Full Paper

Synthesis and Biological Activities of 2-Amino-thiazole-5-carboxylic Acid Phenylamide Derivatives

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In an attempt to develop potent and selective anti-tumor drugs, a series of novel 2-amino-thiazole-5-carboxylic acid phenylamide derivatives were designed based on the structure of dasatinib. All compounds were synthesized by a systematic combinatorial chemical approach. Biological evaluation revealed that *N*-(2-chloro-6-methylphenyl)-2-(2-(4-methylpiperazin-1-yl)acetamido)thiazole-5-carboxamide (**6d**) exhibited high antiproliferative potency on human K563 leukemia cells comparable to dasatinib. Against mammary and colon carcinoma cells **6d** was either inactive (MDA-MB 231) or distinctly less active (MCF-7 and HT-29: $IC_{50} = 20.2$ and 21.6 μM, respectively). Dasatinib showed at each cell line $IC_{50} < 1$ μM. The results of this structure activity relationship study clearly documented that the pyrimidin-4-ylamino core of dasatinib is responsible for the anti-tumor activity against non-leukemia cell lines.

Keywords: Antiproliferative / Dasatinib / Protein tyrosine kinases / Synthesis

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Introduction

Tyrosine kinases are a subgroup of the larger class of protein kinases that can catalyze the transfer of γ-phosphoryl groups from ATP to tyrosine hydroxyls of proteins. Phosphorylation of proteins by kinases is an important mechanism in signal transduction for regulation of cellular activity [1, 2]. These protein tyrosine kinases (PTKs) are classified as receptor PTKs (e.g., insulin receptor and EGF receptor) and non-receptor PTKs (e.g., BCR-ABL and SRC) [3, 4]. They play a key role in the regulation of cell proliferation, differentiation, metabolism, migration, and survival. Overexpression or high activation of PTKs occurs frequently in tumor tissues [5]. The strong correlation of aberrant or over-expressed PTKs with a number of proliferative diseases has raised the possibility that PTK inhibitors may afford new approaches toward anti-cancer therapeutics [6, 7].

The introduction of the BCR–ABL kinase inhibitor imatinib revolutionized the treatment of chronic myeloid leukemia (CML). However, most patients with CML receiving imatinib still harbor molecular residual disease. Even worse, some patients develop resistance associated with ABL kinase domain mutations [8].

Dasatinib (Fig. 1), a potent dual inhibitor of SRC and ABL kinase, was recently approved for the oral treatment of CML and Philadelphia chromosome-positive acute lymphoblastic leukemia [9–16]. This drug has a good therapeutic effect on a wide range of tumors, especially it can inhibit HMC-1 cell proliferation and prevent self-Kit kinase phosphorylation [10]. In the cell test, the inhibitory effect of dasatinib on

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Abbreviations: 5-FU, 5-fluorouracil; **CML,** chronic myeloid leukemia; **CYP3A4,** cytochrome P450 3A4; **PBS,** phosphate buffered saline; **PTKs,** protein tyrosine kinases.

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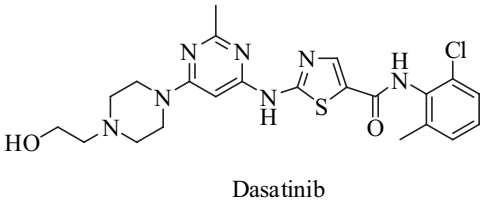


Dasatinib

Figure 1. The structure of dasatinib.

variability of BCR-ABL kinase is stronger than that of imatinib, and has a high degree of selectivity to normal hematopoietic cells and leukemia cells [11]. Preclinical data have indicated that this drug is metabolized primarily through cytochrome P450 3A4 (CYP3A4) [12, 13]. Currently dasatinib is in clinical trials for the treatment of solid tumors and gastrointestinal stromal tumors [12, 13].

As a second-generation BCR–ABL inhibitor, dasatinib has shown significant activity after imatinib failure in clinical trials, but still face similar obstacles to imatinib, including negligible activity against the frequent BCR–ABL T315I mutation and modest effects in advanced phases of CML [8].

The three-dimensional structure of ABL kinase, SRC kinase complexed with dasatinib showed that a pair of hydrogen bonds was formed in the hinge region of the ATP-binding site between the 2-amino hydrogen of dasatinib and the carbonyl oxygen of Met318, between the 3-nitrogen of the thiazole ring of dasatinib and the amide nitrogen of Met318. A hydrogen bond was also formed between the hydroxyl oxygen of Thr315 and the amide nitrogen of dasatinib. However no hydrogen bonds was found between the pyrimidine of dasatinib and protein despite it may account for the increased binding affinity [14–16].

Encouraged by the successful development of dasatinib, our research was focused on the synthesis of 2-aminothiazole-5-carboxylic acid phenylamide derivatives. In order to investigate whether the pyrimidin-4-ylamino moiety is critical for activity, acetyl substitution was adopted as alternate scaffold at the 2-amino group. For comparison, we also developed some derivatives with the absence of the chloro atom at the phenyl ring moiety.

All compounds were synthesized by a systematic combinatorial chemical approach which is recently widespread as tool for the discovery of new therapeutic agents [17] and screened for cytotoxic activities against different cancer cell lines.

