Supporting Information:

Fragment-Based Design of a Potent MAT2a Inhibitor and *in Vivo* Evaluation in an MTAP Null Xenograft Model

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Additional figures:

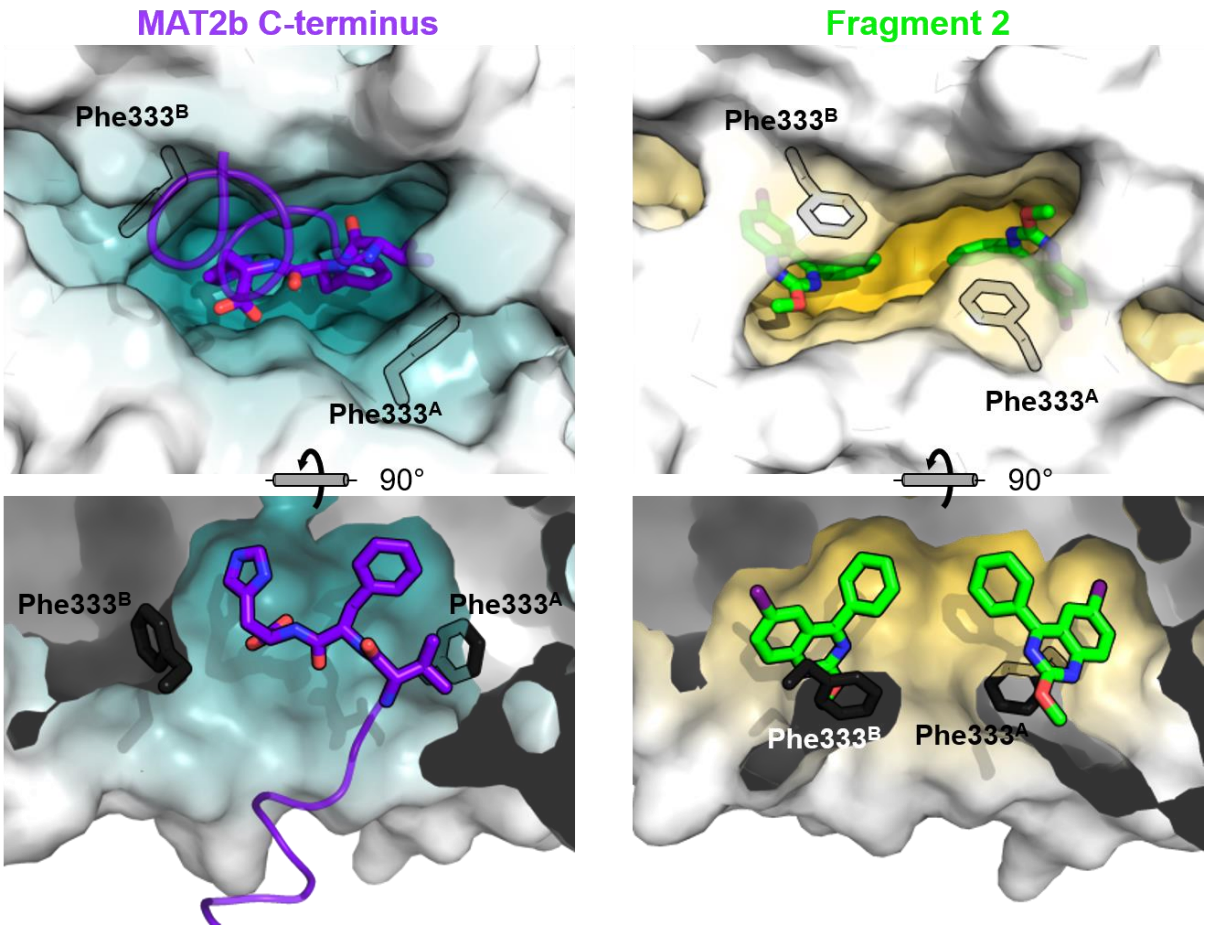


Figure S1: Ligand-induced changes to the allosteric site of MAT2a, which is displayed viewed from solvent (top), and in cross section (bottom). **Left:** View of the allosteric site bound to MAT2b, with Phe333 (highlighted) constricting the width of the site. **Right:** The crystal structure of **2** (PDB 7bhs, 1.1 Å) shows two molecules of the ligand occupying the symmetric site. Ligand binding re-orients the side chain of Phe333, which re-shapes the site into a long and narrow cleft and partially occludes solvent access.

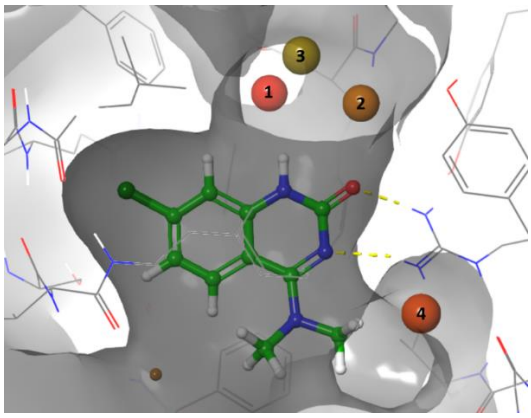


Figure S2: WaterMap analysis (see Computational Details for references) of **5** bound to MAT2a. The calculated excess free energies ΔG of water sites 1-4 are +6.8, +4.1, +3.0, and +5.4 kcal/mol, respectively.

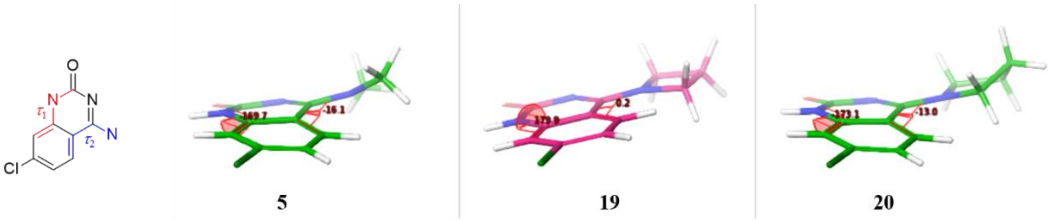


Figure S3: QM optimised structures of 4-aminoquinazolinones **5** (nonplanar), **19** (planar), and **20** (nonplanar). The calculated dihedral angles τ_1 and τ_2, defined to the left, are -169.7 and -16.1, 179.9 and 0.2, and -173.1 and -13.0 degrees for **5**, **19**, and **20**, respectively. For **5** bound to MAT2a, the corresponding experimental values of τ_1 and τ_2 are -169.8 and -11.4 degrees, as determined by X-ray crystallography (Figure 3).

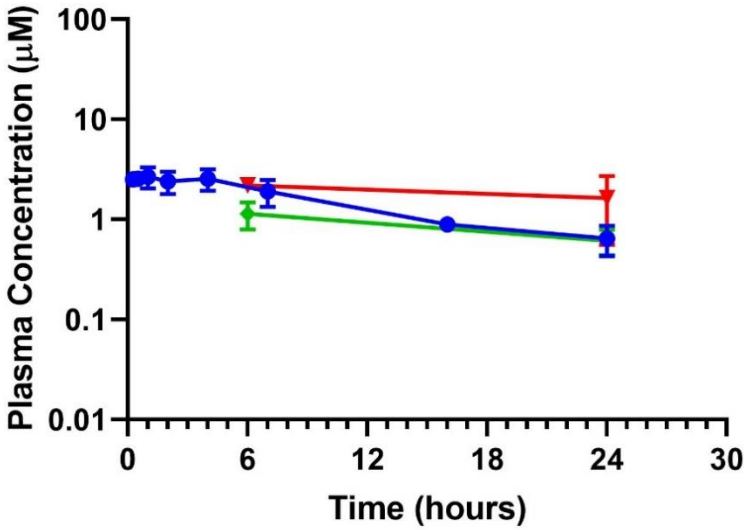


Figure S4: Mouse plasma concentrations of **28** after a single SC dose at 100 mg/kg (blue circles), 14 once daily SC doses of 100 mg/kg (red triangles), and 14 once daily SC doses of 50 mg/kg (green diamonds).

Equation S1:

$$\text{Methionine} + \text{ATP} + H_2O \rightarrow \text{SAM} + \text{pyrophosphate} + \text{phosphate}$$

Table S1: Crystallographic data collection and refinement statistics. Values in parentheses are for the highest resolution shell.

Protein	**MAT2a**	**MAT2a**	**MAT2a**	**MAT2a**
Compound	**1**	**2**	**5**	**26**
PDB ID	7BHR	7BHS	7BHT	7BHU
Data collection				
DLS beamline	I04	I04	I03	I03
Date	2018-07-13	2018-11-30	2020-09-22	2019-05-10
Detector	Pilatus 6M	Pilatus 6M	Eiger X 16M	Eiger X 16M
Wavelength (Å)	0.9750	0.9763	0.8969	0.9763
Data processing				
Software	DIALS/Aimless	XDS/Staraniso	XDS/Staraniso	XDS/Staraniso
Space group	I222	I222	I222	I222
Cell dimensions				
a, *b*, *c* (Å)	67.43	67.92	67.87	68.24
	94.21	94.02	93.93	94.17
	117.28	117.09	116.97	117.30
α, β, γ (°)	90	90	90	90
	90	90	90	90
	90	90	90	90
Resolution (Å)	58.64—1.08	40.11—1.05	19.82—1.05	58.98—1.15
	(1.11—1.08)	(1.15—1.05)	(1.14—1.05)	(1.19—1.15)
R_{pim}	0.027 (0.529)	0.058 (0.597)	0.042 (0.570)	0.025 (0.319)
Reflections, total	892550	798752	1618327	677640
Reflections, unique	144857 (6068)	132040 (6603)	120837 (6042)	119589 (5980)
I / σI	11.6 (1.1)	9.4 (2.2)	12.1 (1.8)	19.1 (2.3)
Completeness, spherical (%)	91.2 (52.4)	76.4 (16.9)	70.3 (16.3)	90.6 (47.2)
Completeness, ellipsoidal (%)		93.8 (49.1)	94.9 (72.3)	91.4 (49.1)
Multiplicity	6.2 (3.9)	6.0 (4.0)	13.4 (14.4)	5.7 (2.5)
CC 1/2	0.997 (0.626)	0.998 (0.535)	0.999 (0.736)	0.999(0.741)
Refinement (Buster)				
Resolution (Å)	58.64—1.08	40.11—1.05	19.82—1.05	16.90—1.15
R_{work} / R_{free}	0.167 / 0.183	0.170 / 0.185	0.156 / 0.175	0.153 / 0.169
No. atoms				
Protein	2943	2939	2983	2945
Inhibitor	13	19	15	20
SAM	27	27	27	27
Water	322	286	400	357
B-factors (Å^2)				
Protein	14.3	11.4	11.8	12.6
Inhibitor	13.9	16.7	11.3	10.8
SAM	14.3	10.8	9.3	12.7
Water	25.7	23.6	23.5	24.4
R.m.s. deviations				
Bond lengths (Å)	0.010	0.010	0.009	0.010
Bond angles (°)	1.100	1.110	1.102	1.093
Model Validation (MolProbity)				
Ramachandran favoured (%)	97.88	97.62	97.62	97.62
Ramachandran allowed (%)	2.12	2.38	2.38	2.38
Ramachandran outliers (%)	0.00	0.00	0.00	0.00
Ramachandran Z-score	0.34	0.10	-0.15	0.12

