Research Article

Design, Synthesis, and Anticancer Activities of Novel 2-Amino-4-phenylthiazole Scaffold Containing Amide Moieties

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Appropriately substituted 2-amino-4-phenylthiazole derivatives were designed and synthesized according to the structural characteristics of crizotinib. The obtained compounds were characterized using 1H NMR, 13C NMR, and HRMS. The target compounds 5a–o were evaluated for their in vitro antiproliferative activity against A549, HeLa, HT29, and Karpas299 human cancer cell lines. Based on results of biological studies, some of these compounds exhibited significant antiproliferative activity. Compound 5b possessed outstanding growth inhibitory effects on the four cell lines, especially for HT29 cell with IC50 value of 2.01 µM. Along with the biological assay data, a molecular docking study suggests that the target compounds were a potential inhibitor.

1. Introduction

The 2-aminothiazole scaffold has played an important role in medicinal chemistry and drug discovery research. The derivatives of 2-aminothiazole are recognized by a wide range of pharmacological activities, including anticancer [1–3], anticonvulsant [4, 5], antidiabetic [6, 7], antihypertensive [8], anti-inflammatory [9, 10], antiviral [11], antimicrobial [12, 13], and antileishmanial [14] activities and neuroprotective agents [8]. Numerous drugs and active compounds (Figure 1) containing 2-aminothiazole or 2-amino-4-phenylthiazole scaffold have been exhibiting a wide range of strong antitumor activities. Compound 1 showed low nanomolar in vitro antitumor activity and selectivity toward cancer cells over normal phenotype cells, which were identified as Hec1/Nek2 inhibitor [3]. Compound 2 (CYC-116, Cyclacel) was an effective Aurora A/B inhibitor and a slightly weaker inhibitor of VEGFR2 and was currently in Phase I clinical trials [15, 16]. Compound 3 (dasatinib, BMS-354825) was a novel, highly potent, and multitargeted inhibitor of Abl, Src, and c-Kit kinase, which was more potent than imatinib against unmutated BCR-ABL [17]. Developed by Pfizer Co., crizotinib was a potent and selective small-molecule dual inhibitor of the mesenchymal-epithelial transition factor (c-Met) kinase and anaplastic lymphoma kinase (ALK), possessing effective tumor growth inhibition and good pharmaceutical properties. In general, the crystal structure of crizotinib deposited in c-Met (PDB code 2WGJ, Figure 2(a)) revealed that a hydrophobic 2,6-dichloro-3-fluorobenzyl moiety formed π–π stacking interaction with Tyr1230 of c-Met kinase. In addition, this aryl fragment was extended into the U-shaped binding site, which adopted an ideal conformation for involvement in a hydrophobic effect with Met1211 and Gly1163. Furthermore, the 2-aminopyridine moiety formed two hydrogen-bonding interactions with the Met1160 and Pro1158 of the hinge region [18–20]. The 4-(1H-pyrazol-1-yl)piperidine moiety faced to the solvent accessible region, indicating that this hydrophobic fragment was relatively prone to bioisosteric replacement [19].
To design a novel scaffold of c-Met inhibitor (Figure 2(b)), a bioactive 2-amino-4-phenylthiazole scaffold was used as the skeleton. In the meanwhile, 2-aminopyridine moiety in crizotinib was replaced by the amide moiety, and the ability to efficiently form hydrogen bonds was retained. As the cocrystal structure of crizotinib has demonstrated that the halogen substitution at the phenyl ring is most favorable, we decided to modify group R1 of the terminal phenyl ring so as to investigate whether the cellular activity can be improved. Moreover, a flexible 2-morpholinoacetamido motif was introduced instead of the piperidinyl moiety in crizotinib. According to Figure 2(a), the possible additional hydrogen bond may be established in this way. In the proposed study, a novel 2-amino-4-phenylthiazole scaffold containing amide moieties was synthesized and evaluated for its anti-proliferative activities.

