Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

SUPPLEMENTARY INFO

EVALUATION OF AROMATIC 6-SUBSTITUTED THIENOPYRIMIDINES AS SCAFFOLDS AGAINST PARASITES THAT CAUSE TRYPANOSOMIASIS, LEISHMANIASIS, AND MALARIA.

Jennifer L. Woodring,[a] Gautam Patel,[a] Jessey Erath,[b] Ranjan Behera,[c] Patricia J. Lee,[d] Susan E. Leed,[d] Ana Rodriguez,[b,e] Richard J. Sciotti,[d] Kojo Mensa-Wilmot,[c] and Michael P. Pollastri[*a]

[a]*Northeastern University Department of Chemistry & Chemical Biology, 360 Huntington Avenue, Boston, MA USA. Tel: 617-373-2703; E-mail: m.pollastri@neu.edu*
[b]*New York University School of Medicine, Department of Microbiology, Division of Parasitology, 341 E. 25th St. New York, NY USA*
[c] *University of Georgia Department of Cellular Biology, Athens, GA USA.*
[d] *Experimental Therapeutics, Walter Reed Army Institute for Research,2460 Linden Lane, Silver Spring, MD, 20910*
[e] *Anti-Infectives Screening Core, New York University School of Medicine, New York, NY 10010;*

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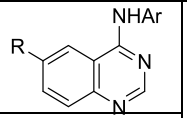
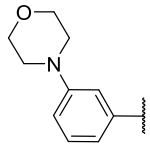
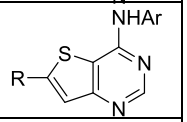
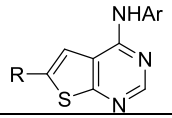
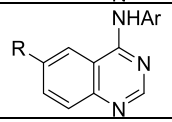
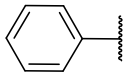
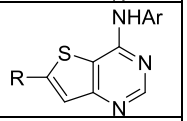
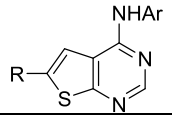
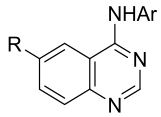
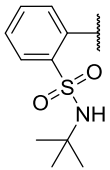
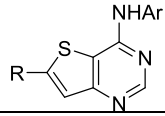
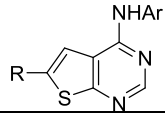
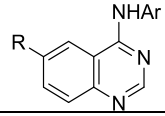
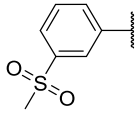
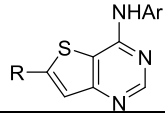
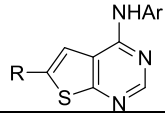
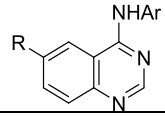
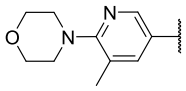
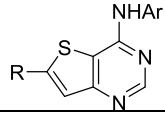
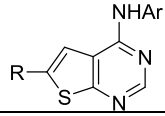
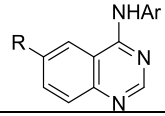
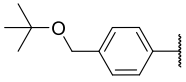
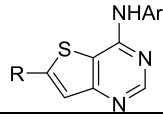
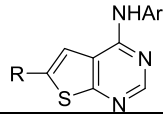
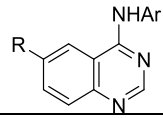
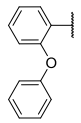
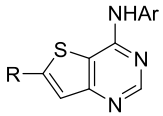
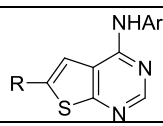
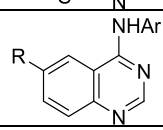
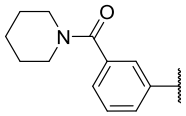
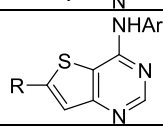
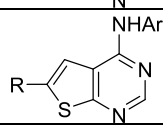
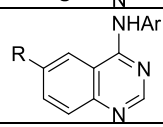
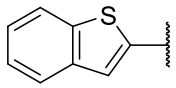
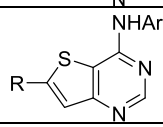
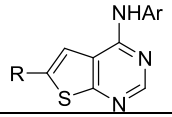
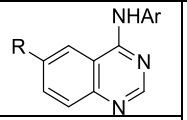
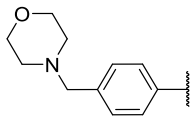
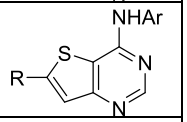
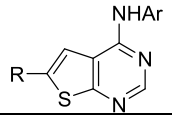
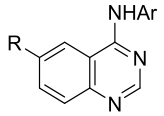
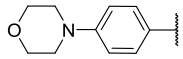
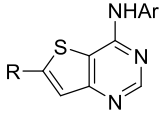
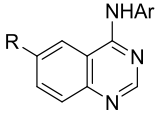
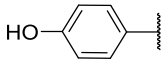
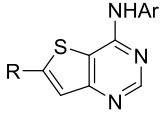
Biological Assay Data Tables

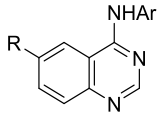
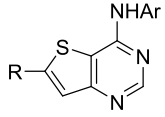
Table S1: Antiparasitic activities of compounds against *T. brucei, T. cruzi, L. major*, and *P. falciparum.*

Cmpd	NEU Number	Scaffold[a]	R	*T. brucei* EC_{50} (μM) or % inh (at 5 μM)[b]	*T. cruzi* EC_{50} (μM) or % inh (at 10 μM)[b]	*L. major*		*P. falciparum*
						Amast EC_{50} (μM)[c]	Promast EC_{50} (μM)[c]	D6 EC_{50} (μM)[d]
2[e]	NEU-617			0.042 ± 0.010	1.8 ± 0.9	7.98 r^2 = 0.75	2.97 r^2 = 0.76	0.23 r^2 = 0.99
11a	NEU-815			42%	>50	5.4 r^2 = 0.81	>20	0.86 r^2 = 0.98
18a	NEU-715			0.40 ± 0.05	2.2 ± 0.4	2.60 r^2 = 0.94	>20	0.52 r^2 = 0.99
4b[e]	NEU-706			3.9 ± 0.3	1.8 ± 0.1	>15	4.05 r^2 = 0.85	0.79 r^2 = 0.90
11b	NEU-747			2.8 ± 0.1	0%	>15	8.14 r^2 = 0.86	2.97 r^2 = 0.98
18b[f]	NEU-739			1.7 ± 0.2	42%	>15	>20	>20

4c[e]	NEU-620			4.7 ± 0.2	6.4 ± 4.0	nd[g]	nd[g]	n[g]
11c	NEU-773			1.2 ± 0.1	0.67 ± 0.06	2.37 r^2 = 0.92	>20	1.07 r^2 = 0.96
18c	NEU-742			0.5 ± 0.2	>50.0	>15	>20	>15
4d[e]	NEU-633			3.2 ± 0.1	5.0 ± 0.0	4.67 r^2 = 0.91	3.49 r^2 = 0.82	0.52 r^2 = 0.98
11d	NEU-774			1.4 ± 0.0	2.7 ± 2.3	>3	>3	0.65 r^2 = 0.96
18d	NEU-743			1.3 ± 0.1	2.4 ± 1.0	>15	11.75 r^2 = 0.90	0.52 r^2 = 0.97
4e	NEU-632			1.0 ± 0.1	49%	1.14 r^2 = 0.85	>20	0.26 r^2 = 0.96
11e	NEU-775			0.084 ± 0.0	3.3 ± 1.2	>3	>3	0.23 r^2 = 0.98
18e	NEU-744			0.42 ± 0.1	29%	>3	>3	0.28 r^2 = 0.99
4f	NEU-635			0.22 ± 0.00	2.2 ± 0.4	4.09 r^2 = 0.92	>20	0.60 r^2 = 0.96

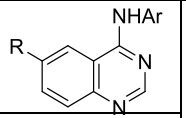
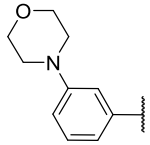
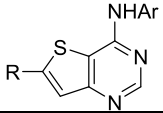
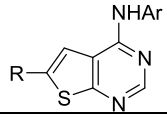
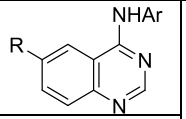
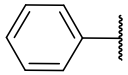
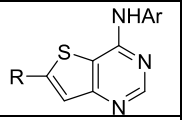
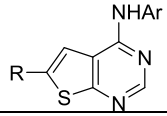
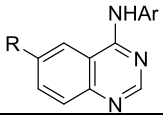
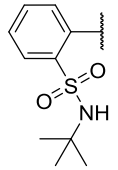
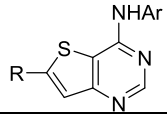
11f	NEU-776			0.89 ± 0.10	30 ± 13	>15	>20	1.65 r^2 = 0.98
18f	NEU-745			1.6 ± 0.0	17%	2.03 r^2 = 0.84	>20	7.79 r^2 = 0.93
4g[e]	NEU-372			5.3 ± 0.2	Nd	>15	6.86 r^2 = 0.78	5.79 r^2 = 0.98
11g	NEU-812			10%	>50.0	3.50 r^2 = 0.84	>20	6.21 r^2 = 0.46
18g	NEU-741			1.2 ± 0. 3	45%	>15	>20	10.78 r^2 = 0.94
4h[e]	NEU-639			0.14 ± 0.1	>50.0	>15	>3	0.51 r^2 = 0.98
11h	NEU-813			46%	3.2 ± 0.5	3.10 r^2 = 0.93	>20	1.17 r^2 =0.94
18h	NEU-746			0.33 ± 0.03	>50.0	>15	>20	2.11 r^2 = 0.98
4i[e]	NEU-371			6.0 ± 0.2	>50.0	>15	>20	0.91 r^2 = 0.99
11i	NEU-814			8%	>50.0	>15	>20	>15

