

## SUPPORTING INFORMATION

# Structure-guided design of a series of MCL-1 inhibitors with high affinity and selectivity

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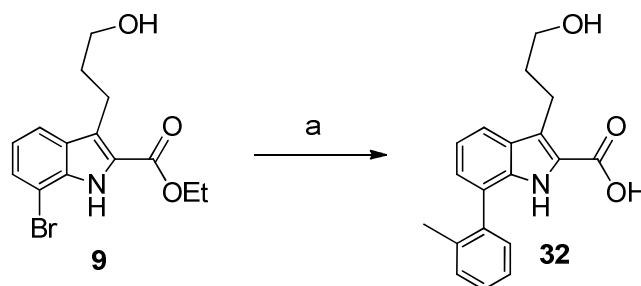
### Chemistry protocols and synthetic routes

<sup>1</sup>H NMR spectra were obtained on a Varian UNITY or Inova (500 MHz), Varian UNITY (400 MHz), or Varian UNITY plus or Mercury (300 MHz) instrument. Chemical shifts are reported as  $\delta$  values (ppm) downfield relative to TMS as an internal standard, with multiplicities reported in the usual manner. Mass spectral analyses were performed on a Finnigan SSQ7000 GC/MS mass spectrometer using different techniques, including electrospray ionization (ESI), desorption chemical ionization (DCI) and atmospheric pressure chemical ionization (APCI), as specified for individual compounds. Exact mass measurements were performed on a Finnigan FTMS Newstar T70 mass spectrometer. The compound is determined to be "consistent" with the chemical formula if the exact mass measurement is within 5.0 ppm relative mass error (RME) of the exact monoisotopic mass.

Analytical LC-MS were performed on a Finnigan Navigator mass spectrometer and Agilent 1100 HPLC system running Xcalibur 1.2 and Open-Access 1.3 software. The

mass spectrometer was operated under positive APCI ionization conditions. The HPLC system comprised an Agilent Quaternary pump, degasser, column compartment, autosampler and diode-array detector, with a Sedere Sedex 75 evaporative light-scattering detector. The column used was a Phenomenex Luna Combi-HTS C8(2) 5  $\mu\text{m}$  100 $\text{\AA}$  (2.1mm  $\times$  30mm). TFA Method (Method A): A gradient of 10-100% acetonitrile (solvent 1) and 0.1% trifluoroacetic acid in water (solvent 2) was used, at a flow rate of 2 mL/min (0-0.1 min 10% solvent 1, 0.1-2.6 min 10-100% solvent 1, 2.6-2.9 min 100% solvent 1, 2.9-3.0 min 100-10% solvent 1). Ammonium Method (Method B): A gradient of 10-100% acetonitrile (solvent 1) and 10 mM  $\text{NH}_4\text{OAc}$  in water (solvent 2) was used, at a flow rate of 1.5 mL/min (0-0.1 min 10% solvent 1, 0.1-3.1 min 10-100% solvent 1, 3.1-3.9 min 100% solvent 1, 3.9-4.0 min 100-10% solvent 1).

