Discovery of Hybrid Dual N-Acylhydrazone and Diaryl Urea Derivatives as Potent Antitumor Agents: Design, Synthesis and Cytotoxicity Evaluation

Xin Zhai, Qiang Huang, Nan Jiang, Di Wu, Hongyu Zhou and Ping Gong *

Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, Liaoning, China

* Author to whom correspondence should be addressed; E-Mail: gongpinggp@126.com; Tel./Fax: +86-24-2398-6429.

Received: 7 January 2013; in revised form: 19 January 2013 / Accepted: 25 January 2013 / Published: 4 March 2013

Abstract: Based on the hybrid pharmacophore design concept, a novel series of dual diaryl urea and N-acylhydrazone derivatives were synthesized and evaluated for their in vitro cytotoxicity by the standard MTT assay. The pharmacological results indicated that most compounds exhibited moderate to excellent activity. Moreover, compound 2g showed the most potent cytotoxicity against HL-60, A549 and MDA-MB-231 cell lines, with IC50 values of 0.22, 0.34 and 0.41 μM, respectively, which was 3.8 to 22.5 times more active than the reference compounds sorafenib and PAC-1. The promising compound 2g thus emerges as a lead for further structural modifications.

Keywords: diaryl ureas; N-acylhydrazone; cytotoxicity

1. Introduction

Design of single chemical compounds that simultaneously modulate multiple biological targets in a specific manner is the current focus of new drug development and is becoming more popular. An effective approach is to take existing individual compounds, each known to have pharmacological structural features and high selectivity against the particular targets of interest, and combine them into a single molecule. Sorafenib (Figure 1), a diaryl urea analogue [1], is a small molecular inhibitor of several tyrosine protein kinases (VEGFR, PDGFR and B-Raf) [2,3] and unique in targeting the
Raf/Mek/Erk pathway (MAPK pathway) [4], was approved by FDA for the treatment of advanced renal cell carcinoma and advanced hepatocellular carcinoma [5,6]. PAC-1 (Figure 1), the first preferential small molecule procaspase-3 activating compound with N-acylhydrazone pharmacophore, is promising as a new anti-tumor drug that can directly influence the apoptotic machinery or suicide of cells and has shown good results in mouse models [7–9].

**Figure 1.** Structures of sorafenib, PAC-1 and target compounds.

In an attempt to discover new antitumor agents with multiple molecular mechanisms, we combined the diaryl urea moiety from sorafenib and N-acylhydrazone based in a hybrid pharmacophore design. The use of thiazolyl and amido moieties as linkers has been reported in our preliminary study [10], and the thiazolyl ring is retained in this paper for its better antitumor potency. Thus a series of diaryl urea derivatives bearing an N-acylhydrazone moiety (Figure 1) were designed and synthesized. Various substituted ureido-linked phenyl (Ar1) and hydrazone-linked phenyl (Ar2) groups were introduced to explore the influence of electronic and steric effects on the anticancer activity. 2-Hydroxyl substitution was retained for the Ar2 ring for the reason that only with the hydroxyl group on Ar2 did the PAC-1 derivatives display antitumor activity *in vitro* [11]. 4-and 5-Benzylxyl groups were introduced to Ar2, respectively, to investigate the effect of the extension of the hydrophobic region. Furthermore, Ar2 was replaced with a substituted chromenonyl or imidazolindionyl groups, which are often associated with a variety of biological activity, to note the effect of each discreet change on the biological activity of the resulting compounds.

2. Results and Discussion

2.1. Chemistry

The synthesis of target compounds 1a–g, 2a–k, 3a–e and 4a–c is described in Scheme 1. Commercially available 4-aminobenzonitrile reacted with triphosgene in dioxane at 80 °C for 24 h to give 4-isocyanatobenzonitrile (5) as a colorless oil. Compound 5 was treated with various substituted anilines to obtain diaryl ureas 6a–o [12], whose cyano group was reduced to a thioamide moiety using magnesium chloride and sodium hydrogensulfide in N,N-dimethylformamide to afford the corresponding derivatives 7a–o. Cyclization of 7a–o with 1,3-dichloroacetone in tetrahydrofuran at 50 °C readily afforded thiozoles 8a–o, which reacted with piperazine in ethanol by nucleophilic substitution to give 9a–o. Consequently, treatment of 9a–o with ethyl chloroacetate in ethanol in the presence of potassium carbonate and sodium iodide afforded esters 10a–o, which were turned into
acylhydrazines 11a–o via hydrazinolysis in 80% hydrazine hydrate for 48 h. Finally, target compounds 1a–g, 2a–k, 3a–e and 4a–c were prepared via condensation of 11a–o with substituted benzaldehydes, various aromatic aldehydes (12a–d, 13a–c or 15) as well as imidazolindiones 19a–b, respectively, and isolated as the corresponding dihydrochlorides [7].

Scheme 1. Synthesis of target compounds 1a–g, 2a–k, 3a–e and 4a–c.

Reactions and conditions: (a) triphosgene, dioxane, 80 °C, 24 h; (b) ArNHNH2, THF, r.t.; (c) MgCl2, NaSH, DMF, overnight; (d) C1H2COCH2Cl, THF, 50 °C, 7 h; (e) piperazine, EtOH, r.t., 2 h; (f) ClCH2COOEt, 15, HCl, EtOH, then HCl-EtOH; (g) 80% NH2NH2·H2O, EtOH, 50 °C, 48 h; (h) i. ArCHO, 12a–d, 13a–c or 15, EtOH, then HCl-EtOH; ii. 19a–b, HCl, EtOH, then HCl-EtOH.

As shown in Scheme 2, aryloxybenzaldehydes 12a–d and 13a–e were prepared from 2,5-(or 2,4-) dihydroxybenzaldehyde via regioselective O-alkylation reactions with benzyl chlorides in acetonitrile in the presence of sodium hydrogen carbonate and potassium iodide. Cyclization of m-dihydroxybenzene with ethyl acetooacetate in sulfuric acid at 10 °C, followed by formylation with urotropine in glacial acetic acid and sulfuric acid in sequence, namely a Duff reaction, provided chromenealdehyde 15 as a white solid [13]. Imidazolindiones 19a–b were synthesized from substituted anilines, which were turned into phenyl isocyanates 16a–b in a similar manner as described for compound 5. Subsequent treatment of 16a–b with glycine methyl ester hydrochloride in the presence of triethylamine in dichloromethane gave ureas 17a–b. Cyclization of 17a–b in the presence of concentrated hydrochloride gave rise to 18a–b, which were further condensed with N,N-dimethylformamide dimethylacetal (DMF-DMA) to obtain 19a–b [14].
Scheme 2. Synthesis of intermediates 12a–d, 13a–c, 15 and 19a–b.

Reactions and conditions: (a) ArCH2Cl, NaHCO3, CH3CN, r.f., 30 h; (b) ArCH2Cl, NaHCO3, CH3CN, r.f., 30 h; (c) ethyl acetoacetate, H2SO4, 10 °C, 4 h; (d) urotropine, HOAc, 115 °C, 4 h; (e) triphosgene, dioxane, 80 °C, 24 h; (f) Glycine methyl ester hydrochloride, TEA, CH2Cl2, r.t., 1 h, 0 °C, 4 h, r.t. 1 h; (g) conc. HCl, acetone, 4 h; (h) DMF-DMA, CH3CN, r.f., 4 h.

