Discovery of Type II inhibitors of TGF-beta-activated kinase 1 (TAK1) and Mitogen-activated protein kinase kinase kinase kinase 2 (MAP4K2)

Supplemental Information

Li Tan[1,2], Tyzoon Nomanbhoy[3], Deepak Gurbani[4], Matthew Patricelli[3], John Hunter[4], Jiefei Geng[5], Lina Herhaus[6], Jianming Zhang[1,2], Eduardo Pauls[6], Youngjin Ham[1,2], Hwan Geun Choi[1], Ting Xie[1,2], Xianming Deng[1,2], Sara J. Buhrlage[1], Taebo Sim[7], Philip Cohen[6], Gopal Sapkota[6], Kenneth D. Westover[4], Nathanael S. Gray[1,2]*

1. Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, 02115, USA

2. Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, 02115, USA

3. ActivX Biosciences, La Jolla, CA 92037, USA

4. Departments of Biochemistry and Radiation Oncology, The University of Texas, Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390, USA

5. Department of Cell Biology, Harvard Medical School, Boston, Massachusetts, 02115, USA

6. MRC Protein Phosphorylation and Ubiquitylation Unit, College of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK

7. Chemical Kinomics Research Center, Korea Institute of Science and Technology, 39-1, Hawolgok-dong, Seongbuk-gu, Seoul, 136-791, Korea

*Correspondence: nathanael_gray@dfci.harvard.edu (NSG)

Contents: Figure S1-S6 (page S2-S4); Table S1-S4 (page S5-S6); Structures and spectral data of compounds **3**-**26** (page S7-17).

Figure S1. Kinases profiling data of **1** at 100 nM. Lower score means higher inhibition.

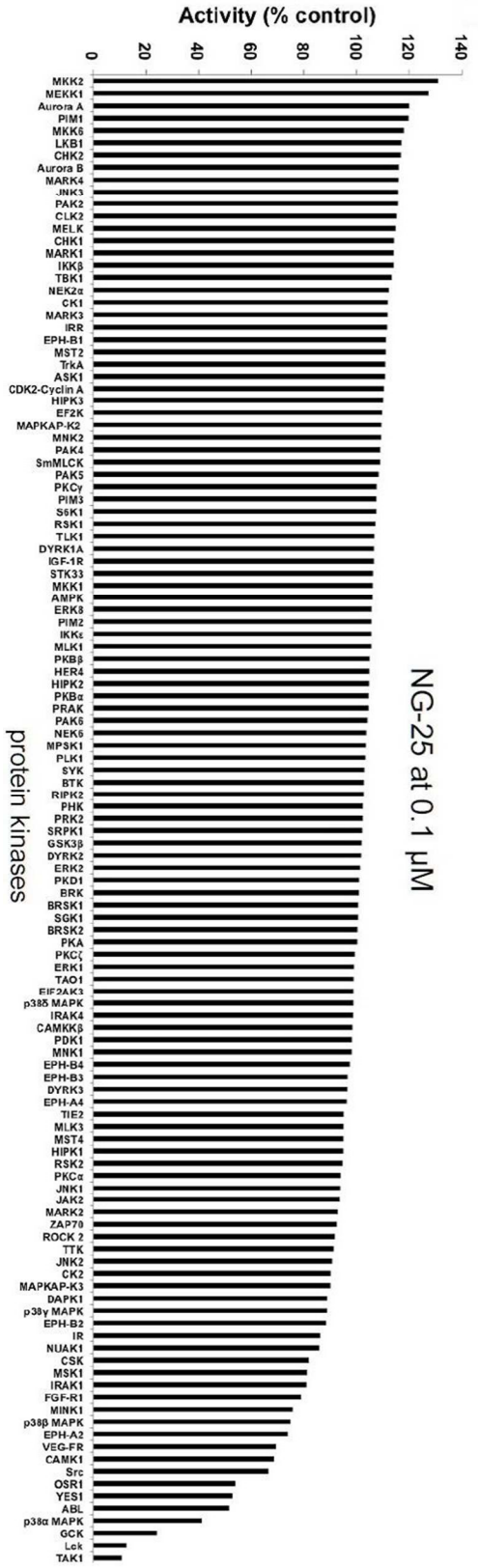


Figure S2. X-ray structure of **1** in complex with TAK1. A) The compound structure is superimposed on a Fo-Fc difference map contoured at 2.5σ (grey mesh). The map was calculated using initial phases after molecular replacement without including **1** in the model to avoid bias. B) Schematic overview of interactions between the TAK1 active site and **1**. Hydrogen bonds are depicted by dashes and hydrophobic interactions by green contours.

