Supplementary Information

Design, synthesis and biological evaluation of 2-amino-N-(2-aminophenyl)thiazole-5-carboxamide derivatives as novel Bcr-Abl and histone deacetylase inhibitors dual inhibitors

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Table of content

General experimental ..............................................................2
Functional assay .................................................................2
Synthetic Procedure and analytic data ....................................3
General experimental

All chemicals were reagent grade and purchased from commercial suppliers. Melting points were determined in open capillaries on a WRS-1A digital melting point apparatus (Shenguang). $^1$H NMR spectra was recorded in CDCl$_3$ and DMSO–d$_6$ on a Bruker DRX–300 (300 MHz) using TMS as internal standard. The chemical shifts are reported in ppm ($\delta$) and coupling constants ($J$) values are given in Hertz (Hz). The IR spectra were performed on a FTIR-8400S (Shimadzu) in KBr pellets; the frequencies are expressed in cm$^{-1}$. Mass spectra were obtained from Agilent 1100 LC/MSD (Agilent) or Q-tof micro MS (Micromass) and the high-resolution (HR) electrospray ionization-time of flight (ESI-TOF)-MS was recorded on Agilent 6224 A (TOF) LC/MS. The purity of all tested compounds was established by HPLC to be >95.0%. HPLC analysis was performed at room temperature using an Agilent Eclipse XDB–C18 (250 mm $\times$ 4.6 mm) and 30% MeOH/H$_2$O as a mobile phase and plotted at 254 nm. All cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

Functional assay

Computational Methods

All computational work was performed in Discovery Studio (2.5). Docking was conducted using cdocker based on the cocrystal of Abl and dasatinib (PDB: 2GQG). Abl was used as receptor. The cavity occupied by Dasatinib was selected as the ligand binding site. The docking sphere radius value based on dasatinib is default. Water molecules outside the binding pocket were excluded. The energy minimization for compound 6a was performed by Powell's method for 1000 iterations using Tripos force field and with Gasteiger-Hückel charge. The other docking parameters were kept at default. The same approach is used to the cocrystal of HDAC1 homolog protein and SAHA (PDB: 1C3R).

HDAC enzymatic assay in vitro

All three full-length recombinant human HDACs (rhHDACs) 1, 3, 6 and 8 were expressed in insect High5 cells using a baculoviral expression system, and all His6-tagged and GST-fusion proteins was purified using Ni-NTA (QIAGEN). The deacetylase activity of HDAC1, 3 and 8 were assayed with a HDAC substrate (Ac-Lys-Tyr-Lys(e-acetyl)-AMC), and HDAC6 was assayed with another HDAC substrate (Boc-Lys(e-acetyl)-AMC).The total HDAC assay volume was 25 µL and all the assay components were diluted in HEPES buffer (25 mM HEPES, 137 mM NaCl,
2.7 mM KCl and 4.9 mM MgCl₂, pH = 8.0). The reaction was carried out in the 384-well plate (OptiPlateTM-384F, PerkinElmer). In brief, the HDAC assay mixture contained the substrate (5-50 μM, 5 μL), rhHDAC isoforms (20-200 nM) and inhibitor (1 μL). Positive controls contained all the above components except the inhibitor. The negative controls contained neither enzyme nor inhibitor. The HDAC6 assay components were incubated at room temperature for 3 h, and HDAC1, 3 and 8 were incubated for 24 h. The reaction was quenched with the addition of 25 μL Trypsin with the final concentration of 0.31%. After 30 min incubation at room temperature, the 384-well plate was read at wavelengths 355 nm (excitation) and 460 nm (emission) using Envision (PerkinElmer). Each experiment was done in triplicate.

*Bcr-Abl enzymatic assay in vitro*

The in vitro enzymatic assays of compounds 6a-n were evaluated by the ADP-Glo™ kinase assay (Promega, Madison, WI), with Dasatinib as the positive controls. General procedures are as the following: Kinases (4 ng/μL) were incubated with substrates (0.2 μg/μL), compounds (3×10⁻⁵ - 3×10⁻¹⁰ M) and ATP (25 μM) in a final buffer of Tris 40 mM, MgCl₂ 10 mM, BSA 0.1mg/mL, DTT 1 mM in 384-well plate with the total volume of 5 μL. The assay plate was incubated at 30ºC for 1 h. After the plate cooled for 5 min at room temperature, 5 μL of ADP-Glo reagent was added into each well to stop the reaction and consume the remaining ADP within 40 min. At the end, 10 μL of kinase detection reagent was added into the well and incubated for 30 min to produce a luminescence signal. The luminescence was read by VICTOR X multi label plate reader. The signal was correlated with the amount of ATP present in the reaction and was inversely correlated with the kinase activity.

