Supplementary Information

Inhibition Studies on *Mycobacterium tuberculosis* 
* N-acetylglucosamine-1-phosphate uridyltransferase (GlmU)

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1. Development and optimisation of *M. tuberculosis* GlmU uridyltransferase kinetic assay:

a. Variation of coupling enzyme concentration:

In 96-well plate format, the kinetic assay mix contained the following (total volume 200 µL: 50 mM Tris.HCl (pH 7.6), 5 mM MgCl₂, 1 mM DTT, 0.2 mM 7-methyl-6-thioguanosine (MESG), 47 nM *Mtb* GlmU uridyltransferase, 10-194 µM UTP and varying concentrations of purine nucleoside phosphorylase (PNPase) and inorganic pyrophosphatase as follows:

Experiment 1 (control): 1 U/mL PNPase and 2.4 U/mL inorganic pyrophosphatase.

Experiment 2: 2 U/mL PNPase and 4.8 U/mL inorganic pyrophosphatase.

Experiment 3: 0.4 U/mL PNPase and 0.96 U/mL inorganic pyrophosphatase.

The assay mix was incubated at 25 °C for 10 minutes. The assays were performed in duplicate and were initiated by the addition of 1 mM GlcNAc-1-P and monitored for 6 minutes at 360 nm on an Omega BMG LabTech microplate reader. The data obtained was fitted to a Michaelis-Menten model by non-linear regression using GraphPad Prism (version 5.03 for Windows, Figure S1). The results indicate that the rate of reaction was not dependent on the concentrations of the coupling enzymes, demonstrating that they were not rate limiting in the assay and that the kinetics of GlmU was being monitored.

**Figure S1.** Least square fitted curve to the Michaelis-Menten model for *M. tuberculosis* GlmU uridyltransferase with varying concentrations of coupling enzymes.
b. Dependence of *M. tuberculosis* GlmU uridyltransferase initial velocity on concentration of enzyme:

Using our established protocol (see 1a), initial rates were measured in the presence of varying concentrations of GlmU uridyltransferase (23-188 nM) and 53 µM GlcNAc-1-P and 1 mM UTP. This study showed a linear dependence of reaction rate on GlmU concentration (Figure S2).

![Figure S2](image)

**Figure S2.** Dependence of reaction rate on GlmU concentration.
2. Synthesis of compound 1:

(S)-tert-butyl-3-amino-4-(((2R,3R,4R,5R)-3,4-bis((tert-butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-4-oxobutanoate (S3)

To a solution of Fmoc-Asp(OtBu)-OH (105 mg, 0.25 mmol), HOBt (34 mg, 0.25 mmol) and PyBOP (130 mg, 0.25 mmol) in DMF (1.7 mL) was added 1 N-N-diisopropylethylamine (45 µL, 0.25 mmol) at 0 °C. Amine S21 (60 mg, 0.13 mmol) was added and the reaction was allowed to warm to room temperature and was stirred for 1.5 h. The reaction was diluted with ethyl acetate (40 mL) and washed successively with 1 M HCl (2 × 40 mL), saturated aqueous NaHCO3 solution (5 × 50 mL) and brine (2 × 40 mL). The organic layer was dried over anhydrous MgSO4 and the solvent was removed in vacuo to give a crude residue that was purified by column...
chromatography (98: 2 v/v CH₂Cl₂ : MeOH → 95: 5 v/v CH₂Cl₂ : MeOH) to afford
the Fmoc-protected amide as a white foam.

Fmoc-protected amide (99 mg, 0.11 mmol) was treated with 20% (vol/vol) piperidine
in a mixture of dichloromethane and methanol (1:1 v/v CH₂Cl₂: MeOH, 1.4 mL). The
reaction was allowed to stir at room temperature for 3.5 h. The solvent was removed
in vacuo to afford a crude residue that was purified by column chromatography (95: 5
v/v CH₂Cl₂ : MeOH → 9: 1 v/v CH₂Cl₂ : MeOH) to afford amine S3 as a white foam
(46 mg, 67% over 2 steps).

¹H NMR (400 MHz, CD₃OD): δ 7.73 (1H, d, J 8.1 Hz, H-6'), 5.84 (1H, d, J 6.4 Hz,
H-1), 5.76 (1H, d, J 8.1 Hz, H-5'), 4.40 (1H, dd, J 6.5, 4.5 Hz, H-2), 4.14-3.99 (2H,
m, H-3 + CH), 3.67-3.58 (1H, m, H-4), 3.51 (2H, app. d, J 6.1 Hz, CH₂), 2.67 (1H,
dd, J 16.4, 5.4 Hz, H-5), 2.57 (dd, J 16.3, 6.9 Hz, H-5), 1.45 (9H, s, CO₂CMes), 0.94
(9H, s, Me₂CSi), 0.89 (9H, s, (CH₃)₂CSi), 0.146 (3H, s, CH₃Si), 0.132 (3H, s, CH₃Si),
0.090 (3H, s, CH₃Si), 0.028 (3H, s, CH₃Si). ¹³C NMR (101 MHz, CD₃OD): δ 175.1
(C=O), 170.8 (C=O), 164.5 (C=O), 151.0 (C=O), 142.1, 101.8, 89.7, 84.1, 80.8, 73.8,
73.0, 51.6, 41.1, 39.9, 27.0, 25.0, 24.9, 17.5, 17.4, -5.6, -5.6, -5.8, -6.0.

(S)-3-tert-butyl-3-amino-4-(((S)-1-(((2R,3R,4R,5R)-3,4-bis((3tert-
butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-
yl)tetrahydrofuran-2-y)methyl)amino)-4-(2-butoxy)-1,4-dioxobutan-2-
yl)amino)-4-oxobutanoate (S4)

To a solution of Fmoc-Asp(O'Bu)-OH (60 mg, 0.14 mmol), HOBt (20 mg, 0.14
mmol) and PyBOP (76 mg, 0.14 mmol) in DMF (1.7 mL) was added N,N-
diisopropylethylamine (27 µL, 0.14 mmol) at 0 °C. Amine S3 (46 mg, 0.072 mmol)
was added and the reaction was allowed to warm to room temperature and was stirred
for 1.5 h. The reaction was diluted with ethyl acetate (40 mL) and washed
successively with 1 M HCl (2 × 40 mL), saturated aqueous NaHCO₃ solution (5 × 50
mL) and brine (2 × 40 mL). The organic layer was dried over anhydrous MgSO₄ and
the solvent removed in vacuo to give a crude residue that was purified by column chromatography (98 CH₂Cl₂ : 2 MeOH → 95 CH₂Cl₂ : 5 MeOH) to afford Fmoc-protected amide as a white foam.

Fmoc-protected amine (67 mg, 0.065 mmol) was treated with 20% (vol/vol) piperidine in a mixture of dichloromethane and methanol (1:1 v/v CH₂Cl₂: MeOH, 1.4 mL). The reaction was allowed to stir at room temperature for 3.5 h. The solvent was removed in vacuo to afford a crude residue that was purified by column chromatography (95: 5 v/v CH₂Cl₂: MeOH → 9: 1 v/v CH₂Cl₂: MeOH) to afford amine S4 as a white foam (40 mg, 67% over 2 steps).

