International Organization of Research & Development (IORD)
ISSN: 2348-0831
Vol 13 Issue 01 | 2025

Covalent Fragment-Based Drug Discovery: Mechanistic Approaches for Target Engagement and Reactivity Profiling

Nisha (Ph. D Research Scholar), Dr. Vinita Kumari (Associate Professor)
Department – Chemistry, Shri Jagdish Prasad Jhabarmal Tibrewala University, Chudela, Jhunjhunu

DOI: 10.5281/zenodo.19814078

ABSTRACT

Covalent fragment-based drug discovery has emerged as a significant strategy in modern medicinal chemistry because it combines the advantages of fragment-based screening with the durable target engagement offered by covalent binding. Traditional fragment discovery identifies small, low-molecular-weight compounds that interact weakly with biological targets, while covalent fragment strategies incorporate electrophilic groups capable of forming reversible or irreversible bonds with nucleophilic amino acid residues. This approach is valuable for identifying ligandable pockets, discovering new binding sites, and developing selective inhibitors against proteins that are difficult to modulate through conventional non-covalent approaches. The success of this strategy depends not only on binding affinity but also on mechanistic understanding of reactivity, residue selectivity, target engagement, residence time, and off-target risk.

The present article examines the mechanistic foundations of covalent fragment-based drug discovery with special emphasis on target engagement and reactivity profiling. It discusses the conceptual basis of covalent fragments, electrophilic warheads, protein nucleophiles, kinetic parameters, screening methods, and validation techniques. The article also highlights the role of mass spectrometry, chemoproteomics, crystallography, activity-based probes, thermal shift assays, and computational modeling in evaluating covalent fragment behavior. It argues that an effective covalent fragment program requires a careful balance between chemical reactivity and biological selectivity. Highly reactive fragments may produce non-specific binding, whereas overly weak electrophiles may fail to engage the target. Therefore, mechanistic profiling becomes essential for transforming preliminary fragment hits into reliable lead compounds. The article concludes that covalent fragment-based drug discovery offers considerable promise for drugging challenging targets, provided that chemical design is supported by rigorous mechanistic evaluation, safety assessment, and systematic optimization.

Keywords:Covalent fragments; Fragment-based drug discovery; Target engagement; Reactivity profiling; Electrophilic warheads; Chemoproteomics; Covalent inhibitors; Medicinal chemistry

1. INTRODUCTION

Drug discovery has experienced substantial methodological change in recent decades due to the increasing need for selective, potent, and mechanistically well-characterized therapeutic agents. Conventional high-throughput screening often relies on large compound libraries and biological readouts to identify molecules with measurable activity. Although this approach has produced many successful candidates, it can be limited when targets possess shallow pockets, weak binding surfaces, or dynamic conformations. Fragment-based drug discovery was developed as a more efficient alternative that begins with small chemical entities rather than large drug-like molecules. These fragments interact with targets through limited but efficient contacts, allowing researchers to explore chemical space more economically and identify starting points for rational optimization.

Covalent fragment-based drug discovery extends this logic by introducing electrophilic functionality into fragment molecules. Such fragments can form covalent bonds with nucleophilic residues such as cysteine, serine, lysine, tyrosine, histidine, or threonine in protein environments. The covalent interaction can stabilize weak fragment binding and provide direct evidence of target engagement. This makes covalent fragments especially useful for mapping ligandable sites, identifying cryptic pockets, and interrogating proteins previously considered difficult to target. The method has gained attention in kinase research, oncology, infectious disease research, protein-protein interaction studies, and chemical biology.

The value of covalent fragment discovery, however, depends on a delicate balance. A fragment must be sufficiently reactive to engage an appropriate protein residue, but not so reactive that it binds indiscriminately to many proteins. Covalent bond formation alone is not sufficient evidence of drug-like promise. A meaningful covalent hit should display measurable target engagement, context-dependent selectivity, rational structure-activity relationships, and a reactivity profile compatible with safety and pharmacological development. For this reason, mechanistic evaluation is central to the field. Researchers must determine how the fragment binds,

which residue is modified, how rapidly modification occurs, whether the reaction is reversible, and whether the same fragment reacts with unwanted proteins.