*Cell culture and Cytotoxicity/proliferation assay*

The antiproliferative activities of compounds 6a-d, 6f, 6g and 6m were evaluated against K562, Hepg2 and DU145 cell lines by the standard MTT assay in vitro, with MS-275 and Dasatinib as the positive control. The cancer cell line was cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS). Approximate 2.5×10³ cells, suspended in RPMI 1640 medium, were plated into each well of a 96-well plate and incubated in 5% CO₂ at 37ºC for 24 h. The tested compounds at the indicated final concentrations were added to the culture medium and incubated for 48 h. Fresh MTT was added to each well at the terminal concentration of 0.5 mg/mL, and incubated with cells at 37ºC for 4 h. After the supernatant was discarded, 150 μL DMSO was added to each well, and the absorbance values were determined by a microplate reader (Bio-Rad, Hercules, CA, USA) at 490 nm.
Synthetic Procedure and analytic data

![Chemical structure of ethyl 2-((6-chloropyrimidin-4-yl)amino)thiazole-5-carboxylate (3)](image)

Ethyl 2-((6-chloropyrimidin-4-yl)amino)thiazole-5-carboxylate (3)
4,6-dimethylpyrimidine (3.75 g, 0.03 mol), ethyl 2-aminothiazole-5-carboxylate (4.25 g, 0.0247 mol) and Cs$_2$CO$_3$ (15 g, 0.046 mol) were stirred in 40 mL DMF at room temperature for 24 h. The mixture was filtered and the filtrate was poured into water, after which a solid was precipitated. Then the precipitant was filtered and purified by recrystallization in ethyl acetate to afford compound 3 as a yellow solid (4.81 g, 64%).

$^1$H-NMR (300 MHz, DMSO-$d_6$) δ 12.46 (s, 1H, -NH-), 8.83 (d, $J = 0.9$ Hz, 1H, $H$-pyrimidine), 8.15 (s, 1H, $H$-thiazole), 7.14 (s, 1H, $H$-pyrimidine), 4.29 (q, $J = 7.0$ Hz, 2H, -OC$_2$H$_2$CH$_3$), 1.30 (t, $J = 7.1$ Hz, 3H, -OCH$_2$CH$_3$).

General synthesis of ethyl 2-((6-aminopyrimidin-4-yl)amino)thiazole-5-carboxylate (4a-k)
Compound 3 (1.0 equiv), amine (1.1 equiv) and DIPEA (4.0 equiv) were stirred in 1-butanol at 120°C for 6 h. The reaction mixture was cooled to room temperature and then the solvent was evaporated under vacuum. The resulting residue was further purified by column chromatography to afford white solids 4a-k.

![Chemical structure of ethyl 2-((6-(4-ethylpiperazin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxylate (4a)](image)

Ethyl 2-((6-(4-ethylpiperazin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxylate (4a)
Compound 4a was synthesized as a white solid (0.88 g, 69%) by treatment of compound 3 (1 g, 3.5 mmol), 1-ethylpiperazine (0.45 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 12.46 (s, 1H, -NH-), 8.42 (s, 1H, $H$-pyrimidine), 8.25 (s, 1H, $H$-thiazole), 6.22 (s, 1H, $H$-pyrimidine), 4.26 (q, $J = 7$ Hz, 2H, -CH$_2$CH$_3$), 3.53 (t, $J = 4.6$ Hz, 4H, -CH$_2$-×2), 2.51-2.49 (m, 4H, -CH$_2$-×2), 2.43-2.32 (m, 2H, -CH$_2$-), 1.27 (t, $J = 7.14$ Hz, 3H, -CH$_2$CH$_3$), 0.89 (t, $J = 11.2$ Hz, 3H, -NCH$_2$CH$_3$). MS m/z: 363.4 [M+H]$^+$
Ethyl 2-((6-(4-(2-(benzyloxy)ethyl)piperazin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxylate (4b)

Compound 4b was synthesized as a white solid (0.96 g, 59%) by treatment of compound 3 (1 g, 3.5 mmol), 1-(2-(benzyloxy)ethyl)piperazine (0.86 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.55 (s, 1H, -NH), 8.41 (s, 1H, H-pyrimidine), 8.06 (s, 1H, H-thiazole), 7.38 (s, 5H, H-Ar), 6.23 (s, 1H, H-pyrimidine), 4.76 (s, 2H, -CH\(_2\)-), 4.26 (q, \(J = 7.0 \text{ Hz}\), 2H, -CH\(_2\)CH\(_3\)), 3.63-3.56 (m, 6H, -CH\(_2\)-×3), 2.51-2.49 (m, 6H, -CH\(_2\)-×3), 1.27 (t, \(J = 7.14 \text{ Hz}\), 3H, -CH\(_3\)). MS m/z: 469.5 [M+H]\(^+\)

Ethyl 2-((6-(benzylamino)pyrimidin-4-yl)amino)thiazole-5-carboxylate (4c)

Compound 4c was synthesized as a white solid (0.84 g, 67%) by treatment of compound 3 (1 g, 3.5 mmol), phenylmethanamine (0.42 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.49 (s, 1H, -NH), 8.41 (s, 1H, H-pyrimidine), 8.05 (s, 1H, H-thiazole), 7.35-7.31 (m, 5H, H-Ar), 6.23 (s, 1H, H-pyrimidine), 4.46 (s, 2H, -CH\(_2\)-), 4.21 (q, \(J = 7.0 \text{ Hz}\), 2H, -CH\(_2\)CH\(_3\)), 1.29 (t, \(J = 7.1 \text{ Hz}\), 3H, -CH\(_3\)). MS m/z: 356.4 [M+H]\(^+\)