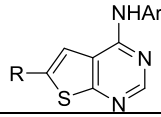
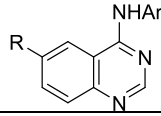
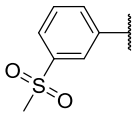
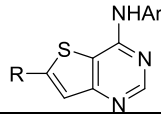
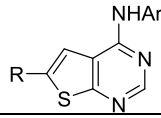
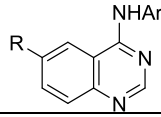
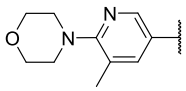
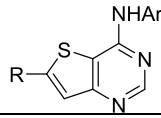
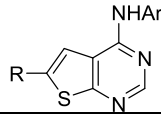
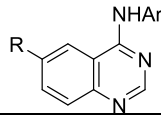
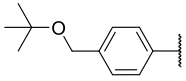
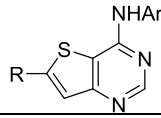
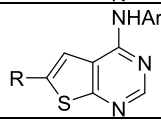
18i	NEU-740			2.9 ± 0.3	49%	>3	>3	>4
4j[e,f]	NEU-636			1.0 ± 0.05	0.60 ± 0.15	5.91 r^2 = 0.94	2.74 r^2 = 0.88	0.063 r^2 =0.99
11j	NEU-1033			1.1 ± 0.0	>50.0	>15.0	0.22 r^2 = 0.84	0.027 r^2 = 0.97
18j	NEU-811			60%	1.7 ± 0.1	4.20 r^2 = 0.91	>20	0.089 r^2 = 0.81
4k[e,f]	NEU-733			1.9 ± 0.9	10.1%	>15	4.40 r^2 = 0.82	0.27 r^2 = 0.98
11k	NEU-749			0.47 ± 0.25	0%	>15	>20	3.10 r^2 = 0.98
4l[e]	NEU-374			6.5 ± 1.0	>50.0	>15	>20	>20
11l	NEU-772			2.2 ± 0.1	0.75 ± 0.02	1.58 r^2 = 0.83	>20	0.44 r^2 = 0.98

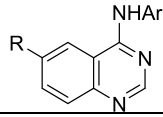
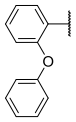
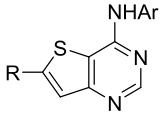
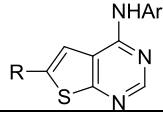
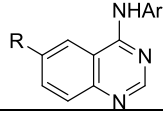
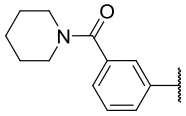
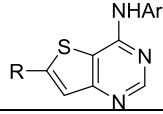
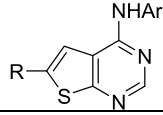
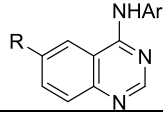
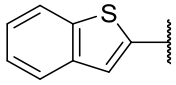
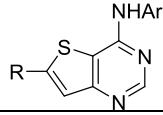
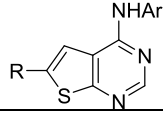
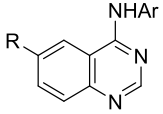
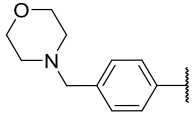
4m	NEU-1010		H	1.3 ± 0.1	2.4 ± 0.2	12.36 r^2 = 0.78	1.75 r^2 = 0.77	5.33 r^2 = 0.69
11m	NEU-920			1%	27 ± 3.5	>15	5.68 r^2 = 0.94	16.4 r^2 = 0.93

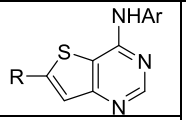
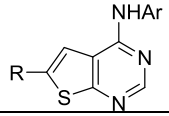
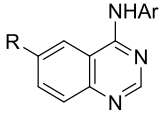
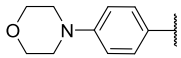
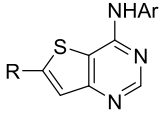
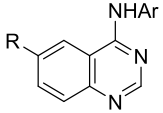
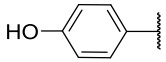
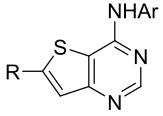
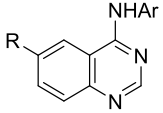
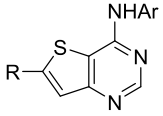
[a]Ar is defined as 3-chloro-4-((3-fluorobenzyl)oxy)phenyl. [b]Compounds showing >75% growth inhibition at 5 or 10 µM for *T. brucei* or *T. cruzi,* respectively, were tested for EC_{50} values. *T. brucei* EC_{50} values are the result of duplicate experiments, within ± 25%, with the exception of **11e** (± 33%), and **11k** (± 52%). *T. cruzi* EC_{50} values are the result of duplicate experiments, within ± 50%, with the exception of **4c** (± 63%), and **11d** (± 85%). [c]Compounds screened against *L. major* amastigotes and promastigotes had r^2 values >0.75. [d]Compounds were tested in duplicate against *P. falciparum* (D6 strain) *and L. major* had r^2 values >0.90 except for **11g** (r^2 = 0.46) and **18j** (r^2 = 0.81) against *P. falciparum.* [e]Previously reported data.[1] [f]All compounds were inactive against HepG2 cell lines except for **4b** (TC_{50} = 4.9 µM), **4j** (12.9 µM), **4k** (9.6 µM), and **18b** (10.0 µM). [g]Not determined due to low solubility in the assay conditions.

Table S2: Inhibition profiles of resistant malaria (*P. falciparum*) strains D6, W2 and C235.

Cmpd	NEU Number	Core[a]	R	D6 EC_{50} (μM)[b]	W2 EC_{50} (μM)[b]	C235 EC_{50} (μM)[b]
2	NEU-617			0.23 r^2 = 0.99	0.68 r^2 = 0.99	0.37 r^2 = 0.98
11a	NEU-815			0.86 r^2 = 0.98	2.40 r^2 = 0.96	1.05 r^2 = 0.98
18a	NEU-715			0.52 r^2 = 0.99	1.14 r^2 = 0.98	0.57 r^2 = 0.97
4b	NEU-706			0.79 r^2 = 0.90	2.59 r^2 = 0.91	1.16 r^2 = 0.92
11b	NEU-747			2.97 r^2 = 0.98	4.22 r^2 = 0.71	2.84 r^2 = 0.97
18b	NEU-739			>20	>20	>20
4c	NEU-620			nd[c]	nd[c]	nd[c]
11c	NEU-773			1.07 r^2 = 0.96	0.69 r^2 = 0.96	1.68 r^2 = 0.96

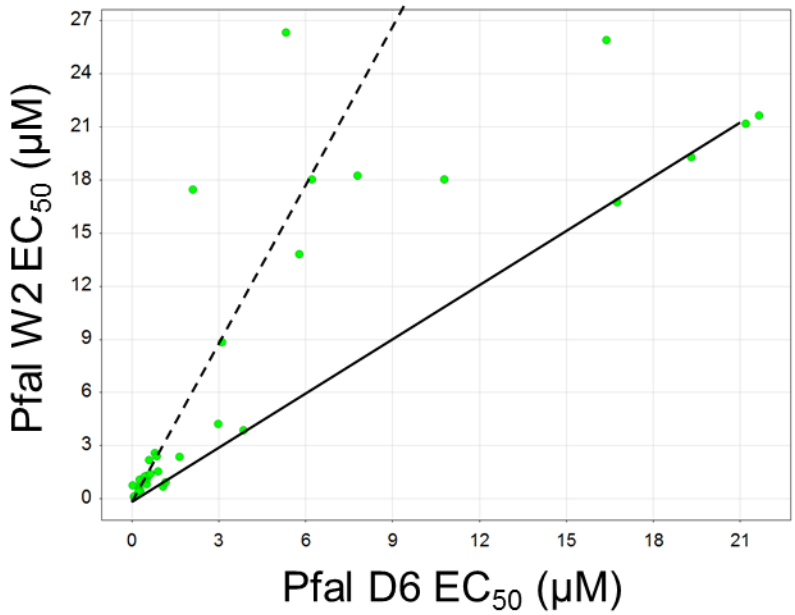
18c	NEU-742			>15	>15	>15
4d	NEU-633			0.52 r^2 = 0.98	1.29 r^2 = 0.98	0.62 r^2 = 0.99
11d	NEU-774			0.65 r^2 = 0.96	1.41 r^2 = 0.95	1.62 r^2 = 0.94
18d	NEU-743			0.52 r^2 = 0.97	1.29 r^2 = 0.98	0.44 r^2 = 1.00
4e	NEU-632			0.26 r^2 = 0.96	0.54 r^2 = 0.96	0.36 r^2 = 0.98
11e	NEU-775			0.23 r^2 = 0.98	0.38 r^2 = 0.94	0.37 r^2 = 0.99
18e	NEU-744			0.28 r^2 = 0.99	0.43 r^2 = 0.97	0.29 r^2 = 0.99
4f	NEU-635			0.60 r^2 = 0.96	2.19 r^2 = 0.91	0.69 r^2 = 0.98
11f	NEU-776			1.65 r^2 = 0.98	2.37 r^2 = 0.87	1.23 r^2 = 0.98
18f	NEU-745			7.79 r^2 = 0.93	>15	7.34 r^2 = 0.93

4g	NEU-372			5.78 r^2 = 0.98	13.8 r^2 = 0.91	7.20 r^2 = 0.99
11g	NEU-812			6.21 r^2 = 0.46	>15	>15
18g	NEU-741			10.78 r^2 = 0.94	>15	>15
4h	NEU-639			0.51 r^2 = 0.98	0.84 r^2 = 0.96	0.53 r^2 = 0.94
11h	NEU-813			1.17 r^2 =0.94	0.94 r^2 = 0.97	3.13 r^2 = 0.98
18h	NEU-746			2.11 r^2 = 0.98	>15	2.06 r^2 = 0.97
4i	NEU-371			0.91 r^2 = 0.99	1.5 r^2 = 0.98	1.04 r^2 = 0.99
11i	NEU-814			>15	>15	>15
18i	NEU-740			>4	>4	>4
4j	NEU-636			0.063 r^2 =0.99	0.10 r^2 = 0.97	0.085 r^2 = 0.98

11j	NEU-1033			0.027 r^2 = 0.97	0.76 r^2 = 0.99	0.037 r^2 = 0.97
18j	NEU-811			0.089 r^2 = 0.81	0.15 r^2 = 0.98	0.068 r^2 = 0.99
4k	NEU-733			0.27 r^2 = 0.98	1.07 r^2 = 0.97	0.29 r^2 = 0.99
11k	NEU-749			3.10 r^2 = 0.98	8.83 r^2 = 0.95	3.29 r^2 =0.98
4l	NEU-374			>20	>20	>20
11l	NEU-772			0.44 r^2 = 0.98	1.27 r^2 = 0.99	0.57 r^2 = 0.98
4m	NEU-1010		H	5.33 r^2 = 0.69	>25	>25
11m	NEU-920			16.4 r^2 = 0.93	>20	>20

[a]Ar is defined as 3-chloro-4-((3-fluorobenzyl)oxy)phenyl. [b]Compounds screened against *P. falciparum* D6, W2 and C235 strains had r^2 values >0.90 except for **11g** (r^2 = 0.46) and **18j** (r^2 = 0.81) for D6, and **11b** (r^2 = 0.71) and **11f** (r^2 = 0.87) for W2. [c]Not determined due to low solubility in the assay conditions.

