The Development of Biologically Important Spirooxindoles as New Antimicrobial Agents

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Abstract: Background: Antibiotic resistance is one of the biggest threats to global health today, leading to higher medical costs and increased mortality. Because of the emergence and rapid spread of new resistance mechanisms globally, a growing number of infections are becoming harder to treat as the antibiotics used to treat them become less effective. Therefore, the development of new effective antimicrobial agents is still urgently needed. In last decades, a large number of structurally novel spirooxindoles have been synthesized mainly based on the ylide intermediates generated \textit{in situ} and further assessed for their antimicrobial activity against different types of bacteria, leading to the discovery of some potent lead compounds with antimicrobial potentials.

Objective: The aim of this review to summarize recent advances on the synthesis, structure-activity relationships studies (SARs) and antimicrobial activity of spirooxindoles.

Method: Peer-reviewed research work on spirooxindoles with antimicrobial activity were downloaded from bibliographic databases and analyzed based on their chemotypes.

Results: 50 papers were retrieved from the literature databases, of which 20 papers described the synthesis and antimicrobial activity of spirooxindoles.

Conclusion: This review highlights the importance of spirooxindoles as potential antimicrobial agents. The antimicrobial activity of spirooxindoles against different types of bacteria is less studied, mainly centering on primary antimicrobial assessment, some of these compounds have showed interesting antimicrobial activity. However, the current study is only limited to primary antimicrobial assessment, no detailed modes of action are investigated.

Keywords: Spiro compounds, Spirooxindoles, Oxindoles, Chemical synthesis, Antimicrobial activity, Antifungal activity.

1. INTRODUCTION

Antibiotic resistance is one of the biggest threats to global health today, leading to higher medical costs and increased mortality [1]. Because of the emergence and rapid spread of new resistance mechanisms globally, a growing number of infections are becoming harder to treat as the antibiotics used to treat them become less effective. The US Centers for Disease Control and Prevention (CDC) estimates that there are about 23,000 people who die every year from antibiotic-resistant infections in the US. There is currently a shortage of effective therapies, lack of successful prevention measures, and only a few new antibiotics, which require
The discovery of new structural scaffolds has recently been recognized as an efficient strategy to find biologically promising molecules that can target some biological sites and explore more chemical space [4, 5] as some already known scaffolds have failed to target biologically relevant sites, especially the undruggable targets [6]. Of particular interest are spiro compounds, which have attracted ever increasing attention due to their highly pronounced biological properties [7, 8], interesting conformational features and unique 3D structural features. It is well recognized that spirocyclic compounds have a reduced conformational entropy upon binding to a protein target and conformational restriction, which make them the promising scaffolds in drug discovery [9]. In particular, spirooxindoles have emerged as attractive synthetic targets because of their prevalence in a large number of biologically validated natural alkaloids and pharmaceutically relevant molecules [10-15]. The key structural characteristic of these compounds is the spiro ring fused at the C3 position of the oxindole core with varied heterocyclic motifs. These spirooxindoles seem to be promising candidates for drug discovery, since they incorporate both oxindoles and other heterocyclic moieties simultaneously. Spirooxindole is a privileged heterocyclic motif that exists in pharmaceutical candidates and naturally occurring compounds. Spirooxindole (Highlighted in red in Fig. 1) containing compounds have exhibited diverse biological properties, such as anticancer [16-21], antimicrobial [22, 23], antivirus [24], etc. Representative examples are NITD609 (also known as Cipargamin) [25], CFI-400945 (the first PLK4 inhibitor) [26, 27], SAR405838 (MDM2 inhibitor) [28, 29] and APG-115 (MDM2 inhibitor) (Fig. 1) [30], which are currently being evaluated in clinic for the treatment of malaria and human cancers, respectively. In last decades, much efforts have been devoted to the development of new spirooxindole-based antimicrobial agents [31]. In this review, we first summarized the advances of antimicrobial spirooxindoles from the chemistry and biology perspectives with a hope of providing an overview of spirooxindoles with antimicrobial potentials and possible directions for designing more potent antimicrobial agents. This review is organized based on the spiro rings attached to the C3 position of the oxindole scaffold.