\(^1\)H NMR (400 MHz, CD₃OD): δ 7.70 (1H, d, J 8.1 Hz, H-6'), 5.86 (1H, d, J 6.8 Hz, H-1), 5.80 (1H, d, J 8.0 Hz, H-5'), 4.75 (1H, t, J 6.0 Hz, CH), 4.30 (1H, dd, J 6.8, 4.6 Hz, H-2), 4.14 (1H, dd, J 4.7, 2.3 Hz, H-3), 4.05 (1H, ddd, J 6.7, 4.8, 2.3 Hz, H-4), 3.67 (1H, t, J 6.5 Hz, CH), 3.57 (1H, d, J 14.2, 6.4 Hz, CH₂), 3.43 (1H, dd, J 14.2, 4.8 Hz, CH₂), 2.75 (3H, m, CH₂ + H-5), 2.61 (1H, dd, J 16.8, 6.4 Hz, H-5), 1.46 (9H, s, CO₂C(CH₃)₂), 1.45 (9H, s, CO₂C(CH₃)₃), 0.93 (9H, s, (CH₃)₃CSi), 0.88 (9H, s, (CH₃)₃CSi), 0.15 (3H, s, CH₃Si), 0.13 (3H, s, CH₃Si), 0.09 (3H, s, CH₃Si), 0.03 (3H, s, CH₃Si). \(^1\)C NMR (101 MHz, CD₃OD): δ 174.5 (C=O), 171.9 (C=O), 171.2 (C=O), 170.1 (C=O), 164.5, 151.0, 142.1, 101.9, 88.8, 84.5, 81.2, 81.1, 73.8, 72.9, 51.4, 49.7, 41.3, 39.5, 36.6, 27.0, 27.0, 25.0, 25.0, 17.5, 17.4, -5.5, -5.6, -5.7, -6.0.

(S)-3-(2-(((2S,3R,4R,5S,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)acetamido)-4-(((S)-3-carboxy-1-(((2R,3S,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoic acid (1)

To a solution of acid S5\(^2\) (6.2 mg, 15 µmol) in DMF (150 µL) was added amine S4 (19 mg, 23 µmol), HATU (6.4 mg, 16 µmol) followed by 1-hydroxy-7-azabenzotriazole (2.3 mg, 16 µmol) and N,N-diisopropylethylamine (6.4 µL, 36 µmol). The reaction was allowed to stir at room temperature for 1 h. The reaction was...
subsequently diluted with ethyl acetate (30 mL), washed with water (5 × 10 mL), brine (10 mL). The organic layer was dried over anhydrous MgSO₄ and the solvent was removed in vacuo to give a crude residue that was purified by reverse phase HPLC (0-100% MeCN over 40 min) to afford fully protected uridylpeptide as a white solid.

Fully protected uridylpeptide (10 mg, 8.3 µmol) was dissolved in a 1:1 (v/v) mixture of methanol and water (90 µL). Triethylamine (10 µL, 140 µmol) was added and the reaction was allowed to stir at room temperature for 16 h. The solvent was removed in vacuo to give a residue that was immediately suspended in 90: 5: 5 (v/v/v) TFA: TIS: H₂O (200 µL). The reaction mixture was allowed to stir at room temperature for 16 h. The solvent was removed in vacuo to give a crude residue that was purified by reverse phase HPLC (0-50% MeCN over 40 min) to afford compound 1 as a white solid (1.1 mg, 10% over 3 steps).

¹H NMR (500 MHz, CD₃OD): δ 7.68 (1H, d, J 8.0 Hz, H-6'), 5.83-5.80 (1H, m), 5.76-5.74 (1H, m), 4.80-4.85 (1H, m), 4.29-4.39 (2H, m), 4.07-3.94 (4H, m), 3.88-3.75 (2H, m), 3.75-3.68 (1H, m), 3.65-3.57 (2H, m), 3.57-3.43 (2H, m), 3.46-3.38 (2H, m), 2.93-2.72 (4H, m), 2.03 (3H, s, NHCOCH₃). LRMS [M+H⁺] 735.3. HRMS (ESI) m/z cald for C₂₇H₃₈N₆O₁₈Na [M+Na⁺]: 757.2135, found 757.2137.
3. Synthesis of compound 2:

![Scheme S2](image)

Scheme S2. Synthesis of compound 2 from protected uridylamine S2.

\( N\-(2\-(\text{cyclohexylamino})\-2\-\text{oxoethyl})\-N\-[((2\text{R},3\text{S},4\text{R},5\text{R})\-5\-(2,4\-\text{dioxo}-3,4\-\text{dihydropyrimidin}-1\text{H}-1\text{yl})\-3,4\-\text{dihydroxytetrahydrofuran}-2\-\text{yl})\text{methyl})\-2,3\-\text{dihydroxybenzamide (2)}}\)

To a solution of TBS-protected uridylamine (70 mg, 0.14 mmol) in methanol (1.8 mL) was added paraformaldehyde (4.2 mg, 1.4 mmol). The solution was allowed to stir at room temperature for 30 min. 2,2-Dimethylbenzo[\text{d}]\-[1,3]dioxole-4-carboxylic acid (27 mg, 0.14 mmol) in methanol (180 \text{µL}) was added to the above solution, followed by cyclohexyl isonitrile (17 \text{µL}, 0.15 mmol). The reaction was allowed to stir at room temperature for 72 h at which point, the solvent was removed \text{in vacuo} to give a crude residue that was purified by column chromatography (95:5 \text{v/v} \text{CH}_2\text{Cl}_2: \text{MeOH}) to give amide S6 as a colourless oil (84 mg).

Amide S6 (84 mg, 0.11 mmol) was dissolved in a mixture of trifluoroacetic acid and water (9:1 \text{v/v} \text{TFA: H}_2\text{O}, 4 mL) and the reaction was allowed to stir at room temperature for 4.5 h. The solvent was removed \text{in vacuo} to give a crude residue that
was purified by column chromatography (95:5 v/v CH₂Cl₂: CH₃OH → 85:15 v/v CH₂Cl₂: CH₃OH) to afford compound 2 as an off-white solid.

m.p. 150-154 °C (decomp.); IR (ATR): ν = 3253, 2937, 2860, 1688 cm⁻¹. ¹H NMR (400 MHz, CD₃CN, 330 K): δ 7.28 (1H, app. s, H-6’), 6.91 (1H, dd, J 8.0, 1.7 Hz, Ar-H, H-6”), 6.80 (1H, t, J 7.8 Hz, Ar-H, H-5”), 6.72 (1H, dd, J 7.6, 1.7 Hz, Ar-H, H-4”), 5.71-5.67 (1H, m, H-1), 5.63 (1H, d, J 8.2 Hz, H-5’), 4.20-3.89 (5H, m), 3.84-3.58 (3H, m), 1.87-1.79 (2H, m, CH₂), 1.70-1.68 (2H, m, CH₂), 1.65-1.57 (1H, m, CH₂)1.41-1.31 (2H, m, CH₂), 1.28-1.17 (3H, m, CH₂). LRMS [M+H⁺] 519.3. HRMS (ESI) m/z cald for C₂₄H₃₀N₄O₉Na [M+Na⁺]: 541.1905, found 541.1904. 
[α]₂⁵ = +28° (c = 0.2 in CH₃OH).
4. Synthesis of compound 3:

$$\text{Scheme S3. Synthesis of compound 3 from sulfamoyluridine S7.}$$

$$(2R,3S,4R,5R,6S)-5$-acetamido-$2$-(acetoxymethyl)$-6$-$2$-(((3aR,4R,6R,6aR)$-6$-$2$,$4$-dioxo-$3$,$4$-dihydropyrimidin-$1$(2$H$)-yl)$-2$,$2$-dimethyltetrahydrofuro[3,$4$-$d$][1,$3$]dioxol-4-yl)methoxy)sulfonyl)amino)-2-o xoethoxy)tetrahydro-2$H$-pyran-3,4-diyl diacetate (S8)

To a solution of 2',3'-O-Isopropylidene-5'-O-sulfamoyluridine S7 (130 mg, 0.35 mmol) in dichloromethane (31 mL) was added acid S5 (160 mg, 0.39 mmol), dicyclohexylcarbodiimide (116 mg, 0.56 mmol) and DMAP (71 mg, 0.56 mmol). The reaction was allowed to stir at room temperature for 16 h. The solvent was concentrated to 5 mL before the resulting precipitate was filtered off and washed with a minimal amount of dichloromethane. The solvent was removed in vacuo and the crude residue was purified by column chromatography (10:1 v/v CH$_2$Cl$_2$: MeOH → 7:1 v/v CH$_2$Cl$_2$: MeOH) to afford sulfonamide S8 as a colourless oil (130 mg, 48%).

