Harnessing the cyclization strategy for new drug discovery

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Abstract The design of new ligands with high affinity and specificity against the targets of interest has been a central focus in drug discovery. As one of the most commonly used methods in drug discovery, the cyclization represents a feasible strategy to identify new lead compounds by increasing structural novelty, scaffold diversity and complexity. Such strategy could also be potentially used for the follow-on drug discovery without patent infringement. In recent years, the cyclization strategy has witnessed great success in the discovery of new lead compounds against different targets for treating various diseases. Herein, we first briefly summarize the use of the cyclization strategy in the discovery of new small-molecule lead compounds, including the proteolysis targeting chimeras (PROTAC) molecules. Particularly, we focus on four main strategies including fused ring cyclization, chain cyclization, spirocyclization and macrocyclization and highlight the use of the cyclization strategy in lead generation. Finally, the challenges including the synthetic intractability, relatively poor pharmacokinetics (PK) profiles and the absence of the structural information for rational structure-based cyclization are also briefly discussed. We hope this review, not exhaustive, could provide a timely overview on the cyclization strategy for the discovery of new lead compounds.

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1. Introduction

From the perspective of drug discovery, the optimization of the lead compounds for improving potency, selectivity, and other drug-like properties is one of the challenges facing the medicinal chemists. In general, the lead compounds with five or more rotatable bonds have many molecular conformations and are difficult to bind to the targets in the correct conformation, thereby reducing the affinity of the lead compounds to the targets of interest. Unrestricted conformations may also bind to other biomolecules, resulting in poor selectivity and unexpected toxicity. Therefore, it is necessary to restrict the conformation of the lead compounds and lock it on the pharmacodynamic conformation that interacts with the target molecules, thereby improving the affinity and selectivity of the compounds.

The binding of the drug to its receptor is a complex thermodynamic process. It is estimated that the entropy loss of a small molecule ligand with a molecular weight of 1000 at room temperature during this process is 258 kJ/mol, and the entropy value decreases by 2–3 kJ/mol for every free bond that the ligand restricts. To make the binding free energy (DG) between the ligand and the receptor negative (DG < 0), the energy consumed to reduce the conformational freedom needs to be compensated by the non-bonding interaction between the molecules. In general, conformational restriction on the ligand can reduce the compensation of entropy and increase the binding affinity of the ligand.

There are many approaches to restrict the conformation of the molecule including cyclization, introducing different rigid groups such as double bond, triple bond, amide as well as benzene ring, and increasing steric hindrance by introducing methyl or cyclopropyl. Among these approaches, the cyclization strategy is the most direct method of conformational restriction and has been widely used in drug design, particularly for the peptide design. In this review, we briefly summarize the cyclization strategies for new drug discovery, including ring fusion, chain cyclization, spiro cyclization as well as macrocyclization, and further highlight their advantages such as improving the potency and selectivity, enhancing the pharmacodynamic and kinetic properties of small molecule compounds, and increasing the structural novelty and complexity of compounds.

2. Using the cyclization strategy for new drug discovery

As a commonly used approach, the cyclization strategy has been widely used in drug discovery. Here we summarize some recent representative examples in detail to present applications of diverse cyclization approaches in drug design, including the introduction of fused rings, macrocycles, spiro rings, etc. These conformational restriction strategies have been proved to significantly improve the drug-like properties of lead compounds.

2.1. Fused cyclization in drug discovery

As a heme-containing enzyme that catalyzes the oxidation of L-tryptophan (L-Trp), indoleamine 2,3-dioxygenase 1 (IDO1) has long been of interest to researchers due to its role in immunotherapy. To date, eleven small-molecule IDO1 inhibitors have advanced into clinical trials, including NLG909 for the treatment of advanced solid tumors (ClinicalTrials.gov Identifier: NCT05469490). In the discovery process of clinical candidate NLG-919 (Fig. 2), IDO1 IC50 = 28 nmol/L, Newlink researchers designed tricyclic imidazoisoindoles based on 4-PI (IC50 = 28 μmol/L) incorporating a methylene linker at its N1 and 2’-positions, which was proved to be able to improve potency (2, IDO1 IC50 = 5.7 μmol/L). The X-ray structure of NLG-919 bound to IDO1 displays that due to the reduction of rotatable bonds, the fused imidazoisoindole core coordinates to the heme iron with higher affinity, and the phenyl group also fits in the hydrophobic pocket better.

Furazanyl hydroxamidine 3 was known to be an effective IDO1 heme-binding inhibitor (Fig. 3, IDO1 IC50 = 1.5 μmol/L, HeLa cell IC50 = 1.0 μmol/L), Zhang et al. further designed a series of benzo-fused analogs with varied ring sizes and identified the 6,5-fused oxindole as an optimal motif, and its (S)-isomer 4 showed excellent inhibitory effects in both enzymatic and cellular assays (IDO1 IC50 = 0.052 μmol/L, HeLa cell IC50 = 0.087 μmol/L). Cheng et al. previously identified phenyl benzenesulfonyl hydrazide 5 as a potent IDO1 inhibitor in vitro (Fig. 3, IDO1 IC50 = 130 nmol/mL), but further pharmacokinetic analysis indicated compound 5 was not stable enough with 37% of oral bioavailability and did not inhibit tumor growth. Therefore, further optimization for improving the pharmacokinetic profile was carried out, resulting in cyclized compound 6, a potent and orally bioavailable IDO1 inhibitor with 73% of tumor growth delay in the implanted CT26 model (IDO1 IC50 = 36 nmol/mL, F = 59%). Accordingly, compound 6, an immunotherapeutic anticancer agent, deserved further preclinical evaluation.