**Scheme S-I: Synthesis of compound 32<sup>a</sup>**

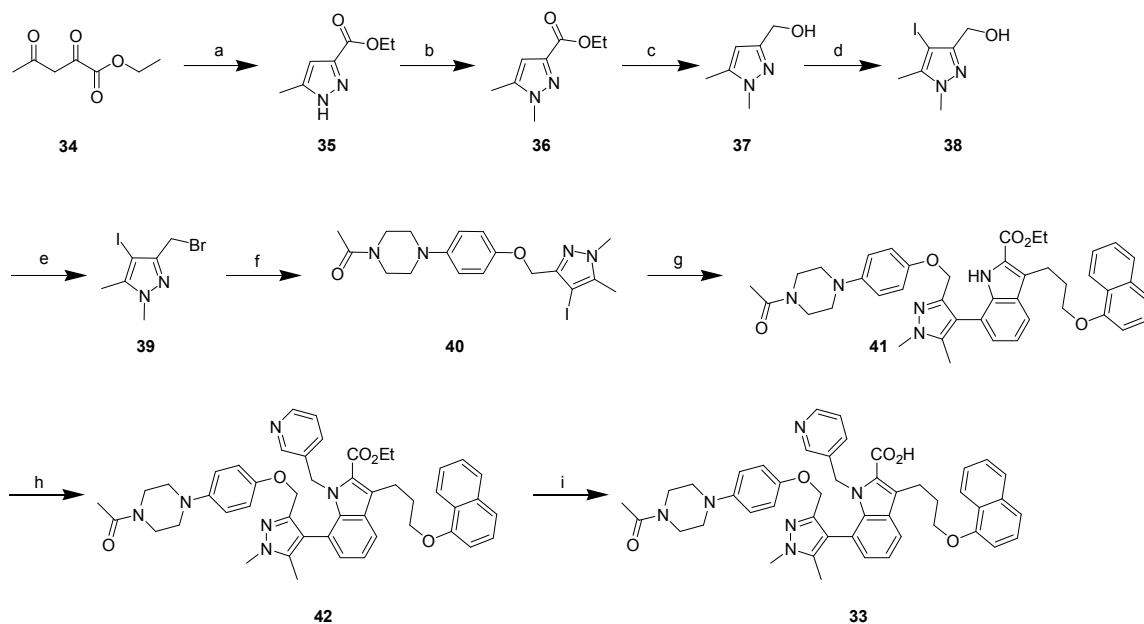


<sup>a</sup>Reagents and conditions: (a)  $(\text{PPh}_3)_2\text{PdCl}_2$ ,  $\text{Na}_2\text{CO}_3$ , *o*-Tolyl-B(OH)<sub>2</sub>, 150  $^\circ\text{C}$ , (33%).

**3-(3-hydroxypropyl)-7-(*o*-tolyl)-1H-indole-2-carboxylic acid (31).** A 5 mL microwave reaction vial was charged ethyl 7-bromo-3-(3-hydroxypropyl)-1H-indole-2-carboxylate (0.05 g, 0.15 mmol), 2M sodium carbonate (0.5 mL, 2 mmol), bis(triphenylphosphine)palladium(II) chloride (0.004 g, 0.006 mmol), and *o*-tolylboronic acid (0.05 g, 0.37 mmol) in 1,2-dimethoxyethane/water/ethanol (7:3:2, 3mL) to give a brown suspension. The vial was sealed and heated in a Biotage Initiator microwave for 30 min at 150  $^\circ\text{C}$ . The reaction was diluted with EtOAc, filtered through a 2.5g Celite SPE column, and washed 2x with brine. The organic layer was dried over  $\text{MgSO}_4$ , filtered, and concentrated. The residue was purified by reverse phase preparative HPLC to provide the title compound (33%). <sup>1</sup>H NMR (500 MHz,  $\text{DMSO-}d_6$ )  $\delta$  10.36 (br s, 1H) 7.68 (d,  $J = 7.93$  Hz, 1H) 7.30 - 7.36 (m, 2H) 7.24 - 7.29 (m, 1H) 7.22 (d,  $J = 7.32$  Hz, 1H) 7.11 - 7.16 (m, 1H) 7.02 - 7.06 (m, 1H) 4.47 (br s, 1H) 3.45 (t,  $J = 6.41$  Hz, 2H) 3.08 (t,  $J = 7.32$  Hz, 2H) 2.06 (s, 3H) 1.74 - 1.82 (m, 2H). MS (ESI)  $m/z = 310.0$  ( $\text{M} + \text{H}^+$ ).