Figure S1: Correlation of EC_{50} values for W2 or C235 versus D6 of malaria resistant strains. In each plot, the solid line denotes equal potency between drug sensitive (D6) and drug resistant (W2 or C235) strains. The dotted line is the 3-fold sensitivity line; compounds between these two lines are essentially equipotent between drug sensitive and drug resistant strains.



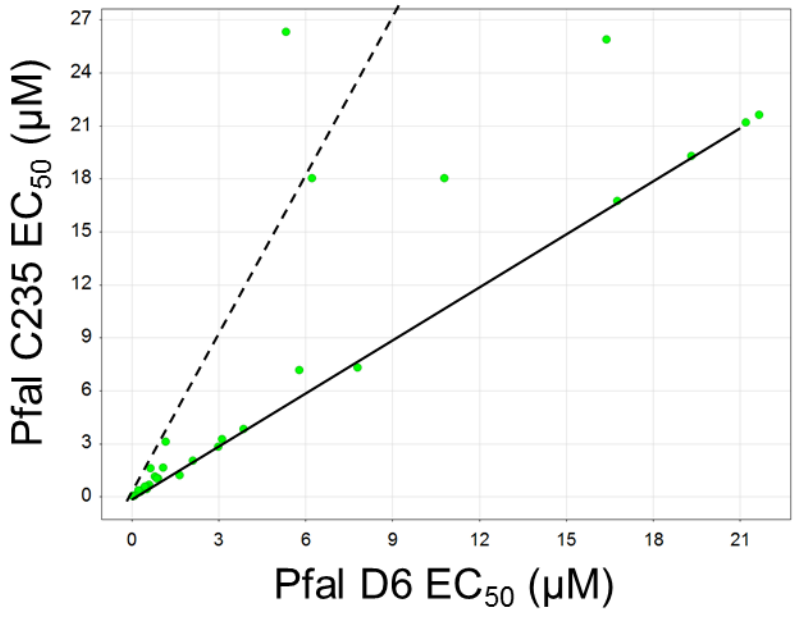
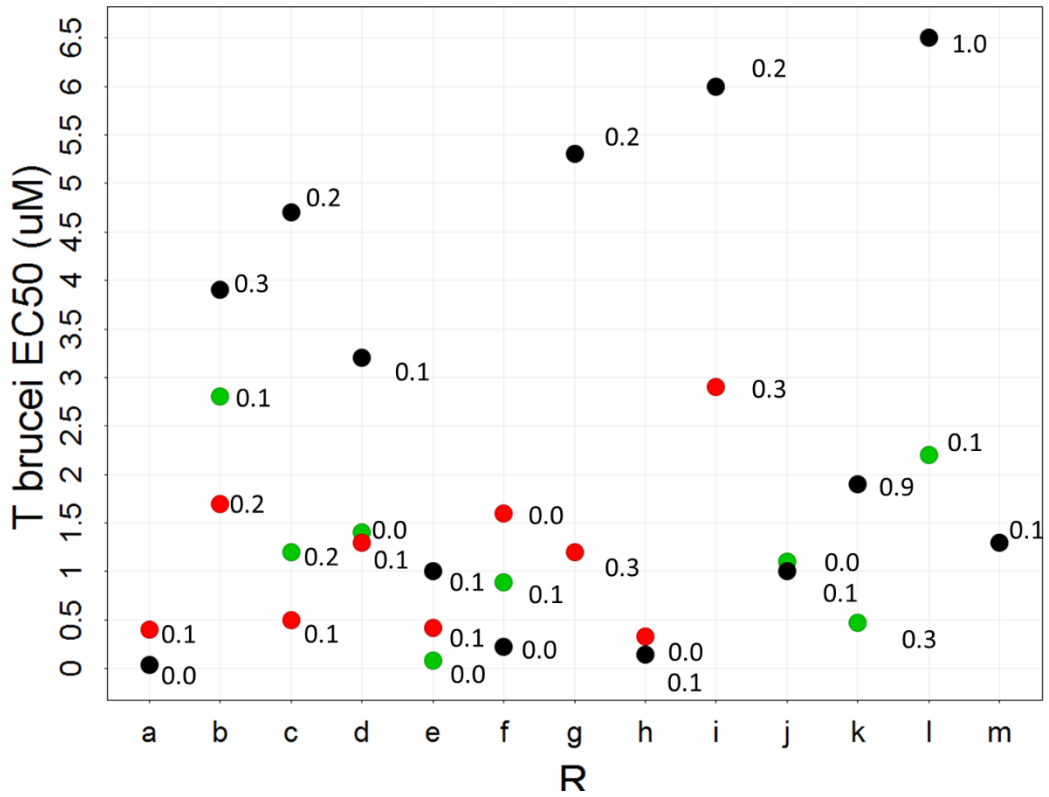


Figure S2. Manuscript Figure 2, annotated with SEM values.



ADME Data Tables

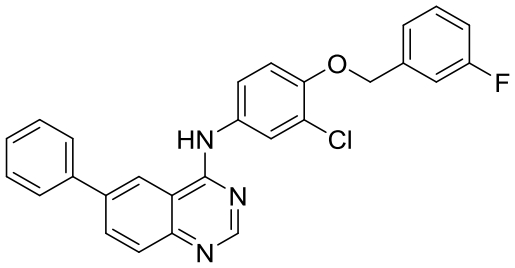
Table S3: ADME properties of compound **2** and **11e**

Compound	Molecular Weight	clogP	ACDLogD	Aqueous Solubility (μM)	Human Plasma Protein Binding %	Human Microsome Cl_{int} (μL/min/mg)	Rat Hepatocytes Cl_{int} (μL/min/10^6 cells)
2	541	7.6	7.3	<1.0	>99.0	63.03	44.5
11e	562	7.4	5.6	<1.0	>99.0	135.2	58.9

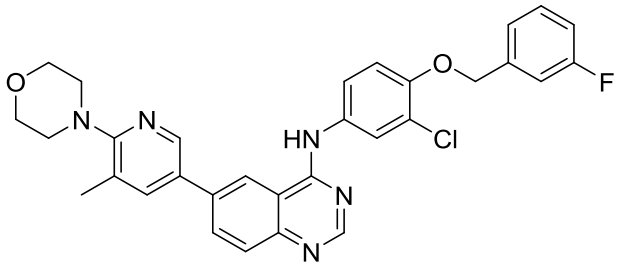
Chemical Synthesis

Most reagents were obtained from Sigma-Aldrich, Inc. (St. Louis, MO), except the boronic esters and acids which were purchased from Frontier Scientific, Inc. (Logan, UT), and all reagents were used as received. NMR spectra were obtained on Varian NMR systems, operating at 400 MHz and 500 MHz for ^{1}H acquisitions. LCMS analysis was performed using a Waters Alliance reverse-phase HPLC, with single-wavelength UV-visible detector and LCTPremier time-of-flight mass spectrometer (electrospray ionization). GCMS analysis was performed using an Agilent gas chromatograph with a transmission quadrupole mass spectrometer (electron impact). All newly synthesized compounds were deemed >95% pure by LCMS analysis.

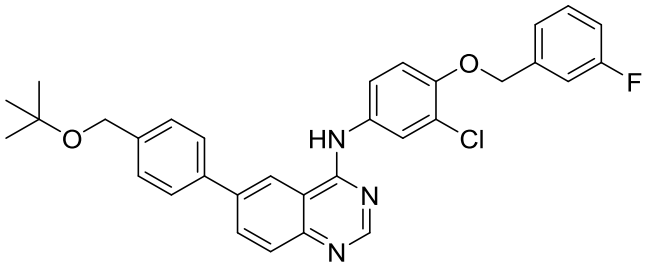
Quinazoline compounds **2** and **4b-m** were synthesized according to previous published methods.[1] Compounds not characterized and disclosed in that reference are listed below.



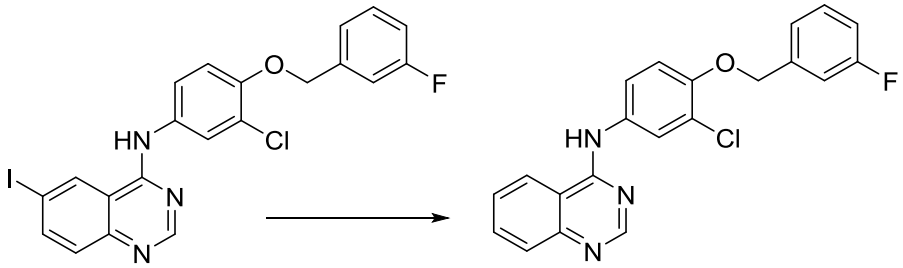
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-phenylquinazolin-4-amine (4b, NEU-706). (Yield: 59%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.27 (s, 2H), 7.19 (td, *J* = 8.7, 2.2 Hz, 1H), 7.31 (m, 3H), 7.46 (m, 2H), 7.56 (t, *J* = 7.8 Hz, 2H), 7.76 (m, 1H), 7.87 (m, 3H), 8.03 (d, *J* = 2.4 Hz, 1H), 8.19 (dd, *J* = 8.8, 1.5 Hz, 1H), 8.60 (s, 1H), 8.81 (d, *J* = 1.0 Hz, 1H), 9.93 (s, 1H). LCMS found 456.0, $[M+H]^+$.



