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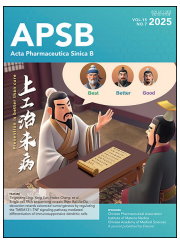

REVIEW

Unraveling the therapeutic landscape of approved non-peptide macrocycles

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Abstract Non-peptide macrocyclic drugs possess unique structural advantages that allow them to target various biomolecules of interest and thus show therapeutic potential against various diseases such as cancer, infectious diseases, etc. This review article examines 34 non-peptide macrocyclic drugs approved between 2000 and 2024, with a particular focus on the optimization process of representative macrocyclic drugs such as natural macrocycles, natural product-inspired macrocycles, and *de novo*-designed macrocycles. We discuss their structural characteristics, highlighting how conformational rigidity and enhanced target specificity contribute to their efficacy. Design details of these new macrocyclic drugs are illustrated through successful examples, offering insights for optimizing macrocycles. Of note, macrocyclization of U-shaped lead structures represents a novel molecular skeleton editing strategy in *de novo* macrocycle drug design.

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1. Introduction

Small molecules have long dominated the drug discovery landscape due to their favorable oral bioavailability and pharmacological profiles, which allow for high potency and selectivity in therapeutical applications[1,2]. However, they face significant limitations when targeting large and flat binding site, as well as undruggable targets like protein−protein interactions[3]. This has led to a renewed interest in exploring alternative structural classes, particularly macrocyclic compounds characterized by 12- or more-membered ring architecture[4]. These macrocycles have drawn significant attention in medicinal chemistry due to their unique structural properties and therapeutic potential[4,5]. In addition, macrocyclic natural products, such as erythromycin, rapamycin, and vancomycin, have demonstrated considerable therapeutic efficacy[6,7]. However, macrocycles have relatively been underexplored, largely due to challenges related to synthetic intractability and perceived non-drug-like properties[8].

From a structural perspective, macrocyclic compounds have a high degree of conformational rigidity compared to linear counterparts, and this architecture enables a molecule to achieve a degree of structural pre-organization, allowing key functional groups to interact across extended binding sites in proteins without a major entropic loss upon binding[9,10]. This pre-organization can result in high affinity and selectivity, which are key parameters for new drug design. Because of their rigid structural properties, macrocyclic compounds provide a balance between pre-organization and flexibility. This enables them to adapt to target surfaces and enhance binding interactions[5]. Moreover, cyclization can convert linear peptides or small molecules into a more rigid and constrained structure, effectively locking them into a specific conformation that enhances their ability to bind biomolecules[11-13]. As macrocyclic drugs continue to advance rapidly, understanding how they interact with biomolecules will greatly enhance the design and discovery of these drugs[1,14].

Among macrocyclic drugs, there are two significant subclasses: peptide macrocyclic macrocycles and non-peptide macrocycles. Peptide macrocycles, composed of amino acids linked in a cyclic structure, have been extensively studied for their unique ability to mimic natural peptides and their potential in therapeutic applications. However, they often face challenges regarding stability and bioavailability, particularly suffering from poor bioavailability due to rapid degradation by proteolytic enzymes. In contrast, non-peptide macrocycles have gained particular attention due to their diverse therapeutic applications and structural versatility. Constructed from a wide range of building blocks, non-peptide macrocycles offer greater structural diversity and the ability to tailor the properties of the molecules to achieve desired pharmacological outcomes[1,8]. These non-peptide macrocycles can interact with a variety of biological targets, including proteins and nucleic acids, enabling them to disrupt protein−protein interactions, module enzymatic activity, and affect cellular pathways in ways that traditional small molecules might not[7,15,16].

The landscape of non-peptide macrocyclic drugs has expanded significantly, with some macrocycles receiving regulatory approval for clinical use[17]. These drugs have demonstrated efficacy in a broad range of therapeutic areas, including oncology, infectious diseases, and autoimmune disorders, etc. The purpose of this Perspective is to highlight the non-peptide macrocyclic drugs approved from 2000 to 2024, including natural macrocycles, natural product inspired macrocycles, and *de novo* designed macrocycles, while macrocyclic peptides, metal-based macrocycles and diagnostic reagents have been extensively reviewed and thus are not discussed here in details[18-20].

2. Overview of approved non-peptide macrocyclic drugs from 2000 to 2024

The therapeutic potential of macrocyclic drugs is underscored by the increasing number of approved macrocyclic drugs over the past two decades. By searching scientific literatures and manually examining drugs approved by regulatory agencies including the United States Food and Drug Administration (FDA), China's National Medical Products Administration (NMPA), European Medicines Agency (EMA), and Pharmaceuticals and Medical Devices Agency of Japan (PMDA), we have analyzed macrocyclic drugs to provide a landscape of macrocyclic drug discovery (Table 1).

Fig. 1A depicts the annual approval trend of non-peptide macrocyclic drugs along with small molecule drugs from 2000 to 2024. In this period, 34 non-peptide macrocyclic drugs were approved, significantly fewer than the 649 small molecule drugs approved. The peak years were 2011 and 2018, with four approvals each, followed by three in both 2017 and 2022. These non-peptide macrocyclic drugs span various therapeutic areas (Fig. 1B). Oncology is the leading therapeutic area, accounting for 38.2%. The drugs within this category mainly target tubulin (Ixabepilone, Eribulin, and Mirvetuximab Soravtansine), CXC chemokine receptor type 4 (CXCR4, Plerixafor), activin A receptor type I (ACVR1, Pacritinib) and kinases such as anaplastic lymphoma kinase (ALK, Lorlatinib), mammalian target of rapamycin (mTOR, Temsirolimus and Everolimus), neuro trophin receptor kinase (NTRK, Repotrectinib), and epidermal growth factor receptor (EGFR, Icotinib). Besides, two porphyrin-derived drugs such as Temoporfin and Haematoporphyrin are utilized in photodynamic therapy for cancer treatment. These drugs highlight the broad target space of macrocycles in oncological interventions. In addition, antivirals account for about 20.6% of the indications. They primarily focus on the treatment of hepatitis C virus (HCV) by targeting HCV NS3/4A protease, with representative drugs including Simeprevir, Vaniprevir, Paritaprevir, Grazoprevir, Voxilaprevir, Glecaprevir, and Danoprevir. Antibiotics represent 11.8% of the indications. These drugs mainly interfere with the classic ribosomes and RNA polymerase, with examples being Telithromycin, Rifaximin, Fidaxomicin, and Rifamycin. In addition, diagnostic agents (Gadobutrol, Dotarem, Padeliporfin, and Gadopiclenol), characterized by metal complex structures, hold a share of 11.8%, highlighting the emerging role of macrocycles in diagnostic applications. The remaining 17.6% of indications encompass a diverse array of therapeutic utilizations such as eye diseases (Verteporfin), skin conditions (Pimecrolimus and Hemoporfin), blood disorders (Hydroxocobalamin), parasitic infections (Spinosyn A and Moxidectin), cardiovascular disease (Zotarolimus). These distributions underscore the versatility and potential of non-peptide macrocyclic drugs in addressing a wide range of medical conditions, showing their significance in modern drug discovery and development efforts.

Fig. 1C shows the structural classification of these non-peptide macrocyclic drugs, categorizing them into natural products, natural product derivatives, metal complexes, and *de novo* designed macrocycles. Notably, natural product inspired macrocycles and *de novo* designed macrocycles stand out as the predominant

Table 1 Approved macrocyclic drugs from 2000 to 2024.

Drug	Therapeutic indication	Mechanism of action or Target	Company	Approval date	Regulatory authority
Verteporfin	Eye disease	Photo-induced apoptosis	QLT PhotoTherapeutics, Inc. and CIBA Vision Co.	2000	FDA
Pimecrolimus	Skin disease	Calcineurin	Novartis Pharma AG	2001	FDA
Temoporfin	Head and neck squamous cell carcinoma	Photo-induced necrosis and apoptosis	Biolitec Pharma Ltd.	2001	EMA
Haematoporphyrin	Cancer	Photo-induced apoptosis	Chongqing Milelonge Pharma.	2003	NMPA
Telithromycin	Antibiotic	Ribosomes	Aventis Pharma	2004	FDA
Rifaximin	Antibiotic	RNA polymerase	Salix Pharma., Inc.	2004	FDA
Hydroxocobalamin	Pernicious anemia	Not available	EMD pharmaceuticals Inc.	2006	FDA
Ixabepilone	Cancer	Tubulin	Bristol-Myers Squibb Co.	2007	FDA
Temsirolimus	Renal cancer	mTOR	Wyeth pharmaceutical Co., Ltd.	2007	FDA
Plerixafor	Cancer	CXCR4	Genzyme Corp.	2008	FDA
Zotarolimus	Coronary artery restenosis	mTOR	Medtronic Inc.	2008	FDA
Everolimus	Cancer	mTOR	Novartis Pharma AG	2009	FDA
Eribulin	Breast cancer	Tubulin	Eisai & Co	2010	FDA
Gadobutrol	Contrast agent	Not available	Bayer HealthCare pharmaceuticals Inc.	2011	FDA
Spinosyn A	Head lice	nAChRs, GABA	ParaPRO LLC	2011	FDA
Fidaxomicin	Diarrhea	RNA polymerase	Optimer pharmaceuticals	2011	FDA
Icotinib	NSCLC	EGFR	Betta pharmaceuticals Co., Ltd.	2011	NMPA
Hemoporfin	Port-wine Stain	Photo-induced apoptosis	Shanghai Fudan Zhangjiang Bio pharmaceutical Co., Ltd.	2012	NMPA
Simeprevir	Hepatitis C	HCV NS3/4A protease	Janssen research & development, LLC.	2013	FDA
Dotarem	Contrast agent	Not available	Guerbet LLC	2013	FDA
Vaniprevir	Hepatitis C	HCV NS3/4A protease	Merck & Co., Inc.	2014	PMDA
Paritaprevir (a part of Viekira Pak)	Hepatitis C	HCV NS3/4A protease	AbbVie Inc.	2014	FDA
Grazoprevir	Hepatitis C	HCV NS3/4A protease	Merck & Co., Inc.	2016	FDA
Voxilaprevir	Hepatitis C	HCV NS3/4A protease	Gilead Sciences,Inc.	2017	FDA
Glecaprevir	Hepatitis C	HCV NS3/4A protease	AbbVie Inc.	2017	FDA
Padeliporfin	Prostate cancer	Vascular-acting photosensitizer	Steba Biotech Ltd.	2017	EMA
Rifamycin	Travelers diarrhea	RNA polymerase	Cosmo technologies Ltd.	2018	FDA
Moxidectin	Oncocerciasis	Glutamate-gated chloride ion channels	Medicines development for global health	2018	FDA
Danoprevir	Hepatitis C	HCV NS3/4A protease	Ascletis Pharma Inc.	2018	NMPA
Lorlatinib	NSCLC	ALK, ROS1	Pfizer	2018	FDA
Pacritinib	Myelofibrosis	JAK, FLT3	CTI BioPharma Corp.	2022	FDA
Mirvetuximab Soravtansine	Ovarian cancer	FRα, tubulin	ImmunoGen Inc.	2022	FDA
Gadopiclenol	Contrast agent	Not available	Guerbet LLC.	2022	FDA
Repotrectinib	NSCLC	ROS1/TRK/ALK	Bristol-Myers Squibb Co.	2023	FDA

categories, representing 38.2% and 35.3% of the total, respectively. On the other hand, natural products constitute 11.8% of the structural classification, indicating the continued relevance of naturally occurring compounds in macrocyclic drug discovery. Additionally, metal complex-based macrocyclic drugs account for about 14.7% of the total. The prominence of natural product inspired macrocycles and *de novo* designed macrocycles suggests an advancement in leveraging both nature inspired compounds and innovative rational design to develop macrocyclic therapeutics.

3. Natural non-peptide macrocyclic drugs

Natural products are a valuable source for drug discovery[21,22]. Their structural novelty and diverse biological activities make them promising leads for developing new macrocyclic drugs. Between 2000 and 2024, there are four natural macrocycles approved for therapeutic use.

Spinosyn A is a natural insecticide found in the soil bacterium *Saccharopolyspora spinosa* (Fig. 2). It is characterized by its complex tetracyclic macrolide structure. The core structure is derived from polyketides and consists of a unique arrangement that includes a 12-membered lactone fused with tricyclic moiety. In addition, spinosyn A features an aglycone with two prominent saccharide substituents: a neutral saccharide and an aminosugar[23]. Originally known for its effectiveness in agricultural pest control, spinosyn A was approved by the FDA in 2011 for medical use in the topical treatment of head lice in patients aged 4 years or older[23,24]. The main mechanism of spinosyn A was proposed to target the

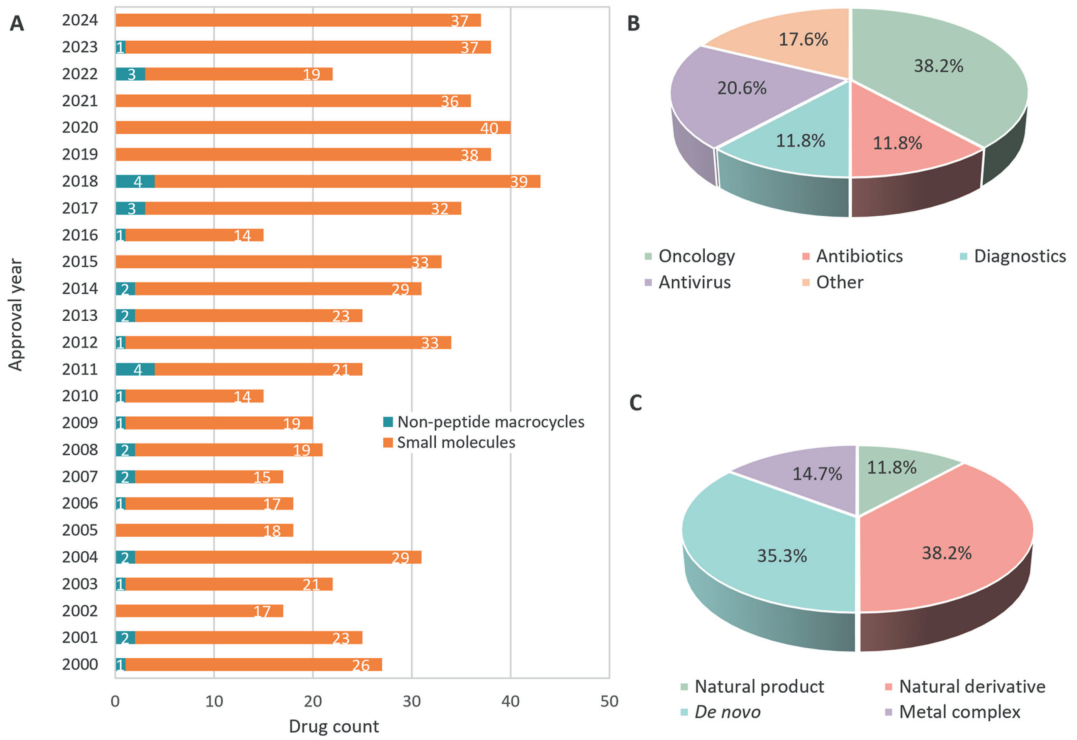


Figure 1 An overview of approved from 2000 to 2024. (A) Annual numbers of approved non-peptide macrocycles and small molecules; Macrocyclic drugs classified by therapeutic indications (B) and origins (C).

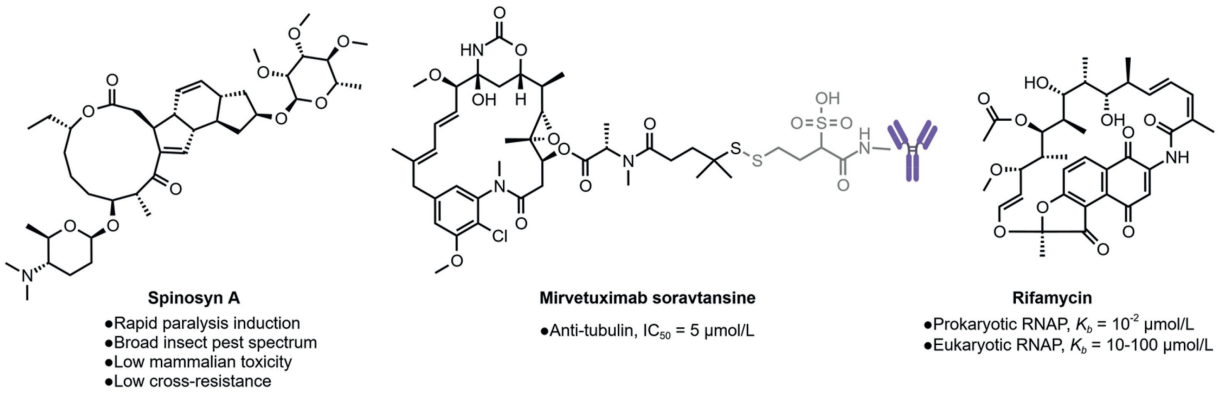


Figure 2 Examples of approved natural non-peptide macrocyclic drugs.

insect nervous systems through its indirect interaction with nicotinic acetylcholine receptor (nAChR) and gamma-aminobutyric acid (GABA), although the precise modes of action remain not fully understood[25-28]. Due to its low toxicity, high efficacy, and low residual properties, spinosyn A is considered an environmentally friendly biopesticide. The most common adverse effects of spinosyn A include application site reactions such as erythema, irritation, and ocular hyperemia. Serious side effects are rare[29]. Beyond its insecticidal activity, spinosyn A has also demonstrated significant anti-tumor activity. Studies conducted by Zou et al. showed that spinosyn A can selectively activate expression of argininosuccinate synthase (ASS1), a key enzyme involved in arginine synthesis in the urea cycle that is commonly downregulated in various cancers including breast, melanoma, liver, and prostate cancers, while the low expression of ASS1 is closely related to the occurrence and poor prognosis of multiple tumors[30-33].