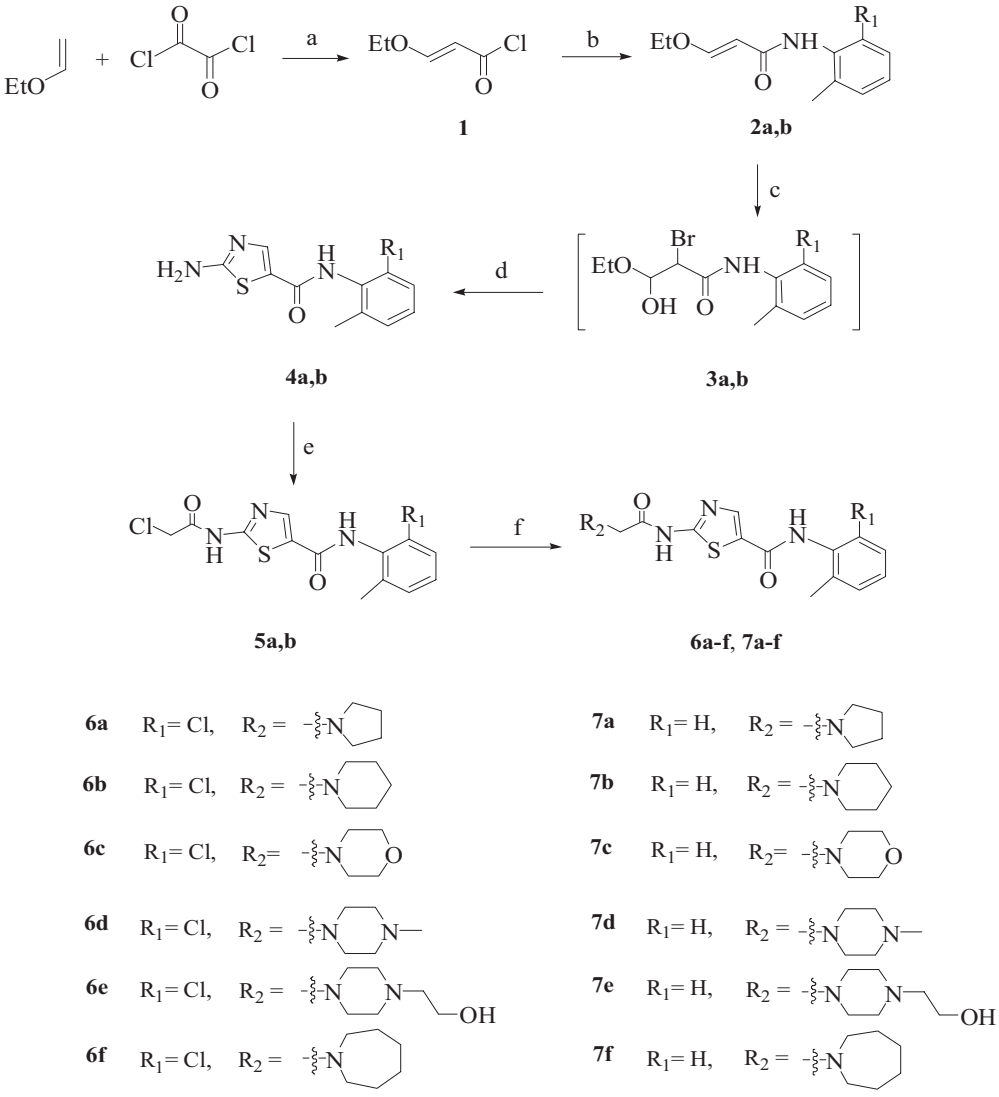
Result and Discussion

Chemistry

The synthetic route to the target compounds was similar to the synthesis of dasatnib and outlined in Scheme 1 [16, 18]. Thus, (E)-3-ethoxyacryloyl chloride **1** was prepared by reaction of ethoxyethene and oxalyl chloride with subsequent decarbonylation in 73% yield under vacuum distillation. Then, treatment of **1** with either 2-methylaniline or 2-chloro-6-methylaniline in THF using pyridine as base afforded the substituted (E)-*N*-phenyl-3-ethoxyacrylamides **2a**,**b** in 74–78% yields. Treatment of **2a**,**b** with *N*-bromosuccinimide (NBS) in a mixture of water and 1,4-dioxane gave the crude α-formyl-α-bromoacetate hemiacetals **3a**,**b**. Addition of thiourea *in situ* to crude **3a**,**b** and heating the resulting mixture at 80°C for 2 h afforded the 2-amino-thiazole-5-carboxylic acid phenylamides **4a**,**b** which were isolated in about 70% yield. With an efficient method available for the large scale synthesis of **4a**,**b**, we next turned our attention to the direct coupling of **4a**,**b** with chloroacetyl chloride. Using K_2CO_3 as base and slowly dropping the chloroacetyl chloride, the coupling reaction took place in THF at 0°C. The reaction was complete after refluxing for 3 h affording the key intermediates **5a**,**b** in a yield of about 80%. Finally, **5a**,**b** reacted with secondary amines to give the desired products **6a–f**, **7a–f** in 58–89% yields.

Biological activity

All target compounds were first evaluated for growth inhibitory activity against MCF-7 and MDA-MB 231 breast cancer and HT-29 colon cancer cell lines according to a previous method [19]. In addition to the target compounds **6a–f**, **7a–f**, gefitinib, dasatinib as well as the established cytostatic agent 5-fluorouracil (5-FU) were used as additional references. IC_{50} values for the reference compounds were calculated (OriginPro 8) and are presented in Table 1. Gefitinib was as active as 5-FU at all cell lines, while dasatinib reduced the cell growth more effective with $IC_{50} < 1$ μM. Out of the new compounds only **6d** and **7a** were able to reduce the cell growth (all other compounds possessed $IC_{50} > 40$ μM). Both were active at HT-29 cells ($IC_{50} = 21.6$ and 21.7 μM, respectively) while at the MCF-7 cell line only **6d** showed an $IC_{50} < 40$ μM (20.2 μM). The excellent growth inhibitory effects of dasatinib at the cell lines used agreed with its actual clinical development for the treatment of solid tumors. It is very likely that for this indication a pyrimidin-4-ylamine core is necessary. Exchange by an acetylamide drastically reduced the activity (compare dasatinib with **6e**). Structural modifications cannot increase the growth inhibitory efficacy. For the investigations on leukemia cells we selected compounds with residues at the acetylamide very similar to that of dasatinib (-(2-hydroxyethyl)piperazin-1-yl), gefitinib (-morpholino) and imatinib (4-methylpiperazin-1-yl). Therefore, **6c**, **6d**, **6e**, **7d**, and **7e** were tested against human K563 leukemia cells by using the MTT method [20]. Compounds **6d** (16.3 μM) and **6e** (17.8 μM) showed IC_{50} values comparable to dasatinib (11.08 μM) (Fig. 2). The fluorescence spectroscopic evaluation of K563 cells indicated a decreased amount of survival cells after incubation with compounds **6d**, **6e**, and dasatinib at