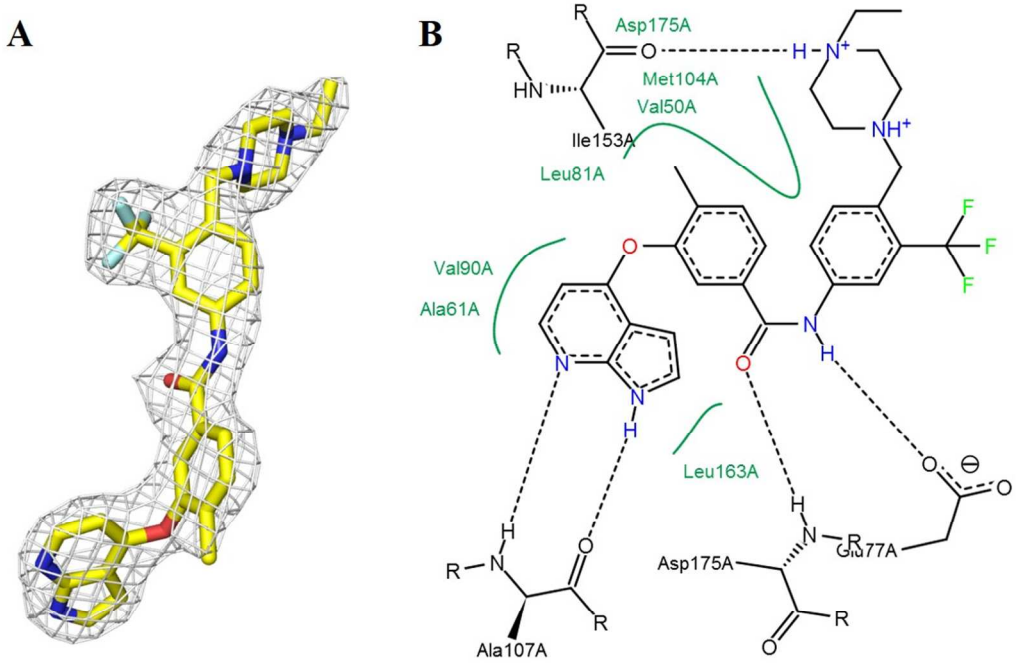


Figure S3. L929 cells were pre-treated with: A) indicated TAK1 (**37**), TAK1/MAP4K2 (**1**), selective MAP4K2 (**12**, **16**, **17**) and p38 inhibitors (**38**) at the concentration of 1 μM for 30 min; B) **1** at 0.1 μM and selective p38 inhibitor **39** at 0.1 and 1.0 μM and then TNFα was added and incubated for 5 min. Samples were collected and subjected to Western blot.

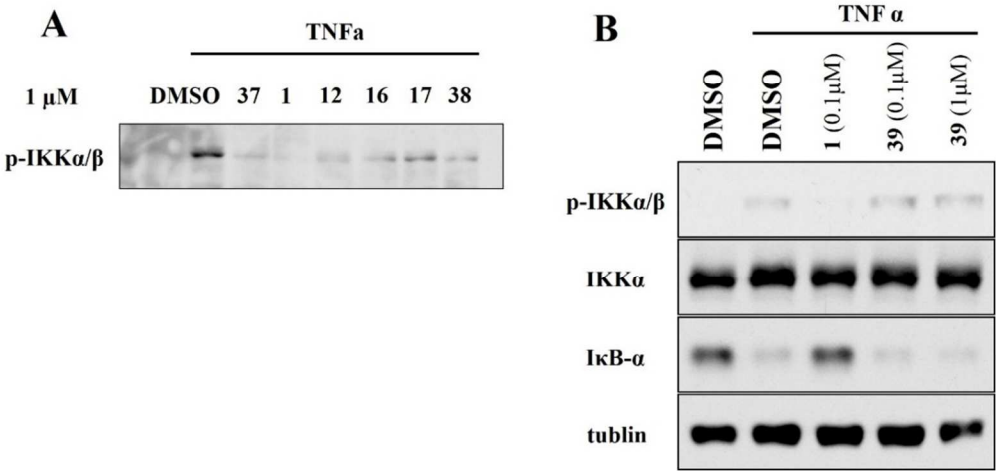


Figure S4. Structure of **S1**.

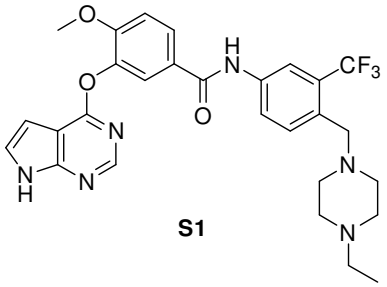


Figure S5. Wild type and TAK1-null MEF cells were pre-treated with **1** (A, B) or **37** (C) for 1 hour, then stimulated with TGFß (A, C) or IL-1α (B, C). Samples were collected and subjected to Western blot.

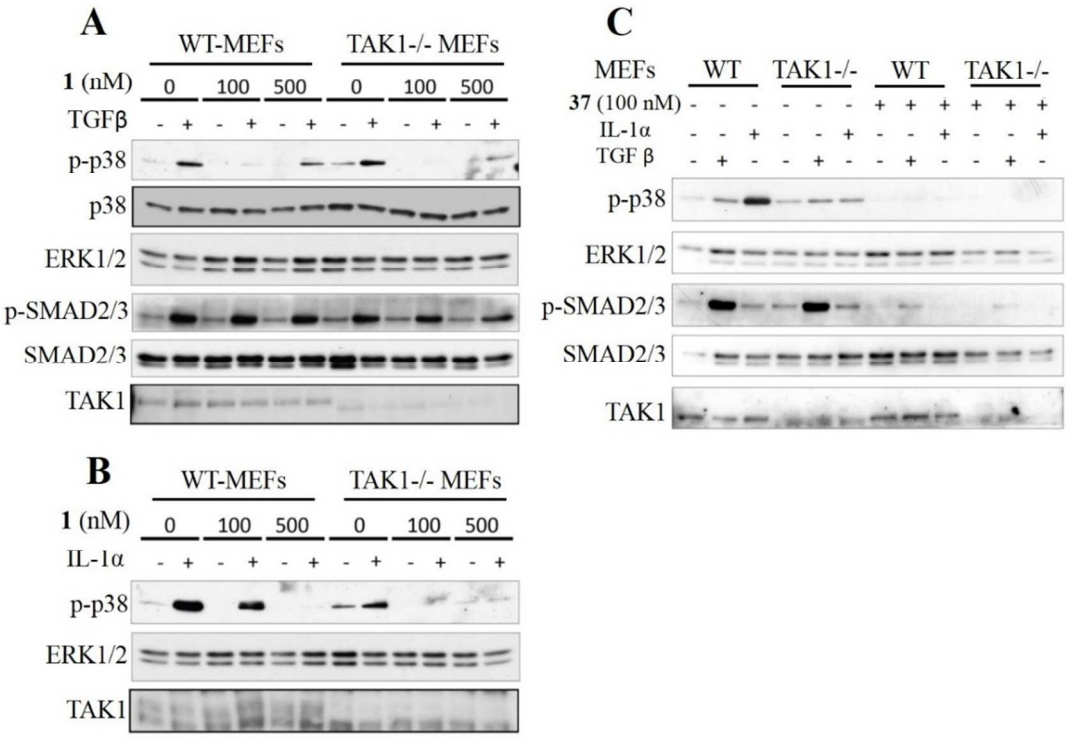


Figure S6. Structrues of **37**-**39**.

