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Discovery of indoline derivatives as anticancer agents via inhibition of tubulin polymerization

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Abstract

Human esophageal squamous cell carcinoma (ESCC) is one of the most lethal cancers in human digestive system. It is necessary to discover novel antitumor agents for the treatment of esophageal cancers because of its poor prognosis. Indoline has been reported as an efficient anticancer fragment to design novel anticancer agents. In this work, indoline derivatives were designed, synthesized and explored their anticancer activity. Compound **9d**, which exhibited potent antiproliferative activity with IC_{50} values of 1.84 μM (MGC-803 cells), 6.82 μM (A549 cells), 1.61 μM (Kyse30 cells), 1.49 μM (Kyse450 cells), 2.08 μM (Kyse510 cells) and 2.24 μM (EC-109 cells), respectively. The most active compound **9d** was identified as a tubulin inhibitor targeting colchicine binding site with an IC_{50} value of 3.4 μM. Compound **9d** could

strongly suppress the tubulin polymerization in Kyse450 cells. The results of molecular docking also suggested compound **9d** could tightly bind into the colchicine binding site of β-tubulin. Besides, compound **9d** inhibited the growth of KYSE450 cells in time and dose-dependent manners. All the results suggest that the indoline derivatives might be a class of novel tubulin inhibitors with potential anticancer activity and is worthy of further study.

Keywords: Esophageal squamous cell carcinoma; Indoline; Anticancer activity; Tubulin; Colchicine binding site

Human esophageal squamous cell carcinoma (ESCC) is one of the most fatal malignant tumors in human digestive system. The great endeavors recently on its treatment seem to have little effect and still carries a poor prognosis[1]. There are more than 500,000 newly diagnosed cases in the world each year, and about 400,000 cases die of ESCC [2]. Although, there are some small molecule drugs under clinical trials for the treatment of ESCC [3-7], no significant improvements have been observed in the overall survival rates of ESCC patients [8]. Thus, there is an urgent need to develop effective therapeutic agents targeting key targets or pathways for the treatment of esophageal cancers.

Microtubules play an important role in the process of mitosis, cell division, signal transduction and material transportation, which has been consider as an attractive and successful drug target in cancer therapy [9-11]. There are several tubulin inhibitors for the clinical treatment, including paclitaxel/taxol, vincristine and vinblastine. However, no colchicine site tubulin inhibitors are approved for clinical use, therefore, there is an urgent need to develop new colchicine site tubulin inhibitors with high antitumor activity and low side effects.

As a simple heterocyclic fusion scaffold, indoline has been widely designed as the core scaffold of bioactive compounds with strong pharmacological significance, especially the anticancer ability[12-15]. Indoline derivatives has also been used to design novel colchicine binding site tubulin inhibitors with potent anticancer acctivity [9, 12, 14, 16-18]. 1-Arylsulfonyl indoline **1** is a potent tubulin and HDAC dual inhibitor with

potential anticancer activity[12]. Compound **1** exhibited striking tubulin inhibition activity and HDAC1 inhibitory potency with IC_{50} values of 1.1 and 0.221 μM, respectively. Besides, compound **1** displayed signifitant antiproliferative activity against KB cells (IC_{50} = 79 nM). 7-Aroyl-aminoindoline-1-sulfonamide **2** potently inhibited the tubulin polymerization (IC_{50} = 1.1 μM) by binding to colchicine binding site. Compound **2** effectiveily suppressed the growth of KB, MKN45, H460, HT29, and TSGH cells with IC_{50} values ranging from 8.6 nM to 10.8 nM[16]. Indoline **3** as a microtubule-targeting agent showed significant antiproliferative activity against HCT-116, PC3, HepG2 and SK-OV-3 cells with IC_{50} values ranging from 0.039 μM to 0.112 μM[18]. Therefore, indoline might be a potential sacfflold for the design of tubulin inhibitors with potential acticancer activity. In addition, trimethoxyphenyl-based derivatives exhibited potent anticaner activity against cancer cells and trimethoxyphenyl group is also an important pharmacophoric point for tubulin binding[10, 19-27]. Compound **4** [19] showed potent antiproliferative activity against SGC-7901 cells (IC_{50} = 0.054 μM) and tubulin inhibitory activity (IC_{50} = 9.17 μM). Compound **5** [20] potently inhibited the tubulin polymerization (IC_{50} = 1.9 μM) and the growth of MGC-803 cells (IC_{50} = 0.035 μM). Compound **6** [27] with a trimethoxyphenyl group displayed low nanomolar antiproliferative efficacy on HeLa cells and potent inhibitory activity of tubulin polymerization (IC_{50} = 1.2 μM) (**Fig. 1**).

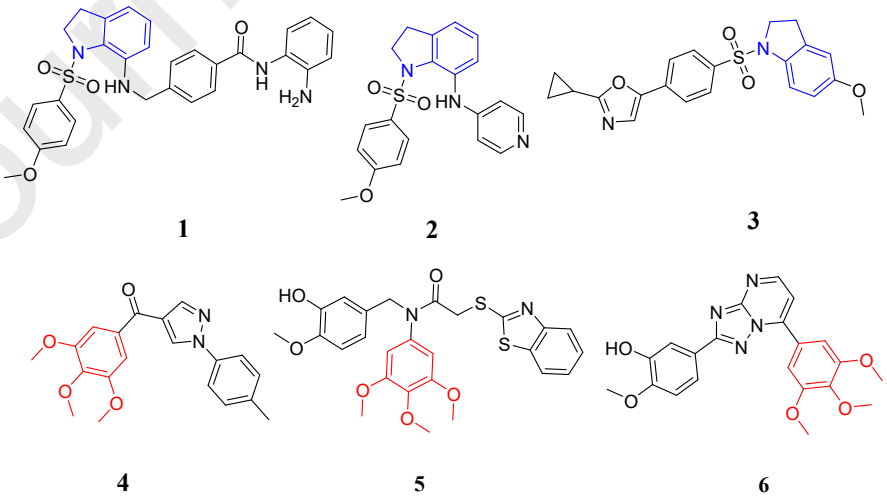


Fig. 1. Structures of indoline-based and trimethoxyphenyl-based derivatives as tubulin inhibitors