Reagents and conditions: (a) 0°C; (b) substituted aniline, pyridine, THF, 0–5°C; (c) *N*-bromosuccinimide, 1,4-dioxane, water, –10–0°C; (d) H_2NCSNH_2, 80°C; (e) K_2CO_3 chloroacetyl chloride THF, 0°C; (f) K_2CO_3, KI, secondary amine, THF, 60°C

Scheme 1. Synthetic route of compounds **6a–f**, **7a–f**.

Table 1. Antiproliferative effects against MCF-7 and MDA-MB 231 human breast cancer and HT-29 colon cancer cell lines.

Compound	Cytotoxicity IC_{50}, [μM][a,b]		
	MDA-MB 231	MCF-7	HT-29
6d	>40	20.2 ± 4.7	21.6 ± 0.6
7a	>40	>40	21.7 ± 0.6
Gefitinib	7.3 ± 0.1	2.3 ± 0.7	12.1 ± 0.1
Dasatinib	<1	<1	<1
5-FU	9.6 ± 0.3	4.7 ± 0.4	7.3 ± 1.0

[a] The IC_{50} values represent the concentration which results in a 50% decrease in cell growth after 72 h incubation. [b] Values are the means of at least 3 experiments.

10 μM for 72 h (Fig. 3). Interestingly, the methylphenyl similarities **7d** and **7e** showed less activity with IC_{50} values beyond 40 μM. The morpholino derivative **6c** exhibited moderate inhibition activities with an IC_{50} value of 27.2 μM. These results indicated that compounds **6d** and **6e** possess high selectivity for leukemia cells and cytotoxic properties comparable to dasatinib.

Conclusion

A series of novel 2-amino-thiazole-5-carboxylic acid phenylamide derivatives were synthesized and their biological

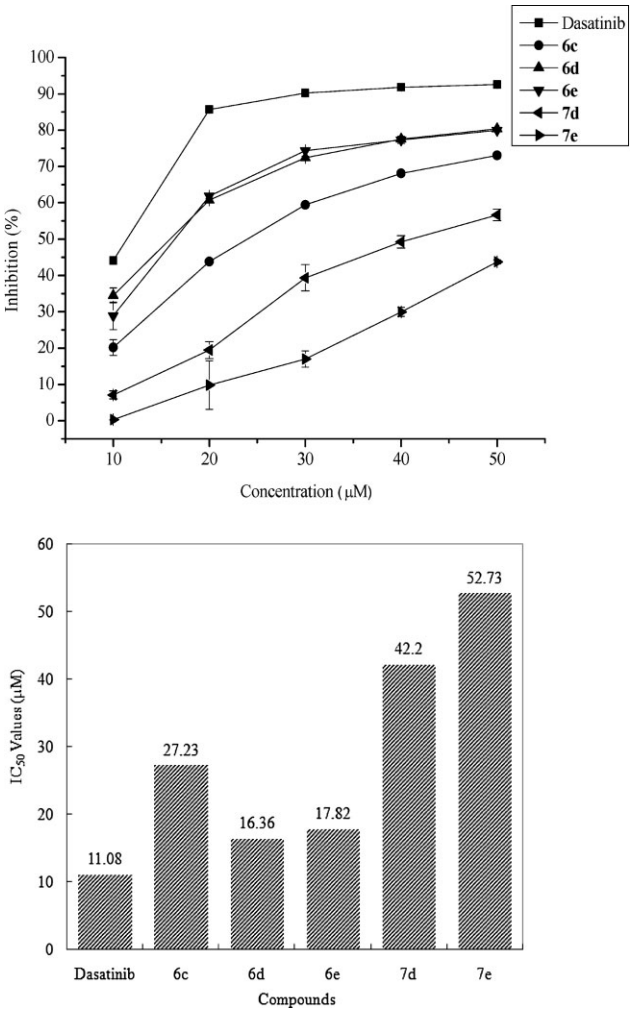


Figure 2. Dose dependent antiproliferative effects and IC_{50} values of compounds and dasatinib on human K563 leukemia cells[a, b, c] [a] The IC_{50} values represent the concentration which results in a 50% decrease in cell growth after 72 h incubation. [b] Values are the means of at least 3 experiments. [c] In some cases the error bars are hidden behind the symbols.

activities were evaluated. *N*-(2-Chloro-6-methylphenyl)-2-(2-(4-methylpiperazin-1-yl)acetamido)thiazole-5-carboxamide (**6d**) exhibited good antiproliferative effects on human K563 leukemia cells. The results of this study documented that the pyrimidin-4-ylamino core seems to be responsible for the high activity of dasatinib against solid tumors. Exchange by an acetamide scaffold terminated nearly completely the effects at MCF-7, MDA-MB 231, and HT-29 cell lines but prevent the activity against leukemia cells (K565). In the next step of our investigations we will focus our attention on the mode of action. Investigations to get insight into the enzyme inhibitory properties as well as an enlarged structure activity relationship are in progress and will give us the opportunity to explain the tumor selectivity more precisely.