**3-(3-hydroxypropyl)-1H-indole-2-carboxylic acid (32).** The title compound was prepared according to the literature protocol.<sup>1</sup> <sup>1</sup>H NMR (300 MHz,  $\text{DMSO-}D_6$ )  $\delta$  12.87 (s, 1 H) 11.36 (s, 1 H) 7.65 (d,  $J = 8.09$  Hz, 1 H) 7.38 (d,  $J = 8.09$  Hz, 1 H) 7.18 - 7.26 (m, 1 H) 7.00 - 7.07 (m, 1 H) 4.41 (t,  $J = 5.15$  Hz, 1 H) 3.36 - 3.46 (m, 2 H) 3.05 (t,  $J = 7.35$  Hz, 2 H) 1.68 - 1.81 (m, 2 H). MS (ESI)  $m/z = 219.9$  ( $\text{M} + \text{H}^+$ ).

**Scheme S-II: Synthesis of Compound 33<sup>a</sup>**



<sup>a</sup>Reagents and conditions: (a)  $\text{NH}_2\text{NH}_2$ , EtOH, r.t., (99%); (b) MeI,  $\text{Cs}_2\text{CO}_3$ , DMF, r.t., (55%); (c)  $\text{LiAlH}_4$ , THF, 0 °C, (93%); (d) NIS, TFA, r.t., (87%); (e)  $\text{PBr}_3$ , DCM, 0 °C, (81%); (f) 1-(4-(4-hydroxyphenyl)piperazin-1-yl)ethanone,  $\text{Cs}_2\text{CO}_3$ , DMF, (53%); (g) **14**,  $\text{Pd}(\text{PPh}_3)_4$ , DMF, 100 °C, (61%); (h) 3-(chloromethyl)pyridine, DCM, TBAB, NaOH, (77%); (i) LiOH, THF,  $\text{H}_2\text{O}$ , (30%).

**Ethyl 5-methyl-1H-pyrazole-3-carboxylate (35).** To a solution of ethyl 2,4-dioxopentanoate (**34**) (76 g, 0.48mol) in ethanol (300mL) was added hydrazine monohydrate (26.45g, 0.53mol) dropwise. After stirring at ambient temperature for 3 h, the reaction mixture was concentrated and washed with brine. The solid was collected and dried under vacuum to give the desired compound **35** as an off-white solid (73 g, 99%). <sup>1</sup>H NMR ( $\text{CDCl}_3$ , 250 MHz):  $\delta$  6.55 (s, 1H), 4.34 (d,  $J = 7.13$  Hz, 2H), 2.35 (s, 3H), 1.33 (t,  $J = 7.13$  Hz, 3H). LC-MS(ESI):  $m/z$  155 ( $\text{M}+\text{H}$ )<sup>+</sup>.

**Ethyl 1,5-dimethyl-1H-pyrazole-3-carboxylate (36).** Into a solution of compound **35** (10g, 0.065mol) in DMF (120ml) was added  $\text{Cs}_2\text{CO}_3$  (25.4g, 0.078mol). Iodomethane (11.1 g, 0.078mol) was then added dropwise while the reaction mixture was stirred at ambient temperature. After stirring at ambient temperature for 5 h, ethyl acetate (150 mL) and water were added to the reaction mixture and the phases separated. The organic phase was washed with brine (3x), dried over  $\text{Na}_2\text{SO}_4$ , and concentrated. The residue was purified by silica-gel column chromatography (10%-50% ethyl acetate/ petroleum ether) to afford compound **36** (6.0 g, 55%) as a yellow oil. <sup>1</sup>H NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  6.51 (d,  $J = 0.9$  Hz, 1H), 4.32 – 4.13 (q,  $J = 7.1$  Hz, 2H), 2.27 (d,  $J = 0.8$  Hz, 3H), 1.26 (t,  $J = 7.1$  Hz, 3H). LC-MS(ESI):  $m/z$  169 ( $\text{M}+\text{H}$ )<sup>+</sup>.

