Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supplementary Information

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

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Table of Contents

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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 $MgCl_2$, 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 ^{o}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.

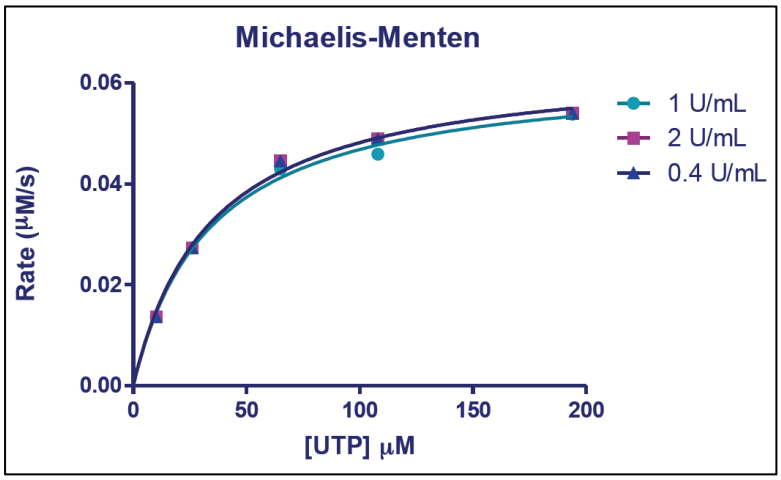

Figure S1. Least square fitted curve to the Michaelis-Menten model for *M. tuberculosis* GlmU uridyltransferase with varying concentrations of coupling enzymes.

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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).

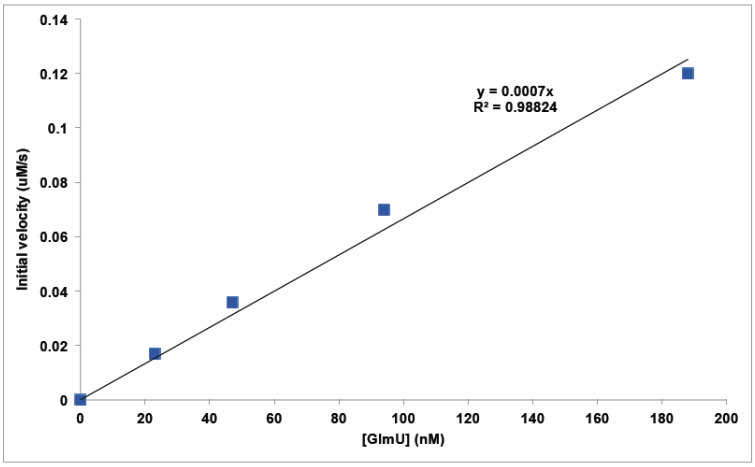
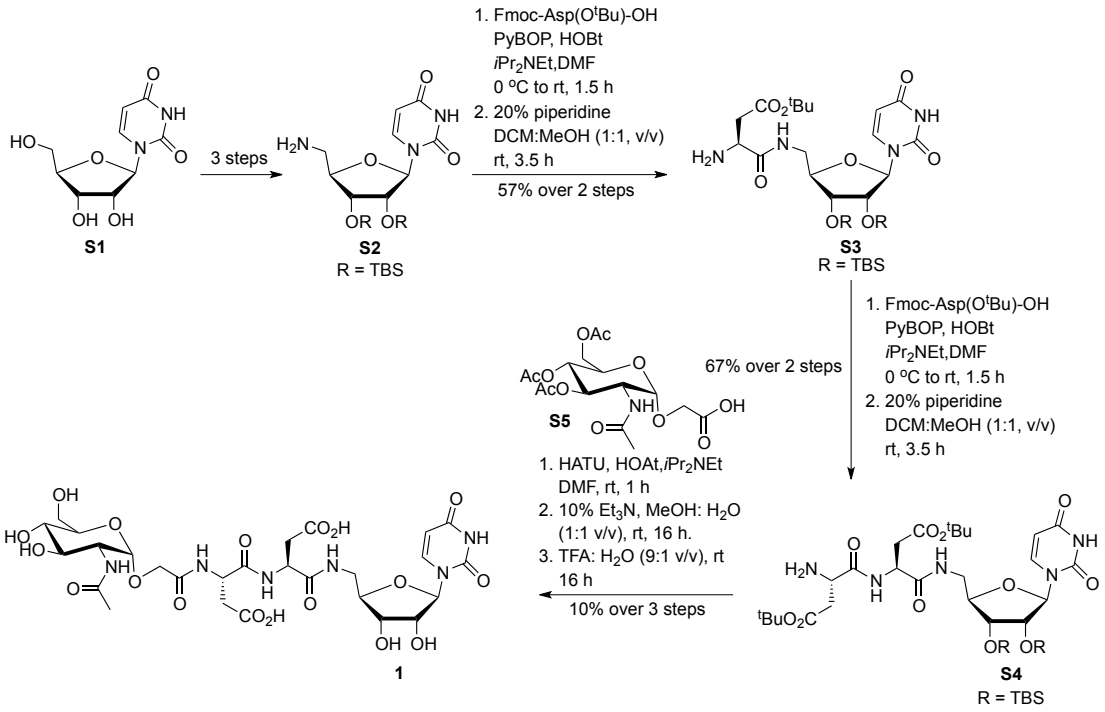


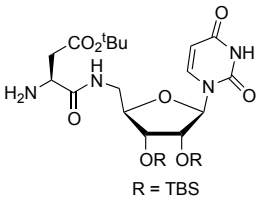
Figure S2. Dependence of reaction rate on GlmU concentration.

2. Synthesis of compound 1:



Scheme S1. Synthesis of compound **1** from uridine

(*S*)-*tert*-butyl-3-amino-4-((((2*R*,3*R*,4*R*,5*R*)-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(O^{t}Bu)-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 *N*-*N*-diisopropylethylamine (45 µL, 0.25 mmol) at 0 °C. Amine **S2**[1] (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 $NaHCO_3$ solution (5 × 50 mL) and brine (2 × 40 mL). The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo* to give a crude residue that was purified by column

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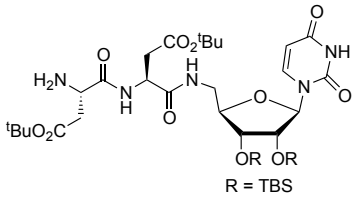
chromatography (98: 2 v/v CH_2Cl_2 : MeOH → 95: 5 v/v CH_2Cl_2 : MeOH) to afford the Fmoc-protected amide as a white foam.

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 CH_2Cl_2: 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 CH_2Cl_2 : MeOH → 9: 1 v/v CH_2Cl_2: MeOH) to afford amine **S3** as a white foam (46 mg, 67% over 2 steps).

^{1}H NMR (400 MHz, CD_3OD): 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, CH_2), 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, $CO_2C\mathit{Me}_3$), 0.94 (9H, s, Me_3CSi), 0.89 (9H, s, $\mathit{(CH_3)}_3CSi$), 0.146 (3H, s, CH_3Si), 0.132 (3H, s, CH_3Si), 0.090 (3H, s, CH_3Si), 0.028 (3H, s, CH_3Si). ^{13}C NMR (101 MHz, CD_3OD): 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*)-*tert*-butyl-3-amino-4-(((*S*)-1-((((2*R*,3*R*,4*R*,5*R*)-3,4-bis((*tert*-butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)amino)-4-(tert-butoxy)-1,4-dioxobutan-2-yl)amino)-4-oxobutanoate (S4)



To a solution of Fmoc-Asp(O^{t}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 ^{o}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 $NaHCO_3$ solution (5 × 50 mL) and brine (2 × 40 mL). The organic layer was dried over anhydrous $MgSO_4$ and

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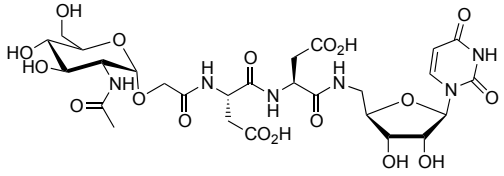
the solvent removed *in vacuo* to give a crude residue that was purified by column chromatography (98 CH_2Cl_2 : 2 MeOH → 95 CH_2Cl_2 : 5 MeOH) to afford Fmoc-protected amide as a white foam.

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 CH_2Cl_2: 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 CH_2Cl_2: MeOH → 9: 1 v/v CH_2Cl_2: MeOH) to afford amine **S4** as a white foam (40 mg, 67% over 2 steps).

^{1}H NMR (400 MHz, CD_3OD): 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, dd, *J* 14.2, 6.4 Hz, CH_2), 3.43 (1H, dd, *J* 14.2, 4.8 Hz, CH_2), 2.75 (3H, m, CH_2 + H-5), 2.61 (1H, dd, *J* 16.8, 6.4 Hz, H-5), 1.46 (9H, s, $CO_2C(CH_3)_3$), 1.45 (9H, s, $CO_2C(CH_3)_3$), 0.93 (9H, s, $(CH_3)_3CSi$), 0.88 (9H, s, $(CH_3)_3CSi$), 0.15 (3H, s, CH_3Si), 0.13 (3H, s, CH_3Si), 0.09 (3H, s, CH_3Si), 0.03 (3H, s, CH_3Si). ^{13}C NMR (101 MHz, CD_3OD): 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-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)acetamido)-4-(((*S*)-3-carboxy-1-((((2*R*,3*S*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-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

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subsequently diluted with ethyl acetate (30 mL), washed with water (5 × 10 mL), brine (10 mL). The organic layer was dried over anhydrous $MgSO_4$ 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: H_2O (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).

^{1}H NMR (500 MHz, CD_3OD): δ 7.68 (1H, d, *J* 8.0 Hz, H-6'), 5.83-5.80 (1H, m), 5.76-5.74 (1H, m), 4.88-4.85 (1H, m), 4.29-4.19 (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, NHCO*CH*$_3$). LRMS [*M*+H^+] 735.3. HRMS (ESI) m/z cald for $C_{27}H_{38}N_6O_{18}Na$ [*M*+Na^+]: 757.2135, found 757.2137.

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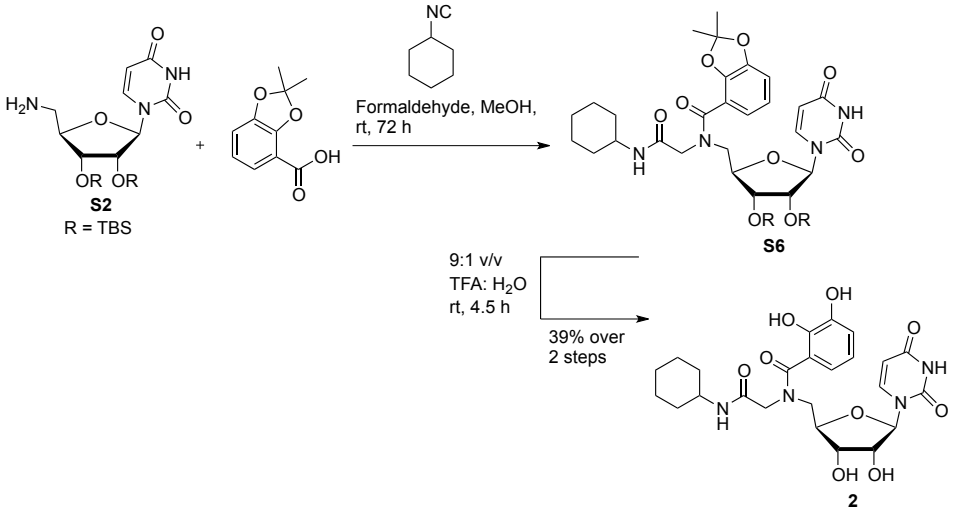
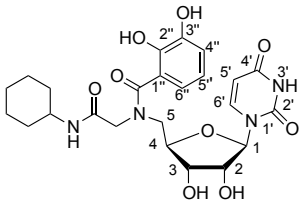
3. Synthesis of compound 2:



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

N-(2-(cyclohexylamino)-2-oxoethyl)-*N*-(((2*R*,3*S*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-2,3-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[*d*][1,3]dioxole-4-carboxylic acid (27 mg, 0.14 mmol) in methanol (180 µL) was added to the above solution, followed by cyclohexyl isonitrile (17 µL, 0.15 mmol). The reaction was allowed to stir at room temperature for 72 h at which point, the solvent was removed *in vacuo* to give a crude a residue that was purified by column chromatography (95:5 v/v CH_2Cl_2: 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 v/v TFA: H_2O, 4 mL) and the reaction was allowed to stir at room temperature for 4.5 h. The solvent was removed *in vacuo* to give a crude residue that

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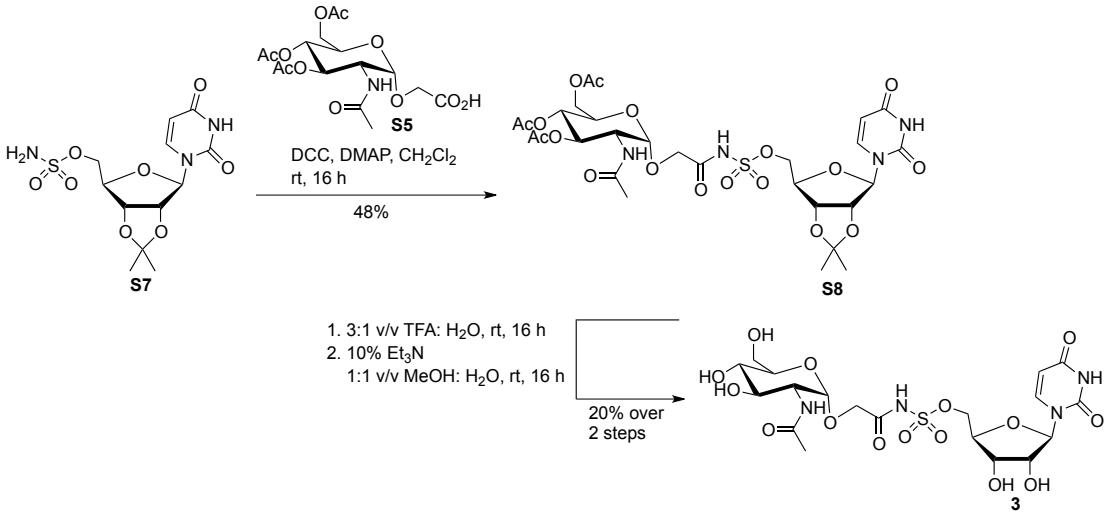
was purified by column chromatography (95:5 v/v CH_2Cl_2: CH_3OH → 85:15 v/v CH_2Cl_2: CH_3OH) to afford compound **2** as an off-white solid.

m.p. 150-154 ^oC (decomp.); IR (ATR): ν = 3253, 2937, 2860, 1688 cm^{-1}. ^1H NMR (400 MHz, CD_3CN, 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, CH_2), 1.70-1.68 (2H, m, CH_2), 1.65-1.57 (1H, m, CH2),1.41-1.31 (2H, m, CH_2), 1.28-1.17 (3H, m, CH_2). LRMS [*M*+H^+] 519.3. HRMS (ESI) m/z cald for $C_{24}H_{30}N_4O_9Na$ [*M*+Na^+]: 541.1905, found 541.1904.

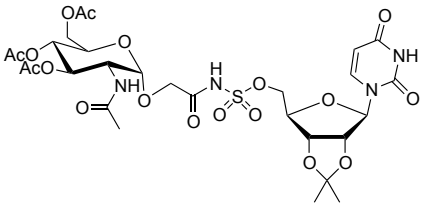
$[\alpha]_D^{25}$ = +28^o (c = 0.2 in CH_3OH).

4. Synthesis of compound 3:



Scheme S3. Synthesis of compound **3** from sulfamoyluridine **S7**.

(2*R*,3*S*,4*R*,5*R*,6*S*)-5-acetamido-2-(acetoxymethyl)-6-(2-(((((3a*R*,4*R*,6*R*,6a*R*)-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-oxoethoxy)tetrahydro-2*H*-pyran-3,4-diyl diacetate (S8)



To a solution of 2',3'-O-Isopropylidene-5'-O'-sulfamoyluridine **S7**[3] (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 dichoromethane. The solvent was removed *in vacuo* and the crude residue was purified by column chromatography (10:1 v/v CH_2Cl_2: MeOH → 7:1 v/v CH_2Cl_2: MeOH) to afford sulfonamide **S8** as a colourless oil (130 mg, 48%).

IR (ATR): ν = 3400, 1678 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ 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),

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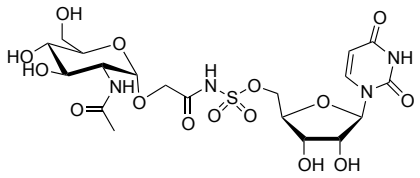
4.92 (1H, dd, *J* 6.3, 3.0 Hz), 4.87 (1H, d, *J* 3.6 Hz), 4.45-4.36 (1H, m), 4.35-4.22 (4H, m), 4.16 (1H, d, *J* 15.5 Hz, C*H*H), 4.12-4.06 (2H, m), 4.03 (1H, d, *J* 15.5 Hz, CH*H*), 2.06 (3H, s, NHAc), 2.01 (3H, s, OAc), 1.96 (6H, s, 2 × OAc), 1.54 (3H, s, CH_3), 1.35 (3H, s, CH_3). ^{13}C NMR (126 MHz, CD_3OD): δ 175.9, 172.2, 171.0, 170.6, 170.0, 164.6, 150.7, 142.4, 113.8, 101.6, 97.6, 92.7, 84.3, 84.2, 81.0, 71.4, 68.6, 67.8, 61.9, 51.5, 38.9, 26.2, 24.2, 21.3, 19.3, 19.2.

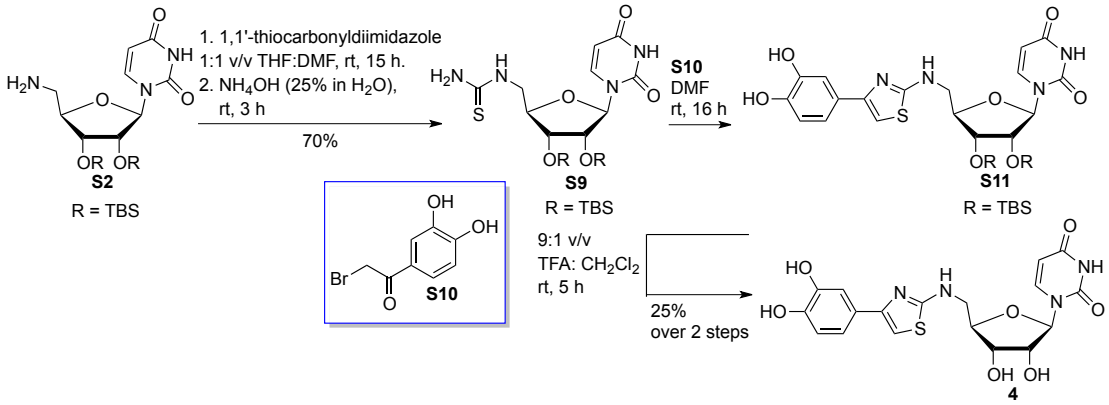
((2*R*,3*S*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (2-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)acetyl)sulfamate (3)



Sulfonamide **S8** (100 mg, 0.13 mmol) was dissolved in a mixture of trifluoroacetic acid and water (3:1 v/v TFA: H_2O, 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).

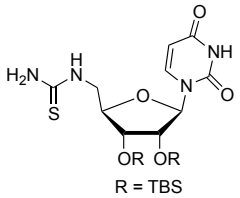
IR (ATR): ν = 3355, 1683 cm^{-1}. 1H NMR (500 MHz, CD_3OD): δ 7.72 (1H, d, *J* 8.2 Hz, H-6'), 5.87 (1H, d, *J* 4.3 Hz, H-1), 5.76 (1H, d, *J* 8.1 Hz, H-5'), 4.89-4.81 (1H, m, H-1", overlapping with HDO signal in CD_3OD as determined by HSQC experiment), 4.59 (1H, dd, *J* 11.3, 2.6 Hz, H-5), 4.52 (1H, dd, *J* 11.2, 3.7 Hz, H-5), 4.29-4.12 (5H, m, CH_2 + 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). ^{13}C NMR (126 MHz, CD_3OD): 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 $C_{19}H_{29}N_4O_{15}S$ [*M*+H^+]: 585.1345, found 585.1345. $[\alpha]_D^{25}$ = +92° (c = 0.2 in CH_3OH).

5. Synthesis of compound 4:



Scheme S4. Synthesis of compound **4** from uridylamine **S2**.

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

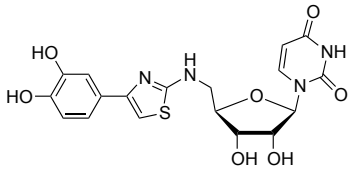


Amine **S2** (0.26 g, 0.55 mmol) was dissolved in 1:1 (v/v) mixture of THF and DMF (5 mL). 1,1'-Thiocarbonyldiimidazole (110 mg, 0.61 mmol) was added and the reaction was stirred for 16 h at room temperature. At this point, aqueous ammonia solution (0.61 mL, 25% in water) was added and the reaction mixture 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 $MgSO_4$ and the solvent was removed *in vacuo* to give a crude residue that was purified by column chromatography (95:5 v/v CH_2Cl_2: MeOH) to afford thiourea **S9** as an off-white foam (200 mg, 70%).

^{1}H NMR (400 MHz, CD_3OD): 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-5_a or H-5_b), 3.74 (1H, dd, *J* 5.2, 13.2 Hz, H-5_a

or H-5$_b$), 0.95 (9H, s, $(CH_3)_3$CSi), 0.88 (9H, s, $(CH_3)_3$CSi), 0.18-0.09 (12H, m, CH_3Si).^{13}C NMR (101 MHz, CD_3OD): δ 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-((2*R*,3*R*,4*S*,5*R*)-5-(((4-(3,4-dihydroxyphenyl)thiazol-2-yl)amino)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (4)



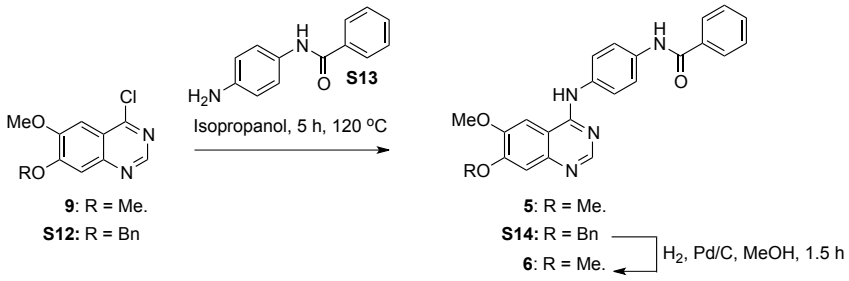
To a solution of thiourea **S9** (163 mg, 0.31 mmol) in DMF (1 mL) was added α-bromoketone **S10**[4] (84 mg, 0.55 mmol) and the reaction was allowed to stir at room temperature for 16 h. The reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (100 mL). The organic layer 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 *in vacuo* to give a crude residue that was purified by column chromatography (100:1 v/v CH_2Cl_2: MeOH → 95:5 v/v CH_2Cl_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 *in vacuo* to give a crude residue that was purified by column chromatography (9:1 v/v CH_2Cl_2: MeOH) to afford compound **4** as a pale yellow oil (34 mg, 25% over 2 steps).

IR (ATR): ν = 3322, 1675 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ 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_3OD): δ 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 $C_{18}H_{18}N_4O_7SNa$ [M+Na^+]: 457.0788, found 457.0790. $[\alpha]_D^{25}$ = +33° (c = 0.4 in CH_3OH).

6. Synthesis of compounds 5 and 6

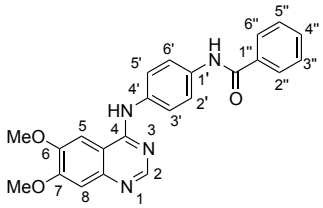


Scheme S5. Synthesis of compounds **5** and **6**.

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-dimethoxyquinazolin-4-yl)amino)phenyl)benzamide (5)

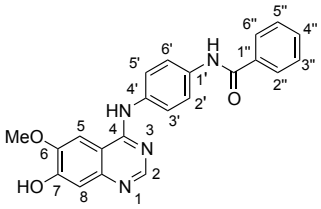


4-chloro-6,7-dimethoxyquinazoline **10** (126 mg, 0.56 mmol) was reacted with *N*-(4-aminophenyl)benzamide **S13** (119 mg, 0.56 mmol) in isopropanol (8 mL) for 5 h according to general procedure **S1** to obtain **5** as a yellow solid (168 mg, 75%).

m.p. = 258-259 °C (decomp.); IR (ATR): ν = 2921, 1627 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 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_3), 3.99 (3H, s, OCH_3). LRMS

[*M*+H$^+$] 401.1. HRMS (ESI) m/z cald for $C_{23}H_{21}N_4O_3$ [*M*+H$^+$]: 401.1608, found 401.1609.

***N*-(4-((7-hydroxy-6-methoxyquinazolin-4-yl)amino)phenyl)benzamide (6)**



7-(benzyloxy)-4-chloro-6-methoxyquinazoline **S12**[5] (15 mg, 0.050 mmol) was reacted with *N*-(4-aminophenyl)benzamide **S13**[6] (12 mg, 0.055 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 **S14** (21 mg, 0.044 mmol) in methanol (10 mL) was hydrogenated (1 atm) over 10% Pd/C (10 mg) for 1.25 h. The reaction mixture was subsequently filtered over Celite® and the filtrate concentrated *in vacuo* to give a crude solid that was purified by column chromatography (95: 5 v/v CH_2Cl_2: MeOH → 9:1 v/v CH_2Cl_2 : MeOH) to afford **6** as a yellow solid (10 mg, 50% over 2 steps).

m.p. 148-152 ^oC (decomp.). IR (ATR): ν = 3000, 1701 cm^{-1}.^{1}H NMR (400 MHz, CD_3OD): δ 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 × OCH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 167.4 (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. LRMS [*M*+H$^+$] 387.3. HRMS (ESI) m/z cald for $C_{22}H_{19}N_4O_3$ [*M*+H$^+$]: 387.1452, found 387.1450.

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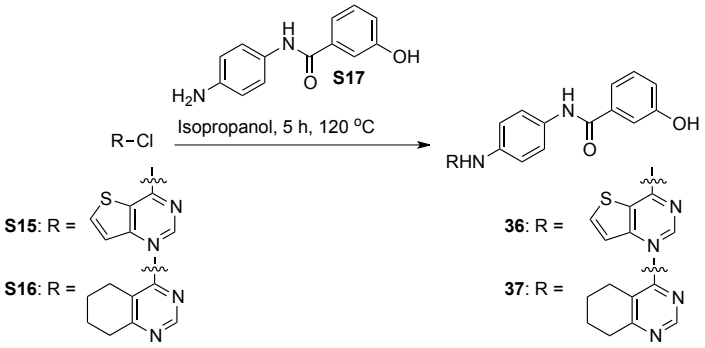
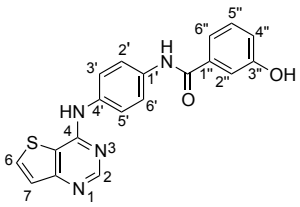
7. Synthesis of compounds 36-38:



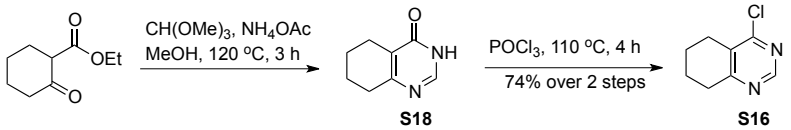
Scheme S6. Synthesis of compounds **36** and **37** *via* chloroquinazoline **S15** and **S16**.

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



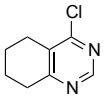
4-chlorothieno[*3,2-d*]pyrimidine **S15** (59 mg, 0.35 mmol) was reacted with the corresponding 4-aminocarboxamide **S17** (78 mg, 0.35 mmol) in isopropanol (8.6 mL) according to general procedure **S1** to obtain compound **36** (as TFA salt), after reverse phase HPLC purification (0 to 100% MeCN over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

m.p. = 125-126 °C (decomp.). IR (ATR): ν = 3248, 1672 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 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"); ^{13}C NMR (101 MHz, DMSO- d_6): 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 $C_{19}H_{15}N_4O_2S$ [*M*+H^+]: 363.0910, found 363.0910.



Scheme S7. Synthesis of chloroquinazoline **S16.**

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

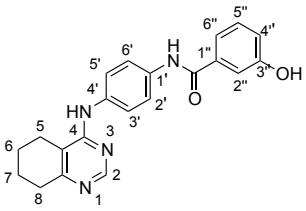


To a solution of ethyl 2-cyclohexanone carboxylate (810 µL, 5.1 mmol) in methanol (4 mL) was added trimethyl orthoformate (2.20 mL, 20.3 mmol) and ammonium acetate (1.56 g, 20.3 mmol). The reaction was heated at 120 °C for 3 h. The reaction was allowed to cool to room temperature, water (40 mL) was added 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 $NaHCO_3$ solution (3 × 50 mL) and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo* to afford a crude residue that was purified by column chromatography (95:5 v/v CH_2Cl_2:MeOH) to afford **S16** as a yellow solid (630 mg, 74% over 2 steps).

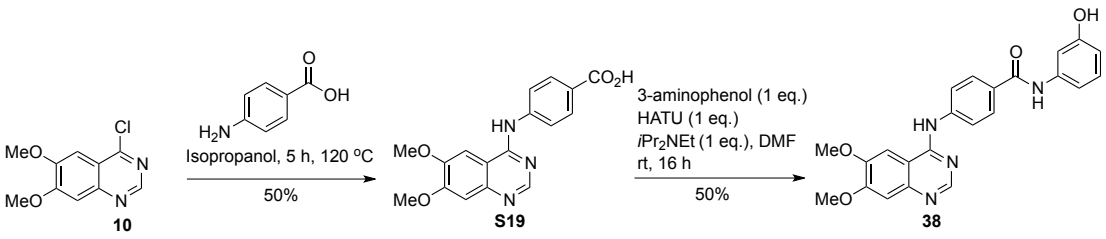
m.p. 86-87 °C (decomp.); IR (ATR): ν = 2939, 2869, 1530 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.66 (1H, s, Ar-H, H-2), 2.87-2.83 (2H, m, CH_2), 2.75-2.70 (2H, m, CH_2), 1.87-1.82 (4H, m, 2 × CH_2. ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.0, 161.2, 155.2, 129.3, 32.3, 25.6, 21.9, 21.8. LRMS [M+H^+] 169.1. HRMS (ESI) m/z cald for $C_8H_{10}ClN_2$ [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 ^{o}C (decomp.). IR (ATR): ν = 3248, 1684, 1675 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 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). ^{13}C NMR (101 MHz, DMSO-d_6): 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 $C_{21}H_{21}N_4O_2$ [*M*+H^{+}]: 361.1659, found 361.1660.



Scheme S8. Synthesis of compound **38** from chloroquinazoline **10.**

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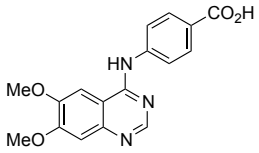
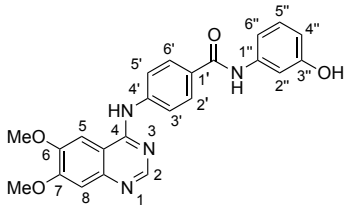
4-((6,7-dimethoxyquinazolin-4-yl)amino)benzoic acid (S19)



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-d_6): 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, OCH_3), 3.99 (3H, s, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6): 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 $C_{17}H_{16}N_3O_4$ [*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 $MgSO_4$. The solvent was subsequently removed *in vacuo* to give a crude a 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-d_6): 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, OCH_3), 4.00 (3H, s, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6): 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 $C_{23}H_{21}N_4O_4$ [M+H^+]: 417.1557, found 417.1556.

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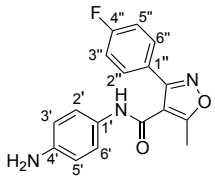
8. General procedure S2: Synthesis of 4-aminocarboxamides 20, S20, S21, S23 and S25-27

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*-diisopropylethylamine (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 $MgSO_4$. 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*-diisopropylethylamine (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 ^{o}C (decomp.). IR (ATR): ν = 3262, 2967, 2922, 1670, 1609 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 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, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): 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 $C_{17}H_{15}FN_3O_2$ [*M*+H^+]: 312.1143, found 312.1143.

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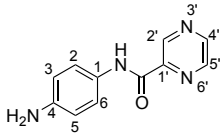
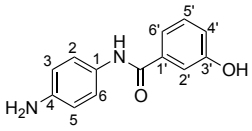
_N_-(4-aminophenyl)pyrazine-2-carboxamide (S21)



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 ^{o}C (decomp.). IR (ATR): ν = 3348, 2639, 1685 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 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). ^{13}C NMR (101 MHz, DMSO-d_6): 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 $C_{11}H_{11}N_4O$ [*M*+H$^+$]: 215.0927, found 215.0928.

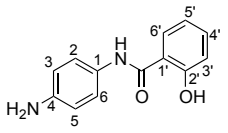
_N_-(4-aminophenyl)-3-hydroxybenzamide (S17)



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 $MgSO_4$. The solvent was removed *in vacuo* to give a crude residue that was purified by column chromatography (95:5 v/v CH_2Cl_2: CH_3OH → 9:1 v/v CH_2Cl_2: CH_3OH) to afford 4-aminocarboxamide **S17** as a white solid (220 mg, 65%).

m.p. 178-181 °C (decomp.). IR (ATR): ν = 3288, 2923, 1672 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 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'). ^{13}C NMR (126 MHz, DMSO-d_6): 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 $C_{13}H_{13}N_2O_2$ [*M*+H^+]: 229.0972, found 229.0973.

