Supporting Information

Discovery of Potent KIFC1 Inhibitors Using a Method of Integrated High-Throughput Synthesis and Screening

Bin Yang, Michelle L. Lamb, Tao Zhang, Edward J. Hennessy, Gurmit Grewal, Li Sha, Mark Zambrowski, Michael H. Block, James E. Dowling, Nancy Su, Jiaquan Wu, Tracy Deegan, Keith Mikule, Wenxian Wang, Rüdiger Kaspera, Claudio Chuaqui, Huawei Chen*

Oncology Innovative Medicine Unit, AstraZeneca R&D Boston, Waltham, MA 02451

Keywords: KIFC1, HSET, kinesin, combinatorial chemistry, parallel synthesis, AZ82, centrosome amplification, cancer.

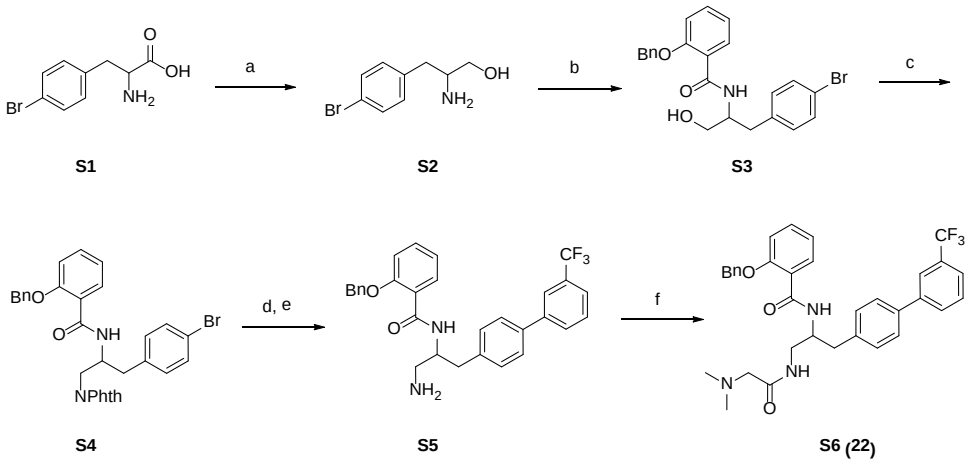
*Phone: 781-839-4135. Fax: 781-839-4230. E-mail: bin.yang@astrazeneca.com.

Table of contents:

Annotation of the table of contents graphic:

KifC1 inhibition by 0.4 uM AZ82 triggers spindle pole declustering in centrosome-amplified breast cancer cells. Fluorescent images of BT-549 cells fixed and stained for DNA (Hoechst, blue), centrosomes (pericentrin, green), mitotic cells (cyclin B, red)

Scheme S1



Reagents and conditions: (a) $BH_3 \cdot Me_2S$, THF, 70°; (b) 2-BnO-PhCOO(C_6F_5), DIPEA, DMF, rt; (c) phthalimide, DIAD, Ph_3P, THF, rt; (d) *m*-CF_3-PhB$(OH)_2$, $Pd(PPh_3)_4$, Cs_2CO_3, 1,4-dioxane/water, reflux; (e) hydrazine, EtOH, reflux; (f) Me_2NCH_2COOH, EDC, DIPEA, DMF, rt.

The *N*,*N*-dimethyl glycinamide side chain of GSK-923295 was incorporated into the phenylalanine scaffold. The reduction of the carboxylic acid group of the bromo phenylalanine compound **S1** using borane dimethyl sulfide complex generated the amino alcohol **S2**. Coupling **S2** with the benzoic activated ester *ortho*-BnO-PhCOO(C_6F_5) gave the amide compound **S3**. Employing a Mitsunobu reaction, the hydroxyl group of compound **S3** was replaced by a phthalimide group to produce compound **S4**. After Suzuki coupling with *meta*-trifluorophenyl boronic acid and subsequent phthalimide deprotection, compound **S4** was converted to the biaryl primary amine compound **S5**. The amide coupling reaction of **S5** with *N*,*N*-dimethylglycine successfully produced compound **22**.