Molecular hybridization strategy is a common method to design new compounds by the combination of different biologically active parts to produce novel hybrids with higher affinity and effectivenes. As the continuation of our group work on colchicine binding site tubulin inhibitors and anticancer agents, we designed and synthesized a series of indoline derivatives by the combination of anticancer scaffold indoline and anti-tubulin pharmacodynamic trimethoxyphenyl group into one hybrid (**Fig. 2**). Among of these compounds , (6-bromoindolin-1-yl)(3,4,5-trimethoxyphenyl)methanone (**9d**) was identified as a tubulin inhibitor. Compound **9d** exhibited potent antiproliferative activity against six tested human cancer cells. In the further studies suggested compound **9d** inhibited tubulin polymerization via binding to the colchicine site and inhibited Kyse450 cells in time and dose-dependent manners.

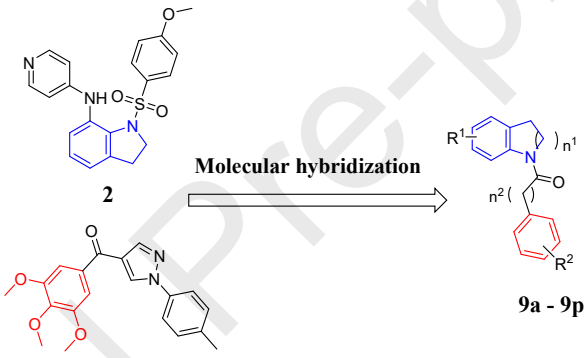
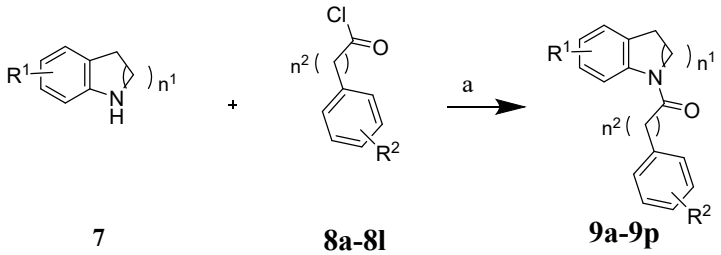


Fig. 2. Design of indoline derivatives in this work.

The synthetic route of indoline derivatives is shown in **Scheme 1**. Commercially available indolines (**7**) as the starting materials reacted with substituted acyl chlorides (**8a-8l**) presence of K_2CO_3 in acetone at 60 °C to give target compounds **9a-9p**. Finally, all the target compounds were fully characterized by NMR.



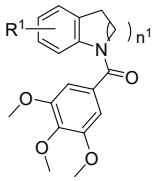
Reagents and conditions: a) K_2CO_3, acetone, 60 °C, 6 h

Scheme 1. Synthetic route of indoline derivatives

The *in vitro* antiproliferative activity of new target compounds **9a-9p** were evaluated against three human cell lines (MGC-803, Kyse30, and A549 cells) using CCK8 assays and the famous tubulin inhibitor **Colchicine** as a positive drug. The following **Table 1**, **Table 2** and **Table 3** depicted the results of *in vitro* antiproliferative activity.

To explore the relationships between chemical groups and antiproliferative activity, compound **9a-9e** were designed and synthesized. Representative chemical features of indoline derivatives **9a-9e** are a 3,4,5-trimethoxypheny moiety linked to a substituted indoline ring with different substituents such as bromine atom, nitro, or H. Most of indoline derivatives exhibited certain antiproliferative potency against three human cancer cell lines. Particularly, compound **9d** exhibited the better growth inhibition of the tested three human cancer cell lines (MGC-803, Kyse30, and A549) with IC_{50} values of 1.84 μM, 6.82 μM and 1.61 μM, respectively.Campared compounds **9a** with **9b** and **9c**, compounds with a indoline ring exhibited better antiproliferative activity than the 1,2,3,4-tetrahydroquinoline ring (**9b)** and 2,3,4,5-tetrahydro-1H-benzo[b]azepine ring (**9c)**. Moreover, the substituent groups of indoline ring was important for antiproliferative activity (5-Br > 5-NO_2 > H).

Table 1. IC_{50} values of compounds **9a-9e** against three human cancer cell lines.



9a-9e

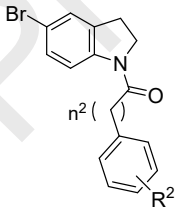
Compounds.	R_1	n_1	IC_{50} (μmol/L) [a]		
			MGC-803	A549	Kyse30
9a	H	1	7.57 ± 0.06	22.0 ± 0.05	7.72 ± 0.19
9b	H	2	4.41 ± 0.08	23.7 ± 0.11	6.72 ± 0.17
9c	H	3	4.3 ± 0.14	21.7 ± 0.07	16.55 ± 0.09
9d	5-Br	1	1.84 ± 0.03	6.82 ± 0.05	1.61 ± 0.06
9e	5-NO_2	1	8.32 ± 0.14	17.7 ± 0.17	6.55 ± 0.09

colchicine	-	-	1.07 ± 0.11	0.19 ± 0.04	0.28 ± 0.04

[a]Antiproliferative activity was assayed by exposure for 48 hours.