Ethyl 2-((6-((pyridin-4-ylmethyl)amino)pyrimidin-4-yl)amino)thiazole-5-carboxylate (4d)

Compound 4d was synthesized as a white solid (0.76 g, 61%) by treatment of compound 3 (1 g, 3.5 mmol), pyridin-4-ylmethanamine (0.42 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.51 (s, 1H, -NH), 8.59-8.51 (m, 4H, H-pyridine), 8.44 (s, 1H, H-pyrimidine), 8.10 (s, 1H, H-thiazole), 6.22 (s, 1H, H-pyrimidine), 4.47 (s, 2H, -CH\(_2\)-), 4.24 (q, \(J = 7.0 \text{ Hz}\), 2H, -CH\(_2\)CH\(_3\)), 1.30 (t, \(J = 7.0 \text{ Hz}\), 3H, -CH\(_3\)). MS m/z: 357.2 [M+H]\(^+\)
Ethyl 2-((6-((pyridin-3-ylmethyl)amino)pyrimidin-4-yl)amino)thiazole-5-carboxylate (4e)

Compound 4e was synthesized as a white solid (0.78 g, 63%) by treatment of compound 3 (1 g, 3.5 mmol), pyridin-3-ylmethanamine (0.42 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.55 (s, 1H, -NH-), 8.59-8.48 (m, 2H, H-pyridine), 8.46 (s, 1H, H-pyrimidine), 8.10 (s, 1H, H-thiazole), 7.93-7.88 (m, 1H, H-pyridine), 7.49-7.44 (m, 1H, H-pyridine), 6.22 (s, 1H, H-pyrimidine), 4.49 (s, 2H, -CH\(_2\)-), 4.23 (q, J = 7.0 Hz, 2H, -CH\(_2\)CH\(_3\)), 1.30 (t, J = 7.0 Hz, 3H, -CH\(_3\)). MS m/z: 357.2 [M+H]\(^+\)

Ethyl 2-((6-morpholinopyrimidin-4-yl)amino)thiazole-5-carboxylate (4f)

Compound 4f was synthesized as a white solid (0.76 g, 65%) by treatment of compound 3 (1 g, 3.5 mmol), morpholine (0.34 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.55 (s, 1H, -NH-), 8.43 (s, 1H, H-pyrimidine), 8.10 (s, 1H, H-thiazole), 6.24 (s, 1H, H-pyrimidine), 4.23 (q, J = 7.0 Hz, 2H, -CH\(_2\)CH\(_3\)), 3.82-3.75 (m, 4H, -CH\(_2\)-×2), 3.65-3.58 (m, 4H, -CH\(_2\)-×2), 1.30 (t, J = 7.0 Hz, 3H, -CH\(_3\)). MS m/z: 336.5 [M+H]\(^+\)

Ethyl 2-((6-(4-(hydroxymethyl)piperidin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxylate (4g)

Compound 4g was synthesized as a white solid (0.69 g, 55%) by treatment of compound 3 (1 g, 3.5 mmol), piperidin-4-ylmethanol (0.45 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.51 (s, 1H, -NH-), 8.41 (s, 1H, H-pyrimidine), 8.12 (s, 1H, H-thiazole), 6.26 (s, 1H, H-pyrimidine), 4.24 (q, J = 7.0 Hz, 2H, -CH\(_2\)CH\(_3\)), 3.69-3.57 (m, 6H, -CH\(_2\)-×3), 1.75-1.71 (m, 5H, -CH\(_2\)-×2, -CH-piperidine), 1.30 (t, J = 7.0 Hz, 3H, -CH\(_3\)). MS m/z: 364.3 [M+H]\(^+\)
Ethyl 2-(((thiazol-2-ylmethyl)amino)pyrimidin-4-yl) amino) thiazole-5-carboxylate (4h)

Compound 4h was synthesized as a white solid (0.70 g, 58%) by treatment of compound 3 (1 g, 3.5 mmol), thiazol-2-ylmethanamine (0.44 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$ 12.54 (s, 1H, -NH-$-$), 8.44 (s, 1H, H-pyrimidine), 8.12 (s, 1H, H-thiazole), 7.88 (s, 1H, H-thiazole), 7.82 (d, $J = 7.7$ Hz, 1H, H-thiazole), 6.28 (s, 1H, H-pyrimidine), 5.02 (br, 1H), 4.32-4.24 (m, 4H), 1.30 (t, $J = 7.0$ Hz, 3H). MS $m/z$: 363.3 [M+H]$^+$

Ethyl 2-(((3-morpholinopropyl)amino)pyrimidin-4-yl) amino) thiazole-5-carboxylate (4i)