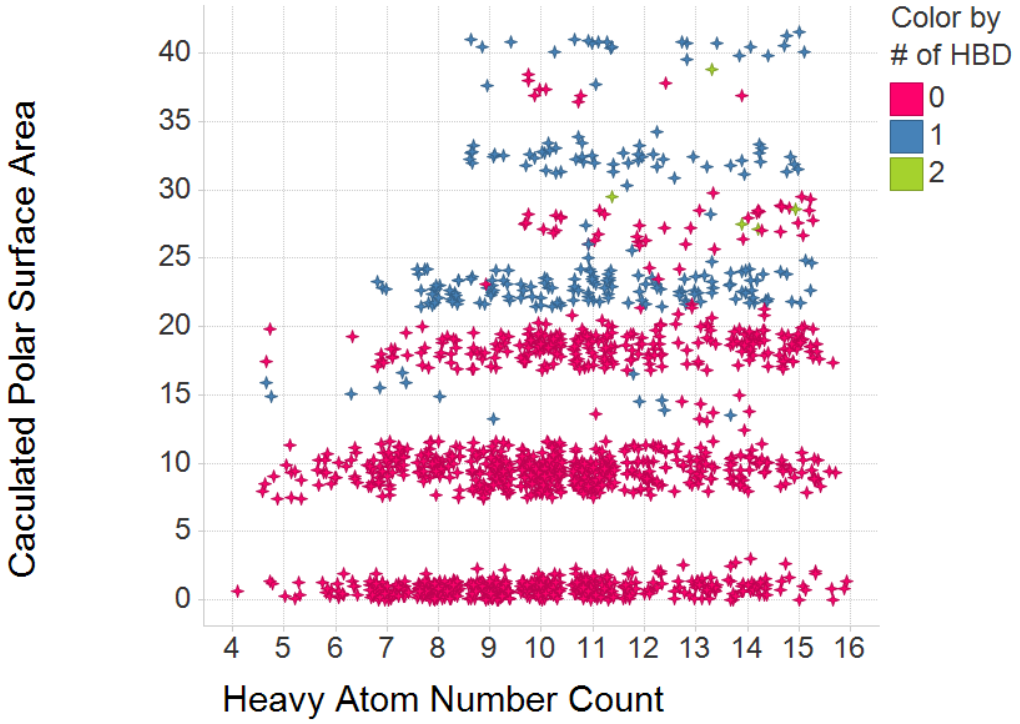
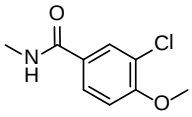


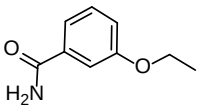
Figure S1. Calculated properties of the R_1 groups (excluding the carboxylic acid group) selected in the high-throughput synthesis

Figure S2. Products detected in the majority of the reactions

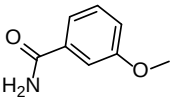
Table S1. IC_{50}s measured for both the isolated pure compounds and their corresponding crude samples synthesized in the high throughout synthesis method.



S23



S24



S25

	Plate Sample IC_{50} (μM)*	Pure compound IC_{50} (μM)
24	2.24	1.3
25	2.45	2.1
26	3.85	2.1
23	4.34	1.9
S7	4.89	5.2
S8	5.60	4.32
S9	7.09	5.2
S10	10.53	7.3
S11	15.55	11.8
S12	16.63	10.2
S13	17.17	12.7
S14	31.80	26.8

	Plate Sample IC_{50} (μM)[a]	Pure compound IC_{50} (μM)
S15	>33.33	44.3
S16	>33.33	34.5
S17	>33.33	74.8
S18	>33.33	>100
S19	>33.33	>100
S20	>33.33	>100
S21	>33.33	>100
S22	>33.33	>100
S23	>33.33	>1000[b]
S24	>33.33	>1000[b]
S25	>33.33	>1000[b]

a. The maximum tested concentration of the plate sample is 33.33 μM

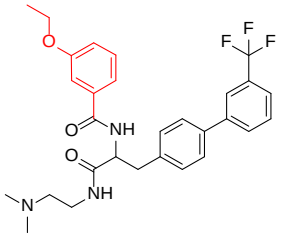
b. *These three amide compounds are used as negative controls in the plate synthesis as they are not reactive towards the amide coupling according* ***Scheme 2****. These amides are also tested separately as fragments at maximum concentration at 1 mM.*

