SUPPORTING INFORMATION

Establishment of a structure-activity relationship of the 1*H*-imidazo[4,5-c]quinoline-based kinase inhibitor NVP-BEZ235 as a lead for African sleeping sickness

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COMPOUND DATA TABLES

Table S1. Biochemical Selectivity data

					PI3K IC_{50} (μM)				
Cmpd	**NEU Number**	**Tbb EC_{50} (μM)**	**HepG2 TC_{50} (μM)**	**Cell Sel.**	**delta**	**gamma**	**beta**	**alpha**	**mTOR IC_{50} (μM)**
1	NEU-0000038	0.0025	nd[b]	-	0.013	0.100	0.316[c]	0.020	0.004
16b	NEU-0001073	0.091	>50	>548	0.079	0.126	>30	0.100	0.038
16d	NEU-0001075	0.088	>50	>567	6.310	0.100	3.162	0.316	0.071
16e	NEU-0001076	0.200	4.780	24	2.512	0.251	>30	0.631	0.468
16g	NEU-0001078	0.166	11.200	67	2.512	0.316	7.943	0.501	0.380
16h	NEU-0001079	0.082	2.340	28	1.259	0.251	3.981	1.000	0.063
17b	NEU-0001083	0.182	>50	>274	0.251	0.158	>30	0.251	nd[a]
17d	NEU-0001084	0.200	>50	>250	1.259	0.063	1.000	0.631	nd[a]
20	NEU-0001068	0.759	>50	>65	0.501	0.316	2.512	1.585	0.513
4a	NEU-0001086	0.135	3.430	25	0.398	0.398	1.259	0.398	0.036
4b	NEU-0001087	0.016	0.575	35	0.251	0.040	0.251	0.040	0.019
4c	NEU-0001088	0.158	1.820	11	nd[a]	nd[a]	nd[a]	0.251	0.069
4e	NEU-0001090	0.051	0.631	12	0.794	0.126	7.943	0.398	1.175
5b	NEU-0001093	2.042	>50	>24	nd[a]	nd[a]	>30	nd[a]	21.380
9	NEU-0001064	0.617	>50	>81	15.849	1.995	>30	3.981	1.622

[a] nd=data not obtained. [b] not obtained due to low solubility. [c]one replicate experiment showed an IC_{50} >30 μM.

Table S2. Summary of physicochemical properties data for compounds.

NEU Number	Compd	Tbb EC_{50} (μM)	HepG2 TC_{50} (μM)	MPO	Solubility (μM)[1]	chromLogD[2]	chromLogP	HSA binding (%)[3]	Permeability (nm/sec)	PFI	cLogP
NEU-0000038	**1**	0.002		3.22	19	4.75		95.7		10.75	5.65
NEU-0001065	**10**	0.008	0.14	3.40							4.75
NEU-0001072	**16a**	0.102	>25	3.21							5.73
NEU-0001073	**16b**	0.091	>50	3.52	7	4.28	4.33	98.2		10.28	4.71
NEU-0001074	**16c**	0.024	>25	3.70							4.86
NEU-0001075	**16d**	0.088	>50	4.83	18	2.67	2.68	90.4	150	7.67	3.18
NEU-0001076	**16e**	0.200	4.78	4.07	17	3.5	3.5	93.8	470	8.5	3.99
NEU-0001077	**16f**	0.072		4.84							3.34
NEU-0001078	**16g**	0.166	11.2	4.57	22	3.03	3.04	92.4	860	8.03	3.32
NEU-0001079	**16h**	0.082	2.34	4.22	20	3.64	3.72	93.9		8.64	3.59
NEU-0001080	**16i**	0.316	>7.6	4.39							3.37
NEU-0001081	**16j**	3.311	>7.6	3.91	275				16		3.22
NEU-0001082	**16k**	3.273	>20	6.00							1.82
NEU-0001083	**17b**	0.182	>50	3.45	7	3.64	3.64	96.1		9.64	4.67
NEU-0001084	**17d**	0.200	>50	4.61	1	2.13	2.13	91.9	<10	7.13	3.13
NEU-0001085	**17h**	0.042	>50	4.17							3.54
NEU-0001066	**18**	6.166	19.05	4.56	55	3.62	3.63	93.4	710	7.62	3.11
NEU-0001067	**19**	9.333	3	4.89	33	4.25	4.38	92.2	670	8.25	3.16
NEU-0001068	**20**	0.759	>50	3.00							5.34
NEU-0001069	**21**	9.886	>50	4.65	103	1.47	1.47	96.5	16	6.47	4.64
NEU-0001070	**22**	1.303	>50	4.51	58	4	4	92.8	550	8	3.56
NEU-0001071	**23**	5.495	>50	5.02	404	3.45	3.45	89	900	7.45	3.05
NEU-0001086	**4a**	0.135	3.43	3.86		2.99	3.1	91.9		7.99	4.28
NEU-0001087	**4b**	0.016	0.575	3.86					530		4.28
NEU-0001088	**4c**	0.158	1.82	3.58							5.49
NEU-0001089	**4d**	0.054	>25	3.09							5.54
NEU-0001090	**4e**	0.051	0.631	4.08							3.92
NEU-0001091	**4f**	0.054	>25	3.18							6.37
NEU-0001092	**5a**	9.772	>50	4.08	158	3.06	3.46	84.8	460	7.06	3.80
NEU-0001093	**5b**	2.042	>50	4.20	95.000	3.83	3.82	85	550	7.83	3.74
NEU-0001061	**6**	10.593	>50	5.08	172	2.8	2.79	80.8	450	6.8	3.02
NEU-0001062	**7**	9.016	>50	4.59	142	2.78	2.78	78.5	190	6.78	3.08
NEU-0001063	**8**	1.995	>50	4.24	298, 81	4.58	4.79	91.6	640	8.58	4.36
NEU-0001064	**9**	0.617	>50	4.56					570		3.85

