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Synthesis and anti-tumor activity of marine alkaloids

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ABSTRACT

Marine alkaloids were divided into five categories from the perspective of anti-tumor activity. The optimization process, chemical synthesis, anti-tumor activity evaluation and structure–activity relationship of various compounds were discussed.

Introduction

Alkaloids are natural compounds (natural products) that contain nitrogen and have significant biological activities [1]. They generally have chemical properties similar to bases. Some nitrogenous compounds derived from plants that are not alkaline, but still be classified as alkaloids because of their obvious biological activity [2–4]. It can be seen from the chemical structure of alkaloids that most alkaloids have complex nitrogen heterocyclic structure and a few alkaloids are organic amine compounds of not nitrogen heterocyclic structure [5–9]. The definition of alkaloids by the international union of pure and applied chemistry (IUPAC) is not been strictly and exactly. For example, some vitamins, amino acids, peptides and other nitrogenous compounds derived from natural products do not belong to the category of alkaloids [10]. Alkaloids are widely distribute in nature, especially in plants, and also in animals, microorganisms and marine organisms. There are many known types of alkaloids, about 10,000 of them, and some structural formulas have not been fully determined [11,12]. As there are many types of alkaloids, each of which has a different structural formula, their properties will be different from each other. Although the amount of alkaloids contained in living organisms is small, but it is closely related to human beings. It has long been recognized that some plants or extracts containing alkaloids can cure diseases or be used as poisons [13]. Since 1806, the German pharmacist Serturner isolated morphine from poppy, there have been>6,000 alkaloids isolated from plants and animals. Through the joint efforts of medical chemists, pharmacologists and other scholars, people have found that a variety of alkaloids have anti-tumor, antibacterial, anti-viral and other aspects of biological activities [14–16]. So far, nearly 100 alkaloid compounds have been used or used in clinical trials. In the treatment of many diseases, many alkaloids are good medicine, but some alkaloids can be used to make pesticides for agriculture [17–19]. Because of the wide range of biological activities, the alkaloids have been widely concerned.

The ocean is the birthplace of life and have rich in biological resources, it is a huge natural products screening resources [20]. So far, researchers have found>300,000 species of marine organisms in the ocean, and more than a one million new species are expect to remain undiscovered [21–24]. Marine organisms are secondary metabolites that have formed and accumulated a large number of special chemical structures and significant biological activities in the process of long-term evolution and metabolism in special environments such as high salt, high pressure, low temperature, hypoxia, and lack of light [25]. Its in the anti-viral, anti-inflammatory and anti-tumor and other aspects of the role of significant. Marine alkaloids are a secondary metabolite of marine organisms, mainly derived from marine organisms such as sponges, algae, coelenterates and tunica [26–29]. Marine alkaloids are a kind of alkaline natural products with important biological activities including amine nitrogen functional groups and complex carbon skeleton ring structure [30]. The main biological activities of marine alkaloids have anti-tumor, anti-fungus, anti-virus, anti-malaria, anti-fungal and anti-osteoporosis. Many marine alkaloids may be used as anti-tumor, anti-viral and anti-fungal clinical drugs or as the lead compounds for structural modification, it have good medicinal prospects [31]. In recent years, the marine drugs research has been paid more and more attention by scholars, especially in the field of marine alkaloid drugs. Many kinds of marine alkaloids have been found and extracted from marine organisms, but most of the marine alkaloids extraction quantity is small and the efficiency is low [32–35]. Representative compounds of these marine

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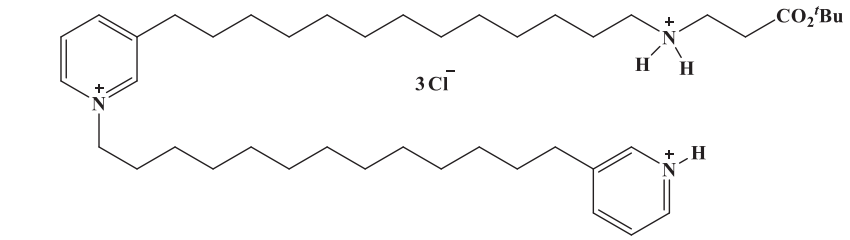
alkaloids include Viscosaline compound **1**, Theonelladin C compound **2**, Makaluvamine A compound **3**, Ellipticine compound **4**, Neoamphimedine compound **5**, Dispacamide compound **6** , Polyandrocarpamine A and B compounds **7**–**8**, Leucettamine B compound **9**, Granulatimide compound **10**, Isogranulatimide compound **11**, Rigidin A-D compounds **12**–**15**, Coscinamide A-C compounds **16**–**18**,



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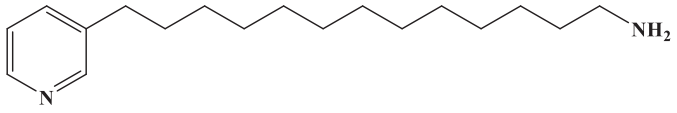

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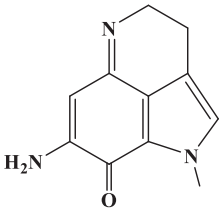

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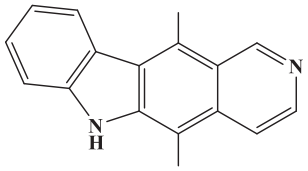

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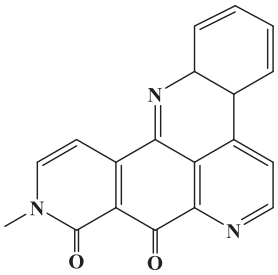

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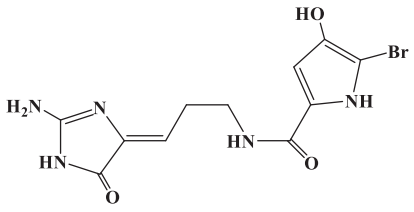

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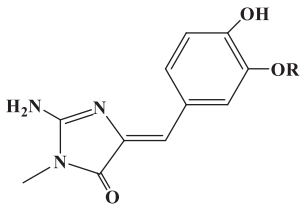
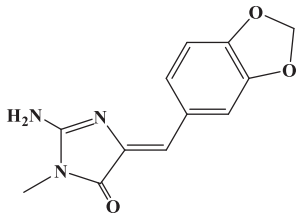


7 R=Me
8 R=H



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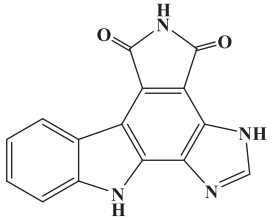

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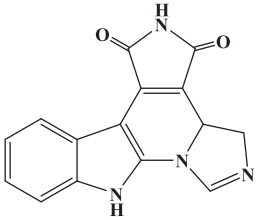

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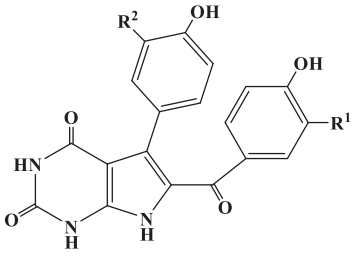
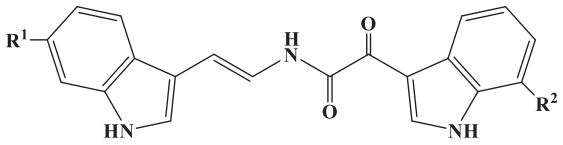
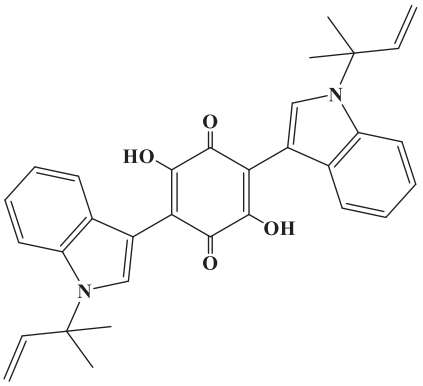


12 R^1=R^2=H
13 R^1=OMe, R^2=H
14 R^1=H, R^2=OMe
15 R^1=R^2=OMe

Fig. 1. The chemical structure of marine alkaloids.



