Design, Synthesis and Anticancer Activities of Diaryl Urea Derivatives Bearing *N*-Acylhydrazone Moiety

Bei Zhang,[a] Yanfang Zhao,[a] Xin Zhai,[a] Lihui Wang,[b] Jingyu Yang,[b] Zehui Tan,[a] and Ping Gong*[,a]

[a] *Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University; and* [b] *School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University; Shenyang, Liaoning 110016, P. R. China.*
Received April 16, 2012; accepted May 18, 2012

A new series of diaryl urea derivatives bearing *N*-acylhydrazone moiety were designed and synthesized. All the target compounds were evaluated for their cytotoxic activities *in vitro* against human lung adenocarcinoma epithelial cell line (A549), human breast cancer cell line (MDA-MB-231) and human leukemia cell line (HL-60) by standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Several compounds (1a, 1f and 1h) were further evaluated against human embryonic fibroblast, lung-derived cell line (WI38). The pharmacological results indicated that some compounds exhibited promising anticancer activities. In particular, compound 1f showed the most potent cytotoxicity against the tested three cell lines with IC_{50} values of 0.41 μM, 0.24 μM and 0.23 μM, respectively.

Key words diaryl urea; *N*-acylhydrazone; anticancer activity

During the past decade, a large number of multitargeted inhibitors were reported to exhibit anticancer activities with various mechanisms of action as distinct from those cytotoxic drugs and mono-targeted inhibitors.[1–4] Some of them have been developed successfully such as sorafenib (Nexavar), sunitinib (Sutent) and lapatinib (Tykerb). Sorafenib (Fig. 1), the first orally bioavailable multitargeted receptor tyrosine kinase inhibitor, has a reasonable enzyme potency against Raf, vascular endothelial growth factor receptor (VEGFR) and platelet derived growth factor receptor (PDGFR) and pathway inhibition in cells (IC_{50} values of 40–1200 nM depending on the cell line).[5–9] It was approved by Food and Drug Administration (FDA) for the treatment of advanced renal cell carcinoma in December 2005 and hepatocellular carcinoma in November in 2007, respectively.[10,11]

PAC-1 (Fig. 1) is the first small molecular procaspase-3 activator that induces apoptotic death in tumor cell lines and retards tumor growth *in vivo*.[12,13] In an attempt to discover new anticancer agents with multi-targeted molecular mechanisms, we combined the diaryl urea moiety from sorafenib and *N*-acylhydrazone, the pharmacophore of PAC-1, with a thiazolyl ring (structure **1**) or an amide bond (structure **2**) as the linker. Thus a series of diaryl urea derivatives bearing *N*-acylhydrazone moiety (Fig. 2) were designed and synthesized. Various substituted ureido-linked phenyl (Ar^1) and hydrazone-linked phenyl (Ar^2) groups were introduced to explore the influence of electronic and steric effects on the anticancer activity. Since only with the hydroxyl group on the phenyl ring Ar^2 did the PAC-1 derivatives display antitumor activity *in vitro*,[12] 2-hydroxyl substitution was retained for the phenyl ring Ar^2. Four- and 5-benzoxyl groups were respectively introduced to the phenyl ring Ar^2 to investigate the effect of the extension of the hydrophobic region. Furthermore, the phenyl group at Ar^2 was replaced with a substituted chromenonyl group which was often associated with a variety of biological activities to explore whether such a replacement would bring a significant rise in anticancer activity.

Results and Discussion

Chemistry The synthetic route of target compounds **1a**–**i** is described in Chart 1. The commercially available 4-aminobezonitrile reacted with triphosgene in dioxane to obtain 4-isocyanatobenzonitrile **3**, which was treated with different substituted anilines to get diaryl ureas **4a**–**h**. **4a**–**h** were then turned into thioamides **5a**–**h** under the condition of magnesium chloride and sodium bisulfide in *N*,*N*-dimethylformamide. Cyclization of **5a**–**h** with 1,3-dichloroacetone in tetrahydrofuran readily afforded thiozoles **6a**–**h**, which reacted with piperazine in ethanol by nucleophilic substitution to get **7a**–**h**. Consequently, ethyl chloroacetate was added to a solution of **7a**–**h** in ethanol to afford esters **8a**–**h**, followed by hydrazinolysis to obtain acylhydrazines **9a**–**h**. Finally, target compounds **1a**–**i** were prepared *via* respective condensation of **9a**–**h** with various benzaldehydes and benzyloxybenzaldehydes **17** and **18**.

The synthesis of target compounds **2a**–**i** is described in

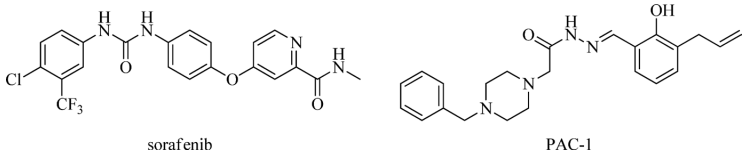


Fig. 1. Structures of Sorafenib and PAC-1

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: gongpinggp@126.com

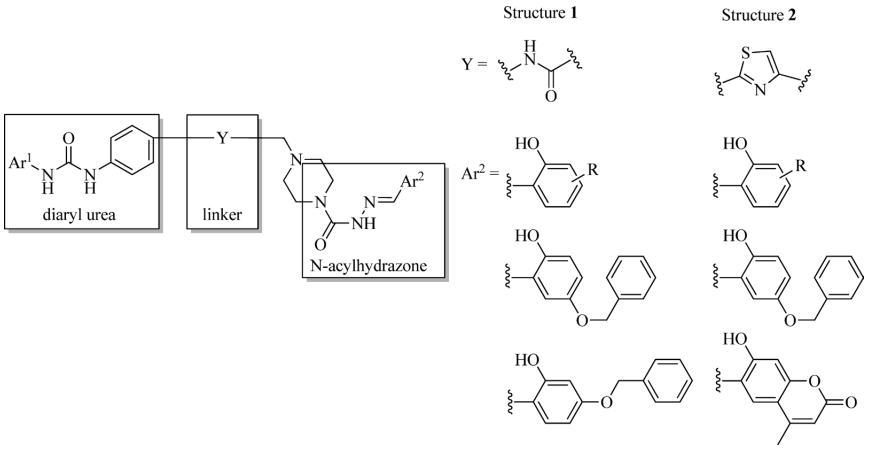
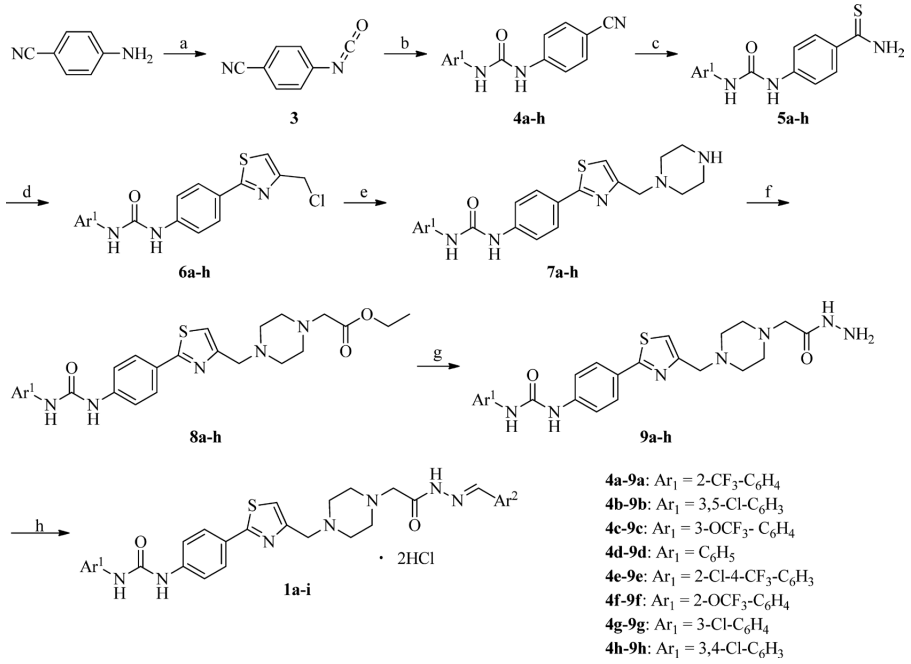