LIPIDOMICS RESULTS

Figure S1 Positive ion mode survey scans from 600-1000 m/z A) DMSO (control), B) **1**, C) **4e**, D) **16g**, E) **16e**,

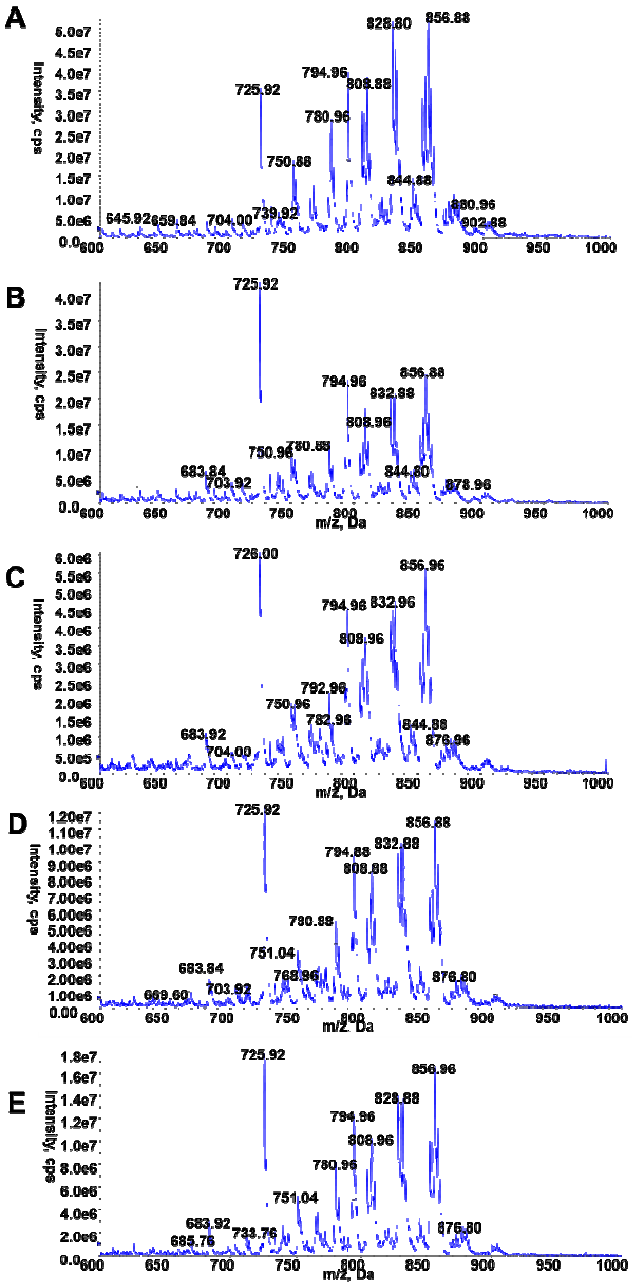
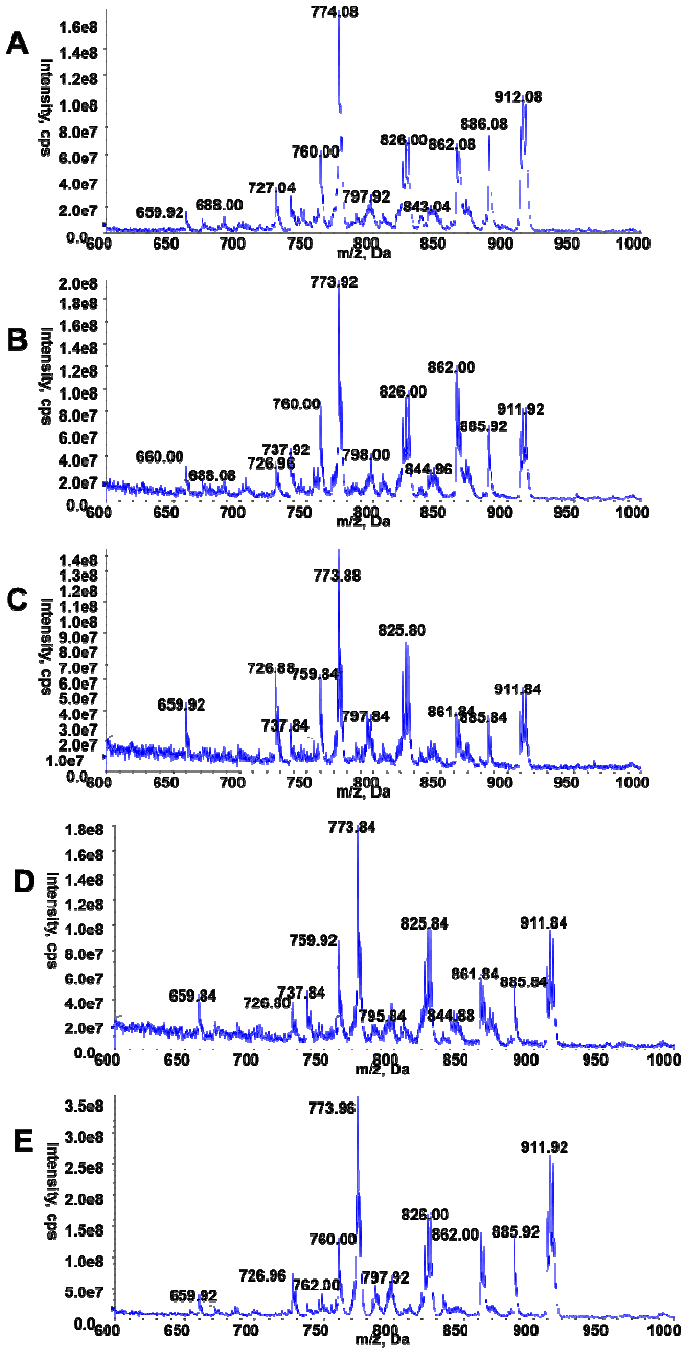
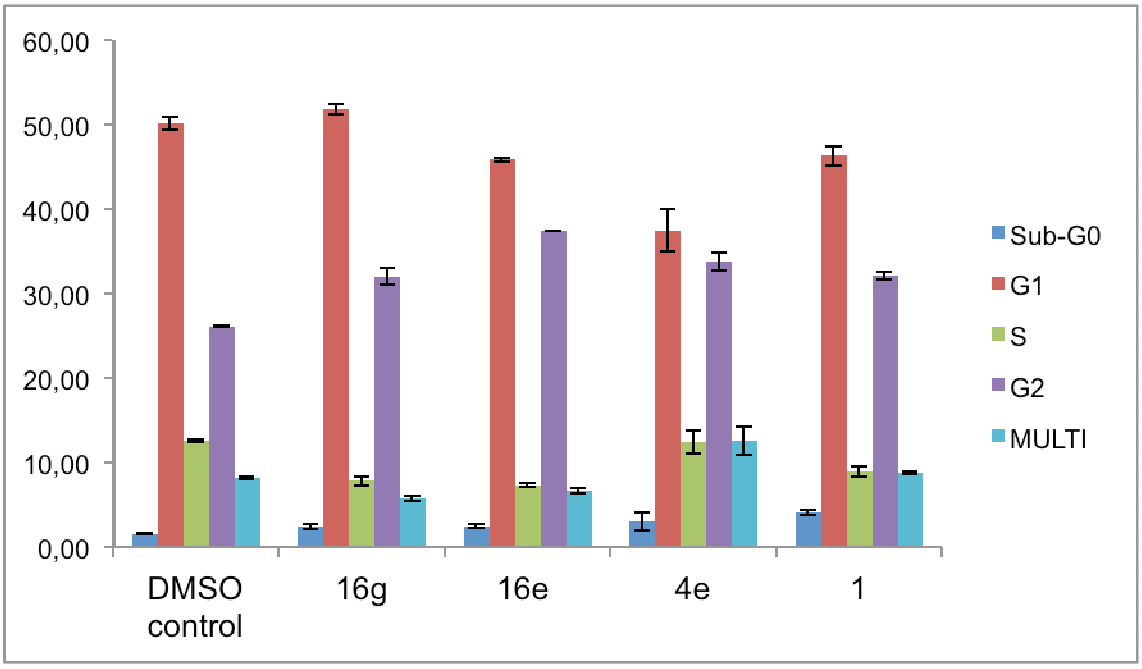


