ChemistryOpen

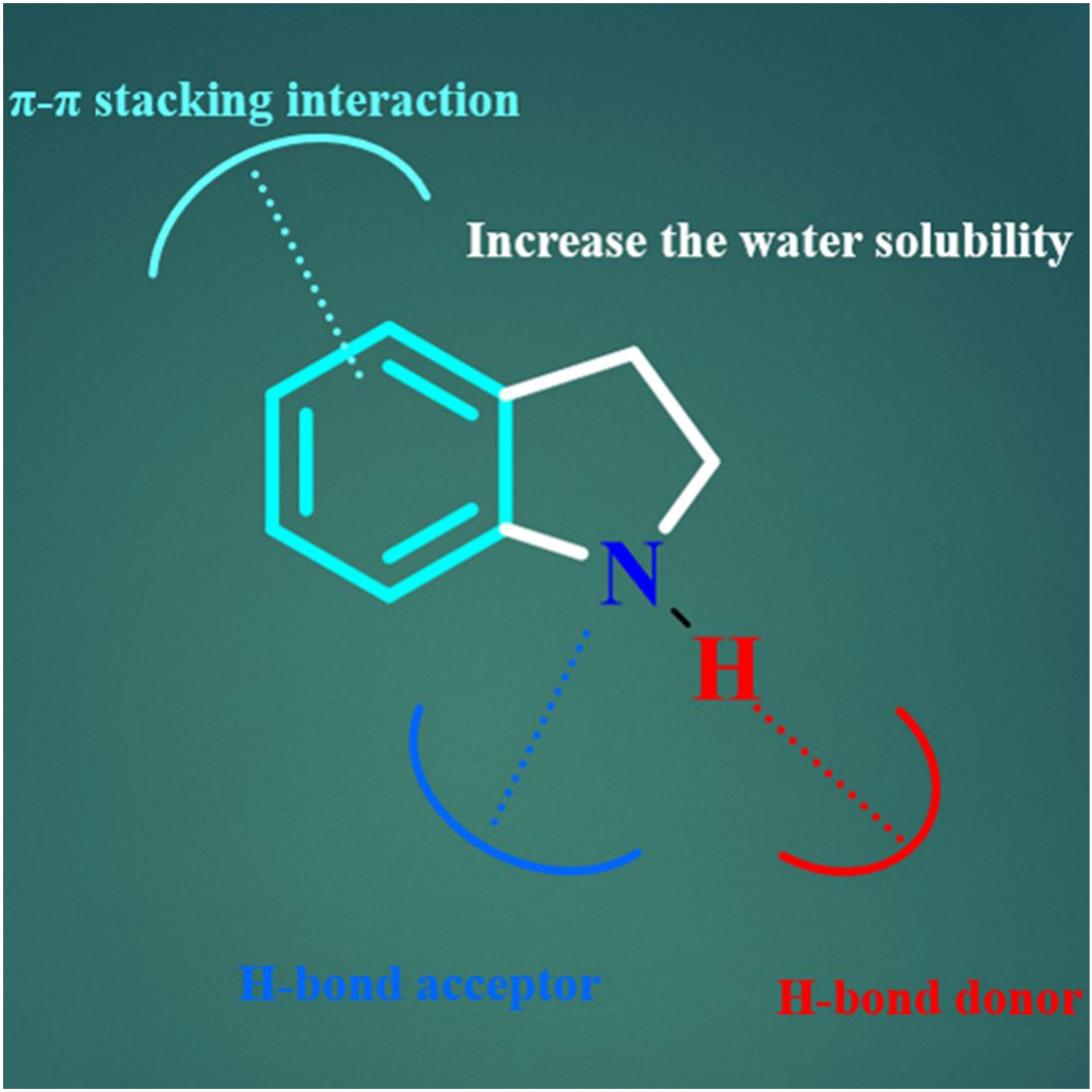
Review
doi.org/10.1002/open.202200235

Chemistry Europe
European Chemical Societies Publishing

www.chemistryopen.org

Development and Application of Indolines in Pharmaceuticals

Huixin Wei, Bo Li, Ning Wang, Yitian Ma, Jingyan Yu, Xuena Wang, Jiayi Su, and Dan Liu*[a]



In recent years, the incidence of cancer is high around the world, and the resistance of bacteria is increasing. To cope with the potentially adverse side effects of cancer chemotherapy and surgery, researchers are turning to the construction of new drug scaffolds. The indoline structure exists in a huge number of natural products, but drugs with indoline have only been formally studied in recent years. With the deepening of research, drugs containing indoline have played important roles in more disease treatment aspects, such as anti-tumor, anti-bacterial, anti-inflammatory and have been used as analgesics, to treat cardiovascular diseases and so on. The synthesis and pharmacological activity of indoline derivatives is summarized in this review in order to support the addition of the indoline component to the toolbox of medicinal chemists. This review focuses on the advantages of indoline compounds in development and synthesis of and for the use as anticancer drugs, antibacterial drugs, to treat cardiovascular diseases and as anti-inflammatory and analgesic drugs. Indoline structures are commonly found in natural and synthetic compounds with medicinal value and are now beginning to be exploited as the basic backbone of various drugs. As research continues, dihydroindoles and their derivatives will play a greater role in the medical field.

1. Introduction

Indoline, also named 2,3-dihydroindole, whose structure consists of a benzene ring fused with a five-membered nitrogenous ring to form a two-ring heterocyclic ring, is chemically named benzopyrrolidine (or 2, 3-dihydro-1*H*-indole or 1-azacinole).[1] It is a bicyclic organic heterocyclic compound with aromatic and weakly basic properties.[2] Considering the low stability of indoline, a protective group is usually attached to the nitrogen atom of the pyrrole ring. The Indoline moiety is widely used in drug design because of its special structure and properties. The benzene ring of indoline can interact with the amino acid residues of proteins by hydrophobic manner, NH acts as a hydrogen bond donor and hydrogen bond acceptor with the amino acid residues of proteins, and the aromatic heterocyclic ring of indoline can improve the physicochemical properties of the compounds compared to other bicyclic structures (e.g., the indole ring), because the two rings are non-coplanar, increasing the water solubility and decreasing the lipid solubility of the compounds.

Indoline-related alkaloids have been fully developed as antibiotics, and the activities on anti-tumor, anti-hypertension and cardiovascular protection are mainly studied now.[1] For conventional chemotherapy, the drugs used in clinical practice have poor selectivity, adverse side effects on normal cells, and are prone to drug resistance developments. The study of Lim[3] and Ndongoab[4] showed that indoline alkaloids exhibited high anticancer effects with low toxicity. High cholesterol in blood is one of the main causes of cardiovascular diseases, such as atherosclerosis, while the novel indoline derivatives as cholesterol ester protein (CETP) inhibitors was synthesized and showed the powerful effect of reducing cholesterol in blood.[5] In another study, an indoline-derived compound was reported that could markedly reduce body weight and lower the blood sugar level in mouse models.[6] The indoline structure is widely found in natural compounds and has been widely reported as the focus of drug design in recent years. When it is structurally modified to join the indoline moiety in a fused or bonded form, new varieties with powerful pharmacological properties are usually produced.[1–2] These characteristics determine the advantages of the indoline skeleton in drug development.

2. Synthesis

Indoline can be synthesized in various ways. There are three traditional methods to prepare indolines, including reduction from indole, intramolecular Diels–Alder synthesis and catalytic synthesis. In addition to those methods, stereoselective syntheses are more suitable for specific indoline isomers. These four methods will be introduced in this review.

2.1. Reduction from indole

Many methods for reducing indole to indoline have been reported, such as catalytic hydrogenation and other reduction conditions including borane-pyridine in hydrogen chloride, zinc powder, tin or zinc amalgam in hydrochloric acid, sodium ammonia, and so on.[2] Taking indole as starting material, Raney-Nickel as catalyst, 90–100 °C, 8.6 MPa (H_2), indoline could be obtained in industry. Actually, catalytic hydrogenation shows certain limitations, such as the low yield, low selectivity, especially there are some sensitive groups on the indole ring. In metal-acid reduction, the indole ring is often destroyed because of strong acid medium, which would affect the reaction effects.[2]

In addition, the reduction of indole to indoline was carried out using sodium borohydride reduction, which would yield the different product in different conditions. The indolines with NH was obtained in acetic acid (Scheme 1a), while the acetylated indolines was obtained in tetrahydrofuran in the presence of trifluoroacetic acid (Scheme 1b).[7]

The *N*-Boc indoles were reduced to indolines with 10% palladium as catalyst, polymethylhydrosiloxane (PMHS) as reducing agent, and the reaction was conducted at room

[a] *H. Wei, B. Li, N. Wang, Y. Ma, J. Yu, X. Wang, J. Su, Prof. Dr. D. Liu*
Key Laboratory of Structure-Based Drugs Design & Discovery of Ministry of Education
Shenyang Pharmaceutical University
Shenyang 110016 (P. R. China)
E-mail: sammyld@163.com

temperature (Scheme 2). PMHS is a cheap reagent with air and humidity stability, so it is generally considered a safe and non-toxic agent compared to other reducing agents. Substituted indolines can also be synthesized by this method, and the yield is more than 80%, while the better results would be achieved starting from indoles without substitution.[8]

2.2. Intramolecular Diels-Alder synthesis

The indoline skeleton could be synthesized by an intramolecular Diels-Alder reaction from 3-alkynylalkylamino-1,2-diazine in 1,3,5-triisopropyl benzene (TIPB, bp. 232–236 °C) at high temperatures (200–230 °C) (Scheme 3). In this study, substitution ($R_1 \neq H$) of 1,2-diazine has little effect on the rate of cycloaddition, while alkyne substitution ($R_2 \neq H$) and the appropriate

Huixin Wei was born in Anshan, Liaoning Province, China in 2001. She entered Shenyang Pharmaceutical University in 2019 and is currently working toward the B.S. degree in Pharmaceutical Engineering from the Shenyang Pharmaceutical University, Shenyang, China. Her major courses include Organic Chemistry, Medicinal Chemistry, Pharmacology, Analytical Chemistry, Pharmaceutical Analysis, Physical Chemistry, Biochemistry, Human Anatomy, Chemical and Pharmaceutical Technology and Physiology and related experimental courses.

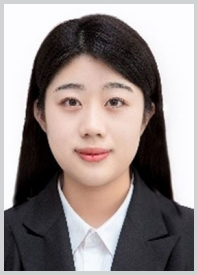
Bo Li received the B.S. degree in Pharmacy from Shenyang Pharmaceutical University, shenyang, China, in 2016 and the M.S. degree in Medicinal Chemistry from, Shenyang, China, in 2019. He is currently working toward the Ph.D. degree in Medicinal Chemistry from Shenyang Pharmaceutical University, Shenyang China, with the School of ShenYang Pharmaceutical University, Shenyang, China. His research interests mainly focused on discovery and design targeted anti-tumor drug research.

Ning Wang received the B.S. degree in Pharmaceutical Engineering from Jining Medical College, Jining, China in 2021. At present, he is studying for a M.S. degree in pharmacy at the School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, China. His research interests are mainly anti-tumor drugs.

Yitian Ma, was born in 2001 in Shenyang, Liaoning Province, China. She entered Shenyang Pharmaceutical University in 2019 and is studying undergraduate courses and working toward the B.S. degree in pharmaceutics, Shenyang, China, with the School of Shenyang Pharmaceutical University, Shenyang, China. Her major courses include Pharmacy, Medicine Chemistry, Medicine Analysis, Pharmacology, Organic Chemistry, Physical Chemistry, Biochemistry, Inorganic chemistry, Pharmaceutical Chemistry, Microbiology, Immunology, Human anatomy, Physiology and related experimental courses.

