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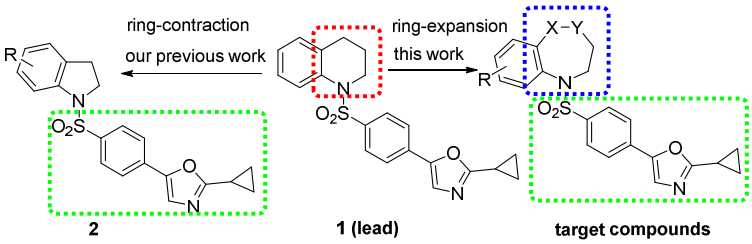
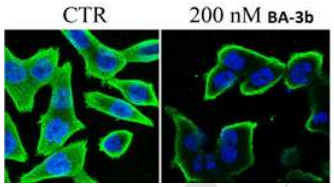
Graphical abstract





BA-3b, X=O, Y=CH_2, R=7-F

IC_{50} 7~36 nM against 7 cancer cell lines

Synthesis, anti-cancer evaluation of benzenesulfonamide derivatives as potent tubulin-targeting agents

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Abstract:

A series of benzenesulfonamide derivatives were synthesized and evaluated for their anti-proliferative activity and interaction with tubulin. These new derivatives showed significant activities against cellular proliferative and tubulin polymerization. Compound **BA-3b** proved to be the most potent compound with IC_{50} value ranging from 0.007 to 0.036 μM against seven cancer cell lines, and three drug-resistant cancer cell lines, which indicated a promising anti-cancer agent. The target tubulin was also verified by dynamic tubulin polymerization assay and tubulin intensity assay.

Keywords

Tubulin-targeting agents, sulfonamides, benzoxazepine, benzodiazepine, benzothiazepine.

1. Introduction:

Microtubules are hollow tubes formed by the polymerization of α, β-tubulin heterodimers, which are essential in a diverse array of eukaryotic cell functions, like intracellular organelle transport, cell motility and mitosis[1-4]. Numbers of clinically used compounds such as vinca alkaloids, colchicines, paclitaxel, and epothilone attack microtubules by interfering with the dynamics of the tubulin polymerization and depolymerization, resulting in mitotic arrest[5-8]. Undoubtedly, targeting tubulin is a successful strategy for cancer chemotherapy, However, there are still many problems existing in clinical use of these anti-tubulin agents, like toxicity, poor water solubility, poor bioavailability and multi-drug-resistant (MDR)[9-11]. Therefore, it is essential to develop small molecular agents which can be effective not only in treating MDR tumors but also in inhibiting tubulin polymerization.

Figure 1: New tubulin-targeting drug design

Stockwell et al identified compound **1** (Fig.1) as a highly potent tubulin-targeting agent after analyzing more than 1 million simple synthetic compounds[11]. The lead compound **1** was proved to be a highly potent structure by our previous work. Our group has actively engaged in searching novel anticancer agents that target tubulin and have synthesized a series of 4-azaheterocycle benzenesulfonamide derivatives (**2**, the ring contraction series), which show excellent activities against a panel of cancer cell lines[12]. Our precious work revealed that cyclopropyl-oxazole moiety was crucial for cytotoxicity. Moreover, benzodiazepine, benzoxazepine and benzothiazepine skeleton as crucial pharmacophore cores have attracted much attention in the past years owning to its broad spectrum of biological activities especially anticancer[13-17],

anticonvulsant[18], CNS activities[19,20] and others[21]. To continue our earlier work[12], we designed the ring expansion series (**3**, **Fig. 1**) of the lead compound **1**, expecting an improvement in drug potency and water solubility. We herein describe the rationale for the design, concise synthesis, and structure-activity relationships (SAR) of a series of benzenesulfonamide derivatives as potent antitubulin agents.

2. Results and discussion

2.1 Chemisty

The reference compound 1 was synthesized following the pathway depicted in **Scheme 1**. The general syntheses of benzenesulfonamide derivatives are shown in **Scheme 2**. The 2,5-disubstituted oxazole was prepared from acetophenone and cyclopropylnitrile using $PhI(OAc)_2$ as an oxidant, which was subsequently treated with chlorosulfonic acid to yield compound **BA-1**[22]. Compounds **BA-2** were synthesized from 2-aminophenol, 2-aminothiophenol or 2-aminobenzylalcohol with 1,3-dibromopropane or 1,2-dibromoehane in DMF, and then reacted with **BA-1** in presence of pridine to give the target compounds **BA-3(a-l), BA-4(a-b),** respectively.

Scheme 1. Reagents and conditions: a) $PhI(OAc)_2$, TfOH, cyclopropanecarbonitrile, DCE, 80°C; b) Chlorosulfonic acid, DCM, 50°C; c) Pyridine, DCM, rt, 2h.

Scheme 2. a) m=1, 1,2-dibromoethane, K_2CO_3/DMF, 60°C; m=2, 1,3-dibromopropane, K_2CO_3/DMF, 60°C; b) **BA-1**, pyridine, DCM, rt, 2-3h.

The synthetic route for compound **BA-2m** was different from other precursor compounds **BA-2**, which prepared from commercially available tetrahydronaphthalene following four steps with a 36% total yield according to the reference methods[23], as shown in **Scheme 3**.

Scheme 3. a）$FeCl_3{\cdot}6H_2O$, TBHP(70%), pyridine, 82°C; b) $NaBH_4$/MeOH, 0°C; c) triethylamine, methanesulfonyl chloride, CH_2Cl_2, then NaN_3, DMF; d) $BF_3{\cdot}OEt_2$, anhydrous CH_2Cl_2. -78°C, then $NaBH_4$, NaOH(15% aqueous).

In order to improve the compounds' water solubility, we designed three compounds, **BA-3n**, **BA-3o** and **BA-3p**. Compounds **BA-3n**, **BA-3o** were expected to introduce amino and tert-ammonia to form salt with suitable acid, which can largely increase water solubility. Compound **BA-3k** was oxidized to sulfone[24], sulfoxide[25], which were expected to improve the water solubility. These compounds' general synthesis routes are shown respectively in **Scheme 4-6**.

Scheme 4. The route of introducing amine:a) Et_3N, HCOOH, Pd/C, EtOH, 80°C.

Scheme 5. The route of introducing tert-amine: a) Sarcosine, DMSO, 180°C; b) $LiAlH_4$, THF, 0°C; c) **BA-1**, pyridine, DCM.

Scheme 6. Synthesis of sulfone and sulfoxide from sulfide: a) 2.10 equiv mCPBA, DCM, r.t; b) 1.05 equiv mCPBA, DCM, rt

2.2 Bilogical results

2.2.1 In vitro cell growth Inhibitory Activity

Preciously, we synthesized four lead compounds and evaluated for antiproliferative activities against four types of human cancer cell lines, colorectal carcinoma HCT-116 cells, prostate carcinoma PC3 cells, liver cancer HepG2 cells and ovarian cancer SK-OV-3 cells. As a result, benzoxazepine derivative **BA-3a** showed highest potent than benzothiazepine, benzoxazine and benzothiazine, and the lead compound **1** (see **Table1**). So we designed a series of benzoxazepine derivatives and evaluated their cytotoxic potency, as shown in **Table 2**. All compounds exhibited excellent cytotoxic activities.

