Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting information

Design, Synthesis and Biological Evaluation of New Molecules Inhibiting Epidermal Growth Factor Receptor Threonine$^{790} \rightarrow$ Methionine^{790} mutant

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General Information:

Reagents and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed using silica gel (200-300 mesh). All reactions were monitored by TLC, silica gel plates with fluorescence F_{254} were used and visualized with UV light. All products were characterized by their NMR, LC/MS. NMR spectras were recorded at 20 °C on a Bruker AC 400 MHz spectrometer working at 400 MHz and 125 MHz for ^{1}H and ^{13}C, respectively. The firstorder peak patterns were indicated as s (singlet), d (doublet), t (triplet), q (quartet) dd (double doublet) and br (broad). Complex non-first-order signals were indicated as m (multiplet). High resolution mass spectral (HRMS) analyses were recorded on AB SCIEX (USA) QSTAR Elite Hybrid LC/MS/MS mass spectrometer using electronspray ionization.

Cell Lines and Reagents:

H1975 (NSCLC, $EGFR^{L858R/T790M}$) and HCC827 (NSCLC, $EGFR^{DEL\ E746\text{-}A750}$) cells were obtained from ATCC. The cells were maintained at 37 ℃ in a 5% CO2 incubator in RPMI 1640 (Gibco, Invitrogen) containing 10% fetal bovine serum (Gibco, Invitrogen). HL-7702 (diploid human liver cell) cells were gifts from Prof. Duanqing Pei. The HL-7702 was cultured in RPMI 1640 containing 10% FBS. The EGFR gene of every cell line was sequenced before use. Gefitinib and WZ4002 were synthesized in our chemistry laboratory.

In Vitro Enzymatic Activity Assay:

Wild-type and different EGFR mutants (T790M, L858R, L861Q and L858R/T790M) and the Z'-Lyte Kinase Assay Kit were purchased from Invitrogen. Ten concentration gradients from 5.1×10^{-11} to 1.0×10^{-6} mol/L were set for all the tested compounds. The experiments were performed according to the instructions of the manufacturer.

Cell Proliferation and Growth Inhibition Assay:

Cell proliferation was assessed by MTS assay. The cells were exposed to treatment for 72 h, and the number of cells used per experiment for each cell line was adjusted

to obtain an absorbance of 1.3 to 2.2 at 490nm. Six concentrations (0.1 nM to 10 μM) were set for the compounds. At least six parallels of every concentration were used. All experiments were repeated at least four times. The data was calculated using GraphPad Prism version 4.0. The IC_{50} were fitted using a nonlinear regression model with a sigmoidal dose-response.

Antibodies and Western Blotting:

Cells were plated in 6-cm dishes. After 24-h growth, the cells were treated with the compounds under the indicated concentrations for 24 h. Cell lysates were collected with 1×lysis buffer (CST) and were briefly sonicated. Western blot analyses were conducted after separation by SDS/PAGE electrophoresis and transfer to PVDF membranes. Membranes were blocked in 5% bovine serum albumin/TBST and probed with the indicated antibodies, followed by a peroxidase-conjugated antimouse or rabbit secondary antibody. Blots were developed by enhanced chemiluminescence (Thermo). Anti-EGFR, anti-phospho specific EGFR (pY1068) and anti-actin antibodies were obtained from Cell Signaling Technology.

Kinase Profiling Results

LeadHunter DISCOVERY SERVICES

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GUA012-01-s-00001 Study Results

Table 1 - Matrix of Kds for GUA012-01-s-00001.

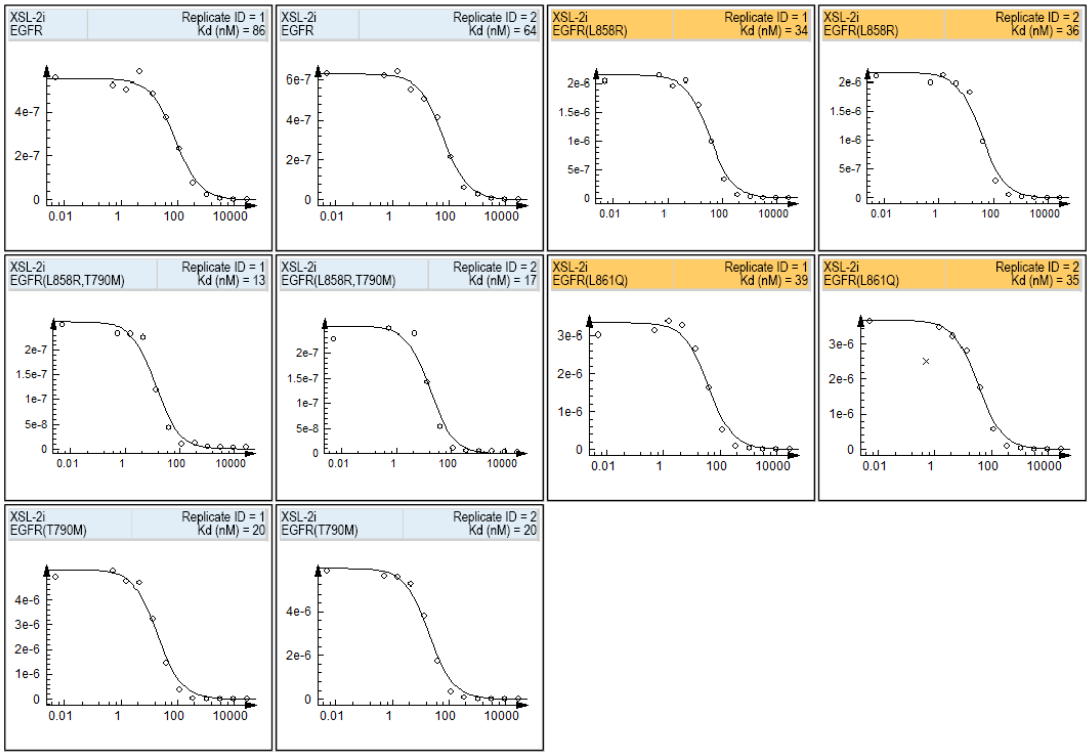
Target	XSL-2i
Gene Symbol	Kd (nM)
EGFR	75
EGFR(L858R)	35
EGFR(L858R,T790M)	15
EGFR(L861Q)	37
EGFR(T790M)	20

Kd Legend

x<100nM	100nM≤x<1uM	x≥1uM	No Binding	Not Requested

GUA012-01-s-00001 Curve Images

Table 2 - Curve Images for GUA012-01-s-00001. The amount of kinase measured by qPCR (Signal; y-axis) is plotted against the corresponding compound concentration in nM in log10 scale (x-axis). Data points marked with an "x" were not used for Kd determination.



General Method for the Synthesis of Compounds and Analytical Data:

1. ethyl 4-(3-(tert-butoxycarbonylamino) phenylamino)-2-(methylthio) pyrimidine-5-carboxylate (5)

To a solution of **3** (12.63 g, 54.27mmol) in DMF (200 mL) was added **4** (11.29 g, 54.27 mmol) and potassium carbonate (15 g, 108.54 mmol). The resulting solution was stirred at 80℃ overnight. After cooled to room temperature, the reaction mixture was added with ice-water (500 mL). The precipitate was filtered, and the filtered cake was rinsed with additional cool water and dried in vacuum oven to give the title compound (21.3 g, 97% yield), which was used without further purification. MS (ESI) m/z 405.0 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 8.77 (s, 1H), 7.92 (s, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 7.17 (dd, *J* = 0.8, 8.8 Hz, 1H), 6.49 (s, 1H), 4.38 (q, *J* = 7.2 Hz, 2H) 2.56 (s, 3H), 1.52 (s, 9H), 1.41 (t, *J* = 7.2 Hz, 3H)

2. 4-(3-(tert-butoxycarbonylamino)phenylamino)-2-(methylthio)pyrimidine-5-carboxylic acid (6)

To a solution of **5** (7 g, 17.33 mmol) in THF (200 mL) and H_2O (100 mL) was added $LiOH·H_2O$ (1.46 g, 34.66 mmol). The mixture was stirred at room temperature overnight. Most solvents were evaporated in vacuo. The residue was acidified with 1M hydrochloric acid till PH = 2. The solid was collected by suction filtration and washed with H_2O (100 mL) to afford a white powder (6.39 g, 98% yield), which was dried in vacuum oven without further purification. MS (ESI) m/z 377.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 13.76 (br, 1H), 10.56 (s, 1H), 9.45 (s, 1H), 8.72 (s, 1H), 7.90 (s, 1H), 7.38 (d, *J* = 9.2 Hz, 1H), 7.27 (t, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 8.0 Hz, 1H), 2.53 (s, 3H), 1.50 (s, 9H).

3. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)acetate (7a)

General Procedure for Synthesis of 7a-m.