2.2. Biological Results and Discussion

All target compounds 1a–g, 2a–k, 3a–e and 4a–c were evaluated for their cytotoxicity in vitro against the human leukemia cell line (HL-60), human lung adenocarcinoma epithelial cell line (A549) and human breast cancer cell line (MDA-MB-231) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, taking sorafenib and PAC-1 as references. The results expressed as IC50 values are summarized in Table 1. The IC50 values are the average of at least three independent experiments. As listed in Table 1, phenylhydrazones 1a–g and 2a–k, with the exception of 1c and 1d, exhibited moderate to excellent cytotoxicity towards the tested cell lines with IC50 values ranging from 0.22 to 6.0 μm. Generally, phenyl groups Ar2 substituted with chromenyl and imidazolidinyl moieties gave rise to two series of compounds 3a–e and 4a–c with a dramatic decrease or even a loss in antitumor potency, indicating that Ar2 was critical for the optimal activity and it is not tolerant ofr bulky and rigid heteroaromatic rings in this region.
Table 1. Structures and cytotoxicity of compounds 1a–g, 2a–k, 3a–e and 4a–c against HL-60, A549 and MDA-MB-231 cell lines.

| Compd. | Ar¹       | Ar²       | IC₅₀ (μmol/L) |
|--------|-----------|-----------|---------------|
|        |           |           | HL-60 | A549 | MDA-MB-231 |
| 1a     | ND        |           | 0.64 ± 0.12 | 1.9 ± 0.16 |
| 1b     |           |           | 0.56 ± 0.04 | 0.78 ± 0.02 | 0.48 ± 0.02 |
| 1c     |           |           | 13.0 ± 0.37 | 0.48 ± 0.06 | 0.26 ± 0.01 |
| 1d     |           |           | 8.8 ± 0.31  | 5.1 ± 0.25  | 8.5 ± 0.44  |
| 1e     |           |           | 0.82 ± 0.08 | 1.6 ± 0.41  | 0.92 ± 0.24 |
| 1f     |           |           | 0.63 ± 0.17 | 1.3 ± 0.16  | 0.82 ± 0.05 |
| 1g     |           |           | 6.0 ± 0.09  | 0.50 ± 0.04 | 0.58 ± 0.03 |
| 2a     |           |           | 0.55 ± 0.09 | 1.6 ± 0.14  | 0.73 ± 0.06 |
| 2b     |           |           | 0.51 ± 0.01 | 1.2 ± 0.05  | 0.73 ± 0.02 |
| 2c     |           |           | 2.6 ± 0.11  | 0.59 ± 0.02 | 0.71 ± 0.01 |
| 2d     |           |           | 3.8 ± 0.13  | 1.7 ± 0.12  | 0.53 ± 0.02 |
| 2e     |           |           | 2.3 ± 0.11  | 0.49 ± 0.05 | 0.35 ± 0.02 |
| 2f     |           |           | 4.7 ± 0.19  | 2.8 ± 0.21  | 0.48 ± 0.05 |
| 2g     |           |           | 0.22 ± 0.01 | 0.34 ± 0.01 | 0.41 ± 0.3  |
| Compd. | Ar$^1$ | Ar$^2$ | IC$_{50}$ (μmol/L) |
|--------|--------|--------|---------------------|
|        |        |        | HL-60 | A549 | MDA-MB-231 |
| 2h     | ![Structure](image) | ![Structure](image) | 0.50 ± 0.004 | 1.8 ± 0.04 | 0.90 ± 0.006 |
| 2i     | ![Structure](image) | ![Structure](image) | 0.38 ± 0.01 | 0.54 ± 0.06 | 0.44 ± 0.04 |
| 2j     | ![Structure](image) | ![Structure](image) | 0.31 ± 0.14 | 0.96 ± 0.20 | 2.0 ± 0.12 |
| 2k     | ![Structure](image) | ![Structure](image) | 2.0 ± 0.11 | 2.3 ± 0.08 | 0.22 ± 0.04 |
| 3a     | ![Structure](image) | ![Structure](image) | 15.2 ± 0.22 | 17.0 ± 0.52 | 5.6 ± 0.36 |
| 3b     | ![Structure](image) | ![Structure](image) | 3.6 ± 0.12 | >50 | 3.8 ± 0.28 |
| 3c     | ![Structure](image) | ![Structure](image) | 3.3 ± 0.25 | 6.4 ± 0.42 | 3.6 ± 0.28 |
| 3d     | ![Structure](image) | ![Structure](image) | 4.0 ± 0.33 | 1.7 ± 0.15 | 1.8 ± 0.07 |
| 3e     | ![Structure](image) | ![Structure](image) | 4.5 ± 0.13 | 19.0 ± 0.57 | 8.9 ± 0.41 |
| 4a     | ![Structure](image) | ![Structure](image) | 12.0 ± 0.32 | 37.2 ± 0.46 | 7.0 ± 0.18 |
| 4b     | ![Structure](image) | ![Structure](image) | 25.5 ± 0.29 | 3.4 ± 0.10 | 13.3 ± 0.32 |
| 4c     | ![Structure](image) | ![Structure](image) | >50 | 7.8 ± 0.20 | 13.1 ± 0.37 |
| Sorafenib | | | ND | 1.3 ± 0.06 | 2.7 ± 0.11 |
| PAC-1  | | | 4.5 ± 0.03 | 2.8 ± 0.10 | 2.0 ± 0.05 |

ND: not determined.
Compounds with alkyl groups on the phenyl ring Ar² (compounds 1a–g) exhibited moderate activity, however, the introduction of 4- or 5-benzyloxyl groups to the phenyl ring Ar² resulted in a remarkable increase in the activity (2a–c, 2e, 2g–k). Moreover, compounds substituted with a 5-(2-fluorobenzyloxyl) group (2g and 2j) exhibited the most potent cytotoxicity, especially compound 2g which displayed prominent activity with IC₅₀ values of 0.22, 0.34 and 0.41 μM, respectively, which were 3.8- to 20.5-fold higher than those of sorafenib and PAC-1.

As for substituents on the ureido-linked phenyl Ar¹, the introduction of electron-withdrawing substituents on Ar¹ was beneficial to the improvement of cytotoxicity, and the trifluoromethoxyl group produced the best potency. A case in point is that compound 2g with a trifluoromethoxyl group at the meta-position of Ar¹ showed more potent cytotoxicity against all the three tested cell lines than compound 2h with no substituent. Similarly, 3b and 3d with 3-trifluoromethyl and 3,5-di-trifluoromethyl groups on Ar¹, respectively, displayed potency against two or three cell lines than 3a with no substituent, while 3e with an electron-donating 3,4-dimethyl group showed a decline in antitumor activity against the A549 and MDA-MB-231 cell lines.

3. Experimental

3.1. Chemistry

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

3.2. 4-Isocyanatobenzonitrile (5)

4-Aminobezonitrile (100 g, 0.847 mol) was treated with excess hydrogen chloride-ethanol and the resulting 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 80 °C and stirred for 24 h. The resulting mixture was concentrated in vacuo and distilled under reduced pressure to give 5 (103 g, 84.4%) as colorless oil. B.p.: 130–131 °C (15 mmHg).

3.3. General Procedure for Preparation of 1-(4-Cyanophenyl)-3-substituted Phenylureas 6a–o

To a solution of 5 (16 g, 0.111 mol) in tetrahydrofuran (100 mL) was slowly added the corresponding substituted aniline (0.111 mol). The resulted mixture was stirred at room temperature and the reaction was monitored by TLC. The reaction mixture was concentrated in vacuo, and the precipitated product was filtered and dried to obtain 6a–o.