Fig. 2. The General Structures of Target Compounds



Reactions and conditions: (a) triphosgene, dioxane, 80°C, 24 h; (b) Ar^1NH_2, THF, r.t.; (c) $MgCl_2$, NaSH, DMF, overnight; (d) 1,3-dichloroacetone, THF, 50°C, 7 h; (e) piperazine, ethanol, r.t., 2 h; (f) ethyl chloroacetate, K_2CO_3, NaI, ethanol, 50°C, 2 h; (g) 80% hydrazine hydrate, ethanol, 50°C, 48 h; (h) i. Ar^2CHO or **17** or **18**, ethanol; ii. HCl-ethanol.

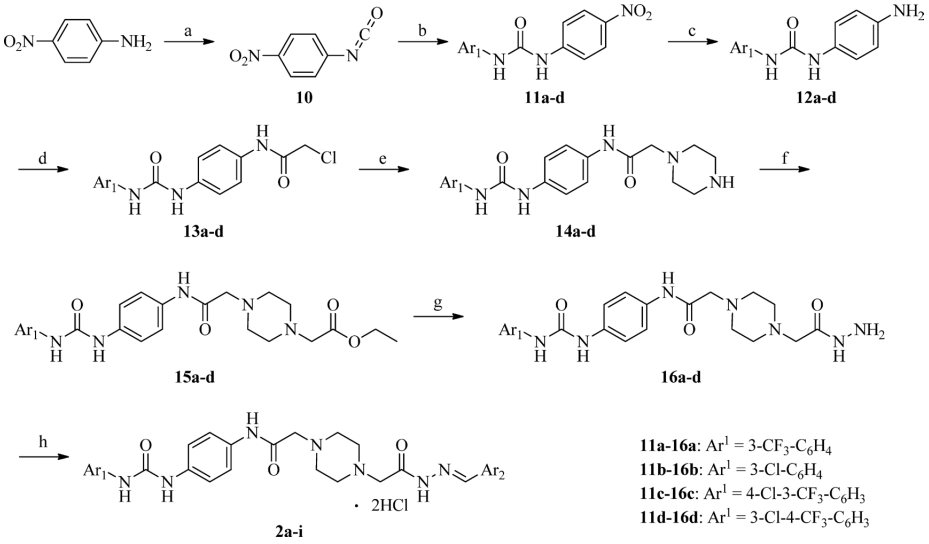
Chart 1. Synthesis of Compounds **1a**–**i**

Chart 2. 4-Nitroaniline reacted with triphosgene in dioxane to afford 1-isocyanato-4-nitrobenzene **10**, which was treated with corresponding substituted anilines to give diaryl ureas **11a**–**d**. Reduction of compounds **11a**–**d** in the presence of iron powder and ammonium chloride in 95% ethanol gave ureidoanilines **12a**–**d**, followed by reaction with chloroacetyl chloride to afford chloroactetamides **13a**–**d**, which underwent nucleophilic attack by piperazine to give **14a**–**d**. According to the same procedures for the preparation of **1a**–**i**, the desired compounds **2a**–**i** were obtained *via* respective condensation of **16a**–**d** with various benzaldehydes, benzyloxybenzaldehyde **17** and chromenealdehyde **20**.

As shown in Chart 3, benzyloxybenzaldehydes **17** and **18** were prepared from 2,5-dihydroxybenzaldehyde and 2,4-dihydroxybenzaldehyde respectively through reactions with benzyl chloride in acetonitrile in the presence of sodium bicarbonate and potassium iodide. Chromenealdehyde **20** was synthesized from benzene-1,3-diol *via* a two-step reaction pathway with ethyl acetoacetate and urotropine in sequence.

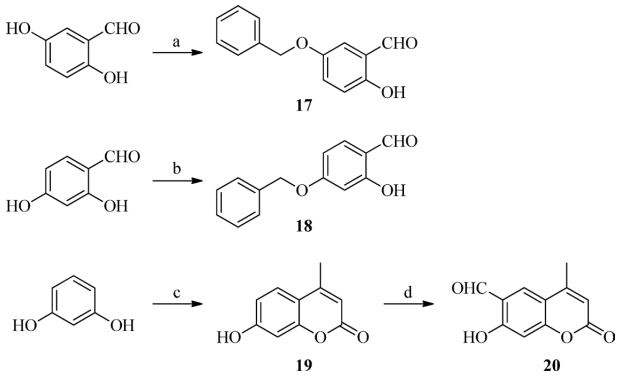
Biological Evaluation The cytotoxicity of target compounds **1a**–**i** and **2a**–**i** were evaluated against human lung adenocarcinoma epithelial cell line (A549), human breast cancer cell line (MDA-MB-231) and human leukemia cell line (HL-60) with sorafenib and PAC-1 as the positive controls. Three compounds (**1a**, **1f** and **1h**) were further evaluated against human embryonic fibroblast, lung-derived cell line (WI38) *in vitro*. The results expressed as IC_{50} values were summarized in Table 1.

As listed in Table 1, most of the target compounds exhibited moderate to strong activities to the tested cell lines. Generally, compounds **1a**–**i** with thiazolyl linker were more potent than compounds **2a**–**i** with amide. It suggested that the heterocyclic ring bridging the two pharmacophores is essential for the activity, and replacement with an acyclic linker may cause a



Reactions and conditions: (a) triphosgene, dioxane, 80°C, 24 h; (b) Ar^1NH_2, THF, r.t.; (c) Fe, NH_4Cl, HOAc, ethanol, 80°C, 5 h; (d) chloroacetyl chloride, TEA, CH_2Cl_2, 0°C, r.t., 5 h; (e) piperazine, ethanol, r.t., 6 h; (f) ethyl chloroacetate, K_2CO_3, NaI, ethanol, 50°C, 2 h; (g) 80% hydrazine hydrate, ethanol, 50°C, 48 h; (h) i. Ar^2CHO or **17** or **20**, ethanol; ii. HCl-ethanol.