Protein	**MAT2a**	**MAT2a**	**MAT2a**
Compound	**28**	**29**	**31**
PDB ID	7BHV	7BHW	7BHX
Data collection			
DLS beamline	I03	I03	I03
Date	2019-04-26	2019-05-10	2020-09-22
Detector	Eiger X 16M	Eiger X 16M	Eiger X 16M
Wavelength (Å)	0.9763	0.9763	0.8153
Data processing			
Software	XDS/Staraniso	XDS/Staraniso	XDS/Staraniso
Space group	I222	I222	I222
Cell dimensions			
a, *b*, *c* (Å)	68.18	68.58	68.04
	93.85	94.20	94.03
	117.07	117.40	117.05
α, β, γ (°)	90	90	90
	90	90	90
	90	90	90
Resolution (Å)	73.23—1.16	59.22—1.16	58.83—1.08
	(1.20—1.16)	(1.21—1.16)	(1.17—1.08)
R_{pim}	0.019 (0.110)	0.021 (0.271)	0.037 (0.537)
Reflections, total	666288	681000	1654788
Reflections, unique	118844 (5943)	113536 (5678)	121124 (6056)
I / σI	27.4 (4.6)	21.5 (2.3)	13.4 (1.8)
Completeness, spherical (%)	91.9 (49.8)	88.1 (40.8)	75.6 (17.4)
Completeness, ellipsoidal (%)	92.2 (50.4)	90.4 (46.9)	94.0 (68.5)
Multiplicity	5.6 (2.1)	6.0 (3.4)	13.7 (13.7)
CC 1/2	0.999 (0.980)	1.000 (0.854)	1.000 (0.743)
Refinement (Buster)			
Resolution (Å)	16.98—1.08	16.90—1.16	15.80—1.08
R_{work} / R_{free}	0.149 / 0.157	0.156 / 0.167	0.158 / 0.173
No. atoms			
Protein	2945	2950	2977
Inhibitor	20	22	21
SAM	27	27	27
Water	357	342	375
B-factors (Å^2)			
Protein	12.6	14.8	13.1
Inhibitor	10.8	11.5	11.1
SAM	12.7	14.4	10.7
Water	24.4	26.2	24.0
R.m.s. deviations			
Bond lengths (Å)	0.010	0.010	0.010
Bond angles (°)	1.078	1.103	1.080
Model Validation (MolProbity)			
Ramachandran favoured (%)	97.62	98.15	97.88
Ramachandran allowed (%)	2.38	1.85	2.12
Ramachandran outliers (%)	0.00	0.00	0.00
Ramachandran Z-score	0.04	0.12	-0.11

Compound Synthesis

Flash column chromatography was carried out using self-packed silica cartridges and C18-flash chromatography was performed with a combine flash (Agela MP 200) using C18 cartridges from Agela.
Preparative reverse phase HPLC was performed on a Waters instrument (2545, 2767 and 2489) fitted with a QDa or SQ Detector 2 ESCi mass spectrometers and a Waters X-Bridge or Waters XSelect or Waters SunFire reverse-phase column (C-18, 5µm, 30 mm diameter and 150 mm length with a flow rate of 60 ml/min or C-18, 5µm, 19 mm diameter and 250 mm length with a flow rate of 25 ml/min) or an Agilent 1290 Infinity II Preparative system equipped with a SQ MS detector (Multimode ESI/APCI source), with a Waters CSH C18 OBD column (5 microns silica, 30 mm diameter, 100 mm length, flow rate of 50 ml/min) using decreasingly polar mixtures of water (containing 0.1 - 0.3% aqueous ammonium) or water (containing 0.1% formic acid) and acetonitrile as eluents.
NMR: NMR spectra were recorded on a Bruker AVANCE III HD 400 (400 MHz) or Bruker AVANCE NEO 400(400 MHz) or Bruker AVANCE III 400 (400 MHz) or Bruker AVANCE II 300 (300 MHz) or Bruker AVANCE III 300 (300 MHz) or Bruker AVANCE III HD 300 (300 MHz). Characteristic chemical shifts (δ) are given in parts-per-million downfield from tetramethylsilane (0.00 ppm) or solvent peaks as the internal reference using conventional abbreviations for designation of major peaks: e.g. s, singlet; d, doublet; t, triplet; q, quarter; m, multiplet; br, broad.
Analytical LCMS: LC/MS experiments were performed using a Shimadzu LCMS-2020 with electrospray ionization in positive ion detection mode with 20ADXR pump, SIL-20ACXR autosampler, CTO-20AC column oven, M20A PDA Detector and LCMS 2020 MS detector. The MS detector was configured with electrospray ionization as ionizable source.

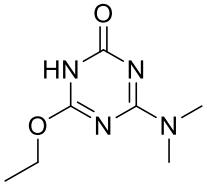
All animal studies were conducted in accordance with the guidelines established by the internal IACUC (Institutional Animal Care and Use Committee) and reported following the ARRIVE (Animal Research: Reporting *In Vivo* experiments) guidelines

General methods:

2,4,7-Trichloroquinazoline is commercially available, but it can also be synthesised according to the following methods:
Method 1: 7-Chloroquinazoline-2,4-diol (600 mg, 3.05 mmol), and DIEA (2.7 mL, 15.3 mmol) were stirred in $POCl_3$ (6.0 mL, 64.4 mmol) at 0 °C for 1 h. The solvent was removed under reduced pressure and the crude product (700 mg) was used in the following step directly without further purification.[1]
Method 2: To a stirred suspension of 7-chloroquinazoline-2,4(1H,3H)-dione (5.00 g, 25.4 mmol) and DIPEA (8.86 mL, 50.9 mmol) in toluene (100 mL) at rt was added $POCl_3$ (3.56 mL, 38.1 mmol) dropwise. The resultant suspension was heated at 90 °C for 3.5 h. The reaction mixture was allowed to cool to rt and was carefully added dropwise to an ice cold solution of saturated $NaHCO_3$ (700 mL) with vigorous stirring. EtOAc was added and the layers were separated. The aqueous was re-extracted with EtOAc and the combined organic phases were

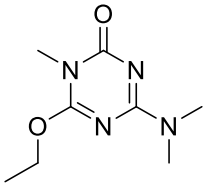
dried by passing through a phase separating cartridge. The filtrate was concentrated to dryness to afford 2,4,7-trichloroquinazoline (4.29 g, 72.2 %) as an orange solid. ^{1}H NMR (400 MHz, DMSO) δ: 7.94 (dd, *J* = 8.9, 2.1 Hz, 1H), 8.22 (dd, *J* = 2.1, 0.5 Hz, 1H), 8.33 (dd, *J* = 8.9, 0.5 Hz, 1H). *m/z*: no mass ion observed.

4-(Dimethylamino)-6-ethoxy-1,3,5-triazin-2(1*H*)-one (1)



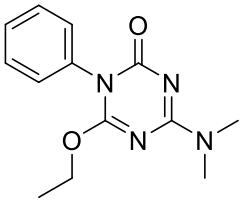
2,4,6-Trichloro-1,3,5-triazine (5.00 g, 27.1 mmol), ethanol (1.27 mL, 27.1 mmol), and K_2CO_3 (3.75 g, 27.1 mmol) were stirred in MeCN (30 mL) at rt for 4 h. Water (489 μL, 27.1 mmol) was then added and the mixture was stirred at rt for 30 min. The solids were filtered out, the solvent was evaporated and the residue (4.78 g) was used directly in the following step. Crude 4-chloro-6-ethoxy-1,3,5-triazin-2(1*H*)-one (120 mg) and dimethylamine (47 μL, 2 M in THF, 0.68 mmol) were stirred in water (5.0 mL) at 50 °C for 3 h. The solvent was evaporated and the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.05%TFA) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4-(dimethylamino)-6-ethoxy-1,3,5-triazin-2(1*H*)-one (30.0 mg, 24% over 2 steps) as a white solid; **1H NMR** (300 MHz, Methanol-d4) δ 1.46 (t, 3H), 3.26 (s, 3H), 3.37 (s, 3H), 4.62 (q, 2H); **m/z** (ES+), [M+H]+ 185.

4-(Dimethylamino)-6-ethoxy-1-methyl-1,3,5-triazin-2(1*H*)-one (3)



A mixture of 4-(dimethylamino)-6-ethoxy-1,3,5-triazin-2(1*H*)-one **1** (1.00 g, 5.43 mmol), iodomethane (0.771 g, 5.43 mmol), and K_2CO_3 (0.750 g, 5.43 mmol) in MeCN (10 mL) was stirred at 50 °C for 4 h. The mixture was cooled to rt and the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.05% $NH_3 \cdot H_2O$) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4-(dimethylamino)-6-ethoxy-1-methyl-1,3,5-triazin-2(1*H*)-one (0.300 g, 28%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 1.35 (t, 3H), 3.03 (s, 3H), 3.11 (s, 3H), 3.16 (s, 3H), 4.43 (q, 2H); **m/z** (ES+), [M+H]+ 199.