As the first discovered histone demethylase, lysine specific demethylase 1 (LSD1) is responsible for mono- and di-methyl modification of histone H3K4 and H3K9 through flavin adenine dinucleotide (FAD)-dependent enzymatic oxidation, being a promising therapeutic target for cancer therapy. There are two types of LSD1 inhibitors reported so far, irreversible inhibitors covalently bonding with the cofactor FAD and reversible inhibitors competing with the substrate binding. Irreversible inhibitors, such as TCP and compound 8 (Fig. 4), can react with the flavin motif of FAD to form a tight-binding adducts and inhibit other homologous FAD-dependent enzymes LSD2 and MAOA.

In 2017, Ji et al. reported a series of conformationally restricted TCP-based derivatives through enantioselective synthesis and chiral resolution. The structure–activity relationships (SARs) studies demonstrated that most cis isomers were found to be more potent than the corresponding trans isomers, and both of the most potent compounds 7a and 7b (IC50 = 6.4 and 2.2 nmol/mL, respectively) showed over 3000-fold increase in LSD1 inhibition compared to TCP and excellent selectivity over MAO-A/B (IC50 > 100 μmol/L). As shown in the co-crystal structure of compound 8 with LSD1 (PDB code: 2XAO), there was a relatively large space between the phenyl ring of FAD-adduct and nearby residues to accommodate substituents or rings. Further cyclization led to the generation of novel indoline derivatives. The representative compound 9 showed potent inhibitory activity against LSD1 (IC50 = 24.43 nmol/mL) and excellent selectivity over MAO-A/B (>4000-fold) and LSD2 (>200-fold). Furthermore, 9 demonstrated a moderate T/C value of 30.89% in vivo.

Compared to irreversible LSD1 inhibitors, reversible LSD1 inhibitors SP-2577 and CC-90011 have already entered clinical trials. Zhao et al. restricted the conformation of compound 10 (SP-2509, the analog of SP-2577) by replacing the acetophenone moiety with 2,3-dihydro-1H-inden-1-one moiety. In consequence, the corresponding products 11a and 11b showed nearly 10-fold increased inhibitory activity (IC50 = 1.4 and 1.7 nmol/mL, respectively). Based on the binding mode of compound 12 with
LSD1, Cheng et al.\textsuperscript{46} employed the cyclization strategy to design novel tetrahydroquinoline-based LSD1 inhibitors (Fig. 5), which not only maintained the U-shaped conformation but also reserved the hydrogen bond interaction between terminal alkaline fragment with D555 and the $\pi$–$\pi$ stacking between arylthiophene fragment and FAD. Compound 13 exhibited excellent LSD1 inhibition ($IC_{50} = 55$ nmol/L), good selectivity over MAO-A/B and moderate antiproliferative activity against MGC-803 cells ($IC_{50} = 1.13 \mu$mol/L). They also designed a series of benzofuran derivatives based on the cyclization strategy of compound 14 ($IC_{50} = 134$ nmol/L). Similarly, the original hydrogen bond interactions between the piperazine ring with D555 and $\pi$–$\pi$ stacking between the 4-cyanophenyl group and FAD were still maintained. In particular, compound 15 showed excellent LSD1 inhibition with an $IC_{50}$ value of 65 nmol/L and an antiproliferative effect against cancer cell lines overexpressing LSD1\textsuperscript{47}.

Figure 1  Selected conformationally constraint lead compounds designed based on the cyclization strategy.

Figure 2  The optimization of NLG-919 using the cyclization strategy (PDB code: 6O3I). The inhibitor NLG-919 is displayed in the yellow stick. The H-bonding interactions between NLG-919 and the IDO1 protein matrix are indicated by the blue dotted lines.
As critical epigenetic modulators of gene expression, histone deacetylases (HDACs) catalyze the removal of acetyl moieties from lysine in histones and non-histone substrates and have been validated as an important class of epigenetic drug targets for the treatment of many cancers, inflammatory and neurodegenerative diseases\(^4\). So far, there are four FDA-approved HDAC inhibitors (HDACis) for the treatment of multiple myeloma and peripheral or cutaneous T-cell lymphoma\(^5\).

Taha et al.\(^5\) developed a novel series of tetrahydroisoquinoline HDAC8 inhibitors based on previous tertiary amine (Fig. 6A). The SAR studies suggested that compound 17 connecting the tertiary amine and the phenyl moiety displayed the highest HDAC inhibition and 135-fold selectivity over HDAC1, which would be further optimized by varying the substitution at the C1 position of the tetrahydroisoquinoline scaffold.

