Published in final edited form as:

Curr Opin Chem Biol. 2016 October ; 34: 110–116. doi:10.1016/j.cbpa.2016.08.011.

Targeting Biomolecules with Reversible Covalent Chemistry

Anupam Bandyopadhyay and Jianmin Gao*

Department of Chemistry, Boston College, 2609 Beacon Street, Chestnut Hill, MA 02467

Abstract

Interaction of biomolecules typically proceeds in a highly selective and reversible manner, for which covalent bond formation has been largely avoided due to the potential difficulty of dissociation. However, employing reversible covalent warheads in drug design has given rise to covalent enzyme inhibitors that serve as powerful therapeutics, as well as molecular probes with exquisite target selectivity. This review article summarizes the recent advances in the development of reversible covalent chemistry for biological and medicinal applications. Specifically, we document the chemical strategies that allow for reversible modification of the three major classes of nucleophiles in biology: thiols, alcohols and amines. Emphasis is given to the chemical mechanisms that underlie the development of these reversible covalent reactions and their utilization in biology.

Graphical abstract

Introduction

In a living cell, various molecules interact with one another in a dynamic and highly selective manner. The exquisite selectivity of molecular interactions underlies essentially every aspect of biology. Similarly, a guiding philosophy in drug discovery has been to develop selective inhibitors of culprit proteins. In order to achieve such targeted molecular recognition, a cognate ligand typically interacts with its target protein via an array of noncovalent interactions including hydrophobic packing, electrostatic interactions, hydrogen bonding, and others. These noncovalent forces collectively stabilize the desired complex over competing structures (Figure 1). The reliance on noncovalent interactions also renders the binding process reversible when needed. Nature rarely uses covalent chemistry to drive molecular interactions in normal physiology; this is perhaps not surprising as dissociation of

*Correspondence can be sent to: jianmin.gao@bc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

a covalent complex may be challenging. One exception is the disulfide bond formation, which presents a prominent feature and a stabilizing mechanism for folded proteins and protein complexes. A disulfide bond can form **reversibly** with redox regulation. It can exchange with free thiols of cysteine side chains to allow thermodynamic control of protein-protein interactions. The reversibility of the disulfide chemistry has been exploited by nature in the redox regulation of transcription factors,[1] as well as in protein glutathionylation.[2] Additional reversible covalent chemistries like the disulfide bond formation would greatly empower chemists toward the development of molecular probes of important biomolecules.

Different from the molecular interactions in normal physiology, which largely rely on noncovalent interactions, considerable success has been achieved in drug discovery by developing covalent inhibitors of target enzymes. In fact, about one-third of all validated enzyme targets have one or more covalent inhibitors approved for therapeutic use.[3] In principle, reversibility is not required, and perhaps should be purposely avoided toward potent inhibition of pathogenic proteins; this makes covalent drugs appealing. However, concerns arise from the “off-target” effect (Figure 1), in which irreversible modification of non-target proteins leads to toxicity.[4] This problem can be potentially mitigated by exploiting **reversible** covalent inhibitors that do not result in permanently labeled proteins.

This review highlights recent advances in reversible covalent chemistry that has been developed to target biomolecules. We focus on the chemistries developed to target abundant nucleophiles in biology including thiols, alcohols, and amines. Particularly, emphasis will be put on the mechanistic underpinnings that have enabled the development and applications of these reversible reactions.

Reversible covalent chemistry targeting thiols

As one of the least abundant amino acids, cysteines play vitally important roles in catalysis, signaling, and redox regulation of gene expression.[5] Not surprisingly, cysteines have attracted much interest in the pursuit of covalent probes and inhibitors of proteins. Taking advantage of the disulfide bond formation, Wells and coworkers have devised a fragment screening strategy, in which protein-specific ligands are selected through disulfide crosslinking of the ligand to a cysteine residue of the target protein.[6] Much work in the field of covalent drug development has focused on targeting cysteines with Michael acceptors. In particular, acrylamide-based inhibitors has found great success, yielding the marketed anticancer drugs ibrutinib and afatinib, which covalently inhibit BTK kinase and EGFR respectively.[3] Directed by the noncovalent interactions between an inhibitor and its cognate protein, the acrylamide warhead irreversibly crosslinks the inhibitor and the target enzyme via Michael addition of a cysteine residue. To better avoid the off-target effects of covalent inhibitors, Taunton and coworkers have recently demonstrated the use of α -cyanoacrylamides (or acrylates) as reversible modifiers of cysteines.[7-10] Inspired by an earlier report,[11] in which conjugation of simple thiols to 2-cyanoacrylate was found to give an unstable product, the Taunton group showed that the reaction between cysteine and compound **1** (Figure 2a) is rapidly reversible, yielding a K_d value of $\sim 10^2 \text{ M}^{-1}$. The facile thiol addition to the α -cyanoacrylate is owing to the two electron withdrawing groups at α -carbon. On the other hand, the increased α -proton acidity of the cyanoacrylate adduct drives