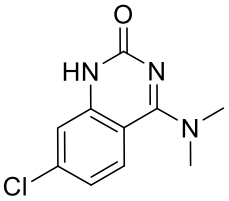
4-(Dimethylamino)-6-ethoxy-1-phenyl-1,3,5-triazin-2(1*H*)-one (4)



Commercially available 4-(dimethylamino)-6-hydroxy-1-phenyl-1,3,5-triazin-2(1H)-one (200 mg, 0.860 mmol), bromoethane (103 mg, 0.950 mmol), and silver carbonate (950 mg, 3.44 mmol) were suspended in toluene (20 mL) and the mixture was stirred at 100 °C for 15 h. The reaction mixture was cooled to rt and filtered through celite and the solvent was removed under

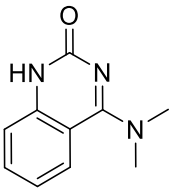
reduced pressure. The crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.05% NH_4HCO_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4-(dimethylamino)-6-ethoxy-1-phenyl-1,3,5-triazin-2(1*H*)-one (60.0 mg, 27%) as a white solid. **1H NMR** (400 MHz, DMSO-d6) δ 1.17 (3H, t), 3.10 (3H, s), 3.19 (3H, s), 4.38 (2H, q), 7.26 (2H, m), 7.40 (1H, m), 7.45 (2 H, dd); **m/z** (ES+), [M+H]+ 261.

7-Chloro-4-(dimethylamino)quinazolin-2(1*H*)-one (5)



Dimethylamine (0.47 mL, 2.0 M in THF, 0.940 mmol) was added to 2,4,7-trichloroquinazoline (200 mg, 0.860 mmol) in THF (2.0 mL) and the solution was stirred at rt for 2 h. The reaction mixture was diluted with EtOAc (50 mL), and washed sequentially with saturated $NaHCO_3$ (20 mL x 2), water (20 mL x 2), and saturated brine (20 mL x 2). The organic layer was dried over Na_2SO_4, filtered, evaporated and the crude product (160 mg) was used in the following step without further purification. Acetic acid (3.0 mL) was added to the crude 2,7-dichloro-*N*,*N*-dimethylquinazolin-4-amine (150 mg, 0.620 mmol) and the resulting mixture was stirred at 100 °C for 12 h. After cooling the reaction mixture to rt, the solvent was removed under reduced pressure. The crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.05% NH_4OH) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(dimethylamino)quinazolin-2(1*H*)-one (40.0 mg, 22% over two steps) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 3.25 (6H, s), 7.10 (1H, dd), 7.20 (1H, d), 7.94 (1H, d), 10.82 (1H, s); **m/z** (ES+), [M+H]+ 224.

4-(Dimethylamino)quinazolin-2(1*H*)-one (6)

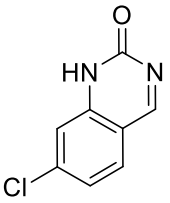


A solution of 2,4-dichloroquinazoline (150 mg, 0.750 mmol), dimethylamine (450 µL, 2 M in THF, 0.900 mmol), DIPEA (0.131 mL, 0.750 mmol) in MeCN (2.1 mL) was heated at 70 °C for 3 h. The reaction mixture was cooled to rt, diluted with EtOAc (10 mL), and washed sequentially with water (10 mL x 2), and saturated brine (10 mL x 2). The organic layer was dried with $MgSO_4$, filtered and evaporated. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 2-chloro-*N*,*N*-dimethylquinazolin-4-amine (125 mg, 80%) as a white solid. **1H NMR** (500 MHz, $CDCl_3$) δ 3.42 (s, 6H), 7.39 (ddd, 1H), 7.69 (ddd, 1H), 7.75 – 7.8 (m, 1H), 7.99 – 8.05 (m, 1H); **m/z** ES+ [M+H]+ 208.

A solution of 2-chloro-*N*,*N*-dimethylquinazolin-4-amine (118 mg, 0.570 mmol) in acetic acid (5.7 mL) was stirred at 90 °C overnight. The reaction mixture was cooled to rt and the solvent was evaporated (as an azeotrope with toluene). The crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 1% $NH_3 \cdot H_2O$) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4-(dimethylamino)quinazolin-2(1*H*)-one (69.8 mg, 65%) as a white solid. **1H NMR** (DMSO-d6,

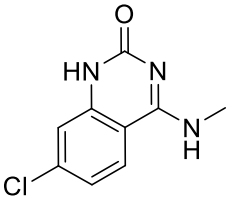
500 MHz) δ 3.24 (6H, s), 7.08 (1H, ddd), 7.18 (1H, dd), 7.53 (1H, ddd), 7.91 (1H, dd), 10.72 (1H, s); **m/z** (ES+), [M+H]+ 190.

7-Chloroquinazolin-2(1*H*)-one (7)



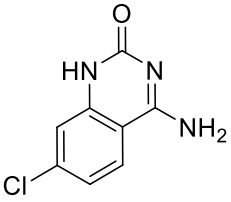
2,2,2-Trichloroacetyl chloride (386 mg, 2.12 mmol) was added to 2-amino-4-chlorobenzaldehyde (300 mg, 1.93 mmol) and DMAP (471 mg, 3.86 mmol) in DCM (5.0 mL) under N_2. The resulting mixture was stirred at rt for 12 h. The reaction mixture was quenched with water (2.0 mL), extracted with DCM (2.0 mL x 2) and the organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by preparative TLC (EtOAc: petroleum ether = 1: 10), to afford 2,2,2-trichloro-*N*-(5-chloro-2-formylphenyl)acetamide (400 mg, 69%) as a white solid; **1H NMR** (DMSO-d6, 300 MHz) δ 7.61 (1H, dd), 8.08 (1H, d), 8.36 (1H, d), 10.03 (1H, d), 12.34 (1H, s); no MS signal detected.
Ammonium acetate (77.0 mg, 1.00 mmol) was added to 2,2,2-trichloro-*N*-(5-chloro-2-formylphenyl)acetamide (150 mg, 0.500 mmol) in DMSO (3.0 mL) under N_2. The resulting mixture was stirred at 80 °C for 12 h. After cooling to rt, the crude product was purified by preparative HPLC using decreasingly polar mixtures of water and MeCN as eluents. Fractions containing the desired compound were freeze-dried to afford 7-chloroquinazolin-2(1*H*)-one (13.0 mg, 14%) as a white solid. **1H NMR** (DMSO-d6, 300 MHz) δ 7.25 – 7.37 (2H, m), 7.93 (1H, d), 9.29 (1H, s), 11.95 (1H, s); **m/z** (ES+), [M+H]+ 181.

7-Chloro-4-(methylamino)quinazolin-2(1*H*)-one (8)



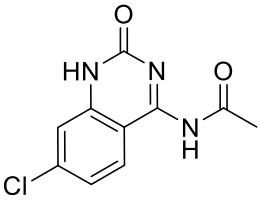
2,4,7-Trichloroquinazoline (400 mg, 1.72 mmol) and methylamine in ethanol (30 mL, 33% w/w) were stirred in MeCN (30 mL) at rt for 30 min. The solvent was removed under reduced pressure and the crude mixture was taken up in formic acid (10 mL) to give a yellow solution. The resulting mixture was stirred at rt for 24 h. The solvent was removed under reduced pressure and the reaction mixture was diluted with MeCN. The precipitate was collected by filtration, washed with MeCN and DMSO and dried under vacuum to afford 7-chloro-4-(methylamino)quinazolin-2(1*H*)-one (250 mg, 70% over two steps) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 3.09 (3H, d), 7.30 (1H, d), 7.39 (1H, dd), 8.44 (1H, d), 10.79 (1H, s), 11.93 (1H, s); **m/z** (ES+), [M+H]+ 210.

4-Amino-7-chloroquinazolin-2(1*H*)-one (9)



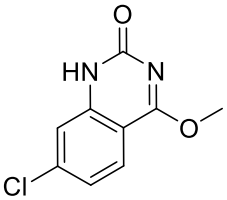
2,4,7-Trichloroquinazoline (200 mg, 0.862 mmol) and ammonia (10 mL, 1 M in THF) were stirred in MeCN (30 mL) at rt for 30 min. The solvent was removed under reduced pressure and the crude product was purified by C18-flash chromatography, elution gradient 10 to 50% MeCN in water. Fractions containing the product were evaporated to dryness providing 100 mg of impure compound. 50 mg of the crude product were purified by preparative HPLC using decreasingly polar mixtures of water (containing 10 mM NH_4HCO_3 + 0.1% $NH_3 \cdot H_2O$) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4-amino-7-chloroquinazolin-2(1*H*)-one (20.0 mg, 24%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 7.13 (2H, m), 7.88 (2H, br s), 7.99 (1H, d), 10.72 (1H, br s); **m/z** (ES+), [M+H]+ 196.

_N_-(7-chloro-2-oxo-1,2-dihydroquinazolin-4-yl)acetamide (10)



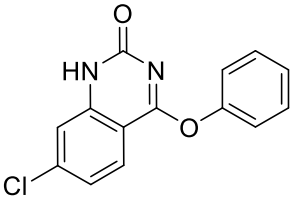
2,4,7-Trichloroquinazoline (200 mg, 0.862 mmol) and ammonia (10 mL, 1 M in THF) were stirred in MeCN (30 mL) at rt for 30 min. The solvent was removed under reduced pressure and the crude product was purified by C18-flash chromatography, elution gradient 10 to 50% MeCN in water. Fractions containing the intermediate were evaporated to dryness providing 100 mg of impure compound. 4-Amino-7-chloroquinazolin-2(1*H*)-one (100 mg, crude) was suspended in acetic anhydride (5.0 mL) and the resulting mixture was stirred at 80 °C for 2 h. The mixture was cooled to rt and a precipitate was observed. The precipitate was collected by filtration, washed with MeCN (75 mL) and dried under vacuum to afford *N*-(7-chloro-2-oxo-1,2-dihydroquinazolin-4-yl)acetamide (36.9 mg, 18% over two steps) as a white solid. **1H NMR** (400 MHz, DMSO-d6) δ 2.27 (3H, s), 7.24 (2H, m), 8.09 (1H, d), 11.61 (1H, s); **m/z** (ES+), [M+H]+ 238.