Chart 2. Synthesis of Compounds **2a**–**i**



Reactions and conditions: (a) benzyl chloride $NaHCO_3$, CH_3CN, r.f., 30 h; (b) benzyl chloride, $NaHCO_3$, CH_3CN, reflux, 30 h; (c) ethyl acetoacetate, H_2SO_4, 10°C, 4 h; (d) urotropine, HOAc, 115°C, 4 h.

Chart 3. Synthesis of Intermediates **17**, **18** and **20**

dramatical decrease in the activity, or even a complete loss.

Compounds **1f**, **1g** and **1i** with trifluoromethoxyl or trifluoromethyl group at the *ortho*-position and dichloro group at the *meta*- and *para*-positions of phenyl ring Ar^1 repectively showed more potent cytotoxicity than compound **1d** with no substituent and **1h** with only chloro group at the *meta*-position. Compounds **2e** and **2g** with trifluoromethyl or more electron-withdrawing groups exhibited comparable cytotoxicity to sorafenib and PAC-1, while compound **2f** with only chloro group at the *meta*-position of phenyl Ar^1 showed no activity against A549 and MDA-MB-231 cell lines. These findings suggested that the electron-withdrawing groups on the phenyl ring Ar^1 are favorable to the activity.

Compounds with alkyl groups or no substituents at the phenyl ring Ar^2, *e.g.* compounds **1c** and **1a**, exhibited comparable activities to sorafenib and PAC-1. Compounds **1f** and **1g** with the benzyloxyl group at the 5-position of the phenyl ring showed a remarkable increase in the activity, especially compound **1f** displayed the most potent activity with IC_{50} values of 0.41 μM, 0.24 μM and 0.23 μM, respectively, which were 3.2- to 19.8-fold higher than sorafenib and PAC-1. However, compounds **1h** and **1i** with the benzyloxyl group at the 4-position of the phenyl ring did not show a similar result. These findings indicated that the aryl substituent at the 5-position of Ar^2 would significantly enhance the cytotoxicity.

To investigate the effect of a heterocylic ring for Ar^2, we replaced the phenyl group with a substituted chromenonyl group to obtained compounds **2h** and **2i**. Unfortunately, these compounds exhibited even lower inhibition activities than the unfavored compounds **2a**–**g**. It suggested that the phenyl group substituted at Ar^2 is critical for the activity.

Compounds **1a**, **1f** and **1h** exerted weaker cytotoxicity against WI38 (the normal cell line) than the three cancer cell lines. The most promising compound **1f** possessed selective indexes (IC_{50} normal cell/IC_{50} cancer cell) of 11, 19 and 20 for A549, MDA-MB-231 and HL-60 cell lines, respectively.

Conclusion

In summary, a new series of diaryl urea derivatives bearing *N*-acylhydrazone moiety were designed and synthesized. All the compounds were screened for their cytotoxicity against A549, MDA-MB-231 and HL-60 cell lines. The pharmacological results indicated that some of the prepared compounds showed promising activities. Compound **1f** showed the most potent cytotoxicity against A549, MDA-MB-231 and HL-60 cell lines with IC_{50} values of 0.41 μM, 0.24 μM and 0.23 μM, repectively, which were 3.2- to 19.8-fold higher than sorafenib and PAC-1. Moreover, compound **1f** exhibited the most selective cytotoxicity against the three cancer cell lines when compared with WI38 cell line. The preliminary strucuture–activity relationships (SARs) showed that the thiazolyl linker and the phenyl group of Ar^2 are favorable for the activity. Moreover, electron-withdrawing groups on Ar^1 and the benzyloxy group at the 5-position of phenyl ring Ar^2 could enhance the activity. These investigations prompt us to carry on further studies on their mechanisms of action.

Experimental

Chemistry Melting points were obtained on a Büchi

Table 1. Structures and Cytotoxicity of Compounds **1a–i** and **2a–i** against A549, MDA-MB-231, HL-60 and WI38 Cell Lines

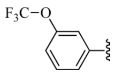
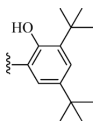
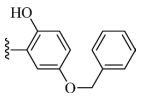
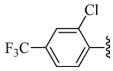
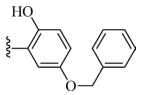
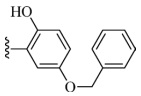
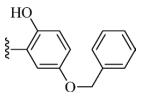
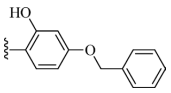
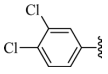
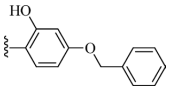
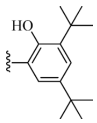
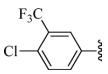
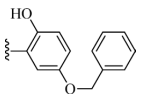
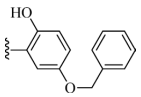
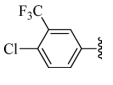
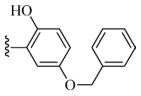
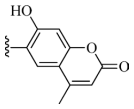
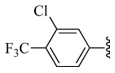
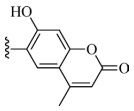
Compd.	Ar^1	Ar^2	IC_{50} (μM)			
			A549	MDA-MB-231	HL-60	WI38
1a			3.88	4.65	2.40	11.67
1b			2.40	1.27	0.33	ND[a)]
1c			1.49	0.90	2.11	ND[a)]
1d			1.67	0.74	0.55	ND[a)]
1e			2.30	0.59	1.03	ND[a)]
1f			0.41	0.24	0.23	4.53
1g			0.45	0.30	0.35	ND[a)]
1h			0.83	0.52	1.18	6.91
1i			0.78	0.47	0.80	ND[a)]
2a			8.97	2.39	4.24	ND[a)]
2b			1.34	0.49	1.29	ND[a)]
2c			52.0	1.18	2.44	ND[a)]
2d			10.7	1.07	4.42	ND[a)]
2e			3.16	1.48	2.66	ND[a)]
2f			>100	>100	1.70	ND[a)]

Table 1. Structures and Cytotoxicity of Compounds **1a–i** and **2a–i** against A549, MDA-MB-231, HL-60 and WI38 Cell Lines

Compd.	Ar^1	Ar^2	IC_{50} (μM)			
			A549	MDA-MB-231	HL-60	WI38
2g			4.02	1.92	1.82	ND[a)]
2h			18.0	>100	5.54	ND[a)]
2i			>100	8.92	3.08	ND[a)]
Sorafenib			1.30	2.70	ND[a)]	10.8
PAC-1			2.81	2.04	4.56	6.63

a) ND: not determined.

Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Mass spectra (MS) were taken in electrospray ionization (ESI) mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, U.S.A.). Proton (^{1}H) nuclear magnetic resonance spectroscopy was performed using Bruker ARX-300, 300 MHz spectrometers (Bruker Bioscience, Billerica, MA, U.S.A.) with tetramethylsilane (TMS) as an internal standard. Unless otherwise noted, all the materials were obtained from commercial available sources and were used without further purification.

4-Isocyanatobenzonitrile (3) 4-Aminobezonitrile (100 g, 0.847 mol) was treated with excess hydrochloride-ethanol and the resulted solution was evaporated to dryness. The hydrochloride salt obtained was then dissolved in dioxane (200 mL) and added dropwise to a solution of triphosgene (125 g, 0.423 mol) in dioxane (200 mL). The reaction mixture was heated to 50°C and stirred for 24 h. The resulting mixture was concentrated *in vacuo* and distilled under reduced pressure to give **3** (103 g, 84.4%) as colorless oil. bp: 130–131°C (15 mmHg).