**(1,5-dimethyl-1H-pyrazol-3-yl)methanol (37).** Compound **36** (9.7 g, 0.058 mol) was dissolved in dry THF (100ml) in a 250 mL three necked bottom flask. The reaction mixture was then cooled to 0°C with an ice/salt bath.  $\text{LiAlH}_4$  (2.63 g, 0.069mol) was added slowly in portions at such rate that the reaction temperature was maintained below 0 °C. After completing the addition, the mixture was stirred at 0°C for 2 h, and the temperature was allowed to increase to room temperature. The reaction mixture was then quenched by slow addition of aqueous sodium hydroxide (15%). The mixture was

extracted with ethyl acetate (3x100mL) and the combined extracts were washed with water, brine and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent afforded the desired compound **37** as an off-white solid (6.8 g, 93%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 5.93 (s, 1H), 4.82 (t, *J* = 5.7 Hz, 1H), 4.29 (d, *J* = 5.7 Hz, 2H), 3.63 (s, 3H), 2.20 (s, 3H). LC/MS(ESI) : m/z 127 (M+H)<sup>+</sup>.

**(4-Iodo-1,5-dimethyl-1H-pyrazol-3-yl)methanol (38)**. A suspension of compound **37** (1.26g, 10mmol) and NIS (2.25g, 10mmol) in trifluoroacetic acid (TFA, 30 mL) was stirred at ambient temperature under nitrogen for 24 h. Upon removal of TFA, the residue was treated with 50 mL of ethyl acetate and washed with brine (3x). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under *vacuo* to give the desired compound **38** as a white solid (2.2 g, 87%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 4.85 (t, *J* = 5.5 Hz, 1H), 4.27 (d, *J* = 5.5 Hz, 2H), 3.76 (s, 3H), 2.23 (s, 3H). LC/MS (ESI): m/z 253 (M+H)<sup>+</sup>.

**3-(Bromomethyl)-4-iodo-1,5-dimethyl-1H-pyrazole (39)**. A solution of compound **38** (2 mmol, 0.5 g) in dichloromethane (15 ml) was cooled to 0°C, then to this solution tribromophosphine (2.4 mmol, 0.64 g) was added dropwise at 0°C. After the addition was complete, the homogeneous solution was stirred for 3 h, with the temperature slowly increased to ambient temperature. After the reaction was complete as judged by TLC and LC-MS, it was quenched by addition of water dropwise. The mixture was extracted with DCM. The collected organic phase was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under *vacuo* to give the desired product as a yellow solid (0.5 g, 81%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 4.47 (s, 2H), 3.79 (s, 3H), 2.24 (s, 3H). LC-MS(ESI): m/z 315(M+H)<sup>+</sup>.

**1-(4-(4-((4-Iodo-1,5-dimethyl-1H-pyrazol-3-yl)methoxy)phenyl)piperazin-1-yl)ethanone (40)**. To a solution of compound **39** (0.83mmol, 260mg) in DMF (10 mL) was added compound 1-(4-(4-hydroxyphenyl)piperazin-1-yl)ethanone (183 mg, 0.83 mmol) and Cs<sub>2</sub>CO<sub>3</sub> (320 mg, 0.996mmol) at room temperature. The mixture was stirred for 10 h. The reaction mixture was partitioned between water (15 mL) and ethyl acetate (25 mL). The aqueous layer was extracted with ethyl acetate (2x). The combined organic layers were washed with brine (3x), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under *vacuo* to give the desired compound as a yellow solid (200 mg, 53%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.09 – 7.01 (m, 2H), 7.04 – 6.96 (m, 2H), 5.02 (s, 2H), 3.82 (s, 3H), 3.61 (s, 4H), 3.05-3.15 (m, 4H), 2.11 (s, 3H), 2.04 (s, 3H). LC/MS(ESI): m/z 455 (M+H)<sup>+</sup>.