1-(4-Cyanophenyl)-3-(3-fluorophenyl)urea (6a): Yield: 87.2%; ESI-MS m/z: 256.1 [M+H]⁺. 1-(4-Cyanophenyl)-3-(3,5-dichlorophenyl)urea (6b): Yield: 78.5%; ESI-MS m/z: 306.0 [M+H]⁺.
Cyanophenyl-3-(3-chloro-4-fluorophenyl)urea (6c): Yield: 84.7%; ESI-MS m/z: 290.0 [M+H]⁺. 1-(4-Cyanophenyl)-3-(4-(trifluoromethyl)phenyl)urea (6d): Yield: 80.5%; ESI-MS m/z: 306.1 [M+H]⁺. 1-(4-Cyanophenyl)-3-(3-(trifluoromethyl)phenyl)urea (6e): Yield: 80.0%; ESI-MS m/z: 306.1 [M+H]⁺. 1-(4-Cyanophenyl)-3-(3-chlorophenyl)urea (6f): Yield: 88.5%; ESI-MS m/z: 272.0 [M+H]⁺. 1-(4-Cyanophenyl)-3-(3,4-dichlorophenyl)urea (6g): Yield: 81.9%; ESI-MS m/z: 306.0 [M+H]⁺. 1-(4-Cyanophenyl)-3-(3-(trifluoromethoxy)phenyl)urea (6h): Yield: 84.8%; ESI-MS m/z: 322.1 [M+H]⁺. 1-(4-Cyanophenyl)-3-phenylurea (6i): Yield: 79.8%; ESI-MS m/z: 238.1 [M+H]⁺. 1-(4-Cyanophenyl)-3-(2-(trifluoromethyl)phenyl)urea (6j): Yield: 83.4%; ESI-MS m/z: 306.1 [M+H]⁺. 1-(4-Cyanophenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)urea (6k): Yield: 80.2%; ESI-MS m/z: 340.0 [M+H]⁺. 1-(4-Cyanophenyl)-3-(3-methoxyphenyl)urea (6l): Yield: 83.0%; ESI-MS m/z: 268.1 [M+H]⁺. 1-(4-Cyanophenyl)-3-(3,5-di(trifluoromethyl)phenyl)urea (6m): Yield: 82.8%; ESI-MS m/z: 373.4 [M+H]⁺. 1-(4-Cyanophenyl)-3-(3,4-dimethylphenyl)urea (6n): Yield: 82.4%; ESI-MS m/z: 266.1 [M+H]⁺. 1-(4-Cyanophenyl)-3-(3-chloro-4-(trifluoromethyl)phenyl)urea (6o): Yield: 83.5%; ESI-MS m/z: 340.0 [M+H]⁺.

3.4. General Procedure for Preparation of 4-(3-Substituted Phenyureido)benzothioamides 7a–o

To a solution of benzonitrile 6a–o (0.086 mol) in N,N-dimethylformamide (250 mL) was added magnesium chloride (22 g, 0.109 mol) and sodium hydrogensulfide (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 hydrochloric acid and filtered. The collected solid was washed with water until the filtrate became neutral and dried to obtain 7a–o.

4-(3-(3-Fuorophenyl)ureido)benzothioamide (7a): Yield: 58.7%; ESI-MS m/z: 290.1 [M+H]⁺. 4-(3-(3,5-Dichlorophenyl)ureido)benzothioamide (7b): Yield: 58.8%; ESI-MS m/z: 340.0 [M+H]⁺. 4-(3-(4-Chloro-4-fluorophenyl)ureido)benzothioamide (7c): Yield: 62.5%; ESI-MS m/z: 324.0 [M+H]⁺. 4-(3-(4-(Trifluoromethyl)phenyl)ureido)benzothioamide (7d): Yield: 60.7%; ESI-MS m/z: 340.1 [M+H]⁺. 4-(3-(3-(Trifluoromethyl)phenyl)ureido)benzothioamide (7e): Yield: 66.4%; ESI-MS m/z: 340.1 [M+H]⁺. 4-(3-(3-Chlorophenyl)ureido)benzothioamide (7f): Yield: 66.4%; ESI-MS m/z: 306.0 [M+H]⁺. 4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)benzothioamide (7g): Yield: 67.0%; ESI-MS m/z: 340.0 [M+H]⁺. 4-(3-(3-Chloro-4-(trifluoromethyl)phenyl)ureido)benzothioamide (7h): Yield: 59.2%; ESI-MS m/z: 356.1 [M+H]⁺. 4-(3-Phenyureido)benzothioamide (7i): Yield: 66.4%; ESI-MS m/z: 272.2 [M+H]⁺. 4-(3-(2-(Trifluoromethyl)phenyl)ureido)benzothioamide (7j): Yield: 67.2%; ESI-MS m/z: 340.1 [M+H]⁺. 4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)benzothioamide (7k): Yield: 61.1%; ESI-MS m/z: 374.2 [M+H]⁺. 4-(3-(3-Methoxyphenyl)ureido)benzothioamide (7l): Yield: 58.5%; ESI-MS m/z: 302.1 [M+H]⁺. 4-(3-(3,5-Di( trifluoromethyl)phenyl)ureido)benzothioamide (7m): Yield: 62.7%; ESI-MS m/z: 408.1 [M+H]⁺. 4-(3-(3,4-Dimethylphenyl)ureido)benzothioamide (7n): Yield: 66.5%; ESI-MS m/z: 300.3 [M+H]⁺. 4-(3-(3-Chloro-4-(trifluoromethyl)phenyl)ureido)benzothioamide (7o): Yield: 63.1%; ESI-MS m/z: 374.2[M+H]⁺.
3.5. General Procedure for Preparation of 1-(4-(4-Chloromethylthiazol-2-yl)phenyl)-3-substituted Phenylureas 8a–o

Arylthioamides 7a–o (0.083 mol) were dissolved in tetrahydrofuran (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 alkalinized to pH 8 with saturated potassium carbonate solution. The precipitates was filtered, washed with water and dried to obtain 8a–o.

1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3-fluorophenyl)urea (8a): Yield: 50.3%; ESI-MS m/z: 362.1 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3,5-dichlorophenyl)urea (8b): Yield: 58.1%; ESI-MS m/z: 412.0 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3-chloro-4-fluorophenyl)urea (8c): Yield: 55.3%; ESI-MS m/z: 396.0 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (8d): Yield: 53.5%; ESI-MS m/z: 412.1 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3-chlorophenyl)urea (8f): Yield: 51.2%; ESI-MS m/z: 378.0 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3,4-dichlorophenyl)urea (8g): Yield: 57.5%; ESI-MS m/z: 412.0 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3-(trifluoromethoxy)phenyl)urea (8h): Yield: 57.6%; ESI-MS m/z: 428.1 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3,4-dichlorophenyl)urea (8i): Yield: 54.3%; ESI-MS m/z: 344.1 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3-chloro-4-(trifluoromethyl)phenyl)urea (8k): Yield: 55.6%; ESI-MS m/z: 446.0 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3-methoxyphenyl)urea (8l): Yield: 57.1%; ESI-MS m/z: 374.2 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3,5-dimethylphenyl)urea (8m): Yield: 52.3%; ESI-MS m/z: 480.0 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3,4-dimethylphenyl)urea (8n): Yield: 50.4%; ESI-MS m/z: 372.2 [M+H]+. 1-(4-(4-(Chloromethyl)thiazol-2-yl)phenyl)-3-(3-chloro-4-(trifluoromethyl)phenyl)urea (8o): Yield: 54.2%; ESI-MS m/z: 446.0 [M+H]+.

3.6. General Procedure for Preparation of 1-Substituted Phenyl-3-(4-(4-(piperazin-1-ylmethyl)thiazol-2-yl)phenyl)ureas 9a–o

To a solution of piperazine (64 g, 0.748 mol) in ethanol was added urea 8a–o 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 9a–o.

1-(3-Fluorophenyl)-3-(4-(4-(piperazin-1-ylmethyl)thiazol-2-yl)phenyl)urea (9a): Yield: 78.1%; ESI-MS m/z: 412.3 [M+H]+. 1-(3,5-Dichlorophenyl)-3-(4-(4-(piperazin-1-ylmethyl)thiazol-2-yl)phenyl)urea (9b): Yield: 79.6%; ESI-MS m/z: 462.1 [M+H]+. 1-(3-Chloro-4-fluorophenyl)-3-(4-(4-(piperazin-1-ylmethyl)thiazol-2-yl)phenyl)urea (9c): Yield: 83.5%; ESI-MS m/z: 446.2 [M+H]+. 1-(4-(Trifluoromethyl)phenyl)-3-(4-(4-(piperazin-1-ylmethyl)thiazol-2-yl)phenyl)urea (9d): Yield: 81.0%; ESI-MS m/z: 463.2 [M+H]+.
3.7. General Procedure for Preparation of Ethyl 2-((2-(4-(3-substituted phenylureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetates 10a–o

To a solution of N-substituted piperazine 9a–o (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 10a–o.