2. Results and Discussion

2.1. General Synthesis of the Target Compounds. The synthetic route of the target compounds in the current work is depicted in Scheme 1. 2-Bromo-1-(3-nitrophenyl)ethanone was warmed with thiourea in ethanol to obtain compound 1 in a good yield. The amido moiety of compound 1 was N-acylated with the excess chloroacetyl chloride in dichloromethane at room temperature to yield compound 2. In search of effective methods to prepare compound 2 by reacting compound 1 with 2-chloroacetic anhydride, we have found that 2-aminothiazole derivatives are quite inactive against a nucleophilic reaction. With abovementioned compounds, the reaction is not complete under reflux for 24 h. Compound 2 reacted with morpholine using Na2CO3 as base in refluxing anhydrous ethanol to afford compound 3. Compound 4 was prepared by treatment of compound 3 with reducing reagent tin (II) chloride dihydrate in refluxing ethanol. Eventually, intermediate 4 reacted with a series of acyl chloride to give the target compounds 5a-o based on the published procedure [21].

Reagents and conditions: (a) thiourea, ethanol, and reflux; (b) chloroacetyl chloride, CH2Cl2, Et3N, r.t.; (c) morpholine, ethanol, K2CO3, r.t.; (d) SnCl2·2H2O, ethanol, reflux; (e) substituted acyl chloride, CH2Cl2, Et3N, r.t.;
2.2. In Vitro Antiproliferative Activity. The obtained compounds were evaluated for antiproliferative activities with the application of the MTT method using human nonsmall cell lung cancer cell (A549), human cervical cancer cell (HeLa), human colon cancer cell (HT29), and human lymphoma cell line (Karpas299). Crizotinib was employed as positive control. The results are shown in Table 1. Some of these compounds displayed better growth inhibitory effects especially for HT29 cell.

In the structure of compounds 5a, 5d, 5e, 5g, and 5j, the metasubstitution on the benzene ring was modified to determine the contribution of this substitution to the antiproliferative activity. In vitro activity of these compounds demonstrated that the meta-halogen- (especially chloro-) substituted benzene ring was favorable for anticancer activity. Compounds 5b and 5d compared with 5c have shown that 3,4-diCl- or 3-Cl-substituted benzene ring (Figure 2(b)) are better than 2,4-diCl substitution on the corresponding benzene ring in terms of inhibitory activity. This result suggested that the ortho substituent on benzene ring 2 are not conducive to antiproliferative activity. Compound 5k, with a furan ring at R (Scheme 1) instead of the benzene ring 2, was only low activity toward four tumor cells, suggesting that the substituted phenyl groups were more beneficial than a furan ring to the inhibitory activity. Compound 5l-o displayed no inhibitory activity toward test cell lines probably due to the lack of the side chains containing aromatic amide groups.

2.3. In Vitro Enzymatic Assays. Finally, the inhibitory effect of compound 5b against c-Met kinase was further examined. According to Table 2, compound 5b exhibited a moderate c-Met enzymatic potency, suggesting that the inhibition of c-Met kinase may be a main mechanism for the antiproliferative activity of the selected compound 5b.

2.4. Western Blot Analysis. The results from cell proliferation activities showed that some target compounds, especially for 5b, strongly inhibited the growth of HT29 cells better than
that of the Karpass299 cells (ALK overexpression). To further elucidate the molecular mechanisms of target compounds, compounds 5b and 5e were selected for further studies. HT29 cell was treated with HGF, and the activation of c-Met was subsequently monitored by western blot analysis. As shown in Figure 3, crizotinib (5 μM) obviously inhibited the HGF-induced c-Met phosphorylation in HT29 cells. When HT29 cell was treated with 5 μM of compounds 5b and 5e for 24 h, it could be observed that the compound 5b obviously blocked the MET signaling pathway. Compound 5e with a relatively low activity has not marked inhibition of the c-Met phosphorylation. These data suggested that the target compounds, especially for compound 5b, might be adopted as a novel c-Met inhibitor.

2.5. Molecular Properties and Drug-likeness. Molecular properties and drug-likeness model score (a combined effect of physicochemical properties, pharmacokinetics, and pharmacodynamics of a compound and is represented by a numerical value) were calculated online by MolSoft software (http://molsoft.com/mprop). Estimation of drug-likeness properties are presented in Table 3.

For most target compounds, the parameters such as molecular weight (MW), number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD) and MolLogP were all in the range of Lipinski’s “rule of five” [22]. The molecular weight of compound 5e and 5f was slightly beyond the range of acceptable limit. Therefore, it was possible that these target compounds might have acceptable physicochemical properties of oral candidate drugs.