**Ethyl 7-(3-((4-(4-acetylpiperazin-1-yl)phenoxy)methyl)-1,5-dimethyl-1H-pyrazol-4-yl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (41)**. A suspension of compound **14** (2.0 g, 3.87 mmol), compound **40** (1.93g, 4.26 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (0.23g, 0.20 mmol), and K<sub>2</sub>CO<sub>3</sub> (1.6g, 12 mmol) in DMF (30ml) was heated at 100 °C for 16 h. After this time the reaction mixture was concentrated. Water was added and the resulted mixture was extracted with ethyl acetate. The combined organic phase was washed with brine, dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to crude product. The crude product was purified by column chromatography (50%-100% ethyl acetate in hexane) to afford the desired compound as a yellow solid (1.7 g, 61%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 10.52 (s, 1H), 8.21 (dd, *J* = 7.2, 2.5 Hz, 1H), 7.94 – 7.79 (m, 1H), 7.71 (dd, *J* = 7.4, 1.8 Hz, 1H), 7.61 – 7.30 (m, 5H), 7.17 – 6.98 (m, 2H), 6.96 – 6.69 (m, 5H), 4.82 (s, 2H), 4.21 (t, *J* = 6.4 Hz, 2H), 3.85 (s, 3H), 3.55 (q, *J* = 4.9 Hz, 4H), 3.34 (dd, *J* = 8.6, 6.3 Hz,

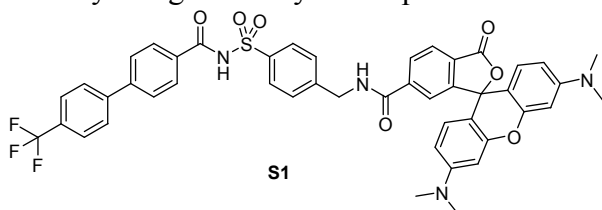
2H), 3.17 (s, 3H), 2.97 (m, 2.92-3.01 Hz, 4H), 2.23 (m, 2H), 2.01 (s, 3H), 2.01 (s, 3H), 1.23 (t, J = 7.0 Hz, 3H). LC-MS(ESI): m/z 700 (M+H)<sup>+</sup>.

**Ethyl 7-(3-((4-(4-acetylpiperazin-1-yl)phenoxy)methyl)-1,5-dimethyl-1H-pyrazol-4-yl)-3-(3-(naphthalen-1-yloxy)propyl)-1-(pyridin-3-ylmethyl)-1H-indole-2-carboxylate (42).** Into a 100 ml three neck flask were added compound **41** (0.3 g, 0.43 mmol), 3-(chloromethyl)pyridine hydrochloride (0.12 g, 0.65 mmol), TBAB (0.03 g, 0.05 mmol), sodium hydroxide (0.2 g, 5 mmol), and dichloromethane (10 ml). The reaction mixture was stirred at ambient temperature for 24 h. Dichloromethane (20 mL) was added and the solution washed with water (10 ml x 2). The organic layer was dried with MgSO<sub>4</sub>. After filtration, the solvent was removed off under reduced pressure to obtain crude product. The crude product was purified by silica gel chromatograph (ethyl acetate/petroleum ether to MeOH/EA 50%-100% to 5%-10%), and concentrated to afford the desired compound as a yellow solid (0.26 g, 77%). LC-MS(ESI): m/z 791 (M+H)<sup>+</sup>.

**7-(3-((4-(4-Acetylpiperazin-1-yl)phenoxy)methyl)-1,5-dimethyl-1H-pyrazol-4-yl)-3-(3-(naphthalen-1-yloxy)propyl)-1-(pyridin-3-ylmethyl)-1H-indole-2-carboxylic acid (33).** To a suspension of compound **42** (0.26 g, 0.33 mmol) in THF (10ml) and water (5ml) was added 0.67 M lithium hydroxide (3 mL, 2 mmol). The reaction mixture was stirred at ambient temperature for 30 h to give a clear solution. The reaction mixture was quenched with aqueous HCl to adjust pH = 6-7. The crude product was extracted with dichloromethane (10 ml x 3), dried and purified by reverse phase Prep HPLC (C-18, 40-100 % MeCN/H<sub>2</sub>O/0.1% TFA) to afford the desired compound **33** as yellow solid (0.076 g, 30 %). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500MHz) δ (ppm) 8.47 (q, 2H), 8.16 (m, 3H), 7.96 (m, 3H), 7.74 (q, 2H), 7.53 (m, 2H), 7.21 (m, 6H), 6.83 (m, 2H), 5.57 (s, 2H), 4.62 (m, 2H), 4.10 (s, 2H), 3.77 (s, 3H), 3.71 (m, 4H), 3.63 (s, 2H), 3.42 (m, 2H), 3.13 (m, 2H), 3.02 (s, 2H), 2.27 (m, 4H). LC-MS(ESI): m/z 763 (M+H)<sup>+</sup>.