16 R^1=Br, R^2=H
17 R^1=R^2=H
18 R^1=Br, R^2=OH



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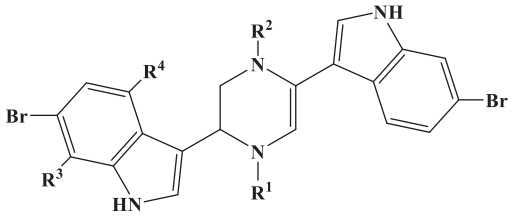
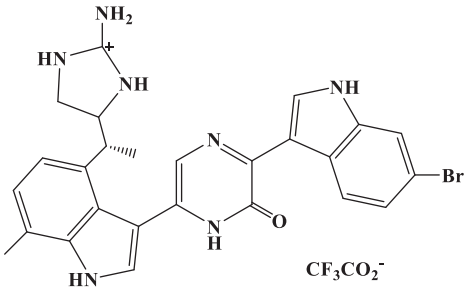


20 R^1=H, R^2=Me, R^3=Br, R^4=OH
21 R^1=R^2=R^3=H, R^4=Me
22 R^1=R^2=Me, R^3=R^4=H
23 R^1=R^2=R^3=R^4=H



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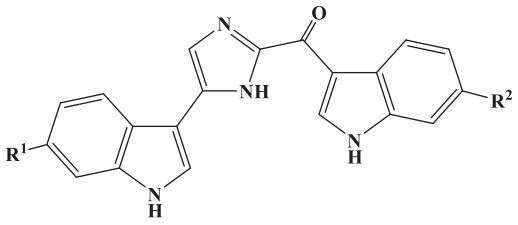
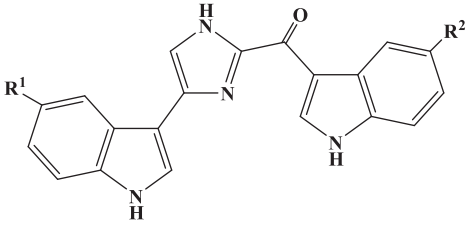


25 R^1=R^2=H
26 R^1=OH, R^2=H
27 R^1=Br, R^2=OH



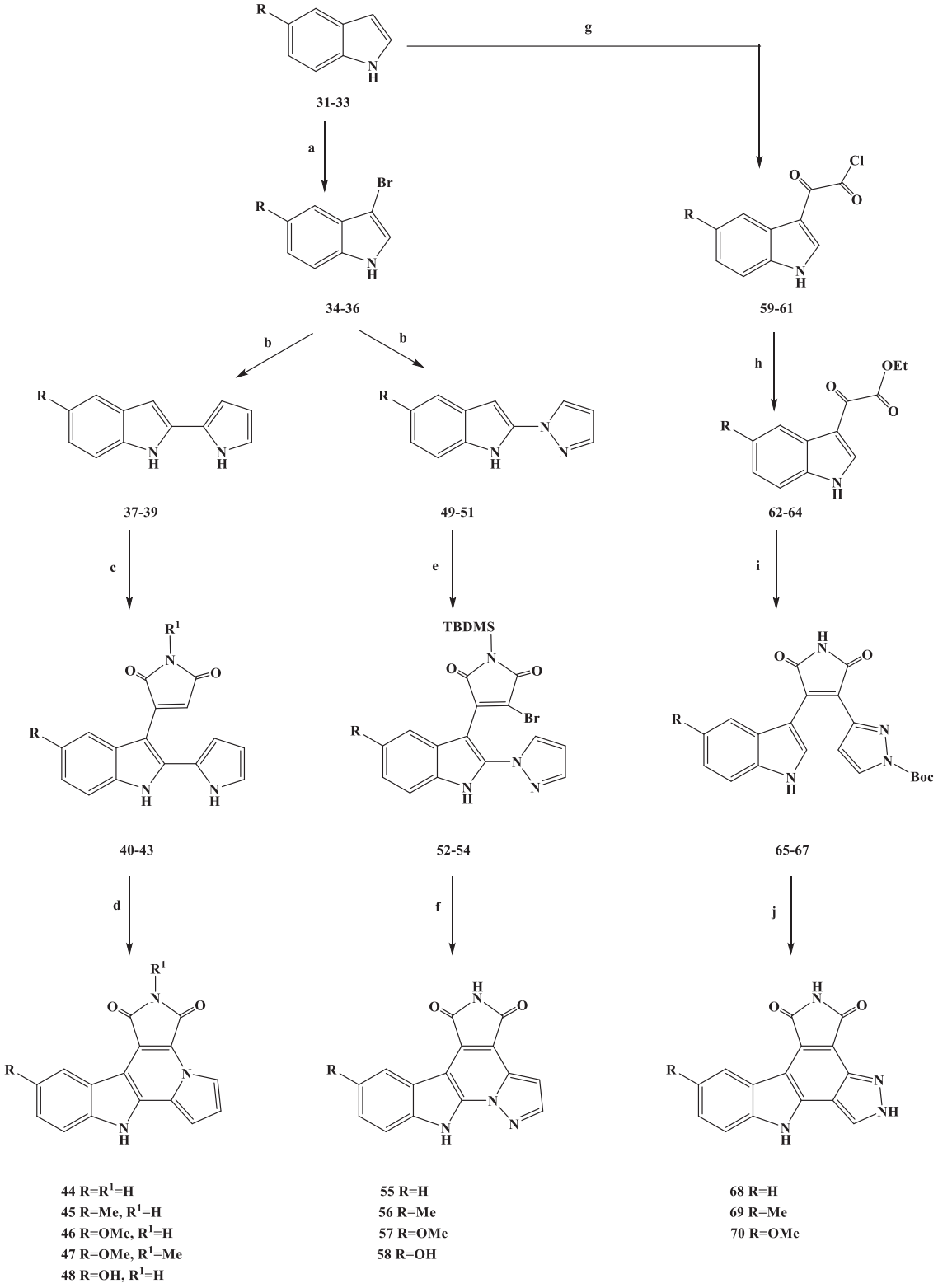
28 R^1=R^2=Br
29 R^1=H, R^2=Br
30 R^1=Br, R^2=H

Fig. 1. (*continued*).

Asterriquinone **19**, Dragmacidin **20**, Dragmacidin A-C **21**–**23**, Dragmacidin D **24**, Topsentin A, B_1 and B_2 compounds **25**–**27**, Nortopsentin A-C compounds **28**–**30** (Fig. 1). In addition, these marine alkaloids have the disadvantages of high cytotoxicity, low selectivity index and low activity [36]. These problems will limit the clinical research and industrial development of marine alkaloid drugs [37–39]. Therefore, it has become an urgent task for research and development to find marine alkaloid analogues and derivatives that are economical and environmentally friendly. A large number of target compounds can be obtained for activity screening by rational drugs design, structural modification of lead compounds and chemical synthesis [40]. In this way, may be to solve the shortage of natural marine alkaloids, and it provides a good platform for the development of marine alkaloid derivatives and analogues [41]. This work system reviews the recent development of marine alkaloid derivatives and analogues in the field of medical chemistry over the last 10 years (20010–2019). We divided marine alkaloid derivatives and analogues into five types from the point of view for biological activity, including the research of anti-tumor, anti-malaria, anti-bacterial, anti-virus (HCV), and other type, and elaborated on these activities. We also briefly discussed the optimization process, chemical synthesis, and anti-tumor activity evaluation of each compounds.

Synthesis and anti-tumor activity

In medicine, the cancer is to point to the malignant tumor that originates from epithelial tissue, it is the most common kind of disease in malignant tumor [42]. The cancer was characterized by abnormal cell differentiation and proliferation, uncontrolled growth, invasion and metastasis [43–45]. Intercellular division is the preparation period for mitosis or meiosis in eukaryotic cells, during which the cell completes the replication of DNA molecules and the synthesis of related proteins. The interphase of cell division is much longer than the interphase of cell



Scheme 1. Synthesis of the marine alkaloids Granulatimide and Isogranulatimid analogues compounds **44**–**48**, **55**–**58** and **68**–**70**. Reagent and conditions: (a) Br_2, DMF, 2 h; (b) TFA, CH_2Cl_2, r.t., 4 h; (c) $SnCl_2$, toluene, reflux, 24 h; (d) Pd, micro wave 300 W, 190℃, 15 min; (e) LiHMDS, THF, −15℃ then r.t., 12 h; (f) hν, CH_3CN, r.t., 5 h; (g) $(COCl)_2$, Et_2O, 25℃, 3 h; (h) EtOH, Et_3N, 78℃, 2 h; (i) i *t*-BuOK, THF, r.t., 12 h; ii HCl; (j) hν, CH_3CN, r.t., 5 h.