In addition, the importance of 3,4,5-trimethoxyphenyl unit and carbon chain length on antiproliferative activity was further explored. As shown in Table 2, the inhibitory potency of compounds **9f-9j** was decreased when the 3,4,5-trimethoxyphenyl unit was replaced by phenyl groups with different substituents such as F, Br, CH_3, OCH_3 or H groups, indicating that the 3,4,5-trimethoxyphenyl unit played an important role in the inhibitory potency for the indoline derivatives. In addition, we also explored the the importance of the carbon chain length on antiproliferative activity. As shown in **Table 2**, when the carbon chain length is 1, compound (**9k- 9o**) including compound **9p** with a 3,4,5-trimethoxyphenyl unit showed weak inhibitory activity against three cancer cells with IC_{50} values > 20 μM. These results indicated that the carbon chain length is also important for the antiproliferative activity.

Table 2. IC_{50} values of compounds **9f-9p** against three human cancer cell lines.



9f - 9p

Compounds.	R_2	n_2	IC_{50} (μmol/L) [a]		
			MGC-803	A549	Kyse30
9f	H	0	28.5 ± 1.17	**>** 40	20.3 ± 1.18
9g	4-F	0	24.1 ± 1.01	18.9 ± 1.23	22.1 ± 1.04
9h	4-Br	0	33.6 ± 1.24	**>** 40	26.8 ± 1.22
9i	4-CH_3	0	**>** 40	38.9 ± 1.23	**>** 40
9j	4-OCH_3	0	18.8 ± 1.18	21. 1 ± 1.67	18.9 ± 1.21
9k	H	1	32.8 ± 2.12	28.5 ± 2.64	**>** 40

9l	4-F	1	**>** 40	**>** 40	**>** 40
9m	4-Br	1	35.8 ± 1.17	**>** 40	20.7 ± 1.28
9n	4-CH_3	1	38.2 ± 1.02	**>** 40	33.2 ± 1.06
9o	4-OCH_3	1	28.7 ± 1.08	24.8 ± 2.13	**>** 40
9p	3,4,5-OCH_3	1	22.4 ± 1.26	24.8 ± 2.13	20.2 ± 1.08
colchicine	-	-	1.07 ± 0.11	0.19 ± 0.04	0.28 ± 0.04

[a]Antiproliferative activity was assayed by exposure for 48 hours.

Compound **9d** exhibited the best antiproliferative potency against there huan cancer cells than other compounds. In addition, Kyse30 cells were more sensitive to the most of compounds including compound **9d** than MGC-803 cells and A549 cells. Therefore, compound **9d** was also chosen to test the antiproliferative activity of another three ESCC cell lines (Kyse450, Kyse510, and EC-109 cells) *in vitro*. As showen in **Table 3**, compound **9d** also displayed potent antiproliferative potency against Kyse450, Kyse510, and EC-109 cells with IC_{50} values of 1.49 μM, 2.08 μM and 2.24 μM, respectively. These results also suggested that Kyse450 cells were more sensitive to the compound **9d** than other cancer cells. Therefore, compound **9d** and Kyse450 cells were used for further detection.

Table 3. IC_{50} values of compound **9d** against four human cancer cell lines.

Tumor type	Cell line	IC_{50} (μM)
Esophagus	Kyse30	1.61 ± 0.06
Esophagus	Kyse450	1.49 ± 0.04
Esophagus	Kyse510	2.08 ± 0.05
Esophagus	EC-109	2.24 ± 0.04

As its potential target protein, activity on tubulin polymerization of compound **9d** was first to be detected. To investigat the effects on microtubules, compound **9d** was selected to do immunofluorescence assay by staining tubulin. The immunofluorescence results in **Fig. 3A** showed compound **9d** inhibited tubulin polymerization starting from the concentration of 1.25 μM in Kyse450 cells. Cell displayed disorganized spindle which was consistent with the effect of another two famous tubulin inhibitors **CA-4P**,

and **Colchicine**. We next evaluated the effects of compound **9d** on tubulin polymerization *in vitro* with **colchicine** as a positive drug. As showen in **Fig. 3B,** when tubulin was incubated with compound **9d** (1 μM, 2 μM and 4 μM), the increased tendency of the fluorescence intensity was obviously slowed down. Compound **9d** potently inhibited tubulin polymerization in a dose-dependent manner with an IC_{50} value of 3.4 μM. The inhibitory tubulin polymerization activity of compound **9** at concentration of 4 μM was stronger than that of **Colchicine** at concentration of 3 μM, while was weaker at 2 μM than that of **Colchicine** at 3 μM. The N, N'-ethylenebis (iodoacetamide) (EBI) assay is usually used test the binding ability of small molecules to β - tubulin at colchicine binding sites. In EBI assay, compound **9d** preincubation prevented the formation of EBI: β-tubulin adduct resulting in the decrease of the adduct band comparing with EBI only (**Fig.3C**), indicating compound **9d** could directly bind to the colchicine binding site of β-tubulin. These results indicated that compound **9d** was a novel tubulin polymerization inhibitor tgargeting the cochicine binding site.

