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REVIEW

An overview of spirooxindole as a promising scaffold for novel drug discovery

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ABSTRACT

Introduction: Spirooxindole, a unique and versatile scaffold, has been widely studied in some fields such as pharmaceutical chemistry and synthetic chemistry. Especially in the application of medicine, quite a few compounds featuring spirooxindole motif have displayed excellent and broad pharmacological activities. Many identified candidate molecules have been used in clinical trials, showing promising prospects.

Areas covered: This article offers an overview of different applications and developments of spirooxindoles (including the related natural products and their derivatives) in the process of drug innovation, including such as in anticancer, antimicrobial, anti–inflammatory, analgesic, antioxidant, antimalarial, and antiviral activities. Furthermore, the crucial structure-activity relationships, molecular mechanisms, pharmacokinetic properties, and main synthetic methods of spirooxindoles-based derivatives are also reviewed.

Expert opinion: Recent progress in the biological activity profiles of spirooxindole derivatives have demonstrated their significant position in present-day drug discovery. Furthermore, we believe that the multidirectional development of novel drugs containing this core scaffold will continue to be the research hotspot in medicinal chemistry in the future.

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Spirooxindole; natural products; bioactivity; anticancer; antimicrobial; structure-activity relationship

1. Introduction

With the tremendous progress of modern medicinal chemistry, drug discovery based on natural products has drawn increasing attention [1]. Although the natural product-derived drug innovation has achieved enormous success during the past century, biological compatibility, oral availability, and the emergence of drug-resistance remain the major restricting factors for the further development of most natural products with potential therapeutic effects. Therefore, chemists and pharmacologists are given a more paramount mission to increase the structural and functional diversification of natural products, with an aim to overcome the existing issues.

Spirooxindole alkaloids, as a family member of oxindole natural products in nature [2], were first isolated from *Rubiaceae* and *Apocynaceae* plants. The privileged scaffold of spirooxindole contains two basic substructure units: one is multiple functionalized oxindole, which can be used as hydrogen bond donors and acceptors to interact with biological targets. The other is a cycloalkyl or heterocyclic moiety fused at the C-3 position of oxindole. It provides an opportunity to regulate the liposolubility and other physicochemical properties of spirooxindole [3]. Accordingly, the unique spatial architecture and significant biological activities of spirooxindole have long captured the great attention of researchers.

Pharmacological effects described over the past decades for spirooxindole-based natural products and the modified derivatives are reported in this review and include evaluations of anticancer, antimicrobial, anti–inflammatory, analgesic, antioxidant, antimalarial, antiviral, antiatherosclerotic, antidiabetic, and insecticidal activities. We also found some reports on the antagonistic/inhibitory action on cholinesterase, DNA cleavage, prolyl hydroxylase 2 (PHD2), mineralocorticoid receptor, and progesterone receptor.

2. The brief review of spirooxindole-based natural products and their potential bioactivities

In medicinal chemistry, natural products are considered as an effective way to obtain lead compounds with prominent biological properties, which plays an essential role in the discovery process of numerous marketed therapeutic drugs. Recently, a multitude of excellent reviews have systematically introduced the importance of compounds derived from natural sources for treating diseases in humans, such as cancer, bacterial/virus infection, and mental disorders [4–6]. Spirooxindole-based alkaloids, as an important member of natural product families, have aroused tremendous attention due to their specific chemical structures (Figure 1) and pharmacological properties.

Horsfiline, Coerulescine, and Elacomine are the simplest compounds with tricyclic spirooxindoles, which were isolated from the roots of *Horsfieldia superba, Phalaris coerulescens*, and *Elaeagnus commutate*, respectively [7,8]. Spirobrassinin and its

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Article highlights

- Spirooxindole, as a unique chiral scaffold consisting of at least three heterocyclic systems, widely exists in the structure of natural products and therapeutic agents.
- The unique three-dimensional scaffold and vast chemical space of spirooxindole are responsible for their broad-spectrum of pharmacological properties.
- Spirooxindoles are of particular interest as lead compounds with antitumor, antimicrobial, anti–inflammatory, antioxidant, antiviral, and other bioactivities.
- Among a variety of active compounds containing spirooxindole, spiro-pyrrolidinyl oxindoles-based derivatives occupy a dominant position in quantity, holding broad and significant biological properties.
- Many modes of action of the spirooxindole-based therapeutic agents have been studied by researchers. Especially, the mechanism of spirooxindole derivatives targeting p53-MDM2 interaction as anticancer agents is extensively investigated.

analog methoxyspirobrassinin, oxindole alkaloids containing dihydrothiazole, are a kind of plant antitoxins with promising bactericidal and antitumor activities [9]. Rhynchophylline is an *N*-methyl-D-aspartate receptor antagonist, which is regarded as a neuroprotective agent (such as antihypertensive, antipyretic, and anticonvulsant medicine) [10]. Strychnofoline can effectively inhibit mitosis of multiple cells, thereby displaying potent inhibitory effects against melanoma and Ehrlich tumor cells [11]. Spindomycin B is a spirooxindole derivative isolated from *Streptomyces* sp. xzqh-9, which exhibits weak inhibition toward tyrosine kinase Bcr-Abl (Philadelphia chromosome) [12].

Gelsemine is a representative example of indole alkaloids isolated from the genus *Gelsemium*. It has been found to possess antihyperlipidemic and antioxidative effects [13], as well as have repairing action against cisplatin-produced nephrotoxicity [14]. Spirotryprostatins A and B are identified as potential anticancer drugs due to their potent inhibition against the G2/M phase of cell division and mouse breast cancer cells tsFT210 [15]. Alstonisine is the first macroline-related oxindole alkaloid isolated from *Alstonia muelleriana* [16], showing moderate *in vitro* antiplasmodial activity.

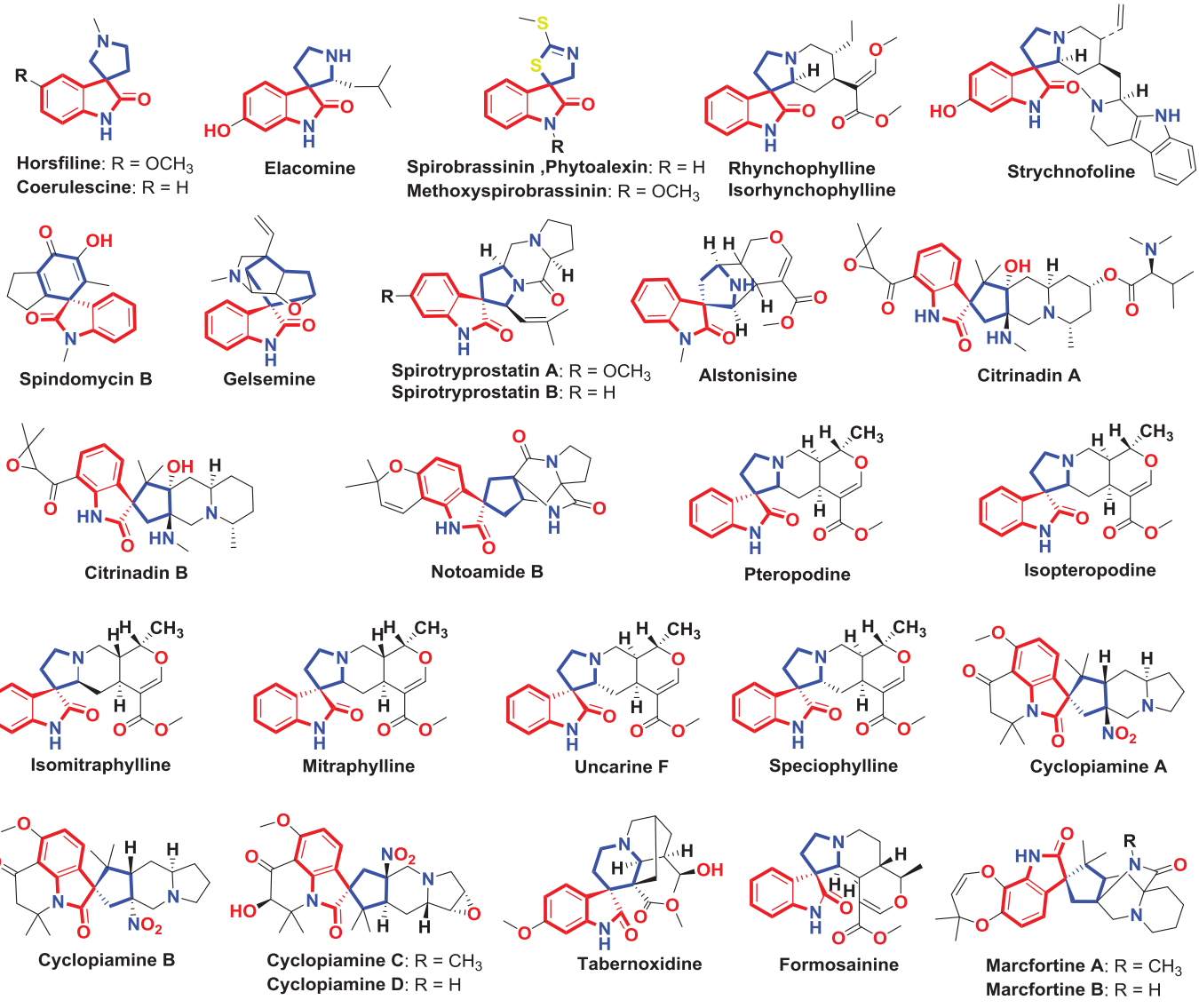

Figure 1. Spirooxindole-based natural products.

Besides, this natural product also can be used to design potential murine double minute 2 (MDM2) inhibitors. Naturally occurring oxindole alkaloid Citrinadin A/B and Notoamide B were isolated from *Penicillium citrinum* N059 strain and *Aspergillus* species, respectively, which have been widely studied because of their remarkable anticancer efficacy [17,18]. Pteropodine, Isopteropodine, Isomitraphylline, Mitraphylline, and Uncarine F were all obtained from *Uncaria tomentosa*. Except for mitraphylline, the other four kinds of alkaloids can control the proliferation of acute lymphoblastic leukemia cells (CEM-C7H2 cells) [19,20]. Besides, Mitraphylline exhibits an *in vivo* controlling effect against the cytokines associated with most inflammation processes. Thus, it is considered to be a novel lead compound for anti–inflammatory therapy [21]. Speciophylline isolated from *Mitragyna inermis* has potential *in vitro* antiplasmodial property [22]. Cyclopiamine A/B and C/D are fungal hexacyclic spiroindolinone alkaloid isolated from *Penicillium cyclopium* in 1979 and soilborne strain coded as *Penicillium* sp. CML 3020 in 2009, respectively. Their related bioactivities are being carried out a detailed investigation [23,24]. Beyond that, there are many other natural products bearing this characteristic structural scaffold (spirooxindole) such as Tabernoxidine, Formosainine, and Marcfortine A/B, which provide abundant structural forms and diverse bioactivities.

3. The biological profiles of the spirooxindole-based natural product analogs

As mentioned above, natural products containing the spirooxindole motif possess multiple types of biological activities. What is more, the investment and breakthroughs in synthetic methods have produced an army of new spirooxindole analogs with structural diversity over the last few decades [25]. The generated spirooxindole library provides a huge opportunity to acquire potent agents with superior pharmaceutical properties (such as high-efficiency, low toxicity, and acceptable bioavailability) [26,27]. Here, some significant bioactivities of these analogs will be reviewed in detail.

3.1. Anticancer activity

Due to the special structure of spirooxindoles, they have a wide range of biological activities, among which the most prominent is their potential anticancer effect. Cancer is the second leading cause of death worldwide. In 2018, there was about 18.1 million new cancer cases and 9.6 million deaths from various cancers. Even more serious is that the number of new cases is expected to increase by around 70% over the next 20 years, which means that cancer remains an urgent public health issue [28,29]. Based on spirooxindole as a core scaffold (Figures 2 and 3), here we will focus on describing their anticancer effect, mechanism of action, pharmacokinetic profiles, and main structure-activity relationships (SARs).

3.1.1. Spiro-cyclopropyl oxindole derivatives

The polo-like kinase (PLK) family of highly conserved serine/threonine kinases has been recently recognized as a promising anticancer target. Especially, PLK4 has been reported to be essential for centriole duplication during the cell cycle, cytokinesis, and maintenance of chromosomal stability [30–33]. In 2015, a new 'star' molecule **1** (CFI-400945) containing spirocyclopropyl oxindole displayed significant antitumor potency with a K_i value of 0.26 nM against PLK4. The SARs data revealed that the introduction of the methoxy group on the indolinone core was conducive to improving potency and kinase selectivity. Besides, the presence of a morpholino group could effectively enhance permeability, cell activity, and pharmacokinetic parameters of the target molecules. Further studies demonstrated that compound **1** could be tolerated in the breast cancer cell lines mouse xenograft model at the doses of 9.4 ~ 3.0 g/kg (once a day for 21 days). These results indicate that this molecule holds the promise as the first potent and orally active PLK4 inhibitor. Nowadays, the fumarate form of CFI-400945 has entered into phase I clinical trials (NCT01954316) for treating solid tumors (breast cancer, particularly) [34,35].