McClure et al.\(^5\) disclosed the allosteric hydrazide-containing HDAC8 inhibitor 18 in 2016 (Fig. 6B). Although the superior inhibitory activity of compound 18, its \(\alpha,\beta\)-unsaturated ketone as a “Michael receptor” might induce promiscuous cellular responses due to the reaction with thiol groups. So they continued to modify the cinnamamide group with an indole ring to deactivate the Michael acceptor\(^5\). Finally, the cyclic compound 19 exhibited improved potency by a factor of 2–5 than 18 against HDAC enzymes (IC\(_{50}\) = 0.43–18.54 \(\mu\)mol/L), excellent PK profile (\(F = 112\%)\) and outstanding \textit{in vivo} anti-AML activity (TGI = 78.9\%).

Bruton’s tyrosine kinase (BTK), a nonreceptor tyrosine kinase belonging to the Tec family of cytoplasmic tyrosine kinases, plays an important role in the pathogenesis of B-cell lymphomas, and increasing evidence suggests that BTK is a validated therapeutic target for the treatment of hematological malignancies\(^5\).

In 2019, Guo et al.\(^5\) synthesized a novel series of compounds with a pseudo pyrimidinone ring through an intramolecular hydrogen bond, and these compounds displayed good BTK potency (Fig. 7A). Encouragingly, the ring-merged compound BGB-3111 (20) possessed unexpected BTK potency (IC\(_{50}\) = 0.3 \(\mu\)mol/L), high selectivity over other kinases as well as outstanding \textit{in vivo} efficacy in OCI-LY10 xenograft models. BGB-3111 (Zanubrutinib) is currently being evaluated in clinical trials.

Ibrutinib (21), the first-generation BTK inhibitor, was approved by FDA in 2013 for the treatment of mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL)\(^6\). As shown in Fig. 7B, ibrutinib is an irreversible inhibitor via a covalent bond formed by its acrylamido moiety with C481 of BTK protein (PDB code: 5P\(9\)). To obtain novel BTK inhibitors, Zhao et al.\(^5\) merged the piperidine ring and the pyrazol[3,4-\(d\)]pyrimidine moiety of 21 into the tricyclic backbone. The critical covalent interaction and three H-bonding interactions with Y474, E475, and M477, were not affected. Finally, they obtained a new BTK inhibitor 22 with comparable potency as ibrutinib (BTK IC\(_{50}\) = 0.4 \(\mu\)mol/L, TMD8 cell IC\(_{50}\) = 16 \(\mu\)mol/L) and effective tumor growth suppression in the TMD8 xenograft model.

Based on previously reported triazine analog 23 (Fig. 7C), Kawahata et al.\(^5\) cyclized the N atom at 1-position and the NH at 2-position of the triazine ring without causing the loss of...
inhibitory activity, which led to the discovery of AS-1763 (24), a potent, selective, and orally available noncovalent BTK inhibitor (IC$_{50}$ = 0.4 nmol/L). Currently, AS-1763 has advanced into phase 1 clinical trial for treating chronic lymphocytic leukaemia (ClinicalTrials.gov Identifier: NCT05365100).

The CXC chemokine receptor 2 (CXCR2) is essential in the recruitment of myeloid-derived suppressor cells (MDSCs) to the tumor microenvironment, making it an attractive drug target for cancer immunotherapy. SB-332235 (25, Fig. 8) featuring a sulfonamide/sulfone moiety adjacent to phenol serves as a starting point for further modification. Dong et al. designed a series of unsaturated benzocyclic sulfone derivatives based on the cyclization strategy. Extensive SAR studies resulted in the discovery of a novel potent CXCR2 antagonist (26) with an IC$_{50}$ value of 34 nmol/L. Molecular docking analysis showed that both the non-cyclic SB-332235 and cyclic 26 occupied the allosteric site of CXCR2 and formed H-bonding interactions with S81, T83, D84 and K320, respectively. In particular, compound 26 did not induce the steric effect compared to SB-332235.

P2Y12 receptor, an important antithrombotic target, plays a crucial role in the amplification of platelet activation, aggregation and stable thrombosis. Among the reported antagonists, the clinical studies of AZD1283 (Fig. 9) were terminated due to the poor metabolic stability of the ester. Yang et al. designed a series of novel bicyclic pyridine analogs of AZD1283 via cyclization of the ester group to the ortho-methyl, which generally possessed comparable enzymatic inhibitory activity and improved metabolic stability. Importantly, compound 27 exhibited significantly enhanced metabolic stability than AZD1283 in rat and human microsomes.

TRAF-2 and NCK-interacting kinase (TNIK) is one of the most promising targets involved in the Wnt/b-catenin pathway. Recently, Li et al. obtained a hit compound 28 (Fig. 10, IC$_{50}$ = 1.337 μmol/L) by virtual screening, further conformation restriction by cyclizing its carbamoyl group and...
methoxy group and lengthening the phenylamine group led to the discovery of the most active compound 29 (IC50 = 0.026 μmol/L) with high selectivity and good PK properties. Moreover, 29 showed excellent antitumor efficacy in the HCT116 xenograft mouse model.