Table 1. IC_{50} values of fist-run compounds over four cancer cell lines[a].

Compared with compounds **BA-3**(g-j), **BA-3**(b-f) generally exhibited better anti-cancer activities, respectively. The result indicated that substituent at the C-7 position is more potent than that at C-8 position except nitro group (**BA-3j** vs **BA-3e**). Furthermore, electron-withdrawing group substitutions, like -F, -Cl, $-NO_2$, showed higher antiproliferative potency than those with electron-donating groups, like methyl and methoxyl. More interesting, the 1,4-oxazepine derivative **BA-3a** is more potent than its 1,3-oxazepine counterpart, **BA-3l**. Of all potent compounds, **BA-3b** and **BA-3f** showed the best activities with IC_{50} values 0.015-0.036 and 0.018-0.039 μM, respectively.

Table 2. Antiproferliferative effects of benzoxazepane derivatives

Of the compounds designed to improve water solubility, only compound **BA-3n** exhibited potent activity. The rest two compounds (**BA-3p** and **BA-3q**) showed a sharply decrease in activity than the benzenothiozepine derivative **BA-3K**, which turned out to be a failure of their structural modification, together with the N-methyl benzodiazepine derivative **BA-3o** (Table 3).

Table 3. Antiproferliferative effects of benzothiazepine and benzodiazepine derivatives

Clinical use of chemotherapeutics, including anti-tubulin agents, multi-drug-resistant problem arose eventually. Compound **BA-3b** and **BA-3g** were chosen to test their potential against several MDR cell lines. As shown in Table 4, compound **BA-3b** and **BA-3g** exhibited strong cytotoxicity against both MDR cell lines K562/A02, KB/Vcr, MCF-7/Adr, and their drug-sensitive parental cell lines.

Table 4. IC_{50} values of compounds in three MDR and drug-sensitive parental cell lines

2.2.2 Inhibition of tubulin polymerization.

To investigate whether the ring-expansion derivatives of lead compound **1** are tubulin-targeting agents, **BA-3b**, **BA-3g** were chosen to undergo tubulin polymerization assay in vitro using

purified porcine brain tubulin[26]. In this assay, tubulin monomer was self-polymerized to microtubules, increasing light scattering at 340 nm. Microtubule-depolymerizing agents, like vinblastine, colchicine, nacodazole, are known to inhibit self-polymerization activity of tubulin, on the contrary, microtubule-stabilizing agent, like Taxol, accelerated polymerizationin this assay[27]. Compounds **BA-3b** and **BA-3g** showed a similar dynamic curve with nacodazole, not Taxol (**Fig. 2**). These results indicated that **BA-3b**, **BA-3g** directly binds to tubulin and induces depolymerization of microtubule network.

Figure 2. Tubulin polymerization in the presence of **BA-3b**, **BA-3g**, nocodazole, and taxol

The effects of these compounds on tubulin polymerization were further examined by fluorescent tubulin intensity assay. Treatment of SW480 cells with compounds **BA-3b**, **BA-3g**, and vincristine, respectively, strong tubulin inhibitory activities were observed (Fig. 3).

Figure 3. Tubulin intensity assay (DMSO as control, Vin for vinblastine)

2.2.3Induction of SW480 cell apoptosis

Considering the potent antiproliferative activity, compound **BA-3b** was selected for flow cytometry analysis. As shown in Fig. 4, compound **BA-3b** could induce early apoptosis in SW480 cells at very low concentration (50 nM) with a ratio of 7.74%, compared with 3.41% in the vehicle control group, in 24 hours.

Figure 4. **BA-3b** induce apoptosis in SW480 cells

The water solubility is a crucial chemo-physical factor for a drug candidate, especially for a tubulin-targeting agent. The solubility of the most potent molecule **BA-3b** is measured as ~10 mg/L in pure water at 25°C, which is 1000-fold higher than its average antiproliferative IC_{50} values.

3. Conclusion

In summary, a series of novel sulfonamide derivatives were synthesized and displayed better potent cytotoxicities than the lead compound **1**. Generally, 7-substituted derivatives showed better activity than those 8-substituted. The most potent compound **BA-3b** displayed excellent cytotoxicity against 7 human tumor cell lines, and 3 MDR cell lines, with an IC_{50} range 0.007-0.036 μM, as well as an excellent antitubulin activity. Moreover, the water solubility of this compound is superior than Docetaxel, which used as an antitumor drug targeting to microtubule in clinical treatment. The above results indicate this new compound might be an orally antitubulin candidate after further investigation.

4. Material and methods

4.1 Chemical synthesis

General

All solvents were of analytical grade.^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker AM-400 MHz spectrometer. The chemical shifts were reported in ppm using TMS as

internal standard. High resolution mass spectrometry data were measured on Bruker Apex IVFTMS. Melting points were measured on an X-5 micro-melting point apparatus and wereuncorrected. Column chromatography was conducted on silica gel 60 (E. Merck, 0.063-0.200mm).

*4.1.1 General procedure for synthesis and purification of precursor compound **BA-2(a-l).***

To a solution of o-aminophenol (1.0 g, 9.2 mmol) in 10mL DMF was added 1, 3-dibromopropane(1.4 ml, 13.8 mmol) and potassium carbonate (6.36 g, 4.6 mmol), the reaction mixture was refluxed overnight. After the reaction was finished monitored by TLC, the suspension was cooled down to room temperature and removed DMF in vacuum. Then extracted with ethyl acetate (3×10 mL) and brine (3×10 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was future purified by column chromatography (petroleum ether/ ethyl acetate=15:1) to obtain precursors compounds **BA-2**(a-l), yields 45-92%.

*4.1.2 General procedure for synthesis and purification of precursor compound **BA-2m**.*

A solution of tetrahydronaphthalene (674 mg, 5.0 mmol) in pyridine (5 mL) was added $FeCl_3{\cdot}6H_2O$ (27 mg, 0.1 mmol) and TBHP (70%) (2.06 mL, 15.0 mmol). The suspension was refluxed for 24 hour, then treated with hydrochloric acid (1M, 10 mL) and exacted with ethyl acetate, the crude product was purified via column chromatography (petroleum ether / ethyl acetate=10:1. The gained compound subsequently was added sodium borohydride (0.34 g, 8.9 mmol) in 9 mL MeOH and stirred at 0°C for 2 hours. When finished, the alcohol (2.0 mmol) was added triethylamine (420 μL, 3.0 mmol) in anhydrous CH_2Cl_2 (5 mL) and followed by methanesulfonyl chloride (2.4 mmol) over a period of 10min. After an additional 15 min at 0°C, the reaction mixture was diluted with CH_2Cl_2(10 mL), and the organic layer was washed with brine (3×5 mL), and then dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the mesylate as yellow liquid. The crude mesylate (1.0 mmol) was re-dissolved in DMF (10 mL), treated with NaN_3 (2 mmol) and stirred at room temperature for 3 hours. The reaction mixture was poured into ice-cold water (10 mL), extracted with brine (3×10 mL), dried and purified by column chromatography (petroleum ether / ethyl acetate=10:1) to give pure benzylicazide. The gained azide was re-dissolved in anhydrous CH_2Cl_2 (5mL) at -78°C, and added $BF_3{\cdot}OEt_2$ (2.2 mmol) dropwise. The resultant mixture was stirred at -78°C for 45 min and then warmed to room temperature. After an additional 45 min reaction at room temperature, the mixture was cooled to 0°C and treated with $NaBH_4$ (265 mg, 7.0 mmol) in 15% aqueous NaOH (3 mL). The reaction was then warmed to room temperature and stirred for 45 min. The mixture was extracted with CH_2Cl_2(2 × 10 mL), brine(2 × 5 mL), dried and purified by column chromatography (petroleum ether / ether acetate =10:1) to give compound **BA-2m**.

