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Perspective

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Overview of Recent Strategic Advances in Medicinal Chemistry

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Abstract: Introducing novel strategies, concepts and technologies that speed up drug discovery and the drug development cycle is of great importance both in the highly competitive pharmaceutical industry as well as in academia. This review aims to present a "big-picture" overview of recent strategic innovations in medicinal chemistry and drug discovery.

Keywords: Drug discovery, Structure optimization, Medicinal chemistry strategies.

Introduction

The process of drug development, from identification of a new bioactive chemical entity to regulatory approval, is complex, costly and time-consuming. It can take 10–15 years or even longer.[1-3] Thus, there is enormous pressure from the pharmaceutical industry, as well as clinicians and patients, to speed up the process. Fortunately, the extremely rapid accumulation of biological data in the postgenomic era as well as the development of computational chemical biology have stimulated an unprecedented revolution in medicinal chemistry, and the paradigm for discovery of pharmacologically interesting molecules has changed over the past few decades from a largely serendipitous, trial-and-error approach to a much more sophisticated and multi-faceted approach, which has greatly improved the efficiency of drug discovery resulting in a significant acceleration of the overall process.[4-7]

This current perspective analyzes the articles that have appeared recently in some top journals of the medicinal chemistry community (primarily in the Journal of Medicinal Chemistry), in order to provide a broad-brush picture of the trends in strategic innovations in the fields of medicinal chemistry, drug discovery and other related

fields (Figure 1), focusing in particular on drug repurposing, diversity-oriented synthesis-facilitated medicinal chemistry, structure-based drug discovery, multiparameter optimization, and biological system-mediated drug delivery. The strategy of bioorthogonal chemistry and photoactivatable-inspired medicinal chemistry with high temporal and spatial precision was first proposed by integrating related innovations in related fields.

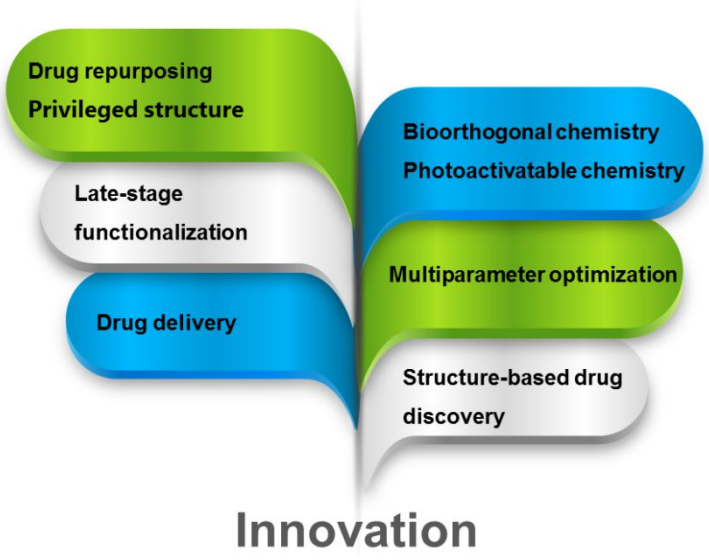


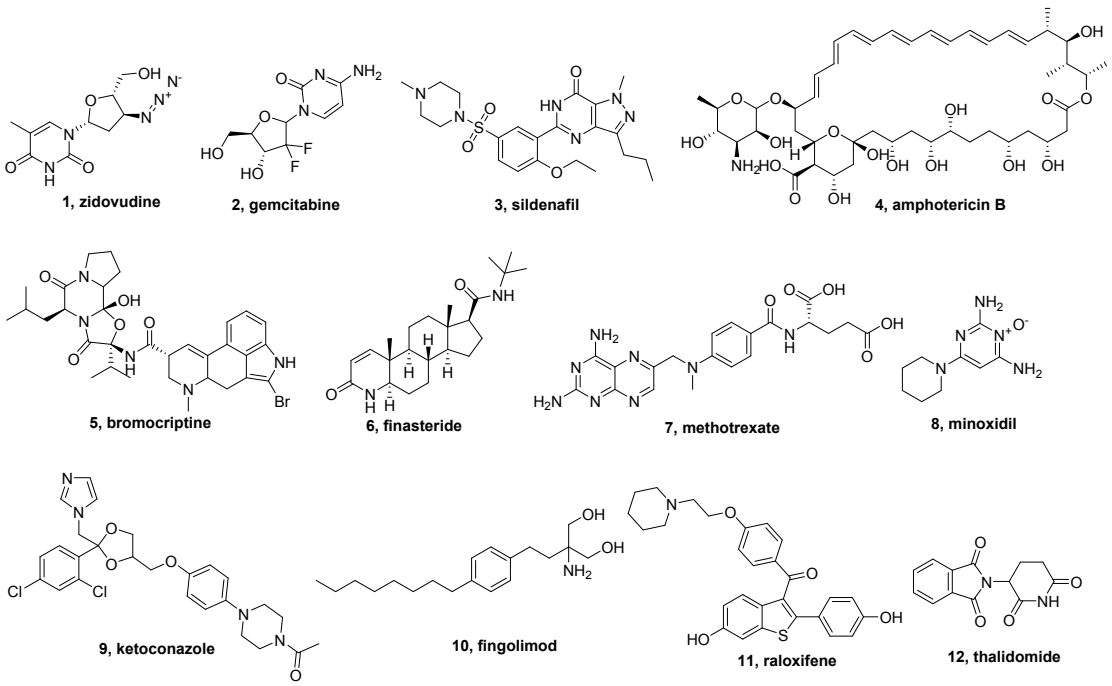
Figure 1. Overview of recent strategic advances in medicinal chemistry.

1. Drug repurposing and "privileged structure" repositioning

Drug repurposing (also called repositioning, redirecting, reprofiling) is a polypharmacology-driven strategy for generating additional value from an existing drug by targeting diseases other than that for which it was originally intended.[8,9] This has significant advantages over new drug discovery since chemical synthesis steps, manufacturing processes, reliable safety and pharmacokinetic properties in early clinical developmental phases (Phase I and Phase IIa trials) are already available. Therefore, repositioning of launched or even failed drugs provides unique translational opportunities, including a substantially higher probability of success to

market as compared with new drugs, and a significantly reduced cost and timeline to clinical availability.[10,11]

Actually, drug repositioning is not a new strategy; many drugs have been successfully repositioned, including zidovudine (**1**, cancer→HIV), gemcitabine (**2**, viral infections →cancer), sildenafil (**3**, heart related chest pain→erectile dysfunction), amphotericin B (**4**, leishmaniasis→fungal infections), bromocriptine (**5**, Parkinson's disease→type 2 diabetes), finasteride (**6**, enlarged prostate → scalp hair loss), methotrexate (**7**, chemotherapy agent → immune system suppressant), minoxidil (**8**, antihypertensive vasodilator medication→androgenic alopecia), ketoconazole (**9**, fungal infections→ Cushing's syndrome), fingolimod (**10**, immunomodulating drug→multiple sclerosis), raloxifene (**11**, osteoporosis → breast cancer), and thalidomide (**12**, antiemetic drug → multiple myeloma) (Figure 2).[12,13]



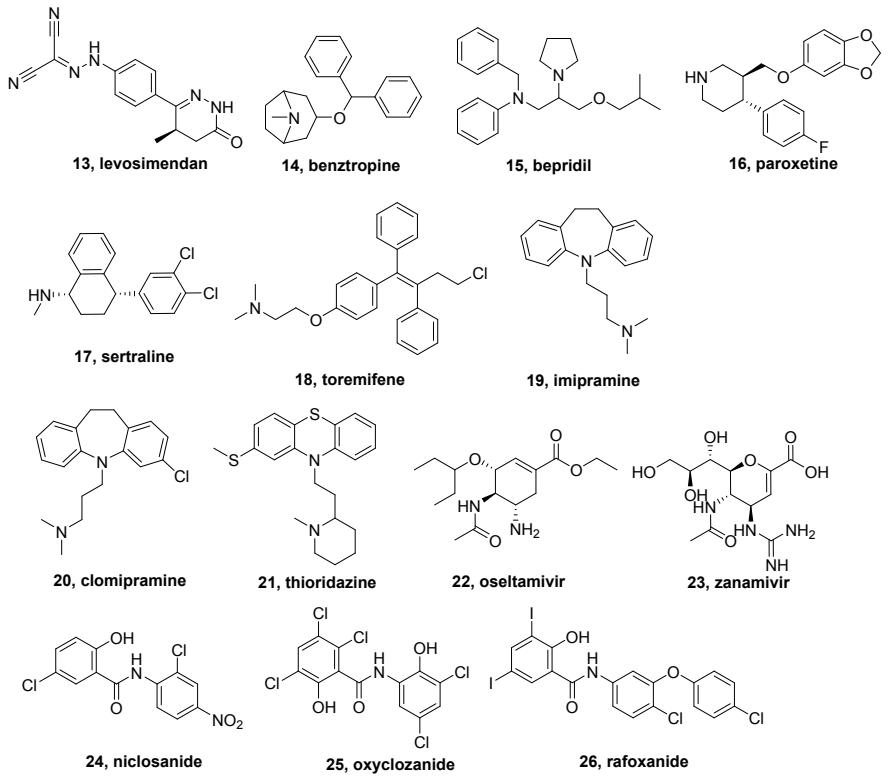


Figure 2. Examples of drugs that have been repositioned: finding and developing new uses for the approved drugs.

Additional successful examples of drug repositioning have been reported. For example, in 2017, levosimendan (**13**, used to treat heart failure) was identified through screening of an FDA-approved drug library as a novel and promising HIV-1 inhibitor targeting viral transcription. Levosimendan displayed robust potency against TNFα-induced HIV-1 reactivation in many cell lines with HIV-1 latency via the HIV-1 Tat-LTR transcriptional axis. Moreover, it inhibited not only acute viral replication but also the reactivation of latent HIV-1 proviruses in primary CD4+ T cells.[14]

In recent years, many chemically diverse FDA-approved drugs with potency of inhibiting the cell entry of Ebola virus; these include benztropine (**14**), bepridil (**15**), paroxetine (**16**), sertraline (**17**), toremifene (**18**), imipramine (**19**), clomipramine (**20**), and thioridazine (**21**), which directly bind with Ebolavirus glycoprotein.[15]

In 2018, pharmacological inhibition of neuraminidase by two anti-influenza agents,

oseltamivir (**22**) and zanamivir (**23**), was found to protect cardiomyocytes and the heart from myocardial injury. Subsequently, a key role of Neu5Ac in acute myocardial infarction was confirmed by functional metabolomics studies, and neuraminidase-1 was identified as a previously unrecognized therapeutic target for coronary artery diseases.[16]

In 2018, through screen of a fragment library (2500 compounds), Urquiza *et al.* identified the antifungal ciclopirox as a binder to and stabilizer of uroporphyrinogen III synthase, a drug target of congenital erythropoietic porphyria (CEP), thereby restoring activity of the enzyme. Also, ciclopirox exhibits orally effective in vivo activity and low toxicity, in a genetic mouse model of CEP.[17]

In 2019, *via* cell-based reporter assays, primary cell culture, and multiple mouse models, it was found that non-nucleoside HIV-1 reverse transcriptase inhibitor efavirenz is a potent pregnane X receptor (PXR)-selective agonist that can efficiently activate PXR to induce hypercholesterolemia and hepatic steatosis.[18]

Via screening 2,486 FDA-approved drugs, new Na^+/K^+ ATPase inhibitors were identified, which enable the dissociation of circulating tumor cells clusters into single cells, resulting in DNA methylation remodeling at critical regions and metastasis suppression.[19]

In 2019, it was reported that three salicylanilide anthelmintic drugs, namely, niclosanide (**24**), oxyclozanide (**25**), and rafoxanide (**26**), showed significant anti-adenovirus potency at low micromolar concentrations with little cytotoxicity. Furthermore, the mechanistic assays show differences in the way the drugs exert anti-adenovirus potency. **24** and **26** target transport of the HAdV particle from the endosome to the nuclear envelope, whilst **25** specifically targets the early gene E1A transcription step of adenovirus.[20]

1.1 New developments on drug repurposing

Today, most strategies for repositioning are based on phenotypic screening, systematic screening (robotic high-throughput screening, HTS) using a panel of approved drugs.[21,22]

Identifying the molecular targets of compounds from phenotypic screening is a challenging but crucial step towards understanding their mechanisms of action. In this context, many target identification methods have been used to successfully elucidate the target proteins of a variety of compounds. For example, chemoproteomics has proven an effective tool to identify protein targets from phenotypic assay and to understand on- and off-target engagement of potential bioactive agents. Mass spectrometry (MS)-based proteomics is the primary technology for target identification.[23] Moreover, photochemistry (live-cell photoaffinity labeling) has been employed recently to identify novel targets for known molecules.[24-26]

In recent years, with the continuous development of bioinformatics and chemical informatics, drug repurposing has gradually developed into a data-driven innovative drug development strategy. Recent work has demonstrated that bioinformatics-based methodologies have the potential to provide the kind of systematic insights into the complicated relationships among diseases, targets, and drugs that are needed for successful repositioning.[27,28]

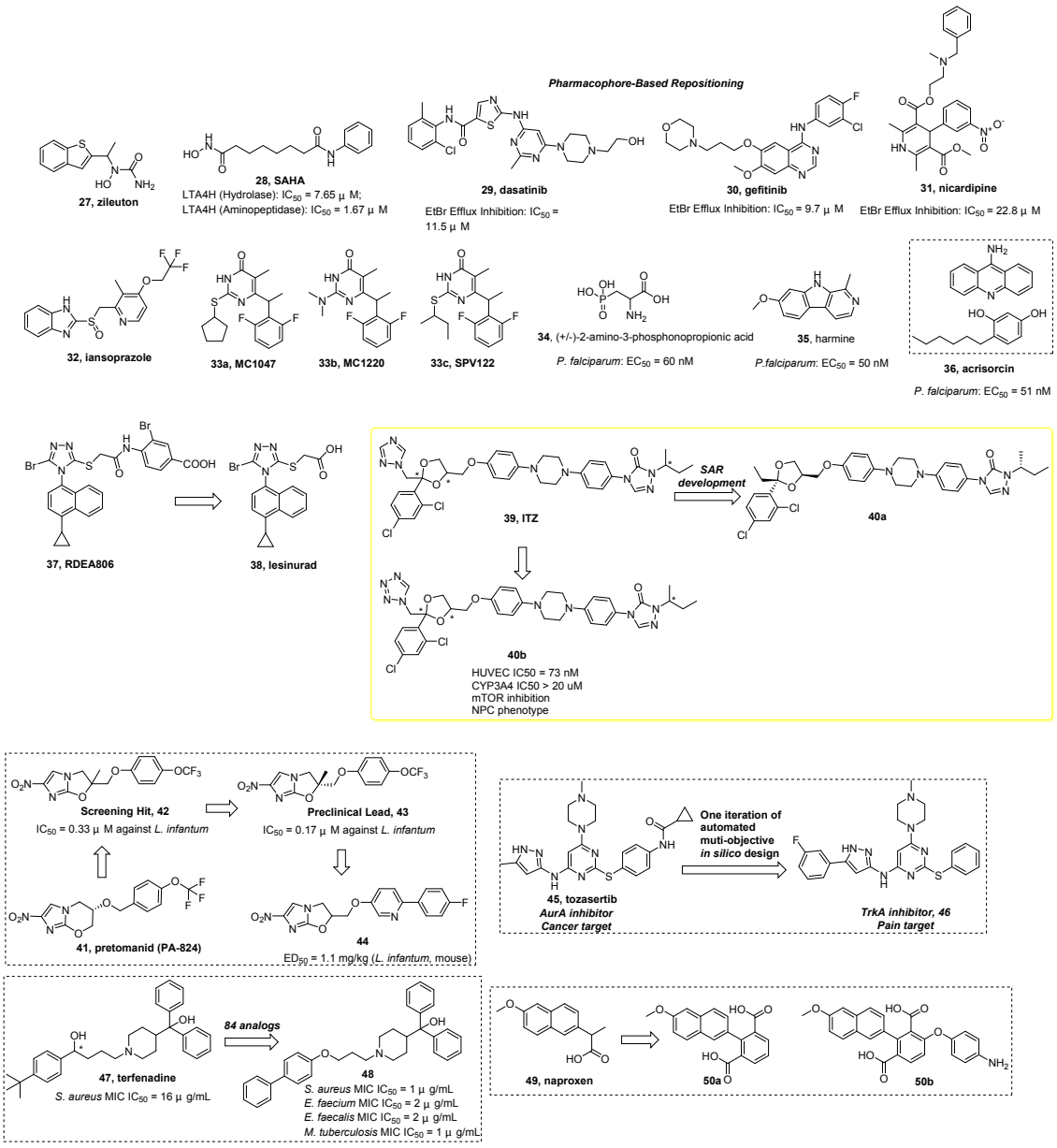


Figure 3. Identifying and developing new uses from existing (investigational) drugs via new approaches or further optimization.

With the guidance of bioinformatics and cheminformatics, recent progress has also been made in exploration of the chemical space of existing drugs for novel bioactivities with translational potential. For example, Zileuton (**27**, trade name ZYFLO) is an orally bioavailable inhibitor of 5-lipoxygenase, and thus inhibits formation of leukotrienes (LTB4, LTC4, LTD4, and LTE4); it is used for the treatment of asthma. Based on previous studies indicating that the leukotriene pathway is involved in human tauopathy (bioinformatics), it was recently shown that

aged tau transgenic mice treated with zileuton exhibit reversal of learning impairments, memory deficits, and neuropathology.[29]

Suberanilohydroxamic acid (SAHA, vorinostat, **28**), an approved histone deacetylase (HDAC) inhibitor, was used for treating cutaneous T cell lymphoma. Previous studies suggested that **28** showed potential anti-inflammatory activity (cheminformatics), though the underlying mechanisms remained unclear. Based on this, Lu *et al.* reported drug repurposing of HDAC inhibitors as agents to alleviate neutrophilic inflammation in idiopathic pulmonary fibrosis and acute lung injury *via* binding with leukotriene A4 hydrolase, thereby inhibiting leukotriene B4 biosynthesis.[30]

Integrating computational prediction and experimental validation has great potential to improve the success of drug repositioning.[31,32] In 2014, Huang *et. al.* reported a combination of systems biology and multiple microarray experimental approaches to find and characterize the function of novel oncogenes associated with hepatocellular carcinoma and lung cancer. This was helpful in drug repositioning discovery.[33]

A plethora of in silico approaches have been developed to facilitate the repositioning of drug-like molecules, including virtual screening, reverse pharmacophore profiling or binding pocket comparisons. For example, NorA is the most important efflux pump of *Staphylococcus aureus* as it confers multi-drug resistance. Astolfi *et al*. constructed a ligand-based 3D-pharmacophore model of efflux pump inhibitors (EPIs) based on the *S. aureus* (ModB and ModC) NorA EPIs library. The best model was screened against approved drugs, leading to the discovery of novel and potent NorA EPIs, including three non-antibiotic approved drugs dasatinib (**29**, used to treat certain cases of chronic myelogenous leukemia and acute lymphoblastic leukemia), gefitinib (**30**, used for certain breast, lung and other cancers.), and nicardipine (**31**, used to treat high blood pressure and angina) that were able to restore the antibacterial activity of

ciprofloxacin against resistant *S. aureus* strains overexpressing NorA.[34]

Using computational screen of an FDA-approved drug library, the proton pump inhibitor lansoprazole (**32**) was repositioned as an anticancer drug by binding to the thioesterase domain of human fatty acid synthase.[35]

Multiple ligand simultaneous docking (MLSD) is a computational tool used to investigate interactions between a biotarget and substrate in the presence of an inhibitor. In 2011, Li *et al.* described a novel approach to drug discovery by combining fragment-based drug design with drug repositioning using MLSD. This led to the identification of celecoxib and its analogues as new inhibitors of signal transducer and activator of transcription 3 (STAT3).[36a] In 2014, they further reported the identification of raloxifene and bazedoxifene as novel inhibitors of IL-6/GP130 protein-protein interaction through MLSD-derived drug-repositioning methodology.[36b] Novel *in silico* drug design approaches, especially those related to machine-learning algorithms, are being utilized for *in silico* drug repositioning, as exemplified by drug profile matching,[37] topological graph theory[38] and other computational methodologies.[39-41]

Because of the limited variety of drugs on the market, it is necessary to expand the selection of drugs or drug-like molecules (investigational compounds). As we know, development of repurposed drugs is also limited by challenges in the regulatory path.[42] Widening the scope of compound library beyond approved drugs can help overcome this shortcoming.

