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Synthesis, antiproliferative activities and in vitro biological evaluation of novel benzofuransulfonamide derivatives

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ABSTRACT

In a cell-based screen of novel antiproliferative agents, the hit compound **1a**, which bears a benzofuransulfonamide scaffold, exhibited broad-spectrum antiproliferative activities against a panel of tumor cell lines. The promising in vitro antiproliferative activity and structural novelty of **1a** prompted us to investigate the synthesis of five analogs of **1a** and test their antiproliferative activities. The most potent analogue, **1h**, exhibited enhanced antiproliferative activities compared with the parent **1a**, and exhibited an IC_{50} value against NCI-H460 cells of 4.13 μM compared with 4.52 μM for the positive control cisplatin. Flow cytometric analysis revealed that **1h** induces significant levels of apoptosis in NCI-H460 cells in vitro at low micromolar concentrations. These results suggest that **1a** and analogs based on its benzofuransulfonamide scaffold may constitute a novel class of antiproliferative agents, which deserve further study.

Cancer is the second most important cause of death in the world and its incidence and resultant mortality continues to increase rapidly.[1] Chemotherapy with various antiproliferative agents is one of the most commonly used clinical treatment options, but side effects and multidrug resistance (MDR) make current treatment regimens far from satisfactory.[2] The development of novel chemotherapeutic agents with excellent antiproliferative activity and high therapeutic indexes therefore remains an important target.

Our research group is interested in the design, screening, synthesis and biological evaluation of novel antiproliferative agents. Recently, a series of promising hit compounds, including **1a**, **1b**,[3] and **1c**,[4] have been found in a cell-based screen of antiproliferative agents (Fig. 1). Among them, compound **1a**, bearing a benzenesulfonamide substituted benzofuran scaffold, exhibited broad-spectrum activity to inhibit the proliferation of a panel of human tumor cell lines in vitro (see Table 1).

Benzofuran derivatives, either natural or synthetic, demonstrate a variety of biological activities.[5] They have been reported to exhibit antiproliferative activity via different mechanisms, such as inhibition of tubulin polymerization[6], inhibition of histone deacetylase enzymes[7], inhibition of angiogenesis[8] and induction of apoptosis[9], among others.[10]

To the best of our knowledge, the antiproliferative activity of benzenesulfonamide substituted benzofuran derivatives such as **1a** was first reported by our group.[11] Given the promising in vitro activity and structural novelty of **1a**, we examined its antiproliferative activity further. Herein, we report the synthesis of **1a** and its analogs, preliminary structure–activity relationships (SARs) and biological evaluation of this novel class of antiproliferative agents.

No report on the synthesis of compound **1a** had been published when we tackled its synthesis, and the synthetic route shown in Scheme 1 was designed and explored. The starting material 4-aminoanisole, **2**, was acetylated with acetic anhydride in dichloromethane to give **3**. Friedel–Crafts acylation of acetamide **3**,[12] followed by condensation with bromoacetophenone accomplished the annulation of benzofuran to yield **1d**. Hydrolysis of the amide group of **1d** with hydrochloric acid gave the key intermediate **5**. Then, **1a** was obtained by sulfonylation of **5** with *p*-fluorobenzenesulfonyl chloride in dichloromethane with pyridine as the base.

Recently, sulfonamides have been reported to show substantial antiproliferative activity both in vitro and in vivo.[13] To investigate whether the aryl sulfonamide moiety in **1a** is necessary for its antiproliferative activity, analogs **1d** and **1e**, which have different acyl groups at the 5-position, were also synthesized. As shown in Scheme 1, **1d** was an intermediate in the synthesis of **1a**, and **1e** could be conveniently obtained by the reaction of **5** with *p*-fluorobenzoyl chloride.

Efforts were also made to incorporate different groups such as an ester group at the 2-position, to investigate the influence of substituents at this position on antiproliferative activity. The target

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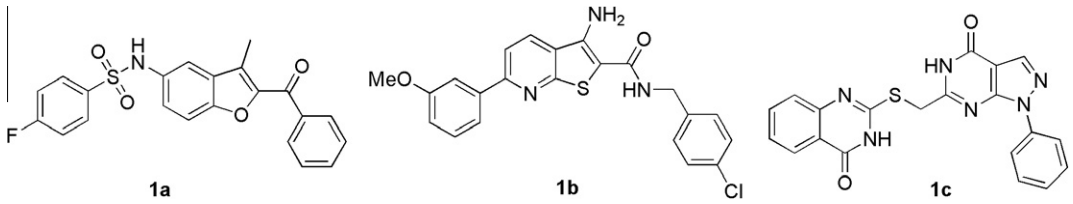


Figure 1. Hit compounds found in a cell-based screening of antiproliferative agents.

Table 1
The antiproliferative activities of compounds **1a**, and **1d**–**h** against various cancer cell lines

Compound	IC_{50}[a] (μM)			
	HepG2	NCI-H460	HeLa	PC-3
1a	24.1 ± 3.3	34.8 ± 2.7	10.6 ± 0.7	38.2 ± 3.4
1d	>40	>40	>40	>40
1e	31.2 ± 2.1	15.6 ± 1.7	18.8 ± 1.4	13.5 ± 0.6
1f	5.68 ± 0.28	8.89 ± 0.51	12.2 ± 1.3	15.9 ± 1.3
1g	32.2 ± 2.9	22.6 ± 1.6	15.4 ± 0.9	22.1 ± 1.5
1h	7.53 ± 0.38	4.13 ± 0.27	9.37 ± 0.44	8.56 ± 0.53
Cisplatin	16.3 ± 0.6	4.52 ± 0.27	3.40 ± 0.14	0.25 ± 0.02

[a] The cytotoxic effect of compounds on various cancer cell lines were determined using the MTT assay.[17] The results were expressed as the IC_{50}, and were the means calculated from three independent experiments.

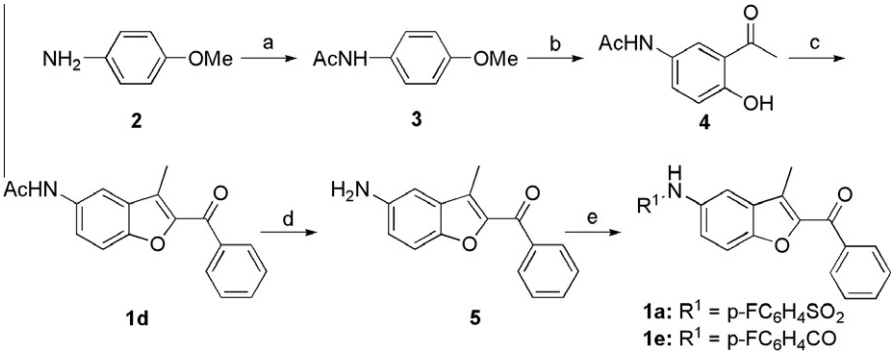
compound **1f** was successfully synthesized using **4** as the starting material (Scheme 2).

As shown in Scheme 2, the solvent-free reaction of **4** and ethyl bromoacetate in the presence of potassium carbonate[14] gave **6**, which was hydrolyzed to afford **7**. Target compound **1f** was then prepared by the sulfonylation of **7** with *p*-fluorobenzenesulfonyl chloride in dichloromethane with pyridine as the base.

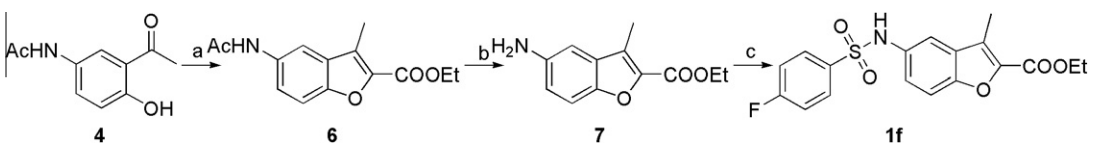
Retro-inverso modification is a useful tool for analog design and drug development, and it prompted us to target **1g** and **1h**, which have a *p*-fluorophenylaminosulfonyl group instead of the *p*-fluorophenylsulfonylamino group at the 5-position found in **1a** and **1f**. The synthesis of **1g** and **1h** was achieved by the acylation of *p*-fluoroaniline with substituted benzofuransulfonyl chloride, as illustrated in Scheme 3.

o-Hydroxyacetophenone was condensed with bromoacetophenone in the presence of potassium carbonate in DMF, or reacted with ethyl bromoacetate under solvent-free conditions to generate annulated product **9**. This was chlorosulfonated with chlorosulfonic acid in dichloromethane to afford **10**. Then, **1g** and **1h** were generated by the sulfonation of *p*-fluoroaniline with **10**, using pyridine as the base, in dichloromethane.

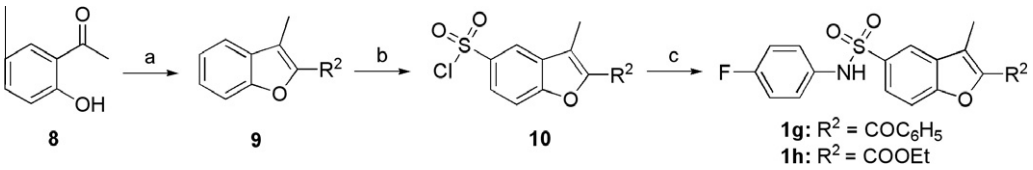
The regioselectivity of the chlorosulfonation of benzofuran **9** is likely to be directed by higher electron density at positions 5 and 6, compared to that at positions 4 and 7. It could be seen by ^{1}H NMR of **10** (R^2 = COOEt) that an AB system of aromatic hydrogens was present, with a coupling constant of 6.6 Hz. The same coupling constant was observed in compounds **1g** and **1h**. The chlorosulfonyl group must therefore have been introduced at the 5- or 6-position, but the exact substitution position remained unconfirmed. Although methyl 3-methylbenzofuran-2-carboxylate has been reported to be chlorosulfonated to give methyl 5-chlorosulfonyl-3-methylbenzofuran-2-carboxylate,[15] we do not believe that the regioselectivity of the chlorosulfonylation can be unambiguously assigned by ^{1}H NMR alone, as provided in the literature. As **1h** displayed the most potent antiproliferative activity of the compounds synthesized in the MTT assay, a single crystal of **1h** was cultivated and X-ray single crystal diffraction was carried out.[16] The result (Fig. 2) unambiguously established the position of the substituted side chain as the 5-position of benzofuran, therefore revealing that the chlorosulfonation of benzofuran **9** takes place at the 5-position. Furthermore, the X-ray single crystal diffraction result may also be helpful to determine the regioselectivity of electrophilic substitution on the benzofuran ring with substituents at the 2- and 3-positions.



Scheme 1. Synthetic route adopted to compounds **1a**, **1d**, and **1e**. Reagents and conditions: (a) Ac_2O, CH_2Cl_2, rt, 2 h, 92%; (b) AcCl, $AlCl_3$, CH_2Cl_2, reflux, 4.5 h, 82%; (c) $C_6H_5COCH_2Br$, K_2CO_3, DMF, 120 °C, 1 h, 72%; (d) 3 M HCl, EtOH, 90 °C, 4.5 h, 77%; (e) *p*-$FC_6H_4SO_2Cl$ or *p*-FC_6H_4COCl, Py, CH_2Cl_2, rt, 0.5 h, 70% for **1a** and 68% for **1e**.



Scheme 2. Synthetic route to compound **1f**. Reagents and conditions: (a) $BrCH_2COOEt$, K_2CO_3, N_2, 80 °C for 0.5 h then 160 °C for 1.5 h, 30%; (b) 3 M HCl, EtOH, 90 °C, 4.5 h, 72%; (c) *p*-$FPhSO_2Cl$, Py, CH_2Cl_2, rt, 0.5 h, 54%.



Scheme 3. Synthetic route to compounds **1g** and **1h**. Reagents and conditions: (a) $C_6H_5COCH_2Br$, K_2CO_3, DMF, 120 °C, 1 h, 76%/$BrCH_2COOEt$, K_2CO_3, N_2, 80 °C for 1 h then 160 °C for 2.5 h, 45%; (b) $ClSO_3H$, CH_2Cl_2, 1 h, 41%/39%; (c) *p*-$FC_6H_4NH_2$, Py, CH_2Cl_2, 77%/72%.

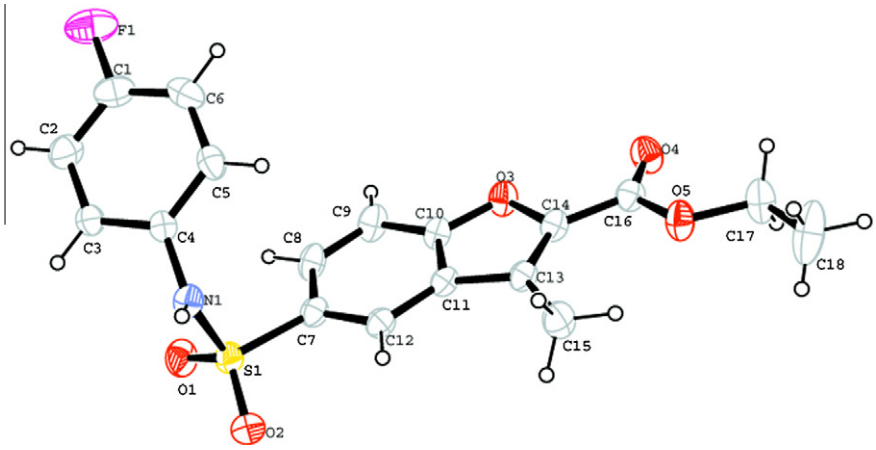


Figure 2. Ortep diagram of the crystal structure of compound **1h**, with displacement ellipsoids drawn at the 50% probability level.

The antiproliferative activities of compounds **1a**, and **1d–h** were examined in four cancer cell lines: the human hepatoma HepG2 cell line, large cell lung cancer cell line NCI-H460 (H460), cervical cancer cell line HeLa, and prostate cancer cell line PC-3, using an MTT assay. Cisplatin was used as a positive control. The experimental data are summarized in Table 1.

As can be seen from Table 1, the hit compound **1a** exhibited broad-spectrum activity, inhibiting the proliferation of all four human tumor cell lines tested in vitro. Introduction of a benzamide substituent at the 5-position on the benzofuran ring (**1e**) was well tolerated, whereas introduction of an acetamide group (**1d**) led to almost total loss of activity. Replacement of the benzoyl group at the 2-position with an ester group yielded **1f**, which exhibited significantly enhanced activities, except against HeLa cells. To our delight, retro–inverso modification of the sulfonamide (**1g** and **1h**) does not compromise activity. The most potent analogue, **1h**, exhibited enhanced antiproliferative activities, and in particular, its IC_{50} value against NCI-H460 cells was 4.13 μM compared with an IC_{50} of 4.52 μM for cisplatin, the positive control. These results suggest that the target compound **1a** and its analogs based on the benzofuransulfonamide scaffold may constitute a novel class of antiproliferative agents, which deserve further study.

To further disclose the antiproliferative mechanism of **1h**, flow cytometric analysis was performed to identify and measure the apoptotic cells after Annexin V/propidium iodide (PI) staining.[18] The data are shown in Figure 3.

As can be seen in Figure 3, a 48 h exposure of NCI-H460 cells to compound **1h** showed Annexin-V positivity (the lower right quadrant and the upper right quadrant which contains early and late apoptotic cells, respectively) that was much higher than untreated cells (b: 33.88% and c: 41.09% vs a: 3.82%). These results suggest that compound **1h** induces significant levels of apoptosis in H460 cells in vitro at low micromolar concentrations.

In conclusion, compound **1a** bearing a novel benzofuransulfonamide scaffold exhibited broad-spectrum antiproliferative activities against a panel of tumor cell lines in a cell-based screen of novel antiproliferative agents. In addition, five analogs of **1a** were designed and synthesized. The most potent analogue, **1h**, exhibited enhanced antiproliferative activities, with an IC_{50} value against NCI-H460 cells of 4.13 μM, compared with an IC_{50} of 4.52 μM for

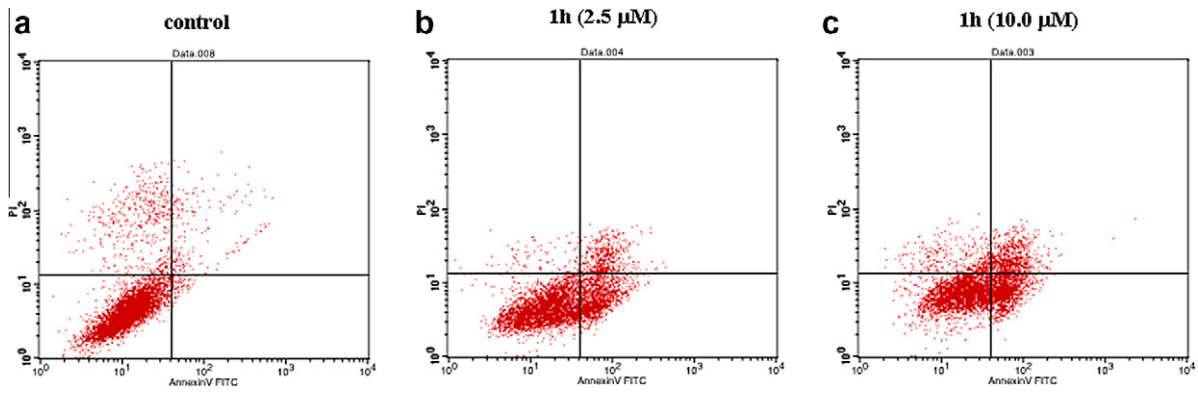


Figure 3. Bivariate annexin V/PI analysis of NCI-H460 cells after 48 h culture in the absence (a: control) or presence of **1h** (b: 2.5 μM and c: 10.0 μM).

cisplatin, the positive control. Flow cytometric analysis revealed that compound **1h** induces significant levels of apoptosis in NCI-H460 cells in vitro at low micromolar concentrations. Further SARs and mechanistic studies of these benzofuransulfonamide-based antiproliferative agents are currently in progress.

Acknowledgment

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Supplementary data

Supplementary data (the list of experimental details and spectroscopic characterization of compounds **1a**, **1d**–**h** and their intermediates) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.007.

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16. *X-ray crystal structure analysis for compound (**1h**)*. Formula $C_{18}H_{16}FNO_5S$, yellow crystal. The crystal is of monoclinic, space group P21/n with a = 12.2470(5) Å, b = 10.2822(4) Å, c = 14.9826(6) Å, β = 112.116(5)°, V = 1747.89(11) Å^3, Z = 4, Dc = 1.434 g/cm^3, F(0 0 0) = 784, T = 293(2) K, μ = 0.225 mm^{-1}, R = 0.0666 and wR = 0.1176 for 5070 observed reflections with $I > 2\sigma(I_0)$. Crystallographic data of **1h** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 814683. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
17. Briefly, cells (2000/well) were seeded in 96-well plates and cultured for 24 h, followed by treatment with the compounds for 48 h. Ten microliters of 10 mg/mL MTT was added per well and incubated for another 2.5 h at 37 °C. Then the supernatant fluid was removed and 150 μL/well DMSO was added for 15–20 min. The absorbance (OD) of each well was measured at 570 nm, using a SpectraMAX M5 microplate spectrophotometer (Molecular Devices).
18. Apoptotic cells were identified and quantified using the Annexin V-FITC kit per instructions of the manufacturer. Each measurement was carried out in quadruplicate and each experiment was repeated at least twice to ensure reproducibility. Adherent cultures served as controls for viability and apoptosis determinations. Annexin V-FITC-positive cells (the lower right quadrant) were scored as early apoptotic cells; both Annexin V-FITC- and propidium iodide-positive cells (the upper right quadrant) were scored as late apoptotic cells; unstained Annexin V-FITC and propidium iodide negative cells (the lower left quadrant) were scored as viable or surviving cells.