the reverse reaction through a β -elimination mechanism. The rapid reversibility and the relatively low K_d value preclude random modification of cysteines in the proteome. Grafting such cyano-substituted acrylate or acrylamide motifs onto a known kinase-binding scaffold yielded potent and highly selective inhibitors of the RSK2 kinase.[7] Crystallography studies clearly revealed the covalent linkage between the inhibitor and the enzyme (Figure 2b), in addition to the noncovalent interactions that stabilize the complex. Interestingly, the covalent linkage rapidly breaks off when the target protein unfolds or is proteolytically degraded, leaving no modified peptide fragments. Despite the potential reversibility, the inhibitory effect of the cyanoacrylamide-based inhibitors was found to be as long-lasting as an irreversible inhibitor. This is presumably due to the cooperative action between the covalent bond formation and various noncovalent interactions that give rise to slow-dissociating drug-enzyme complexes. Unfolding or degradation of the protein abolishes the noncovalent interactions and exposes the cysteine adduct, which rapidly dissociates as expected for quick reversibility and the low millimolar affinity of the reaction (Figure 2a).

The Taunton group further shows the residence time of a reversible covalent drug can be systematically tuned via structural modification of the α -cyanoacrylamide warhead.[10] Specifically, a series of BTK kinase inhibitors displaying end-capped α -cyanoacrylamide warheads were synthesized and found to display biochemical residence times ranging from minutes to seven days (Figure 2c). The reversible conjugation with cysteines is not limited to α -cyanoacrylamides; α -substituted acrylonitriles with various electron-withdrawing groups were found to react with cysteines in a reversible manner as well.[9] The thiol-adduct of these acrylonitriles dissociates with half-life ranging from seconds to days. Reversible cysteine conjugation has also been indicated for benzylidene rhodanine derivatives (Figure 2d),[12-14] although the thermodynamic and kinetic profiles of these cysteine modifiers are less well established.

Reversible chemistry targeting alcohols

Hydroxyl groups are prevalent functionalities across RNAs, proteins and carbohydrates. The boronate ester formation between a boronic acid and a diol functionality is rapidly reversible in aqueous solution[15] and has been extensively explored to target carbohydrates ranging from simple glucose to tumor specific antigens.[16] The recent advances in this area have been nicely summarized by several review articles that appeared in recent literature,[17-19] and therefore will not be discussed in details in this contribution. In addition to the reaction with diols, a boronic acid moiety can also conjugate with single hydroxyl groups, such as the side chains of serines and threonines, to form a boronate (Figure 3a). Boronate conjugates as such present an anionic tetrahedral structure, which effectively mimics the transition state of serine proteases, as well as proteasomes that may use a threonine as the nucleophile. This has generated much impact in medicinal chemistry, yielding the FDA approved drug bortezomib for treating multiple myeloma.[20] This dipeptide-based boronic acid inhibits the 26S proteasome by forging a boronate linkage to a threonine residue in the catalytic site (Figure 3b). It is important to note that the boronate formation requires activated serine or threonine residues, typically in enzyme active sites. In contrast, random surface serine or threonine residues are not expected to conjugate with bortezomib considering their pKa values (~ 16) are significantly higher than that of a boronic acid (pKa ~ 9).[15] Similar to the

