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Synthesis and evaluation of 2-[2-(phenylthiomethyl)-1*H*-benzo[*d*]imidazol-1-yl)acetohydrazide derivatives as antitumor agents

Ting Liu [a,†], Changyan Sun [b,†], Xiumei Xing [a], Lingling Jing [a], Rui Tan [a], Youfu Luo [b], Wencai Huang [a], Hang Song [a], Zicheng Li [a,*], Yinglan Zhao [b,*]

[a] Department of Pharmaceutical and Bioengineering, School of Chemical Engineering, Sichuan University, Chengdu, Sichuan 610065, PR China
[b] State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu, Sichuan 610041, PR China

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ABSTRACT

A novel class of acetylhydrazone derivatives (**5a–x**) containing 2-(phenylthiomethyl)-1*H*-benzo-[*d*]-imidazole moieties are synthesizer, and their antitumor activities against A549, HCT116, HepG2, PC-9, and A375 were determined by the MTT assay. Among them are *N*-(2,4-dihydroxybenzylidene)-2-(2-(phenylthiomethyl)-1*H*-benzo[*d*]-imidazol-1-yl)acetohydrazide (**5a**) and *N*-(5-bromo-2-hydroxybenzylidene)-2-(2-(phenylthiomethyl)-1*H*-benzo[*d*]-imidazol-1-yl)acetohydrazide (**5d**) which displayed excellent cancer inhibitory activity against the tested cancer cells (IC_{50} 4–17 μM), compared with 5-FU and SU11248. The others have moderate to weak inhibitory activity against the tested cancer cell lines.

The benzimidazole unit is the key building block for a variety of compounds, which have crucial roles in the functions of biologically important molecules, its derivatives have a wide range of well known biologic activities, such as anticancer,[1–3] antimicrobial,[4,5] proton pump inhibitor,[6] antiviral,[7,8] etc. Several bibenzimidazole and terbenzimidazole derivativies have recently been identified as topoisomerase II poisons (Hoechst-33258 and Hoechst-33342)[9,10] (Fig. 1). It was found that the topoisomerase II was significantly inhibited by compound **1** with IC_{50} of 17.0 μM,[11] which is higher than etoposide (IC_{50} is 21.8 μM).[12] Abdel-Mohsen et al.[13] reported novel benzimidazole–pyrimidine conjugates with marked potency against twelve cancer cell lines.

The hydrazone compounds have unique biologic activity and strong coordination ability, and they were widely used in the field of sterilization, antibacterial, anti-tuberculosis and anti-tumor. They are also hot topics in the field of pharmaceutical research. Galal et al.[14,15] synthesizer novel benzimidazole-5-carboxylic acid and hydrazide derivatives and complexes with transition metal as topo II inhibitors, among them, two compounds inhibited topo II activity at 10 times lower concentration than etoposide in relaxation assay.

In this Letter, taking into account of the wide bioactivities of benzimidazole derivatives and hydrazones, and basing on analyzing of the structure of compound **1**, the hydrazone group was

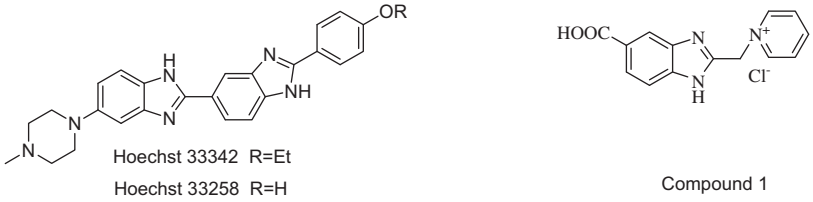

Figure 1. Compounds with good antitumor activity.

* Corresponding authors. Tel./fax: +86 28 85405221.
E-mail addresses: sculzc@scu.edu.cn (Z. Li), alancenxb@sina.com (Y. Zhao).
† These authors contributed equally to this Letter.