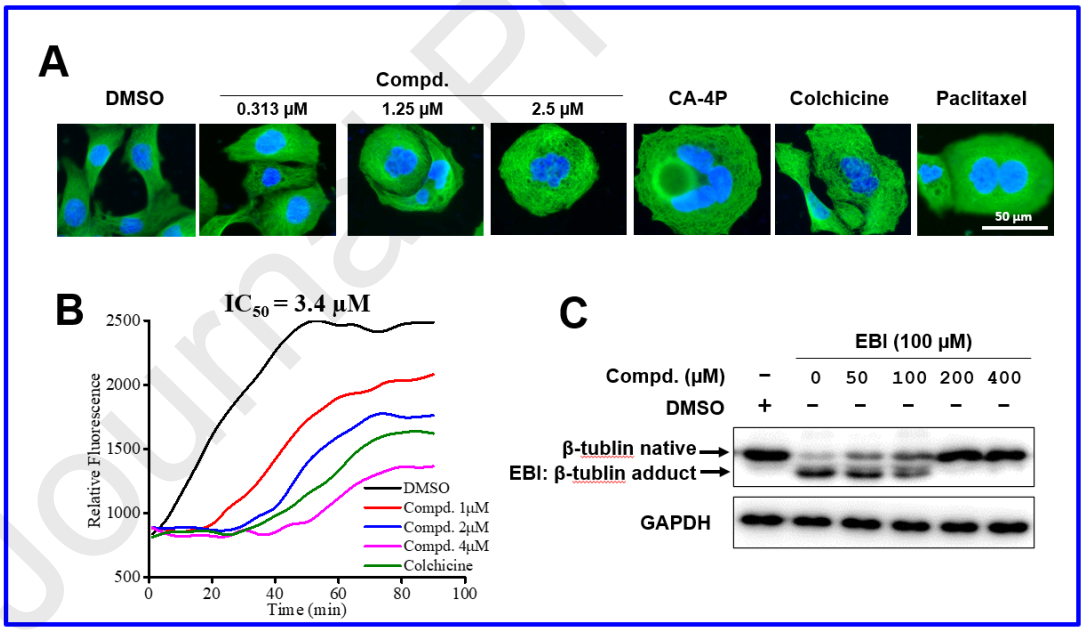


Fig. 3. Compound **9d** inhibited tubulin polymerization. **A.** Immunofluorescence staining of tubulin (green) and nucleus (blue), Kyse450 cells were treated with CA-4P (0.05μM), Colchicine (0.1μM), Paclitaxel (0.05μM) and different concentration of compound **9d** for 48 hours; **B.** Tubulin polymerization inhibitory activity of compound **9d**, concentration of Colchicine was 3 μmol/L; **C.** EBI competition assay on Kyse450 cells;

Compound **9d** displayed the potent tubulin polymerization inhibition activity, and we then selected it as the optimized compound for the molecular docking studies by MOE 2015.10. To investigate the binding site of compound **9d** with the tubulin-microtubule system, PDB code 1SA0 was selected. The docking results were listed in **Fig. 4**. The docking result revealed that compound **9d** could well bind into the colchicine binding site of β-tubulin. The carbonyl group of compound **9d** could form an hydrogen bond with the main chain of Asp249, and the hydrogen bond distance was 2.9 Å. The indoline group of compound **9d** was located in a hydrophobic subpocket consist of Ala180, Val181, Lys252, Thr310, Val313 and Lys350. In addition, the 3,4,5-trimethoxyphenyl group occupied a hydrophobic cavity and involved in hydrophobic interactions with Tyr200, Val236, Leu240, Leu250, Leu253 and Ile368. It was noticed that hydrophobic interaction played a key role in the binding of compound **9d**. These interactions made compound **9d** tightly bind into the colchicine binding site of β-tubulin and exhibit strong inhibitory activity.

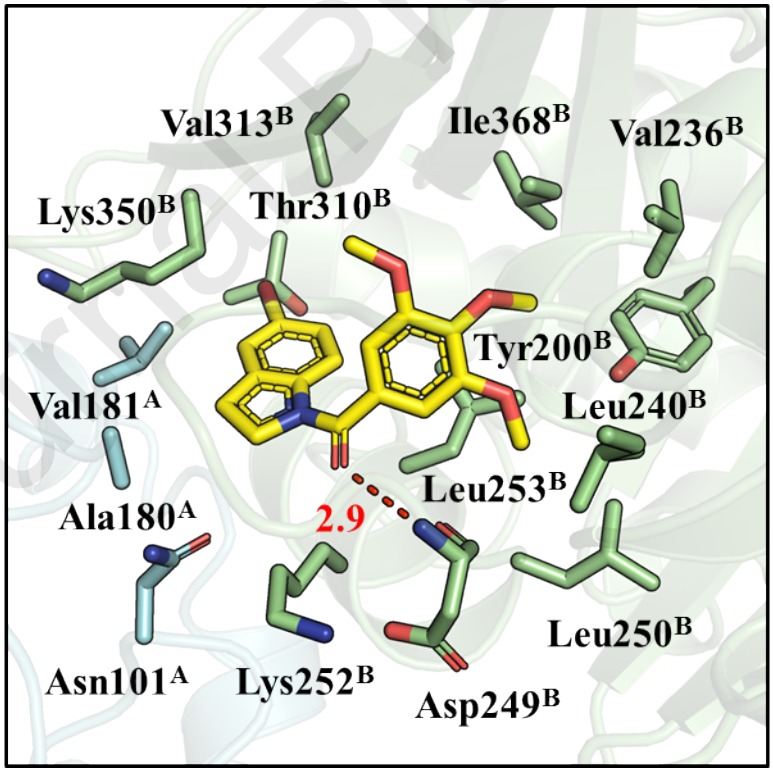


Fig. 4. The proposed binding mode of compound **9d** in the colchicine binding site of tubulin(PDB code: 1SA0). Compound 9d was shown in yellow stick. The hydrogen bond was shown in red dotted lines.