Figure S2: Negative ion mode survey scans from 600-1000 m/z A) DMSO (control), B) **1**, C) **4e**, D) **16g**, E) **16e**.

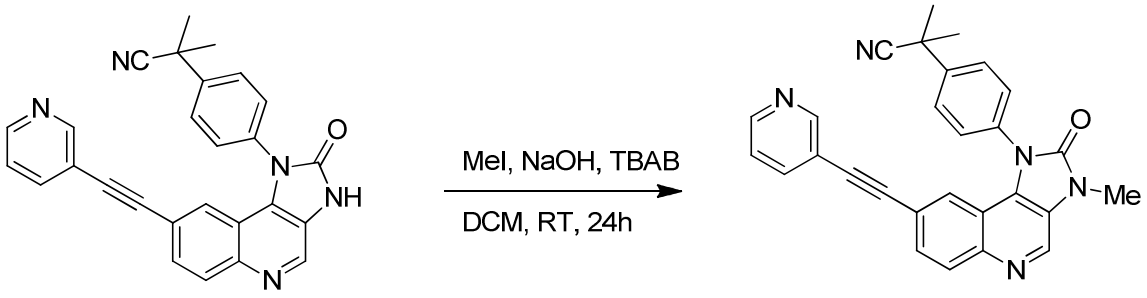


CELL CYCLE ANALYSIS

Figure S3. Cell Cycle analysis of parasites upon dosage with NVP-BEZ235 derivative. Bloodstream form culture of *T. b. brucei* was subjected to different drugs and analyzed by Fluorescence-activated cell sorter (FACS) for DNA content stained by propidium iodide (PI). Cell cultures were incubated during 18 h with various NVP-BEZ235 derivatives using DMSO as a control treatment. Mean ± SD of three independent experiments are shown.