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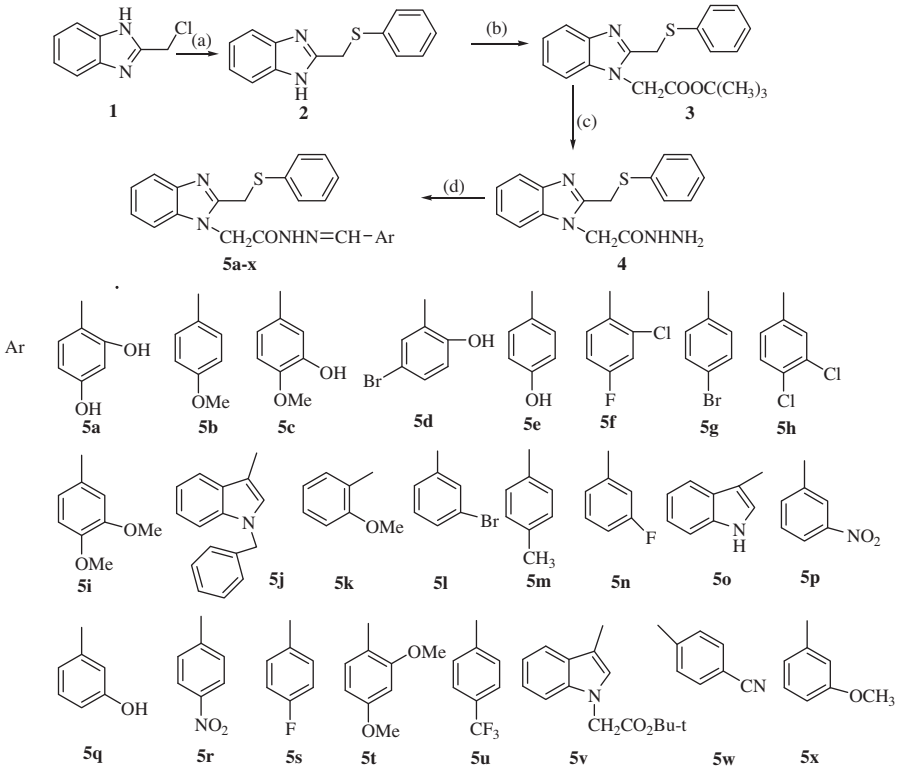

Scheme 1. Synthetic pathway of benzimidazole derivatives (**5a–x**). Reagents and conditions: (a) K_2CO_3 and benzenethiol, stirred for 0.5 h at rt; then compound **1** in DMF was added, and stirred for 2 h at rt; (b) K_2CO_3, *tert*-butyl 2-bromopropanoate, stirred for 6 h at rt; (c) EtOH, NH_2NH_2 H_2O; (d) Ar-CHO, reflux for 2 h in ethanol.

Table 1
Physical data and proliferation inhibitory effect of compounds **5a–x** against tumor cells in vitro