To a solution of **6** (4.5 g, 11.97 mmol) in anhydrous CH_2Cl_2 (200 mL) under argon

was added carefully oxalyl chloride (1.5 mL, 17.96 mmol), followed by DMF (500 μL). The solution slowly turned to a light-yellow homogeneous solution over 4 h. The solution was concentrated in vacuo to give tert-butyl 3-(5- chlorocarbonyl)-2-methylthio)pyrimidin-4-lamino)phenylcarbamate as an yellow solid, which was used immediately in the next reaction without characterization. This yellow solid was dissolved in anhydrous CH_2Cl_2 (200 mL) under argon and cooled to 0 °C (ice bath). N-Methylglycine methyl ester hydrochloride (2.51 g, 17.96 mmol) in CH_2Cl_2 (20 mL) and N,N-diisopropylethylamine (8.3 mL, 47.88 mmol) were added with stirring. The reaction was allowed to warm to room temperature. After stirring for 20 h, TLC analysis indicated completion of the reaction. The mixture was poured into a solution of 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was separated, washed with 10% aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The resultant crude material was purified by column chromatography (SiO_2, CH_2Cl_2/Methanol stepwise elution, 500 :1 to 100:1) to give the title compound. (4.68g, 84.8% yield). MS (ESI) m/z 462.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.19 (s, 1H), 7.94 (s, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.24 (t, *J* = 8.0 Hz, 1H), 7.25 (dd, *J* = 1.2, 8.0 Hz, 1H), 6.48 (s, 1H), 4.25 (s, 2H), 3.83 (s, 3H), 3.16 (s, 3H), 2.56 (s, 3H), 1.51 (s, 9H).

4. methyl 1-(4-(3-(tert-butoxycarbonylamino) phenylamino)-2-(methylthio) pyrimidine-5-carbonyl)pyrrolidine-2-carboxylate (7b)

MS (ESI) m/z 488.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (s, 1H), 8.32 (s, 1H), 7.90 (s, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 7.23 (t, *J* = 8.0 Hz, 1H), 7.11 (d, *J* = 7.6 Hz, 1H), 6.47 (s, 1H), 4.69 (t, *J* = 6.8 Hz, 1H), 3.80 (s, 3H), 3.78-3.69 (m, 2H), 2.56 (s, 3H), 2.40-2.33 (m, 1H), 2.11-1.92 (m, 3H), 1.51 (s, 9H).

5. methyl 1-(4-(3-(tert-butoxycarbonylamino) phenylamino)-2-(methylthio) pyrimidine-5-carbonyl)piperidine-2-carboxylate (7c)

MS (ESI) m/z 502.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.16 (s, 1H), 7.97 (s, 1H), 7.39 (d, *J* = 7.6 Hz, 1H), 7.24 (t, *J* = 8.8 Hz, 1H), 6.99 (dd, *J* = 1.6, 8.4 Hz, 1H), 6.49 (s, 1H), 5.42 (br, 1H), 3.84 (s, 3H), 3.77-3.73 (m, 1H), 3.27 (br, 1H), 2.56 (s, 3H), 2.38 (d, *J* = 13.6 Hz, 1H), 1.85-1.67 (m, 3H), 1.51 (s, 9H), 1.43-1.34 (m,

2H)

6. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)propanoate (7d)

MS (ESI) m/z 476.0 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.17 (s, 1H), 7.93 (s, 1H), 7.33 (d, *J* = 8.8 Hz, 1H), 7.24 (t, *J* = 8.0 Hz, 1H), 6.98 (dd, *J* = 1.2, 8.0 Hz, 1H), 6.48 (s, 1H), 5.06 (br, 1H), 3.81 (s, 3H), 3.05 (s, 3H), 2.56 (s, 3H), 1.56 (d, *J* = 7.2 Hz, 3H), 1.51 (s, 9H).

7. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)-3-methylbutanoate (7e)

MS (ESI) m/z 504.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (br, 1H), 8.22 (s, 1H), 7.88 (s, 1H), 7.24-7.20 (m, 2H), 7.00-6.97 (m, 1H), 6.50 (s, 1H), 4.52 (br, 1H), 3.79 (s, 3H), 3.10 (s, 3H), 2.55 (s, 3H), 2.39-2.29 (m, 1H), 1.51 (s, 9H), 1.02 (s, 3H), 0.94 (s, 3H).

8. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)-4-methylpentanoate (7f)

MS (ESI) m/z 518.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (br, 1H), 8.18 (s, 1H), 7.90 (s, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.23 (t, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 8.0 Hz, 1H), 6.52 (s, 1H), 5.13 (br, 1H), 3.79 (s, 3H), 3.04 (s, 3H), 2.55 (s, 3H), 1.84 (t, *J* = 7.2 Hz, 2H), 1.75-1.64 (m, 1H), 1.51 (s, 9H), 1.00-0.94 (m, 6H).

9. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)-2-phenylacetate (7g)

MS (ESI) m/z 538.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.18 (s, 1H), 7.93 (s, 1H), 7.46-7.40 (m, 3H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.32-7.30 (m, 2H), 7.25 (t, *J* = 8.0 Hz, 1H), 7.02 (dd, *J* = 1.2, 8.0 Hz, 1H), 6.49 (s, 1H), 6.28 (s, 1H), 3.87 (s, 3H), 2.86 (s, 3H), 2.55 (s, 3H), 1.52 (s, 9H).

10. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-2-(methylthio)-N-phenylpyrimidine-5-carboxamido)acetate (7h)

MS (ESI) m/z 524.0 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 7.91 (s, 1H), 7.79 (s, 1H), 7.34-7.31 (m, 2H), 7.28-7.27 (m, 1H), 7.25-7.18 (m, 4H), 7.00 (dt, *J* = 1.6, 7.6 Hz, 1H), 6.48 (s, 1H), 4.58 (s, 2H), 3.82 (s, 3H), 2.47 (s, 3H), 1.52 (s, 9H).

11. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)-3-phenylpropanoate (7i)

MS (ESI) m/z 552.0 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (br. 1H), 7.84 (s, 1H), 7.75 (s, 1H), 7.25-7.19 (m, 7H), 6.99 (dd, *J* = 1.6, 8.8 Hz, 1H), 6.47 (s, 1H), 5.12 (br, 1H), 3.85 (s, 3H), 3.44 (br, 1H), 3.16 (br, 1H), 2.95 (s, 3H), 2.53 (s, 3H), 1.52 (s, 9H).

12. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)-3-(4-methoxyphenyl)propanoate (7j)

MS (ESI) m/z 582.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (br, 1H), 7.86 (s, 1H), 7.70 (s, 1H), 7.23-7.22 (m, 2H), 7.07-7.05 (m, 3H), 6.79 (s, 2H), 6.52 (s, 1H), 5.11 (br, 1H), 3.84 (s, 3H), 3.66 (br, 3H), 3.36 (br, 1H), 3.08 (br, 1H), 2.97 (s, 3H), 2.53 (s, 3H), 1.52 (s, 9H).

13. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)-3-(4-chlorophenyl)propanoate (7k)

MS (ESI) m/z 586.0 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (br, 1H), 7.86 (s, 1H), 7.82 (s, 1H), 7.26-7.22 (m, 4H), 7.13 (s, 2H), 7.01-6.98 (m, 1H), 6.50 (s, 1H), 5.07 (s, 1H), 3.84 (s, 3H), 3.41 (d, *J* = 10.8 Hz, 1H), 3.17-3.11 (m, 1H), 2.95 (s, 3H), 2.53 (s, 3H), 1.52 (s, 9H).

14. methyl 2-(4-(3-(tert-butoxycarbonylamino) phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)-3-(4-fluorophenyl)propanoate (7l)

MS (ESI) m/z 570.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (br, 1H), 7.83 (s, 2H), 7.23-7.22 (m, 2H), 7.16 (s, 2H), 7.00-6.97 (m, 3H), 6.47 (s, 1H), 5.07 (s, 1H), 3.84 (s, 3H), 3.43-3.40 (m, 1H), 3.17-3.11 (m, 1H), 2.96 (s, 3H), 2.53 (s, 3H), 1.52 (s, 9H).

15. methyl 2-(N-benzyl-4-(3-(tert-butoxycarbonylamino) phenylamino)-2-(methylthio)pyrimidine-5-carboxamido)acetate (7m)

MS (ESI) m/z 538.0 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 8.20 (s, 1H), 7.98 (s, 1H), 7.40-7.35 (m, 3H), 7.34-7.31 (m, 1H), 7.28-7.24 (m, 1H), 7.20 (d, *J* = 7.2 Hz, 2H), 7.02 (dd, *J* = 1.2, 8.4 Hz, 1H), 6.50 (s, 1H), 4.68 (s, 2H), 4.15(s, 2H), 3.82 (s, 3H), 2.55 (s, 3H), 1.52 (s, 9H).

16. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)acetic acid (8a)

General Procedure for Synthesis of 8a-m.

To a solution of **7a** (4.98 g, 10.78 mmol) in THF (100 mL) and H_2O (50 mL) was added $LiOH·H_2O$ (0.91 g, 21.56 mmol). The reaction solution was stirred at room temperature overnight. The solution was concentrated in vacuo and acidified with 1M hydrochloric acid till PH = 2. The solid was collected by suction filtration and washed with H_2O (50 mL) to afford a white powder (4.37 g, 90% yield), which was dried in vacuum oven without further purification. MS (ESI) m/z 448.0 $[M+H]^+$. ^1H NMR (400 MHz, DMSO) δ 13.19 (br, 1H), 9.38 (s, 1H), 8.92 (s, 1H), 8.17 (s, 1H), 7.95 (s, 1H), 7.22-7.19 (m, 2H), 7.08 (d, *J* = 6.8 Hz, 1H), 4.19 (s, 2H), 3.02 (s, 3H), 2.46 (s, 3H), 1.47 (s, 9H).