division [46]. The interphase is usually divided into G_1, S and G_2 phases. The mitotic interphase is an important preparatory process for the whole process of mitosis. Studies have shown that G_1 and G_2 checkpoints, if activated during the intermitotic period, can damage the normal replication of DNA, thereby blocking subsequent cell division [47]. In this case, it can provide valuable time to repair normal DNA. The study found that in>50% of cancer cells, the G_1 checkpoint was damaged because of mutations in the p53 anti-oncogene. Relevant researchers suggest that will be able to damage the DNA of the drugs and G_2/M checkpoint inhibitor together, there may be selectively kill cancer cells into premature and mitosis, and shall not affect the normal cell mitosis, and think that this combination was used in the treatment of cancer is a primitive method and promising method of chemotherapy [48]. Under this biological background, the marine alkaloids Granulatimide and Isogranulatimide were isolated and extracted from marine organisms with G_2/M checkpoint of inhibiting cell cycle [49]. The acquisition of these two marine alkaloids has attracted the attention of researchers, and they are a class of inhibitors with great research value. From the perspective of mechanism of action, these marine alkaloids have been identified as inhibitors that can selectively inhibit checkpoint kinase 1 (Chk1), a key enzyme in G_2/M checkpoint [50,51]. Relevant studies have shown that the maleimide groups of these compounds play an important role in affecting biological activity, and they can establish two hydrogen bonds with Glu[85] and Cys[87] in the ATP binding pocket of the enzyme [52]. On this basis, Sebastien Deslandes et al [53] designed three series of Granulatimide and Isogranulatimide analogues for *in vitro* evaluation of anti-tumor activity, a kinase inhibition and G_2/M checkpoint abrogation. It could be seen from the chemical structure of the designed target compounds that the newly designed compounds retain the skeleton structure of the lead compounds, and the main modification was to introduce different substituents into the benzene ring to obtain the active compounds. In the process of synthesizing the target compounds Granulatimide and Isogranulatimide analogues, they used the operation described in Scheme 1. In the first series of the target compounds **45**–**49**, the substituted indoles compounds **31**–**33** were as starting materials and brominated with bromine in *N*, *N*-dimethylformamide (DMF) to obtain 3-bromine derivatives compounds **34**–**36**. The compound **34**–**36** were coupled with pyrrole in methylene dichloride to obtain the intermediate compounds **37**–**39**. The compounds **37**–**39** had a condensation reaction with maleimide or methylmaleimide. In this step, the toluene was used as the reaction solvent and $SnCl_2$ as the reaction catalyst, and the compounds **40**–**43** were obtained by Michael addition. The compounds **40**–**43** were cyclized and prepared by microwave irradiation with Pd as catalyst to obtain the target componds **44**–**47**. Meanwhile, the target compound **46** was demethylated in methylene dichloride with tribromo-based boron to obtain the target compound **48**. In the second series of the target compounds, the compounds **34**–**36** were used as the starting material for reaction, and the intermediate compounds **49**–**51** were obtained by coupling reaction with pyrazole in methylene dichloride. The compounds **49**–**51** condensates with *N*-*t*-butyldimethylsilyl (TBDMS)-dibromo-maleimide in tetrahydrofuran (THF) to obtain low yield intermediate compounds **52**–**54**. The intermediate formed in this step does not form a ring, and the TBDMS protective group does not detach during the reaction. The compounds **52**–**54** were cycloformed by acetonitrile under light conditions to obtain the target compounds **55**–**57.** Meanwhile, the target compound **57** was demethylated in methylene dichloride with tribromo-based boron to obtain the target compound **58**. In the third series of the target compounds synthesis, they first converted compounds **31**–**33** into compounds **62**–**64** in two steps by referring to related literatures. Then compounds **62**–**64** reacted with *N*-Boc-pyrazole-3-acetamide in tetrahydrofuran (THF) to obtain the intermediate compounds **65**–**67**. Finally, the compounds **65**–**67** in acetonitrile, under light conditions to form a ring reaction, and then the protection of the Boc group (cracking reaction), the target compounds **68**–**70** were obtained. This synthesis route used cheap reagent, the synthesis operation was relatively simple, the use of functional group protection was easy to group protection, and in the subsequent process of deprotection was easy to leave. In addition, microwave irradiation was used in the synthesis method, which reduced the reaction time and increased the yield. However, this synthesis route also has some shortcomings, the yield of some compounds was relatively low (3%), which brings great difficulties for industrialization in the future. Further optimization of process conditions is needed to improve the reaction yield and reduce the synthesis cost. In the process of biological activity study, the growth inhibition activity of the synthetic compounds was evaluated *in vitro*. The test results showed that in cancer cells, these compounds showed a degree of sensitivity and resistance to pro-apoptotic stimuli. Of the three series of the target compounds, the second series had the highest average growth inhibition activity on tumor cells, with properties like the target compounds **55** and **58**, and the average IC_{50} growth inhibition concentration was 11 nM and 16 nM, respectively. In addition, the compounds **40**–**43** and **52**–**54** were found to have good anti-tumor activities during the activity screening of intermediates, with an average IC_{50} growth inhibition concentration of about 20 nM, among which the compound **43** had the best activity (IC_{50} = 13 nM). On the basis of *in vitro* screening, the ability of the compounds to eliminate the activated G_2/M checkpoint in HCT116p53 cells was further investigated (Table 1). The test data showed that the target compound **55** had good activity (23.9), while the activity of other compounds was weak. To gain insight into the kinase selectivity of the synthesis compounds, they tested the selectivity of serine/threonine kinases. The results of selectivity test showed that these compounds had weak inhibitory activity and poor selectivity against kinases. In addition, the compounds **55**, **40** and **43** were quantitatively examined by electron microscopy. The results showed that the compounds **55**, **40** and **43** induced cellular inhibition, which reduced cell proliferation by significantly expanding the size of cancer cells, thus reflecting the tissue changes in the actin cytoskeleton. In general, according to the existing data analysis, the target compound **55** and the intermediate compounds **40** and **43** have good anti-tumor activities. These compounds were worthy of further structural modification and other pharmacological studies, so that can reach the true meaning of drugs and become the ideal anti-cancer drugs as soon as possible.

The isolation of biological activity natural products from marine organisms, including the most effective anti-tumor drugs found in them, has become an important source [54–56]. These anti-tumor drugs obtained from marine organisms have structural diversity and complexity. And most of these compounds with anti-tumor activity are alkaloids, which are commonly referred to as marine alkaloids [56–59]. Among these marine alkaloids, a class of marine alkaloids with bis-indole structure is well known [60]. This kind of bis-indole marine alkaloid is usually isolated from the metabolites of the deep-sea sponge, and has been found to have good anti-inflammatory, anti-tumor, anti-viral and anti-bacterial biological activities in the screening process [61]. The chemical structure of this type of bis-indole marine alkaloids was characterized by the fact that they are bound by two indoles through their 3 positions to intermediate ligands (including ring structure and chain structure). Related studies found that Nortopsentins A-C bis-indole marine alkaloids with imidazole as the five-memenon ring as the connector was 4.5–20.7 μM to the IC_{50} of P388

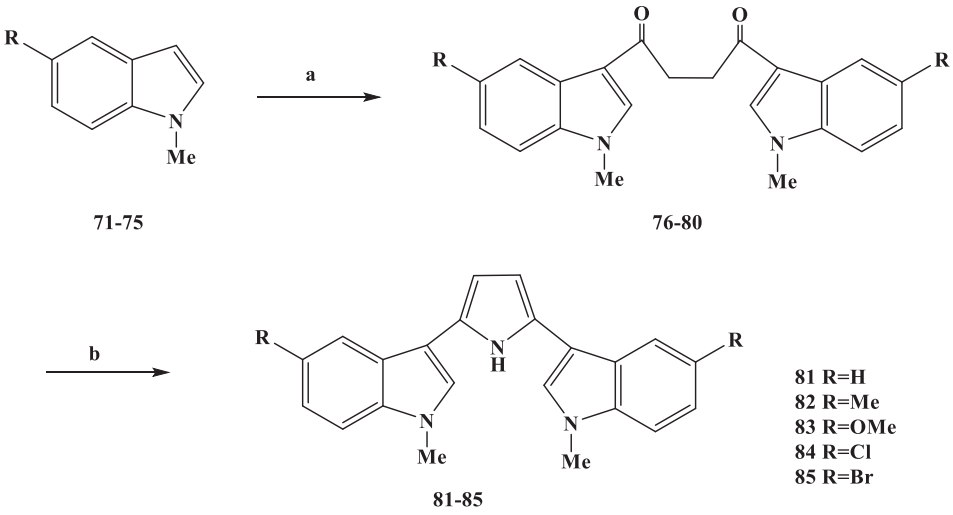
Table 1
Evaluation of the G_2/M checkpoint abrogation.

Compounds	Mitotic index
44	9.6
55	23.9
56	13.9
58	3.9
69	0
38	5.0
39	8.3
53	5.5
Isogranulatimide	17.2
Granulatimide	19.4

cells *in vitro* during the anti-tumor activity test, while the IC_{50} of P388 cells was 0.8–2.1 μM of *N*-methylated derivatives [62–64]. The results of *in vitro* cytotoxicity studies indicated that the methylated derivatives had significantly increased the activity of P388. Nortopsentins, a biological activity compound, was separation from natural products and its weight was small [65]. In order to obtain a large number of compound, several complete synthesis methods of Nortopsentins and analogues have been reported [66–68]. Because of the abundant biological activity of marine alkaloids, people often regarded as new drugs or the lead compounds with biological activity. Therefore, on this basis, the Nortopsentin was structurally modified, and Dragmacidin analogues were synthesized by taking six-element cyclopyridine, pyrimidine, pyrazine and pyrazinone as intermediate ligands. These analogues have been found to have a broad spectrum of anti-tumor activity in human tumor cells. However, the Nortopsentin analogues were synthesized by using five-memorial-heterocyclic imidazole ring as a ligand [69]. In the activity test, it was found that these compounds had significant anti-proliferative activity, and the IC_{50} value was at the sub-micromole level. In the process of modifying the structure of the lead compound, one or two indoles were modified in addition to the bindoles [70,71]. In the activity test results, it was found that 3-indolyl-5-phenylpyridine exhibited anti-proliferative activity in the range of 5–15 μM and effectively inhibited CDK1 at the level of 0.3 ~ 0.7 μM. On the basis of previous work, Anna Carbon et al [72] took Notopentine as the lead compound, modified its structure with pyrrole as the intermediate connector, and modified the substituent on two indoles, and designed Notopentine analogues with 2, 5-bis (3′-indolyl) pyrroles structure. In the process of synthesized the target compounds **81**–**85**, they selected the specific synthesis operation described in Scheme 2. The *N*-methylindole (compounds **71**–**75**) used as starting material, and the compounds were synthesized by two-steps reaction. In the first step reaction, the Vilsmeier-Haack reaction was used to treatment the compounds **71**–**75** with phosphorus oxychloride and tetramethylsuccinamide, and the symmetrical intermediate compounds **76**–**80** was obtained. In the second step, the compounds **76**–**80** was reflux reacted with ammonium acetate and acetic anhydride in acetic acid, and the corresponding of 2, 5-bis (3′-indolyl) pyrroles the target compounds **81**–**85** were obtained after treatment. The synthesis route only used two steps to obtain the target compounds, has the simple operation, the reaction step was few, the total yield was medium to good, the reagent used were cheap and easy to obtain, has laid a solid foundation for the future industrialization. However, these synthesis methods were all classical chemical reactions, and there was no innovation in the synthesis method. In the subsequent process optimization, can be consider other new synthesis methods. In the process of screening the biological activity of these synthesized target compounds **81**–**85**, they used human tumor cell lines for monolayer cell survival and proliferation tests to detect the anti-tumor activity of the target compounds *in vitro*. Using panels of human tumor cell lines of different source/tissue types, they analyzed the anti-tumor activity and selectivity of the tested compounds, and evaluated the eligible target compounds for entry into preclinical studies. *In vitro* anti-tumor activity of the target compounds **81**–**85** against 12 human tumor cell lines was screened by monolayer cell survival and proliferation assay (Table 2). The results of the screening experiments showed that all the target compounds produced cytotoxicity activity at the maximum test concentration of 100 μg/mL, with an average IC_{50} value ranging from 4.4 μg/mL to 0.37 μg/mL. The substituents on the indoles ring in the target compounds **81**–**85** have electron-withdrawing and electron-donating groups. The analysis of the drug structure–activity relationship (SAR) shows that the anti-tumor activity of the electron-donating group on the indoles ring in the target compounds were significantly higher than that of the electron-withdrawing group. At the same time, there was also a SAR that the stronger the ability of electron absorption or electron donation, the better the anti-tumor activity. In addition, the solid space of these substituents will also affect the anti-tumor activity of the target compounds. These target compounds showed good *in vitro* anti-tumor activity, with characteristics like the target compounds **81** and **82**, so the target compounds **81** and **82** were selected for further anti-tumor activity study. The target compounds **81** and **82** were further analyzed in monolayer cultures of 42 human tumor cell lines, including 15 different types of solid tumors. Relevant test results showed that the target compounds **81** and **82** had a concentration-dependent inhibitory effect on tumor cell growth and showed obvious cytotoxicity, with average IC_{50} values of 1.54 μM and 0.67 μM, respectively. Meanwhile, *in vitro* cloning experiments, the target compounds 1a-b showed selective anti-tumor activity for human tumor transplantation, and the sensitive tumor models were dispersed among different tumor tissue types. Therefore,