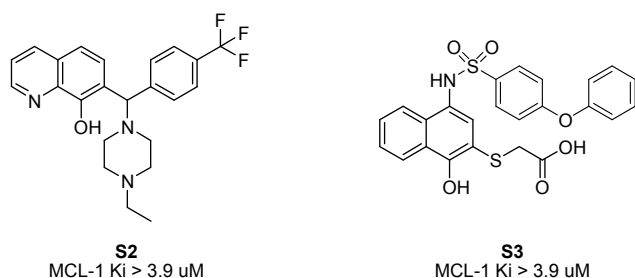
### Domain III binding assay

Domain III protein was obtained as previously described<sup>4</sup> and a fluorescence polarization binding assay performed using probe **S1**. The probe binds to domain III with  $K_D = 1.4$  μM in PBS buffer with 4% DMSO. MCL-1 compounds were assayed in the competition assay using 350 nM domain III protein and 20 nM probe. Compounds were pre-diluted in DMSO, solution containing domain III protein and probe was added to the compound plate with final DMSO concentration of 4%, then the mixtures were incubated for 5 min before reading on an Analyst 96-well plate reader (LJL; Molecular Dynamics, Sunnyvale, CA). Dissociation constants were determined from titration curves with in-house written software by using the analytical expressions of Wang.<sup>5</sup>



**Figure S1.** Domain III probe S1.

## MCL-1 binding data for literature compounds with reported cellular activity



**Figure S2.** Literature compounds S2 and S3 along with MCL-1 binding affinity (TR-FRET).

**S2**<sup>2</sup> and **S3**<sup>3</sup> were recently reported to exhibit on-target cellular effects. In our hands, both compounds exhibit binding  $K_i$  values  $> 3$  micromolar in a TR-FRET binding assay. This level of affinity for MCL-1 would not support on-target pharmacological activity.

## X-ray crystallographic data

**Table S-1. Crystallographic Statistics Table**

	<b>MCL-1: Compound 5</b>	<b>MCL-1: Compound 14</b>	<b>MCL-1: Compound 33</b>
<b>Data collection</b>			
Space group	P 2(1)	C 2 2 2 (1)	P 3 2
Cell dimensions	a, b, c (Å):46.89 82.93 48.00 $\alpha, \beta, \gamma$ (deg): 90.00, 117.50, 90.00	a, b, c (Å):48.36 80.60 89.36 $\alpha, \beta, \gamma$ (deg): 90.00, 90.00, 90.00	a, b, c (Å): 63.92 63.92 91.40 $\alpha, \beta, \gamma$ (deg): 90.00, 90.00, 120.0
Resolution (Å)	2.25	1.95	2.31
Completeness (%)	80.1	92.6	99.4
Redundancy	3.2	6.3	4.9
I/ $\sigma$ I	12.7	18.3	11.3
<b>Refinement</b>			
Resolution (Å)	2.25	1.95	2.31
R-work/R-free (%)	21.8/25.8	22.2/26.0	21.4/23.2
RMSD bonds (Å)	0.01	0.01	0.01
RMSD angles (°)	1.06	0.95	1.08
No. non-hydrogen atoms			
Protein	2412	1256	2324
Other	76	56	162

water	94	93	60
B-factors (Å <sup>2</sup> )			
Protein	43.5	40.2	49.8
Other	35.8	36.1	38.9
water	45.3	49.6	56.8
Ramachandran favoured (%)	96.3	98.1	96.1
Ramachandran allowed (%)	2.4	1.3	1.8
Ramachandran disallowed (%)	1.4	0.7	2.1