17. 1-(4-(3-(tert-butoxycarbonylamino)phenylamino)-2-(methylthio)pyrimidine-5-carbonyl)pyrrolidine-2-carboxylic acid (8b)

MS (ESI) m/z 472.1 $[M+H]^+$. ^1H NMR (400 MHz, DMSO) δ 12.95 (br 1H), 9.39 (s, 2H), 8.31 (s, 1H), 7.88 (s, 1H), 7.24 (d, *J* = 6.8 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 6.8 Hz, 1H), 4.50 (s, 1H), 3.61 (s, 2H), 2.50 (s, 3H), 2.33-2.28 (m, 1H), 1.89 (s, 3H), 1.47 (s, 9H).

18. 1-(4-(3-(tert-butoxycarbonylamino)phenylamino)-2-(methylthio)pyrimidine-5-carbonyl)piperidine-2-carboxylic acid (8c)

MS (ESI) m/z 488.1 $[M+H]^+$. ^1H NMR (400 MHz, DMSO) δ 13.40 (br, 1H), 9.36 (s, 1H), 8.80 (s, 1H), 8.10 (s, 1H), 7.93 (s, 1H), 7.22-7.18 (m, 2H), 7.10 (d, *J* = 6.8 Hz, 1H), 5.24 (br, 1H), 3.57 (s, 1H), 3.20-3.17 (m, 1H), 2.46 (s, 3H), 2.19 (s, 1H), 1.71-1.60 (m, 3H), 1.47 (s, 9H), 1.31-1.25 (m, 2H).

19. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)propanoic acid (8d)

MS (ESI) m/z 462.0 $[M+H]^+$. ^1H NMR (400 MHz, DMSO) δ 13.11 (br, 1H), 9.36 (s, 1H), 8.89 (s, 1H), 8.14 (s, 1H), 7.89 (s, 1H), 7.22-7.19 (m, 2H), 7.08 (d, *J* = 6.8 Hz, 1H), 4.89 (br, 1H), 2.93 (s, 3H), 2.46 (s, 3H), 1.47 (s, 9H), 1.43 (d, *J* = 6.8 Hz, 3H).

20. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-N-methyl-2-(methylthio)py

rimidine-5-carboxamido)-3-methylbutanoic acid (8e)

MS (ESI) m/z 490.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 13.12 (br, 1H), 9.37 (s, 1H), 8.88 (s, 1H), 8.17 (br, 1H), 7.85 (s, 1H), 7.20 (t, *J* = 7.6 Hz, 2H), 7.08 (d, *J* = 7.2 Hz, 1H), 4.56 (br, 1H), 3.00 (s, 3H), 2.45 (s, 3H), 2.25-2.18 (m, 1H), 1.47 (s, 9H), 0.86 (s, 6H).

21. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-N-methyl-2-(methylthio)py rimidine-5-carboxamido)-4-methylpentanoic acid (8f)

MS (ESI) m/z 504.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO, a mixture of rotamers) δ 13.32 (br, 1H), 9.37 (s, 1H), 9.02 (br, 1H), 8.20 (s) and 8.04 (s) (together 1H), 7.92 (s) and 7.85 (s) (together 1H), 7.22-7.15 (m, 2H), 7.09 (d, *J* = 7.2 Hz, 1H), 4.99 (s, 0.67H) and 4.29 (s, 0.33H), 2.92 (s, 3H), 2.46 (s, 3H), 1.81 (s, 1H), 1.75 (s, 1H), 1.63 (s, 1H), 1.47 (s, 9H), 0.93 (s) and 0.68 (s) (together 6H)

22. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-N-methyl-2-(methylthio)py rimidine-5-carboxamido)-2-phenylacetic acid (8g)

MS (ESI) m/z 524.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 13.49 (br, 1H), 9.38 (s, 1H), 9.01 (s, 1H), 8.18 (s, 1H), 7.94 (s, 1H), 7.46-7.39 (m, 5H), 7.24-7.19 (m, 2H), 7.10-7.07 (m, 1H), 5.99 (s, 1H), 2.70 (s, 3H), 2.47 (s, 3H), 1.48 (s, 9H).

23. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-2-(methylthio)-N-phenylpy rimidine-5-carboxamido)acetic acid (8h)

MS (ESI) m/z 510.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 13.27 (br, 1H), 9.36 (s, 1H), 9.19 (s, 1H), 7.96 (s,1H), 7.80 (s, 1H), 7.33-7.27 (m, 4H), 7.23-7.13 (m, 3H), 7.08 (d, *J* = 7.6 Hz, 1H), 4.56 (s, 2H), 2.36 (s, 3H), 1.48 (s, 9H).

24. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-N-methyl-2-(methylthio)py rimidine-5-carboxamido)-3-phenylpropanoic acid (8i)

MS (ESI) m/z 538.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO, a mixture of rotamers) δ 13.34 (br, 1H), 9.38 (s, 1H), 8.64 (s) and 8.19 (s) (together 1H), 7.87-7.53 (m, 2H), 7.30-7.07 (m, 8H), 5.11 (s, 0.59H) and 4.81 (s, 0.41H), 3.32-3.17 (m, 2H), 2.85 (s, 3H), 2.45 (s, 3H), 1.48 (s, 9H).

25. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-N-methyl-2-(methylthio)py rimidine-5-carboxamido)-3-(4-methoxyphenyl)propanoic acid (8j)

MS (ESI) m/z 568.1 $[M+H]^+$. ^1H NMR (400 MHz, DMSO, a mixture of rotamers) δ 13.42 (br, 1H), 9.37 (s, 1H), 8.62 (s) and 8.26 (s) (together 1H), 7.83 (s) and 7.66 (s) (together 2H), 7.22-7.18 (m, 2H), 7.16-7.07 (m, 3H), 6.84 (s, 1H), 6.69 (s, 1H), 5.07 (s) and 4.79 (s) (together 1H), 3.57 (s, 3H), 3.24 (s, 1H), 3.17 (s, 1H), 2.86 (s, 3H), 2.46 (s, 3H), 1.47 (s, 9H).

26. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)-3-(4-chlorophenyl)propanoic acid (8k)

MS (ESI) m/z 572.1 $[M+H]^+$. ^1H NMR (400 MHz, DMSO, a mixture of rotamers) δ13.39 (br, 1H), 9.37 (s, 1H), 8.64 (s) and 8.24 (s) (together 1H), 7.86 (s) and 7.73-7.67 (m) (together 2H), 7.35-7.06 (m, 7H), 5.09 (s) and 4.86 (s) (together 1H), 3.24 (s) and 3.08 (s) (together 2H), 2.86 (s, 3H), 2.46 (s, 3H), 1.48 (s, 9H).

27. 2-(4-(3-(tert-butoxycarbonylamino)phenylamino)-N-methyl-2-(methylthio)pyrimidine-5-carboxamido)-3-(4-fluorophenyl)propanoic acid (8l)

MS (ESI) m/z 556.2 $[M+H]^+$. ^1H NMR (400 MHz, DMSO, a mixture of rotamers) δ 13.44 (br, 1H), 9.38 (s, 1H), 8.67 (s) and 8.26 (s) (together 1H), 7.83 (s) and 7.63 (s) (together 2H), 7.34 (s, 1H), 7.22-7.06 (m, 5H), 6.96 (s, 1H), 5.08 (s) and 4.82 (s) (together 1H), 3.86 (s, 2H), 2.86 (s, 3H), 2.45 (s, 3H), 1.48 (s, 9H).

28. 2-(N-benzyl-4-(3-(tert-butoxycarbonylamino)phenylamino)-2-(methylthio)pyrimidine-5-carboxamido)acetic acid (8m)

MS (ESI) m/z 524.0 $[M+H]^+$. ^1H NMR (400 MHz, DMSO) δ 13.30 (br, 1H), 9.43 (s, 1H), 9.37 (s, 1H), 8.14 (s, 1H), 8.06 (s, 1H), 7.36-7.33 (m, 2H), 7.30-7.26 (m, 3H), 7.22-7.18 (m, 3H), 7.11 (s, 1H), 4.54 (s, 2H), 3.99 (s, 2H), 2.45 (s, 3H), 1.47 (s, 9H).

29. tert-butyl 3-(6-methyl-2-(methylthio) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9a)

General Procedure for Synthesis of 9a-m.

To a solution of **8a** (6.85 g, 15.3 mmol) in anhydrous CH_2Cl_2 (1000 mL) was added HATU (17.5 g, 45.9 mmol) at 0 °C (ice bath). N,N-diisopropylethylamine (8 mL, 45.9 mmol) was added dropwise. Then the solution was allowed to warm to room temperature and stirred for 24 h under argon atmosphere. The solution was poured on a solution of 10% $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was

separated, washed with 10% aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The resultant crude material was purified by column chromatography (SiO_2, Petroleum ether/CH_2Cl_2 stepwise elution, 1:1 to 1:3) to give the title compound. (5.25g, 80% yield). MS (ESI) m/z 430.1 $[M+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.46 (s, 1H), 7.33 (t, *J* = 8.4 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.65 (s, 1H), 4.12 (s, 2H), 3.31 (s. 3H), 2.13 (s, 3H), 1.49 (s, 9H).

30. tert-butyl 3-(2-(methylthio) -5,10-dioxo-8,9,9a,10-tetrahydro-5H-pyrimido [4,5-e]pyrrolo[1,2-a][1,4]diazepin-11(7H)-yl)phenylcarbamate (9b)

MS (ESI) m/z 456.1 $[M+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 7.45 (s, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 7.6 Hz, 1H), 6.55 (s, 1H), 4.30-4.28 (m, 1H), 3.85-3.80 (m, 1H), 3.70-3.63 (m, 1H), 2.83-2.79 (m, 1H), 2.16 (s, 3H), 2.13-2.04 (m, 3H), 1.49 (s, 9H).