Table 2
In vitro activity of compounds **81**–**85**.

Compounds	IC_{50} (μg/mL)
81	0.37
82	0.37
83	3.4
84	3.4
85	4.4
Adriamycin	0.007



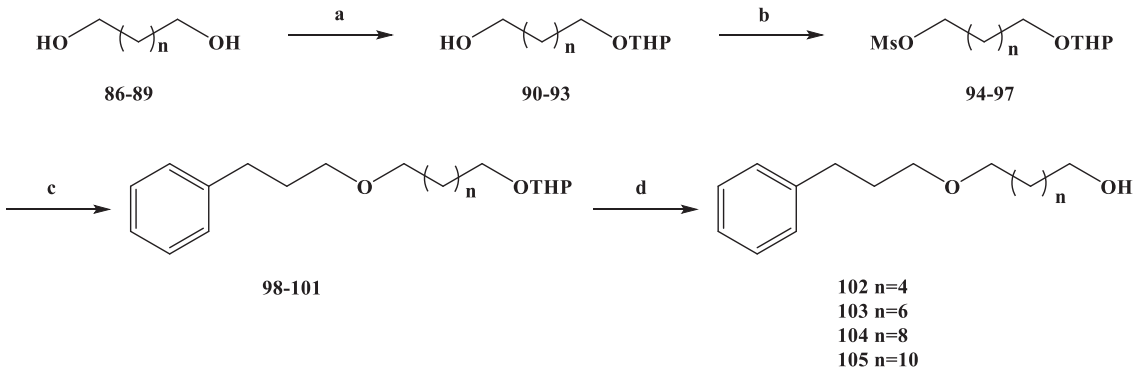
Scheme 2. Synthesis of 2, 5-bis (3′-Indolyl) pyrroles analogues compounds **81**–**85**. Reagent and conditions: (a) $POCl_3$, *N, N, N′, N′*-tetramethylsuccinamide, then 55-60℃, 8 h or r.t., 20 h; (b) NH_4OAc, $(CH_3CO)_2O$, CH_3COOH, reflux, 4 h.

combined with these relevant data, the results indicate that the target compounds **81** and **82** were likely to become a new anti-tumor drug, which needs further studies, including *in vivo* anti-tumor activity evaluation and pharmacokinetic properties.

Cancer is one of the most important health problems in the world, and the number of deaths from it remains high, especially in developing countries [73]. In order to better treat cancer, it is very urgent to find new drugs with small toxicity and side effects and good anti-tumor activity, as well as ways to treatment cancer [74–76]. In the search for new anticancer drugs, people have set their sights on natural products. In recent years, many new compounds have been found in the secondary metabolites of marine organisms [77]. These marine organisms have been shown to be an important source of new compounds. Many new compounds obtained from marine organisms have been found to have a good inhibitory effect on tumor cell lines during the activity screening process. The secondary metabolites of sponges are abundant in marine organisms, and the discovery of these metabolites has been widely reported by researchers [78–80]. Some compounds were found to have anti-tumor activity during *in vitro* activity screening, and on this basis, analogues of these metabolites were synthesized [81–83]. Many compounds were also found to have anti-tumor activity in the activity evaluation. Rich structural diversity and bioactive peptides were separation from marine sponge, and these compounds include the cyclopeptide perthamides G–K, glycopeptide theonellamides A-G and 3-alkylpyridine alkaloids (3-APAs) and Theonelladines A-D [84–86]. Different kinds of metabolites have been separation from marine sponges, and these compounds and synthetic analogues have been widely concerned for their cytotoxicity activity on human tumor cell lines. Theonelladine C is an analogue of 3-alkylpyridine alkaloid, which was found to have anti-protozoa activity and pro-apoptotic effect on human colon cancer (RKO-AS-45-1) cell line in the activity screening. Therefore, on this basis, Alessandra Mirtes Marques et al [87] was further modified the structure of Theonelladine C to obtain target compounds with anti-tumor activity. During structural design, they introduced oxygen-containing carbon chains into the lead compound 3-alkyl chain and changed the length of the chain to alter its anti-tumor activity [88,89]. They designed a marine alkaloid analogues of 3-alkylpyridine with alkyl chains ranging in length from 6 to 12 carbon atoms. At the same time, a hydroxyl group was attached to the end of the alkyl chain to improve the water solubility of the analogues and to provide the necessary preparation for further structural modification in the future. In the synthesis route selection of the new 3-APA analogues compounds **102–105**, they used Scheme 3 description for the synthesis operation. From the synthesis route, it could be seen that the key step in the synthesis of these analogues was the Williamson etherification reaction under mild conditions using phase transfer catalysis (PTC). The first step of this synthesis route was to selectively protect different diols (compounds **86–89**) to produce corresponding tetrahydropyronic acetal (compounds **90–93**) in a yield of 74%-89%. The compounds **90–93** were further treated in dichloromethane with methyl sulfonyl chloride to obtain intermediates compounds **94–97**. Next, the compounds **94–97** were etherized with 3-(pyrid-3-yl) propan-1-ol by a phase transfer catalyst tetrabutyl ammonium bromide, and the compounds **98-101**with protective group were obtained. Finally, the protective group of the compounds **98–101** were removed with hydrochloric acid, and the target compounds **102–105** were obtained with the yield of 71–100%. The synthesis route has the advantages of simple operation, low cost and easy availability of reagents. At the same time, the yield of each step of the synthesis route was relatively high, and the reaction conditions were relatively mild, which was conducive to industrial production in the future. In the course of the anti-tumor biological activity study, they evaluated the anti-tumor activity of all the synthesized target compounds **102–105** and intermediates **98–101** *in vitro* for colon cancer RKO-AS-45–1 and uterine cancer HeLa (Table 3). Meanwhile, the target compounds **102–105** and intermediates **98–101** were tested in non-cancerous human lung fibroblasts WI-26VA4 to evaluate the selectivity index (SI) of the compounds. *In vitro* the MTT assay showed that the compounds **100** and **101**, **104** had good anti-tumor activity. In RKO-AS-45–1 cells, the IC_{50} values were 5.1, 3.2 and 19.1 μM, respectively. In anti HeLa cells, the IC_{50} values ranged from 4.0 to 9.4 μM. The drug structure activity relationship (SAR) analysis showed that the relationship between the IC_{50} value and the length of the alkyl chain of the compounds could be established. With the increasing length of the carbon chain of the alkyl chain, the IC_{50} value of the two tumor cell lines decreased continuously, among which the compounds with 10 carbon atoms had the best anti-tumor activity (compounds **100** and **104**). However, as the carbon chain continued to lengthen, its anti-tumor activity decreased when the carbon chain exceeded 10 carbon atoms. In addition, the selectivity of compound **104** in the *in vitro* activity evaluation was found to be the highest among the compounds tested, with the SI of 5.18 for RKO-AS-45–1 and 11.65 for HeLa cells. On this basis, the compounds **100** and

Table 3
In vitro cytotoxic activity.

