

Article

Synthesis and Biological Evaluation of Novel Benzothiazole-2-thiol Derivatives as Potential Anticancer Agents

Xuan-Hong Shi, Zhao Wang, Yong Xia, Ting-Hong Ye, Mei Deng, You-Zhi Xu, Yu-Quan Wei and Luo-Ting Yu *

State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, China

* Author to whom correspondence should be addressed; E-Mail: yuluot@scu.edu.cn; Tel: +86-28-8550-3817; Fax: +86-28-8516-4060.

Received: 22 February 2012; in revised form: 20 March 2012 / Accepted: 26 March 2012 / Published: 30 March 2012

Abstract: A series of novel benzothiazole-2-thiol derivatives were synthesized and their structures determined by ^1H -NMR, ^{13}C -NMR and HRMS (ESI). The effects of all compounds on a panel of different types of human cancer cell lines were investigated. Among them, pyridinyl-2-amine linked benzothiazole-2-thiol compounds **7d**, **7e**, **7f** and **7i** exhibited potent and broad-spectrum inhibitory activities. Compound **7e** displayed the most potent anticancer activity on SKRB-3 ($\text{IC}_{50} = 1.2 \text{ nM}$), SW620 ($\text{IC}_{50} = 4.3 \text{ nM}$), A549 ($\text{IC}_{50} = 44 \text{ nM}$) and HepG2 ($\text{IC}_{50} = 48 \text{ nM}$) and was found to induce apoptosis in HepG2 cancer cells.

Keywords: anticancer; synthesis; apoptosis; benzothiazole-2-thiol derivatives

1. Introduction

A number of benzothiazole derivatives have exhibited interesting biological activities [1–3] and attracted continuing interest for further molecular exploration as useful anticancer agents [4,5]. Our preceding studies had found that two benzothiazole-2-thiol compounds (compounds **1** and **2**) displayed good anticancer activities and induced HepG2 cell apoptosis *in vitro* [6]. In order to develop more potent tumor growth inhibitors as novel anticancer agents, we designed and synthesized a series of novel benzothiazole-2-thiol derivatives through incorporation of heterocyclic rings (pyridine, pyrimidine and thiazole) to benzothiazole-2-thiol derivatives with the activity and safety advantages of the

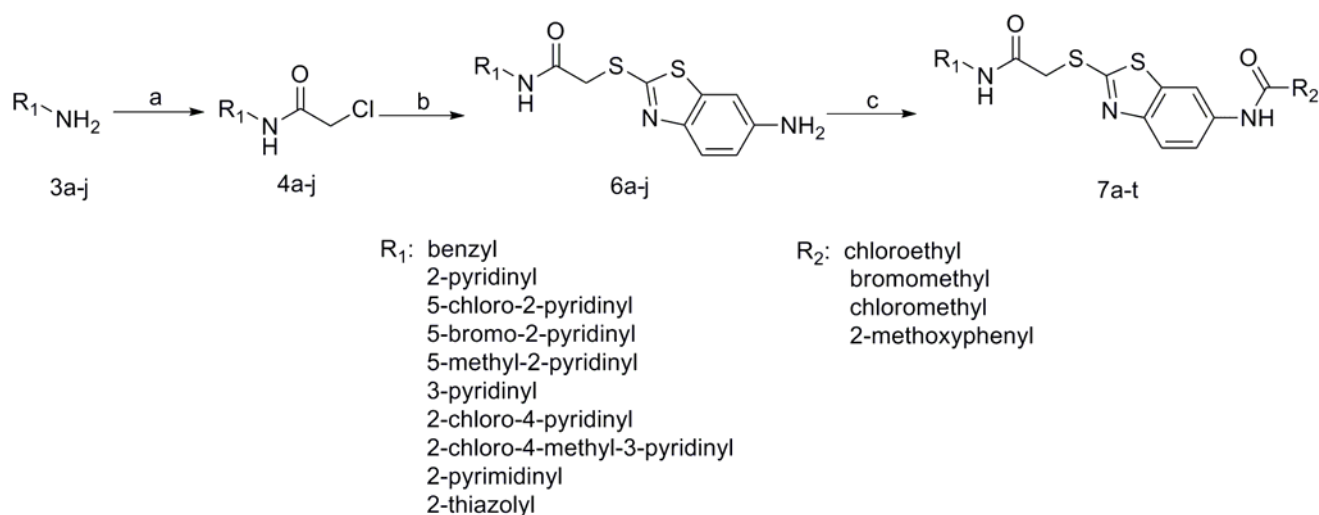
heterocyclic ring structures [7,8]. The effects of all the novel compounds on a panel of different types of human cancer cell lines were investigated by the MTT assay and compound **7e** was selected to examine apoptosis on HepG2 cell cells by flow cytometry. As a result, the pyridinyl-2-amine linked benzothiazole-2-thiol compounds exhibited potent anticancer activities and compound **7e** inhibited the proliferation of HepG2 cell via inducing apoptosis.

2. Results and Discussion

2.1. Chemistry

Twenty novel benzothiazole-2-thiol derivatives linked with heterocyclic rings were designed and synthesized by the route shown in Scheme 1.

Scheme 1. Synthetic route for **7a–t**.



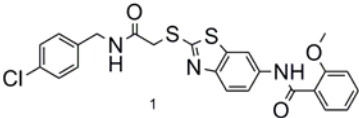
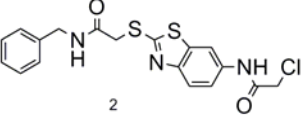
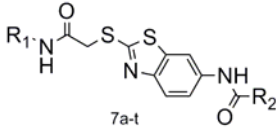
Reagents and conditions: (a) 2-chloroacetyl chloride, CH_2Cl_2 , K_2CO_3 , 4 h, rt, 85%–93%; (b) 6-aminobenzothiazole-2-thiol (compound **5**), THF, Et_3N , reflux, 4–6 h; (c) 2-methoxybenzoyl chloride/2-chloroacetyl chloride/3-chloropropanoyl chloride/2-bromoacetyl bromide, Et_3N , THF, rt, 4–12 h, 63%–91%.