Target engagement and reactivity profiling are therefore two central pillars of covalent fragment-based drug discovery. Target engagement confirms that a compound physically interacts with the intended protein in a biochemical, cellular, or proteomic context. Reactivity profiling determines whether that interaction is selective, controlled, and mechanistically appropriate. Together, these approaches help distinguish meaningful covalent fragments from artifacts caused by excessive electrophilicity, aggregation, assay interference, or non-specific protein modification. The present article provides a conceptual and analytical discussion of these mechanistic approaches and their relevance for covalent fragment development.

2. REVIEW OF LITERATURE

The literature on covalent drug discovery shows a clear shift from viewing covalent binding as undesirable to recognizing it as a powerful design strategy when controlled appropriately. Earlier concerns were linked to the possibility of irreversible protein modification, immune reactions, and toxicity. However, several clinically successful covalent drugs demonstrated that covalent mechanisms can be safe and effective when reactivity is tuned and target selectivity is achieved. This created renewed interest in electrophilic warheads, covalent inhibitors, and targeted residue engagement.

Fragment-based drug discovery has also developed as a mature approach in medicinal chemistry. Its strength lies in the use of small molecules with high ligand efficiency that can be grown, linked, or merged into stronger binders. Non-covalent fragments often display weak affinities, requiring sensitive techniques such as NMR, X-ray crystallography, surface plasmon resonance, or thermal shift assays. Covalent fragments overcome part of this limitation by producing a more durable signal through residue modification. This allows researchers to detect weak but meaningful binding events that might otherwise be missed.

Recent studies emphasize that electrophilic warheads must be evaluated not simply by chemical reactivity but by context-dependent biological behavior. Acrylamides, chloroacetamides, sulfonyl fluorides, nitriles, boronic acids, cyanoacrylamides, and other emerging warheads differ significantly in intrinsic reactivity, reversibility, residue preference, and protein microenvironment sensitivity. The same warhead may behave differently depending on pocket polarity, residue pKa, neighboring amino acids, solvent exposure, and conformational flexibility. Therefore, mechanistic evaluation is necessary for understanding why a fragment reacts with one residue but not another.

The literature also highlights the growing role of chemoproteomics in covalent fragment discovery. Instead of studying only one purified protein, chemoproteomic methods allow researchers to assess fragment reactivity across a proteome. Competitive activity-based protein profiling and mass spectrometry-based workflows can identify proteins and specific residues modified by covalent compounds. These methods help evaluate selectivity, discover new targets, and detect off-target liabilities at an early stage. Such approaches are particularly important because covalent fragments are intentionally reactive and must be tested against broader biological complexity.

Overall, existing scholarship suggests that covalent fragment discovery is most successful when chemistry, biology, and analytical technology are integrated. Hit identification must be followed by residue mapping, kinetic measurement, structural validation, cellular target engagement, and comparative reactivity assessment. The field is moving away from simple covalent screening toward mechanistically informed design. This article builds on that direction by presenting target engagement and reactivity profiling as essential components of a reliable covalent fragment discovery workflow.

3. OBJECTIVES OF THE STUDY

- To explain the conceptual foundation of covalent fragment-based drug discovery in modern medicinal chemistry.
- To analyze the role of electrophilic warheads and protein nucleophiles in covalent target engagement.
- To examine key mechanistic approaches used for validating target engagement in biochemical and cellular systems.
- To discuss reactivity profiling methods for evaluating selectivity, intrinsic electrophilicity, and off-target risk.
- To highlight the importance of mass spectrometry, chemoproteomics, structural biology, and computational modeling in covalent fragment evaluation.
- To identify major challenges, limitations, and future research directions in the development of covalent fragment libraries.

4. RESEARCH METHODOLOGY

The present article is conceptual and analytical in nature. It is based on a qualitative synthesis of secondary sources related to covalent drug discovery, fragment-based screening, electrophilic warheads, chemoproteomics, target engagement assays, and medicinal chemistry optimization. The study does not involve primary experimental data; instead, it organizes and interprets established scientific concepts to present a coherent research-oriented discussion on mechanistic approaches used in covalent fragment discovery.