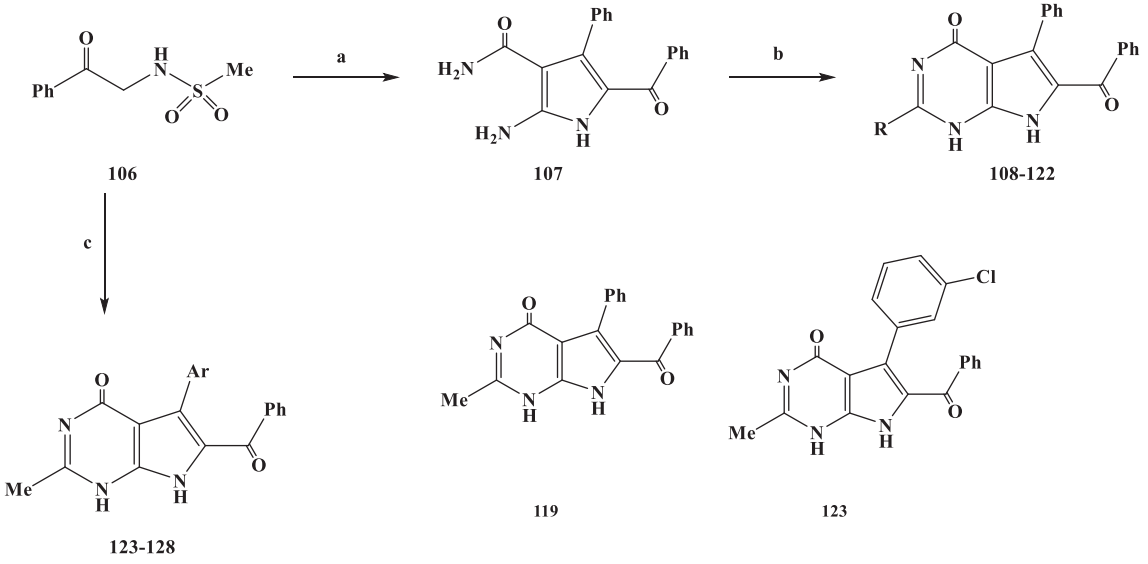
Compounds	IC_{50} (μM) ± SD			SI	
	PKO AS-45–1	HeLa	WL-26VA4	PKO AS-45–1	HeLa
98	>300	>300	>300	nd	nd
99	23.7 ± 1.7	8.1 ± 2.7	37.8 ± 5.0	1.59	4.66
100	5.1 ± 1.1	4.0 ± 0.8	6.4 ± 0.7	1.25	1.60
101	3.2 ± 1.7	9.4 ± 0.7	11.3 ± 1.4	3.53	1.20
102	>400	>400	>400	nd	nd
103	>300	191.8 ± 10.1	167.0 ± 10.5	nd	0.87
104	19.1 ± 4.4	8.5 ± 2.4	99.1 ± 11.2	5.18	11.65
105	131.9 ± 16.8	8.8 ± 1.9	34.1 ± 6.5	0.25	3.87
Etoposide	1.4 ± 0.6	2.7 ± 0.4	nd	-	-



Scheme 3. Synthesis of 3-alkylpyridine marine alkaloid analogues compounds **102–105**. Reagent and conditions: (a) $NaHSO_4$, DHP, DMSO, hexane, 40 °C, 16 h; (b) MsCl, Et_3N, CH_2Cl_2, r.t., 10 h; (c) 3-(pyrid-3-yl) propan-1-ol, NaOH/H_2O, $Bu_4N^+Br^-$, Et_2O, r.t., 72 h; (d) MeOH, HCl, r.t., 12 h.

104 were selected for further related activities. Micronucleus and TUNEL tests showed that the compounds **100** and **104** had mutational effects and induced apoptosis. At the same time, the compound **104** also altered the myogenic RKO-AS-45–1 cells in the cytoskeleton. In general, according to the analysis of the existing test results, the compounds **100** and **104** could be used as potential anti-tumor drug candidates, or as lead compounds for structural modification, which needs further research and development.

Anti-tumor drugs are a class of drugs used to treat cancer, including chemotherapy drugs and biological agents [90]. In recent years, the development of molecular oncology and molecular pharmacology has gradually clarified the nature of tumors [91–93]. The invention and application of advanced technologies such as large-scale rapid screening, combinatorial chemistry and genetic engineering have accelerated the process of drug development, and the research and development of anti-tumor drugs have entered a new era [94]. After years of development, many important advances have been made in the development of anti-tumor drugs. However, in the face of the most serious threat to human life and health, which accounts for>90% of malignant tumors, there is still a lack of effective and highly specific drugs [95]. On the one hand reflects the difficulty in the development of anti-tumor drugs, on the other hand, it also means that the development of anti-tumor drugs still needs the application of new ideas, new technologies and new methods. At present, drug treatment has become one of the important methods for the clinical treatment of cancer, and the sales of anti-tumor drugs have been increasing year by year due to the high incidence and mortality of cancer [96]. The marine alkaloids with pyrrole structure obtained from marine organisms have abundant biological activities found in the activity screening process. Among these marine alkaloids, Rigidins A, B, C, and D are one kind of them. Related studies have shown that these compounds have some potential for biological activity, but few researchers have conducted in-depth studies to explore more biological activity. Liliya V Frolova et al [97] have completed the total synthesis of Rigidins A, B, C and D and carried out a comprehensive study on their biological activities. It was found that L1210 cells of mouse leukemia had good anti-proliferation activity, but the activity of cultured human cancer cells was very low. On this basis, the structure of 7-deazahypoxanthine, 7-deazaadenine and 7-deazapurine were synthesized by modifying 7-azathathine skeleton of Rigidins. In the study of the activity of its compounds, the anti-proliferation activity of tumor cells has been greatly improved. These compounds have also been found to damage the microtubule tissue in cancer cells by binding to the colchicine site of β-tubulin. On the basis of previous work, Robert Scott et al [98] was further modified the structure of the lead compound and introduced different substituents on the *C*2 position to change the biological activity of the target compounds. In structural design, two series of the target compounds were designed. During the synthesis of these target compounds **108–128**, they selected Scheme 4 detailed description for the synthesis operation. In the first series, the target compounds were obtained by two-steps reaction. The *N*-methylsulfonamidoacetophenone (compound **106**) was used as the starting material of the reaction. The reaction was stirred with benzaldehyde and cyanoacetamide in ethanol, the potassium carbonate as acid dressing agent, and the intermediate compound **107** was obtained. In the second step, the ring formation reaction was carried out. The sodium ethanol was prepared by reacting Na with ethanol, and the compound **107** was reacted with the corresponding ester to obtain the target compounds **108–122**. In the second series, the target compounds **123–128** were synthesized in one step. Also, the compound **106** was used as the starting material of the reaction, and the corresponding aldehydes, cyanoacetamide and $MeC(OEt)_3$ were synthesized in ethanol by pot method. In this step, potassium carbonate was used as an acid dressing agent. The reaction temperature of 90℃ was used for 24 h, followed by the reaction temperature of 150℃ for 3–6 h, and the yield of 40–65% could be obtained. The synthesis route has the advantages of simple operation, low cost and easy availability of reagents, and the desired target compounds could be obtained by one-step or two-steps reaction. The one-pot method was applied to the synthesis of target compounds or intermediates, which greatly improves the reaction efficiency and reduces the synthesis cost. However, this synthesis route also has some shortcomings. The yield of the reaction was generally low, especially the yield of the target compound **111** was only 18%, which brings great difficulties for future industrialization. Therefore, it was necessary to further optimize the reaction conditions in the subsequent process optimization to increase the yield of the reaction. During the activity study, HeLa cell line was used as the model of human cervical adenocarcinoma, and MCF-7 cells were used as the model of breast adenocarcinoma to evaluate the anti-tumor proliferation activity of the first series of target compounds *in vitro*. *In vitro* screening showed that except for the target compound **108**, most of the other target compounds introduced at *C*2 had anti-tumor cell proliferation activity. Activity of this series of compounds for drug structure–activity relationship (SAR) analysis found that *C*2-aryl (compounds **111** and **112**), *C*2-OEt (compound **114**) and branched *C*2-alkyl (compound **115**) activity was only two digits to a single-digit micromolar, linear *C*2-alkyl



Scheme 4. Synthesis of marine alkaloid Rigidins analogues compounds **108–128**. Reagent and conditions: (a) benzaldehyde, cyanoacetamide, K_2CO_3, EtOH, reflux, 14 h; (b) corresponding ester, Na, EtOH, reflux, 10 h; (c) corresponding aldehydes, cyanoacetamide, $MeC(OEt)_3$, K_2CO_3, EtOH, 90 °C, 24 h, then 150℃, 3–6 h.