Compound 4i was synthesized as a white solid (0.75 g, 58%) by treatment of compound 3 (1 g, 3.5 mmol), 2-(piperazin-1-yl)ethan-1-ol (0.56 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$ 12.53 (s, 1H, -NH-$-$), 8.44 (s, 1H, H-pyrimidine), 8.12 (s, 1H, H-thiazole), 6.92 (br, 1H, -NH-$-$) 6.28 (s, 1H, H-pyrimidine), 4.32-4.24 (q, $J = 7.0$ Hz, 2H, -CH$_2$CH$_3$), 3.58 (t, $J = 6.7$ Hz, 2H, -CH$_2$-), 3.40-3.36 (m, 4H, -CH$_2$-×2), 2.35-2.33 (m, 6H, -CH$_2$-×3), 1.78-1.75 (m, 2H, -CH$_2$-). 1.32 (t, $J = 7.0$ Hz, 3H, -CH$_3$). MS $m/z$: 393.3 [M+H]$^+$

Ethyl 2-(((6-(4-(2-hydroxyethyl)piperazin-1-yl)pyrimidin-4-yl)amino) thiazole-5-carboxylate (4j)

Compound 4j was synthesized as a white solid (0.63 g, 50%) by treatment of compound 3 (1 g, 3.5 mmol), 3-morpholinopropan-1-amine (0.51 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$ 12.53 (s, 1H, -NH-$-$), 8.44 (s, 1H, H-pyrimidine), 8.12 (s, 1H, H-thiazole), 6.28 (s, 1H, H-pyrimidine), 4.24 (q, $J = 7.0$ Hz, 2H, -CH$_2$CH$_3$), 3.66-3.59 (m, 6H, -CH$_2$-×3), 2.77-2.69 (m, 6H, -CH$_2$-×3), 1.30 (t, $J = 7.0$ Hz, 3H, -CH$_3$). MS $m/z$: 379.3 [M+H]$^+$
Ethyl 2-((6-((4-fluorobenzyl)amino)pyrimidin-4-yl)amino)thiazole-5-carboxylate (4k)

Compound 4k was synthesized as a white solid (0.66 g, 54%) by treatment of compound 3 (1 g, 3.5 mmol), (4-fluorophenyl)methanamine (0.49 g, 3.9 mmol) and DIPEA (2.3 mL, 14 mmol) according to above-mentioned general procedure. $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$ 12.53 (s, 1H, -NH-), 8.44 (s, 1H, $H$-pyrimidine), 8.12 (s, 1H, $H$-thiazole), 7.38-7.33 (m, 4H, $H$-Ar), 6.28 (s, 1H, $H$-pyrimidine), 4.25-4.20 (m, 4H, -CH$_2$-$\times$2), 1.30 (t, $J = 7.0$ Hz, 3H, -CH$_3$). MS m/z: 374.3 [M+H]$^+$

Ethyl 2-((6-(phenylamino)pyrimidin-4-yl)amino)thiazole-5-carboxylate (4l)

Compound 3 (1g, 3.5 mmol), aniline (0.36 g, 3.9 mmol) were stirred in 40mL TFA at 80℃ for 6 h. The reaction mixture was cooled to room temperature and then poured into the water. The solution was extracted with ethyl acetate and the organic layer was concentrated under reduced pressure. The crude product was further purified by column chromatography to afford a white solid 4l (0.74 g, 62%). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$ 12.53 (s, 1H, -NH-), 8.44 (s, 1H, $H$-pyrimidine), 8.12 (s, 1H, $H$-thiazole), 7.28-7.24 (m, 5H, $H$-Ar), 6.28 (s, 1H, $H$-pyrimidine), 4.25 (q, $J = 7.0$ Hz, 2H, -CH$_2$CH$_3$),1.30 (t, $J = 7.0$ Hz, 3H, -CH$_3$). MS m/z: 342.3 [M+H]$^+$

General synthesis of 2-((6-aminopyrimidin-4-yl)amino)thiazole-5-carboxylic acid (5a-l)

Compounds 5a-l (2.8 mmol), NaOH (11 mmol) and 10 mL H$_2$O were stirred in 15 mL methanol at 60℃ for 4 h. The reaction mixture was cooled to 0℃ and acidified with dilute HCl (pH 5-6). The precipitant was filtered and washed by water to give the corresponding compound, which was used directly in the next step.

General synthesis of N-(2-aminophenyl)-2-((6-aminopyrimidin-4-yl)amino)thiazole-5-carboxamide (6a-l) and N-(2-amino-4-fluorophenyl)-2-((6-aminopyrimidin-4-yl)amino)thiazole-5-carboxamide (6m-n)
A mixture of 5a-l (3.0 mmol), 1,2-benzenediamine (3.3 mmol) or 4-fluorobenzene-1,2-diamine (3.3 mmol), HATU (3.3 mmol), Et$_3$N (12 mmol) in 30 mL DMF was stirred at room temperature for 12 h. Then it was poured into 100 mL water and extracted with ether acetate (100 mL×3) and the organic layer was washed with brine, dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude product was further purified by column chromatography to afford white solids 6a-n.

