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Review

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Therapeutic Potential of Spirooxindoles as Antiviral Agents

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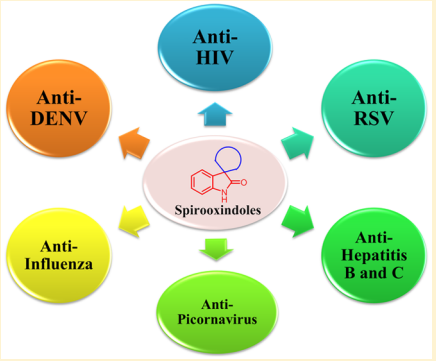
ABSTRACT: Antiviral therapeutics with profiles of high potency, low resistance, panserotype, and low toxicity remain challenging, and obtaining such agents continues to be an active area of therapeutic development. Due to their unique three-dimensional structural features, spirooxindoles have been identified as privileged chemotypes for antiviral drug development. Among them, spiro-pyrazolopyridone oxindoles have been recently reported as potent inhibitors of dengue virus NS4B, leading to the discovery of an orally bioavailable preclinical candidate (*R*)-**44** with excellent in vivo efficacy in a dengue viremia mouse model. This review highlights recent advances in the development of biologically active spirooxindoles for their antiviral potential, primarily focusing on the structure–activity relationships (SARs) and modes of action, as well as future directions to achieve more potent analogues toward a viable antiviral therapy.



KEYWORDS: *spirooxindoles, antiviral agents, HIV, RSV, DENV, influenza virus*

Over the past two decades, remarkable progress has been made toward the discovery and development of antiviral agents that could selectively inhibit viruses without causing toxicity. These antiviral agents inhibit the viral infection cycle through distinct mechanisms, including blocking the synthesis of viral proteins and nucleic acids, receptor recognition, entry, uncoating, virion assembly, and maturation.[1,2] A number of effective antiviral agents are now available in the market. Such success is exemplified by more than 30 clinically approved anti-AIDS drugs.[3] However, the options for available antiviral chemical space, including the diversity of chemical entities and new scaffolds, remain limited and effective against only a small group of pathogens, including human immunodeficiency virus (HIV), herpes simplex virus (HSV), varicella-zoster virus (VZV), human cytomegalovirus (HCMV), influenza virus, and hepatitis B and C viruses (HBV and HCV, respectively).[4] Moreover, these drugs are plagued by the increasing emergence of drug resistance, limited therapeutic efficacy, and severe drug side effects.[1,5,6] Additionally, for many other emerging and/or neglected life-threatening viral diseases (e.g., dengue fever and Zika virus), the lack of approved medications for clinical use represents an unmet medical need.[7] Hence, it is imperative to develop antiviral agents with novel chemotypes, antiresistance, panserotype activity, high efficacy, and low toxicity profiles, as well as increased affordability.

Spirocyclic compounds are notable for their unique three-dimensional structures as well as their broad biological activity.[8] In particular, spirocyclic oxindoles characterized by varied spiro rings fused at the C3 position of the oxindole core represent privileged scaffolds with prevalence in numerous natural products (e.g., **1**–**4**, Figure 1) and pharmacologically relevant drugs (e.g., **5**–**10**, Figure 1).[8−10] Their conformational restriction, imparted by the spiro-carbon, provides an excellent strategy to not only enforce the desired conformation for ligand–protein binding to enforce specificity and potency but also potentially increase molecular complexity to reduce P450 inhibition for better bioavailability and metabolic stability.[11,12] Moreover, owing to the synthetic challenges associated with this unique structure, there have been notable synthetic methodology advancements[13−15] that have provided a means by which spirooxindoles can be pursued as candidates for drug discovery. Although a number of spirooxindoles have been reported with different biological activities, fortunately, this class of molecules is not promiscuous such as those troublesome pan-assay interference compounds (PAINS).[16−19] Actually, some of them (e.g., compound **44** discussed later on) have been identified to be quite target-specific. To the best of our knowledge, the various bioactivities of spirooxindoles are primarily distinguished by the pattern and size of the spiro rings fused at the C3 position of the oxindole scaffold as well as specific substituent moieties on the spirocyclic oxindole core, thereby leading to the diverse but relatively specific pharmacological profiles of this class of molecules.

By using structure-based drug design and computational docking, natural alkaloids such as spirotryprostatin A (**1**) and alstonisine (**2**) were found to fit poorly into the mouse double minute 2 homologue (MDM2) protein cleft due to steric hindrance.[20] Thus, further structural simplification led to the identification of spiro-pyrrolidinyl MI-888 (**5**) as a potent nonpeptide inhibitor of p53/MDM2 interaction (K_i = 0.44 nM), which was reported to achieve rapid, complete, and long-