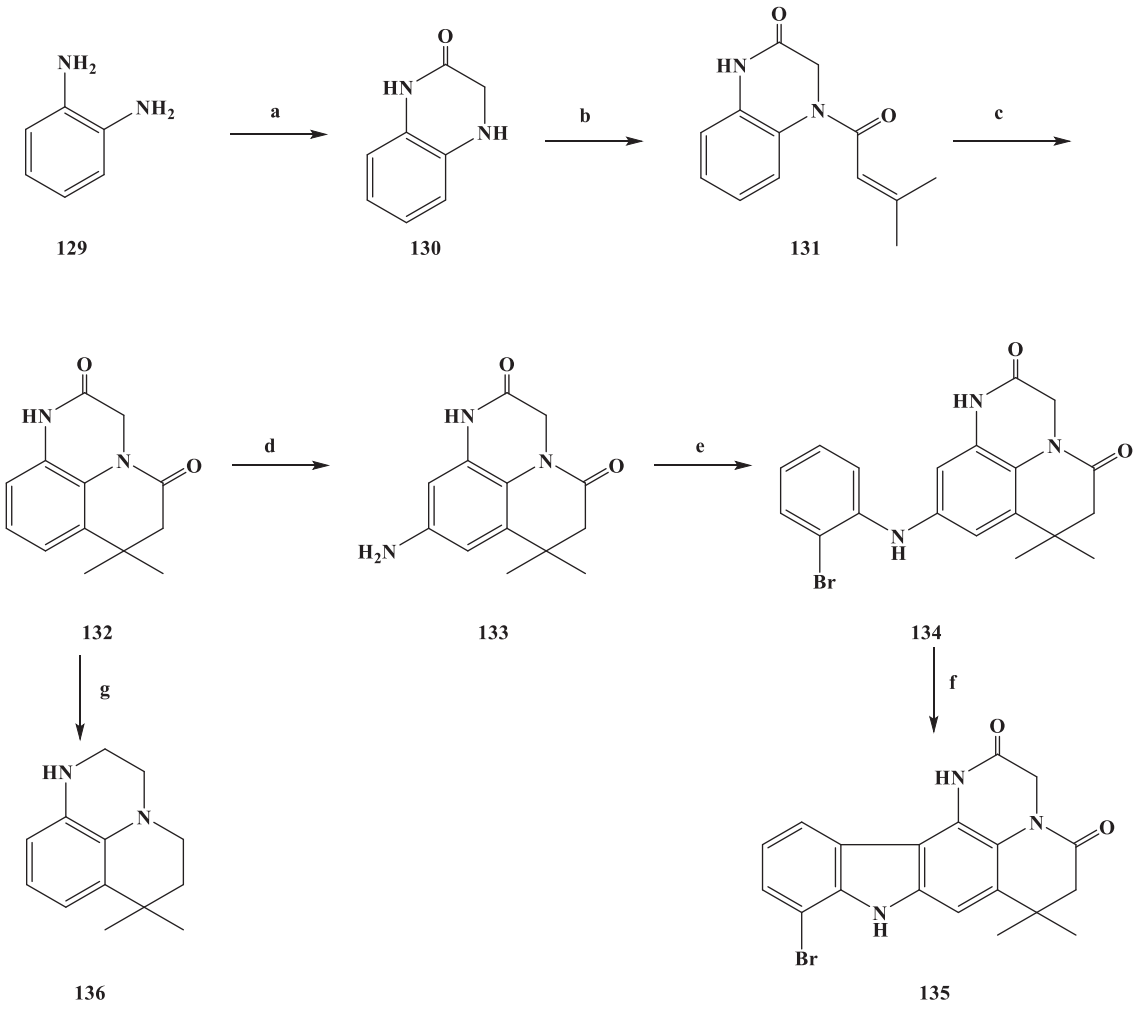
derivatives compounds**116-118** in sub-micromolar concentrations showed inhibitory effect, and *C*2-methyl compound (compound **119**) under the nanomolar could also show activity of inhibiting tumor cell proliferation. When fluorine-containing groups were introduced on *C*2-Me, the activity gradually decreased with the increase of the space of *C*2 position, that were CH_3 (compound **119**) > CHC_2F (compound **120**) > CHF_2 (compound **121**) > CF_3 (compound **123**). It could be seen from the activity results of these target compounds that the anti-proliferative activity of this series of compounds was determined by the spatial properties of the *C*2 substituents rather than by the electronic properties. *In vitro* screening of the second series of compounds, the anti-proliferative activity of the target compounds against U87 and A549 were added. The results showed that the anti-proliferation activity of this series of compounds was lower than that of the target compound **119** (Table 4), and most of the target compounds exhibited inhibitory effect at the sub-micromolarity concentration, except for the target compound **126**. The second series of target compounds introduce different substituents on the C7 site on the basis of target compound **119**. The result of such activity was that the polarity of the target compound was reduced, which damages the permeability of the cells and thus reduces the anti-tumor proliferation activity of the target compounds. On the basic basis, the target compound **119** with the best activity was selected to study the effect on microtubules in cells. The results showed that the target compound **119** could achieve the anti-proliferation activity of tumor cells by acting on microtubule-targeted proteins. According to the existing data, *C*2-aryl and *C*2-alkyl-denitroxanthines marine alkaloid analogues have good anti-tumor activities. In particular, the target compound **119** could be used as a candidate for anti-tumor drugs and has the potential to develop into a new anti-tumor drug, which needs further research and development in the future.

There are many ways to discovery drugs, and natural products are an important way to get new drugs or lead compounds [99]. Sources of natural products include compounds with certain biological activity obtained from plant, animal, and microbial metabolites by modern separation techniques. The isolation of biological activity drugs from naturally occurring secondary metabolites has become an important field in the development of new drugs [100–102]. These natural products have unique chemical structure, play an important role in drug discovery and innovation, and have irreplaceable significance in the treatment and prevention of human diseases. In the natural products separation and extracted by people, the alkaline compounds and alkaloids are widely concerned by researchers. Marine organism has species diversity and biological activity diversity [103]. So far, researchers have found>300,000 species in the ocean, and it was estimated that>1 million new species of ocean have not been found. With this quantitative advantage, obtaining new biological activity compounds from marine organisms has become an important pathway. At present, the research direction of people has begun to turn to marine organisms, many biological activity marine alkaloids have been separation from marine organisms [104]. So far, the anti-tumor alkaloids separation from marine sponges include Ellipticine, Neoamphimedine and Makaluvamine A. Between 2001 and 2010, most of the compounds separation from marine sponges were alkaloids (marine alkaloids) and macrocyclic compounds. During this period, a marine alkaloids with pyrrolidone structure was separation from the marine sponge [105,106]. This substance was found to have significant cytotoxicity effects on a variety of tumor cells *in vitro* in the course of biological activity studies, as well as potential biological activity against topoisomerase II .Based on the previous work, Sebastien Boucle et al [107] designed compounds with praziquantel structure for anti-tumor activity. As could be see from the chemical structure, the new compounds were Makaluvamine A analogues compounds **135** and **136**. Meanwhile, the target compounds of pentacyclic structure were investigated as potential anti-topoisomerase II inhibitors. In the synthesis process of the target compounds, they chose the specific operation described in Scheme 5 to synthesize the target compounds. To obtain the target compounds **135** and **136**, they used 1, 2-phenylenediamine (compound **129**) as the starting material to reaction with bromoacetylbromide in tetrahydrofuran (THF) to produce dihydroquinoxalinone compound **130**. The condensation reaction of compound **130** and dimethylacryloylchloridel in pyridine produces the compound **131**. The compound **131** was cyclized in methylene dichloride using anhydrous aluminum chloride as catalyst, and the tricyclic intermediate compound **132** (yield 83%) was obtained. The intermediate compound **133** containing amino group was obtained by two-steps reaction of nitrification and catalytic hydrogenation of compound **132**. Nitrification was the compound **132** with nitric acid in methylene dichloride. Then the product was reduced by catalytic hydrogenation without separation, and the compound **133** was obtained. The compound **133** reacts with 2-bromoiodobenzene in anhydrous dioxane to obtain the compound **134**, with a yield of 71%. Finally, the compound **134** underwent cyclinization, and through the Heck reaction generated pentacyclic compound **135**. In the reaction process, anhydrous dioxane was used as the solvent, palladium acetate as the catalyst and potassium carbonate as the acid dressing agent, and the yield was up to 40%. At the same time, the compound **132** reduced with boron/tetrahydrofuran, and the target compound **136** was obtained with the yield of 81%. The synthesis route was characterized by simple operation, inexpensive and readily available raw materials, and moderate to good yield per step. However, this synthesis route also has some shortcomings, and used the precious metal Pd as the reaction catalyst, which brings certain difficulties for the future industrialization. At the same time, in the process of the compound **133** synthesis, the yield of this step was only 29%, the low yield will increase the synthesis cost, which needs to be improved in the subsequent process optimization. In the process of studying the biological activity of the synthesized compounds, they evaluated the anti-tumor activity of these compounds and the inhibition efficiency of topoisomerase II. First, they tested the compounds against different cancer cell lines to assess its anti-tumor effects (Table 5). They then investigated the inhibitory efficiency of the synthesized compounds against topoisomerase II in order to determine the mechanism of action of these newly synthesized marine alkaloid analogues. They selected different human cancer cell lines CACO-2, HCT-116, HUH-7, MDA-MB-231, PC-3, and NCI for *in vitro* cytotoxicity activity evaluation. *In vitro* anti-tumor activity evaluation test results showed that the compounds **134–136** had good anti-tumor activity, among which the compound **136** had cytotoxicity to CACO-2, HCT-116, PC-3 and NCI cell lines of 15, 15, 15 and 10 μM, respectively, but had no obvious toxicity to human fibroblasts. However, the compounds **134** and **135** also have certain inhibitory effects on human fibroblasts cell, so there was no obvious selectivity for cell inhibition. In the process of screening the anti-topoisomerase II activity of the synthesized compounds, the concentration of the compounds used for electrophoretic analysis was 100 μM. The results of anti-topoisomerase II activity test of the compounds showed that the compound **135** inhibited the activity of human DNA topoisomerase II better than the positive control doxorubicin at 100 μM. Other synthetic compounds showed no inhibition of topoisomerase II. By *in vitro* anti-tumor activity of synthetic compounds screening and test of topoisomerase II activity, could be on the basis of compounds **135** or **136** further design create more analogue, the structure activity relationship (SAR) and drug research, in order to choose the has stronger anti-tumor activity and higher selectivity of target compounds.

The natural products are rich in biological activity compounds which have the potential to be developed into drugs [108–111]. With the research

Table 4
Anti-proliferative activities of synthesized compounds.