N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(5-methyl-6-morpholinopyridin-3-yl)quinazolin-4-amine (4e, NEU-632). (Yield: 50%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.39 (s, 3 H), 3.16 (m, 4H), 3.77 (m, 4 H), 5.28 (s, 2H), 7.20 (td, *J* = 8.7, 2.2 Hz, 1H), 7.33 (m, 3 H), 7.48 (m, 1H), 7.73 (m, 1H), 7.86 (d, *J* = 8.8 Hz, 1H), 8.04 (d, *J* = 2.0 Hz, 2H), 8.21 (dd, *J* = 8.8, 1.5 Hz, 1H), 8.60 (s, 1H), 8.67 (d, *J* = 2.0 Hz, 1H), 8.79 (d, *J* = 1.50 Hz, 1H), 9.85 (s, 1H). LCMS found 556.2, $[M+H]^+$.

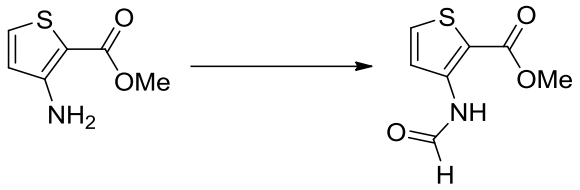


6-(4-(tert-butoxymethyl)phenyl)-N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)quinazolin-4-amine (4f, NEU-635). (Yield: 33%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.28 (s, 9H), 4.54 (s, 2H), 5.28 (s, 2H), 7.19 (m, 1H), 7.32 (m, 3H), 7.41 (d, *J* = 7.3 Hz, 1H), 7.51 (m, 2H), 7.75 (m, 3H), 7.88 (d, *J* = 8.8 Hz, 1H), 8.04 (d, *J* = 2.9 Hz, 1H), 8.18 (dd, *J* = 8.8, 1.5 Hz, 1H), 8.60 (s, 1H), 8.80 (s, 1H), 9.94 (s, 1H). LCMS found 542.3, $[M+H]^+$.

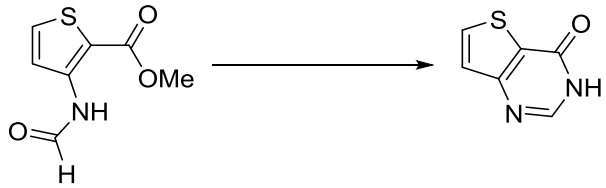


N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)quinazolin-4-amine (4m, NEU-1010). In a microwave tube was added N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-iodoquinazolin-4-amine (0.027 g, 0.054 mmol), potassium carbonate (0.015 g, 0.107 mmol), triphenylphosphine (0.003 g, 0.011 mmol), palladium (II) acetate (0.001 g, 0.003 mmol), and 1 mL of n-butanol. The tube was sealed, purged with nitrogen, and heated in the microwave at 100°C for 45 minutes. The reaction mixture was concentrated under reduced pressure and purified by column chromatography (hexanes/ethyl acetate) to obtain the product in a 26% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.26 (s, 2 H), 7.18 (td, *J* = 8.8, 2.4 Hz, 1 H), 7.27 (d, *J* = 8.8 Hz, 1 H), 7.32 (m, 2 H), 7.47 (m, 1 H), 7.64 (t, *J* = 7.3 Hz, 1 H), 7.74 (dd, *J* = 8.8, 2.4 Hz, 1 H), 7.79 (d,

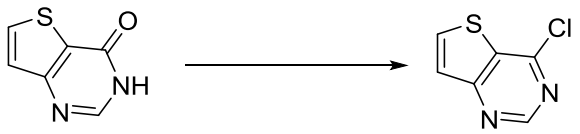
J = 8.3 Hz, 1 H), 7.86 (t, *J* = 8.3 Hz, 1 H), 8.05 (d, *J* = 2.4 Hz, 1 H), 8.50 (d, *J* = 8.3 Hz, 1 H), 8.59 (s, 1 H), 9.79 (s, 1 H). LCMS found 380.0, $[M+H]^+$.



Methyl 3-formamidothiophene-2-carboxylate (6).[2] To a solution of formic acid (25.6 mL, 668 mmol) and acetic anhydride (25.5 mL, 270 mmol) cooled to 0°C was added methyl 3-aminothiophene-2-carboxylate (5.0 g, 31.8 mmol) in small portions over 10 minutes. The reaction stirred for an additional 5 minutes and then brought to room temperature while stirring for 12 hours. Water was added to the reaction mixture and the precipitate was collected by vacuum filtration to obtain the product in 79% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.91 (s, 3 H), 7.50 (d, *J* = 5.4 Hz, 1 H), 8.12 (*J* = 5.4 Hz, 1 H), 8.43 (s, 1 H), 10.11 (s, 1 H). GCMS EI: 185.3, $[M]^{\bullet+}$.

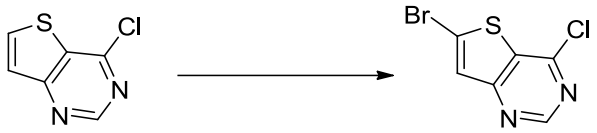


Thieno[3,2-d]pyrimidin-4-ol (7).[2] Formamide (23.25 mL, 583 mmol) and ammonium formate (4.78 g, 76.0 mmol) was heated to 150°C. Methyl 3-formamidothiophene-2-carboxylate (4.32 g, 23.34 mmol) was added portion-wise over 10 minutes and the reaction mixture continued at 150°C for 5 hours and then cooled to room temperature for an additional 12 hours. The precipitate was collected by vacuum filtration to obtain the product in 56% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.39 (d, *J* = 5.4 Hz, 1 H), 8.15 (s, 1 H), 8.17 (d, *J* = 5.4 Hz, 1 H), 12.23 (s, 1 H). LCMS found 152.9, $[M+H]^+$.

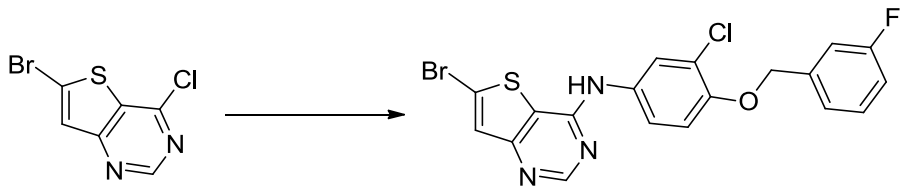


4-chlorothieno[3,2-d]pyrimidine (8). A solution of thieno[3,2-d]pyrimidin-4-ol (0.50 g, 3.30 mmol) in phosphorus oxychloride (3.3 mL, 35.4 mmol) was refluxed at 110°C while under

nitrogen for 12 hours. The reaction was slowly quenched by cooling to 0°C and adding a solution of saturated sodium bicarbonate dropwise. The organic layer was extracted with dichloromethane three times, dried over sodium sulfate, and concentrated under reduced pressure. The desired product was obtained in 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.62 (d, *J* = 5.4 Hz, 1 H), 8.06 (d, *J* = 5.4 Hz, 1 H), 9.01 (s, 1 H). LCMS found 171.0, $[M+H]^+$.

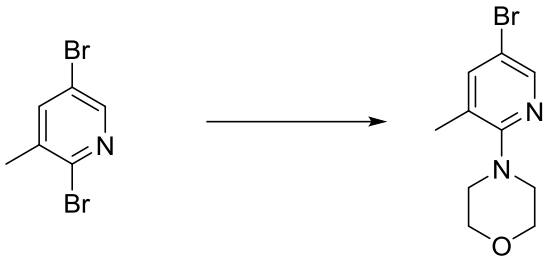


6-bromo-4-chlorothieno[3,2-d]pyrimidine (9).[3] To a flame dried flask that was purged with nitrogen and cooled to 0°C was added anhydrous tetrahydrofuran (5 mL) and diisopropylamine (0.519 mL, 3.64 mmol). N-butyllithium (2.0 molar, 1.734 mL, 3.47 mmol) was added dropwise while under nitrogen and the reaction mixture stirred at 0°C for 20 minutes and then brought to -78°C. In a separate flame dried flask purged with nitrogen was added 4-chlorothieno[3,2-d]pyrimidine (0.493 g, 2.89 mmol) and anhydrous tetrahydrofuran (5 mL). This solution was added to the first reaction flask dropwise at -78°C and stirred for an additional 20 minutes. 1,2-dibromo-1,1,2,2-tetrafluoroethane (0.444 mL, 4.05 mmol) was added to the reaction dropwise and continued stirring at -78°C for 20 minutes, and then room temperature for 12 hours. The reaction was quenched with water, the organic layer was extracted with dichloromethane three times, dried over sodium sulfate, and concentrated under reduced pressure to obtain the product in 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.60 (s, 1 H), 8.92 (s, 1 H). LCMS found 248.9, $[M+H]^+$.