Additionally, the number of potential hydrogen bond donors and acceptors in the ligand must be appropriate. The difference existing in HBA between the target compounds and crizotinib is presented in Table 3. An excessive number of hydrogen bond acceptors in the target compounds may lead to redundant affinities and intramolecular hydrogen bonds. Besides, these analyses were consistent with the observation that the biological activity of the target compounds was worse than crizotinib.

2.5. Molecular Docking. To elucidate the active compound to c-Met, we determined the c-Met kinase-binding domain (PDB code 2WGJ) in complex with the representative compound 5b. Figure 4 showed the superimposition of crizotinib and the docked compound 5b. The two compounds were embedded in the “U shape” pocket of c-Met. The thiazole ring of compound 5b formed a π–π interaction with Tyr 1230 of c-Met protein. The similar interaction between crizotinib and Tyr1230 has been described in Figure 2(a). Additionally, the compound 5b also formed two hydrogen bonds in the active site with Asp1164 and Met1160, respectively. However, the phenyl ring 2 (Figure 2(b)) of compound 5b faced the solvent accessible region, similar to the hydrophobic 4-[(1H-pyrazol-1-yl)piperidine moiety of crizotinib, which may explain the cause of slightly worse antiproliferative activity of the target compounds.

Table 3: Molecular properties and drug-likeness.

| Compound | MW  | HBA | HBD | MolLogP | MolPSA | Score |
|----------|-----|-----|-----|---------|--------|-------|
| 5a       | 436.16 | 6   | 2   | 3.36    | 67.89  | 0.66  |
| 5b       | 490.06 | 6   | 2   | 4.27    | 67.89  | 1.07  |
| 5c       | 490.06 | 6   | 2   | 4.27    | 67.89  | 1.16  |
| 5d       | 456.10 | 6   | 2   | 3.67    | 67.89  | 0.78  |
| 5e       | 500.05 | 6   | 2   | 3.81    | 67.89  | 0.57  |
| 5f       | 500.05 | 6   | 2   | 3.81    | 67.89  | 0.96  |
| 5g       | 440.13 | 6   | 2   | 3.23    | 67.89  | 1.12  |
| 5h       | 440.13 | 6   | 2   | 3.23    | 67.89  | 0.71  |
| 5i       | 490.13 | 6   | 2   | 4.20    | 67.89  | 0.61  |
| 5j       | 490.13 | 6   | 2   | 4.20    | 67.89  | 0.66  |
| 5k       | 412.12 | 7   | 2   | 2.19    | 76.47  | 0.73  |
| 5l       | 390.14 | 7   | 2   | 1.12    | 76.38  | 0.70  |
| 5m       | 428.19 | 6   | 2   | 3.16    | 68.09  | 0.77  |
| 5n       | 360.13 | 6   | 2   | 1.52    | 68.07  | 0.60  |
| 5o       | 394.09 | 6   | 2   | 1.74    | 67.95  | 0.50  |
| Crizotinib | 449.12 | 4   | 3   | 3.95    | 62.56  | 0.36  |

MW: molecular weight (acceptable range ≤500); HBA: number of hydrogen bond acceptors (acceptable range ≤10); HBD: number of hydrogen bond donors (acceptable range ≤5); MolLogP: LogP value predicted by MolSoft (acceptable range ≤5); MolPSA: topological polar surface area, (acceptable range ≤140 Å²); score: drug-likeness model score. Compounds having zero or negative value should not be considered as drug-like candidate.

3. Materials and Methods

3.1. Chemicals, Reagents, and Instruments. Melting points were obtained in open capillaries using a WRS-1B melting point apparatus and were uncorrected. The 1H and 13C NMR spectra were recorded on a 400/54 Premium Shielded NMR Magnet System. Mass spectral data were collected from an Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System B.05.01 (B5125) in positive ion modes.

4-(3-nitrophenyl)thiazol-2-amine 1: The mixture of 2-bromo-1-(3-nitrophenyl)ethanone (24.41 g, 0.10 mol) and thiourea (8.37 g, 0.11 mol) in anhydrous ethanol (200 mL) was heated under reflux. After reaction was complete, the solvent was removed under vacuum, and saturated NaHCO3 was added to make the mixture basic (pH = 8–9). The precipitate was collected by filtration, washed with water, and dried to yield the product.