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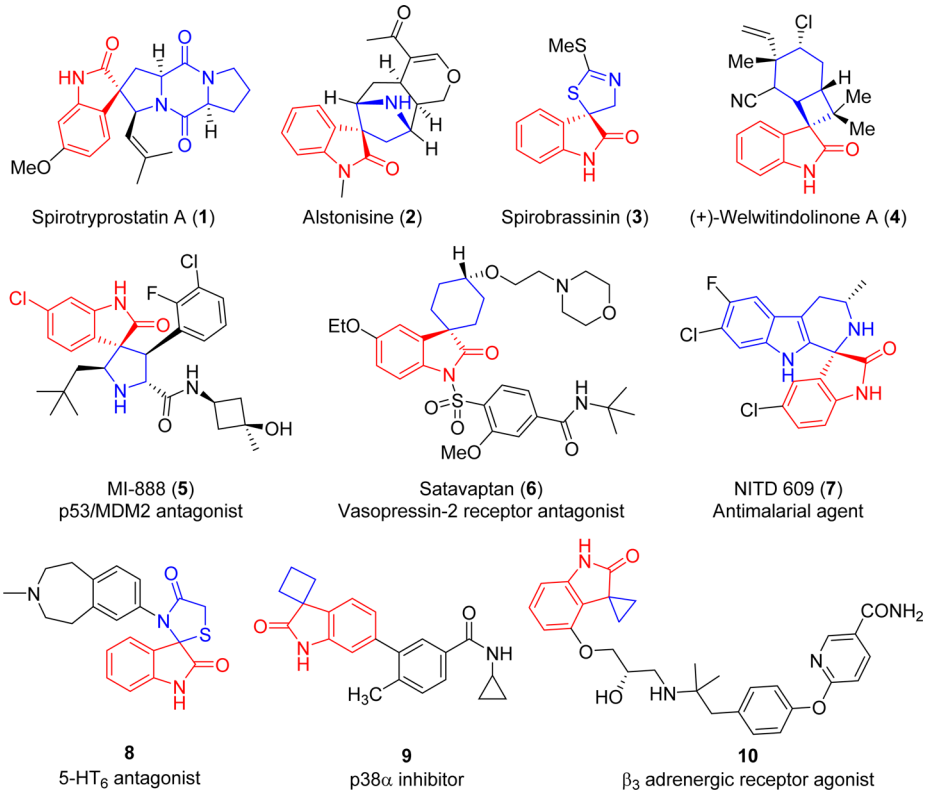

Figure 1. Selected examples of natural products and pharmaceutical drugs or lead compounds with spirooxindole scaffold, which is depicted with red and blue representing two key moieties.

lasting tumor regression in two types of xenograft models of human cancer, with oral administration.[21] Spiro-cyclohexyl satavaptan (**6**) was designed by Sanofi-Aventis and is a vasopressin-2 receptor antagonist under development for the treatment of hyponatremia[22] and ascites.[23] Spiro-tetrahydro-β-carboline NITD609 (**7**) is a synthetic antimalarial drug, an alternative to artemisinin derivatives, and eliminates the blood stages of *Plasmodium falciparum* and *Plasmodium vivax* clinical isolates at a low nanomolar concentration by inhibiting protein synthesis.[24] Given its good physicochemical properties and promising pharmacokinetic and efficacy profile, compound **7** has recently completed phase II studies in humans (NCT01860989; NCT01836458; NCT01524341). Spiro-thiazolidinyl cruciferous phytoalexin spirobrassinin (**3**) was found to have an antiproliferative effect against T-Jurkat leukemic cells,[25] whereas a similar synthetic framework, **8**, was identified as a potent 5-HT_6 antagonist after the introduction of benzazepine fragments.[26] Likewise, antifungal (+)-welwitindolinone A (**4**) is a densely functionalized and compact spiro-fused cyclobutyl oxindole alkaloid that was isolated from the marine blue-green algae *Hapalosiphon welwitschii* to overcome multiple drug resistance,[27] whereas its simplified analogue **9** is a potent and selective p38α mitogen-activated protein (MAP) kinase inhibitor and functions as a novel anti-inflammatory agent.[28] In addition, spirocylic oxindoles such as spiro-cyclopropyl **10** were reported to have therapeutic value for obesity, diabetes, and gastrointestinal and urinary diseases, functioning via a β_3 adrenoceptor agonism.[29]

Intriguingly, spirooxindoles have attracted much attention in the area of antiviral drug discovery and development in recent years, owing to the high number of positive hits encountered with this scaffold. Herein, we seek to review the current progress of spirooxindoles in the antiviral drug research field, including human immunodeficiency virus (HIV), human respiratory syncytial virus (RSV), dengue (DENV), and influenza virus.

■ ANTI-HIV AGENTS

HIV is a retrovirus that infects cells of the immune system, destroying or impairing their function. The most advanced stage of HIV infection is acquired immunodeficiency syndrome (AIDS), which is a global health threat and the leading cause of death due to infectious diseases by a single infectious agent. Since the beginning of the epidemic in 1981, almost 78 million people have been infected with the HIV virus and nearly 39 million people have died from HIV.[30] Combination anti-retroviral therapy (cART), especially once-daily fixed-dose combination (FDC) such as Abacavir/Lamivudine, Atazanavir/Cobicistat, and Darunavir/Cobicistat,[31−33] is the current standard of care regimen for AIDS patients and has led to a significant halt in the progression of AIDS and a decline of the mortality rates.[34,35] Nevertheless, the side-effect profile and the prevalence of drug resistance during long-term therapy may cause treatment failure.[36]

Many efforts have been made to develop new inhibitors specifically to overcome drug resistance problems that occur during the treatment of AIDS. Given that oxindoles currently utilized in several FDA-approved drugs may also interact and fill the P_2' region of HIV protease active site effectively, Ghosh et al.[37] designed and synthesized novel oxindole-derived inhibitors with the aid of a high-resolution X-ray crystal structure of the HIV protease with Darunavir (**11**, Figure 2). This work resulted in a second-generation protease inhibitor approved by the FDA on June 23, 2006, to combat mutant