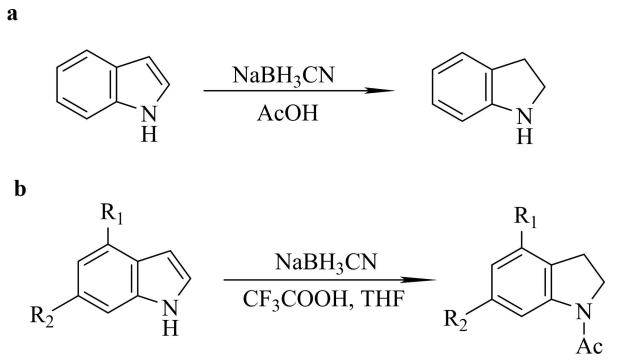
Jingyan Yu was born in the countryside of Huludao, Liaoning Province in 2001. She entered the Shenyang Pharmaceutical University, shenyang, China, in 2019, majoring in Pharmaceutical Engieering. She is currently working toward the B.S. degree in pharmaceutical engieering, Shenyang, China, with the School of Shenyang Pharmaceutical University, Shenyang, China. Her major courses include Pharmacology, Medicine Analysis, Medicine Chemistry, Organic Chemistry, Biological Chemistry, Physical Chemistry, Analytical Chemistry, Physiology and related experimental courses.

Xuena Wang was born in the countryside of Liaoyang, Liaoning Province, China in 2000. She pass the college entrance examination and entered the ShenYang Pharmaceutical University, Shenyang, China, in 2019, majoring in pharmaceutical engineering. She is currently working toward the B.S. degree in pharmaceutical engieering, Shenyang, China, with the School of Shenyang Pharmaceutical University, Shenyang, China. Her major courses include Pharmacology, Medicine Analysis, Medicine Chemistry, Organic Chemistry, Biological Chemistry, Physical Chemistry, Analytical Chemistry, Physiology and related experimental courses.

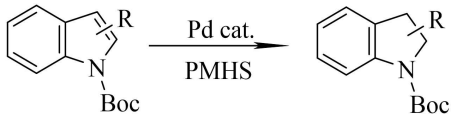


Jiayi Su was born in Liaoyang, Liaoning Province, China in 2001. She entered Shenyang Pharmaceutical University in 2019 and is currently working toward the B.S. degree in pharmacy, Shenyang, China, with the School of Shenyang Pharmaceutical University, Shenyang, China. Her major courses include Pharmacy, Medicine Analysis, Medicine Chemistry, Organic Chemistry, Natural Pharmaceutical Chemistry, Physical Chemistry, Immunology and related experimental courses. Her research interests major in cytological research of photodynamic therapy carrier.

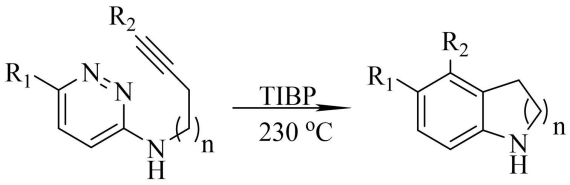
Dan Liu obtained her Ph.D. degree from Shenyang Pharmaceutical University in 2008. Currently, she works at the Shenyang Pharmaceutical University as a full professor and her research interests include design and research of targeted anti-tumor drugs based on epigenetic pathway, development of novel processes for drug synthesis. As the project leader, she presided over 2 NSFC projects. She has authored and co-authored over 30 papers.



Scheme 1. Synthesis of indoline by reduction with $NaBH_3CN$.



Scheme 2. Synthesis of (substituted) indoline using palladium.



Scheme 3. Intramolecular Diels-Alder synthesis of indoline.

length of the alkyne side chain (n = 1) are crucial to the success of the reaction.[9]

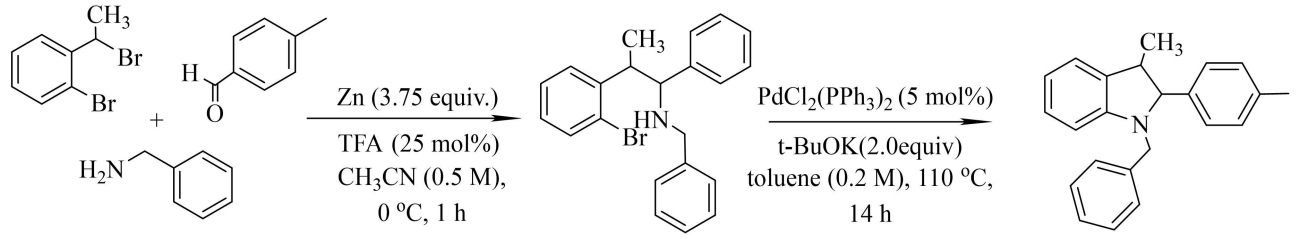
2.3. Catalytic synthesis

The catalytic synthesis methods are introduced. In the first example, a substituted bromobenzene is formed by Mannich reaction, followed by aromatic amination with an intramolecular amine moiety catalyzed by palladium. In the first step, the products as Mannich base were formed by 2-bromobenzyl bromide reacting with primary amines and aldehydes in the presence of zinc powder. A variety of primary amines and aldehydes, including aromatic, alloaromatic and aliphatic derivatives, can be used, but the yield should be guaranteed. The product of the first step was then cyclized by an aromatic amination catalyzed by palladium (Scheme 4). According to statistics, the yield of the reaction was higher than 50% in all attempted reactions. This method was reported for the synthesis of 1,2-disubstituted and 1,2,3-trisubstituted indolines using commercially available materials, making it a versatile and readily usable method.[10]

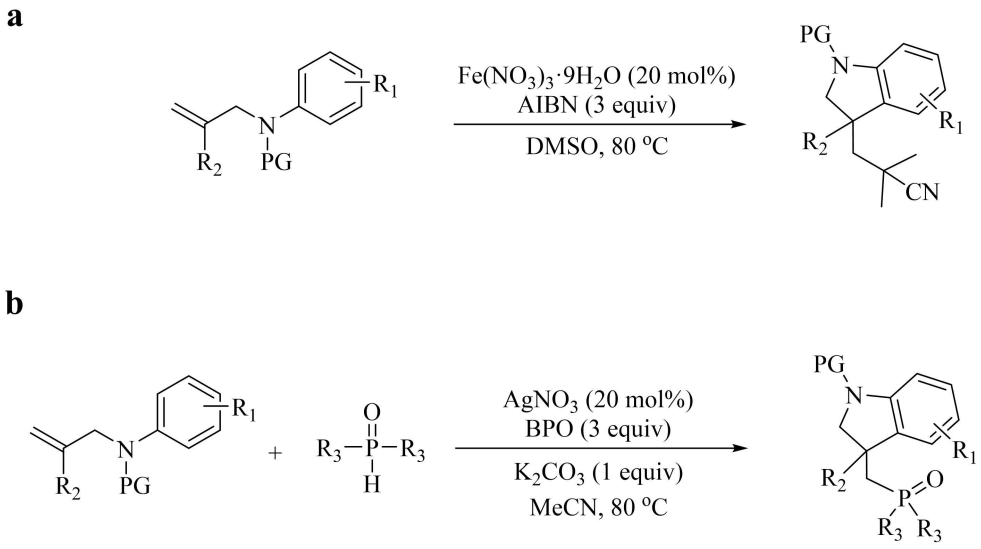
Recently, an iron-catalyzed cyanation reaction using azodiisobutyronitrile (AIBN) was reported by Li,[11] which provided a direct access to obtain 2-unsubstituted-3-cyanoalkyl indolines with a tertiary nitrile moiety. An unactivated double bond acts as the radical acceptor; *N*-allylaniline underwent a cyanoisopropylation/cyclization cascade. This is the first iron-catalyzed cyanoalkylation reaction using AIBN, and excellent *exo*-selectivity was achieved in all cases (Scheme 5a). Liang[12] and coworkers developed a one-pot silver-catalyzed radical cyclization of *N*-allylated anilines to prepare 3-phosphonoalkyl indolines, also using an unactivated double bond acted as the radical acceptor while H-phosphonates or -phosphine oxides served as the radical precursor (Scheme 5b). This protocol features simple operation, broad substrate scope, and great *exo*/*endo* selectivity.

2.4. Stereoselective synthesis

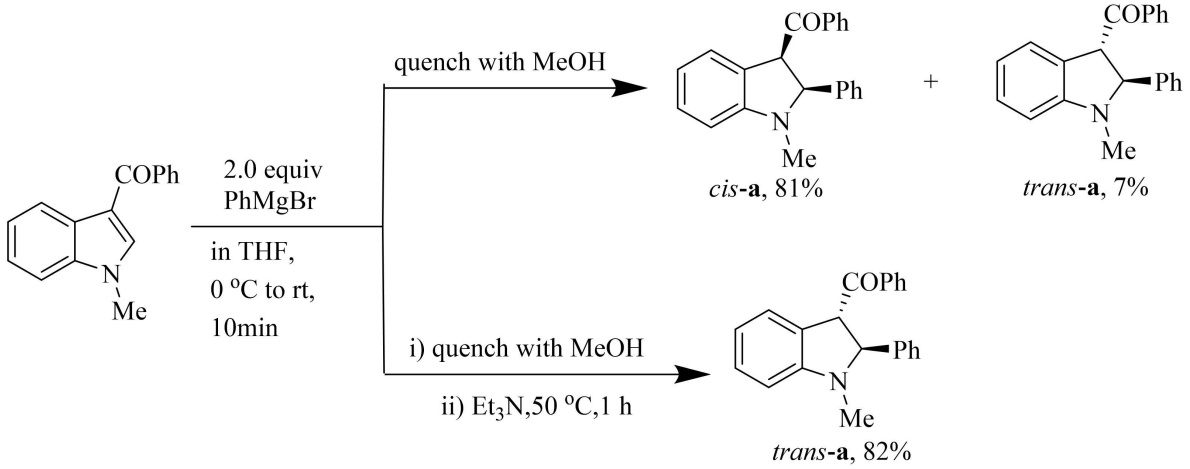
Stereoselective synthesis is mostly used to prepare 2,3-disubstituted indoline. Highly substituted indolines were synthesized by reacting substituted 3*H*-indole with a Brønsted acid, dihydropyridine and chiral nonmetallic catalysts. The final yields usually exceeds 85%, and products are obtained with high ee values. It has been found that 3-acyl glycosides can react with Grignard reagent in Michael addition mode, resulting in the dearomatization of the indole ring and the formation of highly substituted dihydroindole with high stereochemical control level. By choosing a suitable quenching method, high stereoselectivity is obtained for both *cis* and *trans* diastereomers (Scheme 6). For example, first quenching the reaction with methanol, then adding Et_3N, stirring at 50 °C for 1 h, *cis*-**a** (Scheme 6) can be completely converted into the *trans*-isomer, *trans*-**a**, the yield being 82%. The reaction effects would be affected by different substituents on the nitrogen atom of the indole ring. The presence of electron-withdrawing groups such as Boc or $CONMe_2$ on indole nitrogen did not affect the reaction. However, the yield for an Ac-protected indole as the reaction substrate was only 58%. In terms of the nucleophile



Scheme 4. Catalytic synthesis of indoline.



Scheme 5. Catalytic synthesis of indoline (PG = Protecting group).