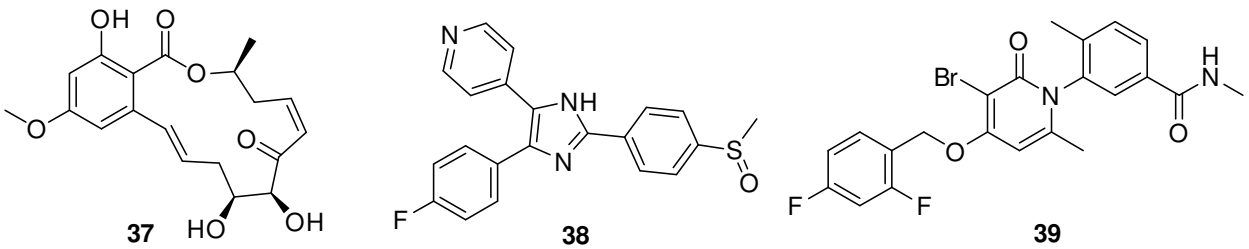


Table S1. Crystallographic data and structure statistics

TAK1-**1** complex	
Wavelength (Å)	0.979
Space Group	I222
Unit cell dimension (Å)	58.2, 132.9, 145.5
Resolution (Å)	50 - 2.4 (2.43 - 2.39)
Unique Reflections	22,388
Completeness (%)	98.6 (95.7)
Redundancy	7.0 (6.3)
R_{merge}(%)	8.2 (93.1)
I/sigma	16.1 (2.2)
Wilson B-factor (Å^2)	46.35
R_{cryst}/R_{free}	20.3 / 22.5
PDB accession	4O91

Table S2. SAR for p38α.

ID	KiNativ* Inhibition (%)	Emzymatic IC50 (nM)
1	**51.9**	**102**
2	**69.2**	**238**
3	**85.2**	**130**
4	**98.6**	**29.8**
5	**82.8**	**149**
6	**98.6**	**250**
7	**95.4**	**45.8**
9	**93.1**	**69.6**
10	**85.8**	**35.9**
11	**88.4**	**19.8**
14	**91.8**	**70.1**
15	**97.3**	**659**
18	**85**	**140**
19	**90.5**	**63.6**
21	**98.8**	**5.27**
22	**95.9**	**555**

*** 1** and **2** were profiled at 0.5 µM on A375 live cell while all others were at 1.0 µM on HUH7 lysate.

Table S3. SAR for ABL.

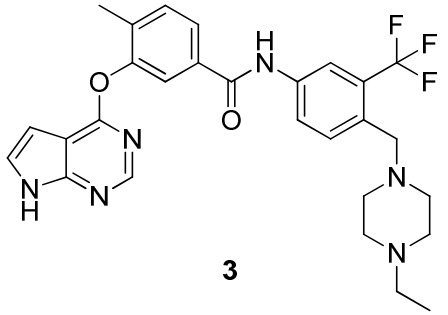
ID	KiNativ* Inhibition (%)		Ba/F3 IC50 (uM)	
	Lys1	ACT	Parental	Bcr/Abl
1	85.5	>98	7.725	0.018
2	>90	>98	4.018	0.01
3		74.2	8.157	0.055
4		93	7.909	0.055
5		59.6	>10	0.02
6	58.4	74.1	>10	0.046
7		74.6	>10	0.085
8	78.8	80.6	>10	0.092
10		>96	7.443	0.01
14	77.2	92.8	4.81	0.01
15	>80	96	8.281	0.039
18	47.5	66.6	5.472	0.01
19	>85	94.5	7.567	0.01
20	71.5	62.2	3.296	0.01
21	80.4	92.2	4.922	0.01
22	>80	90.3	>10	0.016
23	83	66.4	>10	0.387
24	82	89.6	7.103	0.01
25	75.2	80.7	9.743	0.181
26	>80	96.3	7.706	0.01

* **1** and **2** were profiled at 0.5 µM on A375 live cell while all others were at 1.0 µM on HUH7 lysate.

Table S4. SAR for other kinases.

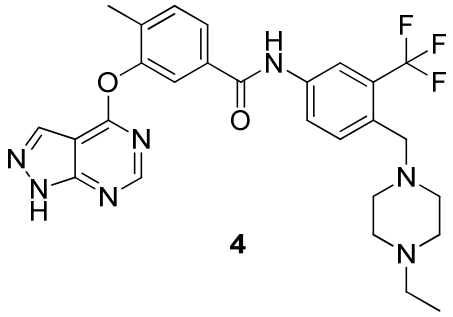
ID	KiNativ* Inhibition (%)					Enzymatic IC50 (nM)				
	ZAK	CSK	EPHA2	FES	FER	ZAK	CSK	EPHA2	FES	FER
1	93	-14.9	27.9			698	56.4	773		82.3
2	98.9	94	96.8			469	80.9	77.1		
4	95.2					127				
5					82.7				69.7	74.8
6				75.8	78.5				252	577
7	89.6					546				
8				92.9	94.7				50.8	36.1
9	82.7					447				
14	>96	89.9	80.9			72.5	12.5	77.1		
15		84					59			
18		75.6					25			
20	95.8	75.7				103	25.1			
21	95.6					98.2				
22	78.4					179				
23	78					301				
24	75.3					396				

* **1** and **2** were profiled at 0.5 uM on A375 live cell while all others were at 1.0 µM on HUH7 lysate.