Commercially available amines (compounds **3a–j**) were first reacted with 2-chloroacetyl chloride in the presence of potassium carbonate as the base in dichloromethane to give crude compounds **4a–j**. The raw products **4a–j** were purified by recrystallization from ethyl acetate/petroleum ether. The compounds **6a–j** were prepared by reacting **4a–j** with 6-aminobenzothiazole-2-thiol (compound **5**) and triethylamine (TEA) as the base in tetrahydrofuran (THF) under reflux. Compounds **6a–j** respectively were thus obtained and could be used directly for the next step without further purification. The reaction mixtures of compounds **6a–j** were further reacted with 3-chloropropyl chloride, 2-bromoacetyl bromide, 2-chloroacetyl chloride and 2-methoxybenzoyl chloride in the presence of triethylamine (TEA), respectively. The precipitates were collected by filtration and washed with water to yield the crude products (compounds **7a–t**). Each compound was purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent. The structures of all compounds were determined by ^1H -NMR, ^{13}C -NMR and HRMS (ESI).

2.2. Biological Activities

The twenty novel synthesized benzothiazole-2-thiol derivatives were investigated for anticancer activity *in vitro* on cancer cell lines by the MTT assay with compounds **1** and **2** as positive controls. Compounds **1** and **2** showed good activity against human colon adenocarcinoma cell line (SW480), human cervical cancer cell line (HeLa) and human hepatocellular carcinoma cell line (HepG2) in our preceding studies. Herein, we selected firstly the three human cell lines to test these compounds and the results were presented in Table 1.

Table 1. The anti-proliferative activities for compounds **1**, **2** and **7a–t**.

			  		
Compd.	R ₁	R ₂	IC ₅₀ (μM) ^a		
			HepG2	SW480	HeLa
1	4-chlorobenzyl	2-methoxyphenyl	0.7	5.6	4.0
2	benzyl	chloromethyl	1.0	5.2	4.6
7a	benzyl	chloroethyl	5.6	15.7	8.6
7b	benzyl	bromoethyl	6.6	14.9	48.1
7c	2-pyridinyl	chloromethyl	0.7	2.3	3.1
7d	5-chloro-2-pyridinyl	chloromethyl	0.26	0.46	0.035
7e	5-bromo-2-pyridinyl	chloromethyl	0.048	0.68	0.02
7f	5-methyl-2-pyridinyl	chloromethyl	0.091	1.0	0.03
7g	3-pyridinyl	chloromethyl	6.6	6.4	12.6
7h	2-chloro-4-pyridinyl	chloromethyl	5.0	2.9	1.9
7i	2-chloro-4-methyl-3-pyridinyl	chloromethyl	0.4	2.4	0.4
7j	2-pyrimidinyl	chloromethyl	24.3	54.8	24.3
7k	2-thiazolyl	chloromethyl	13.1	7.1	14.5
7l	2-pyridinyl	2-methoxyphenyl	>100	>100	>100
7m	5-chloro-2-pyridinyl	2-methoxyphenyl	>100	>100	0.6
7n	5-bromo-2-pyridinyl	2-methoxyphenyl	23.5	23.8	23.5
7o	5-methyl-2-pyridinyl	2-methoxyphenyl	45.6	31.1	0.8
7p	3-pyridinyl	2-methoxyphenyl	>100	>100	>100
7q	2-chloro-4-pyridinyl	2-methoxyphenyl	>100	>100	>100
7r	2-chloro-4-methyl-3-pyridinyl	2-methoxyphenyl	23.1	>100	34.6
7s	2-pyrimidyl	2-methoxyphenyl	27.2	>100	>100
7t	2-thiazolyl	2-methoxyphenyl	>100	>100	>100

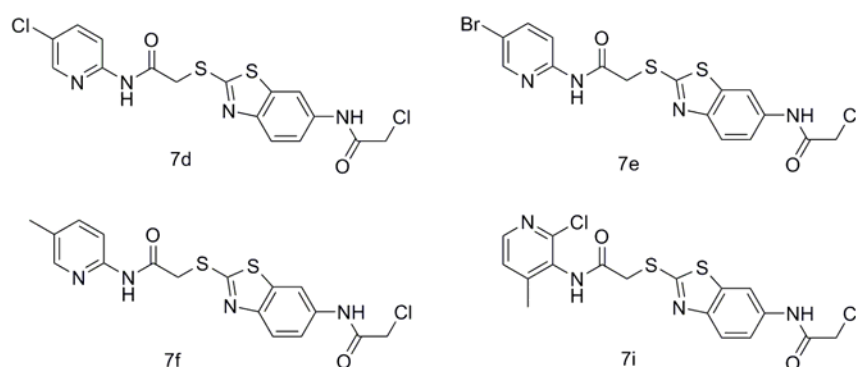
^a Values are means of three experiments.

These novel compounds showed great variation of IC₅₀ values on the three cell lines and compounds **7d**, **7e**, **7f** and **7i** exhibited potent inhibitory activities. To further study the cytotoxic profile, the potent analogues **7d**, **7e**, **7f** and **7i** were selected for further evaluation of inhibitory activities against other eleven types of human cancer cell lines, including colon cancer cell lines HCT-116 and SW620, lung cancer cell line A549, prostate cancer cell line PC-3, pancreatic cancer cell

line BxPC-3, breast cancer cell line BT474, epidermoid cancer cell line A431, ovarian cancer cell line SKOV-3, non-small cell lung cancer cell line H460, breast cancer cell line MDA-MB-468 and SKRB-3.

As shown in Table 2, compounds **7d**, **7e**, **7f** and **7i** exhibited potent and broad-spectrum anticancer activities which were much better than compounds **1** and **2**. Among them, compound **7d** showed the most potent antitumor activities against A431 (IC_{50} = 20 nM) and compound **7e** displayed the most potent anticancer activity on SKRB-3 (IC_{50} = 1.2 nM), SW620 (IC_{50} = 4.3 nM), A549 (IC_{50} = 44 nM) and HepG2 (IC_{50} = 48 nM). The antitumor activities of compounds **7d** and **7e** were about 10–1,000 times greater than compounds **1** and **2** (SW620, A549, SKRB-3 and HepG2). These results suggested that pyridin-2-amine linking benzothiazole-2-thiol derivatives have potent and broad-spectrum anti-cancer activities and be worth being further investigated as candidate of anticancer agent.