Focal adhesion kinase (FAK) is a tyrosine kinase overexpressed in highly aggressive and metastatic cancers including acute myeloid leukemia (AML). While oncogenic mutations in FMS-like tyrosine kinase 3 (FLT3) occur most frequently in AML. A recent study indicated that the FAK inhibitors targeting FLT3 could be used to overcome resistance driven by FLT3 mutations. Therefore, Cho et al. utilized the fused thieno[3,2-d]pyrimidine ring as a novel scaffold for developing FAK inhibitors based on the reported PF-562271 (Fig. 11A), of which the hydrophobic thiophene moiety could mimic the trifluoromethyl group. After several rounds of structural optimizations, they finally obtained a promising dual inhibitor 30, which displayed excellent inhibitory activities against FLT3-D835Y mutant (IC50 < 0.5 nmol/L) and FAK (IC50 = 9.7 nmol/L). The molecular docking model with FAK protein (PDB code: 2JKK) revealed that compound 30 formed two hydrogen bonds with C502, and its thiophene moiety also contributed to enhanced hydrophobic interactions (Fig. 11B). The docking results with FLT3 protein (PDB code: 5X02) showed that 30 also formed the H-bonding interaction with C694 in the hinge region (Fig. 11C).

Enhancer of zeste homolog 2 (EZH2) is a histone methyltransferase, which regulates the normal physiological function of cells by catalyzing the methylation of lysine 27 of histone H3 (H3K27) to control the expression of various genes. Based on the EZH2 inhibitor 31 (Fig. 12, IC50 = 5700 nmol/L), Kung et al. found that the cyclization of the amide linker can improve the binding property of 31. Subsequent structural optimizations resulted in the lactam analog 32, which showed significantly improved ligand efficiency (K = 0.7 nmol/L) and potency (IC50 < 5 nmol/L) and robust in vivo antitumor effects.

The receptor activator of nuclear factor Kappa-B ligand (RANKL) is a member of the tumor necrosis factor superfamily, a key factor responsible for osteoclast differentiation, activation and apoptosis. The small-molecule inhibitors of RANKL can be used in the treatment of osteoporosis. In 2019, Jiang et al. reported that the compound Y1599, a potent small-molecule RANKL inhibitor, showed good osteoclastogenesis inhibition in vitro (Fig. 13, 32% at 1 μmol/L) but failed to exhibit oral efficacy in vivo. Interestingly, cyclization of compound Y1599 gave the β-carboline analog Y1693, which exhibited excellent osteoclastogenesis inhibition (89% at 1 μmol/L) and low toxicity. Y1693 markedly reversed OVX-induced osteoporosis after oral administration and could be used as a promising candidate for antiresorptive therapies.

2.2. Chain cyclization in drug discovery

Chain cyclization is another widely used strategy in drug design. As shown in Fig. 14, based on the antipsychotic drug chlorpromazine, many derivatives have been developed. Considering the flexibility of the side chain, cyclizing the terminal dimethylamino group in chlorpromazine may reduce the flexibility and give methdilazine and prochlorperazine with improved antiemetic effect.

Based on the structure of chidamide (33) approved by the NMPA for treating recurrent and refractory peripheral T-cell lymphoma in 2015, Cui et al. optimized its amide region of 33 by forming a six-member ring to improve its PK profiles and in vivo efficacy (Fig. 15). Among these compounds, the most potent compound 34 (HDAC1 IC50 = 84.9 nmol/L) exhibited significantly improved oral bioavailability (F = 92%) and significant in vivo antitumor efficacy in a TMD-8 xenograft model (TGI = 77%) without obvious toxicity. The docking results showed that the cyclization did not impact original key interactions between chidamide with HDAC1, including the H-
bonding with D168 and \( \pi-\pi \) stacking interactions with F141 as well as F198.

FPR2 can resolve chronic inflammation and promote the wound healing processes due to its important role in both host defense and inflammation\(^93\). In 2019, Asahina et al.\(^96\) firstly obtained the carbamoyl derivative \( 35 \) with excellent potency (Fig. 16, \( \text{IC}_{50} = 0.4 \text{ nmol/L} \)). The subsequent introduction of a conformation-restricted pyrrolidinone moiety led to a series of lactam derivatives, and the final optimization of lactam substituents generated the compound \( 36 \) (BMS-986235, \( \text{IC}_{50} = 0.4 \text{ nmol/L} \)), an FPR2 selective agonist with 7000-fold selectivity over FPR1. While treated with \( 36 \) in a mouse heart failure model, the corresponding cardiac structure and functional improvements were observed.