Yellow solid; yield, 96.6%; m.p.: 269.2–270.8°C; HRMS (m/z): calcd. for C19H14N3O2S (neutral M + H) 222.03372, found 222.03385.
2-chloro-N-(4-(3-nitrophenyl)thiazol-2-yl)acetamide 2: To a solution of compound 1 (2.22 g, 0.01 mol) and triethylamine (1.52 g, 0.015 mol) in anhydrous CH₂Cl₂ (40 mL), chloroacetyl chloride (1.35 g, 0.012 mol) in CH₂Cl₂ (20 mL) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature. When the reaction was finished, saturated Na₂CO₃ was added to the solution until the pH became weakly basic. The mixture was separated with a separatory funnel, and the organic layer was washed with cold water, dried, and evaporated under vacuum to yield the product.

Yellow solid; yield, 82.6%; m.p.: 218.1 °C–219.9 °C; HRMS (m/z): calcd. For C₁₅H₁₇N₄O₄S (neutral M + H) 349.09705, found 349.10016.

2-morpholino-N-(4-(3-nitrophenyl)thiazol-2-yl)acetamide 3: A mixture of compound 2 (2.97 g, 0.01 mol), morpholine (0.96 g, 0.011 mol), potassium iodide (0.17 g, 0.001 mol), and anhydrous potassium carbonate (4.14 g, 0.03 mol) in anhydrous ethanol (50 mL) was heated under reflux for 12 h. The solution (1 mol/L, 3 × 20 mL), and tin (II) chloride dihydrate (0.45 g, 0.002 mol) were added. The mixture was refluxed for 4 hours, and tin (II) chloride dihydrate (0.45 g, 0.002 mol) was added. The mixture was refluxed for 4 hours, and the solvent was evaporated under vacuum. The residue was crystallized from ethanol to give the product.

Yellow solid; yield, 89.6%; m.p.: 189.7 °C–191.2 °C; HRMS (m/z): calcd. For C₁₁H₉ClN₄O₃S (neutral M + H) 298.00531, found 298.00506.

General procedure for preparation of compounds 5: To a solution of compound 4 (50 mg, 0.123 mmol) and triethylamine (25 mg, 0.246 mmol) in CH₂Cl₂ (20 mL), the substituted acyl chloride (0.184 mmol) was added, and the mixture was stirred for 8 h at room temperature. Then, water (10 mL) was added and extracted with CH₂Cl₂. The combined organic layer was dried and evaporated under vacuum. The crude product was purified by column chromatography on silica gel to give a target compound.

3-methyl-N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)benzamide 5a: White solid; yield, 75.1%; m.p.: 158.4°C–160.1°C; ¹H NMR (400 MHz, DMSO-d₆) δ 12.07 (s, 1H), 10.46 (d, J = 3.5 Hz, 1H), 8.8 (s, 1H), 8.22 (t, J = 2.4 Hz, 1H), 7.96–7.92 (m, 1H), 7.82–7.79 (m, 1H), 7.67–7.53 (m, 4H), 3.62–3.53 (m, 4H), 3.28 (d, J = 3.5 Hz, 2H), 2.50 (d, J = 4.9 Hz, 4H), 2.38 (d, J = 3.5 Hz, 3H); ¹³C NMR (101 MHz, DMSO-d₆) δ 168.98, 166.07, 157.87, 149.24, 140.00, 138.14, 135.28, 130.65, 132.63, 129.38, 128.76, 128.55, 125.27, 121.55, 120.34, 118.41, 108.72, 66.57, 60.80, 53.39, 21.43; HRMS (m/z): calcd. For C₂₃H₂₅N₄O₃S (neutral M + H) 437.16474, found 437.16675.

3,4-dichloro-N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)benzamide 5b: White solid; yield, 75.1%; m.p.: 158.4°C–160.1°C; ¹H NMR (400 MHz, DMSO-d₆) δ 12.07 (s, 1H), 10.46 (d, J = 3.5 Hz, 1H), 8.38 (s, 1H), 8.22 (t, J = 2.4 Hz, 1H), 7.96–7.92 (m, 1H), 7.82–7.79 (m, 1H), 7.67–7.53 (m, 4H), 3.58 (q, J = 4.1 Hz, 4H), 3.29 (d, J = 3.2 Hz, 2H), 2.50 (d, J = 4.4 Hz, 4H); ¹³C NMR (101 MHz, DMSO-d₆) δ 168.98, 163.56, 157.56, 149.11, 139.55, 135.53, 135.14, 134.85, 131.73, 131.23, 130.04, 129.48, 128.52, 121.94, 120.41, 118.49, 108.85, 66.58, 60.80, 53.39; HRMS (m/z): calcd. For C₂₃H₂₃N₄O₃S (neutral M + H) 491.07114, found 491.07121.