In 2019, Rodriguez *et al*. discovered that cyclopropane-containing bis-spirooxindole derivatives displayed strong anticancer potency against two isogenic human triple-negative breast cancer MDA-MB-231 and MDA-MB-231-Br cell lines. With the in-depth investigation of these synthesized compounds, four modified compounds **2a**–**2d** (Figure 1) were identified as the optimal hits for the subsequent biological screenings (EC_{50} ranged from 2 to 22 μM against the test cell lines). These compounds showed favorable physicochemical properties, such as meeting Lipinski's rule of five, the capability of penetrating the blood-brain barrier, and metabolic stability. In order to characterize their molecular mechanism, RNA sequencing combined with an advanced detecting mechanism of action by network dysregulation transcriptome analysis was carried out. The assay results suggested that compound **2b** disrupted ribosome function and led to the inhibition of mRNA protein translation, thereby limiting the proliferation of rapidly dividing cancer cells [36]. These findings will accelerate the identification of fire-new effective therapeutic agents for treating cancer.

3.1.2. Spiro-(five-membered carbocyclic and heterocyclic) oxindole derivatives

Structurally, spiro-(five-membered carbocyclic and heterocyclic) oxindoles mean that the C-3 position of oxindole is directly fused by a mono/complexed five-membered cycle such as cycloalkyl, cycloalkenyl, and pyrrolidinyl. In a slice of related researches, some of these spirooxindole derivatives featuring the above five-membered ring have been proved to possess promising antitumor activity. And the structures of representative compounds **3**–**33** are shown in Figure 2.

Utilizing a stepwise *C*-Piancatelli rearrangement reaction, Huang *et al*. synthesized a class of spiropentenoneoxindoles and then tested their antiproliferative activity against human tumor cell lines, such as Du145, LNCaP, and PC3 cells. Among this series, compound **3a** was the best cytotoxic agent against Du145 cells (GI_{50} = 0.60 μM). Moreover, compound **3b** possessed apparent cytotoxicity against LNCaP cells and PC3 (GI_{50} = 0.71 μM and 2.1 μM, respectively). The SAR results revealed that changing substitutions on the aryl ring of the

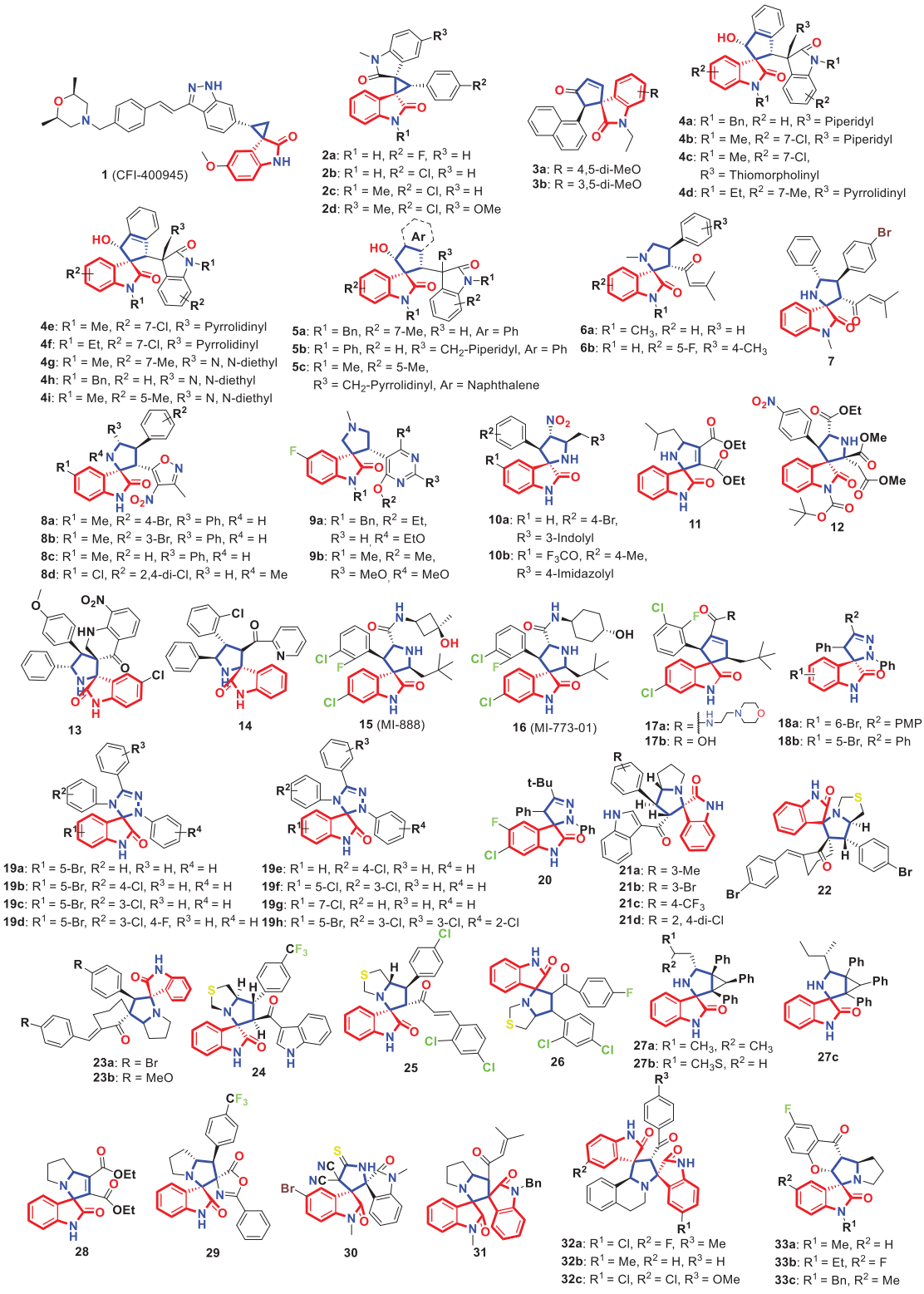

Figure 2. Spirooxindole compounds **1**–**33** as promising anticancer agents.

above parent structure could affect the cytotoxicity significantly [37].

A family of five-membered carbocyclic spirooxindoles complexed with substituted oxindoles was synthesized by Zhou's group, which have been evaluated as active anticancer agents against human leukemia cell line K562, lung cancer cell line A549, and prostate cancer cell line PC-3. Representative compounds **4a**–**4i** showed a comparable or stronger inhibitory effect (IC_{50} = 7.4 to 32.8 μM) against human leukemia cells K562 compared with cisplatin (up to 3.4-fold). Compounds **4f** and **4h** possessed equivalent inhibition toward A549 cell lines. And compounds **4h** and **4g** had a slight enhancement of

inhibitory activity against prostate cancer PC-3 cell lines as compared to cisplatin [38]. Another three similar spirooxindole derivatives **5a–5c** obtained by these researchers exhibited potent efficacy toward K562 cells (IC_{50} = 29.3 μM, 27.4 μM, and 34.2 μM, respectively), which were equipotent to cisplatin (IC_{50} = 26.8 μM) [39].

Liu and his coworkers synthesized several turmerone motif-fused spiro-pyrrolidinyl oxindoles via a [3 + 2] cycloaddition reaction. Furthermore, they examined the *in vitro* antitumor potency of these compounds against A549 lung cancer cells and K562 leukemia cells. The results were presented that compound **6a** displayed the best activity in inhibiting K562 leukemia cells (IC_{50} = 31.1 μM), which was close to that of cisplatin (IC_{50} = 29.2 μM). And compound **6b** showed the best inhibition against A549 lung cancer cells (IC_{50} = 54.1 μM, IC_{50} of cisplatin = 23.7 μM). These data offered a potential pathway for further optimization [40]. As a continuous work, the subsequently synthesized compound (**7**) without methyl group substituted at pyrrolidinyl but bearing one more phenyl had promising efficacy against K562 leukemia cells (IC_{50} = 37.5 μM) as well, which was slightly weaker than cisplatin [41]. This research group used the same synthetic method to further splice isoxazole or alkoxy-pyrimidine into 3-spiropyrrolidine oxindoles in 2016. It has been found that isoxazole-containing compounds **8a, 8b**, and **8c** respectively exhibited favorable inhibitory activities against K562, A549, and PC-3 cancer cells (their corresponding IC_{50} values are 16.0 μM, 24.4 μM, and 21.2 μM, independently), which were comparable to that of cisplatin (IC_{50} = 26.1 μM, 27.2 μM and 25.6 μM, respectively). In parallel, compound **8d** also displayed a positive inhibitory effect (IC_{50} = 10.7 μM) against K562 cells, which was superior to cisplatin [42,43]. Besides, alkoxy-pyrimidine-containing hybrids **9a** and **9b** reported in 2017 also exhibited equipotent anticancer activity with cisplatin toward K562 cancer cell line (IC_{50} = 24.1 and 32.4 μM, respectively) [44].

Spiroheterocycles containing pyrrolidine, indole/imidazole, and spirooxindole moieties were prepared by Arumugam *et al*. [45]. The related anticancer activity and nuclear morphology studies demonstrated that this series compounds could inhibit the proliferation of FaDu epithelial cell line via apoptotic cell death with a moderate potency. Especially, derivatives **10a** and **10b** exhibited excellent cytotoxicity (IC_{50} = 30 μM and 40.1 μM, respectively) against the FaDu cell line.

Mali *et al*. reported that compound **11** bearing two ester groups exhibited a significant controlling effect against lung cancer A549 cell line (IC_{50} = 4.5 μM). Moreover, compound **11** displayed potent inhibition toward MCF-7 breast cancer cells (IC_{50} = 5.0 μM) as well [46]. Huang and coworkers synthesized another batch of spiro-pyrrolidinyl oxindoles and then evaluated *in vitro* cytotoxicities against the mouse breast cancer cells 4T1, colon cancer cells CT26, human liver cancer cells HepG2, and lung cancer cells A549. The produced compounds displayed high cytotoxicity toward HepG2 cells. Among them, compound **12** substituted by four ester groups displayed the most potency in controlling the growth of CT26 cells by 50.9% at a concentration of 50 μg/mL. Furthermore, the cell viability assay revealed that this compound was nontoxic to normal fibroblast 3T3 cells [47]. In the same year, Shyamsivappan and coworkers synthesized a range of spirooxindoles containing pyrrolidine 8-nitroquinolone. These compounds exhibited significant potency against human cervical cancer cell line HeLa and insignificant toxicity to normal cells *in vitro*. Besides, the most potent compound **13** (IC_{50} = 16 μM) could induce apoptosis of HeLa cells through reactive oxygen species (ROS) influx and caspase-3 activation [48]. Five spirooxindole-pyrrolidines synthesized by Tumskiy *et al*. demonstrated cytotoxic activity toward some cancer cell lines. Of which compound, **14** possessed moderate selectivity (3-fold) between HeLa cancer cells and Vero healthy cells, which can be used as a potential source for discovering novel anticancer therapeutic agents [49].

Carcinogenesis is a highly complex multistep induction process induced by multiple kinds of carcinogens. Malignant tumors may invade nearby healthy cells, and even spread to the system or blood in distant parts of the body through lymph nodes [50]. Significant advances have been made in the development of cancer therapeutic agents by targeting p53-MDM2 interaction. p53 is a tumor suppressor protein that plays a vital role in the regulation of DNA repair, cell cycle, apoptosis, and senescence, which is honored as a guardian of the genome [51,52]. It can prevent genome mutation and conserve genomic stability [53]. In response to the stress signals of anticancer therapy, p53 triggers cell cycle arrest and cell death by apoptosis, thus inhibiting the development of tumor [54,55]. Usually, the level and activity of p53 are tightly regulated by a protein called MDM2. In unstressed cells, the continuous degradation of p53 by MDM2 keeps p53 at low levels. The deregulation of MDM2/p53 balance usually leads to the malignant transformation of cells. Most human cancers and tumors are triggered by expression of mutant p53 or MDM2 enhanced degradation of p53 [56,57]. Recently, a host of promising inhibitors inhibiting p53-MDM2 interaction and activating p53 have been developed for cancer therapy. Some reviews have been comprehensively presented regarding the spirooxindoles as anticancer/tumor agents, especially p53-MDM2 interaction inhibitors [58–60]. The p53-MDM2 interaction is primarily mediated by three hydrophobic residues (Trp23, Phe19, and Leu26) of p53 peptide and a small deep hydrophobic cleft in MDM2. The indole ring of the Trp23 residue is buried deep inside the hydrophobic cavity in MDM2, and its NH group forms an H-bond with the backbone carbonyl in MDM2. Spiro-pyrrolidinyl oxindole-containing compounds Spirotryprostatins A and Alstonisine are regarded as possible inhibitors because these compounds can insert into the MDM2 structure and mimic the residue Trp23 of p53 in both hydrophobic interaction and H-bonding formation with MDM2. And the spiro-pyrrolidinyl oxindole ring gives a rigid scaffold, from which two hydrophobic groups can be projected to mimic the side chain of Leu26 and Phe19 [61,62]. Therefore, the spiro-pyrrolidinyl oxindoles scaffold is considered as the potential p53-MDM2 interaction inhibitors.