31. tert-butyl 3-(2-(methylthio) -5,11-dioxo-7,8,9,10,10a,11-hexahydropyrido [1,2-a]pyrimido[4,5-e][1,4]diazepin-12(5H)-yl)phenylcarbamate (9c)

MS (ESI) m/z 470.1 $[M+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.41 (s, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 6.81 (d, *J* = 7.6 Hz, 1H), 6.54 (s, 1H), 4.53-4.48 (m, 1H), 4.34 (s, 1H), 3.09-3.02 (m, 1H), 2.36-2.32 (m, 1H), 2.16 (s, 3H), 1.99-1.60 (m, 5H), 1.50 (s, 9H).

32. tert-butyl 3-(6,7-dimethyl-2-(methylthio) -5,8-dioxo-7,8-dihydro-5H-pyrimido [4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9d)

MS (ESI) m/z 444.1 $[M+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.42 (s, 1H), 7.34 (t, *J* = 8.0 Hz, 1H), 7.25 (s, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.54 (s, 1H), 4.39 (s, 1H), 3.14 (s, 3H), 2.16 (s, 3H), 1.61 (s, 3H), 1.50 (s, 9H).

33. tert-butyl 3-(7-isopropyl-6-methyl-2-(methylthio) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9e)

MS (ESI) m/z 472.1 $[M+H]^+$ ^1H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.00 (s) and 8.96 (s) (together 1H), 7.38-7.29 (m, 3H), 6.82-6.76 (m, 1H), 6.56 (s, 1H), 3.84 (d, *J* = 11.6 Hz, 0.79H) and 3.69 (d, *J* = 10.8 Hz, 0.21H), 3.35 (s) and 3.12 (s) (together 3H), 2.19 (s) and 2.10 (s) (together 3H), 1.97-1.86 (m, 1H), 1.49 (s, 9H),

1.06 (d, *J* = 6.4 Hz, 3H), 0.98 (d, *J* = 6.4 Hz, 3H).

34. tert-butyl 3-(7-isobutyl-6-methyl-2-(methylthio) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9f)

MS (ESI) m/z 486.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 8.99 (s) and 8.98 (s) (together 1H), 7.37-7.28 (m, 3H), 6.79 (d, *J* = 7.6 Hz, 1H), 6.55 (s, 1H), 4.35 (t, *J* = 8.0 Hz, 0.30H) and 4.18 (t, *J* = 7.2 Hz, 0.70H), 3.33 (s) and 3.10 (s) (together 3H), 2.18 (s) and 2.10 (s) (together 3H), 2.05-2.00 (m), 1.90-1.83 (m) and 1.60-1.55 (m) (together 2H), 1.70-1.62 (m, 1H), 1.49 (s, 9H), 0.97-0.92 (m, 6H).

35. tert-butyl 3-(6-methyl-2-(methylthio) -5,8-dioxo-7-phenyl-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9g)

MS (ESI) m/z 506.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 7.40-7.37 (m, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.28 (t, *J* = 7.2 Hz, 2H), 7.24-7.16 (m, 3H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.60 (s, 1H), 5.60 (s, 1H), 3.55 (s, 3H), 2.00 (s, 3H), 1.51 (s, 9H).

36. tert-butyl 3-(2-(methylthio) -5,8-dioxo-6-phenyl-7,8-dihydro-5H-pyrimido [4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9h)

MS (ESI) m/z 492.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 7.50 (d, *J* = 7.6 Hz, 3H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.38-7.29 (m, 3H), 6.88 (d, *J* = 7.6 Hz, 1H), 6.61 (s, 1H), 4.54 (s, 2H), 2.18 (s, 3H), 1.50 (s, 9H).

37. tert-butyl 3-(7-benzyl-6-methyl-2-(methylthio) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9i)

MS (ESI) m/z 520.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.13 (s) and 8.97 (s) (together 1H), 7.35-7.24 (m, 7H), 7.08 (d, *J* = 7.2 Hz, 1H), 6.75-6.69 (m, 1H), 6.53 (d, *J* = 8.0 Hz, 1H), 4.57-4.47 (m, 1H), 3.61-3.56 (m), 3.28-3.23 (m) and 3.07-3.02 (m) (together 2H), 3.17 (s) and 2.98 (s) (together 3H), 2.14 (s) and 2.13 (s) (together 3H), 1.50 (s, 9H).

38. tert-butyl 3-(7-(4-methoxybenzyl)-6-methyl-2-(methylthio) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9j)

MS (ESI) m/z 550.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.12 (s) and 8.97 (s) (together 1H), 7.36-7.29 (m, 2H), 7.23 (s, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 6.99 (d, *J* = 8.8 Hz, 1H), 6.87 (t, *J* = 8.4 Hz, 2H), 6.76-6.69 (m, 1H), 6.52 (d,

J = 7.2 Hz, 1H), 4.52-4.48 (m) and 4.41 (br) (together 1H), 3.80 (s, 3H), 3.56-3.51 (m), 3.19 (s) and 2.98-2.89 (m) (together 2H), 3.16 (s) and 3.01 (s) (together 3H), 2.14 (s) and 2.13 (s) (together 3H), 1.50 (s, 9H).

39. tert-butyl 3-(7-(4-chlorobenzyl)-6-methyl-2-(methylthio) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9k)

MS (ESI) m/z 554.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.11 (s) and 8.97 (s) (together 1H), 7.40-7.27 (m, 4H), 7.25-7.19 (m) and 7.03 (d, *J* = 8.4 Hz) (together 3H), 6.73 (d, *J* = 8.4 Hz) and 6.68 (d, *J* = 7.6 Hz) (together 1H), 6.54 (d, *J* = 8.8 Hz, 1H), 4.53-4.49 (m) and 4.43-4.39 (m) (together 1H), 3.62-3.56 (m), 3.22-3.18 (m) and 3.01-2.90 (m) (together 2H), 3.17 (s) and 3.03 (s) (together 3H), 2.14 (s, 3H), 1.50 (s, 9H).

40. tert-butyl 3-(7-(4-fluorobenzyl)-6-methyl-2-(methylthio) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9l)

MS (ESI) m/z 538.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.12 (s) and 8.97 (s) (together 1H), 7.42-7.29 (m, 2H), 7.25-7.18 (m, 2H), 7.07-6.99 (m, 3H), 6.73 (d, *J* = 7.6 Hz) and 6.68 (d, *J* = 7.6 Hz) (together 1H), 6.56 (s) and 6.53 (s) (together 1H), 4.52-4.48 (m) and 4.43-4.39 (m) (together 1H), 3.61-3.55 (m), 3.23-3.19 (m) and 2.99-2.90 (m) (together 2H), 3.17 (s) and 3.02 (s) (together 3H), 2.14 (s, 3H), 1.50 (s) and 1.49 (s) (together 9H).

41. tert-butyl 3-(6-benzyl-2-(methylthio) -5,8-dioxo-7,8-dihydro-5H-pyrimido [4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (9m)

MS (ESI) m/z 506.1 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.45 (s, 1H), 7.38-7.32 (m, 6H), 7.23 (d, *J* = 8.4 Hz, 1H), 6.82 (d, *J* = 7.6 Hz, 1H), 6.56 (s, 1H), 4.87 (s, 2H), 4.03 (s, 2H), 2.15 (s, 3H), 1.50 (s, 9H).

42. tert-butyl 3-(6-methyl-2-(methylsulfonyl)-5,8-dioxo-7,8-dihydro-5H-pyrimido [4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10a)

General Procedure for Synthesis of 10a-m.

To a solution of **9a** (4.59 g, 10.7 mmol) in anhydrous CH_2Cl_2 (50 mL) was added 85% m-CPBA (6.52 g, 32.1 mmol) at 0 °C under argon. The reaction mixture was allowed to warm to room temperature, stirred for 4 h. The solution was diluted with CH_2Cl_2,

and was then treated with 50% $Na_2S_2O_3$/$NaHCO_3$ solution. The organic phase was washed with saturated aqueous NaCl, was dried over Na_2SO_4, and was then filtered. After removal of solvent under vacuum, The resulting white solid (4.44 g, 90% yield) was recrystallized from a minimum amount of ethyl acetate and petroleum ether, which was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (s, 1H), 7.56 (s, 1H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.19 (dd, *J* = 1.2, 8.0 Hz, 1H), 6.85 (dd, *J* = 1.2, 8.0 Hz, 1H), 6.62 (s, 1H), 4.18 (s, 2H), 3.36 (s, 3H), 2.99 (s, 3H), 1.49 (s, 9H).

43. tert-butyl 3-(2-(methylsulfonyl)-5,10-dioxo-8,9,9a,10-tetrahydro-5H-pyrimido [4,5-e]pyrrolo[1,2-a][1,4]diazepin-11(7H)-yl)phenylcarbamate (10b)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 7.55 (s, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.17 (dd, *J* = 1.2, 8.0 Hz, 1H), 6.83 (d, *J* = 8.0Hz, 1H), 6.64 (s, 1H), 4.34 (d, *J* = 6.4 Hz, 1H), 3.92-3.86 (m, 1H), 3.73-3.66 (m, 1H), 2.96 (s, 3H), 2.87-2.84 (m, 1H), 2.25-2.10 (m, 3H), 1.49 (s, 9H).