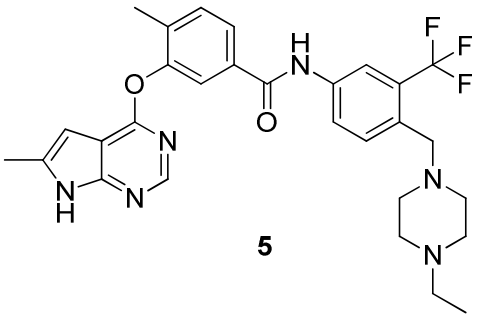
3

3-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)-*N*-(4-((4-ethylpiperazin-1- yl)methyl)-3-(trifluoromethyl)phenyl)-4-methylbenzamide (3). ^{1}H NMR (400 MHz, DMSO) δ 12.24 (bs, 1H), 10.42 (s,1H), 8.26 (s, 1H), 8.15 (s, 1H), 8.03 (d, *J* = 8.8 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.82 (s, 1H), 7.66 (d, *J* = 8.8 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.48 (dd, *J* = 3.6, 2.4, 1H), 6.53 (dd, *J* = 3.6, 2.0, 1H), 3.57 (s, 2H), 2.53-2.32 (m, 8H), 2.47 (q, *J* = 7.2 Hz, 2H), 2.14 (s, 3H), 1.04 (t, *J* = 7.2 Hz, 3H). MS (ESI) *m/z* 539 $(M+H)^{+}$.



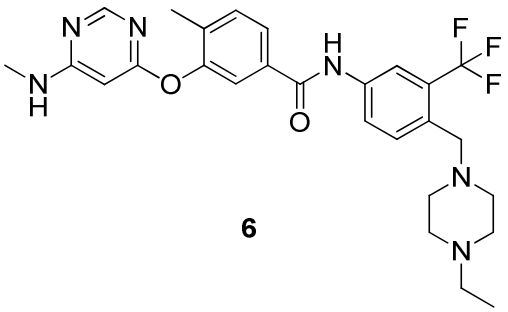
4

3-((1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)-*N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-4-methylbenzamide (4). ^{1}H NMR (400 MHz, DMSO) δ 10.43 (s,1H), 8.49 (s, 1H), 8.23 (s, 1H), 8.15 (d, *J* = 2.0 Hz, 1H), 8.01 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.88 (s, 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.55 (d, *J* = 8.0, 1H), 3.54 (s, 2H), 2.48-2.26 (m, 8H), 2.33 (q, *J* = 6.8 Hz, 2H), 2.17 (s, 3H), 0.96 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 164.83, 162.80, 157.36, 155.45, 150.80, 138.51, 135.29, 134.04, 132.57, 132.06, 131.94, 131.64, 126.16, 123.99, 122.00, 117.76, 117.70, 101.59, 57.88, 53.23, 52.80, 52.00, 16.39, 12.40. MS (ESI) *m/z* 540 $(M+H)^{+}$.



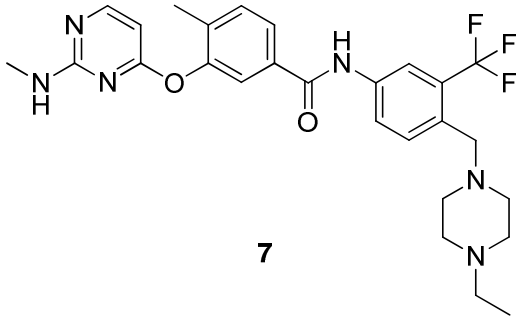
5

***N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-4-methyl-3-((6-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)oxy)benzamide (5).** ^{1}H NMR (400 MHz, DMSO) δ 12.09(bs, 1H), 10.43 (s,1H), 8.20 (s, 1H), 8.18 (s, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.82 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 8.4, 1H), 6.22 (s,1H), 3.55 (s, 2H), 2.48-2.26 (m, 8H), 2.41 (s, 3H), 2.30 (q, *J* = 7.2 Hz, 2H), 2.15 (s, 3H), 0.97 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 164.97, 160.32, 154.34, 151.58, 149.67, 138.55, 136.42, 135.44, 133.74, 132.53, 131.70, 131.62, 125.47, 123.99, 122.01, 117.75, 117.69, 105.42, 95.40, 57.90, 53.27, 52.82, 52.02, 16.48, 13.78, 12.44. MS (ESI) *m/z* 553 $(M+H)^+$.



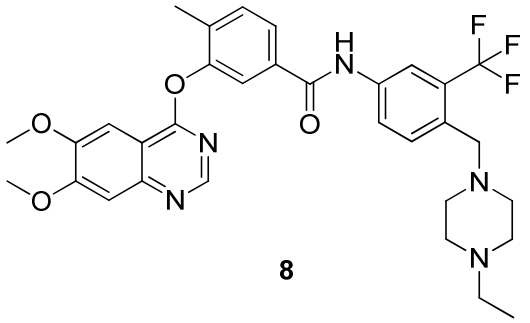
6

***N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-4-methyl-3-((6-(methylamino)pyrimidin-4-yl)oxy)benzamide (6).** ^{1}H NMR (400 MHz, DMSO) δ 10.39 (s,1H), 8.11 (s, 1H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.64 (s, 1H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.41 (d, *J* = 8.0, 1H), 7.33 (bs, 1H), 3.60 (s, 2H), 3.38 (m, 2H), 2.97-2.79 (m, 6H), 2.71 (bs, 3H), 2.37-2.22 (m, 2H), 2.09 (s, 3H), 1.12 (t, *J* = 6.8 Hz, 3H). MS (ESI) *m/z* 529 $(M+H)^+$.