It must be mentioned that compound **15** (MI-888) discovered by Wang's research group demonstrated the enormous potential of spirooxindole-based agents for treating cancer clinically. This compound was identified as a potent p53-MDM2 interaction inhibitor with a K_i value of 0.44 nM, which possessed a superior pharmacokinetic profile and favorable

in vivo efficacy. At an oral dose of 25 mg/kg, the C_{max} (the maximum compound concentration from oral dosing) and AUC (area under the curve) values of compound **15** were 1.73 mg/L and 14.87 mg L^{-1} h^{-1}, respectively. Besides, its $T_{1/2}$ value was 3.2 h. Compound **15** was capable of achieving rapid, complete, and durable tumor regression in xenograft tumor models of two human cancers. More specifically, when the oral dose was at 100 and 200 mg/kg daily for seven days, the phenomenon of complete tumor regression would be observed. Even four weeks after the last treatment, all the test mice treated with compound **15** at 200 mg/kg remained tumor-free status. Meanwhile, there was no toxicity or significant weight loss for the mice treated during the experiment [63]. An analog of compound **15**, named MI-773-01 (**16**, SAR405838), was designed by optimizing the amide side chain of **15**. The *in vivo* assays disclosed that compound **16** also had promising oral pharmacokinetics in mice, and could activate p53 in extracted tumor tissues. The phase I clinical trials of this compound in patients with terminal solid tumors (NCT01636479) manifested that it had an excellent safety profile, and it's MTD (maximum tolerated dose) was 300 mg once daily [64].

In order to avoid the undesired epimerization process in the structure of indoline-3,3′-pyrrolidine of MI-888, Marinetti's research team replaced the original pyrrolidine unit with a cyclopentene ring to yield several analogs. In homogeneous time-resolved fluorescence assays *in vitro* showed that target compound **17a** displayed inferior activity to its intermediate **17b**. However, **17a** exhibited a higher antiproliferative effect against p53 wild-type cancer cell lines than **17b**, which was comparable to the positive control nutlin-3a [65].

In 2014, Santos's group discovered some novel spiropyrazoline oxindoles analogs and then screened their *in vitro* cytotoxicity against MCF-7 breast cancer cell lines. The most active compounds were further carried out for the evaluation of MDA-MB-231 cell lines. Compounds **18a** and **18b** showed the most potency (GI_{50} < 7.4 μM) against MCF-7 cells and high selectivity between MCF-7 tumor cells and MDAMB-231 tumor cells (>10-fold). Notably, these two compounds were noncytotoxic against normal human HEK 293T cell line [66]. Encouraged by these remarkable results, in 2017, Santos's team further synthesized a series of new spirotriazoline oxindoles compounds featuring the hybrids of 1,2,4-triazole and oxindole, which also possessed excellent anticancer potency. In the evaluation of antiproliferative activity, four representative compounds **19a**–**19d** (IC_{50} = 3.5 to 6.7 μM) bearing a -Br at the five position of oxindole were selective against MDA-MB -231 cells (≥2.4-fold) relative to the other four cell lines (MCF-7, HEK 293T, HCT-116 $p53^{(+/+)}$, and HCT-116 $p53^{(-/-)}$). And compounds **19e, 19f, 19a**, and **19g** exhibited higher selectivity (≥2.5-fold) toward the four cancer cell lines than HEK 293T. Especially, compound **19f** achieved a 5.6-fold high-selectivity (IC_{50} = 17.9 μM against HCT-116 $p53^{(-/-)}$; IC_{50} > 100 μM against HEK 293T). Compounds **19h** and **19f** were selected for studying the mechanism of action. As a consequence, both of them induced apoptosis and cell cycle arrest in G0/G1 phase, while decreasing expression levels of MDM2 in the HCT-116 cells. This result suggested the ability of these spirooxindoles to modulate p53-MDM2 interaction [67]. Structurally, compound **19a** is almost the same with **18b**, while the cytotoxic selectivity is reversed. An obvious SAR can be summarized that introducing nitrogen atom into the spiropyrazoline ring may transfer the selectivity toward MCF-7 to MDA-MB-231 cells. Based on the above research on the modification of spirooxindoles with anticancer activity, this research group continued to synthesize a new kind of spiropyrazoline oxindoles, and further assessed their antiproliferative activity in 2018. Among them, compound **20** had selectivity to the cancer cell line expressing wild type p53 and low cytotoxicity toward some normal cells. It induced neural stem cell differentiation through reduced SOX2 (a marker of multipotency) level and increased βIII-tubulin (a marker of neural differentiation), suggesting that it showed great potential as a nontoxic inducer of cell differentiation. This compound could increase p53 steady-state levels. Also, it was able to reduce symmetric renewal divisions in these cells. These findings mean that this compound can be used as a potent agent to exert anticancer activity by activating p53 [68].

In 2018, Assem's group developed a family of functionalized spirooxindoles. Representative compounds **21a**–**21c** exhibited potent cytotoxic activity (IC_{50} = 7 μM, 9 μM, and 9 μM, respectively) against HCT-116 cells and high selectivity index (SI) toward normal cells (SI >2) [69]. Compound **21d** displayed high cytotoxic activity and selectivity against PC-3 and HepG2 (IC_{50} = 2 μM and 2 μM, respectively; SI = 4.5 and 4.5, respectively), which were superior to cisplatin (IC_{50} = 5 μM and 5.5 μM, respectively). Phosphodiesterases (PDEs) consists of 11 isoenzymes (PDE1−PDE11), which play a crucial regulator to the intracellular level of the second messenger cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). It is reported that the increase in concentrations of cAMP may inhibit the interaction of the tumor suppressor p53 protein with its regulator MDM2 [70,71]. Using phosphodiesterase inhibitors could elevate the level of cAMP, thus overcoming tumor drug-resistance as well as reducing side effects on normal cells. Notably, compound **21d** showed favorable inhibition toward phosphodiesterase enzyme (PD-1) at 2 μM, with 74.2%, which could be used for the treatments of multiple solid tumors.

Barakat's team has carried out if not most meaningful works in the discovery of potent anticancer agents containing pyrrolidinyl moiety. In 2017, spirooxindole **22** bearing pyrrolothiazole motif was conferred considerable antiproliferative potential. It was proved to be significantly bioactive against MCF-7 and K562 cancer cells (IC_{50} = 15.32 μM and 14.74 μM, respectively), which was superior to 5-fluorouracil (an anticancer drug, IC_{50} = 78.28 μM and 38.58 μM, respectively) [72]. Next year, this team converted pyrrolothiazole to pyrrolidine and then synthesized a series of corresponding derivatives. Among them, compound **23a** showed more potent potency (IC_{50} = 15.49 μM) against MCF-7 than 5-fluorouracil. And compound **23b** showed stronger potency (IC_{50} = 13.38 μM) against leukemia associated with K562 cells than the control. The SARs study further revealed that carbonyl in the oxindole part and *p*-bromophenyl arm took a crucial determinant in the protein interaction, which resulted in stronger hydrophobicity than

the positive drug [73]. In 2019, compound **24** with a 3-acylindole scaffold was prepared by Barakat's team, which was found to possess higher cytotoxic activity against liver cancer cells HepG2 (IC_{50} = 5.5 μM) and HCT-116 (IC_{50} = 7 μM) in comparison with cisplatin (IC_{50} = 5.5 μM and 12.6 μM, respectively). In the case of prostate cancer cells PC-3, this compound was less active (IC_{50} = 6 μM, SI = 4.3) than cisplatin (IC_{50} = 5 μM, SI = 1.0), but more selective against normal cells as compared to cisplatin. Compound **24** was performed to molecular simulation with MDM2 binding site for p53. Consequently, it showed good binding affinities [74]. As an on-going work, these researchers synthesized a pyrrolothiazole-spirooxindole bearing a 3-cinnamoyl motif. The results of anticancer activities evaluation described that compound **25** was the most active against HCT-116, HepG2, and PC-3 cells (IC_{50} < 4 μM). The selectivity index for the cancer cells versus the normal cells was greater than 2. Further studies conclusively indicated that compound **25** could inhibit colony formation, cell migration, arrest cancer cell growth at the G2/M phase and induce apoptosis through intrinsic and extrinsic pathways [75]. After that, Barakat's team prepared a new compound (i.e. **26**) and then evaluated its preliminary biological activities against Hela cell lines in 2019. However, this compound still showed weaker anticancer efficiency against Hela cell lines (IC_{50} = 11.2 μM) than doxorubicin (an anticancer agent, IC_{50} = 1.2 μM) [76].

Filatov *et al*. designed a series of 3-spiro[cyclopropa[*a*]pyrrolizine]- and 3-spiro[3-azabicyclo[3.1.0]hexane]oxindoles. The *in vitro* measurement of antitumor activity revealed that these compounds exhibited inhibition to a certain extent against K562 cancer cell lines. The relevant cytotoxicity of representative compounds **27a**–**27c** was in the micromolar range, which was at the same level as the commercial drug imatinib [77]. Various hybrids of pyrrolizine 1, 2-dicarboxylate and spirooxindole were produced by Meshram's group, which denoted moderate to excellent growth control against the test cancer cell lines. It is worth mentioning that compound **28** exhibited significant potency against A549 cell lines (IC_{50} = 2.0 μM), which was comparable to that of doxorubicin (IC_{50} = 1.8 μM). Besides, it also displayed excellent potency against Hela cancer cell line (IC_{50} = 8.3 μM) [46].

Dong *et al*. reported a kind of oxazolone moiety in their research, which existed in a multitude of pharmaceutical inhibitors such as tyrosine kinase inhibitors and antibiotic linezolid. By linking spiropyrrolidine pyrrolidines, pyrrolizidines, and pyrrolothiazoles to the C-3 site of spirooxindoles, their group constructed a small molecule library. The *in vitro* antitumor activity screening confirmed that all the compounds exhibited favorable cytotoxicity activities against eight kinds of the test cell lines. The evaluation of the most active compound **29** showed that it displayed similar efficacies with the positive controls gefitinib and sorafenib against the selected human breast cancer cells and hepatocellular carcinoma cells (IC_{50} = 3.4 to 8.7 μM). The further experiment was indicative that the apoptosis triggered by compound **29** underwent the poly ADP-ribose polymerase pathway in MCF-7 cancer cells. This work offers a promising lead compound for anticancer drug discovery [78]. Hou *et al*. discovered that dispiropyrrolidinyl oxindoles possessed a good antitumor effect against human K562 cells by MTT assays. In particular, compound **30** exhibited the best activity, which was close to cisplatin (IC_{50} = 36.3 μM *vs* 20.1 μM) [79].

Sesquiterpene turmerone I and (*S*)-aromatic turmerone II were isolated from the rhizome of turmeric. It was reported that they possessed anti–inflammatory, anticancer, and antivenom activities [80–83]. By incorporating turmerone motif with 3,3′-pyrrolidinyl-dispirooxindoles, Zhou's group designed a series of hybrids. Among these compounds, **31** exhibited equipotent potency with cisplatin in controlling K562 cancer cells (IC_{50} = 27.9 μM *vs* 22.5 μM) [84]. Then, Yan's group synthesized another kind of dispirooxindole derivatives. The obtained compounds were found to be bioactive *in vitro* against human HepG2 cell line related to cancer. Of which compounds, **32a** was the most potent to inhibit the growth of the HepG2 cells to 44% at a concentration of 50 μg/mL. The cytotoxicity of compounds **32a, 32b**, and **32c** on 3T3 cells at 200 mg/mL illustrated that they were toxic to this kind of cancer cells but not to normal cells [85]. Pyrrolidinyl-spirooxindoles containing chromanone motif were also endowed with *in vitro* antitumor activity. Lin's team found that representative compounds **33a**–**33c** displayed a cytotoxic effect against K562 cancer cells (IC_{50} = 46.3 to 69.4 μM), but inferior to cisplatin (IC_{50} = 23.7 μM) [86].