Tubulin polymerization inhibition usually leads to cell inhibition, therefore, the inhibition activity against Kyse450 cells of compound **9d** was further detected. As shown in **Fig. 5A**, with the increase of the concentrations of compound **9d**, the cell viability decreased evidently. In addition, the cell viability were also inhibited in a time-dependent manner with the treatment of different concentrations of compound **9d** (**Fig. 5B**). Compound **9d** also inhibited the proliferation of Kyse450 cells in a dose-dependent manner (**Fig. 5C**). After treatment with a high concentration of compound **9d** for 48 hours, the cell growth was less than 2 times, while the control group was more than 3.5 times (**Fig. 5C**). Some other obvious changes were also observed in Kyse450 cells after the treatment with compound **9d** for 48 hours. In morphology, Kyse450 cells became rounder; the nucleus became smaller and concentrated; Kyse450 cells were induced to death and the cell density decreased (**Fig. 5D**). The antiproliferation activity of compound **9d** was also evaluated by colony formation assay. As shown in **Fig. 5E**, compound **9d** inhibited the ability of Kyse450 cells after a 7 days treatment even with a low concentration from 0.188 μM (**Fig. 4E**). These results suggested that the compound **9d** inhibited the proliferation of MGC-803 cells in dose-and time-dependent manners.

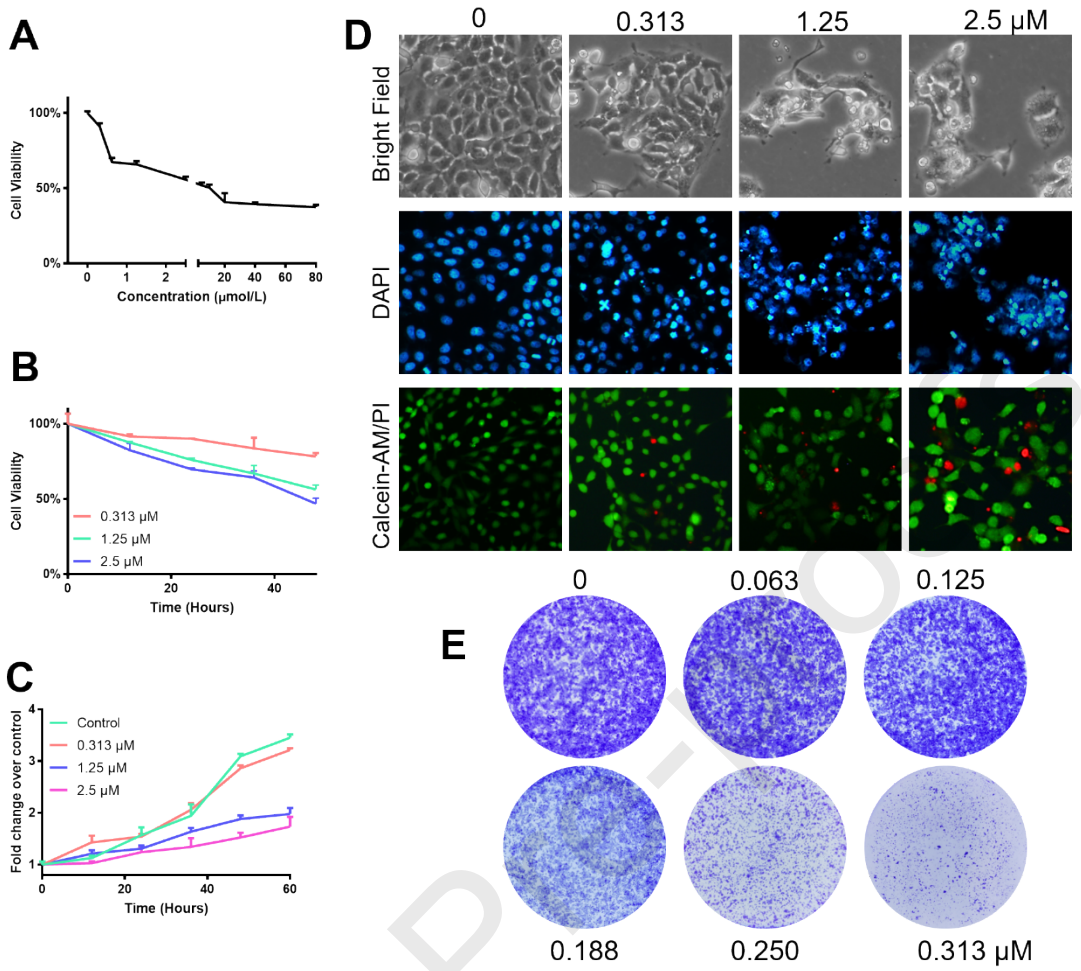


Fig. 5. Compound **9d** inhibited the activity of Kyse450 cells. **A & B.** Kyse450 cell viability. in (**A**) cells were treated with indicated concentrations of compound **9d** for 48 hours; **C.** Growth curve of Kyse450 cells; **D.** Kyse450 cellular morphologies (upper panel), nucleus (mid panel) and cell death by compound **9d** induced (lower panel, dead cells were stained red). cells were treated with different concentration of Avermectin for 48 hours, cells were stained with different dyes; **E.** Cell colony formation assay. Kyse450 cells were treated with indicated concentrations of compound **9d** for 7 days.

In this study, we reported indoline derivatives and their antiproliferative activity against three human cancer cell lines (MGC-803, Kyse30 and A549). Compound **9d** showed potently inhibitiory activity against six human cancer cells, especially Kyse30 and Kyse450 cells with IC_{50} values of 1.61 and 1.49 μM, respectively. Compound **9d** exhibited broad-spectrum antiproliferative activity of ESCC cell lines especially Kyse450 cells. According to the results of antiproliferative activity, compound **9d** and

Kyse450 cells were selected to do the further researches. Compound **9d** was identified as novel tubulin polymerization inhibitor with an IC_{50} value of 3.4 μM. Immunofluorescence results indicated that compound **9d** inhibited tubulin polymerization starting from the concentration of 1.25 μM in Kyse450 cells. Compound **9d** produced a dose-dependent inhibitory effect of tubulin polymerization *in vitro*. EBI assay results suggested compound **9d** could directly bind to the colchicine binding site of β-tubulin. The results of molecular docking also suggested compound **9d** tightly bind into the colchicine binding site of tubulin. Further results indicated compound **9d** inhibited Kyse450 cells in time and dose-denpendent manners. This work is a part of indoline derivatives as anticancer anticancer agents, which suggests that the indoline derivatives mighe be a class of potential anticancer compounds as novel tubulin inhibitors.