Akt (protein kinase B, PKB), a serine/threonine kinase that belongs to the AGC family of kinases, is an important component in the PI3K/Akt signaling pathway\(^97\). GSK-795 is an ATP-competitive Akt inhibitor with excellent PK profiles and now is in early clinical trials\(^100\). Dong et al.\(^101\) designed a series of 3,4-disubstituted piperidine derivatives based on GSK-795 by cyclizing its amino group with the methylene moiety of the benzyl group, which generally showed excellent \textit{in vitro} and \textit{in vivo} antitumor efficacy but with high toxicity (Fig. 17). Further optimization by introducing diverse side chains ultimately led to the identification of compound \( 37 \). The compound not only showed increased inhibitory activity against Akt1 (\( \text{IC}_{50} = 1.4 \text{ nmol/L} \)) and \textit{in vivo} antitumor efficacy (TGI >90%), but also possessed remarkably improved safety profiles (MTD >80 mg/kg).

### 2.3. Spirocyclization in drug discovery

Due to the three-dimensional structural characteristic and conformational restriction, spiro compounds can bind to the

![Figure 10](attachment:image_url) The discovery of novel selective TNIK inhibitors via cyclization strategy (PDB code: 5AX9). The inhibitor \( 28 \) is displayed in the yellow stick.

![Figure 11](attachment:image_url) (A) The discovery of thieno[3,2-d]pyrimidine \( 30 \) as the dual inhibitor of FAK and FLT3. (B) Proposed docking model of \( 30 \) with FAK. The inhibitor \( 30 \) is displayed in the yellow stick. The H-bonding interactions between \( 30 \) and the FAK protein are indicated by the red dotted lines. (C) Proposed docking model of \( 30 \) with FLT3. The H-bonding interactions between \( 30 \) and the FLT3 protein matrix are indicated by the red dotted lines.
The introduction of conformational restriction by spiro ring formation can not only modulate binding potency and specificity but also potentially improve bioavailability and metabolic stability. Furthermore, the conformational restriction by spirocyclization may reduce off-target activities\textsuperscript{103,104}. The fraction of sp\textsuperscript{3} carbons and chiral carbon count have been used as descriptors of molecular complexity, thus spirocyclization can provide a useful method of increasing molecular complexity and may offer greater benefits than the introduction of flat rings\textsuperscript{105}.

Polo-like kinase 4 (PLK4) is present in proliferating tissues and up-regulated in breast cancer, high PLK4 levels are associated with poor outcomes\textsuperscript{106}. Pauls et al.\textsuperscript{11,109} reported the first PLK4 clinical candidate CFI-400945 via the bioisosteric replacement of the alkene linker of compound 38 (Fig. 18, IC\textsubscript{50} = 0.61 nmol/L) with a cyclopropane ring. Compared to the indolinone 38, this structural modification resulted in a substantial improvement in PK properties (\textit{F} = 66\% in dogs) without significant loss of inhibitory activity (IC\textsubscript{50} = 2.8 nmol/L). Currently, the fumarate of CFI-400945 is being studied in phase II clinical trials for the treatment of advanced cancer, including breast cancer (ClinicalTrials.gov identifier: NCT04176848 and NCT03624543), prostate cancer (ClinicalTrials.gov identifier: NCT03385655) as well as acute myeloid leukemia (ClinicalTrials.gov identifier: NCT03187288, NCT01954316, NCT04730258).

Cholesteryl ester transfer protein (CETP), a glycoprotein produced primarily by the liver, could mediate the exchange of triglycerides from the very low density lipoprotein (VLDL) with cholesteryl ester from high density lipoprotein (HDL). Inhibition of CETP is considered to be one of the most effective ways for increasing high density lipoprotein-cholesterol (HDL-C) levels\textsuperscript{110-112}. Previous studies have revealed that the binding pocket of CETP is hydrophilic, which means that the potent CETP inhibitors should be hydrophilic as well. The compounds with high lipophilic properties often impact on solubility, disposition and promiscuity, thus possessing poor drug-like properties\textsuperscript{113-115}.
Starting from the CETP inhibitor 39, Trieselmann et al.117 designed the analog 40 by spirocyclization of the fluorobenzyl group, this structural modification not only reduced the lipophilicity of 40 (Fig. 19, clogP = 4.6) but also increased its inhibitory activity against CETP (IC50 = 18 nmol/L). In addition, 40 effectively increased the level of HDL-C, decreased the level of low density lipoprotein cholesterol (LDL-C) in CETP transgenic mice and had no significant effect on blood pressure and electrocardiogram.

As a non-receptor protein tyrosine phosphatase encoded by the PTPN11 gene, the Src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP2) is ubiquitously expressed and mediates several intracellular oncogenic signaling pathways118-120. In recent years, the development of SHP2 targeting small molecule inhibitors has been highly pursued121-123. The spirocyclization strategy has also been successfully employed by Novartis’s researchers to develop new SHP2 allosteric inhibitors. Compound 41 showed good inhibitory activity (Fig. 20, SHP2 IC50 = 0.034 μmol/L), but had relatively low cellular (p-ERK IC50 = 0.355 μmol/L) and antiproliferative activities (KYSE520 IC50 = 13.49 μmol/L). After introducing the spirocyclic system carrying the exocyclic amine, the corresponding product 42 showed improved cellular efficacy (p-ERK IC50 = 0.012 μmol/L, KYSE520 IC50 = 0.167 μmol/L) due to increased lipophilicity while maintaining enzymatic activity (SHP2 IC50 = 0.028 μmol/L)124.