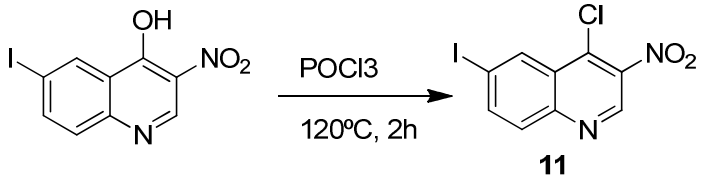


SYNTHETIC CHEMISTRY



10

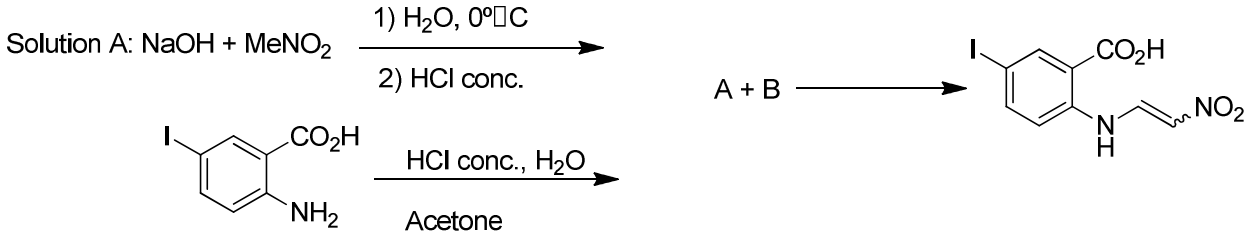
2-methyl-2-(4-(3-methyl-2-oxo-8-(pyridin-3-ylethynyl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (10).[4] Prepared from 2-methyl-2-(4-(2-oxo-8-(pyridin-3-ylethynyl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile using General Procedure D.[4] Purification was carried out by flash column chromatography over silica gel (eluent: CH_2Cl_2/MeOH 100/0 to 95/5) to give **10** as a yellow solid (Yield: 20%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.04 (s, 1H), 8.61 (d, J = 1.4 Hz, 1H), 8.57 (dd, J = 4.9, 1.4 Hz, 1H), 8.05 (d, J = 8.8 Hz, 1H), 7.85 (m, 3H), 7.72 (m, 2H), 7.65 (dd, J = 8.8, 1.9 Hz, 1H), 7.43 (m, 1H), 7.04 (d, J = 1.5 Hz, 1H), 3.60 (s, 3H), 1.79 (s, 6H). LCMS found 444, $[M+H]^+$.



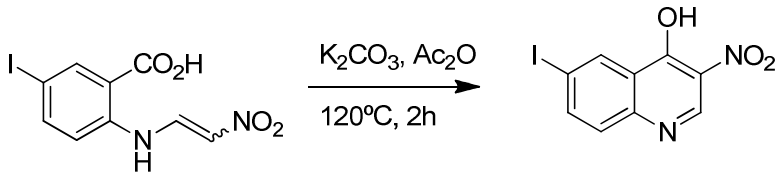
11

Preparation of 4-chloro-6-iodo-3-nitroquinoline (11).[4] A mixture of 6-iodo-3-nitroquinolin-4-ol (9.7 g, 30.7 mmol) in $POCl_3$ (7 mL) was stirred at 120 ºC for 2 h. The mixture was cooled down to rt, concentrated under reduced pressure to give a residue which was dissolved in CH_2Cl_2 (220 mL), washed with cold brine, dried over $MgSO_4$, filtered and concentrated under vacuum to give **11** as a brown solid (Yield: 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.38 (s, 1H), 8.72

(s, 1H), 8.32 (m, 1H), 7.96 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 168.9, 162.8, 143.7, 141.6, 134.8, 131.5, 130.4, 122.9, 91.5.

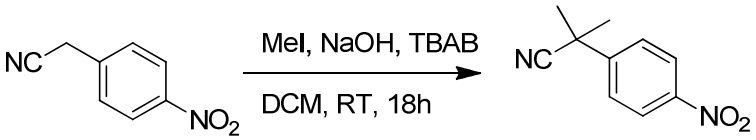


Preparation of 5-iodo-2-((2-nitrovinyl)amino)benzoic acid. Ice (30 g) was added to a solution of NaOH (13.2 g, 326 mmol) in H_2O (17.2 mL) and cooled down to 0 ºC in an ice bath before neat $MeNO_2$ (13.9 mL, 255 mmol) was added over 5 min. The resulting mixture was stirred at 0 ºC for 30 min, and then hydrochloric acid (35 mL, ACS reagent 37%) was added dropwise and stirred for 10 min. The resultant mixture was then added to another mixture of 2-amino-5-iodo-benzoic acid (27 g, 102 mmol), hydrochloric acid (17.2 mL, ACS reagent 37%), H_2O (350 mL) and acetone (300 mL) and stirred for 2 h. The resulting precipitate was filtered out, washed with H_2O (2x400 mL), 2N aqueous hydrochloric acid (150 mL) and left to dry overnight under vacuum. The title compound was obtained as a yellow solid (32.4 g, 97 mmol, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.91 (s, 1H), 8.23 (s, 1H), 8.03 (d, 6.7 Hz 1H), 7.96 (d, 8.9 Hz, 1H), 7.56 (d, 8.9 Hz, 1H), 6.76 (d, 6.7 Hz, 1H), 3.34 (brs, 1H). LCMS found 335, $[M+H]^+$.[1]

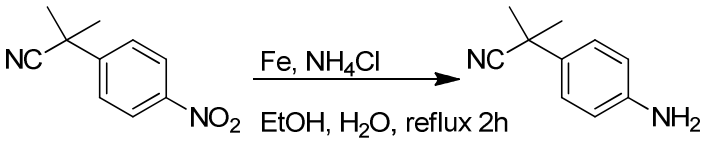


[1] Data for major stereoisomer was given.