44. tert-butyl 3-(2-(methylsulfonyl)-5,11-dioxo-7,8,9,10,10a,11-hexahydropyrido [1,2-a]pyrimido[4,5-e][1,4]diazepin-12(5H)-yl)phenylcarbamate (10c)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (s, 1H), 7.52 (s, 1H) 7.36 (t, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.59 (s, 1H), 4.55-4.50 (m, 1H), 4.33 (s, 1H), 3.13-3.06 (m, 1H), 3.00 (s, 3H), 2.39-2.35 (m, 1H), 2.00-1.57 (m, 5H), 1.49 (s, 9H).

45. tert-butyl 3-(6,7-dimethyl-2-(methylsulfonyl) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10d)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 7.52 (s, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.20 (dd, *J* = 1.2, 8.0 Hz, 1H), 6.83 (d, *J* = 8.0, 1H), 6.62 (s, 1H), 4.38 (d, *J* = 4.8 Hz, 1H), 3.18 (s, 3H), 3.00 (s, 3H), 1.65 (s, 3H), 1.49 (s, 9H).

46. tert-butyl 3-(7-isopropyl-6-methyl-2-(methylsulfonyl) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10e)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.41 (s) and 9.35 (s) (together 1H), 7.43-7.34 (m, 2H), 7.28-7.22 (m, 1H), 6.83-6.78 (m, 1H), 6.60 (s, 1H), 3.92 (d, *J* = 12.0 Hz, 0.78H) and 3.66 (d, *J* = 10.8 Hz, 0.22H), 3.39 (s) and 3.16 (s) (together

3H), 3.02 (s) and 2.93 (s) (together 3H), 1.83-1.73 (m, 1H), 1.49 (s, 9H), 1.11-1.00 (m, 6H).

47. tert-butyl 3-(7-isobutyl-6-methyl-2-(methylsulfonyl) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10f)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.41 (s) and 9.37 (s) (together 1H), 7.44 (s, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 9.2 Hz, 1H), 6.81 (d, *J* = 7.6 Hz, 1H), 6.59 (s, 1H), 4.43 (t, *J* = 8.0 Hz, 0.25H), and 4.15 (t, *J* = 7.2 Hz, 0.75H), 3.38 (s) and 3.14 (s) (together 3H), 3.02 (s) and 2.94 (s) (together 3H), 2.09-2.04 (m), 1.92-1.89 (m), and 1.56-1.55 (m) (together 2H), 1.69-1.63 (m, 1H), 1.49 (s, 9H), 0.99-0.93 (m, 6H).

48. tert-butyl 3-(6-methyl-2-(methylsulfonyl) -5,8-dioxo-7-phenyl-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10g)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H), 7.52 (s, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.31-7.22 (m, 4H), 7.15 (s, 2H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.65 (s, 1H), 5.68 (s, 1H), 3.60 (s, 3H), 2.82 (s, 3H), 1.50 (s, 9H).

49. tert-butyl 3-(2-(methylsulfonyl)-5,8-dioxo-6-phenyl-7,8-dihydro-5H-pyrimido [4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10h)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 7.59 (s, 1H), 7.51-7.45 (m, 4H), 7.41-7.35 (m, 2H), 7.24 (d, *J* = 8.4 Hz, 1H), 6.89 (d, *J* = 7.6 Hz, 1H), 6.65 (s, 1H), 4.58 (s, 2H), 3.01 (s, 3H), 1.49 (s, 9H).

50. tert-butyl 3-(7-benzyl-6-methyl-2-(methylsulfonyl)-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10i)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.52 (s) and 9.35 (s) (together 1H), 7.46-7.28 (m, 5H), 7.24-7.22 (m, 2H), 7.18 (d, *J* = 8.4 Hz) and 7.05 (d, *J* = 6.8 Hz) (together 1H), 6.73-6.68 (m, 1H), 6.59 (s, 1H), 4.66-4.61 (m, 0.30H) and 4.49-4.45 (m, 0.70H), 3.64-3.58 (m), 3.32-3.27 (m) and 2.91-2.81 (m) (together 2H), 3.21 (s) and 3.04 (s) (together 3H), 2.97 (s, 3H), 1.49 (s, 9H).

51. tert-butyl 3-(7-(4-methoxybenzyl)-6-methyl-2-(methylsulfonyl)-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10j)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.50 (s) and 9.34 (s) (together

1H), 7.43-7.31 (m, 2H), 7.25-7.16 (m, 2.40H) and 6.96 (d, *J* = 8.8 Hz, 0.60H), 6.87 (t, *J* = 8.0 Hz, 2H), 6.75-6.67 (m, 1H), 6.58 (s, 1H), 4.61-4.57 (m) and 4.40-4.37 (m) (together 1H), 3.80 (s) and 3.79 (s) (together 3H), 3.59-3.53 (m), 3.23-3.19 (m) and 2.86-2.80 (m) (together 2H), 3.21 (s) and 3.07 (s) (together 3H), 2.97 (s) and 2.96 (s) (together 3H), 1.49 (s, 9H).

52. tert-butyl 3-(7-(4-chlorobenzyl)-6-methyl-2-(methylsulfonyl) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10k)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.52 (s) and 9.34 (s) (together 1H), 7.50-7.28 (m, 4H), 7.24-7.14 (m, 2.60H) and 7.05 (d, *J* = 8.0 Hz, 0.4H), 6.73 (d, *J* = 8.0 Hz) and 6.67 (d, *J* = 8.0 Hz) (together 1H), 6.59 (s, 1H), 4.62-4.58 (m) and 4.41-4.38 (m) (together 1H), 3.65-3.59 (m), 3.26-3.24 (m) and 2.90-2.76 (m) (together 2H), 3.22 (s) and 3.09 (s) (together 3H), 2.97 (s, 3H), 1.49 (s, 9H).

53. tert-butyl 3-(7-(4-fluorobenzyl)-6-methyl-2-(methylsulfonyl) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10l)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.51 (s) and 9.32 (s) (together 1H), 7.51-7.31 (m, 2H), 7.24-7.20 (m, 2H), 7.15-7.00 (m, 3H), 6.73-6.65 (m, 1H), 6.61 (s, 1H), 4.61-4.57 (m, 0.23H) and 4.43-4.39 (m, 0.77H), 3.64-3.58 (m), 3.26-3.25 (m) and 2.90-2.84 (m) (together 2H), 3.21 (s) and 3.08 (s) (together 3H), 2.95 (s, 3H), 1.49 (s, 9H).

54. tert-butyl 3-(6-benzyl-2-(methylsulfonyl)-5,8-dioxo-7,8-dihydro-5H-pyrimido [4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (10m)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 7.42 (s, 1H), 7.29-7.22 (m, 5H), 7.14 (s, 1H), 7.06 (dd, *J* = 1.2, 8.0 Hz, 1H), 6.70 (d, *J* = 8.0 Hz, 1H), 6.48 (s, 1H), 4.78 (s, 2H), 3.95 (s, 2H), 2.86 (s, 3H), 1.37 (s, 9H).

55. tert-butyl 3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (11a)

General Procedure for Synthesis of 11a-m.

To a solution of **10a** (1 g, 2.17 mmol), in 2-butyl alcohol (10 mL) was added 2-methoxy-4-(4-methylpiperazin-1-yl)aniline (529 mg, 2.39 mmol), followed by

trifluoroacetic acid (177 μL, 2.39 mmol). The reaction mixture was stirred for 18 h at 110 ℃ in a seal tube. The reaction mixture was cooled to room temperature and poured into a solution of 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was separated, washed with 10% aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The resultant crude material was purified by column chromatography (SiO_2, CH_2Cl_2/Methanol stepwise elution, 100 :1 to 10 :1) to give the title compound. (0.88g, 67% yield). MS (ESI) m/z 603.2 $[M+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.82 (s, 1H), 7.49 (s, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.32 (s, 1H), 7.07 (d, *J* = 6.8 Hz, 1H), 6.89 (d, *J* = 7.6 Hz, 1H), 6.64 (s, 1H), 6.42 (s, 1H), 6.10 (s, 1H), 4.12 (s, 2H), 3.81 (s, 3H), 3.28 (s, 3H), 3.12 (t, *J* = 4.8 Hz, 4H), 2.57 (t, *J* = 4.8 Hz, 4H), 2.35 (s, 3H), 1.48 (s, 9H).

56. tert-butyl 3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl) phenylamino)-5,10-dioxo-8,9,9a,10-tetrahydro-5H-pyrimido[4,5-e]pyrrolo[1,2-a][1,4]diazepin-11(7H)-yl)phenylcarbamate (11b)

MS (ESI) m/z 629.2 $[M+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.82 (s, 1H), 7.46-7.40 (m, 2H), 7.32 (s, 1H), 7.09 (s, 1H), 6.89 (d, *J* = 7.2 Hz, 1H), 6.64 (s, 1H), 6.41 (s, 1H), 6.12 (s, 1H), 4.33-4.32 (m, 1H), 3.81 (s, 3H), 3.79-3.76 (m, 1H), 3.69-3.64 (m, 1H), 3.27 (br, 4H), 2.83 (br, 4H), 2.80 (s, 1H), 2.53 (s, 3H), 2.14-2.03 (m, 3H), 1.48 (s, 9H).