2,4-dichloro-N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)benzamide 5c: White solid; yield, 70.1%; m.p.: 199.7°C–200.2°C; ¹H NMR (400 MHz, DMSO-d₆) δ 12.16 (s, 1H), 10.66 (s, 1H), 8.43 (s, 1H), 7.81 (d, J = 1.9 Hz, 1H), 7.61–7.58 (m, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.55–7.52 (m, 1H), 7.43 (t, J = 7.9 Hz, 1H), 3.63 (t, J = 4.6 Hz, 4H), 3.34 (s, 2H), 2.55 (t, J = 4.3 Hz, 4H); ¹³C NMR (101 MHz, DMSO-d₆) δ 168.98, 164.49, 157.92, 149.06, 139.53, 136.15, 135.28, 131.65, 130.77, 129.64, 129.60, 127.92, 121.90, 119.60, 117.59, 108.94, 66.57, 60.77, 53.37; HRMS (m/z): calcd. For C₂₂H₂₁Cl₂N₄O₃S (neutral M + H) 491.07114, found 491.07446.
3-chloro-N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)benzamide 5d: White solid; yield, 74.5%; m.p.: 164.3°C–166.9°C; 1H NMR (400 MHz, DMSO-d6) δ 12.07 (s, 1H), 10.42 (d, J = 3.4 Hz, 1H), 8.39 (s, 1H), 8.01 (s, 1H), 7.95–7.88 (m, 1H), 7.69–7.49 (m, 5H), 7.41–7.36 (m, 1H), 3.58 (q, J = 4.1 Hz, 4H), 3.29 (s, 2H), 2.50 (d, J = 4.6 Hz, 4H); 13C NMR (101 MHz, DMSO-d6) δ 168.98, 164.47, 157.89, 149.15, 139.69, 137.23, 135.12, 133.65, 131.89, 130.89, 129.45, 127.84, 126.95, 121.84, 120.41, 118.47, 108.82, 66.57, 60.79, 53.39; HRMS (m/z): calcd. For C22H22ClN4O3S (neutral M + H + K) 457.1101, found 457.1248.

3-bromo-N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)benzamide 5e: White solid; yield, 74.6%; m.p.: 223.3°C–224.4°C; 1H NMR (400 MHz, DMSO-d6) δ 12.13 (s, 1H), 10.47 (s, 1H), 8.45 (s, 1H), 8.07 (s, 1H), 8.01–7.89 (m, 2H), 7.68 (d, J = 9.5 Hz, 3H), 7.61 (s, 1H), 7.44 (d, J = 6.9 Hz, 1H), 3.63 (s, 4H), 3.34 (d, J = 4.1 Hz, 2H), 2.56 (s, 4H); 13C NMR (101 MHz, DMSO-d6) δ 168.98, 164.47, 157.89, 149.15, 139.68, 137.22, 135.11, 133.64, 132.99, 131.89, 131.05, 130.89, 129.44, 129.24, 123.88, 123.74, 126.95, 121.83, 120.41, 118.47, 108.81, 66.57, 60.79, 53.39; HRMS (m/z): calcd. For C22H22BrN4O3S (neutral M + H + Li) 501.05960, found 501.06355, 503.06461.

4-bromo-N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)benzamide 5f: White solid; yield, 76.7%; m.p.: 146.9°C–148.8°C; 1H NMR (400 MHz, DMSO-d6) δ 12.07 (s, 1H), 10.61 (s, 1H), 8.41 (s, 1H), 8.16 (d, J = 7.7 Hz, 2H), 7.90 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 3.1 Hz, 1H), 3.79 (t, J = 3.7 Hz, 1H), 1.38 (d, J = 4.6 Hz, 4H), 3.29 (d, J = 3.2 Hz, 2H), 2.50 (d, J = 4.8 Hz, 4H); 13C NMR (101 MHz, DMSO-d6) δ 169.05, 164.82, 165.13, 149.11, 139.19, 138.66, 131.95, 131.64, 129.46, 129.07, 125.90, 125.87, 125.79, 125.71, 124.52, 123.00, 121.92, 120.46, 118.52, 108.80, 66.58, 60.84, 53.39; HRMS (m/z): calcd. For C22H22F3N4O3S (neutral M + H + Li) 491.13647, found 491.15257.