7-Chloro-4-methoxyquinazolin-2(1*H*)-one (11)



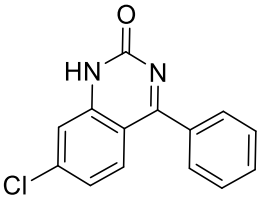
Methanol (0.188 mL, 4.65 mmol) was added to a solution of 2,4,7-trichloroquinazoline (200 mg, 0.862) and DIEA (0.650 mL, 3.72 mmol) in MeCN (10 mL). The resulting mixture was stirred at rt for 30 min. The solvent was removed under reduced pressure, NaOH (186 mg, 4.65 mmol) in water (10 mL) was added and the resulting mixture was stirred at rt for 2 days. The crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.05% $NH_3 \cdot H_2O$) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-methoxyquinazolin-2(1*H*)-one (40.0 mg, 22% over two steps) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 4.01 (3H, s), 7.24 (2H, m), 7.84 (1H, dd), 11.44 (1H, s); **m/z** (ES+), [M+H]+ 211.

7-Chloro-4-phenoxyquinazolin-2(1*H*)-one (12)



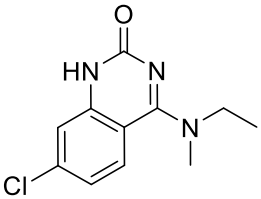
2,4,7-Trichloroquinazoline (150 mg, 0.646 mmol), DIEA (0.487 mL, 2.79 mmol), and phenol (263 mg, 2.79 mmol) were stirred in MeCN (20 mL) at rt for 30 min. The solvent was removed under reduced pressure. The reaction mixture was diluted with MeCN. The precipitate was collected by filtration, washed with MeCN and dried under vacuum to afford 7-chloro-4-phenoxyquinazolin-2(1*H*)-one (140 mg, 80%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 7.14 (1H, dd), 7.27 (4H, m), 7.48 (2H, t), 7.98 (1H, d); **m/z** (ES+), [M+H]+ 273.

7-Chloro-4-phenylquinazolin-2(1*H*)-one (13)



Phenylmagnesium bromide (4.92 mL, 1.0 M in THF, 4.92 mmol) was added dropwise to 2-amino-4-chlorobenzonitrile (250 mg, 1.64 mmol) in THF (20 mL) at 25 °C under N_2. The resulting mixture was stirred at 50 °C for 10 h. After cooling the reaction mixture to 0 °C, methyl chloroformate (155 mg, 1.64 mmol) was added and the resulting mixture was stirred at rt for 16 h. The solvent was removed under reduced pressure, water was added and the mixture was extracted with DCM (3 x 75 mL). The organic layers were dried over Na_2SO_4, filtered and evaporated to afford a yellow solid. The residue was suspended in MeCN and the precipitate was collected by filtration, washed with DMSO and dried under vacuum to afford 7-chloro-4-phenylquinazolin-2(1*H*)-one (173 mg, 41%) as a yellow solid. **1H NMR** (300 MHz, DMSO-d6+CF_3COOD) δ 7.28 (1H, m), 7.44 (1H, d), 7.63 (6H, m); **m/z** (ES+), [M+H]+ 257.

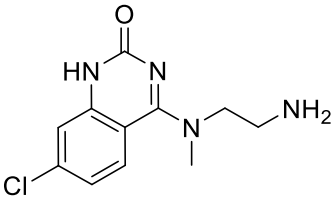
7-Chloro-4-(ethyl(methyl)amino)quinazolin-2(1*H*)-one (14)



2,4,7-Trichloroquinazoline (150 mg, crude, synthesised according to method 1) and *N*-methylethanamine (0.100 mL, 1.16 mmol) were stirred in MeCN (20 mL) for 30 min at rt. The crude product was purified by flash C18-flash chromatography, elution gradient 20 to 50% MeCN in water. Pure fractions were evaporated to dryness to afford 2-({bis[ethyl(methyl)amino]phosphoryl}oxy)-7-chloro-*N*-ethyl-*N*-methylquinazolin-4-amine.[2] (110 mg, 42% over two steps from 7-chloroquinazoline-2,4-diol) as a colourless oil. **1H NMR** (300 MHz, DMSO-d6) δ 1.07 (6H, t), 1.28 (3H, t), 2.64 (6H, m), 3.05 (4H, m), 3.36 (3H, s), 3.74 (2H, q), 7.35 (1H, dd), 7.58 (1H, d), 8.12 (1H, d); **m/z** (ES+), [M+H]+ 400.
2-({bis[ethyl(methyl)amino]phosphoryl}oxy)-7-chloro-*N*-ethyl-*N*-methylquinazolin-4-amine (100 mg, 0.250 mmol) and formic acid (5.0 mL, 130 mmol) were stirred at rt for 3 days and 45 °C for 3 h. The solvent was removed under reduced pressure. The crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.05% $NH_3 \cdot H_2O$)

and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(ethyl(methyl)amino)quinazolin-2(1*H*)-one (50.0 mg, 84%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 1.25 (3H, t), 3.22 (3H, s), 3.64 (2H, q), 7.10 (1H, dd), 7.20 (1H, d), 7.86 (1H, d), 10.73 (1H, s); **m/z** (ES+), [M+H]+ 238.

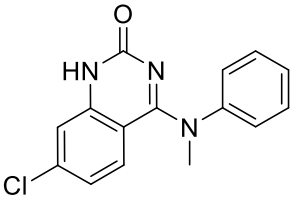
4-((2-Aminoethyl)(methyl)amino)-7-chloroquinazolin-2(1*H*)-one (15)



2,4,7-Trichloroquinazoline (180 mg, 0.776 mmol), DIEA (0.585 mL, 3.35 mmol), and benzyl (2-(methylamino)ethyl)carbamate (697 mg, 3.35 mmol) were stirred in MeCN (15 mL) at rt for 30 min. The solvent was removed under reduced pressure and the crude product was purified by flash C18-flash chromatography, elution gradient 30 to 70% MeCN in water. Pure fractions were evaporated to dryness to afford benzyl (2-((7-chloro-2-hydroxyquinazolin-4-yl)(methyl)amino)ethyl)carbamate (50.0 mg, 17%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 3.40 (t, 2H) , 3.42(s, 3H), 3.86 (t, 2H); 4.98 (s, 2H), 7.40 – 7.20 (m, 4H), 7.52 – 7.41 (m, 2H), 7.72 (d, 1H), 7.95 (dd, 1H), 8.26 – 8.17 (m, 1H), 8.34 (d, 1H); **m/z** (ES+), [M+H]+ 387

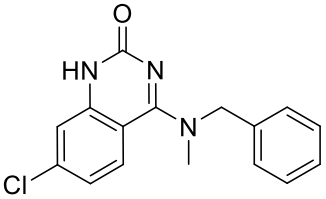
Benzyl (2-((7-chloro-2-hydroxyquinazolin-4-yl)(methyl)amino)ethyl)carbamate (50.0 mg, 0.130 mmol) and acetic acid (5.0 mL, 87.3 mmol) were stirred at 100 °C for 20 h. After cooling the mixture to rt, the solvent was removed under reduced pressure. The crude product was purified by flash C18-flash chromatography, elution gradient 30 to 70% MeCN in water. Pure fractions were evaporated to dryness to afford 4-((2-aminoethyl)(methyl)amino)-7-chloroquinazolin-2(1*H*)-one (25.0 mg, 76%) as a white solid. **1H NMR** (400 MHz, DMSO-d6) δ 2.60 (3H, s), 3.17 (2H, t), 3.73 (2H, d), 7.20 (2H, m), 8.10 (1H, d), 8.70 (2H, s), 10.87 (1H, s); **m/z** (ES+), [M+H]+ 253.

7-Chloro-4-(methyl(phenyl)amino)quinazolin-2(1*H*)-one (16)



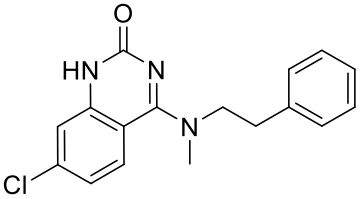
2,4,7-Trichloroquinazoline (200 mg, 0.862), DIEA (0.487 mL, 2.79 mmol) and *N*-methylaniline (299 mg, 2.79 mmol) were stirred in MeCN (20 mL) at rt for 30 min. The solvent was removed under reduced pressure and subsequently formic acid (20 mL) was added. The resulting solution was stirred at rt for 20 h. The solvent was removed under reduced pressure and the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 10 mM NH_4HCO_3 + 0.1% $NH_3 \cdot H_2O$) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(methyl(phenyl)amino)quinazolin-2(1*H*)-one (120 mg, 49% over two steps) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 3.44 (3H, s), 6.60 (1H, d), 6.72 (1H, dd), 7.18 (1H, d), 7.33 (3H, m), 7.45 (2H, dd), 11.14 (1H, s); **m/z** (ES+), [M+H]+ 286.

4-(Benzyl(methyl)amino)-7-chloroquinazolin-2(1*H*)-one (17)



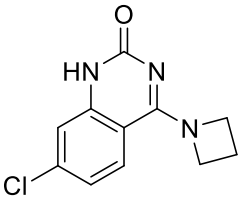
2,4,7-Trichloroquinazoline (150 mg, 0.646 mmol) and *N*-methyl-1-phenylmethanamine (100 μL, 0.780 mmol) were stirred in MeCN (20 mL) at rt for 30 min. The solvent was removed under reduced pressure and the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 10 mM NH_4HCO_3 + 0.1% $NH_3 \cdot H_2O$) and MeCN as eluent. Fractions containing the desired compound were evaporated to dryness to afford 4-(benzyl(methyl)amino)-7-chloroquinazolin-2(1*H*)-one (60.0 mg, 31%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 3.21 (3H, s), 4.92 (2H, s), 7.07 (1H, dd), 7.23 (1H, d), 7.35 (5H, m), 7.84 (1H, d), 10.92 (1H, s); **m/z** (ES+), [M+H]+ 300.