For example, three pyrimidinone derivatives **33a-c**, previously known as non-approved HIV-1 non-nucleoside reverse transcriptase inhibitors, were reported to inhibit cell proliferation and facilitate cell differentiation via inhibiting (a non-telomeric) endogenous reverse transcriptase.[43]

Robotic HTS of a natural compound library (Spectrum), a library of pharmacologically active compound (LOPAC), and an FDA-approved drug library (Prestwick) against *Plasmodium falciparum* Hsp90 (PfHsp90) resulted in three hits: (±)-2-amino-3-phosphonopropionic acid (**34**) (APPA) from LOPAC, and harmine (harmaline) (**35**), and acrisorcin (**36**, a combination of the active ingredients 9-aminoacridine and 4-hexylresorcinol) from Spectrum. These compounds were identified as selective PfHsp90 inhibitors, with IC_{50} values in the nanomolar level in a cell-based antimalarial assay, and showed synergistic potency in the presence of chloroquine, a drug used to prevent and to treat malaria.[44]

Selective optimization of off-target activities represents an original alternative to HTS. For example, the 1,2,4-triazol-3-ylthioacetanilide RDEA806 (**37**) was once advanced into phase IIa clinical trials as a promising novel anti-HIV-1 drug candidate in Ardea Biosciences (a subsidiary of AstraZeneca). Meanwhile, it also showed uric acid lowering potency and potential for the chronic management of hyperuricemia and gout. In a follow-up study, 1,2,4-triazol-3-ylthioacetic acid RDEA594 (**38**), a major metabolite of RDEA806 was identified, which retained all the uric acid lowering effects of RDEA806, but no antiviral potency. Compound **38** is an orally bioavailable inhibitor of urate-anion exchanger transporter 1. In December 2015, **38** was approved by the USA FDA, named Lesinurad, ZURAMPIC® as combination therapy with a xanthine oxidase inhibitor for treating hyperuricaemia and gout.[45,46]

Depending on the new indication, the repurposed (experimental) drugs may need further medicinal chemistry optimization to improve their potency and selectivity as potential clinical candidates for clinical therapy. For example, Itraconazole (ITZ, **39**) is a clinically efficacious antifungal agent. Recent drug-repurposing projects identified ITZ as a potent anticancer agent through its off-target activity, including the

vascular endothelial growth receptor 2 (VEGFR2), mTOR signaling and hedgehog (Hh) signaling pathways. To fully investigate the structural requirements for these anticancer properties, various ITZ derivatives were prepared and evaluated for anti-Hh and antiangiogenic activities (exemplified by **40a**). The results suggest that the triazole functionality is indispensable to ITZ-mediated inhibition of angiogenesis, but that it is not required for inhibition of Hh signaling.[47] Further optimization suggested that optimization of the *sec*-butyl side chain of ITZ can result in improvement of the pharmacological activity (HUVEC proliferation or VEGFR2 glycosylation) of itraconazole.[48a] In an effort to eliminate ITZ's inhibition of CYP3A4 (the drug metabolizing enzyme) while retaining anti-angiogenic activity, a series of derivatives were designed and prepared in which the 1,2,4-triazole ring was replaced with a set of azoles and other rings. Among these analogues, **40b** with tetrazole in place of 1,2,4-triazole displayed the best effect on HUVEC proliferation with an IC_{50} of 73 nM with extremely weak inhibition of CYP3A4 (EC_{50} > 20 μM). Similar to itraconazole, **40b** could induce Nieman-Pick C phenotype and inhibit AMPK/mTOR signaling.[48b]

Pretomanid (**41**, PA-824) is an experimental anti-tuberculosis drug. Phenotypic screening of pretomanid derivatives towards kinetoplastid diseases unexpectedly resulted in the discovery of DNDI-VL-2098 (**42**) as a promising first-in-class drug candidate for visceral leishmaniasis. Additional SAR studies led to the discovery of phenylpyridine derivatives (**43**, **44**) with significantly improved potency in a mouse model of acute *Leishmania donovani* infection.[49]

Tozasertib (**45**) was originally developed as an anti-cancer agent targeting AurA. One iteration of automated muti-objective *in silico* design (computational chemistry and machine learning) was sufficient to shift the selectivity of Tozasertib toward the pain

target TrkA. **46** was selected as a top-ranked molecule from a focused library derived from tozasertib, which showed 10,000-fold improved selectivity *versus* AurA, cellular activity at nanomolar concentration, and high selectivity for TrkA *versus* a kinase panel.[50]

A HTS approach identified the antihistamine terfenadine (**47**) as possessing previously unreported antimicrobial potency against *Staphylococcus aureus* (*S. aureus*) and other Gram-positive bacteria. Mechanism of action investigations by Perlmutter *et al.* indicated that terfenadine-based analogues (exemplified by **48**) displayed antibacterial potency, at least in part, through inhibition of bacterial type II topoisomerases.[51]

The nucleoprotein (NP) of influenza A virus is a promising target for new antivirals. Previously, naproxen (**49**) was disclosed as a dual inhibitor of NP and cyclooxygenase COX2, with antiviral and anti-inflammatory potency by in silico screening. Very recently, further optimization using traditional medicinal chemistry strategies to remove COX2 inhibition potency afforded derivatives **50a** and **50b** with improved antiviral potency in infected cells, without inhibiting COX2. This improved antiviral potency probably results from these two derivatives inhibiting the interactions between NP and RNA and polymerase acidic subunit N-terminal, respectively.[52]

Biology-oriented drug synthesis has great potential to explore compounds derived from commercial pharmaceutical drugs for new and diversified biological potential by adopting simple chemical transformations.[53]

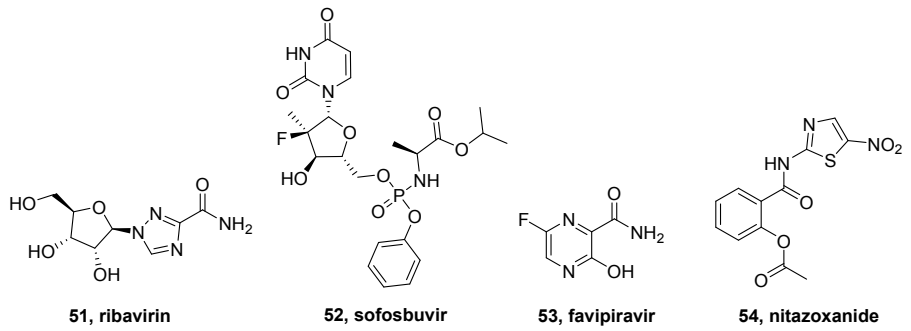


Figure 4. The representative broad-spectrum antiviral drugs.

Overall, drug repurposing, combined with innovative methods for drug validation, has greatly contributed in recent years to the discovery of novel antiviral molecules, broad-spectrum antiviral agents (BSAA), and targets for therapeutic intervention.[54]

In particular, the discovery and development of BSAA has been a key aim of antiviral research,[55] and various broad-spectrum nucleoside derivatives, host-targeting antiviral agents and immune-modulating agents have been reported (Figure 4). For example, ribavirin (**51**), a nucleoside analogue with broad-spectrum antiviral potency, is effective against a mammalian bornavirus, avian bornaviruses, and Borna disease virus.[55] Sofosbuvir (**52**), a clinically approved anti-HCV drug, also inhibits Zika virus RNA polymerase.[56] Favipiravir (**53**, T-705), a broad-spectrum antiviral compound approved in Japan for treating influenza virus infection, has already been used off-label to treat patients infected with Ebola virus and Lassa virus.[57] Nitazoxanide (**54**), a US FDA-licensed drug for treatment of enteritis due to protozoa, parasites, and anaerobic bacteria, also displays a variety of antiviral potencies and is currently in phase II/III clinical trials for treatment of infections caused by influenza viruses, HCV, norovirus and rotavirus.[58]

Host-based antiviral agents could interfere with viral pathogenesis by targeting host cellular factors required for viral infections or innate immune responses.[59] Several potent inhibitors of inosine-5'-monophosphate dehydrogenase[60-63] and inhibitors of phosphatidylinositol 4-kinase IIIβ,[64] have in vitro antiviral potency against a range of

DNA and RNA viruses (HCV, human rhinovirus, and coxsackievirus B3, Zika virus, norovirus, influenza virus and dengue virus). Recently, it was reported that ITZ also displayed broad-spectrum antiviral potency against enteroviruses, cardioviruses and HCV, via targeting oxysterol-binding protein, a cellular lipid shuttling machinery. The core structure bearing five rings, and the *sec*-butyl chain are crucial to antiviral potency, whereas the triazole ring, which is indispensable to antifungal potency, is not.[65]

1.2 "Privileged structure" repositioning

The hit rates by repositioning of commercially available approved or experimental drug libraries are usually quite low, and the hits often have low structural diversity. As a supplement, compound collections based on privileged structures offer the potential of libraries encompassing favorable physicochemical profiles and containing privileged scaffolds known to target various cellular targets, thereby increasing the success rate of discovering selective molecules that inhibit specific targets. In this section, it is intended to be illustrative rather than comprehensive, and the examples are chosen to convey the range of opportunities available and the current state of the art of "privileged structure" repositioning, highlighting its contributions to new drug discovery.

1.2.1 Diversity-oriented "privileged structure" repositioning

Diversity-oriented synthesis (DOS) based on privileged structures employed in existing drugs have frequently other, undiscovered activities. This observation highlights opportunities for drug discovery through screening of drug (lead)-like compound libraries; the resulting hits are likely to be safer and less expensive to develop than conventional hits, as well as having a shorter development timeline.[66,67]

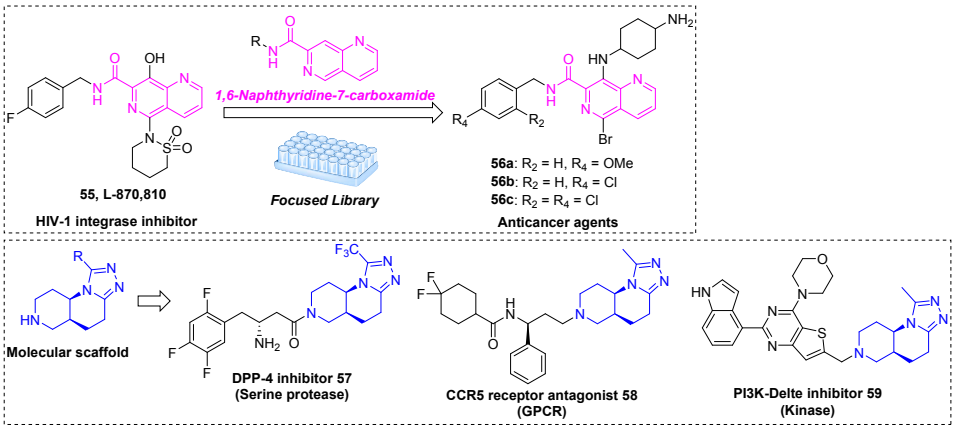


Figure 5. Discovery of bioactive molecules via diversity-oriented "privileged structure" repositioning approach.

1,6-Naphthyridine-7-carboxamide (in pink) has been regarded as a promising motif with drug-like properties. Among structurally diverse HIV-1 integrase inhibitors, the 8-hydroxy[1,6]naphthyridine L-870,810 (**55**) was a promising anti-HIV drug candidate, but in spite of its pharmacological activity, the development of **55** was halted during phase I clinical studies (reasons unknown). Nevertheless, because of its desirable drug-like properties, novel derivatives of **55** (substitutions at the 5- and 8-positions) were designed to overcome the limitations of naphthyridine-7-carboxamides as antiviral compounds and to reposition them as novel cytotoxic anti-cancer agents. Finally, further structural decoration of the 5,8-disubstituted-[1,6]naphthyridines (on 7-carboxamide) afforded novel molecules **56a-c** with remarkable cytotoxicity towards a set of cancer cell lines and high potency against selected oncogenic kinases (Figure 5).[68]

In addition, a newly synthesized tractable tricyclic scaffold has been incorporated into new analogues of bioactive drug candidates across multiple target families, affording the potent serine peptidase DPP-4 inhibitor **57**, a CCR5 receptor antagonist **58**, and the highly selective PI3K δ isoform inhibitor **59**.[69] Overall, the platform described in this section is widely applicable to accelerate drug discovery by using privileged

structures from drugs approved for other indications.

1.2.2 Target similarity-inspired "privileged structure" repositioning

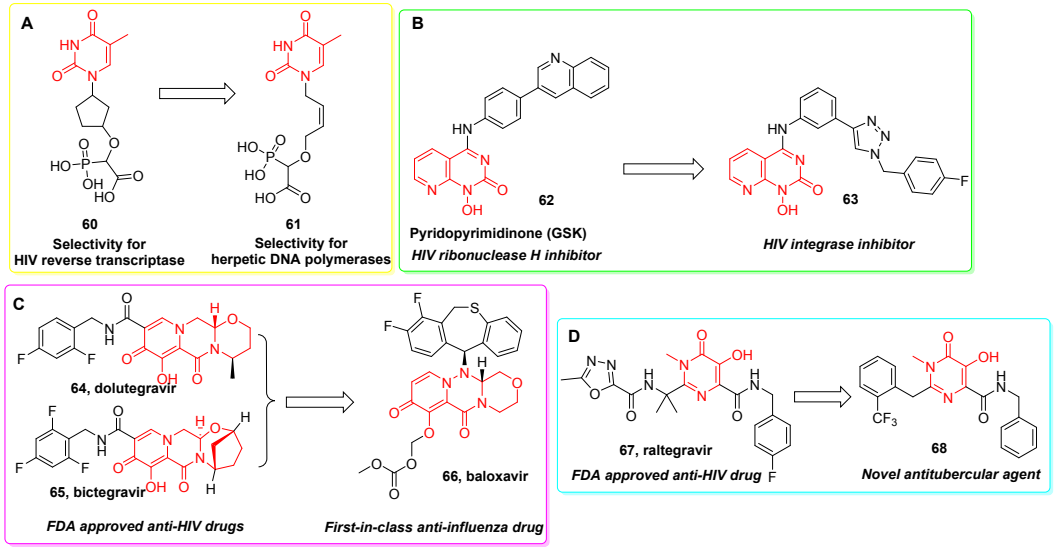


Figure 6. Discovery of bioactive molecules *via* target similarity-inspired "privileged structure" repositioning.

Taking into account the structural understanding of targets, the design and screening of focused libraries (target-oriented synthesis, TOS) based on privileged structure platforms is an efficient strategy to explore chemical space and to discover novel hits. For example, α-carboxynucleoside phosphonate (α-CNP) **60** with a cyclopentyl linker between the nucleobase and α-carboxyphosphonate, inhibits HIV-1 reverse transcriptase preferentially (50 to 100 fold) over herpetic DNA polymerases. Interestingly, modifications in the linker moiety (compound **61**) resulted in pronounced shift of the inhibition target (~50 to 100 fold) from HIV reverse transcriptase to herpetic DNA polymerases (Figure 6).[70]

Ribonuclease H (RNase H)-like superfamily, also called retroviral integrase superfamily (including some metalloenzymes: HIV integrase, HIV RNase H, HBV RNase H, human cytomegalovirus pUL89 endonuclease, the NS1 protein of parvovirus B19, influenza virus polymerase acidic endonuclease), has attracted great attention as a potential drug target. The metalloenzyme activity is dependent on metal

ions located in the catalytic site.[71] The shared enzymatic mechanism employed by many di-cation dependent polynucleotide metabolizing enzymes provides a huge opportunity for a multi-pronged drug discovery effort. Divalent metal ion chelators, such as N-hydroxyimide, diketo acid, α-hydroxytropolone, pyrimidinol carboxylic acid chemotypes, *etc.,* were reported as broad-spectrum antiviral agents via chelation of active-site divalent metal ions of metalloenzymes (as exemplified by **62** and **63**).[72-74] For example, some anti-HIV RNase H compounds can inhibit HIV integrase, and vice versa.[72,74] Compounds prepared during anti-HIV RNase H screening should be screened against HBV, moreover toxicity data of some of these molecules is known.[72] The endonuclease domain (pUL89-C) has an ribonuclease H/integrase-like fold. Like ribonuclease H, pUL89-C endonuclease function is dependent on metal ions in the catalytic site. pUL89-C endonuclease function was inhibited by HIV integrase inhibitor raltegravir.[73e]

Baloxavir marboxil (**66**, trade name Xofluza) is a cap-dependent endonuclease inhibitor for treatment of influenza A and influenza B FDA-approved in October 2018 and given via oral single dose once/day. It shares a similar metal-chelating pharmacophore (red) in the structures of HIV integrase inhibitors dolutegravir (**64**) and bictegravir (**65**), which was prepared from readily derivatizable building blocks with well-established preparation methods.[75]

Analogously, based on integrase inhibitor-like pharmacophore (N-alkyl-5-hydroxypyrimidinone carboxamide in Raltegravir (**67**)), target similarity-inspired "privileged structure" repositioning resulted in the discovery of N-alkyl-5-hydroxypyrimidinone carboxamide **68** as a novel antitubercular agent against Mycobacterium tuberculosis.[76]

2. Bioorthogonal chemistry-inspired drug discovery

Bioorthogonal chemistry, refers to the chemical reaction that can occur at physiological conditions without interference from biomolecules, has been widely used in chemical biology and gradually used in drug discovery.[77] Several chemical reactions have been developed that fulfill the criteria of bioorthogonality, as exemplified by commonly used Cu(I)-catalyzed or strain-promoted azide–alkyne cycloaddition, Staudinger ligation, or the 1,3-dipolar cycloaddition between cyclooctynes and nitrones, thiol–ene click chemistry, oxime/hydrazone formation from ketones and aldehydes, which have greatly facilitated the hit identification.

2.1 Rapid assembly and screening of focused combinatorial fragment libraries

In current drug discovery, parallel HTS or phenotypic screening of large compound collections, is considered an effective approach to exploit chemical space and rapidly find hit compounds. But the time-consuming and labor-intensive compound separation processes prior to evaluation have been bottlenecks in drug discovery.[78] In recent years, the rapid assembly and direct screening of focused combinatorial fragment collections in microtiter plates (or in parallel reactors) using bioorthogonal reaction (predominantly, click chemistry) has been developed as a robust and efficient method to establish structure-activity relationships (SARs) and for discovering bioactive molecules (Figure 7),[79] as exemplified by the discovery of HIV-1 PR inhibitors **69-71**,[80] highly selective and potent epigenetic inhibitors **72-76** (HDACs and SIRTs),[81] JAK inhibitor **77**,[82] and glucocerebrosidase inhibitor **78**.[83] In our recent review, the inherent limitations and challenges facing this methodology were critically discussed.[79]

Undoubtedly, the success rate of this fragment-based drug discovery methodology relies on the information of detailed binding conformation and binding affinity of a range of small fragments bind to their respective targets. Besides, exploration of other

HTS-amenable organic reactions suitable for efficient synthesis and screening of diverse chemical libraries, such as copper-free click chemistry, is crucial in overcoming these problems.[84-86]

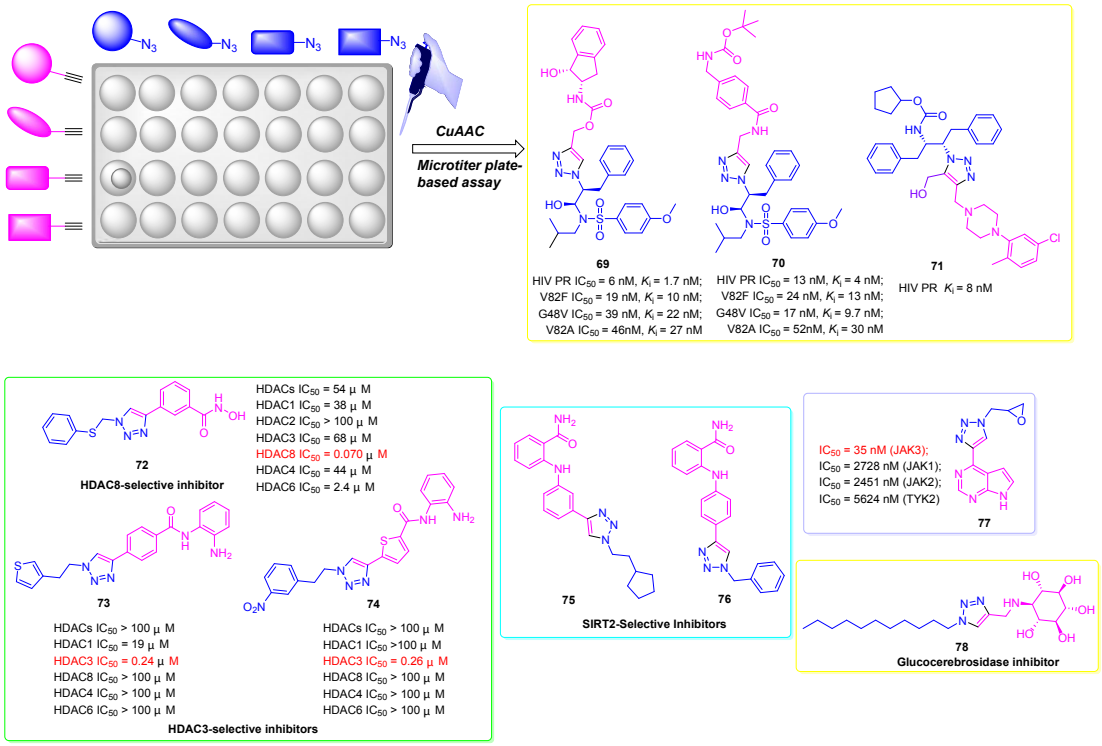


Figure 7. Discovery of highly potent and selective inhibitors through click chemistry-based combinatorial fragment assembly. CuAAC: copper(I)-catalyzed azide-alkyne [3+2] dipolar cycloaddition.