***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-aminocarboxamide **S22** (TFA salt) as a white solid (45 mg, 20%).

m.p. 169-171 °C (decomp.). IR (ATR): ν = 3427, 1679, 1625 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.88 (1H, OH), 10.36 (1H, s, NH), 7.95 (1H, dd, *J* 8.0, 1.7 Hz, Ar-H, H-6'), 7.63 (2H, d, *J* 8.7 Hz, Ar-H, H-3 + H-5), 7.44 (1H, ddd, *J* 8.5, 7.2, 1.7 Hz, Ar-H, H-4'), 7.07 (2H, d, *J* 8.8 Hz, Ar-H, H-2 + H-6), 6.99-6.93 (2H, m, Ar-H, H-3' + H-5'). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.2 (C=O), 159.2, 134.2 (×2), 129.5 (×2), 122.9, 120.4, 119.6, 117.9, 117.8. LRMS [*M*+H^+] 229.0. HRMS (ESI) m/z cald for $C_{13}H_{13}N_2O_2$ [*M*+H^+]: 229.0972, found 229.0971.

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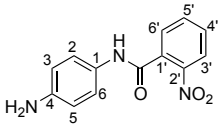
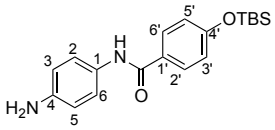
_N_-(4-aminophenyl)-2-nitrobenzamide (S23)



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 ^{o}C (decomp.). IR (ATR): ν = 3288, 1678 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23-8.07 (1H, m, Ar-H, H-3'), 7.87 (1H, t, *J* 7.5 Hz, 1H, Ar-H), 7.79-7.71 (2H, m, Ar-H), 7.66 (2H, d, *J* 8.4 Hz, H-2 + H-6), 7.16 (2H, d, *J* 8.4 Hz, H-3 + H-5). ^{13}C NMR (101 MHz, DMSO-d_6): δ 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 $C_{13}H_{12}N_3O_3$ [*M*+H^{+}]: 258.0873, found 258.0874.

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

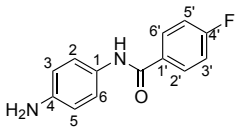


A stirring solution of 4-((*tert*-butyldimethylsilyl)oxy)benzoic acid (500 mg, 3.62 mmol) in dichloromethane (15 mL) was allowed cooled to 0 ^{o}C for 10 min. Oxalyl chloride (735 µL, 8.69 mmol) was added dropwise to this solution, followed by DMF (10 µL). The mixture was allowed to warm up to room temperature over 2 h. The solvent was removed *in vacuo*, and the residue was immediate suspended in THF (15 mL) and cooled to 0 ^{o}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_2Cl_2:

CH_3OH → 98:2 v/v CH_2Cl_2: CH_3OH) to afford 4-aminocarboxamide **S24** as a pale yellow oil (779 mg, 63%) .

IR (ATR): ν = 3337, 3037, 2955, 1638, 1604 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 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-2 + H-6), 6.89 (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, $(CH_3)_3CSi$), 0.23 (6H, s, 2 × CH_3Si); ^{13}C NMR (101 MHz, $CDCl_3$): δ 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 $C_{19}H_{27}N_2O_2Si$ [*M*+H^+]: 343.1836, found 343.1837.

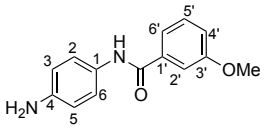
_N_-(4-aminophenyl)-4-fluorobenzamide (S25)



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 µL) 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): ν = 3350, 3339, 1675, 1643 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 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): δ 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 $C_{13}H_{12}FN_2O$ [*M*+H^+]: 231.0928, found 231.0928.

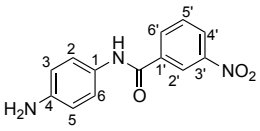
_N_-(4-aminophenyl)-3-methoxybenzamide (S26)



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 µL) 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_2Cl_2: CH_3OH).

IR (ATR): ν = 3350, 3341, 2955, 2942, 1631 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 9.82 (1H, s, NH), 7.54-7.47 (1H, m, Ar-H, H-6'), 7.47-7.43 (1H, m, Ar-H, H-2'), 7.40 (1H, t, *J* 7.9 Hz, Ar-H, H-5'), 7.36 (2H, d, *J* 8.4 Hz, Ar-H, H-2 + H-6), 7.11 (1H, ddd, *J* 8.2, 2.4, 1.1 Hz, Ar-H, H-4'), 6.54 (2H, d, *J* 8.5 Hz, Ar-H, H-3 + H-5), 3.82 (3H, s, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 164.8 (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 [*M*+H^+] 243.1. HRMS (ESI) m/z cald for $C_{14}H_{15}N_2O_2$ [*M*+H^+]: 243.1128, found 243.1130.

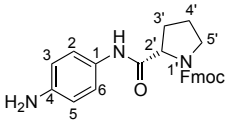
***N*-(4-aminophenyl)-3-nitrobenzamide (S27)**



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 µL) 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): ν = 3080, 2932, 2637, 1667 cm^{-1}. ^1H NMR (400 MHz, CD_3CN): δ 8.73 (1H, s, Ar-H, H-2'), 8.39 (1H, ddd, *J* 8.3, 2.2, 1.0 Hz, Ar-H, H-6'), 8.28 (1H, dt, *J* 8.1, 1.3 Hz, Ar-H, H-4'), 7.75 (1H, t, *J* 8.0 Hz, Ar-H, H-5'), 7.58 (2H, d, *J* 8.8 Hz, Ar-H, H-2 + H-6), 6.95 (2H, d, *J* 8.7 Hz, H-3 + H-5). ^{13}C NMR (101 MHz, CD_3CN): δ 163.4 (C=O), 148.3, 138.0, 136.7, 133.5, 132.3, 129.9, 126.0, 122.3, 122.2, 117.9. LRMS [*M*+H^+] 258.0. HRMS (ESI) m/z cald for $C_{13}H_{12}N_3O_3$ [*M*+H^+]: 258.0873, found 258.0874.

(*S*)-(9*H*-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 CH_2Cl_2: CH_3OH → 95:5 v/v CH_2Cl_2: CH_3OH).

IR (ATR): ν = 3309, 2937, 1666, 1641 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 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, CH_2), 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'$_{eq}$). ^{13}C NMR (101 MHz, DMSO-d_6): 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*-CO_2] 384.0. HRMS (ESI) m/z cald for $C_{26}H_{26}N_3O_3$ [*M*+H^+]: 428.2332, found 428.2333. $[\alpha]_D^{25}$ = -100° (c = 0.2 in CH_2Cl_2).

9. Numbering for aminoquinazoline-based inhibitors

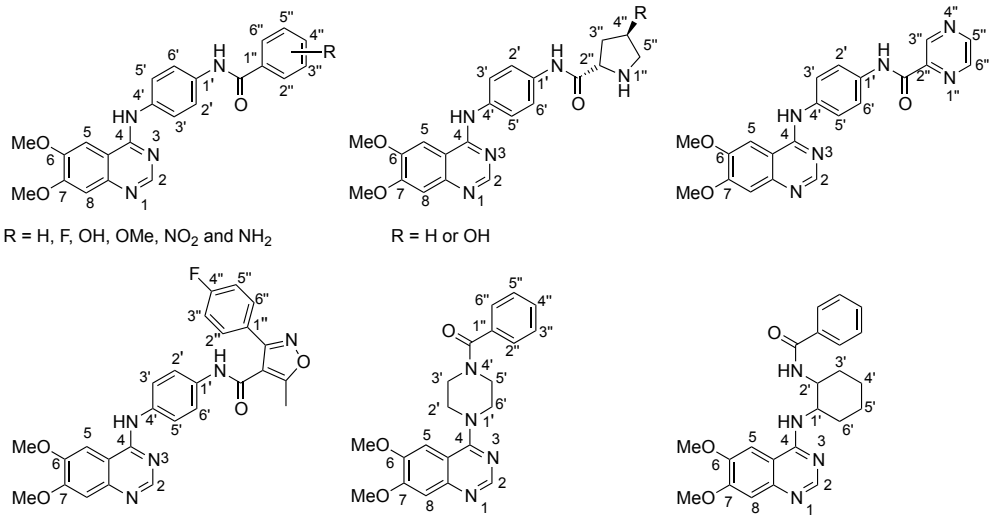


Figure S3. Carbons numbering for aminoquinazoline-based inhibitors.

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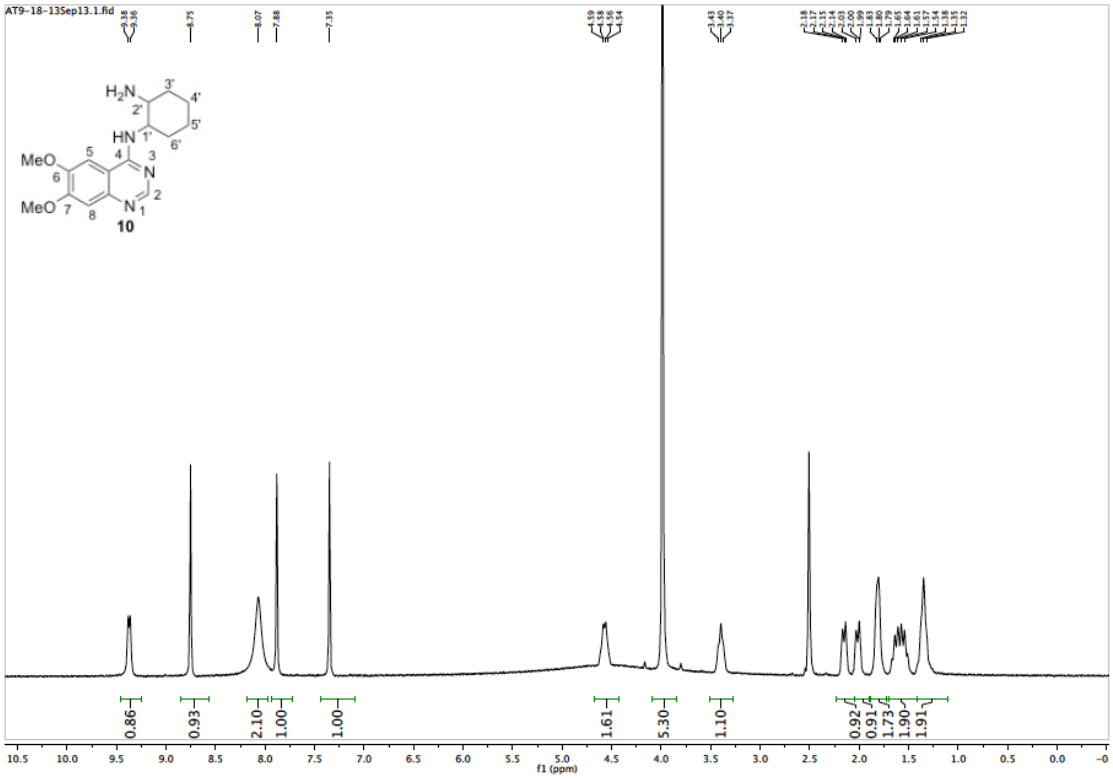
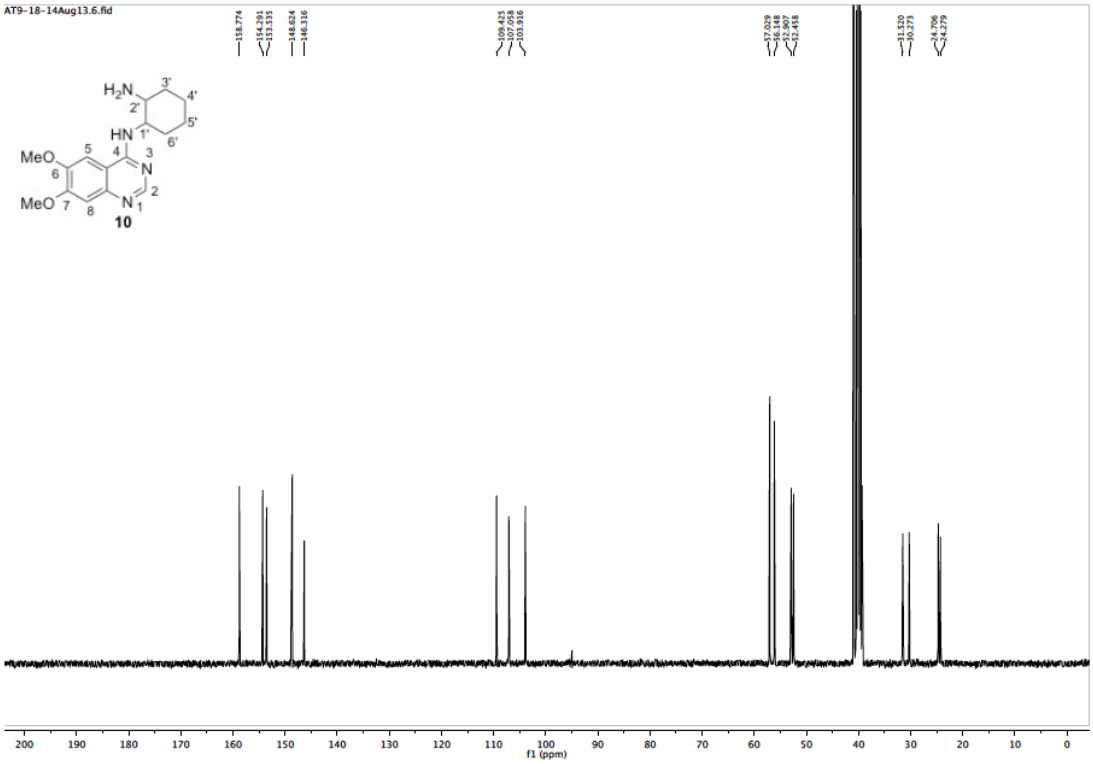
10. ^{1}H, ^{13}C NMR of 10, 11, 13-38, 20, S17, S20-S27 and analytical HPLC traces of 10-38:

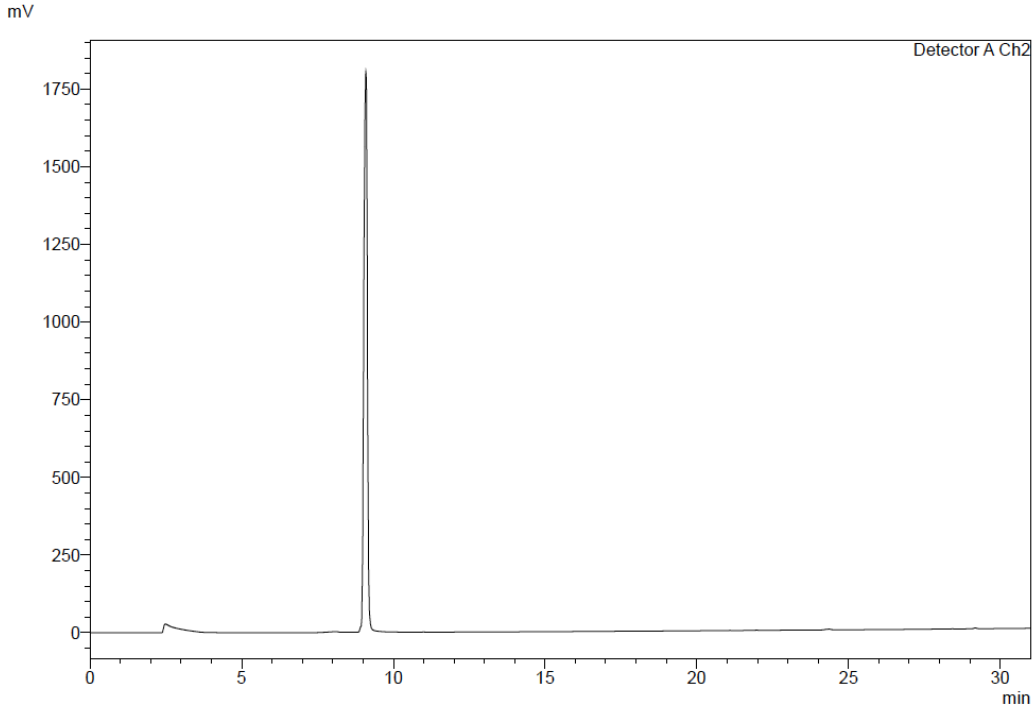
^{1}H NMR (400 MHz, DMSO-d_6)



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



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



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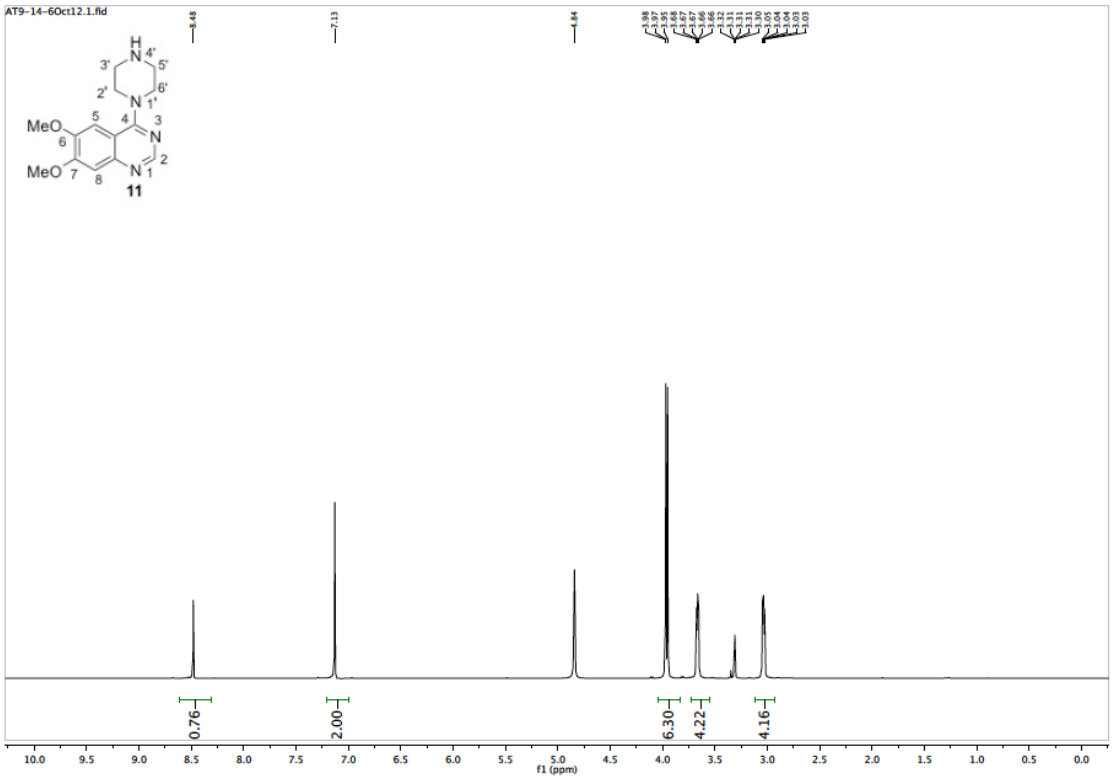
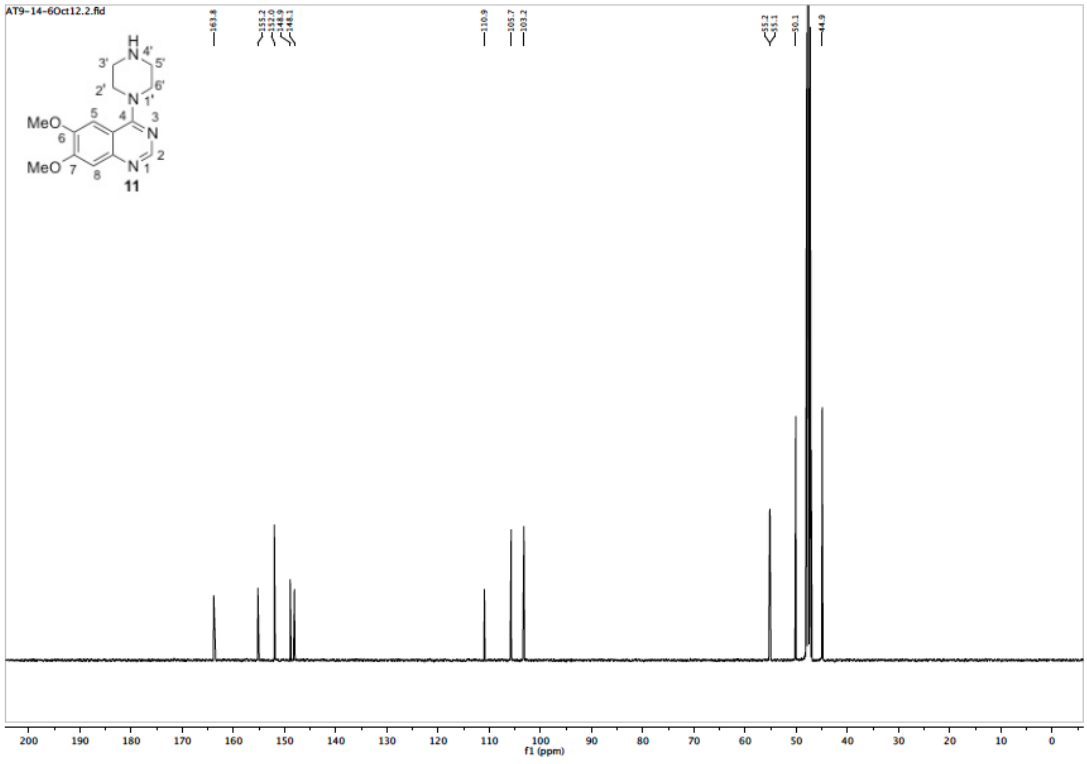
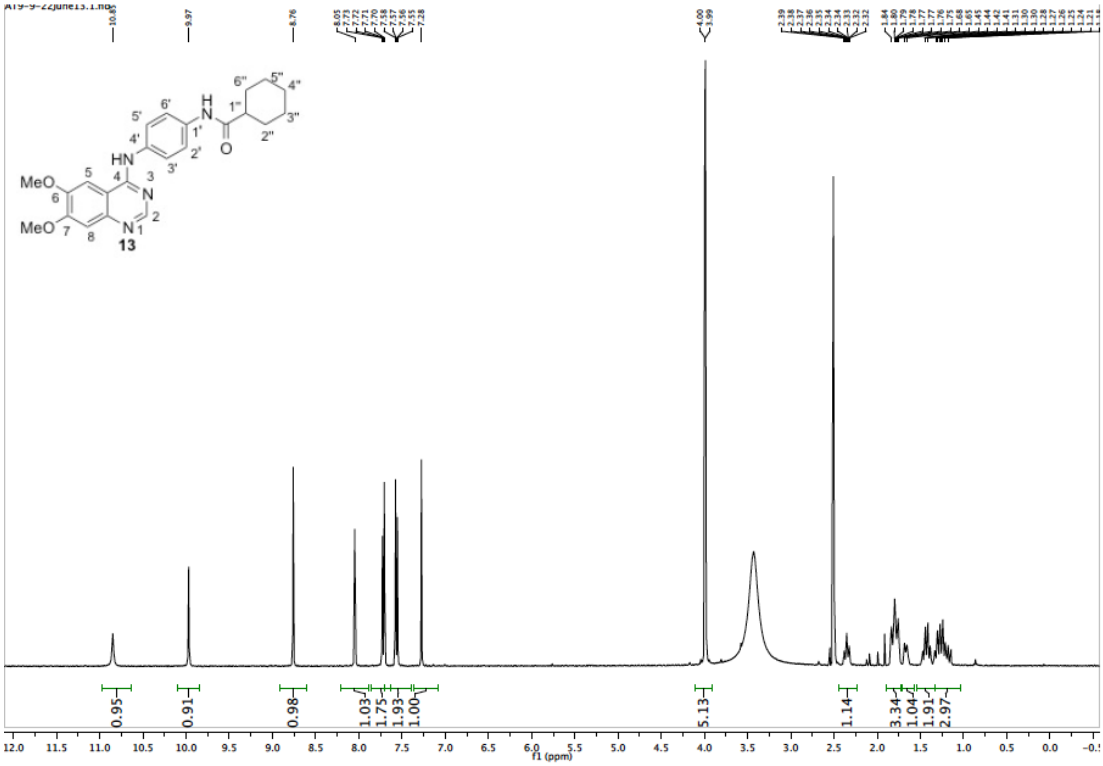
^{1}H NMR (400 MHz, CD_3OD)



^{13}C NMR (101 MHz, CD_3OD)



^{1}H NMR (400 MHz, DMSO-d_6)



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

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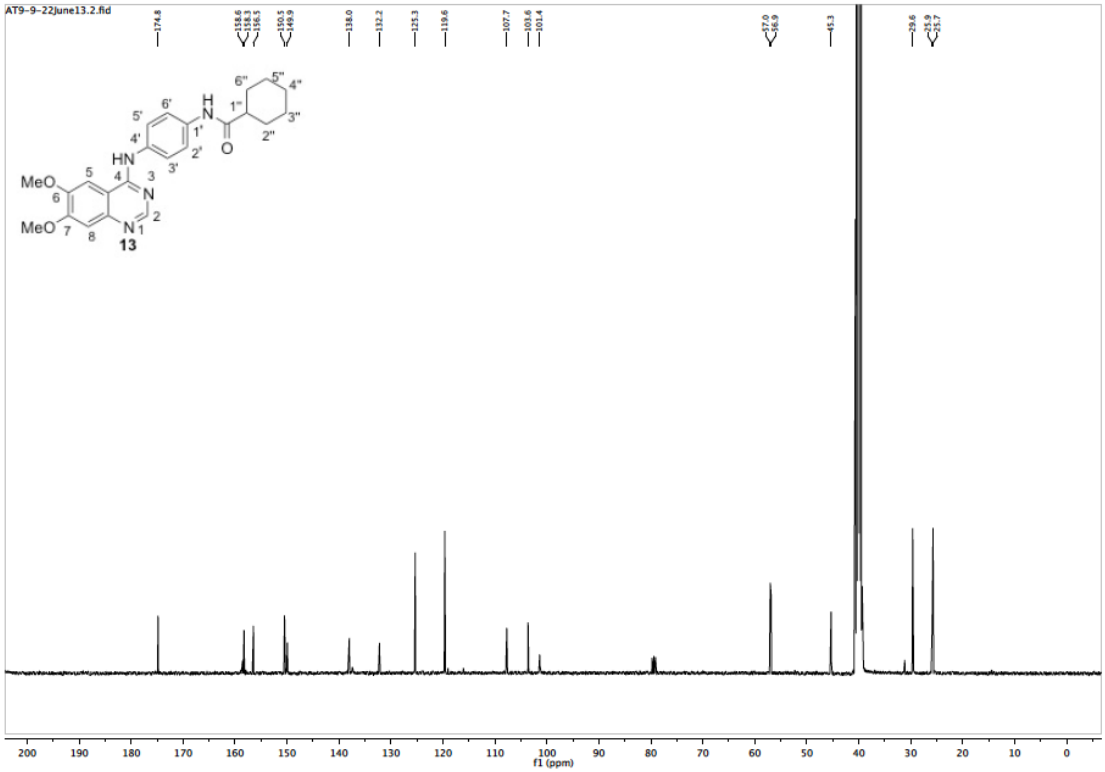
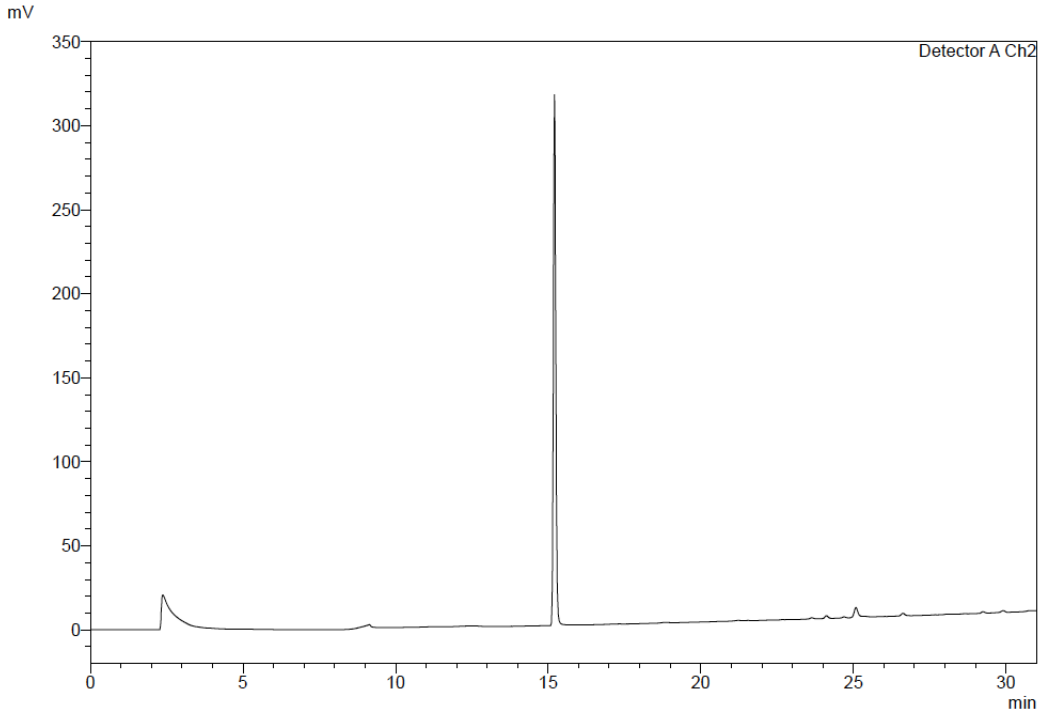
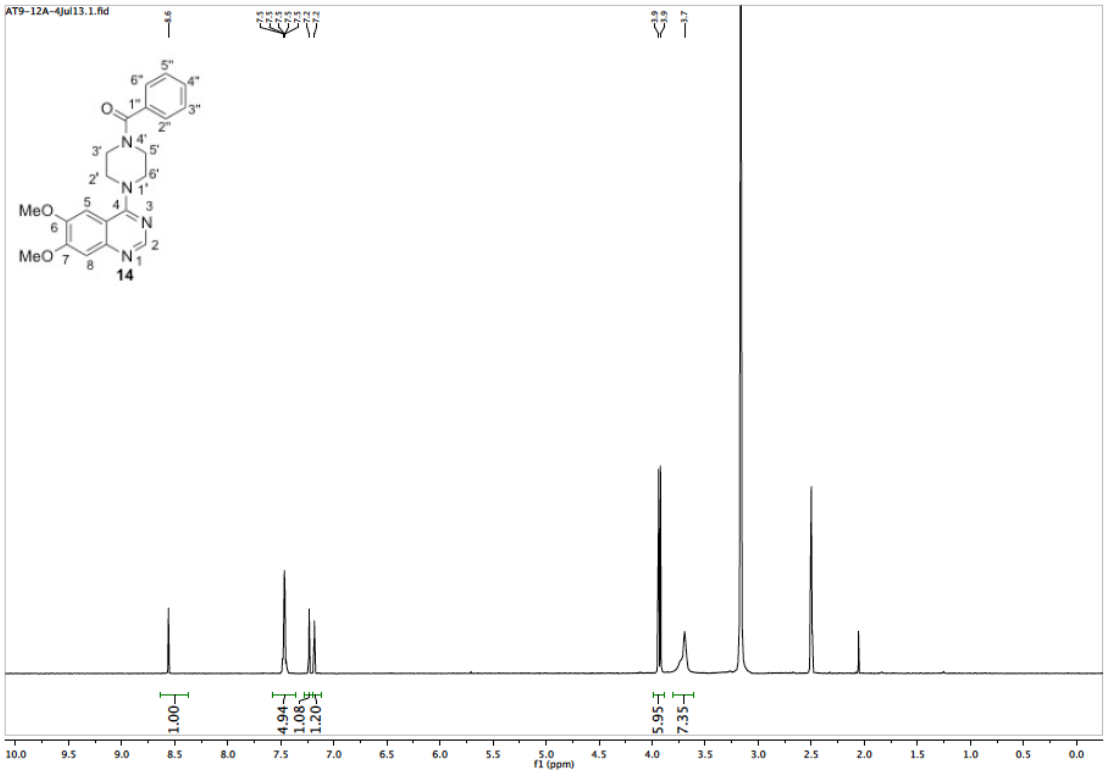


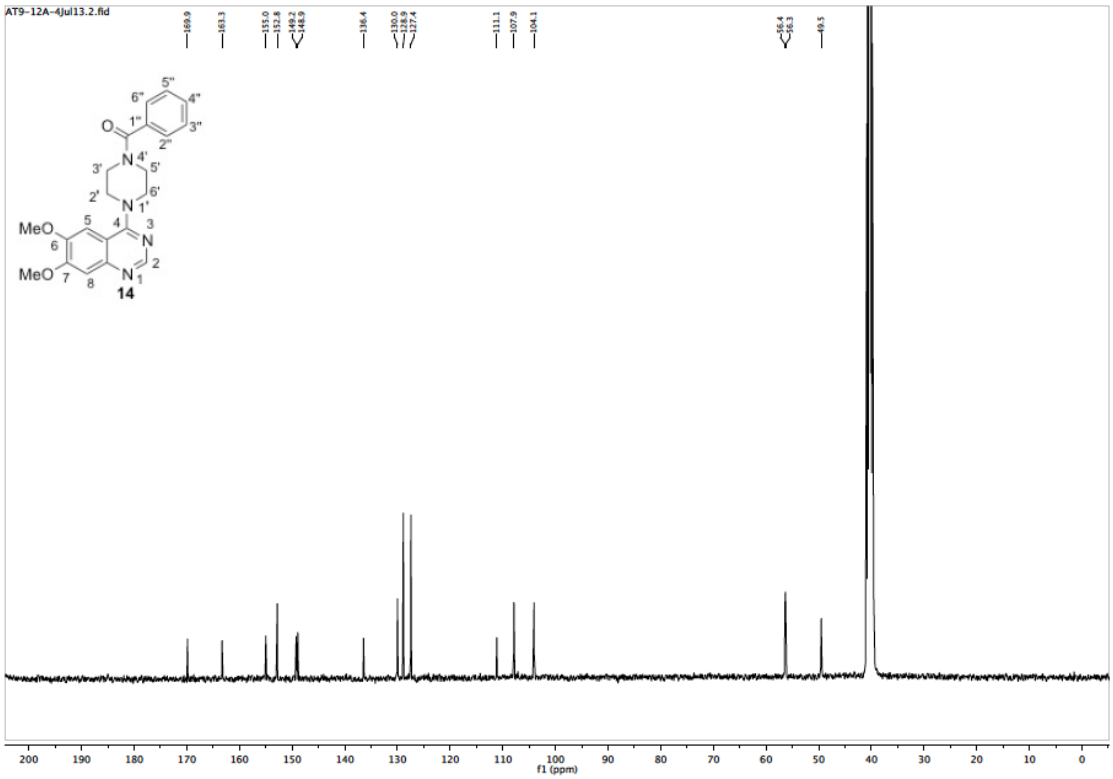
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