IR (ATR): $\nu = 3400, 1678$ cm$^{-1}$. $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 7.78 (1H, d, $J$ 8.5 Hz, H-6'), 5.90 (1H, d, $J$ 2.8 Hz, H-1), 5.73 (1H, d, $J$ 8.1 Hz, H-5'), 5.31 (1H, dd, $J$ 10.7, 9.4 Hz, H-3), 5.02 (1H, dd, $J$ 10.4, 9.4 Hz, H-4), 4.96 (1H, dd, $J$ 6.3, 2.8 Hz),
Sulfonamide S8 (100 mg, 0.13 mmol) was dissolved in a mixture of trifluoroacetic acid and water (3:1 v/v TFA: H₂O, 10 mL) and the reaction was allowed to stir at room temperature for 16 h. The solvent was removed in vacuo and azeotroped with toluene to a crude residue that was dissolved up in 10% triethylamine in 1:1 (v/v) MeOH and water (1.4 mL). The reaction was stirred at room temperature for 16 h. The solvent was removed in vacuo to give a residue that was purified by reverse phase HPLC (0 to 50% MeCN over 50) to afford sulfonamide 3 as a white solid (15 mg, 20% over 2 steps).

IR (ATR): ν = 3355, 1683 cm⁻¹. 1H NMR (500 MHz, CD₃OD): δ 7.72 (1H, d, J 8.2 Hz, H-6”), 5.87 (1H, d, J 4.3 Hz, H-1), 5.76 (1H, d, J 8.1 Hz, H-5”), 4.89-4.81 (1H, m, H-1”), overlapping with HDO signal in CD₃OD as determined by HSQC experiment), 4.59 (1H, dd, J 11.3, 2.6 Hz, H-5), 4.52 (1H, dd, J 11.2, 3.7 Hz, H-5), 4.29-4.12 (5H, m, CH₂ + H-2 + H-3 + H-4), 3.96 (1H, dd, J 10.7, 3.6 Hz, H-2”), 3.83 (1H, dd, J 11.9, 2.3 Hz, H-6”), 3.72-3.63 (2H, m, H-6” + H-3”), 3.60 (1H, ddd, J 9.9, 5.7, 2.2 Hz, H-5”), 3.37 (1H, dd, J 10.0, 8.9 Hz, H-4”), 2.03 (3H, s, NHAc). ¹³C NMR (126 MHz, CD₃OD): δ 172.6, 169.0, 164.8, 151.0, 141.2, 101.8, 97.9, 90.1, 81.4, 73.7, 73.3, 71.4, 71.2, 70.8, 69.8, 65.4, 61.3, 53.7, 21.5. LRMS [M+H⁺] 585.2. HRMS (ESI) m/z cald for C₁₉H₂₉N₄O₁₅S [M+H⁺]: 585.1345, found 585.1345. [α]D²⁵ = +92° (c = 0.2 in CH₃OH).
5. Synthesis of compound 4:

Scheme S4. Synthesis of compound 4 from uridylamine S2.

1-(((2R,3R,4R,5R)-3,4-bis((tert-butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)thiourea (S9)

Amine S2 (0.26 g, 0.55 mmol) was dissolved in 1:1 (v/v) mixture of THF and DMF (5 mL). 1,1'-Thiocarbonyldiimidazole (110 mg, 0.61 mmol) was added and the reaction was stirred for 16 h at room temperature. At this point, aqueous ammonia solution (0.61 mL, 25% in water) was added and the reaction mixture was allowed to stir at room temperature for a further 3 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (30 mL). The organic layer was washed with water (5 × 5 mL) and the aqueous phase was back extracted with ethyl acetate (2 × 25 mL). The combined organic layers were dried over anhydrous MgSO₄ and the solvent was removed in vacuo to give a crude residue that was purified by column chromatography (95:5 v/v CH₂Cl₂: MeOH) to afford thiourea S9 as an off-white foam (200 mg, 70%).

¹H NMR (400 MHz, CD₃OD): δ 7.70 (1H, d, J 8.0 Hz, H-6’), 5.87 (1H, d, J 6.8 Hz, H-1), 5.77 (1H, d, J 8.0 Hz, H-5’), 4.45-4.42 (1H, m, H-2), 4.12-4.06 (2H, m, H-3 + H-4), 3.97 (1H, dd, J 5.2, 13.2 Hz, H-5ₐ or H-5₏), 3.74 (1H, dd, J 5.2, 13.2 Hz, H-5ₐ
or H-5b), 0.95 (9H, s, \((CH_3)_3CSi\)), 0.88 (9H, s, \((CH_3)_3CSi\)), 0.18-0.09 (12H, m, \(CH_3Si\)).\(^{13}\)C NMR (101 MHz, CD\(_3\)OD): \(\delta\) 163.4, 151.0, 142.2, 102.0, 89.3, 85.1, 73.4, 72.9, 25.0, 25.0, 17.5, 17.4, -5.5, -5.6, -5.6, -6.1.

1-((2R,3R,4S,5R)-5-((4-(3,4-dihydroxyphenyl)thiazol-2-yl)amino)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (4)

![Chemical structure](image)

To a solution of thiourea S9 (163 mg, 0.31 mmol) in DMF (1 mL) was added \(\alpha\)-bromoketone S10\(^4\) (84 mg, 0.55 mmol) and the reaction was allowed to stir at room temperature for 16 h. The reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with water (5 × 20 mL) and the aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried over anhydrous MgSO\(_4\). The solvent was removed \textit{in vacuo} to give a crude residue that was purified by column chromatography (100:1 v/v CH\(_2\)Cl\(_2\): MeOH → 95:5 v/v CH\(_2\)Cl\(_2\): MeOH) to afford aminothiazole S11 as a pale yellow oil (100 mg).

Aminothiazole S11 (100 mg, 0.15 mmol) was dissolved in a mixture of trifluoroacetic acid and dichloromethane (9:1 v/v TFA: DCM, 9 mL) and the reaction was allowed to stir at room temperature for 5 h. The solvent was removed \textit{in vacuo} to give a crude residue that was purified by column chromatography (9:1 v/v CH\(_2\)Cl\(_2\): MeOH) to afford compound 4 as a pale yellow oil (34 mg, 25% over 2 steps).

IR (ATR): \(\nu = 3322, 1675\) cm\(^{-1}\). \(^1\)H NMR (400 MHz, CD\(_3\)OD): \(\delta\) 7.63 (1H, d, \(J = 8.1\) Hz, H-6\(^{'}\)), 7.13 (1H, d, \(J = 2.1\) Hz, Ar-H, H-2\(^{''}\)), 7.04 (2H, dd, \(J = 2.1, 8.2\) Hz, Ar-H, H-6\(^{''}\)), 6.80 (1H, d, \(J = 8.2\) Hz, Ar-H, H-5\(^{''}\)), 5.75 (1H, d, \(J = 4.2\) Hz, H-1), 5.62 (1H, d, \(J = 8.1\) Hz, H-5\(^{'}\)), 4.30 (1H, dd, \(J = 4.2, 5.8\) Hz, H-2), 4.12-4.06 (2H, m, H-3 + H-4), 3.97 (1H, dd, \(J = 3.6, 13.2\) Hz, H-5\(_a\) or H-5\(_b\)), 3.74 (1H, dd, \(J = 3.6, 13.2\) Hz, H-5\(_a\) or H-5\(_b\)); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD): \(\delta\) 170.4, 164.6, 150.8, 146.1, 145.5, 145.2, 142.1, 123.5, 117.8 (× 2), 115.1, 113.0, 101.6, 91.7, 82.3, 73.0, 70.6, 46.8. LRMS [M+H\(^{+}\)]
435.3. HRMS (ESI) m/z cald for C\textsubscript{18}H\textsubscript{18}N\textsubscript{4}O\textsubscript{7}SNa [\textit{M+Na}^+] = 457.0788, found 457.0790. \([\alpha]_D^{25} = +33^\circ\) (c = 0.4 in CH\textsubscript{3}OH).