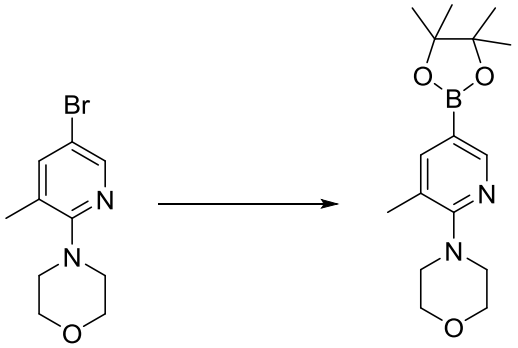


6-bromo-N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)thieno[3,2-d]pyrimidin-4-amine (10). 6-bromo-4-chlorothieno[3,2-d]pyrimidine (0.676 g, 2.71 mmol) and 3-chloro-4-((3-fluorobenzyl)oxy)aniline (0.751 g, 2.98 mmol) were dissolved in 25 mL of 2-propanol and heated to 85°C for 12 hours. The resulting precipitate was collected by vacuum filtration to obtain the product in 88% yield. . ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.28 (s, 2 H), 7.19 (td, *J* = 8.1, 2.2

Hz, 1 H), 7.31 (m, 3 H), 7.47 (m, 1 H), 7.57 (dd, *J* = 8.8, 2.2 Hz, 1 H), 7.74 (s, 1 H), 7.87 (d, *J* = 2.9 Hz, 1 H), 8.71 (s, 1 H), 10.64 (s, 1 H). LCMS found 463.9, $[M+H]^+$.

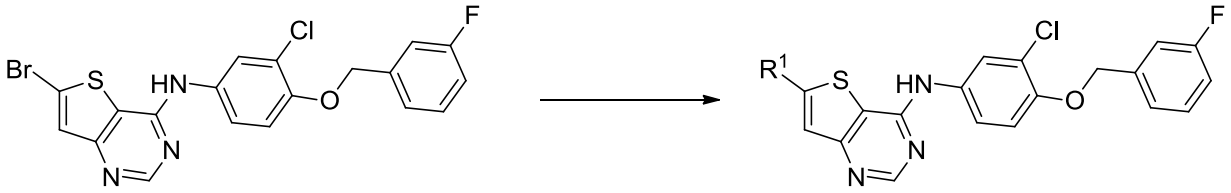


4-(5-bromo-3-methylpyridin-2-yl)morpholine.[4] In a sealed tube, morpholine (1.990 mL, 23.00 mmol) and 2,5-dibromo-3-methylpyridine (0.398 g, 1.586 mmol) were heated in the microwave at 210°C for 10 minutes. The reaction mixture was diluted with water and the organic layer was extracted three times with ethyl acetate. The combined organic layers were concentrated under reduced pressure and the product was separated by column chromotagraphy (hexanes/ethyl acetate) to obtain the product in 97% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.20 (s, 3 H), 3.01 (m, 4 H), 3.70 (m, 4 H), 7.68 (m, 1 H), 8.16 (d, *J* = 2.2 Hz, 1 H). LCMS found 257.0, $[M+H]^+$.

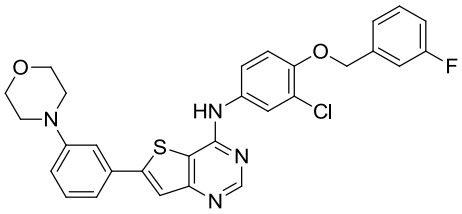


4-(3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)morpholine. A mixture of 4-(5-bromo-3-methylpyridin-2-yl)morpholine (0.738 g, 2.87 mmol), bis(pinacolato)diboron (1.093 g, 4.30 mmol), potassium acetate (0.986 g, 10.04 mmol), and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane adduct (0.117 g, 0.143 mmoL) in dioxane (10 mL) was heated at 85°C for 12 hours. The contents were filtered on celite and concentrated under reduced pressure. The mixture was acidified with 1M HCl and diluted with ethyl acetate. The aqueous layer was extracted three times and combined. Then it was basified with 2M NaOH, diluted with ethyl acetate, and the aqueous layer was extracted three times. The combined aqueous layers were adjusted to pH of 7, diluted with ethyl acetate,

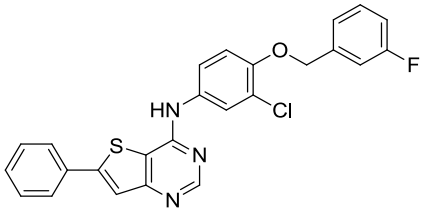
and the organic layer was extracted three times. The combined organic layers were concentrated under reduced pressure. It was then diluted in hexanes and a precipitate was allowed to form overnight. The precipitate was filtered off via vacuum filtration and discarded. The remaining filtrate was concentrated under reduced pressure to obtain the product in 91 % yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.27 (s, 12 H), 2.22 (s, 3 H), 3.12 (m, 4 H), 3.71 (m, 4 H), 7.67 (d, J = 1.0 Hz, 1 H), 8.31 (d, J = 1.5 Hz, 1 H). LCMS found 223.1, $[M+H]^+$, hydrolysis of boronic ester.



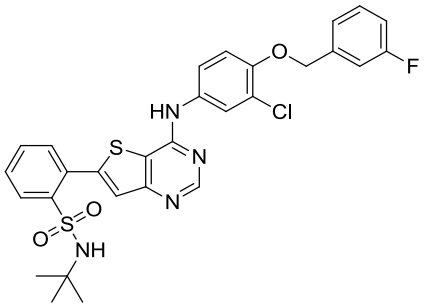
General procedure for Suzuki coupling. 6-bromo-N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)thieno[3,2-d]pyrimidin-4-amine (0.055 g, 0.117 mmol), a 2.0 molar solution of sodium carbonate (0.352 mL, 0.705 mmol) in water, tetrakis(triphenylphosphine)palladium(0) (0.010 g, 0.008 mmol), dimethoxyethane (2 mL), ethanol (1.3 mL), and the respective boronic ester or acid (1.2 equiv) was added together and heated to 80-85^{o}C for 12 hours. The resulting mixture was concentrated under reduced pressure and the product was separated by silica column chromatography (hexanes/ethyl acetate) and purified by reverse phase chromatography (water/acetonitrile).



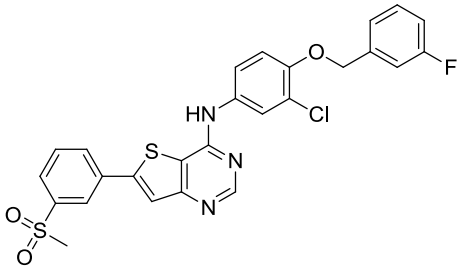
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(3-morpholinophenyl)thieno[3,2-d]pyrimidin-4-amine (11a, NEU-815). (Yield: 12%). **^{1}**H NMR (500 MHz, DMSO-d_6) δ ppm 3.23 (m, 4 H), 3.78 (m, 4 H), 5.25 (s, 2 H), 7.08 (dd, *J* = 7.8, 2.4 Hz, 1 H), 7.18 (td, *J* = 9.3, 2.4 Hz, 1 H), 7.25 (m, 2 H), 7.32 (m, 2 H), 7.37 (d, *J* = 7.8 Hz, 1 H), 7.40 (m, 1 H), 7.47 (m, 1 H), 7.67 (dd, *J* = 8.8, 2.4 Hz, 1 H), 7.94 (s, 1 H), 8.01 (d, *J* = 2.4 Hz, 1 H), 8.59 (s, 1 H), 9.68 (s, 1 H). LCMS found 547.0, $[M+H]^+$.



N-(3-chloro-4-((3-fluorobenzyl)oxy)pheny)-6-phenylthieno[3,2-d]pyrimidin-4-amine (11b, NEU-747). (Yield: 10%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.26 (s, 2 H), 7.18 (td, *J* = 9.2, 2.2 Hz, 1 H), 7.26 (d, *J* = 9.5 Hz, 1 H), 7.32 (m, 2 H), 7.50 (m, 5 H), 7.66 (dd, *J* = 8.8, 2.9 Hz, 1 H), 7.88 (m, 2 H), 7.93 (s, 1 H), 7.99 (m, 1 H), 9.76 (s, 1 H). LCMS found 462.0, $[M+H]^+$.

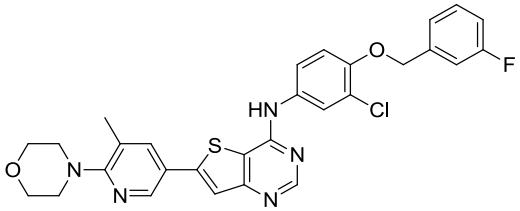


N-(tert-butyl)-2-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)thieno[3,2-d]pyrimidin-6-yl)benzenesulfonamide (11c, NEU-773). (Yield: 18%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.04 (s, 9 H), 5.25 (s, 2 H), 7.18 (td, *J* = 8.4, 2.2 Hz, 1 H), 7.26 (d, *J* = 8.8 Hz, 1 H), 7.32 (m, 2 H), 7.47 (m, 1 H), 7.67 (dd, *J* = 9.0, 2.7 Hz, 1 H), 7.83 (t, *J* = 7.8 Hz, 1 H), 8.03 (m, 2 H), 8.13 (s, 1 H), 8.23 (m, 1 H), 8.36 (t, *J* = 1.7 Hz, 1 H), 8.62 (s, 1 H), 9.79 (s, 1 H). LCMS found 597.0, $[M+H]^+$.

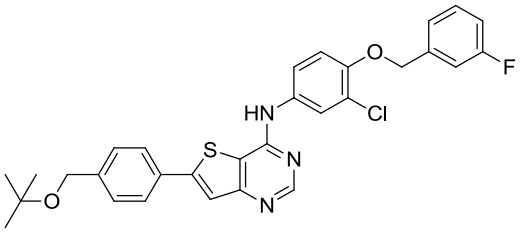


N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(3-(methylsulfonyl)phenyl)thieno[3,2-d]pyrimidin-4-amine (11d, NEU-774). (Yield: 32%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.35 (s, 3H), 5.24 (s, 2 H), 7.18 (td, *J* = 8.4, 2.2 Hz, 1 H), 7.26 (d, *J* = 8.8 Hz, 1 H), 7.32 (m, 2 H), 7.47 (m, 1 H), 7.67 (dd, *J* = 9.0, 2.7 Hz, 1 H), 7.83 (t, *J* = 7.8 Hz, 1 H), 8.03 (m, 2 H), 8.13 (s,

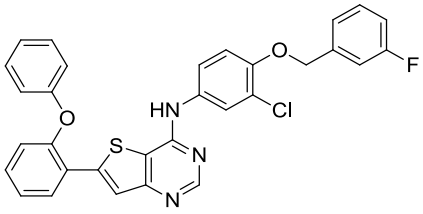
1 H), 8.23 (m, 1 H), 8.36 (t, *J* = 1.7 Hz, 1 H), 8.62 (s, 1 H), 9.79 (s, 1 H). LCMS found 540.0, $[M+H]^+$.