Mirvetuximab Soravtansine is an antibody−drug conjugate (ADC) specially targeting folate receptor-alpha (FRα)-positive ovarian cancer (Fig. 2). This drug received accelerated approval from the FDA in 2022[34]. The cytotoxic component of Mirvetuximab Soravtansine is DM4, also known as ravtansine which belongs to the maytansinoid class. DM4 is a complex polycyclic macrocycle, featuring a fused ring system and a C3 ester side chain. Key functional groups include a dimethyl hindered thiol substituent at the C3 position, which improves reactivity and binding affinity[35]. DM4 is a potent antitubulin regulator to inhibit division and growth of tumor cells[36-38]. FRα is highly expressed in up to 90% of ovarian patients and maintains a relatively stable expression level even after chemotherapy[39]. This stability makes FRα a reliable biomarker for both diagnosis and disease progression, thereby establishing it as an effective therapeutic target for platinum-resistant ovarian cancer. Mirvetuximab Soravtansine

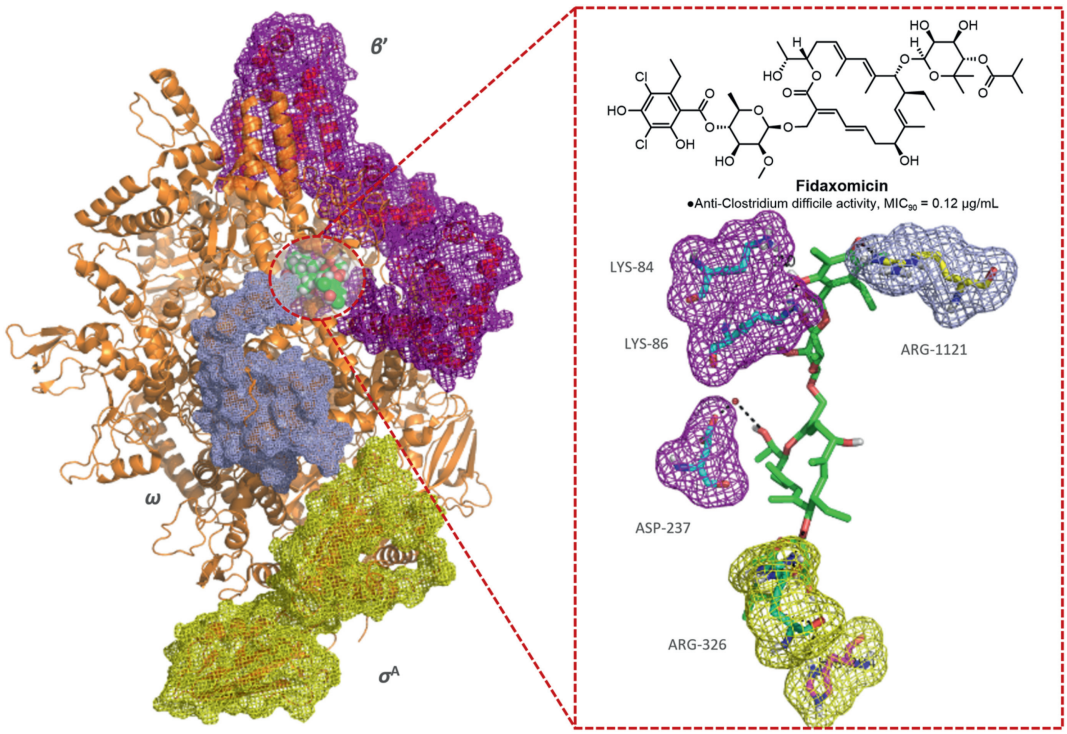


Figure 3 Fidaxomicin and its complex structure with *Cdiff* $E\sigma^A$ (PDB: 7L7B). 3D visualizations were performed using PyMOL (Pymol.org).

has demonstrated significant anti-tumor activity in ovarian cancer, particularly in cases resistant to platinum-based chemotherapy[40]. Although this medication presents a targeted therapeutic option for ovarian cancer but comes with a range of potential adverse effects, particularly ocular toxicities. In clinical trials, 61% of patients reported ocular adverse effects, and other safety concerns include peripheral neuropathy (36%) and pneumonitis (10%). Additionally, as a CY93A4 substrate, mirvetuximab soravtansine is at risk for drug interactions with strong CY93A4 inhibitors, necessitating close monitoring. These safety issues underscore the importance of ongoing collection of long-term safety data and continued research efforts in this area[41].

Rifamycin is a polyketide belonging to the ansmycin family of antibiotics, extracted from the fermentation products by *Amycolatopsis rifamycinica* (Fig. 2)[42]. It features a large 24-membered macrocycle that includes a naphthoquinone framework and an ansa bridge. Rifamycin exhibits broad-spectrum antimicrobial activities against both Gram-positive bacteria and a subset of Gram-negative bacteria[43]. Rifamycin was approved by the FDA in 2018 for the treatment of adult patients with travelers' diarrhea, specifically those caused by non-invasive strains of *Escherichia coli*[44]. Its mechanism of action involves direct steric blocking of transcription elongation by selectively binding to β-subunit of bacterial RNA polymerase, effectively inhibiting bacterial DNA-dependent RNA polymerase and preventing the synthesis of messenger RNA. This specificity allows rifamycin to have potent inhibitory activity against prokaryotic RNA polymerase ($K_b = 10^{-2}$ μmol/L), with minimal effects on eukaryotic RNA polymerases ($K_b = 10-100$ μmol/L). Furthermore, rifamycin does not exhibit cross-resistance with other clinically used antibiotics[45-47]. Despite its efficacy against bacteria resistant to other antibiotics, rifamycin itself is subject to a considerable resistance, and thereby it is typically administered in combination with other antimicrobial agents[48,49].

Fidaxomicin, an 18-membered macrolide antibiotic, was approved by the FDA in 2011 for the treatment of *Clostridioides difficile* (Cdiff) infections (Fig. 3)[50]. This compound is a fermentation byproduct of *Actinoplanes deccanensis* and *Dactylosporangium aurantiacum*. Fidaxomicin selectively targets pathogenic Cdiff populations ($MIC_{90} = 0.12$ μg/mL) while exerting minimal impact on a diverse array of healthy intestinal flora[51,52]. The study conducted by Cao et al.[53] revealed the molecular basis of fidaxomicin's selective action on Cdiff. Their results showed that fidaxomicin formed unique interaction with the RNA polymerase of Cdiff, particularly involving the formation of a crucial salt bridge between the β'K84 residue and phenolic ring of fidaxomicin. The positive state at β'K84 is essential for fidaxomicin sensitivity in gram-positive bacteria such as Cdiff, while neutral or negative charged residues at this position in gram-negative bacteria are associated with resistance to the antibiotic. Additionally, fidaxomicin interacts with other key residues in Cdiff RNA polymerase, such as Lys86, Asp237, Arg326, and Arg1121. These residues contribute to a network of interactions to stabilize the binding of fidaxomicin, thereby enhancing its inhibitory effect on transcription initiation. These findings not only disclose the mechanistic basis of fidaxomicin's action but also offer a new framework for the development of novel antimicrobial agents. In clinical trials, adverse effects were reported at similar rates for fidaxomicin and vancomycin, with 68.3% and 65.5% of subjects experiencing at least one adverse effect, respectively. Regarding hematologic safety, anemia was reported in 2% of subjects, while leukopenia occurred in 2.5% of fidaxomicin, with no apparent bone marrow toxicity observed in the nonclinical studies. The overall findings suggest that fidaxomicin presents a

favorable tolerability profile among patients, particularly due to its minimal absorption from the gastrointestinal tract[54].

4. Natural product-derived macrocyclic drugs

In addition to naturally occurring macrocyclic drugs, modifications of macrocyclic natural products include adding/changing functional groups or simplifying complex structures. Semi-synthetic methods enable the enhancement of drug properties while preserving the essential structural features that are responsible for biological activities.

4.1. From complexity to simplicity: the discovery of eribulin from halichondrin B

Structural simplification is crucial in the discovery and synthesis of complex natural products. This approach simplifies intricate natural compounds into their basic components, which helps identify core structures related to biological activity and aids in lead optimization, therefore accelerating the development of drug candidates from complex natural compounds[55]. Eribulin is a chemotherapeutic drug that was approved by the FDA in 2010 for the treatment of metastatic breast cancer, and it was the first monotherapy drug approved for this indication. Eribulin is derived from a marine natural product Halichondrin B, a complex polyether macrolide compound isolated from a rare Japanese sponge *Halichondria lkadai* (Fig. 4)[56]. While Halichondrin B shows promising results in preclinical studies, the compound itself is not used as a treatment due to challenges in isolating and large-scale synthesis for clinical use. In contrast, eribulin, a synthetic derivative based on the total synthesis of Halichondrin B, was found to show improved antitumor activity and good metabolic stability with a long half-life of 40 h. Eribulin was then developed as an approved chemotherapy drug for cancers. Eribulin works by sequestering tubulin into non-productive aggregates to prevent tubulin assembly (IC_{50} = 6 μmol/L) within tumor cells[57]. Unlike other microtubule inhibitors such as colchicine and paclitaxel, eribulin shows a unique binding preference, primarily associating with a limited number of high-affinity sites at the plus ends of preexisting microtubules, thereby setting it apart from other inhibitors in this class. This mechanism of action may reduce the likelihood of resistance development, offering a more reliable treatment option in oncology[58,59]. Its safety profile is considered manageable, with common treatment-related adverse events including neutropenia (58.6%), alopecia (50.4%), nausea (35.7%), peripheral neuropathy (30.6%), and fatigue (30.4%) as reported in pooled analyses and real-world studies. In clinical settings, neutropenia and peripheral neuropathy consistently emerge as common adverse events, highlighting the need for dose adjustments in patients with hepatic and renal impairments[60].

Eribulin is considered as one of the most challenging natural products to synthesis and manufacture. Its discovery was driven by a systematic strategy aimed at simplifying the complex structure of Halichondrin B, which features 32 chiral carbon atoms and a molecular weight exceeding 1100. In 1992, a landmark total synthesis of halichondrin B was achieved by Kishi and colleagues, requiring over 100 synthetic transformations[61]. However, this extensive synthetic work laid the foundation for the subsequent development of eribulin. Deconstruction of the halichondrin B into simpler fragments resulted in the creation of several key

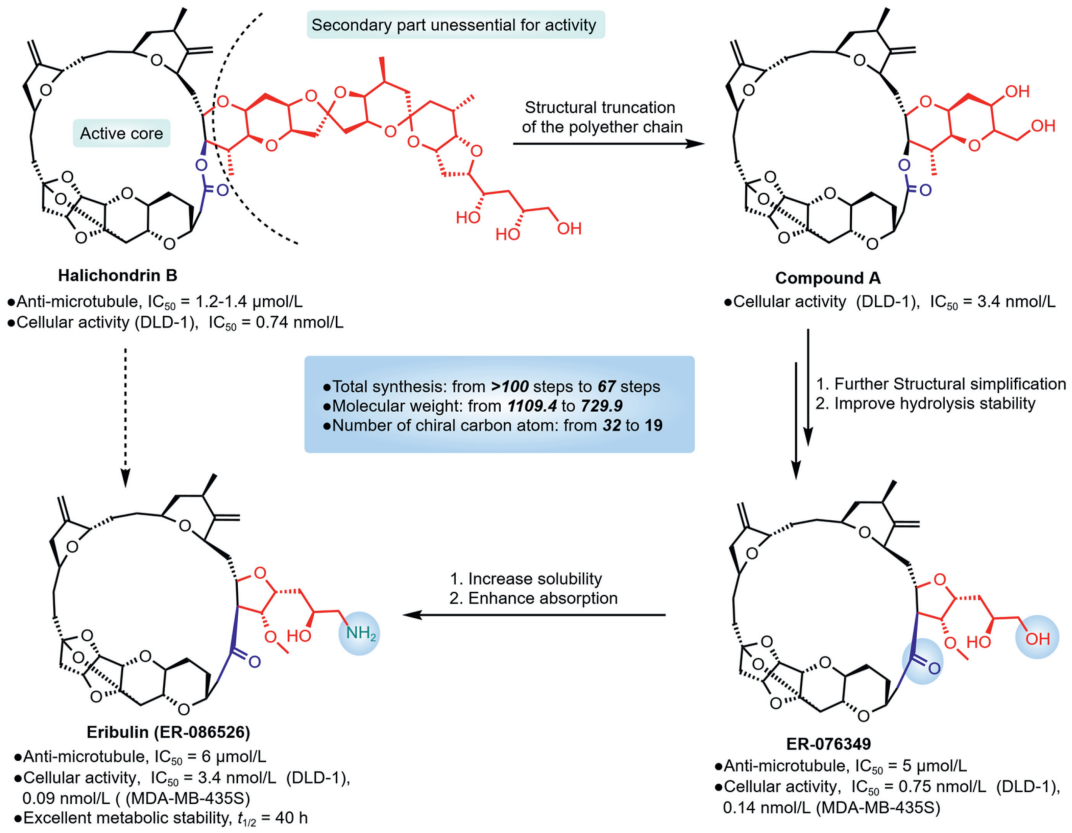


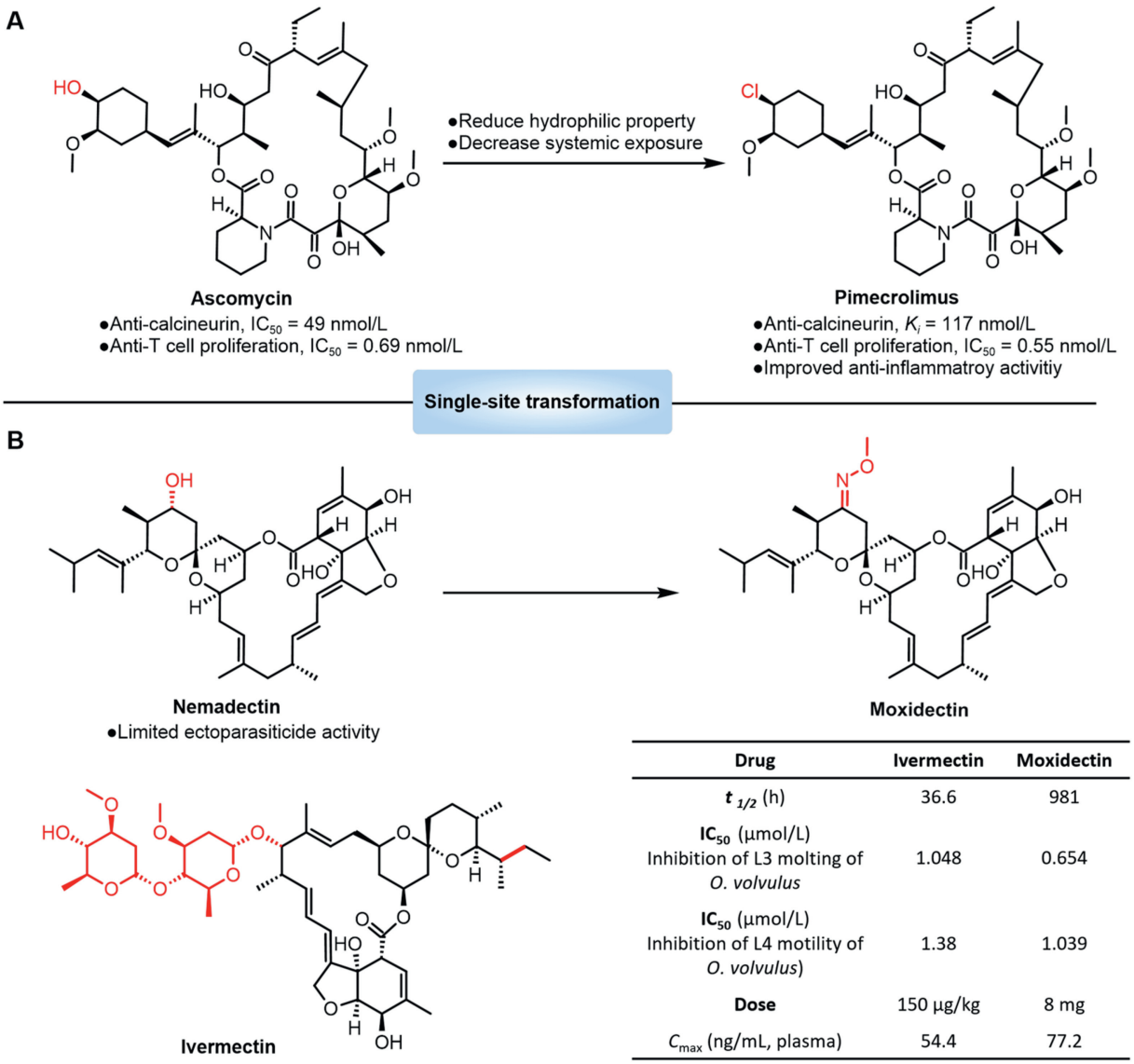
Figure 4 Chemical structure and discovery of eribulin *via* simplification of halichondrin B.

intermediates such as compound **A**, which retained significant nanomolar cellular activity while simplifying the original structure. Subsequent optimization efforts focused on enhancing the hydrolytic stability and solubility of the derivatives, leading to the development of compound ER-076349 and ER-086526 with methylene group in replacement of the lactone and further simplified side chain[62]. Both compounds exhibited strong broad-spectrum cellular activity and anti-microtubule ability. *In vivo* experiments revealed that ER-086526 effectively inhibited tumor growth at doses ranging from 0.05 to 1 mg/kg, with a notably lower recurrence rate post-treatment compared to ER-076349. In addition, ER-086526 has a favorable pharmacokinetic profile as it can form salts with acids to enhance its solubility and absorption, thus contributing to its overall improved therapeutic window. These findings underscore the potential of ER-086526 as a more effective candidate for cancer treatment. Notably, the final synthesis of eribulin represented a complex 62-step process. This marks an approximate 35% reduction in synthesis steps, molecular volume, and the number of chiral centers compared to Halichondrin B. The successful development of eribulin exemplify the effective integration of synthetic and medicinal chemistry[63,64].

4.2. *Single-site modification of macrolides*

Single-site transformation referring to the variation of one functional group, plays a crucial role in the discovery of natural drugs, as it enables targeted modification at specific position of a molecule. This approach can significantly enhance the molecule's biological activity and selectivity while minimizing side effects, therefore facilitating the development and clinical transformation of natural products.

Pimecrolimus is a topical immunomodulatory agent developed by Novartis for the treatment of mild to moderate atopic dermatitis (eczema) in individuals without immune deficiency[65,66]. It is a 23-membered macrolactam derived from ascomycin by chlorinating the hydroxyl group of ascomycin, which helps to reduce the compound's hydrophilic property (Fig. 5A). Ascomycin, isolated



Drug	Ivermectin	Moxidectin
$t_{1/2}$ (h)	36.6	981
IC_{50} (μmol/L) Inhibition of L3 molting of *O. volvulus*	1.048	0.654
IC_{50} (μmol/L) Inhibition of L4 motility of *O. volvulus*)	1.38	1.039
Dose	150 μg/kg	8 mg
C_{max} (ng/mL, plasma)	54.4	77.2

Figure 5 Chemical structures and discovery of pimecrolimus (A) and moxidectin (B).

from *Streptomyces hygroscopicus* var. *ascomycetus* as a fermentation product, was initially investigated for its antifungal properties before later exploration into its immunomodulatory properties[67,68]. With its higher lipophilicity, Pimecrolimus exhibits a strong affinity for skin, effectively and safely managing the symptom of facial seborrheic dermatitis. It can provide a rapid relief from itching for patients with dermatitis and eczema and reduce the area of skin lesions. Pimecrolimus is a highly potent non-steroidal calcineurin inhibitor with a K_i value of 117 nmol/L. Mechanistically, Pimecrolimus can specifically bind to the cytoplasmic receptor macrophilin-12 to inhibit calcineurin, a calcium-dependent serine−threonine phosphatase required for the activation of T cells. By preventing the activation of nuclear factor of activated T-cell, it inhibits the transcription of pro-inflammatory cytokines, thereby preventing the release of inflammatory factors and exhibiting its anti-inflammatory activity[69,70].