2. THE DEVELOPMENT OF NEW SPIROOXINDOLES AS ANTIMICROBIAL AGENTS

2.1. Spiropyrrrolidine Oxindoles

In 2014, Askri et al. synthesized a series of spiro[pyrrolidin-2,3-oxindoles] 5a-r through the exo-selective 1,3-dipolar cycloaddition reaction of the stabilized azomethine ylides 3 with various (E)-3-arylidene-
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Scheme 1. Synthesis of bis-spirooxindoles 5a-r with antimicrobial activity.

Scheme 2. Synthesis of new spirooxindoles as antibacterial and anti-fungal agents.

1-phenyl-pyrrolidine-2,5-diones 4. The ylides 3 were generated in situ by thermal prototropy of the corresponding iminoesters 2 with (E)-3-arylidene-1-phenyl-pyrrolidine-2,5-diones 4 as dipolarophiles (Scheme 1) [32]. Interestingly, other possible diastereoisomers 6a-r were not observed. All products were obtained in good yields (63-95%) and with high regio- and stereoselectivity regardless of the electronic properties of the substituents at the para-position of the aryl groups. Among these series, compounds 5i (R1 = Br, R2 = H, Ar = p-CIPh) and 5p (R1 = H, R2 = Me, Ar = Ph) showed comparable activity with ampicillin against Escherichia coli with the MIC value of 62.5 µg/mL. In the case of Pseudomonas aeruginosa, compound 5r (R1 = H, R2 = Me, Ar = 4-CIPh) was found to be the most active in vitro with the MIC of 62.5 µg/mL against MTCC 1688. Compound 5f (R1 = Br, R2 = H, Ar = Ph) was found to be the most active derivative in vitro against Staphylococcus aureus MTCC 96 (MIC = 62.5 µg/mL). The in vitro antifungal activity was also evaluated, showing that compounds 5n ((R1 = NO2, R2 = H, Ar = 4-CIPh)) and 5p displayed the highest activity against Griseofulvin with the MIC value of 200 µg/mL against Candida albicans (MTCC 227).

The Perumal group synthesized a series of novel spirooxindoles using the 1,3-dipolar cycloaddition of an azomethine ylide generated from isatin and sarcosine or L-proline with the dipolarophile 1,4-naphthoquinone as the key step (Scheme 2) [33]. The antimicrobial activities of synthesized compounds were screened against eight bacteria and three fungi using in vitro disc diffusion method. Among these compounds, compound 7n (R1 = acetyl, R2 = H) was 1.6 times more active against S. aureus (MIC = 31.25 µg/mL) than streptomycin and ciprofloxacin, 6.4 times more active against M. luteus (MIC = 15.62 µg/mL) and S. typhimurium (MIC = 15.62 µg/mL) than ciprofloxacin, and more than 3.2 times more active against C. albicans fungi (MIC = 31.25 µg/mL) than fluconazole. The authors claimed that compound 7n could be potentially used to develop potent antibacterial and anti-fungal agents.

Meshram et al. developed a simple one-pot three-component aqueous phase protocol for the synthesis of functionalized spirooxindole derivatives by the reaction of isatin, β-nitrostyrene and benzyl amine/α-amino acids in water under microwave irradiation. The protocol allows facile construction of a library of spirooxindoles 9a-v in moderate to good yields (up to 92%) with good diastereoselectivity (Scheme 3) [34]. The antimicrobial activity was assessed against Escherichia coli ATCC 10536, Candida tropicalis ATCC 750, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 15442 with reference standard ciprofloxacin.
using the agar well diffusion method. Interestingly, the majority of the synthesized compounds showed good to excellent activity, comparable to that of ciprofloxacin (MIC = 0.3 µg/mL). The pyrrolidine NH and attached NO2 groups provide functional synthetic handles which can be utilized to construct compound library of pharmaceutically and medicinally significant.

Mabkhot et al. reported the highly regioselective synthesis of new derivatives 10a–j and 12a–j through a 1,3-dipolar cycloaddition reaction of azomethine ylides generated in situ from isatin, sarcosine, and L-proline through the decarboxylative route with dipolarophile (Scheme 4) [35]. However, the corresponding bis-adducts were not formed possibly due to the steric hindrance and fixing of the geometry of the spiropyrrolidine ring. All of the newly synthesized compounds were evaluated for their antimicrobial activities. Compounds 12b (Ar = 4-NO2Ph), 12e (Ar = 4-MeOPh), 12g (Ar = 2,4-diFPh) and 10c (Ar = 4-CIPh) exhibited the best inhibitory effect against Bacillus subtilis (RCMB 010067) with the IC50 values of 2.92, 1.34, 1.36, and 1.24 µg/mL, respectively. SARs studies showed that the electron-withdrawing groups attached to the aryl rings were preferred over the electron-donating groups for the antimicrobial effects.

