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Research paper

# 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.



## 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.

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. Chemistry

The reference compound 1 was synthesized following the

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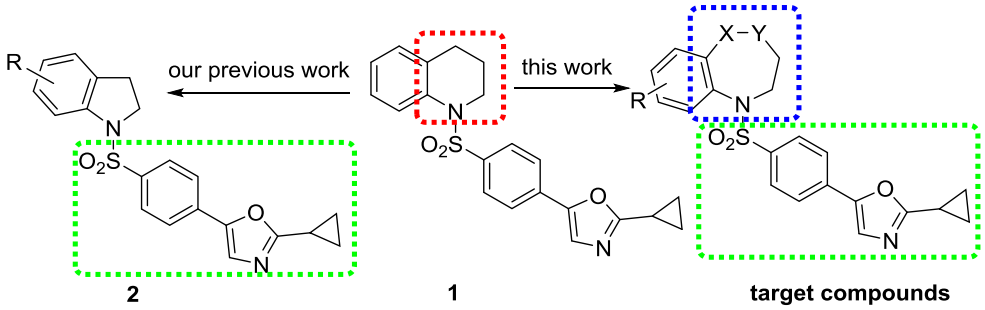



**Fig. 1.** New tubulin-targeting drug design.

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.

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.

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 Schemes 4–6.

## 2.2. Biological 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 Table 1). So we designed a series of benzoxazepine derivatives and evaluated their cytotoxic potency, as shown in Table 2. All compounds exhibited excellent cytotoxic activities.

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.

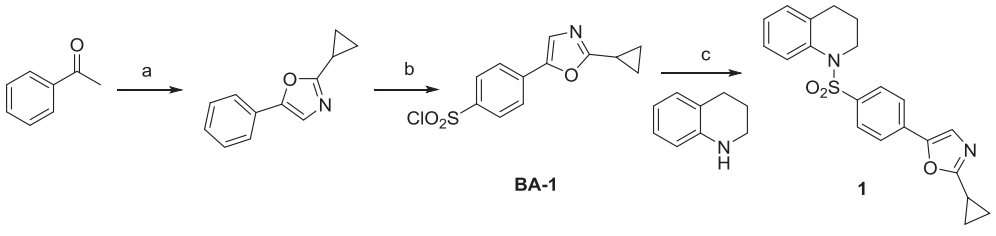
Of the compounds designed to improve water solubility, only compound **BA-3n** exhibited potent activity. The rest two compounds (**BA-3p** and **BA-3q**) showed a sharply decrease in activity than the benzenothiozepine derivative **BA-3K**, which turned out to be a failure of their structural modification,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

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.

### 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 polymerization in 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.

The effects of these compounds on tubulin polymerization were




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