*4.1.3 General procedure for synthesis and purification of precursor compound **BA-2o**.*

To a stirred solution of isatoic anhydride (333 mg, 2.0 mmol) in 4 mL DMSO was added sarcosine (182 mg, 2.0 mmol), the reaction mixture was refluxed for 5 hours. After the reaction was finished, the mixture was cooled down to 0°C and allowed to stand overnight. The solid

product was collected by filtration, dried and recrystallized from ethanol. Then the solid (90 mg) was dissolved in THF (4 mL) at 0°C and added $LiAlH_4$ (79 mg, 2.0 mmol). The reaction mixture was stirred for 6 hours and then warmed to room temperature. To the solution was added water (3 mL), extracted with CH_2Cl_2 (3×5 mL), dried and purified by column chromatography (CH_2Cl_2: MeOH=30:1) to give compound **BA-2o**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16-7.04 (m, 2H), 6.84 (t, J = 7.4 Hz, 1H), 6.74 (d, J = 7.7 Hz, 1H), 3.90 (s, 1H), 3.71 (s, 2H), 3.25-3.10 (m, 2H), 2.94-2.82 (m, 2H), 2.40 (s, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ 150.16, 131.09, 128.76, 128.02, 120.77, 118.62, 62.09, 59.93, 45.93, 43.53. ESI MS: Calcd for $C_{10}H_{14}N_2$ $[M+H]^+$: 163.12; found: 163.28.

*4.1.4 General procedure for synthesis and purification of compounds **BA-3(a-m)**, **BA-3o**and **BA-4(a-b).***

The precursor compound BA-1 was synthesized according to the reference methods [22], illustrated above in Chemistry section. Then **BA-1**(1.0 mmol) was added with compounds **BA-2**(1.2 mmol) and pyridine (2.0 mmol) in CH_2Cl_2 (5 mL), the reaction was stirred at room temperature for 2h, and then purified by column chromatography (petroleum ether/ ethyl acetate=5:1) to obtain compounds **BA-3(a-m)**, **BA-3o** and **BA-4(a-b)**.

*4.1.5 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepine (**BA-3a**)*

White solid, yield 85%. M.p. 87-88°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69-7.56 (m, 4H), 7.52 (d, J=8.0 Hz,1H), 7.29 (s, 1H), 7.23 (t, J=8.0Hz, 1H), 7.10 (t, J=8.0 Hz, 1H), 6.97 (d, J= 8.0 Hz,1H), 4.03-3.67 (m, 4H), 2.13 (tt, J=8.2, 4.4 Hz, 1H), 1.94-1.80 (m, 2H), 1.19-1.06(m, 4H). ^{13}C NMR (100MHz, $CDCl_3$) δ 166.90, 156.54, 148.74, 139.62, 132.22, 131.92, 131.20, 129.39, 128.01, 124.45, 124.03, 123.65, 122.39, 70.88, 48.84, 29.38, 9.09, 8.65. HR-ESI-MS: Calcd for $C_{21}H_{20}N_2O_4S$ $[M+H]^+$: 397.12220; found: 397.12071.

*4.1.6 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-7-fluoro-2,3,4,5-tetrahydrobenzo[b][1,4] oxazepine (**BA-3b**)*

White solid, yield: 72%. M.p. 117-118°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71-7.59 (m, 4H), 7.30 (s, 1H), 7.28-7.23(m 1H), 6.99-6.88 (m, 2H), 3.98-3.71(m, 4H), 2.20-2.08 (m, J=8.2, 5.2 Hz, 1H), 1.94-1.80 (m, 2H), 1.20-1.07 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.07, 156.87, 148.63, 139.23, 132.12, 128.00, 124.58, 123.74, 123.10, 123.01, 117.87, 117.63, 116.08, 115.85, 70.96, 48.81, 29.33, 9.10, 8.70. HR-ESI-MS: Calcd for $C_{21}H_{19}FN_2O_4S$ $[M+H]^+$: 415.11278; found: 415.11265.

*4.1.7 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-7-methoxy-2,3,4,5-tetrahydrobenzo[b][1, 4]oxazepine (**BA-3c**)*

Brown oil, yield: 83%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71-7.54(m, 4H), 7.29 (s, 1H), 7.05 (d, J=3.1 Hz, 1H), 6.89 (d, J=8.8 Hz, 1H), 6.78 (dd, J=8.8, 3.1 Hz, 1H), 3.97-3.69 (m, 7H), 2.21-2.06 (m,1H), 1.91-1.76 (m, 2H), 1.18-1.05 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.94, 155.58, 150.37, 148.72, 139.58, 132.89, 131.89, 128.04, 124.41, 123.62, 122.73, 115.55, 115.33, 71.01, 55.74, 48.95, 29.47, 9.08, 8.65. HR-ESI-MS: Calcd for $C_{22}H_{22}N_2O_5S$ $[M+H]^+$: 427.13277; found: 427.13146 .

*4.1.8 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-7-methyl-2,3,4,5-tetrahydrobenzo[b][1,4] oxazepine (**BA-3d**)*

Yellow solid, yield: 85%. M.p. 66-67°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (q, J=8.7 Hz, 4H), 7.34 (d, J=1.8 Hz, 1H), 7.29 (s, 1H), 7.03 (dd, J=8.2, 1.8 Hz, 1H), 6.86 (d, J=8.2 Hz, 1H), 4.02-3.58 (m, 4H), 2.33 (s, 3H), 2.17-2.10 (m, 1H), 1.91-1.78 (m, 2H), 1.18-1.06 (m, 4H). ^{13}C NMR (100MHz, $CDCl_3$) δ 166.93, 154.34, 148.75, 139.69, 133.83, 131.90, 131.83, 131.65, 130.05, 128.03, 124.40, 123.61, 122.01, 70.97, 48.90, 29.37, 20.70, 9.10, 8.68. HR-ESI-MS: Calcd for $C_{22}H_{22}N_2O_4S$ $[M+H]^+$: 411.13785; found: 411.13688.