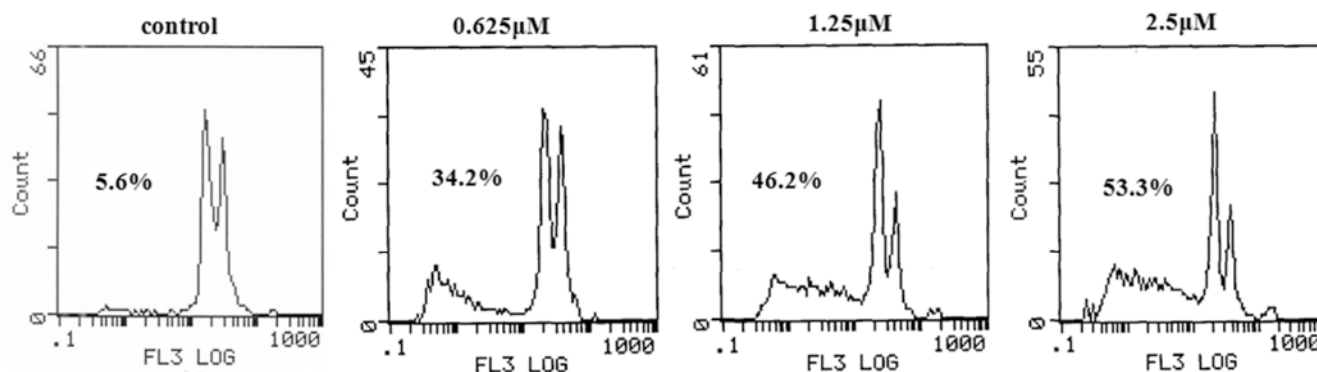
Table 2. The anti-proliferative activities for compounds **7d**, **7e**, **7f**, **7i**, **1** and **2** against various cancer cell lines.



Compd.	IC_{50} (μ M) ^a										MDA-MB-468	SKRB-3
	HCT116	BT474	SW620	H460	PC-3	BXPC-3	A431	A549	SKOV-3			
7d	0.8	4.3	0.033	3.7	7.9	0.1	0.02	0.2	2.7	1.5	0.5	
7e	0.3	0.6	0.0043	0.3	6.0	0.3	0.2	0.044	0.4	0.9	0.0012	
7f	0.6	5.3	1.5	4.2	6.8	0.5	0.1	0.3	3.5	2.5	1.9	
7i	2.2	5.2	6.5	14.6	8.6	9.3	4.8	8.3	5.8	6.8	11.3	
1	1.6	>100	4.1	39.7	4.8	4.7	1.0	2.0	6.0	2.4	1.5	
2	1.2	7.8	1.3	10.0	6.0	6.6	4.5	4.4	4.6	4.0	8.0	

^a Values are means of three experiments.

In order to investigate the apoptosis effects of the benzothiazole-2-thiol derivatives, we selected compound **7e** to examine apoptosis effects on HepG2 cells by flow cytometry. Flow cytometric analysis was performed to measure the apoptotic cells and the cell cycle after propidium iodide (PI) staining [9]. From Figure 1, the percentages of apoptotic cells were 34.2%, 46.2% and 53.3%, respectively, with 0.625 μ M, 1.25 μ M, and 2.5 μ M compound **7e** treatment for 24 h. The results indicated that compound **7e** inhibited the proliferation of HepG2 cell via inducing apoptosis on a concentration-dependent manner. The exact and further biological mechanism of compound **7e** is under investigation in our laboratory.

Figure 1. Compound **7e** concentration-dependently induced apoptosis in HepG2 cancer cells.

3. Experimental

3.1. General

The human cancer cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Dulbecco's modified Eagle medium (DMEM) and RPMI 1640 were purchased from Gibco (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from Hyclone (Logan, UT, USA). Column chromatography was carried out on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China). Thin layer chromatography (TLC) was performed on TLC silica gel 60 F254 plates. Melting points were measured using a Kofler hot stage apparatus and were uncorrected. ^1H -NMR spectroscopy was performed using a Varian Unity Inova-400 spectrometer. The chemical shift values are reported in δ units (ppm) relative to internal standard tetramethylsilane (TMS). ^{13}C -NMR spectra were recorded on a Bruker AV II-600 MHz spectrometer. Mass spectrometry was carried out on a Waters Q-TOF Premier mass spectrometer. All solvents were dried and freshly distilled prior to use according to standard procedures. All the chemicals used were of analytical grade and commercially available. The purity of compound screened in biological assays was determined to be $\geq 90\%$ by HPLC analysis with a photodiode array detector (Waters, Milford, MA, USA). An Atlantis C₁₈ (150 mm $\times$ 4.6 mm, i.d. 5 μ m) (Waters) was used with a gradient elution of methanol and HPLC-grade water as mobile phase at a flow rate of 1 mL/min.

3.2. Preparation of Compounds **4a–j**

Compounds **4a–j** were prepared following the literature procedure [10]. 2-Chloroacetyl chloride (0.75 mol) was added dropwise to a mixture of **3a–j** (0.5 mol) and potassium carbonate (1.0 mol) in dichloromethane (550 mL) at 0 $^{\circ}\text{C}$ with stirring. After removal of the dichloromethane and vacuum filtration, the solid was washed with water and dried under vacuum for 12 h at 25–30 $^{\circ}\text{C}$. The title compounds **4a–j** were purified by recrystallization with petroleum ether/ethyl acetate.

3.3. Preparation of Compounds **6a–j**

Compounds **6a–j** were synthesized according to a literature method [11] with some modifications. Briefly, 6-aminobenzothiazole-2-thiol (35.0 mmol) was added to a mixture of **4a–j** (38.5 mmol) and

triethylamine (TEA, 70.0 mmol) in tetrahydrofuran (THF, 480 mL) at room temperature with stirring for 5 h. The title compounds **6a–j** were obtained, respectively, and could be used directly for the next step without further purification.

3.4. General Procedure for Preparing Compounds **7a–t**

The reaction mixtures of compounds **6a–j** (3 mmol) were further reacted with acyl chloride (3.6 mmol) in the presence of triethylamine (TEA, 4.5 mmol) using tetrahydrofuran (THF, 30 mL) as solvent. The completion of the reaction was monitored by TLC and took 4–26 h. The precipitate was collected by filtration and washed with water to yield the crude product. Compounds **7a–t** were purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent.