General Procedure for the Preparation of 1-(4-Cyanophenyl)-3-substituted Phenylureas (4a–h) To a solution of **3** (16 g, 0.111 mol) in tetrahydrofuran (100 mL) was added the corresponding substituted aniline (0.111 mol) slowly. The resulted mixture was stirred at room temperature and the reaction was monitored by TLC. The reaction mixture was concentrated *in vacuo*. the product that precipitated was filtered and dried to obtain **4a–h**.

1-(4-Cyanophenyl)-3-(2-trifluoromethylphenyl)urea (**4a**): Yield 85.8%. ESI-MS *m/z*: 306.1 $(M+H)^+$.

1-(4-Cyanophenyl)-3-(3,5-dichlorophenyl)urea (**4b**): Yield 83.4%. ESI-MS *m/z*: 306.1 $(M+H)^+$.

1-(4-Cyanophenyl)-3-(3-trifluoromethoxyphenyl)urea (**4c**): Yield 79.7%. ESI-MS *m/z*: 322.2 $(M+H)^+$.

1-(4-Cyanophenyl)-3-phenylurea (**4d**): Yield 87.1%. ESI-MS *m/z*: 238.1 $(M+H)^+$.

1-(2-Chloro-4-trifluoromethylphenyl)-3-(4-cyanophenyl)urea (**4e**): Yield 85.3%. ESI-MS *m/z*: 340.0 $(M+H)^+$.

1-(4-Cyanophenyl)-3-(2-trifluoromethoxyphenyl)urea (**4f**): Yield 80.4%. ESI-MS *m/z*: 322.1 $(M+H)^+$.

1-(3-Chlorophenyl)-3-(4-cyanophenyl)urea (**4g**): Yield 77.5%. ESI-MS *m/z*: 272.0 $(M+H)^+$.

1-(4-Cyanophenyl)-3-(3,4-dichlorophenyl)urea (**4h**): Yield 82.5%. ESI-MS *m/z*: 306.0 $(M+H)^+$.

General Procedure for the Preparation of 4-(3-Substituted phenylureido)benzothioamides (5a–h) To a solution of benzonitrile (**4a–h**) (0.086 mol) in *N*,*N*-dimethylformamide (250 mL) was added magnesium chloride (22 g, 0.109 mol) and sodium bisulfide (12.2 g, 0.218 mol). The reaction mixture was stirred at room temperature overnight and then was added to 1 L water, acidified to pH 4 with dilute hydrochloride and filtered. The collected solid was washed with water until the filtrate became neutral and dried to obtain **5a–h** (47.6–59.2%).

General Procedure for the Preparation of 1-(4-(4-Chloromethylthiazol-2-yl)phenyl)-3-substituted Phenylureas (6a–h) Benzothioamide (**5a–h**) (0.083 mol) was dissolved in THF (300 mL) and heated to 50°C. To the stirred solution was added 1,3-dichloroacetone (10.5 g, 0.083 mol). The reaction mixture was stirred for 7 h. The resulting mixture was evaporated *in vacuo* to remove most of the solvent, cooled and filtered off. The residue was suspended in 1 L water and the suspension was stirred and basified to pH 8 with saturated potassium carbonate solution. The precipitates was filtered, washed with water and dried to obtain **6a–h** (74.2–80.0%).

General Procedure for the Preparation of 1-Substituted Phenyl-3-(4-(4-(piperazin-1-ylmethyl)thiazol-2-yl)phenyl)-ureas (7a–h) To a solution of piperazine (64 g, 0.748 mol) in ethanol was added urea (**6a–h**) in portions. The reaction mixture was stirred at room temperature for 2 h. The resulting mixture was evaporated *in vacuo* to remove most of the solvent and poured into 1.5 L water. The white precipitates was filtered, washed with water and dried to obtain **7a–h**.

1-(4-(4-(Piperazin-1-ylmethyl)thiazol-2-yl)phenyl)-3-(2-trifluoromethylphenyl)urea (**7a**): Yield 58.2%. ESI-MS *m/z*: 462.2 $(M+H)^+$.

1-(3,5-Dichlorophenyl)-3-(4-(4-(piperazin-1-ylmethyl)-thiazol-2-yl)phenyl)urea (**7b**): Yield 55.4%. ESI-MS *m/z*: 462.2 $(M+H)^+$.

1-(4-(4-(Piperazin-1-ylmethyl)thiazol-2-yl)phenyl)-3-(3-trifluoromethoxyphenyl)urea (**7c**): Yield 63.7%. ESI-MS *m/z*: 478.3 $(M+H)^+$.

1-Phenyl-3-(4-(4-(piperazin-1-ylmethyl)thiazol-2-yl)phenyl)-

urea (**7d**): Yield 55.9%. ESI-MS *m/z*: 394.2 $(M+H)^+$.

1-(2-Chloro-4-trifluoromethylphenyl)-3-(4-(4-(piperazin-1-ylmethyl)thiazol-2-yl)phenyl)urea (**7e**): Yield 51.8%. ESI-MS *m/z*: 496.1 $(M+H)^+$.

1-(4-(4-(Piperazin-1-ylmethyl)thiazol-2-yl)phenyl)-3-(2-trifluoromethoxyphenyl)urea (**7f**): Yield 58.4%. ESI-MS *m/z*: 478.1 $(M+H)^+$.

1-(3-Chlorophenyl)-3-(4-(4-(piperazin-1-ylmethyl)thiazol-2-yl)phenyl)urea (**7g**): Yield 50.7%. ESI-MS *m/z*: 428.1 $(M+H)^+$.

1-(3,4-Dichlorophenyl)-3-(4-(4-(piperazin-1-ylmethyl)-thiazol-2-yl)phenyl)urea (**7h**): Yield 53.0%. ESI-MS *m/z*: 462.0 $(M+H)^+$.

General Procedure for the Preparation of Ethyl 2-(4-((2-(4-(3-Substituted phenylureido)phenyl)thiazol-4-yl)-methyl)piperazin-1-yl)acetates (8a–h) To a solution of *N*-substituted piperazine (**7a**–**h**) (0.063 mol) in ethanol (300 mL) was added potassium carbonate (5.2 g, 0.038 mol), ethyl chloroacetate (7.7 g, 0.063 mol) and sodium iodide (cat.). The reaction mixture was heated to 50°C and stirred for 2 h. The reaction mixture was concentrated *in vacuo* and cooled. The product precipitated was filtered off, washed with ethanol and water, and dried to obtain **8a**–**h** (87.5–92.1%).

General Procedure for the Preparation of 1-Substituted Phenyl-3-(4-(4-((4-(2-hydrazinyl-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)ureas (9a–h) To a solution of ester (**8a**–**h**) (0.044 mol) in ethanol (250 mL) was added 80% hydrazine hydrate (27.7 g, 0.443 mol). The reaction mixture was heated to 50°C and stirred for 48 h. The reaction mixture was evaporated to remove most of the solvent. The residue was filtered off, washed with water, and dried to obtain **9a**–**h**.