Experimental Section:

The preparation procedure of compound **22** is adopted from the synthesis of GSK923295.[8]

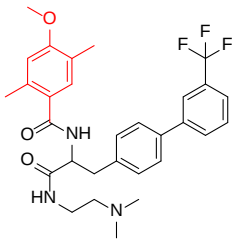
2-(benzyloxy)-N-(1-(2-(dimethylamino)acetamido)-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)benzamide (**22**): ^{1}H NMR (300 MHz, METHANOL-d4) δ ppm 7.75 - 7.87 (m, 3 H), 7.55 - 7.65 (m, 2 H), 7.31 - 7.55 (m, 8 H), 7.13 - 7.29 (m, 3 H), 6.97 - 7.11 (m, 1 H), 5.16 - 5.28 (m, 2 H), 3.42 (d, J = 4.71 Hz, 1 H), 3.23 (d, J = 9.04 Hz, 1 H), 2.81 - 2.93 (m, 3 H), 2.14 - 2.25 (m, 6 H); LCMS (M + H) = 590.

The compounds reported below were used to validate the integrated high throughput synthesis and screening method. All of the products were prepared from compound **11** and the corresponding benzoic acids and the reaction condition utilized is described in general procedure of the amide coupling step (**Scheme 1**, step d). The isolated yield after HPLC purification varies from 40-90%, and the purities of the compounds were established to be > 95% based on LCMS.



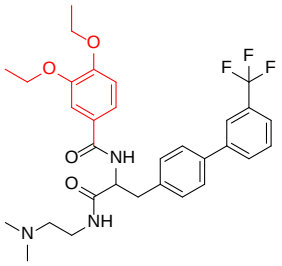
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-3-ethoxybenzamide (**26,** 46.1% yield, 95% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.27 - 9.48 (m, 1 H), 8.68 (d, *J* = 8.10 Hz, 1 H), 8.36 (t, *J* = 5.56 Hz, 1 H), 7.88 - 8.01 (m, 2 H), 7.68 (d, *J* = 7.91 Hz, 4 H), 7.29 - 7.51 (m, 5 H), 6.96 - 7.14 (m, 1 H), 4.69 (d, *J* = 2.83 Hz, 1 H), 4.05 (q, *J* = 6.97 Hz, 2 H), 3.41 - 3.48 (m, 2 H), 3.06 - 3.25 (m, 4 H), 2.82 (d, *J* = 3.96 Hz, 6 H), 1.33 (t, *J* = 6.97 Hz, 3 H); LCMS (M + H) = 528.



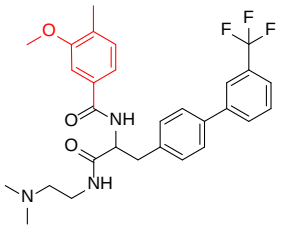
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-4-methoxy-2,5-dimethylbenzamide (**S7**, 40.6% yield, 99% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.23 - 9.44 (m, 1 H), 8.55 (d, *J* = 7.91 Hz, 1 H), 8.28 - 8.43 (m, 1 H), 7.86 - 8.00 (m, 2 H), 7.59 - 7.76 (m, 4 H), 7.32 - 7.51 (m, 4 H), 6.99 (d, *J* = 8.48 Hz, 1 H), 4.61 - 4.74 (m, 1 H), 4.05 (quin, *J* = 6.83 Hz, 4 H), 3.45 (q, *J* = 5.90 Hz, 2 H), 2.98 - 3.26 (m, 4 H), 2.81 (d, *J* = 4.14 Hz, 6 H), 1.33 (t, *J* = 6.97 Hz, 6 H); LCMS (M + H) = 572.