The methodology follows a descriptive and interpretive approach. The descriptive component explains major concepts such as covalent fragments, target engagement, warhead reactivity, ligand efficiency, reversibility, residue selectivity, and reaction kinetics. The interpretive component evaluates how these concepts are applied in practical drug discovery workflows. Special attention is given to mechanistic tools such as intact protein mass spectrometry, peptide mapping, activity-based protein profiling, cellular thermal shift assays, X-ray crystallography, kinetic assays, and molecular docking.

The analysis is organized around two central themes. First, it examines target engagement as a requirement for confirming that a covalent fragment interacts with the intended protein target. Second, it examines reactivity profiling as a requirement for determining whether the fragment is selectively and safely reactive. These two themes are then connected to broader questions of hit validation, lead optimization, off-target evaluation, and translational relevance. The article is limited by its conceptual character, but it provides a useful framework for scholars and researchers developing covalent fragment libraries or designing studies around electrophilic warheads.

Conceptual Basis of Covalent Fragment-Based Drug Discovery

Covalent fragment-based drug discovery is built on the principles of fragment-based design but introduces a reactive functional group capable of forming a covalent bond with a target protein. Fragments are generally small molecules with low molecular weight and limited structural complexity. Their small size allows them to sample chemical space efficiently and bind to pockets that may not accommodate larger molecules. When an electrophilic group is incorporated into such a fragment, it may form a covalent interaction with a suitably positioned nucleophilic residue in the binding site.

This strategy offers several advantages. First, it can enhance the detectability of weak interactions because covalent modification provides a stable analytical signal. Second, it can reveal ligandable residues and pockets that are not obvious from non-covalent screening. Third, it can support the development of potent inhibitors from small starting points. Fourth, it can provide prolonged target engagement, which may be pharmacologically useful when sustained inhibition is desirable. These features make covalent fragment discovery valuable for difficult targets such as protein-protein interaction interfaces, allosteric sites, mutant proteins, and enzymes with catalytic nucleophiles.

Nevertheless, covalent fragment discovery must be guided by chemical discipline. A fragment hit is not valuable merely because it reacts with a protein. It must react in a meaningful binding context. The ideal covalent fragment first recognizes the protein through non-covalent interactions and then positions its electrophilic warhead close enough to react with a specific residue. This two-step recognition mechanism improves selectivity. Without sufficient non-covalent recognition, a fragment may behave as a general reactive species rather than as a selective chemical probe or drug candidate.

Electrophilic Warheads and Protein Nucleophiles

Electrophilic warheads are reactive groups designed to form covalent bonds with nucleophilic amino acid residues. Commonly studied warheads include acrylamides, chloroacetamides, vinyl sulfones, sulfonyl fluorides, nitriles, aldehydes, ketones, boronic acids, cyanoacrylamides, and activated esters. Each warhead has a distinct reactivity profile. Some are irreversible and form stable adducts, while others are reversible and form bonds that can dissociate under certain conditions. The choice of warhead is therefore a central design decision.

Cysteine is the most frequently targeted residue because its thiol group is highly nucleophilic, especially when its pKa is lowered by the surrounding protein environment. However, cysteine is not the only targetable residue. Serine, lysine, tyrosine, threonine, histidine, and aspartate or glutamate side chains may also participate in covalent modification under appropriate conditions. The expansion beyond cysteine has increased the scope of covalent drug discovery, but it also requires more careful mechanistic analysis because residue reactivity can be more context-dependent.

A successful covalent fragment must match warhead reactivity with target residue chemistry. Highly reactive warheads may increase hit rates but also increase non-specific binding. Less reactive warheads may offer better selectivity but require stronger non-covalent positioning. Therefore, warhead selection involves balancing intrinsic chemical reactivity with target-guided proximity effects. This is why covalent fragments are often evaluated through both small-molecule reactivity assays and protein-based engagement assays.

Mechanistic Understanding of Target Engagement

Target engagement refers to direct evidence that a compound interacts with its intended biological target. In covalent fragment discovery, target engagement is usually confirmed by detecting protein modification or by measuring functional consequences of covalent binding. Because covalent fragments can produce strong analytical signals, target engagement can be evaluated through several complementary methods. However, the interpretation must be careful because covalent attachment can occur outside the functional binding site or through non-specific surface labeling.