3.1.3. Spiro-(six-membered Carbon/Sulfur/Nitrogen/Oxygen-containing cyclic) oxindole derivatives

Six-membered heterocyclic spirooxindoles containing Carbon/Sulfur/Nitrogen/Oxygen atom here mean that the C-3 position of oxindole is fused by cyclohexane, pyran, or pyridine derived motif that is pervasive in many promising anticancer chemotherapeutic compounds. The structures of the related compounds **34**–**48** are provided in Figure 3.

In 2019, Morales-Ríos and coworkers synthesized several anancomeric spiranic oxindole-cycloalkane compounds, which were the analogs of neurohormone melatonin. The effect of their stereochemistry and the conformation characteristics on melatoninergic agonist activity were analyzed using computational simulation. These synthesized compounds showed good cytotoxic against human liver cancer HepG2 cell line. The most potent one was compound **34**; the concentration causing 50% cell death (CC_{50}) was 3.3 nM [87]. Recently, Wang *et al*. designed and synthesized a series of chiral tetrahydronaphthalene-spirooxindole-based MDM2/CDK4 (cyclin-dependent kinase 4) dual inhibitors, which showed remarkable anti-glioblastoma activity *in vitro* and *in vivo*. Among these compounds, **35** displayed good inhibitory activities against CDK4 (IC_{50} = 0.25 μM) and MDM2 (IC_{50} = 0.08 μM). Also, this compound could inhibit the growth of glioblastoma xenografts expressing mutant P53, which was better than the known MDM2 inhibitor nutlin-3a. Further assays suggested that compound **35** had a potential low toxicity profile *in vivo* due to its low plasma concentration. The pharmacokinetic studies showed that **35** distributed well in tissues (V_{ss} = 7.36 L/kg) with a moderate plasma clearance rate (CL = 1.21 L·kg/h) after iv injection of 7.5 mg/kg dose, and its absolute oral bioavailability was about 30% [88].

Given that the sulfur atom plays a central role in drug design, chemists successfully constructed multiple types of

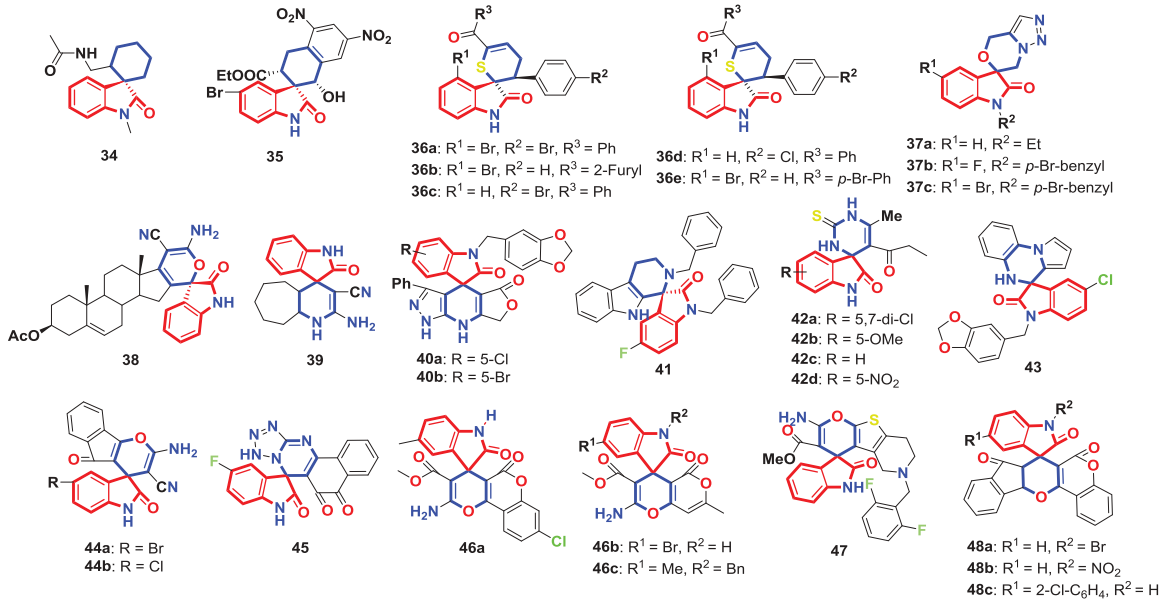

Figure 3. Spirooxindole derivatives **34**–**48** with anticancer activity.

sulfur-containing spirooxindoles. And the related studies were suggestive that spiro-tetrahydrothiopyran oxindoles scaffold was also validated as a class of p53-MDM2 interaction inhibitor [89–92]. Thus, Sheng's research group synthesized five analogs **36a**–**36e** by varying the type of substitutions. The preliminary bioactivities of these tetrahydrothiopyran-grafted spirooxindoles against the selected cancer cell lines (such as A549 lung cancer, MCF-7 breast cancer, and HCT116 colon cancer) were superior or comparable (IC_{50} = 1.5 to 8.8 μM) to that of nutlin-3. Although compound **36c** possessed less potent binding affinity than nutlin-3, it displayed stronger antitumor activity against all three kinds of cancer cell lines. Furthermore, it was validated that **36c** played an anticancer role via inducing A549 cancer cell apoptosis with G0/G1 phase cycle arrest [93].

In 2015, Shankaraiah's team obtained a series of spirooxindole-based morpholine-fused-1,2,3-triazole derivatives. The results of antiproliferative activity against some human tumor cell lines were described that compounds **37a**–**37c** exhibited remarkable growth inhibition against A549 lung cancer cell line (IC_{50} = 1.87 μM, 4.36 μM, and 4.26 μM, respectively), which were comparable to doxorubicin and 5-fluorouracil (IC_{50} = 1.98 μM and 7.24 μM, respectively). When A549 cells were treated by compounds **37a** and **37c**, both of them showed typical apoptotic features and arrested the cells in the G2/M phase of cell cycle. Furthermore, these compounds could lead to the collapse of mitochondrial membrane potential and increased levels of ROS in A549 cells, thus exhibiting a potent inhibitory effect [94]. Shi *et al*. reported a steroidal spirooxindole derivative **38** recently. This compound could induce the apoptosis of human cancer cells EC9706 and MGC-803 cells (IC_{50} = 4.68 and 1.18 μM, respectively), which resulted in the mitochondrial dysfunction and increase of ROS levels. The use of ROS scavenger *N*-acetyl-*L*-cysteine (NAC) could completely restore this phenomenon caused by compound **38**, which revealed the ROS-mediated mechanisms for the observed cell death. More significantly, compound **38** had broad-spectrum and favorable anticancer activity against the test cancer cell lines (IC_{50} < 6.5 μM). Besides, this compound was more potent than 5-fluorouracil and less toxic to normal human cells (IC_{50} > 21 μM). It is worth noting that compound **38** did not have significant acute oral toxicity and showed a favorable *in vivo* anticancer effect [95].

The core structures of dihydropyridine were observed in some of the marketed anticonvulsive and anticancer pharmaceuticals [96–98]. Researchers considered that combining dihydropyridine with spirooxindoles might exhibit synergic properties. Having this strategy in hand, Balaboina and his cooperators synthesized a series of related derivatives and then presented their *in vitro* anticancer activity. Representative compound **39** showed growth inhibitory effects against two cancer cell lines MCF-7 and HepG2 (IC_{50} = 20 μM and 18 μM, respectively) [99]. Kamal and his coworkers found that pyrazolopyridine could be incorporated into spirooxindoles under the catalysis of sulfamic acid, and then constructed a small library of compounds with moderate to good anticancer efficacy. Notably, selected compounds **40a** and **40b** exhibited obvious cytotoxicity against MDA-MB-231 cancer cell lines (IC_{50} = 0.35 μM and 1.92 μM, respectively) [100].

The multicyclic spiroindolinone skeleton is distinguished in medicinal chemistry. In Qi and coworkers' research work, tetrahydro-β-spirooxindole compounds, along with their *in vitro* antitumor activity, were reported [101]. The primary SARs data was observed that introducing substitution on the isatin ring had an evident impact on their IC_{50} value against lung carcinoma cell A549. The fluorine substitution significantly improved the potency, thus making compound **41** become the most active agent (IC_{50} = 5.9 μM) against cancer cell A549 in this series. At the same time, this compound also displayed weak cytotoxicity on normal Chinese hamster lung cells (IC_{50} = 118.20 μM).

Heat shock protein 90 (Hsp90), an adenosine triphosphate (ATP)-dependent molecular chaperone, is a promising target for anticancer therapy. It exerts a key function in protein folding and 3D conformation in cells, as well as balancing the synthesis and degradation of a lot of proteins [102]. Mathew's team reported a small library of spirooxindole-dihydropyrimidinones inhibiting Hsp90 ATPase activity (IC_{50} = 0.18 to 6.80 μM). Four representative compounds **42a**–**42d** showed the most potent inhibitory effect (IC_{50} = 0.18 to 0.55 μM), which were stronger than geldanamycin (IC_{50} = 0.90 μM). Furthermore, compounds **42a** and **42b** displayed significant anticancer potency against HepG2 and MCF-7 cancer cell lines (IC_{50} = 28.43 μM, 21.32 μM, and 20.78 μM, 22.82 μM, respectively). Besides, the SARs results revealed that the presence of *S*-pyrimidone and introducing dichloro substitution at the C-5 and C-7 positions of the isatin were considered to be crucial for conferring their inhibitory efficacy toward Hsp90 [103].

In 2016, a series of pyrrolospirooxindoles were evaluated as potent antiproliferative compounds by Kamal *et al*. Especially compound **43** exhibited significant cytotoxicity against DU-145 human prostate cancer cell lines, which induced cell cycle arrest in G0/G1 phase and inhibited CDK4 expression level thus leading to cell apoptosis [104]. In the same year, Patravale *et al*. prepared a series of indeno-fused spirooxindoles and then tested for the cytotoxicities against normal Vero monkey cell lines and breast carcinoma cell lines (MCF7, MDA-MB-435). Two specific compounds **44a** and **44b** showed better potency against MDA-MB-435 cancer cell lines over the normal cell lines. In estrogen negative cancer cells, the *in vitro* confocal microscopy cell imaging results of these compounds displayed the cellular shrinkage and apoptosis as well as less significant potency toward estrogen positive receptor cell line (MCF7), which implied that these spirooxindoles could be explored as safe and selective estrogen negative receptors [105]. In 2018, a family of novel spirooxindole derivatives was constructed by Wu *et al*. [106]. As a continuing work, they found that these synthesized compounds were identified as high bioactive agents against cancer cell line HepG2, but not damage the normal cell line LO2. The most active compound **45** showed a 20.6-fold selectivity for HepG2 cell lines (IC_{50} = 2.9 μM) than LO2 cell lines, which was safer than tanshinone IIA (IC_{50} = 37.5 μM, selectivity ratio = 2.7). The further SARs analysis found that introducing small steric groups (e.g. -F, -Me) on the indole exhibited more potent potency than those with large volume groups (e.g. -Ph).

Sirtuins consist of a series of NAD^+-dependent protein deacetylases (SIRT1 to SIRT7), which participate in a host of cellular functions. SIRT2, a member of this family, is expressed in various tissues (especially in the adult brain). It has been reported to exert an effect on epigenetic regulation, cell metabolism, and aging. Also, it participates in the pathogenesis and development of several types of cancers. Therefore, designing SIRT2 inhibitors may contribute to developing new candidates for the treatment of cancer, Huntington, and Parkinson's diseases [107–109]. Hasaninejad *et al*. discovered that quite a few spirooxindole and spiroacenaphthylene derivatives could be considered to be potential SIRT2 inhibitors. More specifically, three spirooxindole derivatives **46a, 46b**, and **46c** showed moderate level inhibition (IC_{50} = 118 μM, 124 μM, and 126 μM, respectively) toward SIRT2, but weaker than sirtinol (a reference drug, IC_{50} = 67 μM) [110].

In 2015, diversely modified spirooxindoles containing thienopyridine moiety were presented by Perumal's research group. The cytotoxicity evaluation toward COLO320 adenocarcinoma colorectal cancer cells confirmed that compound **47** bearing a 2,6-difluorobenzyl group was the most active among the screened compounds [111]. In 2018, several spirochromeno indoline-triones were synthesized by Kumar *et al*. [112]. To their delight, the newly reported compounds were identified to be promising anti-prostate cancer agents because they could inhibit alkaline phosphatases and treat prostate cancer. The *in vitro* anticancer activity evaluation revealed that some of the compounds were as active as bicalutamide (an anticancer agent, IC_{50} = 1.25 μM) against PC-3 prostate cancer cells. More prominently, compounds **48a, 48b**, and **48c** (IC_{50} = 0.025 μM, 0.05 μM, and 0.081 μM, respectively) were proved to be more potent than bicalutamide.