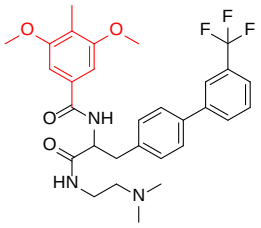
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-3,4-diethoxybenzamide (**S8**, 44.2% yield, 99% purity).

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.23 - 9.44 (m, 1 H), 8.55 (d, *J* = 7.91 Hz, 1 H), 8.28 - 8.43 (m, 1 H), 7.86 - 8.00 (m, 2 H), 7.59 - 7.76 (m, 4 H), 7.32 - 7.51 (m, 4 H), 6.99 (d, *J* = 8.48 Hz, 1 H), 4.61 - 4.74 (m, 1 H), 4.05 (quin, *J* = 6.83 Hz, 4 H), 3.45 (q, *J* = 5.90 Hz, 2 H), 2.98 - 3.26 (m, 4 H), 2.81 (d, *J* = 4.14 Hz, 6 H), 1.33 (t, *J* = 6.97 Hz, 6 H); LCMS (M + H) = 572.



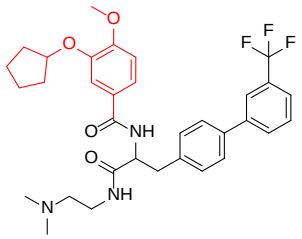
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-3-methoxy-4-methylbenzamide (**S9**, 46.2%, 95% purity).

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.25 - 9.44 (m, 1 H), 8.65 (d, *J* = 7.91 Hz, 1 H), 8.36 (s, 1 H), 7.86 - 8.02 (m, 2 H), 7.68 (d, *J* = 7.91 Hz, 4 H), 7.46 (d, *J* = 8.29 Hz, 2 H), 7.30 - 7.42 (m, 2 H), 7.21 (d, *J* = 7.72 Hz, 1 H), 4.61 - 4.81 (m, 1 H), 3.83 (s, 3 H), 3.45 (d, *J* = 6.22 Hz, 2 H), 2.99 - 3.24 (m, 4 H), 2.82 (d, *J* = 4.33 Hz, 6 H), 2.17 (s, 3 H); LCMS (M + H) = 528.



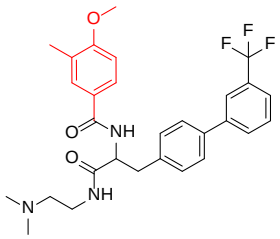
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-3,5-dimethoxy-4-methylbenzamide (**S10**, 45.1% yield, 97% purity).

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.26 - 9.47 (m, 1 H), 8.71 (d, *J* = 8.10 Hz, 1 H), 8.30 - 8.43 (m, 1 H), 7.87 - 8.03 (m, 2 H), 7.61 - 7.79 (m, 4 H), 7.47 (d, *J* = 8.10 Hz, 2 H), 7.08 (s, 2 H), 4.71 (d, *J* = 4.14 Hz, 1 H), 3.82 (s, 6 H), 3.43 - 3.49 (m, 2 H), 3.02 - 3.27 (m, 4 H), 2.82 (d, *J* = 3.77 Hz, 6 H), 2.01 (s, 3 H); LCMS (M + H) = 558.



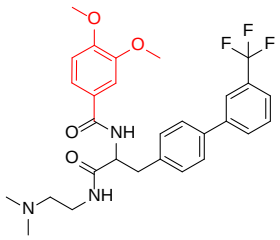
3-(cyclopentyloxy)-N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-4-methoxybenzamide (**S11**, 40% yield, 95% purity).

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.21 - 9.39 (m, 1 H), 8.54 (d, *J* = 8.29 Hz, 1 H), 8.34 (s, 1 H), 7.86 - 8.01 (m, 2 H), 7.61 - 7.76 (m, 4 H), 7.46 (d, *J* = 8.29 Hz, 3 H), 7.33 (d, *J* = 1.88 Hz, 1 H), 6.99 (d, *J* = 8.67 Hz, 1 H), 4.80 (br. s., 1 H), 4.59 - 4.72 (m, 1 H), 3.78 (s, 3 H), 3.45 (m, *J* = 6.20 Hz, 2 H), 3.03 - 3.23 (m, 4 H), 2.82 (br. s., 6 H), 1.78 - 1.97 (m, 2 H), 1.62 - 1.78 (m, 4 H), 1.48 - 1.62 (m, 2 H); LCMS (M + H) = 598.