Plasmodium falciparum histone deacetylases (PfHDACs) are widely expressed and transcribed in multiple life cycle stages of P. falciparum and are involved in many biological functions critical for the survival and reproduction of helminths125,126. Based on quisinostat, an HDAC inhibitor in phase II clinical trial127, Li et al.128 designed a series of novel spirocyclic hydroxamic acid derivatives by introducing the spirocyclic linker. Among them, compound 43 displayed better potency against two multi-resistant malarial parasites than quisinostat (Fig. 21, Pf3D7 IC50 = 5.2 nmol/L, PfDd2 IC50 = 7.1 nmol/L) and attenuated cytotoxicity against two human cell lines (HepG2 IC50 = 264 nmol/L, 293T IC50 = 241 nmol/L). Further optimization gave compound JX35, which showed a stronger triple-stage (the blood stage, liver stage, and gametocyte stage) antimalarial effect (Pf3D7 IC50 = 1.26 nmol/L, PfDd2 IC50 = 1.61 nmol/L), increased safety (HepG2 IC50 = 1.02 μmol/L, 293T IC50 = 1.21 μmol/L), and good pharmacokinetic properties129.

Human mitochondrial peptide deformylase (HsPDF) is a metalloenzyme responsible for removing the N-terminal formyl group in mitochondrial proteins and plays an important role in maintaining mitochondria function130-132. As a naturally occurring antibiotic obtained from Streptomyces species, Actinonin was first reported in 2000 as an HsPDF inhibitor with a metal ion-
bound hydroxamic acid moiety (Fig. 22). It showed acceptable inhibitory activity (HsPDF IC\textsubscript{50} = 31.6 nmol/L, HCT116 IC\textsubscript{50} = 22.6 μmol/L) and provided a framework for the development of novel HsPDF inhibitors. In 2020, Hu et al.\textsuperscript{134} reported the cyclized derivative 44 with significantly improved enzymatic activity (IC\textsubscript{50} = 4.1 nmol/L) and good antiproliferative activity (HCT116 IC\textsubscript{50} = 1.9 μmol/L). It also displayed better antitumor efficacy in HCT116 xenograft mouse models with tolerable toxicity compared to actinonin.

2.4. Macrocyclization in drug discovery

Macrocycles refer to the compounds with more than 12-membered rings, and macrocyclic compounds are believed to achieve good conformation restriction while maintaining relative molecular weight and good lipophilic efficiency (LipE)\textsuperscript{135,136}.

Crizotinib, developed by Pfizer in 2011, is an anaplastic lymphoma kinase (ALK) inhibitor\textsuperscript{137}. However, drug resistance and difficulty in penetrating the blood–brain barrier have emerged. To overcome these defects, Pfizer developed a new generation of ALK inhibitor lorlatinib (Fig. 23, 45) with a macrocyclic ring for the treatment of ALK-positive lung cancer patients\textsuperscript{138}. Compared to crizotinib (ALK IC\textsubscript{50} = 80 nmol/L, ALK-L1196M IC\textsubscript{50} = 843 nmol/L), the macrocyclic inhibitor lorlatinib exhibited excellent inhibitory activities against wild-type ALK (ALK IC\textsubscript{50} = 1.3 nmol/L) and clinical mutants (ALK-L1196M IC\textsubscript{50} = 21 nmol/L) and also had improved CNS penetration (MDR BA/AB = 1.5 vs. 45) as well as kinase selectivity.

The protein–protein interaction (PPI) between B-cell lymphoma 6 (BCL6) and its corepressors has been proposed as an attractive drug target for the treatment of diffuse large B-cell lymphoma (DLBCL) cancers\textsuperscript{139}. In 2017, starting with a hit fragment 46 (Fig. 24, K\textsubscript{d} = 689 μmol/L), McCoull et al.\textsuperscript{140} discovered the macrocycle pyrazolo[1,5-α]pyrimidine BCL6 binder 47 (K\textsubscript{d} = 0.0065 μmol/L) with 100,000-fold increased binding affinity through the structure-based drug design. The X-ray structure of 47 bound to BCL6 demonstrated that several hydrogen bonds were formed by the OH group with Y58, the pyrazole N atom with M51 as well as the lactam oxygen with E115, which were reinforced by the geometry of macrocyclic linker (PDB code: 5N1Z).
The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that transduces mitogenic signals. T790M and C797S mutations are two prevalent EGFR mutations in NSCLC tumors, which could cause ligand-independent EGFR activation and sensitize NSCLC tumors to EGFR tyrosine kinase inhibitors (TKIs). In 2019, Engelhardt et al. identified the aminobenzimidazole as a promising lead compound, demonstrating moderate binding affinity toward mutated EGFR (EGFRdel19/T790M/C797S IC50 = 250 nmol/L) and no affinity to EGFRWT (EGFRWT IC50 > 100 nmol/L) as well as acceptable cellular activity (BaF3 cells IC50 = 300 nmol/L). From the cocrystal structure of 48, they also observed several H-bonding interactions between the carbonyl oxygen group of 48 and benzimidazole NH and M793, respectively, as well as the pyridine N atom with K745. The isobutyl hydroxy group filled the sugar pocket and formed H-bonding interaction with C797. Additionally, there was a two-point hinge binding between the two aromatic rings (40°), which was proved to be a low-energy conformation and important for our further optimization. Similar to lorlatinib, the macrocyclization strategy was also successfully applied to the discovery of BI-4020, a noncovalent EGFR inhibitor with excellent in vitro and in vivo inhibitory potency (EGFRdel19 T790M C797S IC50 = 0.6 nmol/L, BaF3 cells IC50 = 0.2 nmol/L). Furthermore, 49 displayed excellent kinase selectivity, while sparing ample EGFRWT (EGFRWT IC50 > 400 nmol/L).