N-(2-(2-(Benzylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)-3-chloropropanamide (**7a**). Yellow powder, yield 72%, purity 99.8%, mp 177.2–178.1 °C; ¹H-NMR (DMSO-*d*₆) δ: 2.87 (t, *J* = 6.2 Hz, 2H), 3.91 (t, *J* = 6.2 Hz, 2H), 4.19 (s, 2H), 4.33 (d, *J* = 6.0 Hz, 2H), 7.22–7.28 (m, 5H), 7.55 (dd, *J* = 2.0, 4.4 Hz, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 8.40 (d, *J* = 2.0 Hz, 1H), 8.82 (t, *J* = 5.8 Hz, 1H), 10.33 (s, 1H); ¹³C-NMR (DMSO-*d*₆) δ: 36.53, 39.49, 40.76, 42.54, 111.35, 118.49, 121.04, 126.76, 127.10, 128.19, 131.70, 135.45, 135.81, 138.80, 148.68, 148.80, 164.35, 166.43, 168.09; HRMS (ESI) *m/z*: Calcd. for C₁₉H₁₈ClN₃O₂S₂ 420.0602; Found: 420.0592 (M-H⁺).

N-Benzyl-2-(6-(2-bromoacetamido)benzo[d]thiazol-2-ylthio)acetamide (**7b**). Yellow powder, yield 75%, purity 98.0%, mp 155.6–159.7 °C; ¹H-NMR (DMSO-*d*₆) δ: 4.08 (s, 2H), 4.20 (s, 2H), 4.33 (d, *J* = 6.0 Hz, 2H), 7.22–7.28 (m, 5H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 8.38 (s, 1H), 8.83 (s, 1H), 10.64 (s, 1H); ¹³C-NMR (DMSO-*d*₆) δ: 28.72, 39.48, 42.54, 111.56, 118.65, 121.10, 126.86, 127.32, 128.29, 131.86, 135.48, 135.59, 138.94, 148.74, 148.81, 165.23, 167.36, 169.70; HRMS (ESI) *m/z*: Calcd. for C₁₈H₁₆BrN₃O₂S₂ 449.9940; Found: 449.9929 (M-H⁺).

2-Chloro-*N*-(2-(2-oxo-2-(pyridin-2-ylamino)ethylthio)benzo[d]thiazol-6-yl)acetamide (**7c**). Yellow powder, yield 70%, purity 92.0%, mp 168.4–172.9 °C; ¹H-NMR (DMSO-*d*₆) δ: 4.29 (s, 2H), 4.45 (s, 2H), 7.54–7.58 (m, 2H), 7.78 (d, *J* = 8.8 Hz, 1H), 8.18–8.19 (m, 2H), 8.37 (d, *J* = 16.0 Hz, 1H), 8.89 (s, 1H), 10.35 (s, 1H), 10.54 (s, 1H); ¹³C-NMR (DMSO-*d*₆) δ: 39.46, 43.49, 111.69, 113.46, 118.75, 119.69, 121.12, 135.42, 135.57, 138.32, 148.02, 148.91, 151.59, 164.63, 164.79, 166.20; HRMS (ESI) *m/z*: Calcd. for C₁₆H₁₃ClN₄O₂S₂ 393.0241; Found: 393.0241 (M-H⁺).

2-Chloro-*N*-(2-(2-(5-chloropyridin-2-ylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)acetamide (**7d**). Yellow powder, yield 70%, purity 96.8%, mp 217.2–218.8 °C; ¹H-NMR (DMSO-*d*₆) δ: 4.29 (s, 2H), 4.44 (s, 2H), 7.50–7.54 (m, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 8.41 (s, 1H), 7.50–8.41 (m, 3H), 10.54 (s, 1H), 11.10 (s, 1H); ¹³C-NMR (DMSO-*d*₆) δ: 39.48, 43.54, 111.80, 114.52, 118.77, 121.16, 125.38, 135.49, 135.61, 138.01, 148.79, 148.94, 150.27, 164.62, 165.37, 166.35; HRMS (ESI) *m/z*: Calcd. for C₁₆H₁₂Cl₂N₄O₂S₂ 426.9852; Found: 426.9822 (M-H⁺).

N-(5-Bromopyridin-2-yl)-2-(6-(2-chloroacetamido)benzo[d]thiazol-2-ylthio)acetamide (**7e**). Yellow powder, yield 71%, purity 96.0%, mp 221.3–222.5 °C; ¹H-NMR (DMSO-*d*₆) δ: 4.29 (s, 2H), 4.43 (s, 2H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 8.8 Hz, 1H), 8.47 (s, 1H), 8.38 (s, 1H), 8.02 (m, 2H), 10.54 (s, 1H),

11.09 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.98, 44.05, 112.29, 114.26, 115.56, 119.26, 121.66, 135.79, 135.99, 141.22, 149.12, 149.44, 151.07, 165.11, 165.23, 166.88; HRMS (ESI) m/z : Calcd. for $\text{C}_{16}\text{H}_{12}\text{BrClN}_4\text{O}_2\text{S}_2$ 472.7864; Found: 472.7853 (M-H^+).

2-Chloro-N-(2-(2-(5-methylpyridin-2-ylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)acetamide (7f). Yellow powder, yield 90%, purity 97.1%, mp 214.0–214.9 °C; ^1H -NMR (DMSO- d_6) δ : 2.25 (s, 3H), 4.29 (s, 2H), 4.41 (s, 2H), 7.53 (dd, $J = 2.0, 4.4$ Hz, 1H), 7.61 (dd, $J = 2.0, 4.2$ Hz, 1H), 7.77 (d, $J = 9.2$ Hz, 1H), 7.94 (d, $J = 8.4$ Hz, 1H), 8.18 (s, 1H), 8.37 (d, $J = 1.0$ Hz, 1H), 10.54 (s, 1H), 10.82 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 17.22, 39.46, 43.53, 111.75, 113.13, 118.74, 121.14, 128.73, 135.30, 135.47, 139.06, 147.22, 148.95, 149.20, 164.70, 164.74, 166.02; HRMS (ESI) m/z : Calcd. for $\text{C}_{17}\text{H}_{15}\text{ClN}_4\text{O}_2\text{S}_2$ 407.0398; Found: 407.0397 (M-H^+).