Moxidectin is a broad-spectrum anthelmintic drug that belongs to the macrolide milbemycin family (Fig. 5B). It is well-known for its efficacy in treating and controlling parasitic infections across a variety of species, including livestock, pets, and humans[71]. Moxidectin is derived from nemadectin, a naturally occurring fermentation product of *Streptomyces cyaneogriseus* subsp. *Noncyanogenus*. Nemadectin exhibits strong anthelmintic properties but displays limited effectiveness as an ectoparasiticide. Modifying nemadectin at C23 oximation gave Moxidectin, which showed enhanced pharmacokinetic properties, duration of action, and improved potency. The mechanism of action of moxidectin primarily involves binding to glutamate-gated chloride channels and γ-aminobutyric acid receptor/adenosine triphosphate (ATP) binding cassette transporters in the nervous system of parasites, which increases membrane permeability to chloride ions. This action leads to hyperpolarization, paralysis, and eventually, the death of the parasite[72]. Moxidectin was originally utilized as veterinary medicine, and was later approved by the FDA in 2018 for the treatment of onchocerciasis (River blindness) in adults and adolescents aged in 12 and older[73]. It is effective against *Onchocerca volvulus* microfilariae. Although it does not directly kill adult worms, it blocks embryonic development and microfilariae release. Moxidectin exhibits different activity against various stages of *Onchocerca volvulus*. It effectively inhibits the molting of L3 larvae with an IC_{50} value of 0.654 μmol/L, while it has a relatively weak inhibitory activity against the molting of L4 larvae (IC_{50} = 1.039 μmol/L). In comparison to the FDA-approved ivermectin (Fig. 5B), moxidectin has a significantly longer half-life of approximately 40 days and requiring only a single 8 mg dose regardless of body weight. Clinical trials have demonstrated that moxidectin achieves more complete and prolonged suppression of microfilaridermia compared to ivermectin, with significantly higher rates of undetectable skin microfilariae at follow-up intervals. While both drugs can cause Mazzotti reactions due to rapid microfilarial death, moxidectin is associated with a lower incidence of severe adverse events and exhibits less inter-individual variability in treatment response. Overall, moxidectin's superior efficacy and longer duration of action make it a promising alternative to ivermectin in treating this condition[74,75].

4.3. Single-atom transformation of macrolides

In addition to the single-site transformation, the single-atom change also plays an important role in the discovery of macrocyclic drugs. For instance, conversion of a macrolide into a lactam by replacing the oxygen atom with nitrogen atom, usually does not alter the binding conformation of the compound, while improving the stability and pharmacological properties. For example, ixabepilone, a semi-synthetic analog of epothilone B, was first identified for its antifungal and cytotoxic properties[76,77]. Despite its promising anti-cancer activity, epothilone B faced challenges due to poor metabolic stability and pharmacokinetic properties, having a half-life of less than 5 h. Converting the lactone ring of epothilone B into the lactam ring yielded ixabepilone, which showed significant advantages over epothilone B, particularly in metabolic stability, efficacy, and susceptibility to multidrug resistance (MDR) (Fig. 6A). Ixabepilone exhibits a dramatically increased half-life of 52 h, as well as a remarkable metabolic stability with a hydrolysis rate of only 0.01 nmol/min/mg, compared to epothilone B's rate of 1.02 nmol/min/mg. In terms of efficacy, ixabepilone shows a log cell kill (LCK) of 2.1 in preclinical models, significantly higher than epothilone B's LCK of 0.4, indicating a superior antitumor activity. Additionally, ixabepilone has a lower MDR susceptibility ratio of 7.77, compared to paclitaxel's ration exceeding 100. This profile enables ixabepilone to effectively target tumors that are resistant to standard therapies, including those expressing P-glycoprotein and βIII-tubulin, therefore offering a promising treatment option for patients with limited alternatives. In addition, Ixabepilone surpasses taxane in several areas including a wider range of anticancer effects, increased antitumor activity, favorable safety profile, and enhanced water solubility[78,79]. Furthermore, a crystallographic analysis reveals that ixabepilone and epothilone B share a significant overlap in their binding conformations (Fig. 6B and C). Both compounds bind to tubulin at the same site and establish favorable hydrogen bonds with nearby residues including Gln279, Thr274 and Asp224. These findings suggest that the transformation from lactone to lactam did not change the compound's binding conformation with tubulin but improved its antitumor efficacy and pharmacokinetic characteristics[80]. Ixabepilone was approved by the FDA in 2007 for use alone or in combination with capecitabine for the treatment of primary and metastatic breast cancer. It has demonstrated effectiveness in patients facing the common challenge of multidrug resistance in microtubule-targeting agents[81]. The most common adverse effects include sensory neuropathy and hematological abnormalities, particularly neutropenia. Peripheral neuropathy occurs frequently, with an incidence ranging from 31% to 67% for any grade and up to 24% for grades 3 and 4. Moreover, the tolerability of ixabepilone can vary, with high rates of toxicity when used in combination with drugs such as trastuzumab and cetuximab. Thus, it is significantly important to consider its potential adverse effects and drug interactions, particularly when co-administered with other therapies[82].

4.4. Macrocyclic ketolide, a productive scaffold in drug development

In addition to the aforementioned macrolide and macrolactam natural drugs, the macrocyclic ketolide structures characterized by functional groups like ketones and ethers, represent a significant variation within the natural antibiotic class. Ketolides play a crucial role in enhancing the antibacterial spectrum and resistance of these drugs by targeting bacterial ribosomes. Telithromycin is a macrocyclic ketolide antibiotic derived from erythromycin, and it was approved for the treatment of mild to moderate community-acquired pneumonia[83,84]. Telithromycin is a semi-synthetic derivative of erythromycin (Fig. 7)[85]. The first modification site is

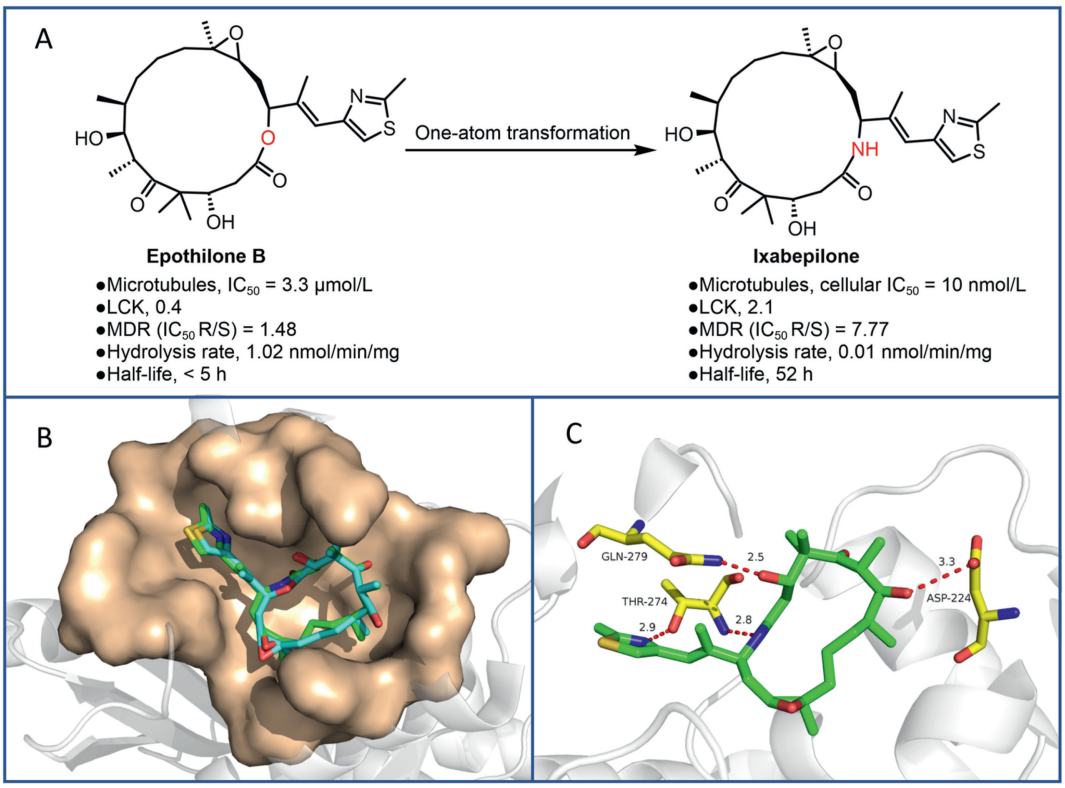


Figure 6 (A) Design strategy for ixabepilone based on epothilone B; (B) Comparison of the active site overlap between ixabepilone (PDB: 7DAF, shown in green) and epothilone B (PDB: 7DAE, shown in cyan) on Tubulin. (C) Analysis of the binding interaction between ixabepilone and Tubulin. Hydrogen bonds are highlighted in red dash lines. MDR (IC_{50} R/S), the ratio of IC_{50} values in MDR resistant *versus* sensitive cell lines. 3D visualizations were performed using PyMOL (Pymol.org).

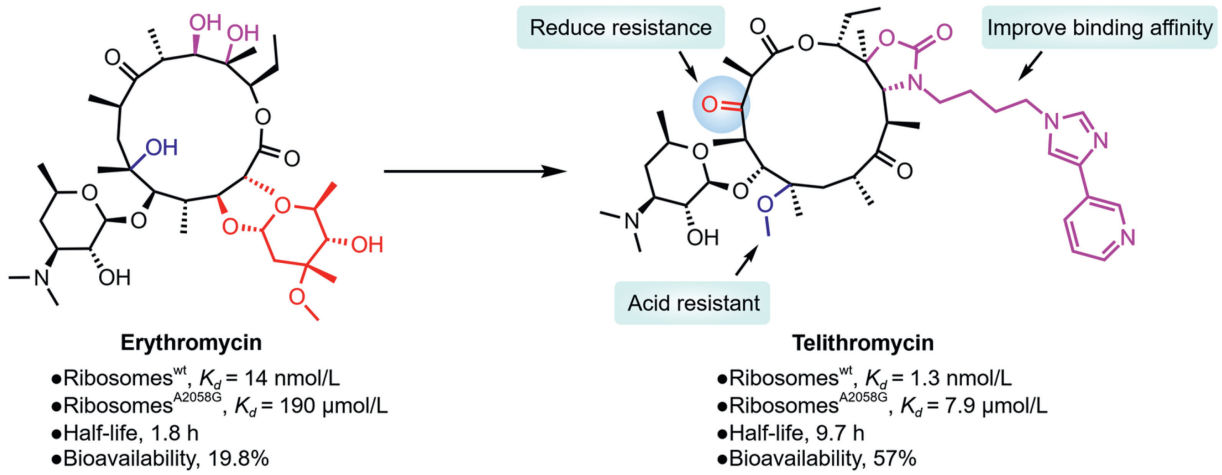


Figure 7 Chemical structure and discovery of telithromycin derived from erythromycin.

the 3-cladinose sugar moiety, which is replaced with a keto group. This change may enhance activity against erythromycin-resistant strains[86]. The second modification involves adding a cyclic carbamate group to the C11−C12 position, enhancing telithromycin's affinity for binding to the ribosome. The third modification takes place at the C6 position of telithromycin, where the hydroxyl group is changed to a methoxyl group to improve telithromycin's acid stability and prevent internal hemiketalization[63,87]. Telithromycin exhibits a binding affinity up to 10 times higher (K_d = 1.3 nmol/L) than erythromycin (K_d = 14 nmol/L), which is attributed to improved interactions between its alkyl-heteroaryl chain and the hairpin loop in domain II of rRNA. Even with the A2058G mutation, telithromycin maintains a strong binding affinity of 7.9 μmol/L, significantly higher than erythromycin's 190 μmol/L. Telithromycin has a dual targeting mechanism by interacting with two domains of the 23S rRNA (domains II and V), enhancing its efficacy and reducing the likelihood of resistance development[88]. Moreover, telithromycin

has a longer half-life of 9.7 h and higher bioavailability at 57%, in contrast to erythromycin which has a shorter half-life of 1.8 h and lower bioavailability at 19.8%. However, the clinical use of telithromycin was limited because it caused severe side effects like liver failure, visual disturbances, and worsening of myasthenia gravis. These effects are likely due to its pyridine-containing side chain acting as an antagonist on different nicotinic acetylcholine receptors (nAChRs)[89,90].

Moreover, macrocyclic ketolides are also of interest in drug research of cancer and immune related diseases. Rapamycin, also known as sirolimus, was originally isolated from *Streptomyces hygroscopicus* (Fig. 8)[91,92]. It exhibits a range of pharmacological activities such as antifungal, immunosuppressive, anticancer properties, primarily through its potent inhibition of the mammalian target of rapamycin (mTOR) with an IC_{50} value of 0.1 nmol/L[93,94]. The mechanism of action involves the formation of a complex between rapamycin and FK506-binding protein 12 (FKBP12), which then binds to the FKBP12-rapamycin binding (FRB) domain on mTOR. The resultant rapamycin−FKBP12−mTOR complex disrupts mTOR signaling pathways, thereby leading to its pharmacological effects on cellular signaling pathways[95,96]. The crystal structure of rapamycin and mTOR reveals that rapamycin binds specifically to FKBP12 by establishing multiple hydrogen bonds between its polyketide segment (highlighted in bule in rapamycin) and residuals including Try82, Asp37, Ile56, and Glu54, while its polyene segment effectively interacts hydrophobically within specific regions of mTOR. The cyclohexane ring is located on the outer surface of the pocket, providing opportunities for further structural modifications[97,98].

Zotarolimus (ABT-578, Fig. 8), a rapamycin derivative, features an C42 tetrazole substitution[99]. As an immunosuppressant, zotarolimus indirectly binds to mTOR by forming a complex with the immunophilin FKBP12[100], leading to an effective blockade of mTOR activity (IC_{50} = 2.8 nmol/L) and inhibition of the phosphorylation of certain key proteins involved in cell proliferation[101]. In 2008, the FDA approved zotarolimus for use in drug-eluting stents (DES) to prevent coronary artery restenosis, owing to its several advantageous properties: a shorter *in vivo* half-life of 35 h compared with 46−78 h of rapamycin, which reduces local adverse effects; high lipophilicity that facilitates its penetration across lipid-rich arterial barrier, as well as sustained and controlled drug release from stents[99,102]. These attributes make zotarolimus particularly effective in reducing neointimal hyperplasia and maintaining vessel patency post-angioplasty, thereby positioning it as an important therapeutic option in the management of coronary artery disease. Additionally, everolimus and temsirolimus are additional two rapamycin derivatives and exert pharmacological effects by interfering with the PI3K-Akt-mTOR signaling pathway[103]. Compared to rapamycin, everolimus has a hydroxyethyl group at the C42 position and exhibits high selectivity for mTORC1. It has an IC_{50} of 1.6 nmol/L against FKBP12, a half-life of approximately 30 h, and a relatively low bioavailability of 20%. Everolimus was approved by the FDA in 2009 for the treatment of various malignancies, including advanced renal cancer, breast cancer, and progressive neuroendocrine tumors[104]. In contrast, temsirolimus bearing the dimethylolpropionic ester has improved water solubility and bioavailability[105]. While its IC_{50} for mTOR is significantly lower at 1.76 μmol/L than rapamycin, it has a favorable half-life of 9−27 h and shows excellent bioavailability when given intravenously. Temsirolimus was approved in 2007 for advanced renal cell carcinoma[106]. In addition, beyond the established roles in cancer treatment, these macrocyclic mTOR inhibitors also exert potential in neurodegenerative diseases such as Alzheimer's disease and Parkinson' diseases[107]. These studies highlight the versatile therapeutic potentials of rapamycin and its derivatives, expanding their clinical applications in diverse disease therapy[108].

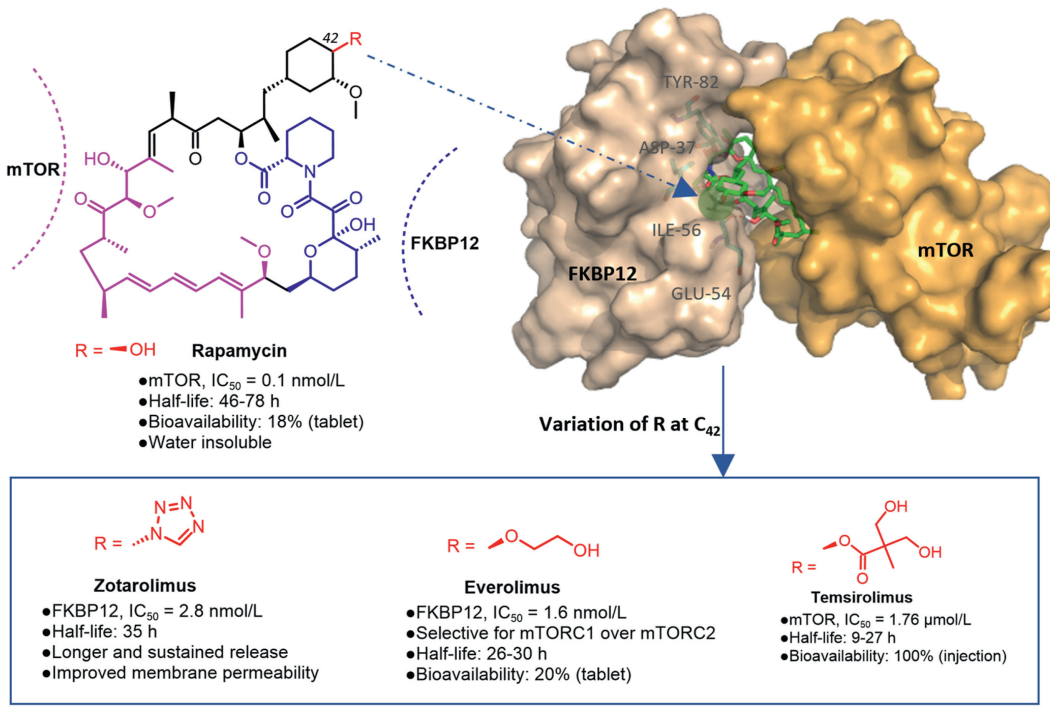


Figure 8 Structures of rapamycin and its analogs including zotarolimus, everolimus, and temsirolimus. The interaction of rapamycin with FKBP12 and mTOR (PDB: 1FAP) is indicated. 3D visualizations were performed using PyMOL (Pymol.org).