Scheme 6. Stereoselective synthesis of substituted indolines.

alkylmagnesium bromide, vinyl magnesium bromides as well as substituted vinylmagnesium bromides were suitable nucleophiles, and the reaction rate decreased if the nucleophile contains electron-withdrawing groups.[8]

3. Anticancer Drug Research

Target-based drug design and discovery has become the mainstream of anticancer drug discovery and development. With the advances in molecular biology, many molecular targets for anticancer drugs have been identified. And due to the versatility of the indoline structure, many effective compounds bearing the indoline core have been reported acting on these targets.

3.1. Kinase inhibitors

Protein kinases are a class of phosphotransferases responsible for transferring the γ-phosphoacyl group of ATP to specific amino acid residues in the substrate and phosphorylating proteins. They can be classified into five groups according to the type of amino acid residues in which their substrate proteins are phosphorylated: serine/threonine kinases, tyrosine kinases, histidine kinases, tryptophan kinases, and aspartate/glutamyl kinases. More than 500 genes encode protein kinases in the human kinase group, and at least 30% of human proteins are phosphorylated by protein kinases. These data suggest that kinases have a wide range of effects in human physiology and pathology. Most protein kinases are involved in cell proliferation, survival and migration, but their overexpression is directly related to tumorigenesis. In addition, advances in molecular studies of cancer mechanisms have demonstrated the involvement of various kinases in carcinogenesis and metastasis.

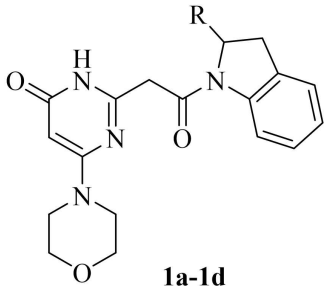
Therefore, the development of kinase inhibitors, including single and multiple targets as well as synthetic and natural chemical entities, are currently considered as one of the potential therapeutic strategies for the treatment of a range of cancers.[1]

Phosphoinositol 3-kinase (PI3 K) of class IA phospholipids is associated with human cancer, and aberrant PI3 K pathway activation plays a major role in cancer. PI3 K includes 4 isoforms: PI3Kα, PI3 Kβ, PI3Kδ and PI3 Kγ. Studies have confirmed that isoform-specific PI3 K inhibitors may exhibit better safety profiles and that future structure-related pharmacological properties could be used in combination with other targeted therapies or standard-of-care agents. Based on the study of the structure and activity of known PI3 K inhibitors, it was found that dihydroindolamide (**1 a**; Table 1) showed a high inhibitory effect on PI3 Kβ, but its water solubility was not high. Taking **1 a** as a lead compound, a series of derivatives with C2/C4 substitutions were synthesized. The results showed that the compound had better solubility when the C2 position was substituted by a methyl group (**1 b**). It was found that the enantiomers could display different selectivity. Pharmacokinetic studies showed that the *S*-enantiomer (**1 c**) was orally absorbed with good PK properties in dogs. The antitumor research showed that compound **1 c** could effectively inhibit pAkt-S473 after 6 h at a dosage of 100 and 200 mg/kg, respectively.[13] Compound **1 c** was entered into phase I/Ib clinical trials for advanced tumors or lymphomas as a monotherapy in 2012, but discontinued by Sanofi because of rapid clearance in 2014.[14]

Aurora kinases are a family of serine/threonine kinases which play key regulatory roles in many processes of mitosis. Two of its three subtypes, Aurora A and Aurora B, are overexpressed in both solid tumors and leukemia, which are considered to be effective targets for novel anticancer drugs. The novel pyrrole-indoline-2-ones with the side chain bearing pyrrole (**2 a**, Figure 1) were designed and synthesized, which can effectively inhibit Aurora kinases. The benzenesulfonamide group in C-5 position on the scaffold had a high inhibition effect on Aurora A and Aurora B, and the inhibition effect was reduced when the substitutions were replaced by benzyl sulfonamide group or 2-naphthalenyl sulfonamide group. The selective inhibition of Aurora A was enhanced by carboxyethyl group (X=COOH) at position C-3′of the pyrrole ring. However, some potent compounds lacked anti-proliferation activity in HCT-116 cancer cells. The team then investigated the structural basis of the different selective inhibition profiles and found that the equivalent residues Arg220 and Thr217 in Aurora A and Lys164 and Glu161 in Aurora B were responsible for the different inhibition ability. Therefore, targeting residues will facilitate the development of selective inhibitors of Aurora kinase. Compounds **2 b** and **2 c** were obtained based on the relationship of structure-activity, and two compounds could inhibit cancer cells growth and inhibit phosphorylation of Aurora A and Aurora B substrates at the cellular level.[15]

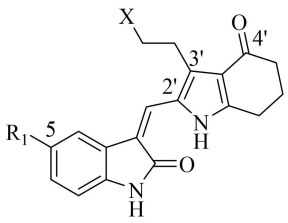
Inhibition of angiogenic kinases VEGFR, PDGFRβ, FGFR1 and TIE-2 can be targeted against different cancers. Hepatocellular carcinoma (HCC) is one of the vascular solid tumors, accounting

Table 1. The inhibitory activities and solubility of compounds **1 a**–**1 d**.

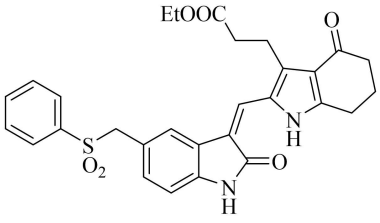


1a-1d

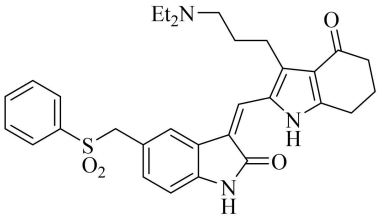
Compd.	R	MW	PI3α IC_{50} [nM]	PI3β IC_{50} [nM]	PI3δ IC_{50} [nM]	PI3γ IC_{50} [nM]	PAkt[a]I C_{50} [nM]	Solubility at pH 7.4 [nM]
1 a	H	340	460	4	28	10000	15	12
1 b	Me	354	322	4	36	9149	6	2754
1 c	(*S*)-Me	354	1539	23	468	10000	49	928
1 d	(*R*)-Me	354	569	6	6	3315	12	2497



2a



2b

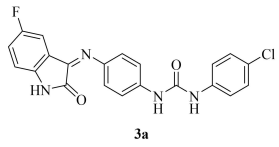


2c

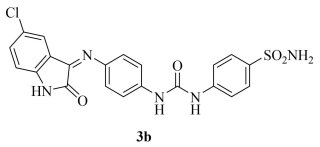
Figure 1. Chemical structures of **2 a**–**c**.

for 70%–85% of liver-related tumors. Isatin (1*H*-indole-2,3-dione) is a special and common scaffold with high activity and tolerance in human body. To identify drugs with potential antiproliferative activity against VEGFR-2-targeting HepG2 hepatocellular carcinoma cells, a series of novel indolines containing a diphenylurea moiety were designed and synthesized based on the developed drug structures. Based on the bioactivity of compound **3a** ($IC_{50} = 1.81 \pm 0.14$ μM), it was determined to be the most active member against HepG2 by studying the effect of substituents on the benzene ring. Compound **3b** (Table 2) was found to be the most effective counterpart against VEGFR-2 with an IC_{50} value of 0.31 ± 0.04 μM.[16]

Table 2. The HepG2 and VEGFR2 inhibitory activities of compounds **3a**–**3b**.

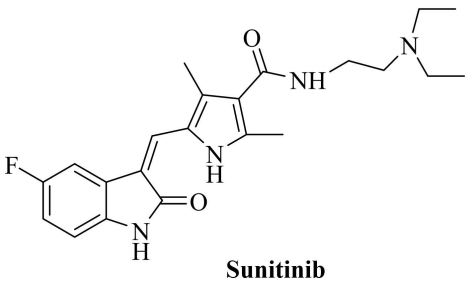


3a



3b

Compound	IC_{50} [μM] HepG2	VEGFR2
3a	1.81 ± 0.14	1.93 ± 0.07
3b	3.15 ± 0.36	0.31 ± 0.04



Sunitinib

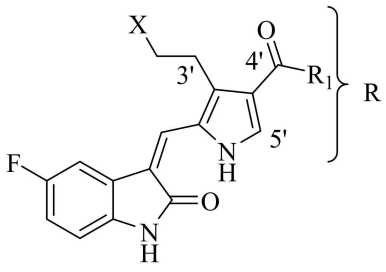
Figure 2. Chemical structure of Sunitinib.

Based on studies of the anti-cancer drug Sunitinib stent (*Z*)-3-[(1*H*-Pyrrol-2-yl) methylene]indolin-2-one (pyrrole-indolin-2-one: Figure 2), the C-5 (2, 6-dichlorobenzyl) sulfonyl substituent was found to inhibit Met kinase with subnanomolar potency. Different compounds were designed and studied, and the specific activities are shown in Table 3. It can be seen that on the basis of scaffold **4**, compounds bearing a C-4′ acetyl group and a C-5′ methyl group have high inhibitory effects on VEGFR2, PDGFR-β and FGFR-1 protein kinases. For FGFR-1 inhibition, the 2-carboxyl ethyl side chain at the C-3′ position (compound **4b**) would enhance the effect on FGFR-1.[17]

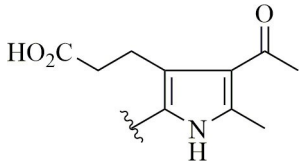
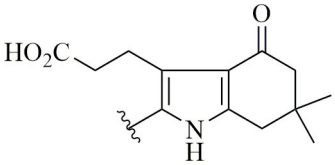
3.2. Histone deacetylase inhibitors

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate the epigenetic processes of core histone acetylation and deacetylation to maintain a balance in normal cells.[18] However, the high expression of HDAC in cancer cells breaks this balance. Epigenetic processes have become the target for treatment of serious disease because of their association with tumorigenesis and inflammatory diseases. HDAC inhibitors inhibit the activity of histone deacetylases by binding to target proteins, promote the acetylation of histones,

Table 3. The inhibitory activities of compounds **4a**–**4d**.