Ethyl 2-((2-(4-(3-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10a): Yield: 87.7%; ESI-MS m/z: 498.2 [M+H]^+. Ethyl 2-((2-(4-(3,5-dichlorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10b): Yield: 88.3%; ESI-MS m/z: 548.2 [M+H]^+. Ethyl 2-((2-(4-(3-chloro-4-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10c): Yield: 90.4%; ESI-MS m/z: 532.2 [M+H]^+. Ethyl 2-((2-(4-(3-chloro-4-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10d): Yield: 93.4%; ESI-MS m/z: 548.2 [M+H]^+. Ethyl 2-((2-(4-(3-chloro-4-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10e): Yield: 92.0%; ESI-MS m/z: 548.2 [M+H]^+. Ethyl 2-((2-(4-(3-chloro-4-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10f): Yield: 89.1%; ESI-MS m/z: 514.2 [M+H]^+. Ethyl 2-((2-(4-(3-chloro-4-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10g): Yield: 87.0%; ESI-MS m/z: 548.1 [M+H]^+. Ethyl 2-((2-(4-(3-chloro-4-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10h): Yield: 88.7%; ESI-MS m/z: 564.2 [M+H]^+. Ethyl 2-((2-(4-(3-chloro-4-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10i): Yield: 90.4%; ESI-MS m/z: 480.2 [M+H]^+. Ethyl 2-((2-(4-(3-chloro-4-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10j): Yield: 91.7%; ESI-MS m/z: 548.2 [M+H]^+. Ethyl 2-((2-(4-(3-chloro-4-fluorophenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10k): Yield: 91.5%; ESI-MS m/z: 582.1 [M+H]^+. Ethyl 2-((2-(4-(3-methoxyphenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10m): Yield: 90.4%; ESI-MS m/z: 548.2 [M+H]^+. Ethyl 2-((2-(4-(3-methoxyphenyl)ureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10n): Yield: 79.4%; ESI-MS m/z: 548.2 [M+H]^+. Ethyl 2-((2-(4-(3-phenylureido)phenyl)thiazol-4-yl)methyl)piperazin-1-yl)acetate (10o): Yield: 83.4%; ESI-MS m/z: 548.2 [M+H]^+.
3.8. General Procedure for Preparation of 1-Substituted phenyl-3-(4-((4-(2-hydrazinyl-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)ureas 11a–o

To a solution of ester 10a–o (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 11a–o.
3.9. General Procedure for Preparation of 5-Benzylxy-2-hydroxybenzaldehydes 12a–d

To a solution of 2,5-dihydroxybenzaldehyde (50 g, 0.362 mol) in acetonitrile (500 mL) was added substituted benzyl chloride (0.471 mol), sodium hydrogen carbonate (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 12a–d.

5-(Benzyloxy)-2-hydroxybenzaldehyde (12a): Yield: 53.5%; ESI-MS m/z: 227.1 [M+H]+.
5-((2-Fluorobenzyl)oxy)-2-hydroxybenzaldehyde (12b): Yield: 48.8%; ESI-MS m/z: 245.1 [M+H]+.
5-((4-Chlorobenzyl)oxy)-2-hydroxybenzaldehyde (12c): Yield: 45.5%; ESI-MS m/z: 261.0 [M+H]+.
5-((3-Chlorobenzyl)oxy)-2-hydroxybenzaldehyde (12d): Yield: 46.9%; ESI-MS m/z: 261.0 [M+H]+.

3.10. General Procedure for Preparation of 4-Benzylxy-2-hydroxybenzaldehydes 13a–c

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 hydrogen carbonate (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 13a–c.

4-((4-Chlorobenzyl)oxy)-2-hydroxybenzaldehyde (13a): Yield: 49.1%; ESI-MS m/z: 260.9 [M+H]+.
4-((2,4-Dichlorobenzyl)oxy)-2-hydroxybenzaldehyde (13b): Yield: 50.4%; ESI-MS m/z: 295.0 [M+H]+.
4-((3-Fluorobenzyl)oxy)-2-hydroxybenzaldehyde (13c): Yield: 49.7%; ESI-MS m/z: 245.1 [M+H]+.

3.11. 7-Hydroxy-4-methyl-2H-chromen-2-one (14)

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 m-dihydroxybenzene (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 14 (67.0 g, 76.1%) as a white solid. ESI-MS m/z: 175.0 [M+H]⁺.

3.12. 7-Hydroxy-4-methyl-2-oxo-2H-chromene-6-carbaldehyde (15)

A suspension of urotropine (20 g, 0.143 mol) in glacial acetic acid (80 mL) was heated to 40 °C and stirred until a clear solution formed. To this solution was added 14 (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 hydrogen carbonate solution, dried over anhydrous sodium sulfate and evaporated to dryness to afford 15 (2.2 g, 38.0%) as a white solid. ESI-MS m/z: 205.0 [M+H]⁺.
3.13. General Procedure for Preparation of Substituted 4-Substituted Phenyl Isocyanates 16a–b

To a solution of triphosgene (40 g, 0.135 mol) in dioxane (100 mL) was added substituted aniline (0.270 mol) dropwise. The reaction mixture was heated to 80 °C and stirred for 24 h. The reaction mixture was evaporated in vacuo to remove the solvent. The residue was distilled under reduced pressure to give the desired phenyl isocyanates 16a–b.

1-Isocyanato-4-(trifluoromethyl)benzene (16a): Yield: 70.6%; b.p.: 90–92 °C (20 mmHg).
1-Fluoro-4-isocyanatobenzene (16b): Yield: 73.1%; b.p.: 75–77 °C (20 mmHg).

3.14. General Procedure for Preparation of Methyl 2-(3-(4-Substituted Phenyl)ureido)acetates 17a–b

To a suspension of glycine methyl ester hydrochloride (16 g, 0.130 mol) in dichloromethane (150 mL) was added triethylamine (15.6 g, 0.154 mol) dropwise. The mixture was stirred at room temperature for 1 h and cooled below 0 °C in an ice bath. To the cold mixture was added isocyanate 16a–b (0.118 mol) slowly. The reaction mixture was kept in an ice bath and stirred for 4 h, then another 1 h at room temperature. The reaction mixture was evaporated to dryness, poured into water (300 mL) and stirred. The precipitates were collected by filtration and dried to give 17a–b.

Methyl 2-(3-(4-(trifluoromethyl)phenyl)ureido)acetate (17a): Yield: 80.6%; ESI-MS m/z: 277.1 [M+H]+.
Methyl 2-(3-(4-fluorophenyl)ureido)acetate (17b): Yield: 77.8%; ESI-MS m/z: 227.1 [M+H]+.

3.15. General Procedure for Preparation of 3-(4-Substituted Phenyl)imidazolidine-2,4-diones 18a–b

To a solution of ester 17a–b (0.044 mol) in acetone (100 mL) was added concentrated hydrochloric acid (110 mL). The reaction mixture was stirred and refluxed for 4 h. The reaction mixture was evaporated to remove most of the solvent and cooled. The precipitates were filtered off, washed by water and dried to give 18a–b.

3-(4-(Trifluoromethyl)phenyl)imidazolidine-2,4-dione (18a): Yield: 66.7%; ESI-MS m/z: 245.1 [M+H]+.
3-(4-Fluorophenyl)imidazolidine-2,4-dione (18b): Yield: 61.2%; ESI-MS m/z: 195.1 [M+H]+.

3.16. General Procedure for Preparation of 5-((dimethylamino)methylene)-3-(4-substituted phenyl) imidazolidine-2,4-diones 19a–b

Imidazolindione 18a–b (0.026 mol), N,N-dimethylformamide dimethylacetal (12 g, 0.103 mol) were added to acetonitrile (10 mL). The reaction mixture was stirred and refluxed for 4 h. The reaction mixture was cooled, and precipitates were filtered off, washed by acetonitrile in small portions, dried to give 19a–b.