5. *De novo* designed macrocycles

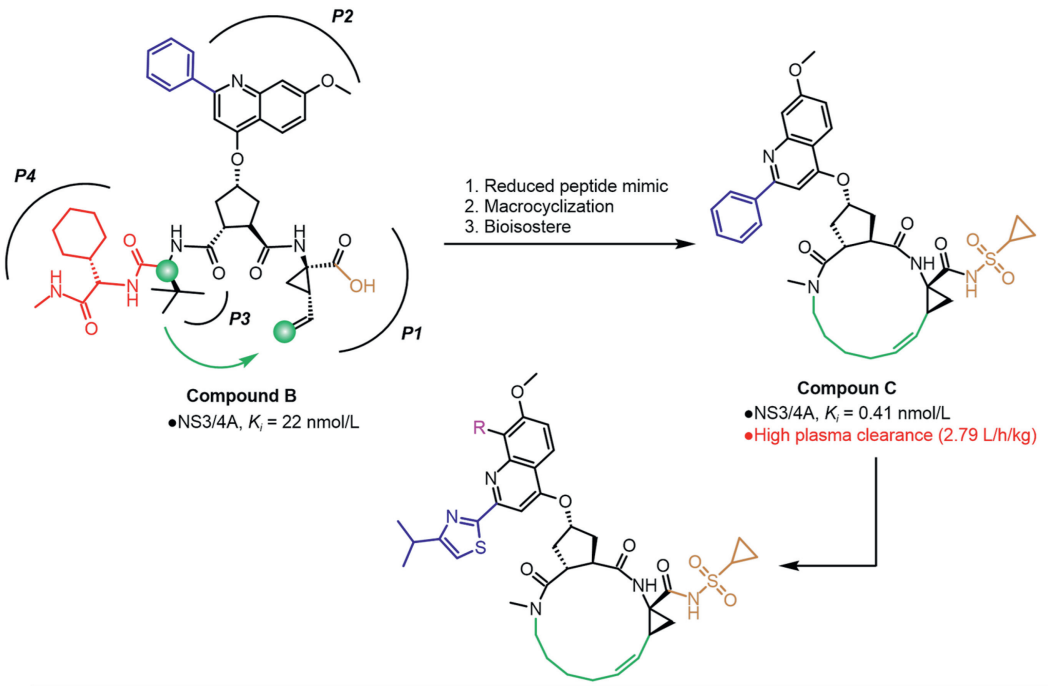
Recent advancements in synthetic chemistry have facilitated the creation of intricate macrocyclic structures with precision and efficiency. Designing and synthesizing macrocycles with tailored structural and functional features has greatly enhanced drug discovery, allowing for the exploration of novel chemical space and the development of targeted therapies.

5.1. *Anti-HCV drugs*

Chronic hepatitis C virus (HCV) is a major global health concern, often resulting in serious complications such as liver cirrhosis, hepatocellular carcinoma, and liver failure[109-111]. The virus is highly variable and classified into multiple genotypes that complicate treatment. Traditional therapies involving pegylated interferon (pegIFN) and ribavirin were plagued by low cure rates and significant side effects, thereby requiring more effective and better tolerated antiviral treatments[112,113]. HCV NS3/4A protease inhibitors target the viral protease essential for HCV replication, significantly reducing viral load and improving treatment outcomes, particularly for genotype 1 infections. The first-generation NS3/4A protease inhibitors such as boceprevir and telaprevir represent a significant advancement in HCV therapy with sustained virologic response rates (SVR) compared to previous regiments. However, their use was accompanied by notable drawbacks, including side effects such as anemia and skin disorders, as well as complex dosing regiments[114]. These limitations underscored the need for newer therapies to enhance cure rates and tolerability, leading to the development of next generation inhibitors and combination therapies for HCV treatment[115].

5.1.1. *Macrocyclization and simplification strategy*

Simeprevir is a potent antiviral medication classified as a HCV protease inhibitor, and it gained the FDA approval in 2013[116]. Simeprevir specially targets the most common genotype of HCV, genotype 1, although its efficacy extends off-label to genotype 4 as well[117]. By inhibiting NS3/4A, simeprevir effectively disrupts the life cycle of viral to reduce viral loads, thereby leading to viral eradication when used as part of a combination therapy[118]. While the first-generation approved inhibitors were linear peptidomimetics that reversibly bind to the NS3/4A protease, simeprevir employed a macrocyclic design for favorable binding profile. Starting from a potent acyclic compound **B** with a K_i value of 22 nmol/L against NS3/4A, a multifaceted design strategy including macrocyclization, reduction of P4 peptidic nature, and P1 bioisosteric replacements, led to a significantly potent cyclic compound **C** with K_i value of 0.41 nmol/L (Fig. 9)[111]. However,



Compound	R	NS3/4A K_i (nmol/L)	HUH7-Rep EC_{50} (nmol/L)	HLM Clint (μL/min/mg)	Bioavailability F (%)
C	-	0.41	9	**46**	**2.5**
D	H	0.84	17	16	-
E	F	0.55	57	-	-
F	Cl	0.10	2.9	9.0	-
Simeprevir	CH_3	0.36	7.8	**<6**	**11-44**

Figure 9 Chemical structure and discovery of simeprevir. The section marked with the green sphere represents the two points where they connect to form a ring.

this compound exhibited a poor rat pharmacokinetic (PK) profile with low bioavailability of only 2.5% and high plasma clearance of 2.79 L/h/kg. Subsequent optimization focusing on the phenyl position in the P2 part, resulted in the generation of isopropylthiazol substituted compound **D**, which maintained comparable enzymatic activity (K_i = 0.84 nmol/L) to compound **C**, while also showing improved stability in human liver microsomes (HLM) with an intrinsic clearance (Cl_{int}) of 16 μL/min/mg. To further enhance both activity and PK profiles, modifications were made to at position 8 (R group) of the quinoline ring. By introducing different alkyl and halogen substituents, simeprevir with a methyl substituent stood out by exhibiting comprehensive advantages in terms of activity and PK properties. Simeprevir exhibits a potent inhibitory effect, with a K_i value of 0.36 nmol/L. Its PK reveals approximately 11%−44% oral bioavailability (up to 100% in dogs), a rapid clearance rate of 0.51 L/h/kg, and a volume of distribution at steady state (Vd_{ss}) of 0.49 L/kg, which indicates effective targeting of the liver, as evidenced by a liver/plasma concentration ration of 32. In addition, simeprevir's safety profile is robust, showing over 1000-fold selectivity for the NS3/4A protease compared to human serine proteases, along with low cytotoxicity ($CC_{50} \geq 10$ μmol/L). Clinical outcomes have confirmed that simeprevir is well tolerated, with only mild increases in bilirubin levels attributed to its inhibition of hepatic transport proteins[119,120]. Furthermore, when used in combination with pegIFN and ribavirin, simeprevir significantly enhances SVR rates, positioning it as a promising therapeutic option for HCV. Of note, simeprevir exemplifies molecular chameleonicity through its conformational flexibility, primarily attributed to *N*-methylated amide chain. This flexibility enables simeprevir to adopt various conformations that influence its solvent-accessible three-dimensional polar surface area, thereby enhancing its PK profiles. The capacity of transition between polar and nonpolar conformations further aids in its absorption and effectiveness[121]. In addition, the macrocyclization strategy employed during the optimization of simeprevir reshaped its molecular structure and enhanced its inhibitory activity against HCV NS3/4A, highlighting the importance of cyclization strategy in lead discovery and drug development[122].

Given the emergence of resistance and adverse effects associated with first-generation NS3 protease inhibitors, there is an urgent need for research on novel anti-HCV drugs that exhibit inhibitory activity against broad range of HCV genotypes. Danoprevir is a highly potent and orally available HCV NS3/4A protease inhibitor, which was developed through structure-based drug design and optimization strategies[123,124]. This compound exhibits subnanomolar potency, effectively inhibiting multiple HCV genotypes and key mutant strains. The discovery of danoprevir began with the design of a virtual chemical library followed by docking studies to identify potential lead compound **G** that has an inhibitory activity against HCV protease with an IC_{50} value of 210 nmol/L (Fig. 10)[124]. However, it exhibited poor cellular potency in replicon-based assays, attributed to its high polar surface area (PSA = 187 Å^2) and multiple hydrogen-bond donors, which hindered its cell permeability. To address this issue, the researchers initially employed depeptidization strategy to remove

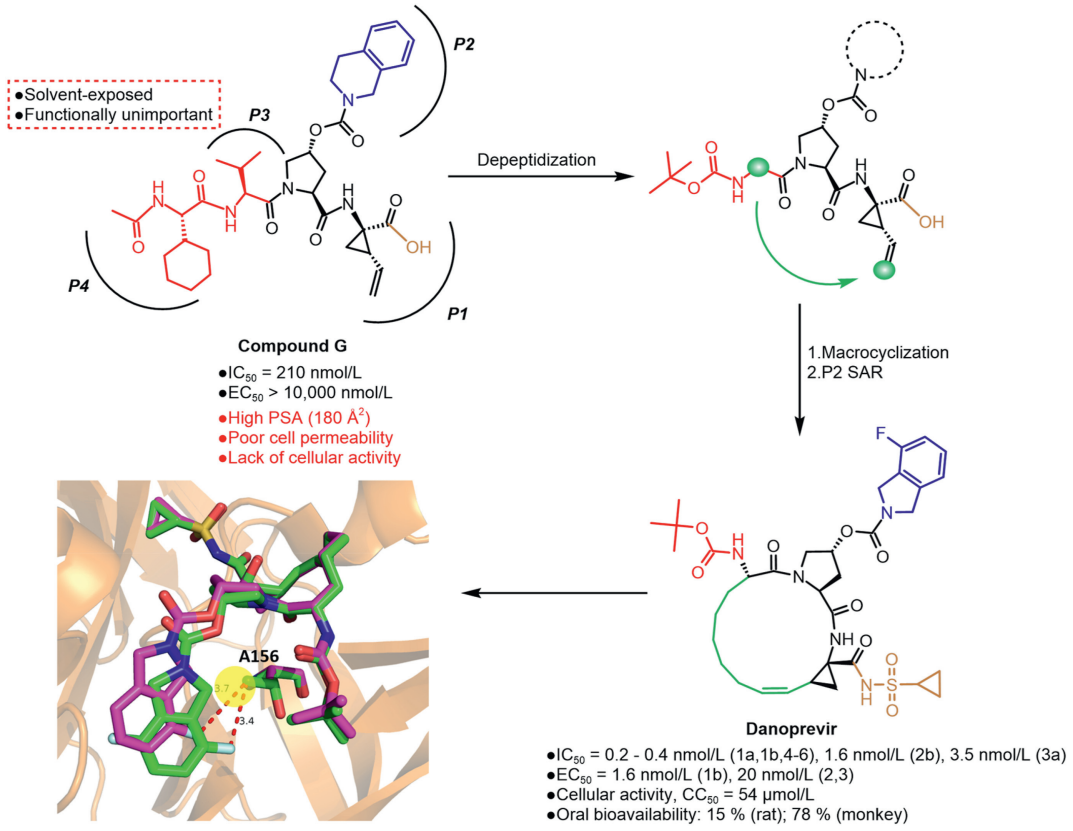


Figure 10 Design strategy of danoprevir and its interactions with wild-type (PDB: 3M5L) and A156T (PDB: 3SU2) NS3/4A proteases. Danoprevir is highlighted in green and purple sticks, respectively. The section marked with the green sphere represents the two points where they connect to form a ring. Hydrogen bonds are highlighted in red dash lines. 3D visualizations were performed using PyMOL (Pymol.org).

the P4 peptide-like elements and macrocyclization approach to reduce the solvent-exposed PSA. In addition, P1 modifications by replacing the carboxylic acid group with a bioisostere acylsulfonamide, further decreased the PSA and led to dramatic increase in both enzymatic and cellular potency. Further optimization at P2 tetrahydroisoquinoline through iterative rounds of preparation and testing, culminated in the development of danoprevir, which shows significantly enhanced potency against HCV genotypes, with IC_{50} values ranging from 0.2 to 3.5 nmol/L. Moreover, danoprevir also exhibits cellular activity with a CC_{50} value of 54 μmol/L as well as favorable oral bioavailability of 78% in monkeys. Additionally, danoprevir retains inhibitory potency against the A156T mutation that is closely associated with drug resistance. Released X-ray crystal structures revealed that danoprevir can adjust its isoindoline cap to avoid steric clashes with this residual mutation, allowing it to interact effectively with the mutated NS3 protease (Fig. 10). In 2018, Ascletis received NMPA approval to market the product[125]. Common adverse effects of danoprevir, particularly when combined with ritonavir, pegIFN, and ribavirin, include anemia, fatigue, fever, and headache. Furthermore, danoprevir's metabolism is significantly influenced by CY93A4, leading to potential drug interactions, especially with inhibitors and inducers of this enzyme. While initial findings suggest considerable efficacy, long-term safety data are still being collected as the HCV therapy landscape shifts towards more tolerable interferon-free and ribavirin-free regimens[126].

5.1.2. *Drug repurposing: from toxicity to bioactivity*

Paritaprevir (ABT-450) is another potent NS3/4A protease inhibitor, and has shown potent inhibitory activity against HCV genotypes 1a and 1b, exhibiting EC_{50} values of 1 and 0.21 nmol/L, respectively, while maintaining moderate efficacy against genotype 2a (EC_{50} = 5.3 nmol/L) and weak activity against genotype 3a (EC_{50} = 19 nmol/L) due to common resistance-associated variants such as D168Q[127]. Furthermore, it retains high potency against genotype 4a (EC_{50} = 0.09 nmol/L) and 6a (EC_{50} = 0.69 nmol/L), indicating a broad spectrum of antiviral efficacy. It is often used in combination with ombitasvir, dasabuvir, and ritonavir, marketed as Viekira Pak[128]. Ritonavir serves as a pharmacokinetic enhancer, boosting paritaprevir levels and preventing resistance-associated variants to improve the regimen's efficacy and reduce virologic breakthrough rates[129]. Paritaprevir and simeprevir are both direct-acting antiviral agents, but they are applied in different ways. While incorporating protease inhibitors like simeprevir into pegIFN and ribavirin therapy has enhanced SVR over pegIFN/ribavirin alone, IFN-based treatments are known for their considerable toxicities that often limit their use. In contrast, paritaprevir-based regimes have demonstrated high SVR and a more favorable side effect profile, including less headache and fatigue compared to older IFN-based treatments[129]. Clinical studies have shown that paritaprevir can reduce viral load by approximately 4 logs in just three days of monotherapy, and higher doses effectively the emergence of resistance-associated mutations. When used in combination with ritonavir and ribavirin for 12 weeks, showing a SVR rate of about 95% in patients with genotype 1[130]. Paritaprevir is derived from an anti-HCV candidate compound ABT-515 that was precluded from further development due to its toxicity (Fig. 11). The development of paritaprevir includes the reductive removal of oxime group followed by incorporating a phenanthridine moiety, and final transformation into a pyrazine amide from the Boc protecting group[131]. The recovery route of ABT-515 not only accelerated the discovery of paritaprevir but also informed the subsequent development of a more efficient synthesis route for commercial production. The optimization of the final synthesis of paritaprevir ultimately achieved an up to 72% overall yield over the final six steps.

5.1.3. *Structure based design strategy: construction of novel macrocycles*

The development of new anti-HCV agents has been a significant challenge due to the issue of drug resistance and the need for broad-spectrum anti-HCV medicines. Ciluprevir (BILN-2061) was an experimental drug developed by Boehringer Ingelheim, and was the first-in-class NS3/4A protease inhibitor to enter clinical development, exhibiting potent competitive inhibition of NS3/4A protease in HCV genotypes 1a and 1b, with K_i values of 0.3 and 0.66 nmol/L, respectively[132]. However, its development was halted during Phase Ib clinical trial due to toxic effects[133]. Despite this setback, the scaffold of ciluprevir was utilized to design new macrocyclic inhibitors. With ciluprevir as a starting point, researchers at Merck & Co. employed a molecular modeling approach to create a new class of macrocyclic inhibitors that incorporated a P2 to P4 macrocyclic constraint, leading to the formation of compound H (Fig. 12). Although this transformation resulted in an activity reduction (compound H's K_i value of 8.5 nmol/L), docking analysis indicated that the original P2

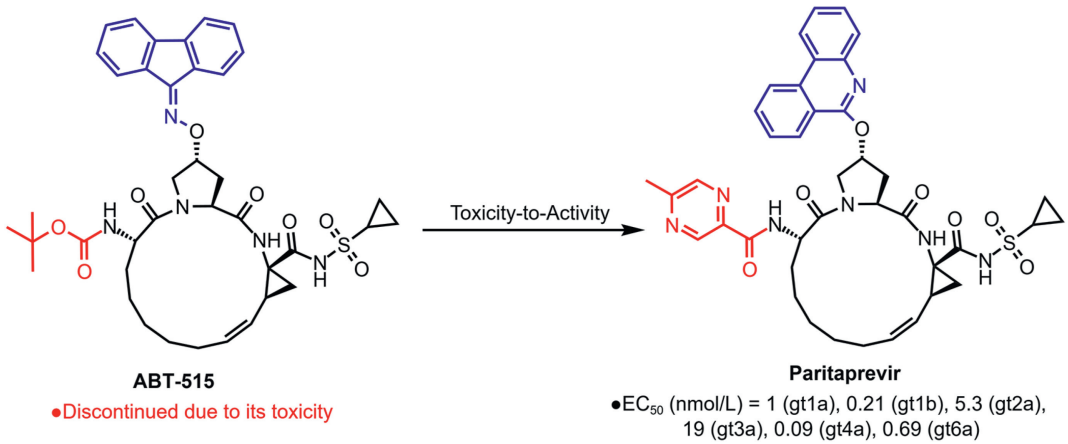


Figure 11 Chemical structure and discovery of paritaprevir.

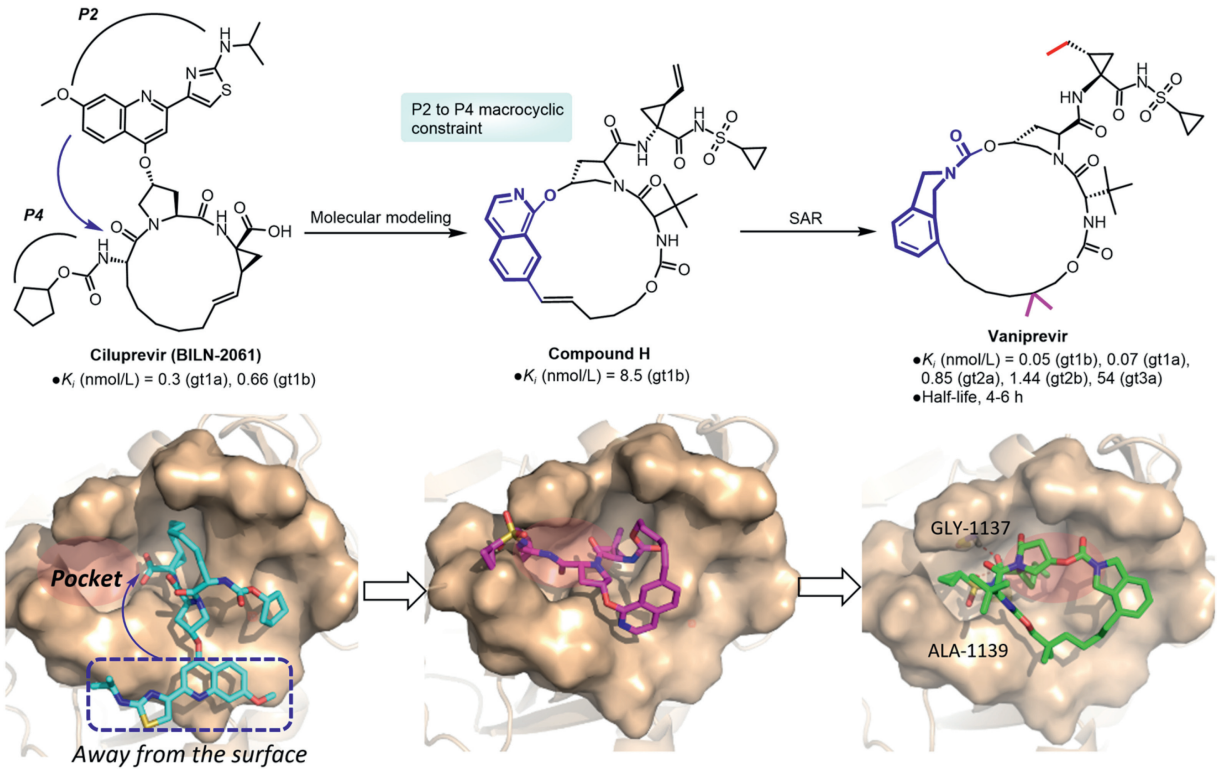


Figure 12 Chemical structure and design strategy of vaniprevir. 3D visualizations of NS3/4A protease (PDB: 5ESB) were performed using PyMOL (Pymol.org).

portion away from the binding surface was positioned closer to a proposed pocket. This strategic redesign laid the ground for subsequent systematic multi-site optimization and SAR studies, ultimately culminating in the development of Vaniprevir (MK-7009)[134,135]. It has shown promising activity against both genotype 1a and 1b NS3/4A protease enzymes, with potent inhibition at subnanomolar concentrations, showing K_i values of 0.07 and 0.05 nmol/L, respectively. It exhibits slightly lower inhibitory activity against genotype 2a and 2b enzymes, with K_i values of 0.85 and 1.44 nmol/L. In contrast, its activity against genotype 3a protease is considerably lower, with a K_i value of 54 nmol/L. Additionally, vaniprevir has shown good plasma exposure, excellent liver exposure, and favorable half-life of 4−6 h in multiple species[134,136]. The drug was approved in Japan in 2014 for the treatment of HCV with the brand name Vanihep.