*4.1.9 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-7-nitro-2,3,4,5-tetrahydrobenzo[b][1,4] oxazepine (**BA-3e**)*

Yellow solid, yield: 68%. M.p. 80-81°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J=2.7 Hz, 1H), 7.99 (dd, J=9.0, 2.7 Hz, 1H), 7.86 (d, J= 8.6 Hz, 2H),7.65 (d, J=8.6 Hz, 2H), 7.31 (s, 1H), 6.88 (d, J=9.0 Hz, 1H), 4.16 (t, J=6.0 Hz, 2H),3.48 (t, J=6.0 Hz, 2H), 2.33 (p, J=6.0 Hz, 2H), 2.21-2.05 (m, 1H), 1.20-1.04 (m,4H). ^{13}C NMR (100 MHz, CDC_{l3}) δ167.42, 152.84, 150.58, 141.82, 137.13, 132.94, 127.97, 126.45, 125.05, 124.04, 121.30, 115.80, 110.78, 67.36, 31.38, 28.99, 9.12, 8.87. HR-ESI-MS: Calcd for $C_{21}H_{19}N_3O_6S$ $[M+H]^+$: 432.10728; found: 432.10678.

*4.1.10 7-chloro-5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-2,3,4,5-tetrahydrobenzo[b][1,4] oxazepine (**BA-3f**)*

Light yellow solid, yield: 86%. M.p. 122-123°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68-7.59 (m, 4H), 7.53 (d, J=2.6 Hz, 1H), 7.31 (s, 1H), 7.19 (dd, J=8.6, 2.6 Hz, 1H), 6.91 (d, J=8.6 Hz, 1H), 4.15-3.58 (m, 4H), 2.20-2.08 (m, 1H), 2.00-1.80 (m, 2H), 1.21-1.07 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.09, 155.10, 148.63, 139.23, 133.15, 132.15, 130.88, 129.28, 128.51, 128.02, 124.59, 123.76, 123.38, 70.95, 48.78, 29.23, 9.10, 8.71. HR-ESI-MS: Calcd for $C_{21}H_{19}ClN_2O_4S$ $[M+H]^+$: 431.08323; found: 431.08182.

*4.1.11 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-8-fluoro-2,3,4,5-tetrahydrobenzo[b][1,4] oxazepine (**BA-3g**)*

White solid, yield: 78%. M.p. 146-147°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 4H), 7.48 (dd, J=8.9, 6.2 Hz, 1H), 7.30 (s, 1H), 6.81 (ddd, J=8.9, 6.2, 2.9Hz, 1H), 6.69 (dd, J=8.9, 2.9 Hz, 1H), 3.97-3.64 (m, 4H), 2.20-2.07 (m, 1H), 1.97-1.78 (m, 2H), 1.20-1.04 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.04, 148.66, 139.35, 135.36, 132.45, 132.34, 132.06, 128.00, 124.56, 123.71, 111.12, 110.90, 109.75, 109.52, 77.34, 77.02, 76.70, 71.18, 48.76, 29.35, 9.09, 8.66. HR-ESI-MS: Calcd for $C_{21}H_{19}FN_2O_4S$ $[M+H]^+$: 415.11278; found: 415.11204.

*4.1.12 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-8-methoxy-2,3,4,5-tetrahydrobenzo[b][1, 4]oxazepine (**BA-3h**)*

Yellow solid, yield: 85%. M.p. 169-171°C. ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.64-7.56 (m, 4H), 7.41 (d, J=8.8 Hz, 1H), 7.30 (s, 1H), 6.65 (dd, J=8.8, 2.8 Hz, 1H), 6.50 (d, J= 2.8 Hz, 1H), 3.79 (s, 7H), 2.17-2.10 (m, 1H), 1.84 (s, 2H), 1.20-1.07 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.50, 160.24, 157.93, 147.16, 139.66, 132.12, 131.81, 128.05, 124.86, 124.37, 123.62, 109.67, 107.41, 71.22, 55.55, 48.96, 29.55, 9.11, 8.69. HR-ESI-MS: Calcd for $C_{22}H_{22}N_2O_5S$ $[M+H]^+$: 427.13277; found: 427.13143.

*4.1.13 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-8-methyl-2,3,4,5-tetrahydrobenzo[b][1,4] oxazepine (**BA-3i**)*

White solid, yield: 86%. M.p.146-147°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68-7.56 (m, 4H), 7.37 (d, J=8.1 Hz, 1H), 7.27 (s, 1H), 6.90 (d, J=8.1, 1H), 6.79(s, 1H), 3.95-3.73 (m, 4H), 2.31 (s, 3H), 2.19-2.08 (m, 1H), 1.91-1.77 (m, 2H), 1.18-1.06 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.91, 156.28, 148.76, 139.83, 139.76, 131.81, 130.84, 129.42, 128.01, 124.76, 124.39, 123.63, 122.82, 70.91, 48.90, 29.47, 21.03, 9.08, 8.65. HR-ESI-MS: Calcd for $C_{22}H_{22}N_2O_4S$ $[M+H]^+$: 411.13785; found: 411.13750.

*4.1.14 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-8-nitro-2,3,4,5-tetrahydrobenzo[b][1,4] oxazepine (**BA-3j**)*

Yellow solid, yield: 65%. M.p. 191-193°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, J=8.8, 2.6 Hz, 1H), 7.83 (d, J=2.6 Hz, 1H), 7.76-7.61 (m, 5H), 7.33 (s, 1H), 4.09-3.80 (m, 4H), 2.25-2.11 (m, 1H), 2.04-1.88 (m, 2H), 1.22-1.05 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.92, 148.38, 147.13, 138.63, 138.10, 132.59, 130.86, 127.90, 124.95, 123.99, 118.58, 118.00, 70.91, 48.71, 28.72, 9.13, 8.82. HR-ESI-MS: Calcd for $C_{21}H_{19}N_3O_6S$ $[M+H]^+$: 442.10728; found: 442.10676 .

*4.1.15 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-2,3,4,5-tetrahydrobenzo[b][1,4] thiazepine (**BA-3k**)*

White solid. yield: 78%. M.p. 129-131°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J=8.6 Hz, 2H), 7.60 (d, J=8.6 Hz, 2H), 7.56-7.47 (m, 2H), 7.32-7.26 (m,2H), 7.21 (dt, J=7.5, 1.4 Hz, 1H), 3.79 (s, 2H), 2.67-2.55 (m, 2H), 2.25-2.01 (m,3H), 1.23-1.01 (m, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ 171.11, 148.87, 142.65,139.73, 136.40, 133.79, 131.89, 130.90, 128.64, 128.36, 128.31, 124.37, 123.59, 49.91, 31.38, 30.74, 9.10, 8.67. HR-ESI-MS: Calcd for $C_{21}H_{20}N_2O_3S_2$ $[M+H]^+$: 413.09936; found: 413.09886.

*4.1.16 1-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-1,2,3,5-tetrahydrobenzo[e][1,4]oxazepine (**BA-3l**)*

Colorless solid, yield: 79%. M.p. 66-67°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.57 (m, 5H), 7.41 (t, J=7.8 Hz, 1H), 7.36 (s, 1H), 7.19 (t, J=7.8 Hz, 1H), 6.51(d, J=7.8 Hz, 1H), 5.00 (d, J=12.5 Hz, 1H), 4.67 (d, J=12.5 Hz, 1H), 4.28-4.07(m, 1H), 3.71-3.52 (m, 1H), 3.48-3.27 (m, 2H), 2.20-2.12 (m, 1H), 1.20-1.08 (m,4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.43, 148.40, 142.49, 136.67, 135.67, 132.72, 131.31, 129.57, 128.78, 128.72, 127.24, 124.95, 123.86, 60.91, 53.78, 28.42, 9.15, 8.88. HR-ESI-MS: Calcd for $C_{21}H_{20}N_2O_4S$ $[M+H]^+$: 397.12220; found: 397.12176.