7-Chloro-4-(methyl(phenethyl)amino)quinazolin-2(1*H*)-one (18)



2,4,7-Trichloroquinazoline (160 mg, 0.690) and *N*-methyl-2-phenylethan-1-amine (100 μL, 1.04 mmol) were stirred in MeCN (20 mL) at rt for 30 min. The solvent was removed under reduced pressure and formic acid (5.7 mL) was added. The resulting solution was stirred at rt for 20 h and the solvent was removed under reduced pressure. The crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 10 mM NH_4HCO_3 + 0.1% $NH_3 \cdot H_2O$) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(methyl(phenethyl)amino)quinazolin-2(1*H*)-one (138 mg, 64% over two steps) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 3.00 (2H, m), 3.27 (3H, s), 3.84 (2H, m), 7.09 (1H, dd), 7.20 (2H, m), 7.28 (4H, d), 7.85 (1H, d), 10.83 (1H, s); **m/z** (ES+), [M+H]+ 314.

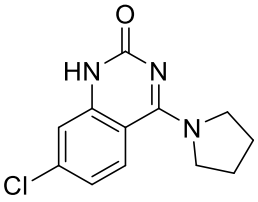
4-(Azetidin-1-yl)-7-chloroquinazolin-2(1*H*)-one (19)



2,4,7-Trichloroquinazoline (150 mg, 0.646 mmol), DIEA (0.487 mL, 2.79 mmol), and azetidine (80.0 mg, 1.40 mmol) were stirred in MeCN (15 mL) at rt for 30 min. A precipitate formed during the reaction and this was collected by filtration, washed with MeCN and dried under vacuum to afford 4-(azetidin-1-yl)-2,7-dichloroquinazoline (60.0 mg) as a white solid, which was used without further purification.

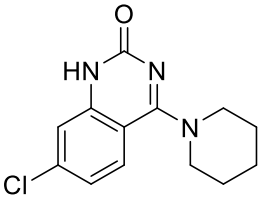
4-(Azetidin-1-yl)-2,7-dichloroquinazoline (55.0 mg) was stirred in acetic acid (5.0 mL) at 110 °C for 2 days. A precipitate formed during the reaction and was collected by filtration, washed with MeCN and dried under vacuum to afford 4-(azetidin-1-yl)-7-chloroquinazolin-2(1*H*)-one (40.0 mg, 29% over two steps) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 2.08 (2H, m), 3.55 (2H, t), 3.94 (2H, t), 7.32 (1 H, d), 7.40 (1 H, dd), 8.11 (1 H, dd); **m/z** (ES+), [M+H]+ 236.

7-Chloro-4-(pyrrolidin-1-yl)quinazolin-2(1*H*)-one (20)



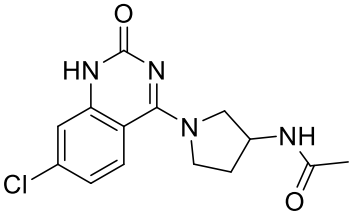
2,4,7-Trichloroquinazoline (150 mg, crude, synthesised according to method 1 and pyrrolidine (0.100 mL, 1.20 mmol) were stirred in MeCN (20 mL) at rt for 30 min. The solvent was removed under reduced pressure to afford 7-chloro-4-(pyrrolidin-1-yl)quinazolin-2-yl di(pyrrolidin-1-yl)phosphinate[2] (200 mg, crude) as a yellow oil. Formic acid (15 mL) was added to 7-chloro-4-(pyrrolidin-1-yl)quinazolin-2-yl di(pyrrolidin-1-yl)phosphinate (195 mg) and the resulting mixture was stirred at rt for 1 day. The solvent was removed under reduced pressure and the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(pyrrolidin-1-yl)quinazolin-2(1*H*)-one (60.0 mg, 40% over three steps from 7-chloroquinazoline-2,4-diol) as a yellow solid. **1H NMR** (300 MHz, DMSO-d6) δ 1.94 (4H, m), 3.78 (4H, m), 7.10 (1H, dd), 7.19 (1H, d), 8.05 (1H, d), 10.74 (1H, s); **m/z** (ES+), [M+H]+ 250.

7-Chloro-4-(piperidin-1-yl)quinazolin-2(1*H*)-one (21)



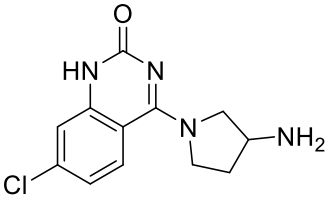
2,4,7-Trichloroquinazoline (150 mg, crude, synthesised according to method 1) and piperidine (0.100 mL, 1.01 mmol) in MeCN (20 mL) were stirred at rt for 30 min. The crude product was purified by flash C18-flash chromatography, elution gradient 50 to 100% MeCN in water. Fractions containing the product were evaporated to dryness to afford 7-chloro-4-(piperidin-1-yl)quinazolin-2-yl di(piperidin-1-yl)phosphinate[2] (110 mg, crude) as a yellow oil. Formic acid (10 mL) was added to 7-chloro-4-(piperidin-1-yl)quinazolin-2-yl di(piperidin-1-yl)phosphinate (100 mg, crude) and the resulting mixture was stirred at 45 °C for 1 day. The solvent was removed under reduced pressure. The crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(piperidin-1-yl)quinazolin-2(1*H*)-one (50.0 mg, 32% over three steps from 7-chloroquinazoline-2,4-diol) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 1.62-1.70 (6 H, m), 3.60 – 3.67 (4 H, m), 7.12 (1H, dd), 7.21 (1H, d), 7.69 (1H, d), 10.92 (1H, s); **m/z** (ES+), [M+H]+ 264.

***N*-(1-(7-chloro-2-oxo-1,2-dihydroquinazolin-4-yl)pyrrolidin-3-yl)acetamide (23)**



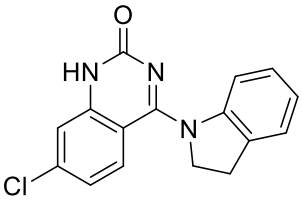
2,4,7-Trichloroquinazoline (200 mg, crude, synthesised according to method 1), K_2CO_3 (643 mg, 4.65 mmol) and *N*-(pyrrolidin-3-yl)acetamide (596 mg, 4.65 mmol) were stirred in MeCN (50 mL) at rt for 1 h. The solvent was removed under reduced pressure and the crude product was purified by flash C18-flash chromatography, elution gradient 20 to 80% MeCN in water. Fractions containing the product were evaporated to dryness to afford *N*-(1-(7-chloro-2-hydroxyquinazolin-4-yl)pyrrolidin-3-yl)acetamide (crude) as a white solid. Half of the crude was used in the following reaction. The other half of the crude product was further purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford *N*-(1-(7-chloro-2-oxo-1,2-dihydroquinazolin-4-yl)pyrrolidin-3-yl)acetamide (30.0 mg, 22% over two steps from 7-chloroquinazoline-2,4-diol) as a yellow solid. **1H NMR** (300 MHz, DMSO-d6) δ 1.81 (3H, s), 1.90 (1H, m), 2.11 (1H, dd), 3.62 (1H, m), 3.92 (3H, m), 4.29 (1H, m), 7.11 (1H, dd), 7.19 (1H, d), 8.00 (1H, d), 8.16 (1H, d), 10.79 (1H, s); **m/z** (ES+), [M+H]+ 307.

4-(3-Aminopyrrolidin-1-yl)-7-chloroquinazolin-2(1*H*)-one (22)



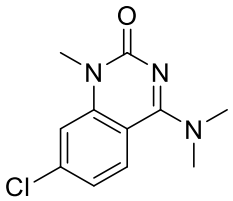
N-(1-(7-chloro-2-hydroxyquinazolin-4-yl)pyrrolidin-3-yl)acetamide **23** (100 mg, 0.33 mmol) and HCl (4.0 mL, 4 M in dioxane, 132 mmol) in water (6.0 mL) were stirred at 50 °C for 3 days. After cooling the reaction to rt, the solvent was removed under reduced pressure and the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 4-(3-aminopyrrolidin-1-yl)-7-chloroquinazolin-2(1*H*)-one (30.0 mg, 31%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 1.94 (1 H, s), 2.15 (1 H, m), 3.83 (5 H, dt), 7.12 (1 H, dd), 7.20 (1 H, d), 8.00 (1 H, d); **m/z** (ES+), [M+H]+ 265.

7-Chloro-4-(indolin-1-yl)quinazolin-2(1*H*)-one (24)



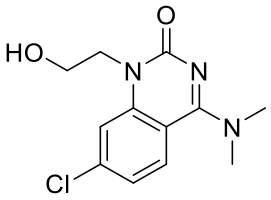
2,4,7-Trichloroquinazoline (200 mg, 0.930 mmol), DIEA (0.487 mL, 2.79 mmol) and indoline (443 mg, 3.72 mmol) were stirred in MeCN (20 mL) at rt for 30 min. The solvent was removed under reduced pressure and formic acid (20 mL) was added. The resulting solution was stirred at rt for 20 h. The solvent was removed under reduced pressure and MeCN (20 mL) was added. The precipitate was collected by filtration, washed with MeCN and dried under vacuum to afford 7-chloro-4-(indolin-1-yl)quinazolin-2(1*H*)-one (50.0 mg, 18% over two steps) as a pale yellow solid. **1H NMR** (300 MHz, DMSO-d6) δ 3.14 (2H, t), 4.38 (2H, t), 7.02 (1H, t), 7.16 (2H, ddd), 7.30 (2H, m), 7.58 (1H, d), 7.96 (1H, d), 11.23 (1H, s); **m/z** (ES+), [M+H]+ 298.

7-Chloro-4-(dimethylamino)-1-methylquinazolin-2(1*H*)-one (25)



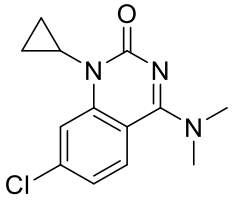
NaH (91.2 mg, ~60% dispersion in mineral oil, 2.28 mmol) was added in small portions to 7-chloro-4-(dimethylamino)quinazolin-2(1*H*)-one **5** (170 mg, 0.760 mmol) in DMF (10 mL) at 0 °C under N_2. The resulting mixture was stirred at 15 °C for 30 min. Iodomethane (324 mg, 2.28 mmol) was added and the resulting mixture was stirred at 15 °C for 5 h. The reaction mixture was quenched with water (20 mL) and extracted with $CHCl_3$ (2 x 25 mL). The combined organic layers were evaporated to afford a yellow oil and the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(dimethylamino)-1-methylquinazolin-2(1*H*)-one (63.0 mg, 35%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 3.22 (6H, s), 3.41 (3H, s), 7.19 (1H, dd), 7.44 (1H, d), 7.95 (1H, d); **m/z** (ES+), [M+H]+ 238.