Compound	mp,(°C)	Yield (%)	*Z*/*E*	IC_{50}[a,b]				
				A549	HCT116	HepG2	PC-9	A375
5a	272–274	86	42/58	12.36	4.86	>20[c]	14.63	5.75
5b	157–160	77	25/75	45.25	48.03	50.24	30.52	43.46
5c	222–224	84	29/71	58.32	76.22	71.7	39.19	>80
5d	194–196	88	29/71	10.75	8.33	17.03	12.2	4.09
5e	267–270	86	27/73	53.1	63.9	78.21	42.03	>80
5f	185–189	76	19/81	41.23	40.92	42.47	38.9	37.15
5g	182	74	23/77	34.98	31.12	32.04	29.84	26.81
5h	201–203	78	20/80	36.07	31.17	33.79	30.15	21.41
5i	198	79	20/80	64	>80	>80	38.58	5.44
5j	215–218	77	23/77	49.06	69.3	>80	10.92	>80
5k	178–180	75	22/78	51.51	55.06	59.93	39.31	53.51
5l	105–107	72	22/78	31.89	30.55	31.55	28.11	21.24
5m	125–127	76	23/77	38.51	43.99	38.2	30.58	42.12
5n	171–175	75	20/40	46.94	49.63	50.85	34.13	40.96
5o	271–273	73	28/72	42.42	40.35	47.31	34.07	45.37
5p	198–199	77	25/75	50.03	44.37	52.36	34.17	58.04
5q	245–248	78	26/74	51.35	77.64	>80	42.61	76.14
5r	201–202	75	20/80	62.16	44.05	61.85	42.21	77.56
5s	120–122	76	20/80	38.11	31.93	44.76	31.03	>80
5t	202	73	20/80	54.41	>80	>80	44	>80
5u	190–192	78	34/66	31.22	10.05	29.55	18.09	18.05
5v	136–138	77	20/80	41.92	40.78	56.54	44.56	52.11
5w	215–218	75	19/81	46.21	62.02	70.01	57.31	>80
5x	160–162	76	22/78	36.89	35.62	48.7	32.81	42.2
5-FU				35.05	31.72	46.31	23.47	46.55
SU11248				12.35	18.35	13.24	10.97	11.58

[a] The IC_{50} values represent the concentration that causes 50% growth inhibition.
[b] The IC_{50} values were the mean values of three repeated experiments.
[c] The concentartion of compounds **5a** and **5d** ranges from 0.625 to 20 μM.

incorporated into the 2-mercapto-1*H*-benzimidazole, novel hydrazone derivatives with benzimidazole moieties have been designed and synthesizer, and their biologic activity against five cancer cells were studied by the MTT assay.

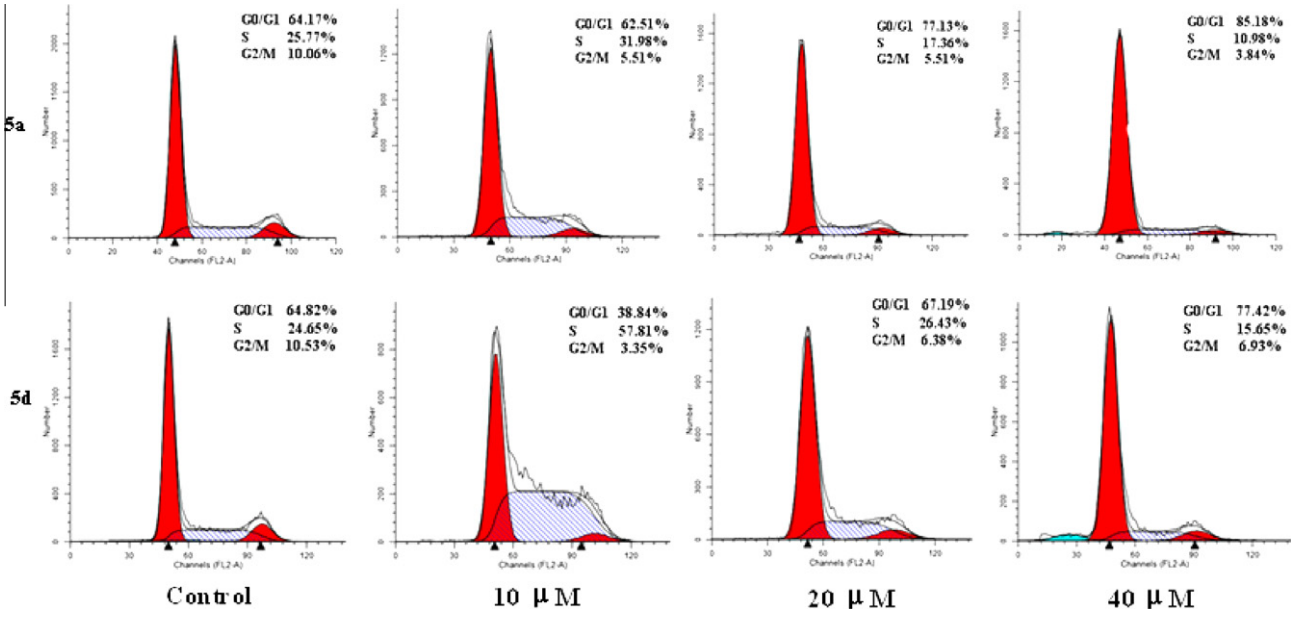

Figure 2. Relative distribution of A375 cells after 24 h treatment with compounds **5a** and **5d** at various concentrations.