Experimental

Chemistry

General

All reagents were purchased from Shanghai Chemical Reagent Company. Dasatinib and gefitinib were synthesized according to previous methods [16, 18, 21]. Column chromatography: Silica gel 60 (200–300 mesh). Thin-layer chromatography: Silica gel 60 F254 plates (250 mm; Qingdao Ocean Chemical Company, China). Melting point: Capillary tube; uncorrected. IR spectra: Shimadzu FTIR-8400S spectrophotometer. NMR spectra: Bruker ACF-300 Q apparatus at 300 MHz for ^{1}H-NMR and Bruker ADX 400 spectrometer at 100 MHz for ^{13}C-NMR (internal standard, TMS). Mass spectrometry: Hewlett–Packard 1100 LC/MSD spectrometer; in *m*/*z*. Elemental analyses: CHN-O-Rapid instrument.

*(E)-3-Ethoxyacryloyl chloride **1** [22]*

Ethoxyethene (30 mL, 313 mmol) was slowly added to oxalyl chloride (36.8 mL, 427 mmol) at 0°C. The reaction mixture was maintained for 2 h at 0°C and was then allowed to warm to room temperature for 12 h. Excess of oxalyl chloride was distilled off, and the residue was heated at 120°C for 30 min and then purified by vacuum distillation, affording **1** (30.7 g, 73.0%) as a colorless oil. Bp: 77–79°C, 12 Torr.

*(E)-N-(2-Chloro-6-methylphenyl)-3-ethoxyacrylamide **2a***

To a cold stirring solution of 2-chloro-6-methylaniline (11.9 g, 840 mmol) and pyridine (10.0 g, 1260 mmol) in THF (60 mL), **1** (16.8 g, 1260 mmol) was added slowly keeping the temperature at 0–5°C. The mixture was then warmed and stirred 3 h at room temperature. Hydrochloric acid (1 N) was added at 0–10°C to adjust a pH of 5. The mixture was diluted with water (62 mL) and the resulting solution was concentrated under vacuum to the thick slurry. The slurry was diluted with toluene (55.0 mL) and stirred for 15 min at 20–22°C then 1 h at 0°C. The solid was collected by vacuum filtration, washed with water (2 × 20 mL) and dried to give **2a** (15.76 g, 78.3% yield). Mp: 98–103°C; ^{1}H-NMR (d_6-DMSO) δ: 9.27 (1H, s, NH). 7.44 (1H, d, J = 12.3 Hz, –CH=), 7.27–7.36 (1H, d, J = 7.5 Hz, Ar-H), 7.10–7.26 (2H, m, Ar-H), 5.58 (1H, d, J = 12.3 Hz, –CH=), 3.93 (2H, q, J = 6.9 Hz, $-CH_2$), 2.15 (3H, s, $-CH_3$), 1.27 (3H, t, J = 6.9 Hz, $-CH_3$); ESI-MS: $[M + H]^+$ 240.

*(E)-N-(2-Methylphenyl)-3-ethoxyacrylamide **2b***

Compound **2b** was synthesized from 2-methylaniline (11.9 g, 840 mmol), pyridine (10.0 g, 1260 mmol) in THF (60 mL), and **1** (16.8 g, 1260 mmol) according to the procedure used to synthesize **2a** in 74.0% yield (12.7 g); Mp: 92–96°C; ESI-MS: $[M + H]^+$ 206.

*2-Amino-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide **4a***

To a mixture of **2a** (3.00 g, 12.50 mmol) in 1,4-dioxane (23 mL) and water (23 mL) *N*-bromosuccinimide (3.90 g, 21.90 mmol) was added at −10 to 0°C. The slurry was warmed and stirred at 20–22°C for 3 h. Thiourea (1.50 g, 19.70 mmol) was added and the mixture heated to 80°C. After 2 h, the resulting solution was

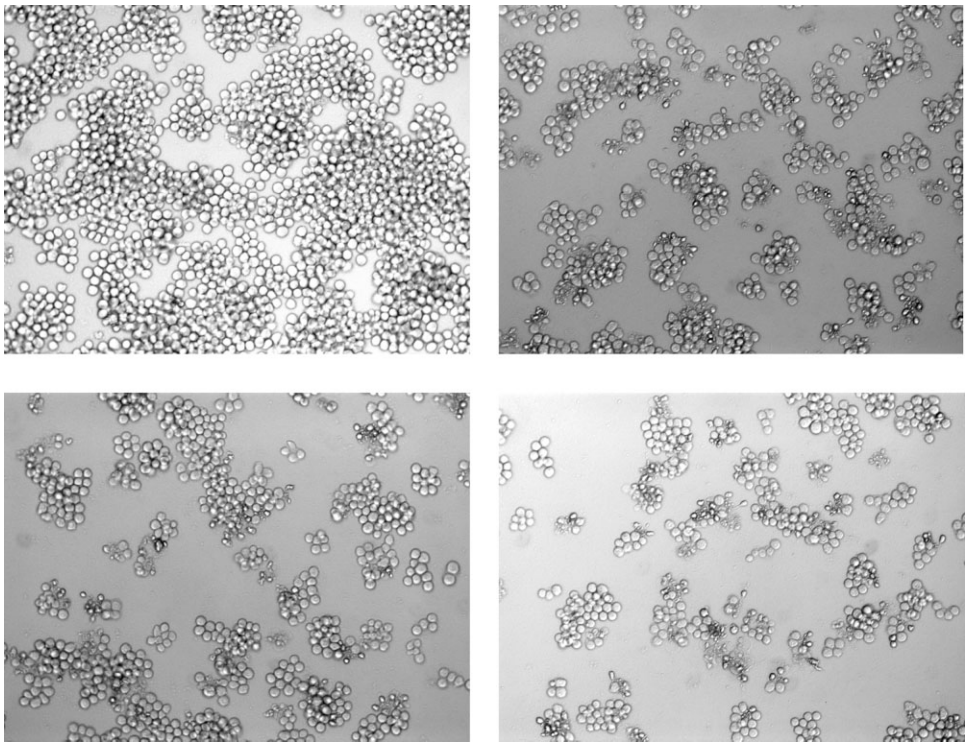


Figure 3. Survival cells after incubation compounds at 10 μM for 72 h. (Control: above left; dasatinib: right above; **6d**: below left; **6e**: below right.)