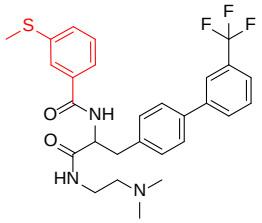
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-4-methoxy-3-methylbenzamide (**S12**, 46% yield, 95% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.24 - 9.47 (m, 1 H), 8.49 (d, *J* = 7.91 Hz, 1 H), 8.33 (t, *J* = 5.46 Hz, 1 H), 7.87 - 8.01 (m, 2 H), 7.61 - 7.76 (m, 6 H), 7.44 (d, *J* = 8.29 Hz, 2 H), 6.98 (d, *J* = 8.48 Hz, 1 H), 4.60 - 4.75 (m, 1 H), 3.83 (s, 3 H), 3.44 (q, *J* = 6.09 Hz, 2 H), 3.00 - 3.26 (m, 4 H), 2.81 (d, *J* = 4.71 Hz, 6 H), 2.17 (s, 3 H); LCMS (M + H) = 528.



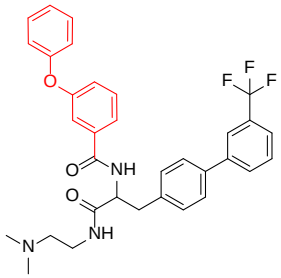
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-2,4-dimethoxybenzamide (**S13**, 50% yield, 95% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.26 - 9.43 (m, 1 H), 8.59 (d, *J* = 8.10 Hz, 1 H), 8.29 - 8.41 (m, 1 H), 7.86 - 8.00 (m, 2 H), 7.68 (d, *J* = 8.29 Hz, 4 H), 7.35 - 7.54 (m, 4 H), 7.01 (d, *J* = 8.48 Hz, 1 H), 4.57 - 4.76 (m, 1 H), 3.81 (d, *J* = 1.00 Hz, 6 H), 3.38 - 3.53 (m, 2 H), 2.98 - 3.24 (m, 4 H), 2.82 (d, *J* = 4.52 Hz, 6 H); LCMS (M + H) = 544.



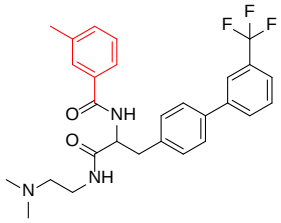
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-3-(methylthio)benzamide (**S14**, 44.2% yield, 99% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.28 - 9.49 (m, 1 H), 8.77 (d, *J* = 7.91 Hz, 1 H), 8.38 (t, *J* = 5.75 Hz, 1 H), 7.89 - 8.00 (m, 2 H), 7.68 (d, *J* = 8.10 Hz, 5 H), 7.52 - 7.63 (m, 1 H), 7.36 - 7.49 (m, 4 H), 4.61 - 4.76 (m, 1 H), 3.40 - 3.50 (m, 2 H), 3.00 - 3.27 (m, 4 H), 2.82 (d, *J* = 3.58 Hz, 6 H), 2.51 (br. s., 3 H); LCMS (M + H) = 530.



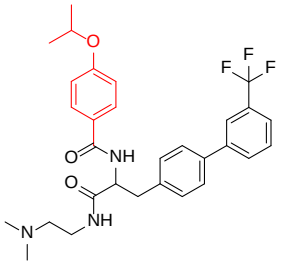
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-3-phenoxybenzamide (**S15**, 35% yield, 99% purity).