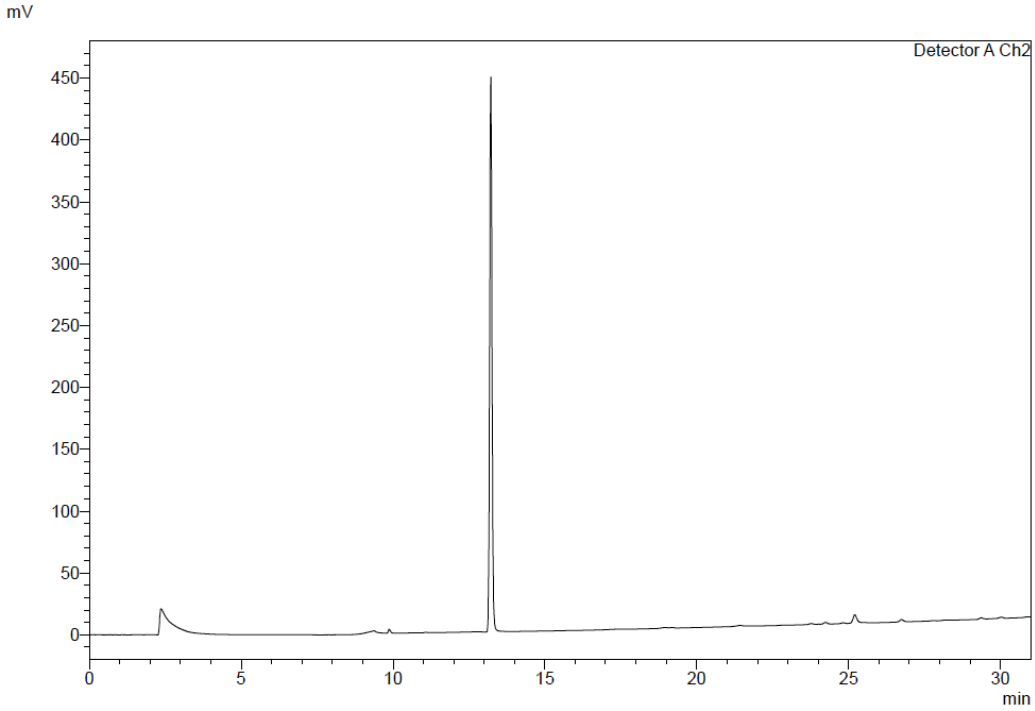
^{1}H NMR (400 MHz, DMSO-d_6)



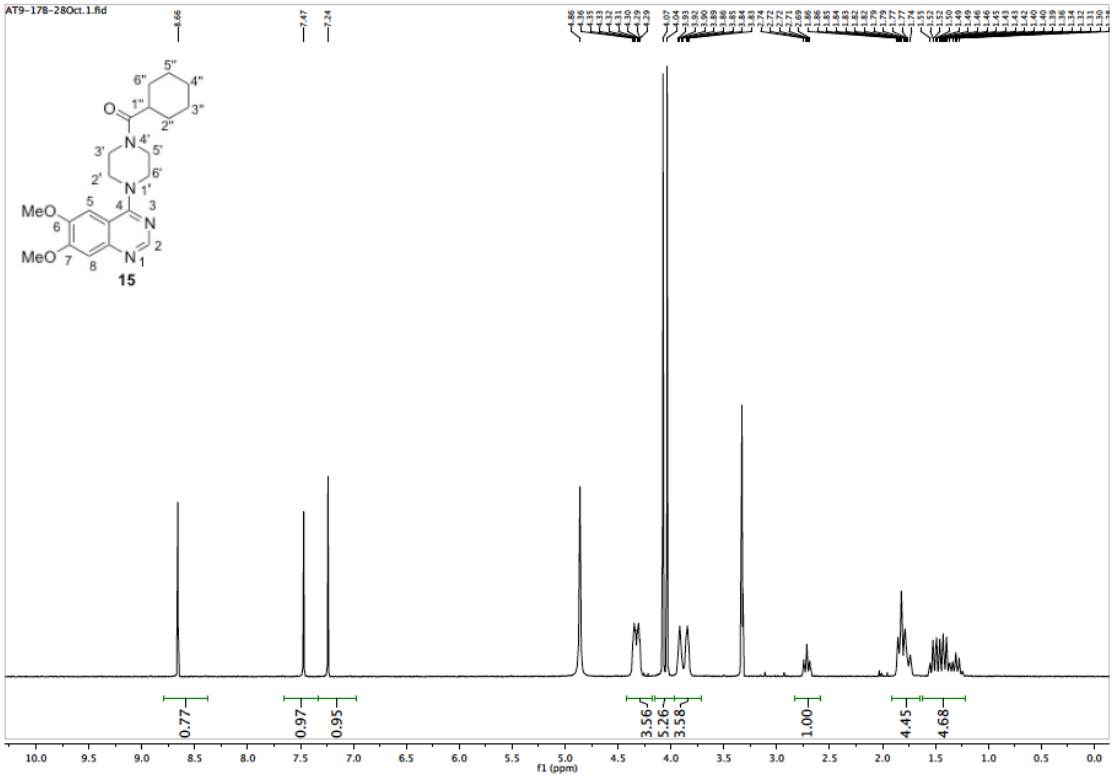
^{13}C NMR (101 MHz, DMSO-d_6)



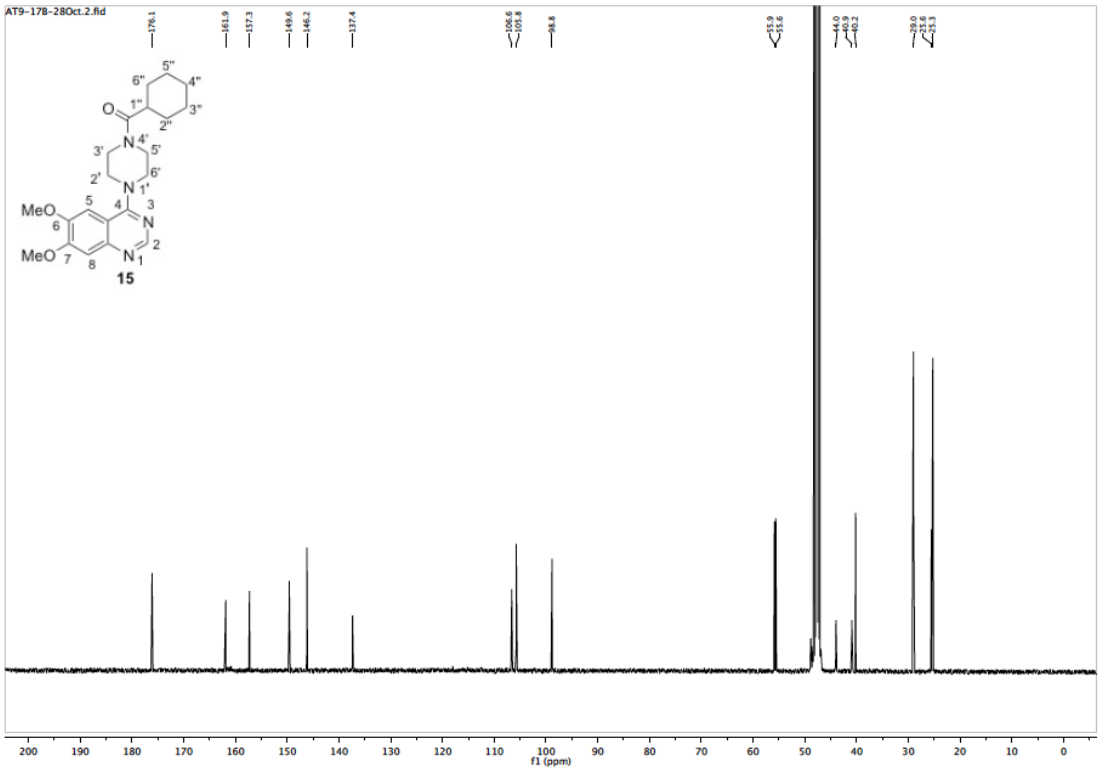
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



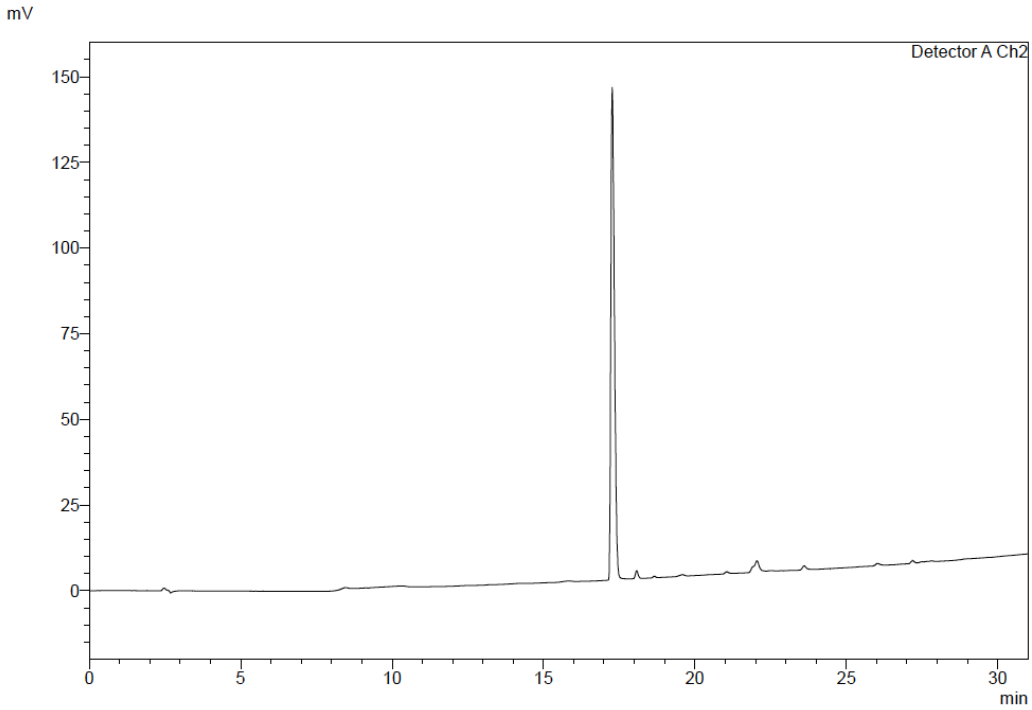
^{1}H NMR (400 MHz, CD_3OD)



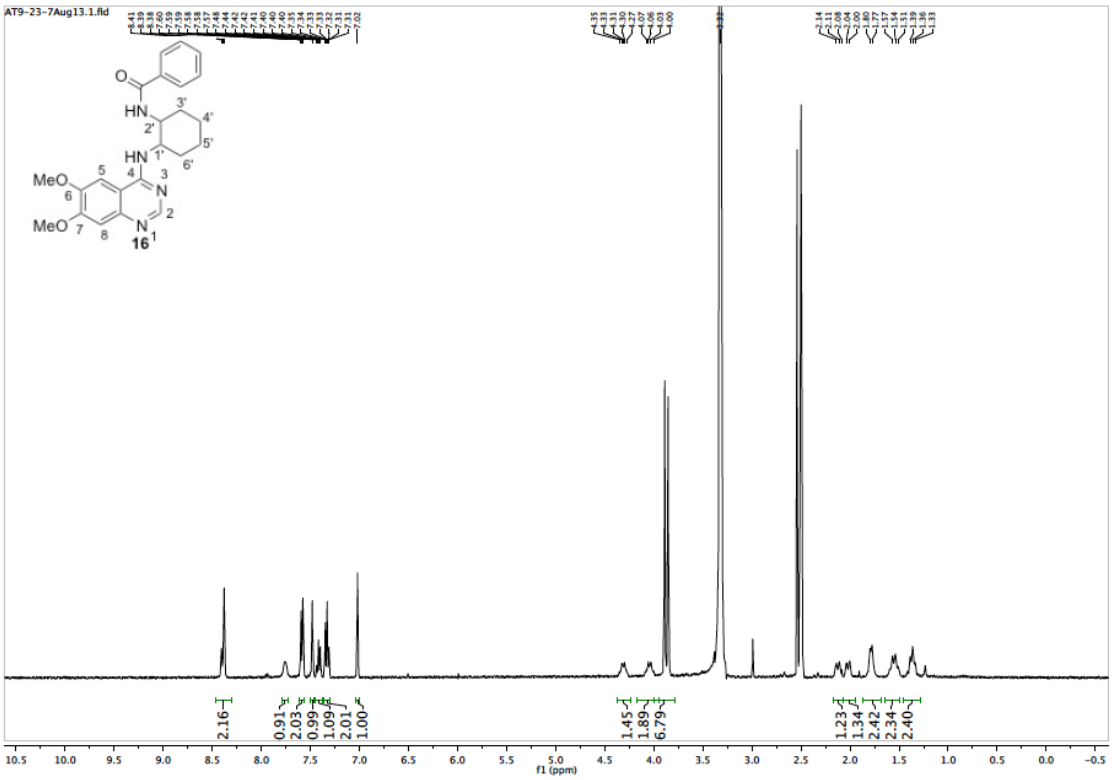
^{13}C NMR (101 MHz, CD_3OD)



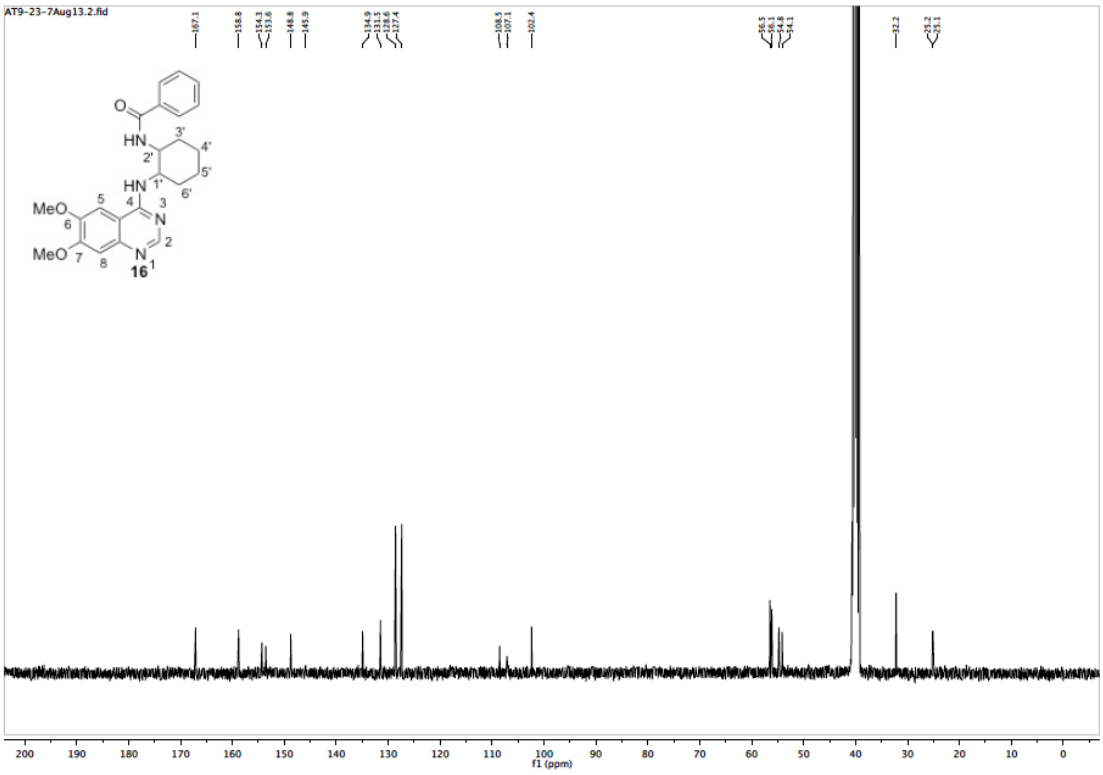
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



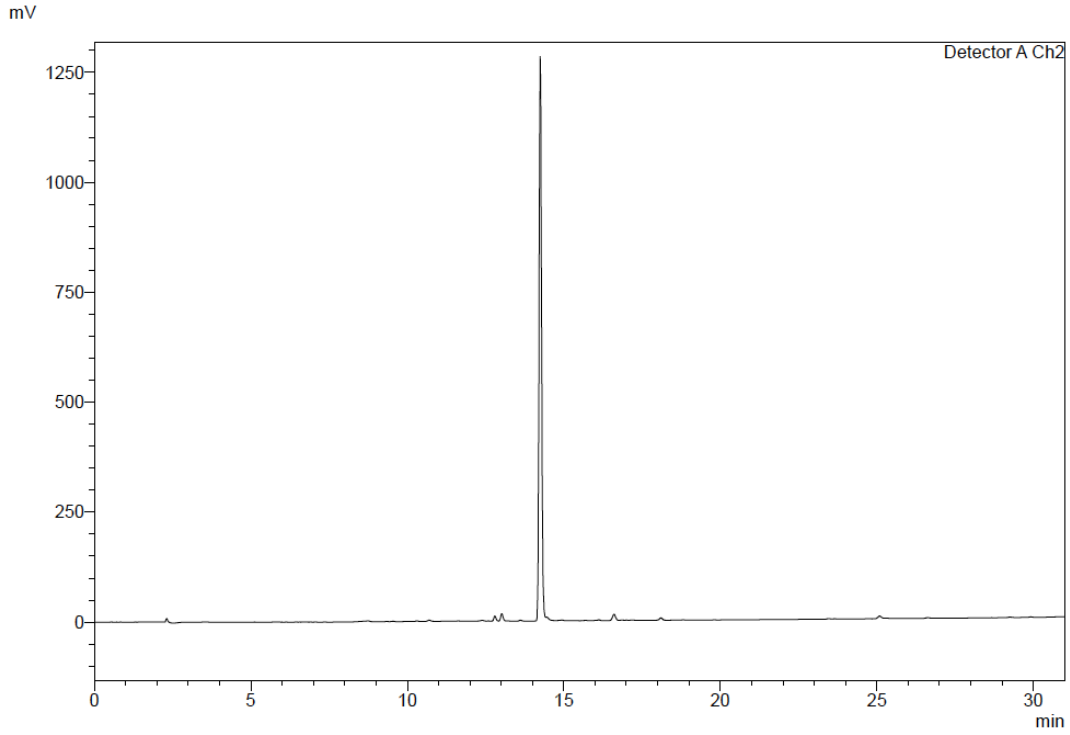
^{1}H NMR (400 MHz, DMSO-d_6)



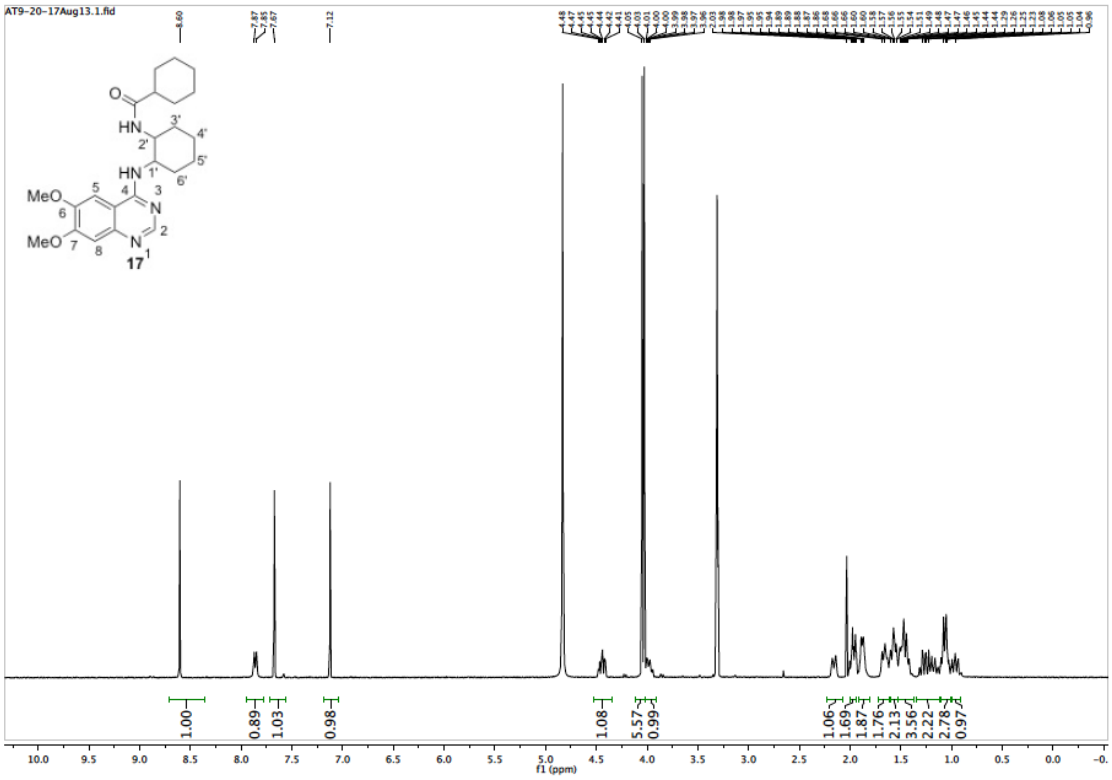
^{13}C NMR (101 MHz, DMSO-d_6)



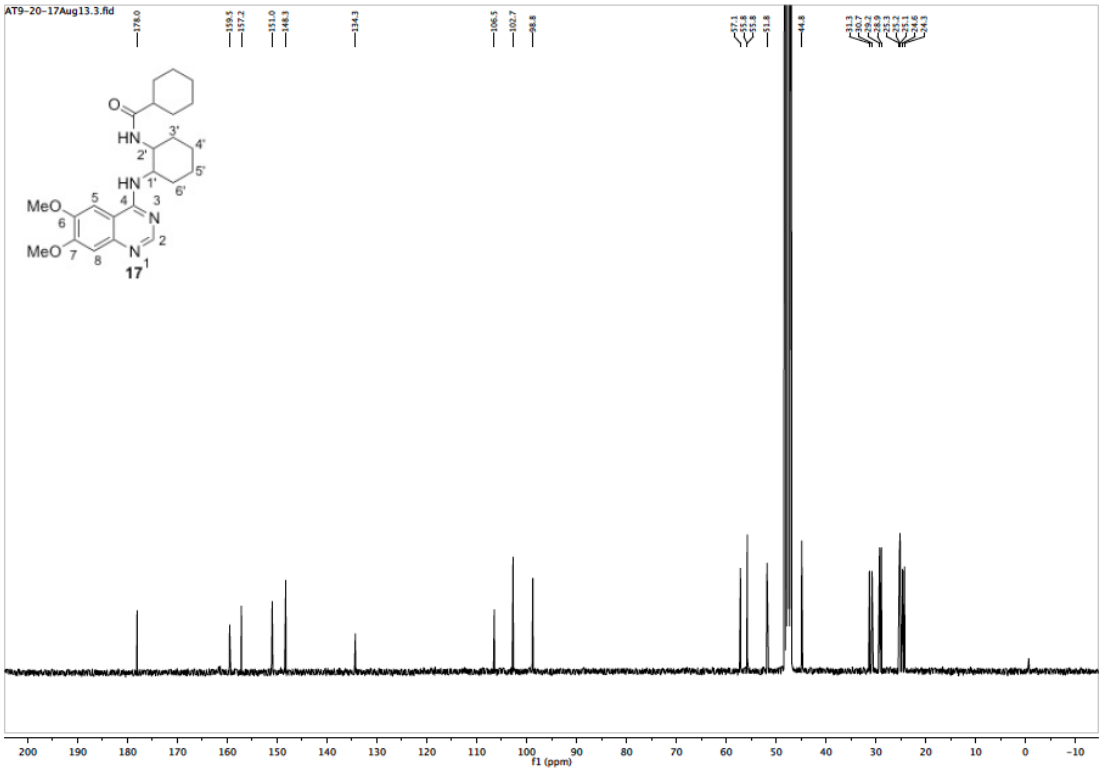
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



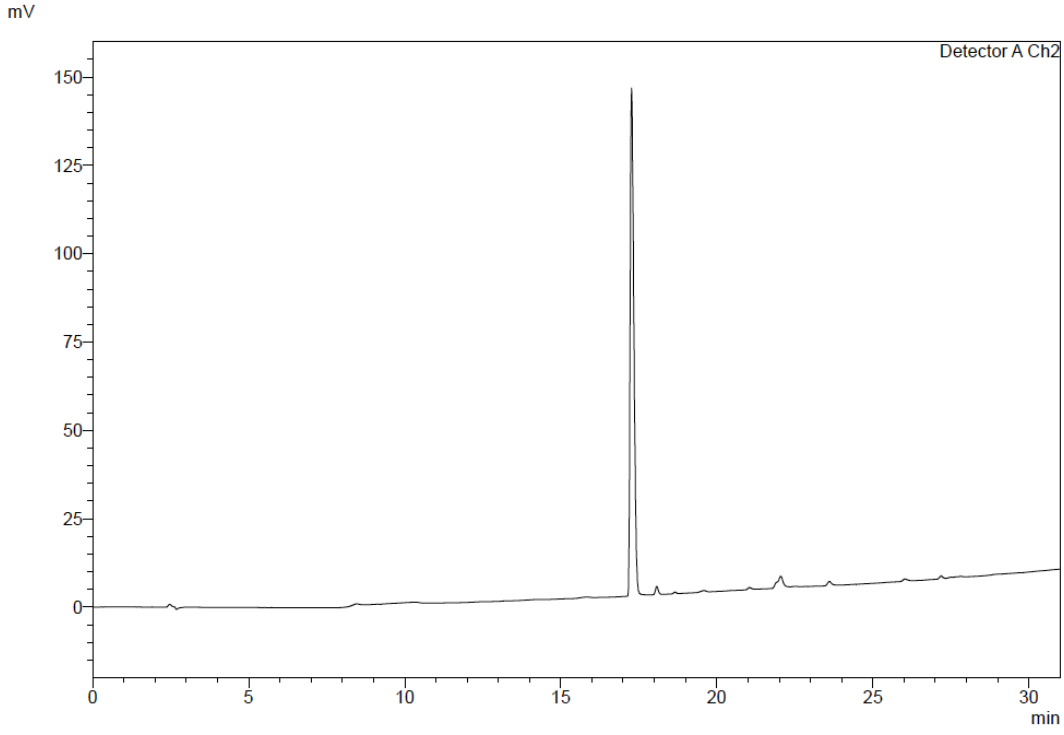
^{1}H NMR (400 MHz, CD_3OD)



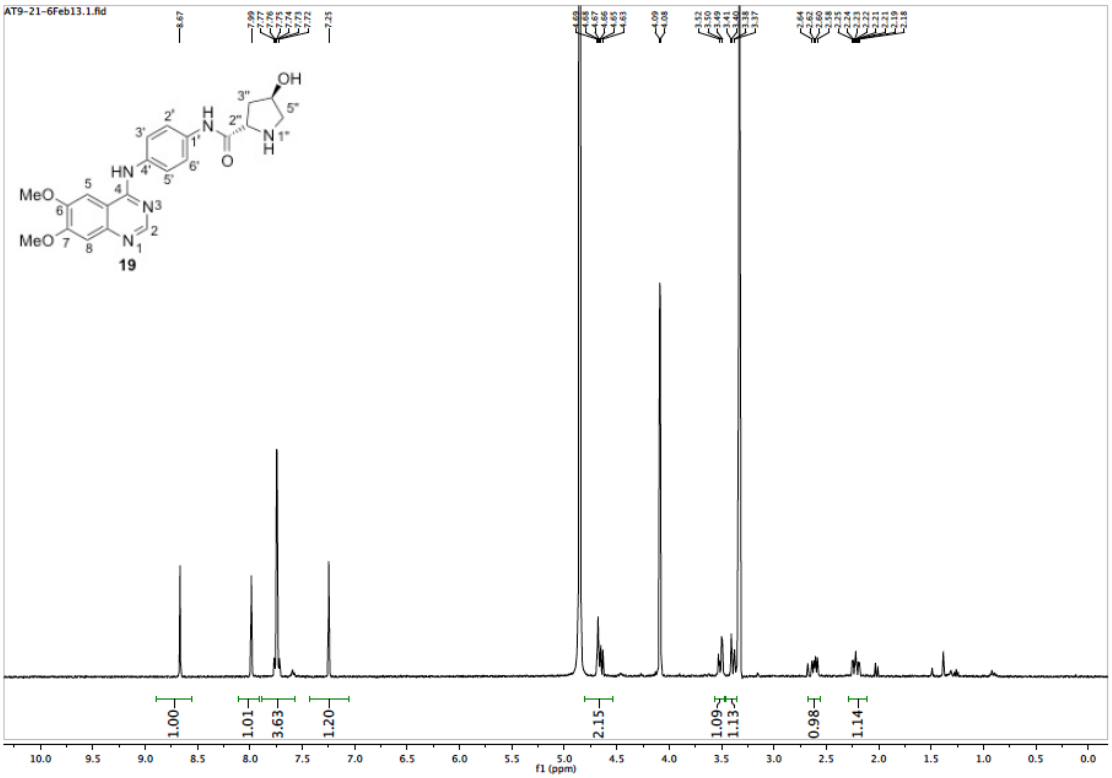
^{13}C NMR (101 MHz, CD_3OD)



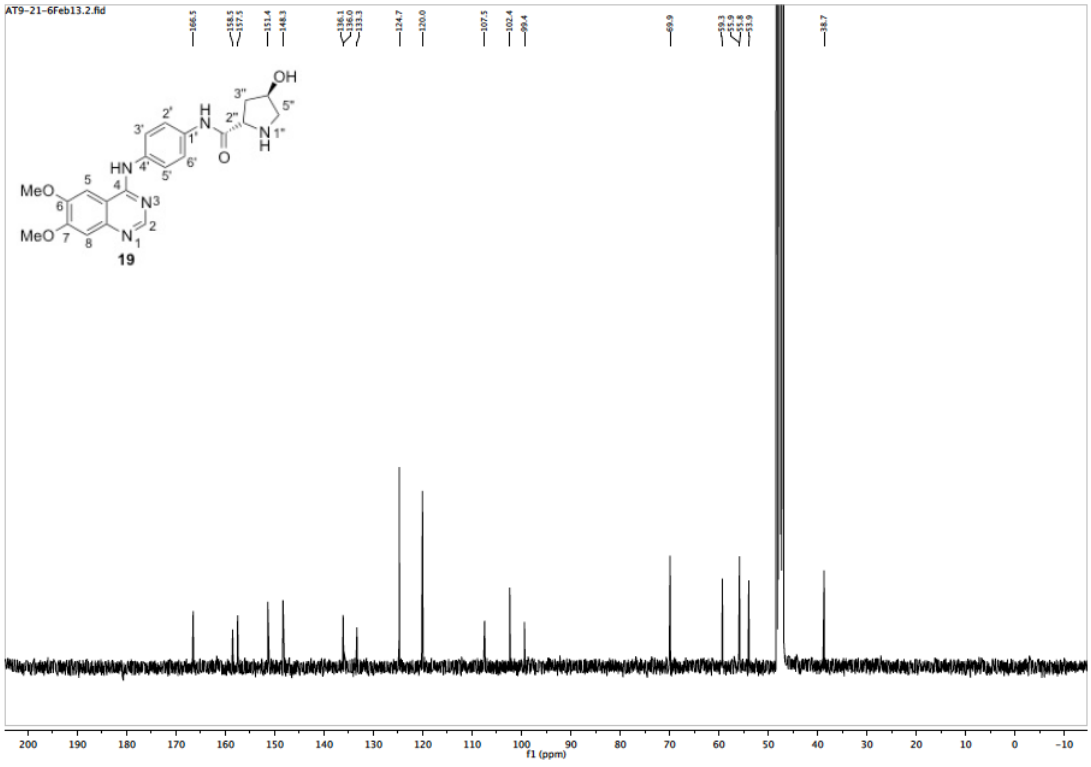
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



^{1}H NMR (400 MHz, CD_3OD)



^{13}C NMR (101 MHz, CD_3OD)