cooled to 20–22°C and conc. ammonium hydroxide (2.85 mL) was added dropwise. The resulting slurry was concentrated under vacuum to about half volume and cooled to 0–5°C. The solid was collected by vacuum filtration, washed with cold water (10 mL) and dried to give **4a** (2.4 g, 71.9% yield). Mp: 258–262°C; ^{1}H-NMR (d_6-DMSO) δ: 9.48 (1H, s, NH), 7.64 (2H, s, NH_2), 7.57–7.51 (1H, m, =N–CH=C–), 7.29–7.15 (3H, m, Ar-H), 2.20 (3H, s, CH_3); ESI-MS: $[M + H]^+$ 268.

*2-Amino-N-(2-methylphenyl)thiazole-5-carboxamide **4b***

Compound **4b** was synthesized from **2a** (2.56 g, 12.50 mmol) in 1,4-dioxane (23 mL) and water (23 mL), *N*-bromosuccinimide (3.90 g, 21.90 mmol), and then added thiourea (1.50 g, 19.70 mmol) and ammonium hydroxide (2.85 mL) according to the procedure used to synthesize **4a** in 70.4% yield (2.05 g); Mp: 226–230°C; ESI-MS: $[M + H]^+$ 234.

*N-(2-Chloro-6-methylphenyl)-2-(2-chloroacetamido)-thiazole-5-carboxamide **5a***

To a stirring solution of **4a** (0.93 g, 3.48 mmol) and K_2CO_3 (0.83 g, 6.00 mmol) in THF (10 mL) chloroacetyl chloride (0.68 g, 6.00 mmol) was added slowly with cooling to keep the temperature at 0°C. The reaction mixture was maintained for 5 min at 0°C and then warmed at 60°C for 3 h followed by cooling to room temperature. Water (50 mL) was added slowly and the mixture was stirred for 0.5 h at 0–5°C. The solid was collected by vacuum filtration, washed with water (15 mL) and dried to give **5a** (1.00 g, 80.6% yield). Mp: 210–212°C; ^{1}H-NMR (d_6-DMSO) δ: 12.78 (1H, s, NH), 9.93 (1H, s, NH), 8.30 (1H, s, =N–CH=C–), 7.32–7.15 (3H, m, Ar-H), 4.44 (2H, s, CH_2), 2.23 (3H, s, CH_3). ESI-MS: $[M + H]^+$ 345.

*N-(2-Chloro-6-methylphenyl)-2-(2-chloroacetamido)-thiazole-5-carboxamide **5b***

Compound **5b** was synthesized from **4b** (0.81 g, 3.48 mmol) and K_2CO_3 (0.83 g, 6.00 mmol) in THF (10 mL), chloroacetyl chloride (0.68 g, 6.00 mmol) according to the procedure used to synthesize **5a** in 88.1% yield (0.94 g); Mp: 200–204°C; ESI-MS: $[M + H]^+$ 310.

*General procedures for the synthesis of the target compounds **6a–f**, **7a–f***

K_2CO_3 (0.138 g, 0.10 mmol) and KI (0.016 g, 0.01mmol) were added to a mixture of **5a** or **5b** (0.60 mmol) and the respective secondary amine (0.69 mmol) in THF (10 mL). The mixture was then warmed at 60°C for 1 h and filtered. The organic layer was dried over Na_2SO_4 and the solvent was distilled off to give the crude product, which was then purified by flash column chromatography with mixture eluent of CH_2Cl_2 and CH_3OH to give the corresponding product.

*N-(2-Chloro-6-methylphenyl)-2-(2-(pyrrolidin-1-yl)-acetamido)thiazole-5-carboxamide **6a***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), (R_f = 0.4); Yield, 81.5%; Mp: 220–222°C; IR (KBr, cm^{-1}): 3231, 2963, 2809, 1699, 1639, 1521, 1288, 1181, 776, 639; ^{1}H-NMR ($CDCl_3$) δ:

8.10 (1H, s, =N–CH=C–), 7.42 (1H, s, –NH–CO–), 7.29 (1H, d, $J = 6.0$ Hz, Ar-H), 7.19 (2H, s, Ar-H), 3.44 (2H, s, $-CH_2-CO-$), 2.73 (4H, br, $-CH_2-N-$), 2.33 (3H, s, $-CH_3$), 1.88 (4H, br, $-CH_2-$); ^{13}C-NMR ($CDCl_3$) δ: 169.6 (C=O, acetamido), 162.2 (S–C=N, thiazole), 160.2 (C=O, carboxamide), 145.1, 142.9, 139.8, 136.4, 130.0, 129.4, 122.3 (–C=), 58.4 (CH_2, acetamido), 54.7 (N–CH_2, pyrrolidin), 24.1 (–CH_2, pyrrolidin), 19.11 (CH_3); ESI-MS: $[M + H]^+$ 379. Anal. calcd. for $C_{17}H_{19}ClN_4O_2S$: C, 53.89; H, 5.05; N, 14.79%; Found C, 53.47; H, 5.42; N, 14.48%.

N-(2-Chloro-6-methylphenyl)-2-(2-(piperidin-1-yl)-acetamido)thiazole-5-carboxamide ***6b***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), ($R_f = 0.4$); Yield, 88.7%; Mp: 246–248°C; IR (KBr, cm^{-1}): 3236, 2931, 2809, 1687, 1506, 1293–1220, 1178, 994, 815; ^{1}H-NMR ($CDCl_3$) δ: 8.01 (1H, s, =N–CH=C–), 7.23 (1H, d, $J = 3.9$ Hz, Ar-H), 7.13 (2H, s, Ar-H), 3.16 (2H, s, $-CH_2-CO-$), 2.49 (4H, s, $-CH_2-N-$), 2.27 (3H, s, $-CH_3$), 1.59 (4H, br, $-CH_2-$), 1.43 (2H, br, $-CH_2-$); ESI-MS: $[M + H]^+$ 393. Anal. calcd. for $C_{18}H_{21}ClN_4O_2S$: C, 55.02; H, 5.39; N, 14.26%; Found C, 54.66; H, 5.71; N, 14.11%.