In addition, the P2 to P4 macrocyclization has also been applied in the development of several other drugs. For example, grazoprevir (MK-5172) is a potent quinoxaline-based NS3/4A protease inhibitor with inhibitory activity across a broad-spectrum of HCV genotypes, particularly against the genotype 3a enzyme and genotype 1 mutants[137,138]. The development of grazoprevir involves the structural insight of P2 and P4 macrocyclization and systematic modifications at the P2 position of the quinoline scaffold (Fig. 13)[138,139]. Replacement of dimethyl groups of compound **I** with small alkyl rings like cyclopropyl is crucial for its broad activity profile against genotypes and key mutants as well as liver exposure of grazoprevir. It has demonstrated significant inhibitory potency with K_i values ranging from 0.01 to 0.9 nmol/L, showing a substantial improvement over compound **I** (K_i = 26 nmol/L). In addition, the introduction of the quinoxaline heterocycle at P2 effectively addresses issues related the disproportionation of pharmaceutical salts, thereby improving solubility and the overall pharmacokinetic profile of grazoprevir, with a half-life of 31 h. Grazoprevir was approved by the FDA in 2016 as part of the combination therapy Zepatier (elbasvir/grazoprevir) for the treatment of chronic HCV genotypes 1 and 4 in adults[140]. Glecaprevir and voxilaprevir, two another quinoxaline-based protease inhibitors, share structural similarities and exhibit broad-spectrum activity across HCV genotypes (Fig. 13)[141,142]. Specifically, voxilaprevir demonstrates a K_i value of 0.038 nmol/L against HCV genotype 1b and 0.066 nmol/L against genotype 3a, while glecaprevir has a K_i value of 0.94 nmol/L for genotype 1b and 1.9 nmol/L for genotype 3a. Furthermore, voxilaprevir has a half-life of 33 h, compared to glecaprevir's shorter half-life of 6−9 h. However, similar to grazoprevir, both glecaprevir and voxilaprevir face challenges in potency against certain HCV variants with resistance-associated substitutions at positions A156 and D168[143,144]. This highlights a common trend among these quinoxaline-based inhibitors in tacking with specific mutant variants of HCV.

The discovery of anti-HCV drugs highlights the innovative strategies employed in developing non-peptide macrocyclic inhibitors targeting the NS3/4A protease. A key strategy in optimizing these drugs is macrocyclization, which significantly enhances conformational rigidity and binding affinity. For example, compounds such as simeprevir, danoprevir and grazoprevir exhibit activity enhancements ranging from several hundred to thousands of times greater than that of their original lead compounds. Additionally, the depeptidization strategy plays an important role in simplifying the structure, improving drug-like properties, and enhancing metabolic stability by removing the functionally non-essential peptide elements. The design of these

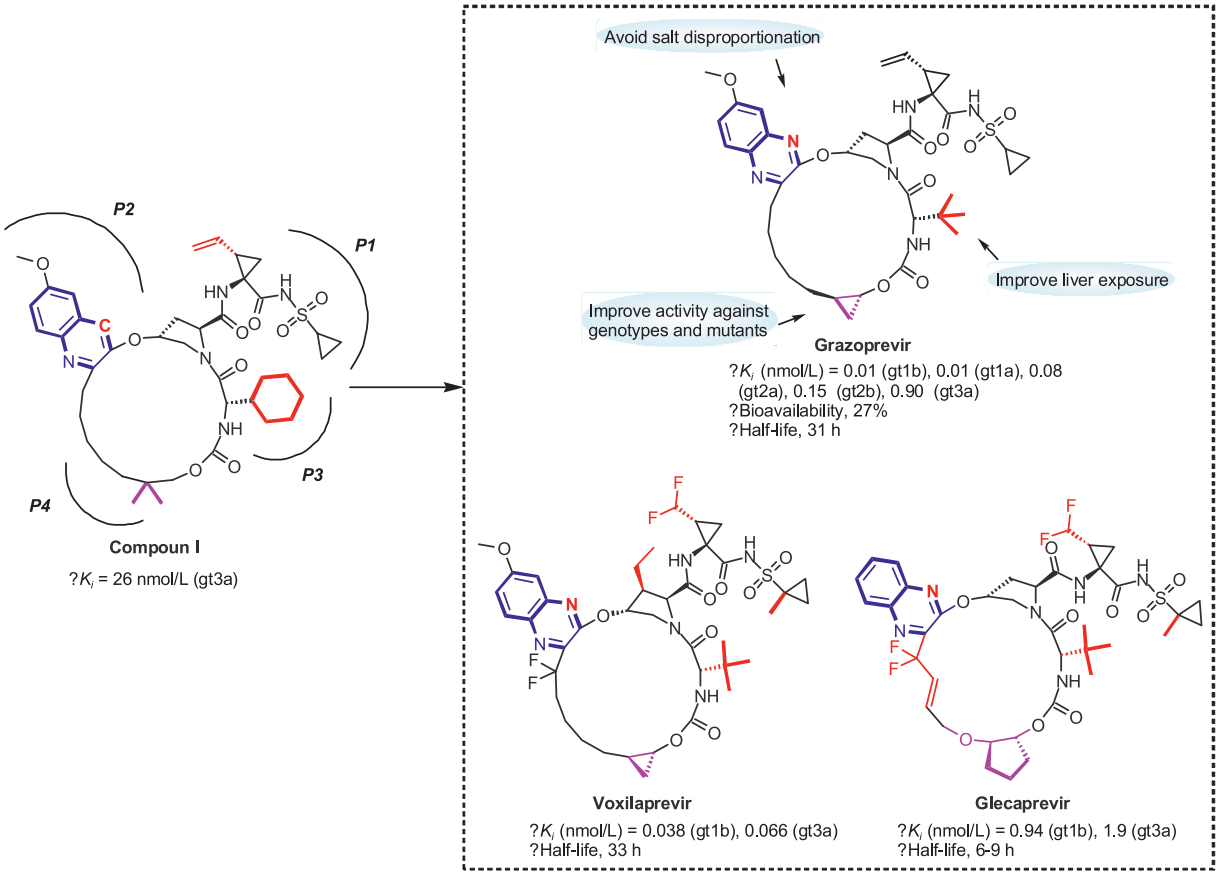


Figure 13 Chemical structures and design strategy of grazoprevir, voxilaprevir, glecaprevir.

drugs effectively addresses HCV variability and the emergence of resistance, with grazoprevir and danoprevir specially engineered to maintain potency against resistant variants. Overall, the development of anti-HCV drugs exemplifies the successful implementation of macrocyclization, structure-based design, and other innovative strategies. This has led to the creation of effective treatments against a complex and variable virus, greatly improving potency and selectivity while addressing resistance and tolerability challenges. These progress significantly advances the therapeutic landscape for HCV and other related diseases.

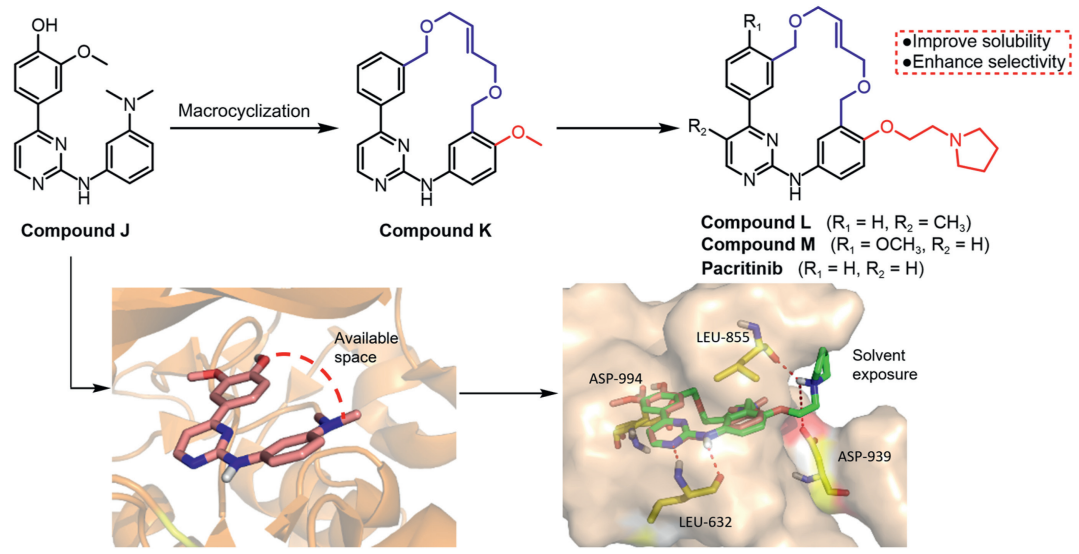
5.2. *Anti-cancer drugs*

5.2.1. *Macrocyclization driven by the need for proprietary drug*

In the pharmaceutical development, the quest for innovative and proprietary drugs has led researchers to explore various design strategies[145]. One key approach is macrocyclization, which is essential for creating structurally novel drug candidates. As the pharmaceutical industry aims to maintain a competitive advantage, utilizing macrocyclization to develop proprietary drugs is a pivotal strategy for bring novel therapeutics to market.

Pacritinib is a macrocyclic protein kinase inhibitor, approved in 2022 for the treatment of myelofibrosis[146]. It targets both Janus kinase 2 (JAK2) and Fms-like tyrosine kinase 3 (FLT3), which are key proteins involved in the development of myelofibrosis, a rare bone marrow disorder characterized by abnormal blood cell production[147,148]. The design of pacritinib began with an acyclic lead compound **J**, which had broad kinase inhibition but was limited by its ubiquitous pyrimidine-based motifs, making it difficult to develop proprietary compounds (Fig. 14)[149]. To overcome this, researchers aimed to create macrocyclic compounds by connecting the open ends of compound **J**. Through initial linker optimization, compound **K** with unsaturated dibenzylic ether chain, was obtained, demonstrating promising inhibitory activity against JAK2 with an IC_{50} value of 70 nmol/L as well as excellent selectivity over JAK1 and JAK3 (IC_{50} values are 1900 and 1200 nmol/L, respectively) but low inhibitory activity against FLT3 (IC_{50} value is 190 nmol/L). Furthermore, the replacement of methoxy group of compound **K** with aminoethylether side-chain like *N*-ethyl pyrrolidine, led to significant improvements in both the inhibitory activity and solubility of the compounds. Compound **L**, which incorporated a 5-methyl substitution on the pyrimidine ring, had a remarkable increase in both potency against JAK2 (IC_{50} is 7 nmol/L) and solubility (>150 μg/mL), but exhibited reduced selectivity against JAK3, possibly due to the resultant torsion angle between the pyrimidine ring and benzene ring. Additionally, compound **M** with a methoxy group at R1 position, generally maintained the inhibitory potency against JAK2 (IC_{50} is 19 nmol/L) while demonstrating selectivity over JAK1, JAK3 and CDK2. However, it has decreased solubility (60.83 μg/mL) compared to that of compound **L**. The iterative optimization culminated in the identification of pacritinib, which exhibited potent inhibitory activity against JAK2, with IC_{50} values of 23 nmol/L for the wild-type form and 19 nmol/L for the V617F mutation, and it inhibits FLT3 with an IC_{50} value of 22 nmol/L.

Crystallographic analysis of its interaction with the JAK2 ATP binding site shows the binding mode, including hinge binding between the amino-pyrimidine moiety and Leu632, and a hydrogen



Compound	IC$_{50}$ (nmol/L)						Solubility (μg/mL)
	JAK2	JAK2^{V617F}	JAK1	JAK3	CDK2	FLT3	
K	70	-	1900	1200	860	190	-
L	7	-	1000	89	>10000	19	>150
M	19	-	>10000	890	>10000	92	60.83
Pacritinib	23	19	1280	520	3900	22	>150

Figure 14 Chemical structure and design strategy of pacritinib. The diagrams illustrate the interaction between pacritinib (highlighted in green) and JAK2 (PDB: 8BPV), as well as the overlap of compound **J** (highlighted in red). Hydrogen bonds are highlighted in red dash lines. 3D visualizations were performed using PyMOL (Pymol.org).

bond of the cyclic linker with Asp994. Notably, compound **J** exhibits a characteristic U-shaped conformation, with two phenyl rings connected to the pyrimidine ring situated within a large cavity and positioned closely, which facilitates the effective cyclization of the molecular ends. It is noteworthy that the two phenyl rings exhibit a certain torsion angel, which presents a challenge in balancing flexibility and rigidity for the design of the linker. The unsaturated chain in pacritinib effectively addressed this issue. In addition, pacritinib exhibits an evident overlap with the scaffold of compound **J**, indicating that the formation of the macrocycle does not alter the conformation of the aromatic region. Furthermore, the pyrrolidine moiety is located in an open solvent-exposed area, and the pyrrolidine's basic nitrogen forms a salt bridge with Asp939, contributing to its potency against JAK2 and improved drug-like properties. These insights emphasize the potential of macrocyclic structures in developing potent and selective compounds. *In vivo* studies showed that pacritinib effectively reduced splenomegaly and hepatomegaly in a Ba/F3−JAK2^{V617F} leukemia model, normalizing spleen and liver weights. In xenograft studies with MV4-11 cells containing FLT3-ITD mutations, pacritinib also provided significant survival benefits at well-tolerated doses. Pharmacokinetically, pacritinib demonstrated rapid absorption and oral favorable bioavailability with rates of 39% in mice and 24% in dogs. Pacritinib has demonstrated promising preclinical and clinical data, ultimately leading to its approval for clinical use in treating hematological malignancies. The development of pacritinib from a patented lead compound to a proprietary macrocyclic inhibitor underscores the importance of macrocyclization in follow-on drug discovery and development[149,150].

5.2.2. Structure-based macrocyclization leading to the evolution of anti-NSCLC drugs

Non-small lung cancer (NSCLC) is a prevalent form of lung cancer, characterized by the presence of specific genetic mutations. Among these, mutations in the EGFR gene play a crucial role, as they are associated with increased sensitivity to EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib known as the first-generation EGFR inhibitors[151,152]. However, despite initial responses, many patients eventually develop acquired resistance, often due to secondary mutations like T790M. Icotinib is a highly selective EGFR TKI that was approved in China for first-line monotherapy in patients with advanced NSCLC harboring somatic EGFR mutations[153,154]. It has shown comparable efficacy to gefitinib and erlotinib but with potentially better safety profiles[155]. A study involving 120 treatment-experienced patients with advanced NSCLC evaluated the efficacy and safety of icotinib and erlotinib. The results indicated that icotinib achieved a 30% objective response rate (ORR) and 65% disease control rate (DCR), while erlotinib had 25% ORR and 56.7%. Median progression-free survival was longer for icotinib at 179 days *versus* 121 days for erlotinib[156]. According to the study by Zhang et al.[157] the occurrence of adverse drug reactions showed a notable decline after 6 months of treatment, with rates dropping from 65.4% during the initial period to 24.3% in long-term use. The most frequently observed adverse effects included rash (16.4%) and diarrhea (5.3%). In elderly patients, these adverse effects were similarly lower over time, with incidents of rash at 15.7% and diarrhea at 4.7% in the long-term treatment phase. This evidence suggests icotinib as a feasible long-term

therapeutic option for NSCLC, particularly in the aging population.

Icotinib shares structural similarities with erlotinib, and is a macrocyclic derivative of erlotinib (Fig. 15)[158]. Both drugs also share comparable mechanism of action and clinical efficacy for treating NSCLC. Erlotinib inhibits EGFR with an IC_{50} value of 2 nmol/L, while icotinib shows a comparable IC_{50} of 5 nmol/L. Besides, both icotinib and erlotinib exhibit similar bioavailability with values of 52% and 60%, respectively. However, their half-life differs significantly, with icotinib having a half-life of approximately 5.5 h compared to erlotinib's 36.2 h. Furthermore, icotinib exhibits inhibitory effects against various mutant forms of EGFR, including $EGFR^{L858L/T790M}$, $EGFR^{T790M}$, and $EGFR^{L861Q}$. As illustrated in Fig. 15, erlotinib displays a distinct U-shaped bioactive conformation in its binding to EGFR. The ends of two ether chains are positioned closely together, at a distance of 3.9 Å, providing potential sites for cyclization. Additionally, the quinazoline skeletons with ethynylbenzene parts of both compounds are well overlapped at the same site and form tight hydrogen-bond with the residue Met-769 with bond lengths of 2.0 Å for erlotinib and 2.2 Å for icotinib, respectively, indicating that the formation of macrocycle in icotinib did not affect the overall binding conformation. While there are slight variations in the binding conformations of the side chains of compounds, these differences are considered to have a minimal impact on their activity. This is because the side chains are located in a solvent-exposed region, leading to little effect on their inhibitory activity against EGFR. This optimization not only preserves the activity of icotinib but also enhances its pharmacological profiles with activity against various EGFR mutant forms. In addition, it also achieves better safety outcomes compared to erlotinib. The overall success of icotinib highlights the importance of macrocyclization in drug development. This technique not only broadens drug structures but also has the potential to help create more effective drugs for treating complex diseases[14].