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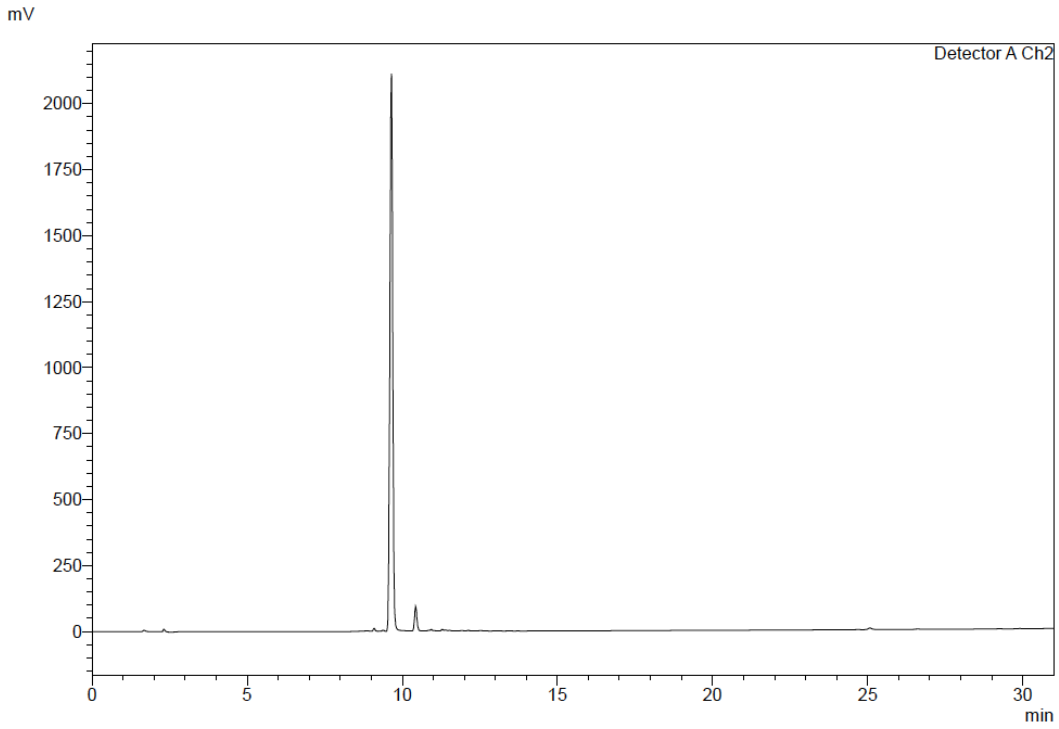
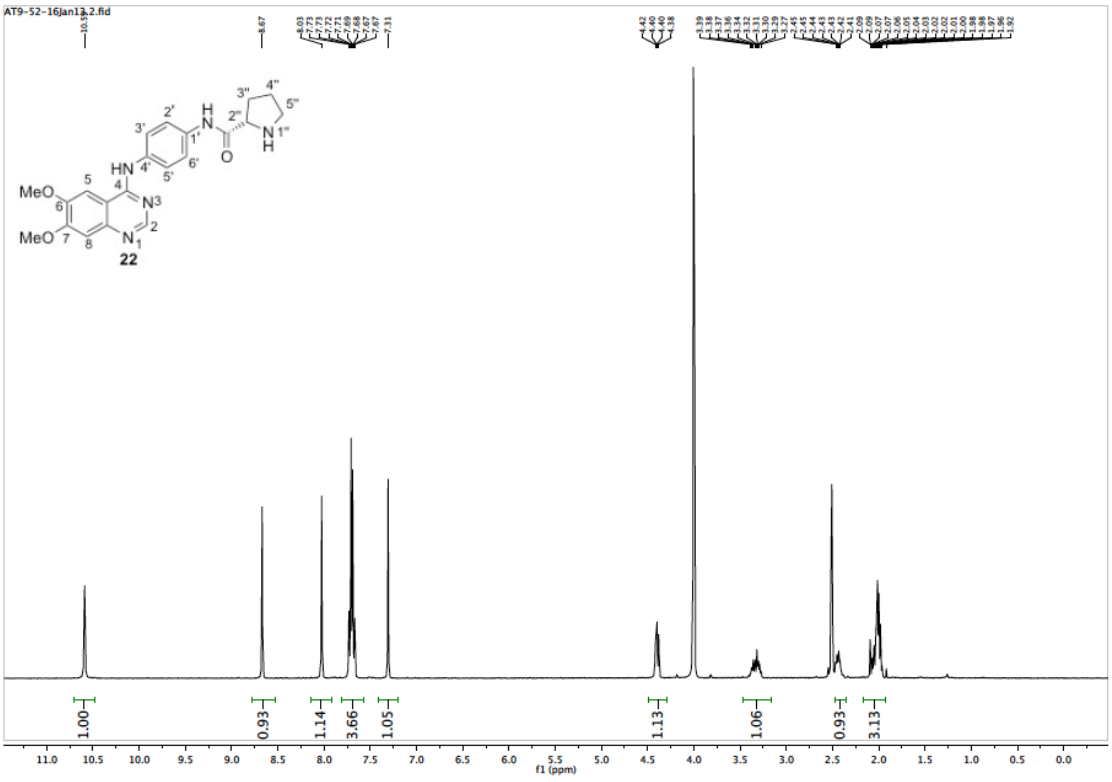
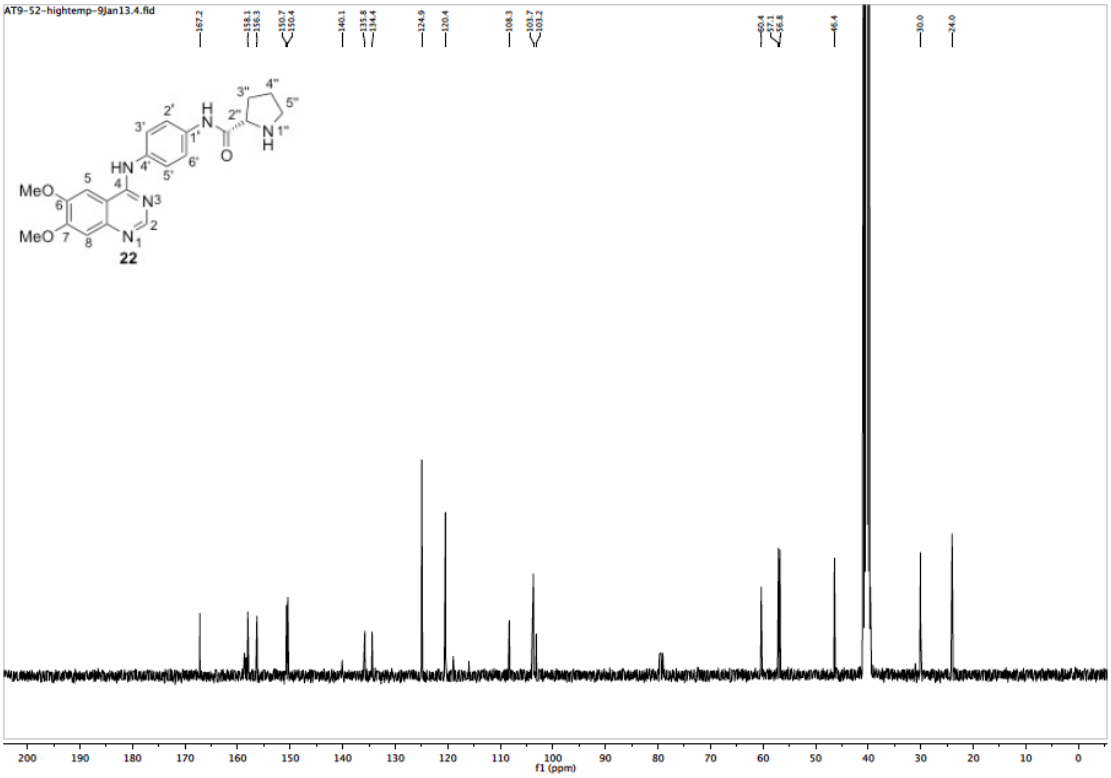
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



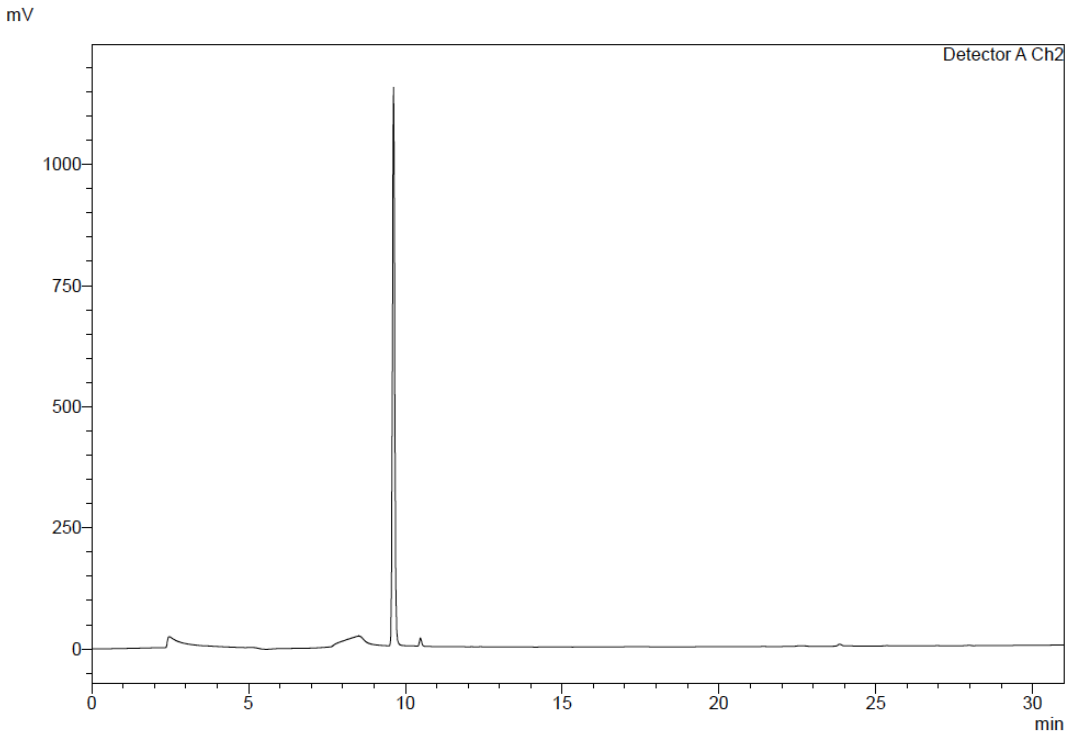
^{1}H NMR (400 MHz, DMSO-d_6, T = 330 K)



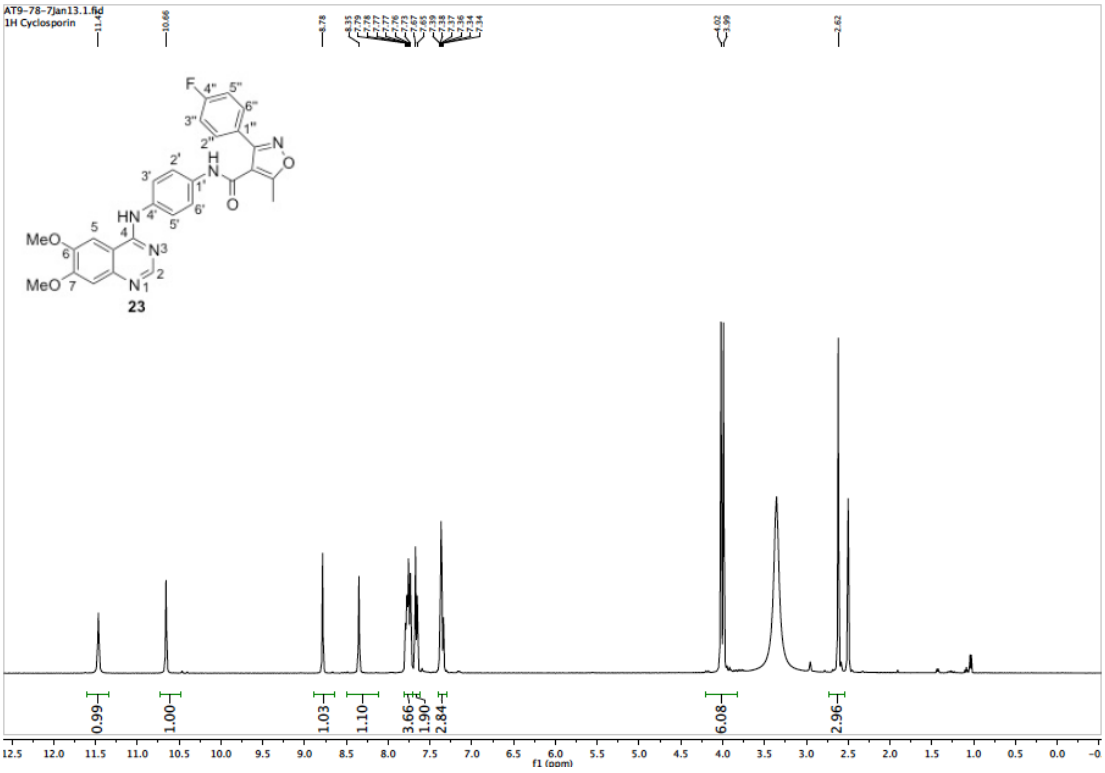
^{13}C NMR (101 MHz, DMSO-d_6, T = 330 K)



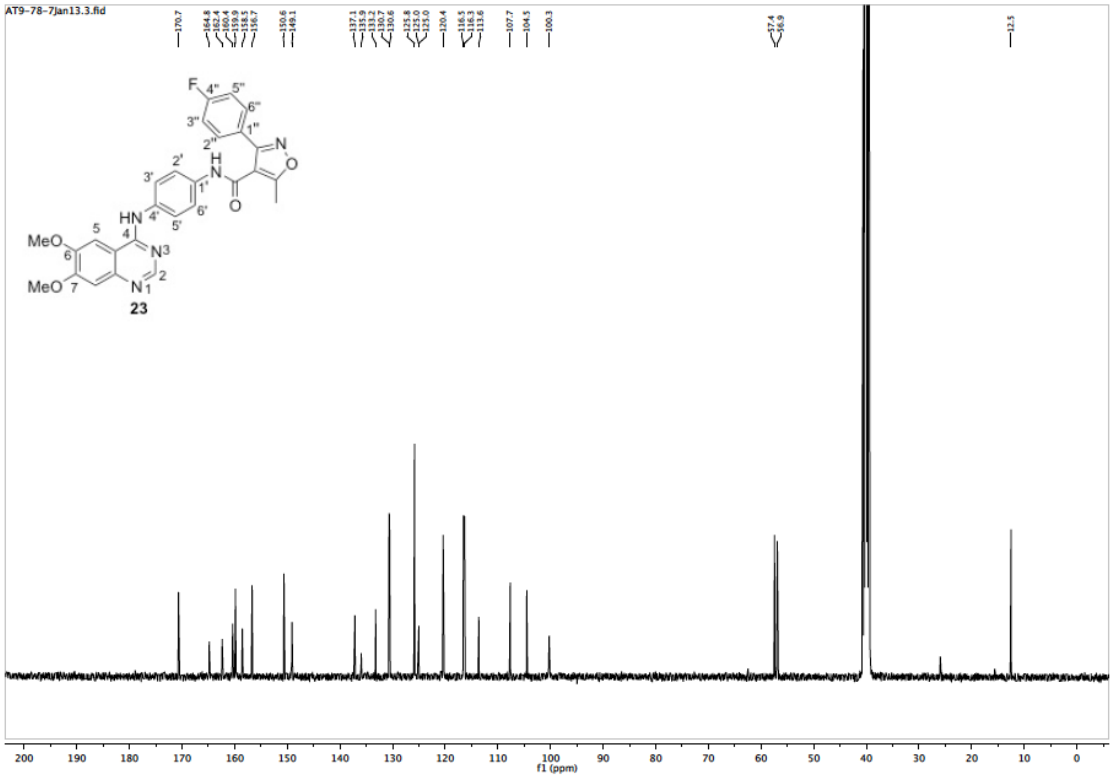
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



^{1}H NMR (400 MHz, DMSO-d_6)



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



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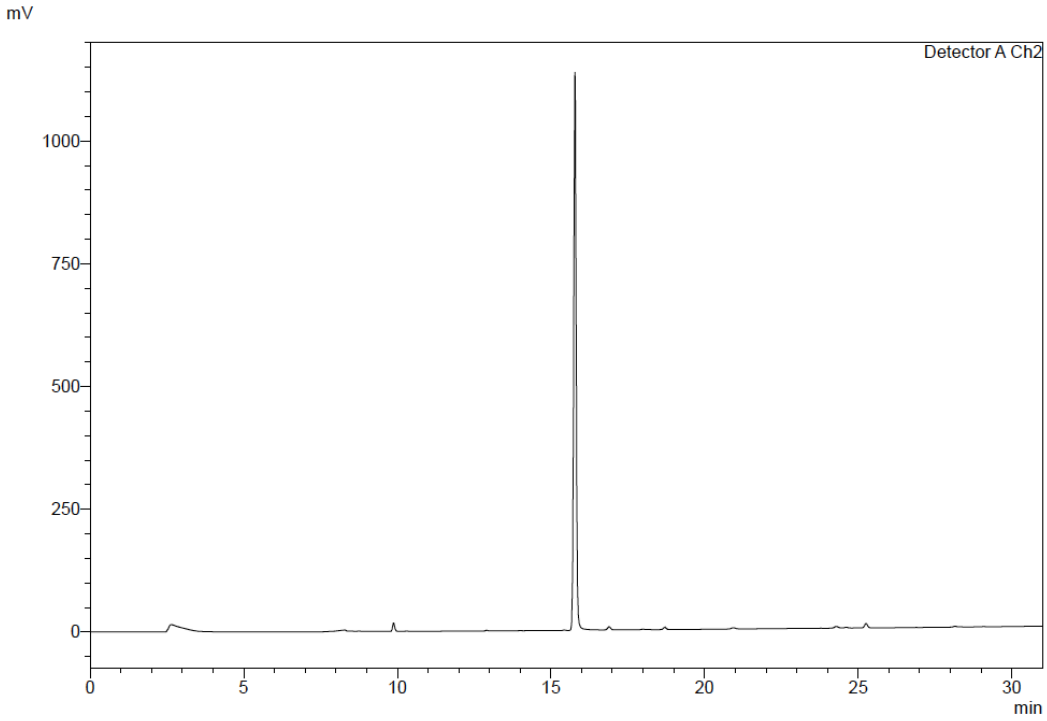
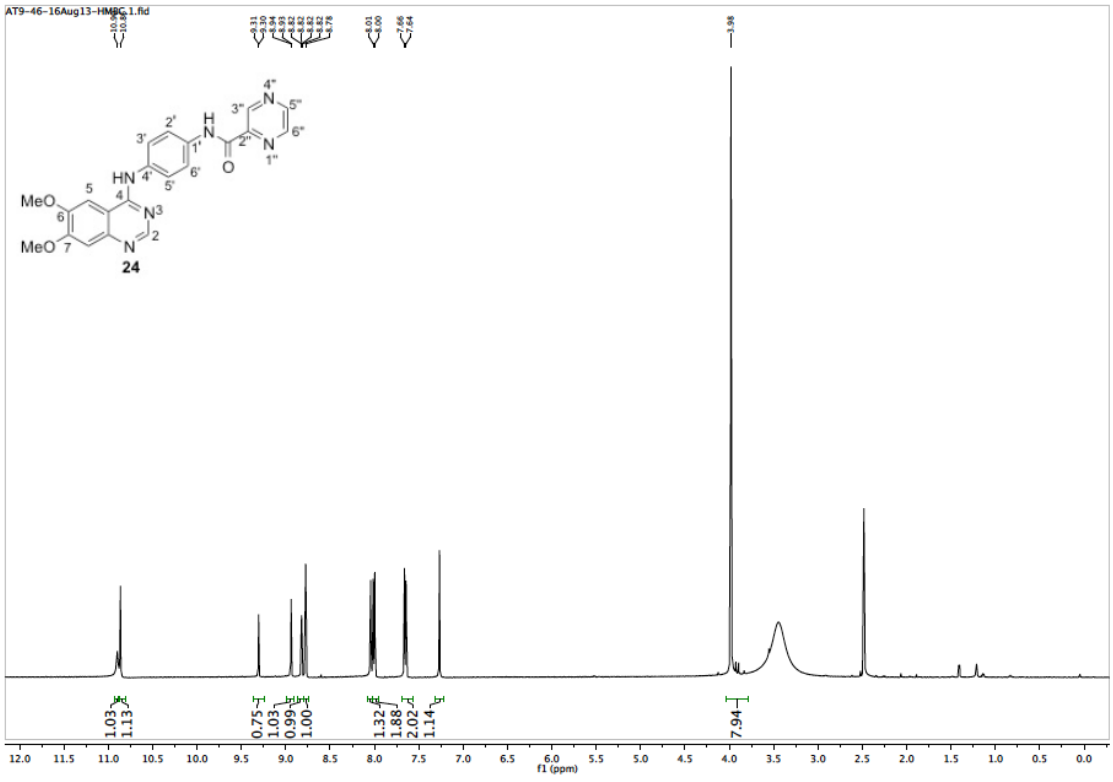
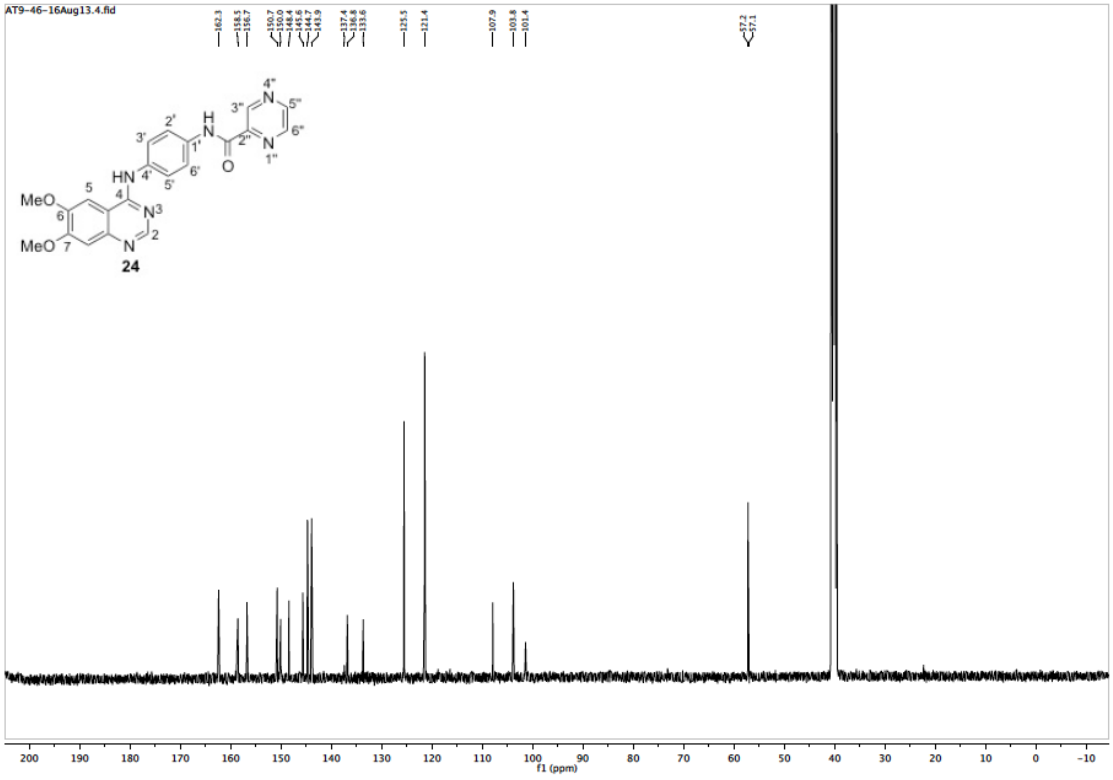
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



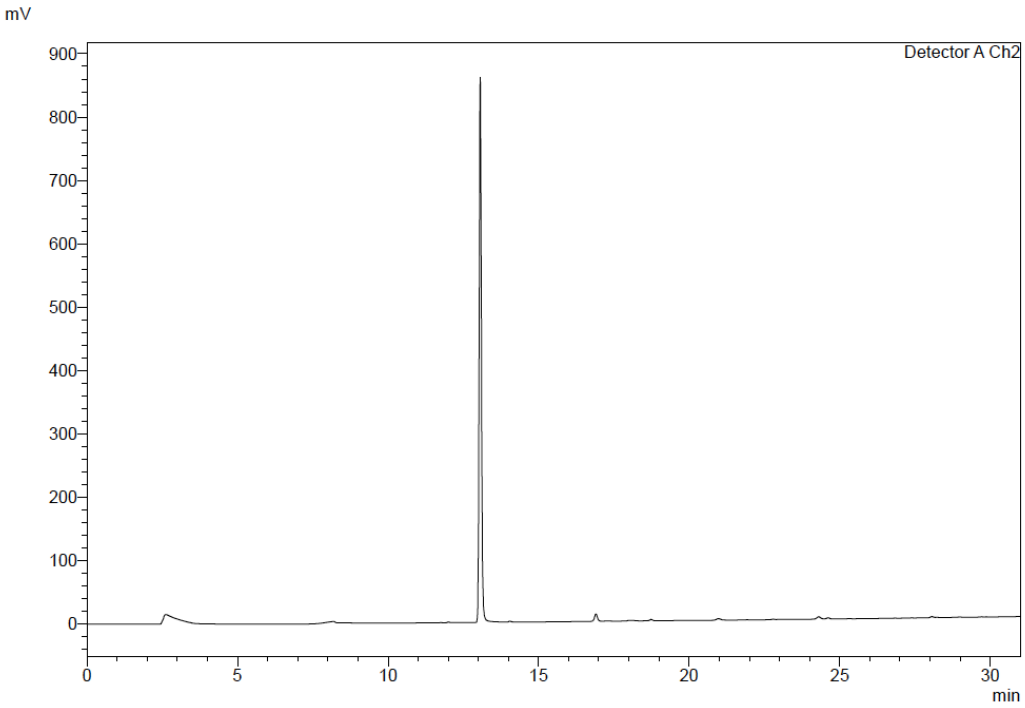
^{1}H NMR (400 MHz, DMSO-d_6)



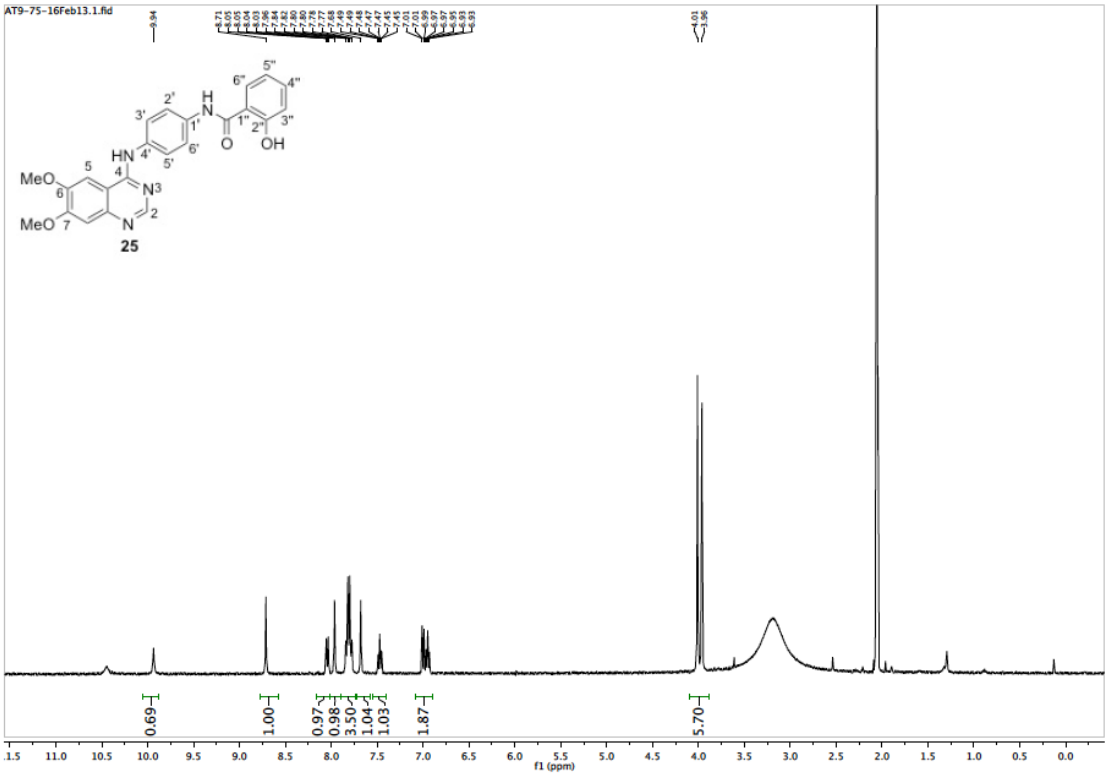
^{13}C NMR (101 MHz, DMSO-d_6)



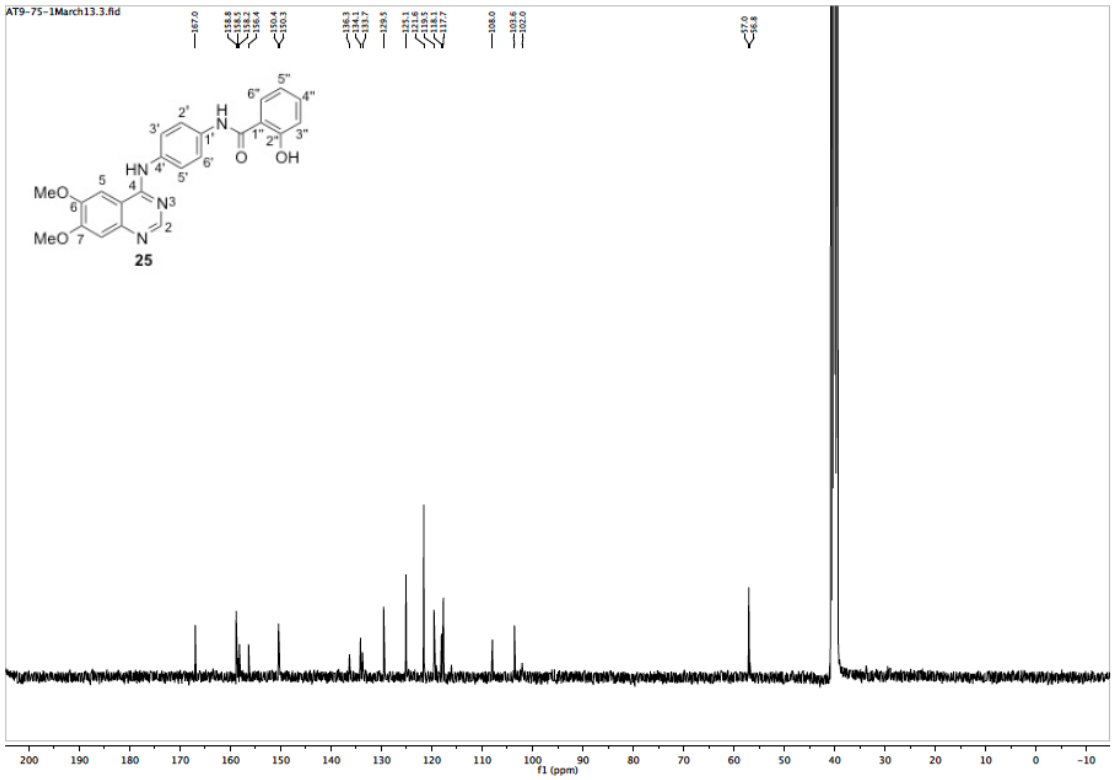
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



^{1}H NMR (400 MHz, Acetone-d_6)



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



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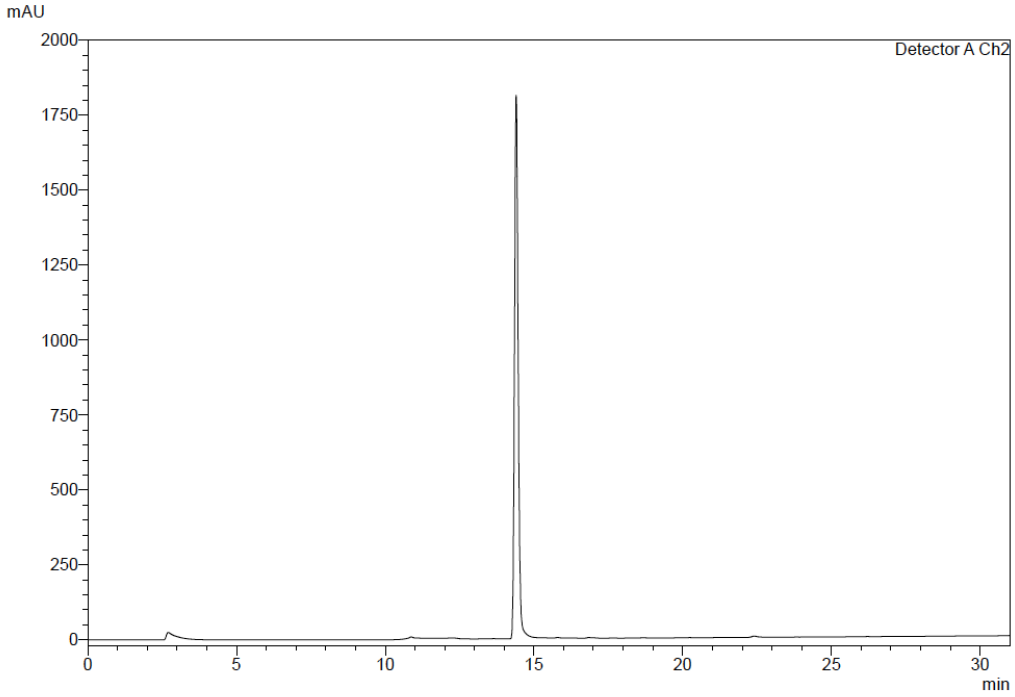
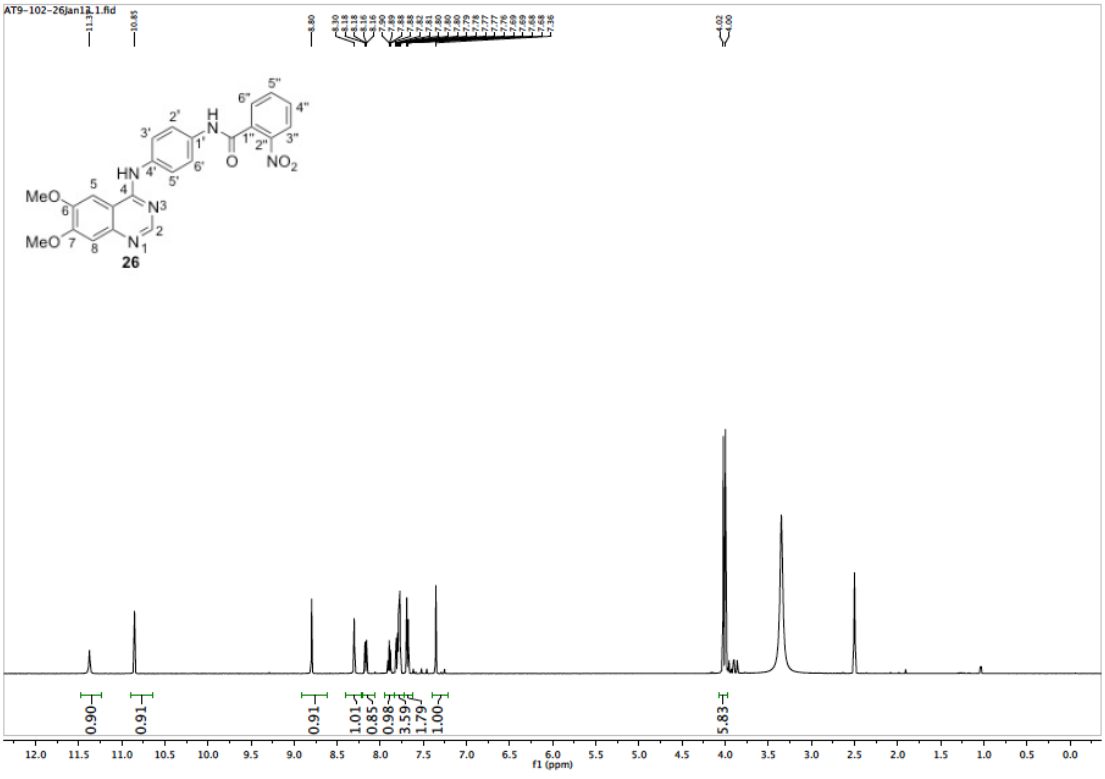
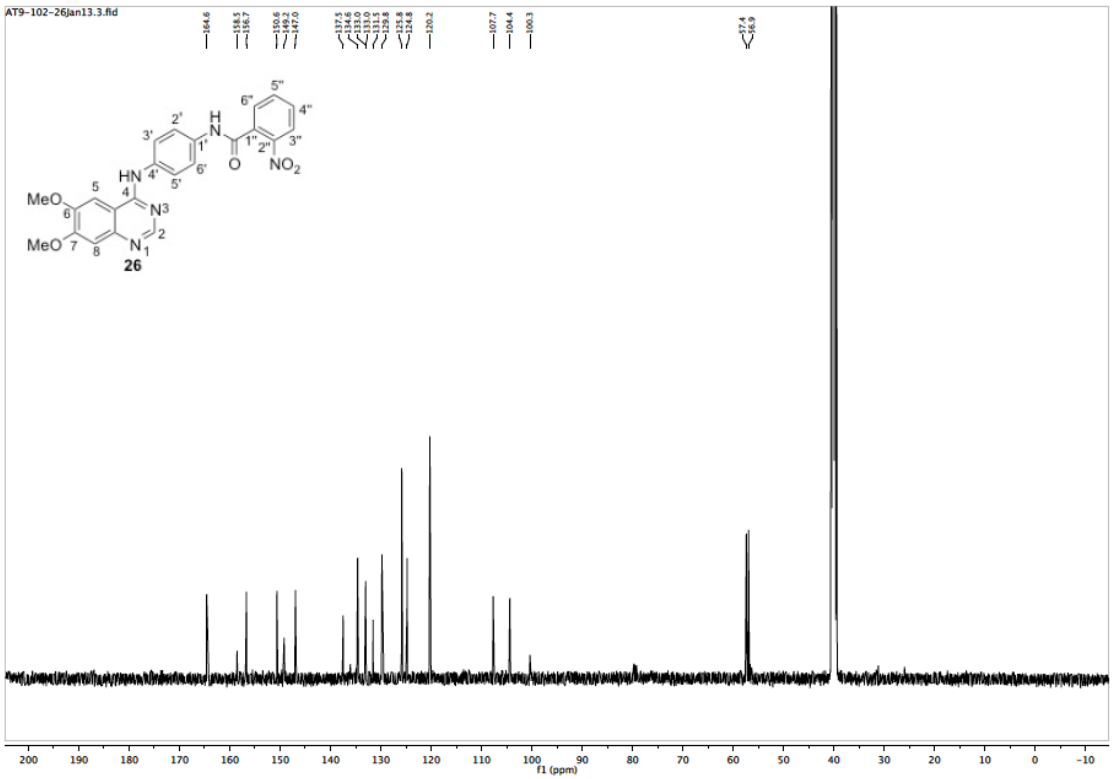
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