4a-4b

Compd.	R	Kinase inhibtory IC_{50} [μM] VEGFR2	PGFr-β	FGFR-1
4a		0.040	0.061	0.72
4b		0.025	0.042	0.26
Sunitinib	–	0.0054	0.021	>0.50

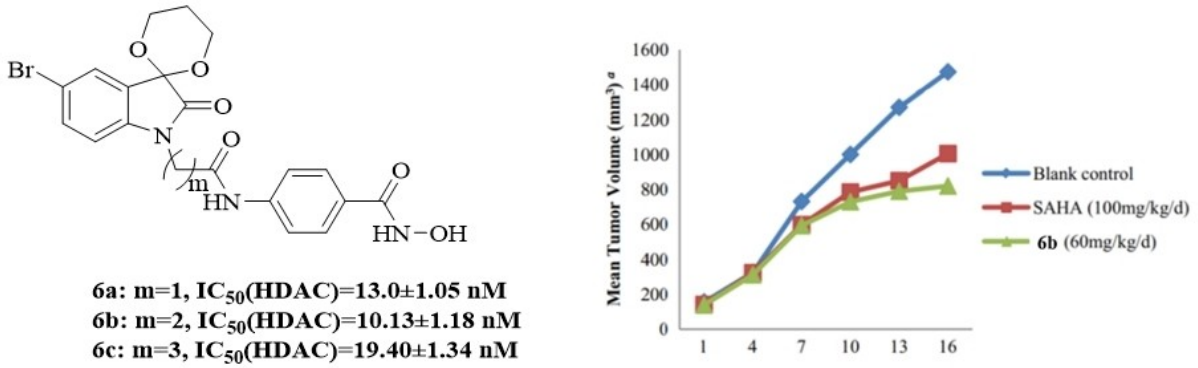


Figure 3. Structures of **6a**–**6c**; antitumor activity of **6b** and SAHA against H7402 human tumor xenografts implanted in nude mice. Reproduced with permission from Ref. [21]. Copyright (2015), with permission from Elsevier Ltd.

and induce the expression of tumor suppressor genes such as p21, thereby inhibiting tumor growth.[19]

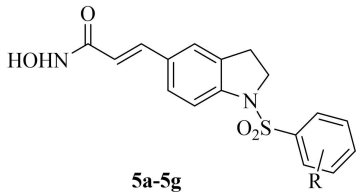
The 1-arylsulfoyl-5-(*N*-hydroxyacrylamide) indoles proved to be effective HDAC inhibitors by the study of indole-linked isohydroxamic acid and benzenesulfonamide derivatives. In this research, the dihydroindoles (**5**; Table 4) were obtained from transformation of indole ring, and the evaluation results showed that the inhibitory activity of HDAC was greatly improved. The compound (**5b**) with a nitro group at position 4 had a certain inhibitory effect, but when the nitro group was converted to an amino group (**5c**), the HDAC activity was significantly decreased. The HDAC inhibitor effects was increased twofold by substituting F at C4-position (**5f**). The remaining 3-OCH_3 (**5e**), 4-OCH_3 (**5d**), 4-Cl (**5g**) substituted 5-(*N*-hydroxyacrylamide)-1-phenylsulfonyl dihydroindole increased the HDAC inhibitor effects 4-6-fold.[20]

On the basis of previous studies, a series of dihydroindole-2,3-diketone derivatives were designed and synthesized. By studying the HDAC inhibitory effects of different side chain lengths, substituents in indoline and different ketospiracles, most of them have good potentials, especially compounds **6a, 6b** and **6c**. As demonstrated by anti-proliferation assays in vitro, compound **6b** had a more potent inhibitory effect, showing a low nanomolar IC_{50} value. In addition, the antitumor assays were evaluated in a H7402 xenograft mouse model in vivo and found that **6b** was more effective than the FDA-approved drug SAHA (Figure 3).[21]

Most HDAC inhibitors are non-selective, which may lead to toxicity issues associated with clinical use of these compounds, so efforts are underway to develop isoform-selective HDAC inhibitors. In this regard, HDAC6, the largest enzyme in the HDAC family, consisting of 1216 amino acids, has emerged as a potential target due to its substrate specificity and cellular localization. Existing studies have demonstrated that the benzohydroxamic acid motif is beneficial for the effective and selective inhibition of HDAC6. Based on the indoline skeleton as an important part of the non-selective HDAC inhibitor MPT0E028, a series of *N*-(4-hydroxyaminoformylbenzyl)alkyl-substituted dihydroindoles have been developed as highly active and selective HDAC6 inhibitors. Seven compounds (**7a**–**7g**; Table 5) were designed and synthesized, and the inhibition rates of HDAC6 ranged from 98 to 100% when the concentration was 10 μM.[22]

Hydroxamic acids of benzothiazole, which are heterocyclic analogues of SAHA, are cytotoxic in addition to inhibiting HDAC activity. On this basis, a series of new 2-oxydihydroindolyl

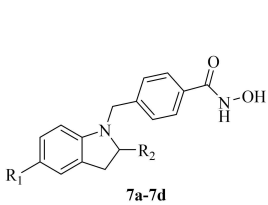
Table 4. The HDAC inhibitory activities of compounds **5a**–**5g**.



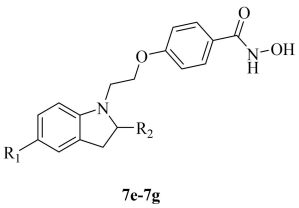
5a-5g

Compound	R	IC_{50} (nM ± SEM)
5a	H	26.49 ± 4.70
5b	4-NO_2	22.02 ± 4.06
5c	4-NH_2	> 1000
5d	4-OCH_3	4.17 ± 0.53
5e	3-OCH_3	5.77 ± 1.25
5f	4-F	11.70 ± 3.07
5g	4-Cl	6.20 ± 1.22

Table 5. The HDAC6 inhibitory activities of compounds **7a**–**7g**.



7a-7d



7e-7g

Compd.	R_1	R_2	%HDAC6 inhibition (at 5 μM)
7a	H	H	98
7b	H	Me	100
7c	Br	H	98
7d	NO_2	H	100
7e	H	H	98
7f	Br	H	100
7g	NO_2	H	98
Trichostatin A	–	–	100

isohydroxamic acids (**8a**–**8h**, **9a**, **9b**; Table 6) were designed. The cytotoxicity of all compounds was evaluated, and it was found that the 5-position or 7-position substituted by halogen, $-NO_2$, $-CH_3$ or $-OCH_3$ in the dihydroindole fraction resulted in enhanced cytotoxicity, and the 7-position substitution was more effective than the 5-position substitution. The HDAC inhibition of the compounds was additionally evaluated using purified HDAC2 enzyme. The results showed that all compounds exhibited an inhibitory effect comparable to that of SAHA on HDAC2 enzyme, and some even 4–6-fold higher than SAHA. The results showed that 2-oxodihydroindolyl substituted isohydroxamic acid could inhibit HDAC and achieve antitumor effects.[23]

3.3. Apoptosis inducers and apoptotic protein inhibitors

Apoptosis, also called programmed cell death, is a process that automatically ends life of cells. There are two major cell-intrinsic pathways for the induction of apoptosis, one beginning with the attachment of cell surface death receptors and the other involving the mitochondrial release of cytochrome C.[24] Previous studies have shown that anticancer drugs can induce apoptosis in malignant cells in vitro, and that cysteine proteases are integral to the apoptotic machinery. The main chemotherapeutic agents or radiation therapies currently used to treat cancer invade the target cells mainly through apoptosis induction.[3]

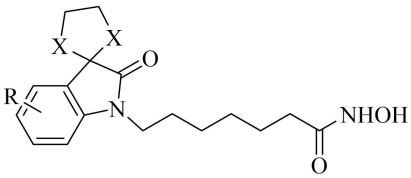
Noxa plays an important role in apoptosis induction as a member of the B-cell lymphoma 2 (Bcl-2) family, promoting apoptosis upon activation. Based on the dihydroindole backbone, several novel compounds were designed and evaluated for their antiproliferative activity. The data showed that, when the dihydroindole ring was replaced by a 1,2,3,4-tetrahydroquinoline ring or a 2,3,4,5-tetrahydro-1*H*-benzo[*b*]aza ring, compounds **10a**–**10e** were obtained with IC_{50} values as shown in the Table 7, indicating that the small size of the dihydroindole ring plays an important role in antiproliferative activity. Apoptosis experiments on compound **10d** revealed that it triggered cysteinase-related apoptosis in ESCC cells through an intrinsic pathway relying on the involvement of Noxa. It could be used as a lead compound for the development of compounds regarding Noxa-mediated apoptosis inducers against esophageal squamous cell carcinoma.[25]

The 2-substituted dihydroindole derivatives have antitumor activity, and imidazolium salt hybrids can induce cell cycle arrest and tumor cell apoptosis. On this basis, 25 novel 2-substituted dihydroindole imidazole derivatives were synthesized and evaluated for their anti-proliferative activities in vitro, and it was found that benzimidazoles with 4-methylbenzyl (**11a**), 2-bromobenzyl (**11b**), 2-naphthylmethyl (**11c**) or naphthoyl substituents (**11d**) at the 3-position exhibited higher cytotoxic activity (Table 8). For compound **11e** at 1, 2, 4 μM for 48 h, the apoptotic cell rate was 7.13 ± 1.25%, 9.14 ± 1.82% and 25.67 ± 2.98%, respectively, which were statistically different from the control (2.23 ± 0.42%). It was determined to induce G2/M phase cell cycle arrest and apoptosis in SMMC-7721 cell lines.[26]

A variety of anticancer drugs with alkoxy side chains in the methoxy neighborhood and with indoline backbones have achieved success in clinical studies. Based on this, researchers have attempted to synthesize a series of 3-((4′-alkylaminoalkoxy)benzyl)indolin-2-ones. Compounds **12a**, **12b** and **12c** have strong estrogenic effects and induce apoptosis via the ROS-dependent mitochondrial pathway in breast cancer MDA-MB-231 cells, as well as exhibit potent antiproliferative effects (Table 9).[27]

It was found that p53 gene is the tumor suppressor gene with the highest relevance to human tumors, which has important roles in DNA transcription, cell growth and prolifer-

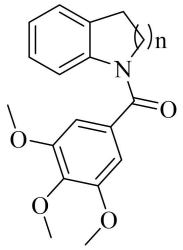
Table 6. The Log*P*, HDAC2 inhibition and anti-proliferative activities of compounds **8a**–**8h**, **9a** and **9b**.



8a-8h, X=O
9a-9b, X=S

Compd.	R	Molecular Formula	Molecular Weight	Log*P*	HDAC2 Inhibition (IC_{50}, [μM])	Cytotoxicity (IC_{50} [μM]) SW620	PC-3	AsPC-1
8a	H	$C_{17}H_{22}N_2O_5$	334.37	1.15	0.16	2.68	2.90	1.60
8b	5′-F	$C_{17}H_{21}FN_2O_5$	352.36	1.35	0.06	1.48	1.86	1.01
8c	5′-Cl	$C_{17}H_{21}ClN_2O_5$	368.81	1.79	0.03	1.04	0.53	0.49
8d	5′-Br	$C_{17}H_{21}BrN_2O_5$	431.26	2.04	0.03	0.50	0.34	0.22
8e	5′-NO_2	$C_{17}H_{21}N_3O_7$	379.36	1.37	0.08	1.11	0.86	0.74
8f	5′-CH_3	$C_{18}H_{24}N_2O_5$	348.39	1.70	0.16	1.48	0.92	0.68
8g	5′OCH_3	$C_{18}H_{24}N_2O_6$	364.39	1.23	0.12	0.05	0.07	0.07
8h	7′-Cl	$C_{17}H_{21}FN_2O_5$	352.36	1.79	0.15	0.07	0.09	0.09
9a	-H	$C_{17}H_{22}N_2O_3S_2$	366.50	3.95	0.32	0.84	0.89	0.86
9b	5′-CH_3	$C_{18}H_{24}N_2O_4S_2$	396.52	4.50	0.26	0.84	0.39	0.36

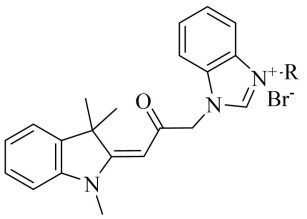
Table 7. The anti-proliferative activities of compounds **10a**–**10e**.