2-Chloro-N-(2-(2-(2-oxo-2-(pyridin-3-ylamino)ethylthio)benzo[d]thiazol-6-yl)acetamide (7g). Yellow powder, yield 68%, purity 92.0%, mp 160.6–165.4 °C; ^1H -NMR (DMSO- d_6) δ : 4.29 (s, 2H), 4.45 (s, 2H), 7.55 (d, $J = 8.0$ Hz, 2H), 7.78 (d, $J = 8.8$ Hz, 1H), 8.19 (d, $J = 8.4$ Hz, 1H), 8.37 (s, 2H), 8.89 (s, 1H), 10.66 (s, 1H), 11.20 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.43, 43.50, 111.67, 118.52, 118.72, 121.18, 130.46, 135.43, 135.51, 140.30, 148.83, 148.89, 164.36, 164.80, 166.47; HRMS (ESI) m/z : Calcd. for $\text{C}_{16}\text{H}_{13}\text{ClN}_4\text{O}_2\text{S}_2$ 393.0241; Found: 393.0247 (M-H^+).

2-Chloro-N-(2-(2-(2-chloropyridin-4-ylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)acetamide (7h). Yellow powder, yield 91%, purity 95.7%, mp 192.5–197.1 °C; ^1H -NMR (DMSO- d_6) δ : 4.29 (s, 2H), 4.43 (s, 2H), 7.47–7.54 (m, 2H), 7.75–7.78 (m, 2H), 8.29 (d, $J = 5.6$ Hz, 1H), 8.39 (s, 1H), 10.55 (s, 1H), 11.08 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.48, 43.53, 111.81, 112.46, 112.62, 118.76, 121.15, 135.34, 135.49, 147.83, 148.85, 150.47, 151.02, 164.34, 164.75, 166.93; HRMS (ESI) m/z : Calcd. for $\text{C}_{16}\text{H}_{12}\text{Cl}_2\text{N}_4\text{O}_2\text{S}_2$ 426.9852; Found: 426.9883 (M-H^+).

2-Chloro-N-(2-(2-(2-chloro-4-methylpyridin-3-ylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)acetamide (7i). Yellow powder, yield 80%, purity 98.0%, mp 186.4–188.3 °C; ^1H -NMR (DMSO- d_6) δ : 2.22 (s, 3H), 4.29 (s, 2H), 4.41 (s, 2H), 7.34 (d, $J = 4.8$ Hz, 1H), 7.67 (t, $J = 8.8$ Hz, 2H), 8.19 (d, $J = 4.8$ Hz, 1H), 8.55 (s, 1H), 10.24 (s, 1H), 10.37 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 17.81, 39.54, 43.53, 111.87, 118.83, 121.08, 125.10, 130.49, 135.30, 135.56, 147.02, 148.67, 148.77, 148.99, 164.43, 164.76, 165.62; HRMS (ESI) m/z : Calcd. for $\text{C}_{17}\text{H}_{14}\text{Cl}_2\text{N}_4\text{O}_2\text{S}_2$ 441.0008; Found: 440.9992 (M-H^+).

2-Chloro-N-(2-(2-(2-oxo-2-(pyrimidin-2-ylamino)ethylthio)benzo[d]thiazol-6-yl)acetamide (7j). Brownish red powder, yield 63%, purity 90.0%, mp 172.6–174.9 °C; ^1H -NMR (DMSO- d_6) δ : 4.29 (s, 2H), 4.59 (s, 2H), 7.22 (t, $J = 4.8$ Hz, 1H), 7.54 (d, $J = 4.4$ Hz, 1H), 7.78 (d, $J = 8.8$ Hz, 1H), 8.38 (s, 1H), 8.69 (d, $J = 2.4$ Hz, 2H), 10.58 (s, 1H), 11.04 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.46, 43.52, 109.98, 117.43, 121.60, 136.22, 147.29, 157.33, 157.66, 158.40, 166.22; HRMS (ESI) m/z : Calcd. for $\text{C}_{15}\text{H}_{12}\text{ClN}_5\text{O}_2\text{S}_2$ 394.0194; Found: 394.0109 (M-H^+).

2-Chloro-N-(2-(2-(2-oxo-2-(thiazol-2-ylamino)ethylthio)benzo[d]thiazol-6-yl)acetamide (7k). Yellow powder, yield 78%, purity 95.0%, mp 218.3–224.7 °C; ^1H -NMR (DMSO- d_6) δ : 4.29 (s, 2H), 4.46 (s, 2H), 7.25 (d, $J = 3.6$ Hz, 1H), 7.50–7.55 (m, 2H), 7.77 (d, $J = 8.8$ Hz, 1H), 8.38 (s, 1H), 10.55 (s, 1H),

12.53 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.46, 43.54, 111.81, 113.79, 118.77, 121.18, 135.33, 135.52, 137.71, 148.87, 157.75, 164.32, 164.74, 165.64; HRMS (ESI) m/z : Calcd. for $\text{C}_{14}\text{H}_{11}\text{ClN}_4\text{O}_2\text{S}_3$ 398.9806; Found: 398.9816 (M-H^+).

2-Methoxy-N-(2-(2-oxo-2-(pyridin-2-ylamino)ethylthio)benzo[d]thiazol-6-yl)benzamide (7l). Yellow powder, yield 70%, purity 92.0%, mp 154.2–158.9 °C; ^1H -NMR (DMSO- d_6) δ : 3.90 (s, 3H), 4.45 (s, 2H), 7.08 (t, $J = 7.4$ Hz, 1H), 7.14 (t, $J = 6.0$ Hz, 1H), 7.19 (d, $J = 8.4$ Hz, 1H), 7.49–7.54 (m, 1H), 7.63–7.69 (m, 2H), 7.80 (dd, $J = 7.0, 6.4$ Hz, 2H), 8.35 (d, $J = 3.6$ Hz, 1H), 8.04 (d, $J = 8.4$ Hz, 1H), 8.54 (s, 1H), 10.35 (s, 1H), 10.91 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.46, 111.69, 118.75, 121.12, 135.41, 135.96, 148.91, 164.19, 164.57, 166.22; HRMS (ESI) m/z : Calcd. for $\text{C}_{22}\text{H}_{18}\text{N}_4\text{O}_3\text{S}_2$ 451.0893; Found: 451.0887 (M-H^+).