1-(4-(4-((4-(2-Hydrazinyl-2-oxoethyl)piperazin-1-yl)methyl)-thiazol-2-yl)phenyl)-3-(2-trifluoromethylphenyl)urea (**9a**): Yield 89.8%. ESI-MS *m/z*: 534.2 $(M+H)^+$.

1-(3,5-Dichlorophenyl)-3-(4-(4-((4-(2-hydrazinyl-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (**9b**): Yield 88.0%. ESI-MS *m/z*: 534.2 $(M+H)^+$.

1-(4-(4-((4-(2-Hydrazinyl-2-oxoethyl)piperazin-1-yl)methyl)-thiazol-2-yl)phenyl)-3-(3-trifluoromethoxyphenyl)urea (**9c**): Yield 92.6%. ESI-MS *m/z*: 550.2 $(M+H)^+$.

1-(4-(4-((4-(2-Hydrazinyl-2-oxoethyl)piperazin-1-yl)methyl)-thiazol-2-yl)phenyl)-3-phenylurea (**9d**): Yield 88.7%. ESI-MS *m/z*: 466.2 $(M+H)^+$.

1-(2-Chloro-4-trifluoromethylphenyl)-3-(4-(4-((4-(2-hydrazinyl-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)-phenyl)urea (**9e**): Yield 85.2%. ESI-MS *m/z*: 568.1 $(M+H)^+$.

1-(4-(4-((4-(2-Hydrazinyl-2-oxoethyl)piperazin-1-yl)methyl)-thiazol-2-yl)phenyl)-3-(2-(trifluoromethoxy)phenyl)urea (**9f**): Yield 90.4%. ESI-MS *m/z*: 550.3 $(M+H)^+$.

1-(3-Chlorophenyl)-3-(4-(4-((4-(2-hydrazinyl-2-oxoethyl)-piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (**9g**): Yield 87.5%. ESI-MS *m/z*: 500.2 $(M+H)^+$.

1-(3,4-Dichlorophenyl)-3-(4-(4-((4-(2-hydrazinyl-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (**9h**): Yield 86.7%. ESI-MS *m/z*: 534.1 $(M+H)^+$.

1-Isocyanato-4-nitrobenzene (10) 4-Nitroaniline (100 g, 0.725 mol) was treated with excess hydrochloride-ethanol and evaporated to dryness. The hydrochloride salt obtained was then dissolved in dioxane (200 mL) and added dropwise to a solution of triphosgene (108 g, 0.362 mol) in dioxane (200 mL). The reaction mixture was heated to 50°C and stirred for 24 h. The resulting mixture was concentrated *in vacuo* and distilled under reduced pressure to give **10** (70 g, 58.9%) as yellow solid. bp: 168–170°C (15 mmHg).

General Procedure for the Preparation of 1-(4-Nitrophenyl)-3-substituted phenylureas (11a–d) To a solution of **10** (40 g, 0.244 mol) in tetrahydrofuran (500 mL) was added the corresponding substituted aniline (0.244 mol) slowly. The resulted mixture was stirred at room temperature and the reaction was monitored by TLC. The reaction mixture was concentrated *in vacuo* and filtered. The solid was dried to obtain **11a**–**d**.

1-(4-Nitrophenyl)-3-(3-(trifluoromethyl)phenyl)urea (**11a**): Yield 75.7%. ESI-MS *m/z*: 326.2 $(M+H)^+$.

1-(3-Chlorophenyl)-3-(4-nitrophenyl)urea (**11b**): Yield 80.1%. ESI-MS *m/z*: 292.1 $(M+H)^+$.

1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-nitrophenyl)-urea (**11c**): Yield 81.4%. ESI-MS *m/z*: 360.0 $(M+H)^+$.

1-(3-Chloro-4-(trifluoromethyl)phenyl)-3-(4-nitrophenyl)-urea (**11d**): Yield 72.4%. ESI-MS *m/z*: 360.0 $(M+H)^+$.

General Procedure for the Preparation of 1-(4-Amino-phenyl)-3-substituted phenylureas (12a–d) To a stirred solution of ureidonitrobenzene (**11a**–**d**) (0.185 mol) in 95% ethanol (500 mL) was added glacial acetic acid (5 mL), ammonium chloride (15 g, 0.280 mol) and iron powder (62 g, 1.107 mol). The mixture was heated to 80°C and stirred for 5 h. The reaction mixture was filtered hot. The filtrate was concentrated *in vacuo* and poured into water (500 mL). The precipitates were filtered off and dried to afford **12a**–**d** (93.1–98.2%).

General Procedure for the Preparation of 2-Chloro-*N*-(4-(3-substituted phenylureido)phenyl)acetamides (13a–d) A solution of amine (**12a**–**d**) (0.183 mol) and triethylamine (28 g, 0.277 mol) in dichloromethane (500 mL) was cooled below 0°C in an ice-salt bath and chloroacetyl chloride (25 g, 0.221 mol) was added dropwise. The ice-salt bath was removed and the resulting mixture was stirred at room temperature for 5 h. The precipitates were filtered off, washed with dichloromethane and dried to afford **13a**–**d** (88.6–91.2%).

General Procedure for the Preparation of 2-(Piperazin-1-yl)-*N*-(4-(3-substituted phenylureido)phenyl)acetamides (14a–d) To a stirred solution of piperazine (194 g, 1 mol) in ethanol (500 mL) was added chloroactetamide (**13a**–**d**) (0.500 mol) in portions. The reaction mixture was stirred at room temperature for 6 h. The resulting mixture was evaporated *in vacuo* to remove the solvent and then poured into water (500 mL). The precipitates were filtered off, washed with water and dried to give **14a**–**d**.

2-(Piperazin-1-yl)-*N*-(4-(3-(3-trifluoromethyl)phenylureido)-phenyl)acetamide (**14a**): Yield 68.9%. ESI-MS *m/z*: 422.2 $(M+H)^+$.

N-(4-(3-(3-Chlorophenyl)ureido)phenyl)-2-(piperazin-1-yl)-acetamide (**14b**): Yield 72.1%. ESI-MS *m/z*: 388.1 $(M+H)^+$.

N-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenyl)-2-(piperazin-1-yl)acetamide (**14c**): Yield 65.4%. ESI-MS *m/z*: 456.1 $(M+H)^+$.

N-(4-(3-(3-Chloro-4-(trifluoromethyl)phenyl)ureido)-phenyl)-2-(piperazin-1-yl)acetamide (**14d**): Yield 69.6%. ESI-MS *m/z*: 456.1 $(M+H)^+$.

General Procedure for the Preparation of Ethyl 2-(4-(2-Oxo-2-((4-(3-substituted phenylureido)phenyl)amino)-ethyl)piperazin-1-yl)acetates (15a–d) To a solution of *N*-substituted piperazine (**14a**–**d**) (0.157 mol) in ethanol (500 mL)

was added sodium dicarbonate (20 g, 0.238 mol) and ethyl chloroacetate (21 g, 0.171 mol). The reaction mixture was heated to 65°C and stirred for 20 h. The mixture was concentrated to about 200 mL *in vacuo* and cooled to room temperature. The precipitates were filtered off and dried to afford **15a–d** (82.3–86.4%).