7-Chloro-4-(dimethylamino)-1-(2-hydroxyethyl)quinazolin-2(1*H*)-one (26)



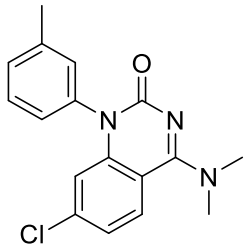
Cs_2CO_3 (1.16 g, 3.58 mmol) was added to 7-chloro-4-(dimethylamino)quinazolin-2(1*H*)-one **5** (200 mg, 0.890 mmol) and 2-((tert-butyldimethylsilyl)oxy)ethan-1-ol (473 mg, 2.68 mmol) in 1,4-dioxane (10 mL) under N_2. The resulting mixture was stirred at 100 °C for 30 h. The reaction mixture was allowed to cool to rt and the crude product was purified by flash C18-flash chromatography, elution gradient 25 to 80% MeCN in water. Fractions containing the product were evaporated to dryness to afford 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)-7-chloro-4-(dimethylamino)quinazolin-2(1*H*)-one (200 mg, crude) as a yellow oil. HCl (10 mL, 4M in dioxane, 40.0 mmol) was added to 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)-7-chloro-4-(dimethylamino)quinazolin-2(1*H*)-one (150 mg, crude) and the resulting mixture was stirred at rt for 1 h. The solvent was removed under reduced pressure and the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 10 mM NH_4HCO_3 + 0.1% $NH_3 \cdot H_2O$) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(dimethylamino)-1-(2-hydroxyethyl)quinazolin-2(1*H*)-one (60.0 mg, 34% over two steps) as a white solid. **1H NMR** (400 MHz, DMSO-d6) δ 3.21 (6H, s), 3.61 (2H, t), 4.09 (2H, t), 4.83 (1H, s), 7.17 (1H, dd), 7.60 (1H, d), 7.93 (1H, d); **m/z** (ES+), [M+H]+ 268.

7-Chloro-1-cyclopropyl-4-(dimethylamino)quinazolin-2(1*H*)-one (27)



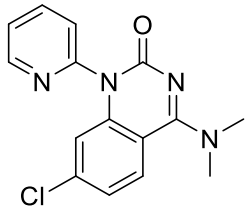
7-Chloro-4-(dimethylamino)quinazolin-2(1*H*)-one **5** (200 mg, 0.900 mmol), potassium cyclopropyltrifluoroborate (662 mg, 4.48 mmol), K_2CO_3 (370 mg, 2.68 mmol) and copper (II) acetate (81.2 mg, 0.440 mmol) were stirred in water (6.0 mL) and toluene (20 mL) under an atmosphere of O_2 at 1 atm and 70 °C for 2 days. The mixture was filtered, evaporated, and purified by flash C18-flash chromatography, elution gradient 30 to 60% MeCN in water. Pure fractions were evaporated to dryness to afford 7-chloro-1-cyclopropyl-4-(dimethylamino)quinazolin-2(1*H*)-one (8.90 mg, 4%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 0.62 (2H, m), 1.14 (2H, td), 2.76 (1H, tt), 3.19 (6H, s), 7.18 (1H, dd), 7.63 (1H, d), 7.88 (1H, d); **m/z** (ES+), [M+H]+ 265.

7-Chloro-4-(dimethylamino)-1-(*m*-tolyl)quinazolin-2(1*H*)-one (29)



K_2CO_3 (204 mg, 1.48 mmol) was added to 7-chloro-4-(dimethylamino)quinazolin-2(1*H*)-one **5** (110 mg, 0.490 mmol), 1-iodo-3-methylbenzene (214 mg, 0.980 mmol), copper(I) iodide (94.0 mg, 0.490 mmol) and copper (31.3 mg, 0.490 mmol) in DMA (2.0 mL) under N_2. The resulting mixture was stirred at 140 °C for 20 h. The reaction mixture was filtered through celite and the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 10 mM NH_4HCO_3 + 0.1% $NH_3 \cdot H_2O$) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(dimethylamino)-1-(m-tolyl)quinazolin-2(1*H*)-one (25.0 mg, 16%) as a white solid. **1H NMR** (400 MHz, DMSO-d6) δ 2.38 (3H, s), 3.3 (6H, s), 6.35 (1H, d), 7.09 (2H, m), 7.19 (1H, dd), 7.33 (1H, d), 7.48 (1H, t), 8.03 (1H, d); **m/z** (ES+), [M+H]+ 314.

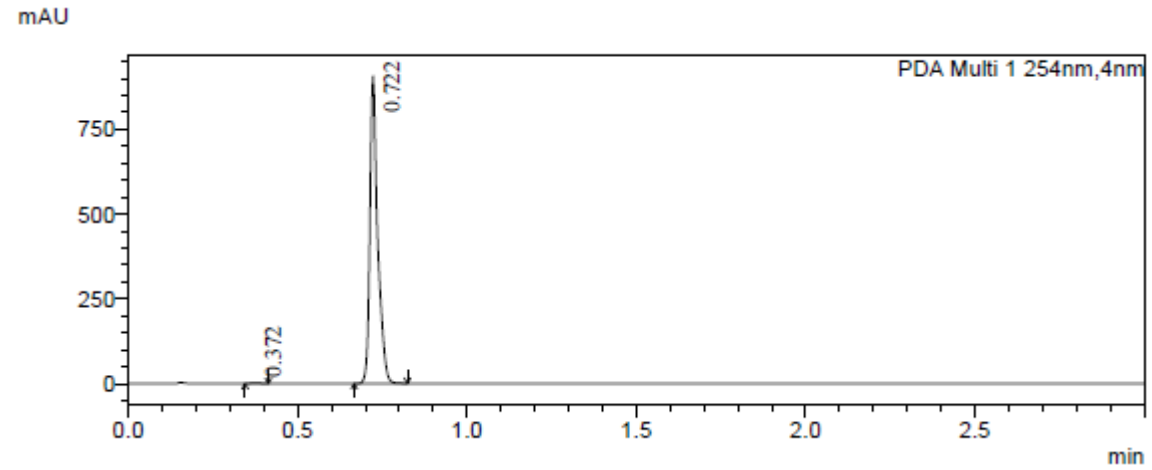
7-Chloro-4-(dimethylamino)-1-(pyridin-2-yl)quinazolin-2(1*H*)-one (30)

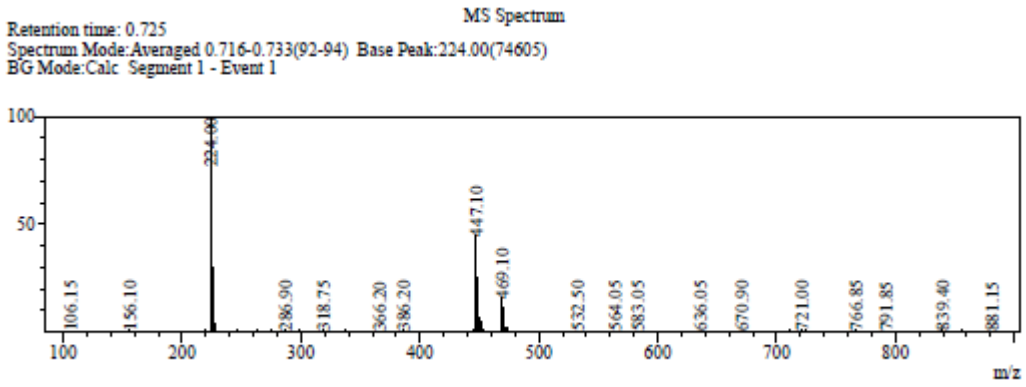


K_2CO_3 (226 mg, 1.64 mmol) was added to 7-chloro-4-(dimethylamino)quinazolin-2(1*H*)-one **5** (122 mg, 0.550 mmol), 2-iodopyridine (224 mg, 1.09 mmol), copper(I) iodide (104 mg, 0.550 mmol) and copper (34.7 mg, 0.550 mmol) in DMA (8.0 mL) under N_2. The resulting mixture was stirred at 140 °C for 20 h. After cooling the mixture to rt, the crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.05% $NH_3 \cdot H_2O$) as MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-chloro-4-(dimethylamino)-1-(pyridin-2-yl)quinazolin-2(1*H*)-one (65.0 mg, 40%) as a white solid. **1H NMR** (300 MHz, DMSO-d6) δ 3.30 (6H, s), 6.29 (1H, d), 7.21 (1H, dd), 7.54 (2H, m), 8.05 (2H, m), 8.67 (1H, dd); **m/z** (ES+), [M+H]+ 301.