N-(2-(2-(5-Chloropyridin-2-ylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)-2-methoxybenzamide (7m). Yellow powder, yield 70%, purity 97.0%, mp 148.5–154.1 °C; ^1H -NMR (DMSO- d_6) δ : 3.90 (s, 3H), 4.45 (s, 2H), 7.19 (d, $J = 8.4$ Hz, 1H), 7.2 (t, $J = 7.4$ Hz, 2H), 7.63–7.75 (m, 2H), 7.77 (d, $J = 8.8$ Hz, 1H), 7.92 (d, $J = 4.4$ Hz, 1H), 8.08 (d, $J = 8.4$ Hz, 1H), 8.41 (d, $J = 2.4$ Hz, 1H), 8.54 (s, 1H), 10.35 (s, 1H), 11.11 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.46, 55.84, 111.83, 111.93, 114.52, 119.17, 120.46, 120.99, 124.68, 125.38, 129.66, 132.09, 135.41, 135.95, 137.99, 146.43, 148.73, 150.28, 156.46, 164.17, 164.53, 166.37; HRMS (ESI) m/z : Calcd. for $\text{C}_{22}\text{H}_{17}\text{ClN}_4\text{O}_3\text{S}_2$ 485.0504; Found: 485.0521 (M-H^+).

N-(2-(2-(5-Bromopyridin-2-ylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)-2-methoxybenzamide (7n). Yellow powder, yield 75%, purity 99.0%, mp 178.4–183.5 °C; ^1H -NMR (DMSO- d_6) δ : 3.90 (s, 3H), 4.45 (s, 2H), 7.07 (t, $J = 7.4$ Hz, 1H), 7.19 (d, $J = 8.4$ Hz, 1H), 7.51 (t, $J = 7.8$ Hz, 1H), 7.66 (d, $J = 10.8$ Hz, 2H), 7.77 (d, $J = 8.8$ Hz, 1H), 7.95–8.03 (m, 2H), 8.48 (s, 1H), 8.54 (s, 1H), 10.35 (s, 1H), 11.10 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.49, 55.87, 111.86, 111.95, 113.78, 115.08, 119.19, 120.48, 121.01, 124.69, 129.68, 132.11, 135.41, 135.96, 148.63, 148.74, 150.59, 156.48, 164.17, 164.54, 166.41; HRMS (ESI) m/z : Calcd. for $\text{C}_{22}\text{H}_{17}\text{BrN}_4\text{O}_3\text{S}_2$ 528.9998; Found: 529.0070 (M-H^+).

2-Methoxy-N-(2-(2-(5-methylpyridin-2-ylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)benzamide (7o). Yellow powder, yield 76%, purity 92.0%, mp 182.7–188.5 °C; ^1H -NMR (DMSO- d_6) δ : 2.28 (s, 3H), 3.90 (s, 3H), 4.45 (s, 2H), 7.08 (t, $J = 7.6$ Hz, 1H), 7.19 (d, $J = 8.4$ Hz, 1H), 7.50–7.62 (m, 2H), 7.68 (t, $J = 10.2$ Hz, 2H), 7.78 (d, $J = 8.8$ Hz, 1H), 7.95 (s, 1H), 8.18 (s, 1H), 8.52 (d, $J = 2.0$ Hz, 1H), 10.31 (s, 1H), 10.76 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 17.24, 39.49, 55.86, 111.84, 111.96, 112.99, 119.18, 120.47, 121.00, 124.71, 128.61, 129.67, 132.10, 135.41, 135.95, 138.57, 147.80, 148.79, 149.49, 156.48, 164.33, 164.55, 165.90; HRMS (ESI) m/z : Calcd. for $\text{C}_{23}\text{H}_{20}\text{N}_4\text{O}_3\text{S}_2$ 465.1050; Found: 465.1071 (M-H^+).

2-Methoxy-N-(2-(2-oxo-2-(pyridin-3-ylamino)ethylthio)benzo[d]thiazol-6-yl)benzamide (7p). Yellow powder, yield 75%, purity 95.0%, mp 154.8–159.5 °C; ^1H -NMR (DMSO- d_6) δ : 3.90 (s, 3H), 4.45 (s, 2H), 7.08 (t, $J = 7.6$ Hz, 1H), 7.19 (d, $J = 8.4$ Hz, 1H), 7.50–7.62 (m, 2H), 7.55 (d, $J = 8.0$ Hz, 1H), 7.70 (t, $J = 6.6$ Hz, 1H), 7.78 (d, $J = 8.8$ Hz, 1H), 8.29 (d, $J = 8.4$ Hz, 1H), 8.46 (d, $J = 4.4$ Hz, 1H), 8.39 (s, 1H), 8.95 (s, 1H), 10.36 (s, 1H), 11.12 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.44, 55.62, 111.90, 112.33, 119.21, 119.95, 120.99, 121.24, 124.69, 126.23, 129.67, 132.97, 135.41, 135.96, 140.75, 144.51,

148.75, 156.47, 158.03, 164.19, 164.57, 167.32; HRMS (ESI) m/z : Calcd. for $C_{22}H_{18}N_4O_3S_2$ 451.0893; Found: 451.0833 ($M-H^+$).

N-(2-(2-(2-Chloropyridin-4-ylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)-2-methoxybenzamide (**7q**). Yellow powder, yield 70%, purity 90.0%, mp 177.9–180.8 °C; 1H -NMR (DMSO- d_6) δ : 3.90 (s, 3H), 4.44 (s, 2H), 7.07 (t, $J = 7.4$ Hz, 1H), 7.19 (d, $J = 8.0$ Hz, 1H), 7.47–7.53 (m, 2H), 7.53–7.62 (m, 2H), 7.76 (t, $J = 5.8$ Hz, 1H), 7.78 (s, 1H), 8.29 (d, $J = 5.6$ Hz, 1H), 8.55 (s, 1H), 10.35 (s, 1H), 11.06 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.44, 55.84, 111.90, 111.93, 112.47, 112.62, 119.20, 120.46, 120.99, 124.67, 129.65, 132.11, 135.41, 135.97, 148.65, 150.47, 151.03, 156.45, 163.91, 164.57, 166.95; HRMS (ESI) m/z : Calcd. for $C_{22}H_{17}ClN_4O_3S_2$ 485.0504; Found: 485.0491 ($M-H^+$).