Preparation of 6-iodo-3-nitroquinolin-4-ol. 5-Iodo-2-((2-nitrovinyl)amino)benzoic acid (16.2 g, 46 mmol), potassium acetate (5.2 g, 55 mmol) and acetic anhydride (220 mL) were mixed and stirred at 120 ºC for 1.5 h. The resultant precipitate was filtered off, washed with acetic acid until the filtrate was colourless, then with H_2O and dried under vacuum to give the title compound as a tan solid (9.8 g, 31 mmol, 69%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 13.1 (s, 1H), 9.21 (s, 1H), 8.49 (d, 2.0 Hz, 1H), 8.07 (dd, 8.6, 2.0 Hz, 1H), 7.51 (d, 8.6 Hz, 1H). LCMS found 317, $[M+H]^+$.



2-Methyl-2-(4-nitrophenyl)propanenitrile (**S1**) To a solution of 4-nitrophenylacetonitrile (8 g, 49.3 mmol), *tetra*-butylammonium bromide (874 mg, 2.7 mmol) and methyl iodide (10 mL, 162 mmol) in CH_2Cl_2 (63 mL) was added dropwise a solution of NaOH (5.3 g, 133 mmol) in H_2O (63 mL). The resulting mixture was stirred 18 h at rt. After separation, the organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuum. The residue obtained was then dissolved in CH_2Cl_2 and treated with black charcoal, stirred for 1 h and then filtered through celite. Volatiles were removed under vacuum to give **S1** as a yellow solid (Yield: 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.27 (m, 2H), 7.68 (m, 2H), 1.78 (s, 6H).[5]



2-(4-Aminophenyl)-2-methylpropanenitrile (**S2**) Prepared from 2-Methyl-2-(4-nitrophenyl)propanenitrile using General Procedure B affording **S2** as a yellow solid (420 mg, 2.6 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.25 (m, 2H), 6.68 (m, 2H), 3.71 (brs, 2H), 1.68 (s, 6H)

BIOCHEMICAL ASSAY DETAILS.

Mammalian Target of Rapamycin (mTOR) assay. FRAP1 (mTOR) Recombinant Human Protein, green fluorescent protein 4E-BP1 (GFP-4E-BP1), LanthaScreen® Tb-p4E-BP1 (pThr46) antibody and TR-FRET dilution buffer were supplied by Invitrogen, a part of Life Technologies Corporation. EDTA was supplied by Gibco. All other chemicals were obtained from Sigma-Aldrich.

11 point, 3-fold serial dilutions of test compounds were prepared in DMSO and 50 nL of these solutions were dispensed into 384-well, low volume, black, assay plates using an Echo 555 acoustic dispenser. In each assay plate two columns of wells (16 wells per column) were used for controls, with column 6 containing DMSO only (high control) and column 18 containing a FRAP1(mTOR) inhibitor at 10μM final assay concentration (low control).

2.5μL of 2 x enzyme solution (assay buffer (50mM HEPES, 1mM CHAPS, 5mM $MnCl_2$, 0.01% w/v BSA and 5mM DTT) containing 0.8μM GFP-4E-BP1 and 1nM FRAP1(mTOR)) was added to all wells and incubated at room temperature for 30 mins. To initiate the reaction 2.5μL of 2 x substrate solution (assay buffer containing 0.8μM ATP) was added to all wells and incubated at room temperature for 20 mins. 5μL of 2 x detection solution (TR-FRET dilution buffer containing 50m EDTA and 2nM Tb-p4E-BP1 (pThr46) antibody) was added to all wells and incubated for 60 mins before reading on the Envision (Excitation 340nm, Emission (Donor)495nm/(Acceptor) 520nm).

Ratiometric data was analysed within ActivityBase and concentration response curves fitted to the equation below:

$$Inhibition\ (\%) = \frac{(a-d)}{1+\left(\frac{[I]}{IC_{50}}\right)^{S}} + d$$

Where a is the uninhibited response, d is the fully inhibited response, [I] is the inhibitor concentration, IC50 is [I] that gives 0.5x(a-d) and S is the Hill slope.