3.2. Anti-microbial/bacterial/fungal activities

3.2.1. Spiro-(five-membered heterocyclic) oxindole derivatives

It is well accepted that microorganisms are very diverse and include all the bacteria and some fungi. And they can give rise to multiple infectious diseases. For example, respiratory infections induced by bacteria are the most common fatal bacterial diseases for humans with tuberculosis (caused by *Mycobacterium tuberculosis*) alone killing about 1.5 million people a year [113]. The infection of fungal pathogens (e.g. *Valsa mali, Fusarium semitectum*, and *Fusarium graminearum*) is a major cause of many plant diseases, which lead to great losses to agriculture and horticulture crop production annually [114]. Hence, it is imperative to develop antimicrobial agents to prevent these diseases. A brief review of the antimicrobial property of spirooxindole derivatives **49**–**76** is given here, which are described in Figure 4.

In Davis's research, the bioactivity of spirocycle derivatives derived from the substituted oxindoles and cyclopentadiene was evaluated against the cytochrome P450 enzyme CYP121 from *Mycobacterium tuberculosis*. Some of the compounds were found to be bond with CYP121 and displayed Type I binding interactions with the heme group. Using an indirect titration, compound **49** was identified as a promising antimicrobial agent having an apparent K_D value of 360 μM [115]. Rajaraman *et al*. reported a series of pyrrolidine-containing spirooxindoles in 2018. Among these synthesized compounds, **50a** demonstrated the best antibacterial activity (MIC = 12.5 μM for *Klepsilae pneumonia, Bacillus cereus*, and *Salmonella typhi*). And compound **50b** showed the most potent *in vitro* antifungal effect (MIC = 3.13 μM for *Aspergillus niger* and *Candida albicans*). These two compounds were comparable to the respective positive controls streptomycin and ketoconazole (MIC = 12.5 μM and 6.25 μM, respectively). A brief SAR was summarized that introducing methyl group or H to the R position of the core structure exhibited

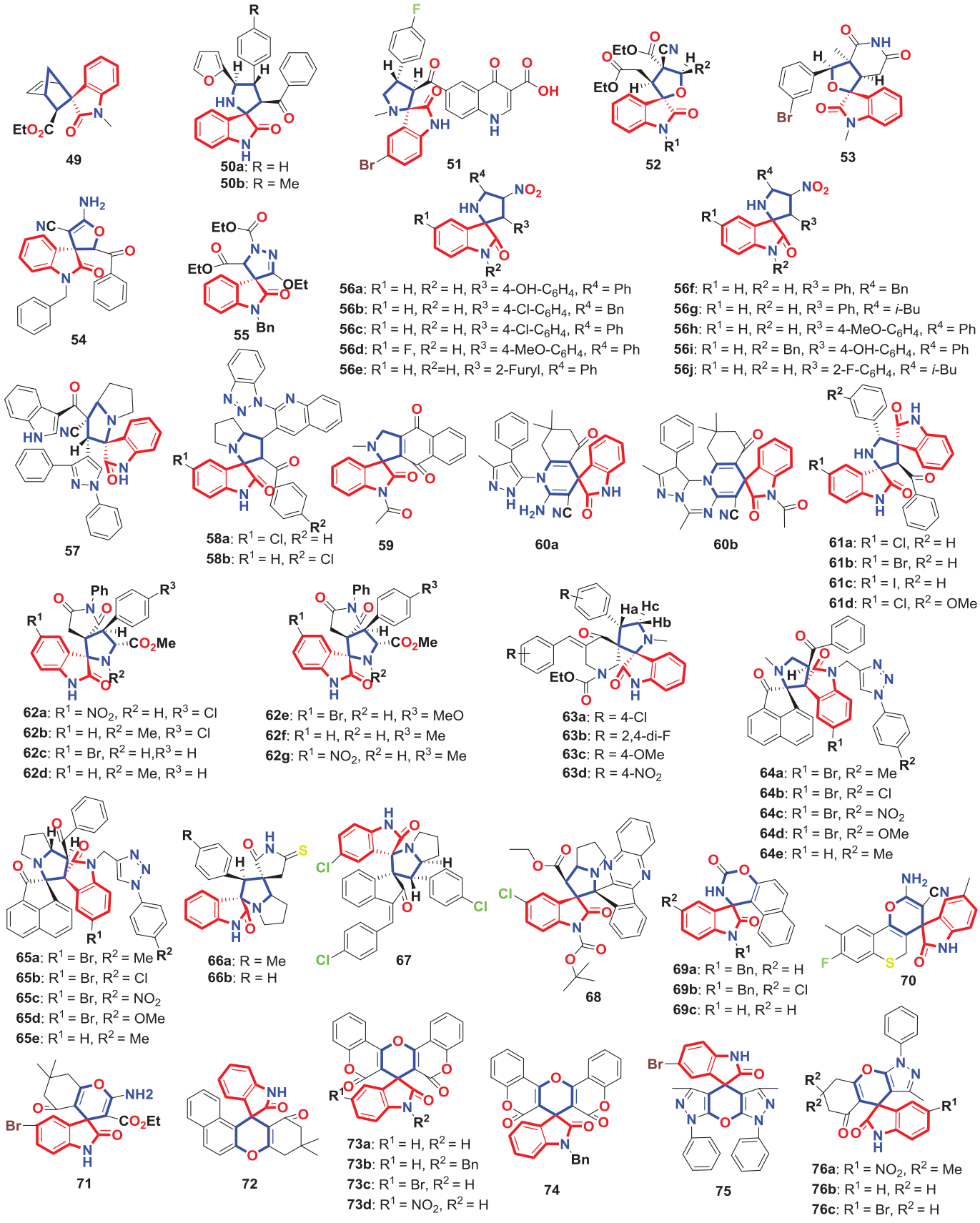

Figure 4. Spirooxindole derivatives **49**–**76** possessing antimicrobial activity.

more superior potency against the test bacteria than other substitutions [116].

A class of spirooxindole-pyrrolidines containing 4-quinolone-3-carboxylic acid moiety was synthesized by Arasakumar *et al*. Representative compound **51** displayed a positive inhibitory effect against the test bacteria. Besides, it also showed significant *in vitro* cytotoxic activity against MCF-7 breast cancer cell lines (IC_{50} = 18.35 μM), which was comparable to that of doxorubicin (IC_{50} = 15.00 μM) [117]. Wu *et al*. synthesized tetrahydrofuran-containing spirooxindoles **52** and further obtained a series of related octahydrofuro[3,4-*c*]pyridine derivatives. The optimized compounds resulted in the enhancement of activity in inhibiting the growth of *Fusarium graminearum* and *Valsa mali*. The best compound **53** showed similar inhibition on *Fusarium graminearum* (IC_{50} = 3.31 μM) with that of the positive control cycloheximide (IC_{50} = 3.30 μM) [118]. Spirooxindoles containing dihydrofuran ring also displayed potent antibacterial activity in controlling the growth of pathogenic strains *Escherichia coli* and *Bacillus subtilis*. The highlighted compounds were selected for the further study of inhibitory effect against eight different bacterial strains. As a result, compound **54** enjoyed the most active (MIC ≤ 50 μM for all the tested strains) [119].

Novel spirooxindole-pyrazolines were developed by Yang *et al*. [120]. The bioassay demonstrated that some of these compounds exhibited excellent fungicidal activity against the test fungi at a concentration of 50 μg/mL. The most active compound **55** showed higher *in vitro* activities against *Gibberellazeae* and *Pellicularia sasakii* than azoxystrobin. Furthermore, spirobenzofuranone-pyrazolines that the nitrogen atom was replaced by oxygen in oxindole displayed high efficacies against all the nine kinds of fungi, which were superior or close to that of the positive control agent. The observed SARs suggested that this series compounds have great potential for discovering new fungicide via modification of the oxindole core. A family of spiro[indoline-3,2′-pyrrolidin]-2-ones was synthesized under microwave irradiation by Meshram's team. Thereinto compounds **56a** and **56b** showed the highest activity against *Escherichia coli* ATCC 10536 (MIC = 9 μM). And compounds **56c**–**56g** possessed the most control effect against *Candida tropicalis* ATCC750 (MIC = 5 μM). Also, compounds **56h** and **56i** exhibited the highest inhibition against *Staphylococcus aureus* ATCC 25923 (MIC = 5 μM). Against *Pseudomonas aeruginasa* ATCC 15442, compound **56j** displayed excellent potency, which was comparable to that of ciprofloxacin (a standard antimicrobial drug). In this series, compounds with corresponding *α*-amino acids rather than benzylamine, and fluorine substituted rather than hydroxy (or) methoxy substituted nitro styrenes exhibited better activity against most of the test bacteria [121].

In 2015, Duraipandiyan's group synthesized a series of 3, 2′-spiropyrrolidine-oxindoles. Most compounds possessed weaker *in vitro* antimicrobial activity than the standard antimicrobial agent. And only compound **57** showed excellent and broad-spectrum activity for the test bacteria. Compound **57** revealed the best binding energy (−11.66 kcal/mol) with DNA Topoisomerase IV receptor. This compound could form two polar interactions with the receptor and interact with two amino acids [122]. Pogaku *et al*. synthesized a range of benzotriazoloquinolinyl spirooxindolopyrrolizidine derivatives. It is worth mentioning that most compounds displayed significant antimycobacterial activity. In particular, compound **58a** (MIC = 1.27 μM) was more active than ethambutol (a standard drug, MIC = 7.64 μM). In respect to antiproliferative activity, **58b** exhibited more significant activity against A549 (IC_{50} = 5.70 μM) and HeLa S3 (IC_{50} = 11.60 μM) cell lines than cisplatin (IC_{50} = 9.80 μM and 20.10 μM, respectively). What's more, these two derivatives displayed less toxicity against normal RAW 264.7 cells, which hold the promise to be antimycobacterial and antiproliferative candidate compounds [123].

Bhaskar *et al*. found that the newly synthesized spirooxindoles containing 1,4-naphthoquinone moiety possessed outstanding antimicrobial activity against eight kinds of bacteria and three kinds of fungi. Especially compound **59** was found to be 1.6 to 6.4 folds effect against the Gram-positive bacteria (*Staphylococcus aureus, Micrococcus luteus*), Gram-negative bacteria (*Salmonella typhimurium*), and fungi (*Candida albicans*) than ciprofloxacin [124]. Based on the versatile bioactivities of spirooxindole and 2-amino-tetrahydroquinolin-5-one, Ghozlan *et al*. got some target compounds via incorporating these two scaffolds. Among them, representative compounds **60a** and **60b** exhibited certain controlling effects against *Bacillus subtilis* (MIC = 0.24 μM and 0.49 μM), *Streptococcus pneumoniae* (MIC = 0.49 μM and 0.98 μM), and *Escherichia coli* (MIC = 0.49 μM and 0.98 μM). In addition, all the obtained compounds in this series exhibited moderate to excellent bioactivity against *Aspergillus fumigatus*, but no inhibition toward *Candida albicans* [125].

A series of functionalized bis-spirooxindoles were prepared by Ramesh *et al*., which showed favorable *in vitro* antimicrobial activity against several bacteria. They were more active against the Gram-positive organisms compared with the Gram-negative organisms and *Candida albicans*. In most cases, halogen substituted derivatives (e.g. **61a**–**61c**), methoxy substituted derivatives (e.g. **61d**), and dihalogenated derivatives possessed superior antibacterial activity against *Micrococcus luteus*. However, compounds **61a** and **61b** lost their efficacy toward the Gram-negative bacteria (*Pseudomonas aeruginosa* and *Salmonella typhi*) [126].

Haddad and coworkers synthesized a class of spirooxindole-pyrrolidine derivatives. Several compounds were found to display remarkable *in vitro* biological activities involving antibacterial and antifungal effects. Against Gram-negative bacteria *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 1688), representative compounds **62a** and **62b** showed comparable activities (MIC = 50 μM and 62.5 μM, respectively) as compared to the standard antibiotic chloramphenicol (MIC = 50 μM and 50 μM, respectively). Moreover, compounds **62b** and **62c** were the most active for Gram-positive bacteria *Staphylococcus pyogenus* (MTCC 442) and *Staphylococcus aureus* (MTCC96) (MIC = 100 μM and 62.5 μM), respectively. In the study of antifungal effects against *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 282), compounds **62a, 62d**, and **62e** displayed the highest inhibition in this series. Besides, the further assay demonstrated that derivatives **62f** and **62g** were proven to be active agents against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTCC 200) strain [127].