N-(2-(2-(2-Chloro-4-methylpyridin-3-ylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)-2-methoxybenzamide (**7r**). Yellow powder, yield 77%, purity 90.0%, mp 166.5–168.1 °C; 1H -NMR (DMSO- d_6) δ : 2.22 (s, 3H), 3.90 (s, 3H), 4.41 (s, 2H), 7.08 (t, $J = 7.2$ Hz, 1H), 7.19 (d, $J = 8.4$ Hz, 1H), 7.34 (d, $J = 4.8$ Hz, 1H), 7.52 (t, $J = 7.8$ Hz, 1H), 7.64 (d, $J = 7.2$ Hz, 1H), 7.69 (d, $J = 8.8$ Hz, 1H), 7.79 (d, $J = 8.8$ Hz, 1H), 8.19 (d, $J = 4.8$ Hz, 1H), 8.55 (s, 1H), 10.24 (s, 1H), 10.37 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 17.84, 39.51, 55.87, 111.91, 111.97, 119.22, 120.49, 120.92, 124.76, 129.65, 130.51, 132.11, 135.47, 135.96, 147.05, 148.70, 148.76, 156.48, 164.03, 164.61, 165.67; HRMS (ESI) m/z : Calcd. for $C_{23}H_{19}ClN_4O_3S_2$ 500.0123; Found: 500.0124 ($M-H^+$).

2-Methoxy-*N*-(2-(2-oxo-2-(pyrimidin-2-ylamino)ethylthio)benzo[d]thiazol-6-yl)benzamide (**7s**). Yellow powder, yield 70%, purity 90.0%, mp 169.2–171.8 °C; 1H -NMR (DMSO- d_6) δ : 3.90 (s, 3H), 4.46 (s, 2H), 7.08 (t, $J = 7.4$ Hz, 1H), 7.18–7.24 (m, 2H), 7.52 (t, $J = 7.6$ Hz, 1H), 7.64–7.69 (m, 2H), 7.78 (d, $J = 8.8$ Hz, 1H), 8.54 (s, 1H), 8.69 (d, $J = 8.4$ Hz, 2H), 10.35 (s, 1H), 11.04 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.46, 55.86, 111.84, 111.95, 119.17, 120.48, 121.00, 124.67, 129.68, 132.12, 135.38, 135.90, 148.80, 156.48, 157.37, 158.41, 164.45, 164.55, 166.18; HRMS (ESI) m/z : Calcd. for $C_{21}H_{17}N_5O_3S_2$ 452.0846; Found: 452.0821 ($M-H^+$).

2-Methoxy-*N*-(2-(2-oxo-2-(thiazol-2-ylamino)ethylthio)benzo[d]thiazol-6-yl)benzamide (**7t**). Yellow powder, yield 68%, purity 92.0%, mp 237.7–239.1 °C; 1H -NMR (DMSO- d_6) δ : 3.90 (s, 3H), 4.47 (s, 2H), 7.08 (t, $J = 7.4$ Hz, 1H), 7.19 (d, $J = 8.4$ Hz, 1H), 7.25 (d, $J = 3.6$ Hz, 1H), 7.52 (t, $J = 6.4$ Hz, 2H), 7.64–7.69 (m, 2H), 7.77 (d, $J = 8.8$ Hz, 1H), 8.54 (s, 1H), 10.36 (s, 1H), 12.55 (s, 1H); ^{13}C -NMR (DMSO- d_6) δ : 39.37, 56.37, 112.39, 112.47, 114.32, 119.71, 120.98, 121.54, 125.24, 130.15, 132.60, 135.95, 136.50, 138.24, 149.19, 156.97, 158.27, 164.38, 165.07, 166.18; HRMS (ESI) m/z : Calcd. for $C_{20}H_{16}N_4O_3S_3$ 457.0458; Found: 457.0457 ($M-H^+$).

3.5. Cell Culture

Cell lines HepG2, HeLa, HCT116, SW620, SKOV-3 and MDA-MB-468 were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 mg/L). Cell lines BT474, A431 and SKRB-3 were maintained in Dulbecco's modified Eagle medium (DMEM) containing 20% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 mg/L). Cell lines H460, PC-3, A549, SW480, and BxPC-3 were

maintained in RPMI 1640 containing 10% FBS, penicillin (100 U/mL) and streptomycin (10 mg/L). Cells were grown in a 5% CO₂ incubator at 37 °C.

3.6. Cell Proliferation Assay (MTT Assay)

Cells ($3\text{--}5 \times 10^3$ /well) were seeded in 200 µL of medium/well in 96-well plates (Costar Corning, Rochester, NY, USA) and cultured for 24 h. The compounds dissolved in dimethylsulfoxide (DMSO) were added to final concentration of 40 µM, 20 µM, 10 µM, 5 µM, 2.5 µM, 1.25 µM, respectively. Compounds **1** and **2** were used as positive control. After 48 h exposure, a volume of 10 µL of 10 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added per well and incubated for another 4 h at 37 °C, then the supernatant fluid was removed and DMSO (150 µL) was added 150 µL/well to dissolve formazan crystals for 15–20 min. The light absorptions (OD) were measured at 570 nm with SpectraMAXM5 microplate spectrophotometer (Molecular Devices). The effect of compounds on tumor cells viability was expressed by IC₅₀ of each cell line. Values shown are the % viability vs. ctrl + SD, n = six independent experiments in triplicate.

3.7. Apoptosis Analysis by Flow Cytometry (FCM)

Cells were seeded into 1 mL of medium/well cell in 6-well plates (Costar Corning) culture bottles at 10×10^6 cells 24 h before treatment. Then cells were treated with compound **7e** for 24 h: 0 µM (control), 0.625 µM, 1.25 µM, 2.5 µM. After 24 h incubation, floating and adherent cells were collected, washed three times with PBS (pH 7.4) and fixed for 24 h with cool alcohol at 4 °C. 1 mL cell suspension (10^6 /mL) was washed three times with cooled PBS, treated with RNase for 30 min at 37 °C, stained it with PI for 30 min at 37 °C in a dark environment, and taken for flow cytometry analysis.