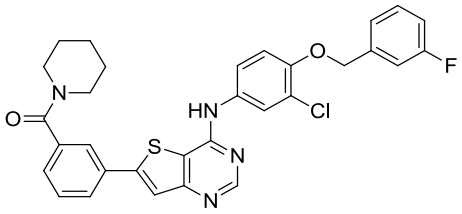
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(5-methyl-6-morpholinopyridin-3-yl)thieno[3,2-d]pyrimidin-4-amine (11e, NEU-775). (Yield: 11%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.34 (s, 3 H), 3.18 (m, 4 H), 3.75 (m, 4 H), 5.25 (s, 2 H), 7.18 (td, *J* = 8.8, 2.4 Hz, 1 H), 7.25 (d, *J* = 8.8 Hz, 1 H), 7.32 (m, 2 H), 7.47 (m, 1 H), 7.65 (dd, *J* = 9.0, 2.7 Hz, 1 H), 7.85 (s, 1 H), 8.00 (dd, *J* = 8.5, 2.2 Hz, 2 H), 8.58 (m, 2 H), 9.7 (s, 1 H). LCMS found 562.1, $[M+H]^+$.



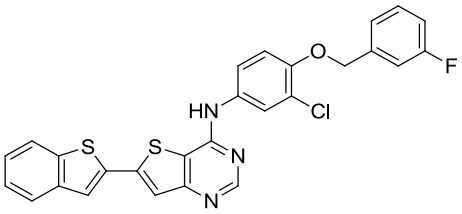
6-(4-(tert-butoxymethyl)phenyl)-N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)thieno[3,2-d]pyrimidin-4-amine (11f, NEU-776). (Yield: 8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.25 (s, 9 H), 4.48 (s, 2 H), 5.25 (s, 2 H), 7.18 (td, *J* = 8.8, 1.0 Hz, 1 H), 7.25 (d, *J* = 8.8 Hz, 1 H), 7.33 (m, 2 H), 7.47 (m, 3 H), 7.66 (m, 1 H), 7.83 (d, *J* = 8.1 Hz, 2 H), 7.89 (s, 1 H), 8.00 (d, *J* = 2.9 Hz, 1 H), 8.58 (s, 1 H), 9.71 (s, 1 H). LCMS found 548.1, $[M+H]^+$.



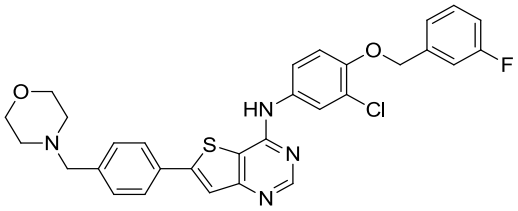
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(2-phenoxyphenyl)thieno[3,2-d]pyrimidin-4-amine (11g, NEU-812). (Yield: 39%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.23 (s, 2 H), 7.02 (d, *J* = 9.3 Hz, 1 H), 7.09 (d, *J* = 7.8 Hz, 2 H), 7.18 (m, 3 H), 7.32 (m, 3 H), 7.45 (m, 4 H), 7.62 (d, *J* = 8.8 Hz, 1 H), 7.94 (m, 2 H), 8.09 (d, *J* = 7.3 Hz, 1 H), 8.56 (s, 1 H), 9.66 (s, 1 H). LCMS found 554.0, $[M+H]^+$.



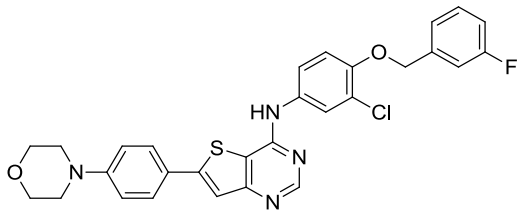
(3-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)thieno[3,2-d]pyrimidin-6-yl)phenyl)(piperidin-1-yl)methanone (11h, NEU-813). (Yield: 46%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.59 (m, 6 H), 2.54 (s, 2 H) 3.62 (s, 2 H), 5.25 (s, 2 H), 7.18 (td, *J* = 8.5, 2.4 Hz, 1 H), 7.25 (d, *J* = 8.8 Hz, 1 H), 7.32 (m, 2 H), 7.46 (m, 2 H), 7.6 (t, *J* = 8.3 Hz, 1 H), 7.66 (dd, *J* = 8.8, 2.4 Hz, 1 H), 7.84 (m, 1 H), 7.92 (m, 1 H), 7.98 (d, *J* = 2.4 Hz, 1 H), 8.00 (s, 1 H), 8.59 (s, 1 H), 9.74 (s, 1 H). LCMS found 573.0, $[M+H]^+$.



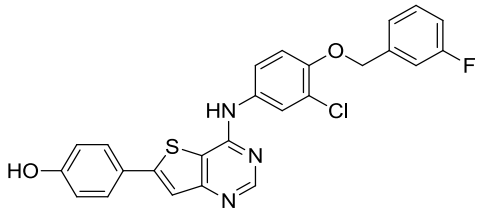
6-(benzo[b]thiophen-2-yl)-N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)thieno[3,2-d]pyrimidin-4-amine (11i, NEU-814). (Yield: 19%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.26 (s, 2 H), 7.18 (td, *J* = 8.3, 2.4 Hz, 1 H), 7.26 (d, *J* = 9.3 Hz, 1 H), 7.33 (m, 2 H), 7.46 (m, 3 H), 7.66 (dd, *J* = 8.8, 2.4 Hz, 1 H), 7.83 (s, 1 H), 7.95 (m, 1 H), 7.99 (d, *J* = 2.9 Hz, 1 H), 8.01 (s, 1 H), 8.05 (m, 1 H), 8.60 (s, 1 H), 9.78 (s, 1 H). LCMS found 518.0, $[M+H]^+$.



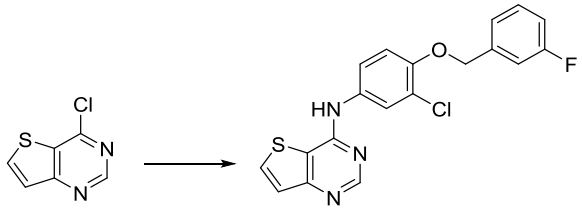
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(4-(morpholinomethyl)phenyl)thieno[3,2-d]pyrimidin-4-amine (11j, NEU-1033). (Yield: 42%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.38 (s, 4 H), 3.52 (s, 2 H), 3.59 (m, 4 H), 5.24 (s, 2 H), 7.18 (td, *J* = 8.5, 2.4 Hz, 1 H), 7.25 (d, *J* = 9.3 Hz, 1 H), 7.32 (m, 2 H), 7.46 (m, 3 H), 7.66 (dd, J = 8.8, 2.4 Hz, 1 H), 7.83 (d, *J* = 8.3 Hz, 2 H), 7.88 (s, 1 H), 7.99 (d, *J* = 2.9 Hz, 1 H), 8.58 (s, 1 H), 9.70 (s, 1 H). LCMS found 561.0, $[M+H]^+$.



N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(4-morpholinophenyl)thieno[3,2-d]pyrimidin-4-amine (11k, NEU-749). (Yield: 9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.23 (m, 4 H), 3.76 (m, 4 H), 5.25 (s, 2 H), 7.07 (d, *J* = 8.8 Hz, 2 H), 7.18 (td, *J* = 9.3, 2.0 Hz, 1 H), 7.24 (d, *J* = 9.3 Hz, 1 H), 7.32 (m, 2 H), 7.47 (m, 1 H), 7.65 (dd, *J* = 8.8, 2.4 Hz, 1 H), 7.72 (m, 2 H), 7.74 (m, 1 H), 7.98 (d, *J* = 2.4 Hz, 1 H), 8.54 (s, 1 H), 9.60 (s, 1 H). LCMS found 547.1, $[M+H]^+$.



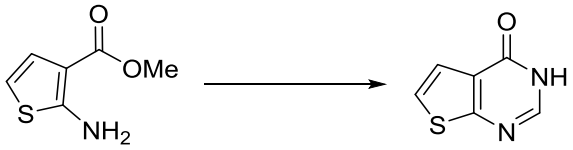
4-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)thieno[3,2-d]pyrimidin-6-yl)phenol (11l, NEU-772). (Yield: 20%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.25 (s, 2 H), 6.90 (d, *J* = 8.8 Hz, 2 H), 7.18 (td, *J* = 8.5, 2.4 Hz, 1 H), 7.24 (d, *J* = 8.8 Hz, 1 H), 7.32 (m, 2 H), 7.47 (m, 1 H), 7.65 (dd, *J* = 8.8, 2.4 Hz, 1 H), 7.70 (m, 3 H), 7.98 (d, *J* = 2.4 Hz, 1 H), 8.54 (s, 1 H), 9.61 (s, 1 H), 10.02 (s, 1 H). LCMS found 478.0, $[M+H]^+$.