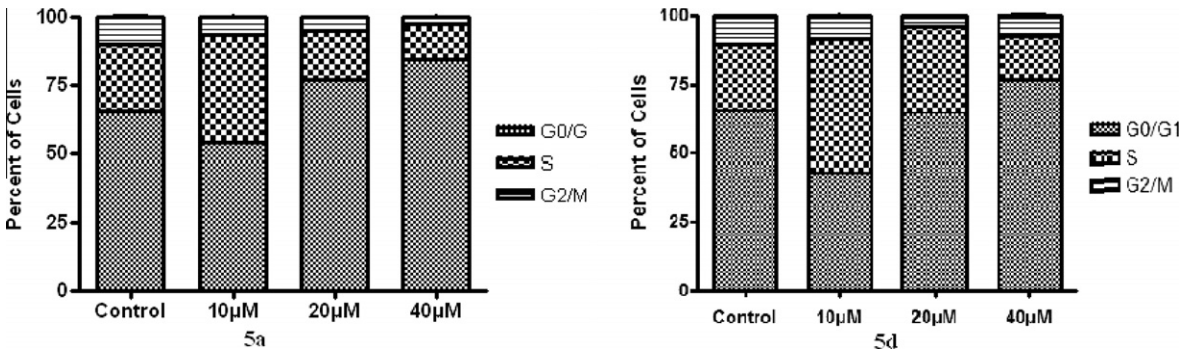

Figure 3. Statistic results of the effect of **5a**/**5d** on G0/G1, S and G2/M phase in A375 cells. Data were obtained from three independent experiments, with triplicates in each experiment.

In this Letter, taking into account of the wide bioactivities of benzimidazole derivatives and hydrazones, and basing on analyzing of the structure of compound **1**, the hydrazone group was incorporated into the 2-mercapto-1*H*-benzimidazole, novel hydrazone derivatives with benzimidazole moieties have been designed and synthesizer, and their biologic activity against five cancer cells were studied by the MTT assay.

The target compounds **5a–x** were synthesizer via the general synthetic route shown in Scheme 1. Compound **2** was prepared from potassium benzenethiolate reacted with 2-(chloromethyl)-1*H*-benzo[*d*]-imidazole (**1**), which was produced from *o*-phenylenediamine and 2-chloroacetic acid by the procedures reported,[17,18] Compound **3** was obtained by combining of **2** and *tert*-butyl 2-bromoacetate in dry acetone in the presence of anhydrous K_2CO_3[13,14], which was then treated with 85% hydrazine hydrate to afford **4**; the target compounds **5a–x** were obtained by the condensation of compound **4** with corresponding phenylcarbaldehyde in the presence of glacial acetic acid[19,20], and then recrystallized from methanol. The structures of 5**a–x** were characterized by ^{1}H NMR, IR, and ESI-HRMS analysis and all the analytical data were documented in Supplementary data. The purity of all compounds was above 97.0%, which is determined by HPLC normalization method.