57. tert-butyl 3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl) phenylamino)-5,11-dioxo-7,8,9,10,10a,11-hexahydropyrido[1,2-a]pyrimido[4,5-e][1,4]diazepin-12(5H)-yl)phenylcarbamate (11c)

MS (ESI) m/z 643.2 $[M+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 7.81 (s, 1H), 7.49 (d, *J* = 5.2 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 7.14 (s, 1H), 6.87 (d, *J* = 7.6 Hz, 1H), 6.66 (s, 1H), 6.41 (s, 1H), 6.12 (s, 1H), 4.52-4.47 (m, 1H), 4.41-4.38 (m, 1H), 3.81 (s, 3H), 3.16 (t, *J* = 4.8 Hz, 4H), 3.08-3.01 (m, 1H), 2.64 (t, *J* = 4.8 Hz, 4H), 2.40 (s, 3H), 2.34-2.30 (m, 1H), 1.98-1.93 (m, 1H), 1.85-1.59 (m, 4H), 1.48 (s, 9H).

58. tert-butyl 3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl) phenylamino)-6,7-dimethyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl

carbamate (11d)

MS (ESI) m/z 617.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.82 (s, 1H), 7.49 (s, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 7.14 (s, 1H), 6.88 (d, *J* = 7.6 Hz, 1H), 6.62 (s, 1H), 6.41 (s, 1H), 6.12 (s, 1H), 4.44 (dd, *J* = 6.8, 13.6 Hz, 1H), 3.82 (s, 3H), 3.24 (br, 4H), 3.11 (s, 3H), 2.77 (br, 4H), 2.50 (s, 3H), 1.59 (s, 3H), 1.48 (s, 9H).

59. tert-butyl 3-(7-isopropyl-2- (2-methoxy-4- (4-methylpiperazin -1-yl) phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9 (6H)-yl)phenylcarbamate (11e)

MS (ESI) m/z 645.3 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 8.97 (s) and 8.92 (s) (together 1H), 7.90-7.82 (m, 1H), 7.58 (s, 1H), 7.47-7.42 (m, 1H), 7.18 (s, 1H), 6.99 (s, 1H), 6.87 (d, *J* = 6.8 Hz, 1H), 6.65 (s) and 6.63 (s) a(together 1H), 6.41 (s, 1H), 6.09 (s, 1H), 3.90-3.86 (m, 1H), 3.81 (s, 3H), 3.46 (s) and 3.32 (s) (together 3H), 3.17 (br, 4H), 2.65 (br, 4H), 2.41 (s, 3H), 2.11-2.04 (m, 1H), 1.48 (s, 9H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.4 Hz, 3H).

60. tert-butyl 3-(7-isobutyl-2- (2-methoxy-4- (4-methylpiperazin -1-yl) phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9 (6H)-yl)phenylcarbamate (11f)

MS (ESI) m/z 659.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 8.93 (s) and 8.91 (s) (together 1H), 7.83 (s, 1H), 7.55 (s, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.21 (s, 1H), 7.13 (s) and 6.98 (s) (together 1H), 6.87 (d, *J* = 7.6 Hz, 1H), 6.61 (d, *J* = 6.8 Hz, 1H), 6.41 (s, 1H), 6.12 (s, 1H), 4.32 (t, *J* = 7.6 Hz, 0.3H) and 4.22 (t, *J* = 6.0 Hz, 0.7H), 3.82 (s, 3H), 3.26 (br, 4H), 3.09 (s, 3H), 2.80 (br, 4H), 2.08-2.01 (m, 1H), 1.87-1.80 (m, 1H), 1.70-1.65 (m, 1H), 1.48 (s, 9H), 0.96-0.94 (m, 6H).

61. tert-butyl 3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl -5,8-dioxo-7-phenyl-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)pheny lcarbamate (11g)

MS (ESI) m/z 679.0 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.61 (s, 2H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.27-7.24 (m, 3H), 7.19 (s, 3H), 6.97 (d, *J* = 8.0 Hz, 2H), 6.62 (s, 1H), 6.38 (s, 1H), 6.09 (s, 1H), 5.58 (s, 1H), 3.76 (s, 3H), 3.54 (s, 3H), 3.14 (t, *J* = 4.0 Hz, 4H), 2.62 (t, *J* = 4.0 Hz, 4H), 2.39 (s, 3H), 1.49 (s, 9H).

62. tert-butyl 3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-5,8-dioxo-6-phenyl-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (11h)

MS (ESI) m/z 665.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (s, 1H), 7.88 (s, 1H), 7.55-7.50 (m, 3H), 7.46-7.41 (m, 3H), 7.35 (s, 1H), 7.30 (d, *J* = 7.2 Hz, 1H), 7.11 (d, *J* = 7.2 Hz, 1H), 6.94 (d, *J* = 7.6 Hz, 1H), 6.66 (s, 1H), 6.43 (S, 1H), 6.12 (s, 1H), 4.55 (s, 2H), 3.83 (s, 3H), 3.15 (br, 4H), 2.60 (br, 4H), 2.38 (s, 3H), 1.49 (s, 9H).

63. tert-butyl 3-(7-benzyl-2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (11i)

MS (ESI) m/z 693.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.05 (s) and 8.90 (s) (together 1H), 7.89 (s) and 7.80 (s) (together 1H), 7.54 (s, 1H), 7.44-7.20 (m, 6H), 7.11-7.03 (m, 2H), 6.81 (d, *J* = 8.4 Hz, 0.36H) and 6.76 (d, *J* = 8.0 Hz, 0.64H), 6.59 (s) and 6.56 (s) (together 1H), 6.43 (s) and 6.40 (s), (together 1H), 6.10 (s) and 6.07 (s) (together 1H), 4.57-4.49 (m, 1H), 3.83 (s) and 3.80 (s) (together 3H), 3.61-3.56 (m), 3.25-3.22 (m) and 3.07-3.04 (m) (together 2H), 3.18 (br, 4H), 3.14 (s) and 2.97 (s) (together 3H), 2.65 (br, 4H), 2.42 (s) and 2.41 (s) (together 3H), 1.48 (s, 9H).

64. tert-butyl 3-(2-(2-methoxy-4- (4-methylpiperazin-1-yl) phenylamino)-7-(4-methoxybenzyl)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbamate (11j)

MS (ESI) m/z 723.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.04 (s) and 8.89 (s) (together 1H), 7.89 (s) and 7.80 (s) (together 1H), 7.53 (s, 1H), 7.43-7.37 (m, 1H), 7.18-7.00 (m, 4H), 6.87-6.75 (m, 3H), 6.66 (s) and 6.63 (s) (together 1H), 6.42 (s) and 6.40 (s) (together 1H), 6.10 (s) and 6.06 (s) (together 1H), 4.49-4.44 (m, 1H), 3.83 (s) and 3.79 (s) (together 3H), 3.79 (s) and 3.77 (s) (together 3H), 3.15 (s, 3H), 3.12 (br, 4H), 2.99 (s, 2H), 2.62 (br, 4H), 2.39 (s) and 2.38 (s) (together 3H), 1.48 (s, 9H).

65. tert-butyl 3-(7-(4-chlorobenzyl) -2-(2-methoxy-4-(4-methylpiperazin -1-yl) phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9

(6H)-yl)phenylcarbamate (11k)

MS (ESI) m/z 727.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.04 (s) and 8.89 (s) (together 1H), 7.90 (s) and 7.81 (s) (together 1H), 7.50 (s, 1H), 7.40 (dd, *J* = 8.4, 16.8 Hz, 1H), 7.33-7.27 (m, 2H), 7.22-7.19 (m, 2H), 7.13-7.04 (m, 2H), 6.81 (d, *J* = 7.20 Hz, 0.3H) and 6.75 (d, *J* = 7.60 Hz, 0.7 Hz) (together 1H), 6.60 (s) and 6.57 (s) (together 1H), 6.43 (s) and 6.40 (s) (together 1H), 6.10 (s) and 6.06 (s) (together 1H), 4.50-4.46 (m, 1H), 3.83 (s) and 3.80 (s) (together 3H), 3.61-3.55 (m), 3.19-3.17 (m) and 3.05 (br) (together 2H), 3.15 (s) and 3.02 (s) (together 3H), 3.14 (br, 4H), 2.62 (br, 4H), 2.41 (s) and 2.39 (s) (together 3H), 1.48 (s, 9H).

66. tert-butyl 3-(7-(4-fluorobenzyl) -2-(2-methoxy-4-(4-methylpiperazin-1-yl) phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9 (6H)-yl)phenylcarbamate (11l)

MS (ESI) m/z 711.3 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 7.90 (s) and 7.81 (s) (together 1H), 7.52-7.36 (m, 2H), 7.25-7.22 (m, 2H), 7.12-6.97 (m, 4H), 6.80 (d, *J* = 6.8 Hz, 0.32H) and 6.74 (d, *J* = 8.0 Hz, 0.68H), 6.62 (s) and 6.58 (s) (together 1H), 6.43 (s) and 6.39 (s) (together 1H), 6.10 (s) and 6.06 (s) (together 1H), 4.49-4.46 (m, 1H), 3.83 (s) and 3.80 (s) (together 3H), 3.61-3.55 (m), 3.25-3.22 (m) and 3.06-3.03 (m) (together 2H), 3.18 (br, 4H), 3.16 (s) and 3.00 (s) (together 3H), 2.69 (br, 4H), 2.45 (s) and 2.44 (s) (together 3H), 1.48 (s, 9H).