PI 3-kinase assays. Standard enzyme reactions were performed using kinases expressed within GlaxoSmithKline: PI3Kα (Gene ID 5295) β (Gene ID 5291), γ (Gene ID 5294),and δ (5293). The compounds were tested in a TR-FRET assay in 50 mM HEPES pH 7.0, 150 mM NaCl, 10 mM $MgCl_2$, 2.3 mM sodium cholate 10 μM CHAPS and 1 mM dithiothreitol containing 80 μM ATP, 10 μM diC8 PtdIns(4,5)P_2, 40 nM biotinylated diC6 PtdIns(3,4,5)P_3 and 2 ng of PI 3-kinase in a total reaction volume of 6 μL. Inhibitors were added to dry wells in 0.1 μL DMSO giving a final DMSO concentration of 1.6% in the assay and allowed to preincubate with enzyme before initiating the reaction upon addition of the substrates. Reactions were stopped after 1 hr by adding 3 μL detection solution containing 50 nM Streptavidin-APC, 4 nM Europium-anti-GST and 15 nM GST-tagged PH domain diluted in 50mM HEPES pH 7.0, 150mM NaCl, 30mM EDTA, 2.3 mM sodium cholate, 10 μM CHAPS 40mM potassium fluoride and 1mM DTT. These were left to equilibrate for 1 hr before reading using a Perkin Elmer Envision plate reader. All concentrations quoted are final assay concentrations.

PHYSICOCHEMICAL PROPERTIES ASSAYS

CLND kinetic solubility assay. 5 µL of 10mM DMSO stock solution diluted to 100ul with pH7.4 phosphate buffered saline, equilibrated for 1 hour at room temperature, filtered through Millipore Multiscreen$_{HTS}$-PCF filter plates (MSSL BPC). The filtrate is quantified by suitably calibrated flow injection Chemi-Luminescent Nitrogen Detection.[1] The standard error of the CLND solubility determination is ±30 µM, the upper limit of the solubility is 500 µM when working from 10 mM DMSO stock solution.

GSK in-house Artificial Membrane Permeability Assay: A 1.8% lipid (phosphatidyl choline, egg) in 1% cholesterol decane solution was applied to a Millicell 96-well, 0.4um, PCF culture plate. 250 µL and 100 µL 50 mM phosphate buffer pH 7.4 with 0.5% encapsin was applied to the donor and receiver compartments, respectively. 2.5 µL of a 10 mM stock solution of compound in DMSO was added to the donor compartment. The assay was incubated at RT for 3 hours. Samples from both donor and receiver compartments were analyzed by HPLC with UV detection at 215 and 254 nm and permeability was calculated. The permeability ($logP_{app}$) measuring how fast molecules pass through the black lipid membrane is expressed in nm/s.

ChromlogD assay. The Chromatographic Hydrophobicity Index (CHI)[2] values were measured using reversed phase HPLC column (Luna C18 (2),Phenomenex, UK) with fast acetonitrile gradient at starting mobile phase of pH = 7.4. CHI values are derived directly from the gradient retention times by using a calibration line obtained for standard compounds. The CHI value approximates to the volume % organic concentration when the compound elutes. CHI is linearly transformed into ChromlogD by least-square fitting of experimental CHI values to calculated ClogP values for over 20K research compounds using the following formula: ChromlogD = 0.0857CHI-2.00. The average error of the assay is ±3 CHI unit or ±0.25 ChromlogD.

Protein binding assay. Chemically bonded Human Serum Albumin (HSA) and Alpha-1-acidglycoprotein HPLC stationary phases (Chiral Technologies, France) were used for measuring compounds' binding to plasma proteins, applying linear gradient elution up to 30% iso-propanol. The run time was 6 minutes including the re-equilibration of the stationary phases with the 50 mM pH7.4 ammonium acetate buffer. The obtained gradient retention times were standardised using a calibration set of mixtures as described in the references.[3] The average standard error of the assay depends on the binding strength and kinetics of the compounds. It ranges from ±5% in the medium binding range which reduces to 0.1% at binding above 99% with fast kinetics.

Phospholipid binding assay (IAM). Compounds binding to immobilised artificial membrane (IAM) has been measured using commercially available IAM PC DD (Regis Analytical, West Lafayette, USA) HPLC column. Applying acetonitrile gradient up to 70% the gradient retention times of the compounds were converted to Chromatographic Hydrophobicity Indices (CHI IAM) using a calibration set of compounds as described in reference 6. The CHI IAM values then were converted to the logarithmic retention factors using the following formula: log k IAM = 0.046*CHI IAM + 0.42, that was obtained from the correlation of isocratic and gradient retention time.[6]

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