N-(2-aminophenyl)-2-((6-(4-ethylpiperazin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6a)

The title compound was synthesized as a white solid (0.78 g, 61%) by treatment of compound 5a (1.0 g, 3.0 mmol), 1,2-benzenediamine (0.69 g, 3.3 mmol), HATU (1.14 g, 3.3 mmol), Et$_3$N (1.21 g, 12 mmol). Mp: 238-240°C; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 11.45 (s, 1H, -NH-), 9.52 (s, 1H, -CONH), 8.40 (d, $J = 0.9$ Hz, 1H, H-pyrimidine), 8.18 (s, 1H, H-thiazole), 7.12 (d, $J = 6.6$ Hz, 1H, H-Ar), 6.99-6.94 (m, 1H, H-Ar), 6.79-6.76 (m, 1H, H-Ar), 6.31 (s, 1H, H-pyrimidine), 4.89 (s, 2H, -N$H_2$), 3.50 (t, $J = 4.6$ Hz, 4H, -CH$_2$-×2), 2.51-2.49 (m, 4H, -CH$_2$-×2), 1.03 (t, $J = 11.2$ Hz, 3H, -CH$_3$). IR (KBr cm$^{-1}$): 3434, 1640, 1534, 1532, 1482, 1454, 741, 595, 520; HRMS (ESI): m/z calcd for C$_{20}$H$_{24}$N$_8$OSNa$^+$ (M + Na$^+$): 447.1686, found: 447.1698.

N-(2-aminophenyl)-2-((6-(4-(2-(benzyloxy)ethyl)piperazin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6b)

The title compound was synthesized as a white solid (0.77 g, 48%) by treatment of compound 5b (1.3 g, 3.0 mmol), 1,2-benzenediamine (0.69 g, 3.3 mmol), HATU (1.14 g, 3.3 mmol), Et$_3$N (1.21 g, 12 mmol). Mp: 262-264°C; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 11.50 (s, 1H, -NH-), 9.52 (s, 1H, -CONH), 8.40 (d, $J = 0.9$ Hz, 1H, H-pyrimidine), 8.19 (s, 1H, H-thiazole), 7.36 (s, 5H, H-Ar), 7.12 (d, $J = 7.0$ Hz, 1H, H-Ar), 6.96-6.81 (m, 1H, H-Ar), 6.77 (d, $J = 7.9$ Hz, 1H, H-Ar), 6.59 (m, 1H, H-Ar), 6.22 (s, 1H, H-pyrimidine), 4.91 (s, 2H, -NH$_2$), 4.49 (s, 2H, -OCH$_2$Ph), 4.13-4.07 (m, 4H, -CH$_2$-×2), 3.60-3.52 (m, 6H, -CH$_2$-×3), 2.60-2.56 (m, 2H, -CH$_2$-); IR (KBr cm$^{-1}$): 3430, 1740,
1605, 1523, 1493, 13, 1316, 1288, 1102, 1090, 785, 643; HRMS (ESI): m/z calcd for C_{27}H_{30}N_{8}O_{2}Na^{+} (M + Na^{+}): 553.2105, found: 553.2136.

N-(2-aminophenyl)-2-((6-(benzylamino)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6c)
The title compound was synthesized as a white solid (0.76 g, 61%) by treatment of compound 5c (0.98 g, 3.0 mmol), 1,2-benzenediamine (0.69 g, 3.3 mmol), HATU (1.14 g, 3.3 mmol), Et_3N (1.21 g, 12 mmol). Mp: 263-265 °C; ^1H-NMR (300 MHz, DMSO-d_6) δ 11.46 (s, 1H, -NH-), 9.58 (s, 1H, -CONH-), 8.34 (s, 1H, H-pyrimidine), 8.18 (s, 1H, H-thiazole), 7.93-7.89 (m, 1H, H-Ar), 7.31 (m, 5H, H-Ar), 7.14 (d, J = 7.6 Hz, 1H, H-Ar), 6.99 (t, J = 7.6 Hz, 1H, H-Ar), 6.81 (d, J = 7.8 Hz, 1H, H-Ar), 6.64 (s, 1H, H-pyrimidine), 6.12 (s, 1H, -CH_2NH-), 5.85 (s, 2H, -NH_2), 4.49 (s, 2H, -CH_2-); IR (KBr cm⁻¹): 3756, 1840, 1615, 1490, 1450, 1300, 1258, 1152, 1150, 1096, 1030, 756, 423; HRMS (ESI): m/z calcd for C_{21}H_{19}N_{7}O_{2}SNa^{+} (M + Na^{+}): 440.1264, found: 440.1287.