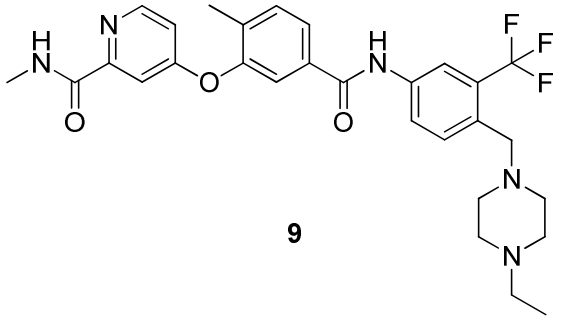
7

***N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-4-methyl-3-((2-(methylamino)pyrimidin-4-yl)oxy)benzamide (7).** ^{1}H NMR (400 MHz, DMSO) δ 10.39 (s,1H), 8.18 (bs, 1H), 8.16 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.75 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 8.4, 1H), 3.54 (s, 2H), 2.76-2.59 (m, 2H), 2.52 (s, 3H), 2.45-2.23 (m, 4H), 2.28 (q, *J* = 7.2, 2H), 2.16 (s, 3H), 0.96 (t, *J* = 7.2 Hz, 3H). MS (ESI) *m/z* 529 $(M+H)^+$.



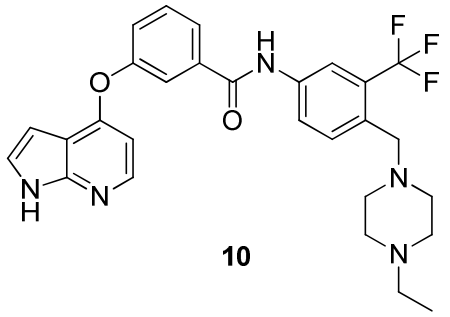
8

3-((6,7-dimethoxyquinazolin-4-yl)oxy)-*N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-4-methylbenzamide (8). ^{1}H NMR (400 MHz, DMSO) δ 10.40 (s,1H), 8.18 (d, *J* = 9.2 Hz, 1H), 8.06 (d, *J* = 5.6 Hz, 1H), 7.88 (s, 1H), 7.68 (d, *J* = 5.6 Hz, 1H), 7.41 (d, *J* = 8.8, 1H), 7.34 (s, 1H), 7.17 (s, 1H), 7.09 (s, 1H), 7.00 (s, 1H), 3.98 (s, 3H), 3.86 (s, 3H), 3.65 (s, 2H), 3.43 (m, 2H), 3.12(m, 2H), 3.01-2.87 (m, 4H), 2.36 (q, *J* = 7.2, 2H), 2.17 (s, 3H), 1.19 (t, *J* = 7.2 Hz, 3H). MS (ESI) *m/z* 610 $(M+H)^+$.



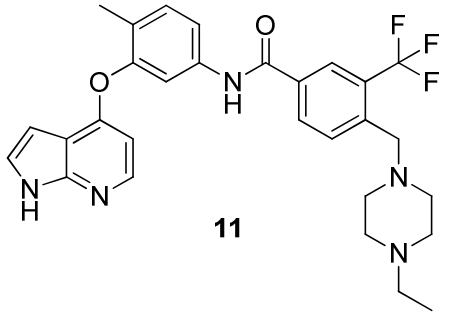
9

4-(5-((4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)carbamoyl)-2-methylphenoxy)-*N*-methylpicolinamide (9). ^{1}H NMR (600 MHz, DMSO) δ 10.50 (s,1H), 8.77 (m, 1H), 8.53 (d, *J* = 5.6 Hz, 1H), 8.16 (s, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.78 (s,1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.60 (d, *J* = 8.4, 1H), 7.31(s, 1H), 7.15 (d, *J* = 5.6 Hz, 1H), 3.65 (s, 2H), 3.43 (m, 2H), 3.12(m, 2H), 3.02-2.82 (m, 4H), 2.76 (d, *J* = 4.8 Hz, 3H), 2.35 (q, *J* = 7.2, 2H), 2.18 (s, 3H), 1.18 (t, *J* = 7.2 Hz, 3H). MS (ESI) *m/z* 556 $(M+H)^+$.



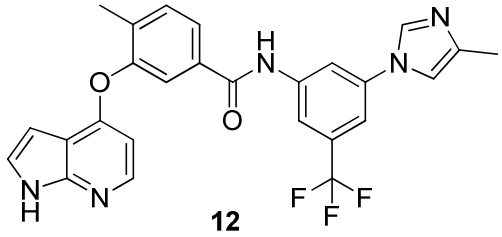
10

3-((1*H*-pyrrolo[2,3-b]pyridin-4-yl)oxy)-*N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoro-methyl)phenyl)benzamide (10). ^{1}H NMR (400 MHz, DMSO) δ 11.80 (bs, 1H), 10.54 (s, 1H), 8.19(s, 1H), 8.13 (d, *J* = 5.6 Hz, 1H), 8.02 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.80 (m,1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.64 (dd, *J* = 8.0,7.6 Hz, 1H), 7.38 (dd, *J* = 8.0, 2.4, 1H), 7.39 (m, 1H), 6.52 (d, *J* = 5.6, 1H), 6.21 (m, 1H), 3.57 (s, 2H), 2.52-2.30 (m, 10H), 2.24 (s, 3H), 1.01 (t, *J* = 7.2 Hz, 3H). MS (ESI) *m/z* 524 $(M+H)^+$.



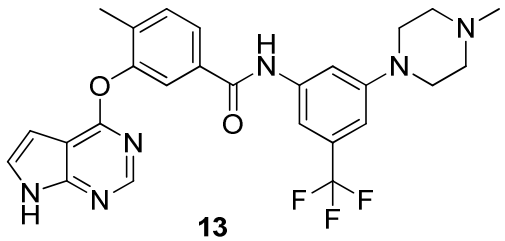
11

***N*-(3-((1*H*-pyrrolo[2,3-b]pyridin-4-yl)oxy)-4-methylphenyl)-4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)benzamide (11).** ^{1}H NMR (600 MHz, DMSO) δ 11.72 (bs, 1H), 10.44 (s,1H), 8.20 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.08 (d, *J* = 5.6 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.59 (s, 1H), 7.37 (d, *J* = 8.0, 1H), 7.36 (s, 1H), 6.36 (d, *J* = 5.2, 1H), 6.23 (m, 1H), 3.66 (s, 2H), 2.53-2.26 (m, 8H), 2.32 (q, *J* = 7.2 Hz, 2H), 2.14 (s, 3H), 0.98 (t, *J* = 7.2 Hz, 3H). MS (ESI) *m/z* 538 $(M+H)^+$.