cysteine-targeting covalent inhibitors, bortezomib acquires proteasome selectivity by using a combination of covalent and noncovalent interactions, which are clearly seen in the crystal structures of bortezomib-bound proteasomes (Figure 3b).[21] Also to mimic the tetrahedral transition state of serine proteases, hemiketal formation has been employed for the design of reversible covalent inhibitors.[3] This line of efforts has yielded two FDA approved drugs, telaprevir and boceprevir; both target HCV proteases with α -ketoamide warheads (Figure 3c). Another class of reversible covalent inhibitors of serine proteases employs a nitrile moiety, which reacts with an active site serine to form an imidate (Figure 3d).[22] Medicinal chemistry investigations of this strategy have yielded reversible covalent inhibitors of dipeptidyl peptidase-4 (DPP4), vildagliptin and saxagliptin, which have been approved to treat diabetes (Figure 3e, f). We note that the two α -ketoamide based drugs, telaprevir and boceprevir, have been recently suspended due to toxicity and less optimal efficacy, which highlights the challenges for choosing appropriate reversible covalent chemistries for drug design.

Reversible covalent chemistry targeting amines

Imine or Schiff base formation is a well-known reversible reaction of amines. In fact, several families of enzymes take advantage of imine formation in their catalytic mechanisms including aldolases and pyridoxal phosphate (PLP)-dependent enzymes.[23,24] However, imine formation in water typically displays unfavorable thermodynamic equilibrium, yielding only trace amount of imine conjugates. For example, the conjugation of glycine to benzaldehyde gives an estimated association constant (K_a) of $3.3 \times 10^{-3} \text{ M}^{-1}$. [24] A similar value was obtained for the imine conjugation between glycine methyl ester

and acetone.[23] The poor thermodynamic stability of imines presents a challenge in utilizing aldehydes or ketones as warheads to target biological amines. Fortunately, the thermodynamic profiles of imine formation can be tuned by altering the neighboring structures of the ketone or aldehyde functionality. As a matter of fact, PLP was found to conjugate with glycine with a K_a of 833 M^{-1} , which is greater than that of benzaldehyde by several orders of magnitude.[24] The enhanced imine stability is owing to the iminium ion forming a hydrogen bonded ion pair with the *ortho*-phenoxide moiety (Figure 4a). The phosphate group provides additional stabilization via electrostatic interaction with the iminium ion as well.

Inspired by the PLP-promoted imine formation, chemists have been devising strategies to favor imine formation toward the goal of targeting biological amines with reversible covalent chemistry. To develop fluorescent reporters of small molecule metabolites, Glass and coworkers have utilized the coumarin 3-aldehyde scaffold (Figure 4b) that conjugates with various biomolecules like glutamate, kynurenine, and serotonin via imine formation.[25-28] The association constants with the analytes range from single digits to over 1000 M^{-1} . In comparison to benzaldehyde, coumarin 3-aldehyde favors imine formation with the hydrogen bond that form between the iminium ion and the α -carbonyl group (Figure 4b). Incorporating a phenylboronic acid moiety into the coumarin 3-aldehyde scaffold affords a highly selective fluorescent sensor for dopamine[29] and glucosamine.[30] The sensor-analyte pairs give K_a values over 4000 M^{-1} , reflecting the additional thermodynamic

stabilization provided by the boronate ester formation (Figure 4c). Also based on the coumarin 3-aldehyde scaffold, a clever design by Buccella and coworkers exploits imine formation in an intramolecular setting (Figure 4d), which allows facile detection of histone deacetylase activity in live cells.[31]