6. Synthesis of compounds 5 and 6

![Scheme S5. Synthesis of compounds 5 and 6.](image)

**General procedure S1: Aminoquinazoline-based inhibitors 5 and 6**

To a solution of 4-chloro-6,7-dimethoxyquinazoline 9 (1 eq.) in isopropanol was added the corresponding 4-aminocarboxamide (1 eq.) and the reaction mixture was heated at 120 °C for 5 h. The reaction was subsequently filtered and washed with diethyl ether (2 × 10 mL) to obtain compounds 5 and 6 which were purified by column chromatography or used without further purification.

**N-(4-((6,7-dimethoxyquainzolin-4-yl)amino)phenyl)benzamide (5)**

4-chloro-6,7-dimethoxyquinazoline 10 (126 mg, 0.56 mmol) was reacted with \(N\)-(4-amino-phenyl)benzamide S13 (119 mg, 0.56 mmol) in isopropanol (8 mL) for 5 h according to general procedure S1 to obtain 5 as a yellow solid (168 mg, 75%).

m.p. = 258-259 °C (decomp.); IR (ATR): \(\nu = 2921, 1627\) cm\textsuperscript{-1}. \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta 11.22\) (1H, s, NH), 10.42 (1H, s, NH), 8.80 (1H, s, Ar-H, H-2), 8.23 (1H, s, Ar-H, H-8), 7.99 (2H, d, \(J = 7.4\) Hz, Ar-H, H-2’ + H-6’), 7.90 (2H, d, \(J = 8.6\) Hz, Ar-H, H-2” + H-6”), 7.66 (2H, d, \(J = 8.7\) Hz, Ar-H, H-3” + H-5”), 7.62-7.53 (5H, m, Ar-H), 7.31 (1H, s, Ar-H, H-5), 4.01 (3H, s, OCH\textsubscript{3}), 3.99 (3H, s, OCH\textsubscript{3}). LRMS
[\text{M+H}^+] \ 401.1. \text{HRMS (ESI)} \text{ m/z} \text{ cald for C}_{23}\text{H}_{21}\text{N}_{4}\text{O}_{3} \ [\text{M+H}^+]: \ 401.1608, \text{found} \ 401.1609.

\text{N-(4-} \text{((7-hydroxy-6-methoxyquinazolin-4-yl)amino)phenyl)benzamide (6)}

7-(benzylxy)-4-chloro-6-methoxyquinazoline \text{ S12}^5 (15 \text{ mg}, 0.050 \text{ mmol}) was reacted with \text{N-(4-aminophenyl)benzamide S13}^6 (12 \text{ mg}, 0.055 \text{ mmol}) in isopropanol (1 mL) according to general procedure S1 for 5 h to obtain benzyl ether S14 as a yellow solid which was used in the next step without purification.

A mixture of benzyl ether \text{S14} (21 \text{ mg}, 0.044 \text{ mmol}) in methanol (10 mL) was hydrogenated (1 atm) over 10\% \text{Pd/C} (10 \text{ mg}) for 1.25 h. The reaction mixture was subsequently filtered over Celite® and the filtrate concentrated \textit{in vacuo} to give a crude solid that was purified by column chromatography (95: 5 v/v CH\textsubscript{2}Cl\textsubscript{2}: MeOH \rightarrow 9:1 v/v CH\textsubscript{2}Cl\textsubscript{2}: MeOH) to afford 6 as a yellow solid (10 mg, 50\% over 2 steps).

\text{m.p.} \ 148-152 \degree \text{C} (decomp.). \text{IR (ATR):} \ \nu = 3000, \ 1701 \text{ cm}^{-1}. \text{\textsuperscript{1}H NMR (400 MHz, CD\textsubscript{3}OD):} \ \delta \ 8.34 (1H, s, Ar-H, H-2), \ 7.97-7.94 (2H, m, Ar-H, H-2’ + H-6’), \ 7.76-7.69 (5H, m, Ar-H), \ 7.59-7.50 (3H, m, Ar-H), \ 7.06 (1H, s, H-5), \ 4.05 (6H, s, 2 \times \text{OCH}_3). \text{\textsuperscript{13}C NMR (100 MHz, CD\textsubscript{3}OD):} \ \delta \ 167.4 (\text{C=O}), \ 157.3, \ 154.4, \ 152.0, \ 149.5, \ 145.7, \ 135.4, \ 135.0, \ 134.9, \ 131.4, \ 128.2, \ 127.2, \ 123.2, \ 121.2, \ 108.6, \ 108.2, \ 101.1, \ 55.4. \text{LRMS} \ [\text{M+H}^+] \ 387.3. \text{HRMS (ESI)} \text{ m/z} \text{ cald for C}_{22}\text{H}_{19}\text{N}_{4}\text{O}_{3} \ [\text{M+H}^+]: \ 387.1452, \text{found} \ 387.1450.
7. Synthesis of compounds 36-38:

![Scheme S6. Synthesis of compounds 36 and 37 via chloroquinazoline S15 and S16.](image)

3-hydroxy-N-(4-(thieno[3,2-d]pyrimidin-4-ylamino)phenyl)benzamide (36)

4-chlorothieno[3,2-d]pyrimidine S15 (59 mg, 0.35 mmol) was reacted with the corresponding 4-aminocarboxamide S17 (78 mg, 0.35 mmol) in isopropanol (8.6 mL) according to general procedure S1 to obtain compound 36 (as TFA salt), after reverse phase HPLC purification (0 to 100% MeCN over 40 min), as an off-white solid (47 mg, 28%).

m.p. = 125-126 °C (decomp.). IR (ATR): ν = 3248, 1672 cm⁻¹. ¹H NMR (400 MHz, DMSO-ｄ₆): δ 10.55 (1H, s, NH), 10.27 (1H, s, NH), 8.75 (1H, s, Ar-H, H-2), 8.35 (1H, d, J 5.5 Hz, Ar-H, H-6), 7.84 (2H, d, J 8.9 Hz, Ar-H, H-2’ + H-6’), 7.74-7.59 (2H, m, Ar-H, H-3’ + H-5’), 7.50 (1H, d, J 5.5 Hz, Ar-H, H-7), 7.39 (1H, dt, J 7.6, 1.3 Hz, Ar-H, H-6’’), 7.35-7.33 (2H, m, Ar-H, H-2” + H-5”’), 6.98 (1H, ddd, J 7.9, 2.5, 1.1 Hz, Ar-H, H-4’’); ¹³C NMR (101 MHz, DMSO-ｄ₆): δ 166.0 (C=O), 157.8, 156.7, 154.5, 152.0, 137.7, 137.3, 136.8, 133.4, 129.9, 124.8, 121.8, 121.0, 119.0, 118.6, 115.7, 115.0. LRMS [M+H⁺] 363.2. HRMS (ESI) m/z cald for C₁₀H₁₅N₄O₂S [M+H⁺]: 363.0910, found 363.0910.
Scheme S7. Synthesis of chloroquinazoline S16.

4-chloro-5,6,7,8-tetrahydroquinazoline (S16)

To a solution of ethyl 2-cyclohexanone carboxylate (810 µL, 5.1 mmol) in methanol (4 mL) was added trimethyl orthoformate (2.20 mL, 20.3 mmol) and ammonium acetate (1.56 g, 20.3 mmol). The reaction was heated at 120 °C for 3 h. The reaction was allowed to cool to room temperature, water (40 mL) was added and the suspension was stirred for an additional 1 h. The solution was subsequently filtered to afford S18 as a white solid (800 mg).