N-(2-aminophenyl)-2-((6-((pyridin-4-ylmethyl)amino)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6d)
The title compound was synthesized as a white solid (0.61 g, 48%) by treatment of compound 5d (0.99 g, 3.0 mmol), 1,2-benzenediamine (0.69 g, 3.3 mmol), HATU (1.14 g, 3.3 mmol), Et_3N (1.21 g, 12 mmol). Mp: 270-272 °C; ^1H-NMR (300 MHz, DMSO-d_6) δ 11.50 (s, 1H, -NH-), 9.56 (s, 1H, -CONH-), 8.54 (s, 2H, H-pyridine), 8.33 (s, 1H, H-pyrimidine), 8.18 (s, 1H, H-thiazole), 7.36 (s, 2H, H-pyridine), 7.12 (d, J = 6.6 Hz, 1H, H-Ar), 6.92 (m, 1H, H-Ar), 6.72-6.70 (m, 1H, H-Ar), 6.65-6.60 (m, 1H, H-Ar), 6.54 (s, 1H, H-pyrimidine), 6.18 (s, 1H, -NHCH_2-), 4.56 (s, 2H, -CH_2-); IR (KBr cm⁻¹): 3335, 1648, 1625, 1500, 1495, 1422, 1345, 1100, 1050, 727, 613; HRMS (ESI): m/z calcd for C_{20}H_{18}N_{8}OSNa^{+} (M + Na^{+}): 441.1216, found: 441.1234.
N-(2-aminophenyl)-2-((6-((pyridin-3-ylmethyl)amino)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6e)

The title compound was synthesized as a white solid (0.57 g, 45%) by treatment of compound 5e (0.99 g, 3.0 mmol), 1,2-benzenediamine (0.69 g, 3.3 mmol), HATU (1.14g, 3.3 mmol), Et₃N (1.21g, 12mmol). Mp: 250-252°C; ¹H-NMR (300 MHz, DMSO-d₆) δ 11.50 (s, 1H, -NH-), 9.62 (s, 1H, -CONH-), 8.60 (s, 1H, H-pyridine), 8.52 (d, J = 0.9 Hz, 1H, H-pyrimidine), 8.34 (s, 1H, H-pyrimidine), 8.19 (s, 1H, H-thiazole), 7.98 (s, 1H, H-pyrimidine) 7.83 (d, J = 7.7 Hz, 1H, H-pyrimidine), 7.49-7.44 (m, 1H, H-Ar), 7.14 (s, J = 7.9 Hz, 1H, H-Ar), 7.00 (m, J = 6.7 Hz, 1H, H-Ar), 6.82 (d, J = 7.7 Hz, 1H, H-Ar), 6.68-6.63 (m, 1H, H-pyrimidine), 6.16 (br, 1H, -NHCH₂-), 4.55 (s, 2H, -CH₂-); IR (KBr cm⁻¹): 3501, 1841, 1615, 1529, 1450, 1411, 1345, 1320; HRMS (ESI): m/z calcd for C₂₀H₁₈N₈O₂SNa⁺ (M + Na⁺): 441.1216, found: 441.1245.

N-(2-aminophenyl)-2-((6-morpholinopyrimidin-4-yl)amino)thiazole-5-carboxamide (6f)

The title compound was synthesized as a white solid (0.57 g, 48%) by treatment of compound 5f (0.97 g, 3.0 mmol), 1,2-benzenediamine (0.68 g, 3.3 mmol), HATU (1.14g, 3.3 mmol), Et₃N (1.21g, 12mmol). Mp: 258-260°C; ¹H-NMR (300 MHz, DMSO-d₆) δ 11.50 (s, 1H, -NH-), 9.49 (s, 1H, -CONH-), 8.42 (s, 1H, H-pyrimidine), 8.15 (s, 1H, H-thiazole), 7.10 (m, 2H, H-Ar), 6.52 (s, 1H, H-Ar), 6.41-6.20 (m, 2H, H-pyrimidine and H-Ar), 4.94 (s, 2H, -NH₂), 3.61 (t, J = 4.6 Hz, 4H, -CH₂-×2), 3.82-3.74 (m, 4H, -CH₂-×2); IR (KBr cm⁻¹): 3512, 1841, 1615, 1529, 1450, 1411, 1345, 1320; HRMS (ESI): m/z calcd for C₁₈H₁₉N₇O₂SNa⁺ (M + Na⁺): 420.1213, found: 420.1235.

N-(2-aminophenyl)-2-((6-(4-(hydroxymethyl)piperidin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6g)
The title compound was synthesized as a white solid (0.65 g, 51%) by treatment of compound 5g (1.00 g, 3.0 mmol), 1,2-benzenediamine (0.68 g, 3.3 mmol), HATU (1.14 g, 3.3 mmol), Et3N (1.21g, 12mmol). Mp: 228-230°C; 1H-NMR (300 MHz, DMSO-d6) δ 11.40 (s, 1H, -NH), 9.59 (s, 1H, -CONH), 8.38 (s, 1H, H-pyrimidine), 8.19 (s, 1H, H-thiazole), 7.15 (d, J = 7.7 Hz, 1H, H-Ar), 6.99-6.97 (m, 1H, H-Ar), 6.82 (d, J = 7.8 Hz, 1H, H-Ar), 6.65 (t, J = 7.2 Hz, 1H, H-Ar), 6.24 (s, 1H, H-pyrimidine), 4.27-4.23 (m, 3H, -OH, -NH2), 3.27 (d, J = 5.3 Hz, 1H, -CH2-), 2.93-2.84 (m, 3H, H-piperidine), 1.75-1.71 (m, 4H, H-piperidine), 1.11-1.07 (m, 2H, H-piperidine). IR (KBr cm⁻¹): 3425, 1610, 1600, 1500, 1425, 1347, 1289, 1202, 1152, 1106, 1090, 740, 620; HRMS (ESI): m/z calcd for C20H23N7O2SNa⁺ (M + Na⁺): 448.1526, found: 448.1549.