67. tert-butyl 3-(6-benzyl-2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino) -5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenylcarbama te (11m)

MS (ESI) m/z 679.2 $[M+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.85 (s, 1H). 7.49 (s, 1H), 7.43-7.31 (m, 7H), 7.09 (s, 1H), 6.88 (d, *J* = 7.6 Hz, 1H), 6.65 (s, 1H), 6.41 (s, 1H), 6.11 (s, 1H), 4.86 (s, 2H), 4.04 (s, 2H), 3.81 (s, 3H), 3.19 (br, 4H), 2.68 (br, 4H), 2.43 (s, 3H), 1.48 (s, 9H).

68. N-(3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl-5,8-dio xo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2a)

General Procedure for Synthesis of 2a-m.

To a solution of **11a** (687 mg, 1.14 mmol) in CH_2Cl_2 (5 mL) was added trifluoroacetic

acid (0.85 mL, 11.4 mmol). The reaction mixture was stirred for 4 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, and basified with saturated aqueous $NaHCO_3$ till PH = 9 and extracted with CH_2Cl_2. The organic layer was separated, washed with 10% aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The resultant crude material was purified by column chromatography (SiO_2, CH_2Cl_2/Methanol stepwise elution, 100 :1 to 10 :1) to give 9-(3-aminophenyl)-2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl-6,7-dihydro-5H-pyrimido[4,5-e][1,4]diazepine-5,8(9H)-dione **12a** as an yellow solid (487 mg, 85% yield), which was used in the next reaction without characterization. This yellow solid **12a** (428 mg, 0.85 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) under argon and cooled to 0 °C (ice bath). N,N-diisopropylethylamine (221 μL, 1.28 mmol) and acryloyl chloride (105 μL, 1.28 mmol) were added with stirring. The reaction was allowed to warm to room temperature. After stirring for 4 h, the reaction mixture was poured into a solution of 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was separated, washed with 10% aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The resultant crude material was purified by column chromatography (SiO_2, CH_2Cl_2/Methanol stepwise elution, 100 :1 to 10 :1) to give the title compound. (388 mg, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.98 (s, 2H), 7.74 (s, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.40 (s, 1H), 7.00-6.91 (m, 2H), 6.43-6.39 (m, 2H), 6.31-6.25 (m, 1H), 6.08 (d, *J* = 8.0 Hz, 1H), 5.74 (dd, *J* = 1.2, 10.0 Hz, 1H), 4.06 (s, 2H), 3.81 (s, 3H), 3.26 (s, 3H), 3.18 (t, *J* = 4.4 Hz, 4H), 2.71 (s, 4H), 2.46 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.70, 165.16, 163.33, 162.97, 159.35, 158.47, 148.89, 147.78, 139.21, 139.16, 130.99, 130.22, 128.12, 124.29, 120.22, 120.08, 119.83, 119.57, 109.46, 107.53, 99.35, 55.60, 55.07, 53.65, 49.47, 46.07, 35.96. HRMS (ESI) Calcd for $[M+H]^+$ = 557.2619, found: $[M+H]^+$ = 557.2621.

69. N-(3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-5,10-dioxo-8,9,9a,10-tetrahydro-5H-pyrimido[4,5-e]pyrrolo[1,2-a][1,4]diazepin-11(7H)-yl)phenyl) acrylamide (2b)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.91 (s, 1H), 7.78 (s, 1H), 7.73 (s, 1H),

7.44 (t, *J* = 7.6 Hz, 1H), 7.38 (s, 1H), 7.00 (s, 1H), 6.93 (d, *J* = 7.2 Hz, 1H), 6.42-6.38 (m, 2H), 6.22-6.16 (m, 1H), 6.09 (s, 1H), 5.74 (d, *J* = 10.0 Hz, 1H), 4.34 (d, *J* = 5.2 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 1H), 3.67-3.65 (m, 1H), 3.12 (br, 4H), 2.79 (s, 1H), 2.60 (br, 4H), 2.38 (s, 3H), 2.14-2.05 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.16, 163.27, 162.40, 159.46, 158.28, 148.79, 147.51, 140.23, 139.17, 131.05, 130.10, 127.96, 124.35, 120.46, 120.31, 119.64, 119.53, 113.04, 110.43, 107.76, 99.61, 58.04, 55.60, 55.02, 49.42, 47.00, 45.92, 26.95, 23.81. HRMS (ESI) Calcd for $[M+H]^+$ = 583.2776, found: $[M+H]^+$ = 583.2773.

70. N-(3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-5,11-dioxo-7,8,9,10,10a,11-hexahydropyrido[1,2-a]pyrimido[4,5-e][1,4]diazepin-12(5H)-yl)phenyl) acrylamide (2c)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 7.97 (s, 1H), 7.90 (s, 1H), 7.77 (s, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.40 (s, 1H), 7.08 (s, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.40-6.36 (m, 2H), 6.27-6.20 (m 1H), 6.09 (s, 1H), 5.72 (d, *J* = 10.8 Hz, 1H), 4.50-4.47 (m, 1H), 4.43-4.40 (m, 1H), 3.79 (s, 3H), 3.15 (br, 4H), 3.06-2.99 (m, 1H), 2.67 (br, 4H), 2.42 (s, 3H), 2.27 (s, 1H), 1.95-1.90 (m, 1H), 1.83-1.62 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.68, 166.07, 163.26, 162.49, 159.44, 158.26, 148.78, 147.61, 139.42, 139.06, 131.02, 130.05, 127.95, 124.47, 120.39, 120.33, 119.70, 119.47, 110.55, 107.69, 99.55, 55.60, 55.02, 52.63, 49.51, 46.01, 39.88, 23.27, 22.84, 18.83. HRMS (ESI) Calcd for $[M+H]^+$ = 597.2932, found: $[M+H]^+$ = 597.2929.

71. N-(3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6,7-dimethyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2d)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.04 (s, 1H), 7.88 (s, 1H), 7.77 (s, 1H), 7.47-7.41 (m, 2H), 7.06 (s, 1H), 6.92 (d, *J* = 7.6, 1H), 6.41-6.23 (m, 3H), 6.15-6.06 (m, 1H), 5.72 (dd, *J* = 1.2, 10.0 Hz, 1H), 4.45 (dd, *J* = 6.8, 13.6 Hz, 1H), 3.80 (s, 3H), 3.17 (br, 4H), 3.10 (s, 3H), 2.72 (br, 4H), 2.45 (s, 3H), 1.57 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.25, 165.99, 163.45, 162.65, 159.50, 158.16, 148.85, 147.17, 139.61, 139.30, 134.60, 131.13, 129.99, 127.92, 124.25, 120.94, 120.40, 119.69, 110.35, 108.11, 100.08, 55.61, 54.69, 52.47, 49.12, 45.36, 28.37, 12.65. HRMS (ESI)

Calcd for $[M+H]^+$ = 571.2776, found: $[M+H]^+$ = 571.2770.

72. N-(3-(7-isopropyl-2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2e)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 8.97 (s) and 8.92 (s) (together 1H), 8.04-7.96 (m) and 7.80 (s) (together 3H), 7.51-7.33 (m, 2H), 7.04 (d, *J* = 6.8Hz) and 6.91 (d, *J* = 6.8Hz) (together 2H), 6.41-6.35 (m, 2H), 6.20-6.06 (m, 2H), 5.73-5.70 (m, 1H), 3.82 (s, 1H), 3.80 (s, 3H), 3.45 (s, 0.70H) and 3.33 (s, 2.30H), 3.10 (br, 4H), 2.59 (br, 4H), 2.37 (s, 3H), 2.13-2.05 (m, 1H), 1.07-0.96 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.45, 163.60, 163.28, 162.62, 162.42, 157.16, 148.77, 147.49, 140.26, 139.57, 131.08, 130.24, 130.12, 127.87, 124.47, 120.42, 120.19, 119.71, 119.39, 107.73, 99.52, 74.31, 55.61, 54.99, 49.39, 45.93, 39.73, 28.76, 20.12, 19.96, 19.48, 19.28. HRMS (ESI) Calcd for $[M+H]^+$ = 599.3089, found: $[M+H]^+$ = 599.3087.

73. N-(3-(7-isobutyl-2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2f)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 8.92 (s) and 8.90 (s) (together 1H), 7.92-7.79 (m, 3H), 7.49-7.42 (m, 1H), 7.36 (s, 1H), 7.05-6.91 (m, 2H), 6.41-6.37 (m, 2H), 6.26-6.19 (m, 1H), 6.10 (s, 1H), 5.73 (d, *J* = 10.4 Hz, 1H), 4.32 (t, *J* = 7.6 Hz, 0.3H) and 4.27 (t, *J* = 7.6 Hz, 0.7H), 3.80 (s, 3H), 3.32 (s) and 3.08 (s) (together 3H), 3.14 (br, 4H), 2.66 (br, 4H), 2.41 (s, 3H), 2.07-1.61 (m, 3H), 0.96-0.91 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.82, 168.67, 166.38, 163.81, 163.35, 162.76, 162.60, 158.31, 148.86, 147.38, 139.58, 139.26, 131.10, 130.05, 127.90, 124.38, 120.57, 120.27, 119.80, 107.92, 99.81, 55.62, 55.25, 54.88, 49.30, 45.71, 38.83, 38.59, 35.42, 28.84, 25.69, 25.18, 22.59, 22.32. HRMS (ESI) Calcd for $[M+H]^+$ = 613.3245, found: $[M+H]^+$ = 613.3237.