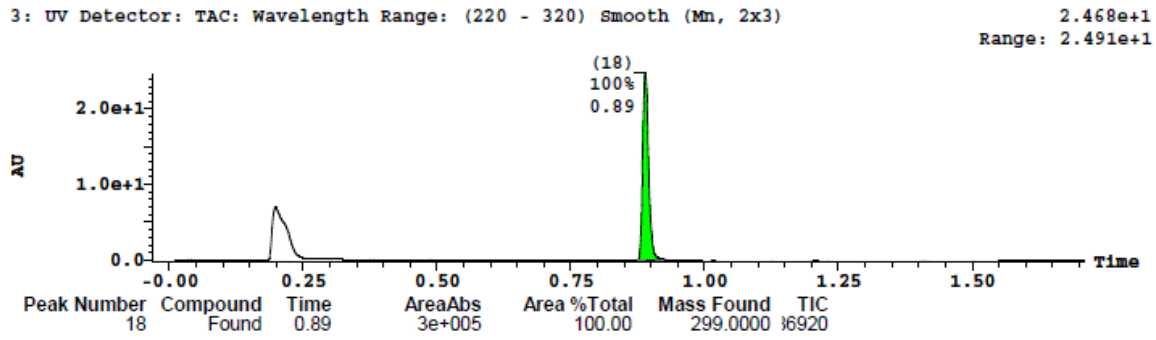
HPLC/UPLC traces for key compounds

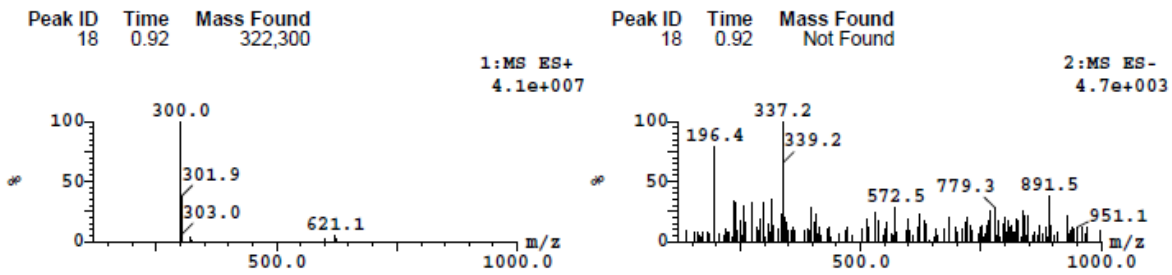
7-Chloro-4-(dimethylamino)quinazolin-2(1*H*)-one (5)



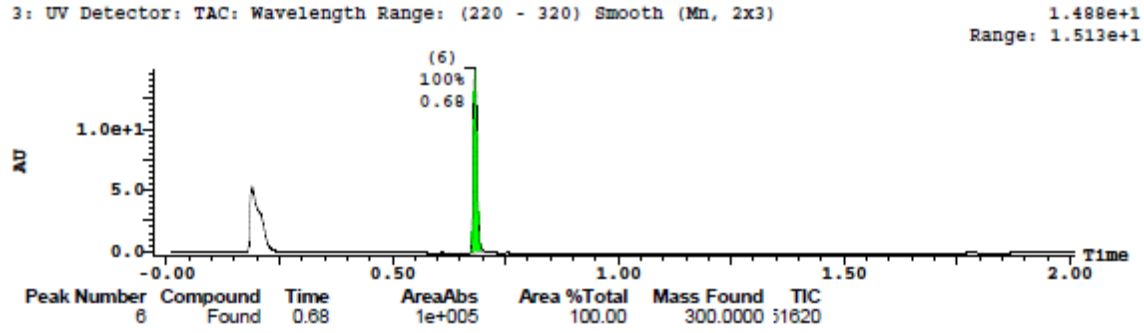


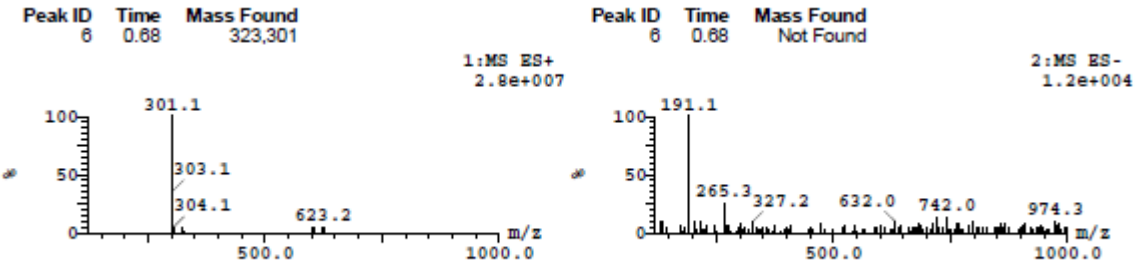
7-Chloro-4-(dimethylamino)-1-phenylquinazolin-2(1*H*)-one (28)



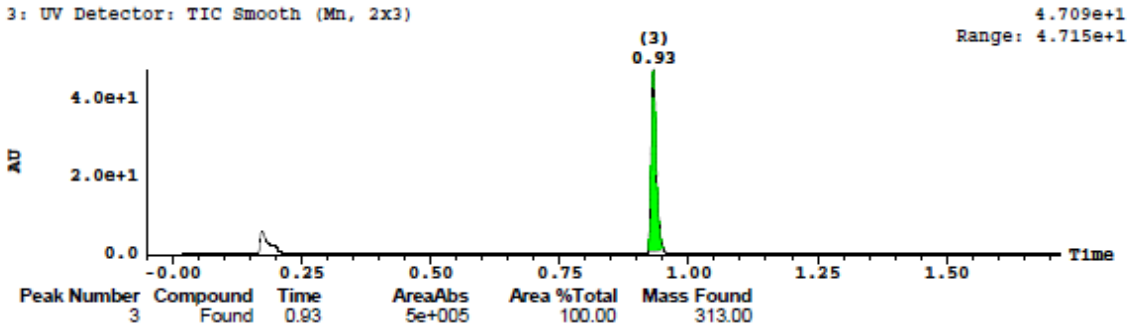


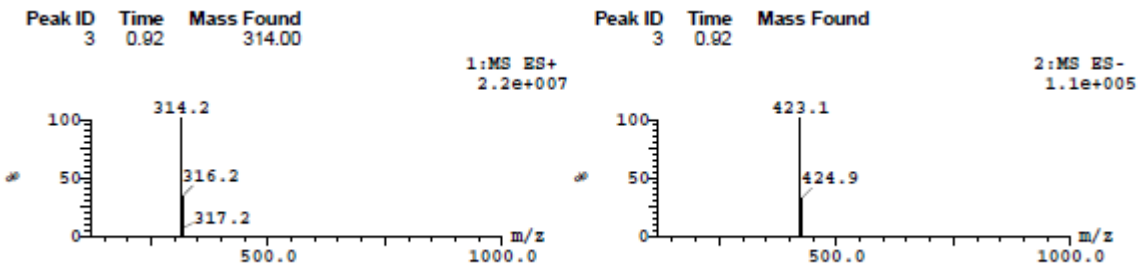
7-Chloro-4-(dimethylamino)-1-(pyridin-3-yl)quinazolin-2(1*H*)-one (**31**)



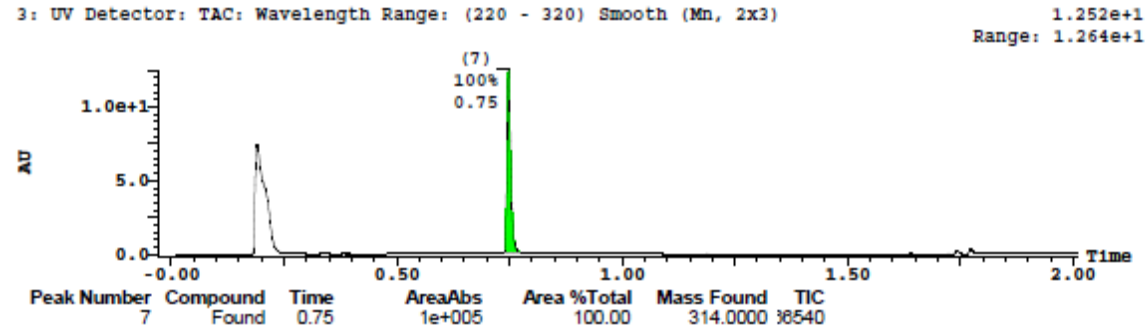


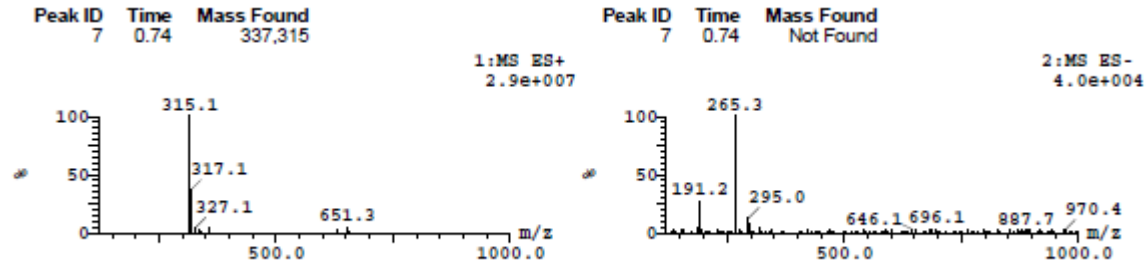
7-Chloro-4-(ethyl(methyl)amino)-1-phenylquinazolin-2(1*H*)-one (**32**)





7-Chloro-4-(ethyl(methyl)amino)-1-(pyridin-3-yl)quinazolin-2(1*H*)-one –(33)