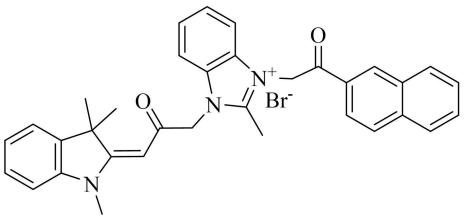
10a-10e

Compd.	n	IC_{50} [μM] Hela	A549	HepG2	KYSE30
10a	2	8.46 ± 0.04	> 80	8.09 ± 0.05	10.42 ± 0.04
10b	1	5.98 ± 0.04	20.71 ± 0.08	3.61 ± 0.02	17.67 ± 0.11
10c	2	7.57 ± 0.06	34.05 ± 0.05	13.73 ± 0.09	7.92 ± 0.03
10d	1	4.32 ± 0.05	18.41 ± 0.08	23.72 ± 0.11	12.76 ± 0.07
10e	3	> 80	31.38 ± 0.14	21.74 ± 0.07	6.55 ± 0.09

Table 8. The anti-proliferative activities of compounds **11a**–**11d**.



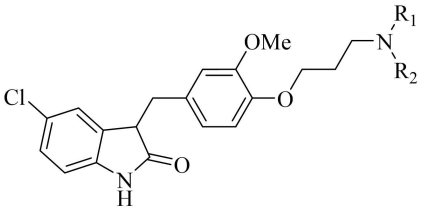
11a-11d



11e

Compd.	R	IC_{50} [μM] HL-60	SMMC-7721	A549	MCF-7	SW480
11a	4-methylbenzyl	0.70	1.98	2.93	2.96	4.54
11b	2-bromobenzul	0.39	1.10	1.21	3.96	2.54
11c	2-naphthylmethyl	0.47	1.10	1.40	1.91	2.23
11d	naphthylacyl	0.67	2.13	2.85	4.47	2.85
11e	-	0.24	1.09	0.98	1.13	1.18

Table 9. The anti-proliferative activities of compounds **12a**–**12c**.



12a, NR^1R^2=dimethylmorpholino
12b, NR^1R^2=4-(3-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl
12c, NR^1R^2=4-methylpiperazin-1-yl

Compd.	IC_{50} [μM ± SD] A549	DU-145	BT-549	MDA-MB-231	MCT-10A
12a	6.25 ± 0.25	2.71 ± 0.11	2.77 ± 0.2	1.99 ± 0.1	54.65 ± 2.53
12b	9.53 ± 0.55	1.61 ± 0.05	1.53 ± 0.06	1.26 ± 0.1	41.29 ± 0.47
12c	11.34 ± 0.7	2.15 ± 0.1	2.17 ± 0.1	1.75 ± 0.1	43.64 ± 0.45

ation, and many metabolic processes, and can effectively fight against tissue proliferation and promote apoptosis, with the function of maintaining genome stability and inhibiting or preventing cell transformation. p53 is a key negative regulator of MDM2 gene, which maintains a fine balance, and blocking

the binding of p53 to MDM2 can be used as a target to effectively inhibit tumorigenesis.[1]

After the development of p53-MDM2 interaction inhibitors based on the spiro(oxindole-3,3′-thiazolidine) structure, compound **13a** considered as a basic scaffold for further modification. A new series of 2-oxospiro(indoline-3,2′-thiazolidine) derivatives were synthesized. Compounds **13a**–**13e** (Table 10) were obtained with IC_{50} values ranging from 0.04–2.15 μM (human breast cancer MCF-7 cells) and 0.07–3.69 μM (human colorectal carcinoma HT29 cells). It is indicated that the nature of the substituents of the oxindole moiety significantly influenced the anti-proliferative activity. Among them, the presence of an electron-withdrawing group at the C-5 position is good for increasing the activity, and the conformation of the carbon atom at the 2′ position at (2′*R*, 4′*R*) was more active than with (2′*S*, 4′*R*) configuration. The introduction of a methyl group at the N-1 position also enhanced the activity. Taken together, the most potent compound **13e** had powerful cancer cell growth inhibition and could inhibit 30% of p53-MDM2.[28]

To overcome the slow isomerization of earlier spirooxindole inhibitors in solution, new spirooxindolines containing two identical substituents at C-2 of the pyrrolidine ring were designed as MDM2 inhibitors. Among them, compound **14** (Figure 4; A domain: cyclohexyl group, R domain : bicyclo[2.2.2]octane, 1-carboxylic acid-4-) exhibited high affinity to MDM2 with $K_i < 1$ nM, powerful anti-proliferative activity, good solution stability and tolerability. Compound **14** could inhibit cancer cell growth effectively no matter p53 was loss or not.[29]

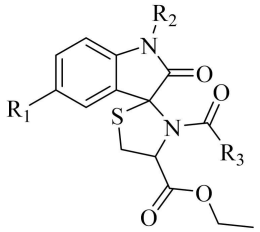
Dihydroindolo-3,4-pyrazolo[3,4-*b*]pyridine derivatives have been shown to possess potent anti-proliferative activities. Consistent with anticancer activity, compounds **15a** and **15b** (Figure 5) could inhibit p53-MDM2 interactions effectively in vitro.[30]

3.4. Microtubule protein inhibitors

Microtubules play a key role in the formation of the spindle body during tumor cell division, and in the support and movement of tumor cells. The cytoskeleton of tumor vascular endothelium is also composed of microtubules. If the microstructure is disrupted, tumor cells cannot draw nutrients and growth is inhibited. Microtubules also play an important role in signal transduction in cancer cells, which is related to the growth, proliferation and invasion of cancer cells. Based on these points, microtubules have become one of the effective targets for antitumor drugs. Microtubule protein inhibitors are important antitumor drugs.

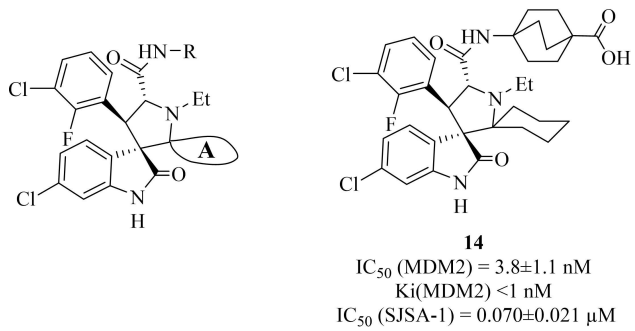
A series of 7-anilinodihydroindole-*N*-benzenesulfonamide compounds **16a**–**16j** (Table 11) were designed and synthesized, and cell growth inhibitory activities were studied in vitro. It was

Table 10. The anti-proliferative activities of compounds **13a**–**13e**.



13a-13e

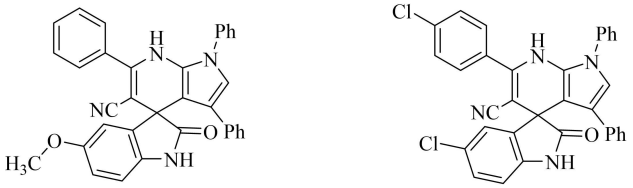
Compd.	R_1	R_2	R_3	IC_{50} [μM ± SD] MCF-7	HT29
13a	H	H	H	1.21 ± 0.6	1.60 ± 0.4
13b	H	H	C_6H_4(4-Cl)	2.15 ± 0.7	3.69 ± 0.9
13c	CH_3	H	C_6H_4(4-Cl)	2.12 ± 0.7	1.09 ± 0.6
13d	Br	H	C_6H_4(4-Cl)	0.90 ± 0.2	0.11 ± 0.09
13e	Br	CH_3	cyclohexyl	0.04 ± 0.01	0.07 ± 0.01



14

IC_{50} (MDM2) = 3.8±1.1 nM
Ki(MDM2) <1 nM
IC_{50} (SJSA-1) = 0.070±0.021 μM

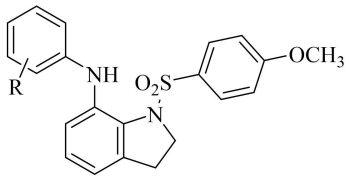
Figure 4. Chemical structures and IC_{50} values of compound class **14**.



15a **15b**

Figure 5. Chemical structures of **15a** and **15b**.

Table 11. The anti-proliferative activities of compounds **16a**–**16j**.



16a-16j

Compd.	R	Cell type IC_{50} [μM ± SD] KB	HT29	MKN45
16a	4-OH	239 ± 55	511 ± 60	493 ± 85
16b	3-OH	342 ± 43	343 ± 60	187 ± 102
16c	4-OCH_3	1200 ± 300	803 ± 296	487 ± 170
16d	4-Cl	1100 ± 500	658 ± 194	463 ± 56
16e	4-F	557 ± 272	588 ± 27	563 ± 298
16f	3,4-di-F	412 ± 64	968 ± 66	726 ± 239
16g	4-NO_2	101 ± 2.4	191 ± 8	107 ± 1
16h	4-CN	49.7 ± 15	149 ± 51	92 ± 34
16i	4-$COOCH_3$	93.5 ± 22	678 ± 267	487 ± 51
16j	4-COOH	846 ± 183	2377 ± 548	1394 ± 390

found that the bioactivity would be good when there was electron-withdrawing group on the phenylamino group, and the sequence was as follows: –CN > –$COOCH_3$ > –NO_2 > –OH > C1. Among them, **16g**, **16h** and **16i** showed higher inhibition of microtubulin polymerization compared to colchicine at 1 μM and 5 μM concentrations. Compound **16h** with a cyano group showed the highest affinity (86.4%), competitively bound to the colchicine binding site of microtubulin, and almost completely disrupted microtubulin assembly at 10.0 μM.[31]

A series of new compounds **17a**–**17e** (Table 12) were designed after the discovery of trimethoxyphenyl as an important pharmacodynamic group acting with microtubulin, and it was found that compound **17d** exhibited the best anti-proliferative ability against KYSE-30 cells than other cancer cells. Further evaluation revealed that it was an effective inhibitor of microtubulin polymerization in a dose-dependent manner with an IC_{50} value of 3.4 μM. In an EBI assay, it was shown that compound **17d** could directly bind to the colchicine binding site of β-microtubulin to exert an inhibitory effect. These results suggest that the anticancer activity of compound **17d** was achieved by microtubulin inhibition.[32]

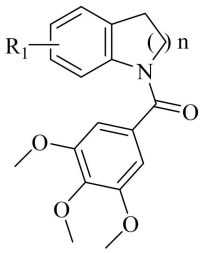
3.5. Multi-target agents

Tumor growth involves multiple factors, and a single target often cannot effectively kill cancer cells and is prone to drug resistance, and recurrence sometimes occurs with single-target drugs[1]. In order to overcome these problems, researchers have designed multi-target inhibitors, which have produced good results.[33]

The isopropylresorcinol fragment substitutes the benzene-sulfonyl group in compounds **18a** and **18b** (Table 13) to induce dual inhibition of HDAC and HSP90. By determining the effect of different alkyl chain lengths and the *N*-benzyl junction with zinc-binding groups on antiproliferative effects in vitro, compound **18c** was found to have high antiproliferative activity against human cancer cells and to be an effective inhibitor of the HSP90 protein. As a potent dual-target inhibitor, compound **18e** showed high selectivity for HDAC1, 2, 3 and HDAC6 with an IC_{50} value of 46.3 nM for HSP90, producing cytotoxic effects against cancer cell lines A549, HCT116, HL60 cells.[34]

The structure-activity relationship of a series of 1-aryl sulfonyl dihydroindolyl benzamides was investigated, and the results of cytotoxicity studies showed that compounds **19a**, **19b** and **19c** (Table 14) exhibited powerful antiproliferative

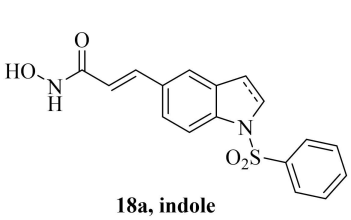
Table 12. The anti-proliferative activities of compounds **17a**–**17e**.