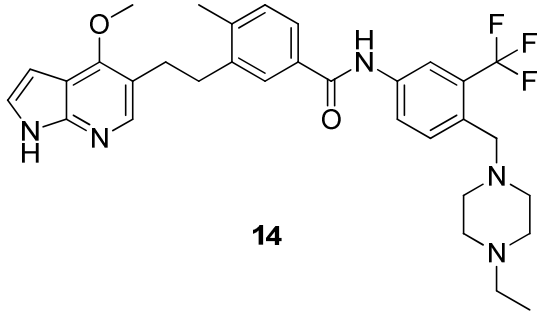
12

3-((1*H*-pyrrolo[2,3-b]pyridin-4-yl)oxy)-4-methyl-*N*-(3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl)benzamide (12). ^{1}H NMR (400 MHz, DMSO) δ 11.72 (bs, 1H), 10.54 (s, 1H), 8.18 (s, 1H), 8.12 (s, 1H), 8.04 (s, 1H), 8.03 (d, *J* = 5.6 Hz, 1H), 7.85 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.65 (s, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.40 (s, 1H), 7.31 (m, 1H), 6.27 (d, *J* = 5.2, 1H), 6.15 (dd, *J* = 3.6, 2.0 Hz, 1H), 2.19 (s, 3H), 2.10 (s, 3H). MS (ESI) *m/z* 492 $(M+H)^+$.



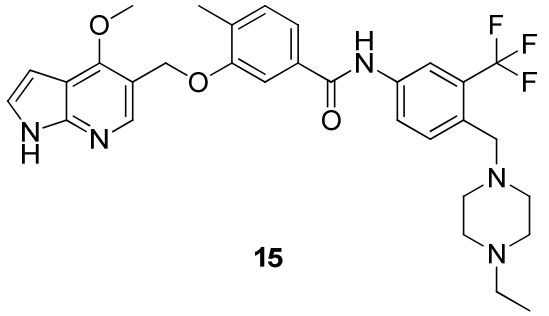
13

3-((7*H*-pyrrolo[2,3-d]pyrimidin-4-yl)oxy)-4-methyl-*N*-(3-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)phenyl)benzamide (13). ^{1}H NMR (400 MHz, DMSO) δ 10.03 (s,1H), 8.30 (s, 1H), 8.15 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.84 (s, 1H), 7.66 (s, 1H), 7.60 (s, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 3.6, 1H), 6.93 (s, 1H), 6.55 (d, *J* = 3.2, 1H), 3.19 (t, *J* = 5.2 Hz, 4H), 2.45 (t, *J* = 5.2 Hz, 4H), 2.22 (s, 3H), 2.17 (s, 3H). MS (ESI) *m/z* 511 $(M+H)^+$.



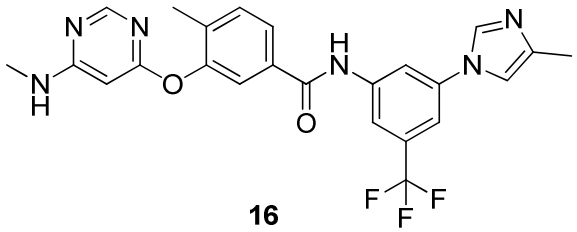
14

***N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)ethyl)-4-methylbenzamide (14).** ^{1}H NMR (600 MHz, DMSO) δ 11.56 (br, 1H), 10.47 (s, 1H), 8.28 (s, 1H), 8.13 (m, 1H), 7.98 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.90 (m, 2H), 7.39 (m, 2H), 6.79 (s, 1H), 4.34 (s, 3H), 3.66 (s, 2H), 2.95 (m, 4H), 2.63-2.44 (m, 10H), 2.43 (s, 3H), 1.08 (m, 3H). MS (ESI) *m/z* 580 $(M+H)^+$.



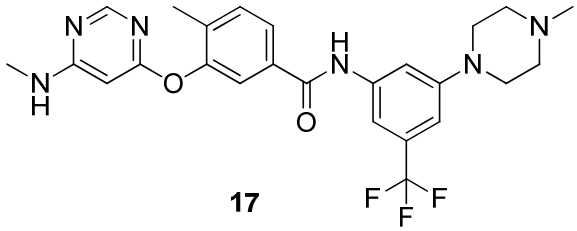
15

***N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-((4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)methoxy)-4-methylbenzamide (15).** ^{1}H NMR (600 MHz, DMSO) δ 12.23 (br, 1H), 10.63 (s, 1H), 9.81 (br, 1H), 8.40 (s, 1H), 8.17 (m, 1H), 7.85 (m, 1H), 7.78 (m, 1H), 7.48 (m, 2H), 7.37 (m, 1H), 7.20 (s, 1H), 5.37 (s, 2H), 4.50 (s, 3H), 3.66 (s, 2H), 3.29-2.92 (m, 10H), 2.55 (s, 3H), 1.34 (m, 3H). MS (ESI) m/z 582 $(M+H)^+$.



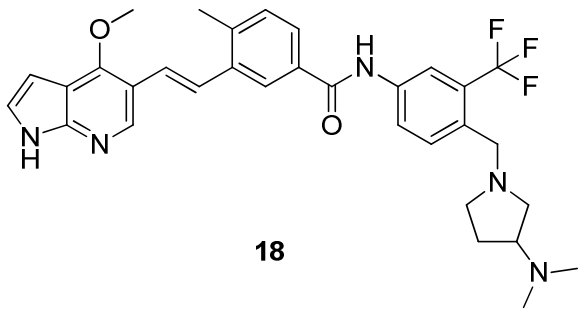
16

4-methyl-*N*-(3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl)-3-((6-(methylamino)pyrimidin-4-yl)oxy)benzamide (16). ^{1}H NMR (400 MHz, DMSO) δ 10.57 (s, 1H), 8.26 (s, 1H), 8.22 (s, 1H), 8.12 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.75 (s, 1H), 7.74 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.36 (m, 1H), 2.79 (s, 3H), 2.50 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 165.22, 151.56, 141.62, 139.07, 138.29, 135.72, 135.39, 133.35, 131.88, 131.42, 131.10, 125.43, 125.33, 121.76, 115.54, 114.86, 114.74, 113.39, 112.21, 16.48, 13.86. MS (ESI) *m/z* 483 $(M+H)^{+}$.