5-((Dimethylamino)methylene)-3-(4-(trifluoromethyl)phenyl)imidazolidine-2,4-dione (19a): Yield: 47.7%; ESI-MS m/z: 277.1 [M+H]+.
5-((Dimethylamino)methylene)-3-(4-fluorophenyl)imidazolidine-2,4-dione (19b): Yield: 52.4%; ESI-MS m/z: 227.1 [M+H]+.
3.17. General Procedure for the Preparation of Target Compounds 1a–g, 2a–k, and 3a–e

To a solution of acethydrazide 11a–o (0.002 mol) in ethanol (10 mL) was added appropriate benzaldehyde or the prepared aromatic aldehyde 12a–d, 13a–c or 15. 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 excess hydrogen chloride-ethanol and stirred for 1 h. Ether was added to the mixture above. The precipitates were filtered off and dried to afford 1a–g, 2a–k, and 3a–e as dihydrochlorides.

1-(3-Fluorophenyl)-3-(4-(4-((4-(2-(2-(2-hydroxy-4-methylbenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (1a). Yield: 80.1%; M.p.: 185–187 °C; ESI-MS m/z: 602.1 [M−2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 11.66 (s, 1H), 11.50 (s, 1H), 10.44 (s, 1H), 9.03 (s, 1H), 8.97 (d, 2H), 7.86 (d, 2H), 7.59 (d, 1H), 7.41 (d, 1H), 7.32 (dd, 1H), 7.15 (d, 1H), 7.04 (s, 1H), 6.98 (s, 1H), 6.80 (t, 1H), 3.66 (s, 2H), 3.34 (s, 1H), 3.32 (s, 2H), 3.12 (s, 1H), 2.54 (s, 6H), 2.22 (s, 3H). Anal. Calcd for C31H32FN7O3S (%): C, 61.88; H, 5.36; N, 16.30; Found (%): C, 61.81; H, 5.42; N, 16.28.

1-(3,5-Dichlorophenyl)-3-(4-(4-((4-(2-(2-(2-hydroxy-3-(2-methylallyl)benzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (1b). Yield: 76.1%; M.p.: 178–180 °C; ESI-MS m/z: 691.7 [M−2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 11.89 (s, 1H), 9.20 (s, 1H), 8.99 (s, 1H), 7.86 (m, 3H), 7.57 (m, 3H), 7.41 (s, 1H), 7.25 (d, 1H), 7.18–7.14 (m, 2H), 6.88 (t, 1H), 4.75 (s, 1H), 4.63 (s, 1H), 3.66–3.58 (m, 4H), 3.13 (s, 2H), 2.53 (s, 2H), 1.67 (s, 3H). Anal. Calcd for C34H35Cl2N7O3S (%): C, 58.96; H, 5.09; N, 14.16; Found (%): C, 58.94; H, 5.02; N, 14.22.

1-(3-Chloro-4-fluorophenyl)-3-(4-(4-((4-(2-(2-(3,5-di-tert-butyl-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (1c). Yield: 82.7%; M.p.: 231–232 °C; ESI-MS m/z: 734.6 [M−2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 9.09 (s, 1H), 8.47 (s, 1H), 7.91 (d, 3H), 7.81 (d, 1H), 7.62 (d, 2H), 7.44 (d, 1H), 7.38–7.33 (m, 2H), 7.22 (s, 1H), 4.45 (s, 2H), 3.67 (brs, 10H), 1.41 (d, 9H), 1.28 (d, 9H). Anal. Calcd for C38H45ClFN7O3S (%): C, 62.15; H, 6.18; N, 13.35; Found (%): C, 62.17; H, 6.11; N, 13.28.

1-(4-(4-((4-(2-(2-(2-Hydroxy-6-isopropyl-3-methylbenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)urea (1d). Yield: 77.4%; M.p.: 185–187 °C; ESI-MS m/z: 694.5 [M−2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 9.01 (s, 1H), 8.54 (s, 1H), 8.37 (s, 1H), 7.98–7.93 (m, 3H), 7.70–7.64 (m, 6H), 7.33 (t, 1H), 6.90 (d, 1H), 4.67 (s, 2H), 4.56 (s, 2H), 3.60 (brs, 8H), 1.28 (d, 9H), 1.28 (d, 9H). Anal. Calcd for C35H38F3N7O3S (%): C, 60.59; H, 5.52; N, 14.13; Found (%): C, 60.61; H, 5.51; N, 14.18.

1-(4-(4-((4-(2-(5-(tert-Butyl)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)urea (1e). Yield: 75.1%; M.p.: 215–218 °C; ESI-MS m/z: 694.4 [M−2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 12.51 (s, 1H), 11.70 (s, 1H), 10.00 (s, 1H), 9.95 (s, 1H), 8.90 (s, 1H), 7.89 (s, 3H), 7.75–7.62 (m, 7H), 7.14 (d, 1H), 6.75 (d, 1H), 4.27–4.09
(m, 2H), 3.35 (s, 1H), 3.17 (s, 4H), 3.07 (s, 2H), 2.87 (s, 4H), 2.13 (s, 3H), 1.21 (s, 6H). Anal. Calcd for C_{35}H_{38}F_{3}N_{7}O_{3}S (%): C, 60.59; H, 5.52; N, 14.13; Found (%): C, 60.51; H, 5.48; N, 14.16.

1-(4-((4-(2-(2-Hydroxy-5-methoxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)urea (1f). Yield: 70.7%; M.p.: 198–200 °C; ESI-MS m/z: 667.9 [M−2HCl+H]+; ¹H-NMR (DMSO-d₆) δ (ppm): 12.00 (s, 1H), 10.22 (s, 1H), 10.12 (s, 1H), 8.52 (s, 1H), 8.35 (s, 1H), 7.98–7.92 (m, 4H), 7.70–7.63 (m, 7H), 7.28 (s, 1H), 6.89 (s, 2H), 4.67 (s, 1H), 4.57 (s, 2H), 3.72 (s, 3H), 3.64 (brs, 9H). Anal. Calcd for C_{32}H_{33}F_{3}N_{7}O_{4}S (%): C, 57.56; H, 4.83; N, 14.68; Found (%): C, 57.53; H, 4.85; N, 14.71.

1-(4-(4-((4-(2-(2-(2-Hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (1g). Yield: 78.4%; M.p.: 205–207 °C; ESI-MS m/z: 638.1 [M−2HCl+H]+; ¹H-NMR (DMSO-d₆) δ (ppm): 11.94 (s, 1H), 10.14 (s, 1H), 9.84 (s, 1H), 9.74 (d, 1H), 8.51 (s, 1H), 8.36 (s, 1H), 8.02 (s, 1H), 7.93 (d, 2H), 7.89–7.88 (m, 1H), 7.75 (d, 1H), 7.66–7.50 (m, 4H), 7.34–7.24 (m, 2H), 6.94–6.84 (m, 2H), 4.54 (s, 1H), 4.45 (s, 2H), 3.51 (brs, 8H). Anal. Calcd for C_{31}H_{30}F_{3}N_{7}O_{3}S (%): C, 58.39; H, 4.74; N, 15.38; Found (%): C, 58.36; H, 4.75; N, 15.36.

1-(4-(4-((4-(2-(2-(5-(Benzyloxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(3-fluorophenyl)urea (2a). Yield: 74.9%; M.p.: 190–192 °C; ESI-MS m/z: 694.5 [M−2HCl+H]+; ¹H-NMR (DMSO-d₆) δ (ppm): 11.41 (s, 1H), 10.66 (s, 1H), 9.72 (s, 1H), 9.09 (s, 1H), 8.47 (s, 1H), 7.85 (d, 2H), 7.59 (d, 2H), 7.50 (d, 1H), 7.47–7.25 (m, 7H), 7.23–7.08 (m, 2H), 6.97 (dd, 1H), 6.85–6.77 (m, 2H), 5.04 (s, 2H), 3.64 (d, 2H), 3.31 (s, 4H), 2.52 (s, 6H). Anal. Calcd for C_{37}H_{36}FN_{7}O_{4}S (%): C, 64.05; H, 5.23; N, 14.13; Found (%): C, 64.02; H, 5.24; N, 14.09.