Similarly, the Perumal group also achieved the efficient synthesis of structurally novel 1-methyl-3-[(E)-arylmethylidene]tetrahydro-4(1H)-pyridinones 14-16 by the reaction of 3-substituted 1-methyl-4-piperidones, isatin and L-proline, sarcosine or benzyl amine under reflux (Scheme 5) [36]. The products were obtained in excellent yields and with high regio- and stereoselectivities. The compounds were screened for their in vitro activity against Mycobacterium tuberculosis H37Rv (MTB), multi-drug resistant M. tuberculosis (MDR-TB) and Mycobacterium smegmatis (MCsem) using the agar dilution method. Compound 15e (Ar = 2,4-diCIPh) showed the best potency with the MIC values of 1.76 and 0.88 µM, respectively against MTB and MDR-TB, more potent than control anti-TB drugs Isoniazid, Ethambutol and Pyrazinamide. For MDR-TB, compounds 14c (Ar = 2-CIPh) and 14e (Ar = 2,4-diCIPh) were also active with the IC50 values of 3.58 and 1.66 µM, respectively, possibly suggesting the importance of the 2-Cl group of the phenyl ring for the observed activity against MDR-TB. Interestingly, all these compounds 14-16 showed good to excellent inhibition against MTB, most of them were much more potent than reference drug pyrazinamide, underscoring the importance of such scaffolds for the activity against MTB. In contrast, compounds 14-16 exerted weak inhibition toward MCsem.
Scheme 5. Synthesis of structurally new spiropyrrolidyl oxindoles 14-16.


Similar to above work, Raghunathan et al. synthesized three series of spirooxindoles 17-19 from isatin, sarcosine, and the corresponding cyclohexanone-based dipolarophiles via the regioselective 1,3-dipolar cycloaddition reactions (Scheme 6) [37]. Some of the compounds showed certain antibacterial and antifungal activity.

Following their previous work on the identification of new antmycobacterial agents [36], Perumal et al. reported an atom economic and stereoselective synthesis of several spiro-piperidin-4-ones 20-22 through the 1,3-dipolar cycloaddition of azomethine ylides generated in situ from isatin and amino acids (L-proline, phenylglycine, and sarcosine) (Scheme 7) [38]. The compounds were screened for their in vitro and in vivo activity against MTB, MDR-TB and MC². All compounds showed moderate to excellent in vitro activity against MTB with MIC ranging from 0.07-53.30 µg/mL. Compound 22e (Ar = 4-FPh) was found to be the most active in vitro with the MIC value of 0.07 µg/mL against MTB and showed 5.1 and 67.2 times potency than isoniazid and ciprofloxacin, respectively. Compound 22e also potently inhibited MDR-TB with the MIC value of 0.16 µg/mL. Collectively, for MTB, MDR-TB and MC², compound 22e was the most potent compound, suggesting the importance of the core structure and
Compounds such as Escherichia coli, Aspergillus niger found to be active against MTB and MDR-TB with IC\textsubscript{50} values of 0.17, 0.08 and 1.47 µg/mL, respectively, but was less potent than isoniazid at the same dose level. Besides, compound 22e was nontoxic up to 62.5 µg/mL (111.41 µM) and showed good selectivity index (IC\textsubscript{50}/MIC) of 1634. Additionally, compound 21f (Ar = 2-ClPh) displayed the best potency against MTB, MDR-TB and MC\textsuperscript{2} with the IC\textsubscript{50} values of 0.17, 0.08 and 1.47 µg/mL, respectively, significantly more potent than other compounds of this series. Taken above data into consideration, the substituents attached to the pyrrolidinyl ring played an essential role in the antimicrobial activity.