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 8.66 (d, *J* = 8.29 Hz, 1 H), 7.87 - 8.07 (m, 3 H), 7.53 - 7.74 (m, 5 H), 7.31 - 7.51 (m, 6 H), 7.11 - 7.23 (m, 2 H), 6.95 - 7.07 (m, 2 H), 4.68 (d, *J* = 3.20 Hz, 1 H), 2.94 - 3.25 (m, 4 H), 2.27 (t, *J* = 6.59 Hz, 2 H), 2.14 (s, 6 H); LCMS (M + H) = 576.



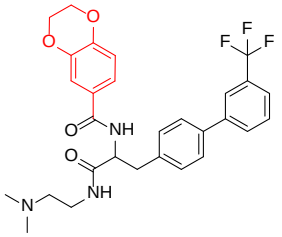
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-3-methylbenzamide (**S16**, 44.2% yield, 95% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.30 - 9.47 (m, 1 H), 8.63 (d, *J* = 8.10 Hz, 1 H), 8.34 (t, *J* = 5.75 Hz, 1 H), 7.87 - 8.02 (m, 2 H), 7.68 (d, *J* = 8.10 Hz, 4 H), 7.44 (d, *J* = 8.29 Hz, 2 H), 6.95 (d, *J* = 2.26 Hz, 2 H), 6.60 (t, *J* = 2.17 Hz, 1 H), 4.68 (br. s., 1 H), 4.03 (q, *J* = 6.97 Hz, 4 H), 3.37 - 3.53 (m, 2 H), 3.00 - 3.26 (m, 4 H), 2.82 (d, *J* = 4.33 Hz, 6 H), 1.31 (t, *J* = 6.97 Hz, 6 H); LCMS (M + H) = 572.



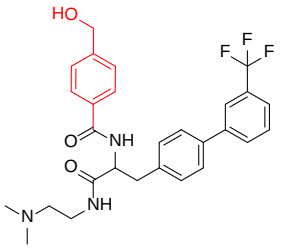
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-4-isopropoxybenzamide (**S17**, 62% yield, 99% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.23 - 9.43 (m, 1 H), 8.53 (d, *J* = 7.91 Hz, 1 H), 8.33 (t, *J* = 5.65 Hz, 1 H), 7.88 - 8.01 (m, 2 H), 7.80 (d, *J* = 8.67 Hz, 2 H), 7.61 - 7.74 (m, 4 H), 7.45 (d, *J* = 8.10 Hz, 2 H), 6.95 (d, *J* = 8.85 Hz, 2 H), 4.58 - 4.74 (m, 2 H), 3.44 (q, *J* = 6.09 Hz, 2 H), 3.02 - 3.23 (m, 4 H), 2.82 (d, *J* = 4.52 Hz, 6 H), 1.27 (d, *J* = 6.03 Hz, 6 H); LCMS (M + H) = 542.



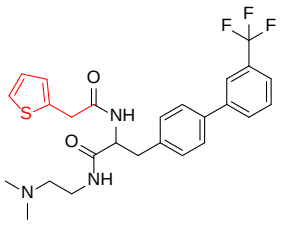
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-2,3-dihydrobenzo[b][1,4]dioxine-6-carboxamide (**S18**, 44.4% yield, 95% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.22 - 9.45 (m, 1 H), 8.53 (d, *J* = 7.91 Hz, 1 H), 8.32 (t, *J* = 5.65 Hz, 1 H), 7.88 - 8.01 (m, 2 H), 7.61 - 7.74 (m, 4 H), 7.30 - 7.50 (m, 4 H), 6.90 (d, *J* = 8.48 Hz, 1 H), 4.57 - 4.71 (m, 1 H), 4.27 (br. s., 4 H), 3.44 (q, *J* = 6.03 Hz, 2 H), 2.98 - 3.23 (m, 4 H), 2.81 (d, *J* = 4.52 Hz, 6 H); LCMS (M + H) = 542.