1-(4-(4-((4-(2-(2-(5-(Benzyloxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(3-chlorophenyl)urea (2b). Yield: 78.3%; M.p.: 188–190 °C; ESI-MS m/z: 709.9 [M−2HCl+H]+; ¹H-NMR (DMSO-d₆) δ (ppm): 11.45 (s, 1H), 10.67 (s, 1H), 9.53 (d, 2H), 8.48 (s, 1H), 8.17 (s, 1H), 7.85 (d, 2H), 7.72 (s, 1H), 7.59 (d, 2H), 7.50 (d, 1H), 7.47–7.25 (m, 7H), 7.23–7.08 (m, 2H), 6.97 (dd, 1H), 6.85–6.77 (m, 2H), 5.04 (s, 2H), 3.64 (d, 2H), 3.32 (s, 4H), 2.54 (s, 6H). Anal. Calcd for C_{37}H_{36}ClN_{7}O_{4}S (%): C, 62.57; H, 5.11; N, 13.80; Found (%): C, 62.55; H, 5.14; N, 13.73.

1-(4-((4-(2-(4-(4-Chlorobenzyl)oxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(3-chlorophenyl)urea (2c). Yield: 84.4%; M.p.: 218–220 °C; ESI-MS m/z: 744.1 [M−2HCl+H]+; ¹H-NMR (DMSO-d₆) δ (ppm): 11.80 (s, 1H), 9.76 (s, 1H), 9.72 (s, 1H), 9.67 (s, 1H), 8.41 (s, 1H), 8.26 (s, 1H), 7.93 (s, 1H), 7.90 (s, 2H), 7.71 (s, 1H), 7.63 (d, 2H), 7.46 (s, 4H), 7.31 (s, 1H), 7.30 (s, 1H), 7.02–7.01 (m, 1H), 6.59–6.54 (m, 2H), 5.11 (d, 2H), 4.51 (s, 1H), 4.45 (s, 2H), 3.58 (brs, 9H). Anal. Calcd for C_{37}H_{35}Cl_{2}N_{7}O_{4}S (%): C, 59.68; H, 4.74; N, 13.17; Found (%): C, 59.64; H, 4.70; N, 13.13.

1-(3-Chlorophenyl)-3-(4-(4-(2-(4-(2,4-dichlorobenzyl)oxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(3-chlorophenyl)urea (2d). Yield: 75.2%; M.p.: 218–220 °C; ESI-MS m/z: 778.0 [M−2HCl+H]+; ¹H-NMR (DMSO-d₆) δ (ppm): 11.82 (s, 1H), 9.78 (s, 1H), 9.74 (s, 1H), 9.70 (s, 1H), 8.27 (s, 1H), 7.94 (s, 2H), 7.91 (s, 2H), 7.71–7.70 (m, 3H), 7.66–7.59 (m, 4H), 7.50–7.45 (m, 1H), 7.31 (s, 1H), 7.30 (s, 1H), 7.04–7.01 (m, 1H), 6.61–6.56 (m, 2H), 5.15 (d, 2H), 4.51 (s, 1H), 4.45 (s, 2H), 3.58 (brs, 9H). Anal. Calcd for C_{37}H_{36}Cl_{2}N_{7}O_{4}S (%): C, 59.68; H, 4.74; N, 13.17; Found (%): C, 59.64; H, 4.70; N, 13.13.
4.53 (s, 1H), 4.46 (s, 2H), 3.66 (brs, 9H). Anal. Calcd for C\textsubscript{37}H\textsubscript{34}Cl\textsubscript{3}N\textsubscript{7}O\textsubscript{4}S (%): C, 57.04; H, 4.40; N, 12.58; Found (%): C, 57.06; H, 4.47; N, 12.51.

1-(3,4-Dichlorophenyl)-3-(4-((4-(2-(4-(3-fluorobenzyl)oxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl/thiazol-2-yl)phenyl)urea (2e). Yield: 79.4%; M.p.: 209–210 °C; ESI-MS m/z: 761.8 [M−2HCl+H]+; 1H-NMR (DMSO-\textsubscript{d}6) δ (ppm): 11.79 (s, 1H), 9.81 (s, 1H), 9.77 (s, 1H), 9.74 (s, 1H), 9.70 (s, 1H), 8.26 (s, 1H), 7.93–7.88 (m, 4H), 7.62 (d, 3H), 7.53 (d, 1H), 7.46–7.41 (m, 1H), 7.35 (dd, 1H), 7.29–7.25 (m, 2H), 7.20–7.14 (m, 1H), 6.61–6.55 (m, 2H), 5.15 (s, 1H), 5.12 (s, 1H), 4.50 (s, 1H), 4.45 (s, 2H), 3.52 (brs, 9H). Anal. Calcd for C\textsubscript{37}H\textsubscript{34}Cl\textsubscript{2}FN\textsubscript{7}O\textsubscript{4}S (%): C, 58.27; H, 4.49; N, 12.86; Found (%): C, 58.26; H, 4.47; N, 12.89.

1-(4-(4-((4-(2-(2-(4-((4-Chlorobenzyl)oxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(3,4-dichlorophenyl)urea (2f). Yield: 73.5%; M.p.: 227–228 °C; ESI-MS m/z: 777.7 [M−2HCl+H]+; 1H-NMR (DMSO-\textsubscript{d}6) δ (ppm): 11.78 (s, 1H), 9.79 (s, 1H), 9.75 (s, 1H), 9.72 (s, 1H), 9.69 (s, 1H), 8.41 (s, 1H), 8.25 (s, 1H), 7.93–7.88 (m, 4H), 7.62 (d, 2H), 7.53 (d, 2H), 7.46 (s, 4H), 7.35 (dd, 1H), 6.59–6.53 (m, 2H), 5.12 (s, 1H), 5.09 (s, 1H), 4.49 (s, 1H), 4.43 (s, 2H), 3.53 (s, 9H). Anal. Calcd for C\textsubscript{37}H\textsubscript{34}Cl\textsubscript{3}N\textsubscript{7}O\textsubscript{4}S (%): C, 57.04; H, 4.40; N, 12.58; Found (%): C, 57.06; H, 4.47; N, 12.59.

1-(4-(4-((4-(2-(2-(5-((2-Fluorobenzyl)oxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(3-(trifluoromethoxy)phenyl)urea (2g). Yield: 71.2%; M.p.: 165–168 °C; ESI-MS m/z: 778.5 [M−2HCl+H]+; 1H-NMR (DMSO-\textsubscript{d}6) δ (ppm): 11.42 (s, 1H), 10.71 (s, 1H), 9.75 (s, 1H), 9.09 (s, 1H), 9.08 (s, 1H), 8.48 (s, 1H), 8.17 (s, 1H), 7.86 (d, 2H), 7.71 (s, 1H), 7.59 (d, 2H), 7.54 (d, 1H), 7.44–7.39 (m, 3H), 7.33–7.14 (m, 3H), 7.01–6.95 (m, 2H), 6.85 (d, 1H), 5.08 (s, 2H), 3.66 (s, 2H), 3.47 (s, 1H), 3.10 (s, 1H), 2.54 (s, 8H). Anal. Calcd for C\textsubscript{38}H\textsubscript{35}F\textsubscript{4}N\textsubscript{7}O\textsubscript{5}S (%): C, 58.68; H, 4.54; N, 12.61; Found (%): C, 58.66; H, 4.59; N, 12.58.