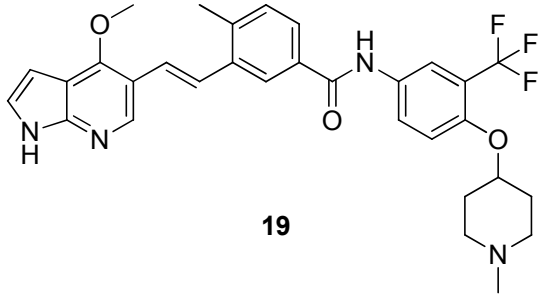
17

4-methyl-3-((6-(methylamino)pyrimidin-4-yl)oxy)-*N*-(3-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)phenyl)benzamide (17). ^{1}H NMR (600 MHz, DMSO) δ 10.23 (s, 1H), 8.10 (br, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.69 (s, 1H), 7.64 (s, 1H), 7.60 (s, 1H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 3.6 Hz, 1H), 6.92 (s, 1H), 5.74 (br, 1H), 3.18 (t, *J* = 4.8 Hz, 4H), 2.76 (br, 3H), 2.44 (t, *J* = 4.8 Hz, 4H), 2.21 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 164.92, 152.04, 151.48, 141.05, 135.28, 133.83, 131.74, 130.27, 125.24, 121.63, 110.26, 110.10, 107.05, 54.79, 48.02, 46.12, 16.44. MS (ESI) *m/z* 500 $(M+H)^{+}$.



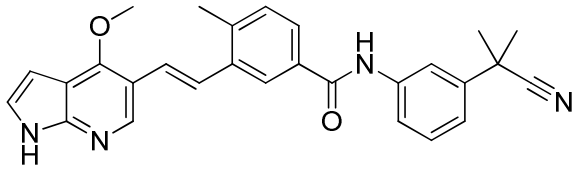
18

(*E*)-*N*-(4-((3-(dimethylamino)pyrrolidin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)vinyl)-4-methylbenzamide (18). ^{1}H NMR (600 MHz, DMSO) δ 11.92 (s, 1H), 10.56 (s, 1H), 8.53 (s, 1H), 8.24 (s, 1H), 8.20 (s, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.77(d, *J* = 8.4 Hz, 1H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.46-7.37 (m, 4H), 6.88 (s, 1H), 4.37 (s, 3H), 3.74 (s, 2H), 3.00-2.01 (m, 7H), 2.76 (s, 6H), 2.46 (s, 3H). MS (ESI) m/z 582 $(M+H)^+$.



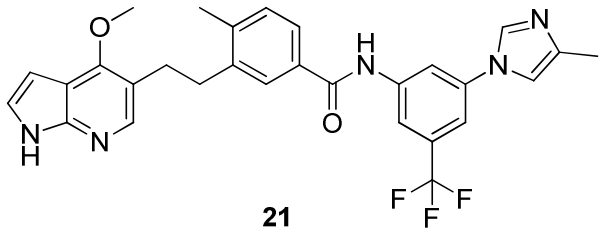
19

(*E*)-3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)vinyl)-4-methyl-*N*-(4-((1- methylpiperidin-4-yl)oxy)-3-(trifluoromethyl)phenyl)benzamide (19). ^{1}H NMR (600 MHz, DMSO) δ 11.82 (br, 1H), 10.38 (s, 1H), 9.41 (br, 1H), 8.50 (s, 1H), 8.18 (s, 1H), 8.13 (m, 1H), 8.02 (s, 1H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.41 (d, *J* = 5.4 Hz, 1H), 7.40 (m, 3H), 6.84 (s, 1H), 4.35 (s, 3H), 3.42 (m, 1H), 3.07 (m, 2H), 2.81 (s, 3H), 2.46 (s, 3H), 2.25 (m, 2H), 2.13 (m, 2H), 2.00 (m, 2H). MS (ESI) m/z 565 $(M+H)^+$.



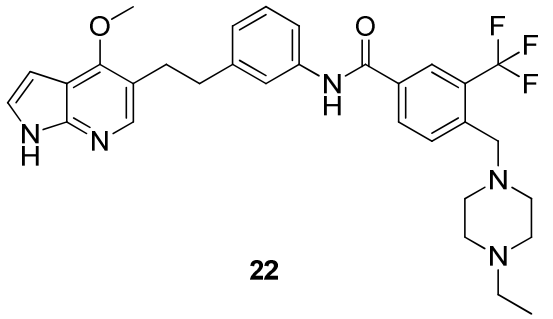
20

(*E*)-*N*-(3-(2-cyanopropan-2-yl)phenyl)-3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*] pyridin-5-yl)vinyl)-4-methylbenzamide (20). ^{1}H NMR (600 MHz, DMSO) δ 11.92 (br, 1H), 10.54 (br, 1H), 8.36 (s, 1H), 7.98 (s, 1H), 7.76 (s, 1H), 7.58 (m, 2H), 7.24-7.15 (m, 4H), 7.02 (s, 1H), 6.74 (s, 1H), 4.20 (s, 3H), 2.23 (s, 3H), 1.48 (s, 6H). MS (ESI) m/z 451 $(M+H)^+$.