More recently, a new strategy for promoting imine formation has been demonstrated, in which the imine product is stabilized by a nitrogen-boron dative bond. Specifically, benzaldehyde or acetophenone with an *ortho*-boronic acid substituent were found to rapidly conjugate with biological amines to form iminoboronates (Figure 4e). Yatsimirsky and coworkers reported that 2-formyl-phenylboronic acid (2-FPBA) readily conjugates with aminosugars (e.g., glucosamine) at sub to low millimolar concentrations, while the regioisomers 3-FPBA and 4-FPBA give no conjugation under the same conditions.[32] The Gois group showed that 2-FPBA, as well as the ketone analogue (2-acetyl phenylboronic acid, 2-APBA), conjugates with protein lysine side chains to form iminoboronates (Figure 4f).[33,34] The protein modification can be reversed with addition of small molecule competitors such as dopamine, glutathione and fructose. Our group later showed that the iminoboronate formation is spontaneously reversible under physiologic conditions and the reversibility does not require competing small molecules.[35,36] In other words, the iminoboronate formation is under thermodynamic control similar to the disulfide chemistry in a redox buffer. Further, by incorporating the 2-APBA moiety into synthetic peptides, we have developed peptide probes that preferentially label gram-positive bacteria (Figure 4g). Specifically, a cationic peptide incorporating the 2-APBA warhead (dubbed Hlys-AB1) effectively labels *S. aureus* cells in blood serum, while bypassing mammalian cells and gram-negative bacteria.[35] The cell selectivity is partly owing to the 2-APBA warhead eliciting iminoboronate formation of amine-presenting lipids (phosphoethanolamine (PE) and lysyl phosphoglycerol (Lys-PG)) that are enriched on the surfaces of gram-positive bacteria.[37] This capability of Hlys-AB1 to detect bacteria in blood serum is rather remarkable considering the high concentrations of lysine side chains in serum proteins. We attribute the lack of protein interference to the combination of the reversible iminoboronate formation and the cationic peptide preferring bacterial cell membranes that display a large number of negative charges.[37] Our work clearly demonstrates that the iminoboronate chemistry can be used for targeting biomolecules in complex biological systems, complementing the canonical noncovalent mechanisms (e.g., hydrogen bonds) for molecular recognition.

Conclusions and future directions

Specific molecular recognition is a ubiquitous and essential feature of biological systems. While nature largely utilizes noncovalent driving forces (e.g., hydrogen bonds) to program molecular interactions, reversible covalent chemistry has been increasingly recognized as a powerful strategy for targeted recognition of biomolecules. Summarized above are the reversible covalent chemistries that have been developed for the three major classes of nucleophiles in biology. Implementing these covalent mechanisms in ligand design has yielded an impressive array of molecular probes/sensors for biological research. Further, a number of FDA approved drugs have been shown to work by inhibiting their target enzymes via reversible modification of nucleophilic residues.

In comparison to the repertoire of irreversible conjugation reactions of biomolecules, their reversible covalent chemistry is still in its infancy, with only a handful of reactions available for all bio-nucleophiles combined. Expanding the reversible covalent chemistry arsenal will greatly empower chemical biologists and medicinal chemists to develop synthetic probes and inhibitors for important players of biology. Further, it is nontrivial to implement such reversible covalent mechanisms in probe or inhibitor design: the warhead groups described above, including the cyanoacrylamides and 2-APBA moieties, are sterically bulky and the reactions have stringent requirement of geometry. It is challenging to devise synthetic ligands that incorporate such warhead motifs, yet able to fit into target proteins for covalent modification. Excitingly, a computational docking protocol has been developed for Michael acceptors to target cysteines.[38] It remains to be seen how easily adaptable such algorithm is to a variety of covalent warheads. Despite the technical challenges, we believe the concept of reversible covalent binding will generate much more excitement in biological research and medicine.

Acknowledgement

We thank the National Institutes of Health (Grant GM102735) for supporting our research.