1-(4-(4-((4-(2-(2-(5-((4-Chlorobenzyl)oxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-phenylurea (2h). Yield: 78.4%; M.p.: 202–203 °C; ESI-MS m/z: 694.4 [M−2HCl+H]+; 1H-NMR (DMSO-\textsubscript{d}6) δ (ppm): 11.40 (s, 1H), 10.70 (s, 1H), 10.33–10.26 (m, 3H), 8.50 (s, 1H), 8.33 (s, 1H), 8.26 (s, 1H), 7.86 (d, 2H), 7.84 (s, 1H), 8.48 (s, 1H), 7.84 (d, 2H), 7.59–7.52 (m, 3H), 7.47 (d, 2H), 7.40 (s, 2H), 7.32–7.20 (m, 4H), 7.16 (d, 1H), 7.01–6.97 (m, 2H), 6.85 (d, 1H), 5.08 (s, 2H), 3.66 (s, 1H), 3.29 (s, 4H), 3.10 (s, 1H), 2.54 (s, 8H). Anal. Calcd for C\textsubscript{37}H\textsubscript{36}FN\textsubscript{7}O\textsubscript{4}S (%): C, 64.05; H, 5.23; N, 14.13; Found (%): C, 64.02; H, 5.20; N, 14.18.

1-(4-(4-((4-(2-(5-((2-Fluorobenzyl)oxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-(3-(trifluoromethoxy)phenyl)urea (2i). Yield: 72.6%; M.p.: 247–249 °C; ESI-MS m/z: 778.0 [M−2HCl+H]+; 1H-NMR (DMSO-\textsubscript{d}6) δ (ppm): 12.03 (s, 1H), 10.65 (s, 1H), 10.33–10.26 (m, 3H), 8.50 (s, 3H), 8.33 (s, 1H), 7.86 (s, 1H), 7.71 (s, 2H), 7.47 (s, 3H), 7.36–7.29 (m, 4H), 6.98 (dd, 1H), 6.89 (d, 1H), 5.06 (s, 2H), 4.67 (s, 2H), 4.52 (s, 2H), 3.92–3.32 (s, 8H). Anal. Calcd for C\textsubscript{38}H\textsubscript{35}ClF\textsubscript{3}N\textsubscript{7}O\textsubscript{4}S (%): C, 58.63; H, 4.55; N, 12.61.
1-(4-Chloro-3-(Trifluoromethyl)phenyl)-3-(4-(4-(2-(2-(5-((2-fluorobenzyl)oxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (2j). Yield: 74.5%; M.p.: 165–167 °C; ESI-MS m/z: 796.5 [M–2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 11.42 (s, 1H), 10.71 (s, 1H), 9.47 (s, 1H), 9.47 (s, 1H), 8.49 (s, 1H), 8.13 (s, 1H), 7.83 (d, 2H), 7.66–7.52 (m, 6H), 7.40 (s, 2H), 7.23–7.17 (m, 3H), 6.99 (d, 1H), 6.85 (d, 1H), 5.08 (s, 2H), 3.66 (s, 1H), 3.64 (s, 1H), 3.49 (s, 1H), 3.31 (s, 1H), 3.11 (s, 2H), 2.54 (s, 6H). Anal. Calcd for C38H34ClF4N7O4S (%): C, 57.32; H, 4.30; N, 12.31; Found (%): C, 57.33; H, 4.35; N, 12.25.

1-(3-Chloro-4-fluorophenyl)-3-(4-(4-((4-(2-(2-(5-((3-chlorobenzyl)oxy)-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (2k). Yield: 73.3%; M.p.: 194–195 °C; ESI-MS m/z: 762.2 [M–2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 11.42 (s, 1H), 10.67 (s, 1H), 9.75 (s, 1H), 9.09 (s, 1H), 8.98 (s, 1H), 8.47 (s, 1H), 7.86–7.80 (m, 4H), 7.58 (d, 2H), 7.51 (s, 1H), 7.41 (s, 4H), 7.34 (d, 2H), 7.15 (d, 1H), 7.00–6.96 (m, 1H), 6.84 (d, 1H), 5.07 (s, 2H), 3.66 (s, 2H), 3.32 (s, 2H), 3.10 (s, 2H), 2.54 (s, 6H). Anal. Calcd for C37H34Cl2FN7O4S (%): C, 58.27; H, 4.49; N, 12.86; Found (%): C, 58.25; H, 4.45; N, 12.92.

1-(4-(4-((4-(2-(2-((7-Hydroxy-4-methyl-2-oxo-2H-chromen-6-yl)methylene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)-3-phenylurea (3a). Yield: 75.2%; M.p.: 243–245 °C; ESI-MS m/z: 652.1 [M–2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 12.19 (s, 1H), 9.46 (d, 1H), 9.18 (d, 1H), 9.00 (s, 1H), 8.61 (s, 1H), 7.91 (d, 3H), 7.71 (d, 1H), 7.62 (d, 2H), 7.29 (t, 2H), 7.00–6.95 (m, 2H), 6.26 (s, 1H), 4.47 (s, 2H), 3.47 (d, 10H), 2.41 (s, 3H). Anal. Calcd for C34H33N7O5S (%): C, 62.66; H, 5.10; N, 15.04; Found (%): C, 62.61; H, 5.13; N, 15.02.

1-(4-(4-(2-(2-((7-Hydroxy-4-methyl-2-oxo-2H-chromen-6-yl)methylene)methylene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (3b). Yield: 71.7%; M.p.: 242–243 °C; ESI-MS m/z: 682.3 [M–2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 12.53 (s, 1H), 12.16 (s, 1H), 10.99 (s, 1H), 10.99 (s, 1H), 9.96 (s, 1H), 9.87 (s, 1H), 8.98 (s, 1H), 8.61 (s, 1H), 8.01 (s, 1H), 7.93 (d, 3H), 7.72 (t, 1H), 7.68–7.57 (m, 3H), 7.52 (t, 1H), 7.32 (d, 1H), 6.98–6.95 (m, 1H), 6.26 (s, 1H), 4.52 (s, 2H), 3.80 (s, 2H), 3.59 (s, 8H), 2.41 (s, 3H). Anal. Calcd for C33H32F3N7O6S (%): C, 58.41; H, 4.48; N, 13.62; Found (%): C, 58.43; H, 4.43; N, 13.68.

1-(3,5-bis(Trifluoromethyl)phenyl)-3-(4-(4-(2-(2-((7-Hydroxy-4-methyl-2-oxo-2H-chromen-6-yl)methylene)methylene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (3d). Yield: 70.1%; M.p.: 225–226 °C; ESI-MS m/z: 788.3 [M–2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 12.68 (s, 1H), 10.53 (d, 1H), 10.01 (d, 1H), 8.98 (s, 1H), 8.13 (s, 2H), 8.00–7.89 (m, 4H), 7.73 (t, 1H), 7.66 (d, 3H), 7.57 (t, 1H), 7.41 (s, 4H), 7.34 (d, 2H), 7.26 (s, 1H), 6.26 (s, 1H), 6.17 (m, 1H), 4.47 (s, 2H), 3.72 (s, 3H), 3.61 (s, 10H), 2.41 (s, 3H). Anal. Calcd for C33H32N7O6S (%): C, 61.66; H, 5.17; N, 14.38; Found (%): C, 61.13; H, 5.14; N, 14.35.
7.06–6.88 (m, 1H), 6.26 (d, 1H), 4.11–3.56 (m, 8H), 2.41 (s, 3H). Anal. Calcd for C_{36}H_{31}F_{6}N_{7}O_{5}S (%): C, 54.89; H, 3.97; N, 12.45; Found (%): C, 54.88; H, 3.94; N, 12.40.