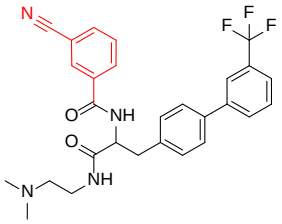
N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)-4-(hydroxymethyl)benzamide (**S19**, 44.9% yield, 95% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.25 - 9.47 (m, 1 H), 8.65 (d, *J* = 8.10 Hz, 1 H), 8.36 (t, *J* = 5.37 Hz, 1 H), 7.89 - 8.02 (m, 2 H), 7.81 (m, *J* = 8.29 Hz, 2 H), 7.68 (d, *J* = 7.91 Hz, 4 H), 7.45 (d, *J* = 8.29 Hz, 2 H), 7.38 (m, *J* = 8.29 Hz, 2 H), 4.63 - 4.78 (m, 1 H), 4.54 (s, 2 H), 3.45 (q, *J* = 6.15 Hz, 2 H), 3.01 - 3.22 (m, 4 H), 2.82 (d, *J* = 4.33 Hz, 6 H); LCMS (M + H) = 514.



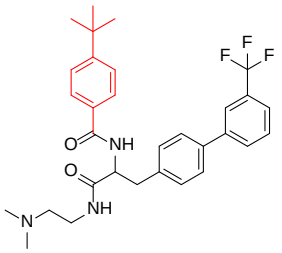
N-(2-(dimethylamino)ethyl)-2-(2-(thiophen-2-yl)acetamido)-3-(3'-(trifluoromethyl)biphenyl-4-yl)propanamide (**S20**, 45.4% yield, 99% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 8.43 (d, *J* = 8.10 Hz, 1 H), 8.12 - 8.20 (m, 1 H), 7.87 - 8.01 (m, 2 H), 7.68 - 7.77 (m, 2 H), 7.64 (d, *J* = 8.29 Hz, 3 H), 7.23 - 7.39 (m, 3 H), 6.88 (dd, *J* = 5.27, 3.39 Hz, 1 H), 6.73 - 6.84 (m, 1 H), 4.51 (d, *J* = 4.71 Hz, 1 H), 3.65 (s, 2 H), 3.20 - 3.28 (m, 2 H), 3.05 (dd, *J* = 13.66, 5.18 Hz, 1 H), 2.85 (dd, *J* = 13.47, 9.32 Hz, 1 H), 2.69 (br. s., 2 H), 2.48 (br. s., 6 H); LCMS (M + H) = 504.



3-cyano-N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)benzamide (**S21**, 43.% yield, 95% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.29 - 9.45 (m, 1 H), 8.97 (d, *J* = 8.10 Hz, 1 H), 8.41 (s, 1 H), 8.28 (s, 1 H), 8.07 - 8.16 (m, 1 H), 7.99 - 8.06 (m, 1 H), 7.90 - 7.99 (m, 2 H), 7.63 - 7.75 (m, 5 H), 7.45 (d, *J* = 8.29 Hz, 2 H), 4.64 - 4.80 (m, 1 H), 3.41 - 3.49 (m, 2 H), 3.02 - 3.27 (m, 4 H), 2.82 (d, *J* = 4.52 Hz, 6 H); LCMS (M + H) = 509.



4-tert-butyl-N-(1-(2-(dimethylamino)ethylamino)-1-oxo-3-(3'-(trifluoromethyl)biphenyl-4-yl)propan-2-yl)benzamide (**S22,** 48.6% yield, 95% purity)

^{1}H NMR (300 MHz, DMSO-*d6*) δ ppm 9.24 - 9.43 (m, 1 H), 8.62 (d, *J* = 7.91 Hz, 1 H), 8.33 (s, 1 H), 7.90 - 7.99 (m, 2 H), 7.88 (d, *J* = 8.48 Hz, 1 H), 7.78 (d, *J* = 8.67 Hz, 2 H), 7.68 (d, *J* = 8.10 Hz, 4 H), 7.52 (d, *J* = 8.48 Hz, 1 H), 7.40 - 7.49 (m, 4 H), 4.61 - 4.77 (m, 0 H), 3.45 (d, *J* = 5.84 Hz, 2 H), 3.00 - 3.27 (m, 4 H), 2.82 (d, *J* = 4.14 Hz, 6 H), 1.29 (s, 9 H); LCMS (M + H) = 540.