2.2. Target-guided synthesis (TGS) approaches

Target-guided synthesis (TGS) has proven a robust strategy in recent years for its original concept: using the biological target itself to assemble its selective ligands directly from a pool of fragments bearing complementary reactive functional groups. The approaches, bridging the gap between chemical synthesis and bioactivity assays, are divided into two major approaches: the kinetic TGS (KTGS) approach (Figure 8A), namely, the kinetically controlled reactions involving irreversible bond formation,[87] and the thermodynamically controlled reactions involving reversible reactions (also known as dynamic combinatorial chemistry, DCC) (Figure 8B).[88] Both strategies have been extensively and successfully implemented for hit finding for

receptors and enzymes,[89] such as neuraminidase,[90] carbonic anhydrase,[91] kinase/phosphatase,[92] cyclooxygenase-2,[93] *etc*.

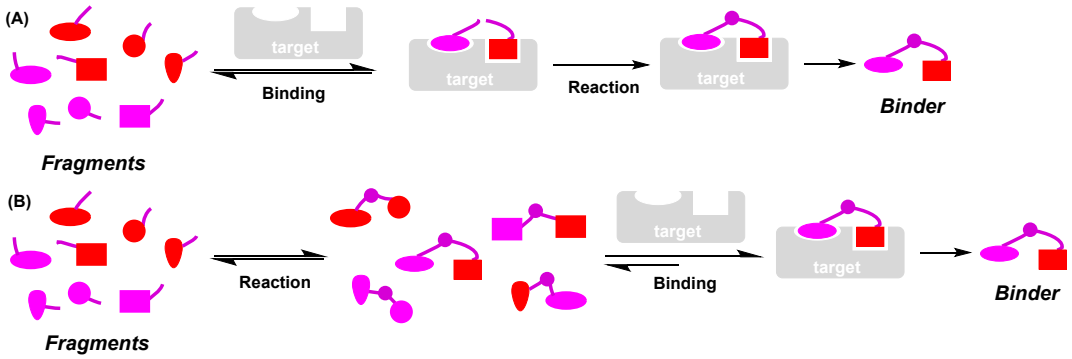


Figure 8. (A) KTGS principle. best building blocks from each binding pocket of the biomolecule would preferentially react together because of their spatial proximity, and afforded the corresponding dimeric binder displaying synergistic bioactivity. (B) Thermodynamically controlled TGS or DCC principle. From appropriate fragments under equilibrium conditions, suitable dynamic combinatorial libraries (DCLs) are built up, allowing the generation of all possible library members in a thermodynamically controlled distribution (via selection pressure of the bio-target).

Particularly, structure-guided fragment linking of precursors that display weak affinity to the target (the KTGS approach) is considered a robust way to rapidly find potent inhibitors, based on cooperative binding. In KTGS approach, the bio-target accelerates the irreversible reaction (*in situ* click chemistry) between complementary fragments by bringing them into close proximity in proper orientation.[89] In 2017, Wang *et al.*[94] reported two cell-permeable O-GlcNAc transferase (OGT) inhibitors (**79** and **80**, IC_{50} = 139μM and 66.7 μ M, respectively), developed from low-activity components (IC_{50} > 1 mM) *via* a kind of dynamic combinatorial chemistry, namely, "tethering *in situ* click chemistry" (Figure 9A). The discovery of these compounds supports the idea that tethering *in situ* click chemistry can be utilized to search novel lead molecules from weak-binding fragments.[94]

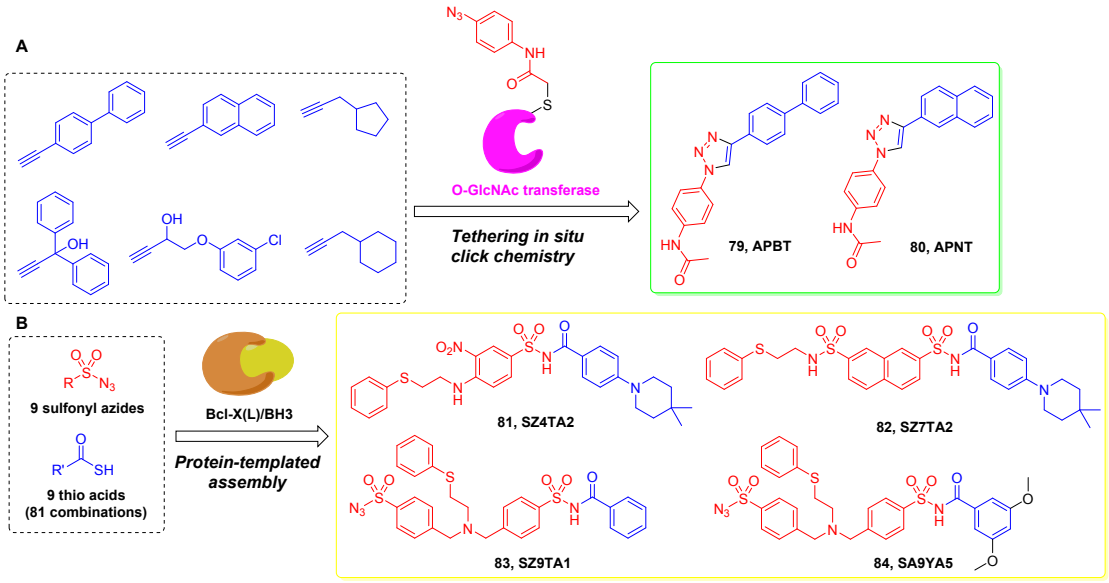


Figure 9. Kinetic target-guided synthesis of (A) cell-permeable O-GlcNAc transferase inhibitors and (B) Bcl-X(L)/BH3 protein-protein interaction modulators.

In 2011, Manetsch *et al.* reported the use and validation of KTGS via the sulfo-click reaction between sulfonyl azides and thio acids as a valuable tool for the discovery of potent modulators targeting protein-protein interaction (as exemplified by Bcl-X(L)/BH3), acylsulfonamides **81-84** (Figure 9B).[95,96] These results demonstrated that KTGS based on the sulfo-click reaction is a screening and synthesis platform for the straightforward identification of high-quality modulators.[96]

However, in most cases, target-guided synthesis requires purified proteins, which limits its application. In 2018, by applying metabolomics methods, Antti *et al.* demonstrated that target-guided synthesis with target proteins can also be achieved directly in cellular environments.[97] This method opens up new possibilities to screen drug candidates for difficult target proteins in cell-based systems.

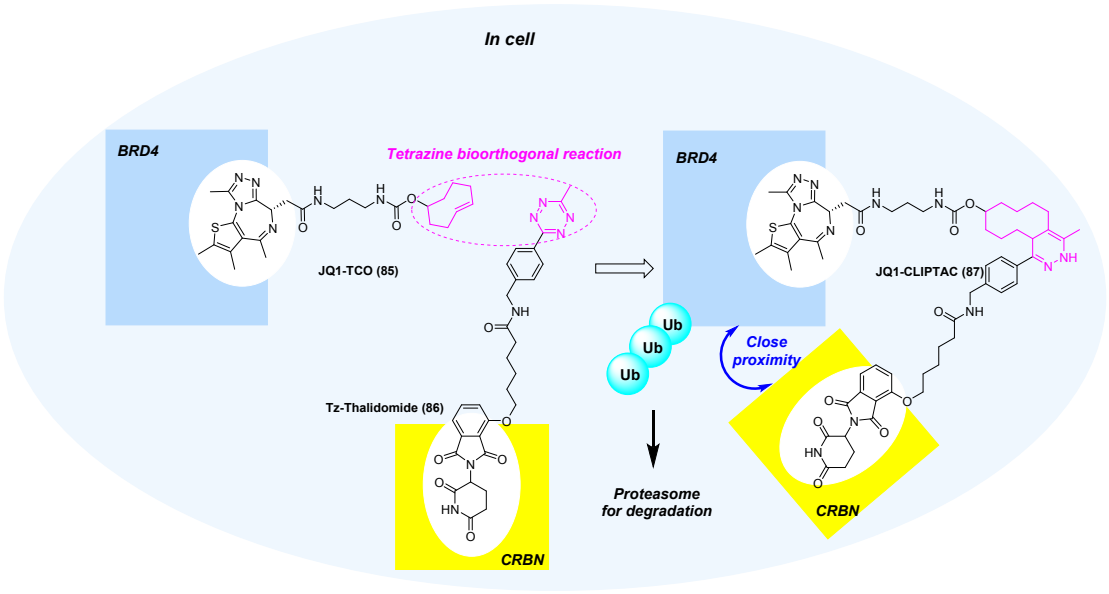


Figure 10. Tetrazine bioorthogonal reaction was used to form drug-like heterobifunctional molecule **87** inside cells, as the BRD4 degrader via the ubiquitin pathway.[98]

Recently, the tetrazine bioorthogonal reaction has been used by Astex Pharmaceutical to stitch together functional proteolysis targeting chimeras (PROTACs) for degrading anticancer drug targets BRD4 and ERK1/2 within living cells (Figure 10). Notably, *in situ* assembly of two small compounds within a cell demonstrated higher efficacy than simply adding the preassembled compound.[98]

On the other hand, DCC represents a promising approach for a highly efficient generation of libraries.[89] As yet, however, this approach is limited by the techniques used for the analysis of protein–binder complexes and the few appropriate reactions. Several techniques have been used to the analysis of protein-directed DCL. These include X-ray crystallography, NMR spectroscopy, HPLC and mass spectrometry. X-ray crystallography and NMR spectroscopy are high-resolution techniques, but are time-consuming. Notably, competitive mass spectrometric (MS) binding assays have proved to be an effective method for the affinity determination of single molecule toward a protein of interest, which has been readily extended to the screening of compound collections as well. In the search of new γ-aminobutyric acid transporter 1

(GAT1) inhibitors, an exploratory research was undertaken in which pseudo-static hydrazone libraries were generated by DCC and screened against GAT1 using MS binding assays (Figure 11). Hydrazone **88** bearing a 2',4'-dichlorobiphenyl moiety was identified as a robust binder with low nanomolar affinity (pK_i = 8.1). Further optimization afforded **89** (pK_i = 6.9) as a stable carba analogue of hydrazone.[99]

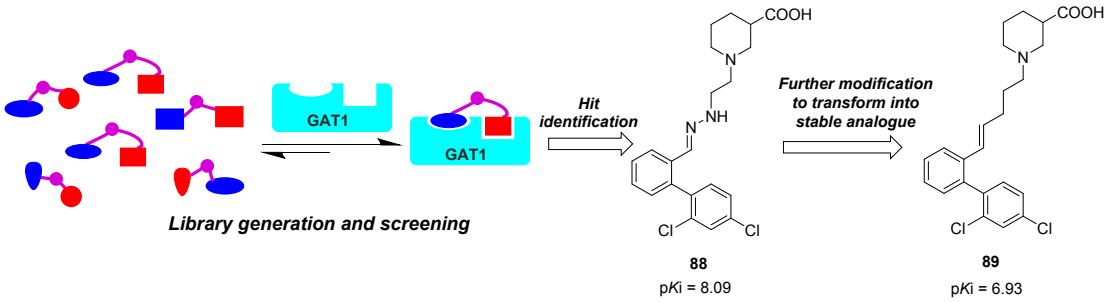


Figure 11. GAT1 inhibitor hits **88** and **89** were obtained by means of library screening and further optimization.

Besides, the efficient dynamic-combinatorial MS technique has the advantages of providing detailed information on mass shifts. In recent years, the disulfide or boronic acid/boronate ester dynamic systems coupled with protein MS analysis has been employed in the rapid discovery of JmjC histone demethylases and nucleic acid demethylases.[100]

To expand the potential and scope of DCC, very recently, a novel multi-protein DCC strategy was developed, which combines the sensitivity of differential scanning fluorimetry and the discriminatory power of zwitterionic 'thermal-tag'. This methodology enables simultaneous identification of subfamily-selective inhibitors against several targets of interest.[101] It was illustrated that from aldehyde and amine fragments, a quadruplex nanotemplate can dynamically select and amplify selective G quadruplex DNA binders, which further enriched the literature in this area and indicate the increasing level of interest in this field.[102]

2.3. Bioorthogonally activated chemotherapy (bioorthogonal uncaging strategies)

Complementary to bioorthogonal reactions that ligate two molecules, there is an increasing interest in reactions that cleave a linker or release a molecule. Such dissociative bioorthogonal reactions have a broad spectrum of uses, including in drug delivery.[103]

To avoid toxic side effects on healthy cells and tissues, much research has been directed at the design of cancer-specific strategies (selective delivery of chemotherapeutic drugs to cancer cells), for example by using prodrugs via controlled activation, which are inactive precursors of cytotoxic agents, but can be biochemically converted into their active forms in a spatially controlled manner. Recent advances in bioorthogonal catalysis are increasing the ability of medicinal chemists to manipulate the fate of molecules in complex biological systems. Consequently, bioorthogonal uncaging (bioorthogonally activated chemotherapy, also termed "click to release") have recently reported as an experimental prodrug therapeutic strategy to control drug release by the application of solid metals (mainly palladium) as implantable activating devices to catalyze various chemical reactions in biocompatible environments, and to modulate the cytotoxicity of anticancer agents in specific biological settings.[104,105] While soluble palladium species such as Pd^{2+} complexes exert inherent cytotoxicity, Pd(0) catalysts are biocompatible and seem to be the safer agents.[105]

Recently, Unciti-Broceta reported palladium-labile biorthogonal prodrugs (**92**-**95**) of several anticancer drugs, including cytotoxic gemcitabine (**2**),[105d] 5-fluorouracil (**90**, 5FU),[105e,f] floxuridine (**91**)[105g] and vorinostat (**28**)[105h] by introducing a Pd(0)-cleavable group (N-propargyloxycarbonyl (N-Poc) promoiety) at positions that are mechanistically relevant for the bioactivity of the original anticancer agents. As shown in Figure 12, such prodrugs are converted into cytotoxic agents selectively in the presence of Pd(0). For example, a Pd(0)-functionalized device could be surgically

implanted in the affected area of tissue or organ, enabling local treatment of the disease with prodrugs, while reducing side effects in distant tissues and organs.[105] Very recently, new Pd-labile NO precursors were reported, which can be effectively uncaged by a biocompatible Pd(0) catalyst *via* bioorthogonal bond cleavage to release NO in living cancer cells, eliciting a potent antiproliferative effect.[106]

There remains great interest in novel bioorthogonal uncaging strategies, as exemplified by the newly disclosed inverse electron-demand Diels-Alder reaction and gold-triggered uncaging chemistry.[107,108]

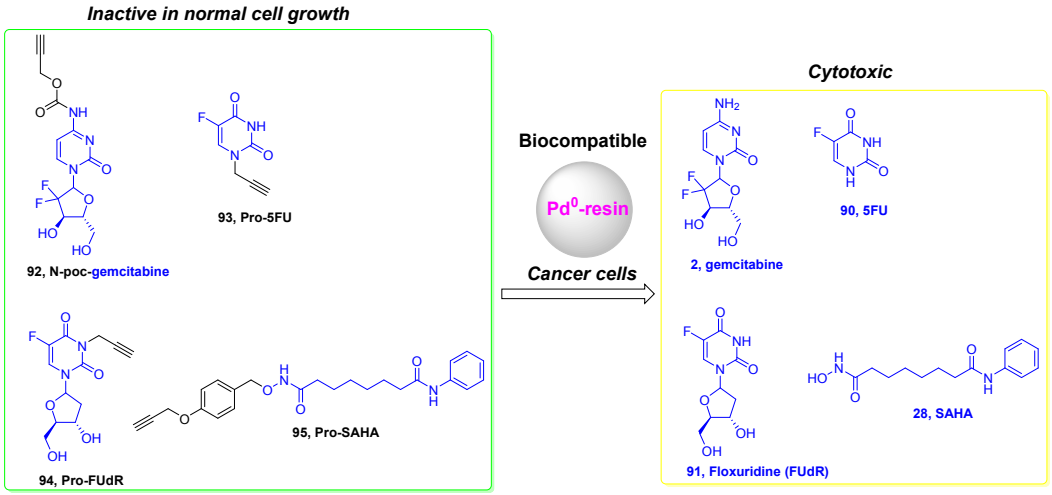


Figure 12. Bioorthogonally activated palladium-labile prodrug strategy and toxigenic mode of action.

Another prominent example is the application of the highly strained alkene transcyclooctene and ene ether to mask functional groups, including amines and alcohols (Figure 13), which is then liberated upon reaction with a tetrazine.[109] In 2018, the group of Wang reported a concentration-sensitive bioorthogonal prodrug activation approach by taking advantage of reaction kinetics-controlled tetrazine–cyclooctyne click reaction, and spontaneous cyclization-based release. This study robustly demonstrated the concept of enrichment-triggered prodrug activation specifically in mitochondria and the critical feasibility of treating the related clinical diseases such as cancer and acute liver injury.[110]

In summary, the use of bioorthogonal reactions to trigger caged groups furnishs a great opportunity to activate on the the human body and could be translatable to patients in theory. We anticipate that more and more investigators will apply "click to release" approaches to trigger the release of drugs in vitro and in vivo.[110b]

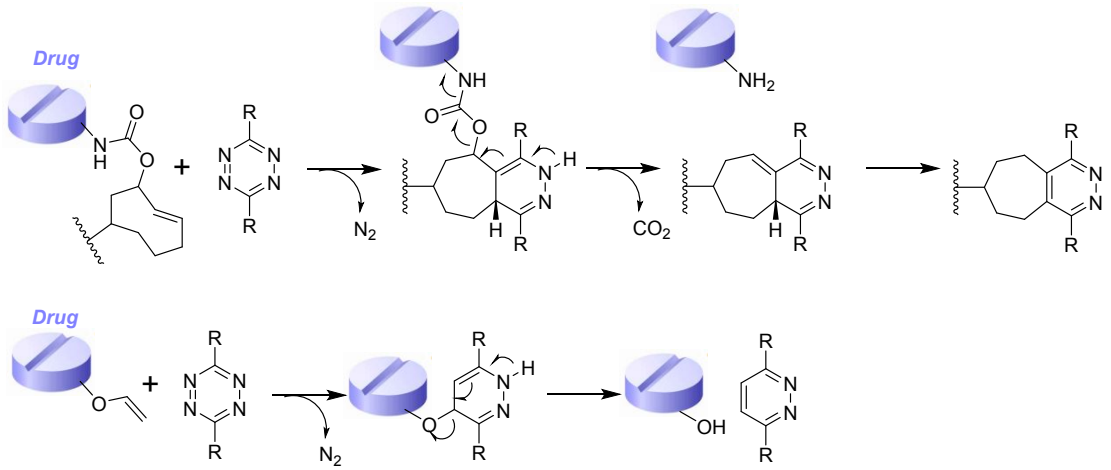


Figure 13. Schematic illustration of the application of the highly strained alkene transcyclooctene and ene ether to mask an amine or alcohol.[109]

3. Photoactivatable medicinal chemistry

Light has been used to suddenly liberate a ligand or substrate by removing a photolabile- protecting group (PPG) from a suitable precursor molecule, to produce reactive oxygen species from photosensitizers or to control the activity of ligands in precise spatiotemporal manner to investigate the structure and function of important biotargets, different concepts may be followed, as exemplified by photoactivatable caged (phototriggering) prodrug, photodynamic therapy and photoswitchable ligands. These are collectively referred to as "photoactivatable medicinal chemistry".