Lorlatinib, a macrolactam anti-cancer drug developed by Pfizer, was approved by the FDA in 2018 for the treatment of NSCLC[159]. It effectively inhibits both anaplastic lymphoma kinase (ALK) and c-ROS oncogene 1 (ROS1), two key kinases involved in cancer development. Lorlatinib has remarkable potency against ALK, ALK^{L1196M} mutation, and ROS1, with IC_{50} values of <0.07, 0.7 and <0.025 nmol/L, respectively. ALK rearrangements, point mutations, or amplifications are closely associated with the initiation and progression of various malignant cancers like NSCLC[160,161]. Lorlatinib was developed based on crizotinib, a first-generation ALK inhibitor approved in 2011 for treating NSCLC. However, crizotinib is prone to develop resistance and has limited ability to cross the blood−brain barrier (BBB)[162,163]. To tackle these challenges, a combined strategy of structure-based drug design and macrocyclization was used to analyze the structure−activity relationship of crizotinib in detail (Fig. 16)[164]. The co-crystal analysis revealed that both lorlatinib and crizotinib bind to the identical active site and share identical hydrogen interactions with residues like Glu1197, Leu1198 and Met1199, showing substantial structural overlap in their pyridine, benzene, and pyrazole rings. These results suggest that the macrocyclization process does not impact the interaction between the compounds and target. Additionally, the incorporation of an amide group into the macrocycle optimizes the lipophilic efficiency of lorlatinib, leading to an enhancement in potency, while the nitrile group on the pyrazole greatly improves the target selectivity of lorlatinib. Clinical outcomes show that lorlatinib exhibits

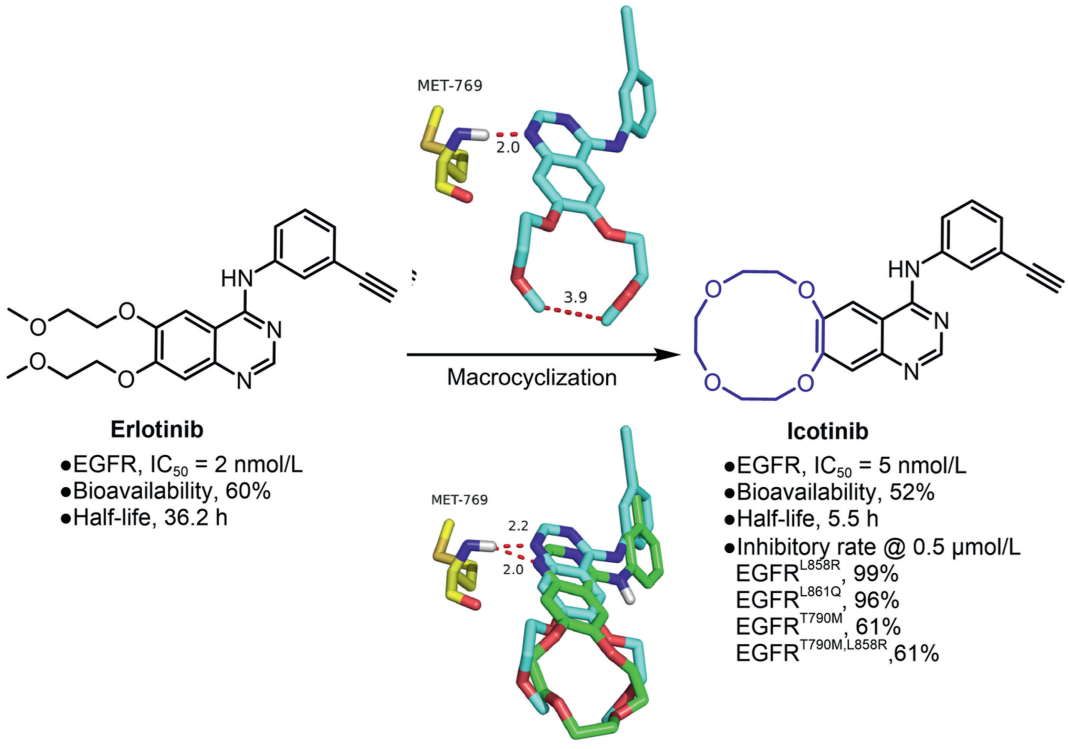


Figure 15 Chemical structure and design strategy of icotinib. The docking diagram illustrates the overlap of icotinib (highlighted in green) and erlotinib (highlighted in cyan) with EGFR (PDB: 4HJO). Hydrogen bonds are highlighted in red dash lines. 3D visualizations were performed using PyMOL (Pymol.org).

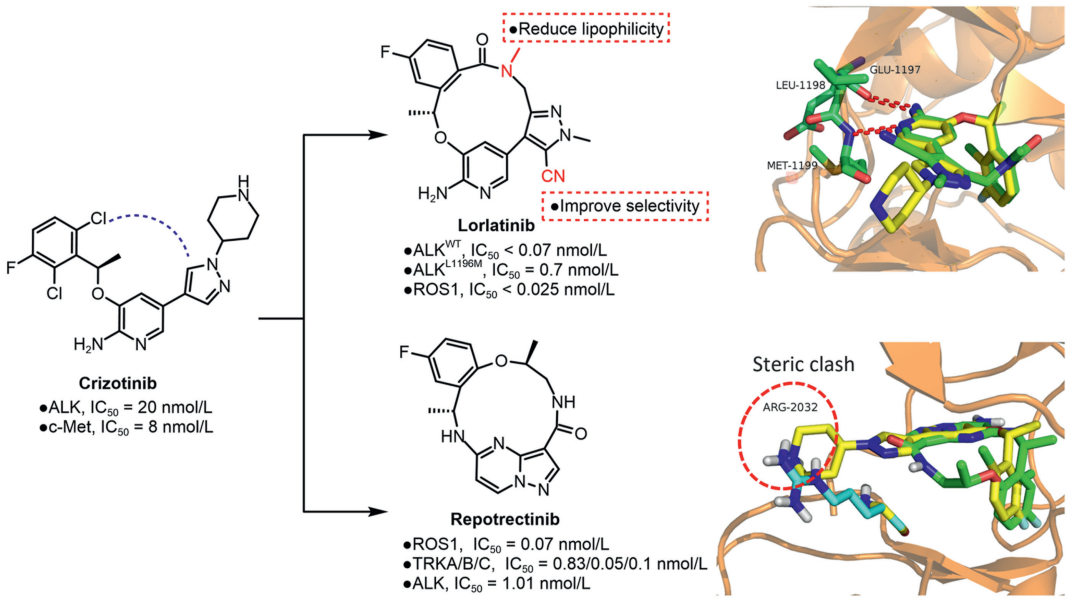


Figure 16 Chemical structures and design strategy of lorlatinib and repotrectinib. The binding diagrams display lorlatinib (green sticks) and crizotinib (cyan sticks) bound to ALK (PDB: 4CLI), as well as repotrectinib (green sticks) and crizotinib (cyan sticks) bound to ROS1 (PDB: 4XUL). Hydrogen bonds are highlighted in red dash lines. 3D visualizations were performed using PyMOL (Pymol.org).

remarkable effectiveness in both preventing and treating brain metastases in NSCLC because of its superior ability to penetrate BBB. Moreover, in patients without existing brain metastases, lorlatinib can effectively prevent their occurrence[159,165].

Repotrectinib is a next-generation TKI designed to address acquired resistance mechanisms in patients with ALK, ROS1, or NTRK rearrangements (Fig. 16)[166]. The development of earlier-generation TKIs for ALK- and ROS1-rearranged NSCLC highlighted the importance of targeting receptor tyrosine kinase fusions in solid tumors. However, resistance often emerges, particularly through on-target kinase domain mutations[167-169]. Repotrectinib was originally designed as a macrocyclic TKI with high selectivity and potency against ROS1 (IC_{50} = 0.07 nmol/L), TRKA/B/C (IC_{50} = 0.83/0.05/0.1 nmol/L), and ALK (IC_{50} = 1.01 nmol/L). Its unique structure allows it to effectively inhibit clinically recalcitrant solvent-front mutations, including ALK^{G1202R}, $ROS1^{G2032R, D2033N}$, $TRKA^{G595R}$, and $TRKC^{G623R}$. By anchoring itself in the ATP adenosine binding site with a predefined conformation, repotrectinib avoids steric hindrance from solvent-front mutations, while crizotinib shows evident steric clash with Arg2032 in ROS1. In preclinical studies, repotrectinib exhibited potent antitumor activity and selectivity against both wild-type and mutant fusion proteins in cellular and animal models. It was approved by the FDA in 2023 for the treatment of adult patients with locally advanced or metastatic NSCLC positive for ROS1. As a TKI, repotrectinib inhibits ROS1 and TRKs to block the signaling pathways in cancer cells, thereby curbing their growth and spread. Clinical outcomes showed that repotrectinib demonstrates remarkable efficacy and good tolerance, offering a new therapeutic option and hope for patients with ROS1-positive NSCLC[170-172].

5.2.3. From impurity to therapeutic versatility: the discovery of plerixafor

In addition to the previously discussed structural design of macrocyclic drugs, the unexpected discovery and optimization of lead compounds play an important role in the new drugs development. Plerixafor (AMD3100, Fig. 17A) is a bicyclam compound that was originally developed as an antagonist for CXCR4 receptor for the treatment of human immunodeficiency virus (HIV). Its discovery began with an accidental identification of an impurity in a cyclam preparation of JM1498. While the mono cyclam JM1498 exhibited very weak anti-HIV activity, its impurity named as JM1657, a bislactam demonstrated significantly higher potency against the virus, with nearly a 3000-fold increase in activity against HIV-1 and about a 150-fold increase against HIV-2[173,174]. This unexpected finding promoted researchers to explore JM1657 as a leading candidate for further SAR investigation. Subsequent structural modification by replacing the short alkyl chain with benzyl group led to the development of AMD3100, which showed a dramatic increase in anti-HIV potency with the IC_{50} values of 4.2 nmol/L against HIV-1 and 5.9 nmol/L against HIV-2, respectively. AMD3100 was subsequently identified as a specific inhibitor (IC_{50} value of 44 nmol/L) for the CXCR4 receptor, a co-receptor for T-lymphotropic HIV strains. Its unique specificity to target CXCR4 proved to be a breakthrough, setting the stage for its potential applications in combating not only HIV but also inflammatory diseases, cancers, and stem cell mobilization. In 2024, Kei Saotome and colleagues utilized cryo-electron microscopy to elucidate the structural basis of CXCR4 modulation and oligomerization, providing significant insights into the binding mechanism of plerixafor[175]. Their study revealed that plerixafor binds to the active conformation of CXCR4 in a diagonal orientation within the orthosteric pocket, thereby effectively blocking the binding of its natural ligand CXCL12 (Fig. 17B). This binding is stabilized by electrostatic interactions between the two positively charged cyclam rings of plerixafor and acidic residues including D262 and E288 in the transmembrane domain of CXCR4. This interaction is crucial for the antagonist's efficacy, as mutations in these residues reduce plerixafor's affinity for CXCR4[176].

While its initial development for the treatment of HIV faced challenges due to issues related to oral availability and cardiac

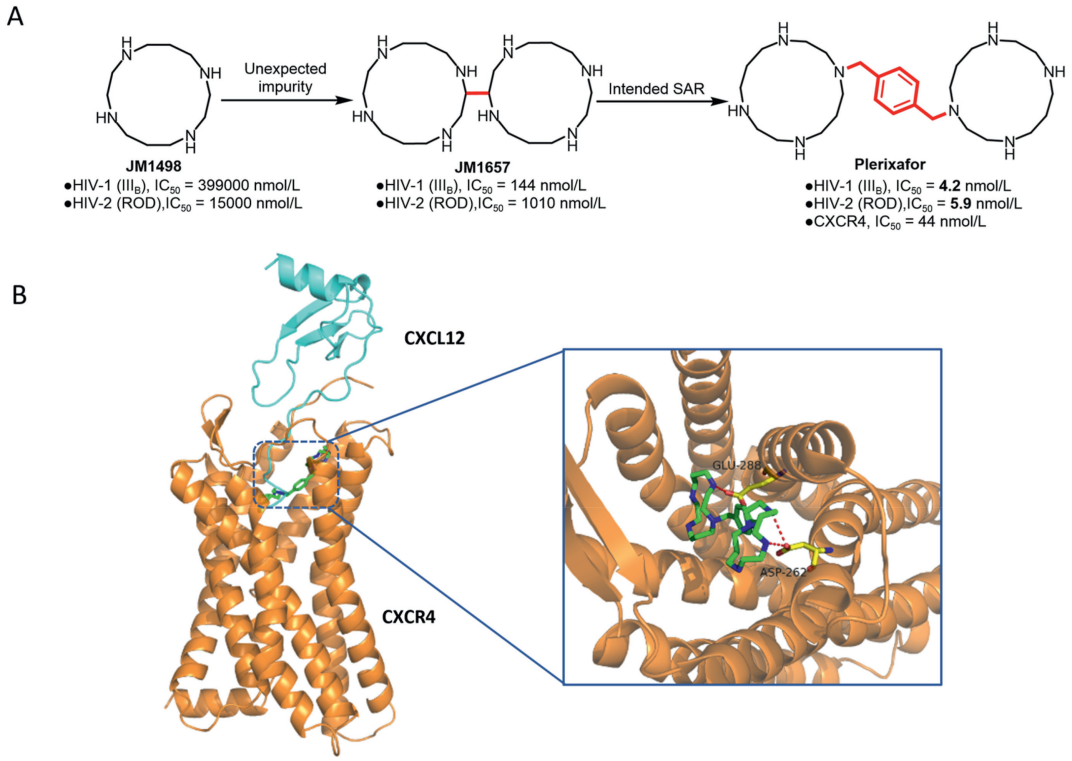


Figure 17 Chemical structure and development of plerixafor derived from JM1498 (A) and the crystal structures of CXCR4 (PDB: 8U4P) with plerixafor. Hydrogen bonds are highlighted in red dash lines. 3D visualizations were performed using PyMOL (Pymol.org).

disturbances, further research endeavors led to a pivotal shift towards its application in cancer treatment[177,178]. Plerixafor emerges as an immunostimulant for its role in mobilizing hematopoietic stem cells into the bloodstream of cancer patients. This process is crucial for harvesting stem cells for transplantation, and particularly beneficial for individuals with lymphoma and multiple myeloma. When combined with granulocyte colony-stimulating factor (G-CSF), plerixafor enables the enhanced efficacy of peripheral blood stem cell mobilization, thereby addressing the limitations faced by a significant portion of patients[173,179].

6. Perspectives and conclusions

Macrocycles are endowed with distinct structural features, their semi-rigid, pre-organized structures enable effective binding to undruggable targets. This characteristic makes them valuable contenders for drug development, paving the way for discovering new and effective treatments. To date, over 30 non-peptide macrocyclic drugs have been approved since 2000 for treating various diseases. In addition to naturally occurring macrocycles, natural product-inspired macrocycles and *de novo* designed macrocycles has further expanded the landscape of macrocyclic drugs. Of note, the macrocyclization of U-shaped structures in *de novo* macrocycle drug design presents a novel molecular skeleton editing strategy that allows for precise control over conformational properties. This strategy maintains structural integrity while enhancing binding affinity, thus promoting increased selectivity and potency in therapeutic applications. By employing structural data from co-crystal complexes and using computational tools, researchers can effectively identify suitable connection points for macrocyclization, leading to enhanced drug-like properties and innovative scaffolding[180]. This macrocyclization strategy has witnessed great success in the discovery of macrocyclic drugs like pacritinib, icotinib, and lorlatinib.

The advancements of approved non-peptide macrocyclic drugs over the past two decades highlight their significance in the therapeutic landscape. The diverse structures and versatile functions of these compounds provide valuable inspiration for future drug discovery. By delving into new chemical realms and harnessing the distinct characteristics of macrocycles, researchers can create innovative treatments with enhanced effectiveness, selectivity, and safety profiles. Historically, macrocyclic drugs were typically discovered by screening of natural and synthetic compounds. Although these methods have produced some effective treatments, they are hindered by their unpredictability and inefficiency, resulting in a slow and expensive drug discovery process. On the other hand, contemporary drug development relies heavily on rational design principles that leverage current knowledge of molecular biology, structural biology, and computational chemistry to intentionally design macrocyclic compounds tailored to specific pharmaceutical requirements. One of the most exciting developments is the *de novo* creation of new macrocyclic drugs. Advances in computational chemistry and co-crystallization techniques have greatly facilitated the design of new macrocyclic structures with precise targeting and effectiveness. Furthermore, *de novo* design, which entails crafting new macrocycles from scratch without using natural templates, has resulted in potent compounds that target previously inaccessible sites.

Additionally, the idea of molecular chameleons introduces an important dimension to the design of macrocyclic drugs[181,182]. These compounds exhibit conformational flexibility, allowing them to dynamically shield or exposure polar functionalities in response environment. This adaptability is crucial for enhancing cell

permeability and aqueous solubility which are important for drug action. The ability of molecular chameleons to adopt different conformations can lead to improved interactions with biological targets, particularly those that are large and featureless, which are often challenging for traditional small molecules to engage.

Developing macrocyclic drugs presents challenges due to the complexity and labor-intensive nature of their synthesis, which often involves multiple steps and specialized techniques. Predicting and controlling the desired conformational rigidity and stability crucial for biological activity is difficult. Additionally, optimizing pharmacokinetic properties such as solubility, permeability, and metabolic stability remains a significant obstacle. Researchers have employed various strategies including privileged scaffolds, conformational constraints like intramolecular hydrogen bonds and steric hindrance, as well as hybrid macrocyclic structures that combine peptidic and non-peptidic elements to address these challenges. The development of macrocyclic drugs relies on combining different fields such as synthetic chemistry, computational modeling, and biological screening. Further investigation into natural products and their derivatives will provide valuable insights into novel macrocyclic structures and mechanisms of action. Moreover, utilizing advanced technologies like artificial intelligence and machine learning will revolutionize the design of macrocyclic drugs, accelerating the discovery of new treatments. While this perspective touches on the synthesis of non-peptide macrocycles, a more critical examination of these challenges and potential solutions is needed. Incorporating recent advances in synthetic methodologies that tackle these challenges would be beneficial. Several published review articles have systematically discussed these issues in detail[180,183-186].

In conclusion, macrocyclic drugs represent a promising and rapidly advancing therapeutic category capable of tackling medical challenges. Their distinctive structural and functional characteristics, combined with advancements in synthetic and computational techniques, have resulted in the successful creation of several FDA-approved macrocyclic drugs. By leveraging these accomplishments and overcoming existing challenges, researchers can unleash the complete potential of macrocycles for future effective treatments.