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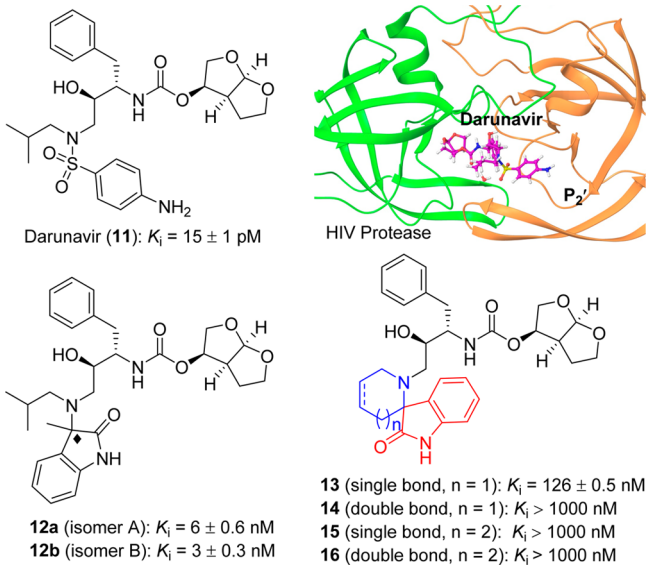

Figure 2. Selected examples of HIV protease inhibitors with spirooxindole scaffold and the cocrystal structure of **11** and HIV protease (PDB code 2IEN).

strains of HIV. Oxindoles **12a** and **12b** have shown antiviral potencies of 6 and 3 nM, respectively, with only a slight difference between the two diastereomers. Considering that constrained rings in the HIV protease active site significantly improved enzyme inhibitory activity,[38−40] the feasibility of spirocyclic oxindole derivatives as P_2'-ligands has also been examined. Unfortunately, there was a dramatic reduction in inhibitory potency for the corresponding six- and seven-membered unsaturated and saturated spirocyclic compounds, such as **13**−**16**. The basis for this potency loss was revealed by docking that showed oxindole carbonyls of the spirocyclic derivatives do not overlap with the sulfone oxygen of **11** that effectively interacts with the tightly bound water molecule in the active site.[37]

Non-nucleoside reverse transcriptase inhibitors have been viewed as therapeutic agents with the potential to solve drug resistance problems. Using a cell-based HIV reporter infection screening assay, hit **17**, an oxindole with a unique spiral cyclopropane moiety (Figure 3), was identified as an HIV

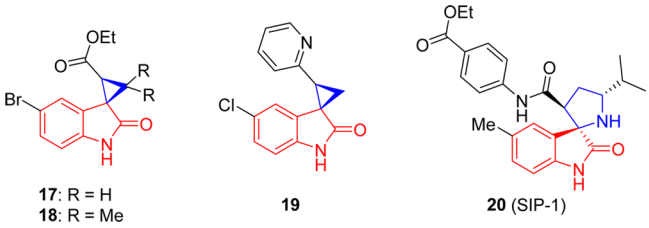

Figure 3. Selected examples of HIV non-nucleoside reverse transcriptase inhibitors with spirooxindole scaffold.

reverse transcriptase inhibitor with an EC_{50} value of ~75 nM.[41] The relative stereochemistry around the cyclopropane moiety, confirmed during a later synthesis, was found to be crucial for its antiviral activity due to its specific interactions with reverse transcriptase.[41] Systematic structural modifications have been conducted to establish preliminary SAR, including multiple substituents on the aromatic ring and various substituents on the cyclopropane, the lactam moiety, and the ester group, leading to the discovery of the early lead compound **18** with a *gem*-dimethyl on the cyclopropane.[41] Although lead **18** displayed significantly improved potency with an EC_{50} of 15 nM, it unfortunately exhibited high clearance, low exposure, and limited oral bioavailability (F = 27%), possibly associated with its poor rat liver microsomal stability in vitro.[42] Considering that the ester moieties may be the cause of its metabolic instability, further efforts to replace them with bioisosteres, including tetrazole, furan, thiophene, pyrrole, thiazole, imidazole, pyridine, and phenyl and biaryl rings, have been carried out. 2-Pyridinyl analogue **19** exhibits a surprisingly potent inhibition of HIV replication, with an EC_{50} of 8 nM. As expected, **19** has enhanced water solubility and metabolic stability in vitro, as well as improved pharmacokinetic properties in vivo, with an F value of 46%. Although it was reported to have antiviral activity against L100I, I135T, I135V, E138K, and F227L mutant viruses, poor antiviral activity was found against K103N, Y188C, and Y188L.[42] Further optimization would be required to achieve potency in all NNRTI-resistant mutant viruses.

In addition, viral protein R (Vpr), a small protein encoded in the central region of the HIV-1 genome, is also an ideal target for inhibiting HIV-1 replication due to its unique functions in nuclear import,[43] induction of cell cycle arrest at the G2 phase,[43] apoptosis regulation,[43−45] and splicing inhibition.[46] Aida et al.[47] developed novel chemical arrays as a useful method to screen compounds that bound recombinant Vpr purified from transfected COS-7 cells. Among these compounds, spirooxindole SIP-1 (**20**, Figure 3) was shown to significantly inhibit Vpr function via binding to its functional domain, thereby displaying high inhibitory activity against HIV with an IC_{50} of 0.5 μM and reducing the levels of p24 by >98% in macrophages after 8 or 12 days of infection.[47] Moreover, **20** has no cytotoxic effects and does not disrupt cell cycle progression or induce apoptosis of Molt-4 and HeLa cell lines as was measured by the MTT assay, flow cytometry analysis, and a caspase-3 assay.

■ ANTI-RSV AGENTS

RSV is a large RNA virus and a member of the Paramyxoviridae family, subfamily Pneumoviridae. It is a major cause of respiratory tract infections in people of all ages worldwide. Because natural human immunity against RSV is incomplete, nearly every child has been infected by RSV at least once before the age of 3. Severe exposures to RSV can cause infection in normal adults and older children, largely upper respiratory tract infections, often leading to bronchiolitis and pneumonia that may require hospitalization. However, exposure to RSV at a young age may cause predominately lower respiratory tract infections, leading to recurrent wheezing and asthma.