Biochemical target engagement can be assessed using intact protein mass spectrometry, where a mass shift indicates covalent adduct formation. Peptide mapping can identify the modified residue after protein digestion. Kinetic assays can determine whether the compound modifies the protein in a time-dependent manner. Activity assays can measure whether modification leads to inhibition or functional modulation. Together, these methods help establish whether the fragment is merely reactive or mechanistically relevant.

Cellular target engagement is more complex because the compound must enter cells, remain stable, avoid excessive sequestration by cellular nucleophiles, and engage the intended target in a crowded proteomic environment. Methods such as cellular thermal shift assay, NanoBRET-based engagement assays, clickable probe labeling, and competitive chemoproteomics can help evaluate engagement under cellular conditions. A compound that modifies a purified protein but fails to engage the same target in cells may lack permeability, stability, selectivity, or sufficient residence time.

Reactivity Profiling and Selectivity Assessment

Reactivity profiling examines how readily and selectively a covalent fragment reacts under chemical and biological conditions. This is essential because covalent fragments contain electrophilic groups by design. A useful fragment should not simply be reactive; it should be appropriately reactive. Intrinsic reactivity can be evaluated using model nucleophiles such as glutathione, cysteine, or synthetic thiols. These assays provide an initial estimate of electrophilic strength, but they cannot fully predict protein selectivity because biological reactivity depends on pocket geometry and residue environment.

Protein-based reactivity profiling provides more meaningful information. A fragment may be tested against a panel of proteins or against a proteome to determine how many sites it modifies. Chemoproteomic profiling is especially valuable for this purpose. In competitive activity-based protein profiling, a covalent fragment competes with a broadly reactive probe for binding to nucleophilic residues. Reduced probe labeling indicates that the fragment has engaged those sites. Mass spectrometry then identifies the affected proteins and residues. This enables researchers to compare target engagement with off-target reactivity.

Selectivity assessment must consider both potency and specificity. A compound that strongly modifies many proteins may show activity in an assay but may be unsuitable for drug development. Conversely, a weakly reactive fragment that selectively modifies a biologically important site may represent a valuable starting point for optimization. Reactivity profiling therefore helps prioritize hits not merely by percentage modification but by mechanistic quality. The goal is to identify fragments whose covalent reactivity is guided by molecular recognition rather than uncontrolled electrophilicity.

Analytical Techniques for Covalent Fragment Evaluation

Mass spectrometry is one of the most important techniques in covalent fragment discovery. Intact protein mass spectrometry can rapidly detect whether a fragment has formed an adduct with a target protein. Tandem mass spectrometry after proteolytic digestion can localize the modified peptide and sometimes the exact residue. Time-course mass spectrometry can provide information about reaction progression and apparent modification rates. These features make mass spectrometry central for hit confirmation and mechanistic interpretation.

X-ray crystallography and cryo-electron microscopy can provide structural evidence for covalent binding. A crystal structure can show how the fragment occupies the binding pocket, how the warhead is oriented, and which non-covalent interactions stabilize the complex. Structural data are especially valuable for fragment growing, scaffold merging, and warhead repositioning. Without structural confirmation, optimization may proceed blindly and produce compounds with increased reactivity but not improved selectivity.

Thermal shift assays, surface plasmon resonance, isothermal titration calorimetry, and enzyme kinetics can also contribute to evaluation. Although some of these methods are more commonly associated with non-covalent binding, they can provide useful evidence when combined with covalent readouts. For example, a thermal shift may indicate stabilization of a target protein after fragment binding, while kinetic assays can measure time-dependent inhibition. No single method is sufficient in all cases; robust evaluation normally requires multiple orthogonal techniques.

Kinetic Parameters and Mechanistic Interpretation

Covalent inhibition is often described through kinetic parameters that combine reversible binding and covalent bond formation. The initial non-covalent association positions the electrophile near the nucleophilic residue, and the subsequent chemical step forms the covalent adduct. Parameters such as KI, kinact, and kinact/KI are used

to evaluate covalent inhibitor performance. KI reflects the reversible binding component, kinact reflects the rate of covalent bond formation, and kinact/KI represents overall covalent efficiency.