Recently, Chen et al. originally reported a series of 4-indolyl-2-phenylaminopyrimidine-based EGFRC797S inhibitors by hybridizing the scaffolds of approved drugs Osimertinib and Brigatinib, and the compound 50 exhibited excellent kinase inhibitory activities against EGFRL858R/T790M/C797S and EGFR19del/T790M/C797S with IC50 values of 4.3 and 25.6 nmol/L, respectively. However, compound 50 had a poor cellular permeability, which caused the unsatisfied antiproliferative effect. The co-crystal structure of EGFRT790M/C797S with 50 showed that compound 50 adopted a “U-shape” conformation, and formed three hydrogen bonds between the N atom of pyrimidine with L792, the NH of aniline with the backbone carbonyl of M793 and the ethyl sulfonate moiety with K745. Importantly, it also revealed that there was a dihedral angle of 30.9° upon binding between the pyrimidine and the 4-(3-indolyl) group, which implied that the conformationally constrained macrocyclization strategy may be used to avoid the entropy-driven binding affinity loss. Further optimizations were carried out based on the structural information. Encouragingly, the macrocyclic compound 51 not only maintained comparable kinase inhibitory activity against EGFRdel19/T790M/C797S (IC50 = 15.8 nmol/L) but also displayed a significantly improved...
antiproliferative activity against Ba/F3 cells (IC$_{50}$ = 0.052 µmol/L vs. 3.156 µmol/L). However, compound 51 exhibited unfavorable oral PK properties (F = 11.9%).

As the activated form of the zymogen factor XI (FXI), trypsin-like serine protease factor XIa (FXIa) plays an important role in the blood coagulation cascade and may contribute to thrombosis risk$^{146,147}$. Finto et al.$^{148}$ have disclosed the phenyl substituted imidazole 52 with excellent FXIa inhibitory activity (Fig. 27, $K_i = 5.8$ nmol/L) and moderate anticoagulant activity (aPTT EC$_{1.5x}$ = 5.3 µmol/L) in 2015. To further improve aPTT potency, they reported a series of novel macrocyclic FXIa inhibitors via linking the benzyl and phenyl groups of compound 52. Among these inhibitors, the macrocycle 53 containing an E-alkene showed picomolar FXIa inhibitory activity ($K_i = 0.03$ nmol/L), improved aPTT potency (EC$_{1.5x}$ = 0.28 µmol/L) and over 1000-fold selectivity over other blood coagulation enzymes$^{149}$. Although the macrocyclic amide linker formed a hydrogen bond with L41 and significantly contributed to the improved FXIa potency, it also had poor oral bioavailability (F = 1%).

Glutaminases (GLSs) including kidney-type glutaminase (GLS1) and liver-type glutaminase (GLS2) are key enzymes that catalyze glutamine to glutamate$^{150}$. CB839 (Fig. 28) is a widely recognized allosteric inhibitor with an IC$_{50}$ of 22 nmol/L, the previously published X-ray crystal structure revealed an U-shaped configuration with a distance of 10.58 Å between the terminal atoms (PDB code: 5HL1), which provided a certain chemical space allowing for the introduction of a macrocyclic linker$^{151,152}$. Based on the structural information, Bian et al.$^{153}$ reported a series of macrocyclic GLS1 inhibitors by linking the terminal aromatic rings of CB839. The most promising compound 54 not only showed robust inhibitory activity in enzymatic and cellular levels (GLS1 IC$_{50}$ = 6 nmol/L, HCT116 IC$_{50}$ = 81 nmol/L), but also induced ROS activation by blocking glutamine metabolism. Compound 54 also exhibited comparable in vivo antitumor effects as CB839.