General Procedure for the Preparation of 2-(4-(2-Hydrazinyl-2-oxoethyl)piperazin-1-yl)-*N*-(4-(3-substituted phenylureido)phenyl)acetamides (16a–d) To a solution of ester (**15a–d**) (0.059 mol) in ethanol (300 mL) was added 80% hydrazine hydrate (37 g, 0.592 mol). The reaction mixture was heated to 50°C and stirred for 48 h. The precipitates were filtered off and dried to afford **16a–d**.

2-(4-(2-Hydrazinyl-2-oxoethyl)piperazin-1-yl)-*N*-(4-(3-(3-(trifluoromethyl)phenyl)ureido)phenyl)acetamide (**16a**): Yield 81.2%. ESI-MS *m/z*: 494.2 $(M+H)^+$.

N-(4-(3-(3-Chlorophenyl)ureido)phenyl)-2-(4-(2-hydrazinyl-2-oxoethyl)piperazin-1-yl)acetamide (**16b**): Yield 85.4%. ESI-MS *m/z*: 460.2 $(M+H)^+$.

N-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenyl)-2-(4-(2-hydrazinyl-2-oxoethyl)piperazin-1-yl)-acetamide (**16c**): Yield 77.5%. ESI-MS *m/z*: 528.2 $(M+H)^+$.

N-(4-(3-(3-Chloro-4-(trifluoromethyl)phenyl)ureido)-phenyl)-2-(4-(2-hydrazinyl-2-oxoethyl)piperazin-1-yl)-acetamide (**16d**): Yield 81.1%. ESI-MS *m/z*: 528.2 $(M+H)^+$.

5-Benzyloxy-2-hydroxybenzaldehyde (17) To a solution of 2,5-dihydroxybenzaldehyde (50 g, 0.362 mol) in acetonitrile (500 mL) was added benzyl chloride (60 g, 0.471 mol), sodium bicarbonate (35 g, 0.413 mol) and potassium iodide (cat.). The reaction mixture was heated to reflux and stirred for 30 h. The mixture was poured into water (500 mL). The precipitates were filtered off, dried, recrystallized from methanol to give **17** (46.5 g, 56.3%) as white solid. ESI-MS *m/z*: 227.1 $(M-H)^-$.

4-Benzyloxy-2-hydroxybenzaldehyde (18) To a solution of 2,4-dihydroxybenzaldehyde (50 g, 0.362 mol) in acetonitrile (500 mL) was added benzyl chloride (60 g, 0.471 mol), sodium bicarbonate (35 g, 0.413 mol) and potassium iodide (cat.). The reaction mixture was heated to reflux and stirred for 30 h. The mixture was poured into water (500 mL). The precipitates were filtered off, dried, recrystallized from methanol to give **18** (43.6 g, 52.8%) as white solid. ESI-MS *m/z*: 227.1 $(M-H)^-$.

7-Hydroxy-4-methyl-2*H*-chromen-2-one (19) Sulfuric acid (500 mL) was cooled below 10°C in an ice-salt bath, and to the cooled acid was slowly added a solution of benzene-1,3-diol (55 g, 0.500 mol) in ethyl acetoacetate (65 g, 0.500 mol). The reaction mixture was stirred for 4 h. The resulting mixture was poured onto cracked ice and stirred. The precipitates were filtered off, washed with water and dried to afford **19** (67.0 g, 76.1%) as white solid. ESI-MS *m/z*: 175.1 $(M-H)^-$.

7-Hydroxy-4-methyl-2-oxo-2*H*-chromene-6-carbaldehyde (20) A suspension of urotropine (20 g, 0.143 mol) in glacial acetic acid (80 mL) was heated to 40°C and stirred to form a clear solution. To this solution was added **19** (5 g, 0.028 mol) in portions. The reaction mixture was stirred for 20 min and then heated to 115°C and stirred for another 2 h. Finally the reaction mixture was cooled to 95°C, 30% sulfuric acid (15 mL) was added and the reaction continued for 1.5 h. The resulting mixture was evaporated to dryness and the dry residue obtained was extracted with ether. The organic layer was then washed with saturated sodium bicarbonate solution, dried over anhydrous sodium sulfate and evaporated to dryness to afford **20** (2.2 g, 38.0%) as white solid. ESI-MS *m/z*: 203.0 $(M-H)^-$.

General Procedure for the Preparation of Target Compounds 1a–i and 2a–i To a solution of acethydrazide (**9a–h**, **16a–d**) (0.002 mol) in ethanol (10 mL) was added appropriate benzaldehyde or the prepared aromatic aldehyde (**17**, **18**, **20**). The reaction mixture was stirred and refluxed for 2 h. The reaction mixture was cooled and precipitates were collected by filtration to obtain the crude product, which was then purified by flash column chromatography. The pure product was dissolved in chloroform. To the solution was added excessive hydrochloride-ethanol and stirred for 1 h. Ether was added to the mixture above. The precipitates were filtered off and dried to afford **1a–i** and **2a–i**.

1-(4-(4-((4-(2-(2-(2-Hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(2-trifluoromethylphenyl)urea Dihydrochloride (**1a**): Yield 55.8%. mp 186–188°C. ESI-MS *m/z*: 638.2 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.96 (s, 1H), 10.15 (s, 1H), 8.42–8.37 (m, 2H), 7.95–7.88 (m, 5H), 7.77–7.64 (m, 5H), 7.34–7.24 (m, 2H), 7.01–6.84 (m, 2H), 4.58 (s, 1H), 4.48 (s, 2H), 3.81 (s, 1H), 3.66 (brs, 8H).

1-(3,5-Dichlorophenyl)-3-(4-(4-((4-(2-(2-(2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea Dihydrochloride (**1b**): Yield 53.3%. mp 230–232°C. ESI-MS *m/z*: 638.3 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.98 (s, 1H), 10.15–10.01 (m, 3H), 8.38 (s, 1H), 7.94 (d, J=8.6 Hz, 3H), 7.76 (d, J=7.8 Hz, 1H), 7.63 (d, J=8.6 Hz, 2H), 7.54 (s, 2H), 7.29–7.23 (m, 1H), 7.16 (s, 1H), 6.95–6.84 (m, 2H), 4.63 (s, 1H), 4.53 (s, 2H), 3.99 (s, 1H), 3.64 (brs, 8H).

1-(4-(4-((4-(2-(2-(3,5-Di-*tert*-butyl-2-hydroxybenzylidene)-hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)-phenyl)-3-(3-trifluoromethoxyphenyl)urea Dihydrochloride (**1c**): Yield 59.7%. mp 221–222°C. ESI-MS *m/z*: 766.2 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.12 (s, 1H), 11.45 (s, 1H), 9.10 (s, 1H), 9.08 (s, 1H), 8.49 (s, 1H), 7.86 (d, J=8.6 Hz, 2H), 7.72 (s, 1H), 7.59 (d, J=8.6 Hz, 2H), 7.44–7.39 (m, 2H), 7.33–7.30 (m, 2H), 7.18 (s, 1H), 6.96 (d, J=7.7 Hz, 1H), 3.67 (s, 2H), 3.32 (s, 1H), 3.14 (s, 2H), 2.56 (s, 8H), 1.39 (s, 9H), 1.27 (s, 9H)

1-(4-(4-((4-(2-(2-(5-Benzyloxy-2-hydroxybenzylidene)-hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)-phenyl)-3-phenylurea Dihydrochloride (**1d**): Yield 62.5%. mp 202–204°C. ESI-MS *m/z*: 676.3 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.94 (s, 1H), 9.71 (d, J=13.4 Hz, 1H), 9.42 (d, J=12.7 Hz, 1H), 8.32 (s, 1H), 7.90 (s, 4H), 7.63 (d, J=8.1 Hz, 3H), 7.49–7.38 (m, 7H), 7.35–7.26 (m, 4H), 6.99 (d, J=7.1 Hz, 2H), 6.87 (d, J=8.8 Hz, 1H), 5.05 (s, 2H), 4.56 (s, 1H), 4.47 (s, 2H), 3.78 (s, 1H), 3.59 (brs, 8H).