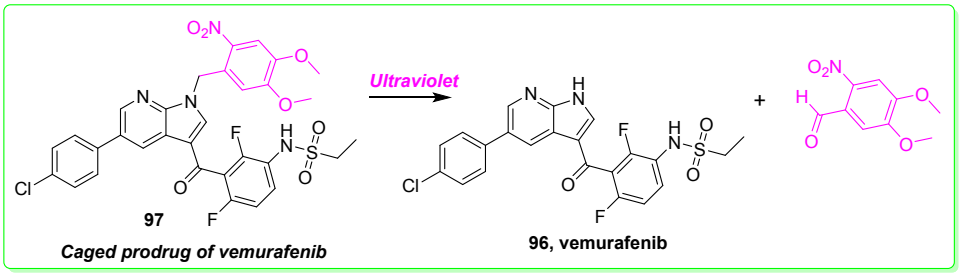
3.1 Photoactivatable caged prodrug

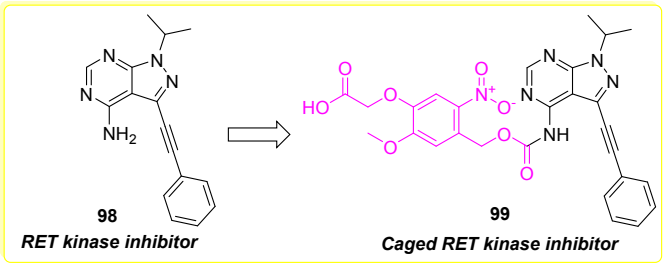
A newly emerging approach to regulate the action of bioactive compounds in a spatial and temporal control manner employs PPGs.[111] The PPG is a chromophore covalently linked to the pharmacophoric group of the bioactive compound, thus inhibiting its bioactivity, a concept known as "caging" (the photo prodrug). The covalent bond

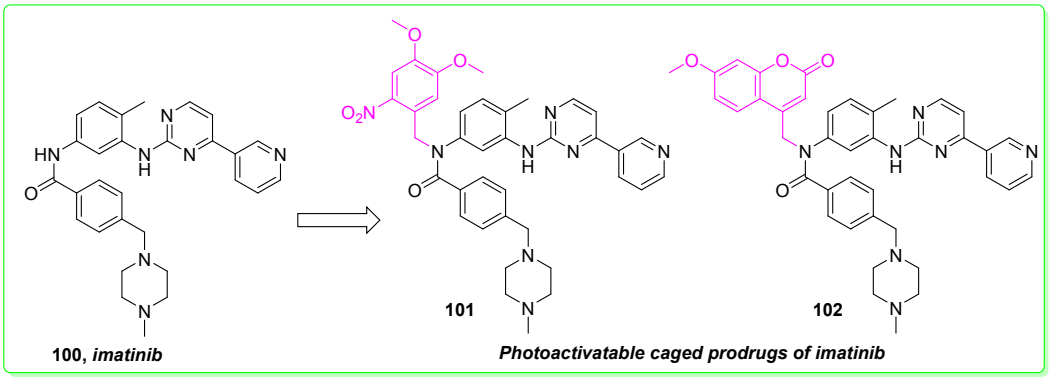
between the PPG and the drug molecule is broken by irradiation with ultraviolet light, resulting in the formation of the parent drug molecule ("uncaging").

The off-target effects of systemically administered anticancer drugs heavily constrained their efficacy and tolerability. The photocaging concept has been used in the delivery of drugs across membranes; it reduces off-target effects, as exemplified by photoactivatable prodrug (**97**) of the anti-melanoma agent vemurafenib (**96**),[112] caged RET kinase inhibitor **99**,[113] photoactivatable caged prodrugs (**101** and **102**) of imatinib (**100**),[114] photocontrolled HDAC inhibitors **103**,**104**[115,116] and **106**, a photoactivatable prodrug of doxazolidine (**105**) targeting exosomes (Figure 14).[117]

Meanwhile, a number of PPGs have been exploited for this purpose, including *p*-nitrobenzyl, 4,5-dimethoxy-2-nitrobenzyl, 7-diethylaminocoumarin-4-ylmethyl, and 6-bromo-7-hydroxycoumarine-4-ylmethyl. The successful application of the photoactivation concept to some kinase inhibitors and HDAC inhibitors afforded further evidence for this concept as a feasible approach in many other fields.[118,119] These photoactivatable caged prodrugs can be useful as pharmacological probes to investigate the impact of the parent molecules toward biological systems.


Ultraviolet
97
Caged prodrug of vemurafenib
96, vemurafenib


98
RET kinase inhibitor
99
Caged RET kinase inhibitor


100, imatinib
101
102
Photoactivatable caged prodrugs of imatinib

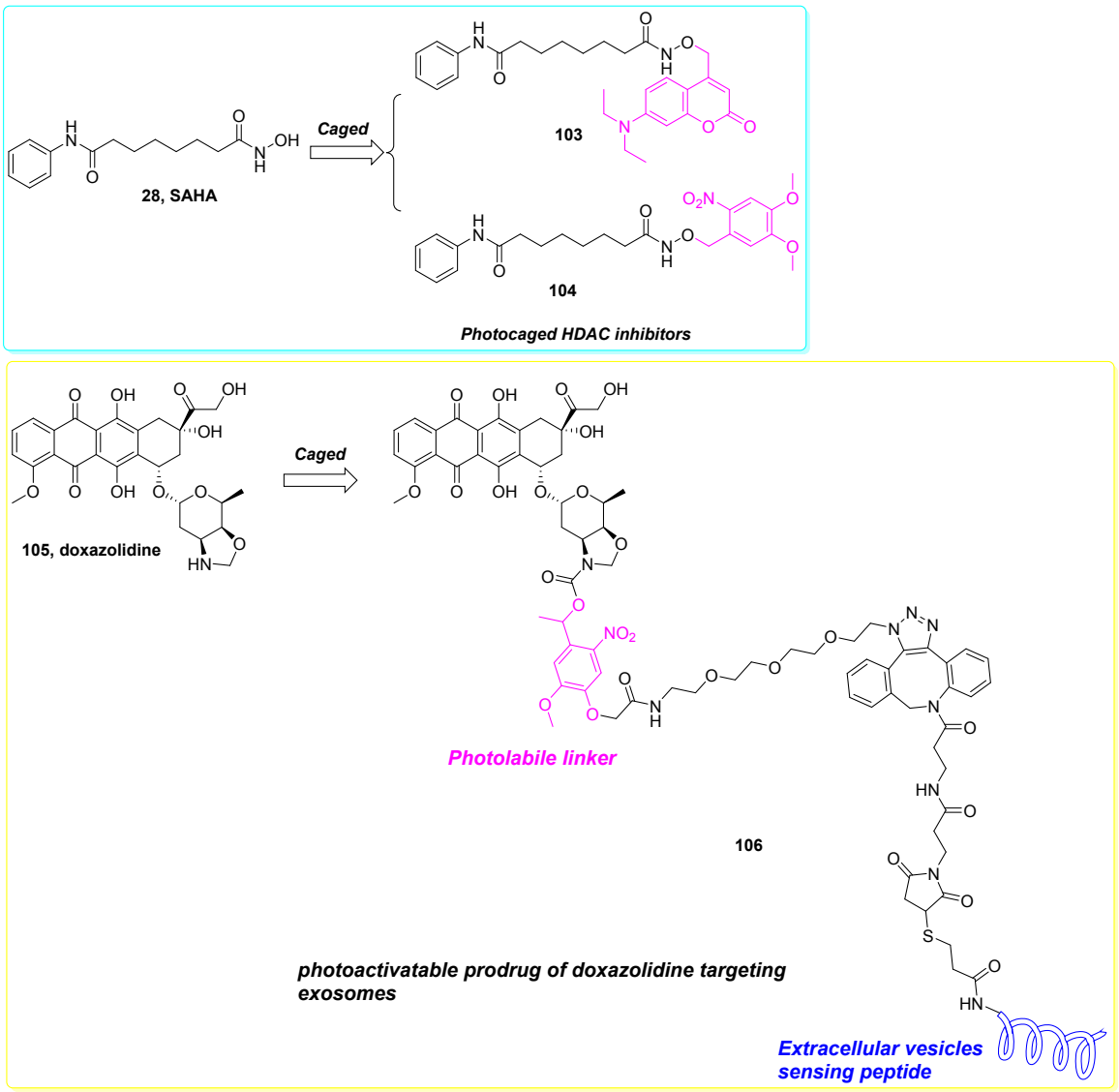


Figure 14. Photoactivatable caged kinase inhibitors, HDAC inhibitors and photoactivatable prodrug of doxazolidine targeting exosomes. Essentially, The photo prodrug concept is based on the mask of a pharmacophoric moiety. The PPG (pink) is therefore attached to the bioactive compound by a covalent bond. The parent bioactive molecules can be released by irradiation with ultraviolet light. Therefore, this method might improve higher drug concentrations in the area of interest sparing other compartments (such as cancer-afflicted tissues) in a rapid and efficient manner with lower side effects.

3.2 Photodynamic therapy

Photodynamic therapy (PDT) is well studied and established in clinical application since the approval of the first drug, porfimer sodium, based on the characteristics of strong metabolism of tumor cells, after injection of photosensitizers (drugs), the

concentration of tumor tissue is notably higher than that of neighboring normal tissues. At appropriate time, light irradiation with specific wavelength could activate photosensitizers, produce reactive oxygen species (such as singlet oxygen), and specifically kill cancer cells and destroy neovascularization.[120] PDT has proven a promising treatment option for various kinds of cancers and non-malignant diseases including infections. Even though several photosensitizers have been clinically approved already, the development of additional photosensitizer with high phototoxicity, low dark-toxicity and favorable aqueous solubility is very challenging for PDT.[121-123]

Several methods have been employed to obtain more efficient and less toxic photosensitizers. For example, conjugation with tumor-specific ligands (including small molecules, peptides and proteins) significantly improves the selectivity of the active photosensitizer toward specific cells. In 2017, the conjugates **107** and **108** were synthesized by coupling zinc phthalocyanine to gonadotropin-releasing hormone (GnRH) analogues. Compared to unmodified zinc phthalocyanine, conjugates **107** and **108** demonstrated higher and more specific phototoxicities against breast cancer cells, and robust in vivo anticancer efficacies (in animal model). Conjugate **108** exhibited high safety profile for its low retention in brain and skin (Figure 15).[124]

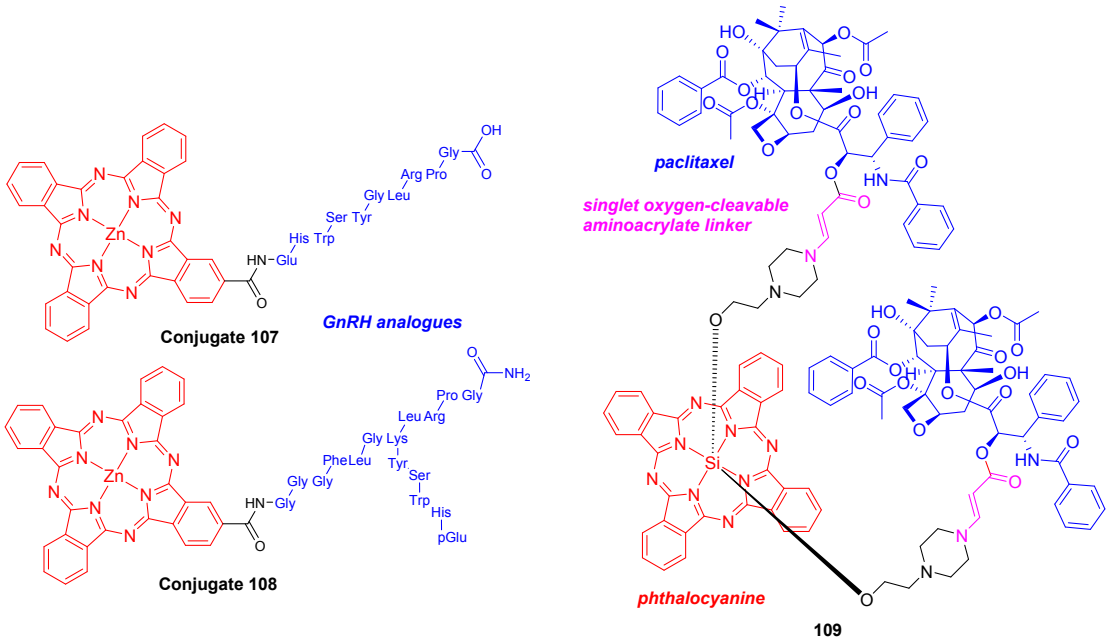


Figure 15. The photosensitizers **107** and **108** mediated by GnRH receptors, and **109**, the far-red light-activatable prodrug of PTX.

In 2016, compound **109**, the far-red light-activatable prodrug of paclitaxel (PTX), was prepared by conjugating the photosensitizer *via* singlet oxygen-cleavable aminoacrylate linker (Figure 15). Through the combined effects of site-specific paclitaxel chemotherapy and photodynamic therapy, the cytotoxicity was significantly reduced.[125]

A recent study shows that hydroxypyridinone and 5-aminolaevulinic acid conjugates could substantially enhance the formation of phototherapeutic metabolite and phototoxicity.[126] In 2018, a novel series of porphyrin-based water-soluble derivatives were reported as potential sensitizers for effective PDT against breast cancer.[127] In 2018, two advanced boron dipyrromethene (BODIPY)-based photosensitizers with a glibenclamide-derived moiety were reported to behave as singlet oxygen provider with high photostability.[128]

This direction will continue to be a hot topic in the field of anticancer drugs.[129] In the

future it would be of high interest to explore if the photodynamic effectiveness may be improved, and at the same time the systemic toxicity may be reduced by photosensitizers that circulate in their inactive form in the normal tissues and are activated only by specific conditions in cancer cells (e.g., lower pH value, reducing environment). Furthermore, research in this direction should pay attention to questions like: a high concentration of glutathione present in the tumor tissues can consume reactive oxygen species; PDT is often followed by recurrence because of incomplete ablation of tumors.

3.3 Photoswitchable ligands: azobenzenes

There is growing interest in designing spatiotemporal control over enzyme activities using noninvasive stimuli, as exemplified by light.[130] Photoswitchable ligands, also termed photochromic ligands, are a class of molecules whose activity at a target of interest can be controlled precisely in a reversible manner by light. When irradiated with light of a certain wavelength, such molecules undergo a configurational change in their structure in a reversible way, which may substantially alter their binding affinity to a specific target.[131]

In recent years, azobenzenes were used as photoswitch due to favorable geometric and photochemical properties. This approach resulted in discovery of potent photoswitchable inhibitors (**110** and **111)** of γ-aminobutyric acid transporter,[132] a potent photochromic antagonist **112** that selectively targets the calcium ion permeable AMPA-type of ionotropic glutamate receptors,[133] phototrexate (**114**), a photochromic analogue of methotrexate (**113**) as the human dihydrofolate reductase inhibitor (Figure 16).[134] All in all, this design approach opens new avenues for optically controlling enzyme function and optochemical biology.[135]

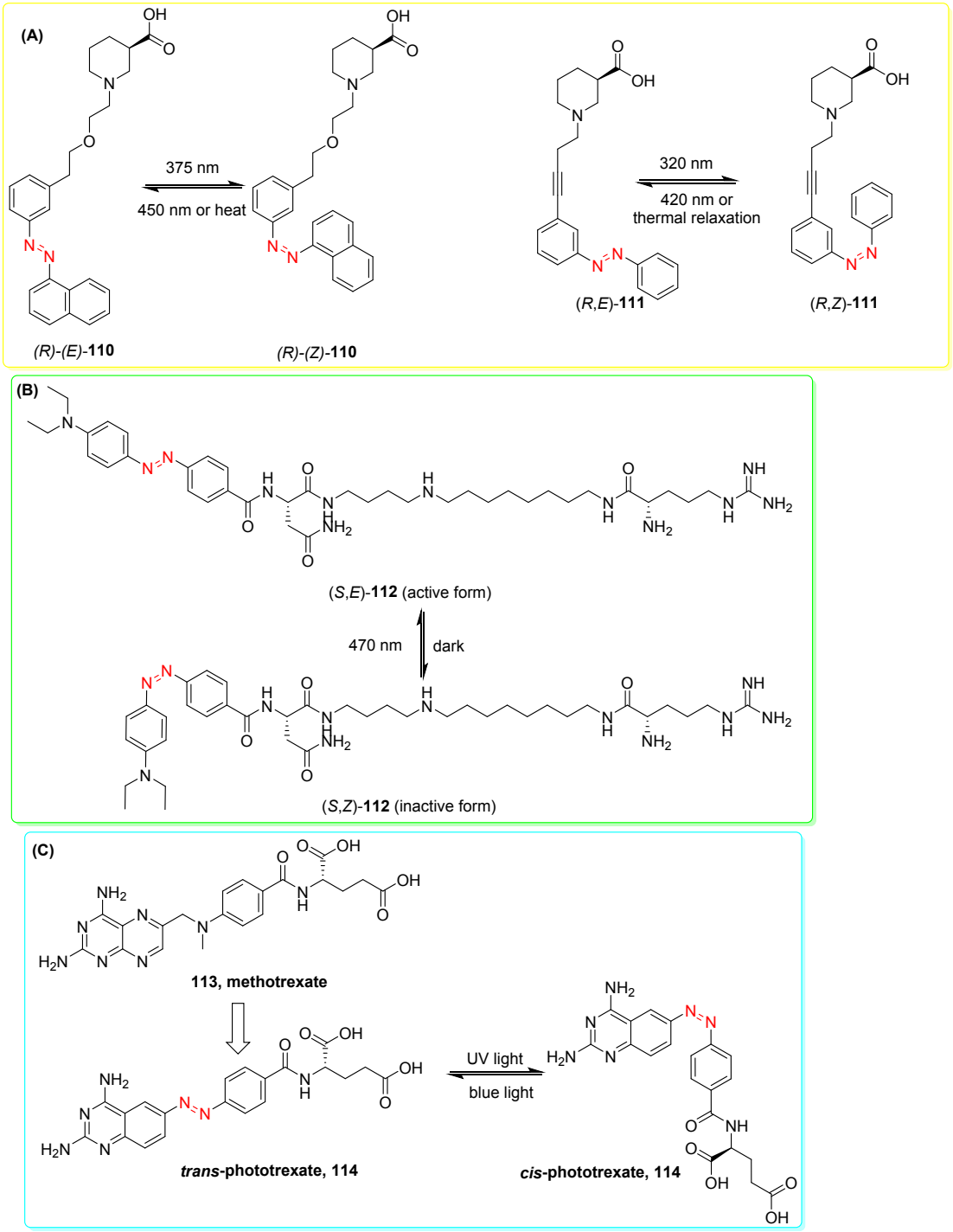


Figure 16. Chemical structures of phototrexate in the trans (in the dark and under blue/white light illumination) and cis (under UVA illumination) configurations.

4. Lead diversification via late-stage functionalization

Several strategies have been employed to generate collections of compounds with complex scaffolds, including diversity-oriented synthesis (DOS).[136] The direct

functionalization of complex scaffolds at a late stage, namely, late-stage functionalization (LSF), is another often used approach to build derivatives efficiently with precise and substantial modifications to the platforms of these molecules without resorting to *de novo* synthesis.[137] In recent years, there is a lot of research on LSF, which has enabled rapid diversification of drug-like molecules or drug candidates to improve their potency and drug-likeness. Many innovative methods have been developed for this endeavor, including late stage C–H functionalization, nucleophilic aromatic substitution, *etc*.