N-(2-aminophenyl)-2-((6-((thiazol-2-ylmethyl)amino)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6h)
The title compound was synthesized as a white solid (0.56 g, 44%) by treatment of compound 5h (1.00 g, 3.0 mmol), 1,2-benzenediamine (0.68 g, 3.3 mmol), HATU (1.14 g, 3.3 mmol), Et3N (1.21g, 12mmol). Mp: 271-273°C; 1H-NMR (300 MHz, DMSO-d6) δ 11.47 (s, 1H, -NH), 9.60 (s, 1H, -CONH), 8.52 (d, J = 0.9 Hz, 1H, H-pyrimidine), 8.30 (s, 1H, H-thiazole), 8.11 (s, 1H, H-thiazole), 7.90 (s, 1H, H-thiazole), 7.85 (d, J = 7.7 Hz, 1H, H-Ar), 7.64 (m, 1H, H-Ar), 7.17 (m, 1H, H-Ar), 6.97 (m, 1H, H-Ar), 6.64-6.62 (m, 1H, -NHCH2-), 6.57 (s, 1H, H-pyrimidine), 6.16 (s, 2H, -NH2), 4.55 (s, 2H, -CH2-); IR (KBr cm⁻¹): 3416, 1845, 1625, 1620, 1452, 1178; HRMS (ESI): m/z calcd for C18H16N8O2S2Na⁺ (M + Na⁺): 447.0781, found: 447.0805.

N-(2-aminophenyl)-2-((6-((3-morpholinopropyl)amino)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6i)
The title compound was synthesized as a white solid (0.73 g, 53%) by treatment of compound 5i (1.10 g, 3.0 mmol), 1,2-benzenediamine (0.68 g, 3.3 mmol), HATU (1.14 g, 3.3 mmol), Et3N (1.21g, 12mmol). Mp: 275-277°C; 1H-NMR (300 MHz, DMSO-d6) δ 11.42 (s, 1H, -NH), 9.52 (s, 1H, -CONH), 8.34 (s, 1H, H-pyrimidine), 8.18 (s, 1H, H-thiazole), 7.45 (s, 1H, H-Ar), 7.12 (d, J = 7.8 Hz, 1H, H-Ar), 6.96-6.94
N-(2-aminophenyl)-2-((6-(4-(2-hydroxyethyl)piperazin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6j)

The title compound was synthesized as a white solid (0.64 g, 48%) by treatment of compound 5j (1.05 g, 3.0 mmol), 1,2-benzenediamine (0.68 g, 3.3 mmol). Mp: 252-255 °C; 1H-NMR (300 MHz, DMSO-d$_6$) δ 11.51 (s, 1H, -N$\text{H}_2$), 9.55 (s, 1H, -CON$\text{H}$), 8.42 (s, 1H, H-pyrimidine), 8.20 (s, 1H, H-thiazole), 7.12 (d, $J = 6.6$ Hz, 1H, H-Ar), 6.97-6.94 (m, 1H, H-Ar), 6.77 (d, $J = 8.1$ Hz, 1H, H-Ar), 6.61-6.59 (m, 1H, H-Ar), 6.26 (s, 1H, H-pyrimidine), 4.78 (s, 2H, -NH$_2$), 3.60 (s, 6H, -CH$_2$-$\times$3), 2.73-2.64 (m, 6H, -CH$_2$-$\times$3). IR (KBr cm$^{-1}$): 3613, 1740, 1648, 1500, 1325, 1316, 1250, 1200, 1147, 1116, 1020, 647, 523; HRMS (ESI): m/z calcd for C$_{20}$H$_{24}$N$_8$O$_2$SNa$^+$(M + Na$^+$): 463.1635, found: 463.1667.