For covalent fragments, these parameters may be difficult to measure precisely because fragments are small and often weak. However, the conceptual framework remains important. A fragment with high kinact but poor binding may react broadly, while a fragment with moderate kinact and meaningful binding may be more selective. Optimization should therefore not aim only to increase reactivity. It should improve the alignment of binding interactions and warhead positioning so that covalent bond formation occurs preferentially at the target site.

Mechanistic interpretation also requires attention to reversibility. Reversible covalent fragments may offer safety advantages by reducing permanent off-target modification. Irreversible covalent fragments may be useful when long target residence time is therapeutically desirable. The choice depends on the target, disease context, pharmacokinetic behavior, and acceptable risk profile. Mechanistic studies help determine whether the covalent interaction supports the intended biological objective.

Computational Modeling in Covalent Fragment Discovery

Computational approaches support covalent fragment discovery by predicting binding poses, residue accessibility, warhead orientation, and potential reactivity. Molecular docking can be adapted for covalent bond formation, allowing researchers to evaluate whether a fragment can place its electrophilic center near a nucleophilic residue. Molecular dynamics simulations can examine whether the binding pose remains stable over time and whether the residue environment favors reaction. Quantum mechanical calculations can estimate warhead reactivity and reaction feasibility.

Computational modeling is particularly useful during library design. It can help identify targetable residues, prioritize fragments with favorable geometry, and avoid warheads that are mismatched with the selected residue. It can also support structure-guided optimization after an initial hit has been identified. However, computational predictions must be experimentally validated. Protein flexibility, solvent effects, cellular context, and unknown off-target interactions can limit purely in silico conclusions.

The strongest use of computational tools is therefore integrative. Modeling can generate hypotheses, guide synthesis, reduce unnecessary experiments, and interpret structural data, but final decisions should depend on biochemical and cellular evidence. In covalent fragment programs, computational insight becomes most valuable when combined with mass spectrometry, chemoproteomics, and structural biology.

5. CHALLENGES AND LIMITATIONS

Covalent fragment-based drug discovery faces several challenges. The first is non-specific reactivity. Because covalent fragments contain electrophilic groups, they may react with abundant cellular nucleophiles or unrelated proteins. This can create false positives and toxicity concerns. Reactivity profiling is necessary to distinguish selective target-guided modification from general chemical reactivity.

The second challenge is assay interference. Some electrophilic molecules may aggregate, degrade, react with assay components, or interfere with detection systems. A fragment may appear active in a biochemical assay for reasons unrelated to meaningful target engagement. Therefore, orthogonal validation methods are essential. Mass spectrometry, structural studies, and cellular assays help reduce the risk of misleading conclusions.

A third challenge is optimization complexity. Improving potency by increasing electrophilicity may reduce selectivity. Improving selectivity by reducing reactivity may reduce engagement. Adding substituents to improve binding may increase molecular weight and reduce fragment-like properties. The medicinal chemist must therefore optimize several variables simultaneously, including binding orientation, warhead positioning, physicochemical properties, cellular permeability, metabolic stability, and safety.

A fourth limitation is target dependence. Not every protein contains an appropriately positioned nucleophilic residue. Even when such a residue exists, it may not be accessible, functionally relevant, or suitable for selective modification. Covalent fragment discovery is most productive when target selection is informed by structural data, residue mapping, and biological rationale.

Future Perspectives

The future of covalent fragment-based drug discovery is likely to involve more selective warheads, broader residue targeting, and deeper integration with proteomics. Emerging electrophilic groups will expand the range of targetable amino acids and allow covalent strategies beyond cysteine-centric design. Reversible covalent chemistry may become increasingly important because it offers a balance between durable engagement and controlled safety. At the same time, improved chemoproteomic technologies will make it easier to evaluate selectivity at the proteome-wide level.

Artificial intelligence and machine learning may also support future development by predicting residue ligandability, fragment reactivity, and off-target profiles. Large datasets from covalent screening and chemoproteomics can help train models for library design. However, the value of such models will depend on

high-quality experimental data. Mechanistic interpretation will remain essential because covalent drug discovery cannot be reduced to prediction alone.