A mixture of quinazolinone S18 (0.44 g, 2 mmol) and phosphorus(V) oxychloride (4.4 mL, 43 mmol) was refluxed for 4 h. The solvent was removed in vacuo and the residue was dissolved in dichloromethane (200 mL) and washed with saturated aqueous NaHCO₃ solution (3 × 50 mL) and brine (50 mL). The organic layer was dried over anhydrous MgSO₄ and the solvent was removed in vacuo to afford a crude residue that was purified by column chromatography (95:5 v/v CH₂Cl₂:MeOH) to afford S16 as a yellow solid (630 mg, 74% over 2 steps).

m.p. 86-87 °C (decomp.); IR (ATR): ν = 2939, 2869, 1530 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ 8.66 (1H, s, Ar-H, H-2), 2.87-2.83 (2H, m, CH₂), 2.75-2.70 (2H, m, CH₂), 1.87-1.82 (4H, m, 2 × CH₂). ¹³C NMR (75 MHz, CDCl₃): δ 168.0, 161.2, 155.2, 129.3, 32.3, 25.6, 21.9, 21.8. LRMS [M+H⁺] 169.1. HRMS (ESI) m/z calcd for C₈H₁₀ClN₂ [M+H⁺]: 169.0527, found 169.0529.
3-hydroxy-N-(4-((5,6,7,8-tetrahydroquinazolin-4-yl)amino)phenyl)benzamide (37)

4-Chloro-5,6,7,8-tetrahydroquinazoline S16 (37 mg, 0.22 mmol) was reacted with 4-aminocarboxamide S17 (50 mg, 0.22 mmol) in isopropanol (8.5 mL) according to general procedure S1 for 4 h to obtain compound 37 (as TFA salt) as a white solid (55 mg, 70%) following reverse phase HPLC purification (0 to 100% MeCN over 40 min).

m.p. 234-237 °C (decomp.). IR (ATR): ν = 3248, 1684, 1675 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆): δ 10.25 (1H, s, NH), 9.79 (1H, s, NH), 8.67 (1H, s, Ar-H, H-2), 7.84-7.79 (2H, m, Ar-H, H-2’ + H-6’), 7.52-7.47 (2H, m, Ar-H, H-3’ + H-5’), 7.38 (1H, dt, J 7.6, 1.4 Hz, Ar-H, H-6”), 7.37-7.26 (2H, m, Ar-H, H-2” + H-5”), 6.98 (1H, ddd, J 7.9, 2.5, 1.2 Hz, Ar-H, H-4”), 2.78-2.73 (2H, m), 2.59-2.56 (2H, m), 1.88-1.78 (4H, m). ¹³C NMR (101 MHz, DMSO-d₆): δ 166.0 (C=O), 160.5, 157.8, 153.5, 150.0, 137.5, 136.7, 133.0, 129.9, 125.4, 120.8, 119.0, 118.6, 115.0, 114.6, 27.2, 22.3, 21.1, 20.5. LRMS [M+H⁺] 361.3. HRMS (ESI) m/z cald for C₂₁H₂₁N₄O₂ [M+H⁺]: 361.1659, found 361.1660.

Scheme S8. Synthesis of compound 38 from chloroquinazoline 10.
4-((6,7-dimethoxyquinazolin-4-yl)amino)benzoic acid (S19)

4-Chloro-6,7-dimethoxyquinazoline 10 (58 mg, 0.26 mmol), 4-aminobenzoic acid (36 mg, 0.26 mmol) in isopropanol (6 mL) were reacted for 5 h according to general procedure S1 to obtain S19 as a off-white solid (21 mg, 50%) which was used without further purification.

m.p. 249-251°C (decomp.); IR (ATR): ν = 3272, 3086, 1676, 1641 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆): δ 10.67 (1H, s, NH), 8.81 (1H, s, Ar-H, H-2), 8.05-8.02 (3H, m, Ar-H, H-2' + H-6' + H-8), 7.90 (2H, d, Ar-H, J 8.40 Hz, H-3' + H-5'), 7.29 (1H, s, Ar-H, H-5), 4.00 (3H, s, OCH₃), 3.99 (3H, s, OCH₃); ¹³C NMR (101 MHz, DMSO-d₆): δ 167.3 (C=O), 157.9, 156.4, 150.6, 150.5, 142.4, 130.5 (× 2), 127.5, 123.4, 108.5, 103.3, 102.8, 57.0, 56.8. LRMS [M+H⁺] 326.0. HRMS (ESI) m/z cald for C₁₇H₁₆N₃O₄ [M+H⁺]: 326.1135, found 326.1135.

4-((6,7-dimethoxyquinazolin-4-yl)amino)-N-(3-hydroxyphenyl)benzamide (38)

To a solution of carboxylic acid S19 (8 mg, 0.024 mmol) in DMF (0.1 mL) was added HATU (9 mg, 0.024 mmol) and N,N-diisopropylethylamine (7 µL, 0.024 mmol). The reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was diluted with ethyl acetate (20 mL), washed with water (5×5 mL), brine (5 mL) and dried over anhydrous MgSO₄. The solvent was subsequently removed in vacuo to give a crude residue that was purified by reverse phase HPLC (0 to 100% MeCN over 40 min) to afford compound 38 (TFA salt) as a white solid (5 mg, 50%).

m.p. 219-221°C (decomp.); IR (ATR): ν = 3307, 1684 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 10.70 (1H, s, NH), 10.11 (1H, s, NH), 9.40 (1H, s, OH), 8.80 (1H, s,
Ar-H, H-2), 8.06-8.04 (3H, m, Ar-H, H-2’ + H-6’ + H-8), 7.90 (2H, d, J 8.8 Hz, Ar-H, H-3’ + H-5’), 7.38-7.36 (1H, m, Ar-H, H-2’’), 7.30 (1H, s, Ar-H, H-5), 7.18 (1H, app. d, J 8.4 Hz, Ar-H, H-5’’), 7.12-7.10 (1H, m, Ar-H), 6.51 (1H, app. d, J 8.0 Hz, Ar-H), 4.01 (3H, s, OCH₃), 4.00 (3H, s, OCH₃); ¹³C NMR (101 MHz, DMSO-d₆): δ 165.3 (C=O), 158.0, 157.9, 156.4, 150.7, 150.5, 141.1, 140.7, 131.9, 129.7, 128.8, 127.4, 123.4, 111.6, 111.2, 108.4, 108.0, 103.3, 102.8, 57.0, 56.8; LRMS [M+H⁺] 417.1. HRMS (ESI) m/z cald for C₂₃H₂₁N₄O₄ [M+H⁺]: 417.1557, found 417.1556.
8. General procedure S2: Synthesis of 4-aminocarboxamides 20, S20, S21, S23 and S25-27

To a solution of carboxylic acid (1.19-1.48 mmol) in dichloromethane (27-34 mL/mmol) was added HATU (1.19-1.48 mmol, 1 eq.), followed by 1,4-phenylenediamine (1.19-1.48 mmol, 1 eq.) and N,N-diisopropylethyamine (1.19-1.48 mmol, 1 eq.). The reaction was allowed to stir at room temperature for 1-1.5 h. The reaction mixture was diluted with dichloromethane (30 mL) and washed with brine (2 × 20 mL). The organic layer was dried over anhydrous MgSO₄. The solvent was removed in vacuo to give 4-aminocarboxamides 20, S20, S21, S23 and S25-27 followed column chromatography or reverse phase HPLC purification.