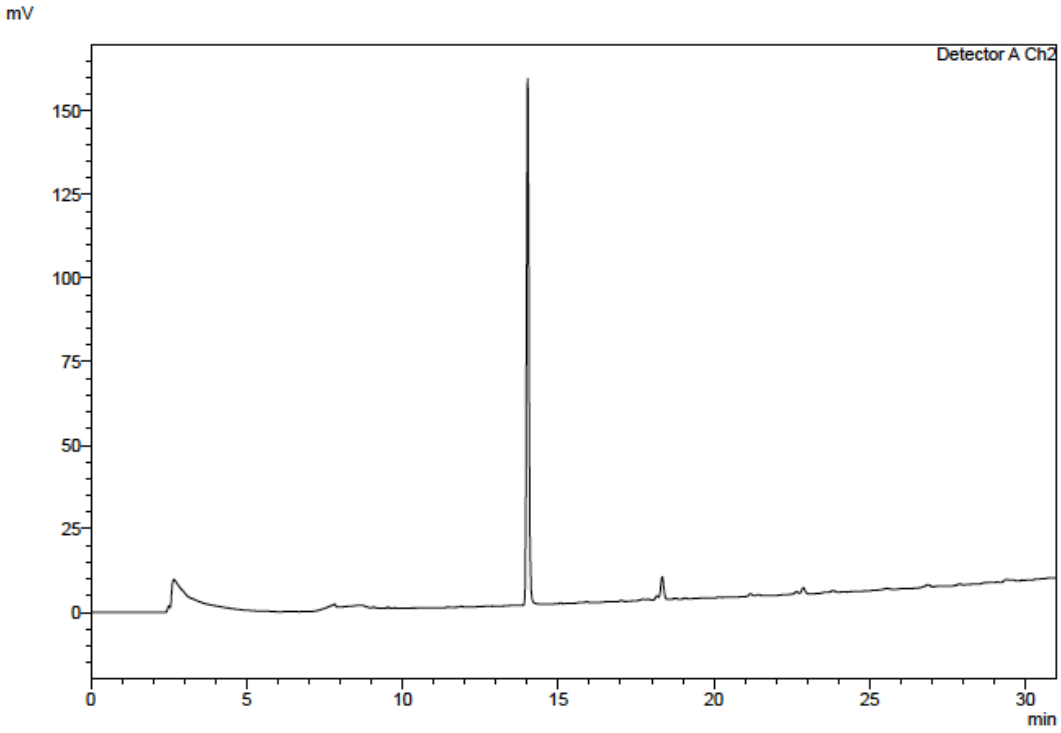
^{1}H NMR (500 MHz, DMSO-d_6)



^{13}C NMR (125 MHz, DMSO-d_6)



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



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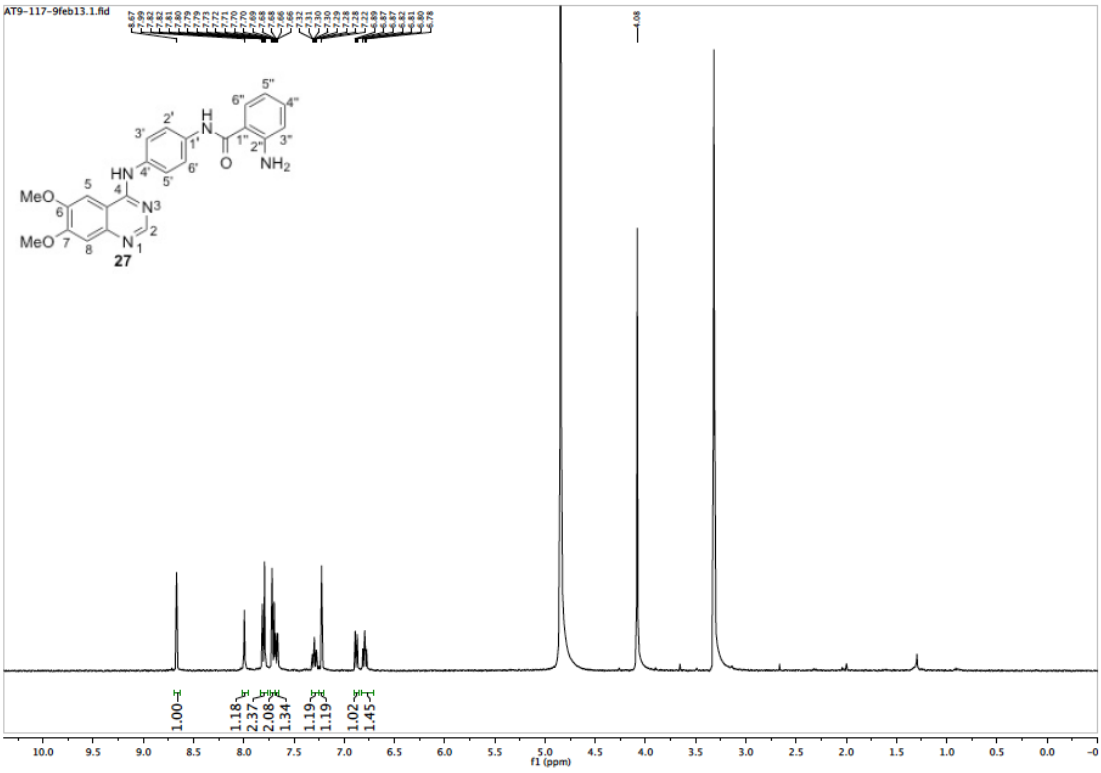
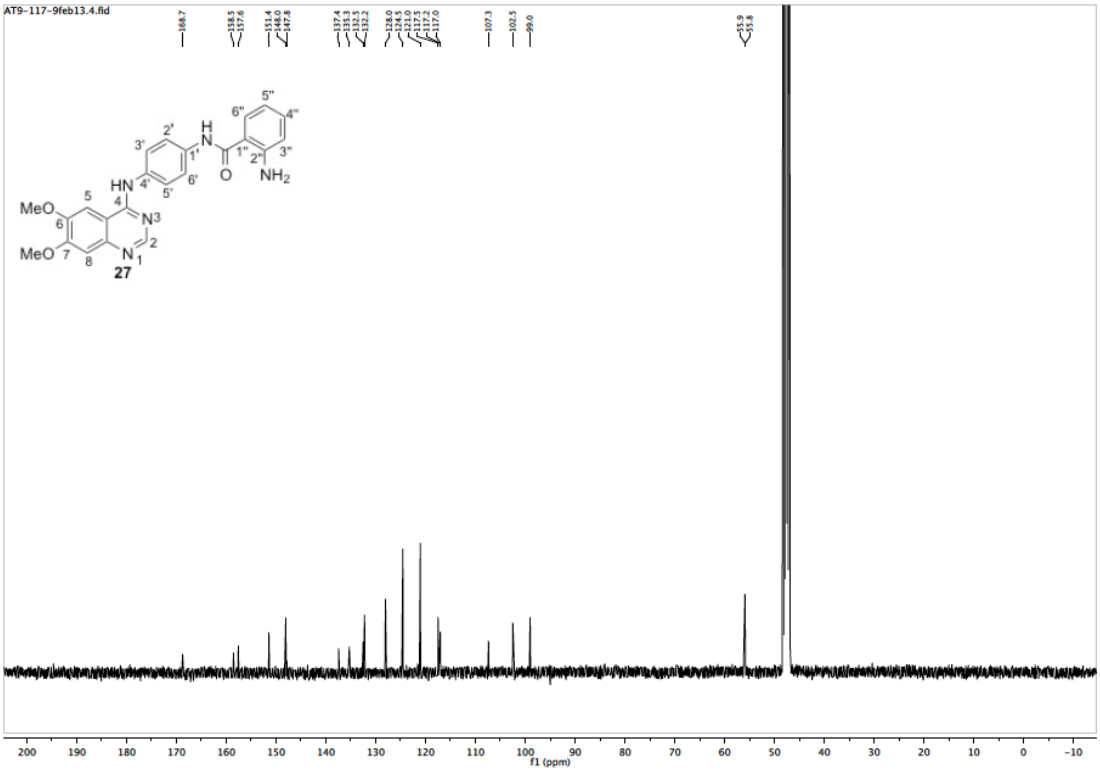
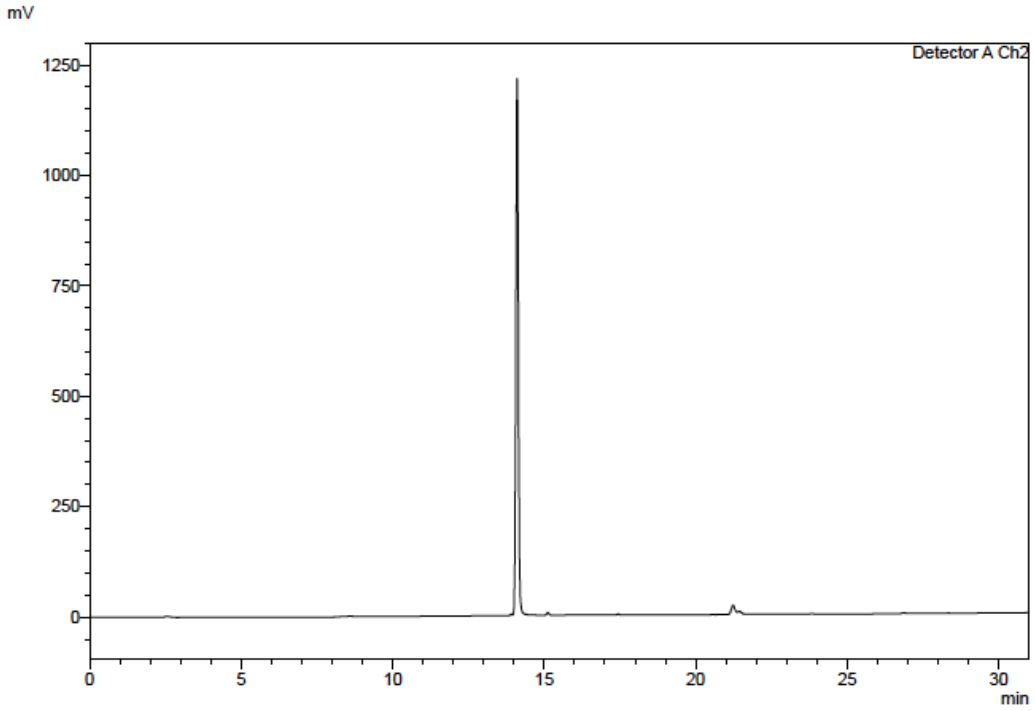
^{1}H NMR (400 MHz, CD_3OD)



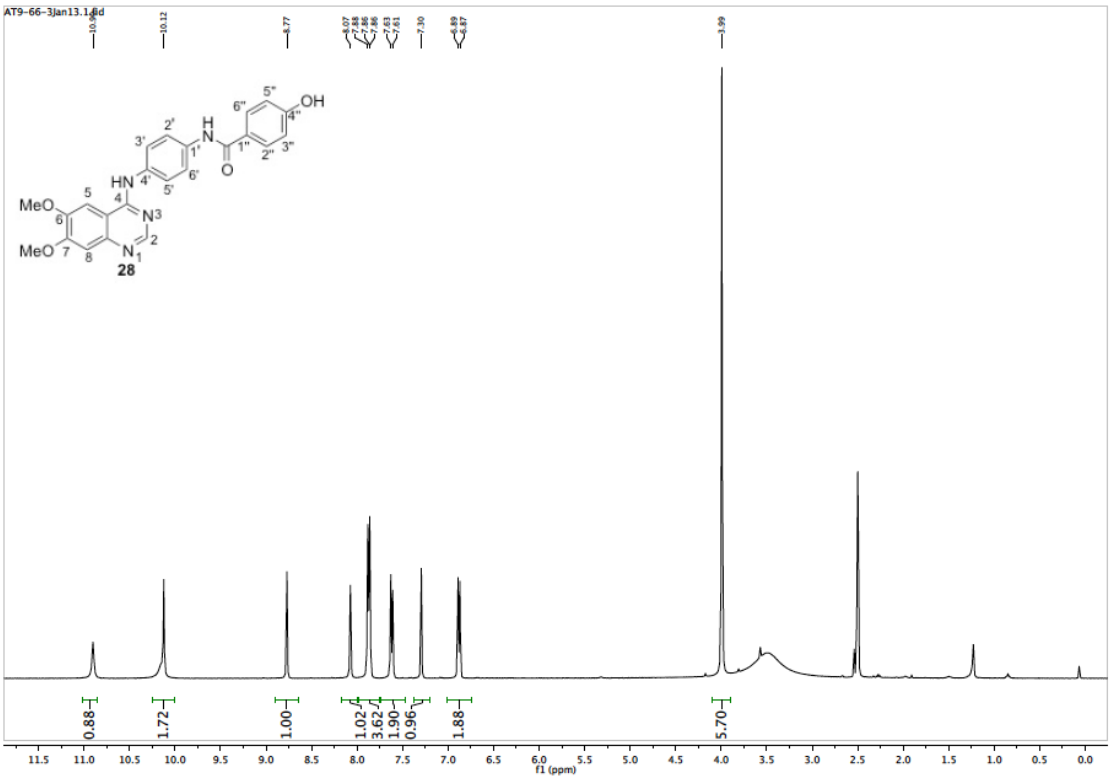
^{13}C NMR (101 MHz, CD_3OD)



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



^{1}H NMR (400 MHz, DMSO-d_6)



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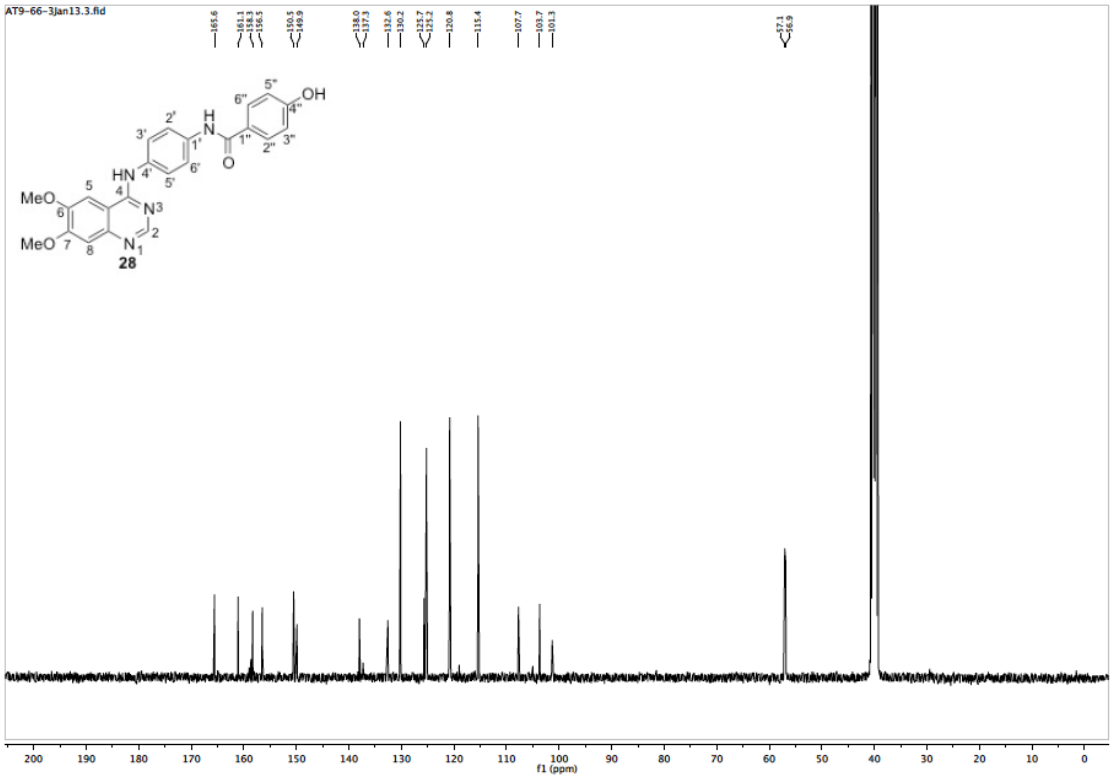
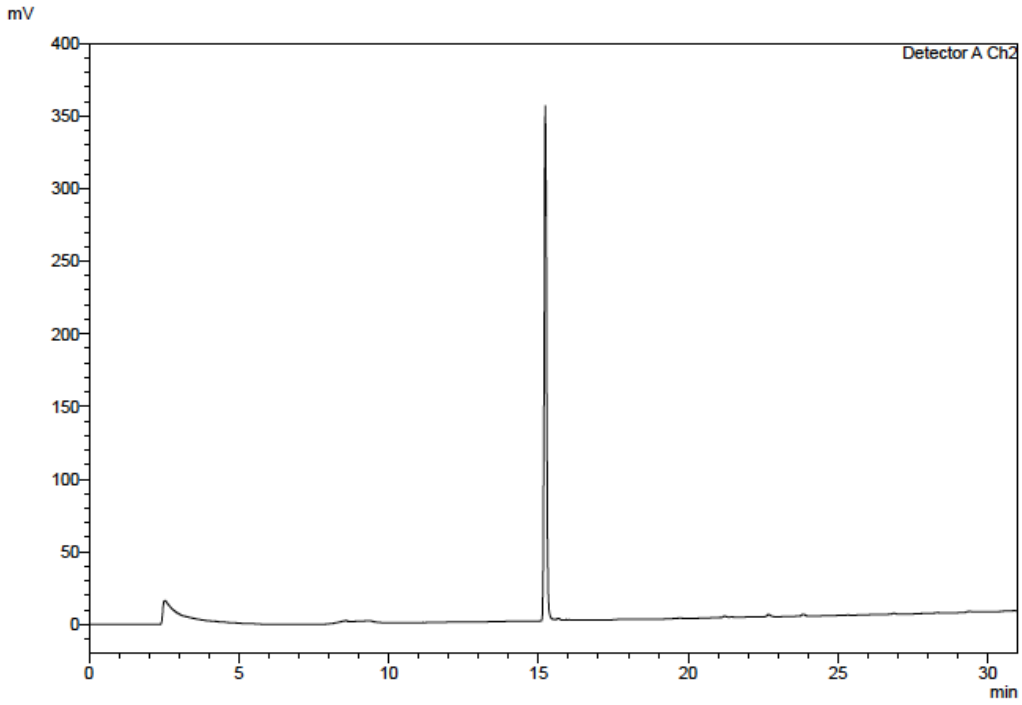
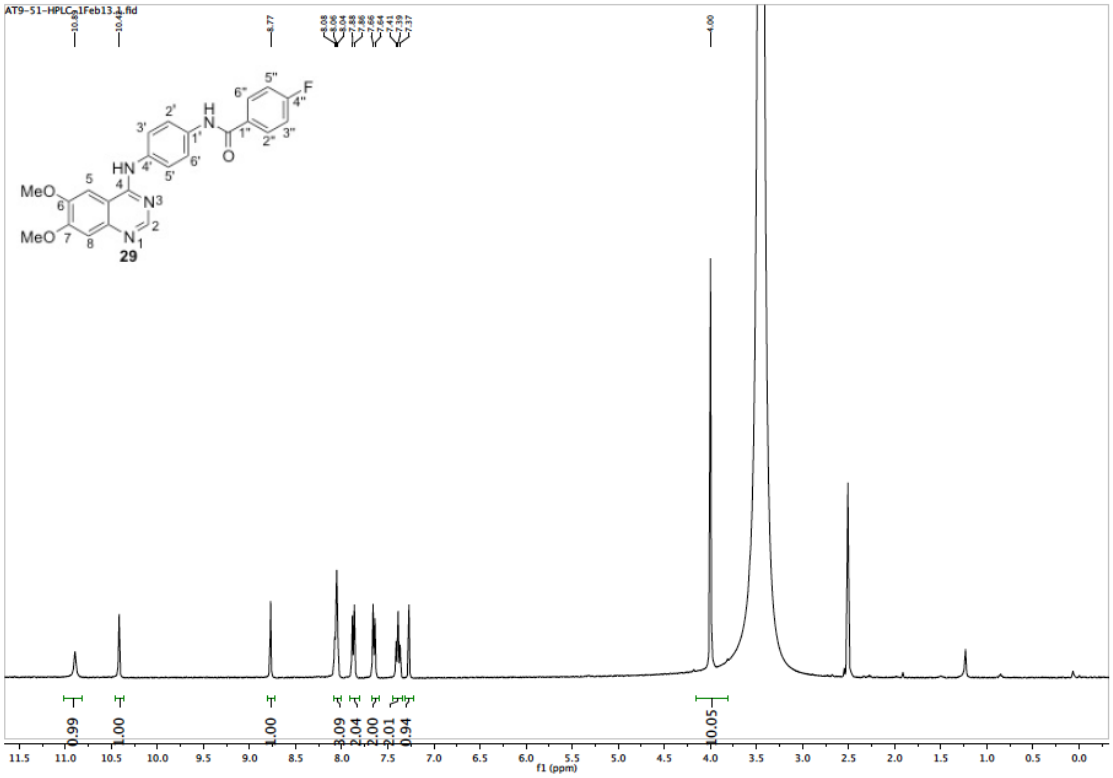
^{13}C NMR (101 MHz, DMSO-d_6)



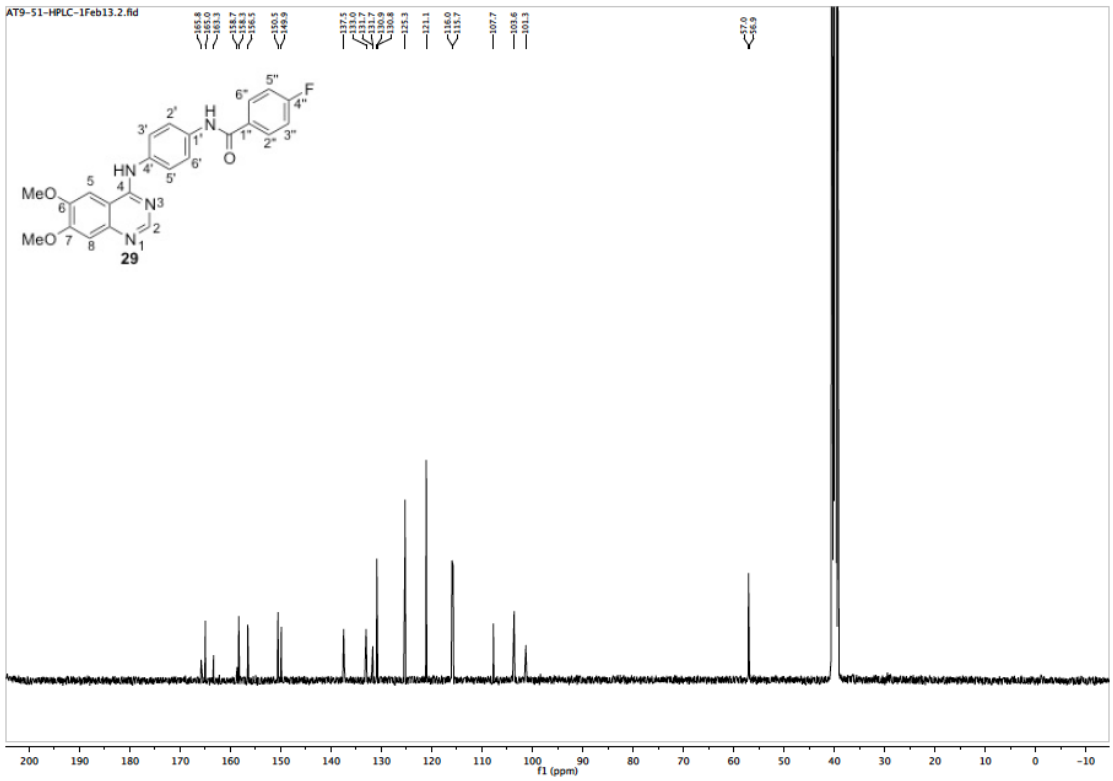
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



^{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, λ = 254 nm)

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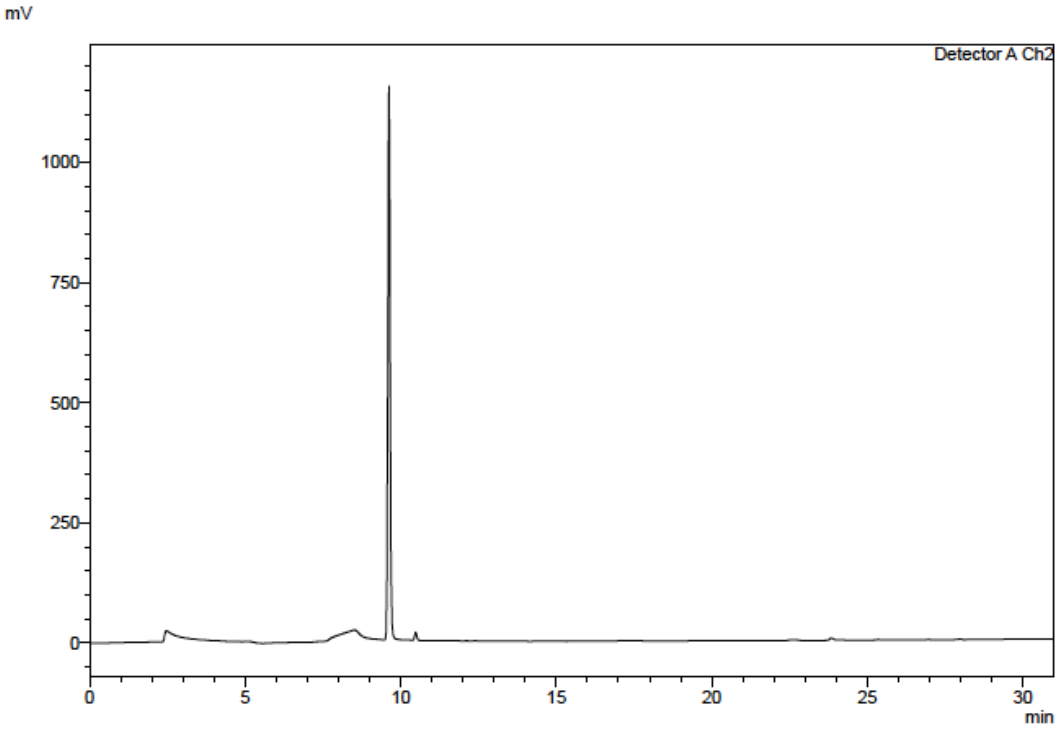
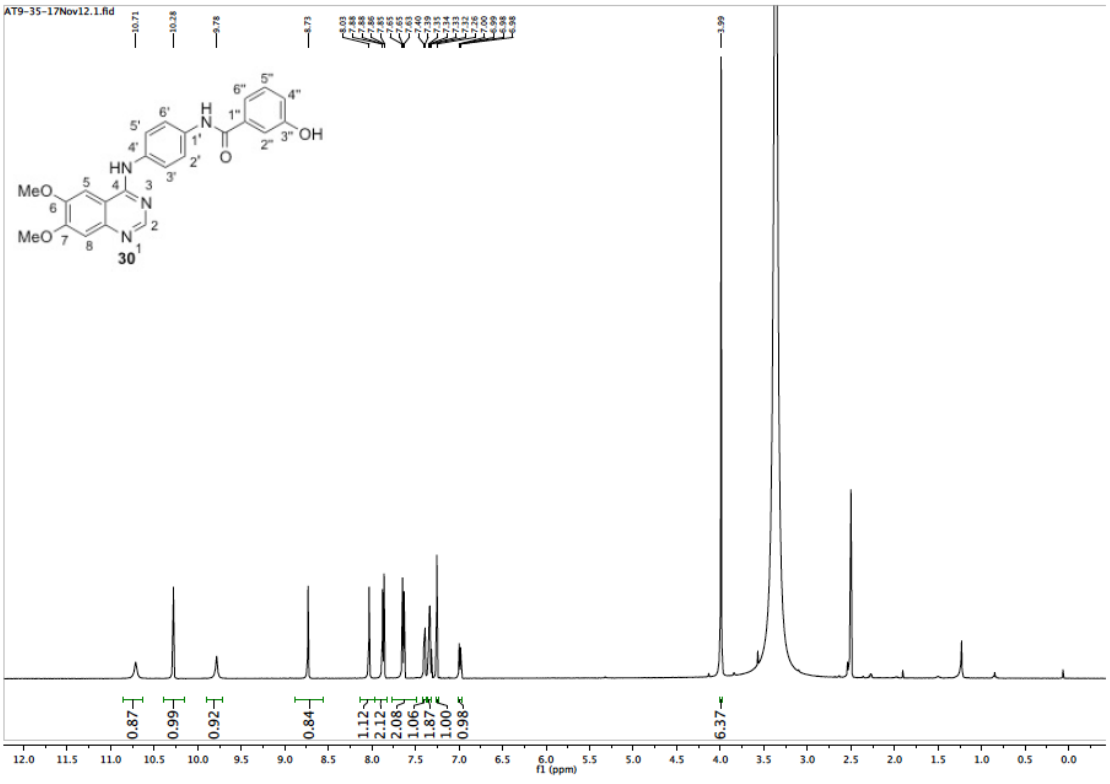
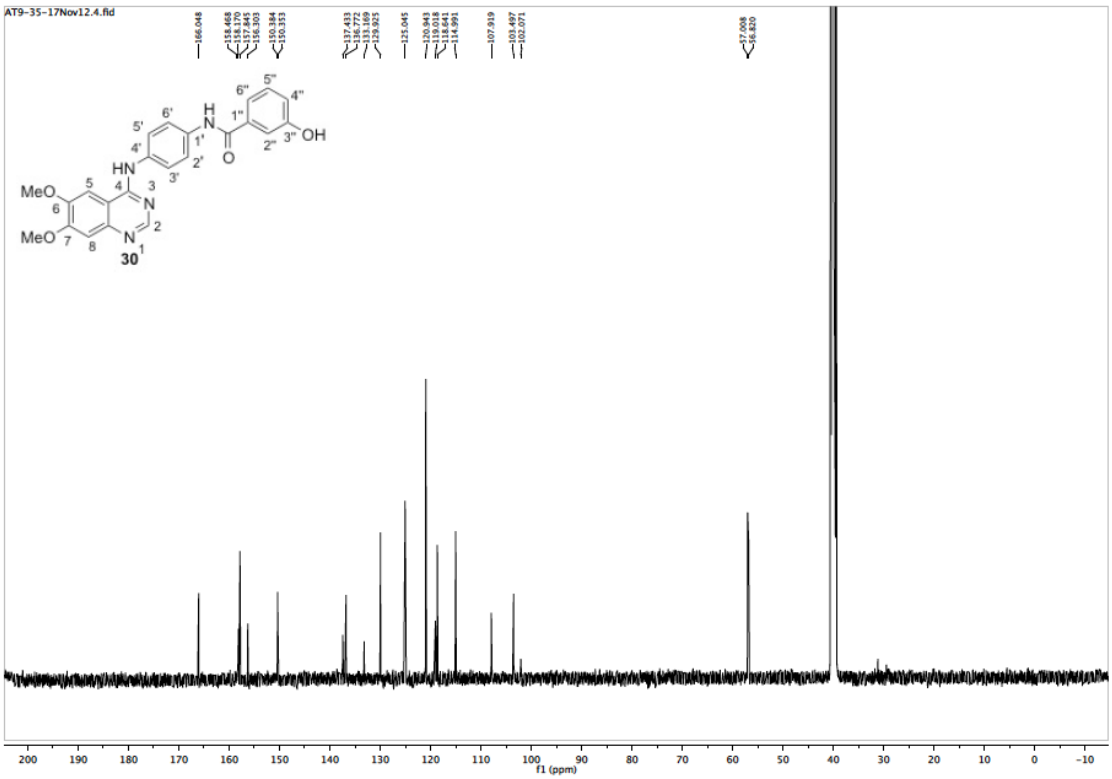