The characteristic data of the target compounds are presented in Table 1. The ratio of *Z*/*E* isomers caused by imine (CH═NH) bond can be calculated from splitting of hydrogen. According to the ^{1}H NMR spectra of compounds **5a–x**, each group of NH, N═CH, CH_2C═O, S—CH_2, OH, OCH_3, CH_3, displaying two single peaks, showed the cis–trans isomerism of the prepared compounds. The ratio of *Z*/*E* of **5a–x** ranged were from 1/4 to 1/3, and the *E* isomer is main isomer, the ratio of *Z*/*E* for **5a** reached 42:58. It seems that the existence of hydroxy group may cause a higher *Z*/*E* value, and the more the hydroxy groups has, the higher the value of *Z*/*E* is, which could be due to the intramolecular hydrogen bond.[9,16]

The proliferation inhibitory effect of all target compounds against cancer cells was determined by the MTT assay.[21] The antitumor screening results, presented in Table 1, revealed that most target compounds were exhibited potent antitumor activity against A549, HCT116, HepG2, PC-9 and A375 cancer cell lines. Among them were **5a** and **5d** exhibiting excellent antitumor activity against HCT116 (IC_{50} = 4.86 μM and 8.33 μM, respectively) and A375 (IC_{50} = 5.75 μM and 4.09 μM, respectively), the result of which is compared with 5-FU and SU11248. Compound **5i** exhibited enchanced antiproliferation activity against A375 cell with IC_{50} of 5.44 μM, **5j** showed moderate cancer inhibitory activity

against PC-9 with IC_{50} of 10.92 μM, and **5u** showed moderate cancer inhibitory activity against HCT116 with IC_{50} of 10.05 μM. These results showed that compounds containing electron withdrawing substituents on 3 place at phenly of hydrazone have moderate activity against cancer cell, and those containing hydroxyl on 2 (and 4) place at phenyl of hydrazone have the highest antitumor activity.

To examine the mechanism of compounds **5a** and **5d** responsible for mediated cell proliferation inhibition, cell cycle distribution was evaluated using flow cytometric analysis as described previously using flow cytometry[22,23] .The data are shown in Figures 2 and 3.

As can be seen in Figure 2, exposure of A375 cells to growth suppressive concentration of compounds **5a** and **5d** resulted in a significant accumulation of cells in G0/G1 phases, which was accompanied by a decrease in cells with S and G2/M DNA content. For example, the percentage of cells in G0/G1 phases was increased to 85.18% after treatment with **5a** at 40 μM for 24 h, whereas the G0/G1 phase cells in control is 64.17%. Moreover, the percentage of cells in G0/G1 phases increased by 12.6% over the control after treatment with **5d** at 40 μM for 24 h.

In summary, a novel class of acetylhydrazone derivatives with benzimidazole moieties were synthesizer and tested for their in vitro antitumor activity against five strains of cancer cell lines. compounds **5a** and **5d** showed excellent cancer inhibitory activity in vitro against the tested cancer cell lines (IC_{50} 4–17 μM). Flow cytometric analysis revealed that compound **5a** and **5d** induce significant levels of apoptosis in A375 cells in vitro at low micromolar concentrations. The hydroxyl at 2 (and 4) position on the phenyl ring of hydrazone play a significant role in the antitumor activity. This kind of benzimidazole derivatives may constitute a novel class of antiproliferative agents, which deserve further study.

Acknowledgments

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Supplementary data

Supplementary data (the list of experimental details and spectroscopic characterization of **5a–x**, and their intermediates) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.061.

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21. Briefly, Cells ($4–5 \times 10^3$/well) were seeded in 96-well plates and cultured for 24 h, followed by various concentration of compounds treatment for 48 h. 10 μl 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 DMSO was added 150 μl/well for 15–20 min. The light absorptions (OD) were measured at 570 nm with SpectraMAX M5 microplate spectrophotometer (Molecular Devices). The effect of compounds on tumor cells viability was expressed by IC_{50} of each cell lines.
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23. A375 (1×10^5cells/well) were seeded in 6-well plates and cultured for 24 h, followed by treatment with **5a** or **5d** at various concentrations for 24 h. Then, cells were harvested and fixed with 70% ethanol for 1 h. The fixed cells were washed with PBS and suspended in 1 mL hypotonic solution containing 50 μg/mL PI in 0.1% sodium citrate plus 0.1% Triton X-100, and then analyzed by Flow Cytometer (FACS-Callibur, BD, USA). The DNA content of the cells was analyzed using ModFit software.