N-(2-Chloro-6-methylphenyl)-2-(2-morpholinoacetamido)-thiazole-5-carboxamide ***6c***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.4$; Yield, 84.2%; Mp: 224–226°C; IR (KBr, cm^{-1}): 3467, 3254, 2855, 1697, 1641, 1520, 1445, 1292, 1186, 869; ^{1}H-NMR ($CDCl_3$) δ: 8.09 (1H, s, =N–CH=C–), 7.30 (1H, s, Ar-H), 7.24 (1H, s, Ar-H), 7.21 (1H, s, Ar-H), 3.83 (4H, m, $-CH_2-O-$), 3.36 (2H, s, $-CH_2-CO-$), 2.70 (4H, br, $-CH_2-N-$), 2.34 (3H, s, $-CH_3$); ^{13}C-NMR ($CDCl_3$) δ: 168.5 (C=O, acetamido), 162.2 (S–C=N, thiazole), 160.0 (C=O, carboxamide), 141.1, 138.2, 132.0, 131.4, 129.4, 128.2, 127.1 (–C=), 66.8 (O–CH_2, morpholino), 61.3 (–CH_2, acetamido), 53.9 (N–CH_2, morpholino), 19.0 (CH_3); ESI-MS: $[M + H]^+$ 395. Anal. calcd. for $C_{17}H_{19}ClN_4O_3S$: C, 51.71; H, 4.85; N, 14.19%; Found C, 51.59; H, 5.21; N, 14.42%.

N-(2-Chloro-6-methylphenyl)-2-(2-(4-methylpiperazin-1-yl)acetamido)thiazole-5-carboxamide ***6d***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.35$; Yield, 80.5%; Mp: 120–122°C; IR (KBr, cm^{-1}): 3476, 3417, 3233, 2937, 2814, 1633, 1500, 1414, 1289, 779; ^{1}H-NMR ($CDCl_3$) δ: 8.15 (1H, s, =N–CH=C–), 7.29 (2H, s, Ar-H), 7.20 (1H, s, Ar-H), 3.43 (2H, s, $-CH_2-CO-$), 2.69 (8H, br, $-CH_2-N-$), 2.40 (3H, s, $-CH_3$), 2.33 (3H, s, $-CH_3$); ESI-MS: $[M + H]^+$ 408, $[M - H]^-$ 406. Anal. calcd. for $C_{18}H_{22}ClN_5O_2S$: C, 53.00; H, 5.44; N, 17.17%; Found C, 53.23; H, 5.01; N, 17.55%.

N-(2-Chloro-6-methylphenyl)-2-(2-(4-(2-hydroxyethyl)-piperazin-1-yl)acetamido)thiazole-5-carboxamide ***6e***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.2$; Yield: 85.7%; Mp: 220–223°C; IR (KBr, cm^{-1}): 3435, 2922, 2750, 1658, 1575, 1533, 1400, 1302, 1196, 766; ^{1}H-NMR (CD_3OD) δ: 8.13 (1H, s, =N–CH=C–), 7.25 (1H, m, Ar-H), 7.14 (2H, d, $J = 6.6$ Hz, Ar-H), 3.61 (2H, t, $J = 10.8$ Hz, $-CH_2-O-$), 3.28 (2H, s, $-CH_2-CO-$), 3.21 (2H, t, $J = 10.8$ Hz, $-CH_2-N-$), 2.49–2.63 (8H, m, $-CH_2-N-$, piperazin), 2.21 (3H, s, $-CH_3$); ESI-MS: $[M]^+$ 437. Anal. calcd. for $C_{19}H_{24}ClN_5O_3S$: C, 52.11; H, 5.52; N, 15.99%; Found C, 51.89; H, 5.42; N, 16.32%.

2-(2-(Azepan-1-yl)acetamido)-N-(2-chloro-6-methylphenyl)-thiazole-5-carboxamide ***6f***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.4$; Yield: 65.4%; Mp: 208–210°C; IR (KBr, cm^{-1}): 3419, 3246, 2927, 2819, 1687, 1647, 1505, 1290, 1194, 755; ^{1}H-NMR ($CDCl_3$) δ: 8.09 (1H, s, =N–CH=C–), 7.31 (1H, m, Ar-H), 7.17 (2H, m, Ar-H), 3.43 (2H, s, $-CH_2-CO-$), 2.82 (4H, m, $-CH_2-N-$), 2.34 (3H, s, $-CH_3$), 1.69 (8H, br, $-CH_2-$); ESI-MS: $[M + H]^+$ 407. Anal. calcd. for $C_{19}H_{23}ClN_4O_2S \cdot 2\ H_2O$: C, 51.52; H, 6.14; N, 12.65%; Found C, 51.93; H, 6.05; N, 12.65%.