In 2017, Hassaneen *et al*. [128] reported the synthesis and bioactivities of spirooxindole-spiropiperidinone-pyrrolidines

/pyrrolizines. These compounds exhibited strong antimicrobial activity against all the test bacteria species, as well as showed a more potent effect against Gram-positive bacteria over negative bacteria. Against *Bacillis subtilis*, the most active compounds **63a**–**63d** were identified. Of which compounds, **63a** and **63b** displayed comparable antimicrobial activity with the control drugs against all the test microorganisms. The results have been proved that compounds bearing phenyl-hexahydro-1*H*-pyrrolizine ring were prone to be more active than compounds containing methyl-substituted pyrrolidine ring. But for the fungi *Candida albicans*, most derivatives denoted no growth inhibition. A similar phenomenon was also described by Ramesh and Bhaskar *et al*. [124,126].

Considering that triazoles display a broad range of bioactivities including antimicrobial, antifungal, and anticancer [129–131], Sakly *et al*. synthesized a series of spirooxindolopyrrolidine and spirooxindolopyrrolizidine-linked 1,2,3-triazole conjugates via an efficient one-pot four-component domino reaction. *In vitro* antifungal and antibacterial effects assay indicated that these new compounds possessed favorable activities. In the details, compounds **64a**–**64c** and **65a**–**65c** exhibited excellent inhibition against *Staphylococcus aureus* and *Escherichia coli* (MIC = 31.25 μM). Likewise, compounds **64d** and **65d** exhibited a positive inhibitory effect against *Staphylococcus pyogenus* (MIC = 62.5 μM). Besides, compounds containing a nitro group (**64 c** and **65 c**) or methyl group (**64e** and **65e**) on the triazole ring showed pleasurable activity against *Candida albicans*. It seemed that some substituents on the aryl ring that was linked to the triazole ring and halogen substituents on indolinone could be beneficial to enhance the potency [132].

Compounds **66a** and **66b** bearing polycyclic heterocycles were synthesized by Ghabbour's research group. Both of them showed antifungal and antimicrobial activities, which were superior to that of four controls fluconazole, Amphotericin A, gentamicin, and ampicillin. More detailly, compound **66b** possessed better efficacy (MIC = 15 μM) than **66a** and gentamicin (MIC = 16 μM and 19 μM, respectively) against *Pseudomonas aeruginosa* [133]. A series of functionalized spirooxindole pyrrolizidines were prepared by Rouatbi *et al*. Representative compound **67** displayed equipotent *in vitro* potency against *Mycobacterium tuberculosis* (MIC = 1.56 μM) with ethambutol. The summarized SARs data showed that the substituents on the isatin had a great influence on their antibacterial activity (such as Cl > CH_3 > propargy) [134]. Ren and coworkers synthesized a series of spirooxindole–indenoquinoxaline derivatives and tested their bioactivities. Among these compounds, **68** showed the best *in vitro* activity against *Escherichia coli* tryptophanyl-tRNA synthetase (TrpRS) with an IC_{50} value of 74 nM. Compound **68** also had a comparable activity with the standard indolmycin against *Staphylococcus aureus* (MIC_{90} = 4 μg/mL). Moreover, it also exhibited a good inhibitory effect against diffuse large B cell lymphoma (DLBCL) cell proliferation. This compound could be an attractive scaffold for the discovery of antibiotics and anticancer agents [135].

3.2.2. Spiro-(fused-pyran) oxindole derivatives

Nishtala *et al*. reported a series of spirooxindolocarbamates and then evaluated their bioactivities. Specifically, the *in vitro* antibacterial activity results displayed that compound **69a** had higher inhibition (MIC = 7.5 μg/mL) against *Escherichia coli* when compared with ciprofloxacin (MIC = 9.25 μg/mL). Compound **69b** displayed potent antioxidant activity (IC_{50} = 7.06 μM), which was comparable to that of the standard ascorbic acid (IC_{50} = 6.63 μM). Besides, compound **69c** exhibited moderate antifungal activity against *Aspergillus niger* (MIC = 17.5 μg/mL) [136].

In the research of Song *et al*., the pyran and thiochromanone moieties possessing potential antifungal and anticancer activities were combined with spirooxindoles to obtain corresponding compound **70**. As expected, this compound was better than fluconazole in inhibiting the activities of *Epidermophyton floccosum, Cryptococcus neoformans*, and *Mucor racemosus* [137]. Moradi and his cooperators reported the synthesis and *in vitro* antimicrobial activity of spirooxindole derivatives in 2019. The bioactivity results showed that representative compound **71** was active against all the eight test microorganisms, and was the most potency against *Proteus vulgaris* with a MIC value of 250 μg/mL in this series [138]. Compound **72** was synthesized by Kong *et al*., which exhibited pleasurable antimicrobial effects against *Staphylococcus aureus, Escherichia coli*, and the other four test bacteria *in vitro* with MIC values to be around 10 μM [139].

In 2015, Parthasarathy *et al*. introduced coumarin into substituted isatins scaffold utilizing the catalyzation of zinc reagent. The antimicrobial evaluation displayed that the activities of three representative compounds **73a**–**73c** were comparable to that of streptomycin against the test bacterial strains. And **73b** was equipotent against fungi *Candida albicans* to ketoconazole. Accidentally, the cytotoxic investigation against COLO320 adenocarcinoma colorectal cancer cell lines manifested that analog **73d** was the most active (IC_{50} = 50.7 μM toward checkpoint kinase 1 receptor). An obvious SAR was observed that an increase in electronegativity and size at the C-5 position of the oxindole core could result in the enhancement of potency [140]. Next year, this team further reported the antimicrobial potency and cytotoxic evaluation of similar compounds synthesized via the catalyzation of gold reagent. Antimicrobial evaluation displayed that the activity of newly yielded compound **74** was also close to that of the corresponding control agent [141].

In 2015, Ziarania and his coworkers prepared a series of pyranodipyrazol-containing spirooxindoles using SBA-15-Pr-SO_3H as a catalyst. Some of these derivatives showed an extent of antimicrobial activity against *Staphylococcus aureus* and *Bacillis subtilis*. Especially, compound **75** exhibited the best efficacy against *Staphylococcus aureus* (MIC = 64 μM) in this series. Unfortunately, none of these compounds displayed an antibiotic effect against *Pseudomonas aeruginosa, Escherichia coli*, or *Candida albicans* [142]. In 2016, this research team further synthesized spirooxindoles containing chromene ring via a similar synthetic approach and then tested their antimicrobial activity. Representative compounds **76a**–**76c** showed expected potency toward *Staphylococcus aureus, Bacillis subtilis*, and *Escherichia coli*. But none of the

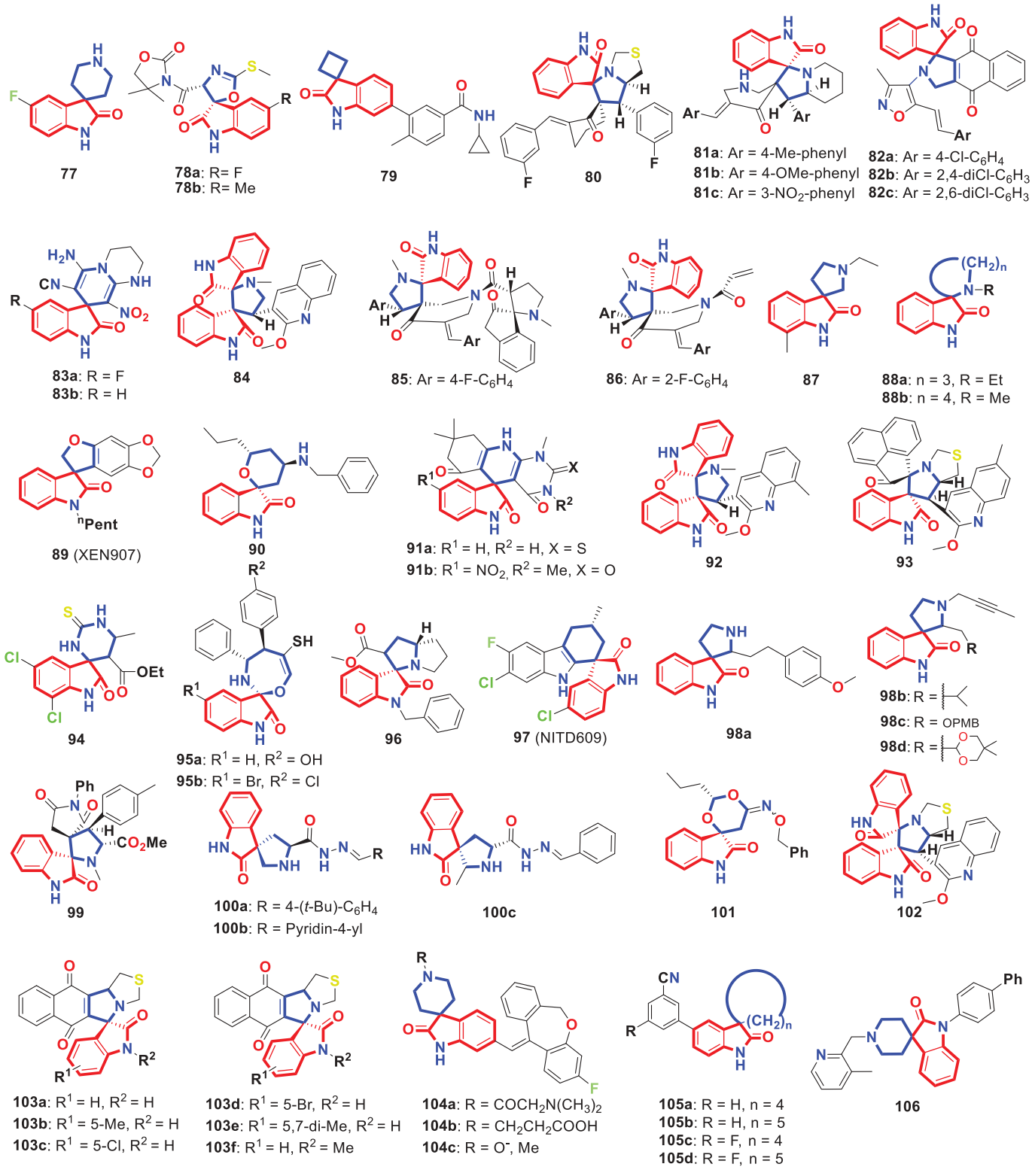

Figure 5. The structures of spirooxindole derivatives **77**–**106.**

compounds exhibited antibiotic activity against *Candida albicans* or *Pseudomonas aeruginosa* [143].

3.3. Anti-inflammatory activity

Inflammatory conditions are a common clinical-pathological process, which can occur in tissues and organs of various parts of the body. Particularly, those that involve in chronic inflammation disorders (such as rheumatoid arthritis, asthma, bronchitis, nephritis, and psoriasis) result in body damage to the suffered patients [144]. In the investigation of anti–inflammatory drugs, spirooxindole-based derivatives have been proved to be potential non-steroidal therapeutic agents. The structures of spirooxindoles **77**–**82** with anti–inflammatory activity are depicted in Figure 5.

Taking spiro-piperidyl oxindole **77** as an example, it possessed anti–inflammatory, antipyretic, and analgesic effects, especially against chronic pain related to inflammation [145]. A kind of new spirooxazolines analogs in particular compounds **78a** and **78b** disclosed by Jiang *et al*. were capable of significantly reducing lipopolysaccharide-induced fever in a model of acute neuroinflammation, which displayed promising antipyretic activity [146]. Eastwood *et al*. designed and synthesized a family of spirooxindole compounds through computation simulation, among which compound **79** was selected as a powerful and high selective p38αmitogen-activated protein (MAP) kinase inhibitor (IC_{50} = 1.3 nM) [147]. In the activated state of p38α, a series of intracellular protein substrates are phosphorylated, which made post-transcriptionally regulate the biosynthesis of cytokines tumor necrosis factor-α (TNF-α) and interleukin-1B (IL-1B). The excessive production of these two cytokines is significant in the development of inflammatory diseases [148–150]. Therefore, the highly selective inhibition of a molecule for p38α may transform it into an anti–inflammatory agent for clinical treatment.

In 2019, Barakat's research team synthesized a series of spirooxindole-pyrrolothiazoles, which had excellent anti–inflammatory activity. Among these compounds, **80** (IC_{50} = 2.4 μM) containing two *meta*-fluorine substituted phenyl rings was about five-fold more active than ibuprofen. This compound has the potential to be further investigated as a lead agent [151]. A series of novel spirooxindole-indolizines containing substituted piperidine moiety were accomplished and then evaluated for the anti–inflammatory efficacy by Kumar and his cooperators. Among them, derivatives **81a**–**81c** showed favorable anti–inflammatory efficacy against some acute and chronic inflammatory models, which could significantly reduce the levels of prostaglandin E2, TNF-α, and nitrite in carrageenan-induced hind paw edema [152].