References

1. Dansen TB, Smits LMM, van Triest MH, de Keizer PLJ, van Leenen D, Koerkamp MG, Szypowska A, Meppelink A, Brenkman AB, Yodoi J, Holstege FCP, Burgering BMT. Redox-sensitive cysteines bridge p300/CBP-mediated acetylation and FoxO4 activity. *Nat Chem Biol.* 2009; 5:664–672. [PubMed: 19648934]
2. Grek CL, Zhang J, Manevich Y, Townsend DM, Tew KD. Causes and consequences of cysteine S-glutathionylation. *J Biol Chem.* 2013; 288:26497–26504. [PubMed: 23861399]
- 3*. Singh J, Petter RC, Baillie TA, Whitty A. The resurgence of covalent drugs. *Nat Rev Drug Discov.* 2011; 10:307–317. This article presents a detailed and systematic analysis of covalent drugs in comparison to their noncovalent counterparts. [PubMed: 21455239]
4. Uetrecht J. Idiosyncratic drug reactions: past, present, and future. *Chem Res Toxicol.* 2008; 21:84–92. [PubMed: 18052104]
5. Pace NJ, Weerapana E. Diverse functional roles of reactive cysteines. *ACS Chem Biol.* 2013; 8:283–296. [PubMed: 23163700]
- 6**. Erlanson DA, Braisted AC, Raphael DR, Randal M, Stroud RM, Gordon EM, Wells JA. Site-directed ligand discovery. *Proc Natl Acad Sci.* 2000; 97:9367–9372. This work introduces the proximity tethering concept in ligand design, which takes advantage of the reversible disulfide chemistry in search for thermodynamically most favorable binders of target proteins. [PubMed: 10944209]
- 7**. Serafimova IM, Pufall MA, Krishnan S, Duda K, Cohen MS, Maglathlin RL, McFarland JM, Miller RM, Frodin M, Taunton J. Reversible targeting of noncatalytic cysteines with chemically tuned electrophiles. *Nat Chem Biol.* 2012; 8:471–476. This paper demonstrates for the first time that reversible covalent targeting of cysteines with α -cyanoacrylamides can afford potent and highly selective kinase inhibitors. The α -cyanoacrylamide-based inhibitors covalently inactivate the target kinase, yet able to dissociate upon unfolding or degradation of the enzyme. [PubMed: 22466421]
8. Miller RM, Paavilainen VO, Krishnan S, Serafimova IM, Taunton J. Electrophilic fragment- based design of reversible covalent kinase inhibitors. *J Am Chem Soc.* 2013; 135:5298–5301. [PubMed: 23540679]

9. Krishnan S, Miller RM, Tian B, Mullins RD, Jacobson MP, Taunton J. Design of reversible, cysteine-targeted Michael acceptors guided by kinetic and computational analysis. *J Am Chem Soc.* 2014; 136:12624–12630. [PubMed: 25153195]
- 10*. Bradshaw JM, McFarland JM, Paavilainen VO, Bisconte A, Tam D, Phan VT, Romanov S, Finkle D, Shu J, Patel V, Ton T, Li X, Loughhead DG, Nunn PA, Karr DE, Gerritsen ME, Funk JO, Owens TD, Verner E, Brameld KA, Hill RJ, Goldstein DM, Taunton J. Prolonged and tunable residence time using reversible covalent kinase inhibitors. *Nat Chem Biol.* 2015; 11:525–531. This contribution reports that the pharmacokinetics of reversible covalent inhibitors can be systematically tuned through structural alterations of the α -cyanoacrylamide warheads. [PubMed: 26006010]
11. Pritchard RB, Lough CE, Currie DJ, Holmes HL. Equilibrium reactions of n-butanethiol with some conjugated heteroenoid compounds. *Can J Chem.* 1968; 46:775–781.
12. Powers JP, Piper DE, Li Y, Mayorga V, Anzola J, Chen JM, Jaen JC, Lee G, Liu J, Peterson MG, Tonn GR, Ye Q, Walker NP, Wang Z. SAR and mode of action of novel non-nucleoside inhibitors of hepatitis C NS5b RNA polymerase. *J Med Chem.* 2006; 49:1034–1046. [PubMed: 16451069]
13. Lee G, Piper DE, Wang Z, Anzola J, Powers J, Walker N, Li Y. Novel inhibitors of hepatitis C virus RNA-dependent RNA polymerases. *J Mol Biol.* 2006; 357:1051–1057. [PubMed: 16476448]
14. Patch RJ, Searle LL, Kim AJ, De D, Zhu X, Askari HB, O'Neill JC, Abad MC, Rentzeperis D, Liu J, Kemmerer M, Lin L, Kasturi J, Geisler JG, Lenhard JM, Player MR, Gaul MD. Identification of diaryl ether-based ligands for estrogen-related receptor α as potential antidiabetic agents. *J Med Chem.* 2011; 54:788–808. [PubMed: 21218783]
- 15*. Springsteen G, Wang BH. A detailed examination of boronic acid–diol complexation. *Tetrahedron.* 2002; 58:5291–5300. This contribution documents a comprehensive investigation of boronate ester formation and provides guidelines for designing boronic acid-based covalent ligands.
16. James TD, Sandanayake KRAS, Shinkai S. Saccharide Sensing with Molecular Receptors Based on Boronic Acid. *Angew Chem, Int Ed.* 1996; 35:1910–1922.
17. Bull SD, Davidson MG, van den Elsen JM, Fossey JS, Jenkins AT, Jiang YB, Kubo Y, Marken F, Sakurai K, Zhao J, James TD. Exploiting the reversible covalent bonding of boronic acids: recognition, sensing, and assembly. *Acc Chem Res.* 2013; 46:312–326. [PubMed: 23148559]
18. Sun X, Zhai W, Fossey JS, James TD. Boronic acids for fluorescence imaging of carbohydrates. *Chem Commun.* 2016; 52:3456–3469.
19. Adamczyk-Wo niak A, Borys KM, Sporyzowski A. Recent developments in the chemistry and biological applications of benzoxaboroles. *Chem Rev.* 2015; 115:5224–5247. [PubMed: 26017806]
20. Bold R, Adams Julian, Kauffman Michael. Development of the proteasome inhibitor Velcade (Bortezomib). *Cancer Invest.* 2004; 22:328–329. Ph.D. M.D., Ph.D. [PubMed: 15199617]
21. Groll M, Berkens CR, Ploegh HL, Ova H. Crystal structure of the boronic acid-based proteasome inhibitor bortezomib in complex with the yeast 20S proteasome. *Structure.* 2006; 14:451–456. [PubMed: 16531229]
22. Bachovchin DA, Cravatt BF. The pharmacological landscape and therapeutic potential of serine hydrolases. *Nat. Rev. Drug Discov.* 2012; 11:52–68. [PubMed: 22212679]
23. Crueiras J, Rios A, Riveiros E, Amyes TL, Richard JP. Glycine enolates: the effect of formation of iminium ions to simple ketones on α -amino carbon acidity and a comparison with pyridoxal iminium ions. *J Am Chem Soc.* 2008; 130:2041–2050. [PubMed: 18198876]
- 24*. Crueiras J, Rios A, Riveiros E, Richard JP. Substituent effects on the thermodynamic stability of imines formed from glycine and aromatic aldehydes: implications for the catalytic activity of pyridoxal-5'-phosphate. *J Am Chem Soc.* 2009; 131:15815–15824. This paper presents a rigorous physical organic chemistry investigation of the PLP-induced imine formation. Comparison of PLP to close structural analogues illustrates the key features of PLP that favor imine formation. [PubMed: 19807092]
25. Hettie KS, Glass TE. Turn-On Near-Infrared Fluorescent Sensor for Selectively Imaging Serotonin. *ACS Chem Neurosci.* 2016; 7:21–25. [PubMed: 26521705]