Substituents attached to the phenyl ring. Further in vivo studies indicated that compound 22e decreased the bacterial load in lung and spleen tissues with 1.30 and 3.73-log 10 protections, respectively, but was less potent than isoniazid at the same dose level. Besides, compound 22e was nontoxic up to 62.5 µg/mL (111.41 µM) and showed good selectivity index (IC\textsubscript{50}/MIC) of 1634. Additionally, compound 21f (Ar = 2-ClPh) displayed the best potency against MTB, MDR-TB and MC\textsuperscript{2} with the IC\textsubscript{50} values of 0.17, 0.08 and 1.47 µg/mL, respectively, significantly more potent than other compounds of this series. Taken above data into consideration, the substituents attached to the pyrrolidinyl ring played an essential role in the antimicrobial activity.

Narayana et al. identified a new series of spirooxindoles that could inhibit methionine tRNA synthase (PDB ID: 1PFV) and glucosamine-6-phosphate synthase (PDB ID: 1JXA) enzymes through the virtual screening (Scheme 8). The compounds were efficiently synthesized from isatin, amino acids, and dipolarophile chalcones by a three-component 1,3-dipolar cycloaddition reaction [22]. These compounds were then found to be active against Staphylococcus aureus, Escherichia coli, Aspergillus niger and Aspergillus flavus. Compounds 23a (R\textsubscript{1}, R\textsubscript{2}, and R\textsubscript{4} = H, R\textsubscript{3} = 4-F), 23e (R\textsubscript{1} = H, R\textsubscript{2} = CH\textsubscript{2}OH, and R\textsubscript{4} = Br, R\textsubscript{3} = 4-F), 23g (R\textsubscript{1} = H, R\textsubscript{2} = i-butyl, and R\textsubscript{4} = Cl, R\textsubscript{3} = 4-F) showed the MIC value of 0.8 µg/mL for their antitubercular activity.

Raghunathan et al. described the synthesis of a series of structurally novel and complex dispiro pyrrolizidines 24 and 25 through the 1,3-dipolar cycloaddition reaction of azomethine ylides generated in situ from secondary amino acids and isatin with bischalcones (Scheme 9) [39]. These compounds were evaluated for their antibacterial activity, and some of them exhibited good antibacterial activity against Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Proteus vulgaris, and Proteus mirabilis. Compounds 24c and 25c (R\textsubscript{1} = H, R\textsubscript{2} = OH) showed good antibacterial activity against the tested pathogens, more potent than Tetracycline.

**2.2. Spirooxindole Tetrahydrofurans and 4H-pyrans**

3-Hydroxyoxindole scaffolds exist in natural products and have proven to possess promising biological activities. 3-Hydroxyoxindoles as versatile precursors have also been used in the total synthesis of natural products and for constructing structurally novel scaffolds [40]. Based on the 3-hydroxyoxindoles, Xie et al.

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**Scheme 7.** Stereoselective synthesis of new spiro-piperidin-4-ones 20-22.

**Scheme 8.** Synthesis of spiro-oxindole derivatives 23.
In general, compounds 26a-p were much more potent than compounds 27a-m against Valsa mali and Fusarium graminearum, unveiling that the incorporation of the piperidine-2,6-dione into the core scaffold was unbeneficial for the activity. Among these compounds, compound 27a (R1 = Me, R2 = Ph) exhibited the best potency against F. g. with an IC50 value of 3.31 µg/mL, comparable to that of the control cycloheximide (IC50 = 3.3 µg/mL).

Song et al. developed an efficient ultrasound-promoted one-pot three-component synthesis of polycyclic spirooxindole 4H-pyran (Scheme 11) [42]. The antifungal activity was then determined by micro dilution method. Compound 28f (R1, R3 = H, R3 = H, R4 = Me) exhibited good inhibitory activity against Cryptococcus neoformans, Epidermophyton floccosum and Mucor racemosus with the MIC values of 16, 8, 16 µg/mL, much more potent than the positive control drug fluconazole.

4-hydroxycoumarin is a structural motif present in various pharmaceuticals and clinical drugs like warfarin, etc. [43, 44]. Based on their unique pharmacological properties, Praveen et al. speculated that combining the structural characteristics of both spirooxindole and 4-hydroxycoumarin moieties by a hybrid pharmacophore approach could remarkably enhance the biological activity. Based on the hypothesis, these two interesting heterocycles were utilized in the development of biologically important spirooxindoles.

Song et al. demonstrated that combining the structural characteristics of both spirooxindole and 4-hydroxycoumarin moieties by a hybrid pharmacophore approach could remarkably enhance the biological activity. Based on the hypothesis, these two interesting heterocycles were utilized in the development of biologically important spirooxindoles.
Scheme 12. Zn(OTf)$_2$ catalyzed synthesis of bis-coumarin containing spirooxindole 4H-pyran.