Appendix A. supplementary data

The supporting information provided the date of NMR and HRMS spectra of **9d**.

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Declaration of competing interest

The authors declared that there was no conflict of interest about this work.

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Declaration of Interest Statement

The authors declare that they have no conflicts of interest.

Discovery of indoline derivatives as anticancer agents via inhibition of tubulin polymerization

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1. Experimental section

1.1. General

All commercial materials were used without further purification. TLC analysis was carried out on silica gel plates GF254 (Qingdao Haiyang Chemical, China). Silica gel chromatography was carried out on 200-300 mesh gel. Anhydrous solvents and reagents were dried by routine protocols. Melting points were determined on an X-5 micromelting apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz and 100 MHz spectrometer, respectively.

1.2. Cell culture

MGC-803, A549, Kyse30, Kyse450, Kyse510, and Ec109 cell lines were cultured in an atmosphere with 5% CO_2 at 37 °C, using RPMI-1640 with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 0.1 mg/ml streptomycin as culture medium.

1.3. Cell viability

Growth inhibition of MGC-803, A549, Kyse30, Kyse 450, Kyse510, Ec109 cell lines was examined by the CCK8 assays. Briefly, human cancer cells were seeded in 96 - well plates at a density of 3000–5000 cells/well and incubated 24 hours. The cells were treated with compounds **9d** or medium for different times and 5 μl CCK8 was added to per well. After incubation for another 4 hours, the absorbance was measured at 450 nM by a microplate reader (MK3, Thermo, Germany). Data are calculated by SPSS version 10.0.

1.4. Colony formation assay

Kyse450, cells were seeded in a 6-well plate at a density of 5 x 103 per well and incubated at 37 °C in 5% CO_2 for 24 h, then they were treated with different concentrations of **9d**. After 7 days, the culture medium was removed, and the cells were washed with PBS and fix with 4% paraformaldehyde. Then stain the cells with 0.1% crystal violet. The image was captured with camera, and the number of colonies were quantified using Image J software (National Institutes)

1.5. Immunostaining and microscopy

Cell slices were placed on the bottom of a 24-well plate. Kyse 450 cells were seeded on the slices and incubated for 24 hours. After 48 hours, slices were fixed with 4% paraformaldehyde for 20 minutes. 0.5 % Triton-X-100 was added and shake for 20 min. 0.1% BSA was used to block for 30 minutes and then removed. The slices were co-incubated with anti-β-tubulin antibody (1:100) for 8 hours at 4 °C. Then slices were washed by PBST 3 times, bind with secondary antibody with FITC signal (1:500) in a dark. DAPI was used to stain for 5 min. After that, images were captured by Laser scanning confocal microscope (Nikon, Japan).

1.6. *In vitro* tubulin polymerization assay

An amount of 5.6 mg/ml tubulin was resuspended in PEM buffer (containing 80 mmol/L PIPES (Ph = 6.9)), 1 mmol/L EGTA, 0.5 mmol/L $MgCl_2$, 1 mmol/L ATP, 10.2%

(v/v) glycerol) and then was preincubated with certain concentrations of **9d**, Colchicine or vehicle DMSO at 0 ℃. The reaction was monitored by a spectrophotometer in absorbance at 420 nm at 37 °C every minute (excitation wavelength is 340 nm).

1.7. EBI competition assay

6-well plates were seeded with Kyse 450 cells at 5 × 105 cells per well. Cells were first incubated with certain concentrations of **9d**, CA-4P, colchicine and paclitaxel or DMSO for 2 hours and afterward treated with EBI (100 μmol/L) for 2 hours. Then the cells were harvested and lysed. Cell extracts were used for Western blotting analysis.

1.8. Western blot analysis

Treated and untreated cells were harvested and lysed. Cell extracts were denatured by loading buffer and resolved by SDS-PAGE and transferred to nitrocellulose membrane. The membranes were incubated with appropriate primary antibodies at 4 °C for 10 hours after blocking with 5% skimmed milk. After conjugated with secondary antibodies, the detection of proteins was carried out with an ECL kit.

1.9. Molecular docking

The molecular docking study was performed using MOE 2015.10. The crystal structure of tubulin (PDB ID: 1SA0) was retrieved from RCSB Protein Data Bank, and then was prepared by adding hydrogen atoms, removing water molecules and repairing the missing side chains. The protonation states of protein residues were calculated in the pKa at 7. The ligand compound **9d** was built in MOE 2015.10 and was prepared by energy minimization and conformational search. The ligand was docked into the colchicine binding site of tubulin and 20 poses were exported for the next analysis.