*4.1.17 2-cyclopropyl-5-(4-(2,3,4,5-tetrahydro-1H-benzo[b]azepin-1-ylsulfonyl)phenyl)oxazole (**BA-3m**)*

White solid, yield: 81%. M.p.112-113°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J=8.6Hz, 2H), 7.63 (d, J=8.6 Hz, 2H), 7.32-7.28 (m, 2H), 7.21-7.16(m, 2H), 7.14-7.09 (m, 3.2 Hz, 1H), 3.99-3.39 (m, 2H), 2.49-2.29 (m, 2H), 2.23-2.08 (m, 1H), 1.88-1.75 (m, 2H), 1.65-1.48 (m, 2H), 1.20-1.07 (m, 4H). ^{13}CNMR (100 MHz, $CDCl_3$) δ 167.03, 148.69, 141.86, 140.61, 139.73, 131.79, 130.20, 129.53, 128.15, 127.79, 126.99, 124.42, 123.83, 50.96, 34.15, 29.69, 25.66, 9.09, 8.68. HR-ESI-MS: Calcd for $C_{22}H_{22}N_2O_3S$ $[M+H]^+$: 395.142394; found: 395.14239.

*4.1.18 2-cyclopropyl-5-(4-(4-methyl-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-1-ylsulfonyl) phenyl)oxazole **(BA-3o)***

Yellow solid, yield: 46%. M.p. 108-110°C. ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.76-7.67 (m, 4H), 7.37 (d, J=7.2 Hz, 1H), 7.32 (s, 1H), 7.27-7.20 (m, 2H), 7.19-7.11 (m, 1H), 3.26 (s, 2H), 3.13-2.68 (m, 4H), 2.22 (s, 3H), 2.16-2.10 (m, 1H), 1.23-1.06 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.14, 148.55, 140.27, 139.36, 132.06, 130.88, 129.12, 128.33, 128.02, 127.78, 124.59, 123.91, 60.34, 57.46, 47.86, 42.70, 9.11, 8.76. HR-ESI-MS: Calcd for $C_{22}H_{23}N_3O_3S$ $[M+H]^+$: 410.15384; found: 410.15406.

*4.1.19 4-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-3,4-dihydro-2H-benzo[b][1,4]oxazine **(BA-4a)***

Yellow solid, yield: 88%. M.p. 114-115°C. ^{1}H NMR (400 MHz, $CDCl_3$)δ7.86 (dd, J=8.2, 1.5 Hz, 1H), 7.67-7.57 (m, 4H), 7.30 (s, 1H), 7.08 (ddd, J=8.2, 7.4, 1.5 Hz, 1H), 6.95 (ddd, J=8.2, 7.4, 1.5 Hz, 1H), 6.80 (dd, J=8.2, 1.5 Hz, 1H),4.01-3.83 (m, 2H), 3.83-3.62 (m, 2H), 2.16-2.06 (m, J=8.1, 5.3 Hz, 1H), 1.18-1.07 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.85, 137.25, 132.68, 127.89, 126.52, 124.90, 124.87, 124.07, 123.67, 120.98, 117.58, 62.63, 44.39, 9.09, 8.75. HR-ESI-MS: Calcd $C_{20}H_{18}N_2O_4S$ $[M+H]^+$: 383.10655; found: 383.10545.

*4.1.20 5-(4-(2H-benzo[b][1,4]thiazin-4(3H)-ylsulfonyl)phenyl)-2-cyclopropyloxazole (**BA-4b**)*

Yellow solid, yield: 86%. M.p. 147-148°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (dd, J= 6.0, 3.6 Hz, 1H), 7.58 (s, 4H), 7.30 (s, 1H), 7.11 (dt, J=7.2, 3.6 Hz, 2H), 7.08-7.03 (m, 1H), 4.08-3.96 (m, 2H), 2.96-2.85 (m, 2H), 2.20-2.08 (m, 1H),1.19-1.07 (m, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ 167.16, 148.28, 138.86, 134.15,132.38, 128.34, 128.21, 127.78, 126.92, 126.77, 124.75, 123.91, 44.82, 25.92, 9.09, 8.73. HR-ESI-MS: Calcd for $C_{20}H_{18}N_2O_3S_2$ $[M+H]^+$: 399.08371; found: 399.08235.

*4.1.21 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-8-amine (**BA-3n**)*

To a solution of **BA-3j** (32 mg, 0.07 mmol) in 3 mL ethanol was added Et_3N (0.31 mmol), formic acid (0.3 mmol) and palladium on activated carbon (5 mg) in sequence. The reaction mixture was refluxed for 2h, then cooled down to room temperature, then filtered and collected the filtrate. After removal of the solvent in vacuum, the crude product was purified by column chromatography (CH_2Cl_2: MeOH=20:1) to obtain compound BA-3n as light yellow solid, yield: 90%. M.p. 91-92°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ7.72-7.54 (m, 4H), 7.36-7.21 (m,2H), 6.39 (dd, J=8.5, 2.6 Hz, 1H), 6.25 (d, J=2.6 Hz, 1H), 4.23-3.59 (m, 5H), 2.23-2.04 (m, 1H), 2.03-1.71 (m, 2H), 1.18-1.04 (m, 4H).^{13}C NMR (100 MHz,$CDCl_3$) δ 166.85, 157.86, 148.86, 147.82, 139.84, 132.27, 131.68, 128.08, 124.28,123.54, 122.43, 110.54, 107.88, 71.13, 49.05, 29.63, 9.07, 8.60. HR-ESI-MS: Calcd for $C_{21}H_{21}N_3O_4S$ $[M+H]^+$: 412.13310; found: 412.13156.

*4.1.22 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-2,3,4,5-tetrahydrobenzo[b][1,4] thiazepine 1,1-dioxide (**BA-3p**)*

To a solution of **BA-3k** (100 mg, 0.24 mmol) in 3 mL CH_2Cl_2 in an ice bath, was added m-CPBA (86 mg, 0.50 mmol). The mixture was stirred at room temperature for 2h. The solvent was removed under reduced pressure and purified by column chromatography (petroleum ether / ether acetate =3:1) to give compound BA-3p as a white solid, yield: 86%. M.p. 203-205°C. ^{1}H

NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J=7.9, 1H), 7.94 (d, J= 8.6 Hz, 2H), 7.75 (d, J=7.9 Hz, 1H), 7.69 (d, J=8.6 Hz, 2H),7.64 (t, J=7.9, 1H), 7.53-7.47 (m, 1H), 7.33 (s, 1H), 3.26-3.04(m, 2H), 2.22-2.04(m, 3H), 1.21-1.04 (m, 4H). ^{13}C NMR (100MHz, $CDCl_3$) δ 148.57, 138.58, 138.36,137.61, 134.41, 132.73, 129.96, 129.28, 129.08, 128.29, 124.84, 123.72, 53.88, 49.70,24.55, 9.10, 8.71. HR-ESI-MS:Calcd for $C_{21}H_{20}N_2O_5S_2$ $[M+H]^+$: 445.08919; found: 445.08926.