There are limited effective treatment options against RSA infection for the above-mentioned patient populations. RespiGam (RSV-IG)[48] and Synagis (palivizumab),[49] developed by MedImmune (CA, USA), are a polyclonal-concentrated RSV neutralizing antibody and a humanized monoclonal antibody against RSV fusion (F) protein, respectively, and are intended for prevention and treatment in preterm and high-risk infants. However, both are very costly, leading to low affordability, and require parenteral administration. Unfortunately, a newer version monoclonal antibody, motavizumab, failed to show additional benefit over palivizumab in recent phase III human clinical trials.[50] Moreover, there is no RSV vaccine available for human use, despite many attempts in

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subunit vaccine and live-attenuated vaccine approaches.[51] The fate of RNAi therapeutics against RSA such as ALN-RSV01 is still pending and relies on the result of phase II clinical trial.[52]

Virazole (ribavirin), a nucleoside analogue, is the only approved antiviral drug as an aerosol treatment of serious RSV infection in hospitalized children. Due to its aerosol administration, low affordability, limited efficacy, and toxicity profile (e.g., teratogenicity), it is rarely used in the clinic. Although a number of small-molecule RSV inhibitors have been discovered, only a few have reached phase I or II clinical trials, including the first nucleocapsid (N) protein inhibitor RSV-604 developed by AstraZeneca.[53]

Nevertheless, safe and effective treatment of RSV disease is in urgent need. Direct-acting antivirals, specifically RSV fusion protein inhibitors, have been the focus of numerous drug development programs.[54] BMS-433771 (**21**, Figure 4) is a benzimidazole-based representative RSV fusion inhibitor and has been reported to exhibit excellent potency in four mammalian species and efficacy in mouse and cotton rat infection models treated by oral administration.[55] Mechanistic studies using a photo affinity probe have established that compound **21** interferes with the formation of the 6-helix bundle of the RSV F1 protein. This 6-helix bundle formation is an essential step in the fusion of virus and host cell membranes that occurs in the latter stages of the entry process.[56] **21** has thus attracted much attention from leading biopharmaceutical entities. Moreover, the recently resolved cocrystal structure of **21** with the assembled trimer of the N-terminal heptad repeat element of the RSV F1 protein[57] has provided a tremendous opportunity to explore the molecular space and possibly discover novel scaffolds based on **21** (Figure 5).

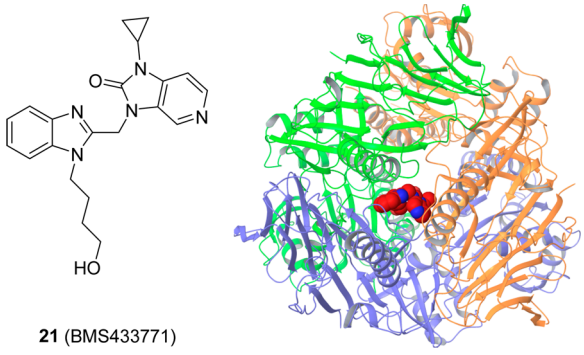

Figure 4. Structure of BMS433771 (**21**) and the cocrystal structure of **21** and 3-fold-symmetric cavity in prefusion RSV fusion protein (PDB code 5EA7).

Viral Ltd.[58] first introduced the spiral exocyclic *N*-*c*-propyl group onto the 3-position of oxindole, which led to a novel series of compounds such as **22**−**24** with very weak activity (EC_{50} > 80 μM; Figure 5), likely due to the lack of hydroxyl group interacting with the viral protein. However, when the terminal hydroxyl group was reintroduced, inhibitory activity against RSV long strain was regained even if the benzimidazole was reduced to tetrahydrobenzimidazole (**25**). **25** displayed an EC_{50} value of <0.1 μM and a CC_{50} value of >100 μM in viral cytopathic effect (CPE) assays.[59] When a methylsulfonyl group was further introduced to the terminal, Roche Ltd.[60] identified compounds with activity and high potency against RSV long strain. Additionally, SAR studies showed that the 5-chloro on the benzimidazole gave the best activity. Further addition of a halogen atom onto the 4-position of the indolone fragment resulted in compounds **26**−**28**, which display excellent inhibitory effects against RSV in the order F > Cl > Br (Figure 5), but all of them were less potent than the 6-aza-oxindole analogue **29** (EC_{50} = 12 nM). Replacement of the benzimidazole ring in **29** with an indole, by deleting a nitrogen atom, resulted in compounds **30**, **34**, and **35**, with 2−4-fold enhanced EC_{50} values. However, modification of the benzimidazole ring in **29** into its structural isomers aza-indoles **31**−**33** led to a minor reduction in potency. A minor extension of the flexible methylsulfonyl linker length of **34** from two to three carbons (**35**) retained potency, with an IC_{50} value of 6 nM.[60] Further studies indicated that inserting a pyridyl ring