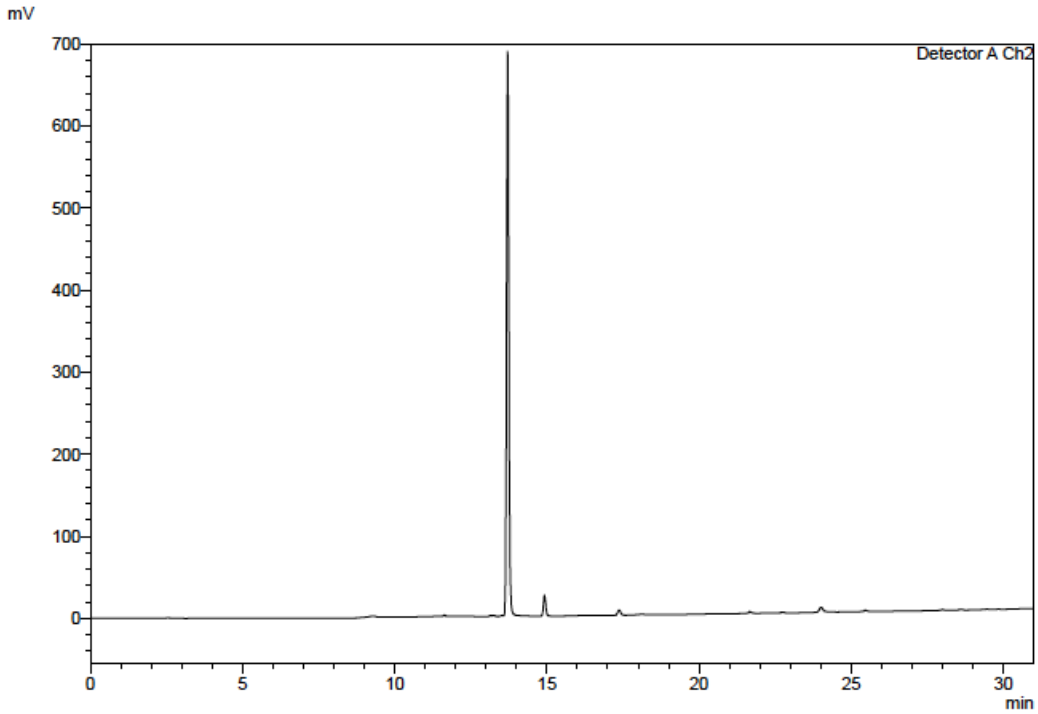
^{1}H NMR (500 MHz, DMSO-d_6)



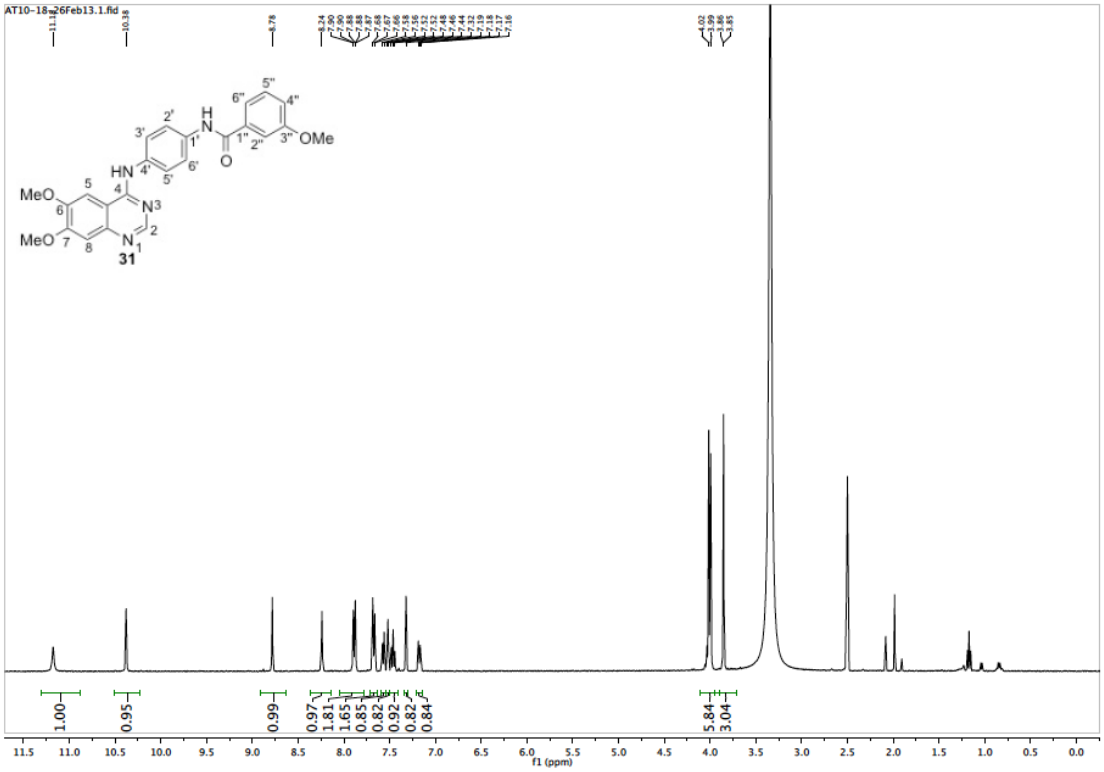
^{13}C NMR (125 MHz, DMSO-d_6)



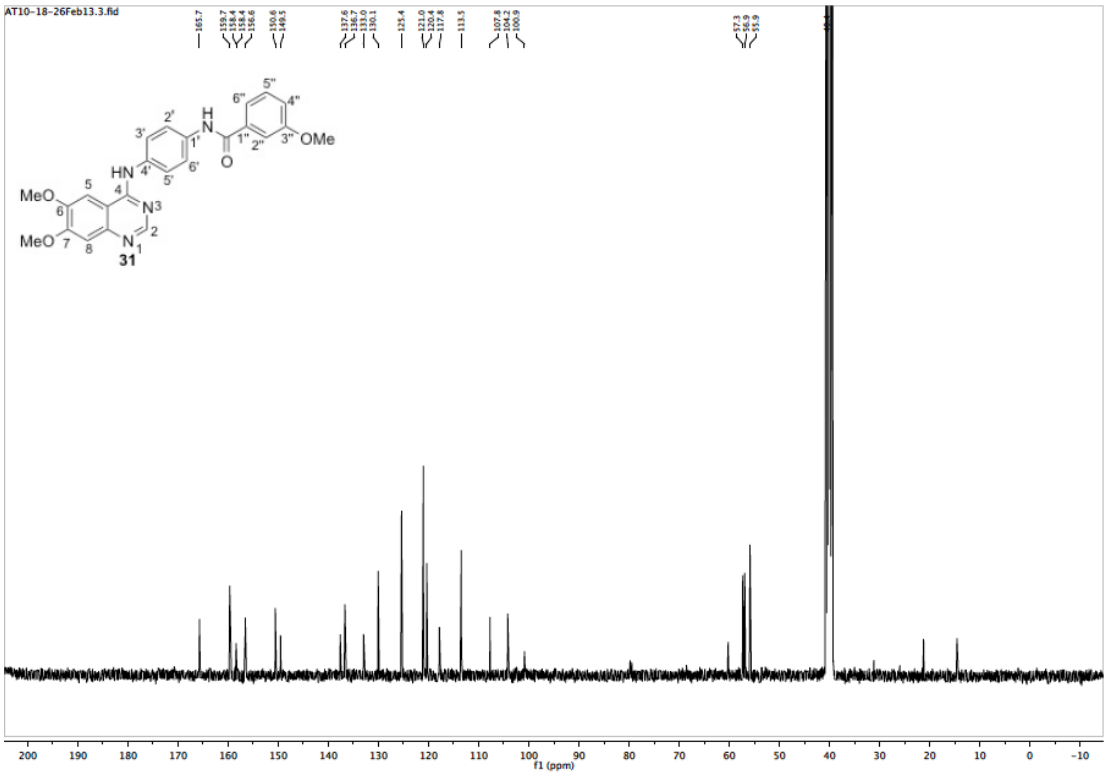
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



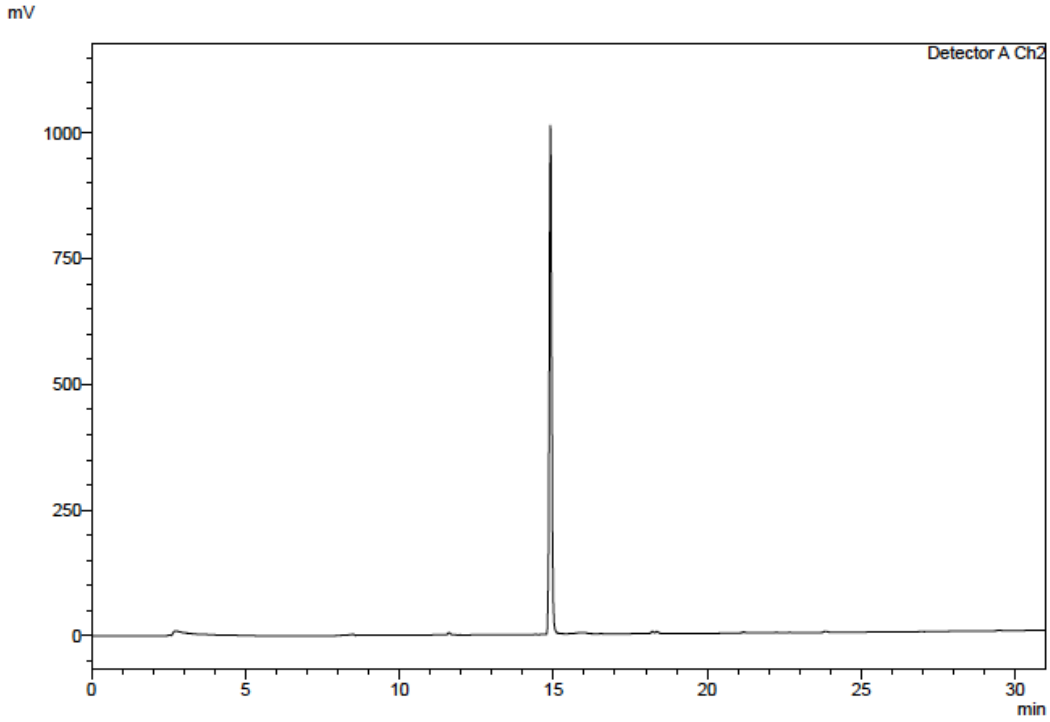
^{1}H NMR (400 MHz, DMSO-d_6)



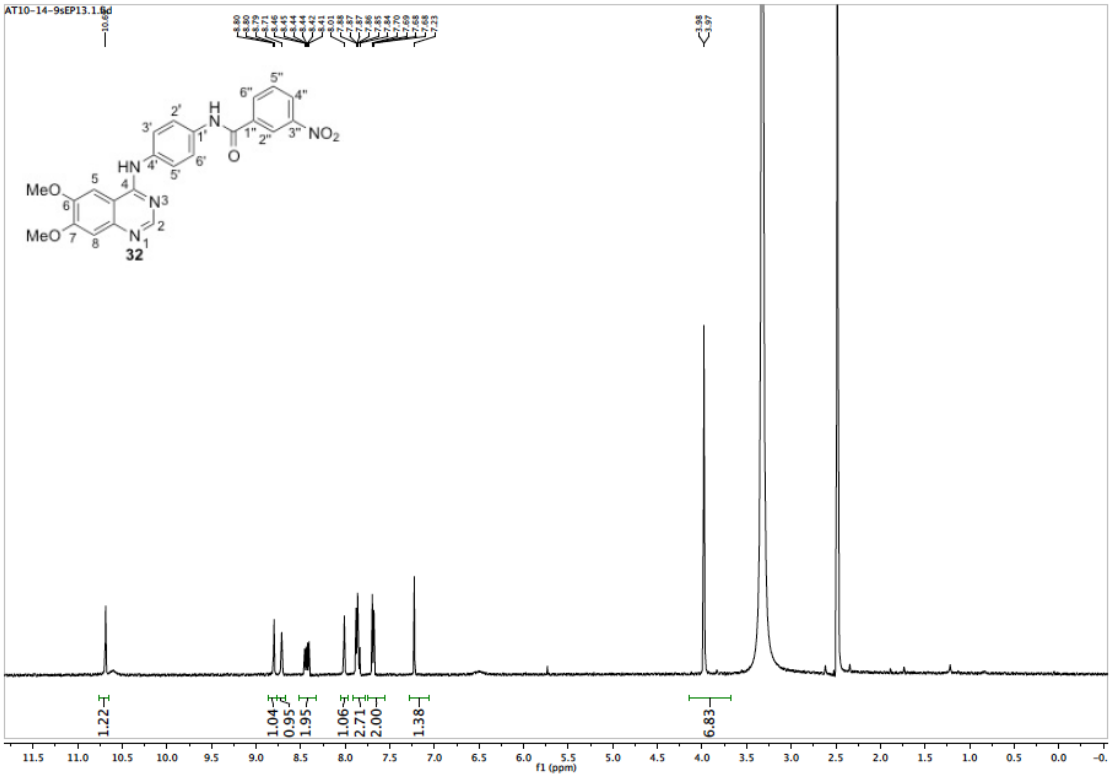
^{13}C NMR (101 MHz, DMSO-d_6)



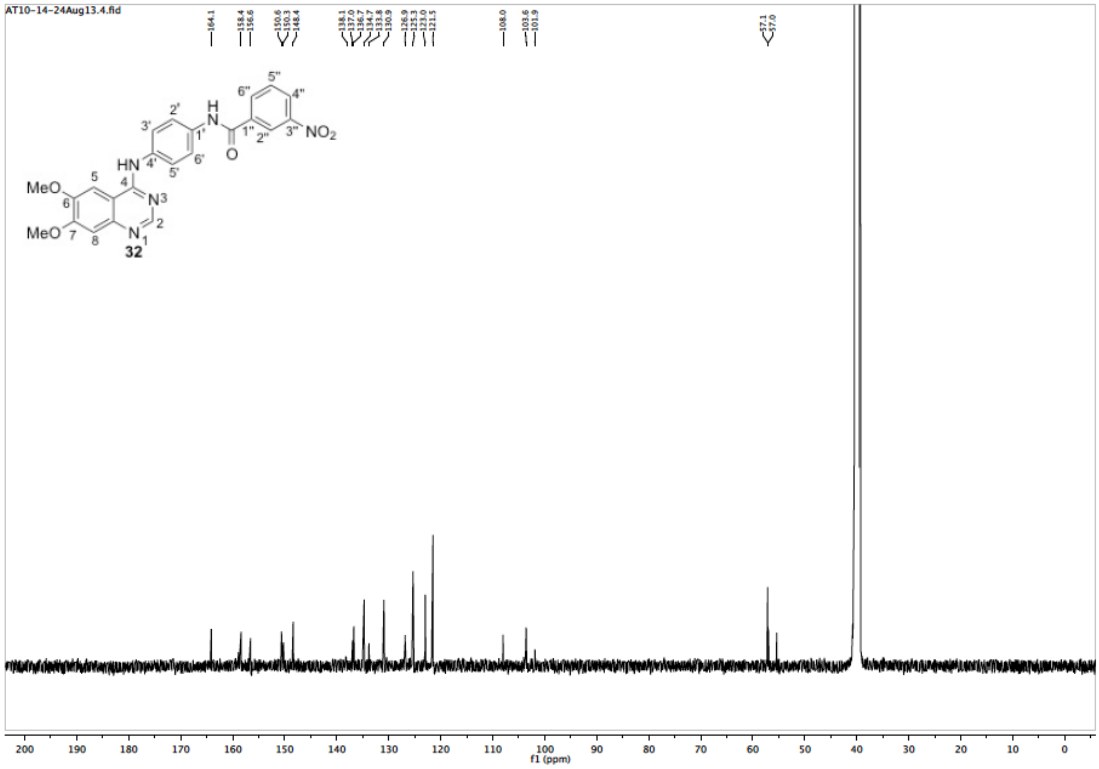
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



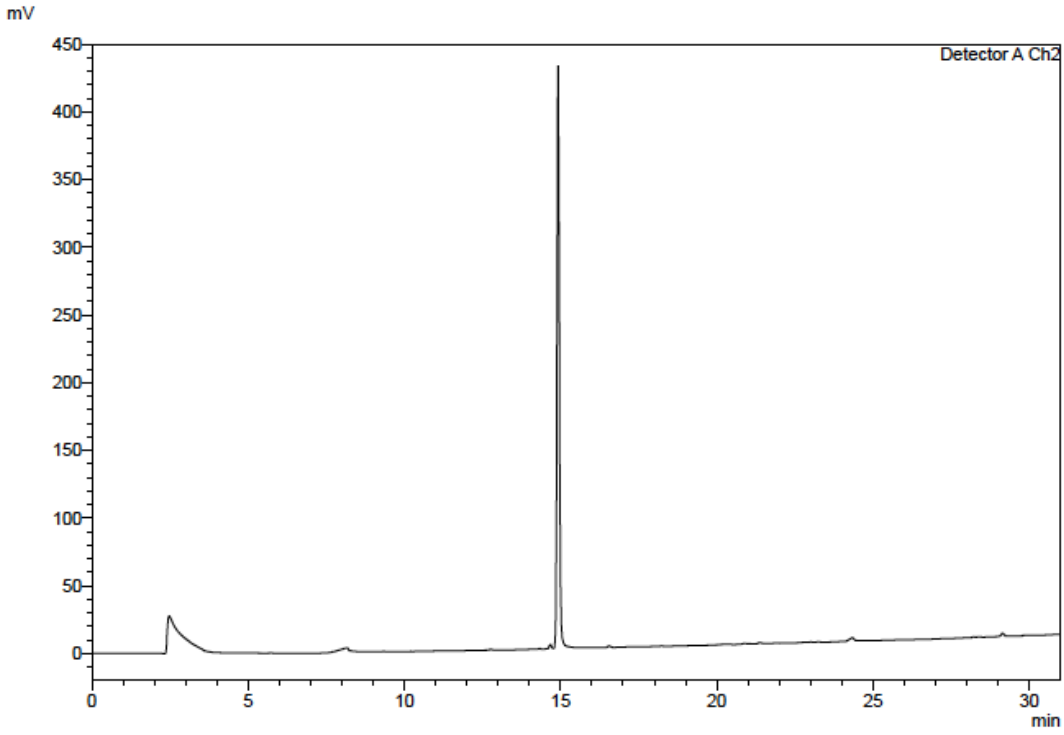
^{1}H NMR (500 MHz, DMSO-d_6)



^{13}C NMR (125 MHz, DMSO-d_6)



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



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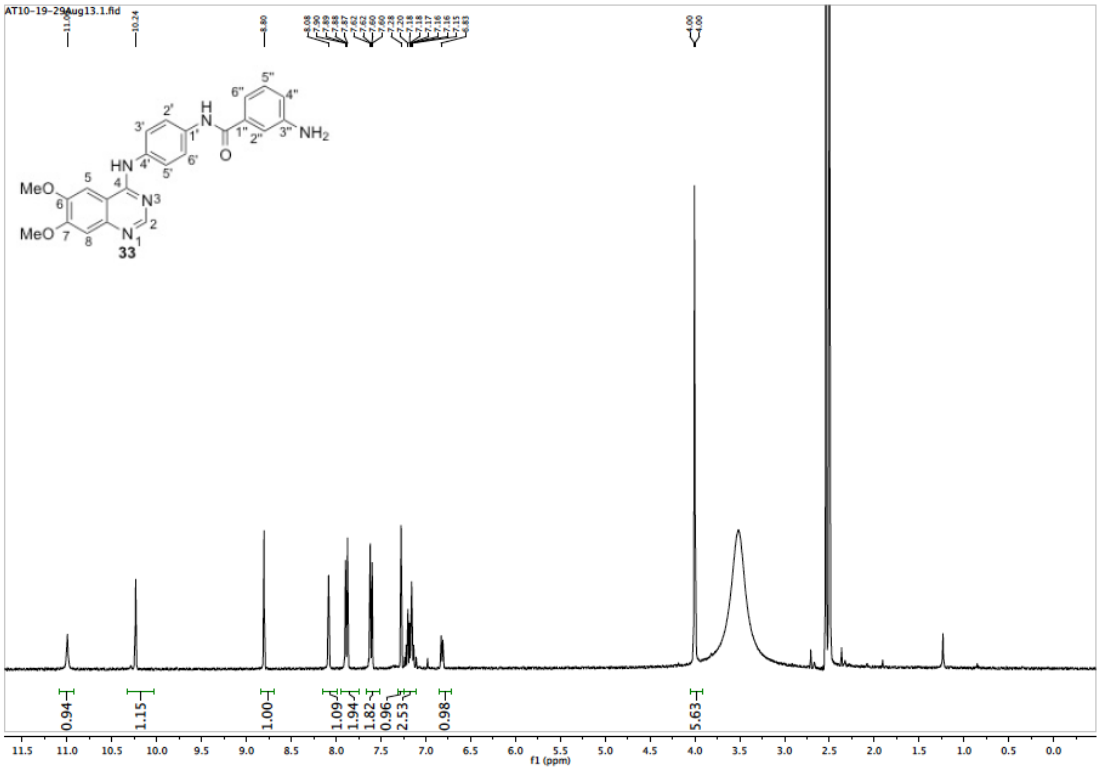
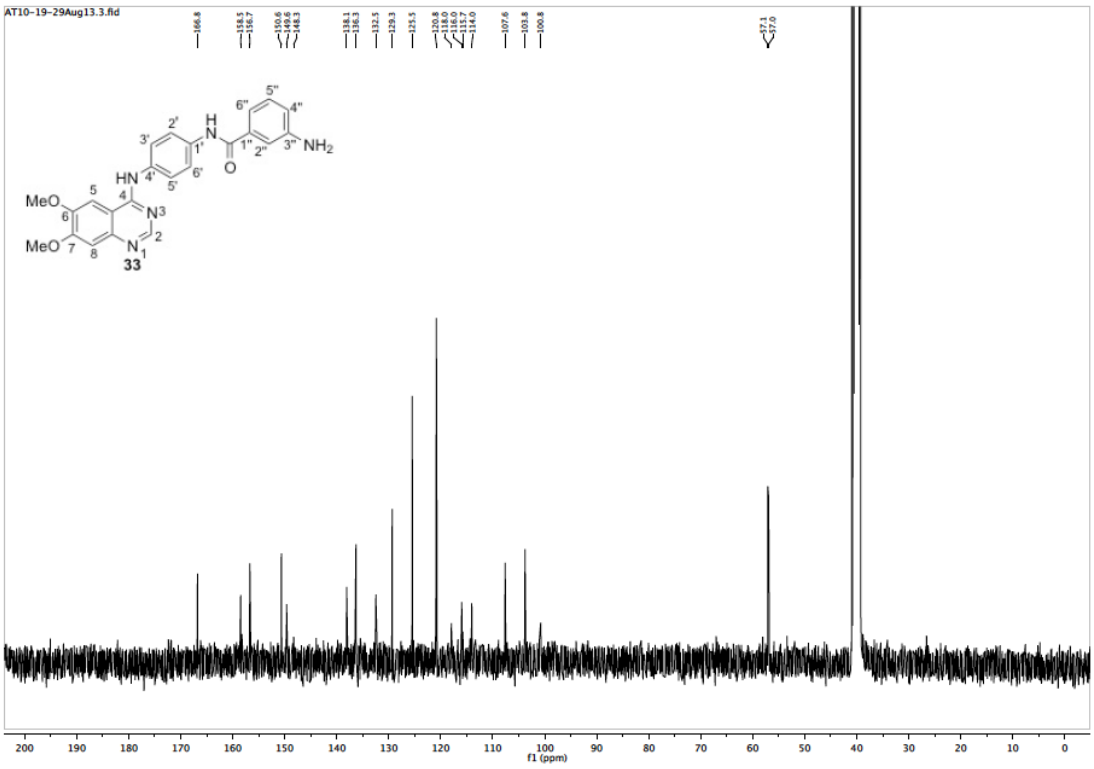
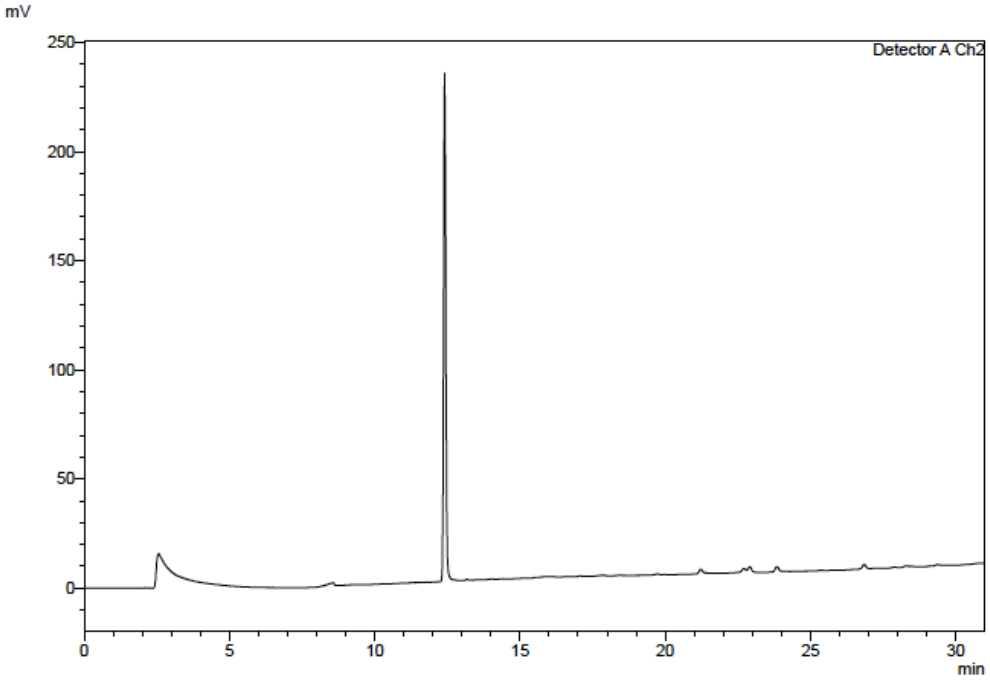
^{1}H NMR (400 MHz, DMSO-d_6)



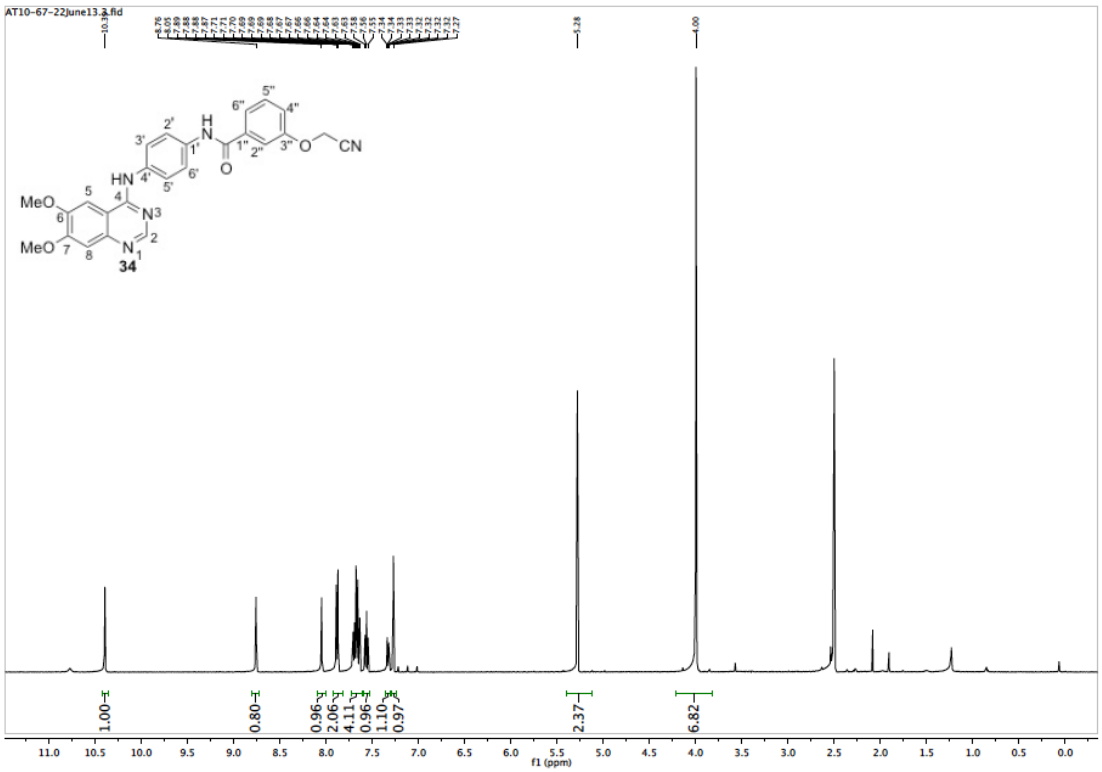
^{13}C NMR (101 MHz, DMSO-d_6)



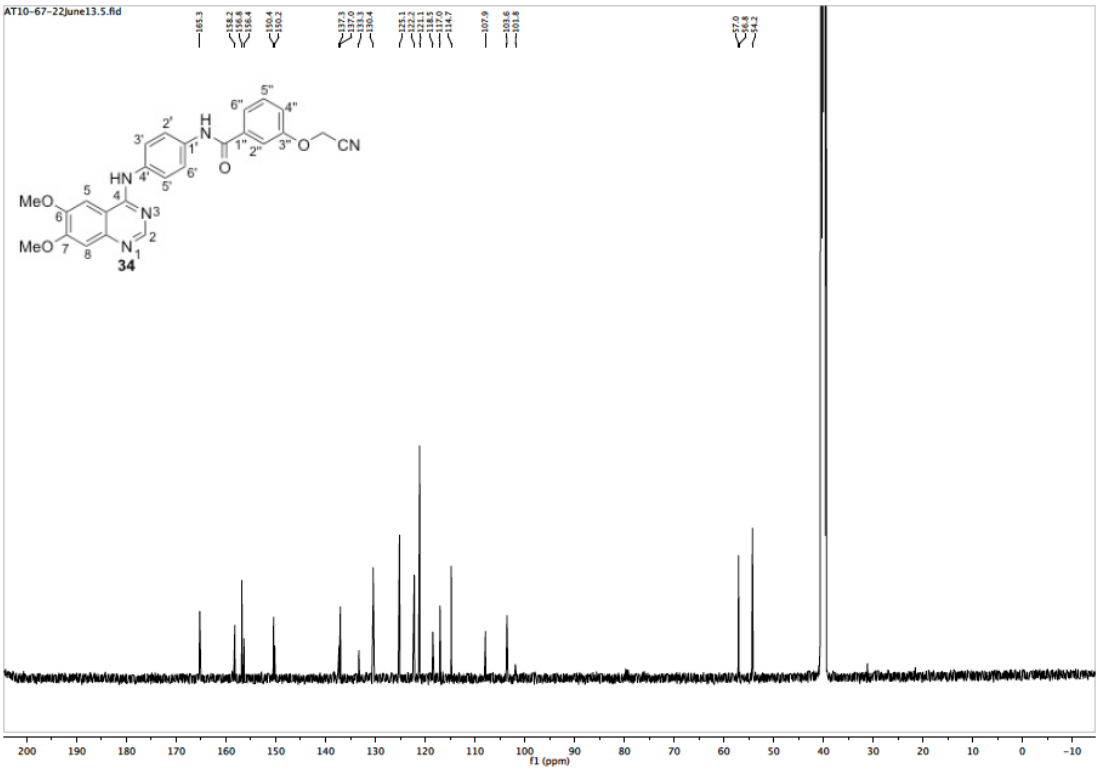
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



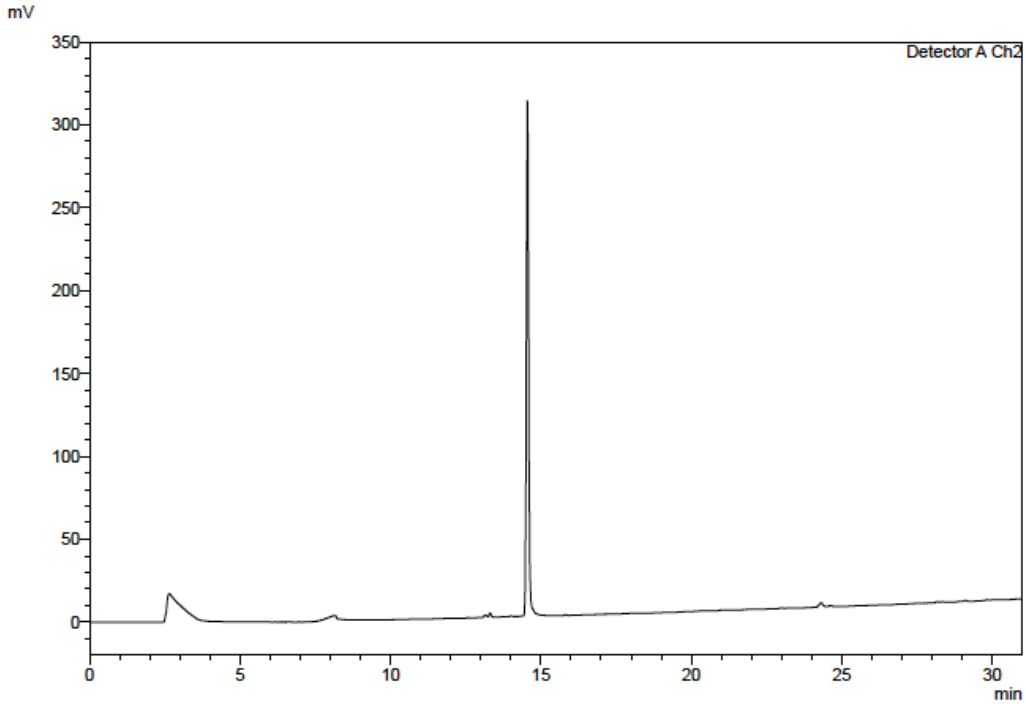
^{1}H NMR (500 MHz, DMSO-d_6)