*4.1.23 5-(4-(2-cyclopropyloxazol-5-yl)phenylsulfonyl)-2,3,4,5-tetrahydrobenzo[b][1,4] thiazepine 1-oxide (**BA-3q**)*

To a solution of BA-3k (100 mg, 0.24 mmol) in 3 mL CH_2Cl_2 in an ice bath, was added m-CPBA (43 mg, 0.25 mmol). The mixture was stirred at room temperature for 2h. After finished, the solvent was removed under reduced pressure and purified by column chromatography (petroleum ether / ether acetate =3:1) to give compound BA-3p as a white solid in 77% yield. M.p. 157-158°C. ^1H NMR (400 MHz, $CDCl_3$) δ7.78-7.80(m, 3H), 7.67-7.70(m, 2H), 7.59-7.63(m, 1H), 7.46-7.53(m, 2H), 7.35(s, 1H), 4.40-4.44(m, 1H), 3.18-3.23(m, 1H), 2.98(t, J=12.0 Hz, 1H), 2.83-2.90(m, 1H), 2.42-2.52(m, 1H), 2.13-2.20(m, 1H), 2.03-2.07(m, 1H),1.12—1.21(m, 4H). ^{13}C NMR (100MHz, $CDCl_3$) δ163.33, 148.38, 144.42, 138.61, 135.52, 132.70, 131.43, 129.73, 129.40, 128.18, 125.20, 124.94, 124.02, 53.43, 48.76, 25.96, 9.11, 8.78. HR-ESI-MS: Calcd for $C_{21}H_{20}N_2O_4S_2$ $[M+H]^+$: 429.09427; found: 429.09253.

4.2 Pharmacological protocols

4.2.1 Cell culture

HCT116, PC-3, HepG2, SK-OV-3, MCF-7, KB, K562 cell lines were from ATCC (ATCC, Rockville, MD). KB/VCR, K562/A02, MCF-7/ADR were from Institute of Hematology and Blood Disease Hospital（Tianjin, CAMS). The cells were maintained in RPMI 1640 medium supplemented with 10% FBS (fetal bovine serum), 1 mmol/L sodium pyruvate, 2 mmol/L L-Glutamine. FBS was heat inactivated for 30 min at 56°C before use. Cell cultures were grown at 37°C, in a humidified atmosphere of 5% CO_2, in a CO_2 incubator.

4.2.2 Cell viability analysis

All cells used in the research were prepared at 3.5×10^4 cells/mL concentration and each 100 mL cells suspension was seeded in 96-well cell imcroplate for 24 h (37 ºC, 5% CO_2). Then each solution was added and incubated for another 72 h. For the control group, equivalent concentration of DMSO (final concentration 0.5%) was added. MTT (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide) method was employed to measure the number of surviving cells and recorded the OD value at 492nm/620 nm. The IC_{50} values were calculated using Prism Graphpad software of the triplicate experiment.

4.2.3 Tubulin polymerization assay

Microtubule-associated protein-rich tubulin (2 mg/mL, bovine brain, Cytoskeleton) in buffer containing 80 mM PIPES (pH 6.9), 2 mM $MgCl_2$, 0.5 mM EGTA, and 5% glycerol was placed in cuvettes, 200 μL/assay, and incubated respectively with DMSO, 1 μM compound BA-3b and BA-3g, 3 μM Nocodazole, and 10 μM Taxol, respectively. Polymerization was started by adding 1 mM GTP and incubating at 37 °C, followed by absorption readings at 340 nm with a Varian Cary 50 series spectrophotometer.

4.2.4 Tubulin intensity assay

A549 cells were treatment for 24h in the presence of 200 nM of BA-3b and BA-3g, 50 nM vinblastine, and stained for α-tubulin (green) with tubulin primary antibody and dylight488 secondary antibody, and nucleus (blue) with Hoechst 33258, images were taken by a fluorescence microscopy.

4.2.5 SW480 cell apoptosis analysis

The effect of compound on apoptosis was assessed by flow cytometer. The human cancer cells SW480 were cultured in 60 mm dishes to 70%-80% confluence. After treated with DMSO, BA-3b for 24 hours, all the cells were harvested and fixed by 70% alcohol. Then the fixed samples were washed twice by cold PBS buffer, incubated in staining solution (5μg/ml propidium iodide, 100 μg/ml RNase, 0.2% Triton X-100) at 37 °C for 30 min and analyzed by flow cytometry.

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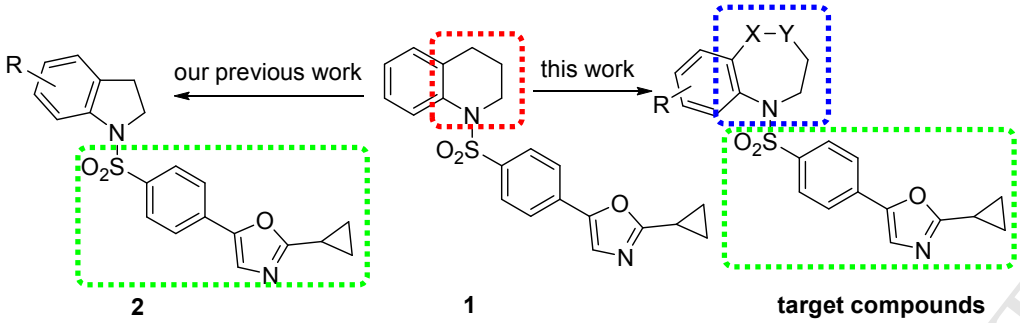
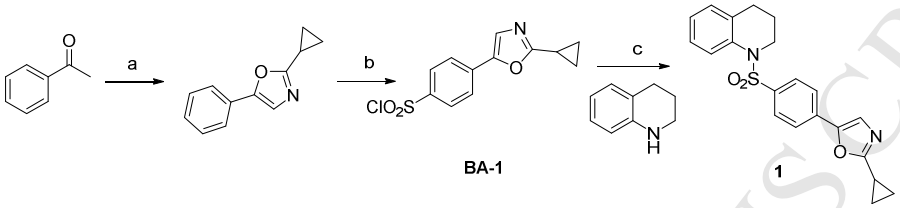
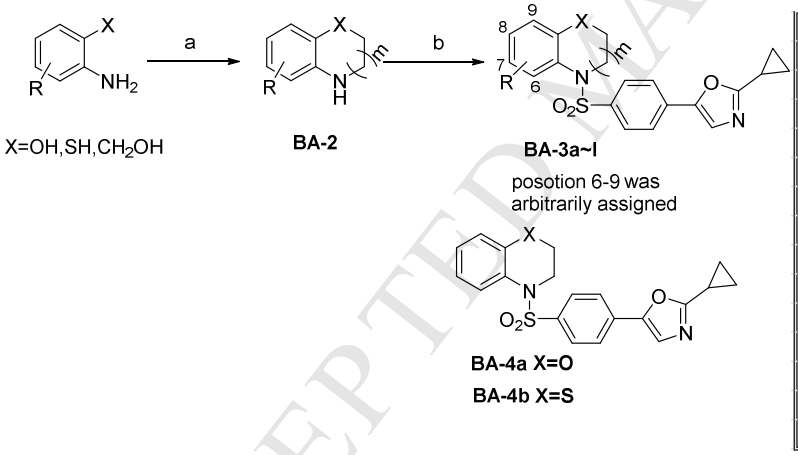


