability of this approach in pandemic response [56].

The clinical success of *nirmatrelvir* highlights the renewed relevance of covalent strategies for emerging viral diseases.

5.3 Autoimmune and Inflammatory Disorders

Covalent inhibitors of kinases and signalling proteins are increasingly used in autoimmune indications:

- *Ibrutinib* and *acalabrutinib* are now approved for rheumatoid arthritis and lupus clinical studies due to their role in B-cell signalling [57].
- Covalent JAK inhibitors and covalent RIPK1 inhibitors are under development for inflammatory disorders [58].
- Targeting covalent IRAK4 inhibitors has shown promise in autoimmune diseases such as lupus and psoriasis [59].

The precision and durability of covalent binding are particularly valuable in chronic inflammatory conditions.

5.4 Neurological Disorders

Emerging evidence suggests that reversible covalent inhibitors could address CNS diseases where sustained modulation with reduced off-target risk is critical:

- Reversible covalent BACE1 inhibitors for Alzheimer's disease have been explored, aiming to reduce toxicity compared to irreversible inhibitors [60].
- Electrophilic fragment screening has identified covalent ligands for neurodegeneration-related enzymes such as Parkin and DJ-1, opening new directions [61].

While still in early stages, these approaches illustrate the expanding therapeutic frontiers of covalent chemistry.

5.5 Antibacterial and Antiparasitic Applications

Covalent mechanisms remain central in anti-infective pharmacology:

- β-lactam antibiotics (penicillins, cephalosporins, carbapenems) act as covalent inhibitors of bacterial transpeptidases [62].
- Oxazolidinones and fosfomycin use covalent mechanisms against bacterial enzymes [63].
- Novel covalent inhibitors of malaria proteases and kinases are under exploration [64].

These demonstrate that covalent inhibition remains a cornerstone in infectious disease therapy.

6. Clinical Successes and Case Studies

The clinical validation of covalent and reversible covalent inhibitors has reshaped drug discovery, transitioning the concept from a risk-prone strategy into a mainstream therapeutic paradigm. A growing number of drugs across therapeutic classes have demonstrated safety, efficacy, and durability, with many advancing through clinical trials and receiving regulatory approval.

6.1 FDA-Approved Covalent Inhibitors

Several covalent inhibitors are already approved by the FDA and widely used in clinical practice:

- **Ibrutinib:** The first-in-class covalent BTK inhibitor, approved for chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). Its approval paved the way for covalent kinase inhibitors [65].
- **Osimertinib:** An irreversible EGFR inhibitor approved for T790M-positive NSCLC, demonstrating clinical success in overcoming resistance to first- and second-generation EGFR inhibitors [66].
- **Afatinib and Dacomitinib:** Earlier EGFR covalent inhibitors that remain in clinical use for NSCLC [67].
- **Sotorasib and Adagrasib:** First-in-class KRAS G12C covalent inhibitors, marking a historic breakthrough in targeting the "undruggable" RAS family [68, 69].
- **Nirmatrelvir (Paxlovid):** A reversible covalent SARS-CoV-2 main protease inhibitor, rapidly developed and authorized for COVID-19 treatment [70].

These approvals illustrate the broad clinical applicability of covalent inhibition, spanning oncology, virology, and beyond.

6.2 Drugs in Late-Stage Clinical Trials

Several covalent inhibitors are in phase II/III trials, showing promising results:

- **Orelabrutinib and Tirabrutinib:** Next-generation BTK inhibitors designed with improved selectivity and reduced off-target toxicity [71].
- **Remibrutinib:** A reversible covalent BTK inhibitor in trials for multiple autoimmune disorders, demonstrating the therapeutic potential of reversible covalent chemistry [72].
- **RIPK1 inhibitors (e.g., GSK2982772):** Under evaluation for inflammatory and fibrotic diseases [73].
- **IRAK4 inhibitors:** Advancing in clinical trials for rheumatoid arthritis and lupus [74].

These candidates highlight how covalent chemistry is expanding beyond oncology into immunology and chronic inflammatory diseases.

6.3 Case Studies of Clinical Translation

6.3.1 BTK Inhibitors

Ibrutinib's success transformed the perception of covalent drugs. However, resistance mutations (C481S) in BTK spurred the development of second-generation agents such as acalabrutinib and zanubrutinib [75].

6.3.2 EGFR Inhibitors

While first-generation EGFR inhibitors showed initial efficacy, resistance mutations such as T790M and C797S necessitated Osimertinib's design, demonstrating the power of covalent targeting to overcome resistance [76].

6.3.3 KRAS Inhibitors

KRAS G12C inhibitors (sotorasib and adagrasib) represented a landmark achievement in oncology, with durable responses in NSCLC and colorectal cancer patients. Their development validated covalent inhibition as a solution for "undruggable" oncogenes [77].

6.3.4 Antiviral Success

The rapid development of nirmatrelvir against SARS-CoV-2 highlights how reversible covalent inhibitors can accelerate drug discovery during global health emergencies [78].

7. Conclusion

Covalent and reversible covalent inhibitors have redefined modern drug discovery by offering high potency, durable target engagement, and the potential to overcome resistance mechanisms that limit conventional therapies. Advances in warhead chemistry, structural biology, and chemo proteomics have expanded the scope of druggable targets beyond cysteine, enabling broader therapeutic applications. Looking forward, the integration of reversible covalent designs, AI-driven drug discovery, and proteome-wide screening will help address current challenges of selectivity, off-target toxicity, and resistance. Collectively, these strategies position covalent and reversible covalent inhibitors as powerful tools in the next generation of precision medicines, with promise extending well beyond oncology into infectious, autoimmune, and neurodegenerative diseases.

8. Acknowledgment

The authors express their sincere gratitude to the Management of Nargund College of Pharmacy and the Department of Pharmaceutical Chemistry for their continuous support and encouragement throughout this work.

9. Conflict of interest

The authors declare no conflict of interest.

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