N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)-4-fluoro-N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)benzamide 5g: White solid; yield, 88.2%; m.p.: 189.5°C–190.8°C; 1H NMR (400 MHz, DMSO-d6) δ 12.12 (s, 1H), 10.29 (s, 1H), 8.38 (t, J = 1.9 Hz, 1H), 7.97 (d, J = 1.6 Hz, 1H), 7.66 (t, J = 1.9 Hz, 1H), 7.64 (t, J = 1.8 Hz, 1H), 7.59 (s, 1H), 7.42 (d, J = 7.9 Hz, 1H), 7.38 (d, J = 3.3 Hz, 1H), 6.74–6.73 (m, 1H), 3.62 (t, J = 4.6 Hz, 4H), 3.33 (s, 2H), 2.54 (t, J = 4.6 Hz, 4H); 13C NMR (101 MHz, DMSO-d6) δ 164.37, 153.28, 152.05, 144.56, 143.27, 141.65, 134.73, 130.50, 124.83, 117.05, 115.77, 113.84, 110.61, 108.01, 104.19, 61.98, 56.20, 48.79; HRMS (m/z): calcd. For C20H21N4O3S (neutral M + H + Li) 413.12835, found 413.13295.

2-methoxy-N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)benzamide 5i: Yellow solid; yield, 85.2%; m.p.: 137.0°C–139.5°C; 1H NMR (400 MHz, DMSO-d6) δ 12.22 (s, 1H), 9.79 (s, 1H), 8.27 (s, 1H), 7.56 (d, J = 7.7 Hz, 1H), 7.52 (d, J = 2.7 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.35–7.30 (m, 1H), 4.14 (d, J = 2.9 Hz, 2H), 3.98 (d, J = 2.9 Hz, 2H), 3.35 (d, J = 1.9 Hz, 1H); 13C NMR (101 MHz, DMSO-d6) δ 169.04, 168.55, 157.75, 149.19, 139.21, 135.08, 129.42, 121.48, 119.78, 117.81, 108.80, 72.09, 70.86, 59.18, 59.09; HRMS (m/z): calcd. For C14H13N2O3S (neutral M + H + Li) 391.14400, found 391.14766.

N-(3-(2-(2-morpholinoacetamido)thiazol-4-yl)phenyl)cylohexanecarboxamide 5m: White solid; yield, 69.1%; m.p.: 165.5°C–166.8°C; 1H NMR (400 MHz, DMSO-d6) δ 20.41 (s, 1H), 9.91 (s, 1H), 8.31 (d, J = 1.8 Hz, 1H), 7.55 (s, 1H), 7.44–7.41 (m, 1H), 7.33 (t, J = 7.9 Hz, 1H), 3.62 (t, J = 4.6 Hz, 4H), 3.33 (s, 2H), 2.54 (d, J = 4.5 Hz, 3H), 2.39–2.31 (m, 1H), 1.87–1.73 (m, 5H), 1.67 (d, J = 11.5 Hz, 1H).
3.2. Primary Anticancer Assay. The cells were seeded in 96-well microplates at a density of $5 \times 10^3$ cells per well and incubated with 5% CO\textsubscript{2} at 37°C overnight. On the next day, the target compounds were added into the culture medium. The final concentration of DMSO in the medium was less than 0.5%. Triplicates of each concentration were used. After the cells were treated with compounds for 24 h, the supernatant was removed, and 5 mg/mL of a fresh prepared solution of MTT was added to each well and incubated with the cells at 37°C for another 4 h. The medium was removed, and 100 \muL of DMSO was added to each well to dissolve formazan. The absorbances at 490 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) were measured by the microplate reader. Cell growth inhibition rates formula is \((1 - \frac{A_{control} - AT}{AC})\times 100\%\) (AC, absorbance value of the blank control group; AT, absorbance value of the experimental group). The IC\textsubscript{50} was calculated using GraphPad Prism version 6.00 software from the nonlinear curve.