Scheme 13. On-water synthesis of spirooxindole 30.

Scheme 14. Green synthesis of bis-spirooxindole containing compounds 31a-u.

Scheme 15. Nano CeO$_2$-catalyzed synthesis of spiro-oxindole dihydroquinazolinones.

evaluation showed that the compounds 29a (R, R’ = H), 29j (R = Bn, R’ = H), and 29m (R = H, R’ = Br) exerted promising antimicrobial activity with the MIC value of 62.5 µM. Docking studies showed that compound 29a was well fitted into the pocket of AmpC-β-lactamase receptor (PDB ID: 3OT3).

Similar to above work, the Wu group developed an efficient on-water synthesis of spirooxindole 4H-pyran 30 (Scheme 13), which showed good activities against Micrococcus tetragenus, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, S. albus and Escherichia coli. with the MIC values of 10, 8, 10, 7, 10 and 10 µg/mL [46].

Hasaninejad et al. developed a highly efficient, one-pot multi-component synthesis of novel bis-spirooxindoles 31a-u featuring bis-spirooxindole scaffold with excellent yields (up to 95% yield) (Scheme 14) [23]. PEG-400 was used as a biodegradable polymeric solvent in the reaction.

2.3. Other Spirocyclic Oxindoles

Ma et al. developed the nano CeO$_2$-catalyzed synthesis of two series of new spiro-oxindole dihydroquinazolinone derivatives (Scheme 15) [47]. The synthesized compounds were evaluated for their in vitro antibacterial activity, and compounds 32i-p showed
considerable antibacterial activities against *E. coli* with the MIC values between 62.5 and 15.6 µg/mL. Further molecular docking studies were performed to predict the interactions of the synthesized compounds in the active site of Biotin Carboxylase (EcBC) enzyme (PDB ID: 2W6O).

Tiwari *et al.* efficiently synthesized two series of new 2,3-dihydrooxazole-spirooxindole derivatives (Scheme 16) and then evaluated their antibacterial and antifungal activity against *Bacillus subtilis, Enterobacter* and *Klebsiella pneumoniae* using Ciprofloxacin as positive control [48]. Among these compounds, compound 33g (R1 = 2,4-diCl) was found to be active against *K. pneumoniae* and *S. fuliginea* at 25 and 14 µg/ml, respectively.

Singh *et al.* developed a new eco-friendly strategy for the synthesis of novel spiro-oxindole derivatives by one-pot multicomponent reaction using isatins, urea and 1,3-dicarbonyls (Scheme 17) [49, 50]. β-Cyclodextrin was used as the catalyst and can be reused for further reaction after recovery. The synthesized compounds were evaluated for their antimicrobial activities against *Escherichia coli, Staphylococcus aureus, Aspergillus niger* and *Candida albicans*, showing that all the synthesized spiro-oxindoles exhibited significant antimicrobial activity, equal to that of standard drug streptomycin.

**CONCLUSION AND OUTLOOK**

Spiro scaffolds have been recognized as privileged scaffolds in drug design due to their highly pronounced biological properties, interesting conformational features and unique 3D structural features. In particular, spirooxindoles have emerged as attractive synthetic targets because of their prevalence in numerous biologically validated natural alkaloids and pharmaceutically relevant molecules. The key structural characteristic of these compounds is the spiro ring fused at the C3 position of the oxindole core with varied heterocyclic motifs. Spirooxindole is a privileged heterocyclic motif that exists in pharmaceutical candidates and naturally occurring compounds. Spirooxindole containing compounds have exhibited diverse biological properties. Antimalarial agent NITD609, PLK4 inhibitor CFI-400945, MDM2 inhibitors SAR405838 and APG-115 have advanced into clinical trials for the treatment of malaria and human cancers. In last decades, a large number of structurally novel spirooxindoles have been synthesized mainly based on the ylide intermediates generated in situ and further assessed for their antimicrobial activity against different types of bacteria, lead-
ing to the discovery of some potent lead compounds with antimicrobial potentials. The structural complexity and diversity of spirooxindoles could be achieved by using diverse dipolarophiles and ylides generated from isatin and amino acid derivatives. However, the current study is only limited to primary antimicrobial assessment, no detailed modes of action are investigated.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

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