1-(3,4-Dimethylphenyl)-3-(4-(4-((4-(2-(2-(7-hydroxy-4-methyl-2-oxo-2H-chromen-6-yl)methylene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (3e). Yield: 72.2%; M.p.: 246–248 °C; ESI-MS m/z: 666.3 [M − 2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 12.56 (s, 1H), 12.16 (s, 1H), 11.00 (s, 1H), 9.71 (d, 1H), 9.26 (d, 1H), 8.98 (s, 1H), 7.93–7.88 (m, 3H), 7.75–7.70 (m, 1H), 7.61 (dd, 2H), 7.24–7.19 (m, 2H), 7.04–6.95 (m, 2H), 6.27–6.25 (m, 1H), 4.52 (s, 2H), 3.81 (s, 2H), 3.60 (brs, 8H), 2.41 (s, 3H), 2.19 (s, 3H). Anal. Calcd for C_{36}H_{37}N_{7}O_{5}S (%): C, 63.61; H, 5.49; N, 14.42; Found (%): C, 63.58; H, 5.54; N, 14.40.

3.18. General Procedure for Preparation of Target Compounds 4a–c

Acethydrazide 11e or 11o (0.002 mol) was dissolved in 1M hydrochloride (5 mL) to obtain the hydrochloride form. The resulting hydrochloride salt was then added to a solution of imidazolindione 19a–b (0.002 mol) in ethanol (10 mL). The reaction mixture was stirred at room temperature for 4 h. The precipitates were filtered off and dried to obtain 4a–c as dihydrochlorides.

1-(3-Chloro-4-(trifluoromethyl)phenyl)-3-(4-(4-((4-(2-(2-((2,5-dioxo-1-(4-(trifluoromethyl)phenyl)imidazolidin-4-yl)methylene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)thiazol-2-yl)phenyl)urea (4a). Yield: 58.4%; M.p.: 239–241 °C; ESI-MS m/z: 822.0 [M − 2HCl+H]+; 1H-NMR (DMSO-d6) δ (ppm): 10.63 (s, 1H), 9.98 (s, 2H), 9.77 (s, 1H), 8.72 (d, 1H), 8.12 (s, 1H), 7.94 (s, 1H), 7.92 (d, J = 3.1 Hz, 2H), 7.84 (d, 2H), 7.67 (d, 2H), 7.65–7.62 (t, 4H), 6.73 (d, 1H), 4.49 (s, 2H), 3.73 (s, 2H), 3.32 (s, 10H). Anal. Calcd for C_{35}H_{30}ClF_{6}N_{9}O_{4}S (%): C, 51.13; H, 3.68; N, 15.33; Found (%): C, 51.18; H, 3.64; N, 15.40.

3.19. Evaluation of the Biological Activity

The cytotoxicity of target compounds 1a–g, 2a–k, 3a–e and 4a–c were evaluated against the A549, MDA-MB-231 and HL-60 cell lines by MTT method in vitro, with sorafenib and PAC-1 as the
positive controls. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately $4 \times 10^3$ 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 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 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 Incorporated Slide Scanner (Bliss) software.

4. Conclusions

In summary, a novel series of dual diaryl urea and N-acylhydrazone derivatives were designed based on the hybrid pharmacophore concept. All the target compounds were synthesized and screened for their cytotoxicity against three human cancer cell lines (HL-60, A549 and MDA-MB-231) by standard MTT assays. The pharmacological results indicated that most compounds exhibited moderate to excellent activity. The preliminary SARs showed that both electron-withdrawing groups on Ar$^1$ and 4- or 5-benzyloxyl groups on Ar$^2$ are favorable for optimal cytotoxicity. Moreover, this encouraging research provides a valuable leading compound 2g with IC$_{50}$ values of 0.22, 0.34 and 0.41 μM against tested cell lines respectively, which were 3.8 to 22.5 times more active than the references sorafenib and PAC-1, and highlights the potential for further development of novel dual diaryl urea and N-acylhydrazone derivatives. Studies on the mechanism of action of these compounds are in progress and will be reported in the near future.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 21002065) and the Young Scholar Growth Plan in University of Liaoning Province (No. LJQ201107).

References

1. Keating, G.M.; Santoro, A. Sorafenib: A review of its use in advanced hepatocellular carcinoma. Drugs 2009, 69, 223–240.
2. Wilhelm, S.M.; Adnane, L.; Newell, P.; Villanueva, A.; Llovet, J.M.; Lynch, M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol. Cancer Ther. 2008, 7, 3129–3140.
3. Smalley, K.S.; Xiao, M.; Villanueva, J.; Nguyen, T.K.; Flaherty, K.T.; Letrero, R.; van Belle, P.; Elder, D.E.; Wang, Y.; Nathanson, K.L.; et al. CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. Oncogene 2009, 28, 85–94.
4. Adnane, L.; Trail, P.A.; Taylor, I.; Wilhelm, S.M. Sorafenib (BAY 43-9006, Nexavar), a dual-action inhibitor that targets RAF/MEK/ERK pathway in tumor cells and tyrosine kinases VEGFR/PDGFR in tumor vasculature. *Methods Enzymol.* **2006**, *407*, 597–612.

5. Hyun, Y.W.; Jeong, H. Sorafenib in liver cancer. *Expert Opin. Pharmacother.* **2012**, *13*, 1059–1067.

6. Strumberg, D. Sorafenib for the treatment of renal cancer. *Expert Opin. Pharmacother.* **2012**, *13*, 407–419.

7. Peterson, Q.P.; Hsu, D.C.; Goode, D.R.; Novotny, C.J.; Totten, R.K.; Hergenrother, P.J. Procaspase-3 activation as an anti-cancer strategy: Structure-activity relationship of PAC-1, and its cellular co-localization with caspase-3. *J. Med. Chem.* **2009**, *52*, 5721–5731.

8. Lucas, P.W.; Schmit, J.M.; Peterson, Q.P.; West, D.C.; Hsu, D.C.; Novotny, C.J.; Dirikolu, L.; Churchwell, M.I.; Doerge, D.R.; Garrett, L.D.; *et al.* Pharmacokinetics and Derivation of an anticancer dosing regimen for PAC-1, a preferential small molecule activator of procaspase-3, in healthy dogs. *Invest. New Drugs* **2011**, *29*, 901–911.

9. Peterson, Q.P.; Goode, D.R.; West, D.C.; Ramsey, K.N.; Lee, J.J.; Hergenrother, P.J. PAC-1 activates procaspase-3 *in vitro* through relief of zinc-mediated inhibition. *J. Mol. Biol.* **2009**, *388*, 144–158.

10. Zhang, B.; Zhao, Y.; Zhai, X.; Wang, L.; Yang, J.; Tan, Z.; Gong, P. Design, synthesis and anticancer activities of diaryl urea derivatives bearing N-acylhydrazone moiety. *Chem. Pharm. Bull.* **2012**, *60*, 1046–1054.

11. Putt, K.S.; Chen, G.W.; Pearson, J.M.; Sandhorst, J.S.; Hoagland, M.S.; Kwon, J.T.; Hwang, S.K.; Jin, H.; Churchwell, M.I.; Cho, M.H.; *et al.* Small-molecule activation of procaspase-3 to caspase-3 as a personalized anticancer strategy. *Nat. Chem. Biol.* **2006**, *2*, 543–550.

12. Kudo, M.; Hanashima, T.; Muranaka, A.; Sato, H.; Uchiyama, M.; Azumaya, I.; Hirano, T.; Kagechika, H.; Tanatani, A. Identification of Absolute Helical Structures of Aromatic Multilayered Oligo(m-Phenylurea)s in Solution. *J. Org. Chem.* **2009**, *74*, 8154–8163.

13. Duff, J.C.; Bills, E.J. Reactions between Hexamethylenetetramine and Phenolic Compounds. Part I. A New Method for the Preparation of 3- and 5-Aldehydosalicylic Acids. *J. Chem. Soc.* **1932**, *1932*, 1987–1988.

14. Muccioli, G.G.; Fazio, N.; Scriba, G.K.; Poppitz, W.; Cannata, F.; Poupaert, J.H.; Wouters, J.; Lambert, J.D. Substituted 2-Thioxoimidazolidin-4-Ones and Imidazolidine-2,4-Diones as Fatty Acid Amide Hydrolase Inhibitors Templates. *J. Med. Chem.* **2006**, *49*, 417–425.

Sample Availability: Samples of the compounds are available from the authors.

© 2013 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/).