Compounds	IC_{50} (μM)			
	HeLa	MCF-7	U-87	A549
119	0.029 ± 0.001	0.035 ± 0.003	0.077 ± 0.002	0.25 ± 0.01
123	0.27 ± 0.01	0.23 ± 0.00	0.90 ± 0.16	0.60 ± 0.23

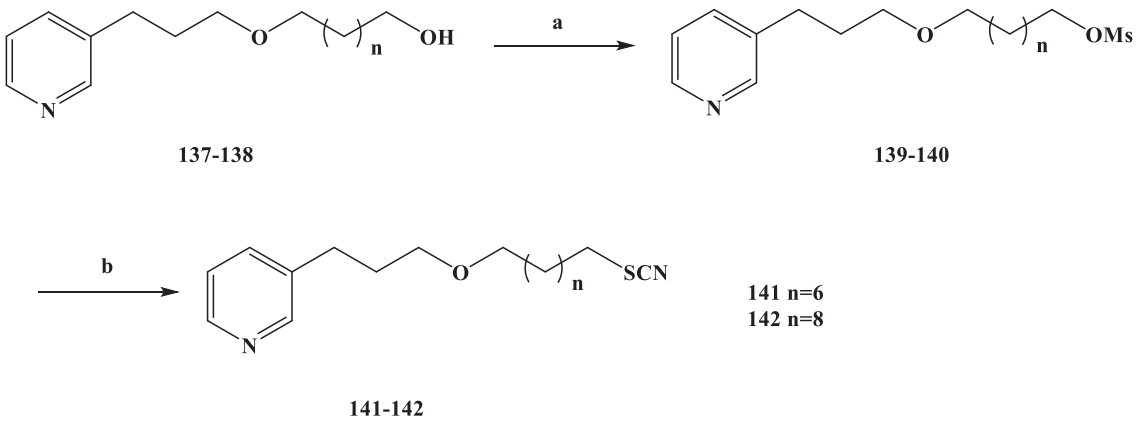


Scheme 5. Synthesis of compounds **135**–**136**. Reagent and conditions: (a) bromoacetylbromide, THF, Et_3N, reflux, 2 days; (b) dimethylacryloylchloride, pyridine, reflux, 2 h; (c) $AlCl_3$, DCM, 24 h; (d) i HNO_3, DCM/DMF, reflux, 24 h; ii H_2, Pd/C, DMF/DMSO, reflux, 18 h; (e) 2-bromoiodobenzene, Pd_2dba_3, Xantphos, K_2CO_3, dioxane, schlenk, 18 h; (f) $Pd(OAc)_2$, Pcy_3HBF_4, K_2CO_3, DMA, reflux, 24 h; (g) BH_3/THF, THF, reflux, 2 h.

Table 5
Cytotoxicity of the synthesized compounds.

Compounds	IC_{50} (μM)						
	HUH-7	CaCo-2	MDA-MB-231	HCT-116	*Pc*-3	NCI	Fib. Hum
134	20	20	>25	25	25	>2	25
135	20	20	20	10	6	5	6
136	20	15	20	15	15	1010	>25

and development of modern drugs, obtaining new drugs or lead compounds from marine organisms has become an important source. Marine sponges have been well studied and many biological activity compounds have been separation from these relatively simple organisms in recent years, including some new structural compounds [112–116]. In the process of screening these compounds for biological activity *in vitro*, the results show that many of them have some specific biological activity and play an important role in the development of new drugs [117] Lithistida is one of the sponge, people separation get rich diversity, structure diversity of biological activity of 3-alkylpyridine alkaloids (3-APAs), such as Viscosaline and Theonelladin A-D [118–121]. From the analysis of the structure of these obtained marine alkaloids, it is found that 3-APAs has a pyridine structure and an alkyl chain with variable length on the side chain, and the position of this alkyl chain is usually in the 1 or 3 position. The discovery that most marine alkaloids have cytotoxicity (anti-tumor activity) has been widely studied and concerned [122–126]. Previous studies have shown that the synthesized 3-APA analogues exhibited two aspects of anti-malarial or anti-tumor biological activity in the evaluation process of biological activity [127]. And in these two aspects of activity showed a certain inhibition potential, and even some of the compounds may become ideal new drugs [128–132]. Tumor is one of the major health problems in the world, which has been concerned by people all over the world [133–135]. In the previous work, 3-APA analogues were found to promote DNA damage, induce apoptosis, and alter two human tumor cell lines: RKO-AS-45–1 and HeLa actin cytoskeleton during active screening [135]. The naturally occurring molecules containing thiocyanate structures are rare, and these functional groups are mainly present in the formation of cruciferae glucosides during the desaccharification of some natural products with anti-tumor activity. It has also been shown that compounds containing thiocyanate groups also have anti-parasite effects, showing high cytotoxicity in anti-parasite studies. In view of these data, Maria Cristina S Barbosa et al [137] designed two new 3-APA analogues to study their anti-tumor activity by using Theoneladin C as the lead compound and by introducing thiocyanate groups into its chemical structure. During the synthesis of the target compounds **141** and **142**, they chose the route described in Scheme 6 to conduct the synthesis operation. They selected the previously reported synthetic product compounds 1a-b as the starting material and synthesized the designed target compounds **141** and **142** in two steps. In the first step, the hydroxyl compounds **137** and **138** were reacted with methyl sulfonyl



Scheme 6. Synthesis of compounds **141** and **142**. Reagent and conditions: (a) MsCl, Et_3N, CH_2Cl_2, r.t., 10 h; (b) TBAB, KSCN, THF, reflux, 3 h.

chloride in methylene dichloromethane to obtain the intermediate compounds **139** and **140**, which could reach the yield of 52%-83% after a simple treatment. The second step was accomplished by the occurrence of S_N2 nucleophilic substitution, in which the intermediate compounds **139** and **140** were converted to the corresponding target compounds **141** and **142** containing thiocyanate groups. In this step, the compounds **139** and **140** was substituted with potassium thiocyanate in tetrahydrofuran (THF) and tetrabutyl ammonium bromide was used as catalyst. The desired target compounds **141** and **142** were obtained by nucleophilic substitution, and the yield was 55–78%. The synthesis route has the advantages of short reaction steps, high yield of each step, easy to control the total yield of the reaction. At the same time, the reagent used were cheap and easy to get, the reaction conditions was mild, which lays a solid experimental foundation for future industrialization. In the activity screening process of the target compounds **141** and **142**, they first evaluated the anti-malarial activity of the target compounds (Table 6). *In vitro* test results showed that target compounds **141** and **142** inhibited the growth of parasites with IC_{50} of 5.5 and 2.3 μM, respectively. These results indicate that these compounds have anti-malarial activity, but the selectivity was not high, which reflects that these compounds have high cytotoxicity to the control cell line and not conducive to proprietary medicine. Because these compounds have good cytotoxicity, they have certain advantages in anti-tumor. Next, they focused on the anti-tumor activity of target compounds **141** and **142**. *In vitro* activity evaluation, they evaluated the cytotoxicity of the compounds to human cancer lines colon cancer (RKO-AS-45–1) and cervical cancer (HeLa) to evaluate the anti-tumor activity of target compounds **141** and **142**. Meanwhile, non-tumor human cell line (lung fibroblast WI-26VA4) was used as a reference to determine the selectivity index of the target compounds. Because the selectivity index was one of the important indexes to evaluate the activity of taeget compounds. The anti-tumor activity of target compounds **141** and **142** were investigated *in vitro*. The IC_{50} values of target compound **141** on RKO-AS-45–1 cells and HeLa cells were 0.8 and 12.4 μM, respectively, and the IC_{50} values of target compound **142** on RKO-AS-45–1 cells and HeLa cells were 6.36 and 3.8 μM, respectively. Anti-tumor drugs need to be able to choose high and effective (active) compounds, in the process of killing tumor cells, should be almost impossible to kill normal cells. In determining the selectivity index of target compounds **141** and **142**, it was found that the target compound **141** was selective for tumor cell line RKO-AS-45–1 (SI = 3.59). In order to further investigate the cytotoxicity mechanism of target compounds **141** and **142**, they conducted a variety of gene toxicity tests *in vitro*, including micronucleus assay, comet assay, Ames assay and annexin-V/propidium iodide staining. The results of relevant experiments showed that the synthetic alkaloids could induce the wrong separation of chromosomes and damage DNA during the cell division of human tumor cells, resulting in cell death. These results preliminarily indicate that the target compound **141** may be a promising candidate for anti-tumor chemotherapy drugs, which needs to be further studied and developed in the future, so as to be an anti-tumor drug for clinical use as soon as possible to treat more cancer patients.

Table 6
In vitro inhibitory concentrations (IC_{50}) and selectivity index (SI) obtained for the human cancer cell lines RKO-AS45-1 and HeLa cells after exposure to different concentrations of the target compounds **141** and **142**.