Figure 1. New tubulin-targeting drug design

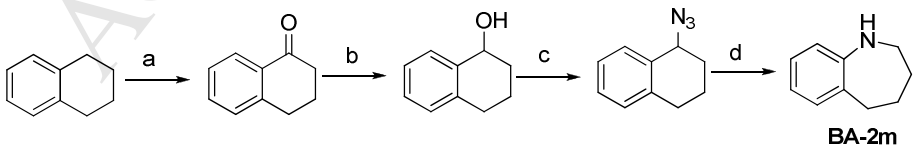


Scheme 1. Reagents and conditions: a) $PhI(OAc)_2$, TfOH, cyclopropanecarbonitrile, DCE, 80°C; b) Chlorosulfonic acid, DCM, 50°C; c) Pyridine, DCM, rt, 2h.

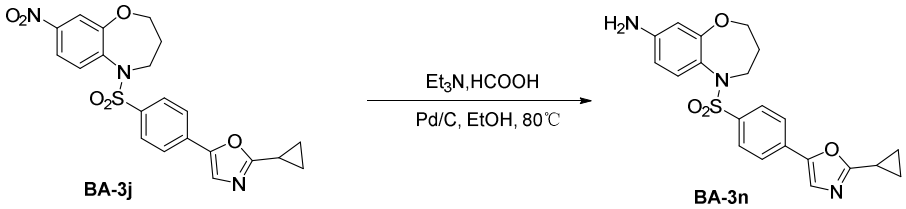


	X	m	R
BA-3a	O	2	H
BA-3b	O	2	7-F
BA-3c	O	2	7-MeO
BA-3d	O	2	7-Me
BA-3e	O	2	7-NO_2
BA-3f	O	2	7-Cl
BA-3g	O	2	8-F
BA-3h	O	2	8-MeO
BA-3i	O	2	8-Me
BA-3j	O	2	8-NO_2
BA-3k	S	2	H
BA-3l	CH_2O	1	H
BA-4a	O	1	H
BA-4b	S	1	H

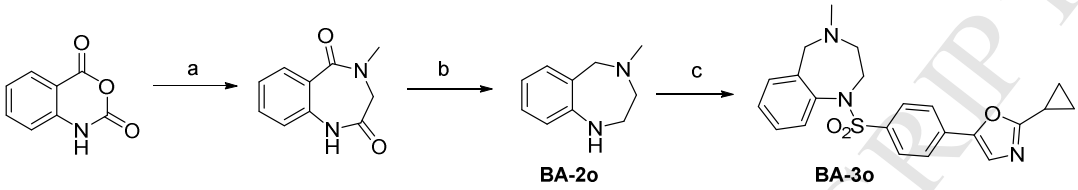
Scheme 2. a) m=1, 1,2-dibromoethane, K_2CO_3/DMF, 60°C; m=2, 1,3-dibromopropane, K_2CO_3/DMF, 60°C; b) **BA-1**, pyridine, DCM, rt, 2-3h.



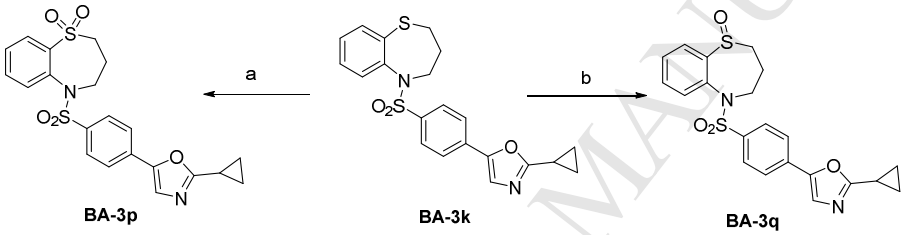
Scheme 3. a）$FeCl_3{\cdot}6H_2O$, TBHP(70%), pyridine, 82°C; b) $NaBH_4$/MeOH, 0°C; c) triethylamine,methanesulfonyl chloride, CH_2Cl_2, then NaN_3, DMF; d) $BF_3{\cdot}OEt_2$, anhydrous CH_2Cl_2. -78°C, then $NaBH_4$, NaOH(15% aqueous).



Scheme 4. The route of introduce amine: a) Et_3N, HCOOH, Pd/C, EtOH, 80°C

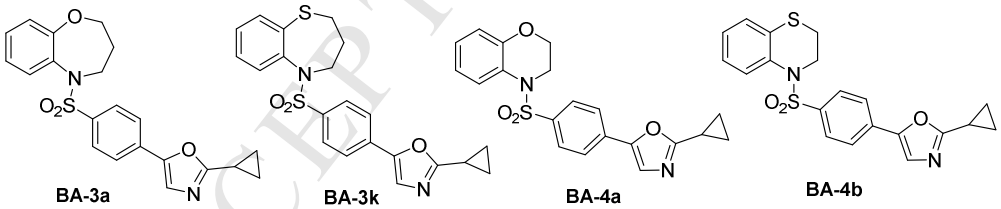


Scheme 5. The route of introduce tert-amine: a) Sarcosine, DMSO, 180°C; b) $LiAlH_4$, THF, 0°C; c) **BA-1**, pyridine, DCM.



Scheme 6. Synthesis of sulfone and sulfoxide from sulfide: a) 2.10 equiv mCPBA, DCM, r.t; b) 1.05 equiv mCPBA, DCM, r.t

Table 1. IC_{50} values of fist-run compounds over four cancer cell lines[a].



Comd No.	IC_{50}(μM)[a]			
	HCT116	PC3	HepG2	SK-OV-3
BA-3a	0.040	0.027	0.040	0.036
BA-3k	0.088	0.034	0.027	0.049
BA-4a	0.071	0.077	0.058	0.047
BA-4b	0.045	0.049	0.048	0.039
1	0.049	0.038	0.029	0.047

[a]MTT assays were used for evaluation, and values were expressed as mean IC_{50} of the triplicate experiment.

Table 2. Antiproferliferative effects of benzoxazepane derivatives

Compd No.	$IC_{50}(\mu M)$[a]			
	HCT116	PC3	HepG2	SK-OV-3
BA-3b	0.024	0.018	0.036	0.015
BA-3c	0.047	0.014	0.049	0.053
BA-3d	0.029	0.041	0.031	0.034
BA-3e	0.133	0.119	0.149	0.053
BA-3f	0.039	0.018	0.028	0.019
BA-3g	0.030	0.042	0.065	0.028
BA-3h	0.223	0.271	0.229	0.185
BA-3i	0.766	0.241	0.795	0.179
BA-3j	0.145	0.072	0.071	0.120
BA-3l	0.187	0.137	0.242	0.175
BA-3m	0.072	0.039	0.073	0.038
BA-3n	0.087	0.033	0.100	0.056

[a]MTT assays were used for evaluation, and values were expressed as mean IC_{50} of the triplicate experiment.