N-(2-aminophenyl)-2-((6-((4-fluorobenzyl)amino)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6k)

The title compound was synthesized as a white solid (0.58 g, 45%) by treatment of compound 5k (1.04 g, 3.0 mmol), 1,2-benzenediamine (0.36 g, 3.3 mmol), HATU (1.14 g, 3.3 mmol), Et$_3$N (1.21g, 12mmol). Mp: 228-230 °C; 1H-NMR (300 MHz, DMSO-d$_6$) δ 11.43 (s, 1H, -NH$_2$), 9.46 (s, 1H, -CONH$-$), 8.32-8.16 (m, 3H, H-pyrimidine, H-thiazole and H-Ar), 7.88 (m, 1H, H-Ar), 7.76 (m, 1H, H-Ar), 7.34 (s, 4H, H-Ar), 7.02 (d, $J = 3.6$ Hz, 1H, H-Ar), 6.38-6.31 (m, 1H, H-pyrimidine), 5.25 (s, 2H, -NH$_2$), 4.02 (s, 2H, -CH$_2$-$\times$3); IR (KBr cm$^{-1}$): 3214, 1741, 1610, 1558, 1400, 1340, 1312, 1234, 1200, 1110, 1090, 659, 603; HRMS (ESI): m/z calcd for C$_{21}$H$_{18}$FN$_2$OSNa$^+$(M + Na$^+$): 458.1170, found: 458.1191.
N-(2-aminophenyl)-2-((6-(phenylamino)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6f)
The title compound was synthesized as a white solid (0.62 g, 51%) by treatment of compound 5l (0.94 g, 3.0 mmol), 4-fluorobenzene-1,2-diamine (0.42 g, 3.3 mmol), HATU (1.25 g, 3.3 mmol), Et3N (1.66 mL, 12mmol). Mp:244-247°C; 1H-NMR (300 MHz, DMSO-d6) δ 11.58 (s, 1H, -NH-), 9.60 (s, 1H, -CONH-), 9.47 (s, 1H, -NH-), 8.44 (s, 1H, H-pyrimidine), 8.18 (s, 1H, H-thiazole), 7.93-7.89 (m, 1H, H-Ar), 7.18 (m, 5H, H-Ar), 7.12 (d, J = 7.6 Hz, 1H, H-Ar), 6.91 (t, J = 7.6 Hz, 1H, H-Ar), 6.71 (d, J = 7.8 Hz, 1H, H-Ar), 5.85 (s, 2H, -NH2); IR (KBr cm⁻¹): 3465, 1840, 1615, 1490, 1050, 1331, 756, 410; HRMS (ESI): m/z calcd for C20H17N7OSNa⁺ (M + Na⁺): 426.1107, found: 426.1123.

N-(2-amino-4-fluorophenyl)-2-((6-(4-ethylpiperazin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6m)
The title compound was synthesized as a white solid (0.66 g, 50%) by treatment of compound 5a (1.00 g, 3.0 mmol), 4-fluorobenzene-1,2-diamine (0.42 g, 3.3 mmol), HATU (1.25 g, 3.3 mmol), Et3N (1.66 mL, 12mmol). Mp:245-247°C; 1H-NMR (300 MHz, DMSO-d6) δ 11.49 (s, 1H, -NH-), 9.47 (s, 1H, -CONH-), 8.41 (s, 1H, H-pyrimidine), 8.18 (s, 1H, H-thiazole), 7.08 (s, 1H, H-Ar), 6.55 (s, 1H, H-Ar), 6.35-6.23 (m, 2H, H-pyrimidine and H-Ar), 5.24 (s, 2H, -NH2), 3.50 (t, J = 4.6 Hz, 4H, -CH2×2), 2.56-2.45 (m, 4H, -CH2×2), 2.47-2.38 (m, 2H, -CH2), 1.03 (t, J = 11.2 Hz, 3H, -CH3); IR (KBr cm⁻¹): 3473, 1694, 1752, 1580, 1550, 1336, 1326, 1382, 1125, 1190, 742, 614; HRMS (ESI): m/z calcd for C20H23FN8OSNa⁺ (M + Na⁺): 465.1592, found: 465.1612.

N-(2-amino-4-fluorophenyl)-2-((6-((4-fluorobenzyl)amino)pyrimidin-4-yl)amino)thiazole-5-carboxamide (6n)

yl)amino)thiazole-5-carboxamide (6n)
The title compound was synthesized as a white solid (0.56 g, 41%) by treatment of compound 5k (1.04 g, 3.0 mmol), 4-fluorobenzene-1,2-diamine (0.42 g, 3.3 mmol), HATU (1.25 g, 3.3 mmol), Et$_3$N (1.66 mL, 12mmol). Mp: 255-257°C; $^1$H-NMR (300 MHz, DMSO-d$_6$) δ 11.44 (s, 1H, -NH-), 9.46 (s, 1H, -CONH-), 8.32 (s, 1H, H-pyrimidine), 8.24 (s, 1H, H-thiazole), 7.91-7.87 (m, 1H, H-Ar), 7.78-7.74 (m, 1H, H-Ar), 7.36-7.32 (m, 4H, H-Ar), 7.02 (d, J = 3.6 Hz, 1H, H-Ar), 6.38-6.31 (m, 1H, H-pyrimidine), 5.26 (br, 2H, -NH$_2$), 4.46 (s, 2H, -CH$_2$-); IR (KBr cm$^{-1}$): 3429, 1699, 1618, 1532, 1490, 1412, 1132, 1090, 720, 620; HRMS (ESI): m/z calcd for C$_{21}$H$_{17}$F$_2$N$_7$OSNa$^+$ (M + Na$^+$): 476.1076, found: 476.1098.