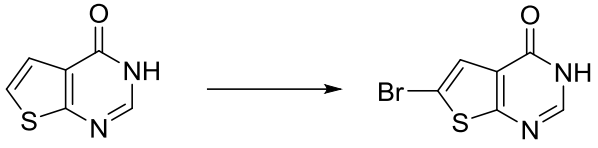
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)thieno[3,2-d]pyrimidin-4-amine (11m, NEU-920). 4-chlorothieno[3,2-d]pyrimidine (0.053 g, 0.312 mmol) and 3-chloro-4-((3-fluorobenzyl)oxy)aniline (0.086 g, 0.344 mmol) were dissolved in 3 mL of 2-propanol and heated to 85°C for 12 hours. The mixture was concentrated under reduced pressure and the product was purified by column chromatography (hexanes/ethyl acetate) in 63% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.24 (s, 2 H), 7.18 (td, *J* = 8.8, 2.9 Hz, 1 H), 7.24 (d, *J* = 8.8 Hz, 1 H),

7.31 (m, 2 H), 7.46 (m, 2 H), 7.65 (dd, *J* = 9.2, 2.6 Hz, 1 H), 7.98 (d, *J* = 2.2 Hz, 1 H), 8.22 (d, *J* = 5.1 Hz, 1 H), 8.58 (s, 1 H), 9.70 (s, 1 H). LCMS found 386.1, $[M+H]^+$.

Methyl 2-aminothiophene-3-carboxylate (13).[5] To a stirred solution of methyl 2-cyanoacetate (8.80 mL, 100 mmol) and 1,4-dithiane-2,5-diol (7.61 g, 50.0 mmol) in 40 mL of DMF was added triethylamine (6.97 mL, 50.0 mmol) dropwise over a period of 10 minutes at 25 °C. The reaction mixture was stirred at 45°C for 2 hours and then cooled to room temperature. The mixture was diluted with 0.4 M acetic acid until the pH was 7, and the organic layer was extracted with diethyl ether (100 mL x 3). The combined organic phases were dried over anhydrous sodium sulfate, and evaporation of solvent afforded the product in 58% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.81 (s, 3 H), 5.90 (s, 2 H), 6.18 (d, *J* = 5.9 Hz, 1 H), 6.96 (d, *J* = 5.9 Hz, 1 H). LCMS found 158.1, $[M+H]^+$.

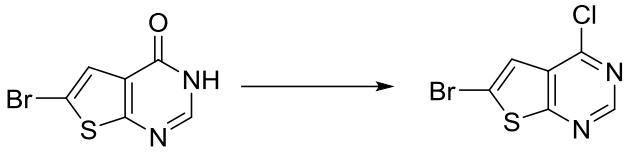


Thieno[2,3-d]pyrimidin-4(3H)-one (14). A solution of methyl 2-aminothiophene-3-carboxylate (6.7 g, 42.6 mmol) and formamide (34.0 mL, 852 mmol) was stirred at 170°C for 6 hours. The reaction mixture was cooled to room temperature and 100 mL of water was added. It was allowed to stand overnight and then filtered, washed thoroughly with water, and air dried to afford the product in a 38 % yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.39 (d, *J* = 5.9 Hz, 1 H), 7.58 (d, *J* = 5.9 Hz, 1 H), 8.12 (s, 1 H), 12.49 (s, 1 H). LCMS found 153.1 $[M+H]^+$.

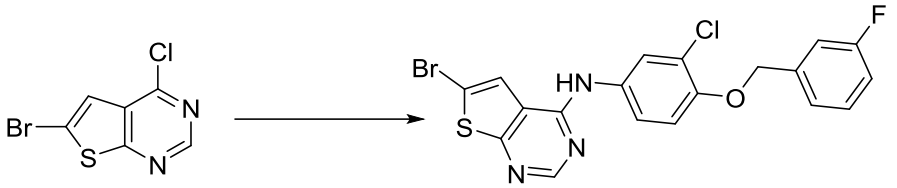


6-bromothieno[2,3-d]pyrimidin-4(3H)-one (15).[6] To a solution of thieno[2,3-d]pyrimidin-4(3H)-one (2.34 g, 15.38 mmol) in acetic acid (30 mL) was added bromine (1.584 mL, 30.8 mmol). The reaction mixture was stirred at 80°C for 2 hours and then cooled to 25°C. The mixture was

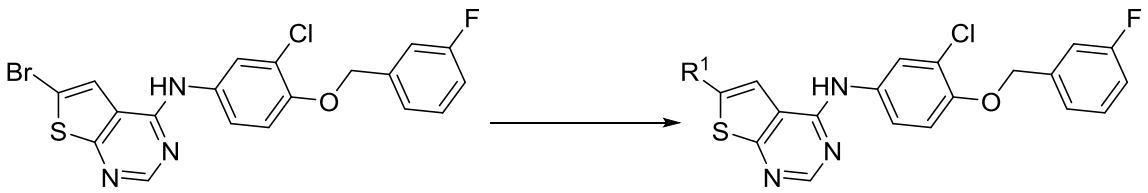
poured over a saturated sodium bicarbonate solution on ice, and the precipitated was filtered, washed with water, and dried to afford the product in 79 % yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.55 (s, 1 H), 8.14 (s, 1 H), 12.65 (s, 1 H). LCMS found 230.9, $[M+H]^+$.



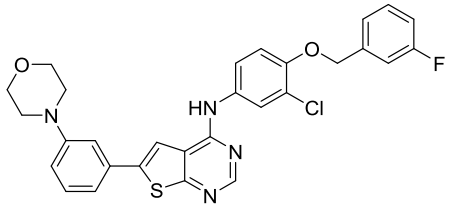
6-bromo-4-chlorothieno[2,3-d]pyrimidine (16).[6] 6-bromothieno[2,3-d]pyrimidin-4(3H)-one (0.800 g, 3.46 mmol) was dissolved in phosphorous oxychloride (5 mL, 53.60 mmol) and heated at 100 °C for 2 hours. The reaction mixture was cooled to room temperature and poured over a saturated sodium bicarbonate solution on ice dropwise. The precipitate was filtered, dried, and purified by flash column chromatography (hexanes/ethyl acetate) to afford the product in a 75 % yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.86 (s, 1 H), 8.93 (s, 1 H). LCMS found 248.9, $[M+H]^+$.



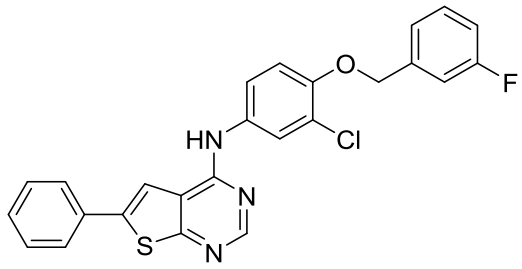
6-bromo-N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)thieno[2,3-d]pyrimidin-4-amine (17).[6] To a solution of 6-bromo-4-chlorothieno[2,3-d]pyrimidine (0.79 g, 3.17 mmol) in 15 mL of 2-propanol was added 3-chloro-4-((3-fluorobenzyl)oxy)aniline (0.797 g, 3.17 mmol) and stirred at 85 °C for 6 hours. The precipitate was filtered off, washed with cold 2-propanol (10 mL), and dried to afford the product in 88 % yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.24 (s, 2H), 7.18 (td, J = 8.6, 2.4 Hz, 1H), 7.29 (m, 3H), 7.46 (m, 1H), 7.67 (dd, J = 9.0, 2.7 Hz, 1H), 8.02 (d, J = 2.4 Hz, 1H), 8.12 (s, 1H), 8.49 (s, 1H), 9.80 (s, 1H). LCMS found 463.8, $[M+H]^+$.



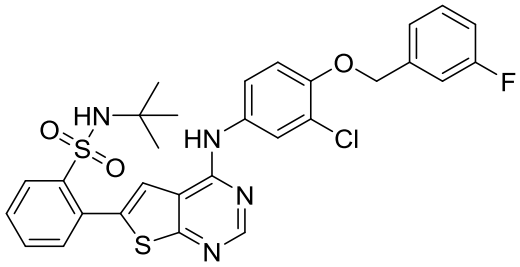
General procedure for Suzuki coupling. 6-bromo-N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)thieno[2,3-d]pyrimidin-4-amine (0.050 g, 0.108 mmol), 2M sodium carbonate (0.323 mL, 0.646 mmol) in water, tetrakis(triphenylphosphine)palladium(0) (0.009 g, 0.008 mmol), 0.4 mL of dimethoxyethane, 0.267 mL of ethanol, and the respective boronic acid or ester (0.118 mmol). The reaction mixture was heated on a shaker plate at 85 °C for 12 hours. Volatile organic substances were evaporated off under reduced pressure. The product was purified by preparative HPLC (water/acetonitrile).



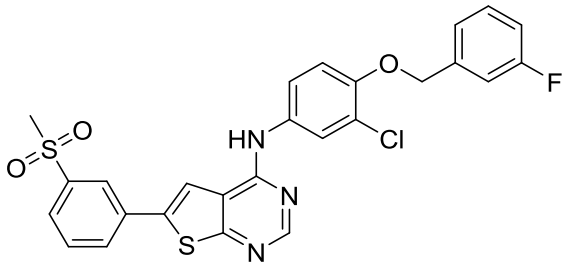
N-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(3-morpholinophenyl)thieno[2,3-d]pyrimidin-4-amine (18a, NEU-715). (Yield: 39%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.21 (m, 4H), 3.78 (m, 4H), 5.25 (s, 2H), 7.04 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.27 (m, 7H), 7.47 (m, 1H), 7.71 (dd, *J* = 8.8, 2.4 Hz, 1H), 8.05 (d, *J* = 2.9 Hz, 1H), 8.18 (s, 1H), 8.49 (s, 1H), 9.64 (s, 1H). LCMS found 547.0, $[M+H]^+$.