C–H activation reactions are valuable tools for medicinal chemists to directly introduce functional groups into a bioactive compound at a late stage of synthesis.[138] For example, in the optimization of NCH-31 (**115**), a histone deacetylase (HDAC) inhibitor, a late-stage C-H coupling approach enabled rapid identification of novel derivatives IYS-10 (**116a**) and IYS-14 (**116b**), as a potent pan-HDAC inhibitor and an HDAC6-insensitive inhibitor, respectively (Figure 17).[139]

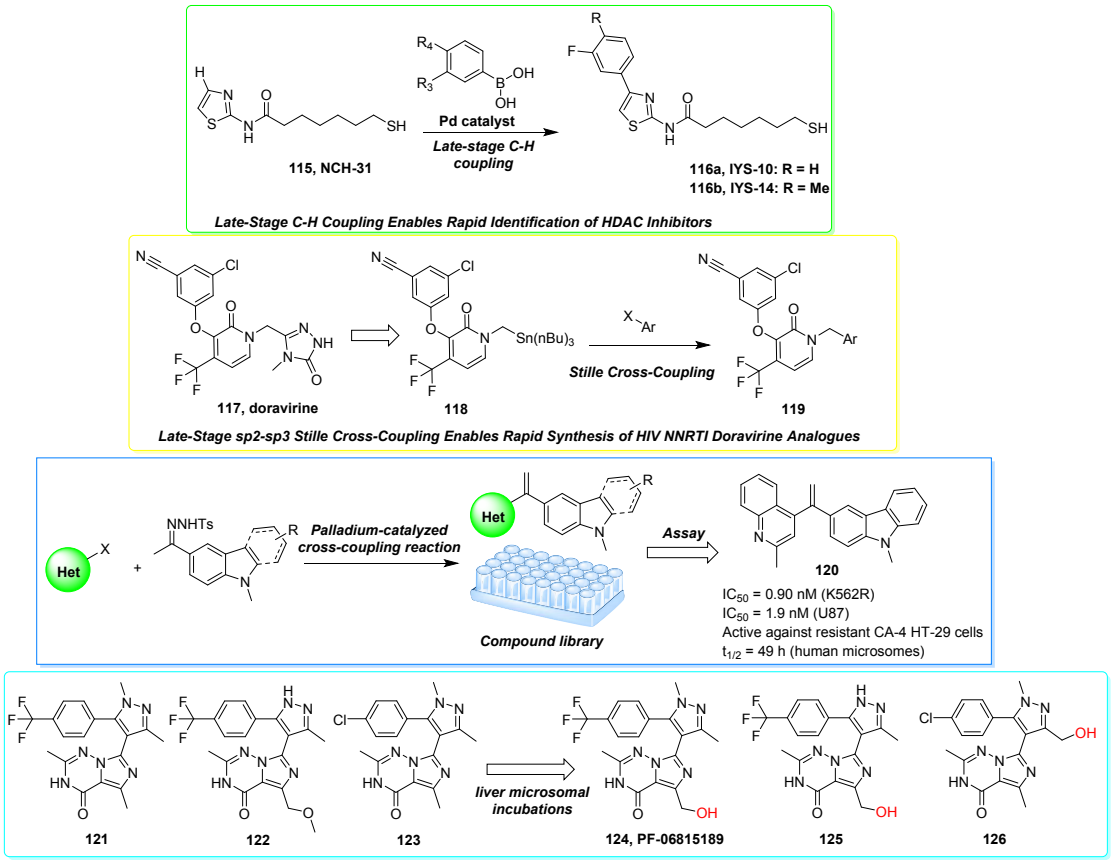


Figure 17. Late-stage functionalization using C–H diversification reactions and cytochrome P450 enabled rapid identification of HDAC inhibitors, HIV non-nucleoside reverse transcriptase inhibitors and phosphodiesterase 2 inhibitors.

In 2015, a stereodivergent and step-economical diversification of the privileged 2-arylcyclopropylamine motif by sequential C(sp^3)-H borylation and Suzuki-Miyaura coupling was developed.[140] Then, in 2017, El Marrouni *et al.* described the successful application of a palladium-catalyzed C(sp^2)-C(sp^3) cross-coupling reaction of a fully elaborated inactivated organostannane compound **118** with a range of aryl halides to achieve rapid, parallel synthesis of HIV non-nucleoside reverse transcriptase inhibitor **119** from doravirine (**117**) (Figure 17).[141]

The palladium-catalyzed cross-coupling reaction between N-tosylhydrazones and heteroaryl halides constitutes a rapid and efficient approach to the preparation of 1,1-diarylethylenes as novel heterocyclic analogues of isoCombretastatin-A4. Among

them, **120** induced G2/M cell cycle arrest. It also exhibited interesting potency against CA-4-resistant colon carcinoma cells and multidrug-resistant leukemia cells, high human microsomal stability (in comparison to isoCA-4) and central nervous system (CNS) permeability.[142]

Direct functionalization of complex molecules still faces several challenges, chemical reactions with high selectivity, high yield, and mild reaction conditions are required. Somewhat unexpectedly, it was found that the number of reactions, dominating the chemical landscape of contemporary medicinal chemistry, is very limited.[143] To expand the chemical space and structure diversity of compound libraries and to facilitate the late-stage derivatization of several complex pharmaceutical compounds, there is a need to develop more LSF compatible reactions that prepare functional compounds with desirable properties.[144,145] Novel organic catalysts and synthetic methodology (including photochemistry, electrochemistry) are particularly promising for late-stage derivatization of complex molecules.[146-148] Sulfur(VI) fluoride exchange (SuFEx) reaction, which relies on readily available building blocks to afford molecules with the sulfonyl fluoride motif, has also expanded the toolbox of organic and medicinal chemists.[149-151]

Metabolomics has been applied not only in the drug discovery process and personalized medicine, but also in lead diversification.[152] In 2018, Obach *et al.*[153] described a rapid and cost-effective late-stage lead functionalization method, whereby lead compounds can be converted into new derivatives by using liver microsomes at a submicromolar scale. Several representative human phosphodiesterase-2 inhibitors were incubated with liver microsomes from various organisms to afford multiple products, which were isolated and analyzed by quantitative cryomicroprobe NMR (qNMR) spectroscopy. The diluted solutions from qNMR analysis were subjected to

biochemical assays, which yielded compounds PF-06815189 (**124**), **125** and **126** with improved potency inhibiting phosphodiesterase-2, physicochemical profile and favorable metabolic properties, compared with the respective parent molecules **121-123** (Figure 17).[153] The examples highlighted herein illustrate the value of organic and biocatalytic C–H functionalization methods in drug discovery.[154]

5. Multiparameter optimization

A high-quality drug should exhibit a good balance of efficacy against its therapeutic targets, physicochemical properties, ADME properties (absorption, distribution, metabolism and elimination) and safety.[155,156] In other words, drug discovery is a multiparameter optimization (MPO) process in which the aim is to find novel pharmaceutical molecules that meet the multiple drug-like criteria. Examples are "rule of 5", "beyond rule of 5", “lead-like drugs”)[157-160] and ligand efficiency metrics (such as lipophilic efficiency).[161] Half of all therapeutic targets cannot be modulated with small-molecules that comply with the rule of 5. Macrocycles have been found to be in "beyond rule of 5" space, and were especially useful in drugging targets that have large, flat, or groove-shaped binding sites.[158]

Avoidance of toxicity and optimization of drug-like properties is a critical issue at the late stage of drug discovery.[162] Thus, in drug optimization, structure-property (or toxicity) relationship studies should focus on a range of targets, not merely activity.[163,164]

Drug discovery for the CNS disorders still faces huge challenges, for example, the optimization of lead compounds into drug candidates is difficult due to the strict physicochemical properties required to penetrate the blood−brain barrier. In 2010, a druglikeness CNS multiparameter optimization (CNS MPO) algorithm designed by Pfizer, which has parameterized medicinal chemistry design space for CNS drug

candidates.[165a] Since then, significant progress has been made in application of this simple-to-use design algorithm.[165b,c]

The significance of understanding the kinetics of the interaction between a ligand and its target has been acknowledged for a long time. Ligand-target residence time (structure-kinetic relationship, SKR) has also been valued as a key drug discovery parameter and it is still receiving sustained attention. Numerous recent research articles from the medicinal chemistry community provide compelling arguments for more widespread assessment of binding kinetics and discussion of SKR.[166-169]

The binding of ligand with target is influenced by multiple factors, including hydrogen bonds and hydrophobic interactions, residual mobility, desolvation, dynamics and the local water molecule.[170] Experimental tools to (un)binding kinetics are nowadays available,[171] but reliable computational methods for predicting kinetics and residence time are still lacking. Most attempts have involved molecular dynamics (MD) simulations, which are CPU-intensive, and not yet particularly accurate.[172,173]

In 2016, Mollica *et al.* reported a new scaled-MD-based protocol, verified by directly comparing computational predictions, experimental kinetics measurements and X-ray crystallography, which seems to have potential for predicting kinetics and drug residence times in drug discovery.[174] In considering structure-property-activity relationships, multiple aspects of ligand-protein binding need to be considered, including surface water networks coating protein-bound ligands[175] and water-mediated ligand functional group cooperativity.[176]

Off-rate screening by surface plasmon resonance (SPR) is an efficient approach to kinetically sample the hit-to-lead chemical space using unpurified reaction products.[177,178] Recent study demonstrated that the lifetime of the drug−target complex is govern by interactions in the transition state for ligand binding rather than

the ground state of the enzyme−ligand complex, and the on-rates can play a key role in drug−target residence time.[179] In 2018, an efficient computational method, for the ranking of drug candidates by their residence time and giving insights into ligand-target dissociation mechanisms, was reported.[180]

Innovations in characterizing lead quality and compound prioritization have allowed more informed decision-making by medicinal chemists.[181] We anticipate that these new methodologies and technologies will dramatically improve the efficiency of early-stage in drug discovery.

6. Biological system-mediated drug delivery

6.1 Antibody-recruiting molecules

Synthetic immunology, i.e., the development of synthetic systems to modulate immunological functions, is a newly established field. One focus of research has been to find synthetic small-molecular agents, named antibody-recruiting molecules (ARMs) that can enhance antibody binding to disease-relevant viruses or cells, thus promoting their immune-mediated clearance.[182,183]

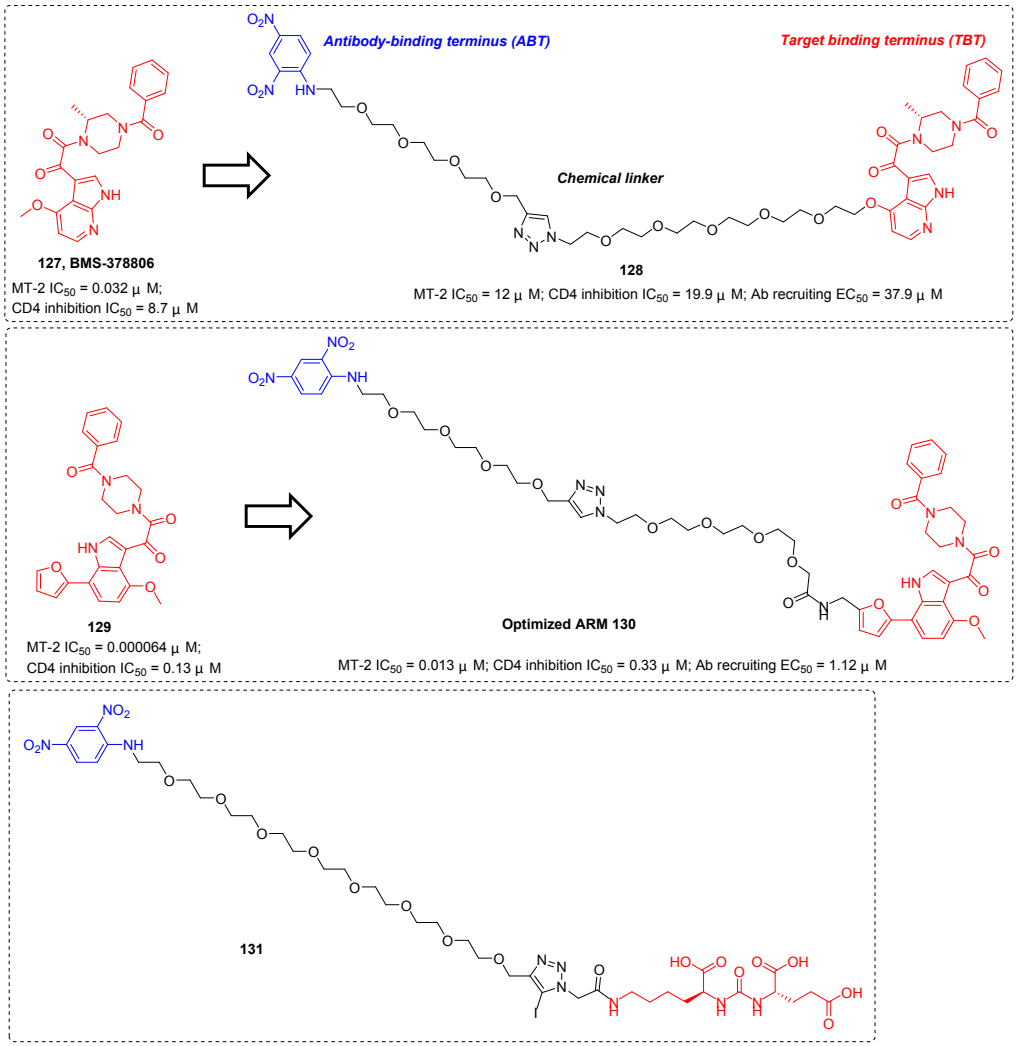


Figure 18. Antibody-recruiting small molecules **128** and **130** that target HIV gp120 and **131** that targets prostate-specific membrane antigen.

Early in 2009, Spiegel *et al.* reported several ARMs that target prostate cancer.[184] They designed new bifunctional ARMs to bind to HIV-1 gp120 and anti-dinitrophenyl (DNP) antibodies, simultaneously. Anti-DNP antibodies are abundant in the human bloodstream. By connecting these two fragments together, ARMs (exemplified by **128**, derived from the existing molecule **127** (BMS-378806)) could mediate the formation of a ternary complex, leading to blocking virus entry and antibody-mediated immune clearance of gp120-bearing cells (Figure 18).[185a]

In 2014, computationally driven modification of ARMs targeting HIV-1 gp120 gave

an optimized molecule **130** (derived from **129)**, which was almost 1000-fold more potent than **128** in gp120-binding and cell-based antiviral assay. It was also effective against multiple HIV pseudotypes in laboratory and clinic.[185b]

In 2016, Genady *et al.* reported the discovery of radiolabeled ARMs (exemplified by **131**) that target prostate-specific membrane antigen and anti-DNP antibodies for combined immunotherapy and radiotherapy.[186]

6.2 Human serum albumin-derived drug delivery

Human serum albumin (HSA) is the most abundant protein in sera (30–50 g/L human serum), where it primarily functions as a natural transporter for a myriad of molecules. Being an intrinsic protein of the human blood, it exhibits no immunogenicity. It also has a long circulatory half-life (about 19 days) due to its binding affinity for the recycling neonatal Fc receptor. Thus, HSA is an ideal drug carrier for targeted delivery and for improving the pharmacokinetic profile (half-life extension) of drugs.[187-190] Several albumin-related small-molecular drug delivery technologies have been developed, including in vivo non-covalent or covalent endogenous HSA targeting, coupling of small molecule drugs to exogenous albumin, and encapsulation of drugs into albumin coated nanoparticles.[187]

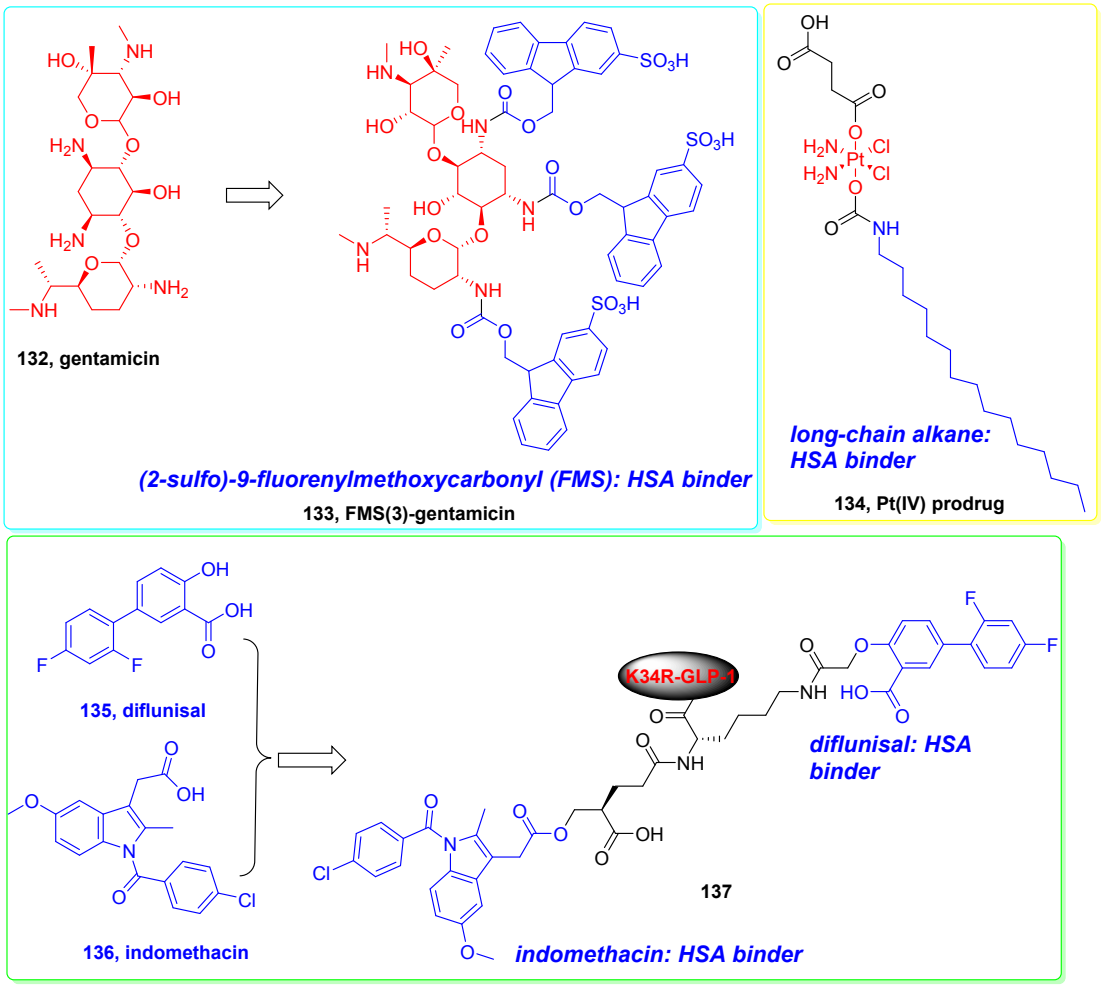


Figure 19. Examples of the in vivo non-covalent endogenous HSA targeting approach.

Most small-molecular drugs are short-lived species in the circulatory system, and can be rapidly eliminated *via* glomerular filtration. Noncovalent binding of small-molecular drugs to HSA could protect them against enzymatic degradation and renal clearance, affording slower clearance and a prolonged half-life in vivo.

For example, FMS(3)-gentamicin (**133**) was developed as a long-acting prodrug derivative by linking three (2-sulfo)-9-fluorenylmethoxycarbonyl (FMS) moieties to three amino moieties of gentamicin (**132**) to provide increased affinity for albumin.[191] **134** is a fatty acid-like platinum(IV) prodrug designed to improve drug delivery via enhanced interaction with HSA.[192] The clinically relevant glucagon-like peptide 1 (GLP-1) was functionalized with diflunisal (**135**, albumin binder) and indomethacin (**136**, albumin binder) to afford a divalent GLP-1 analogue **137** with a longer

circulatory half-life and absorption time compared to its monovalent equivalent (Figure 19).[193]

Kratz *et al.* established an *in vivo* covalent conjugation strategy that exploits endogenous HSA as a drug carrier. In this approach, the prodrug binds selectively and rapidly to the cysteine-34 residue on the surface of HSA after intravenous administration, thereby generating an *in situ* transport form of the drug in the blood (Figure 20A, B).

A proof-of-concept was obtained with the (6-maleimidocaproyl)hydrazone derivative of doxorubicin (**138**, DOXO-EMCH, INNO-206), which is the first albumin-binding prodrug of doxorubicin to enter clinical trials. **139** selectively binds to circulating albumin within just a few minutes (Figure 20C).[194] Inspired by translational research with **138**, many albumin-binding prodrugs have been developed, as exemplified by recently reported in situ covalent-albumin-binding gemcitabine prodrugs **139** and **140**, which offer improved bioavailability and tumor accumulation (Figure 20C).[195] Notably, compound **139** demonstrated remarkably increased bioavailability (21-fold higher than gemcitabine) and efficient tumor accumulation of free-gemcitabine (8-fold greater than gemcitabine).