Several hybrids of isoxazolyl and pentacyclic spirooxindole were reported in Rajanarendar's work. Three compounds **82a**–**82c** possessed remarkable anti–inflammatory activity with decreasing the paw volume. And these compounds also displayed significant analgesic potency and reduced the number of writhings of mice compared with diclofenac. Through the systematic study of SARs, the authors considered that their potent effect was attributed to the substitution of chlorine on the benzene ring [153].

3.4. Spirooxindole-based analogs as cholinesterase inhibitors

Cholinesterase (ChE) is a crucial hydrolase in living organisms, including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which is widely distributed in human brain, blood, and other tissues. They play a pivotal role in the regulation of physiological functions and the formation of Aβ (β-amyloid, one of the most significant biomarkers of Alzheimer's disease (AD)). Both AChE and BChE are considered as therapeutic targets for the different AD pathological stages i.e. slightly to moderate, moderate to severe stage of AD, respectively [154]. Nowadays, some of the spirooxindole analogs are discovered to display potential inhibitory effects toward AChE/BChE. Here we would take compounds **83**–**86** as representative examples to illuminate their cholinesterase inhibitory activity (Figure 5).

In 2016, Maryamabadi *et al*. designed and synthesized a new class of functionalized spirodihydropyridine-spirooxindole hybrids, which showed moderate to high inhibition against AChE and BChE *in vitro* and *in silico*. Among them, compound **83a** possessed more potent inhibition toward AChE than galantamine (IC_{50} = 0.42 μM *vs* 0.61 μM). And compound **83b** possessed comparable activity with the positive control toward BChE (IC_{50} = 8.93 μM *vs* 6.12 μM) [155]. In the same year, Mathusalini *et al*. synthesized a number of dispiropyrrolidinyl-containing bisoxindoles. Most of these compounds showed equivalent potency against AChE as compared to eserine (a reference drug). Especially compound **84** had the highest inhibitory potency (IC_{50} = 12.80 μM) among them. Besides, the main SARs results illustrated that the presence of the methoxy group on the oxindole was beneficial to the improvement of inhibition toward AChE [156].

In Kia's previous effort, via a three-component reaction of 1-acryloyl-3,5-bisbenzylidenepiperidin-4-ones with L-proline and isatin/5-choloroisatin afforded a series of piperidone-grafted functionalized mono- and bisspirooxindoles. These synthesized cycloadducts were active against cholinesterase enzymes with the range of IC_{50} values from 3.36 to 54.04 μM. In most cases, monospiripyrrolizines were more potent than bisspiropyrrolizines [157,158]. As a continuous research, this group obtained a new series of the same type of hybrids in good yields by changing L-proline into sarcosine. As expected, all the compounds displayed favorable inhibitory activity. Against AChE, their IC_{50} values ranged from 2.36 to 9.43 μM. Against BChE, mono-cycloadducts still showed a better inhibitory effect than their bis-cycloadduct analogs. Above all, compounds **85** and **86** were found to be the most active against AChE (IC_{50} = 2.35 μM) and BChE (IC_{50} = 3.21 μM) in this series, respectively. Through studying the mechanism of action, compound **85** was demonstrated as a competitive inhibitor of AChE, while **86** was a mixed-mode inhibitor of BChE. The docking analysis of the corresponding compounds revealed a good correlation between IC_{50} values and free binding energies when the compounds were docked into the active site of *Torpedo californica* AChE and BChE [159]. In 2018, Amiri *et al*. studied QSAR, docking, and molecular dynamics of piperidone-grafted mono and bis-spiro-oxindole-hexahydropyrrolizines as potent BChE inhibitors. The docking results manifested that the hydrogen bond (e.g. between the carbonyl oxygen of Pro285 and the amino hydrogen of oxindole) was a pivotal index in displaying their inhibitory effect in all the studied 33 BChE inhibitors. Molecular dynamics results also demonstrated that these potent compounds had a higher tendency to interact with the BChE, thus changing enzyme structure [160]. The above results offer a solid foundation to design novel spirooxindoles with better ChE inhibitory activity.

3.5. Analgesic activity

The pathology of chronic pain diseases has severely affected the human's life. It is estimated that chronic pain impacts

approximately 20% of adults worldwide. For centuries, treatment with opioid analgesics (e.g. morphine and codeine) has been researched broadly. However, these existing drugs have produced some adverse effects, such as dependence, tolerance, constipation, and respiratory depression [161–163]. Searching for novel agents or therapeutic targets remains an arduous work. Here some of the spirooxindoles **87**–**90** (Figure 5) exhibited outstanding analgesic activity, which given more options to develop new analgesic drugs.

Kornet *et al*. reported the structure and synthesis of oxindole-3-spiropyrrolidines and -piperidines. All the synthesized compounds showed local anesthetic properties by the assessment of rat sciatic nerve block. Among them, 7-methyl substituted compound **87** exhibited the best potency. Notably, indolinones with either a 2′-pyrrolidine **88a** or 2′-piperidine substituent **88b** displayed the lowest normalized toxicities. Besides, an apparent SAR was observed in this series that the increase in the size of the N-alkyl functional group produced an improvement in iv injection toxicity [164].

The human ion channel $Na_V1.7$ subtype (encoded by the SCN9A gene) has aroused much attention as a drug target for treating chronic pain [165]. In 2011, based on an oxindole-containing hit compound obtained from high-throughput screening, some $Na_V1.7$ blockers were discovered by Cadieux's group. And then the structural modification of substituents on the C-3 and N-1 position and the employment of ring formation rigidification strategy afforded a highly potent compound **89** (XEN907) (IC_{50} = 3 nM), which led to a dramatic increase in activity (100-fold) when compared to the initial hit compound. The pharmacokinetic analysis in rats showed that **89** possessed modestly bioavailable (F = 13%). After the initial rapid absorption phase (po T_{max} = 20 min), compound **89** was extensively distributed (V_{ss} was about 600-fold higher than the plasma volume in rats) and rapidly cleared [166]. In 2016, a series of spirooxindole tetrahydropyranones were prepared by Cui *et al*. Moreover, their inhibitory effect against Nav1.7 was evaluated by Tanaka's team. It is worth mentioning that compound **90** was the most potent lead with an IC_{50} value as low as 1.0 μM [167], which implied that compounds containing an epoxy alkyl motif would exert great use in the innovation of pain treatment agents

3.6. Antioxidant activity

Since oxidative stress can damage healthy cell structure and function, it is considered as one of the main factors to induce a multitude of human diseases such as cancers. The application of antioxidants in pharmacology has been intensively studied, particularly for treating stroke and neurodegenerative diseases [168,169].

As depicted in Figure 5, a new series of uracil-fused spirooxindoles were reported by Baharfari *et al*. in 2015. Their antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay [170]. The results illustrated that all the compounds in this series exhibited excellent inhibitory activity. Among them, compounds **91a** and **91b** displayed the highest percentage inhibition of DPPH radical activity (96.2% and 92.5%, respectively), which could be attributed to the presence of NH groups [171,172]. In 2016, Mohan's research group synthesized several mono- and bisoxindole containing dispiropyrrolidinyl/thiapyrrolizidinyl hybrid molecules and further evaluated their *in vitro* antioxidant activity. The tested results of DPPH radical scavenging, lipid peroxidation antioxidant, and metal chelating activities exhibited that highlighted compounds **84**, **92,** and **93** possessed stronger or comparable potency in both primary and secondary antioxidant assays compared with other derivatives and the positive control. The presence of an electron-donating methoxy group at the β-position of the quinoline ring might enhance the stabilization of the resulting oxygen radicals [156]. In 2018, spirooxindole-dihydropyrimidinone **94** obtained by Maddela *et al*. also displayed equipotent antioxidant activity in the DPPH test and H_2O_2 assay (IC_{50} = 20.13 μM and 23.27 μM, respectively) with ascorbic acid (a standard drug, IC_{50} = 19.16 μM and 20.66 μM, respectively) [103]. Recently, Zahedifar *et al*. reported the synthesis, antioxidant, and antibacterial activity of spiroindoline oxazepine derivatives. According to the assay results, most of these compounds exhibited antioxidant activity and antibacterial property. Among them, compound **95a** possessed the highest antioxidant activity against DPPH, and compound **95b** was the most active against *Bacillus subtilis* [173].

3.7. Antileishmanial and antiplasmodial activity

Visceral Leishmaniasis is a terrible parasitic disease, which affects millions of people all over the world, especially tropical and subtropical countries like India and Bangladesh [174,175]. It is an urgent demand for discovering more effective chemotherapeutic targets and drugs owing to a lack of proper vaccine and the emergence of drug-resistant strains [175–177]. The detailed structures of spirooxindoles **96**–**99** with antileishmanial and antiplasmodial activities are provided in Figure 5.

In 2016, Saha *et al*. reported a spirooxindole-based derivative **96**, which could inhibit the catalytic activity of *Leishmania donovani* topoisomerase IB and kill both the wild type and drug-resistant parasite strains. It is well known to all that DNA topoisomerase enzymes of *Leishmania* have been reported as excellent targets for antileishmanial chemotherapy. Unlike camptothecin, compound **96** did not stabilize the DNA-topoisomerase IB cleavage complex instead of hindering the formation of the drug-DNA-enzyme covalent complex. Treatment with compound **96** depolarized the mitochondrial membrane potential and formed reactive oxygen species in the parasite, eventually which led to nuclear DNA fragmentation. The strong antileishmanial efficacy of **96** in the BALB/c mice model of leishmaniasis along with the low cytotoxicity toward host macrophages is suggestive that this compound has great potential to be an antileishmanial therapeutic agent in future [178].

To date, statistics report from the World Health Organization (WHO) shows that malaria infections have given rise to over 435,000 deaths worldwide, notably in Africa. Malaria is caused by single-celled parasites of the genus *Plasmodium*, especially *Plasmodium falciparum* (It accounts for approximately 75% of all human malarial infections and nearly all deaths globally). More seriously, many existing antimalarial therapeutic agents (such as artemisinin) have suffered from drug resistance issues currently [179–181]. In 2010, spirooxindole-based compound **97**

(NITD609) synthesized by Rottmann *et al*. was served as an antimalarial drug that could replace artemisinin. It showed strong inhibitory activity against the clinical isolates of *Plasmodium falciparum*. This compound could inhibit the protein synthesis of *Plasmodium falciparum*. More significantly, compound **97** possessed ideal physicochemical properties and pharmacokinetic characteristics. Through the assessment of intravenous and oral administration in mice, they found that this compound exhibited a moderate volume of distribution (V_{ss} = 3.04 and 2.11 L/kg) and a low total systemic clearance (CL = 3.48 and 9.75 ml min^{-1} kg^{-1}). Compound **97** displayed a long half-life ($T_{1/2}$ = 10 and 27.7 hours) and excellent bioavailability (F = 100%) after orally administered. Besides, it has favorable pharmacological property and excellent safety at a specific dose. Nowadays, this candidate has completed a phase II clinical trial on safety, pharmacokinetic character, and pharmacological activity (NCT01860989; NCT01836458; NCT01524341) [182].

In many of the polycyclic indole alkaloids isolated from *Mitragyna inermis*, speciophylline exhibited potent antiplasmodial activity toward the chloroquine-resistant strain W2. In 2018, Pierrot *et al*. reported a number of simplified speciophylline analogs [183]. The antiplasmodial activity results revealed that representative compound **98a** was the most active (IC_{50} = 10.6 μM) against the chloroquine-resistant *plasmodium falciparum* strain K1. The N-alkylation derivatives of spirooxindoles **98b, 98c**, and their diastereoisomers exhibited much better potency (IC_{50} = 11.2 to 29.4 μM) against K1 strain than the positive control speciophylline (IC_{50} = 42.1 μM). It is interesting to note that compounds **98a**–**98d** displayed moderate *in vitro* cytotoxic activity against HT29 colon adenocarcinoma cancer cell lines. Also, the protected alcohol **98a** and its alkylated analog **98c** exhibited the same cytotoxicity. Besides, the introduction of the alkyne chain on the nitrogen atom could increase the cytotoxicity and antiplasmodial potency to some extent. The discovery of such cheaper and simpler structural analogs possesses significant value for efficiently studying these natural products' mode of action and developing new cures against malaria. Noteworthily, most derivatives of the above-mentioned compound **62** with antifungal effect also displayed good antimalarial potency. In particular, the optimal compound of this series (**99**) possessed better activity than quinine.