Another promising direction is the use of covalent fragments as chemical probes. Even when a fragment does not become a drug candidate, it may help identify functional residues, map allosteric sites, or validate new therapeutic targets. This chemical biology role is important because many disease-relevant proteins remain poorly understood. Covalent fragments can therefore contribute both to drug discovery and to fundamental biological investigation.

Suggested Mechanistic Workflow for Covalent Fragment Evaluation

Stage	Purpose	Preferred Approach
Library design	Select fragment scaffolds and warheads with controlled reactivity	Use fragment rules, warhead diversity, physicochemical filtering and target residue analysis
Primary screening	Identify fragments that modify or inhibit the target	Use intact protein MS, activity assays, thermal shift assays or covalent docking-guided screening
Hit confirmation	Remove false positives and confirm direct engagement	Apply dose-response testing, time-dependence analysis, competition assays and residue mapping
Reactivity profiling	Assess intrinsic and biological selectivity	Use glutathione/cysteine assays, protein panels and chemoproteomic profiling
Structural validation	Understand binding orientation and warhead positioning	Use X-ray crystallography, cryo-EM, covalent docking or molecular dynamics
Lead optimization	Improve potency, selectivity and drug-like properties	Optimize scaffold interactions, warhead placement, permeability and metabolic stability

6. SUGGESTIONS AND IMPLICATIONS

1. Researchers developing covalent fragment libraries should begin with carefully selected warheads of moderate and tunable reactivity rather than relying on highly reactive electrophiles that may generate non-specific hits.
2. Target selection should include structural and biological justification. Proteins with accessible, functionally relevant, and uniquely positioned nucleophilic residues are more suitable for covalent fragment strategies.
3. Hit confirmation should use multiple orthogonal techniques. A covalent mass shift should be supported by residue mapping, functional assays, structural evidence, or cellular engagement data whenever possible.
4. Reactivity profiling should be performed early in the workflow. Model nucleophile assays, protein panels, and chemoproteomics can help identify compounds with unacceptable promiscuity before extensive optimization is attempted.
5. Optimization should prioritize recognition-guided reactivity. Increasing covalent efficiency should come from better binding geometry and residue positioning, not merely from stronger electrophilicity.
6. Future academic and industrial studies should integrate synthetic chemistry, structural biology, proteomics, and computational modeling to improve reliability and translational relevance.

7. CONCLUSION

Covalent fragment-based drug discovery represents an important development in modern medicinal chemistry because it combines efficient exploration of chemical space with the mechanistic power of covalent target engagement. By using small fragments equipped with electrophilic warheads, researchers can detect weak binding events, identify ligandable pockets, and develop starting points for selective inhibitors or chemical probes. The approach is particularly valuable for challenging targets that are difficult to modulate through conventional non-covalent molecules.

The central requirement for success is mechanistic control. Covalent binding must be understood in terms of target engagement, residue specificity, reaction kinetics, reversibility, and proteome-wide selectivity. A fragment that reacts strongly but non-selectively is not a reliable lead. Conversely, a fragment with moderate reactivity and clear target-guided engagement may provide a strong foundation for lead development. Therefore, reactivity profiling is not an optional step but a core requirement of covalent fragment research.

The article has shown that methods such as mass spectrometry, chemoproteomics, crystallography, thermal shift assays, kinetic analysis, and computational modeling provide complementary insights into covalent fragment behavior. These tools help determine whether observed modification is biologically meaningful, structurally rational, and chemically selective. As the field advances, the integration of emerging warheads, reversible covalent chemistry, proteome-wide profiling, and data-driven design will likely strengthen the reliability of covalent fragment-based drug discovery. In conclusion, covalent fragments offer major opportunities, but their value depends on disciplined mechanistic evaluation and careful balance between reactivity and selectivity.

8. REFERENCES

[1] Baillie, T. A. (2016). Targeted covalent inhibitors for drug design. Angewandte Chemie International Edition, 55(43), 13408-13421.