1-(4-(4-((4-(2-(2-(5-Benzyloxy-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(2-chloro-4-(trifluoromethyl)phenyl)urea Dihydrochloride (**1e**): Yield 52.3%. mp 214–215°C. ESI-MS *m/z*: 778.4 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.96 (s, 1H), 10.62 (d, J=14.0 Hz, 1H), 9.75 (s, 1H), 9.02 (d, J=7.0 Hz, 1H), 8.62 (s, 1H), 8.51 (s, 1H), 8.33 (s, 1H), 7.96–7.93 (m, 3H), 7.72 (d, J=8.2 Hz, 2H), 7.68 (s, 1H), 7.44–7.32 (m, 7H), 6.99 (dd, J_1=2.8 Hz, J_2=8.9 Hz, 1H), 6.88 (d, J=8.9 Hz, 1H), 5.05 (s, 2H), 4.58 (s, 1H), 4.50 (s, 2H), 3.84 (s, 1H), 3.68 (brs, 8H).

1-(4-(4-((4-(2-(2-(5-Benzyloxy-2-hydroxybenzylidene)-

hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)-phenyl)-3-(2-trifluoromethoxyphenyl)urea Dihydrochloride (**1f**): Yield 54.1%. mp 225–226°C. ESI-MS *m/z*: 760.3 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.98 (s, 1H), 10.06 (d, J=11.7 Hz, 1H), 9.98 (d, J=10.9 Hz, 1H), 8.51 (s, 1H), 8.34 (s, 1H), 7.96–7.91 (m, 4H), 7.71 (s, 1H), 7.64 (d, J=8.6 Hz, 1H), 7.46–7.31 (m, 8H), 7.22 (d, J=2.9 Hz, 1H), 7.01–6.87 (m, 3H), 5.06 (s, 2H), 4.62 (s, 1H), 4.53 (s, 2H), 3.94 (s, 1H), 3.72–3.51 (m, 8H).

1-(4-(4-((4-(2-(2-(5-Benzyloxy-2-hydroxybenzylidene)-hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)-phenyl)-3-(2-trifluoromethylphenyl)urea Dihydrochloride (**1g**): Yield 53.7%. mp 241–244°C. ESI-MS *m/z*: 744.4 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.00 (s, 1H), 10.14 (d, J=11.9 Hz, 1H), 10.02 (d, J=10.1 Hz, 1H), 8.51 (s, 1H), 8.34 (s, 1H), 8.02–7.92 (m, 4H), 7.67 (s, 1H), 7.63 (d, J=8.7 Hz, 2H), 7.53 (t, J=7.7 Hz, 1H), 7.44–7.30 (m, 7H), 6.99 (dd, J_1=3.0 Hz, J_2=8.9 Hz, 1H), 6.89 (d, J=8.8 Hz, 1H), 5.06 (s, 2H), 4.64 (s, 1H), 4.56 (s, 2H), 4.03 (s, 1H), 3.75–3.58 (m, 8H).

1-(4-(4-((4-(2-(2-(4-Benzyloxy-2-hydroxybenzylidene)-hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)-phenyl)-3-(3-chlorophenyl)urea Dihydrochloride (**1h**): Yield 54.1%. mp 213–215°C. ESI-MS *m/z*: 710.2 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.78 (s, 1H), 9.65 (d, J=10.8 Hz, 1H), 9.59 (d, J=12.4 Hz, 1H), 8.26 (s, 1H), 7.91 (d, J=8.7 Hz, 2H), 7.86 (d, J=9.0 Hz, 1H), 7.71 (s, 1H), 7.66–7.61 (m, 3H), 7.45–7.40 (m, 5H), 7.37–7.33 (m, 2H), 7.31 (d, J=5.2 Hz, 2H), 7.04–7.02 (m, 1H), 6.57–6.55 (m, 2H), 5.12 (s, 1H), 5.09 (s, 1H), 4.48 (s, 1H), 4.43 (s, 2H), 3.46 (brs, 9H).

1-(4-(4-((4-(2-(2-(4-Benzyloxy-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(3,4-dichlorophenyl)urea Dihydrochloride (**1i**): Yield 50.4%. mP 205–207°C. ESI-MS *m/z*: 744.1 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.77 (s, 1H), 9.65 (brs, 2H), 8.25 (s, 1H), 7.93–7.88 (m, 5H), 7.62 (d, J=8.6 Hz, 3H), 7.53 (d, J=8.8 Hz, 1H), 7.45–7.33 (m, 6H), 6.61–6.54 (m, 2H), 5.12 (s, 1H), 5.09 (s, 1H), 4.43 (brs, 3H), 3.45 (brs, 9H).

2-(4-(2-(2-(2-Hydroxybenzylidene)hydrazinyl)-2-oxoethyl)-piperazin-1-yl)-*N*-(4-(3-(3-(trifluoromethyl)phenyl)ureido)-phenyl)acetamide Dihydrochloride (**2a**): Yield 45.1%. mp 245–248°C. ESI-MS *m/z*: 598.4 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.98 (s, 1H), 10.59 (s, 1H), 10.17 (s, 1H), 9.77 (s, 1H), 9.42 (s, 1H), 8.39 (s, 1H), 8.01 (s, 1H), 7.71 (d, J=6.5 Hz, 1H), 7.58–7.53 (m, 4H), 7.50–7.43 (m, 3H), 7.33–7.25 (m, 2H), 6.97–6.85 (m, 2H), 4.59 (s, 1H), 4.04 (s, 2H), 3.86 (s, 1H), 3.54 (s, 8H).

2-(4-(2-(2-(3,5-Di-*tert*-butyl-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)-*N*-(4-(3-(3-(trifluoromethyl)-phenyl)ureido)phenyl)acetamide Dihydrochloride (**2b**): Yield 58.9%. mp 275–277°C. ESI-MS *m/z*: 710.3 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.99 (s, 1H), 10.56 (s, 1H), 9.66 (s, 1H), 9.32 (s, 1H), 8.51 (s, 1H), 8.00 (s, 1H), 7.59–7.43 (m, 6H), 7.32–7.24 (m, 3H), 4.06 (s, 4H), 3.82 (s, 4H), 3.46 (s, 6H), 1.40 (s, 9H), 1.28 (s, 9H).