N-(4-aminophenyl)-3-(4-fluorophenyl)-5-methylisoxazole-4-carboxamide (S20)

3-(4-Fluorophenyl)-5-methylisoxazole-4-carboxylic acid (328 mg, 1.48 mmol) was reacted with 1,4-phenylenediamine (160 mg, 1.48 mmol) in dichloromethane (40 mL) in the presence of HATU (563 mg, 1.48 mmol) and N,N-diisopropylethyamine (260 µL) for 1 h according to general procedure S2 to afford 4-aminocarboxamide S20 (TFA salt) as an off-white solid (219 mg, 47%) which was used without further purification.

m.p. 179-182 °C (decomp.). IR (ATR): ν = 3262, 2967, 2922, 1670, 1609 cm⁻¹.¹H NMR (400 MHz, DMSO-d₆): δ 10.41 (1H, s, NH), 7.80-7.70 (2H, m, Ar-H, H-2’ + H-6’), 7.56 (2H, d, J 8.3 Hz, 1H, Ar-H, H-2 + H-6), 7.38-7.28 (2H, m, Ar-H, H-3’ + H-5’), 7.05 (2H, d, J 8.3 Hz, Ar-H, H-3 + H-4). 2.58 (3H, s, CH₃). ¹³C NMR (101 MHz, DMSO-d₆): δ 170.5, 164.8 (C=O), 161.2 (d, J 221 Hz, ipso-C), 160.3, 159.8, 134.8, 130.5 (d, J 8.7 Hz, meta-C), 125.0 (d, J 3.2 Hz, para-C), 121.6, 120.4, 116.4 (d, J 22 Hz, ortho-C), 113.6, 12.4. LRMS [M+H⁺] 312.1. HRMS (ESI) m/z cald for C₁₇H₁₅FN₃O₂ [M+H⁺]: 312.1143, found 312.1143.
2-Pyrazinecarboxylic acid (184 mg, 1.48 mmol) was reacted with 1,4-phenylenediamine (160 mg, 1.48 mmol) in dichloromethane (40 mL) in the presence of HATU (563 mg, 1.48 mmol) and N,N-diisopropylethylamine (260 µL) for 1.5 h according to general procedure S2 to afford 4-aminocarboxamide S21 as an off-white solid (109 mg, 34%) after reverse phase HPLC purification (0 to 100% MeCN over 40 min).

m.p. 167-171 °C (decomp.). IR (ATR): ν = 3348, 2639, 1685 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆): δ 10.72 (1H, s, NH), 9.30-9.27 (1H, m, Ar-H, H-2’), 8.93 (1H, dd, J 2.5, 1.0 Hz, Ar-H, H-4’), 8.81-8.80 (1H, m, Ar-H, H-5’), 7.84 (2H, d, J 8.7 Hz, Ar-H, H-2 + H-6), 7.09 (2H, d, J 8.4 Hz, H-3 + H-5). ¹³C NMR (101 MHz, DMSO-d₆): δ 161.9 (C=O), 148.1, 145.5, 144.5, 143.7, 134.6, 122.2, 120.5, 120.5. LRMS [M+H⁺] 215.0. HRMS (ESI) m/z cald for C₁₁H₁₁N₄O [M+H⁺]: 215.0927, found 215.0928.

N-(4-aminophenyl)pyrazine-2-carboxamide (S21)

To a solution of 3-hydroxybenzoic acid (205 mg, 1.48 mmol) in dichloromethane (50 mL) was added HATU (563 mg, 1.48 mmol) followed by N,N-diisopropylethylamine (260 µL, 1.48 mmol) and the reaction mixture was allowed to stir at room temperature for 10 min. At this point, 1,4-phenylenediamine (160 mg, 1.48 mmol) was added and the reaction was allowed to stir at room temperature for 1 h. The reaction mixture was diluted with dichloromethane (30 mL) and washed with brine (2 × 20 mL). The organic layer was dried over anhydrous MgSO₄. The solvent was removed in vacuo to give a crude residue that was purified by column chromatography (95:5 v/v CH₂Cl₂: CH₃OH → 9:1 v/v CH₂Cl₂: CH₃OH) to afford 4-aminocarboxamide S17 as a white solid (220 mg, 65%).

N-(4-aminophenyl)-3-hydroxybenzamide (S17)
N-(4-aminophenyl)-2-hydroxybenzamide (S22)

To a stirred solution of salicylic acid (135 mg, 0.97 mmol) in acetonitrile (2.9 mL) at room temperature was added 1-chloro-N,N-2-trimethyl-1-propenylamine (258 µL, 1.95 mmol). The reaction was allowed to stir at room temperature for 2.5 h. The solvent was removed in vacuo to give a residue that was suspended in dichloromethane (2.2 mL) and the solution was allowed to cool to 0 °C. A solution of 1,4-phenylenediamine (106 mg, 1 mmol) in dichloromethane (14.7 mL) was added dropwise, followed by triethylamine (280 µL, 1.94 mmol). The reaction was allowed to stir at 10 °C for 16 h. The solvent was subsequently removed in vacuo and the resulting residue was diluted with ethyl acetate (70 mL), washed with water (30 mL) and brine (30 mL) to give a crude residue that was purified by reverse phase HPLC (0 to 100% MeCN over 40 min) to afford 4-aminocarbamoyl S22 (TFA salt) as a white solid (45 mg, 20%).

m.p. 169-171 °C (decomp.). IR (ATR): ν = 3288, 2923, 1672 cm⁻¹.¹H NMR (500 MHz, DMSO-d₆): δ 7.76 (2H, d, J 8.8 Hz, Ar-H, H-2 + H-6), 7.37 (1H, dt, J 8.0, 1.3 Hz, Ar-H, H-6’), 7.34-7.26 (2H, m, Ar-H, H-4’ + H-5’), 7.15 (2H, d, J 8.8 Hz, Ar-H, H-3 + H-5), 6.97 (1H, ddd, J 8.0, 2.4, 1.1 Hz, Ar-H, H-4’).¹³C NMR (126 MHz, DMSO-d₆): δ 166.1 (C=O), 158.0, 137.0, 136.8, 130.0, 130.0, 122.1, 121.7, 119.1, 118.7, 115.1. LRMS [M+H⁺] 229.0. HRMS (ESI) m/z cald for C₁₃H₁₃N₂O₂ [M+H⁺]: 229.0972, found 229.0973.
**N-(4-aminophenyl)-2-nitrobenzamide (S23)**

2-Nitrobenzoic acid (247 mg, 1.48 mmol) was reacted with 1,4-phenylenediamine (160 mg, 1.48 mmol) in dichloromethane (40 mL) in the presence of HATU (563 mg, 1.48 mmol) and *N*,*N*-diisopropylethylamine (260 µL) for 1 h according to general procedure S2 to afford 4-aminocarboxamide S23 as a white solid (228 mg, 60%) after reverse phase HPLC purification (0 to 100% MeCN over 40 min).

m.p. 98-99 °C (decomp.). IR (ATR): \( \nu = 3288, 1678 \text{ cm}^{-1} \). \(^1\text{H} \) NMR (400 MHz, DMSO-\( d_6 \)): \( \delta \) 8.23-8.07 (1H, m, Ar-H, H-3'), 7.87 (1H, t, \( J = 7.5 \text{ Hz} \), 1H, Ar-H), 7.79-7.71 (2H, m, Ar-H), 7.66 (2H, d, \( J = 8.4 \text{ Hz} \), H-2 + H-6), 7.16 (2H, d, \( J = 8.4 \text{ Hz} \), H-3 + H-5). \(^{13}\text{C} \) NMR (101 MHz, DMSO-\( d_6 \)): \( \delta \) 164.5 (C=O), 146.9, 136.3, 134.5, 133.0, 132.4, 131.5, 129.7, 124.7, 121.6, 121.3. LRMS [\( M+H^+ \)] 258.0. HRMS (ESI) m/z cald for C\(_{13}\)H\(_{12}\)N\(_3\)O\(_3\) [\( M+H^+ \)]: 258.0873, found 258.0874.