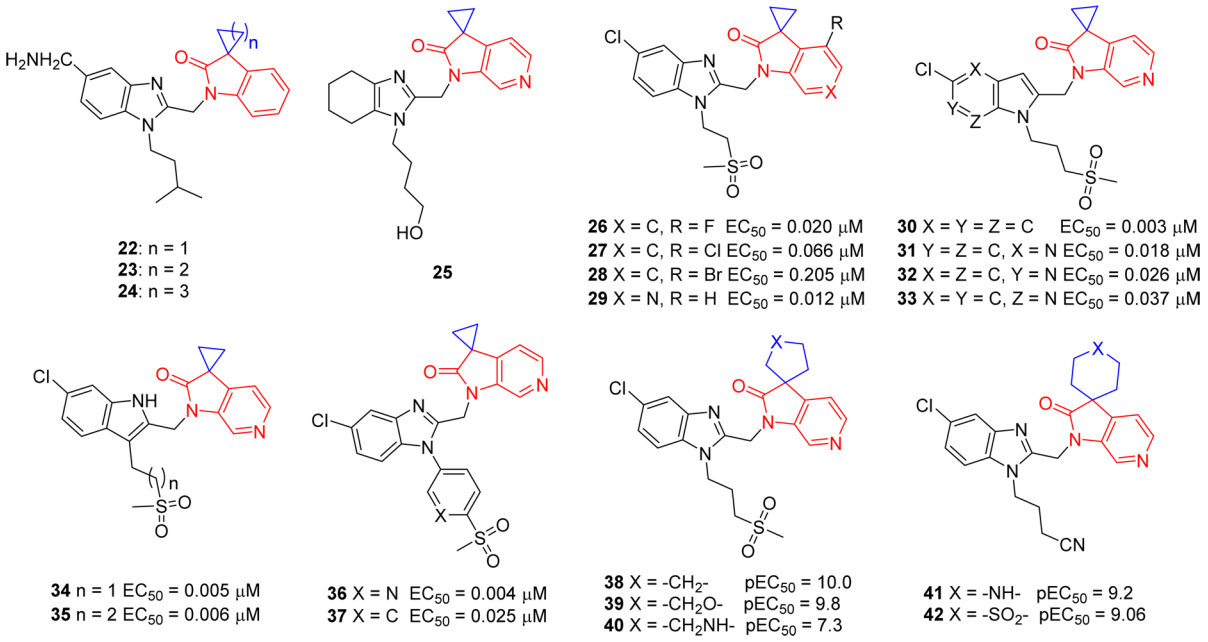

Figure 5. Selected examples of RSV fusion inhibitors with spirooxindole scaffold.

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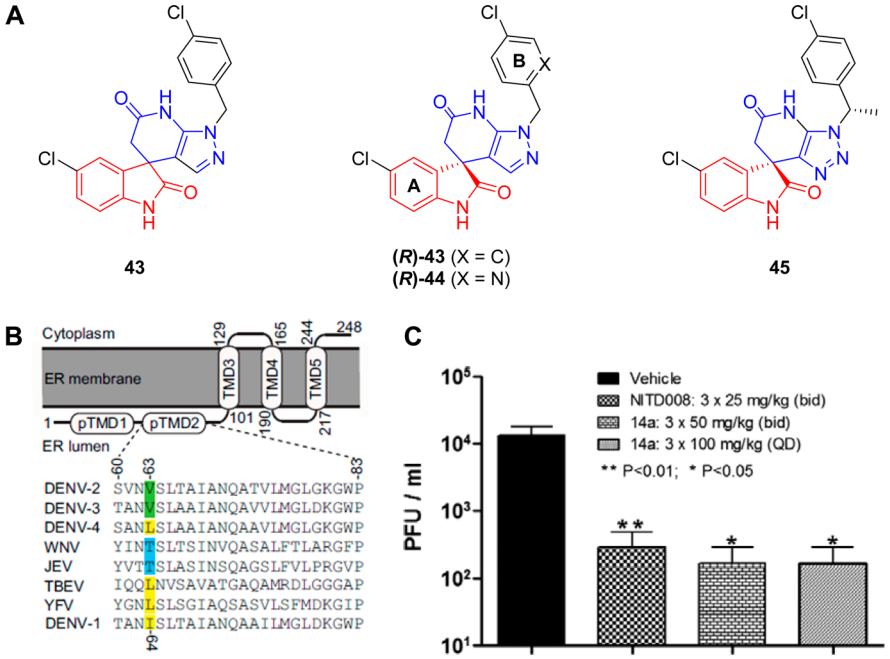

Figure 6. (A) Selected examples of DENV NS4B inhibitors with spirooxindole scaffold. (B) Membrane topology and sequence alignment of the DENV NS4B protein. Reprinted with permission from ref 72. Copyright 2015 American Society for Microbiology Limited. (C) DENV-2 in vivo mouse efficacy of compound (*R*)-**44**. Reprinted with permission from ref 73.

(**36**) as a structural constrained linker continued to retain potency, whereas the phenyl ring addition (**37**) led to a 5-fold decrease in potency.[61] These findings suggest that the length and shape of the linker on a benzimidazole can be well scrutinized and optimized. Janssen Ltd.[62] attempted to enlarge the spiro-cyclopropyl ring to a four to six-membered ring (**38**–**42**), including cyclobutyl, azetidyl, piperidyl, oxetyl, cyclopentyl, tetrahydro-2*H*-pyran, and tetrahydro-2*H*-thiopyran 1,1-dioxide. Interestingly, compound **38** displayed a promising potency, with an EC_{50} value of 0.1 nM. In addition, replacement of the terminal methylsulfonyl (**40**) with a cyano group (**41**) was reported to improve potency by 79-fold against rgRSV224 virus.

■ ANTI-DENV AGENTS

Dengue is the fastest growing mosquito-borne viral disease, threatening roughly half of the world's population and causing nearly 400 million infections each year.[63] Additionally, the incidence of dengue has increased 30-fold over the past 50 years due to expanded urbanization, mobility of populations, and climate changes.[64] There are currently several promising vaccine candidates in clinical trials,[65] whereas the first vaccine, Dengvaxia, developed by Sanofi, was approved in Mexico on December 9, 2015, for the prevention of disease caused by all four dengue virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) in patients 9–45 years of age living in endemic areas.[66] For antiviral development, only repurposed compounds have been tested in dengue clinical trials, including the nucleoside inhibitor balapiravir,[67] α-glucosidase inhibitor celgosivir,[68] malaria drug chloroquine,[69] corticosteroid drug prednisolone,[70] and lovastatin for lowing cholesterol level.[71] However, none of them have shown significant antiviral activity or clinical benefits in dengue patients.