2. ^{1}H, and ^{13}C-NMR of Compound 9a-9p

Indolin-1-yl(3,4,5-trimethoxyphenyl)methanone（9a）

White solid, m.p.: 105~106 ^{o}C, yield:90%. ^{1}H NMR (400 MHz, CDCl3) δ 7.27 (s, 1H), 7.22 (d, J = 7.4 Hz, 1H), 7.12 (s, 1H), 7.02 (t, J = 7.1 Hz, 1H), 6.78 (s, 2H), 4.12 (t, J = 8.0 Hz, 2H), 3.88 (d, J = 14.8 Hz, 9H), 3.13 (t, J = 8.3 Hz, 2H). ^{13}C NMR (100 MHz, CDCl3) δ 168.58, 153.38, 142.51, 139.78, 132.42, 132.20, 127.24, 124.96, 123.95, 123.89, 104.55, 60.98, 56.29, 50.45, 28.06. HRMS (ESI): Calcd. C18H20NO4,

[M+H]+m/z: 314.1392, found: 314.1398.

(3,4-Dihydroquinolin-1(2H)-yl)(3,4,5-trimethoxyphenyl) methanone（9b）

White solid, m.p.: 102~103 °C, yield:60%. ^{1}H NMR (400 MHz, CDCl3) δ 7.06 (d, J = 7.3 Hz, 1H), 6.91 (t, J = 7.3 Hz, 1H), 6.82 (t, J = 7.5 Hz, 1H), 6.66 (d, J = 7.8 Hz, 1H), 6.50 (s, 2H), 3.82 (t, J = 6.5 Hz, 2H), 3.74 (s, 3H), 3.58 (s, 6H), 2.75 (t, J = 6.5 Hz, 2H), 2.06 – 1.85 (m, 2H). ^{13}C NMR (100 MHz, CDCl3) δ 168.60, 151.69, 138.80, 138.51, 130.65, 129.96, 127.16, 124.85, 124.34, 123.59, 105.48, 59.84, 55.01, 43.53, 25.94, 23.22. HRMS (ESI): Calcd. C19H22NO4, [M+H]+m/z: 328.1549, found: 328.1552.

(2,3,4,5-Tetrahydro-1H-benzo[b]azepin-1-yl)(3,4,5-trimethoxyphenyl)methanone（9c）

White solid, m.p.: 144~145 °C, yield:50%. ^{1}H NMR (400 MHz, CDCl3) δ 7.16 (d, J = 7.5 Hz, 1H), 7.02 (t, J = 7.4 Hz, 1H), 6.89 (t, J = 7.5 Hz, 1H), 6.60 (d, J = 7.7 Hz, 1H), 6.38 (s, 2H), 4.95 (d, J = 13.2 Hz, 1H), 3.69 (s, 3H), 3.52 (s, 6H), 2.97 (t, J = 13.0 Hz, 1H), 2.82 (dd, J = 13.6, 4.4 Hz, 1H), 2.71 (t, J = 10.1 Hz, 1H), 2.02 (d, J = 11.5 Hz, 1H), 1.89 (dd, J = 11.8, 7.5 Hz, 2H), 1.45 (d, J = 11.5 Hz, 1H). ^{13}C NMR (100 MHz, CDCl3) δ 167.16, 151.25, 143.46, 138.14, 138.01, 129.72, 128.78, 127.15, 126.31, 126.20, 105.23, 59.78, 54.85, 46.75, 33.85, 28.65, 25.27. HRMS (ESI): Calcd. C20H24NO4, [M+H]+m/z: 342.1705, found: 342.1709.

(5-Bromoindolin-1-yl)(3,4,5-trimethoxyphenyl)methanone （9d）

White solid, m.p.: 126~127 °C, yield:31%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.48 (s, 1H), 7.37 (d, J = 7.9 Hz, 1H), 6.90 (s, 2H), 4.06 (t, J = 8.4 Hz, 2H), 3.82 (d, J = 8.0 Hz, 6H), 3.73 (d, J = 4.9 Hz, 3H), 3.10 (t, J = 8.3 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.29, 153.28, 142.63, 139.31, 136.21, 132.46, 130.03, 128.30, 115.66, 107.00, 105.02, 60.57, 56.58. HRMS (ESI): Calcd. $C_{18}H_{19}BrNO_4$, [M+H]+m/z: 392.0497, found: 392.0499.

(5-bromoindolin-1-yl)(phenyl)methanone (*9f*)

White solid, m.p.: 131~132 °C, yield:44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.52 (m, 2H), 7.47 – 7.39 (m, 2H), 7.30 (d, J = 9.0 Hz, 4H), 3.69 (t, J = 14.6 Hz, 2H),

3.06 (t, J = 14.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.98, 141.89, 136.57, 134.61, 130.52, 130.19, 128.65, 127.91, 127.06, 116.40, 50.80, 28.00.

(5-bromoindolin-1-yl)(4-fluorophenyl)methanone (*9g*)

White solid, m.p.: 128~129 °C, yield: 40%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (dd, *J* = 8.5, 5.4 Hz, 2H), 7.33 (s, 1H), 7.26 (s, 1H), 7.14 (t, *J* = 8.6 Hz, 2H), 4.07 (d, *J* = 7.3 Hz, 2H), 3.12 (t, *J* = 8.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.93, 165.14, 162.64, 141.80, 134.56, 132.59, 130.21, 129.60, 129.52, 127.98, 116.56, 115.89, 115.67, 50.85, 27.95.

(5-bromoindolin-1-yl)(4-bromophenyl)methanone (*9h*)

White solid, m.p.: 123~124 °C, yield: 48%.^{1}H NMR (400 MHz, CDCl3) δ 7.60 (d, J = 8.3 Hz, 2H), 7.43 (d, J = 8.3 Hz, 2H), 7.34 (s, 1H), 7.26 (s, 2H), 4.06 (s, 2H), 3.12 (t, J = 8.3 Hz, 2H). ^{13}C NMR (101 MHz, CDCl3) δ 167.84, 141.68, 131.92, 130.28, 128.84, 128.00, 124.99, 116.69, 50.85, 27.97.