N-(2-Methylphenyl)-2-(2-(pyrrolidin-1-yl)acetamido)-thiazole-5-carboxamide ***7a***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.45$; Yield: 86.6%; Mp: 198–199°C; IR (KBr, cm^{-1}): 3469, 3265, 2799, 1689, 1630, 1507, 1476, 1289, 1155, 751; ^{1}H-NMR ($CDCl_3$) δ: 8.01 (1H, s, =N–CH=C–), 7.82 (1H, d, $J = 7.5$ Hz, Ar-H), 7.43 (1H, s, Ar-H), 7.23 (1H, d, $J_A = J_B = 7.5$ Hz, Ar-H), 7.15 (1H, d, $J_A = J_B = 7.5$ Hz, Ar-H), 3.43 (2H, s, $-CH_2-CO-$), 2.71 (4H, br, $-CH_2-N-$), 2.33 (3H, s, $-CH_3$), 1.87 (4H, br, $-CH_2-$); ^{13}C-NMR ($CDCl_3$) δ: 169.8 (C=O, acetamido), 160.0 (S–C=N, thiazole), 159.3 (C=O, carboxamide), 140.7, 135.1, 130.6, 127.6, 126.9, 125.8, 123.7 (–C=), 58.4 (CH_2, acetamido), 54.7 (N–CH_2, pyrrolidin), 24.1 (–CH_2, pyrrolidin), 17.8 (CH_3); ESI-MS: $[M + H]^+$ 345. Anal. calcd. for $C_{17}H_{20}N_4O_2S \cdot CH_2Cl_2$: C, 50.35; H, 5.16; N, 13.05%; Found C, 50.54; H, 5.02; N, 13.08%.

N-(2-Methylphenyl)-2-(2-(piperidin-1-yl)acetamido)-thiazole-5-carboxamide ***7b***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.45$; Yield: 75.1%; Mp: 179–180°C; IR (KBr, cm^{-1}): 3451, 3266, 2932, 2853, 1692, 1635, 1447, 1292, 1196, 751; ^{1}H-NMR ($CDCl_3$) δ: 8.03 (1H, s, =N–CH=C–), 7.82 (1H, d, $J = 7.5$ Hz, Ar-H), 7.43 (1H, s, Ar-H), 7.22 (1H, m, Ar-H), 7.15 (1H, d, $J = 7.5$ Hz, Ar-H), 3.25 (2H, s, $-CH_2-CO-$), 2.58 (4H, br, $-CH_2-N-$), 3.34 (3H, s, $-CH_3$), 1.66 (4H, m, $-CH_2-$), 1.50 (2H, br, $-CH_2-$); ^{13}C-NMR ($CDCl_3$) δ: 169.5 (C=O, acetamido), 160.0 (S–C=N, thiazole), 159.5 (C=O, carboxamide), 149.7, 135.1, 130.6, 127.7, 126.8, 125.9, 124.2, 124.1(–C=), 61.6 (CH_2, acetamido), 55.0 (N–CH_2, piperidin), 25.9 (–CH_2, piperidin), 23.4 (–CH_2, piperidin), 17.8 (CH_3); ESI-MS: $[M + H]^+$ 359. Anal. calcd. for $C_{18}H_{22}N_4O_2S$: C, 60.31; H, 6.19; N, 15.63%; Found C, 60.69; H, 6.07; N, 15.50%.

N-(2-Methylphenyl)-2-(2-morpholinoacetamido)thiazole-5-carboxamide ***7c***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.4$; Yield: 81.3%; Mp: 209–210°C; IR (KBr, cm^{-1}): 3275, 2925, 2856, 1694, 1492, 1291, 1199, 1022, 869, 747; ^{1}H-NMR ($CDCl_3$) δ: 10.43 (1H, br, –NH–CO–), 8.00 (1H, s, =N–CH=C–), 7.81 (1H, d, $J = 7.2$ Hz, Ar-H), 7.42 (1H, s, Ar-H), 7.35 (1H, m, Ar-H), 7.14 (1H, d, $J = 7.2$ Hz, Ar-H), 3.80 (4H, t, $J = 4.5$ Hz, $-CH_2-O-$), 3.32 (2H, s, $-CH_2-CO-$), 2.67 (4H, br, $-CH_2-N-$), 2.33 (3H, s, $-CH_3$); ^{13}C-NMR ($CDCl_3$) δ: 168.7 (C=O, acetamido), 159.5 (S–C=N, thiazole), 158.9 (C=O, carboxamide), 140.3, 134.9, 130.4, 127.7, 126.8, 125.7 (–C=), 66.5 (O–CH_2, morpholino), 61.1 (–CH_2, acetamido), 53.7 (N–CH_2, morpholino), 17.6 (CH_3); ESI-MS: $[M + H]^+$ 361, $[M + Na]^+$ 383, $[M - H]^-$ 359. Anal. calcd. for $C_{17}H_{20}N_4O_3S \cdot 0.6\ CH_2Cl_2$: C, 51.38; H, 5.19; N, 13.62%; Found C, 51.55; H, 5.35; N, 13.60%.

N-(2-Methylphenyl)-2-(2-(4-methylpiperazin-1-yl)-acetamido)thiazole-5-carboxamide ***7d***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.35$; Yield: 86.5%; Mp: 198–199°C; IR (KBr, cm^{-1}): 3489, 2941, 1645, 1529, 1485, 1296, 1209, 1161, 834, 749; ^{1}H-NMR ($CDCl_3$) δ: 8.01(1H, s, =N–CH=C–), 7.82 (1H, d, $J = 8.1$ Hz, ArH), 7.38 (1H, s, Ar-H), 7.22 (1H, s, Ar-H), 7.14 (1H, m, Ar-H), 3.28 (2H, s, $-CH_2-CO-$), 2.67 (4H, br, $-CH_2-N-$), 2.53 (4H, br, $-CH_2-N-$), 2.30 (3H, s, $-CH_3$), 2.28 (3H, s, $-CH_3$); ESI-MS: $[M + H]^+$ 374, $[M - H]^-$ 372. Anal. calcd. for $C_{18}H_{23}N_5O_2S$: C, 57.89; H, 6.21; N, 18.75%; Found C, 57.98; H, 6.03; N, 18.39%.