On 6 June 2018, Albuvirtide (**141**, ABT), a HIV fusion inhibitor developed by Frontier Biotech, was approved as a new anti-HIV drug in China, with long half-life in vivo (suitable for injection once a week) and potent and broad-spectrum potency against HIV-1 variants resistant to T20.[196] ABT is a 3-maleimimidopropionic acid-modified peptide that can irreversibly conjugate to HSA.[197]

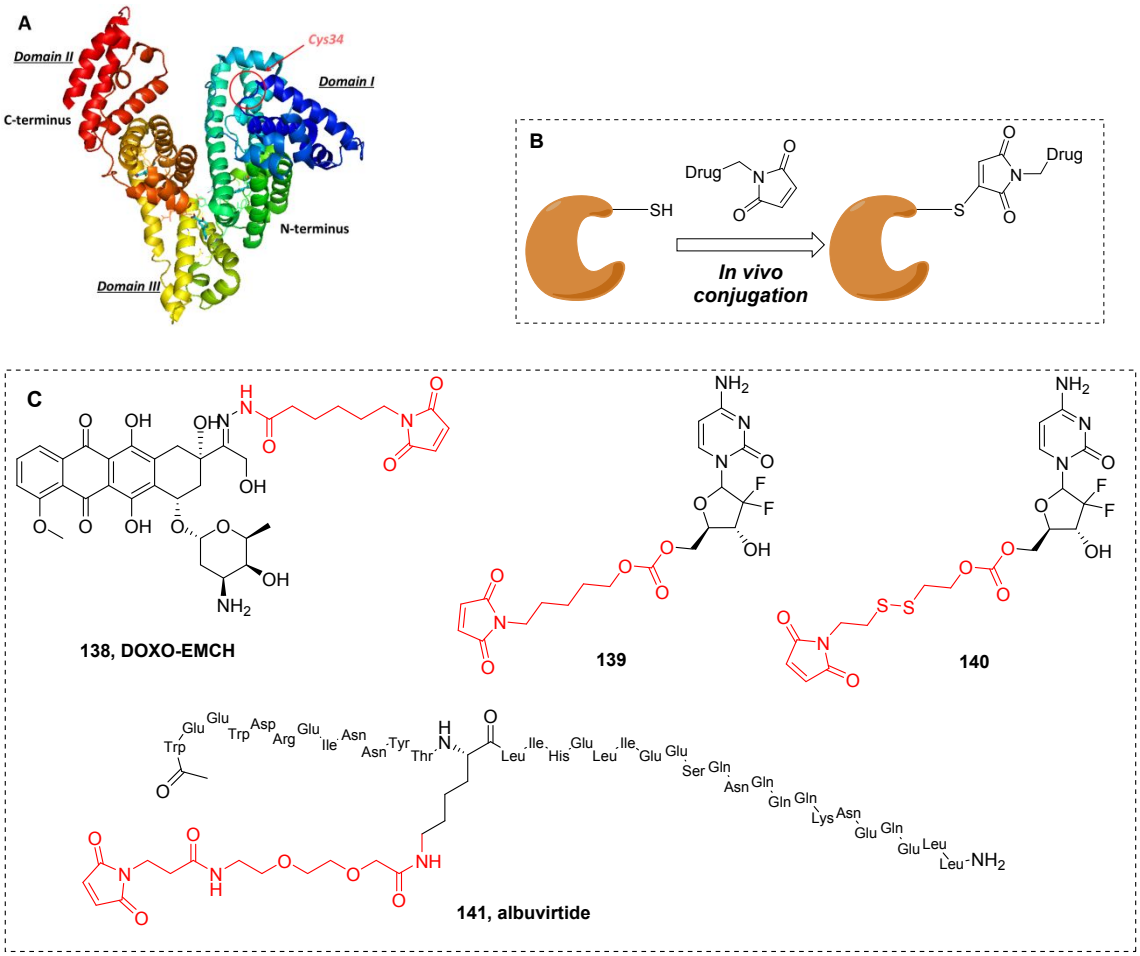


Figure 20. (A) X-ray structure of human serum albumin (bound with diflunisal, PDB ID: 2BXE): the position of cysteine-34 is highlighted; (B) Schematic illustration of in vivo thiol–maleimide conjugation; (C) Chemical structure of doxorubicin-maleimide derivative **138**, gemcitabine-maleimide derivatives **139** and **140**, and albuvirtide.

The macromolecular prodrug strategy of in vivo HSA conjugation is effective to overcome rapid enzyme inactivation, extend half-life (exemplified by ABT) and poor tumor targeting of cytotoxic anticancer agents. It also offers several advantages over in vitro-synthesized drug albumin conjugates: (a) it avoids the need for carefully controlled purification of commercial albumin; (b) the prodrug is relatively inexpensive to manufacture and convenient to store and use; and (c) quality control is simple, being comparable to that needed for any other small-molecular drug candidate.

6.3 "Bio-Oxidizable" Prodrug Strategy

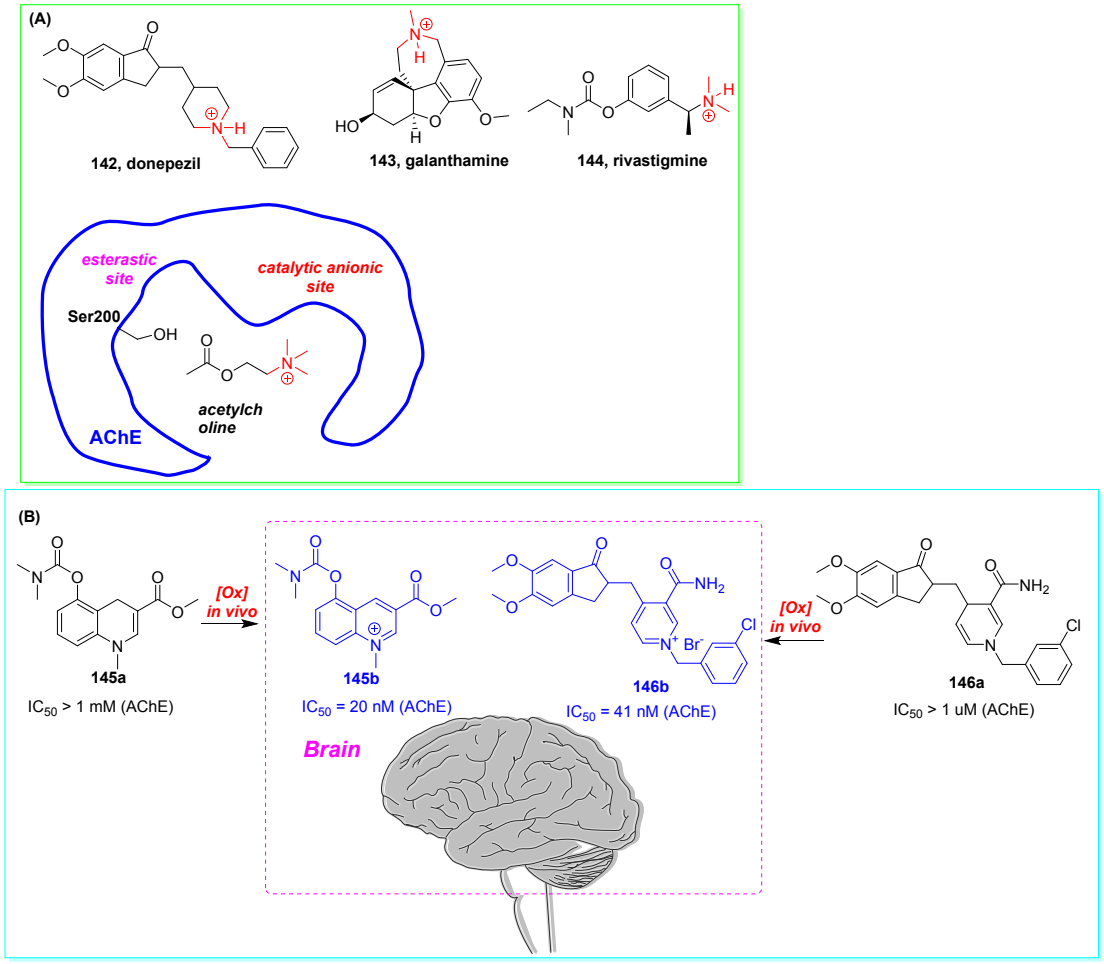


Figure 21. (A) Approved AChE inhibitors for the symptomatic treatment of AD. (A) Schematic representation of the ligand binding site of AChE, the "anionic" and "esterase" sites were highlighted. (B) Rational design of central selective AChE inhibitors via a "bio-oxidizable" prodrug approach.

To improve the efficiency and reduce side effects (arising from peripheral cholinergic activation) of approved acetylcholinesterase (AChE) inhibitors during symptomatic treatment of Alzheimer's disease (AD), Levacher V. *et al.* reported a biological investigation of new AChE inhibitors based on a novel "bio-oxidizable" prodrug approach.[198] The design of this "bio-oxidisable" prodrug originates from an insight into the action mechanism of AChE inhibitors. Whereas donepezil (**142**) and galanthamine (**143**) are competitive inhibitors, rivastigmine (**144**) is a

pseudo-irreversible inhibitor via the carbamylation of the serine-OH located at the "esterasic site" of AChE. All these drugs share in common a tertiary amine moiety which plays an important role in the inhibition of AChE. At physiological pH, this amine was protonated as a positive charge bioactive form, which binds to the "catalytic anionic site" of AChE (Figure 21). Generally, the charged form cannot cross the blood brain barrier through passive diffusion, the acid–base equilibrium permitted the neutral inactive form to penetrate this physiological barrier, which resulted in both central and peripheral cholinergic effects often observed with these drugs. Based on these mechanistic insights, by temporarily masking the positive charge at the periphery, a "bio-oxidisable prodrug" strategy was envisaged to design central specific AChE inhibitors, which afforded dihydroquinoline carbamate **1a** and donepezil-based "bio-oxidizable" prodrugs **1r**.

Putatively, once in the CNS, the prodrugs **145a** and **146a** should be converted into the parent compounds **145b** and **146b** through a redox-activation process mediated by the NAD(P)H/NAD(P)$^+$ coenzyme system. It is expected that the presence of a permanent positive charge in **145b** and **146b** could not only act as AChE inhibitors in the CNS through "locked-in" effect, but also facilitate rapid elimination of the quinolinium salt form in the peripheral system. These studies strongly prove that this attractive "bio-oxidizable" prodrug strategy is ingenious and practical.

6.4 Mitochondrial-targeted agents

Cancer cells principally show higher mitochondrial transmembrane potential and abnormal metabolic pathways. Thus, the targeting and delivery of anticancer agents to the mitochondria could improve therapeutic efficacy.[199] This mitochondrial-targeted strategy was exemplified by introduction of delocalized lipophilic cations to the parent compounds. The most investigation delocalized lipophilic cation is the

triphenylphosphonium cation, and several successful examples have been reported with remarkable cytotoxicity and selectivity, including a chlorambucil derivative **148** (Mito-Chlor),[200] and lupane triterpenoid derivative **150** (Figure 22).[201a] Additionally, a recent study demonstrated that conjugation with triphenylphosphonium cation can restore the antifungal activity of some natural terpenes.[201b]

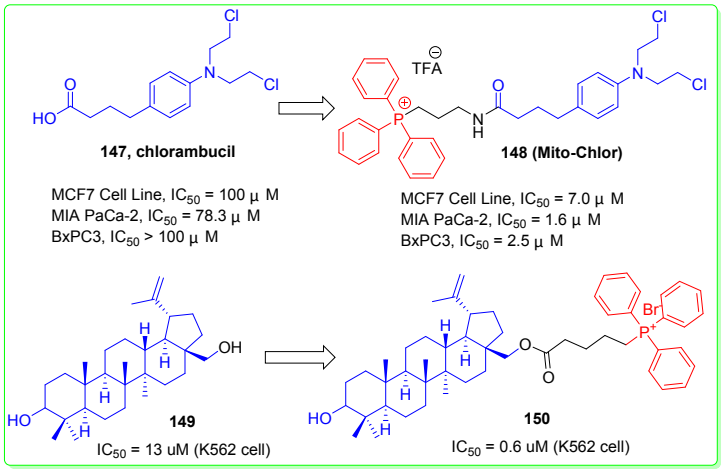


Figure 22. Mitochondria-targeted anticancer agents.

7. Structure-based drug discovery

Structure-based drug discovery is a fundamental strategy for finding and optimizing lead compounds of therapeutic importance. This section will discuss the following topics: covalent inhibitors or probes, bisubstrate inhibitors, exploration of water-binding pockets, drug design to stabilize inactive protein conformations, as these have been highlighted in very recent literature.

7.1 Covalent inhibitors or probes

An attractive increase in the potency and pharmacokinetics of a drug-like compound is to evoke the formation of a covalent bond. Compared with noncovalent inhibitors, the advantages of covalent compounds lie in the following aspects: higher potency, long residence time, and decreased drug resistance. In the past several years, many covalent drugs such as telaprevir, abiraterone, carfilzomib, and afatinib have been used in clinical, ushering in a new era for covalent modifiers.[202,203]

Covalent inhibitors should remain a key focus of contemporary drug discovery, especially in the initial structure-based design optimization, as exemplified by human tissue transglutaminase inhibitor **151**,[204] c-Jun N-terminal kinase 3 inhibitor **152**,[205] janus kinase 3 selective inhibitor **153**,[206] KRAS inhibitor ARS-853 (**154**),[207] FGFR inhibitor **155** (PRN1371),[208] mitogen-activated protein kinase kinase 7 (MKK7) inhibitor **156**, [209] EGFR inhibitor **147**,[210] histone lysine demethylase KDM5A inhibitor **158**,[211] pyruvate dehydrogenase kinase 1 (PDK1) inhibitor **159**,[212] monoacylglycerol lipase (MAGL) inhibitors **160** and **161**[213] (Figure 23).

Complementarily, allosteric modulators are sought after as a means to avoid undesirable side effects of covalent or active site inhibitors. In 2016, the first covalent and potent cannabinoid 1 receptor (CB1R) allosteric modulator **162** was reported, which can be used as an effective chemical probe for characterizing CB1R allosteric ligand-binding motifs.[214] In 2018, the first covalent positive allosteric modulator **163** for the metabotropic glutamate receptor 2 (mGlu2, a class C GPCR) was reported, which advanced the understanding of the mGlu2 PAM interaction.[215]

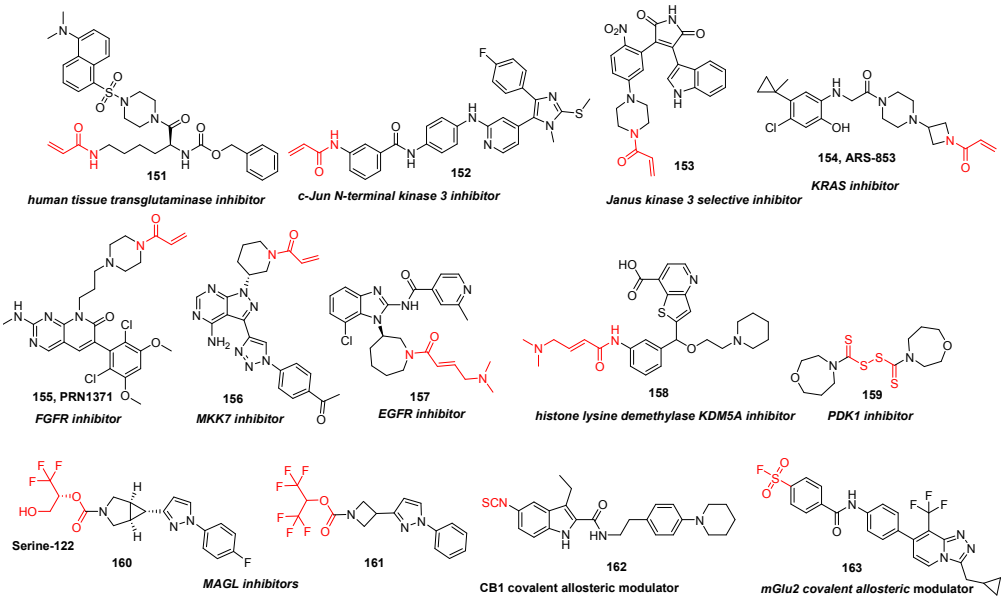


Figure 23. Covalent inhibitors in drug discovery: typical examples. The warheads for covalent binding are highlighted in red.

The main hurdle for covalent inhibitors is the lack of selectivity. Apart from selecting a warhead or modulating of electrophilic warhead reactivity, substantial efforts are required to optimize noncovalent reversible interactions to facilitate target-selective recognition and the overall potency. For example, through crystallography, kinetic, and molecular simulation studies, interaction of cyanamide-based covalent JAK3 inhibitor **164** with residue Cys909 was optimized affording potent and selective JAK3 inhibitors as exemplified by **165**, with substantially enhanced activity and selectivity (Figure 24A).[216]

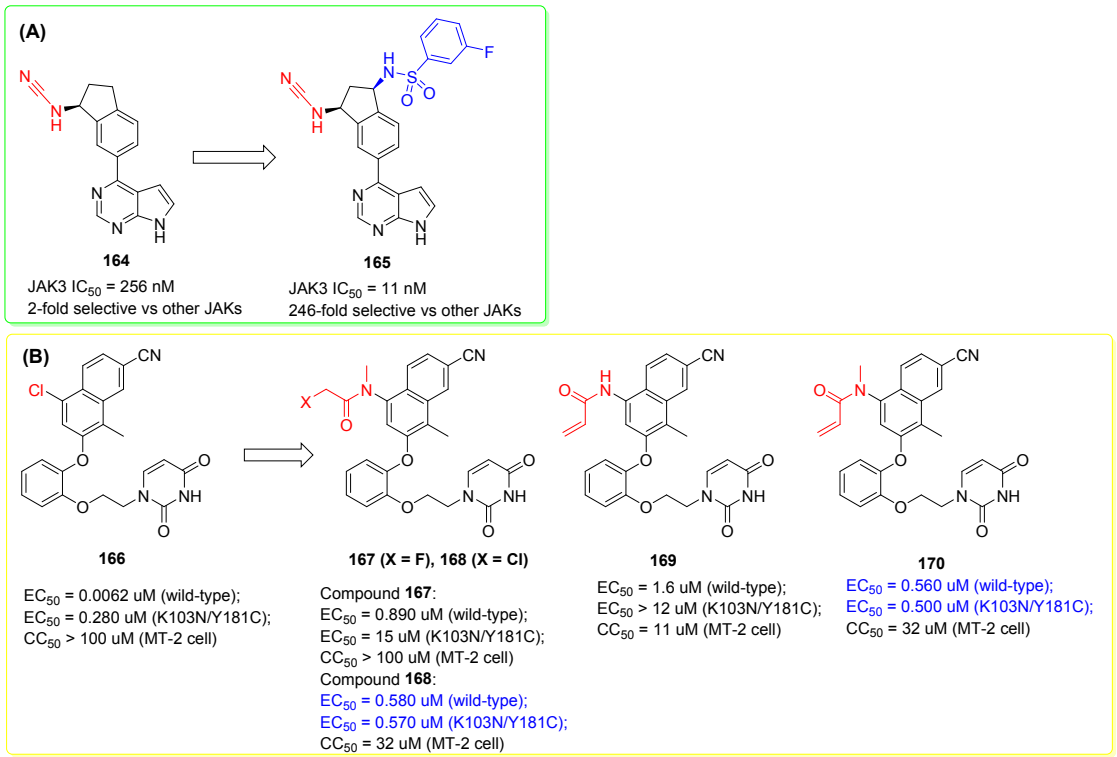


Figure 24. Discovery of covalent inhibitors via (A) optimization of noncovalent reversible interactions and (B) diversity-oriented modification of warheads.

Y181C-mutated HIV-1 strain is one of the key clinically observed mutants. In 2017, Jorgensen's group took Y181C-mutated HIV-1 reverse transcriptase as the target for drug design.[217] Based on the protein crystallography, it was found that the carbon–

chlorine bond catechol in diethers compound **166** is oriented toward Tyr181, its replacement with an electrophilic warhead could make covalent binding of Cys181 variants. Consequently, electrophilic group-bearing compounds **167-170** were designed and synthesized. Especially, compounds **168** and **170** are covalent inhibitors of HIV-1 RT mutants (Y181C and K103N/Y181C) through in vitro assays (MT-2 cells), mass spectrometry, and protein crystallography. This is the first and successful application of the irreversible covalent inhibition strategy to HIV-1 reverse transcriptase (Figure 24B).[217] Based on activity results, it is found that small chemical alterations of warheads often cause significant differences in activity. Therefore, diversity-oriented selection of warheads make possible the systematic exploration of the chemical space.[218]

Besides the most popular acrylamide, additional electrophilic traps have undergone considerable development as "privileged warheads" in chemical biology (exemplified by sulfonyl fluoride and isothiocyanate).[215,219] The exploration of warheads with chemical reactivity towards target enzymes for incorporation into parent compounds is expected to afford novel covalent drugs.