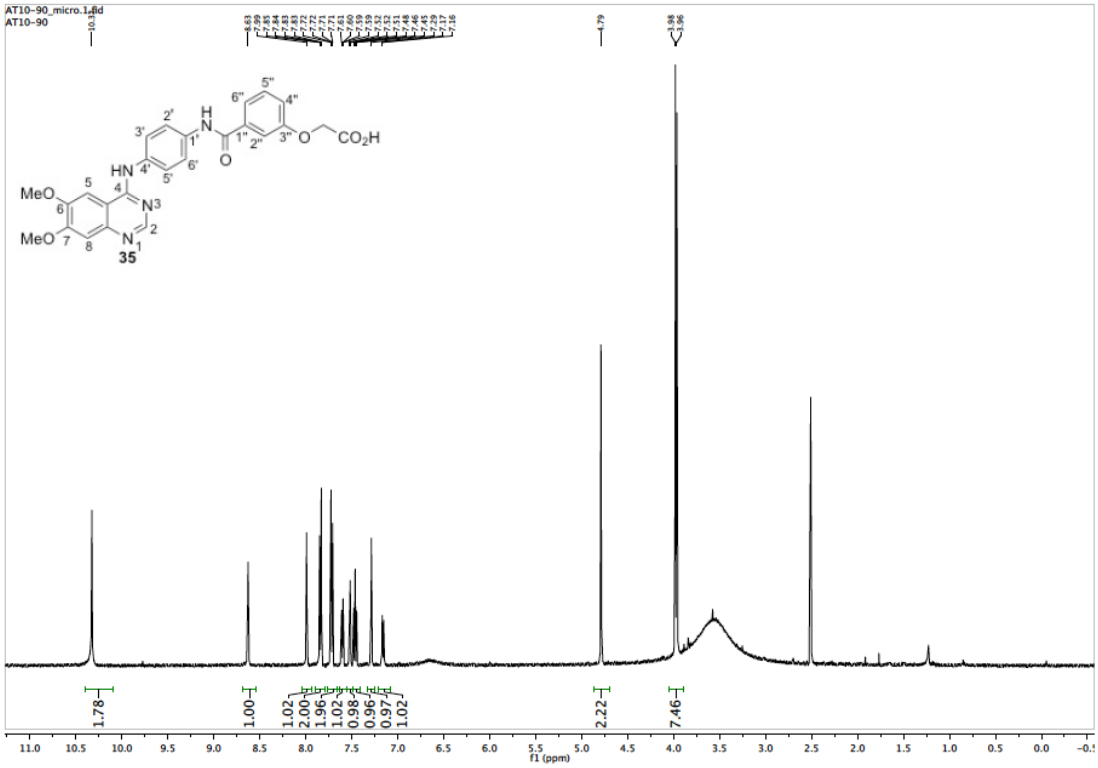
^{13}C NMR (125 MHz, DMSO-d_6)



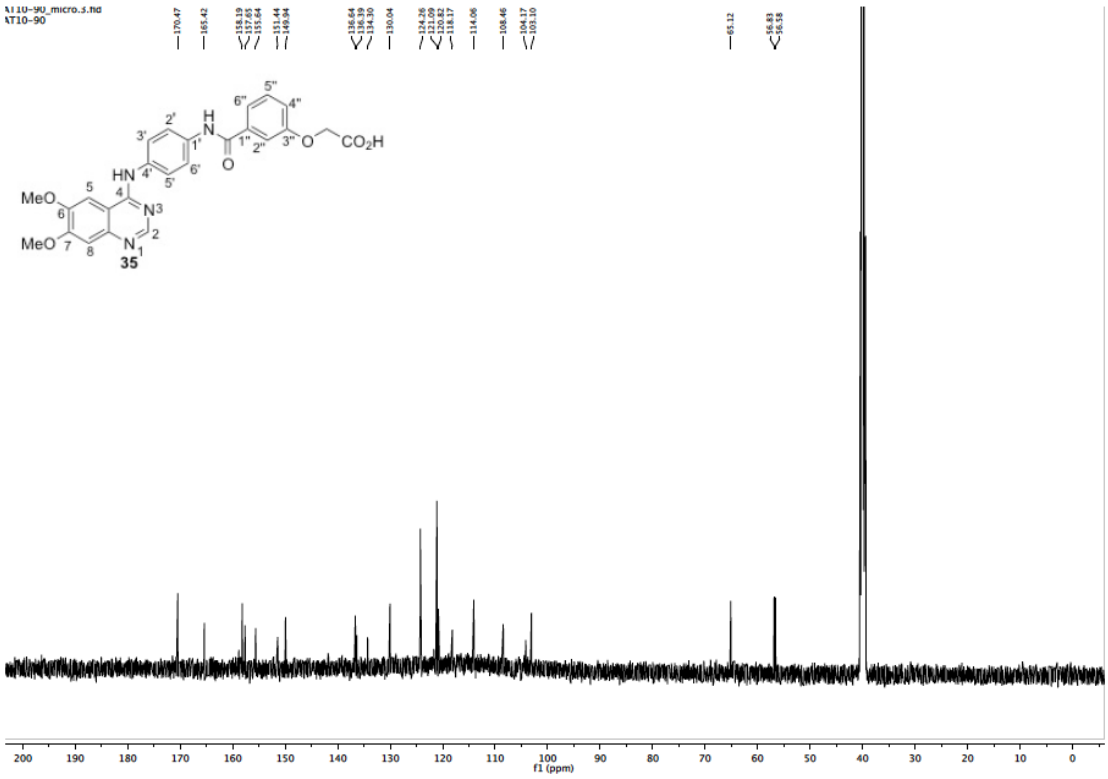
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



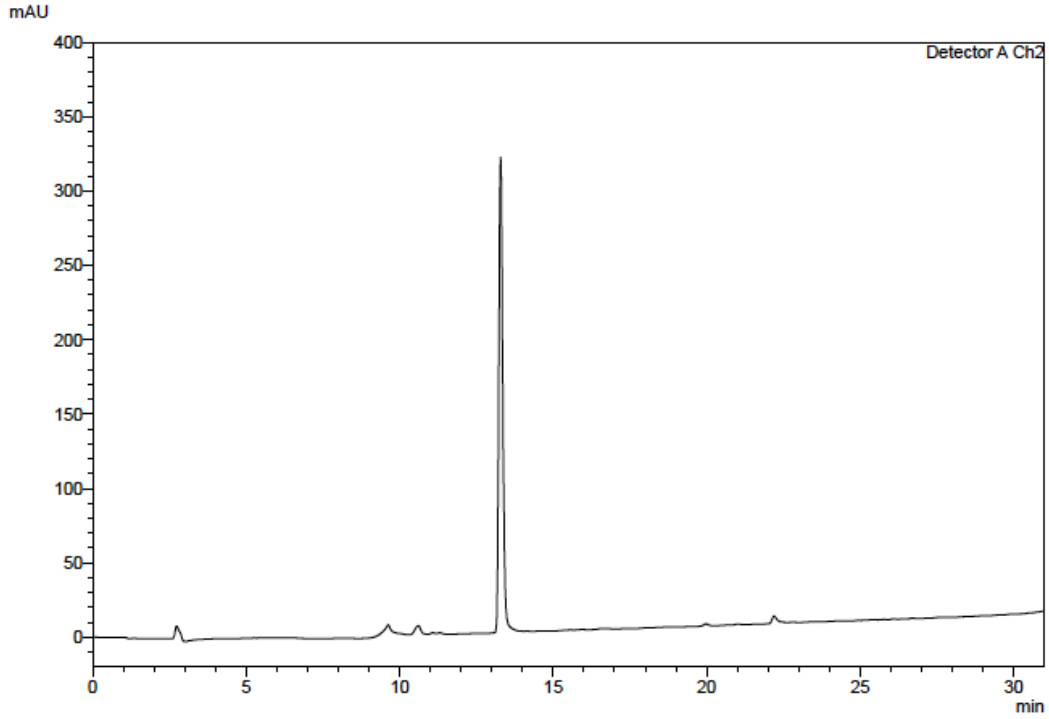
^{1}H NMR (500 MHz, DMSO-d_6)



^{13}C NMR (125 MHz, DMSO-d_6)



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



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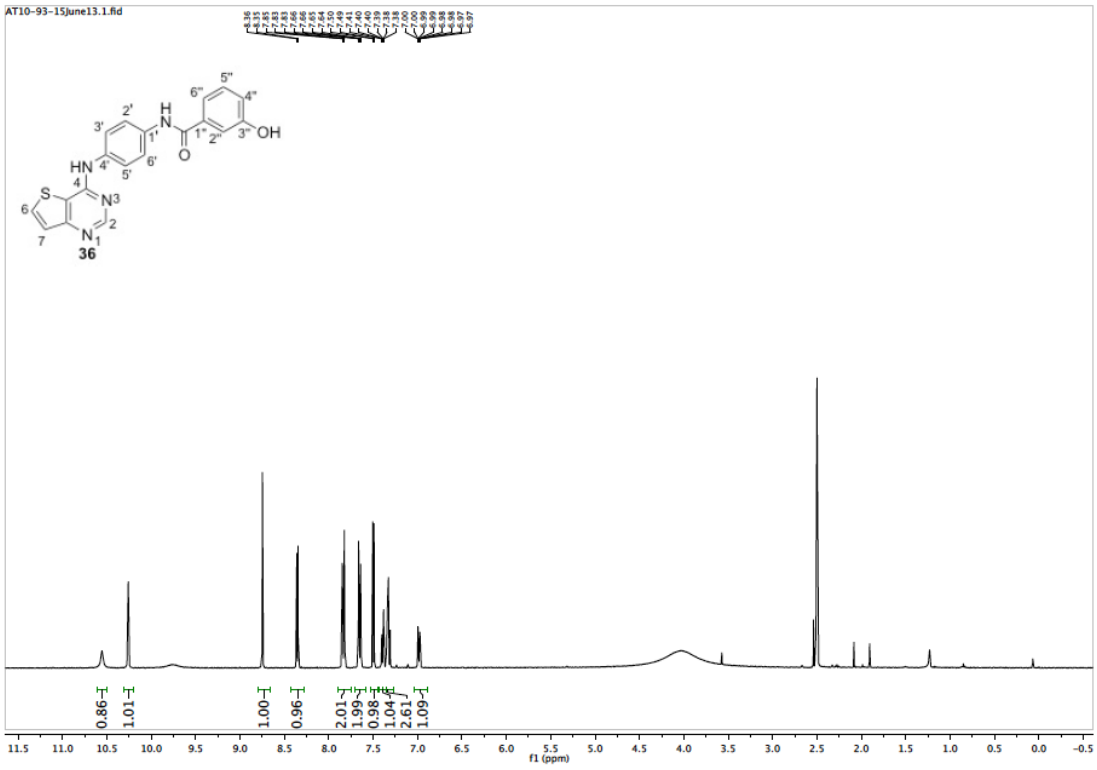
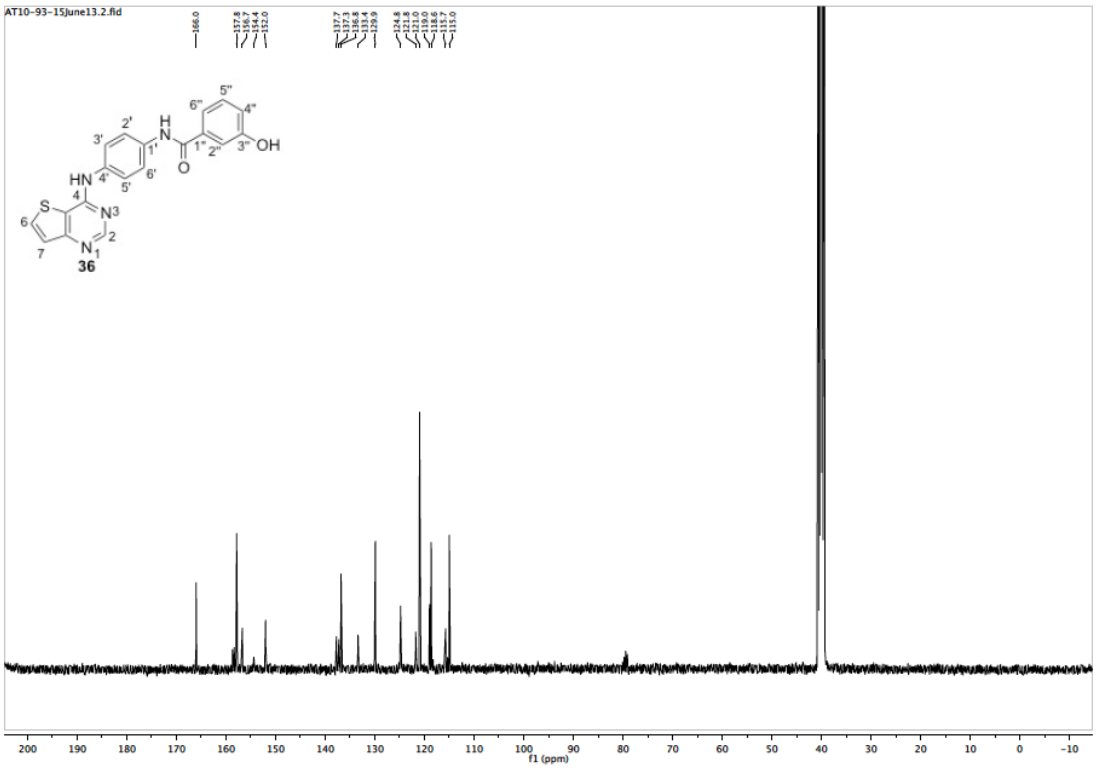
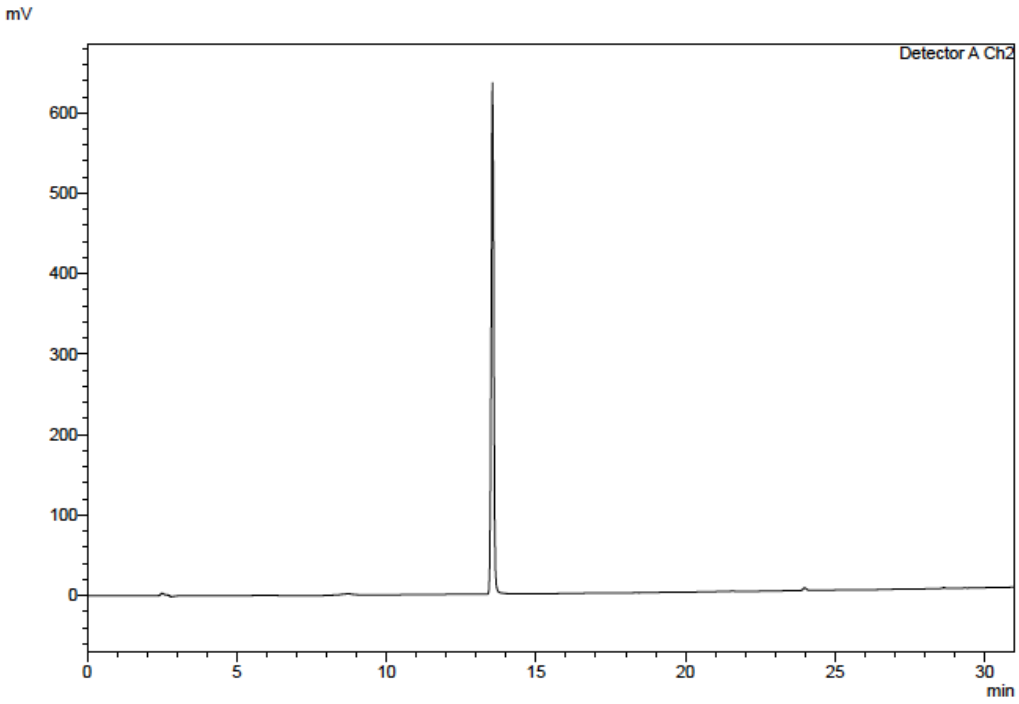


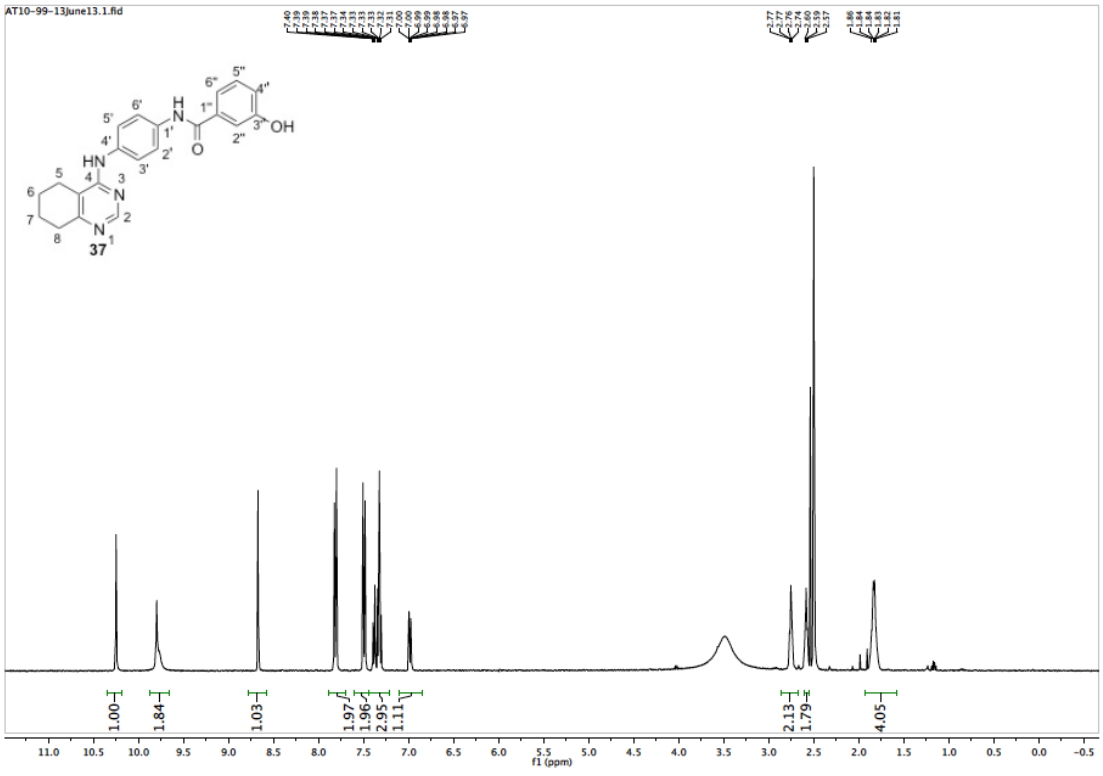
^{13}C NMR (101 MHz, DMSO-d_6)



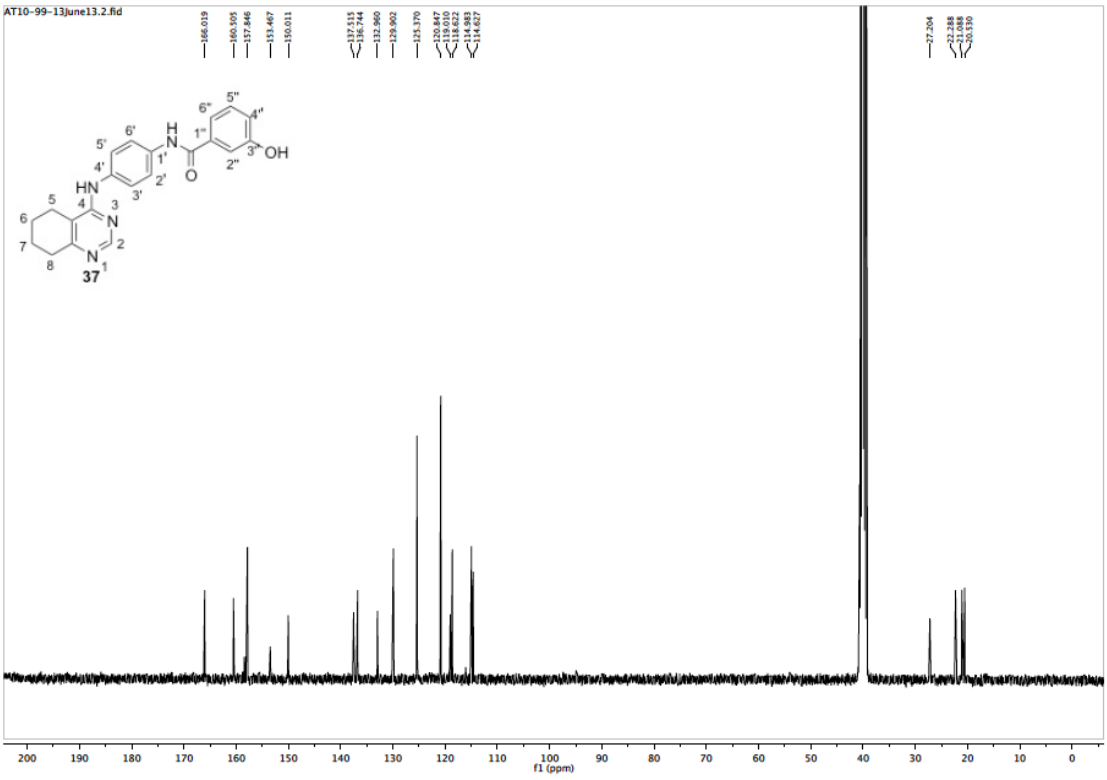
Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



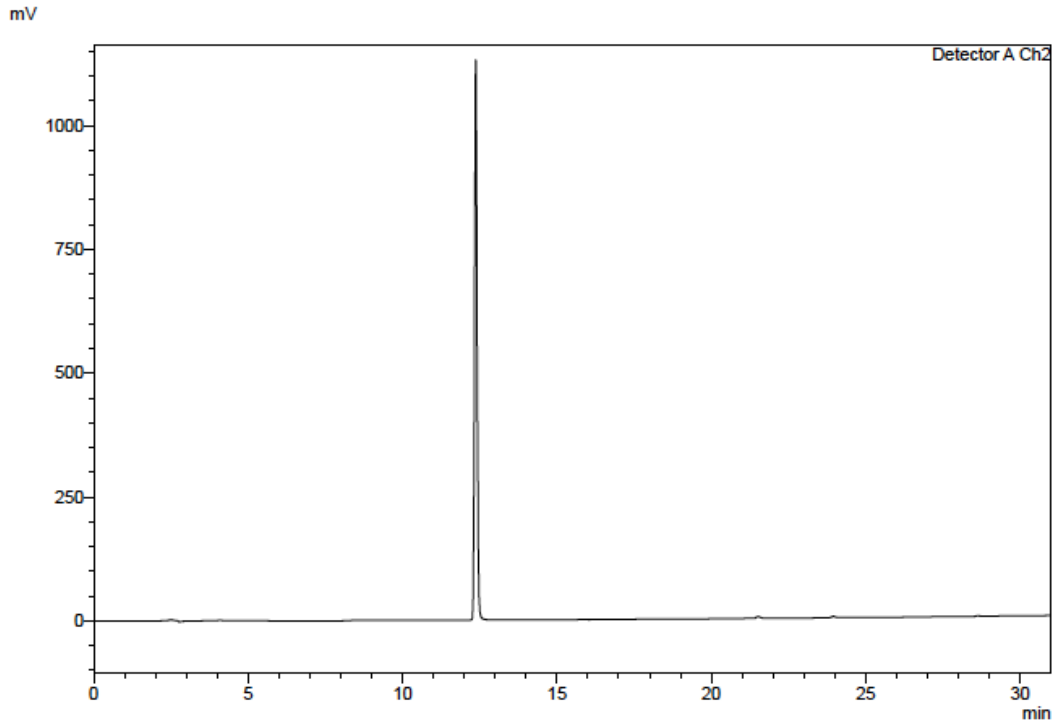
^{1}H NMR (400 MHz, DMSO-d_6)



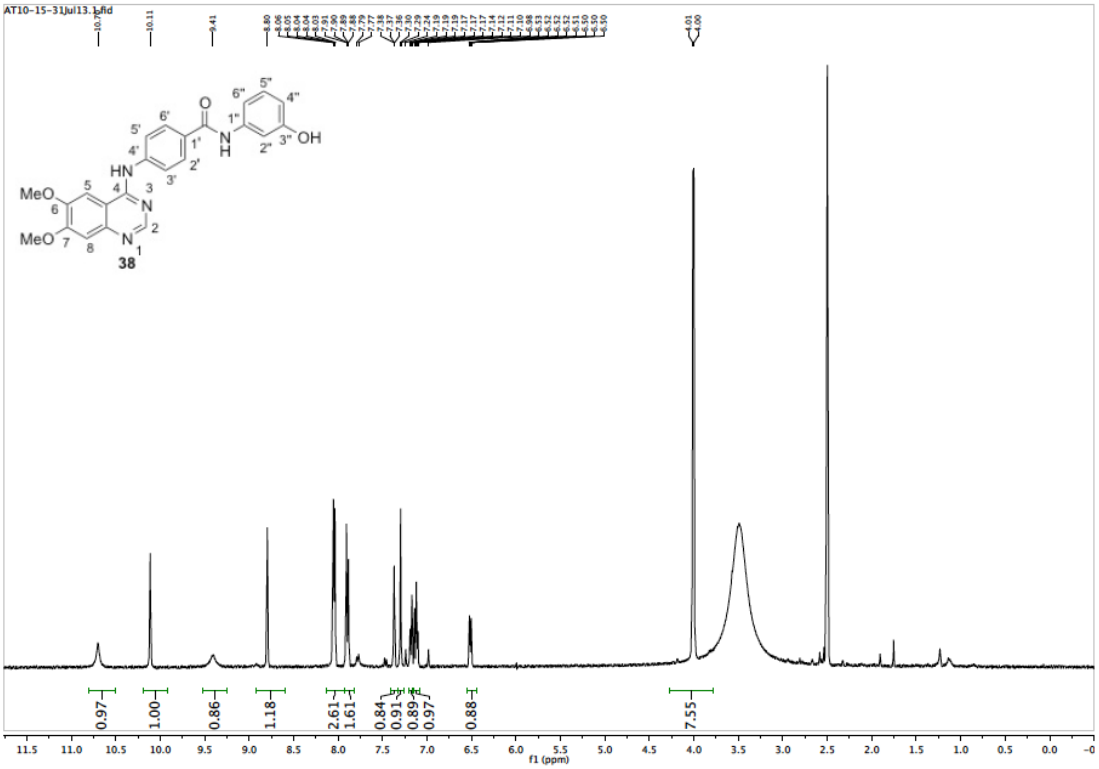
^{13}C NMR (101 MHz, DMSO-d_6)



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



^{1}H NMR (400 MHz, DMSO-d_6)



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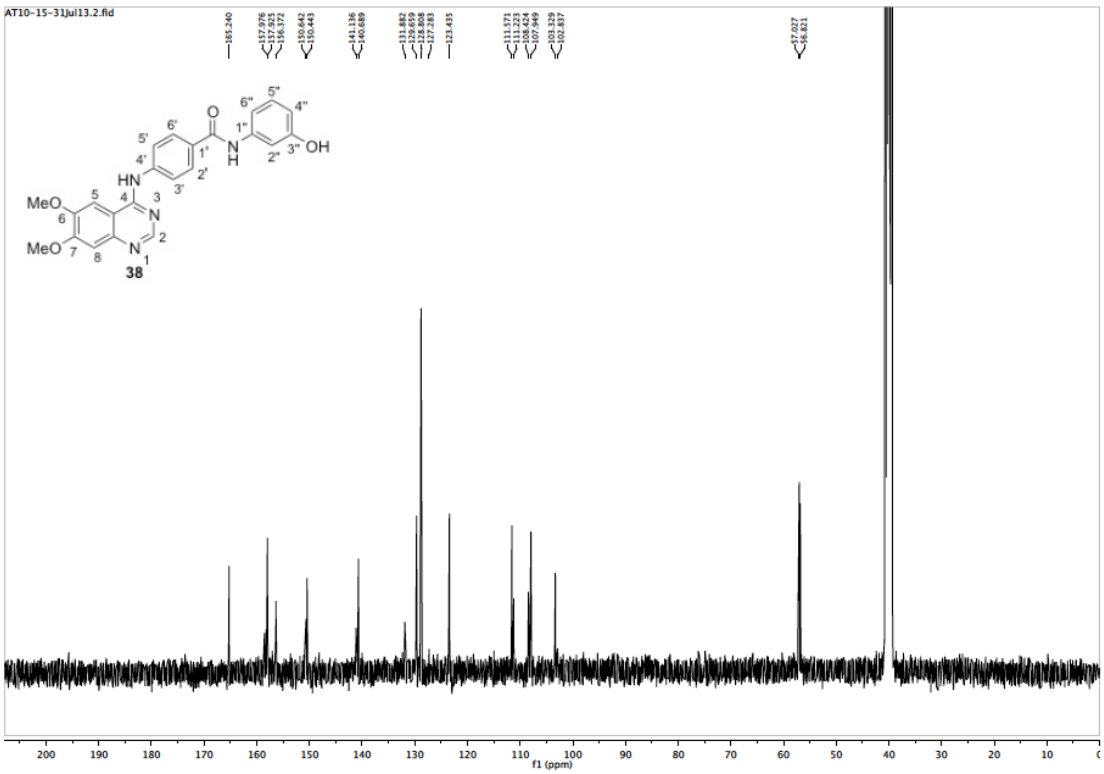
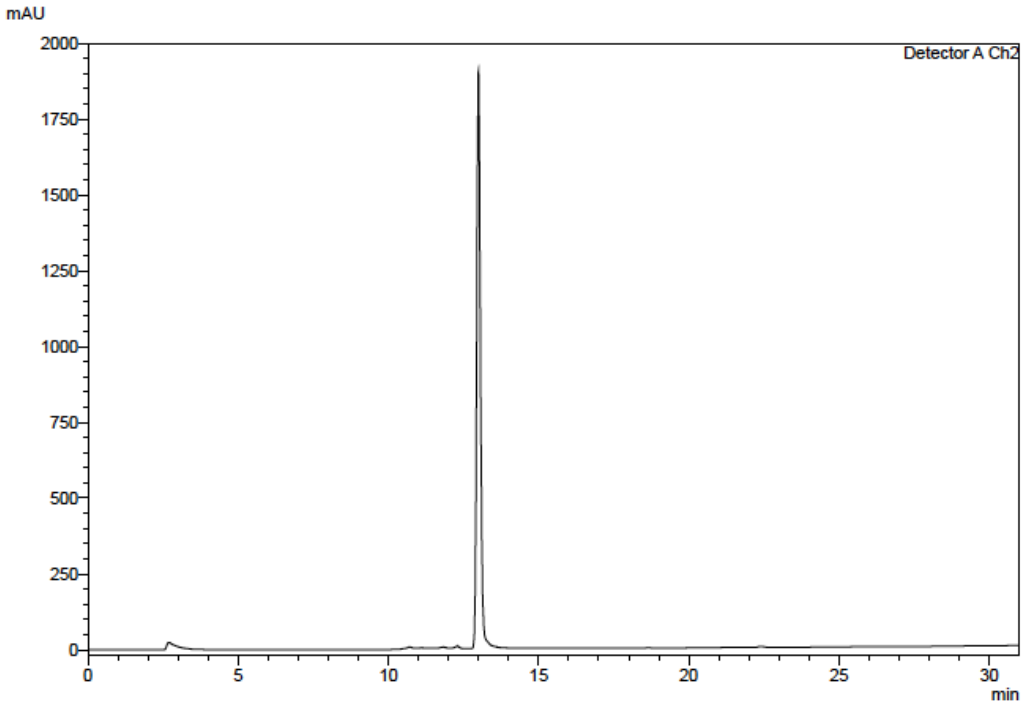


Analytical HPLC trace (0 to 100% MeCN over 30 min, λ = 254 nm)



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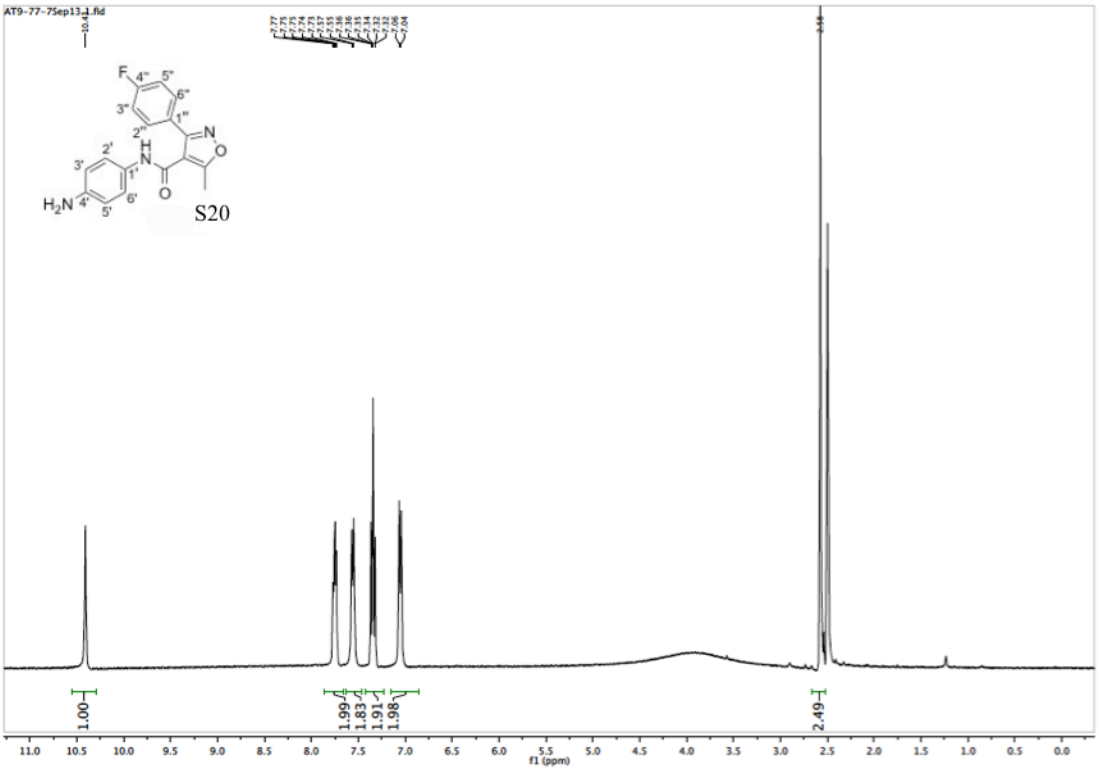