**N-(4-aminophenyl)-4-((tert-butyldimethylsilyl)oxy)benzamide (S24)**

A stirring solution of 4-((tert-butyldimethylsilyl)oxy)benzoic acid (500 mg, 3.62 mmol) in dichloromethane (15 mL) was allowed cooled to 0 °C for 10 min. Oxalyl chloride (735 µL, 8.69 mmol) was added dropwise to this solution, followed by DMF (10 µL). The mixture was allowed to warm up to room temperature over 2 h. The solvent was removed *in vacuo*, and the residue was immediate suspended in THF (15 mL) and cooled to 0 °C. A solution of 1,4-phenylenediamine in THF (0.25 M, 392 mg in 15 mL of THF) was added dropwise, followed by triethylamine (555 µL). The reaction was warmed to room temperature and allowed to stir at this temperature for 16 h. The solvent was subsequently removed *in vacuo* and the resulting residue was diluted with ethyl acetate (70 mL), washed with water (30 mL) and brine (30 mL) to give a crude residue that was purified by column chromatography (100:1 v/v CH\(_2\)Cl\(_2\):
CH$_3$OH $\rightarrow$ 98:2 v/v CH$_2$Cl$_2$: CH$_3$OH) to afford 4-aminocarboxamide S24 as a pale yellow oil (779 mg, 63%).

IR (ATR): $\nu$ = 3337, 3037, 2955, 1638, 1604 cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.75 (2H, d, $J$ 8.2 Hz, Ar-H, H-2' + H-6'), 7.60 (1H, s, NH), 7.37 (2H, d, $J$ 8.2 Hz, Ar-H, H-3' + H-5'), 6.69 (2H, d, $J$ 8.2 Hz, Ar-H, H-3 + H-5), 3.60 (2H, s, NH$_2$), 0.996 (9H, s, (CH$_3$)$_3$CSi), 0.23 (6H, s, 2 $\times$ CH$_3$Si); $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 165.2 (C=O), 158.8, 143.4, 129.5, 128.7, 128.1, 122.3, 120.1, 115.5, 38.6, 25.6, -4.4. LRMS [M+H$^+$] 343.3. HRMS (ESI) m/z cald for C$_{19}$H$_{27}$N$_2$O$_2$Si [M+H$^+$]: 343.1836, found 343.1837.

**N-(4-aminophenyl)-4-fluorobenzamide (S25)**

![Chemical structure image]

4-Fluorobenzoic acid (207 mg, 1.48 mmol) was reacted with 1,4-phenylenediamine (160 mg, 1.48 mmol) in dichloromethane (40 mL) in the presence of HATU (563 mg, 1.48 mmol) and N,N-diisopropylethylamine (260 $\mu$L) for 1 h according to general procedure S2 to afford 4-aminocarboxamide S25 as a white solid (170 mg, 50%) after reverse phase HPLC purification (0 to 100% MeCN over 40 min).

m.p. 179-181 °C (decomp.). IR (ATR): $\nu$ = 3350, 3339, 1675, 1643 cm$^{-1}$. $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 9.86 (1H, s, NH), 7.98-7.96 (2H, m, Ar-H, H-2' + H-6'), 7.34-7.29 (4H, m, Ar-H, H-2 + H-6 + H-3' + H-5'), 6.52 (2H, d, $J$ 8.7 Hz, Ar-H, H-3 + H-5). $^{13}$C NMR (126 MHz, DMSO-$d_6$): $\delta$ 164.8 (d, $J$ 156 Hz, ipso-C), 163.4 (C=O), 145.9, 132.3 (d, $J$ 2.9 Hz, para-C), 130.6 (d, $J$ 9.0 Hz, meta-C), 128.6, 122.9, 115.8 (d, $J$ 22 Hz, ortho-C), 114.3. LRMS [M+H$^+$] 231.0. HRMS (ESI) m/z cald for C$_{13}$H$_{12}$FN$_2$O [M+H$^+$]: 231.0928, found 231.0928.

**N-(4-aminophenyl)-3-methoxybenzamide (S26)**

![Chemical structure image]
3-methoxybenzoic acid (225 mg, 1.48 mmol) was reacted with 1,4-phenylenediamine (160 mg, 1.48 mmol) in dichloromethane (40 mL) in the presence of HATU (563 mg, 1.48 mmol) and \(N,N\)-diisopropylethylamine (260 \(\mu\)L) for 1 h according to general procedure S2 to afford 4-aminocarboxamide S26 as a yellow oil (178 mg, 50%) after column chromatography (95:5 v/v CH\(_2\)Cl\(_2\): CH\(_3\)OH).

IR (ATR): \(\nu = 3350, 3341, 2955, 2942, 1631\) cm\(^{-1}\). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta 9.82 (1\mathrm{H}, \mathrm{s}, \text{NH}), 7.54-7.47 (1\mathrm{H}, \mathrm{m}, \text{Ar-H, H-6’}), 7.47-7.43 (1\mathrm{H}, \mathrm{m}, \text{Ar-H, H-2’}), 7.40 (1\mathrm{H}, \mathrm{t}, J 7.9 \text{ Hz, Ar-H, H-5’}), 7.36 (2\mathrm{H}, \mathrm{d}, J 8.4 \text{ Hz, Ar-H, H-2 + H-6}), 7.11 (1\mathrm{H}, \mathrm{ddd}, J 8.2, 2.4, 1.1 \text{ Hz, Ar-H, H-4’}), 6.54 (2\mathrm{H}, \mathrm{d}, J 8.5 \text{ Hz, Ar-H, H-3 + H-5}), 3.82 (3\mathrm{H}, \mathrm{s}, \text{OCH}_3)\). \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)):\(\delta 164.8 (\text{C=O}), 159.6, 145.7, 137.2, 129.9, 128.5, 122.8, 120.1, 117.3, 114.1, 113.1, 55.7\). LRMS \([\text{M+H}^+]\) 243.1. HRMS (ESI) m/z cald for C\(_{14}\)H\(_{15}\)N\(_2\)O\(_2\) \([\text{M+H}^+]\): 243.1128, found 243.1130.

\(N\)-(4-aminophenyl)-3-nitrobenzamide (S27)

3-Nitrobenzoic acid (250 mg, 1.5 mmol) was reacted with 1,4-phenylenediamine (160 mg, 1.5 mmol) in dichloromethane (40 mL) in the presence of HATU (560 mg, 1.5 mmol) and \(N,N\)-diisopropylethylamine (260 \(\mu\)L) for 1 h according to general procedure S2 to afford 4-aminocarboxamide S27 as an off-white solid (190 mg, 50%) after reverse phase HPLC purification (0 to 100% MeCN over 40 min).

m.p. 170-173 °C (decomp.); IR (ATR): \(\nu = 3080, 2932, 2637, 1667\) cm\(^{-1}\). \(^1\)H NMR (400 MHz, CD\(_3\)CN): \(\delta 8.73 (1\mathrm{H}, \mathrm{s}, \text{Ar-H, H-2’}), 8.39 (1\mathrm{H}, \mathrm{ddd}, J 8.3, 2.2, 1.0 \text{ Hz, Ar-H, H-6’}), 8.28 (1\mathrm{H}, \mathrm{dt}, J 8.1, 1.3 \text{ Hz, Ar-H, H-4’}), 7.75 (1\mathrm{H}, \mathrm{t}, J 8.0 \text{ Hz, Ar-H, H-5’}), 7.58 (2\mathrm{H}, \mathrm{d}, J 8.8 \text{ Hz, Ar-H, H-2 + H-6}), 6.95 (2\mathrm{H}, \mathrm{d}, J 8.7 \text{ Hz, H-3 + H-5}). \(^{13}\)C NMR (101 MHz, CD\(_3\)CN): \(\delta 163.4 (\text{C=O}), 148.3, 138.0, 136.7, 133.5, 132.3, 129.9, 126.0, 122.3, 122.2, 117.9\). LRMS \([\text{M+H}^+]\) 258.0. HRMS (ESI) m/z cald for C\(_{13}\)H\(_{12}\)N\(_3\)O\(_3\) \([\text{M+H}^+]\): 258.0873, found 258.0874.