In an effort to develop safe and effective antiviral therapeutics, Wang et al.[72] identified spiropyrazolopyridones as a novel chemical class of anti-DENV agents by utilizing a high-throughput phenotypic screening of the Novartis compound library. Hit **43** (Figure 6A) was shown to inhibit the DENV-2 replicon with an EC_{50} value of 14 nM. Because **43** is a racemate, two enantiomers were subsequently separated by chiral column HPLC and led to isolation of the *R*-enantiomer (*R*)-**43** with an EC_{50} value of 12 nM, which is 83-fold more potent than the *S*-enantiomer. Further antiviral spectrum analysis of (*R*)-**43** against a panel of flaviviruses surprisingly revealed that (*R*)-**43** inhibits only DENV-2 and -3, but not other viruses including DENV-1 and -4. Resistance analysis and direct ligand-protein binding confirmed that variations at amino acid 63 of DENV nonstructural protein 4B (NS4B), a nonenzymatic transmembrane protein functioning as an essential component of the viral replication complex, were responsible for the observed resistance in DENV-2 and -3 (Figure 6B).[72] Thus, it confirms that (*R*)-**43** specifically targets DENV NS4B to block viral RNA synthesis or accumulation. Although (*R*)-**43** was found to be poorly soluble in aqueous media (11 μM) and highly lipophilic with a LogP value of 4.9, (*R*)-**43** remains a promising starting point for lead optimization efforts in an attempt to improve the solubility while retaining or improving its potency. Utilizing a three-component condensation reaction of aminopyrazoles, commercially available isatin derivatives, and Meldrum's acid, Zou et al. quickly provided access to a series of spiropyrazolopyridone analogues for SAR investigation mainly focused on A- and B-ring modifications.[73] It has been further confirmed by chiral column HPLC separation of racemates that *R* enantiomers tend to be significantly more potent than *S* enantiomers. Meanwhile, it has remained that both of the amide carbonyl groups appear to be important for the activity, whereas neither of the NH groups is critical for the potency. Cytotoxicity studies have indicated that these compounds portray advantageous toxicity selectivity for dengue-infected cells over normal cells.

Preliminary SAR studies following promising initial results have led to the discovery of a new chemical lead, (*R*)-**44**

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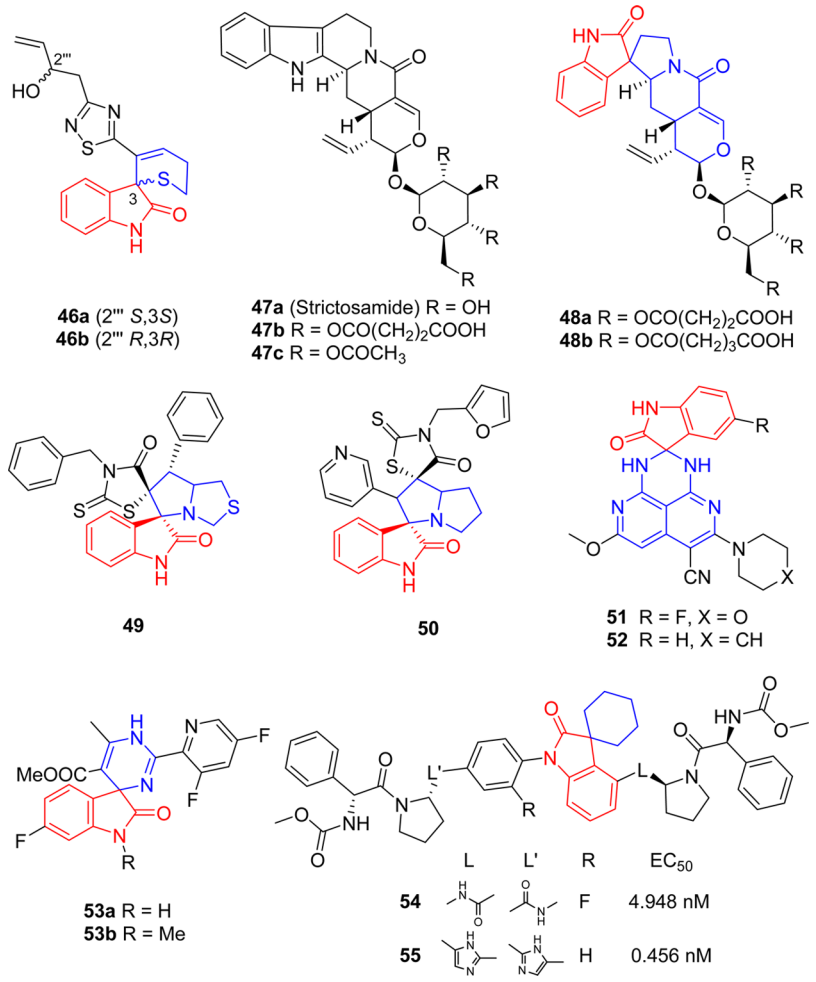

Figure 7. Selected examples of other antiviral inhibitors with the spirooxindole scaffold.