**Table S-2. Kinome selectivity data for 30b<sup>6</sup>**

ENZYME	MEAN IC50 (micromolar)	ENZYME	MEAN IC50 (micromolar)
Rock1	>10	Erk2	>10
Rock2	>10	FAK	>10
Rsk2	>10	FGFR1	>10
SGK1	>10	Flt1	>10
Src	>10	Fyn	>10
STK16	>10	GRK5	>10
STK33	>10	Gsk3a	>10
Syk CatDom	>10	Gsk3b	>10
TAOK2	>10	IGF1R	>10
TBK1	>10	IKKE	>10
TNK2	>10	InsR	>10
TrkA	>10	JAK2	>10
TrkB	>10	JAK3	>10
TrkC	>10	JNK1	>10
TYRO3	>10	JNK2	>10
Wee1	>10	Kdr	>10
Zipk	>10	Lck	>10
Abl	>10	LTK	>10
ACVR1	>10	MAP2K3	>10
Akt1	>10	MAP3K10	>10
ALK	>10	MAP4K2	>10
Aurora1	>10	MAP4K4	>10
Aurora2	>10	MEK1	>10
BRAF	>10	MEK2	>10
BTK	>10	MST1	>10
CAMK1D	>10	Nek2	>10
CAMK2A	>10	p38 alpha	>10
CAMKK2	9.84	PAK4KD	>10
cdk2	>10	PDGFRA V561D	>10
CDK7/Cyclin H/Mat1	>10	PDGFRB	>10
CDK8/Cyclin C	8.53	Pim1	>10
CDK9/Cyclin K	>10	Pim2	>10
CDK11	>10	PKA	>10
Ck1a1pha1	>10	PKCtheta	>10
CLK2	>10	PKCzeta	>10
cMET	>10	PKG1A	>10
CSF1R	>10	PIk3	>10
DDR1	>10	Prkcn	>10
Dyrk1A	>10	RET	>10
DYRK1B	>10		
EGFR	>10		

**Table S-3. Bioprofiling selectivity data for 30b<sup>7</sup>**

RECEPTOR	RESULT_TYPE	RESULT (Micromolar)
5-HT1A_ag	EC50	> 10
5-HT1A_antag	IC50	> 10
5-HT2A_ag	EC50	> 10
5-HT2A_antag	IC50	> 10
5-HT2B_ag	EC50	> 10
5-HT2B_antag	IC50	> 10
A1_ag	EC50	> 10
A1_antag	IC50	> 10
Alpha1A_ag	EC50	> 10
Alpha1A_antag	IC50	> 10
Alpha2A_ag	EC50	> 10
Alpha2A_antag	IC50	> 10
AT1_ag	EC50	> 10
AT1_antag	IC50	> 10
B2_ag	EC50	> 10
B2_antag	IC50	> 10
Beta1_ag	EC50	> 10
Beta1_antag	IC50	> 10
Cav1.2 (L-type)_ag	EC50	> 10
Cav1.2 (L-type)_antag	IC50	> 10
CB1_ag	EC50	> 10
CB1_antag	IC50	> 10
D2L_ag	EC50	> 10
D2L_antag	IC50	> 10
ETA_ag	EC50	> 10
ETA_antag	IC50	> 10
ETB_ag	EC50	> 10
ETB_antag	IC50	> 10
H1_ag	EC50	> 10
H1_antag	IC50	> 10
M2_ag	EC50	> 10
M2_antag	IC50	> 10
Opioid mu_ag	EC50	> 10
Opioid mu_antag	IC50	> 10
P2Y_antag	IC50	> 10
PDE3A_antag	IC50	> 10
PDE4B2_antag	IC50	> 10
PPARgamma_ag	EC50	> 10

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