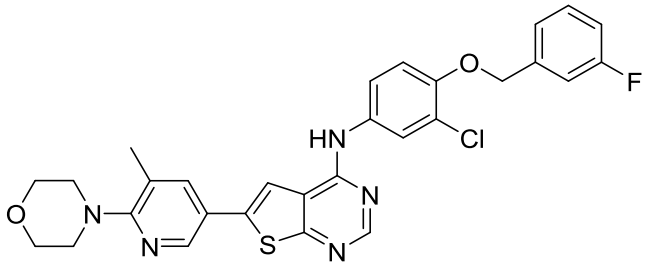
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-phenylthieno[2,3-d]pyrimidin-4-amine (18b, NEU-739). (Yield: 20%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.24 (s, 2H), 7.17 (td, *J* = 8.7, 2.2 Hz, 1H), 7.29 (m, 3H), 7.44 (m, 2H), 7.53 (t, *J* = 7.6 Hz, 2H), 7.69 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.72 (d, *J* = 7.3 Hz, 2H), 8.04 (d, *J* = 2.4 Hz, 1H), 8.21 (s, 1H), 8.49 (s, 1H), 9.66 (s, 1H). LCMS found 461.9, $[M+H]^+$.



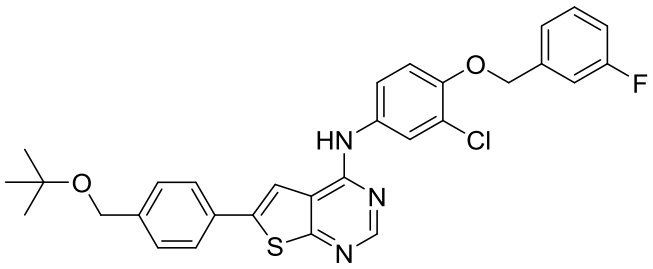
N-(tert-butyl)-2-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)thieno[2,3-d]pyrimidin-6-yl)benzenesulfonamide (18c, NEU-742). (Yield: 25%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.03 (s, 9H), 5.22 (s, 2H), 7.16 (m, 2H), 7.28 (m, 3H), 7.45 (m, 1H), 7.57 (dd, *J* = 7.1, 1.2 Hz, 1H), 7.67 (m, 3H), 7.89 (s, 1H), 8.04 (d, *J* = 2.4 Hz, 1H), 8.11 (m, 1H), 8.51 (s, 1H), 9.65 (s, 1H). LCMS found 597.0, $[M+H]^+$.



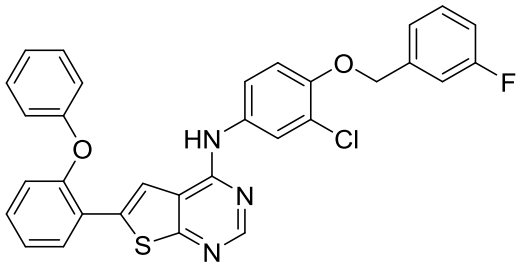
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(3-(methylsulfonyl)phenyl)thieno[2,3-d]pyrimidin-4-amine (18d, NEU-743). (Yield: 36%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.32 (s, 3H), 5.25 (s, 2H), 7.18 (d, *J* = 2.0 Hz, 1H), 7.31 (m, 3H), 7.47 (m, 1H), 7.70 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.81 (m, 1H), 8.00 (m, 2H), 8.06 (d, *J* = 2.4 Hz, 1H), 8.27 (s, 1H), 8.42 (s, 1H), 8.53 (s, 1H), 9.80 (s, 1H). LCMS found 540.0, $[M+H]^+$.



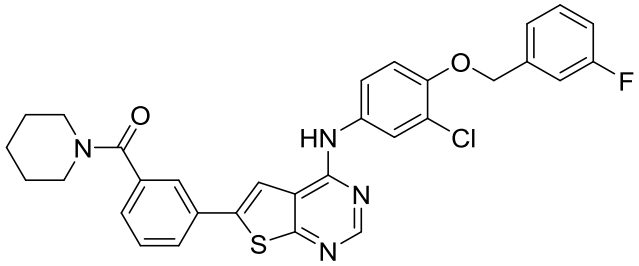
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(5-methyl-6-morpholinopyridin-3-yl)thieno[2,3-d]pyrimidin-4-amine (18e, NEU-744). (Yield: 28%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.32 (s, 3H), 3.13 (m, 4H), 3.73 (m, 4H), 5.22 (s, 2H), 7.16 (m, 1H), 7.28 (m, 3H), 7.45 (m, 1H), 7.68 (m, 1H), 7.79 (d, *J* = 1.5 Hz, 1H), 8.03 (m, 1H), 8.09 (s, 1H), 8.45 (m, 2H), 9.61 (s, 1H). LCMS found 562.0, $[M+H]^+$.



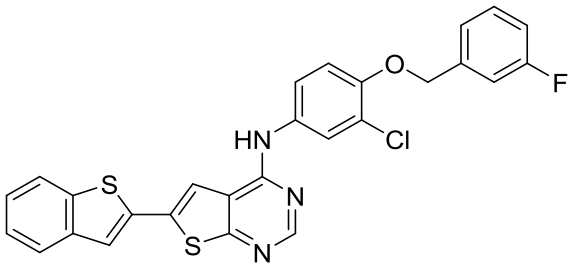
6-(4-(tert-butoxymethyl)phenyl)-N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)thieno[2,3-d]pyrimidin-4-amine (18f, NEU-745). (Yield: 22%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.24 (s, 9H), 4.45 (s, 2H), 5.24 (s, 2H), 7.18 (m, 1H), 7.29 (m, 3H), 7.45 (m, 3H), 7.68 (m, 3H), 8.06 (d, J = 2.4 Hz, 1H), 8.18 (s, 1H), 8.49 (s, 1H), 9.65 (s, 1H). LCMS found 548.0, $[M+H]^+$.



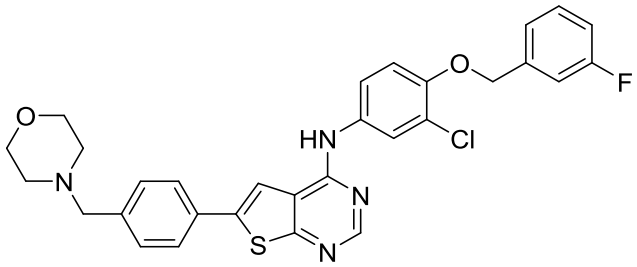
N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(2-phenoxyphenyl)thieno[2,3-d]pyrimidin-4-amine (18g, NEU-741). (Yield: 34%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 5.13 (s, 2H), 6.98 (m, 5H), 7.17 (m, 4H), 7.33 (m, 4H), 7.44 (dd, J = 8.8, 2.4 Hz, 1H), 7.55 (s, 1H), 7.73 (m, 2H), 8.54 (s, 1H). LCMS found 554.0, $[M+H]^+$.



(3-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)thieno[2,3-d]pyrimidin-6-yl)phenyl)(piperidin-1-yl)methanone (18h, NEU-746). (Yield: 38%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.53 (m, 6H), 3.28 (s, 2H), 3.60 (s, 2H), 5.22 (s, 2H), 7.15 (m, 1H), 7.28 (m, 3H), 7.39 (d, J = 7.8 Hz, 1H), 7.45 (m, 1H), 7.56 (m, 1H), 7.68 (m, 2H), 7.73 (d, J = 7.8 Hz, 1H), 8.04 (d, J = 2.4 Hz, 1H), 8.29 (s, 1H), 8.49 (s, 1H), 9.61 (s, 1H). LCMS found 573.1, $[M+H]^+$.



6-(benzo[b]thiophen-2-yl)-N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)thieno[2,3-d]pyrimidin-4-amine (18i, NEU-740). (Yield: 41%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.25 (s, 2H), 7.19 (td, *J* = 8.7, 2.2 Hz, 1H), 7.30 (m, 3H), 7.44 (m, 3H), 7.71 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.78 (s, 1H), 7.90 (dd, *J* = 5.9, 2.9 Hz, 1H), 8.04 (m, 2H), 8.15 (s, 1H), 8.51 (s, 1H), 9.79 (s, 1H). LCMS found 517.9, $[M+H]^+$.



N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(4-(morpholinomethyl)phenyl)thieno[2,3-d]pyrimidin-4-amine (18j, NEU-811). (Yield: 24%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.55 (s, 4H), 3.61 (s, 2H), 3.77 (s, 4H), 5.17 (s, 2H), 6.98 (d, *J* = 8.8 Hz, 1H), 7.03 (td, *J* = 8.4, 2.2 Hz, 1H), 7.22 (m, 2H), 7.36 (m, 2H), 7.44 (d, *J* = 7.8 Hz, 2H), 7.51 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.60 (d, *J* = 7.8 Hz, 2H), 7.81 (d, *J* = 2.4 Hz, 1H), 8.57 (s, 1H). LCMS found 561.2, $[M+H]^+$.

Biological Assays

The protocols for the biological assays of *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major* promastigotes, *Leishmania major* amastigotes, *Plasmodium falciparum* D6, W2, and C235, and HepG2 toxicity were performed as previously described.[7]

References

1. G. Patel, C. E. Karver, R. Behera, P. J. Guyett, C. Sullenberger, P. Edwards, N. E. Roncal, K. Mensa-Wilmot and M. P. Pollastri, *J. Med. Chem.*, 2013, **56**, 3820-3832.
2. M. A. Benish, R. J. A. Budde and M. Lawless, WO2002057271A2, 2002.
3. B. Buchmann, N. Braeuer, M. Koppitz, O. Peters, L. A. Ter, B. Lindenthal, G. Langer and T. Wintermantel, WO2009007421A1, 2009.
4. N. Negoro, Y. Terao, S. Mikami and T. Yukawa, Google Patents, 2010.
5. Y. Huang, S. Wolf, M. Bista, L. Meireles, C. Camacho, T. A. Holak and A. Domling, *Chem Biol Drug Des*, 2010, **76**, 116-129.
6. T. R. Caferro, S. D. Chamberlain, K. H. Donaldson, P. A. Harris, M. D. Gaul, D. E. Uehling and D. E. Vanderwall, WO2003053446A1, 2003.
7. G. Patel, N. E. Roncal, P. J. Lee, S. E. Leed, J. Erath, A. Rodriguez, R. J. Sciotti and M. P. Pollastri, *MedChemComm*, 2014, **5**, 655-658.