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Author contributions

Zhonghua Li wrote the original draft. Bin Yu and Zhenqiang Zhang revised the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Giordanetto F, Kihlberg J. Macrocyclic drugs and clinical candidates: what can medicinal chemists learn from their properties?. *J Med Chem* 2014;**57**:278−95.
2. Hann MM, Keseru GM. Finding the sweet spot: the role of nature and nurture in medicinal chemistry. *Nat Rev Drug Discov* 2012;**11**: 355−65.
3. Zhang G, Zhang J, Gao Y, Li Y, Li Y. Strategies for targeting undruggable targets. *Expet Opin Drug Discov* 2022;**17**:55−69.
4. You L, An R, Liang K, Cui B, Wang X. Macrocyclic compounds: emerging opportunities for current drug discovery. *Curr Pharm Des* 2016;**22**:4086−93.
5. Driggers EM, Hale SP, Lee J, Terrett NK. The exploration of macrocycles for drug discovery—an underexploited structural class. *Nat Rev Drug Discov* 2008;**7**:608−24.
6. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod* 2020;**83**:770−803.
7. Wessjohann LA, Ruijter E, Garcia-Rivera D, Brandt W. What can a chemist learn from nature's macrocycles?—a brief, conceptual view. *Mol Divers* 2005;**9**:171−86.
8. Marsault E, Peterson ML. Macrocycles are great cycles: applications, opportunities, and challenges of synthetic macrocycles in drug discovery. *J Med Chem* 2011;**54**:1961−2004.
9. Delorbe JE, Clements JH, Whiddon BB, Martin SF. Thermodynamic and structural effects of macrocyclization as a constraining method in protein−ligand interactions. *ACS Med Chem Lett* 2010;**1**:448−52.
10. Mallinson J, Collins I. Macrocycles in new drug discovery. *Future Med Chem* 2012;**4**:1409−38.
11. Gongora-Benitez M, Tulla-Puche J, Albericio F. Multifaceted roles of disulfide bonds. peptides as therapeutics. *Chem Rev* 2014;**114**: 901−26.
12. Philippe GJB, Craik DJ, Henriques ST. Converting peptides into drugs targeting intracellular protein−protein interactions. *Drug Discov Today* 2021;**26**:1521−31.
13. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem* 2002;**45**:2615−23.
14. Zhang C, Liu F, Zhang Y, Song C. Macrocycles and macrocyclization in anticancer drug discovery: important pieces of the puzzle. *Eur J Med Chem* 2024;**268**:116234.
15. Dougherty PG, Qian Z, Pei D. Macrocycles as protein−protein interaction inhibitors. *Biochem J* 2017;**474**:1109−25.
16. Kruger DM, Glas A, Bier D, Pospiech N, Wallraven K, Dietrich L, et al. Structure-based design of non-natural macrocyclic peptides that inhibit protein−protein interactions. *J Med Chem* 2017;**60**:8982−8.
17. Garcia Jimenez D, Poongavanam V, Kihlberg J. Macrocycles in drug discovery horizontal line learning from the past for the future. *J Med Chem* 2023;**66**:5377−96.
18. Ji X, Nielsen AL, Heinis C. Cyclic peptides for drug development. *Angew Chem Int Ed Engl* 2024;**63**:e202308251.
19. McFarland SA, Mandel A, Dumoulin-White R, Gasser G. Metal-based photosensitizers for photodynamic therapy: the future of multimodal oncology?. *Curr Opin Chem Biol* 2020;**56**:23−7.
20. Zhang J, Yuan J, Li Z, Fu C, Xu M, Yang J, et al. Exploring and exploiting plant cyclic peptides for drug discovery and development. *Med Res Rev* 2021;**41**:3096−117.
21. Chopra B, Dhingra AK. Natural products: a lead for drug discovery and development. *Phytother Res* 2021;**35**:4660−702.
22. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. *Nat Rev Drug Discov* 2015;**14**:111−29.
23. Kirst HA. The spinosyn family of insecticides: realizing the potential of natural products research. *J Antibiot (Tokyo)* 2010;**63**:101−11.
24. Villegas SC, Breitzka RL. Head lice and the use of spinosad. *Clin Ther* 2012;**34**:14−23.

25. Snyder DE, Meyer J, Zimmermann AG, Qiao M, Gissendanner SJ, Cruthers LR, et al. Preliminary studies on the effectiveness of the novel pulicide, spinosad, for the treatment and control of fleas on dogs. *Vet Parasitol* 2007;**150**:345−51.
26. Sparks TC, Crouse GD, Durst G. Natural products as insecticides: the biology, biochemistry and quantitative structure−activity relationships of spinosyns and spinosoids. *Pest Manag Sci* 2001;**57**: 896−905.
27. McCormack PL. Spinosad: in pediculosis capitis. *Am J Clin Dermatol* 2011;**12**:349−53.
28. Ihara M, Buckingham SD, Matsuda K, Sattelle DB. Modes of action, resistance and toxicity of insecticides targeting nicotinic acetylcholine receptors. *Curr Med Chem* 2017;**24**:2925−34.
29. Cole SW, Lundquist LM. Spinosad for treatment of head lice infestation. *Ann Pharmacother* 2011;**45**:954−9.
30. Zou Z, Hu X, Luo T, Ming Z, Chen X, Xia L, et al. Naturally-occurring spinosyn A and its derivatives function as argininosuccinate synthase activator and tumor inhibitor. *Nat Commun* 2021;**12**: 2263.
31. Qiu F, Chen YR, Liu X, Chu CY, Shen LJ, Xu J, et al. Arginine starvation impairs mitochondrial respiratory function in ASS1-deficient breast cancer cells. *Sci Signal* 2014;**7**:ra31.
32. Delage B, Fennell DA, Nicholson L, McNeish I, Lemoine NR, Crook T, et al. Arginine deprivation and argininosuccinate synthetase expression in the treatment of cancer. *Int J Cancer* 2010;**126**:2762−72.
33. Chen CL, Hsu SC, Ann DK, Yen Y, Kung HJ. Arginine signaling and cancer metabolism. *Cancers (Basel)* 2021;**13**:3541.
34. Roskoski RJ. Properties of FDA-approved small molecule protein kinase inhibitors: a 2023 update. *Pharmacol Res* 2023;**187**:106552.
35. Cassady JM, Chan KK, Floss HG, Leistner E. Recent developments in the maytansinoid antitumor agents. *Chem Pharm Bull (Tokyo)* 2004;**52**:1−26.
36. Cao S, Dong YH, Wang DF, Liu ZP. Tubulin maytansine site binding ligands and their applications as MTAs and ADCs for cancer therapy. *Curr Med Chem* 2020;**27**:4567−76.
37. Trneny M, Verhoef G, Dyer MJ, Ben Yehuda D, Patti C, Canales M, et al. A phase II multicenter study of the anti-CD19 antibody drug conjugate coltuximab ravtansine (SAR3419) in patients with relapsed or refractory diffuse large B-cell lymphoma previously treated with rituximab-based immunotherapy. *Haematologica* 2018;**103**:1351−8.
38. Remillard S, Rebhun LI, Howie GA, Kupchan SM. Antimitotic activity of the potent tumor inhibitor maytansine. *Science* 1975;**189**: 1002−5.
39. Scaranti M, Cojocaru E, Banerjee S, Banerji U. Exploiting the folate receptor α in oncology. *Nat Rev Clin Oncol* 2020;**17**:349−59.
40. Matulonis UA, Lorusso D, Oaknin A, Pignata S, Dean A, Denys H, et al. Efficacy and safety of mirvetuximab soravtansine in patients with platinum-resistant ovarian cancer with high folate receptor α expression: results from the SORAYA study. *J Clin Oncol* 2023;**41**: 2436−45.
41. Dilawari A, Shah M, Ison G, Gittleman H, Fiero MH, Shah A, et al. FDA approval summary: mirvetuximab soravtansine-Gynx for FRα-positive, platinum-resistant ovarian cancer. *Clin Cancer Res* 2023;**29**: 3835−40.
42. Saxena A, Kumari R, Mukherjee U, Singh P, Lal R. Draft genome sequence of the rifamycin producer amycolatopsis rifamycinica DSM 46095. *Genome Announc* 2014;**2**:e00662-14.
43. Sensi P. History of the development of rifampin. *Rev Infect Dis* 1983; **5**:S402−6.
44. Andrei S, Droc G, Stefan G. FDA approved antibacterial drugs: 2018-2019. *Discoveries* 2019;**7**:e102.
45. Calvori C, Frontali L, Leoni L, Tecce G. Effect of rifamycin on protein synthesis. *Nature* 1965;**207**:417−8.
46. Floss HG, Yu TW. Rifamycin-mode of action, resistance, and biosynthesis. *Chem Rev* 2005;**105**:621−32.
47. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, et al. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. *Cell* 2001;**104**:901−12.
48. Dickinson JM, Mitchison DA. *In vitro* activity of new rifamycins against rifampicin-resistant M. tuberculosis and MAIS-complex mycobacteria. *Tubercle* 1987;**68**:177−82.
49. Hopewell PC, Pai M, Maher D, Uplekar M, Raviglione MC. International standards for tuberculosis care. *Lancet Infect Dis* 2006;**6**: 710−25.
50. Hardesty JS, Juang P. Fidaxomicin: a macrocyclic antibiotic for the treatment of clostridium difficile infection. *Pharmacotherapy* 2011; **31**:877−86.
51. Fidaxomicin: difimicin; lipiarmycin; OPT 80. OPT-80; PAR 101; PAR-101. *Drugs R&D* 2010;**10**:37−45.
52. Goldstein EJ, Citron DM, Sears P, Babakhani F, Sambol SP, Gerding DN. Comparative susceptibilities to fidaxomicin (OPT-80) of isolates collected at baseline, recurrence, and failure from patients in two phase III trials of fidaxomicin against clostridium difficile infection. *Antimicrob Agents Chemother* 2011;**55**:5194−9.
53. Cao X, Boyaci H, Chen J, Bao Y, Landick R, Campbell EA. Basis of narrow-spectrum activity of fidaxomicin on *clostridioides difficile*. *Nature* 2022;**604**:541−5.
54. Weiss K, Allgren RL, Sellers S. Safety analysis of fidaxomicin in comparison with oral vancomycin for clostridium difficile infections. *Clin Infect Dis* 2012;**55**:S110−5.
55. Wang S, Dong G, Sheng C. Structural simplification: an efficient strategy in lead optimization. *Acta Pharm Sin B* 2019;**9**:880−901.
56. Yeung BK. Natural product drug discovery: the successful optimization of ISP-1 and halichondrin B. *Curr Opin Chem Biol* 2011;**15**: 523−8.
57. Bai RL, Paull KD, Herald CL, Malspeis L, Pettit GR, Hamel E. Halichondrin B and homohalichondrin B, marine natural products binding in the vinca domain of tubulin. Discovery of tubulin-based mechanism of action by analysis of differential cytotoxicity data. *J Biol Chem* 1991;**266**:15882−9.
58. Wilson L, Lopus M, Miller HP, Azarenko O, Riffle S, Smith JA, et al. Effects of eribulin on microtubule binding and dynamic instability are strengthened in the absence of the βIII tubulin isotype. *Biochemistry* 2015;**54**:6482−9.
59. Smith JA, Wilson L, Azarenko O, Zhu X, Lewis BM, Littlefield BA, et al. Eribulin binds at microtubule ends to a single site on tubulin to suppress dynamic instability. *Biochemistry* 2010;**49**:1331−7.
60. Perez-Garcia JM, Cortes J. The safety of eribulin for the treatment of metastatic breast cancer. *Expet Opin Drug Saf* 2019;**18**:347−55.
61. Thomas DA, Keith RB, Francis GF, Craig JF, Sun HJ, Yoshito K, et al. Total synthesis of halichondrin B and norhalichondrin B. *J Am Chem Soc* 1992;**114**:3162−4.
62. Dean PS, Sean SC, Yoshito K. New synthetic route to the C.14−C.38 segment of halichondrins. *J Org Chem* 1997;**62**:7552−3.
63. Kaghad A, Panagopoulos D, Caballero-Garcia G, Zhai H, Britton R. An α-chloroaldehyde-based rormal synthesis of eribulin. *Nat Commun* 2023;**14**:1904.
64. Nicolaou KC, Pan S, Shelke Y, Rigol S, Bao R, Das D, et al. A unified strategy for the total syntheses of eribulin and a macrolactam analogue of halichondrin B. *Proc Natl Acad Sci USA* 2022;**119**: e2208938119.
65. Prucha H, Schnopp C, Akdis C, Lauener R, Wollenberg A, Ring J, et al. Pimecrolimus, a topical calcineurin inhibitor used in the treatment of atopic eczema. *Expet Opin Drug Metabol Toxicol* 2013; **9**:1507−16.
66. Schopf RE. Pimecrolimus. Novartis. *Curr Opin Invest Drugs* 2002;**3**: 720−4.
67. Paul C, Graeber M, Stuetz A. Ascomycins: promising agents for the treatment of inflammatory skin diseases. *Expet Opin Invest Drugs* 2000;**9**:69−77.
68. Gupta AK, Chow M. Pimecrolimus: a review. *J Eur Acad Dermatol Venereol* 2003;**17**:493−503.
69. Grassberger M, Baumruker T, Enz A, Hiestand P, Hultsch T, Kalthoff F, et al. A novel anti-inflammatory drug, SDZ ASM 981, for the treatment of skin diseases: *in vitro* pharmacology. *Br J Dermatol* 1999;**141**:264−73.

70. Grassberger M, Steinhoff M, Schneider D, Luger TA. Pimecrolimus—an anti-inflammatory drug targeting the Skin. *Exp Dermatol* 2004;**13**:721−30.
71. Old JM, Skelton CJA, Stannard HJ. The use of cydectinn® by wildlife carers to treat sarcoptic mange in free-ranging bare-nosed Wombats (*Vombatus ursinus*). *Parasitol Res* 2021;**120**:1077−90.
72. Wolstenholme AJ, Fairweather I, Prichard R, von Samson-Himmelstjerna G, Sangster NC. Drug resistance in veterinary helminths. *Trends Parasitol* 2004;**20**:469−76.
73. Awadzi K, Opoku NO, Attah SK, Lazdins-Helds J, Kuesel AC. A randomized, single-ascending-dose, ivermectin-controlled, double-blind study of moxidectin in *Onchocerca volvulus* infection. *PLoS Neglected Trop Dis* 2014;**8**:e2953.
74. Jawahar S, Tricoche N, Bulman CA, Sakanari J, Lustigman S. Drugs that target early stages of onchocerca bolvulus: a revisited means to facilitate the elimination goals for onchocerciasis. *PLoS Neglected Trop Dis* 2021;**15**:e0009064.
75. Milton P, Hamley JID, Walker M, Basanez MG. Moxidectin: an oral treatment for human onchocerciasis. *Expert Rev Anti Infect Ther* 2020;**18**:1067−81.
76. Goodin S. Novel cytotoxic agents: epothilones. *Am J Health Syst Pharm* 2008;**65**:S10−5.
77. Lee FY, Borzilleri R, Fairchild CR, Kamath A, Smykla R, Kramer R, et al. Preclinical discovery of ixabepilone, a highly active antineoplastic agent. *Cancer Chemother Pharmacol* 2008;**63**:157−66.
78. Goodin S. Ixabepilone: a novel microtubule-stabilizing agent for the treatment of metastatic breast cancer. *Am J Health Syst Pharm* 2008; **65**:2017−26.
79. Vulfovich M, Rocha-Lima C. Novel advances in pancreatic cancer treatment. *Expert Rev Anticancer Ther* 2008;**8**:993−1002.
80. Szychowski J, Truchon JF, Bennani YL. Natural products in medicine: transformational outcome of synthetic chemistry. *J Med Chem* 2014;**57**:9292−308.
81. Gentile I, Borgia F, Buonomo AR, Zappulo E, Castaldo G, Borgia G. ABT-450: a novel protease inhibitor for the treatment of hepatitis C virus infection. *Curr Med Chem* 2014;**21**:3261−70.
82. Ibrahim NK. Ixabepilone: overview of effectiveness, safety, and tolerability in metastatic breast cancer. *Front Oncol* 2021;**11**:617874.
83. Leiva M, Ruiz-Bravo A, Jimenez-Valera M. Effects of telithromycin in *in vitro* and *in vivo* models of lipopolysaccharide-induced airway inflammation. *Chest* 2008;**134**:20−9.
84. Morinaga Y, Yanagihara K, Miyashita N, Seki M, Izumikawa K, Kakeya H, et al. Azithromycin, clarithromycin and telithromycin inhibit MUC5AC induction by chlamydophila pneumoniae in airway epithelial cells. *Pulm Pharmacol Ther* 2009;**22**:580−6.
85. Douthwaite S. Structure−activity relationships of ketolides *vs.* macrolides. *Clin Microbiol Infect* 2001;**7**:11−7.
86. Bonnefoy A, Girard AM, Agouridas C, Chantot JF. Ketolides lack inducibility properties of MLS(B) resistance phenotype. *J Antimicrob Chemother* 1997;**40**:85−90.
87. Tran MP. Telithromycin: a novel agent for the treatment of community-acquired upper respiratory infections. *SAVE Proc* 2004; **17**:475−9.
88. Eyal Z, Matzov D, Krupkin M, Wekselman I, Paukner S, Zimmerman E, et al. Structural insights into species-specific features of the ribosome from the pathogen staphylococcus aureus. *Proc Natl Acad Sci USA* 2015;**112**:E5805−14.
89. Bertrand D, Bertrand S, Neveu E, Fernandes P. Molecular characterization of off-target activities of telithromycin: a potential role for nicotinic acetylcholine receptors. *Antimicrob Agents Chemother* 2010;**54**:5399−402.
90. Fernandes P, Martens E, Bertrand D, Pereira D. The solithromycin journey-it is all in the chemistry. *Bioorg Med Chem* 2016;**24**:6420−8.
91. Pritchard DI. Sourcing a chemical succession for cyclosporin from parasites and human pathogens. *Drug Discov Today* 2005;**10**: 688−91.
92. Seto B. Rapamycin and mTOR: a serendipitous discovery and implications for breast cancer. *Clin Transl Med* 2012;**1**:29.
93. Edwards SR, Wandless TJ. The rapamycin-binding domain of the protein kinase mammalian target of rapamycin is a destabilizing domain. *J Biol Chem* 2007;**282**:13395−401.
94. Rangaraju S, Verrier JD, Madorsky I, Nicks J, Dunn WJ, Notterpek L. Rapamycin activates autophagy and improves myelination in explant cultures from neuropathic mice. *J Neurosci* 2010; **30**:11388−97.
95. Choi J, Chen J, Schreiber SL, Clardy J. Structure of the FKBP12−rapamycin complex interacting with the binding domain of human FRAP. *Science* 1996;**273**:239−42.
96. Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP. mTOR kinase structure, mechanism and regulation. *Nature* 2013;**497**:217−23.
97. Chen Y, Zhou X. Research progress of mTOR inhibitors. *Eur J Med Chem* 2020;**208**:112820.
98. Guduru SKR, Arya P. Synthesis and biological evaluation of rapamycin-derived, next generation small molecules. *Medchemcomm* 2018;**9**:27−43.
99. Burke SE, Kuntz RE, Schwartz LB. Zotarolimus (ABT-578) eluting stents. *Adv Drug Deliv Rev* 2006;**58**:437−46.
100. Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederrecht G, et al. Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. *J Biol Chem* 1995;**270**: 815−22.
101. Chen YW, Smith ML, Sheets M, Ballaron S, Trevillyan JM, Burke SE, et al. Zotarolimus, a novel sirolimus analogue with potent anti-proliferative activity on coronary smooth muscle cells and reduced potential for systemic immunosuppression. *J Cardiovasc Pharmacol* 2007;**49**:228−35.
102. Coolong A, Kuntz RE. Understanding the drug-eluting stent trials. *Am J Cardiol* 2007;**100**:17−24K.
103. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. *Front Oncol* 2014;**4**:64.
104. Hasskarl J. Everolimus. *Recent Results Cancer Res* 2018;**211**:101−23.
105. Stock C, Zaccagnini M, Schulze M, Teber D, Rassweiler JJ. Temsirolimus. *Recent Results Cancer Res* 2010;**184**:189−97.
106. Schulze M, Stock C, Zaccagnini M, Teber D, Rassweiler JJ. Temsirolimus. *Recent Results Cancer Res* 2014;**201**:393−403.
107. Jiang T, Yu JT, Zhu XC, Tan MS, Wang HF, Cao L, et al. Temsirolimus promotes autophagic clearance of amyloid-β and provides protective effects in cellular and animal models of Alzheimer's disease. *Pharmacol Res* 2014;**81**:54−63.
108. Querfurth H, Lee HK. Mammalian/mechanistic target of rapamycin (mTOR) complexes in neurodegeneration. *Mol Neurodegener* 2021; **16**:44.
109. Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011;**17**:107−15.
110. Brown RS. Hepatitis C and liver transplantation. *Nature* 2005;**436**: 973−8.
111. Rosenquist A, Samuelsson B, Johansson PO, Cummings MD, Lenz O, Raboisson P, et al. Discovery and development of simeprevir (TMC435), a HCV NS3/4A protease inhibitor. *J Med Chem* 2014;**57**: 1673−93.
112. Susser S, Welsch C, Wang Y, Zettler M, Domingues FS, Karey U, et al. Characterization of resistance to the protease inhibitor boceprevir in hepatitis C virus-infected patients. *Hepatology* 2009;**50**: 1709−18.
113. Kieffer TL, Sarrazin C, Miller JS, Welker MW, Forestier N, Reesink HW, et al. Telaprevir and pegylated interferon-α-2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. *Hepatology* 2007;**46**:631−9.
114. Barritt AS, Fried MW. Maximizing opportunities and avoiding mistakes in triple therapy for hepatitis C virus. *Gastroenterology* 2012;**142**:1314−23.
115. Manns MP, von Hahn T. Novel therapies for hepatitis C—one pill fits all?. *Nat Rev Drug Discov* 2013;**12**:595−610.
116. Vaidya A, Perry CM. Simeprevir: first global approval. *Drugs* 2013; **73**:2093−106.