Biological data and errors

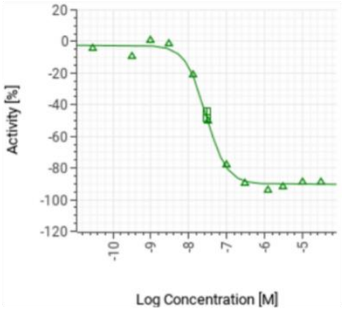
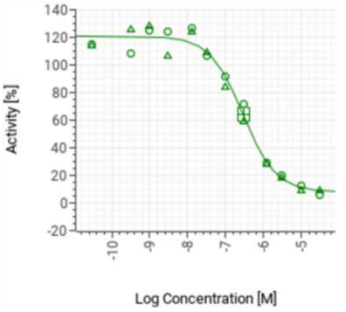
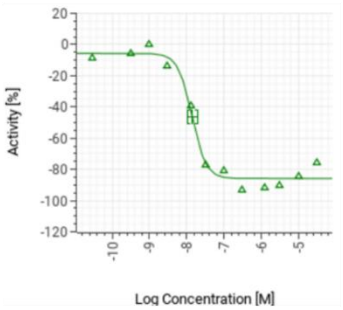
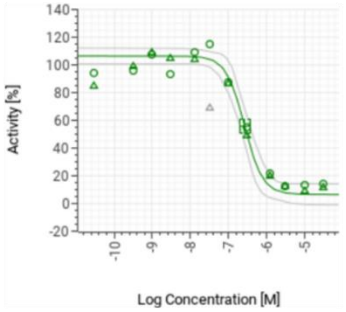
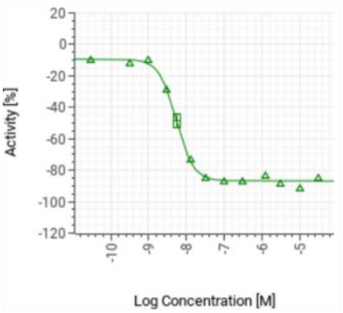
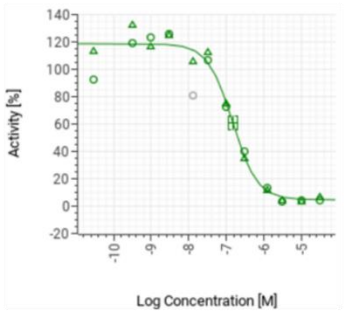
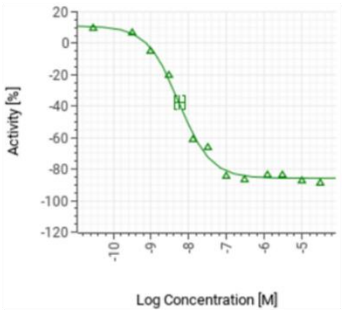
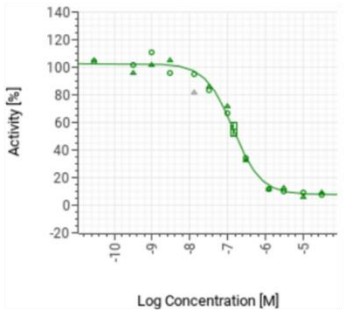
Compound	Activity	Mean pK_d	Std dev	Top conc. (μM)	Replicates
1	A	3.6	0.19	1000	9
2	A	5.2	0.06	100	4
3	N	-	-	200	1
4	A	4.4	0.00	500	3
5	A	5.3	0.11	50	5

Compound	pIC_{50}	Std Dev	Mean Max conc inh	Replicates
1	3.7	0.039	-96	4
2	<3	-	-	1
3	<3	-	-	1
4	3.5	0.29	-84	6
5	5.1	0.075	-99	3
6	<4	-	-	4
7	3.2	0.033	-85	3
8	4.3	0.079	-72	5
9	<4	-	-	1
10	<4	-	-	2
11	<4	-	-	1
12	<3	-	-	1
13	<4	-	-	1
14	6.1	0.010	-110	3
15	<4	-	-	1
16	<3	-	-	1
17	4.2	0.05	-86	3
18	<4	-	-	4
19	<4	-	-	2
20	5.3	0.13	-110	3
21	4.1	0.032	-86	3
22	4.3	0.15	-70	3
23	<4	-	-	3
24	<4	-	-	2

25	3.7	0.35	-65	5
26	5.1	0.029	-100	3
27	5.1	0.082	-99	3
28	7.6	0.087	-110	5
29	7.8	0.14	-110	3
30	4.7	0.21	-78	4
31	7.7	0.16	-110	5
32	7.9	0.18	-110	3
33	7.7	0.083	-110	5

Compound	Mean Cell SDMA pIC_{50}	Std dev	Replicates	Mean Prolif pGI_{50}	Std dev	Replicates
28	7.6	0.22	7	6.5	0.18	10
31	7.8	0.26	4	6.5	0.23	5
32	8.2	0.25	3	6.8	0.13	3
33	8.3	0.18	3	6.8	0.07	2

Representative dose-response curves for cellular SDMA and proliferation assays:

Cmpd	Cell IC_{50} (SDMA levels)	Proliferation IC_{50} (HCT110 MTAP KO)
29		
31		
32		
33		

In vivo Methods

Animals

Female Ncr Nude mice were purchased from Taconic Biosciences. Mice were housed under pathogen-free conditions in individual ventilated cages (IVC) at our AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care) accredited facility in Waltham, MA. All animal manipulations were conducted in a biosafety cabinet maintained under positive pressure. Mice used were 5-6 weeks old at the time of tumour implantation.

Xenograft Efficacy Studies

Ten million HCT116 MTAP KO tumour cells were injected subcutaneously in the right flank of Ncr female mice in a volume of 0.1 mL. Tumour volumes (measured by caliper), animal body weight, and tumour condition were recorded twice weekly for the duration of the study. The tumour volume was calculated (taking length to be the longest diameter across the tumour and width to be the corresponding perpendicular diameter) using the formula: length (mm) x width $(mm)^2/0.52$. For efficacy studies, growth inhibition from the start of treatment was assessed by comparison of the differences in tumour volume between control and treated groups. Because the variance in mean tumour volume data increases proportionally with volume (and is therefore disproportionate between groups), data were log transformed to remove any size dependency before statistical evaluation. Statistical significance was evaluated using a one-tailed, 2-sample *t* test. For efficacy studies, mice were randomized based on tumour volumes using stratified sampling, and enrolled into control and treatment groups. Dosing began when mean tumour size reached approximately 200 mm^3. Compound **28** was formulated in 0.5% hydroxypropyl methylcellulose (HPMC)/ 0.1% Tween 80 in water and dosed subcutaneously at 5 mL/kg using a 25 gauge syringe.

Quantitative LCMSMS to evaluate SAM in tumour: LC/MS/MS quantitation of tumour lysates was performed using an Agilent 6490 triple-quadrupole mass spectrometer with electrospray ionization in positive ion detection mode with an Agilent 1290 UHPLC system consisting of a binary pump, HTS autosampler, and a Waters Acquity UPLC BEH Amide 2.1 x 100 mm, 1.7 µm column. Chromatography was performed under HILIC conditions with a gradient of 97% B to 30% B over 3.5 min, using water and MeCN with 0.1% formic acid. Internal standard was d-citrulline (CDN Isotopes # D-6396) at 1 mg/L in MeCN/methanol/water/formic acid 40/40/20/0.05 v/v/v/v. External standard was *S*-adenosylmethionine (Cayman Chemical # 16376) prepared at concentrations from 5 – 10,000

nM in methanol. MRM transitions for detection were 399.15 > 250, collision energy 10 V (SAM) and 180 > 74, collision energy 28 V (d-citrulline.)

Tumour lysates were prepared by taking 50-100 mg of frozen tissue and adding MeCN/methanol/water 40/40/20 v/v/v to the samples at a concentration of 1 mL/100 mg tissue. Samples were gently agitated for 2 min and then spun at ~21000g at 0 ºC for 10 min. Clear supernatant was transferred, then the solvent addition, shaking, and centrifugation were repeated with the second supernatant added to the original. Prior to LC-MS-MS analysis, 15 µL of each lysate was combined with 135 µL of internal standard then vortexed thoroughly.

MAT2a protein production

Full-length human MAT2a protein was recombinantly expressed in *E.coli* using a plasmid obtained from the Structural Genomics Consortium[1]. *N*-terminally His_6-tagged MAT2a was purified by Ni^{2+}-immobilised metal affinity chromatography (IMAC), proteolytic cleavage of the hexahistidine tag and size exclusion chromatography. Final protein concentration was 20 mg/mL (0.47 mM) in a buffer containing 10 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol and 0.5 mM TCEP.

To allow immobilisation, an expression plasmid containing His_6-TEV-Avi-MAT2a (2-395) was generated and Avi-tagged protein was purified as above. *In vitro* biotinylation was carried out using the BirA biotinylation kit from Avidity according to the manufacturer's instructions. Excess reagents were removed by passing over a desalting column.

Protein was snap-frozen and stored in aliquots until use.

X-ray crystallography of MAT2a

Full-length human MAT2a protein was recombinantly expressed in *E.coli* using a plasmid obtained from the Structural Genomics Consortium[3]. Final protein concentration was 20 mg/mL (0.47 mM) in a buffer containing 10 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol and 0.5 mM TCEP. For crystallisation, a 1.5-fold molar excess of S-adenosylmethionine was added before dispensing sitting drop vapour diffusion experiments with 150 nL protein and 150 nL of a reservoir solution containing 8—12 % PEG 8000, 8—12 % ethylene glycol, 0.1 M Hepes pH 8.0. Bar shaped crystals appeared within minutes, reaching their maximum dimensions within 12 h. Crystallisation reliability was enhanced by micro-seeding. Crystals remained stable for approximately 4 weeks without loss of diffraction quality, although the *S*-adenosylmethionine appears partially hydrolysed in crystals harvested after more than a few

days. Inhibitors were introduced by soaking the crystals in reservoir solution supplemented with 10 % of compound solution (100 mM in DMSO). Soaked crystals were frozen after 2—24 h without further cryoprotection. X-ray diffraction data were collected at Diamond Light Source beamlines I03 and I04, and images were processed with autoproc[4], STARANISO[5] and additional programmes from the CCP4 suite[6]. The structures were solved by molecular replacement using AMoRe[7] and refined with Buster[8] and Coot[9]. Initial ligand restraint dictionaries were generated with Grade[10]. Atomic coordinates and structure factors are available from the Protein Data Bank.

Computational details

Protein structures were prepared before analysis using the Protein Preparation Wizard[11] in Maestro[12]. Ligand tautomers and protonation states were assigned using Epik[13,14], and protonation states of the protein and water orientations were determined and optimised at pH 7.0. Hydrogen atoms of ligand, protein, and water molecules were minimised.

WaterMap[15,16] calculations involved analysing simulated explicit-solvent waters in the binding sites near bound ligands. Water sites within 8 Å of the ligand of interest were analysed. X-ray waters were included and treated as solvent. Simulation time was 2.0 ns.

Quantum-mechanical geometry optimisations were done at DFT level using Jaguar[17] in Maestro[12]. All optimisations were performed using B3LYP-D3 functionals, 6-31G** basis sets, and a PBF solvent (water) model.

References:

(1) In some cases after evaporation the residual $POCl_3$ caused the formation of a phosphorylated species that was hydrolysed in the last step with formic acid or acetic acid to give the final product. The yields account for the by-product.

(2) This phosphorous by-product originates from residual $POCl_3$ (synthesis 2,4,7-trichloroquinazoline, method 1) and the yield is calculated based on the starting material in method 1.

(3) Human Methionine adenosyl transferase II, alpha http://www.thesgc.org/structures/2p02.

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