\((S)-(9H-fluoren-9-yl)methyl 2-((4-aminophenyl)carbamoyl)pyrrolidine-1-carboxylate (20)
Fmoc-L-proline (402 mg, 1.2 mmol) was reacted with 1,4-phenylenediamine (130 mg, 1.2 mmol) in dichloromethane (40 mL) in the presence of HATU (450 mg, 1.5 mmol) and N,N-diisopropylethylamine (200 µL) for 1 h according to general procedure S2 to afford 4-aminocarboxamide 20 as a yellow oil (290 mg, 57%) after column chromatography (98:2 v/v CH2Cl2: CH3OH → 95:5 v/v CH2Cl2: CH3OH).

IR (ATR): ν = 3309, 2937, 1666, 1641 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆): δ 8.73 (1H, s, NH), 7.92 (1H, d, J 7.3 Hz, Ar-H), 7.89-7.85 (2H, m, Ar-J), 7.61 (1H, d, J 7.4 Hz, Ar-H), 7.51-7.42 (1H, m, Ar-H), 7.42-7.33 (1H, m, Ar-H), 7.31 (1H, t, J 7.4 Hz, Ar-H), 6.77 (d, J 8.3 Hz, Ar-H, H-2 + H-6), 6.38 (2H, d, J 8.3 Hz, Ar-H, H-3 + H-5), 4.17 (1H, dd, J 10.8, 5.0 Hz, CH), 3.49-3.43 (1H, m, H-5'), 3.18 (2H, dd, J 11.8, 5.2 Hz, CH₂), 3.11 (1H, dd, J 9.6, 4.9 Hz, H-2”), 2.62 (1H, q, J 8.3 Hz, H-5”), 2.22-2.10 (1H, m, H-3”), 1.94-1.79 (3H, m, H-3' + H-4'ax + H-4'eq). ¹³C NMR (101 MHz, DMSO-d₆): δ 171.1, 146.4, 145.8, 145.3, 141.1, 140.7, 127.8, 127.8, 127.6, 127.5, 127.5, 126.2, 125.0, 120.9, 120.8, 120.5, 114.0, 68.7, 59.2, 53.7, 46.6, 30.1, 24.1. LRMS [M-CO₂] 384.0. HRMS (ESI) m/z cald for C₂₆H₂₆N₃O₃ [M+H⁺]: 428.2332, found 428.2333. [α]D²⁵ = -100° (c = 0.2 in CH₂Cl₂).

9. Numbering for aminquinazoline-based inhibitors

![Figure S3. Carbons numbering for aminquinazoline-based inhibitors.](image-url)
10. $^1$H, $^{13}$C NMR of 10, 11, 13-38, 20, S17, S20-S27 and analytical HPLC traces of 10-38:

$^1$H NMR (400 MHz, DMSO-<i>d</i>$_6$)
$^{13}$C NMR (101 MHz, DMSO-$d_6$)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^{1}$H NMR (400 MHz, CD$_3$OD)

$^{13}$C NMR (101 MHz, CD$_3$OD)
$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (101 MHz, DMSO-$d_6$)
Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)

$^1$H NMR (400 MHz, DMSO-$d_6$)
$^{13}$C NMR (101 MHz, DMSO-$d_6$)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^1$H NMR (400 MHz, CD$_3$OD)

$^{13}$C NMR (101 MHz, CD$_3$OD)
Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (101 MHz, DMSO-$d_6$)
Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)

$^1$H NMR (400 MHz, CD$_3$OD)
$^{13}$C NMR (101 MHz, CD$_3$OD)

[Image of a spectrum with chemical structure]

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^1$H NMR (400 MHz, CD$_3$OD)

$^{13}$C NMR (101 MHz, CD$_3$OD)
Analytical HPLC trace (0 to 100% MeCN over 30 min, \( \lambda = 254 \text{ nm} \))

\[ \text{Detector A Ch2} \]

\( ^1 \text{H NMR (400 MHz, DMSO-}d_6, T = 330 \text{ K)} \)
$^{13}$C NMR (101 MHz, DMSO-$d_6$, T = 330 K)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda$ = 254 nm)
$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (101 MHz, DMSO-$d_6$)
Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)

$^1$H NMR (400 MHz, DMSO-$d_6$)
$^{13}$C NMR (101 MHz, DMSO-$d_6$)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^1$H NMR (400 MHz, Acetone-$d_6$)

$^{13}$C NMR (101 MHz, Acetone-$d_6$)
Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)

$^1$H NMR (500 MHz, DMSO-$d_6$)
$^{13}$C NMR (125 MHz, DMSO-$d_6$)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^1$H NMR (400 MHz, CD$_3$OD)

$^{13}$C NMR (101 MHz, CD$_3$OD)
Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)

$^1$H NMR (400 MHz, DMSO-$d_6$)
$^{13}$C NMR (101 MHz, DMSO-$d_6$)

![C NMR spectrum](image)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)

![HPLC trace](image)

$^1$H NMR (400 MHz, DMSO-$d_6$)
$^{13}$C NMR (101 MHz, DMSO-$d_6$)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^1$H NMR (500 MHz, DMSO-$d_6$)
\[ ^{13}C \text{ NMR (125 MHz, DMSO-}d_6) \]

![13C NMR spectrum](image1)

Analytical HPLC trace (0 to 100% MeCN over 30 min, \( \lambda = 254 \text{ nm} \))

![HPLC trace](image2)

\[ ^1\text{H NMR (400 MHz, DMSO-}d_6) \]
$^{13}$C NMR (101 MHz, DMSO-$d_6$)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
^{1}H NMR (500 MHz, DMSO-\textit{d}_6)
$^{13}$C NMR (125 MHz, DMSO-$d_6$)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^1$H NMR (400 MHz, DMSO-$d_6$)

![H NMR spectrum](image)

$^{13}$C NMR (101 MHz, DMSO-$d_6$)

![C NMR spectrum](image)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^1$H NMR (500 MHz, DMSO-$d_6$)

$^{13}$C NMR (125 MHz, DMSO-$d_6$)
Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)

$^1$H NMR (500 MHz, DMSO-$d_6$)
$^{13}$C NMR (125 MHz, DMSO-$d_6$)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^{1}H$ NMR (400 MHz, DMSO-$d_6$)

$^{13}C$ NMR (101 MHz, DMSO-$d_6$)
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)

$^1$H NMR (400 MHz, DMSO-$d_6$)
$^{13}$C NMR (101 MHz, DMSO-$d_6$)

Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)
$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (101 MHz, DMSO-$d_6$)
Analytical HPLC trace (0 to 100% MeCN over 30 min, $\lambda = 254$ nm)

$^1$H NMR (400 MHz, DMSO-$d_6$)
$^{13}$C NMR (101 MHz, DMSO-$d_6$)

$^1$H NMR (400 MHz, DMSO-$d_6$)
$^{13}$C NMR (101 MHz, DMSO-$d_6$)

$^1$H NMR (500 MHz, DMSO-$d_6$)
$^1$H NMR (500 MHz, DMSO-$d_6$)

$^{13}$C NMR (126 MHz, DMSO-$d_6$)
$^{13}$C NMR (126 MHz, DMSO-$d_6$)

$^1$H NMR (400 MHz, DMSO-$d_6$)
$^{13}$C NMR (101 MHz, DMSO-$d_6$)
$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (101 MHz, DMSO-$d_6$)
$^1$H NMR (500 MHz, DMSO-$d_6$)

$^{13}$C NMR (126 MHz, DMSO-$d_6$)
$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (101 MHz, DMSO-$d_6$)
$^1$H NMR (400 MHz, CD$_3$CN)

$^{13}$C NMR (101 MHz, CD$_3$CN)
$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (101 MHz, DMSO-$d_6$)
11. References: