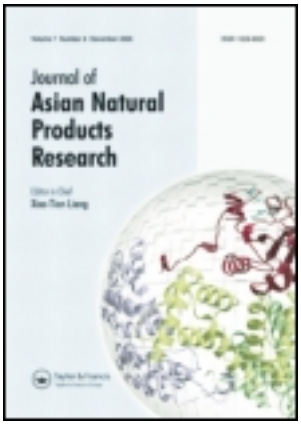

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Synthesis and cytotoxic evaluation of *N*-(4-methoxy-1*H*-benzo[*d*]imidazol-7-yl)-arylsulfonamide and *N*-aryl-(4-methoxy-1*H*-benzo[*d*]imidazol)-7-sulfonamide analogs of combretastatin A-4

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[a] Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100050, China

[b] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, 110016, China

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Synthesis and cytotoxic evaluation of *N*-(4-methoxy-1*H*-benzo[*d*]imidazol-7-yl)-arylsulfonamide and *N*-aryl-(4-methoxy-1*H*-benzo[*d*]imidazol)-7-sulfonamide analogs of combretastatin A-4

Jie Zhou[a†], Yi Zhang[a†], Yi-Wen Cui[ab], Zhan-Mei Li[ab], Hong-Rui Song[b], Jin-Hua Dong[b], Xiao-Guang Chen[a] and Bai-Ling Xu[a]*

[a]*Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China;* [b]*School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, China*

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Two series of novel benzoimidazole sulfonamides as combretastatin A-4 analogs were synthesized. The cytotoxicities of the title compounds were evaluated against five different cancer cell lines. Among the tested compounds, four compounds displayed cytotoxicities against the HCT8 cell line. Compound **6a** has shown the strongest potency against the tested human tumor cell lines with an IC_{50} value ranging from submicromolar to micromolar level.

Keywords: combretastatin; sulfonamide; benzo[*d*]imidazole; cytotoxicity

1. Introduction

Combretastatin A-4 (CA-4) (Figure 1) is a natural product containing a *cis*-stilbene fragment and first isolated from the bark of *Combretum caffrum* in 1982 by Pettit *et al.* [1]. CA-4 exhibits strong cytotoxic activities against a wide variety of cancer cell lines including multidrug-resistant cell lines with an IC_{50} value ranging from 10^{-8} to 10^{-10} M [2,3]. It has been demonstrated that CA-4 can result in the microtubule depolymerization and mitotic arrest of the cancer cells via the interaction with colchicine-binding site on tubulin [4,5]. Most interestingly, CA-4 has shown tumor vascular disrupting activity at a nontoxic dose and was referred as the vascular disrupting agent [6]. However, CA-4 has shown some disadvantages in relation to physicochemical and pharmacokinetic properties, for example the lower aqueous solubility, higher lipophilicity, and metabolic instability, which are caused by the easy isomerization from the active *cis*-isomer to the inactive *trans*-isomer [7,8].

In view of the structural simplicity, the strong *in vitro* potency, the validated microtubule-depolymerization mechanism, and the unique antiangiogenesis activity, considerable efforts have been taken in developing the novel CA-4 analogs with the improved pharmacokinetic properties employing CA-4 as a lead structure [9–11]. Disodium phosphate of CA-4 (CA-4P) (Figure 1), a water-soluble prodrug, is now under Phase II trial in the treatment of anaplastic thyroid cancer[1] [12]. AVE8062 (Figure 1), a serine derivative of AC-7739 (Figure 1), has been tested in the phase IIb/III clinical trial[2] [13].

*Corresponding author. Email: xubl@imm.ac.cn
†Both authors contributed equally to this work.

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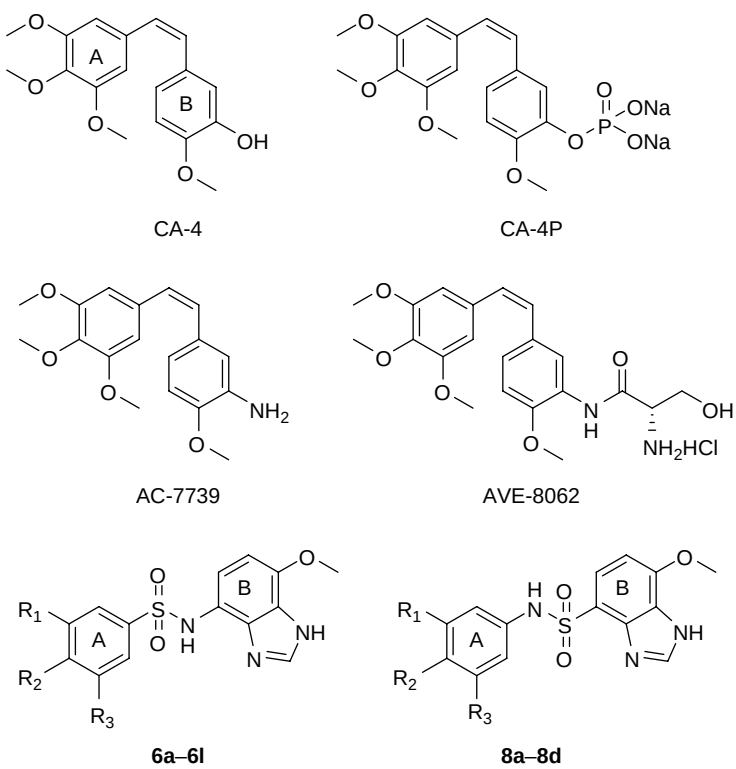
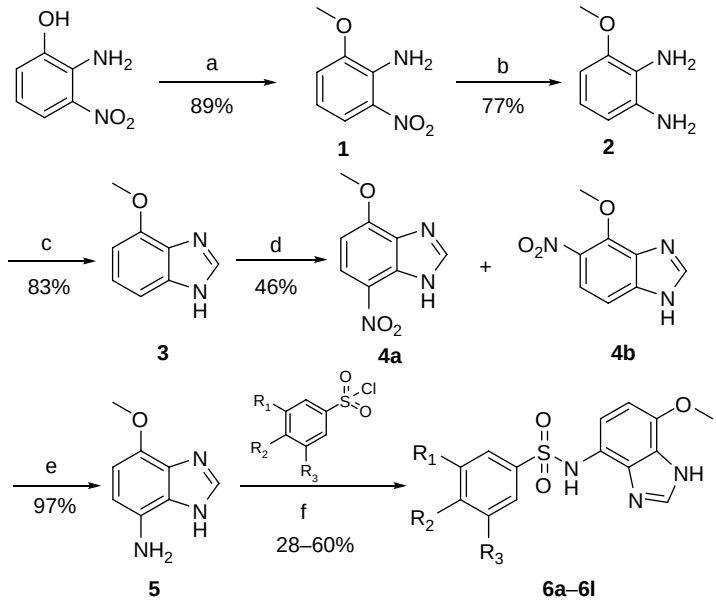


Figure 1. The structures of CA-4, CA-4 analogs and designed sulfonamide derivatives.

The structure–activity relationship (SAR) studies on a number of CA-4 analogs have disclosed that the key structural features of CA-4 derivatives comprise a 3,4,5-trimethoxyl phenyl ring as A ring, a 4-methoxyl group presented on B ring, and the *cis*-orientation of A and B rings. In this work, two series of novel CA-4 analogs containing 4-methoxy-7-amino-1*H*-benzo[*d*]-imidazole as B ring and sulfonamide as linker (**6a**–**6l**, **8a**–**8d**, Figure 1) have been designed. The 4-methoxy group was installed deliberately on the benzoimidazole ring according to the known SAR studies on B ring [9–11]. The sulfonamide linker has ever been efficiently utilized in CA-4 analogs [14–16]. The sulfonamide linker SO_2NH in the series **6a**–**6l** was reversed into $NHSO_2$ relative to A and B rings in compounds **8a**–**8d**. Besides the 3,4,5-trimethoxyl phenyl ring, other substituted phenyl groups were also exploited as A ring to examine the SAR of this new series CA-4

Table 1. Structures of *N*-(4-methoxy-1*H*-benzo[*d*]imidazol-7-yl)-arylsulfonamides (**6a**–**6l**).

Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3
6a	H	OCH_3	H	**6g**	H	OCF_3	H
6b	H	CH_3	CH_3	**6h**	H	H	OCH_3
6c	H	OCH_3	OCH_3	**6i**	H	CH_3	F
6d	H	CH_3	Cl	**6j**	H	OCH_3	F
6e	H	CH_3	NO_2	**6k**	H	Br	CH_3
6f	Cl	OH	Cl	**6l**	OCH_3	OCH_3	OCH_3



Scheme 1. Synthetic route of the *N*-(4-methoxy-1*H*-benzo[*d*]imidazol-7yl)-arylsulfonamides (**6a**–**6l**). Reagents and conditions: (a) CH_3I, K_2CO_3, DMF, rt; (b) 10% Pd/C, H_2, rt; (c) HCOOH, reflux; (d) $NaNO_3$, TFA, 70°C; (e) 10% Pd/C, H_2, rt; (f) Pyridine, DCM, rt.

analogs. The chemical synthesis and cytotoxicities of these novel CA-4 analogs are described as follows.

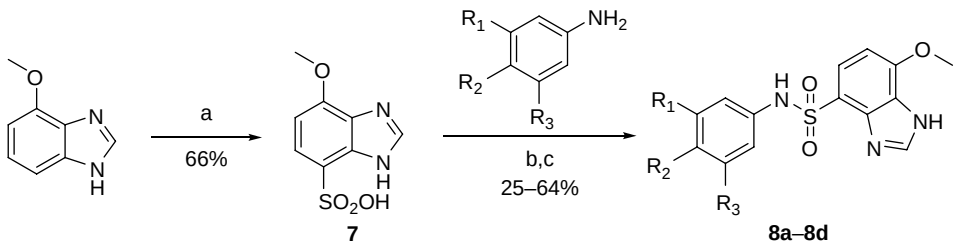
2. Results and discussion

Target arylsulfonamides **6a**–**6l** (Table 1) were synthesized according to Scheme 1. Treatment of 2-amino-3-nitrophenol with methyl iodide and K_2CO_3 provided 2-methoxy-6-nitroaniline (**1**) in 89% yield. The catalytic hydrogenation of compound **1** was performed to give compound **2** in 77% yield. Conversion of *o*-phenylenediamine (**2**) into 4-methoxy-1*H*-benzo[d]imidazole (**3**) was achieved in 83% yield in refluxing HCOOH. The nitration reaction of benzoimidazole **3** with $NaNO_3$ and trifluoroacetic acid (TFA) at 70°C furnished two constitutional isomers 4-methoxy-7-nitro-1*H*-benzo[d]imidazole (**4a**) in 46% yield and 4-methoxy-5-nitro-1*H*-benzo[d]imidazole (**4b**) in 31% yield, respectively. Subsequently, the isolated compound **4a** was reduced into key intermediate 4-methoxy-7-amino-1*H*-benzo[*d*]imidazole (**5**) in almost quantitative yield on treatment with H_2 catalyzed by 10% Pd-C. The coupling reaction of the amino-substituted benzoimidazole **5** with a variety of arylsulfonyl chloride in the presence of pyridine delivered total 12 novel sulfonamides **6a**–**6l** in 28–60% yield.

Table 2. Structures of *N*-aryl-(4-methoxy-1*H*-benzo[*d*]imidazol)-7-sulfonamides (**8a**–**8d**).

Compound	R_1	R_2	R_3
8a	H	OCH_3	H
8b	H	OCH_3	OCH_3
8c	OCH_3	OCH_3	OCH_3
8d	F	F	F

The other series of benzoimidazole-substituted sulfonamides **8a**–**8d** (Table 2) were synthesized as outlined in Scheme 2. Treatment of 4-methoxy-1*H*-benzo[d]imidazole (**3**) with $ClSO_3H$ at −5 to 0°C furnished 4-methoxy-1*H*-benzo[*d*]imidazole-7-sulfonic acid (**7**) in a yield of



Scheme 2. Synthetic route of the *N*-aryl-(4-methoxy-1*H*-benzo[*d*]imidazol)-7-sulfonamides (**8a**–**8d**). Reagents and conditions: (a) $ClSO_3H$, −5 to 0°C; (b) $SOCl_2$/reflux; (c) Et_3N, DCM, rt or pyridine, DCM, rt.

66%. Heating of compound **7** in refluxing $SOCl_2$ afforded the corresponding sulfonyl chloride, which was then reacted with a range of the substituted arylamines in the presence of Et_3N or pyridine at room temperature to produce arylsulfonamides **8a**–**8d** in 25–64% yield.

It is worth mentioning that two conformers originated from the restrict rotation of —SO_2NH— group were observed in a ratio of 4:6 with compound **6c**. The ^1H NMR spectrum of compound **6c** was obtained at various temperatures, 25, 50 and 70°C. At 25°C, in one conformer C_2–H of benzoimidazole ring resonated at 8.05 ppm, and in other conformer the signal of C_2–H of benzoimidazole ring appeared at 8.00 ppm. As the temperature was raised to 50°C, the two peaks of C_2–H from two conformers approached closely, and finally at 70°C two peaks of C_2–H in two conformers were coalescent. Similarly, it was demonstrated by calculations that two stable conformers exist with the NH_2 group eclipsing or staggering the SO_2 group in benzenesulfonamide system [17]. In our cases, it seemed likely that the ratios of two conformers are highly dependent on chemical structure of target molecules, for example with compounds **6c** and **6j**, a mixture of two conformers was observed. By contrast, in other synthesized compounds the restricted conformers were not detected by ^1H NMR spectra.

The cytotoxicities of all target sulfonamides were evaluated by MTT assay, wherein five cancer cell lines were used: HCT-8, Bel7402, BGC803, A549, and A2708. The cytotoxicities are expressed as IC_{50}. CA-4 was used as reference compound. The cytotoxic potency of the active compounds is presented in Table 3.

It was noteworthy that the substitution pattern in compounds **6a**–**6l** is highly preferred over that in reverse sulfonamides **8a**–**8d** because compounds **8a**–**8d** did not

Table 3. *In vitro* cytotoxic activities of compounds **6a**, **6d**, **6k**, **6l**, and CA-4 against five human cancer cell lines.

	Cytotoxicity (IC_{50}, μM)[a]				
Compound	HCT8	Bel7402	BGC823	A549	A2708
6a	2.65	2.15	0.77	2.72	1.61
6d	3.83	27.4	NA	NA	NA
6k	57.8	NA[b]	NA	NA	NA
6l	3.74	NA	NA	NA	NA
CA-4	0.19	0.16	0.012	0.49	4.48

Notes: [a]Compound dose (μM) required to inhibit cell growth by 50%.
[b]NA, not active.

show any activities at all against the tested tumor cell lines. In comparison with compounds **6a** and **6l**, the complete loss of activities of compounds **8a** and **8c** has further demonstrated that the substitution pattern of sulfonamides in compounds **6a** and **6l** plays a crucial role in their interactions with the biological target.

As shown in Table 3, compounds **6a**, **6d**, **6k**, and **6l** are active against HCT8 cancer cells with IC_{50} values ranging from 2.65 to 57.8 μM. Among the tested compounds, compound **6a** is the most potent and showed cytotoxicities against all tested tumor cell lines at lower micromolar level. Compound **6a** exhibited a stronger potency against BGC803 ($IC_{50} = 0.77$ μM) than that of other four tumor cell lines. In addition, the cytotoxicity of compound **6a** against A2708 ($IC_{50} = 1.61$ μM) is comparable to that of CA-4 ($IC_{50} = 4.48$ μM).

3. Conclusions

A variety of novel sulfonamides incorporating 1*H*-benzo[*d*]imidazol fragment were synthesized. The preliminary study on the SAR disclosed that installment of 1*H*-benzo[*d*]imidazol motif on nitrogen atom of sulfonamide is beneficial to the cytotoxicities of the target compounds. In this series, compound **6a** with a *para*-methoxyl group on the phenyl ring is the most potent chemical entity against five cancer cell lines with cytotoxic IC_{50} value ranging from submicromolar to low micromolar level. The investigation on other novel benzoimidazole CA-4 analogs is currently in progress.

4. Experimental

4.1 General experimental procedures

Unless noted otherwise, all reagents and solvents were used as purchased without further purification. Melting points (m.p.) were determined on a Yanaco apparatus and are uncorrected. NMR spectra were obtained on a Varian Mercury 300 spectrometer. Coupling constants (J) are reported in hertz, and chemical shifts are reported in parts per million (δ) relative to $CDCl_3$ (7.26 ppm for ^{1}H) or DMSO-d_6 (2.49 ppm for ^{1}H) or Acetone-d_6 (2.04 ppm for ^{1}H). Molecular weights of compounds were determined by an Agilent Technologies LC/MSD TOF spectrometer, and values are expressed as $[M + H]^+$.

4.2 The preparation procedure of the intermediates

4.2.1 2-Methoxy-6-nitroaniline (1)

The chemical synthesis of compound **1** was according to the procedure described by Zhang *et al.* [18]. The title compound was obtained as salmon pink powder (89%). Mp 68–69°C; ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 7.73 (1H, d, $J = 9.0$ Hz), 6.88 (1H, d, $J = 7.8$ Hz), 6.60 (1H, dd, $J_1 = 9.0$ Hz, $J_2 = 7.8$ Hz), 6.42 (2H, br s), 3.92 (3H, s); HR-MS (ESI) *m/z*: 169.0607 $[M + 1]^+$ (calcd for $C_7H_9N_2O_3$, 169.0608).

4.2.2 3-Methoxy-O-phenylenediamine (2)

The chemical synthesis of compound **2** was according to the procedure described by Doherty *et al.* [19]. The title compound was obtained as off-white powder (77%). Mp 77–78°C; ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 6.68 (1H, t, $J = 7.8$ Hz), 6.42 (1H, d, $J = 7.8$ Hz), 6.40 (1H, d, $J = 7.8$ Hz), 3.84 (3H, s), 3.31 (4H, br s); HR-MS (ESI) *m/z*: 138.0870 $[M + 1]^+$ (calcd for $C_7H_9N_2O$, 138.0866).

4.2.3 4-Methoxy-1H-benzo[d]imidazole (3)

The chemical synthesis of compound **3** was according to the procedure described by Lane and Williams [20]. The title compound was obtained as white powder

(83.9%). Mp 165–167°C; ^{1}H NMR (acetone-d_6, 300 MHz, δ ppm): δ 8.08 (1H, s), 7.21 (1H, d, $J = 7.8$ Hz), 7.11 (1H, t, $J = 7.8$ Hz), 6.73 (1H, d, $J = 7.8$ Hz), 3.97 (3H, s); HR-MS (ESI) *m/z*: 149.0707 $[M + 1]^+$ (calcd for $C_8H_9N_2O$, 149.0709).

*4.2.4 4-Methoxy-7-nitro-1H-benzo[d]-imidazole (**4a**) and 4-methoxy-5-nitro-1H-benzo[d]imidazole (**4b**)*

The mixture of 4-methoxy-1*H*-benzo[d]-imidazole (**3**, 148 mg, 1 mmol) and sodium nitrate (127 mg, 1.5 mmol) in TFA (3 ml) was heated at 70°C for 16 h. The reaction mixture was cooled to room temperature and poured into ice/water (20 ml). Precipitation occurred at pH $\approx$ 8 when the mixture was treated with 10% NaOH aqueous solution. The resulting mixture was extracted with ethyl acetate (10 ml × 3). The solid in the water layer was filtered and washed with water, affording the desired compound **4a** (90 mg, yield 46.6%) as amber powder. Mp 265–267°C; ^{1}H NMR (acetone-d_6, 300 MHz, δ ppm): δ 13.12 (1H, br s), 8.28 (1H, s), 8.17 (1H, d, $J = 8.4$ Hz), 6.94 (1H, d, $J = 8.4$ Hz), 4.09 (3H, s); HR-MS (ESI) *m/z*: 194.0562 $[M + 1]^+$ (calcd for $C_8H_8N_3O_3$, 194.0560). The ethyl acetate phase was concentrated to give a crude product, which was purified by column chromatography with CH_2Cl_2–MeOH (v/v = 10:1). The side product **4b** (60 mg) was afforded as yellow powder with 31% yield. Mp 161–162°C; ^{1}H NMR (acetone-d_6, 300 MHz, δ ppm): δ 11.91 (1H, br s), 8.42 (1H, s), 7.72 (1H, d, $J = 8.7$ Hz), 7.29 (1H, d, $J = 8.7$ Hz), 4.53 (s, 3H); HR-MS (ESI) *m/z*: 194.0563 $[M + 1]^+$ (calcd for $C_8H_8N_3O_3$, 194.0560).

*4.2.5 4-Methoxy-7-amino-1H-benzo[d]-imidazole (**5**)*

The mixture of 4-methoxy-7-nitro-1*H*-benzo[d]imidazole (**4a**, 80 mg, 0.46 mmol) and 10% Pd/C (16 mg) in ethanol (10 ml) was hydrogenated at room temperature and atmospheric pressure for 5 h. The reaction mixture was filtered and the filtrate was evaporated to afford the title compound **5** (65 mg, 97% yield) as purple powder. Mp 150–153°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 7.95 (1H, s), 6.46 (1H, d, $J = 8.1$ Hz), 6.25 (1H, d, $J = 8.1$ Hz), 3.79 (3H, s); HR-MS (ESI) *m/z*: 164.0816 $[M + 1]^+$ (calcd for $C_8H_{10}N_3O$, 164.0818).

*4.2.6 4-Methoxy-1H-benzo[d]imidazole-7-sulfonic acid (**7**)*

4-Methoxy-1*H*-benzo[d]imidazole (**3**, 500 mg, 3.37 mmol) was added in portions into chlorosulfonic acid (1.8 ml, 27 mmol) at −5°C. The mixture was stirred at 0°C for 4 h. After the reaction was quenched with water, the solid was collected by filtration and washed with water to give the crude product, which was purified by recrystallization with ethanol. The desired product **7** (512 mg) was obtained as white powder with 66.7% yield. Mp > 300°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 9.47 (1H, s), 7.66 (1H, d, $J = 8.4$ Hz), 7.10 (1H, d, $J = 8.4$ Hz), 4.03 (3H, s); HR-MS (ESI) *m/z*: 229.0277 $[M + 1]^+$ (calcd for $C_8H_9N_2O_4S$, 229.0278).

4.3 General procedure for the preparation of N-(4-methoxy-1H-benzo[d]-imidazol-7-yl)-arylsulfonamides (6a–6 l)

The reaction mixture of 4-methoxy-7-amino-1*H*-benzo[d]imidazole (**5**, 163 mg, 1 mmol), sulfonyl chloride (1 mmol), and pyridine (0.39 ml, 5 mmol) in CH_2Cl_2 (10 ml) was stirred at room temperature. After the reaction completed, the precipitate was filtered and washed with CH_2Cl_2 and water. The crude product was recrystallized from the mixture of methanol, dichloromethane, and petroleum ether.

4.3.1 *N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-4-methoxybenzenesulfonamide (**6a**)*

Following the general procedure, starting from 4-methoxybenzenesulfonyl chloride (206 mg, 1 mmol), the title compound **6a** (200 mg, 60% yield) was obtained as off-white powder. Mp 191–193°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 9.86 (1H, s), 8.61 (1H, s), 7.62 (2H, d, $J = 8.7$ Hz), 7.01 (2H, d, $J = 8.7$ Hz), 6.70 (2H, s), 3.88 (3H, s), 3.78 (3H, s); HR-MS (ESI) *m/z*: 334.0864 $[M + 1]^+$ (calcd for $C_{15}H_{16}N_3O_4S$, 334.0856).

4.3.2 *N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3,4- dimethylbenzenesulfonamide (**6b**)*

Following the general procedure starting from 3,4-dimethylbenzenesulfonyl chloride (204 mg, 1 mmol), the title compound **6b** (100 mg, 30.3% yield) was obtained as off-white powder. Mp 240–243°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 9.96 (1H, br s), 8.74 (1H, s), 7.52 (1H, s), 7.41 (1H, d, $J = 8.1$ Hz), 7.24 (1H, d, $J = 8.1$ Hz), 6.73 (2H, s), 3.88 (3H, s), 2.23 (3H, s), 2.21 (3H, s); HR-MS (ESI) *m/z*: 332.1061 $[M + 1]^+$ (calcd for $C_{16}H_{18}N_3O_3S$, 332.1063).

4.3.3 *N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3,4-dimethoxybenzenesulfonamide (**6c**)*

Following the general procedure, starting from 3,4-dimethoxybenzenesulfonyl chloride (236 mg, 1 mmol), the title compound **6c** (200 mg, 55% yield) was obtained as white powder. Mp 227–229°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 12.75 (0.4H, s), 12.17 (0.6H, s), 9.75 (0.4H, br s), 9.56 (0.6H, s), 8.05 (0.4H, s), 8.00 (0.6H, s), 7.39 (0.6H, s), 7.32 (0.4H, d, $J = 8.7$ Hz), 7.17–7.19 (m, 1H), 6.99 (0.6H, d, $J = 8.7$ Hz), 6.93–6.98 (1H, m), 6.65 (0.4H, d, $J = 8.1$ Hz), 6.52 (1H, q, $J = 8.1$ Hz), 3.84 (3H, s), 3.77 (1.8H, s), 3.74 (1.2H, s), 3.70 (3H, s); HR-MS (ESI) *m/z*: 364.0959 $[M + 1]^+$ (calcd for $C_{16}H_{18}N_3O_5S$, 364.0962).

4.3.4 *N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3-chloro-4-methylbenzenesulfonamide (**6d**)*

Following the general procedure, starting from 3-chloro-4-methylbenzenesulfonyl chloride (224 mg, 1 mmol), the title compound **6d** (200 mg, 56.9% yield) was obtained as off-white powder. Mp 250–252°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 10.07 (1H, br s), 8.45 (1H, s), 7.73 (1H, s), 7.45–7.51 (2H, m), 6.70 (2H, s), 3.88 (3H, s), 2.34 (3H, s); HR-MS (ESI) *m/z*: 352.0519 $[M + 1]^+$ (calcd for $C_{15}H_{15}ClN_3O_3S$, 352.0517).

4.3.5 *N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3-nitro-4-methylbenzenesulfonamide (**6e**)*

Following the general procedure, starting from 3-nitro-4-methylbenzenesulfonyl chloride (234 mg, 1 mmol), the title compound **6e** (188 mg, 51.9% yield) was obtained as off-white powder. Mp 253–255°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 12.78 (0.5 H, br s), 12.33 (0.5 H, br s), 10.02 (1H, br s), 8.29 (1H, s), 8.06 (1H, s), 7.85 (1H, br s), 7.60 (1H, d, $J = 7.2$ Hz), 6.64–6.93 (2H, m), 3.86 (3H, s), 2.53 (3H, s); HR-MS (ESI) *m/z*: 363.0757 $[M + 1]^+$ (calcd for $C_{15}H_{15}N_4O_5S$, 363.0758).

4.3.6 *N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3,5-dichloro-4-hydroxylbenzene-sulfonamide (**6f**)*

Following the general procedure, starting from 3,5-dichloro-4-hydroxylbenzenesulfonyl chloride (260 mg, 1 mmol), the title compound **6f** (110 mg, 28.4% yield) was obtained as gray powder. Mp 283–285°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 12.11 (1H, br s), 9.95 (1H, br s), 8.14 (1H,

s), 7.61 (2H, s), 6.74 (1H, d, $J = 8.4$ Hz), 6.65 (1H, d, $J = 8.4$ Hz), 3.87 (3H, s); HR-MS (ESI) *m/z*: 387.9918 $[M + 1]^+$ (calcd for $C_{14}H_{12}Cl_2N_3O_4S$, 387.9920).

*4.3.7 N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-4-trifluoromethoxybenzene-sulfonamide (**6g**)*

Following the general procedure, starting from 4-trifluoromethoxybenzenesulfonyl chloride (260 mg, 1 mmol), the title compound **6g** (140 mg, 36.1% yield) was obtained as off-white powder. Mp 234–235°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 12.86 (0.5 H, br s), 12.25 (0.5 H, br s), 9.91 (1H, br s), 7.99 (1H, s), 7.81 (2H, d, $J = 7.5$ Hz), 7.47 (2H, d, $J = 7.5$ Hz), 6.59–6.89 (2H, m), 3.86 (3H, s); HR-MS (ESI) *m/z*: 388.0570 $[M + 1]^+$ (calcd for $C_{15}H_{13}F_3N_3O_4S$, 388.0573).

*4.3.8 N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3-methoxybenzenesulfon-amide (**6h**)*

Following the general procedure, starting from 3-methoxybenzenesulfonyl chloride (206 mg, 1 mmol), the title compound **6h** (130 mg, 39% yield) was obtained as off-white powder. Mp 178–180°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 12.62 (1H, br s), 9.86 (1H, br s), 8.08 (1H, s), 7.38 (1H, t, $J = 8.1$ Hz), 7.25–7.28 (2H, m), 7.11 (1H, dd, $J_1 = 8.1$ Hz, $J_2 = 1.8$ Hz), 6.69 (1H, d, $J = 8.7$ Hz), 6.57 (1H, d, $J = 8.7$ Hz), 3.85 (3H, s), 3.73 (3H, s); HR-MS (ESI) *m/z*: 334.0860 $[M + 1]^+$ (calcd for $C_{15}H_{16}N_3O_4S$, 334.0856).

*4.3.9 N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3-fluoro-4-methylbenzene-sulfonamide (**6i**)*

Following the general procedure, starting from 3-fluoro-4-methylbenzenesulfonyl chloride (208 mg, 1 mmol), the title compound **6i** (180 mg, 53.7% yield) was obtained as off-white powder. Mp 234–236°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 12.78 (0.4 H, br s), 12.27 (0.6 H, br s), 9.88 (1H, br s), 8.03 (1H, s), 7.41 (3H, br s), 6.57–6.82 (2H, m), 3.86 (3H, s), 2.24 (3H, s); HR-MS (ESI) *m/z*: 336.0815 $[M + 1]^+$ (calcd for $C_{15}H_{15}FN_3O_3S$, 336.0813).

*4.3.10 N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3-fluoro-4-methoxybenzene-sulfonamide (**6j**)*

Following the general procedure, starting from 3-fluoro-4-methoxybenzenesulfonyl chloride (226 mg, 1 mmol), the title compound **6j** (120 mg, 34.1% yield) was obtained as off-white powder. Mp 221–223°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 12.76 (0.4 H, br s), 12.23 (0.6 H, br s), 9.92 (0.4H, br s), 9.70 (0.6H, s), 8.04 (0.4H, s), 8.01 (0.6H, s), 7.62 (0.4H, d, $J = 11.1$ Hz), 7.51 (0.4H, d, $J = 9.3$ Hz), 7.48 (0.6H, d, $J = 11.1$ Hz), 7.41 (0.6H, d, $J = 9.3$ Hz), 7.25 (0.6H, t, $J = 8.4$ Hz), 7.19 (0.4H, t, $J = 8.4$ Hz), 6.93 (0.6H, d, $J = 8.4$ Hz), 6.66 (0.4H, d, $J = 8.4$ Hz), 6.50 (1H, s), 3.83–3.87 (6H, m); HR-MS (ESI) m/z: 352.0758 $[M + 1]^+$ (calcd for $C_{15}H_{15}FN_3O_4S$, 352.0762).

*4.3.11 N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3-methyl-4-bromobenzene-sulfonamide (**6k**)*

Following the general procedure, starting from 3-methyl-4-bromobenzenesulfonyl chloride (269 mg, 1 mmol), the title compound **6k** (190 mg, 50% yield) was obtained as off-white powder. Mp 263–266°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 12.46 (1H, br s), 9.93 (1H, br s), 8.04 (s, 1H), 7.69 (2H, d, $J = 8.1$ Hz), 7.43 (1H, d, $J = 8.1$ Hz), 6.68 (1H, br s), 6.58 (1H, d, $J = 8.1$ Hz), 3.86 (3H, s), 2.33 (3H, s); HR-MS (ESI) *m/z*: 396.0008 $[M + 1]^+$ (calcd for $C_{15}H_{15}BrN_3O_3S$, 396.0012).

*4.3.12 N-(4-Methoxy-1H-benzo[d]-imidazol-7-yl)-3,4,5-trimethoxybenzene-sulfonamide (**6l**)*

The reaction mixture of 4-methoxy-7-amino-1*H*-benzo[d]imidazole **5** (124 mg, 0.76 mmol), 3,4,5-trimethoxybenzenesulfonyl chloride (200 mg, 0.7 mmol), and pyridine (0.15 ml, 1.9 mmol) in CH_2Cl_2 (10 ml) and DMF (0.5 ml) was stirred at room temperature. After the reaction completed, the reaction mixture was diluted with water (30 ml) and extracted with ethyl acetate (30 ml × 3). The combined extracts were washed with water and brine, and then dried over anhydrous Na_2SO_4, filtered, and concentrated to give the crude product, which was purified by column chromatography on silica gel with CH_2Cl_2–MeOH (v/v = 10:1). The title compound **6l** (80 mg, 26.8% yield) was obtained as white powder. Mp 183–185°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 8.05 (1H, s), 7.01 (2H, br s), 6.79 (1H, br s), 6.62 (1H, d, $J = 7.2$ Hz), 3.87 (3H, s), 3.72 (6H, s), 3.67 (3H, s); HR-MS (ESI) *m/z*: 394.1071 $[M + 1]^+$ (calcd for $C_{17}H_{20}N_3O_6S$, 394.1067).

4.4 General procedure for the preparation of N-aryl-(4-methoxy-1H-benzo-[d]imidazole)-7-sulfonamides (8a–8d)

4-Methoxy-1*H*-benzo[d]imidazole-7-sulfonic acid (**7**, 150 mg, 0.66 mmol) in thionyl chloride (5 ml, 68.5 mmol) and DMF (5 drops) was refluxed for 4 h. The reaction mixture was concentrated and the crude 4-methoxy-1*H*-benzo[d]imidazole-7-sulfonyl chloride was directly used to the next step without further purification. The arylamine (0.88 mmol) and Et_3N (0.88 mmol) were added to the solution of sulfonyl chloride in CH_2Cl_2 (15 ml) and DMF (1 ml) at room temperature. After the reaction completed, the solvent was evaporated under reduced pressure and the residue was taken up with EtOAc (60 ml). The organic solution was washed with water and brine, then dried over anhydrous Na_2SO_4. The organic phase was concentrated to give the crude product, which was purified by column chromatography with CH_2Cl_2–MeOH (v/v = 20:1).

*4.4.1 N-(4-Methoxyphenyl)-(4-methoxy-1H-benzo[d]imidazole)-7-sulfonamide (**8a**)*

Following the general procedure, starting from 4-methoxybenzenamine (108 mg, 0.88 mmol), the title compound **8a** (140 mg, 63.9% yield) was obtained as brown powder. Mp 181–183°C; ^{1}H NMR (acetone-d_6, 300 MHz, δ ppm): δ 8.56 (1H, br s), 8.18 (1H, br s), 7.49 (1H, d, $J = 8.4$ Hz), 6.98–7.02 (2H, m), 6.79 (1H, d, $J = 8.4$ Hz), 6.70 (1H, d, $J = 8.4$ Hz), 4.04 (3H, s), 3.67 (3H, s); HR-MS (ESI) *m/z*: 334.0862 $[M + 1]^+$ (calcd for $C_{15}H_{16}N_3O_4S$, 334.0856).

*4.4.2 N-(3,4-Dimethoxyphenyl)-(4-methoxy-1H-benzo[d]imidazole)-7-sulfonamide (**8b**)*

Following the general procedure, starting from 3,4-dimethoxybenzenamine (134.6 mg, 0.88 mmol), the title compound **8b** (122 mg, 51% yield) was obtained as brown powder. Mp 198–200°C; ^{1}H NMR (acetone-d_6, 300 MHz, δ ppm): δ 8.58 (1H, br s), 8.19 (1H, br s), 7.54 (1H, d, $J = 8.4$ Hz), 6.81 (1H, d, $J = 8.4$ Hz), 6.73 (1H, d, $J = 2.4$ Hz), 6.68 (1H, d, $J = 8.4$ Hz), 6.57 (1H, dd, $J_1 = 8.4$ Hz, $J_2 = 2.4$ Hz), 4.04 (3H, s), 3.66 (3H, s), 3.60 (3H, s); HR-MS (ESI) *m/z*: 364.0957 $[M + 1]^+$ (calcd for $C_{16}H_{18}N_3O_5S$, 364.0962).

*4.4.3 N-(3,4,5-Trimethoxyphenyl)-(4-methoxy-1H-benzo[d]imidazole)-7-sulfonamide (**8c**)*

Following the general procedure, starting from 3,4,5-trimethoxybenzenamine (120 mg, 0.66 mmol), the title compound **8c** (44 mg, 25.4% yield) was obtained as brown powder. Mp 91–93°C; ^{1}H NMR

(acetone-d_6, 300 MHz, δ ppm): δ 8.76 (1H, br s), 8.21 (1H, br s), 7.63 (1H, d, $J = 8.4$ Hz), 6.84 (1H, d, $J = 8.4$ Hz), 6.43 (2H, s), 4.04 (3H, s), 3.61 (6H, s), 3.56 (3H, s); HR-MS (ESI) *m/z*: 394.1069 $[M + 1]^+$ (calcd for $C_{17}H_{20}N_3O_6S$, 394.1069).

*4.4.4 N-(3,4,5-Trifluorophenyl)-(4-methoxy-1H-benzo[d]imidazole)-7-sulfonamide (**8d**)*

To a solution of 4-methoxy-1*H*-benzo[-*d*]imidazole-7-sulfonyl chloride (250 mg, 1.02 mmol) in CH_2Cl_2 (8 ml) was added 3,4,5-trifluorobenzenamine (179 mg, 1.22 mmol) and pyridine (0.3 ml, 3.05 mmol). The reaction mixture was stirred at room temperature for 5 h. A crude product was obtained by filtration and was recrystallized from methanol. The title compound **8d** (90 mg, 24.7% yield) was obtained as white powder. Mp 225–227°C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): δ 8.34 (1H, s), 7.72 (1H, d, $J = 8.7$ Hz), 7.01 (1H, d, $J = 6.6$ Hz), 6.98 (1H, d, $J = 6.6$ Hz), 6.92 (1H, d, $J = 8.7$ Hz), 4.01 (3H, s); HR-MS (ESI) *m/z*: 358.0472 $[M + 1]^+$ (calcd for $C_{14}H_{11}F_3N_3O_3S$, 358.0468).

5. Biological evaluation

5.1 Cell culture

The human lung adenocarcinoma cell line A549, the human hepatocellular carcinoma cell line Bel7402, the human stomach adenocarcinoma cell line BGC823, the human colon carcinoma cell line HCT8, and the human ovarian carcinoma cell line A2780 were cultured at 37°C in a 5% CO_2 and 95% air atmosphere using RPMI-1640 with 10% FBS and penicillin (100 U/ml). All the cells were purchased from the American Type Culture Collection and the Cell Culture Center of Institute of Basic Medical Science, Chinese Academy of Medical Sciences.

5.2 Cytotoxicity assay

The *in vitro* cytotoxic activities of tested compounds were determined by MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide, Sigma, St Louis, MO, USA] assays. Briefly, cells were seeded into 96-well plates at a density of 1–2×10^3/well (depending on the cell growth rate). Twenty-four hours later, triplicate wells were treated with media and agents. After 72 h incubation at 37°C in 5% CO_2, the medium containing drug was removed and replaced by fresh medium. The cells in each well were then incubated in culture medium with 100 μl of 0.5 mg/ml MTT solution for 4 h. After the medium was removed, 150 μl of DMSO was added to each well. The plates were gently agitated until the color of the reaction was uniform and the OD_{570} was determined using a microplate reader (Wellscan MK3, Labsystems Dragon, Helsinki, Finland). Microsoft® Excel 2003 was used for data analysis. Media-only treated cells served as the indicator of 100% cell viability. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the vehicle in the MTT assay.

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Notes

1. Oxigene company web site: www.oxigene.com (March 2010).
2. Aventis company web site: www.sanofi-aventis.com.

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