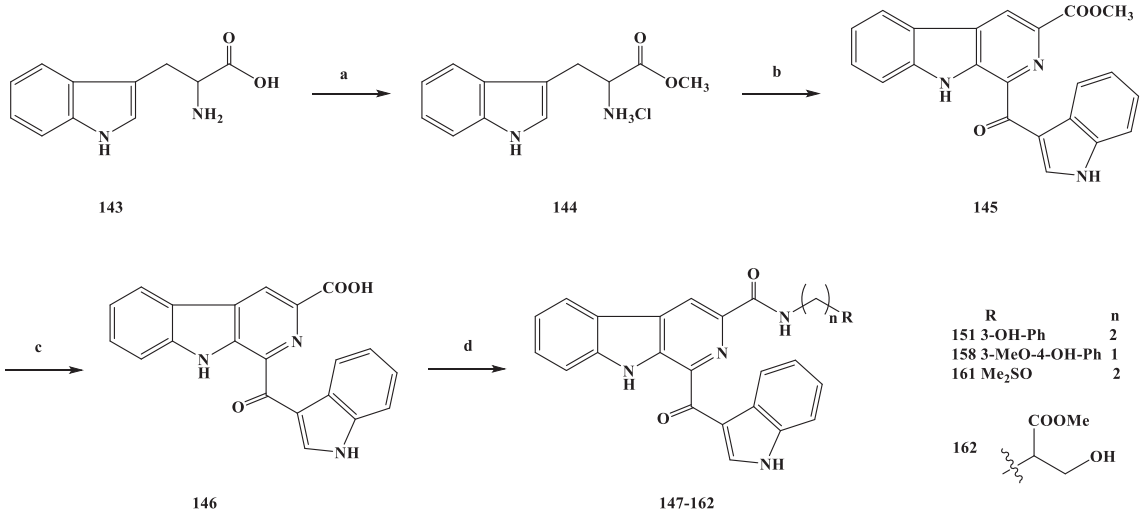
Compounds	IC_{50} (μM) ± SD			SI	
	PKO-AS45-1	HeLa	WI-26VA4	PKO-AS45-1	HeLa
141	0.80 ± 0.11	12.40 ± 2.25	2.87 ± 0.85	3.59	0.23
142	6.36 ± 1.30	3.80 ± 0.58	4.83 ± 1.14	0.76	1.27

Cancer is one of the major diseases that seriously endanger human health in today's society [138–142]. In the treatment of cancer, there are mainly surgical treatment, chemotherapy, radiotherapy and other means, among which chemotherapy is the most important treatment method currently used [143–146]. Research and development of novel compounds with unique chemical structure has become an important research direction as anti-cancer drugs. The discovery of new drugs or lead compounds from natural products provides an important source [147]. On the basis of natural products, many derivatives or analogues with biological diversity have been synthesized, especially in the aspect of anti-tumor activity. Alkaloids are a kind of natural products widely distributed in plants [148–152]. They have a variety of chemical structures and play an important role in medicine. Among these natural alkaloids, the *β*-carboline alkaloids are a kind of widely distributed natural products with biological activities, which belong to the indoles alkaloids and have a unique structure of tricyclic pyrido [3, 4-*b*] indoles ring [153–157]. The Pityriacitrin is a marine alkaloid separation from marine organisms. From the chemical structure analysis, it can be found that Pityriacitrin tricyclic pyrido [3, 4-*b*] indoles ring structure is connected to the indoles ring structure at the C-1 position [158–161]. The Pityriacitrin has been widely studied because of its unique chemical structure. In recent years, some derivatives and analogues of Pityriacitrin have been separation or synthesized by researchers and their biological activities have been studied [162]. It has been found that these compounds have a wide range of biological activities, especially good anti-tumor activity and good cytotoxicity. Natural products are always present in small amounts in nature, and in order to obtain large amounts of compounds it is impossible to extract them by the method of extraction [163–165]. The application of synthetic methods will change this situation, and a large number of desired target compounds can be obtained through synthesis, which can be applied to relevant research or used directly as drugs. Tingting Xu et al [168] used the Pityriacitrin as the lead compound to modify its structure to obtain a large number of cytotoxic compounds.

From the structure of the designed target compounds, it can be seen that the target compounds retains the chemical structure of Pityriacitrin, and different amide groups were introduced in the C-6 position of the tricyclic pyrido [3, 4-*b*] indoles ring, and the design results in the marine alkaloid *β*-carboline analogues. In this work, they combined the pharmacophones of *β*-carboline with the biologically functional amide groups, thus synthesizing target compounds **147**–**162** of the structure of *β*-carboline analogues. During the synthesis route, they selected Scheme 7 detailed description for the synthesis operation. The synthesis of the target compounds **147**–**162** were prepared by four-steps reaction with tryptophan (compound **143**) as the starting material. In the first step, the compound **143** was added to anhydrous methanol and then reacted with sulfoxide chloride to obtain the crude product methyl tryptophan hydrochloride (compound **144**) without separation. In the second step, iodine and 1-(1*H*-indol-3-yl) ethanone were added to dimethylsulfoxide (DMSO) for the reaction, then compound **144** was added to continue the reaction, and 1-(1*H*-indole-3-carbonyl)-9*H*-pyrido [3, 4-*b*] indole-3-carboxylate (compound **145**) was generated by simple condensation ring reaction. In the direct nucleophilic substitution reaction of the compound **145**, it was found that the reaction could not be carried out, so the reaction pathway was changed. The third step was to put compound **145** in the solution of sodium hydroxide. The hydrolysis reaction easily occurs, and the intermediate **146** could be obtained. In the last step, the compound **146** was reacted with different substituted amines. The dimethylformamide (DMF) was used as the reaction solvent and HOBt/EDCI/Et_3N as the reaction catalyst to obtain the target compounds **147**–**162**. The synthesis uses inexpensive and readily available reagents, and the yield of the target compounds were moderate to good through a simple synthesis operation. This synthetic route has laid a certain experimental foundation for future industrialization. However, there was some problems in the green environmental protection of this synthesis route. Reagents such as chlorinated sulfoxide were used in the experiment, and some polluting gases were generated in the reaction process, which cannot meet the requirements of green chemistry, and the subsequent process conditions need to be optimized. In the course of the biological activity study, they tested the cytotoxic effect of the target compounds marine alkaloid *β*-carboline analogues compounds **147**–**162** *in vitro*. The SGC-790, A875, HepG2 and MARC145 cell lines were used as test subjects. *In vitro* cytotoxicity tests result showed that the target compounds exhibited moderate to good inhibitory activity against all four cell lines. The activity of target compounds (**159** and **160**) with direct aromatic groups on amide was lower than that of benzylamine substituted compounds (**153**–**158**) and phenylethylamine substituted compounds (**147**–**152**). The compounds containing 3-hydroxyl group (compound **151**) was significantly more biological activity than containing 3-methoxy substituents compound (compound **148**). In order to further study the biological activity of these target compounds and to screen out the more active compounds, the IC_{50} value of the target compounds was also tested *in vitro*. The results showed that the target compounds **151,158, 161** and **162** showed good inhibitory activity (Table 7). The IC_{50} values of the target compound **161** were 6.82 ± 0.98, 8.43 ± 1.93, 7.69 ± 2.17 and 7.19 ± 1.43 μM, respectively. The results of drug structure–activity relationship (SAR) showed that benzylamine substituted compounds usually had better activity than phenylethylamine substituted compounds, and the activity of these two target compounds were obviously better than that of aromatic amine substituted compounds, which demonstrated the importance of the link chain between aromatic rings and amides. In addition, the biological activity of most of the target compounds was better than that of the *β*-carboline and *β*-carboline B, which indicated that the presence of amide groups in the compounds was beneficial to the anti-tumor activity of the target compounds. In addition, hydroxyl phenyl substituted compounds (**158** and **151**) have significantly higher activity than other corresponding compounds, possibly because the polarity of the hydroxyl phenyl was favorable for binding to the target protein. The target compound **161** was a compound containing a special sulfonyl group and has the highest inhibitory activity. The special properties of hydroxyphenyl and sulfur groups will help to improve the biological activity of the target compounds. On the basis of the dose response analysis of the target compounds **151,158, 161** and **162** the results showed that these target compounds had a concentration-dependent inhibitory effect on cell lines. In general, these target compounds have good cytotoxic activity and can be used as potential cell agents.

Table 7
Cytotoxic activities of the target compounds.

Compounds	IC_{50} (μM)			
	SGC-7901	A875	HepG2	MARC145
9e	17.65 ± 5.84	11.91 ± 0.80	8.63 ± 3.31	13.77 ± 3.75
9 l	16.18 ± 2.31	8.47 ± 2.96	11.08 ± 3.33	21.77 ± 6.94
9o	6.82 ± 0.98	8.43 ± 1.93	7.69 ± 2.17	7.19 ± 1.43
9p	14.30 ± 2.57	10.46 ± 1.34	9.99 ± 1.82	16.27 ± 0.07
5-FU	53.58 ± 1.99	62.12 ± 17.83	66.42 ± 12.99	115.54 ± 8.30



Scheme 7. Synthesis of marine alkaloid oriented *β*-carboline analogues compounds **147**–**162**. Reagent and conditions: (a) $SOCl_2$, MeOH; (b) 1-(1*H*-indol-3-yl) ethanone, I_2, DMSO; (c) NaOH, MeOH, r.t.; (d) $NH_2(CH_2)nR$, HOBt, EDCI, Et_3N, DMF, r.t.

Conclusion and prospect

We discussed the optimization process, chemical synthesis, anti-tumor activity evaluation and structure–activity relationship (SAR) of each type of compounds. On this basis, we evaluated the synthesis route of each type of compounds, so as to provide necessary reference for future to process optimization. The abundant SAR may be provides a reasonable way for the design and develop of novel marine alkaloid derivatives or analogues. It was an important way to obtain the lead compounds from marine alkaloids, and it is very prospect to obtain a large number of derivatives and analogues by structural modification. These derivatives and analogues have anti-tumor activity diversity, which combined with the SAR, the provide a good research platform for the discovery of new drugs in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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