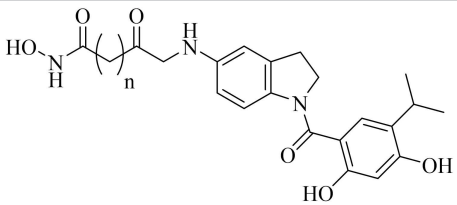
17a-e

Compd.	R_1	n	IC_{50} [μM] MGC-803	A549	Kyse30
17a	H	1	7.57 ± 0.06	22.0 ± 0.05	7.72 ± 0.19
17b	H	2	4.41 ± 0.08	23.7 ± 0.11	6.72 ± 0.17
17c	H	3	4.3 ± 0.14	21.7 ± 0.07	16.55 ± 0.09
17d	5-Br	1	1.84 ± 0.03	6.82 ± 0.05	1.61 ± 0.06
17e	5-NO_2	1	8.32 ± 0.14	17.7 ± 0.17	6.55 ± 0.09

Table 13. The HDAC inhibition and anti-proliferative activities of compounds **18a**–**18c**.



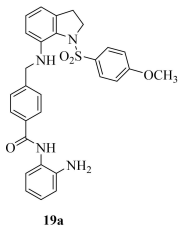
18a, indole
18b, indoline



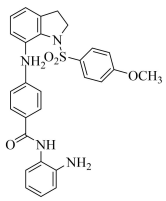
18c-18e

Compd.	n	HSP90 IC_{50} [nM]	Hela HDAC IC_{50} [nM]
18c	2	49.3 ± 1.6	> 10000
18d	5	40.2 ± 5.2	86.9 ± 14.4
18e	6	46.3 ± 7.5	61.2 ± 2.0

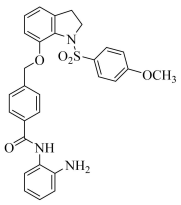
Table 14. The anti-proliferative activities of compounds **19a**–**19c**.



19a



19b



19c

Compd.	$IC_{50} \pm SD$ [nM] KB(Parental)	KB-VIN10(MDR↑)	KB–S15(MDR↑)	KB-7D(MRP↑)
19a	48.6 ± 46.4	64.7 ± 24.5	43.9 ± 2.8	46.6 ± 1.0
19b	116.2 ± 3.9	114.3 ± 4.8	106.0 ± 11.5	78.3 ± 9.2
19c	54.2 ± 6.8	54.2 ± 6.8	55.7 ± 4.6	54.2 ± 6.8

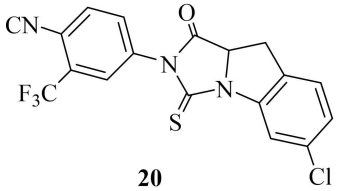
activity against KB-derived MDR-positive KB-VIN10, KB-S15 and KB-7D cells in vitro. Further research revealed that compound **19a** and **19c** were the more potent inhibitors of microtubulin polymerization with IC_{50} values of 1.1 and 1.9 μM, respectively, while compound **19a** inhibited HDAC 1, 2 and 6 as a dual inhibitor. Compound **19a** was also found to show efficacy in human non-small cell lung cancer A549 xenograft model as well as in a xenograft tumor model of BJAB in vivo.[34]

3.6. Receptor modulators

Receptors are biomacromolecules on the cell membrane or within the cell that recognize and bind to bioactive molecules. Changes in genetic and somatic factors disrupt the normal expression of several receptors, leading to the occurrence and development of different types of cancer. Due to the high expression of these receptors, drug resistance can also develop in cancer cells. Therefore, targeting different receptors using receptor modulators as receptor agonists or receptor antagonists is one approach in order to treat cancer cells effectively, while receptor degraders are another recently reported approach.[1]

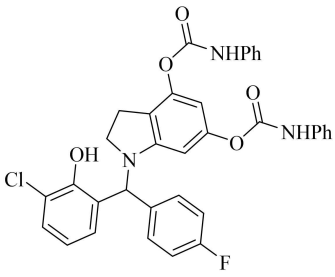
A novel, species-specific class of dihydroindole thioglycolide derivatives were designed and evaluated in vitro as potent antagonists of the androgen receptor (AR). By researching the mechanisms of growth inhibition in cancer cells as well as through SAR studies, it was shown that the initially synthesized compounds exhibited good anti-proliferative activity in LnCaP cells but lacked antagonistic potency. The complete AR antagonist conformation was then investigated and the side chain was optimized to obtain a potent AR antagonist, compound **20**, (Figure 6) which showed comparable effects to enzalutamide (LNCaP $IC_{50\ (\text{compound } \mathbf{20} \text{ vs. enzalutamide})} = 27.9$ μM vs. 12.5 μM) in terms of inhibition of AR transcription, as well as lower toxicity (DU145 $IC_{50\ (\mathbf{20} \text{ vs enzalutamide})} = 200$ μM vs. 46.1 μM).[35]

GPR17 has been identified as a prominent target for the treatment of human glioblastoma multiforme (GBM). The study design identified CHBC (Figure 7) as a potential agonist with high selectivity and specificity. Validatation of GPR17-CHBC



20

Figure 6. Chemical Structure of **20**.



CHBC

Figure 7. Chemical structure of CHBC.

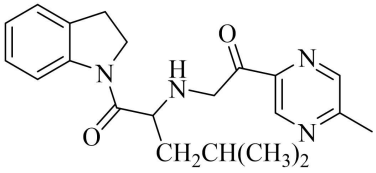
in vitro showed that the ligand CHBC inhibited cAMP and calcium levels in LN229 and SNB19 cells in a dose-dependent manner. Further cell viability assays showed potential growth inhibition of GBM cells by the lead compound, but no effect on non-cancerous MEF cells. Interestingly, the known agonist MDL29,951 had a negligible cytotoxic effect on both GBM cell lines. This suggests that the novel agonist CHBC is a better agonist for GBM therapy, as inhibition of cancer cell proliferation is a prerequisite for the development of potential drug-targeting compounds for cancer therapy.[36]

4. Antibacterial Agents

In recent years, researchers have found that many commonly used antibiotics are less effective against certain diseases due to their toxic response and the spread of microbial resistance, hence there is a need to design more new drugs to deal with resistant bacteria with high specificity. Indolines are mainly found in bioactive alkaloids and therefore have a potential role in therapy, making them suitable candidates for treatment and disease. Indoline scaffolds have been reported as important scaffolds in the field of medicinal chemistry and are used among antibacterial drugs.[37]

4.1. E. coli DNA gyrase inhibitors

Bacterial DNA gyrase B is a common target for the design of antibacterial drugs. Applying a computational approach to 3D conformational analysis, researchers designed compound **21** and found that compound **21** (Figure 8) has a strong binding capacity (KwissDock Score = −9.02 kcal/mol) and interacts with DNA gyrase B in the binding groove (Figure 9). Molecular



21

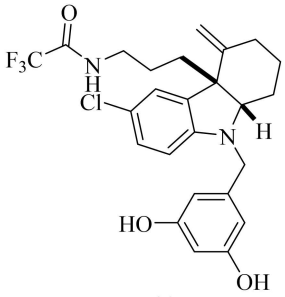
Figure 8. Chemical structure of compound **21**.

dynamics simulations of the compound **21**-DNA gyrase B complex demonstrated that it has low RMSD and RMSF values in a stable and strongly bound state. In addition, the lead compound **21** also has good pharmacological and pharmacokinetic properties such as high oral bioavailability, high tolerability, absence of any skin sensation or side effects, and no carcinogenicity.[37]

4.2. Direct action on Staphylococcus aureus

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prevalent drug-resistant bacteria, and new structures and mechanistic classes of antimicrobial agents need to be developed to combat MRSA. Substitution of TFA with benzyloxycarbonyl (Cbz) and reduction of the side chain by one methylene gave **22k** (Table 15) as the most effective structure with an 8-fold increase in activity. **22k** had GI_{50} value of 8 μg/mL in human cervical adenocarcinoma HeLa cells, which was 2-fold higher than its MIC.[38]

Table 15. Structures and MIC values of compounds **22** against MSSA.



22

Compd.	Z^1	Z^2	R^1	R^2	R^3	R^4	Y	MIC[a,b]
22	TFA[c]	Cl	OH	H	OH	H	$(CH_2)_2$	32
22a	TFA[c]	H	OH	H	OH	H	$(CH_2)_2$	>128
22b	TFA[c]	Cl	OMe	H	OMe	H	$(CH_2)_2$	>128
22c	TFA[c]	Cl	F	H	F	H	$(CH_2)_2$	>128
22d	TFA[c]	Cl	H	H	H	H	$(CH_2)_2$	>128
22e	TFA[c]	Cl	OH	OH	OH	H	$(CH_2)_2$	>128
22f	TFA[c]	Cl	H	H	OH	OH	$(CH_2)_2$	32
22g	TFA[c]	Cl	H	OH	H	OH	$(CH_2)_2$	32
22h	TFA[c]	Cl	OH	H	OH	H	CH_2	16
22i	TFA[c]	Cl	H	COOH	H	OH	CH_2	>128
22j	H	Cl	OH	H	OH	H	$(CH_2)_2$	64
22k	Cbz[d]	Cl	OH	H	OH	H	CH_2	4

[a] ALL MIC values are reported in μg/mL, [b] MSSA stain ATCC 25923, [c] trifluoroacetyl, [d] benzyloxycarbonyl.

Various structural variations of tricyclic dihydroindoles **23** (Figure 10) were prepared by Zhang et al. and analyzed for their ability to enhance β-lactam antibiotics in MRSA. The presence of the sulphonamide group in analogue **23** was most favorable

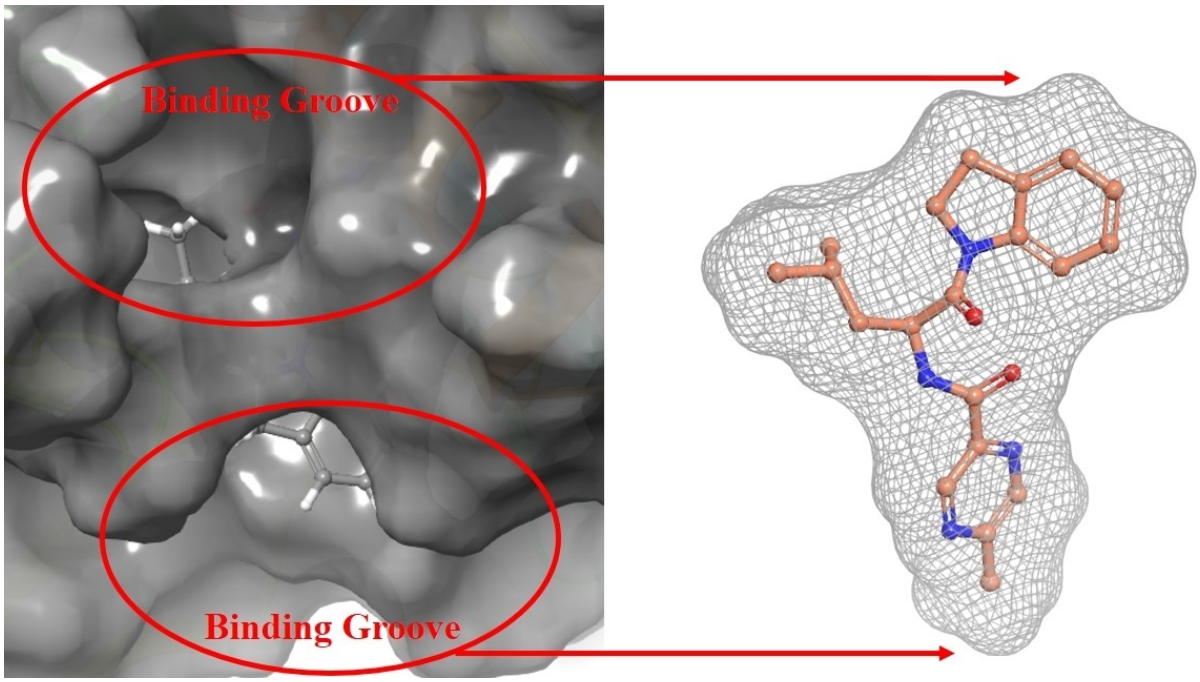


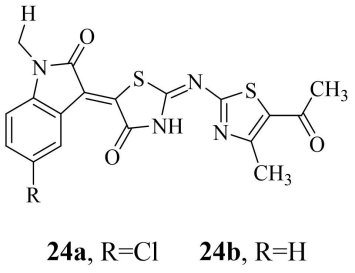
Figure 9. The surface view of molecular interactions of compound **21** to the binding groove of DNA gyrase B enzyme (PDB: 4DUH) at the left side. At the right side, enlarged view of compound **21** with interacting cavity mesh network.