26. Klockow JL, Glass TE. Development of a fluorescent chemosensor for the detection of kynurenine. *Org Lett*. 2013; 15:235–237. [PubMed: 23265271]
27. Hettie KS, Liu X, Gillis KD, Glass TE. Selective catecholamine recognition with NeuroSensor 521: a fluorescent sensor for the visualization of norepinephrine in fixed and live cells. *ACS Chem Neurosci*. 2013; 4:918–923. [PubMed: 23527575]
28. Feuster EK, Glass TE. Detection of amines and unprotected amino acids in aqueous conditions by formation of highly fluorescent iminium ions. *J Am Chem Soc*. 2003; 125:16174–16175. [PubMed: 14692743]
29. Secor KE, Glass TE. Selective amine recognition: development of a chemosensor for dopamine and norepinephrine. *Org Lett*. 2004; 6:3727–3730. [PubMed: 15469334]
30. Tran TM, Alan Y, Glass TE. A highly selective fluorescent sensor for glucosamine. *Chem Commun*. 2015; 51:7915–7918.
31. Rooker DR, Buccella D. *Chem Sci*. 2015; 6:6456–6458.
32. Gutierrez-Moreno NJ, Medrano F, Yatsimirsky AK. Schiff base formation and recognition of amino sugars, aminoglycosides and biological polyamines by 2-formyl phenylboronic acid in aqueous solution. *Org Biomol Chem*. 2012; 10:6960–6972. [PubMed: 22842531]
- 33*. Cal PM, Vicente JB, Pires E, Coelho AV, Veiros LF, Cordeiro C, Gois PM. Iminoboronates: a new strategy for reversible protein modification. *J Am Chem Soc*. 2012; 134:10299–10305. References 32 and 33 independently demonstrate that an ortho-boronic acid substituent can promote imine formation of benzaldehyde and acetophenone under physiologic conditions. [PubMed: 22642715]
34. Cal PM, Frade RF, Cordeiro C, Gois PM. Reversible lysine modification on proteins by using functionalized boronic acids. *Chem -Eur J*. 2015; 21:8182–8187. [PubMed: 25900406]
- 35*. Bandyopadhyay A, McCarthy KA, Kelly MA, Gao J. Targeting bacteria via iminoboronate chemistry of amine-presenting lipids. *Nat Commun*. 2015; 6:6161. This paper provides the first example that the iminoboronate chemistry can be implemented in molecular design to realize selective recognition of biomolecules of interest in complex biological systems. [PubMed: 25635909]
36. Bandyopadhyay A, Gao J. Iminoboronate Formation Leads to Fast and Reversible Conjugation Chemistry of α -Nucleophiles at Neutral pH. *Chem -Eur J*. 2015; 21:14748–14752. [PubMed: 26311464]
37. Roy H. Tuning the properties of the bacterial membrane with aminoacylated phosphatidylglycerol. *IUBMB Life*. 2009; 61:940–953. [PubMed: 19787708]
38. London N, Miller RM, Krishnan S, Uchida K, Irwin JJ, Eidam O, Gibold L, Cimermanic P, Bonnet R, Shoichet BK, Taunton J. Covalent docking of large libraries for the discovery of chemical probes. *Nat Chem Biol*. 2014; 10:1066–1072. [PubMed: 25344815]