N-(2-Methylphenyl)-2-(2-(4-(2-hydroxyethyl)piperazin-1-yl)-acetamido)thiazole-5-carboxamide ***7e***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.3$; Yield: 85.7%; Mp: 220–223°C; IR (KBr, cm^{-1}): 3435, 2922, 2750, 1658, 1575, 1533, 1400, 1302, 1196, 766; ^{1}H-NMR (CD_3OD) δ: 8.21 (1H, s, =N–CH=C–), 7.29 (2H, d, $J = 3.9$ Hz, Ar-H), 7.23 (2H, m, Ar-H), 3.82 (2H, t, $J = 10.8$ Hz, $-CH_2-O-$), 3.47 (2H, s, $-CH_2-CO-$), 3.08 (4H, br, $-CH_2-N-$), 2.96 (2H, t, $J = 10.8$ Hz, $-CH_2-N-$), 2.85 (4H, m, $-CH_2-N-$), 2.31 (3H, s, $-CH_3$); ESI-MS: $[M + H]^+$ 404, $[M - H]^-$ 402. Anal. calcd. for $C_{19}H_{25}N_5O_3S$: C, 56.56; H, 6.25; N, 17.36%; Found C, 56.18; H, 6.44; N, 17.36%.

2-(2-(Azepan-1-yl)acetamido)-N-(2-methylphenyl)-thiazole-5-carboxamide ***7f***

Column chromatography: CH_2Cl_2/CH_3OH (20:1), $R_f = 0.35$; Yield: 57.7%; Mp: 120–122°C; IR (KBr, cm^{-1}): 3267, 2924, 2856, 1693, 1637, 1489, 1291, 1193, 1090, 749; ^{1}H-NMR ($CDCl_3$) δ: 8.01 (1H, s, =N–CH=C–), 7.82 (1H, d, $J = 7.5$ Hz, Ar-H), 7.41 (1H, s, Ar-H), 7.22 (1H, s, Ar-H), 7.13 (1H, t, $J = 14.4$ Hz, Ar-H), 3.41 (2H, s, $-CH_2-CO-$), 2.80 (4H, m, $-CH_2-N-$), 2.33 (3H, s, $-CH_3$), 1.69 (8H, br, $-CH_2-$); ^{13}C-NMR ($CDCl_3$) δ: 169.6 (C=O, acetamido), 163.2 (S–C=N, thiazole), 160.1 (C=O, carboxamide), 140.7, 135.2, 132.9, 130.6, 126.9, 122.4 (–C=), 58.9 (CH_2, acetamido), 56.5 (N–CH_2, azepan), 27.8 (–CH_2, azepan), 26.8 (–CH_2, azepan), 17.8 (CH_3); ESI-MS: $[M + H]^+$ 373. Anal. calcd. for $C_{19}H_{24}N_4O_2S \cdot CH_3OH$: C, 59.38; H, 6.98; N, 13.85%; Found C, 59.74; H, 6.56; N, 13.42%.

Biological Activity

Cell Culture

The human MCF-7, MDA-MB 231 breast cancer, and HT-29 colon cancer cell lines were obtained from the American Type Culture Collection. All cell lines were maintained as a monolayer culture in L-glutamine containing Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose (PAA Laboratories, Austria), supplemented with 5% fetal bovine serum (FBS; Biochrom, Germany) in a humidified atmosphere (5% CO_2) at 37°C.

Growth Inhibitory Effects

The experiments were performed according to established procedures with some modifications [19]. In 96-well plates 100 μL of a cell suspension in culture medium (7500 cells/mL (MCF-7 and MDA-MB 231) or 3000 cells/mL (HT-29)) were plated into each well and were incubated for three days under culture conditions. After the addition of various concentrations of the test compounds, cells were incubated for up to 144 h. Then the medium was removed, the cells were fixed with glutardialdehyde solution (1%) and stored under phosphate buffered saline (PBS) at 4°C. The cell biomass was determined by a crystal violet staining, followed by extracting of the bound dye with ethanol and a photometric measurement at 590 nm. Mean values were calculated and the effects of the compounds were expressed as %Treated/$Control_{corr}$ values according to the following equations:

$$T/C_{corr}[\%] = \frac{T - C_0}{C - C_0} \times 100$$

where C_0, control cells at the time of compound addition; C, control cells at the time of test end; T, probes/samples at the time of test end.

The IC_{50} value was determined as the concentration causing 50% inhibition of cell proliferation and calculated as mean of at least three independent experiments (OriginPro 8).

K-563 cells viability assay

K-563 cells were obtained from Shanghai Institute of Cell Biology (China). The cells were grown in RPMI 1640 (Life Technologies, Inc., USA) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Inc., USA) and cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

The experiments were performed according to established procedures with some modifications [20]. K-563 cells were cultured in medium till mid-log phase, then seeded in 96-well plate at a density of 1×10^4 cells per well in 100 μL medium. After 24 h of incubation, K-563 cells were treated with various concentrations of the test compounds for 72 h. After treatment, 10 μL of 5 mg/mL MTT were added and the cells were incubated for further 4 h at 37°C. The supernatant was discarded and 100 μL of DMSO was added to each well. The mixture was shaken on a mini shaker at room temperature for 10 min and the spectrophotometric absorbance was measured by Multiskan Spectrum Microplate Reader (Thermo) at 570 and 630 nm (absorbance 570 nm, reference 630 nm). Triplicate experiments were performed in a parallel manner for each concentration point and the results were presented as mean ± SD. The net A570 nm – A630 nm was taken as the index of cell viability. The net absorbance from the wells of cells cultured with 0.1% DMSO was taken as the 100% viability value. The percent inhibition of the treated cells was calculated by the following formula:

$$\%\,\text{Inhibition} = \frac{[(A_{570\,nm} - A_{630\,nm})_{control} - (A_{570\,nm} - A_{630\,nm})_{treated}]}{(A_{570\,nm} - A_{630\,nm})_{control}} \times 100\%.$$

The IC_{50} value was determined as the concentration causing 50% inhibition of cell proliferation and calculated as mean of at least three independent experiments (OriginPro 8).

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