Table 3. Antiproferliferative effects of benzothiazepine and benzodiazepine derivatives

Compd No.	$IC_{50}(\mu M)$[a]			
	HCT116	PC3	HepG2	SK-OV-3
BA-3k	0.088	0.034	0.027	0.049
BA-3p	0.263	5.882	2.174	1.616
BA-3q	>1.00	>1.00	>1.00	>1.00
BA-3o	0.256	0.347	0.525	0.199

[a] MTT assays were used for evaluation, and values were expressed as mean IC_{50} of the triplicate experiment.

Table 4. IC_{50} values of compounds in three MDR and drug-sensitive parental cell lines

Compd No.	$IC_{50}(\mu M)\pm$ standard deviation[a]					
	K562	K562/A02	KB	KB/V	MCF-7	MCF-7/A
BA-3b	0.008 *±0.002*	0.013 *±0.002*	0.007 *±0.002*	0.007 *±0.002*	0.021 *±0.007*	0.020 *±0.008*
BA-3g	0.014 *±0.004*	0.046 *±0.007*	0.015 *±0.004*	0.012 *±0.003*	0.006 *±0.002*	0.012 *±0.007*
Dox	0.006 *±0.001*	5.961 *±0.478*	0.141 *±0.019*	1.289 *±1.138*	0.140 *±0.097*	2.940 *±0.257*
VCR	0.004 *±0.002*	0.926 *±0.132*	0.036 *±0.034*	0.871 *±0.366*	0.029 *±0.003*	0.308 *±0.037*

a MTT assays were used for evaluation, and values were expressed as mean IC_{50} of the triplicate experiment.

Dox: doxorubicin，VCR: Vincristine.

K562/A02: K562 resistant to Dox; KB/V: KB resistant to VCR; MCF-7/A: MCF-7 resistant to Dox.

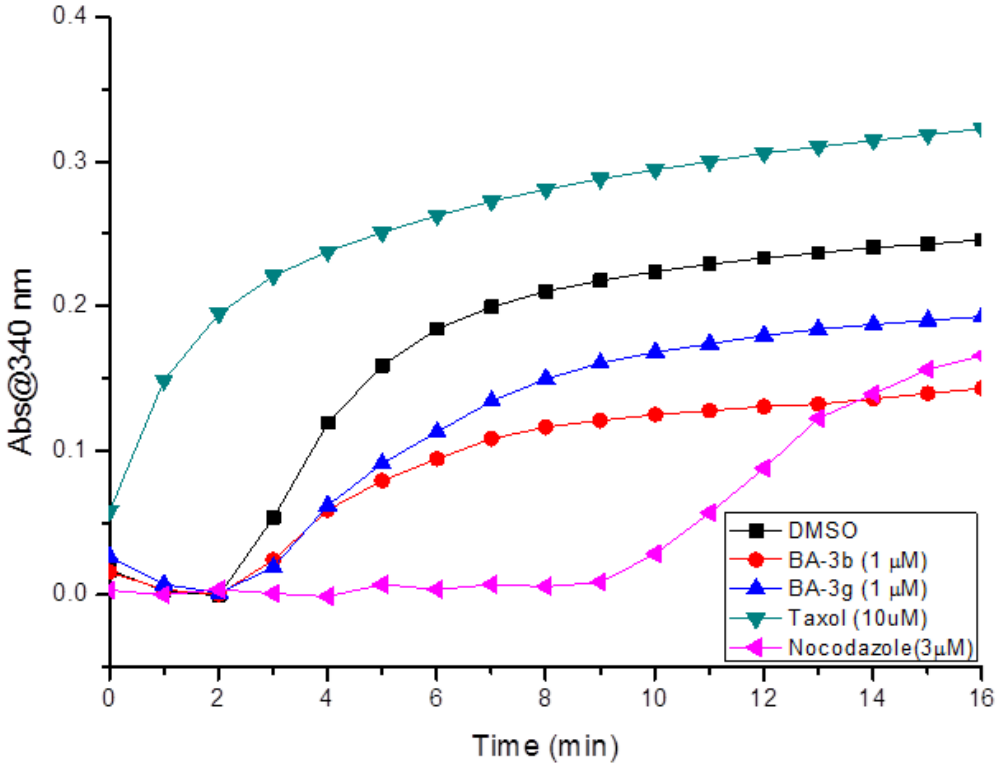


Figure 2. Tubulin polymerization in the presence of **BA-3b**, **BA-3g**, nocodazole, and taxol

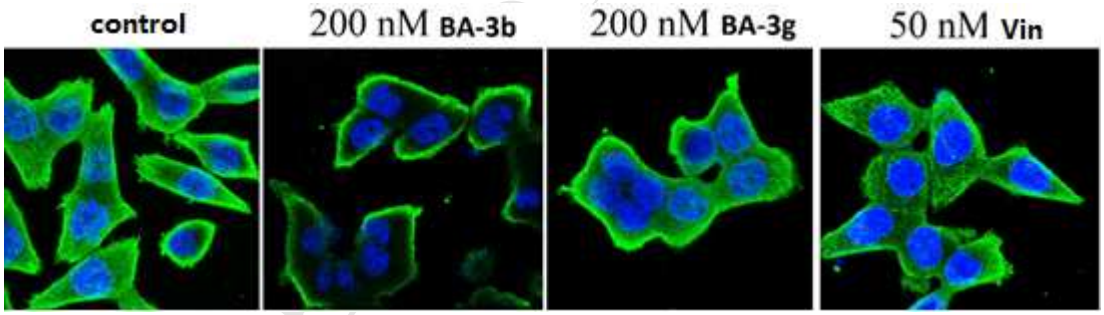
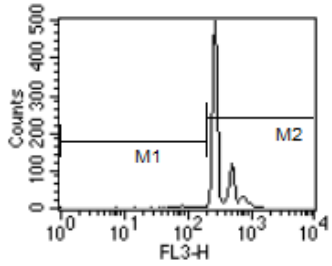


Figure 3. Tubulin intensity assay (DMSO as control, Vin for vinblastine)



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Gated Events: 12084
Total Events: 13376

Marker	Events	% Gated
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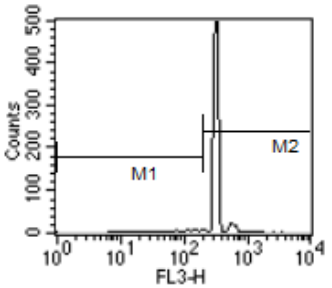
M1	412	3.41
M2	11683	96.68



File: 2015-0407PI.001
Gate: G1
Gated Events: 11283
Total Events: 14340

Marker	Events	% Gated
All	11283	100.00
M1	873	7.74
M2	10412	92.28

Figure 4. **BA-3b** induce apoptosis in SW480 cells

Highlights

- A series of novel sulfonamide derivatives were synthesized.
- The antiproliferative activities against four cancer cell lines were determined.
- These compounds exert antitumor effect via tubulin depolymerization.
- **BA-3b** was very potent against both drug-sensitive and MDR cancer cell lines.