Highlights

- Reversible covalent chemistry expands the tool box for molecular recognition.
- Reversible covalent inhibitors exhibit appealing characteristics as therapeutics.
- α -cyanoacrylamides display tunable profiles (stability and kinetics) for cysteine targeting.
- Boronate ester formation enables selective targeting of activated Ser/Thr nucleophiles.
- Iminoboronate formation enables selective targeting of amine-presenting biomolecules.

Figure 1. Comparison of a) noncovalent, b) irreversible covalent and c) reversible covalent inhibitors.

Figure 2.

Reversible covalent chemistry of thiols. a) Reversible conjugation between an α -cyanoacrylate and cysteine. b) Crystal structure of a reversible covalent inhibitor bound to the kinase RSK2, highlighting the covalent linkage as well as a series of noncovalent interactions that give rise to target specificity. c) End-capped α -cyanoacrylamide inhibitors of the BTK kinase and crystal structure of **2** bound to BTK. d) Benzylidene rhodamine-based reversible covalent inhibitors of Hepatitis C NS5b RNA polymerase (left) and a cocystal structure (right) to illustrate the covalent linkage. The structural images in b, c, and d are adapted with permission from references [7], [10] and [12] respectively.

Figure 3.

Reversible covalent chemistry of alcohols. a) Reversible targeting of serine/threonine using boronic acid. b) Chemical structure of bortezomib, and its cocrystal structure with the yeast 20S proteasome (PDB: 2F16). c) Mechanism of action (left) and examples (right) of α -ketoamide-based reversible inhibitors. d) Three different classes of nitrile derivatives used for covalent drug design. e) Structure of saxagliptin as an example of nitrile-based reversible covalent inhibitors. f) Crystal structure of DPP4 showing covalent modification of Ser592 by saxagliptin.

Figure 4.

Reversible covalent chemistry of amines. a) PLP-elicited imine formation. b) Coumarin 3-aldehyde based sensors for the detection of important amine-presenting metabolites. The table presents the substrate specificity of several sensors. c) A boronic acid-derivatized coumarin 3-aldehyde probe selectively binds glucosamine through cooperative imine and boronate ester formation. d) A molecular probe for the detection of histone deacetylase activity in live cells. e) Reversible iminoboronate formation of biological amines. f) Cartoon illustration of lysine side modification of proteins through iminoboronate formation. g) Illustration of bacterial labeling through iminoboronate formation of bacterial lipids. Shown on the right is a confocal image showing membrane labeling of *S. aureus* cells via iminoboronate chemistry.