for enhancing the antimicrobial effect. The introduction of the F substituent at the C-7 position of dihydroindole (**23 b**) greatly enhanced the RMA efficacy.[39]

Compounds **24 a** and **24 b** (Figure 11) inhibited microbial strains MRSA and VRE with MICs of 3.90 and 7.81 mg/mL, respectively, which was superior to vancomycin (MIC: 1.95 mg/mL) and ciprofloxacin (MIC: 3.90 mg/mL). More importantly, the potent compound **24 a** was non-cytotoxic and had good selectivity against the common lung fibroblast WI-38 cell line.[39]

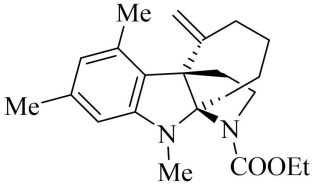
4.3 .Drug-resistance modifiers

In addition to the development of antimicrobial agents, indolines have recently been more frequently investigated as antibiotic resistance modifiers (RMAs). Unlike antibiotics, RMAs do not have bactericidal, bacteriostatic or antibacterial effects. They target non-essential genes or proteins in bacteria by acting synergistically with the antibiotic/antimicrobial agent, binding to the gene responsible for the resistant protein or proteins and preventing them from breaking down the potent antibiotic. Many resistance modifiers have been approved by the FDA and are used in combination with antibiotics. On the basis of known tetracyclic dihydroindoles, the alkyl chain length of the carbamate on the nitrogen is altered by changing to include methyl, ethyl, benzyl and *tert*-butyl. The most effective was found to be ethyl carbamate (**25**) (Figure 12), which was not significantly cytotoxic to mammalian cells and could be used in combination with amoxicillin and cefazolin (MRC of 2 μg/mL) as a novel class of β-lactam-MRSA selective resistance modifiers.[40]



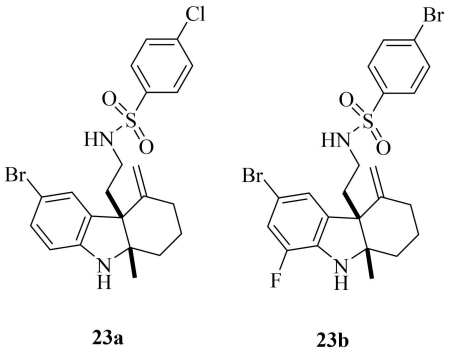
24a, R=Cl **24b**, R=H

Figure 11. Chemical structures of **24 a** and **24 b**.



25

Figure 12. Chemical structure of **25**.



23a **23b**

Figure 10. Chemical structures of **23 a** and **23 b**.

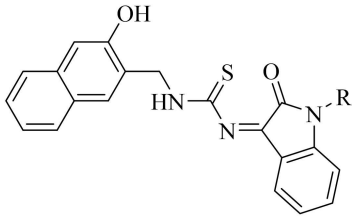
5. Cardiovascular Diseases

5.1 ACE inhibitors

Angiotensin-converting enzyme (ACE) is a major component of the renin-angiotensin system and an influential factor in hypertension (HTN) and congestive heart failure (CHF). It consists of two catalytic structural domains, the C-structural domain (carboxy-terminal structural domain) and the N-structural domain (nitro/amino-terminal structural domain), which hydrolyze angiotensin I (AngI) and bradykinin. The C-structural domain primarily converts AngI into the active vasoconstrictor AngII. In addition to bradykinin degradation, AngII production induces an increase in blood pressure by stimulating vascular or cyclic pressure. ACE competitive inhibitors can be used to treat cardiovascular disease and lower blood pressure. Inhibitors of the ACEc domain have potential therapeutic value over the ACEn domain primarily in the benefit or treatment of cardiovascular disease.[41]

Among a series of the naphthalene-2-ol-indoline-2-one-thiocarbamide derivatives, their cardiovascular protection, thrombolytic efficiency and DNA damage protection were assessed. Docking study found that compound **26 b** (Table 16)

Table 16. Results of in vitro cytotoxicity assay for compounds **26 a–d**.



26a-26d

Compd.	R	IC_{50} [μM ± S.D.][b] MCF-7	% activity
26 a	$-CH_2CH_2Br$	0.092 ± 0.66	82.94
26 b	$-CH_2COOH$	0.082 ± 0.12	89.02
26 d	$-CH_2CH_2COPh$	0.102 ± 0.65	78.12
DOX[a]		0.142 ± 0.94	78.42

[a] DOX-Doxorubicin, Data analyzed by one-way ANOVA followed by Dunnett's't' test, (n = 4), [b] IC_{50} values are the mean of duplicate or triplicate measurements, S.D.-Standard Deviation.

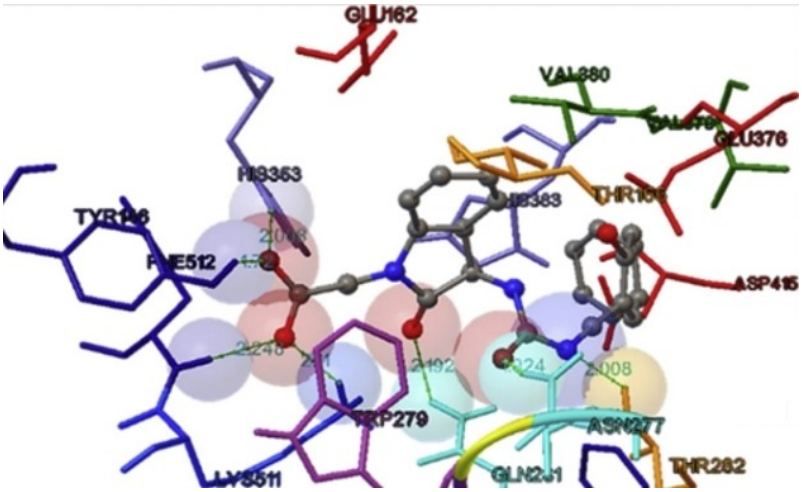


Figure 13. Binding mode of Ligand-Receptor **26 b** with ACE protein (PDB: 2XY9). Reproduced with permission from Ref. [41]. Copyright (2014), with permission from Elsevier Masson SA.

was found to have seven hydrogen bonds with ACE and to be the most active compound, with the highest solubility, polarity and hydrophilicity (Figure 13). Typically, the presence of a hydroxyl group (OH, hydrogen bond acceptor) and the smallest one, a carboxyl group (C=O, hydrogen bond acceptor/donor), which makes the molecule polar/hydrophilic, ensures that the drug has the required solubility. The compounds containing a halogen or C(sp^3) leaving group (**26 a**), containing a carboxylic acid (**26 b**), and containing a carboxyl/*tert*-carboxyl (**26 c** and **26 d**) substituent had the strongest targeting activity. The cytotoxicity results ensured the safety of the most potent ACE inhibitors (**26 a**, **26 b** and **26 d**) in normal breast epithelial MCF-10 cells.[41]

6. Anti-Inflammatory and Analgesic Drugs

Inflammation is a normal response to infection and injury and involves the recruitment of the immune system to neutralize invading pathogens, repair damaged tissue and promote

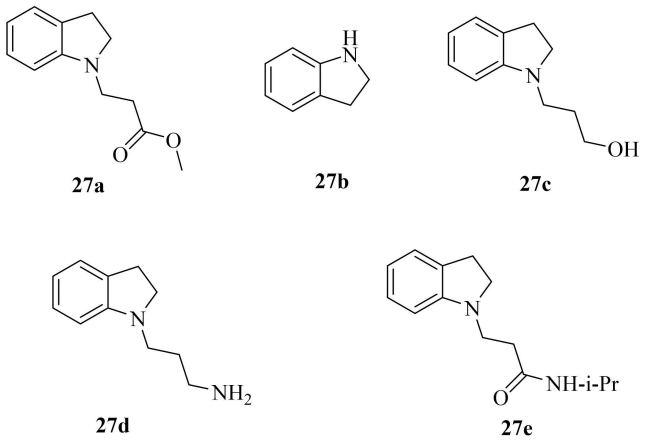


Figure 14. Chemical structures of **27 a**–**e**.

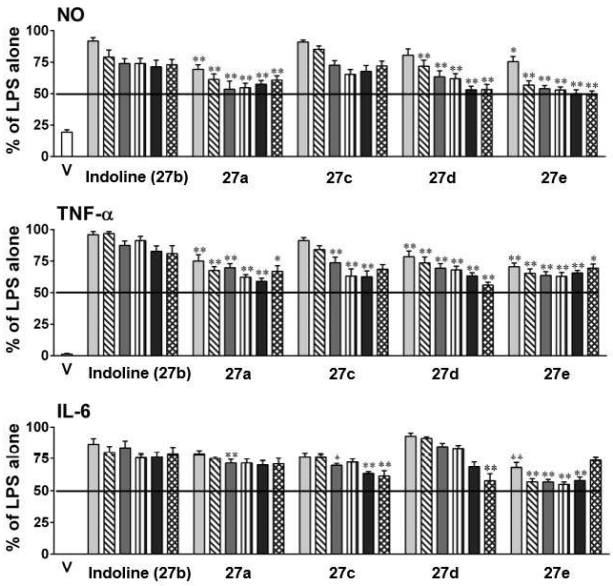


Figure 15. Effect of an **27 a**–**27 e** on the reduction NO, TNF-α, and IL-6 in LPS activated macrophages. Reproduced with permission from Ref. [42]. Copyright (2018), with permission from the American Chemical Society.