3.3. Kinase Assay. The c-Met activity was performed by enzyme-linked immunosorbsent assay (ELISA), and 20 \mug/mL poly (Glu, Tyr) 4:1 (Sigma) was precoated in 96-well plates. Then, 50 \muL of 10 mmol/L ATP solution diluted in kinase reaction buffer (50 mmol/L HEPES, pH 7.4, 50 mmol/L MgCl\textsubscript{2}, 0.5 mmol/L MnCl\textsubscript{2}, 0.2 mmol/L Na\textsubscript{3}VO\textsubscript{4}, and 1 mmol/L DTT) was added to each well. Various concentrations of compound 5b diluted in 1% DMSO (v/v) were added to each reaction well. Blank DMSO (1% v/v) was used as the negative control. The kinase reaction was initiated after the addition of purified tyrosine kinase proteins. After incubation for 60 min at 37°C, the plate was washed three times with phosphate buffered saline (PBS) containing 0.1% Tween 20 (T-PBS). Then antiphosphotyrosine (PY99) antibody (1: 500 diluted in 5 mg/mL BSA T-PBS) was then added. After 30 min incubation at 37°C, the plate was washed three times. Horseradish peroxidase-conjugated goat anti-mouse IgG (1: 2000 diluted in 5 mg/mL BSA T-PBS) was added. The plate was then incubated at 37°C for 30 min and washed three times. Finally, a solution containing 0.03% H\textsubscript{2}O\textsubscript{2} and 2 mg/mL o-phenylenediamine in 0.1 mol/L citrate buffer (pH 5.5) was added. The reaction was terminated by the addition of 2 mol/L H\textsubscript{2}SO\textsubscript{4}, and the plate was read using a multiwell spectrophotometer ((VERSAmax™, Molecular Devices) at 490 nm. The inhibition rate (%) was calculated using the following equation: \((1 - \frac{A_{control}}{A_{490/A490control}})\times 100\%\). IC\textsubscript{50} values were calculated from the inhibition curve.

3.4. Western Blot. Cells were harvested and washed twice with cold PBS, stimulated with HGF (40 ng/mL) for 15 min, then resuspended and lysed in RIPA buffer (150 mmol NaCl, 1% NP-40, 1% SDS, 1 mmol PMSF, 10 \mug/mL leupeptin, 1 mmol aprotinin, 50 mmol Tris-Cl, pH 7.4) at 4°C for 30 min. The protein concentration was determined by the BCA assay. Cell lystate was separated by 10% SDS-PAGE, and then transferred to PVDF membranes. The membranes were blocked with 5% nonfat milk and incubated with primary antibodies at 4°C overnight. Horseradish peroxidase-conjugated secondary antibodies were used against corresponding primary antibody at room temperature. Bound antibodies were revealed by the enhanced chemiluminescence (ECL) technique.

3.5. Molecular Docking. Molecular docking was performed using the CDOCKER protocol (the Discovery Studio 3.5 software package, Accelrys, Co. Ltd). The cocrystal structure of c-Met (PDB code 2WGI) was downloaded from the RCSB Protein Data Bank (http://www.rcsb.org). Protein preparation was carried out using the Prepare Protein protocol, and all bound water was deleted from the protein. The ligand preparation was carried out using the Prepare Ligand protocol. The docking parameters were set as default. The lowest binding energy was taken as the best-docked conformation of the ligand for the protein.

4. Conclusion

A series of new substituted 2-amino-4-phenylthiazole derivatives were designed and synthesized according to the structural characteristics of crizotinib and screened for biological activity. Some of these novel compounds have mild to good anticancer activity. Among these compounds, compound 5b possessed outstanding growth inhibitory effects, especially for HT29 cells with an IC\textsubscript{50} value of 2.01 \muM. Further experimental results support that the target compounds may be potential inhibitors of c-Met kinase.
**Data Availability**

The data used to support the findings of this study are included within the article. The $^1$H NMR and $^{13}$C NMR of synthesized compounds used to support the findings of this study are included within the supplementary information file.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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**Supplementary Materials**

$^1$H NMR and $^{13}$C NMR spectra for the prepared compounds are available in Supplementary Materials. (Supplementary Materials)

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