3.8. Antiviral activity

Viruses can invade different tissues, infect cells, cause sickness, and threaten human health seriously. The development of high efficiency, low toxicity, and low resistant therapeutic antiviral drugs currently remain a challenge. Due to the unique structural features, spirooxindoles have been widely used in the discovery of antiviral drug with a broad spectrum of virus inhibitory activities, such as anti-human immunodeficiency virus, anti-respiratory syncytial virus, anti-dengue virus, anti–influenza virus, and other antiviral activities. Ye and Jin's reviews [28,184] have systematically described the feasibility of these spirooxindoles as antiviral therapeutic agents, here we will mention concerning the activities in brief.

In 2016, Wang's group synthesized a kind of new spirooxindole hybrids containing acylhydrazone moiety and further evaluated their biological activity. Most compounds in this series exhibited good antiviral activity against tobacco mosaic virus. Among them, compounds **100a**–**100c** (Figure 5) displayed the best *in vitro* potency as well as inactivation, curative, and protection activities *in vivo*, which were all the better than the positive controls ribavirin and harmine. Unexpectedly, most of this series compounds displayed more than 60% fungicidal effect against *Physalospora piricola* at 50 mg/kg as well [185].

3.9. Other related biological activities of spirooxindole-based analogs

There is a sea of spirooxindole-based compounds possessing other paramount bioactivities except what we have described above. These derivatives can perform multiple tasks, which have great potential for further transformation into multi-effect or specific effect oriented therapeutically useful drug candidates. This diversity will be demonstrated concisely here.

3.9.1. Antiatherosclerotic activity

The enzyme sterol *O*-acyltransferase (SOAT) can regulate cholesterol metabolism [186], which consists of two isozymes SOAT1 and SOAT2. SOAT1 is ubiquitously expressed in all cells, while SOAT2 is expressed mainly in the hepatocytes [187]. Lack of SOAT1 will result in many harmful effects such as dry eye and cutaneous xanthomatosis. On the contrary, lacking SOAT2 would like to exert antiatherosclerotic and antihypercholesterolemic effects [188–191]. These findings suggest that SOAT2-selective inhibitors have the potential to act as novel antiatherosclerotic agents. In 2016, Tanaka's team reported some spirooxindoles containing tetrahydropyran moiety and systematically evaluated their inhibitory effects against SOAT1 and SOAT2. Beyond expectation, three racemic derivatives, particular compound **101** (Figure 5), could effectively inhibit SOAT2 (IC_{50} = 1.5 μM), which showed 11-fold selectivity for SOAT2 than for SOAT1. This study provides a way to discover promising antiatherosclerotic inhibitors by discriminating isozymes [192].

3.9.2. Antidiabetic activity

In 2016, Mohan's research group evaluated the antidiabetic efficacy of dispiropyrrolidinyl/thiapyrrolizidinyl-spirooxindoles. The results of antidiabetic assay demonstrated that derivative **102** (Figure 5) exhibited higher inhibitory potency against α-amylase with an IC_{50} value of 18.72 μM when compared to the positive control curcumin (IC_{50} = 24.98 μM). The potency of compound **102** might attribute to the presence of NH on the oxindole and methoxy group on the quinoline ring [156].

3.9.3. DNA cleavage activity

In 2018, many hexacyclic spirooxindoles were constructed by Jain's group [193]. These synthesized compounds possessed promising DNA cleavage function in the evaluation of inhibitory potency. All the tested compounds **103a**–**103f** (Figure 5) could completely cleave the DNA of *Staphylococcus aureus* after treatment.

3.9.4. MR and PR antagonistic activity

Nuclear hormone receptor (NHR) family commonly contains mineralocorticoid receptor (MR), progesterone (PR), estrogen (ER), androgen (AR), and glucocorticoid (GR) receptors. These

receptors can regulate the expression of specific genes together with other proteins, thereby controlling the development, homeostasis, and metabolism of the organism. However, abnormal activation of these receptors may result in disease. As an example, abnormal activation of MR can give rise to cardiovascular, hypertension, and chronic kidney disease [194,195]. In this respect, Lotesta *et al*. developed a row of potent MR antagonists by combining the spirooxindole scaffold with dibenzoxazepine. The SARs analysis of these analogs produced several highly active compounds possessing polar solubilizing groups, which interacted with the helix-1L region of the MR ligand-binding domain. Especially compounds **104a**–**104c** (Figure 5) exhibited favorable binding against MR (K_i ranged from 6 to 19 nM) and cellular potencies as well as excellent selectivity (400 to >1000 folds) against the other three members of NHR family (glucocorticoid, progesterone, and progesterone receptors). Additionally, these compounds possessed reasonable cytochrome P450 profiles and excellent human liver microsomal stabilities [196].

PR is a ligand-activated transcription factor, which can regulate physiological processes such as reproduction and morphogenesis. A class of aryloxindoles was prepared by Fensome and coworkers [197]. Moreover, these compounds also were evaluated for PR antagonist activity in the T47D cell progesterone-induced alkaline phosphatase assays and their potency to bind PR in the competition binding assay. Spirocyclopentyl and its derivatives, especially compounds **105a**–**105d** (Figure 5) bearing a -CN group on the benzene ring, showed better potency against alkaline phosphatase than those compounds that the C-3 position of oxindole was not spirocycle. Thereinto, compound **105d** was regarded to be the most active compound (IC_{50} = 51 nM), while spirocyclopentyl analog **105c** was less active (IC_{50} = 230 nM). Therefore, this type of cyclohexyl compound has potential utility as contraceptive or medicine used in the treatment of reproductive disorders (such as endometriosis, uterine leiomyomas, and hormone-dependent tumors) [199,198].

3.9.5. Insecticidal activity

As a representative case of 'agricultural use in medicine [198]', the above-mentioned compound **100** with antiviral activity also possessed promising insecticidal potency. Notably, its derivatives **100b** and **100c** (Figure 5) exhibited potent inhibition against *Culex pipiens pallens* at 0.25 mg/kg. But the enantiomer of compound **100c** at the 5-position displayed a less insecticidal effect (20% mortality against *Culex pipiens pallens* at 5 mg/kg), which suggested the great significance of chirality in the spirooxindole molecules. Some compounds of this series hold not only great potential for medical applications but also agricultural applications [185].

3.9.6. PHD2 inhibition activity

Vachal *et al*. [200] screened out a class of spirooxindole derivatives using affinity selection mass spectrometry (AS-MS) methodology and further systematically optimized for their efficacy and physical-chemical properties. Modified analog **106** (Figure 5) showed 4 nM inhibition *in vitro* toward PHD2. Based on compound **106**, some lead compounds with better therapeutic effects were obtained by further scaffold transition. Finally, they achieved a short-acting PHD inhibitor among the modified compounds, which was proved to be effective in treating anemia.

4. Main synthetic routes and reactions for the construction of spirooxindole derivatives

With the growing reports of the extensive and excellent biological activities regarding the spirooxindole-based natural products and the analogs, the structural modification based on this subject also has captured much attention, which lays an abundant and solid material foundation for the exploration and discovery of pharmaceutical agents. Due to the existence of the chiral carbon atom of spirooxindole and its huge structural alterability, various derivatives can be constructed based on the structure of 2-oxindole via forming different kinds of the non-planar tricyclic or polycyclic scaffold at the C-3 site of oxindole. Its synthetic methods, especially the simple and high-efficiency asymmetric synthesis, have always been a hot field studied by researchers. Actually, numerous synthetic methods of spirooxindoles have been developed, which mainly include 1, 3-dipolar cycloaddition, domino Knoevenagel-Michael-cyclization, Michael-cyclization of isatins, C-H activation, and D-A reaction, etc. The related synthetic chemistry of spirooxindoles has been comprehensively summarized and described by organic chemists in the previous excellent reviews [25,201–212].

5. Conclusion

In summary, a large number of spirooxindole-based natural products and their analogs have been reviewed. Because their various bioactivities have been discovered, the study on the modification of this parent structure is in full swing, all of which have been consolidated by the great efforts of researchers. This overview is an attempt to take the readers on a clear profile of spirooxindole-based scaffold with enormous potential in drug design and discovery. Not only do an army of the related natural products bring hope for further development of agents for disease treatment, but they also provide directions for the further modification of spirooxindole compounds. The unique structure of spirooxindoles endows them with broad-spectrum and excellent pharmaceutical properties, including anticancer, antimicrobial, anti–inflammatory, analgesic, antioxidant, antimalarial, antiviral, and other important biological activities. In recent years, some of the new lead compounds featuring spirooxindole structure have been found continuously in the field of targeted small molecule drugs due to their high target selectivity, suitable physicochemical, and excellent pharmacokinetic properties. At present, a slice of potent candidate agents has entered the clinical research stage. In terms of its development, spirooxindoles will remain an essential structural scaffold in medicinal chemistry.

6. Expert opinion

From the perspective of drug discovery, the investment in numerous researches of spirooxindole will bring endless possibilities in pharmaceutical chemistry due to its vast chemical space. To date, the spirooxindole-based natural products and their derivatives have been reported to possess various

biological activities such as anticancer, antibacterial, anti–inflammatory, analgesic, and antimalarial properties as well as cholinesterase inhibition, etc. It should be pointed out that improving potency, suitable aqueous solubility, and bioavailability should be concerned as the emphasis in carrying out the process of further structural modification.

As is showed in the pharmacological action research of spirooxindole, lots of the analogs mentioned above (such as **62, 84, 98**, and **100**) possess more than a kind of biological activity. This phenomenon may be a 'double-edged sword' in the drug discovery process. On the bright side, this multi-selectivity provides high feasibility to obtain multifunctional drugs in the future. Also, it is conducive for researchers to find out the differences and relationships among different pathologies. On the unfavorable side, it may result in undesirable off-target effects, which may make spirooxindole derivatives generate potential toxicity to normal organisms. High selectivity of the molecule is indispensable when a single target or action site has been considered as the main reason for the pathogenesis of a disease.

Up till now, the molecular mechanism studies of spirooxindole analogs have achieved significant progress. As an instance, inhibitors containing spirooxindole scaffolds have been proved to effectively inhibit the cancer-related p53-MDM2 interaction and other specific targets (such as PLK4, Hsp90 ATPase, CDK4, p38αMAP kinase, and SOAT2). Besides, quite a few medicinal chemists have utilized computational simulation methods to explore the molecular mechanism of spirooxindole derivatives. However, the mode of action of many spirooxindole derivatives is still unknown or ambiguous at present, which limits pharmacological studies and further molecular design. Furthermore, the bioactivity of numerous reported spirooxindoles analogs with novel structure has not yet been investigated owing to the deficiency of experimental conditions or knowledge areas. This limitation makes an ocean of potential candidates lose the opportunity to be identified as agents with medicinal value. We believe extensive and concerted efforts among pharmacologists, biologists, and computational chemists will provide more unequivocal target information, bioactivity, and the related molecular mechanism study of the spirooxindole-based compounds in the future.

It is well established that oriented or synergic biological property can be achieved through the splicing of active pharmacophore and the replacement of bioisostere. In this review, there are two apparent cases verifying the effectiveness of this strategy, i.e. compounds **39** and **64**. As another optimization direction, simplifying the structure of natural products is also regarded as a practical approach to obtain active molecules in view of the creation cost, the difficulty of synthesis, and molecular modifiability [213]. Compound **98** is a successful example through simplifying natural products because it displays stronger antiplasmodial activity than initial speciophylline. These molecule optimization strategies provide a valuable reference for developing new spirooxindole derivatives with more potent and broader biological properties.

Stereoisomerism exists in the vast majority of spirooxindole-based natural products and the analogs. In many cases, the isomers usually exhibit notable bioactive differences (such as representative compounds **15** and **100**). Therefore, the key problem of currently available synthetic methods of spirooxindole (including asymmetric synthesis and resolution of enantiomers or diastereomers) needs to be addressed. Concretely speaking, the presence of carbon quaternary spirocentre in the core spirooxindoles plays a critical part in exhibiting different bioactivities owing to their special spatial effects. Consequently, it is a demand for developing more efficient and high-stereoselective synthetic strategies for constructing spirooxindoles, which will accelerate the discovery of more novel spirooxindole agents.

To summarize, spirooxindole-based compounds occupy a prominent place in medicinal chemistry, especially as anticancer agents. The spirooxindole scaffold with multiple fused structures is broadly and deeply explored, which has produced a considerable number of promising lead compounds and drug candidates. On the basis of spirooxindole, the continuous efforts of chemists and biologists will further afford significant discovery in the fields of pharmaceuticals and agrochemicals. With the developments of pharmaceutical and academic laboratories, probing out the more novel spirooxindole with reliable synthetic methods will expand their application on the drug discovery beyond all doubt.

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