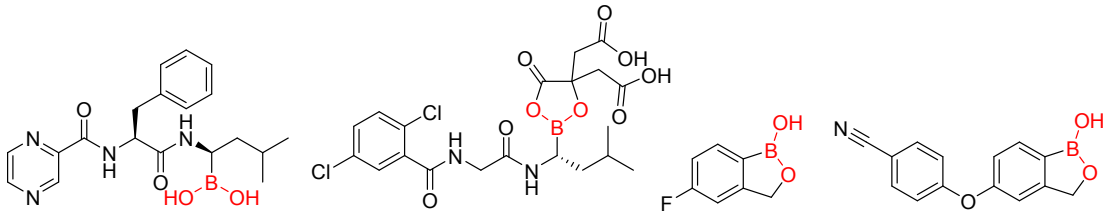


Figure 25. Structures of boron-containing drugs.

Design of boron-containing molecules has recently attracted much attention. Boron differs from carbon in that it has a vacant p-porbital that is receptive to a reversible covalent bond formation with a Lewis base under physiological conditions. Recently, boronic acid was proven a structurally and mechanistically differentiated electrophile

from other cysteine reacting moieties, arising from the ability of boronic acids to generate a reversible covalent bond with with oxygen nucleophiles (Lewis base) of the target protein. Boronic acid-based covalent agents have received widespread attention in the drug design community, with several boron-containing drugs (including bortezomib and ixazomib, tavaborole, and crisaborole) and other recent successful examples that have demonstrated their therapeutic effects (Figure 25).[220]

Raines's team has been engaged in the research of boric acid-based drug discovery and chemical biology for many years.[221] In 2017, Raines *et al.* reported on boronic acid-substituted stilbenes that limit transthyretin (TTR) amyloidosis in vitro. X-ray crystallographic analysis of TTR/**175** complexes (PDB code: 5U4F) demonstrated that a boronic ester was reversibly formed with Ser117, which has a great contribution to the thermodynamics and kinetics of binding (Figure 26).[221a]

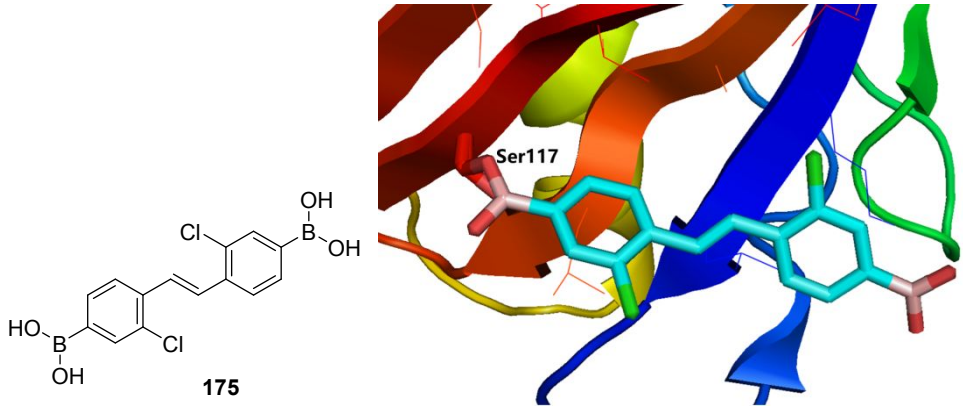
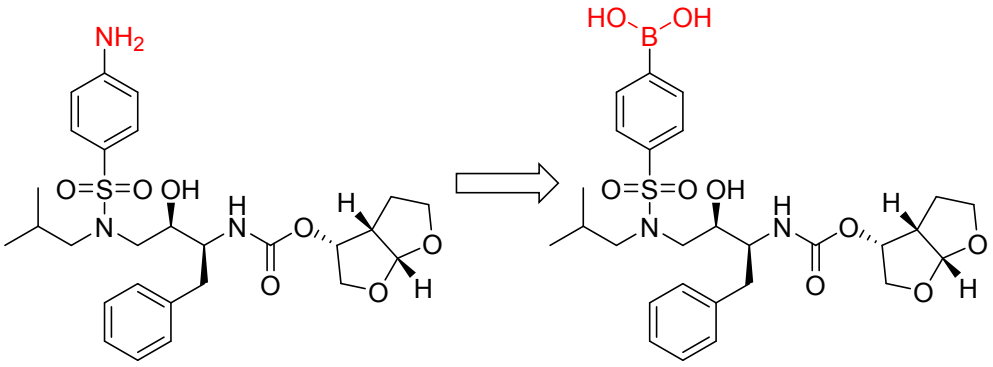


Figure 26. Structure of stilbene boronic acid **175** and its binding mode with TTR (PDB entry 5u4f).

In 2018, based on the 3-D structure and mechanism of HIV-1 protease, this group replaced the aniline group of darunavir (**176**) with phenylboronic acid moiety, which led to the identification of **177** with increased affinity with the protease by 20 times, and a high affinity for HIV-1 protease-resistant strain D30N (Figure 27). X-ray co-crystallization structure demonstrated that boric acid group participated in triple hydrogen bonding, which was superior to the amino group in darunavir and other

derivatives. The hydrogen bond distance between Asp30 (or Asn30) of protease and hydroxyl group of boric acid was shorter than orthodox hydrogen bonding, which had a certain degree of covalence. This is a reasonable explanation for high potency and remarkable anti-resistance profiles of boric acid derivatives.[221b]



176, darunavir
K_i = 10 pM
(PDB code: 4hla)

177
K_i = 0.5 pM
(PDB code: 6c8x)

Figure 27. Discovery of boronic acid-bearing sub-picomolar inhibitors of HIV-1 protease

In 2007, a 100-fold affinity gain was achieved by introduction of a C-terminal boronic acid group into dipeptidic inhibitors (**178a**,**b**) of the West Nile, Zika, and dengue virus proteases. The resulting molecules (as exemplified by **179**) have high binding affinity with K_i values in the two-digit nanomolar level, low cytotoxicity, and virus-inhibitory potency (Figure 28). SARs and a X-ray cocrystal structure of **179** with West Nile virus NS2B-NS3 protease pave the way for the design of advanced covalent-reversible inhibitors targeting emerging flaviviral pathogens.[222]

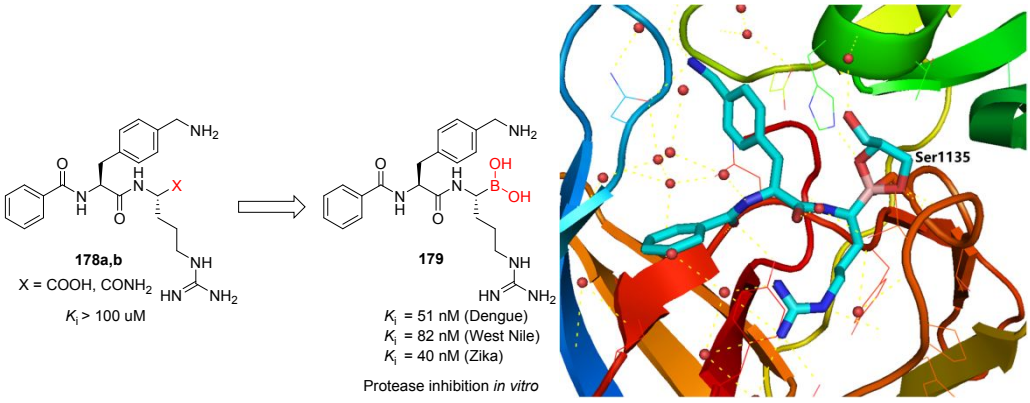


Figure 28. Discovery of **179** as a capped peptide-boronic acid inhibitor of flaviviral proteases and its binding mode with West Nile virus NS2B-NS3 protease (PDB code: 5IDK)

Boronic acid transition-state inhibitors proved to be one of the most promising classes of serine amidohydrolase inhibitors, including *β*-lactamase inhibitors. In 2001, an unexpected tricovalent binding mode of boronic acids within the catalytic site of a penicillin-binding protein was reported.[223] The boron usually adopts a tetrahedral conformation, binds to the nucleophilic serine of the active site and mimicks the transition state of the catalytic reaction. Compound **180** (Ki = 44 nM) represented a promising lead compound against ADC-7 (Acinetobacter-derived cephalosporinase), one of the most critical resistance determinants in *A. baumannii.* The cocrystal structure of the ADC-7/**180** complex suggested that the inhibitor was covalently linked to the catalytic residue Ser64, highlighting the significance of key structural factors for recognition of the boronic acid moiety.[224]

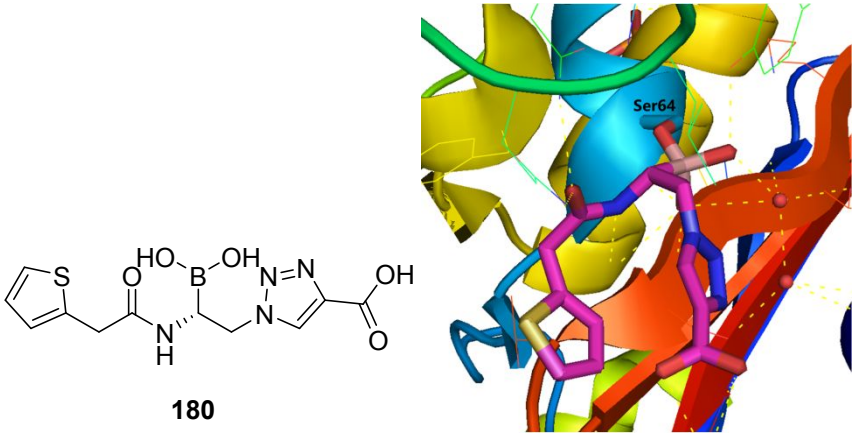


Figure 29. The cocrystal structure of **180** in complex with ADC-7 β-Lactamase (PDB code: 4U0X).

The risk of indiscriminate reactivity of active warheads and the resulting adverse effects can be reduced by incorporating latent electrophiles into irreversible covalent inhibitors. In general, terminal alkyne is considered "inert" toward cellular components and are therefore often applied in bioorthogonal reactions. Recently, an alkyne moiety was introduced as a less reactive electrophilic moiety into cathepsin K inhibitors. Notably, based on crystal structure analysis, alkyne-based compounds **181** and **182** (Figure 30) effectively inhibit the activity of cathepsin K protease by formation of an irreversible covalent bond with the cysteine residue in the catalytic site in a proximity-driven manner. Just because of this, they did not show indiscriminate thiol reactivity. More importantly, based on these proof-of-concept studies, it is foreseeable that latent electrophiles such as the alkyne may be of important prospects in further design of cysteine-targeting irreversible covalent drugs with an improved safety profile.[225] Interestingly, warhead moiety is not necessary for covalent inhibitors. For example, the cyclic piperidine and piperazine aryl ureas (piperidine-bearing PF750 (**183**) and piperazine-bearing JNJ1661010 (**184**)), without any warhead moiety, were reported as potent fatty acid amide hydrolase (FAAH) inhibitors (Figure 30), because FAAH could efficiently hydrolyze the amide bond of these molecules, forming a covalent enzyme-binder adduct.[226]

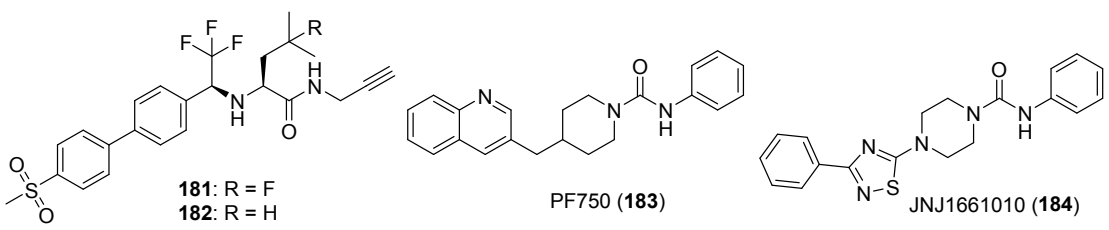


Figure 30. Covalent small molecule inhibitors with a latent electrophile or via a proximity-driven mode.

The binding process covalent inhibitors with related targets consists of multiple processes, which are not necessarily independent of each other. Thus, computer-aided prediction of binding affinity for covalent inhibitors presents a great challenge. The free energy perturbation combined with λ-exchange molecular dynamics technique provided particular advantages in predicting binding selectivity among protein isoforms, which is a major challenge in covalent inhibitor design.[227] We envision that the continued interest in covalent drug discovery will propel further development of new computer programs to predict the binding energetics and binding mode of covalent drugs.[228]

Generally speaking, diversity-oriented structural modification can compensate for the shortcomings of target-based drug design and computational prediction. Under this situation, a fragment-based method combined with a MS covalent ligand screening was developed to discover irreversible covalent inhibitors of cysteine proteases, ubiquitin ligase.[229,230]

7.2 Bisubstrate inhibitors

A bisubstrate inhibitor consists of two covalently connected fragments, each targeting either the substrate or the cofactor binding pocket, thus potentially mimicking the ternary transition state of a bireactant catalytic reaction. This drug design approach has the potential to obtain high potency and selectivity. More than a decade ago, several bisubstrate inhibitors were successfully developed for several proteins, including kinases and acetyltransferases.[231,232]

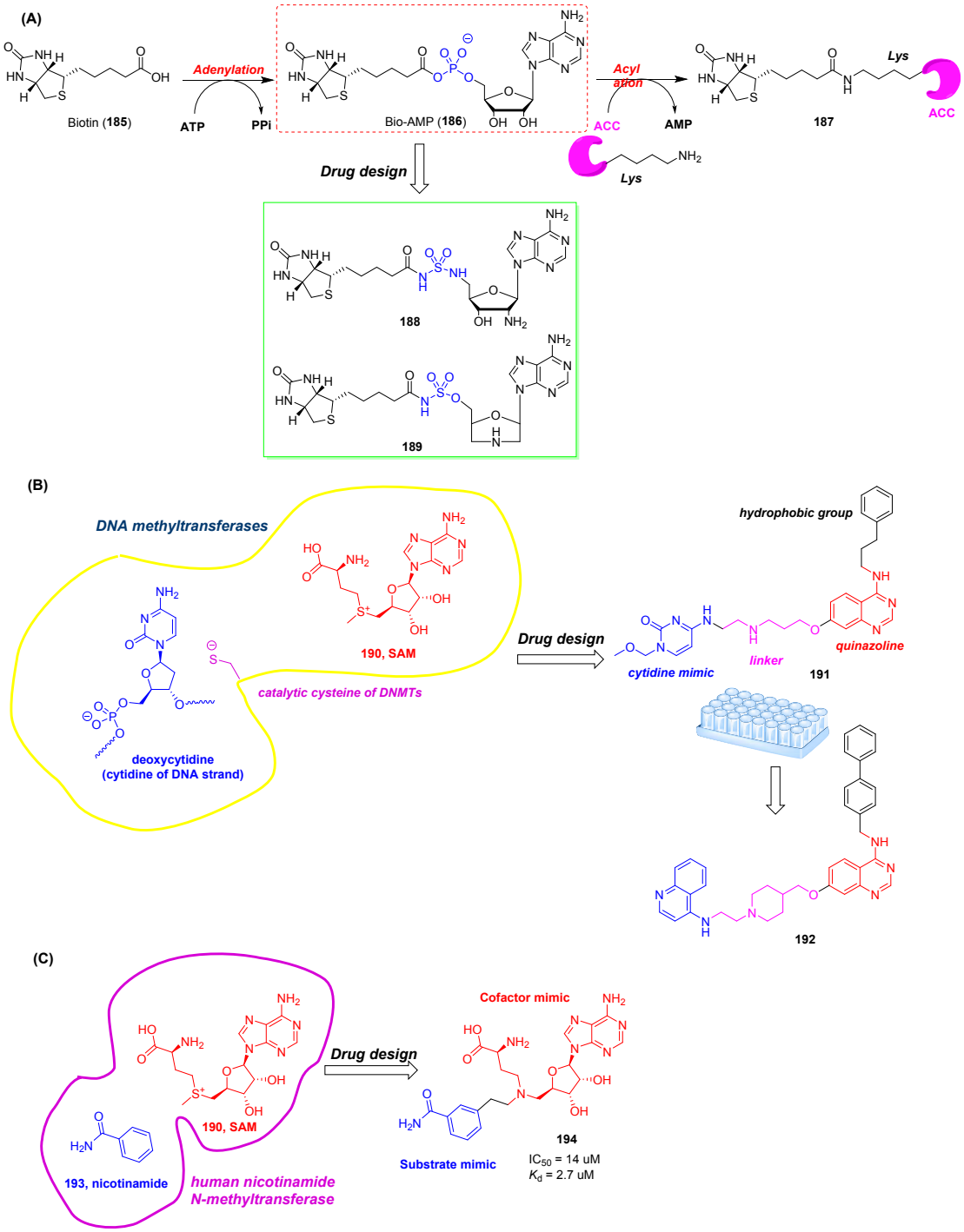


Figure 31. (A) Targeting MtBPL with nucleoside-based bisubstrate adenylation inhibitors. (B) Schematic representation of the transition state of DNMT (DNMT1 and DNMT3A) catalytic site and the chemical modulation strategy. In red is depicted the methyl-donor AdoMet and its mimic 4-aminoquinazoline, in blue the deoxycytidine in the DNA and its analogues, and in pink the linker between the two moieties. Besides, the amino moiety in C4 position of the quinazoline was substituted by phenylpropylamine as a hydrophobic group on the amine at C4 position of the adenosine in AdoMet analogues was probably favorable for DNMT inhibition potency. (C)

Discovery of a bisubstrate inhibitor of NNMT.

There have been some new developments in recent years. Mycobacterial biotin protein ligase (MtBPL) is an indispensable enzyme in mycobacterium tuberculosis (Mtb) and regulates lipid metabolism via the post-translational biotinylation of acyl coenzyme A carboxylases (ACCs). The enzymatic reaction proceeds in two steps by sequential adenylation of biotin (**185**) to generate Bio-AMP (**186**), followed by acylation of the biotin carboxylase carrier protein domain of ACCs to afford holo-ACC (**187**). In 2015, nucleoside-based bisubstrate adenylation MtBPL inhibitors **188** and **189** were discovered, through modifications on the ribofuranosyl ring of the nucleoside (Figure 31A).[233]

In 2017, on the basis of the structure of the catalytic pocket of DNMT (Figure 31B), the bisubstrate analogues-based inhibitors were designed, by mimicking each substrate of DNA methyltransferases (DNMT3A and DNMT1), the S-adenosyl-l-methionine (**190**, SAM) and the deoxycytidine, and linking them together, which resulted in quinazoline-quinoline-derived DNMT3A and DNMT1 inhibitors **191** and **192**, some showing certain isoform selectivity.[234]

Inspired by the recently published ternary crystal structure of human nicotinamide N-methyltransferase (NNMT) in complex with the substrate nicotinamide (**193**) and the cofactor SAM as the methyl group donor, a bisubstrate NNMT inhibitor MS2734 (**194**) was discovered and characterized (Figure 31C). Furthermore, a co-crystal structure of **194** in complex with hNNMT was obtained, which paved the way for further developing more potent and selective NNMT inhibitors.[235]

7.3 Exploring water-binding pockets (structural water molecules) in structure-based design

Water molecules are important components in protein channels, and are often found around ligands in protein crystal structures. Water-mediated interactions, especially hydrogen bonds, play key roles in drug binding.[236,237] Careful examination of these water molecules and their energetics can contribute to successful drug design, as exemplified by neuronal nitric oxide synthase inhibitors, nonpeptidic urea HIV protease inhibitors and benzoxaborole non-nucleoside polymerase inhibitors of HCV.[238-240]

Recently, several structural and computational studies to explore water-binding pockets have been reported.[241-244] One way to systematically improve existing weak binders could focus on identifying and later chemically optimizing those moieties with a particular proximity or orientation to water molecules in the protein−binder complex. For example, the X-ray structure of the antiviral drug Arbidol (**195**) with influenza hemagglutinin revealed a highly ordered water molecule adjacent to Arbidol, and this was exploited in the structure-based design of Arbidol analogues (Figure 32). Addition of *meta*-hydroxyl group to the thiophenol group of Arbidol to replace the water molecule in the binding pocket afforded **196**, which showed significantly increased affinity for both H1 (98-fold) and H3 (1150-fold) hemagglutinin subtypes.[245]

A recent study indicated that the introduction of hydroxyl group to form water-mediated hydrogen bond may not necessarily improve the binding affinity between ligand and target, because hydrophobic effects also play an important role in ligand binding affinity in some cases.[246]

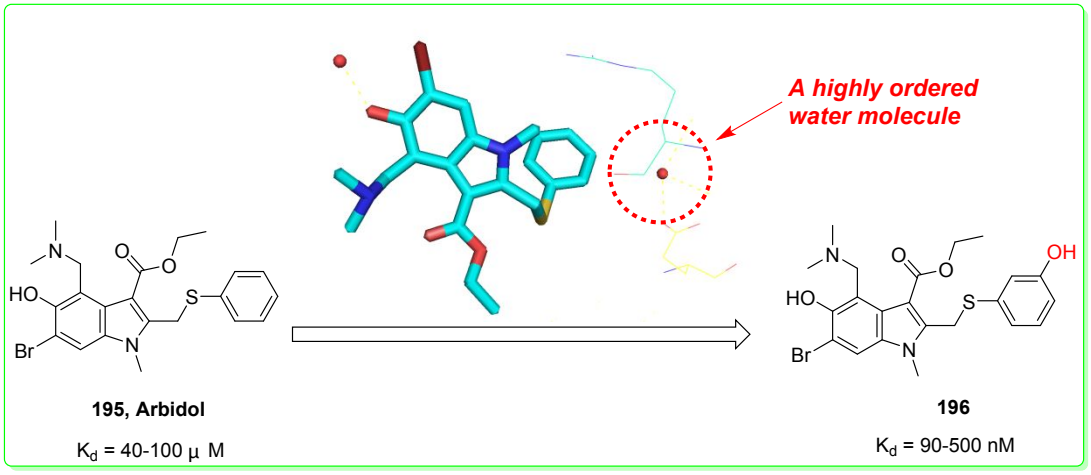


Figure 32. Structure-based optimization based on the X-ray structure of Arbidol bound to influenza virus hemagglutinin (H3 - HK68: A/Hong Kong/1/1968, PDB ID: 5T6N).