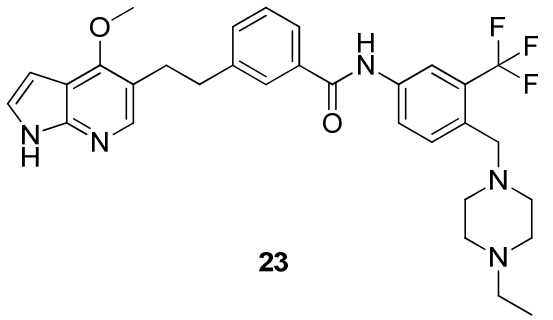
21

3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)ethyl)-4-methyl-*N*-(3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl)benzamide (21). ^{1}H NMR (600 MHz, DMSO) δ 12.02 (br, 1H), 10.77 (s, 1H), 8.63 (s, 1H), 8.51 (m, 1H), 8.45 (m, 1H), 8.36 (s, 1H), 8.26 (s, 1H), 8.20-7.78 (m, 3H), 7.26 (m, 1H), 4.74 (s, 3H), 3.42 (m, 1H), 3.05 (m, 2H), 3.02 (s, 3H), 3.01 (s, 3H), 2.99 (s, 4H), 2.77 (m, 2H), 2.44 (m, 2H), 2.23 (m, 2H). MS (ESI) m/z 567 $(M+H)^+$.



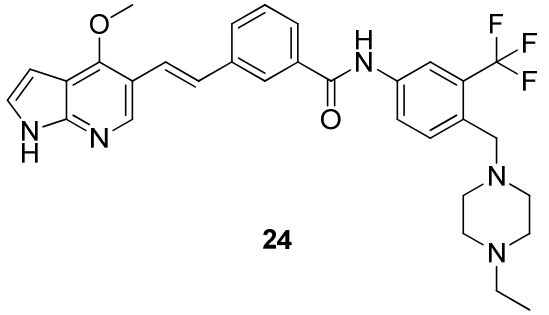
22

4-((4-ethylpiperazin-1-yl)methyl)-*N*-(3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*] pyridin-5-yl)ethyl)phenyl)-3-(trifluoromethyl)benzamide (22). ^{1}H NMR (600 MHz, DMSO) δ 12.11 (br, 1H), 10.46 (s, 1H), 9.44 (br, 1H), 8.36 (s, 1H), 8.03 (m, 1H), 7.72 (s, 1H), 7.48 (m, 1H), 7.40-7.10 (m, 4H), 6.66 (s, 1H), 4.42 (s, 3H), 3.86 (s, 2H), 3.04 (m, 4H), 2.80-2.45 (m, 10H), 1.31 (m, 3H). MS (ESI) m/z 566 $(M+H)^+$.



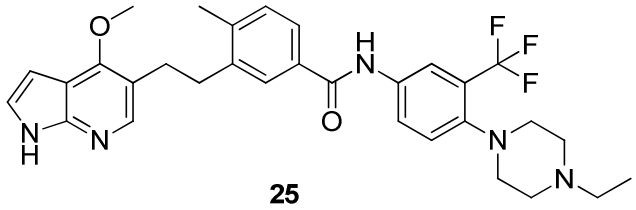
23

***N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)ethyl)benzamide (23).** ^{1}H NMR (600 MHz, DMSO) δ 11.95 (br, 1H), 10.57 (s, 1H), 8.28 (s, 1H), 8.15 (d, *J* = 9.0 Hz, 1H), 8.04 (s, 1H), 7.92 (s, 1H), 7.86 (d, *J* = 7.2 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.51 (m, 3H), 6.91 (s, 1H), 4.38 (s, 3H), 3.72 (s, 2H), 3.00 (s, 4H), 2.98-2.36 (m, 8H), 2.44 (m, 2H), 1.29 (m, 3H). MS (ESI) m/z 566 $(M+H)^+$.



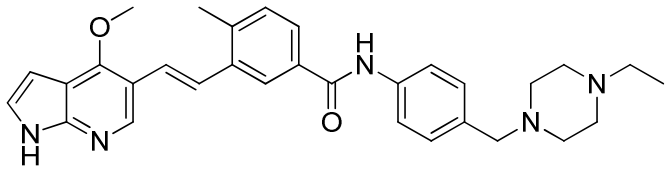
24

(*E*)-*N*-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)vinyl)benzamide (24). ^{1}H NMR (600 MHz, DMSO) δ 11.87 (br, 1H), 10.43 (s, 1H), 8.57 (s, 1H), 8.11 (s, 1H), 7.72 (m, 5H), 7.35 (m, 4H), 6.80 (m, 1H), 4.34 (s, 3H), 3.68 (s, 2H), 3.20-2.48 (m, 10H), 1.19 (s, 3H). MS (ESI) m/z 564 $(M+H)^+$.



25

***N*-(4-(4-ethylpiperazin-1-yl)-3-(trifluoromethyl)phenyl)-3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)ethyl)-4-methylbenzamide (25).** ^{1}H NMR (600 MHz, DMSO) δ 12.36 (s, 1H), 10.41 (s, 1H), 8.17 (s, 1H), 8.13 (s, 1H), 8.09 (d, *J* = 9.0 Hz, 1H), 7.79 (s, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.51 (s, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.00 (s, 1H), 4.40 (s, 3H), 3.56 (m, 2H), 3.23 (m, 2H), 3.08 (m, 6H), 2.90 (s, 4H), 2.36 (s, 3H), 1.24 (t, J = 6.6 Hz, 3H). MS (ESI) m/z 566 $(M+H)^+$.



26

(*E*)-*N*-(4-((4-ethylpiperazin-1-yl)methyl)phenyl)-3-(2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin- 5-yl)vinyl)-4-methylbenzamide (26). ^{1}H NMR (600 MHz, DMSO) δ 11.85 (br, 1H), 10.29 (s, 1H), 8.50 (s, 1H), 8.17 (s, 1H), 7.78 (m, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.41-7.34 (m, 6H), 6.85 (s, 1H), 4.35 (s, 3H), 3.39 (s, 2H), 2.68-2.25 (m, 10H), 2.52 (s, 3H), 1.17 (m, 3H). MS (ESI) m/z 510 $(M+H)^{+}$.