wound healing. During chronic or excessive activation of the immune system, nitric oxide synthase (iNOS) stimulates the release of nitric oxide (NO) and pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6). The acetylcholinesterase (AChE) inhibitors reduces the release of cytokines from α7-activated macrophages by indirectly stimulating the α 7 nicotinic acetylcholine receptor, activating the Jak2/STAT3 pathway and reducing pro-inflammatory cytokines in various animal models of inflammation. 3-(Indolin-1-yl)-*N*-isopropylpropanamide, **27 e** (Figure 14) is 1000-fold more potent than indoline (**27 b**) and was the most potent anti-inflammatory agent, reducing NO and IL-6 by 50% at a concentration of 100 pM. Compounds **27 d** and **27 a** have same in antioxidant activity, but NO and TNF-α were significantly

reduced at 1×10^{-12} M to 1×10^{-7} M. 3-hydroxypropyl derivative **27c** did not reduce NO more than winteroside **27b** at any concentration, but was more effective in reducing TNF- α and IL-6 at the three higher concentrations (Figure 15).[42]

Acknowledgements

This work was supported by the National College Students' Innovation and Entrepreneurship Training Program (No. 202110163037) and the MNR Key Laboratory of Eco-Environmental Science and Technology, China.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: antibacterial · anti-tumor · indoline · pharmacological activity · synthesis

[1] A. Thakur, A. Singh, N. Kaur, R. Ojha, K. Nepali, *Bioorg. Chem.* **2020**, *94*, 103436.
[2] M. J. Nieto, H. K. Lupton, *Current. Med. Chem.* **2021**, *28*, 4828–4844.
[3] K. H. Lim, O. Hiraku, K. Komiyama, T. S. Kam, *J. Nat. Prod.* **2008**, *71*, 1591–1594.
[4] J. T. Ndongo, J. N. Mbing, M. F. Tala, A. Monteillier, D. E. Pegnyemb, M. Cuendet, H. Laatsch, *Phytochemistry* **2017**, *144*, 189–196.
[5] J. E. Wilson, R. Kurukulasuriya, M. Reibarkh, M. Reiter, A. Zwicker, K. Zhao, F. Q. Zhang, R. Anand, V. J. Colandrea, A. M. Cumiskey, A. Crespo, R. A. Duffy, B. A. Murphy, K. Mitra, D. G. Johns, J. L. Duffy, P. Vachal, *ACS Med. Chem. Lett.* **2016**, *7*, 261–265.
[6] M. Tercel, R. N. Marnane, M. A. Tatnell, R. J. Stevenson, A. Halim, G. L. Lu, C. Duchesnes, M. Truong, W. A. Denny, W. R. Wilson, K. G. Mountjoy, *Int. J. Obes.* **2013**, *37*, 685–692.
[7] L. Wang, Y. Shao, Y. Liu, *Org. Lett.* **2012**, *14*, 3978–3981.
[8] S. Chandrasekhar, D. Basu, Ch. Reddy, *Synthesis.* **2007**, 1509–1512.
[9] L. Dale, R. Coleman, *J. Org. Chem.* **1984**, *49*, 2240–2245.
[10] M. Presset, A. Pignon, J. Paul, E. L. Gall, E. Léonel, T. Martens, *J. Org. Chem.* **2017**, *82*, 3302–3310.
[11] Y. Li, Y. Chang, Y. Li, C. Cao, J. Yang, B. Wang, D. Liang, *Adv. Synth. Catal.* **2018**, *360*, 2488.
[12] D. Liang, D. Ge, Y. Lv, W. Huang, B. Wang, W. Li, *J. Org. Chem.* **2018**, *83*, 4681–4691.
[13] V. Certal, J. C. Carry, F. Halley, A. Virone-Oddos, F. Thompson, B. Filoche-Rommé, Y. El-Ahmad, A. Karlsson, V. Charrier, C. Delorme, A. Rak, P. Y. Abecassis, C. Amara, L. Vincent, H. Bonnevaux, J. P. Nicolas, M. Mathieu, T. Bertrand, J. P. Marquette, N. Michot, T. Benard, M. A. Perrin, O. Lemaitre, S. Guerif, S. Perron, S. Monget, F. Gruss-Leleu, G. Doerflinger, H. Guizani, M. Brollo, L. Delbarre, L. Bertin, P. Richepin, V. Loyau, C. Garcia-Echeverria, C. Lengauer, L. Schio, *J. Med. Chem.* **2014**, *57*, 903–920.
[14] P. Bédard, M. A. Davies, S. Kopetz, D. Juric, G. Shapiro, J. Luke, A. Spreafico, B. Wu, C. Castell, C. Gomez, S. Cartot-Cotton, F. Mazuir, M. Dubar, S. Micallef, B. Demers, K. T. Flaherty, *Cancer* **2018**, *124*, 315–324.
[15] C. C. Chiang, Y. H. Lin, S. F. Lin, C. L. Lai, C. Liu, W. Y. Wei, S. Yang, R. W. Wang, L. W. Teng, S. H. Chuang, J. M. Chang, T. T. Yuan, Y. S. Lee, P. Chen, W. K. Chi, J. Y. Yang, H. J. Huang, C. B. Liao, J. J. Huang, *J. Med. Chem.* **2010**, *53*, 5929–5941.
[16] W. M. Eldehna, M. Fares, H. S. Ibrahim, M. H. Aly, S. Zada, M. M. Ali, S. M. Abou-Seri, H. A. Abdel-Aziz, D. A. A. E. Ella, *Eur. J. Med. Chem.* **2015**, *15*, 89–97.
[17] J. J. Huang, Y. H. Lin, C. L. Lai, S. C. Yang, S. F. Lin, J. Y. Yang, H. J. Huang, C. Liu, W. Y. Wei, S. H. Chuang, C. C. Chiang, Y. S. E. Lee, C. B. Liao, C. Y. Chern, *J. Chin. Chem. Soc.* **2020**, *67*, 422–429.
[18] H. Y. Lee, C. R. Yang, M. J. Lai, H. L. Huang, Y. L. Hsieh, Y. M. Liu, T. K. Yeh, Y. H. Li, S. Mehndiratta, C. M. Teng, J. P. Liou, *ChemBioChem* **2013**, *14*, 1248–1254.
[19] G. Kouraklis, S. Theocharis, *Oncol. Rep.* **2006**, *15*, 489–494.
[20] H. Y. Lee, L. T. Wang, Y. H. Li, S. L. Pan, Y. L. Chen, C. M. Teng, J. P. Liou, *Org. Biomol. Chem.* **2014**, *12*, 8966–8976.
[21] K. Jin, S. S. Li, X. G. Li, J. Zhang, W. F. Xu, X. C. Li, *Bioorg. Med. Chem.* **2015**, *23*, 4728–4736.
[22] Y. Depetter, S. Geurs, J. Franceus, D. Knez, T. Desmet, K. D. Bosscher, O. D. Wever, S. Gobec, M. D'hooghe, *ChemistrySelect* **2019**, *4*, 12308–12312.
[23] T. T. L. Huong, D. T. M. Dung, P. T. P. Dung, P. T. Huong, T. K. Vu, H. Hahn, B. W. Han, J. Kim, M. Pyo, S. B. Han, N. H. Nam, *Tetrahedron Lett.* **2015**, *56*, 6425–6429.
[24] S. H. Kaufmann, W. C. Earnshaw, *Exp. Cell Res.* **2000**, *256*, 42–49.
[25] D. H. Fu, M. M. Li, S. Y. Zhang, J. F. Li, B. B. Sha, L. H. Wang, Y. B. Zhang, P. Chen, T. Hu, *Bioorg. Chem.* **2019**, *92*, 103190.
[26] X. L. Xu, C. L. Yu, W. Chen, Y. C. Li, L. J. Yang, Y. Li, H. B. Zhang, X. D. Yang, *Org. Biomol. Chem.* **2015**, *13*, 1550–1557.
[27] K. R. Senwar, T. S. Reddy, D. Thummuri, P. Sharma, S. K. Bharghava, V. G. M. Naidu, N. Shankaraiah, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4061–4069.
[28] A. Bertamino, M. Soprano, S. Musella, M. R. Rusciano, M. Sala, E. Vernieri, V. D. Sarno, A. Limatola, A. Carotenuto, S. Cosconati, P. Grieco, E. Novellino, M. Illario, P. Campiglia, I. G-Monterrey, *J. Med. Chem.* **2013**, *56*, 5407–5421.
[29] A. Aguilar, J. F. Lu, L. Liu, D. Du, D. Bernard, D. McEachern, S. Przybranowski, X. Q. Li, R. J. Luo, B. Wen, D. X. Sun, H. B. Wang, J. F. Wen, G. F. Wang, Y. F. Zhai, M. Guo, D. J. Yang, S. M. Wang, *J. Med. Chem.* **2017**, *60*, 2819–2839.
[30] H. S. Ibrahim, W. M. Eldehna, A. L. Fallacara, E. R. Ahmed, H. A. Ghabbour, M. M. Elaasser, M. Botta, S. M. Abou-seri, H. A. Abdel-Aziz, *Future Med. Chem.* **2018**, *10*, 2771–2789.
[31] S. Mehndiratta, Y. F. Chiang, M. J. Lai, H. Y. Lee, M. C. Chen, C. C. Kuo, C. Y. Chang, J. Y. Chang, J. P. Liou, *Bioorg. Med. Chem.* **2014**, *22*, 4917–4923.
[32] S. Y. Wang, X. Liu, L. W. Meng, M. M. Li, Y. R. Li, G. X. Yu, J. Song, H. Y. Zhang, P. Chen, S. Y. Zhang, T. W. Hu, *Bioorg. Med. Chem.* **2021**, *45*, 128131.
[33] M. J. Lai, R. Ojha, M. H. Lin, Y. M. Liu, H. Y. Lee, T. E. Lin, K. C. Hsu, C. Y. Chang, M. C. Chen, K. Nepali, J. Y. Chang, J. P. Liou, *Eur. J. Med. Chem.* **2019**, *162*, 612–630.
[34] R. Ojha, H. L. Huang, W. H. Fu, Y. W. Wu, K. Nepali, M. J. Lai, C. J. Su, T. Y. Sung, Y. L. Chen, S. L. Pan, J. P. Liou, *Eur. J. Med. Chem.* **2018**, *150*, 667–677.
[35] M. Z. Zuo, X. Xu, Z. L. Xie, R. L. Ge, Z. Y. Zhang, Z. Y. Li, J. L. Bian, *Eur. J. Med. Chem.* **2017**, *125*, 1002–1022.
[36] P. Nguyen, P. Doan, T. Rimpilainen, S. K. Mani, A. Murugesan, O. Yli-Harja, N. R. Candeias, M. Kandhavelu, *J. Med. Chem.* **2021**, *64*, 10908–10918.
[37] A. Singh, N. Kumar, D. Sood, S. Singh, A. Awasthi, V. Tomar, R. Chandra, *Curr. Top. Med. Chem.* **2018**, *18*, 2056–2065.
[38] P. M. Barbour, J. D. Podoll, L. J. Marholz, X. Wang, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5602–5605.
[39] H. L. Qin, J. Liu, W. Y. Fang, L. Ravindar, K. P. Rakesh, *Eur. J. Med. Chem.* **2020**, *194*, 112245.
[40] Y. Zhu, L. Cleaver, W. Wang, J. D. Podoll, X. Wang, *Eur. J. Med. Chem.* **2017**, *125*, 130–142.
[41] M. A. P. Moharil, M. Sathishkumar, R. C. Muñoz-Garay, A. Sivakumar, *Eur. J. Med. Chem.* **2017**, *141*, 417–426.
[42] S. Zeeli, T. Weill, E. Finkin-Groner, C. Bejar, M. Melamed, S. Furman, M. Zhenin, A. Nudelman, M. Weinstock, *J. Med. Chem.* **2018**, *61*, 4004–4019.

Manuscript received: November 8, 2022
Revised manuscript received: December 6, 2022