Mcl-1, a member of the anti-apoptotic protein Bcl-2 family, is a major component of the mitochondrial apoptotic pathway$^{154}$. The Mcl-1 gene is amplified in a variety of human tumors, and the high expression of Mcl-1 is closely related to the development of resistance to chemotherapy$^2$. In tumor cells, Mcl-1 and pro-apoptotic proteins (e.g., Bak, Bid, Bad, etc.) exert anti-apoptotic effects through protein–protein interactions, leading to tumor cell proliferation. However, such PPI has a large contact interface and strong affinity, making the development of small-molecule drugs difficult$^{155}$. The researchers from AstraZeneca found that compound 55 inhibited Mcl-1 with an IC$_{50}$ of 0.042 µmol/L$^{156}$. By analyzing the X-ray crystal structure (Fig. 29, PDB code: 6FS1), they found that 55 adopted a U-shaped configuration and occupied a larger hydrophobic space, its indole-2-carboxylic acid formed an ionic interaction with R263. Particularly, the distance between the pyrazole methyl group and the naphthalene ring was only 3.6 Å, allowing for subsequent structural modifications. They then designed several macrocyclic compounds by linking the pyrazole methyl of 55 to its naphthalene ring. As expected, (R$_a$)-6-Cl indole derivative AZD5991 (56) exhibited significantly improved inhibitory activity (IC$_{50}$ = 0.7 nmol/L) and complete tumor regression (TGI = 100%) in the multiple myeloma model and acute myeloid leukemia model. Currently, the compound is undergoing phase II clinical evaluation for the treatment of acute myeloid leukemia (ClinicalTrials.gov identifier: NCT03218683 and NCT03013998).

The proteolysis targeting chimeras (PROTAC) molecules are bifunctional molecules by recruiting cellular degradation machinery to induce ubiquitination and subsequent proteasomal degradation$^{103,157,158}$. McCoull et al.$^{159}$ first developed a macrocycle-based therapeutic modality combined with PROTAC technology for the development of BCL6 modulators in 2018. They originally...
obtained the macrocycle inhibitor 58 by pursuing a hit-to-lead optimization campaign of hit 57, which demonstrated excellent inhibitory activity against BCL6 (IC$_{50} = 0.0089$ µmol/L, Fig. 30). Nextly, the macrocycle-based PROTAC 59 was designed through the conjugation of 58 with the CRBN binder thalidomide. Although 59 could effectively degrade BCL-6 in a dose-dependent fashion (82% degradation at 1 µmol/L), it did not show a significant anti-proliferative effect and phenotypic response.

In 2019, Testa et al. reported the first BET macrocyclic PROTAC molecule 60 (BRD4$_{BD2}$ $K_d = 15$ nmol/L, Fig. 31) by introducing an ether linker to the BET degrader MZ1. The X-ray co–crystal structure of MZ1 (PDB code: 5T35) revealed that MZ1 adopted an U-shaped configuration, and its two ligand moieties laid in close spatial proximity with a distance of 7.9 Å. Based on this structural information, they designed a macrocyclic PROTAC by adding an ether linker to lock the PROTAC configuration. Encouragingly, the corresponding MacroPROTAC-1 (60) exhibited high binary binding affinity (BRD4$_{BD2}$ $K_d = 180$ nmol/L, VHL $K_i = 33$ nmol/L) and comparable cellular degradation activity to MZ1 (DC$_{50} < 125$ nmol/L). Furthermore, compound 60

Figure 29  The discovery of the macrocyclic Mcl-1 specific inhibitor AZD5991. The inhibitor 56 is displayed in the yellow stick (PDB code: 6FS1).

Figure 30  Discovery of the BCL6 macrocyclic PROTAC 59.
also showed good antiproliferative activity in 22RV1 cells (EC$_{50}$ = 640 nmol/L) and MV4; 11 cells (EC$_{50}$ = 300 nmol/L). These findings support that macrocyclic PROTACs may represent an alternative strategy for the development of new PROTAC degraders.

3. Conclusions and perspectives

The design of highly potent and specific ligands for the targets of interest has been a continued challenge in recent years. As one of the most commonly applied methods in drug discovery, the cyclization strategy has proven to significantly increase the binding affinity and selectivity via stabilizing the binding pattern. Moreover, due to the scaffold change and increased structural novelty of cyclized compounds, this strategy could provide feasible ways to discover new lead compounds based on the patented compounds. The cyclization strategy has been successfully applied to the discovery of novel drug candidates for treating various diseases, some drugs including Dolutegravir have been approved by the FDA.

When designing new cyclized compounds based on existing lead compounds, the newly cyclized scaffold should maintain original key interactions with the targets of interest, the modifiable sites for cyclization should be solvent exposed or do not have key interactions with the proteins of interest. However, there are still several challenges facing the researchers, including (i) the synthetic intractability limits further investigation of the cyclized compounds, particularly for macrocycles. In addition, the development of novel synthetic methods may facilitate the macrocycle-based drug discovery; (ii) the macrocycles are structurally complex and have high molecular weight, thus showing relatively poor PK profiles; (iii) for some proteins, the structural information of the small molecule/protein complexes are rarely reported, making structure-based cyclization less rationale and more challenging. Anyway, we believe the cyclization strategy will find wide applications in drug discovery.

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Author contributions

Bin Yu conceived this project, revised this review extensively, and submitted this review on behalf of other authors. Bin Yu, Kai Tang, Yihui Song, Shu Wang and Wenshuo Gao wrote this review together.

Conflicts of interest

The authors have no conflicts of interest to declare.

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