(Figure 6A). It is worth mentioning that (*R*)-**44** has been found to be quite target-specific in the exploration of off-target effects. Although (*R*)-**44** exhibited slightly decreased potency against DENV-2 in the secondary viral-titer reduction assay (EC_{50} = 42 nM), it possesses an enhanced aqueous solubility (504 μM), an improved LogP value of 2.8 and a moderate mouse microsomal stability (hepatic extraction ratio 71%; $t_{1/2}$ 24 min). These characteristics led to better in vivo pharmacokinetics with higher C_{max} and exposure (AUC) as well as increased oral bioavailability (*F* = 63%) in rats, when compared to those of compound (*R*)-**43**. Furthermore, (*R*)-**44** demonstrates favorable in vivo efficacy in a DENV-2 viremia mouse model (Figure 6C). When compound (*R*)-**44** was orally administered at 50 mg/kg twice daily (bid) for 3 days or orally dosed at 100 mg/kg once daily (QD) for 3 days, compared to vehicle control group, significant viremia reduction in mice was achieved. This result provides a valuable proof of concept that DENV NS4B protein is a valid and druggable antiviral target for dengue drug development.

After validation that DENV NS4B protein is a valid anti-DENV target, further modification was conducted by adding a nitrogen atom within a pyrazole ring and led to a new series of triazole analogues.[74] Among these, compound **45** (Figure 6A) displayed the highest inhibitory activity against DENV-2 and DENV-3 in CFI assays with EC_{50} values of 14 and 2.5 nM, respectively, whereas three additional isomers showed moderate to low activity. Although the potency against DENV-3 nearly matches the DENV-2 activity, the major weakness of (*R*)-**43**−**45** is their lack of efficacy against other serotypes such as DENV-1 and -4. Because the amino acid variation among the four DENV serotypes is 30−35%,[75] it will be a challenging but essential endeavor for medicinal chemists to achieve panserotype activity for successful drug development.

■ ANTI-INFLUENZA VIRUS AGENTS

Influenza virus is a prominent cause of upper respiratory tract infection disease and associated with high morbidity and mortality. In particular, China is considered an area with a high rate of influenza infection and influenza-associated morbidity. Globally, prevention and treatment of influenza currently relies on vaccines and fast-acting antiviral agents. In addition, traditional Chinese therapeutics have been used clinically for influenza therapy.

Isatis indigotica is a biennial herbaceous plant, and as a traditional Chinese medicine it has been used for the treatment of various ailments, especially influenza, cold, fever, and infections.[76] A pair of enantiomers (**46a** and **46b**; Figure 7) characterized by the 5′,6′-dihydrospiro[indoline-3,2′-thiopyran]-2-one and 3″-(2‴-hydroxybut-3‴-en-1‴-yl)-1″,2″,4″-thiadiazole moieties, which had never been presented in a natural product, was isolated by Chen et al. from aqueous extracts of the *I. indigotica* root.[76] In vitro assays demonstrated[77] that compounds **46a** and **46b** show similar antiviral activities against the herpes simplex virus 1 (HSV-1) with IC_{50} values of 33.33

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and 25.87 μM as well as SI values of 2.0 and 3.9, respectively. Compound **46a** also inhibited the influenza virus A/Hanfang/359/95 (H3N2), with IC_{50} and SI values of 33.33 μM and 3.0, respectively, while **46b** was found to be inactive (IC_{50} > 100 μM).

Strictosamide (**47a**) is a glycoalkaloid that has been isolated from leaves and roots of *Sarcocephalus latifolius* (Smith) Bruce (*Nauclea latifolia* Sm.), and it has been used as a medicinal plant in folk medicine due to its antiproliferative, antiparasitic, antimicrobial, antimalarial, anti-inflammatory, antiviral, and other suggested biological activities.[78] However, the poor aqueous solubility, short half-life, and poor efficacy of strictosamide has limited its clinical application. Xu et al. has reported structural modifications that led to two series of strictosamide derivatives represented by **47b** and **47c** and spirooxindoles **48a** and **48b** that were synthesized by a rearrangement reaction of **47c** under the treatment of NBS/H_2O/THF (Figure 7).[79] Compounds **47b** and **47c** were reported to possess high antiviral activities against influenza A virus (A/Jinan/15/90) with IC_{50} values of 4.12 and 12.35 μg/mL, respectively (**47a**: IC_{50} = 25.68 μg/mL). However, compounds **48a** and **48b** have shown very weak antiviral activity against influenza A virus and respiratory syncytial virus (IC_{50} > 110 μg/mL). Surprisingly, compounds **48a** and **48b** were revealed to have moderate antiproliferative effects against five human cancer cell lines (HepG2, A549, KB, MCF-7, and K562), with IC_{50} values in a range of 28.85−39.06 μM.

To efficiently construct the spirooxindole core, Kurbatov et al. earlier designed a polar [3 + 2] cycloaddition reaction of isatin, thiaproline, and arylidene derivatives of rhodanine to synthesize bis-spirocyclic compounds, such as **49**, containing spiro units at the 1,2-positions of the pyrrolizidine (isothiapyrrolizidine) moiety (Figure 7).[80] Later, the 1,3-dipolar addition reaction of unstabilized azomethine ylides generated in situ from isatin and proline (isothiaproline), with hetarylidene-substituted rhodanines, provided another novel bis-spiro heterocycle, such as **50**, with spiro units at the 1,3-positions of the pyrrolizidine (isothiapyrrolizidine) moiety. Among these, compound **50** exhibited the most potent inhibitory activity against the influenza virus A/California/07/09(H1N1)-pdm2009 replication (EC_{50} = 1.9 μg/mL) with high toxicity (CTD_{50} = 37 μg/mL), whereas compound **49** showed the lowest toxicity (CTD_{50} = 415 μg/mL) with moderate inhibition (EC_{50} = 26.0 μg/mL).[80]