(5-bromoindolin-1-yl)(p-tolyl)methanone (*9i*)

White solid, m.p.: 119~120 °C, yield: 50%.^{1}H NMR (400 MHz, CDCl3) δ 7.45 (d, J = 8.0 Hz, 2H), 7.32 (s, 1H), 7.25 (d, J = 10.2 Hz, 4H), 4.09 (t, J = 7.7 Hz, 2H), 3.10 (t, J = 8.3 Hz, 2H), 2.41 (s, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 169.13, 142.05, 140.87, 134.65, 133.63, 130.13, 129.22, 127.87, 127.24, 118.41, 116.23, 50.76, 27.95, 21.51, -0.00.

(5-bromoindolin-1-yl)(4-methoxyphenyl)methanone (*9j*)

White solid, m.p.: 129~130 °C, yield: 38%.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 8.6 Hz, 2H), 7.32 (s, 1H), 7.26 (s, 2H), 6.94 (d, J = 8.7 Hz, 2H), 4.12 (t, J = 8.3 Hz, 2H), 3.86 (s, 3H), 3.10 (t, J = 8.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.82, 161.47, 142.17, 134.67, 130.09, 129.36, 128.55, 127.89, 116.11, 113.82, 55.41, 50.93, 28.01.

1-(5-bromoindolin-1-yl)-2-phenylethan-1-one (*9k*)

White solid, m.p.: 118~119 °C, yield: 54%.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 8.5 Hz, 1H), 7.37 – 7.32 (m, 2H), 7.31 – 7.28 (m, 3H), 7.25 (s, 2H), 4.06 (t, J = 8.5 Hz, 2H), 3.79 (s, 2H), 3.13 (t, J = 8.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.18, 142.22, 133.90, 133.38, 130.41, 129.06, 128.79, 127.53, 127.10, 118.44, 116.12, 48.26, 43.42, 27.77.

1-(5-bromoindolin-1-yl)-2-(4-fluorophenyl) ethan-1-one (*9l*)

White solid, m.p.: 126~127 °C, yield: 49%.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 8.4 Hz, 1H), 7.25 (dd, J = 8.7, 5.5 Hz, 4H), 7.03 (t, J = 8.7 Hz, 2H), 4.08 (t, J = 8.5 Hz, 2H), 3.74 (s, 2H), 3.16 (t, J = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.98, 163.19, 160.75, 142.12, 133.35, 130.78, 130.70, 130.45, 129.62, 129.58, 127.58, 118.42, 116.24, 115.72, 115.50, 48.22, 42.32, 27.77.

1-(5-bromoindolin-1-yl)-2-(4-bromophenyl) ethan-1-one (*9m*)

White solid, m.p.: 126~127 °C, yield: 49%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.4 Hz, 1H), 7.46 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 6.1 Hz, 2H), 7.17 (d, J = 8.2 Hz, 2H), 4.06 (t, J = 8.5 Hz, 2H), 3.72 (s, 2H), 3.15 (t, J = 8.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.53, 142.06, 133.33, 132.89, 131.83, 130.92, 130.46, 127.59, 121.15, 118.41, 116.28, 48.23, 42.57, 27.76.

1-(5-bromoindolin-1-yl)-2-(p-tolyl) ethan-1-one (*9n*)

White solid, m.p.: 121~122°C, yield: 57%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 8.5 Hz, 1H), 7.26 (s, 2H), 7.16 (q, J = 8.0 Hz, 4H), 4.06 (t, J = 8.5 Hz, 2H), 3.75 (s, 2H), 3.13 (t, J = 8.4 Hz, 2H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.43, 142.27, 136.74, 133.36, 130.77, 130.40, 129.49, 128.91, 127.51, 118.44, 116.07, 48.24, 43.06, 27.78, 21.07.

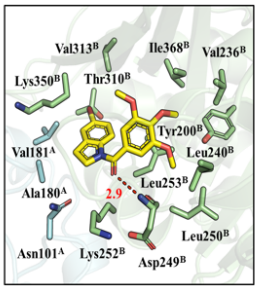
1-(5-bromoindolin-1-yl)-2-(4-methoxyphenyl) ethan-1-one (*9o*)

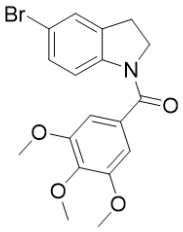
White solid, m.p.: 121~122 °C, yield: 57%.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 8.5 Hz, 1H), 7.26 (s, 2H), 7.21 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 4.07 (t, J = 8.5 Hz, 2H), 3.79 (s, 3H), 3.73 (s, 2H), 3.14 (t, J = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.56, 158.68, 142.27, 133.35, 130.42, 130.11, 127.52, 125.85, 118.44, 116.08, 114.22, 55.29, 48.21, 42.52, 27.80.

1-(5-bromoindolin-1-yl)-2-(3,4,5-trimethoxyphenyl) ethan-1-one (*9p*)

White solid, m.p.: 128~129 °C, yield: 51%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 8.3 Hz, 1H), 7.30 (d, J = 8.4 Hz, 2H), 6.51 (s, 2H), 4.10 (t, J = 8.5 Hz, 2H), 3.84 (d, J = 6.1 Hz, 9H), 3.73 (s, 2H), 3.18 (t, J = 8.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.05, 153.42, 142.18, 137.06, 133.36, 130.48, 129.46, 127.59, 118.45, 116.24, 106.13, 60.85, 56.16, 48.29, 43.65, 27.83.

Graphical Abstract





Compound **9d**

IC_{50} = 1.84 μM (MGC-803 cells)
6.82 μM (A549 cells)
1.61 μM (Kyse30 cells)
1.49 μM (Kyse450 cells)
2.08 μM (Kyse510 cells)
2.24 μM (EC-109 cells)