The M2 proton channel of influenza A is a well-validated target for the antiviral drugs, such as amantadine and rimantadine. Recent disclosed X-ray crystal structures of the M2 proton channel with bound inhibitors reveal that the inhibitors engage in and disrupt transmembrane networks of hydrogen-bonded water, small molecules can enable potent inhibition by targeting key water molecules.[247]

Generally, it is thought that a protein binder can achieve affinity by extending into a region occupied by unfavorable water molecules or lose affinity by displacing water molecules from a region where it was relatively stable. However, the real situation is much more complicated. The prevailing thermodynamic theories of the past few years claim that water was observed largely in terms of an entropic gain after it was displaced by a ligand, which are now known to be too idealistic. In most cases, as water molecules can be difficult to locate by X-ray diffraction methods, especially when they are not tightly bound to biomacromolecules, NMR spectroscopy can be used as a valuable technique to assess those water molecules. By increasing the (perdeuterated) protein concentration, WaterLOGSY titration experiments help to get useful information about the location of protein-bound water in the surroundings of the ligand, and ligand binding modes even in the case of weak binders, which are

extremely beneficial to specific optimization of the ligand to enhance binding affinities.[248]

In view of the complexity and uncertainty of water-mediated interaction, the understanding of water's roles in the underlying structural protein-ligand complexes came at the expense of very careful and detailed dissections of the relevant scenarios with new computational methodologies, and isothermal calorimetric, spectroscopic, crystallographic experiments.[249]

7.4 Stabilization of protein inactive conformations or protein-protein interactions

A large number of medicinal chemists have engaged in the design and development of protein kinases inhibitors. However in comparison, targeting protein kinases with small compounds that bind outside the highly conserved ATP pocket to stabilize inactive protein conformations has been regarded as a fresh approach in kinase-targeted drug design that is worthy to be promoted because these compounds often have improved pharmacological profiles compared to inhibitors exclusively targeting the ATP pocket. On the other hand, traditional screening approaches for kinase inhibitors are often based on enzyme activity, and it has been recognized that they may miss ligands that stabilize inactive protein conformations. An example of ways to overcome this issue is provided by a study to find selective Met tyrosine kinase inhibitors, in which a high-throughput virtual screening of a ChemNavigator compound database was employed for directed discovery of inhibitors targeting the Met tyrosine kinase domain (i.e., compounds that stabilize the kinase domain in its inactive conformation).[250]

In 2010, Klüter *et al.* reported a kinase binding assay using a pyrazolourea type III inhibitor and enzyme fragment complementation technique that is suitable to screen

stabilizers of enzymatically inactive kinases.[251] In the same year, Whelligan *et al*. reported the first systematic exploration of compounds binding to an unusual, inactive conformation of the mitotic kinase Nek2.[252]

Recently, a novel series of competitive shikimate kinase inhibitors that stabilize an inactive open conformation of the enzyme by targeting the dynamic apolar pocket surrounding the natural substrate was disclosed.[253]

Novel pleckstrin homology domain-dependent covalent-allosteric inhibitors of the kinase Akt were identified via structure-based design, which bind covalently to a distinct cysteine residue of the kinase and stabilize the inactive protein conformation.[254] To sum up, stabilization of an inappropriate and inactive conformation for enzymatic catalysis seems an innovative and a promising approach for dissecting conformation-dependent signaling of protein kinases and finding drug candidates.[252] Certainly, there is a significant challenge to adapt screening methodologies and downstream techniques to identify and optimize stabilizers of inactive conformations.

Similarly, stabilization of protein-protein interactions is also a viable strategy for drug discovery, but has not been exploited in a systematic manner. Ottmann' lab has pioneered this field.[255] The potential for high selectivity, the ability to gain access to previously undruggable targets and the list of impressive compounds were reviewed recently.[256]

8. Conclusions and Prospects

Contemporary drug discovery still faces major scientific and economic challenges to identify and provide lead compounds in an efficient manner. The classical methods of drug design, preparation, and evaluation lead to substantial time delays, resulting in inefficient usage of data in the complex drug design process. This article has focused

on exploring strategic approaches to solving issues in lead identification and optimization projects that affect candidate development and quality and which are frequently encountered in drug development campaigns (Figure 33).

Structure-based drug design intends to generate the protein and ligand molecules that have high affinity and specificity. Since the binding cavity of an identified drug is usually large and their possible protein interactions are still required to explore and detailed understanding of drug-protein interactions or selectivity-determining features are needed to increase the effectiveness of structure-based drug design. Precise knowledge of (non-)covalent interactions between proteins and binders provides good foundations for rational design of covalent inhibitors, bisubstrate inhibitors, stabilizers of protein inactive conformations and exploitation of the water-binding pocket (structural water molecules) for structural modification. Notably, covalent inhibitors attract tremendous attention. One advantage of covalent drugs is a reduced risk for the development of resistance, which is a major challenge in the treatment of cancer and infectious diseases. The key factor affecting the target specificity of covalent inhibitor is the warhead moieties reactivity. The ideal warhead moieties should be stable under physiological conditions, and eliminate the off-target reaction with undesired biological targets as far as possible. Undoubtedly, target-induced covalent inhibitors (a proximity-driven reactivity) are an ideal type.

Despite the huge information contributed by academia and industry in the past decades, rational approach using the structure-based drug design with the aid of computational methods remains challenging. There are large flexible variations in the process of protein-ligand binding: there can be, beyond the protein and ligand, cofactors and solvent molecules should be observed. Additionally, entropic contributions can be important because the protein and ligand often possess

significant flexibility.[223] In an recent study, the role of protein conformational entropy and the response of water molecules located around the binding sites and ligand is highlighted in detail.[257]

In most cases, drugs are identified from the biological screening of compound collection, followed by hit to lead optimization toward functional endpoints. Large number of failures in the late stages of drug development underlines the point that drug discovery is a multi-parameter optimization process, and multiple properties should be enhanced to reach the stage where a molecule to be considered for *in vivo* studies. However, if a lead compound is not considered to be efficient, it will never transformed to a drug candidate, irrespective of other properties, which makes the structure-activity and structure-property relationships as a central task during early to mid-stages of lead optimization. Structural diversity within chemical libraries increases the probability of identifying a lead molecule. In phenotype-based drug discovery, molecular diversity is even more significant due to absence of target information. Consequently, the features of screening collections are often balanced between diversity, physicochemical desirability, intrinsic complexity and synthetic feasibility. Even then, multiparameter optimization of the lead compound for efficacy and drug-like properties is challenging not only because of synthetic considerations, but also because of limited ability to predict how compounds will interact with complex biological systems. Given the complexity of lead optimization, this process is mainly driven by knowledge, intuition and experience. In this stage, diversity-oriented synthesis-facilitated medicinal chemistry with combinations of many cheminformatics tools, as exemplified by hierarchical multiple-filter database screening (*in silico* ADMET prediction), is an attractive strategy.

Innovations in synthetic methodologies (exemplified by LSF), synthetic chemistry

techniques (microwave chemistry, continuous flow synthesis, automated synthesis and purification methods) and development of modular, convergent synthetic reactions ("build-couple-pair" approach, bioorthogonal reactions, cascade reactions, multicomponent reactions and late-stage microsomal oxidation) have enabled the diversity-oriented synthesis-facilitated medicinal chemistry, which can greatly facilitate the construction of diverse and drug-like compound libraries.[258-260] As exemplified by bioorthogonal chemistry and photoactivatable-inspired drug discovery, a strong communication between synthetic and medicinal chemists could allow greater impact of novel methodologies and expand the synthetic toolbox of medicinal chemistry in future drug discovery.

Drug repositioning has emerged as an alternative approach to the traditional drug discovery and development due to its efficiency in cost and time. Historically, this approach was accomplished by serendipitous discoveries and the exploitation of unintended side effects. Core concepts that support drug repurposing using network strategies include drug–target interactions, target–disease associations, and drug–indication associations. On the basis of medically inspired phenotypic screening and the modern mechanism-of-action methods, drug repositioning will continue to promote drug discovery.[261] Meanwhile, computational (network-based) repositioning strategies are becoming increasingly popular. However, it should also be noted that the potential of the drug repurposing strategy has not been as widely realized as had been hoped, in part owing to legal and regulatory barriers.

Similarly, "over-represented" features of "privileged structures" in drug-like molecules enable us to design isosters with structural diversity by maintaining the core physicochemical properties. Focused libraries of privileged scaffolds can expand the molecular diversity of synthetic collections. The strategy of "privileged structure"

repositioning has been extensively employed to exploit additional bioactivities by rapid derivatization of key intermediates with well-established synthetic protocols. We envision that fragment-based lead discovery methodology and computational chemistry will significantly facilitate the exploitation of privileged motifs.

The "precise" drug delivery is one of the pivotal aspects of contemporary drug development. Notably, drug delivery systems with high temporal and spatial precision using bioorthogonal uncaging strategies, photoactivatable chemistry, antibody-recruiting molecules, and human serum albumin-derived drug delivery have received significant attention during the past few years, which reflects the deep integration of medicinal chemistry and biology. Challenges such as nonspecific binding, accumulation, degradation, or renal clearance require the development of innovative drug delivery systems that are capable of selective targeting and real-time monitoring of drug release. Bringing the two disciplines chemistry and biology closer and diminishing the boundaries creates new opportunities to identify new drug delivery system. What can not be ignored is that, the use of proteolysis-targeting chimeras (PROTACs), which induce selective degradation of specific target protein through the ubiquitin-proteasome system, found as a novel, though well-known, drug discovery strategy with the capability to offer unique therapeutic advantages, compared with the present approaches.[262] Relevant research papers and reviews are abundant, so this is not covered in this article.

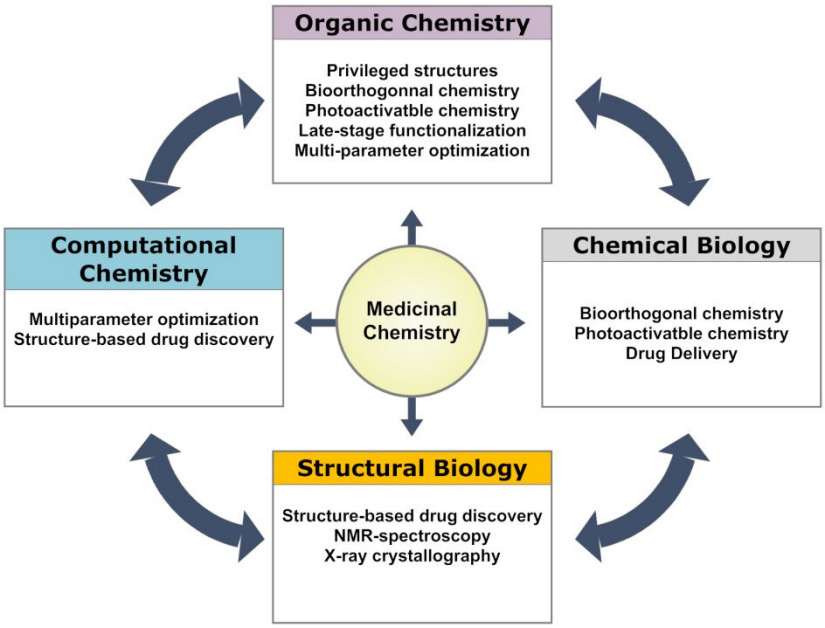


Figure 33. Schematic diagram of interdisciplinary teamwork at the interface between the field of chemistry and biology with the aid of informatics.

All in all, the main goal of medicinal chemists is to change the drug discovery process more efficient by reducing the number and duration of design cycles required to optimize lead compounds into safe and efficacious drug candidates. No single strategy is likely to be a universal panacea.[263,264] The successful development of innovative drugs requires interdisciplinary teamwork at the interface between chemistry, biology, and informatics and multiple techniques working in concert (Figure 18).[265] For example, medicinal chemists are deploying artificial intelligence (machine learning) to discover drugs, which will accelerate the productivity of the drug discovery process *via* automated compound optimization.[266,267] We hope this Perspective will contribute to overcoming the problems associated with drug discovery campaigns by summarizing the many ideas and strategies that are available to researchers in the field.

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Notes

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Abbreviations used:

AChE, acetylcholinesterase; ARMs, antibody-recruiting molecules; BSAA, broad-spectrum antiviral agents; CNS, central nervous system; CuAAC, copper(I)-catalyzed azide-alkyne [3+2] dipolar cycloaddition; DCC, dynamic combinatorial chemistry; DEL, DNA-encoded chemical library; DOS, diversity-oriented synthesis; DPM, drug profile matching; EPIs, efflux pump inhibitors; FDA, Food and Drug Administration; FMS, (2-sulfo)-9-fluorenylmethoxycarbonyl; GAT1, γ-aminobutyric acid transporter 1; HAS, human serum albumin; HDAC, histone deacetylase; Hh, hedgehog; HTS, high-throughput screen(ing); LOPAC, library of pharmacologically active compounds; LSF, late-stage functionalization; MD, molecular dynamics; MLSD, multiple ligand simultaneous docking; MPO, multiparameter optimization; MS, mass spectrometry; NNRTIs, non-nucleoside reverse transcriptase inhibitors; Pf, *plasmodium falciparum*; PROTACs, proteolysis-targeting chimeras; RT, reverse transcriptase; PXR, pregnane X receptor; SARs, structure-activity relationships; SPR, surface plasmon resonance; SKR, structure-kinetic relationship; TGS, Target-guided synthesis; TOS, target-oriented synthesis; TTR, transthyretin; VEGFR2, vascular endothelial growth receptor 2.

Biographies

Gaochan Wu received his bachelor's degree in pharmacy from Hebei University, China, in 2016. Currently he is a master graduate student in medicinal chemistry at the School of Pharmaceutical Sciences, Shandong University, working under the supervision of Professor Xinyong Liu and Associate Professor Peng Zhan. His work

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Jacob Kongsted completed his PhD in Theoretical and Computational Chemistry at the University of Copenhagen in 2005, followed by post-doctoral training at Lund University (Sweden) and University of Aarhus (Denmark). In 2009 he moved to University of Southern Denmark as an Assistant Professor and from 2015 he is Full Professor of computational quantum chemistry at the University of Southern Denmark. Kongsted has a broad research interest in theoretical and computational chemistry and has especially contributed to this field by development of novel quantum chemistry embedding methods as well as the application of these computational models to systems of biophysical and biochemical interest. In recent research, he is using quantum chemistry methods for rational design of light-induced biological functional materials and advanced drug design.

Christophe Pannecouque graduated in pharmaceutical sciences at the Rega Institute for Medical Research of the Katholieke Universiteit Leuven in 1990. He obtained his PhD in medicinal chemistry at the same university in 1995, and joined the group of Professor Erik De Clercq as a postdoctoral fellow. In 2003, he became an associate professor. In the field of virological research, he has unravelled the modes of action of several classes of new products with anti-HIV activity. Recently, his research has been focused on cellular targets interfering with HIV replication and on regulation of the (cyto)pathogenicity of the virus.

Erik De Clercq has MD and PhD degrees and has taught at the Katholieke Universiteit Leuven (and Kortrijk) Medical School, Belgium, where he was Chairman of the Department of Microbiology and Immunology until September 2006. He is currently Emeritus Professor of K.U. Leuven University, Member of the Belgian (Flemish) Royal Academy of Medicine and the Academia Europaea, and Fellow of the American Association for the Advancement of Science. In 2008, he was elected European Inventor of the Year (Lifetime Achievement Award), and in 2010 he, together with Dr. A. S. Fauci, was Laureate of the Dr. Paul Janssen Award for Biomedical Research. He is the (co)inventor of a number of antiviral drugs (valaciclovir, brivudin, cidofovir, adefovir, and tenofovir).

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Peng Zhan obtained his B.S. degree from Shandong University, China, in 2005. Then he earned his MS degree and PhD in medicinal chemistry from Shandong University in 2008 and 2010, respectively. He subsequently joined the research group of Professor Xinyong Liu as a Lecturer (2010-2012). From 2012 to 2014, he worked as a Postdoctoral Fellow funded by JSPS (Japan Society for the Promotion of Science) in the Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Japan. He is currently an associate professor in the Institute of Medicinal Chemistry, Shandong University. His research interests involve the discovery of novel antiviral, anticancer and neurodegenerative diseases-related agents based on rational drug design and combinatorial chemistry approaches.

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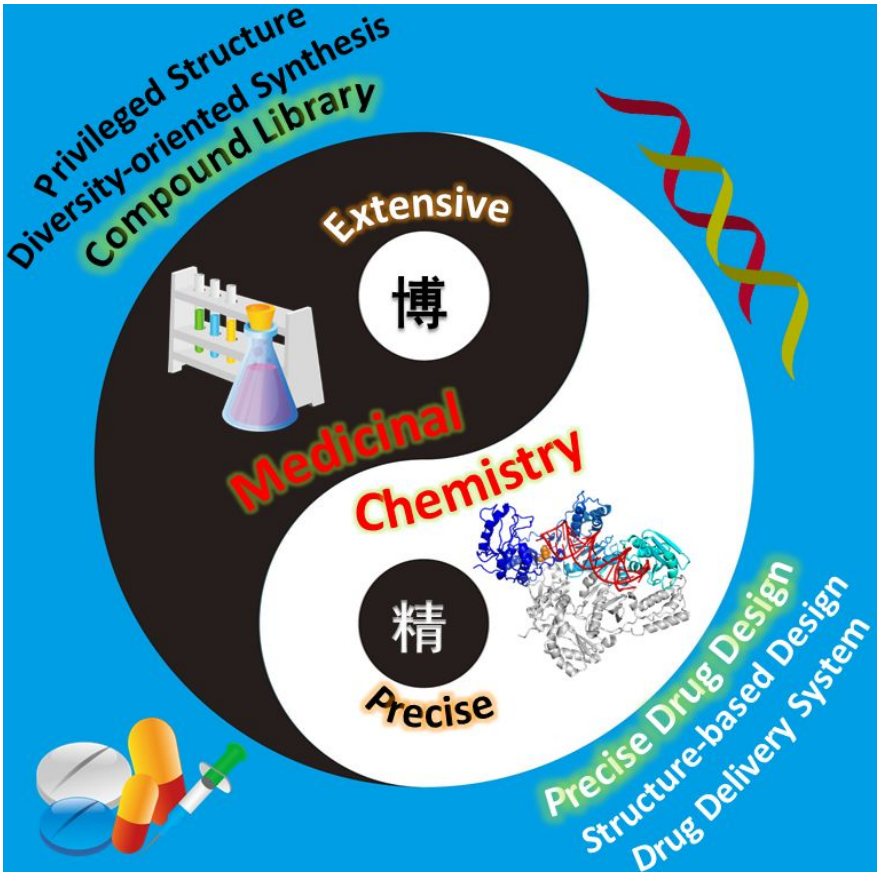
Table of Contents Graphic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