117. Chung RT, Davis GL, Jensen DM, Masur H, Saag MS, Thomas DL, et al. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015;**62**:932−54.
118. Lin TI, Lenz O, Fanning G, Verbinnen T, Delouvroy F, Scholliers A, et al. *In vitro* activity and preclinical profile of TMC435350, a potent hepatitis C virus protease inhibitor. *Antimicrob Agents Chemother* 2009;**53**:1377−85.
119. Moreno C, Berg T, Tanwandee T, Thongsawat S, Van Vlierberghe H, Zeuzem S, et al. Antiviral activity of TMC435 monotherapy in patients infected with HCV genotypes 2-6: TMC435-C202, a phase IIa, open-label study. *J Hepatol* 2012;**56**:1247−53.
120. Manns M, Reesink H, Berg T, Dusheiko G, Flisiak R, Marcellin P, et al. Rapid viral response of once-daily TMC435 plus pegylated interferon/ribavirin in hepatitis C genotype-1 patients: a randomized trial. *Antivir Ther* 2011;**16**:1021−33.
121. Wieske LHE, Atilaw Y, Poongavanam V, Erdelyi M, Kihlberg J. Going viral: an investigation into the chameleonic behaviour of antiviral compounds. *Chemistry* 2023;**29**:e202202798.
122. Tang K, Wang S, Gao W, Song Y, Yu B. Harnessing the cyclization strategy for new drug discovery. *Acta Pharm Sin B* 2022;**12**: 4309−26.
123. Deutsch M, Papatheodoridis GV. Danoprevir, a small-molecule NS3/4A P rotease inhibitor for the potential oral treatment of HCV infection. *Curr Opin Invest Drugs* 2010;**11**:951−63.
124. Jiang Y, Andrews SW, Condroski KR, Buckman B, Serebryany V, Wenglowsky S, et al. Discovery of danoprevir (ITMN-191/R7227), a highly selective and potent inhibitor of hepatitis C virus (HCV) NS3/4A protease. *J Med Chem* 2014;**57**:1753−69.
125. Markham A, Keam SJ. Danoprevir: first global approval. *Drugs* 2018;**78**:1271−6.
126. Miao M, Jing X, De Clercq E, Li G. Danoprevir for the treatment of hepatitis C virus infection: design, development, and place in therapy. *Drug Des Dev Ther* 2020;**14**:2759−74.
127. Lenz O, Vijgen L, Berke JM, Cummings MD, Fevery B, Peeters M, et al. Virologic response and characterisation of HCV genotype 2−6 in patients receiving TMC435 monotherapy (study TMC435-C202). *J Hepatol* 2013;**58**:445−51.
128. Raedler LA. Viekira Pak (ombitasvir, paritaprevir, and ritonavir tablets; dasabuvir tablets): all-oral fixed combination approved for genotype 1 chronic hepatitis C infection. *Am Health Drug Benefits* 2015;**8**:142−7.
129. Pilot-Matias T, Tripathi R, Cohen D, Gaultier I, Dekhtyar T, Lu L, et al. *In vitro* and *in vivo* antiviral activity and resistance profile of the hepatitis C virus NS3/4A protease inhibitor ABT-450. *Antimicrob Agents Chemother* 2015;**59**:988−97.
130. Kowdley KV, Lawitz E, Poordad F, Cohen DE, Nelson DR, Zeuzem S, et al. Phase 2b trial of interferon-free therapy for hepatitis C virus genotype 1. *N Engl J Med* 2014;**370**:222−32.
131. Caspi DD, Cink RD, Clyne D, Diwan M, Engstrom KM, Grieme T, et al. Process development of ABT-450—a first generation NS3/4A protease inhibitor for HCV. *Tetrahedron* 2019;**75**:4271−86.
132. Lamarre D, Anderson PC, Bailey M, Beaulieu P, Bolger G, Bonneau P, et al. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature* 2003;**426**:186−9.
133. Chatel-Chaix L, Baril M, Lamarre D. Hepatitis C virus NS3/4A protease inhibitors: a light at the end of the tunnel. *Viruses* 2010;**2**: 1752−65.
134. McCauley JA, McIntyre CJ, Rudd MT, Nguyen KT, Romano JJ, Butcher JW, et al. Discovery of vaniprevir (MK-7009), a macrocyclic hepatitis C virus NS3/4a protease inhibitor. *J Med Chem* 2010;**53**: 2443−63.
135. Liverton NJ, Holloway MK, McCauley JA, Rudd MT, Butcher JW, Carroll SS, et al. Molecular modeling based approach to potent P2-P4 macrocyclic inhibitors of hepatitis C NS3/4A protease. *J Am Chem Soc* 2008;**130**:4607−9.
136. Liverton NJ, Carroll SS, Dimuzio J, Fandozzi C, Graham DJ, Hazuda D, et al. MK-7009, a potent and selective inhibitor of hepatitis C virus NS3/4A protease. *Antimicrob Agents Chemother* 2010;**54**:305−11.
137. Summa V, Ludmerer SW, McCauley JA, Fandozzi C, Burlein C, Claudio G, et al. MK-5172, a selective inhibitor of hepatitis C virus NS3/4a protease with broad activity across genotypes and resistant variants. *Antimicrob Agents Chemother* 2012;**56**:4161−7.
138. Nageswara Rao D, Zephyr J, Henes M, Chan ET, Matthew AN, Hedger AK, et al. Discovery of quinoxaline-based P1-P3 macrocyclic NS3/4A protease inhibitors with potent activity against drug-resistant hepatitis C virus variants. *J Med Chem* 2021;**64**:11972−89.
139. Martino SD, Petri GL, De Rosa M. Hepatitis C: the story of a long journey through first, second, and third generation NS3/4A peptidomimetic inhibitors. What did we learn?. *J Med Chem* 2024;**67**: 885−921.
140. Lawitz E, Gane E, Pearlman B, Tam E, Ghesquiere W, Guyader D, et al. Efficacy and safety of 12 weeks *versus* 18 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin for hepatitis C virus genotype 1 infection in previously untreated patients with cirrhosis and patients with previous Null response with or without cirrhosis (C-WORTHY): a randomised, open-label phase 2 trial. *Lancet* 2015;**385**:1075−86.
141. Ng TI, Tripathi R, Reisch T, Lu L, Middleton T, Hopkins TA, et al. *In vitro* antiviral activity and resistance profile of the next-generation hepatitis C virus NS3/4A protease inhibitor glecaprevir. *Antimicrob Agents Chemother* 2017;**62**:e01620-17.
142. Lawitz E, Yang JC, Stamm LM, Taylor JG, Cheng G, Brainard DM, et al. Characterization of HCV resistance from a 3-day monotherapy study of voxilaprevir, a novel pangenotypic NS3/4A protease inhibitor. *Antivir Ther* 2018;**23**:325−34.
143. Matthew AN, Leidner F, Newton A, Petropoulos CJ, Huang W, Ali A, et al. Molecular mechanism of resistance in a clinically significant double-mutant variant of HCV NS3/4A protease. *Structure* 2018;**26**:1360−72.
144. Jensen SB, Fahnoe U, Pham LV, Serre SBN, Tang Q, Ghanem L, et al. Evolutionary pathways to persistence of highly fit and resistant hepatitis C virus protease inhibitor escape variants. *Hepatology* 2019; **70**:771−87.
145. Pennington LD, Hesse MJ, Koester DC, McAtee RC, Qunies AM, Hu DX. Property-based drug design merits a Nobel prize. *J Med Chem* 2024;**67**:11452−8.
146. Lamb YN. Pacritinib: first approval. *Drugs* 2022;**82**:831−8.
147. Verstovsek S, Odenike O, Singer JW, Granston T, Al-Fayoumi S, Deeg HJ. Phase 1/2 study of pacritinib, a next generation JAK2/FLT3 inhibitor, in myelofibrosis or other myeloid malignancies. *J Hematol Oncol* 2016;**9**:137.
148. Verstovsek S, Komrokji RS. A comprehensive review of pacritinib in myelofibrosis. *Future Oncol* 2015;**11**:2819−30.
149. William AD, Lee AC, Blanchard S, Poulsen A, Teo EL, Nagaraj H, et al., Discovery of the macrocycle11-(2-pyrrolidin-1-yl-ethoxy)-14,19-dioxa-5,7,26-triaza-tetracyclo. 19.3.1.1(2,6). 1(8,12)]heptacosa-1(25),2(26),3,5,8,10,12(27),16,21,23-decaene (SB1518), a potent janus kinase 2/fms-like tyrosine kinase-3 (JAK2/FLT3) inhibitor for the treatment of myelofibrosis and lymphoma. *J Med Chem* 2011;**54**:4638−58.
150. Kiss R, Sayeski PP, Keseru GM. Recent developments on JAK2 inhibitors: a patent review. *Expert Opin Ther Pat* 2010;**20**:471−95.
151. Janne PA. Challenges of detecting EGFR T790M in gefitinib/erlotinib-resistant tumours. *Lung Cancer* 2008;**60**:S3−9.
152. da Cunha Santos G, Shepherd FA, Tsao MS. EGFR mutations and lung cancer. *Annu Rev Pathol* 2011;**6**:49−69.
153. Tan F, Shen X, Wang D, Xie G, Zhang X, Ding L, et al. Lcotinib (BPI-2009H), a novel EGFR tyrosine kinase inhibitor, displays potent efficacy in preclinical studies. *Lung Cancer* 2012;**76**:177−82.
154. Tan F, Shi Y, Wang Y, Ding L, Yuan X, Sun Y. Icotinib, a selective EGF receptor tyrosine kinase inhibitor, for the treatment of non-small-cell lung cancer. *Future Oncol* 2015;**11**:385−97.
155. Shi Y, Zhang L, Liu X, Zhou C, Zhang L, Zhang S, et al. Icotinib *versus* gefitinib in previously treated advanced non-small-cell lung

cancer (ICOGEN): a randomised, double-blind phase 3 non-inferiority trial. *Lancet Oncol* 2013;**14**:953−61.
156. Ma XH, Tian TD, Liu HM, Li QJ, Gao QL, Li L, et al. Efficacy and influence factors of icotinib hydrochloride in treating advanced non-small cell lung cancer. *Eur Rev Med Pharmacol Sci* 2017;**21**: 266−74.
157. Zhang W, Zhang Y, Zhao Q, Liu X, Chen L, Pan H, et al. Long-term safety of icotinib in patients with non-small cell lung cancer: a retrospective, real-world study. *J Thorac Dis* 2020;**12**:639−50.
158. Luo Z, Gyawali B, Han S, Shi L, Guan X, Wagner AK. Can locally developed me-too drugs aid price negotiation? An example of cancer therapies from China. *Semin Oncol* 2021;**48**:141−4.
159. Shaw AT, Bauer TM, de Marinis F, Felip E, Goto Y, Liu G, et al. First-line lorlatinib or crizotinib in advanced ALK-positive lung cancer. *N Engl J Med* 2020;**383**:2018−29.
160. Roskoski RJ. Anaplastic lymphoma kinase (ALK) inhibitors in the treatment of ALK-driven lung cancers. *Pharmacol Res* 2017;**117**: 343−56.
161. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007;**448**:561−6.
162. Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. *Nat Rev Cancer* 2010;**10**: 760−74.
163. Shaw AT, Yeap BY, Solomon BJ, Riely GJ, Gainor J, Engelman JA, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol* 2011;**12**:1004−12.
164. Johnson TW, Richardson PF, Bailey S, Brooun A, Burke BJ, Collins MR, et al. Discovery of (10*R*)-7-amino-12-fluoro-2,10,16-trimethyl-15-oxo-10,15,16,17-tetrahydro-2*H*-8,4-(metheno) pyrazolo [4,3-*h*][2,5,11]-benzoxadiazacyclotetradecine-3-carbonitrile (PF-06463922), a macrocyclic inhibitor of anaplastic lymphoma kinase (ALK) and C-ROS oncogene 1 (ROS1) with preclinical brain exposure and broad-spectrum potency against ALK-resistant mutations. *J Med Chem* 2014;**57**:4720−44.
165. Solomon BJ, Bauer TM, Mok TSK, Liu G, Mazieres J, de Marinis F, et al. Efficacy and safety of first-line lorlatinib *versus* crizotinib in patients with advanced, ALK-positive non-small-cell lung cancer: updated analysis of data from the phase 3, randomised, open-label CROWN study. *Lancet Respir Med* 2023;**11**:354−66.
166. Drilon A, Camidge DR, Lin JJ, Kim SW, Solomon BJ, Dziadziuszko R, et al. Repotrectinib in fusion-positive non-small-cell lung cancer. *N Engl J Med* 2024;**390**:118−31.
167. Ou SHI, Azada M, Hsiang DJ, Herman JM, Kain TS, Siwak-Tapp C, et al. Next-generation sequencing reveals a novel NSCLC ALK F1174V mutation and confirms ALK G1202R mutation confers high-level resistance to alectinib (CH5424802/RO5424802) in ALK-rearranged NSCLC patients who progressed on crizotinib. *J Thorac Oncol* 2014;**9**:549−53.
168. Friboulet L, Li NX, Katayama R, Lee CC, Gainor JF, Crystal AS, et al. The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer. *Cancer Discov* 2014;**4**:662−73.
169. Drilon A, Ou SHI, Cho BC, Kim DW, Lees J, Lin JJ, et al. Repotrectinib (TPX-0005) is a next-generation ROS1/TRK/ALK inhibitor that potently inhibit ROS1/TRK/ALK solvent-front mutations. *Cancer Discov* 2018;**8**:1227−36.
170. Yun MR, Kim DH, Kim SY, Joo HS, Lee YW, Choi HM, et al. Repotrectinib exhibits potent antitumor activity in treatment-naive and solvent-front-mutant ROS1-rearranged non-small cell lung cancer. *Clin Cancer Res* 2020;**26**:3287−95.
171. Murray BW, Rogers E, Zhai DY, Deng W, Chen X, Sprengeler PA, et al. Molecular characteristics of repotrectinib that enable potent inhibition of TRK fusion proteins and resistant mutations. *Mol Cancer Therapeut* 2021;**20**:2446−56.
172. Dhillon S. Repotrectinib: first approval. *Drugs* 2024;**84**:239−46.
173. De Clercq E. The bicyclam AMD3100 story. *Nat Rev Drug Discov* 2003;**2**:581−7.
174. Bridger GJ, Skerlj RT, Thornton D, Padmanabhan S, Martellucci SA, Henson GW, et al. Synthesis and structure−activity relationships of phenylenebis(methylene)-linked bis-tetraazamacrocycles that inhibit HIV replication. Effects of macrocyclic ring size and substituents on the aromatic linker. *J Med Chem* 1995;**38**:366−78.
175. Saotome K, McGoldrick LL, Ho JH, Ramlall TF, Shah S, Moore MJ, et al. Structural insights into CXCR4 modulation and oligomerization. *Nat Struct Mol Biol* 2024;**32**:315−25.
176. Heusinkveld LE, Majumdar S, Gao JL, McDermott DH, Murphy PM. WHIM syndrome: from pathogenesis towards personalized medicine and cure. *J Clin Immunol* 2019;**39**:532−56.
177. Wang J, Tannous BA, Poznansky MC, Chen H. CXCR4 antagonist AMD3100 (plerixafor): from an impurity to a therapeutic agent. *Pharmacol Res* 2020;**159**:105010.
178. DiPersio JF, Uy GL, Yasothan U, Kirkpatrick P. Plerixafor. *Nat Rev Drug Discov* 2009;**8**:105−6.
179. Wagstaff AJ. Plerixafor: in patients with non-Hodgkin's lymphoma or multiple myeloma. *Drugs* 2009;**69**:319−26.
180. Brudy C, Walz C, Spiske M, Dreizler JK, Hausch F. The missing link (er): a roadmap to macrocyclization in drug discovery. *J Med Chem* 2024;**67**:14768−85.
181. Poongavanam V, Wieske LHE, Peintner S, Erdelyi M, Kihlberg J. Molecular chameleons in drug discovery. *Nat Rev Chem* 2024;**8**: 45−60.
182. Garcia JD, Vallaro M, Rossi SM, Apprato G, D'Agostini G, Rossetti P, et al. Chamelogk: a Chromatographic chameleonicity quantifier to design orally bioavailable beyond-rule-of-5 drugs. *J Med Chem* 2023;**66**:10681−93.
183. Bechtler C, Lamers C. Macrocyclization strategies for cyclic peptides and peptidomimetics. *RSC Med Chem* 2021;**12**:1325−51.
184. Diao Y, Liu D, Ge H, Zhang R, Jiang K, Bao R, et al. Macrocyclization of linear molecules by deep learning to facilitate macrocyclic drug candidates discovery. *Nat Commun* 2023;**14**:4552.
185. Zaretsky S, Yudin AK. Recent advances in the synthesis of cyclic pseudopeptides. *Drug Discov Today Technol* 2017;**26**:3−10.
186. Sengupta S, Mehta G. Macrocyclization *via* C−H functionalization: a new paradigm in macrocycle synthesis. *Org Biomol Chem* 2020;**18**: 1851−76.