4. Conclusions

In conclusion, a series of novel benzothiazole-2-thiol derivatives were synthesized and their anti-proliferative activities were evaluated *in vitro*. The results showed that the pyridinyl-2-amine linked benzothiazole-2-thiol compounds **7d**, **7e**, **7f** and **7i** exhibited increased anticancer activities compared with compounds **1** and **2** on the three human cancer cells (SW480, HeLa and HepG2). Further studies showed that they displayed potent and broad-spectrum anti-proliferative activities against other human cancer cell lines. Among these compounds, compound **7e** displayed the most potent anticancer activity on SKRB-3 (IC₅₀ = 1.2 nM), SW620 (IC₅₀ = 4.3 nM), A549 (IC₅₀ = 44 nM) and HepG2 (IC₅₀ = 48 nM). The results of flow cytometry analysis indicated that compound **7e** induce apoptosis in HepG2 cancer cells on a concentration-dependent manner. These data suggested that compound **7e** may be powerful tumor growth inhibitors via apoptosis as novel anticancer agents and be worth being further investigated as a potential of anticancer agent.

Acknowledgements

This work was supported by National Science and Technology Major Project of China (2009ZX09103-132). The authors gratefully thank Yong-Qiu Mao (State Key Laboratory of Biotherapy, Huaxi Hospital, Sichuan University) for helpful technical assistance and discussion in the flow cytometry analysis.

References and Notes

1. Quiroga, J.; Hernandez, P.; Insuasty, B.; Abonia, R.; Cobo, J.; Sanchez, A.; Nogueras, M.; Low, J.N. Control of the reaction between 2-aminobenzothiazoles and Mannich bases. Synthesis of pyrido[2,1-*b*][1,3]benzothiazoles *versus* [1,3]benzothiazolo[2,3-*b*]quinazolines. *J. Chem. Soc. Perkin Trans. I* **2002**, *1*, 555–559.
2. Kok, S.H.L.; Chui, C.H.; Lam, W.S.; Chen, J.; Lau, F.Y.; Wong, R.S.M.; Cheng, G.Y.M.; Lai, P.B.S.; Leung, R.W.T.; Tang, J.C.O.; *et al.* Synthesis and structure evaluation of a novel cantharimide and its cytotoxicity on SK-Hep-1 hepatoma cells. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1155–1159.
3. Kok, S.H.L.; Chui, C.H.; Lam, W.S.; Chen, J.; Lau, F.Y.; Wong, R.S.M.; Cheng, G.Y.M.; Tang, W.K.; Teo, I.T.N.; Cheung, F.; *et al.* Apoptogenic activity of a synthetic cantharimide in leukaemia: Implication on its structural activity relationship. *Int. J. Mol. Med.* **2006**, *18*, 1217–1221.
4. Kok, S.H.L.; Gambari, R.; Chui, C.H.; Yuen, M.C.W.; Lin, E.; Wong, R.S.M.; Lau, F.Y.; Cheng, G.Y.M.; Lam, W.S.; Chan, S.H.; *et al.* Synthesis and anti-cancer activity of benzothiazole containing phthalimide on human carcinoma cell lines. *Bioorg. Med. Chem.* **2008**, *16*, 3626–3631.
5. Song, E.Y.; Kau, R.N.; Park, M.Y.; Jin, Y.L.; Lee, K.; Kim, G.; Lee, K.Y.; Yang, J.S.; Shin, J.H.; Nam, K.Y.; *et al.* Synthesis of amide and urea derivatives of benzothiazole as Raf-1 inhibitor. *Eur. J. Med. Chem.* **2008**, *43*, 1519–1524.
6. Wang, Z.; Shi, X.H.; Wang, J.; Zhou, T.; Xu, Y.Z.; Huang, T.T.; Li, Y.F.; Zhao, Y.L.; Yang, L.; Yang, S.Y.; *et al.* Synthesis, structure-activity relationships and preliminary antitumor evaluation of benzothiazole-2-thiol derivatives as novel apoptosis inducers. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1097–1101.
7. Guo, X.Z.; Shi, L.; Wang, R.; Liu, X.X.; Li, B.G.; Lu, X.X. Synthesis and biological activities of novel nonpeptide angiotensin II receptor antagonists based on benzimidazole derivatives bearing a heterocyclic ring. *Bioorg. Med. Chem. Lett.* **2008**, *16*, 10301–10310.
8. Liu, Y.; Gerald, N.L. Activation of heterocyclic amines by combinations of prostaglandin H synthase-1 and -2 with *N*-acetyltransferase 1 and 2. *Cancer Lett.* **1998**, *133*, 115–123.
9. Peng, F.; Wei, Y.Q.; Tian, L.; Yang, L.; Zhao, X.; Lu, Y.; Mao, Y.Q.; Kan, B.; Lei, S.; Wang, G.S.; *et al.* Induction of apoptosis by norcantharidin in human colorectal carcinoma cell lines: Involvement of the CD95 receptor/ligand. *Cancer Res. Clin. Oncol.* **2002**, *128*, 223–230.
10. Baraldi, P.G.; Preti, D.; Tabrizi, M.A.; Fruttarolo, F.; Saponaro, G.; Baraldi, S.; Romagnoli, R.; Moorman, A.R.; Gessi, S.; Varani, K.; *et al.* Hybrid molecules between distamycin A and active moieties of antitumor agents. *Bioorg. Med. Chem.* **2007**, *15*, 17–35.

11. Musso, D.L.; Cochran, F.R.; Kelley, J.L.; McLean, E.W.; Selph, J.L.; Rigdon, G.C.; Orr, G.F.; Davis, R.G.; Cooper, B.R.; Styles, V.L.; *et al.* Indanylidenes. 1. Design and synthesis of (*E*)-2-(4,6-difluoro-1-indanylidene)acetamide, a potent, centrally acting muscle relaxant with antiinflammatory and analgesic activity. *J. Med. Chem.* **2003**, *46*, 399–408.

Sample Availability: Samples of the compounds **7a–t** are available from the authors.

© 2012 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).