[2] Backus, K. M., Correia, B. E., Lum, K. M., Forli, S., Horning, B. D., González-Páez, G. E., Chatterjee, S., Lanning, B. R., Teijaro, J. R., Olson, A. J., Wolan, D. W., & Cravatt, B. F. (2016). Proteome-wide covalent ligand discovery in native biological systems. Nature, 534, 570-574.

[3] Bauer, R. A. (2015). Covalent inhibitors in drug discovery: From accidental discoveries to avoided liabilities and designed therapies. Drug Discovery Today, 20(9), 1061-1073.

[4] Boike, L., Henning, N. J., & Nomura, D. K. (2022). Advances in covalent drug discovery. Nature Reviews Drug Discovery, 21, 881-898.

[5] Bollong, M. J., Lee, G., Coukos, J. S., Yun, H., Zambaldo, C., Chang, J. W., Chin, E. N., Ahmad, I., Chatterjee, A. K., Lairson, L. L., Schultz, P. G., & Moellering, R. E. (2018). A metabolite-derived protein modification integrates glycolysis with KEAP1-NRF2 signalling. Nature, 562, 600-604.

[6] Bradshaw, J. M., McFarland, J. M., Paavilainen, V. O., Bisconte, A., Tam, D., Phan, V. T., Romanov, S., Finkle, D., Shu, J., Patel, V., Ton, T., Li, X., Loughhead, D. G., Nunn, P. A., Karr, D. E., Gerritsen, M. E., Funk, J. O., Owens, T. D., Verner, E., Brameld, K. A., Hill, R. J., Goldstein, D. M., & Taunton, J. (2015). Prolonged and tunable residence time using reversible covalent kinase inhibitors. Nature Chemical Biology, 11, 525-531.

[7] Erlanson, D. A., Wells, J. A., & Braisted, A. C. (2004). Tethering: Fragment-based drug discovery. Annual Review of Biophysics and Biomolecular Structure, 33, 199-223.

[8] Gehringer, M., & Laufer, S. A. (2019). Emerging and re-emerging warheads for targeted covalent inhibitors: Applications in medicinal chemistry and chemical biology. Journal of Medicinal Chemistry, 62(12), 5673-5724.

[9] Keeley, A., Petri, L., Ábrányi-Balogh, P., & Keserű, G. M. (2020). Covalent fragment libraries in drug discovery. Drug Discovery Today, 25(6), 983-996.

[10] London, N., Miller, R. M., Krishnan, S., Uchida, K., Irwin, J. J., Eidam, O., Gibold, L., Cimermancic, P., Bonnet, R., Shoichet, B. K., & Taunton, J. (2014). Covalent docking of large libraries for the discovery of chemical probes. Nature Chemical Biology, 10, 1066-1072.

[11] Mullard, A. (2022). Covalent drugs go from fringe field to fashionable endeavour. Nature Reviews Drug Discovery, 21, 170-172.

[12] Parker, C. G., Galmozzi, A., Wang, Y., Correia, B. E., Sasaki, K., Joslyn, C. M., Kim, A. S., Cavallaro, C. L., Lawrence, R. M., Johnson, S. R., Narvaiza, I., Saez, E., & Cravatt, B. F. (2017). Ligand and target discovery by fragment-based screening in human cells. Cell, 168(3), 527-541.

[13] Resnick, E., Bradley, A., Gan, J., Douangamath, A., Krojer, T., Sethi, R., Geurink, P. P., Aimon, A., Amitai, G., Bellini, D., Bennett, J., Fairhead, M., Fearon, D., Powell, A. J., Skyner, R., Ahel, I., & London, N. (2019). Rapid covalent-probe discovery by electrophile-fragment screening. Journal of the American Chemical Society, 141(22), 8951-8968.

[14] Singh, J., Petter, R. C., Baillie, T. A., & Whitty, A. (2011). The resurgence of covalent drugs. Nature Reviews Drug Discovery, 10, 307-317.

[15] Sutanto, F., Konstantinidou, M., & Dömling, A. (2020). Covalent inhibitors: A rational approach to drug discovery. RSC Medicinal Chemistry, 11, 876-884.

[16] Tuley, A., & Fast, W. (2018). The taxonomy of covalent inhibitors. Biochemistry, 57(24), 3326-3337.