OTHER ANTIVIRAL AGENTS

Picornaviruses represent a major health burden, yet antiviral treatments are not available. By using gene-trap mutagenized human haploid cells for screening picornaviral host-dependent factors, Burckstummer et al. found that human cells lacking PLA2G16, a poorly characterized phospholipase A2, are largely resistant to infection by all of the picornaviruses tested, including polio virus, coxsackie viruses, rhinoviruses, and encephalomyocarditis virus. However, those cells did not lose susceptibility to non-picornaviruses, such as vesivular stomatitis virus, influenza virus, adenovirus, and herpes simplex virus.[81] To identify small-molecule inhibitors of PLA2G16 for combating picornavirus infection, a drug discovery program was subsequently initiated. This effort resulted in 25 μM concentration, single-replicate screening of 24000 unrelated and highly diverse compounds, which originated from chemical diversity libraries (Otava and Enamine), focused libraries for kinases, chromatin modifiers, the NIH clinical collection, and others. Spirooxindoles **51** and **52** (Figure 7), identified from the aforementioned screen, were found to exhibit similar inhibitory activities against PLA2G16, with IC_{50} values of 7 and 6 μM, respectively.[82] However, similar compounds such as the 4,4-disubstituted-1,4-dihydropyrimidines **53a** and **53b**, developed by Janssen R&D Ireland, show weak anti-HBV activities using stable transfected cell lines HepG2.2.15 and HepG2.117 (IC_{50} > 25 μM).[83]

Hepatitis C virus (HCV) infects >3% of the world's population, consequently leading to an increased risk of steatosis, cirrhosis, and hepatocellular carcinoma. HCV non-structural protein 5A (NS5A) is a zinc-binding and proline-rich hydrophilic phosphoprotein that plays a key role in the virus replication cycle, facilitating both replication of the genomic RNA and assembly of the virion as well as modulating host cell factors to create an hospitable environment for the virus.[84−86] With the successful discovery and the recent approval of Daclatasvir and Ledipasvir,[87−89] NS5A inhibitors have emerged as a clinically relevant class of HCV therapeutic agents that may offer a promising replacement for the current standard of care, a combination of pegylated interferon alfa and ribavirin, which can be poorly tolerated and is often ineffective against the most prevalent genotype of the virus, genotype 1.[90−94] Therefore, recent successes stimulate considerable interest in the design and development of NS5A inhibitors. Spirooxindoles as unique scalffolds have been used to replace the central biphenyl of Daclatasvir and the fluorene ring of Ledipasvir and have led to a novel series of NS5A inhibitors such as **54** and **55** with excellent EC_{50} values of 4.95 and 0.45 nM, respectively, against HCV1b genotype (Figure 7).[95] Moreover, both analogues have also shown moderate potency against HCV1a genotype (EC_{50} > 400 nM).

CONCLUSIONS AND FUTURE DIRECTIONS

Due to potential problems exhibited by current antiviral drugs including toxicity, mutagenicity, limited efficacy, poor bioavailability, racemization of chiral centers, and difficulty of synthesis, the search for a new generation of effective antiviral agents with better drug properties and affordability continues to be an active area of medicinal chemistry and antiviral research. Spirooxindoles have emerged as attractive scaffolds with unique structural architecture and noticeable biological activity.

Nevertheless, numerous reported spirooxindoles with structural novelty have not been evaluated for antiviral activity, which limits hit discovery for new antiviral agents. Considering that natural products have emerged as a great resource for the discovery of various therapeutic agents,[96] structural modification on the template of oxindole alkaloids such as **46a** and **46b** and strictosamide (**47a**) may provide an alternative method to identify antiviral drug candidates with improved potency, aqueous solubility, and bioavailability. Moreover, although some spirooxindoles have previously been identified with antiviral activity, such as the DENV 4B inhibitors (*R*)-**43** and (*R*)-**44**, their exact molecular target and mechanism of action remain to be elucidated, thereby increasing the difficulty for further rational drug design and discovery. Furthermore, many additional challenges based on currently available synthetic routes need to be addressed for further lead optimization, such as how to efficiently generate the pure enantiomers or diastereomers. Although chiral separation to yield pure enantiomers such as **20** and (*R*)-**43**−**45** is feasible in laboratories, applications in large-scale production or manufacturing could be costly and time-consuming.

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Despite the exciting work and promising results on spirooxindoles as potential antiviral drugs as discussed above, challenges and opportunities remain for pharmacologists, medicinal chemists, virologists, and biologists to make synergistic efforts to eventually advance this class of molecules into a viable antiviral therapy. In terms of future directions, it is imperative to evaluate the antiviral activity of an expanded panel of spirooxindole-based chemical entities and to develop new methodologies, tool molecules, and biomarkers for elucidation of relevant molecular targets and exact mechanisms of action of active compounds. Meanwhile, there is an urgent need for structural biologists to resolve the X-ray crystallography of target−ligand complexes so as to facilitate rational antiviral drug design using modern drug discovery techniques such as structure-based drug design,[97−99] computer-aided drug design,[100−103] and fragment-based drug design (FBDD).[104−107] In addition, extensive efforts from medicinal and organic chemists will provide efficient synthetic approaches for creating novel compound libraries of spirooxindole analogues necessary for significant SAR explorations toward antiviral drug discovery.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

HIV, human immunodeficiency virus; HSV, herpes simplex virus; VZV, varicella-zoster virus; HCMV, human cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; MAP, mitogen activated protein; SI, selection index; AIDS, acquired immunodeficiency syndrome; HAART, highly active antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitors; Vpr, viral protein R; RSV, respiratory syncytial virus; DENV, dengue virus; NS4B, nonstructural protein 4B; NS5A, nonstructural protein 5A; NBS, *N*-bromosuccinimide; THF, tetrahydrofuran; IC_{50}, concentration causing 50% inhibition of antiviral activity; TC_{50}, 50% toxic concentration; CC_{50}, 50% cytotoxicity concentration SI = TC_{50}/IC_{50}

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