74. N-(3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl-5,8-dioxo-7-phenyl-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2g)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.48 (s. 1H), 7.97 (d, *J* = 6.4 Hz, 1H), 7.63 (s, 1H), 7.56 (s, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.24 (d, *J* = 7.2 Hz, 2H), 7.18 (d, *J* = 6.8 Hz, 3H), 6.98 (d, *J* = 7.6 Hz, 1H), 6.87 (s, 1H), 6.39-6.37 (m, 2H), 6.32 (s, 1H), 6.10 (s, 1H), 5.73-5.70 (m, 1H), 5.57 (s, 1H), 3.73 (s, 3H), 3.52 (s, 3H), 3.23 (br, 4H), 2.85 (br, 4H), 2.54 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.99, 165.02, 163.67, 162.41, 158.81, 156.75, 148.66, 146.52, 139.84, 139.61, 133.36, 131.27, 130.05, 129.18, 128.41, 127.89, 124.12, 121.37, 120.36, 119.78, 119.36, 108.41, 100.30, 69.08, 55.57, 54.44, 48.70, 44.86, 29.66. HRMS (ESI) Calcd for $[M+H]^+$ = 633.2932, found: $[M+H]^+$ = 633.2927.

75. N-(3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-5,8-dioxo-6-phenyl-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2h)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.07 (s, 1H), 7.94 (s, 1H), 7.82 (s, 1H), 7.48-7.46 (m, 3H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.31-7.27 (m, 2H), 6.96 (d, *J* = 7.2 Hz, 2H), 6.40-6.36 (m, 2H), 6.19-6.06 (m, 2H), 5.70 (d, *J* = 10.8 Hz, 1H), 4.44 (s, 2H), 3.83 (s, 3H), 3.09 (br, 4H), 2.56 (br, 4H), 2.36 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.30, 164.26, 163.31, 159.35, 158.37, 148.87, 147.86, 141.72, 139.33, 138.96, 131.01, 130.30, 129.27, 127.95, 127.30, 125.35, 124.05, 120.18, 119.95, 119.86, 119.60, 109.75, 107.47, 99.21, 55.62, 55.07, 54.54, 49.33, 46.10. HRMS (ESI) Calcd for $[M+H]^+$ = 619.2776, found: $[M+H]^+$ = 619.2770.

76. N-(3-(7-benzyl-2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2i)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.04 (s) and 8.89 (s) (together 1H), 7.92-7.75 (m, 3H), 7.43-7.39 (m, 1H), 7.35-7.27 (m, 3H), 7.25-7.09 (m, 3H), 6.96 (s, 1H), 6.86-6.81 (m, 1H), 6.40-6.35 (m, 2H), 6.16-6.04 (m, 2H), 5.70 (d, *J* = 10.4 Hz, 1H), 4.61-4.58 (m, 0.64H) and 4.51 (t, *J* = 8.4 Hz, 0.36H), 3.81 (s) and 3.78 (s) (together 3H), 3.63-3.52 (m), 3.26-3.21 (m) and 3.04 (br) (together 2H), 3.14 (s) and 2.98 (s) (together 3H), 3.12-3.06 (m, 4H), 2.60-2.55 (m, 4H), 2.37-2.35 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.88, 167.96, 166.06, 163.64, 163.21, 162.98,

162.69, 158.23, 139.05, 136.06, 135.15, 130.96, 130.17, 129.08, 128.98, 128.89, 128.09, 127.59, 127.17, 124.53, 120.07, 107.65, 107.53, 99.48, 68.88, 58.25, 55.63, 55.58, 55.07, 49.54, 46.11, 38.82, 35.41, 32.87, 29.06. HRMS (ESI) Calcd for $[M+H]^+$ = 647.3089, found: $[M+H]^+$ = 647.3079.

77. N-(3-(2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-7-(4-methoxybenzyl)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2j)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.04 (s) and 8.99 (s) (together 1H), 8.08-7.75 (m, 3H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.34-7.24 (m, 2H), 7.16 (d, *J* = 8.4 Hz, 1H), 7.03-6.82 (m, 4H), 6.41-6.37 (m, 2H), 6.30-6.21 (m, 1H), 6.10-6.06 (m, 1H), 5.73 (d, J = 9.6 Hz, 1H), 4.53-4.45 (m, 1H), 3.84-3.78 (m, 6H), 3.55-3.46 (m), 3.24-3.22 (m) and 2.99 (s) (together 2H), 3.21-3.18 (m, 4H), 3.15 (s) and 3.01 (s) (together 3H), 2.76-2.72 (m, 4H), 2.53-2.46 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.00, 168.09, 166.05, 163.64, 163.37, 163.00, 162.69, 158.99, 158.65, 158.19, 148.95, 147.16, 139.40, 131.10, 130.13, 130.04, 127.95, 126.96, 124.37, 120.10, 119.81, 114.53, 114.32, 108.26, 108.09, 100.19, 100.07, 68.99, 58.50, 55.65, 55.60, 55.28, 54.71, 49.08, 45.38, 38.81, 34.56, 32.02, 29.05. HRMS (ESI) Calcd for $[M+H]^+$ = 677.3194, found: $[M+H]^+$ = 677.3190.

78. N-(3-(7-(4-chlorobenzyl)-2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2k)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.03 (s) and 8.89 (s) (together 1H), 7.96-7.87 (m, 2H), 7.59 (s, 1H), 7.45-7.43 (m, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.25-7.16 (m, 3H), 7.05 (d, *J* = 8.0 Hz, 1H), 6.96 (s, 1H), 6.88-6.82 (m, 1H), 6.43-6.37 (m, 2H), 6.22-6.15 (m, 1H), 6.10-6.03 (m, 1H), 5.75 (d, *J* = 10.8 Hz, 1H), 4.53-4.47 (m, 1H), 3.82 (s) and 3.78 (s) (together 3H), 3.58-3.53 (m), 3.20-3.19 (m) and 3.05 (br) (together 2H), 3.15 (s) and 3.03 (s) (together 3H), 3.09 (br, 4H), 2.58 (br, 4H), 2.39 (s) and 2.37 (s) (together 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.57, 167.84, 166.00, 163.57, 163.30, 162.97, 162.69, 159.53,159.35, 158.13, 157.24, 148.91, 147.64, 139.33, 134.52, 133.63, 133.59, 133.03, 130.97, 130.55, 130.33,

130.15, 129.31, 129.00, 128.01, 124.31, 120.17, 119.88, 119.59, 107.75, 107.61, 99.63, 68.63, 58.20, 55.58, 54.93, 49.37, 45.87, 38.83, 34.81, 32.34, 29.05. HRMS (ESI) Calcd for $[M+H]^+$ = 681.2699, found: $[M+H]^+$ = 681.2692.

79. N-(3-(7-(4-fluorobenzyl)-2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-6-methyl-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl)phenyl)acrylamide (2l)

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 9.04 (s) and 8.89 (s) (together 1H), 7.93-7.66 (m, 3H), 7.44 (s, 1H), 7.36-6.93 (m, 6H), 6.87-6.81 (m, 1H), 6.42 (s) and 6.38 (s) (together 2H), 6.20 (d, *J* = 10.0 Hz) and 6.16 (d, *J* = 10.0 Hz) (together 1H), 6.10-6.03 (m, 1H), 5.74 (d, *J* = 10.4 Hz, 1H), 4.53-4.45 (m, 1H), 3.82 (s) and 3.78 (s) (together 3H), 3.58-3.52 (m), 3.21-3.17 (m) and 3.05-3.02 (m) (together 2H), 3.15 (s) and 3.01 (s) (together 3H), 3.10 (br, 4H), 2.63-2.58 (br, 4H), 2.40 (s) and 2.37 (s) (together 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.88, 166.02, 163.58, 163.30, 162.88, 162.68, 160.92, 158.15, 148.92, 147.69, 139.37, 131.73, 131.70, 130.97, 130.79, 130.72, 130.58, 130.52, 130.13, 128.03, 124.36, 120.17, 119.97, 119.85, 119.63, 116.18, 116.01, 115.83, 115.66, 107.81, 107.66, 99.64, 68.89, 58.38, 55.63, 55.58, 54.96, 49.41, 45.91, 38.81, 34.61, 32.16, 29.04. HRMS (ESI) Calcd for $[M+H]^+$ = 665.2995, found: $[M+H]^+$ = 665.2982.

80. N-(3-(6-benzyl-2-(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)-5,8-dioxo-7,8-dihydro-5H-pyrimido[4,5-e][1,4]diazepin-9(6H)-yl) phenyl) acrylamide (2m)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 7.96 (s, 1H), 7.77 (d, *J* = 13.2 Hz, 2H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.36-7.30 (m, 6H), 7.01-6.96 (m, 1H), 6.90 (d, *J* = 7.6 Hz, 1H), 6.41-6.37 (m, 2H), 6.19-6.10 (m, 1H), 6.07 (d, *J* = 6.4 Hz, 1H), 5.72 (dd, *J* = 1.2, 6.4 Hz, 1H), 4.84 (s, 2H), 4.00 (s, 2H), 3.81 (s, 3H), 3.12 (t, *J* = 4.8 Hz, 4H), 2.60 (t, *J* = 4.8 Hz, 4H), 2.38 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.77, 165.23, 163.22, 159.34, 158.51, 148.86, 147.59, 139.19, 135.90, 131.07, 130.19, 128.90, 128.39, 128.10, 127.95, 124.09, 120.27, 119.85, 119.62, 109.31, 107.71, 99.54, 55.61, 54.99, 51.44, 51.09, 49.34, 45.89. HRMS (ESI) Calcd for $[M+H]^+$ = 633.2932, found: $[M+H]^+$ = 633.2929.