N-(4-(3-(3-Chlorophenyl)ureido)phenyl)-2-(4-(2-(2-(2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)-acetamide Dihydrochloride (**2c**): Yield 57.2%. mp 259–261°C. ESI-MS *m/z*: 564.2 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.43 (s, 1H), 11.21 (s, 1H), 9.60 (s, 1H), 8.84 (s, 1H), 8.68 (s, 1H), 8.53 (s, 1H), 7.71 (s, 1H), 7.54 (d, J=8.9 Hz, 2H), 7.48 (dd, J_1=1.7 Hz, J_2=7.2 Hz, 1H), 7.38 (d, J=8.9 Hz, 2H), 7.32–7.23 (m, 3H), 7.02–6.99 (m, 1H), 6.93–6.88 (m, 2H), 3.33 (s, 1H), 3.14 (s, 1H), 3.12 (s, 2H), 2.59 (s, 8H).

N-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-phenyl)-2-(4-(2-(2-(2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)acetamide Dihydrochloride (**2d**): Yield 51.4%. mp 260–261°C. ESI-MS *m/z*: 632.1 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.99 (s, 1H), 10.69 (s, 1H), 10.06 (s, 1H), 9.53 (s, 1H), 8.40 (s, 1H), 8.11 (s, 1H), 7.77 (d, J=7.3 Hz, 1H), 7.65–7.56 (m, 5H), 7.45 (d, J=8.9 Hz, 2H), 7.33–7.24 (m, 1H), 6.98–6.93 (m, 1H), 6.91–6.85 (m, 1H), 4.62 (s, 1H), 4.12 (s, 2H), 3.95 (s, 1H), 3.64 (brs, 8H).

2-(4-(2-(2-(5-(Benzyloxy)-2-hydroxybenzylidene)-hydrazinyl)-2-oxoethyl)piperazin-1-yl)-*N*-(4-(3-(3-(trifluoromethyl)phenyl)ureido)phenyl)acetamide Dihydrochloride (**2e**): Yield 58.3%. mp 210–212°C. ESI-MS *m/z*: 704.3 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.40 (s, 1H), 10.65 (s, 1H), 9.58 (s, 1H), 8.99 (s, 1H), 8.71 (s, 1H), 8.48 (s, 1H), 8.01 (s, 1H), 7.55–7.50 (m, 4H), 7.47–7.28 (m, 9H), 7.15 (d, J=2.9 Hz, 1H), 6.98 (dd, J_1=3.0 Hz, J_2=8.9 Hz, 1H), 6.84 (d, J=8.8 Hz, 1H), 5.05 (s, 2H), 3.30 (s, 1H), 3.12 (s, 3H), 2.59 (s, 8H).

2-(4-(2-(2-(5-(Benzyloxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)-*N*-(4-(3-(3-chlorophenyl)-ureido)phenyl)acetamide Dihydrochloride (**2f**): Yield 53.1%. mp 203–205°C. ESI-MS *m/z*: 670.4 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.40 (s, 1H), 10.65 (s, 1H), 9.58 (s, 1H), 8.99 (s, 1H), 8.71 (s, 1H), 8.48 (s, 1H), 8.01 (s, 1H), 7.55–7.50 (m, 4H), 7.47–7.28 (m, 9H), 7.15 (d, J=2.9 Hz, 1H), 6.98 (dd, J_1=3.0 Hz, J_2=8.9 Hz, 1H), 6.84 (d, J=8.8 Hz, 1H), 5.05 (s, 2H), 3.30 (s, 1H), 3.12 (s, 3H), 2.59 (s, 8H).

2-(4-(2-(2-(5-(Benzyloxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)-*N*-(4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl)acetamide Dihydrochloride (**2g**): Yield 47.8%. mp 180–182°C. ESI-MS *m/z*: 738.4 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.43 (s, 1H), 10.66 (s, 1H), 9.60 (s, 1H), 9.11 (s, 1H), 8.77 (s, 1H), 8.48 (s, 1H), 8.11 (s, 1H), 7.61–7.54 (m, 4H), 7.46–7.32 (m, 7H), 7.15 (d, J=2.9 Hz, 1H), 6.98 (dd, J_1=2.9 Hz, J_2=8.8 Hz, 1H), 6.84 (d, J=8.8 Hz, 1H), 5.05 (s, 2H), 3.32 (s, 1H), 3.12 (s, 3H), 2.58 (s, 8H).

2-(4-(2-(2-((7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-6-yl)-methylene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)-*N*-(4-(3-(3-(trifluoromethyl)phenyl)ureido)phenyl)acetamide Dihydrochloride (**2h**): Yield 54.2%. mp 261–263°C. ESI-MS *m/z*: 680.3 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.53 (s, 1H), 10.64 (s, 1H), 9.71 (d, J=9.0 Hz, 1H), 9.39 (d, J=7.4 Hz, 1H), 9.36 (s, 1H), 9.01 (s, 1H), 8.00 (s, 1H), 7.75 (d, J=8.9 Hz, 1H), 7.59–7.50 (m, 4H), 7.45 (d, J=8.9 Hz, 2H), 7.29 (d, J=7.4 Hz, 1H), 6.97 (d, J=8.9 Hz, 1H), 6.28 (s, 1H), 4.52 (s, 1H), 4.12 (s, 2H), 3.77 (s, 1H), 3.55–3.27 (m, 8H), 2.42 (s, 3H).

N-(4-(3-(3-Chloro-4-(trifluoromethyl)phenyl)ureido)-phenyl)-2-(4-(2-(2-((7-hydroxy-4-methyl-2-oxo-2*H*-chromen-6-yl)methylene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)acetamide Dihydrochloride (**2i**): Yield 49.0%. mp 249–251°C. ESI-MS *m/z*: 714.0 $(M+H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.47 (s, 1H), 10.61 (s, 1H), 9.85 (d, J=8.9 Hz, 1H), 9.37 (d, J=6.1 Hz, 1H), 9.01 (s, 1H), 8.63 (s, 1H), 8.11 (s, 1H), 7.74 (d, J=8.9 Hz, 1H), 7.61 (s, 2H), 7.55 (d, J=8.9 Hz, 2H), 7.44 (d, J=8.9 Hz, 2H), 6.97 (d, J=8.9 Hz, 1H), 6.27 (s, 1H), 4.49 (s, 1H), 4.10 (s, 2H), 3.74 (s, 1H), 3.52–3.24 (m, 8H), 2.42 (s, 3H).

Cytotoxicity The cytotoxicity of compounds **1a**–**i** and

2a–**i** were evaluated against A549, MDA-MB-231 and HL-60 cell lines, and **1a**, **1f** and **1h** were evaluated against WI38 cell line by the MTT method *in vitro*, with sorafenib and PAC-1 as the positive controls. The cancer cell lines and the normal cell line were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS).

Approximately 4×10^3 cells, suspend in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37°C for 24 h. The tested compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL and incubated with cells at 37°C for 4 h. The formazan crystals were dissolved in 100 μL dimethyl sulfoxide (DMSO) per well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the enzyme-linked immunosorbent assay (ELISA) reader. All of the compounds were tested twice in the cell lines. The results expressed as IC_{50} (inhibitory concentration of 50%) were the averages of two determinations and were calculated by using the Bacus Laboratories Inc. Slide Scanner (Bliss) software.

Acknowledgements This work was supported by the National High Technology Research and Development Program ("863" Program) of China (No. 2012AA020305) and National Natural Science Foundation of China (No. 21002065 and No. 81102470).

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