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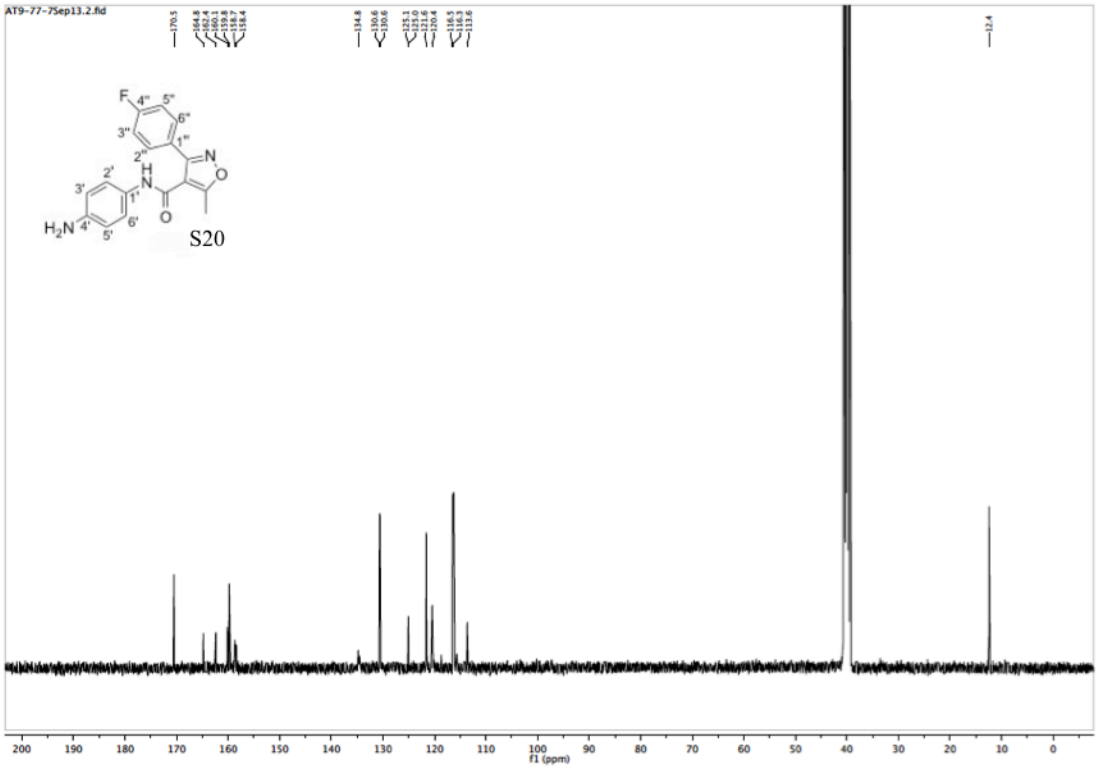

^{1}H NMR (400 MHz, DMSO-d_6)

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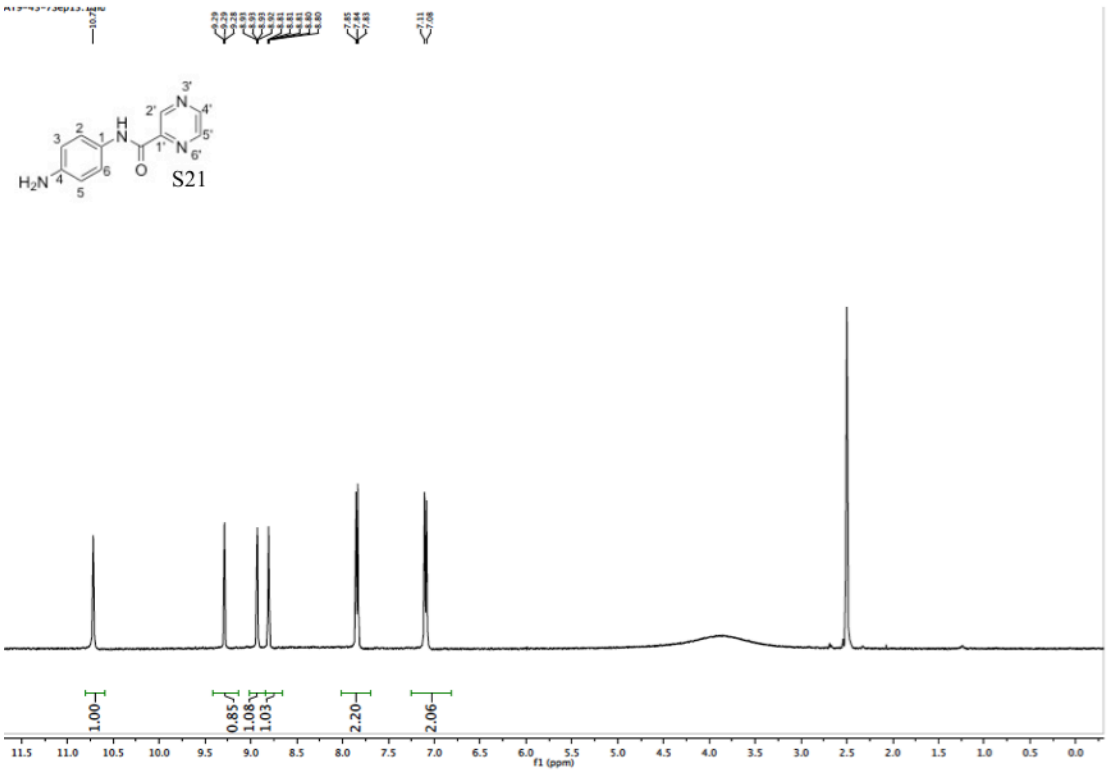

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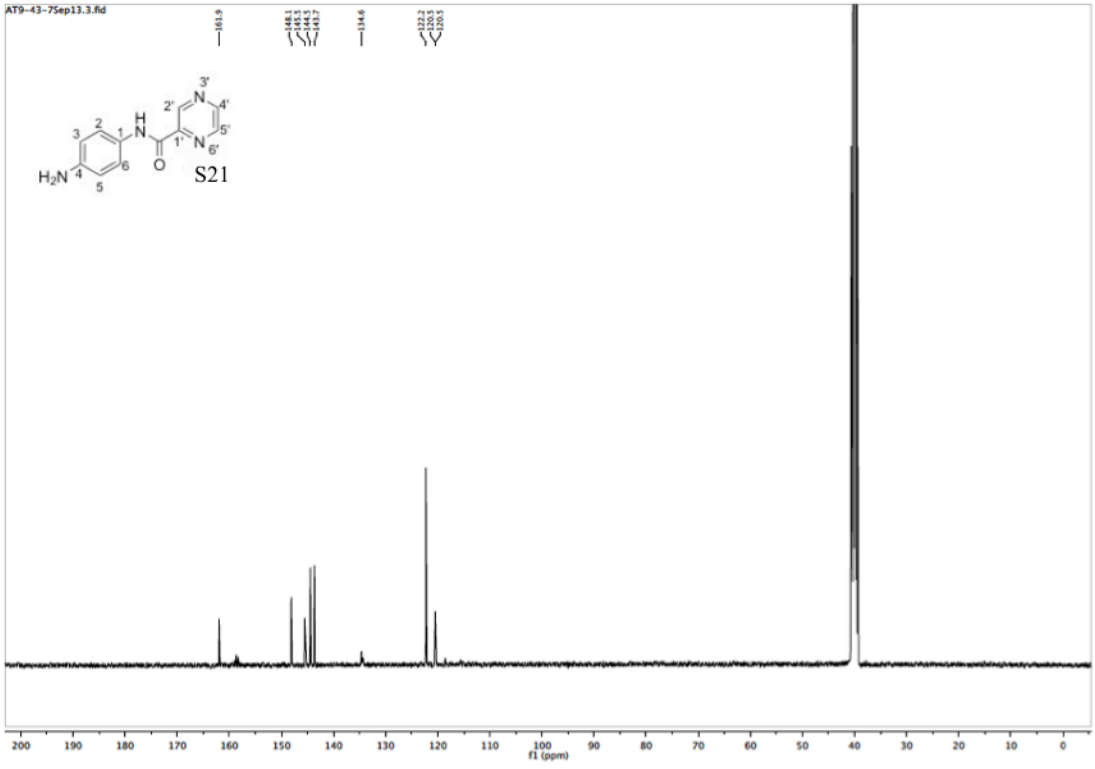
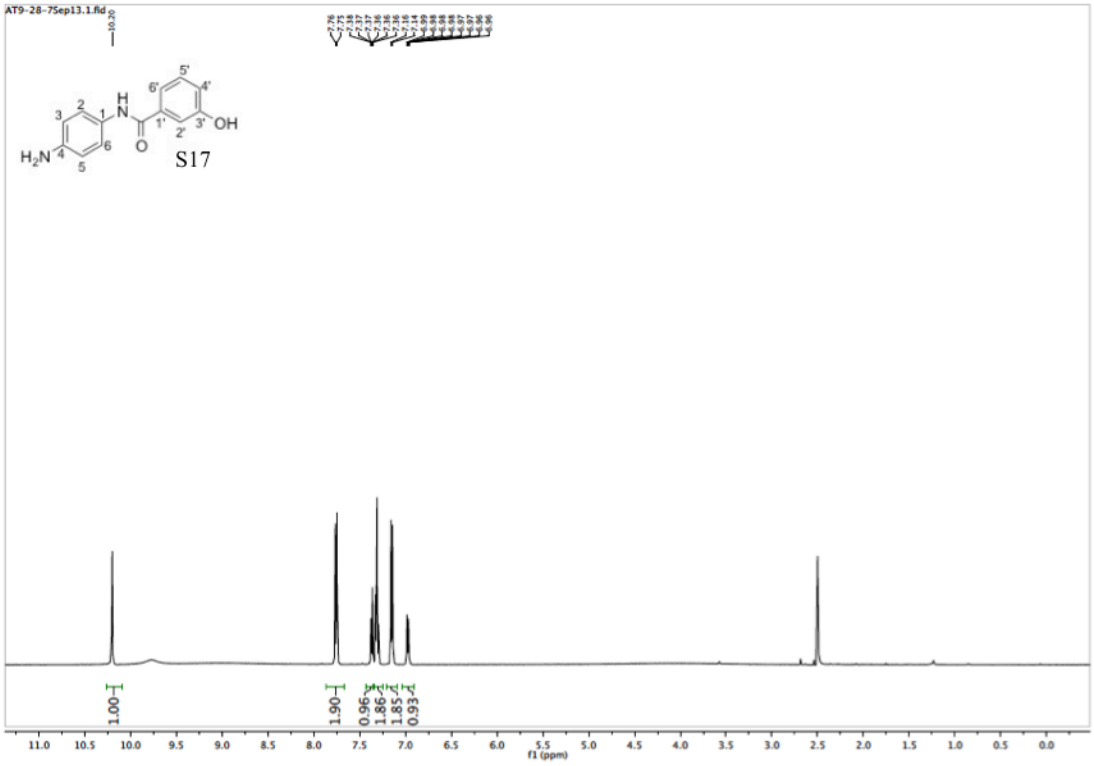
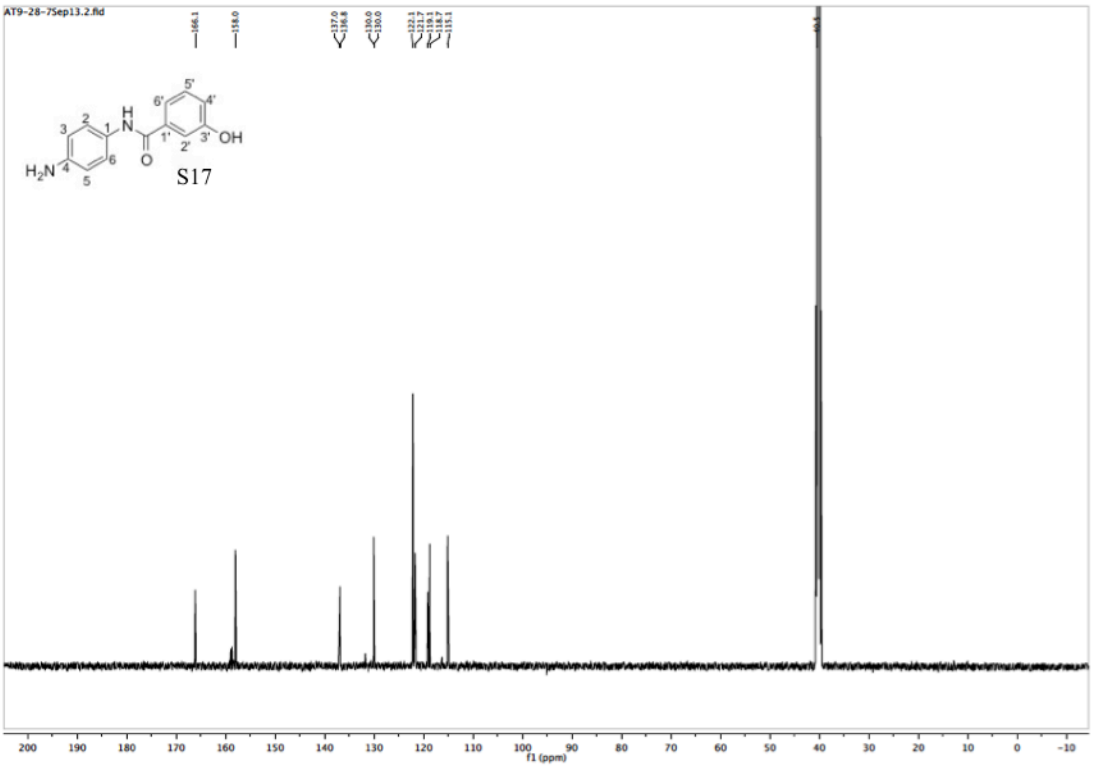


^{1}H NMR (500 MHz, DMSO-d_6)



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



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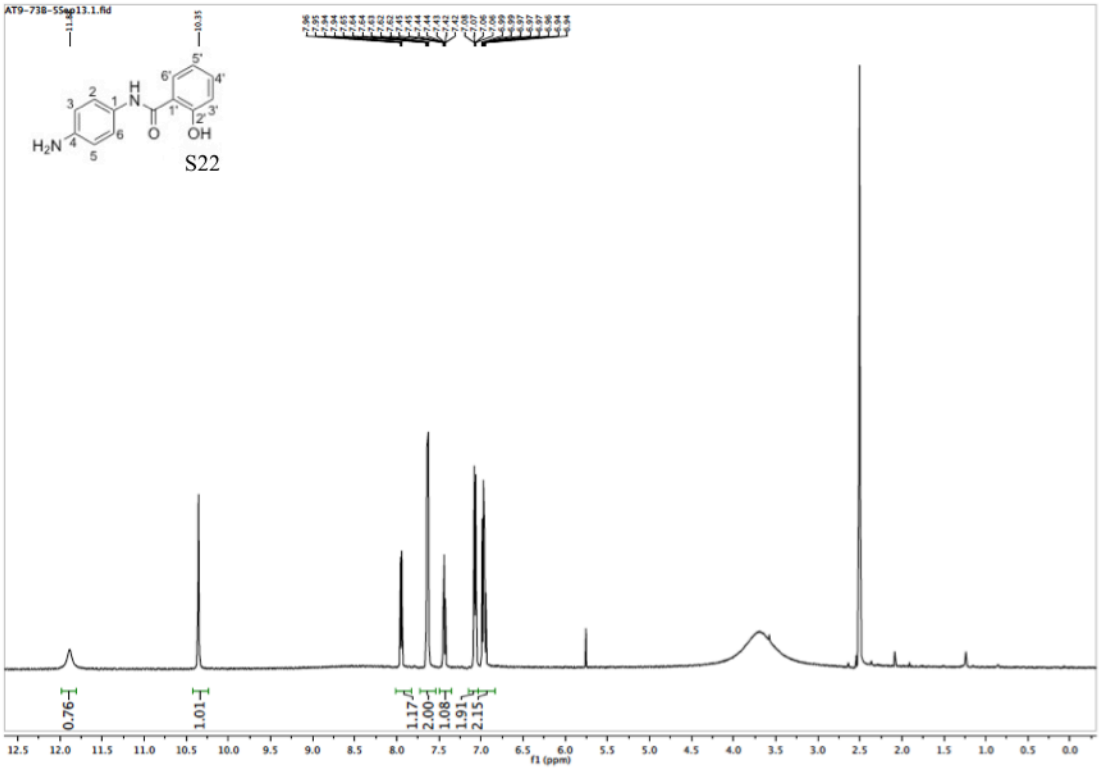
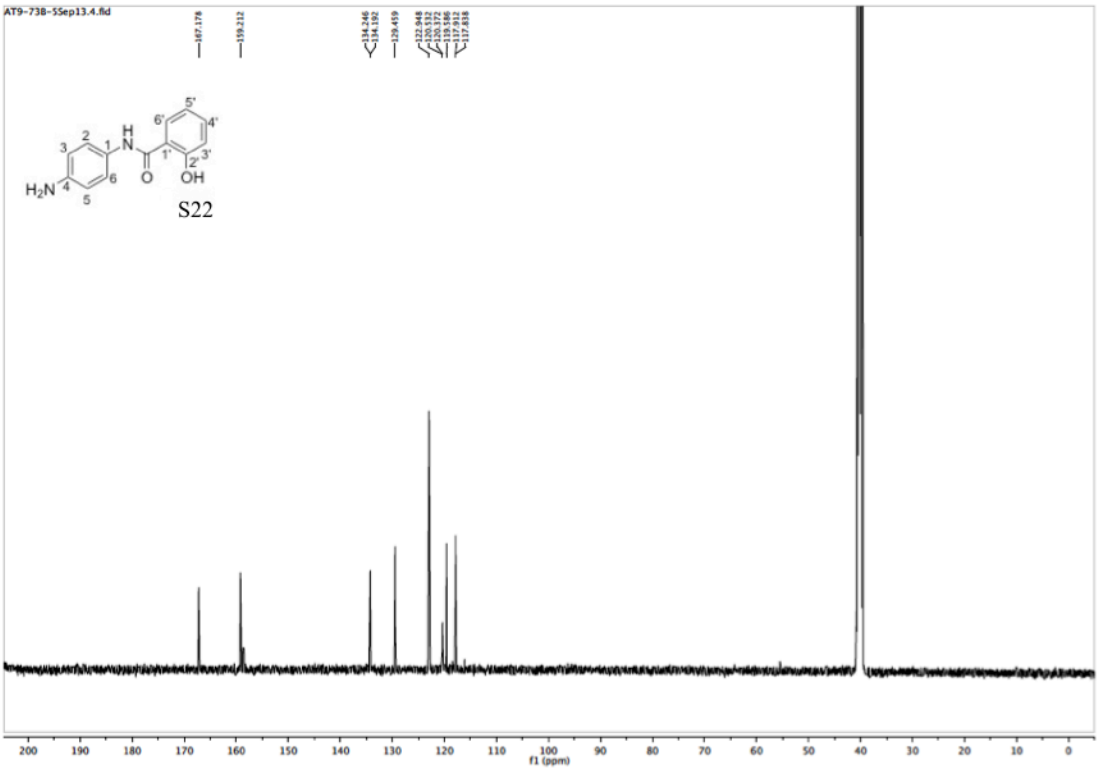


^{13}C NMR (126 MHz, DMSO-d_6)



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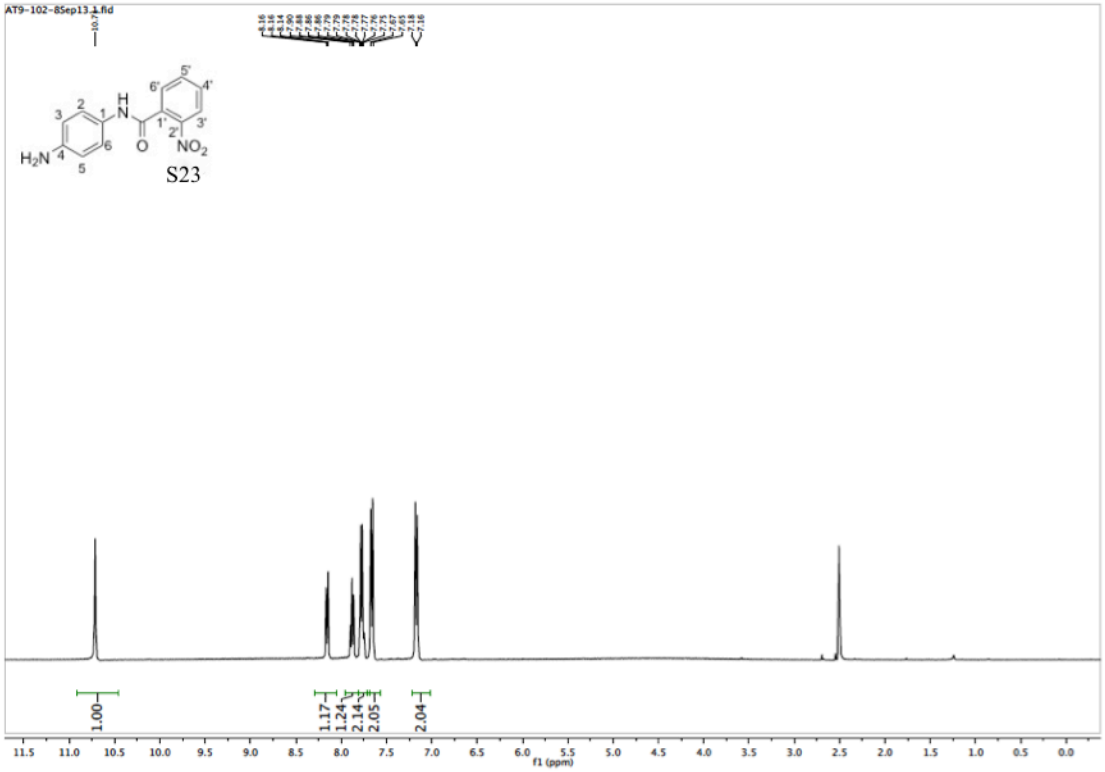
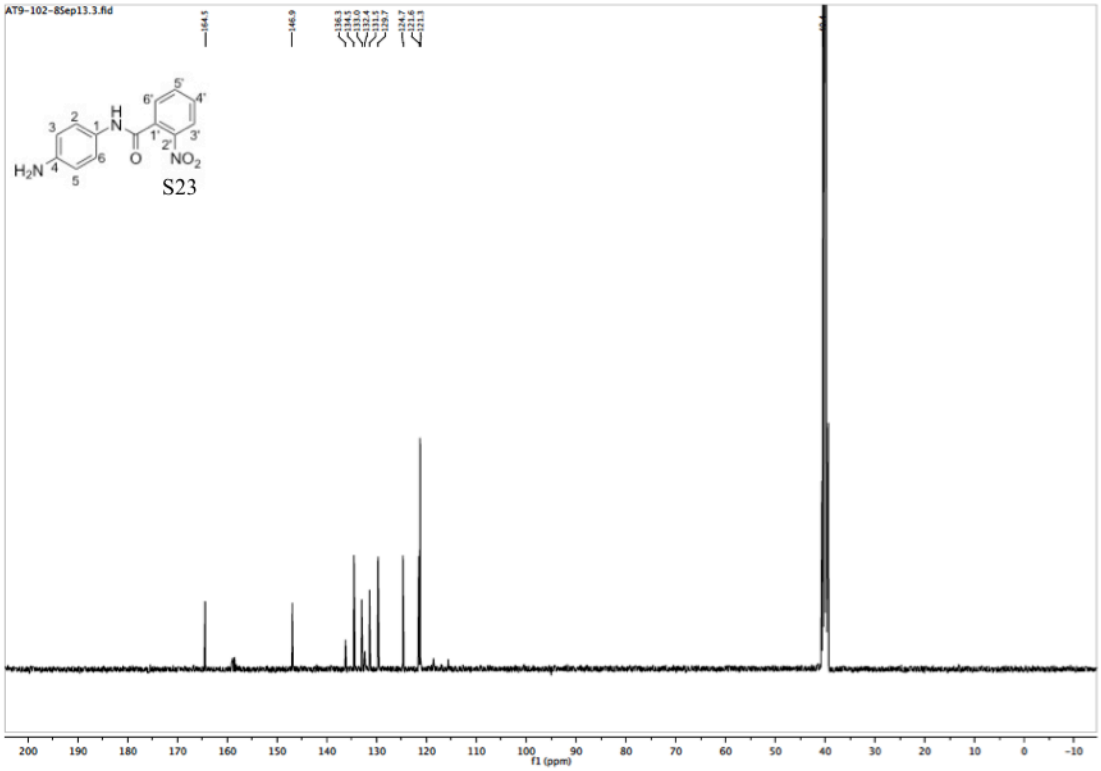


^{13}C NMR (101 MHz, DMSO-d_6)



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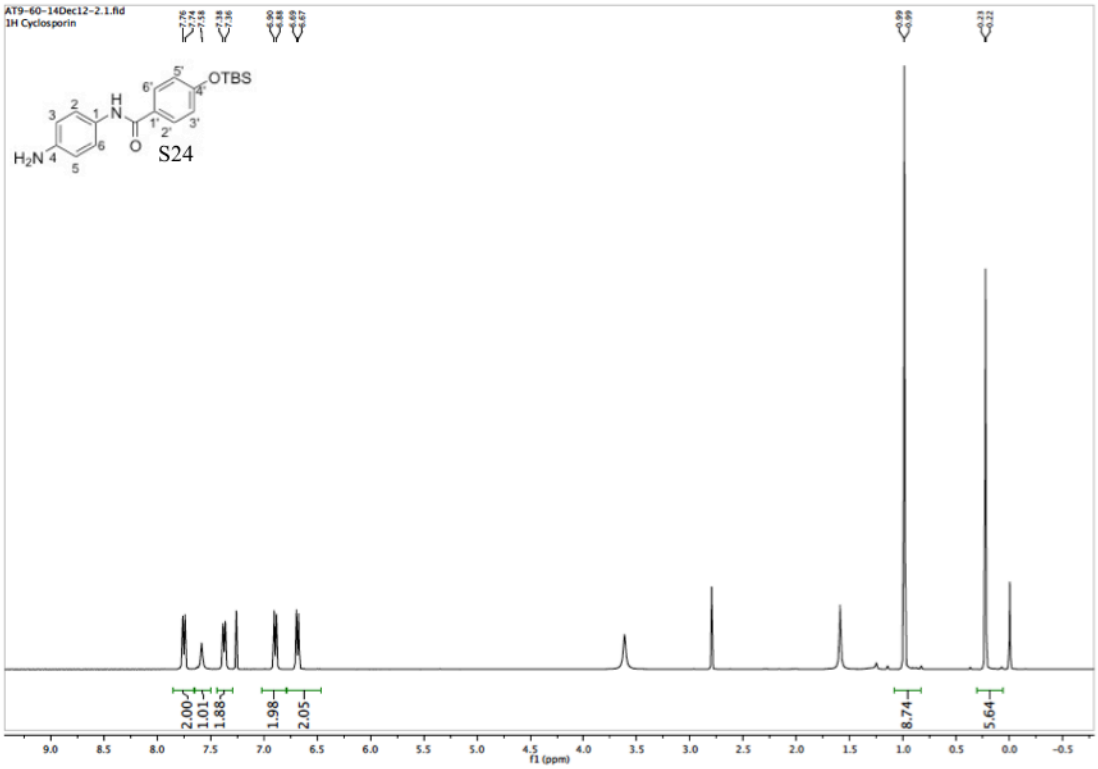
^{1}H NMR (400 MHz, DMSO-d_6)



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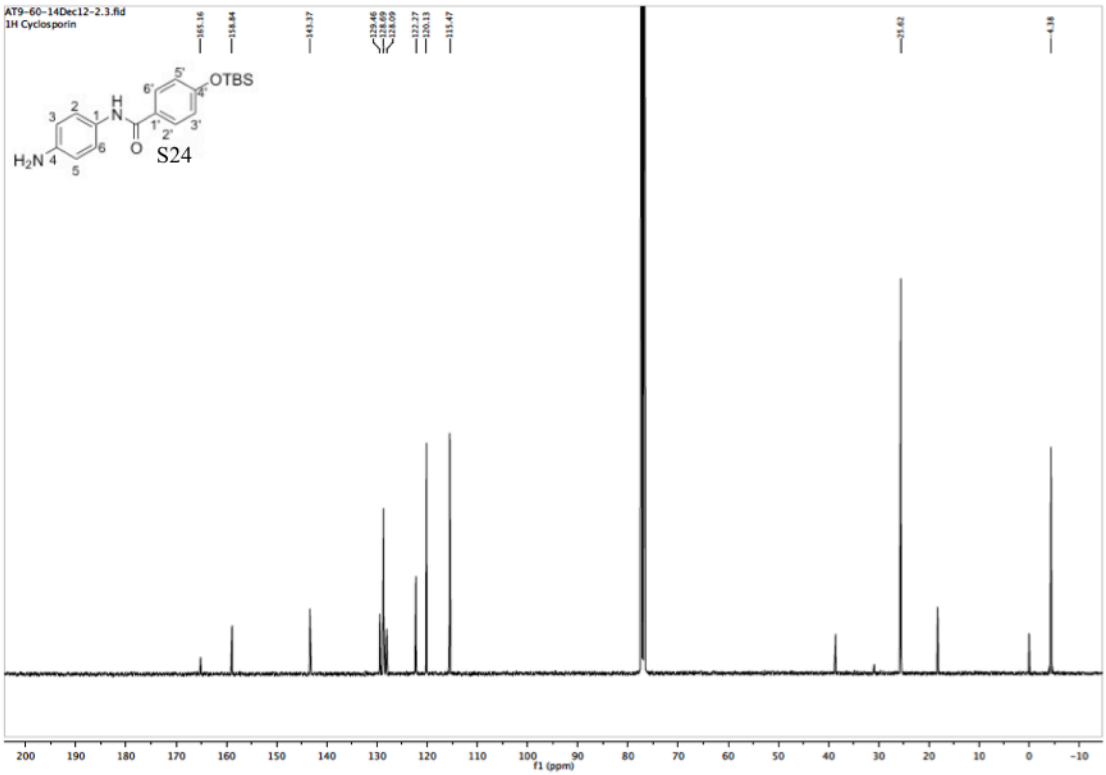

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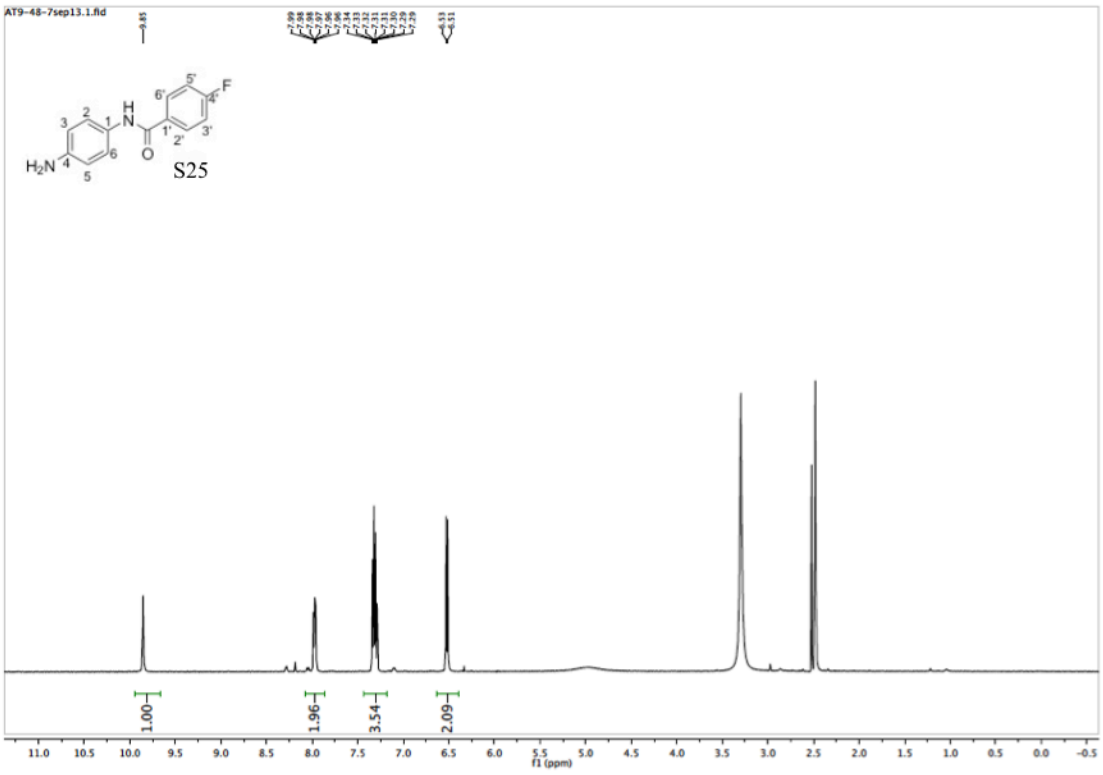
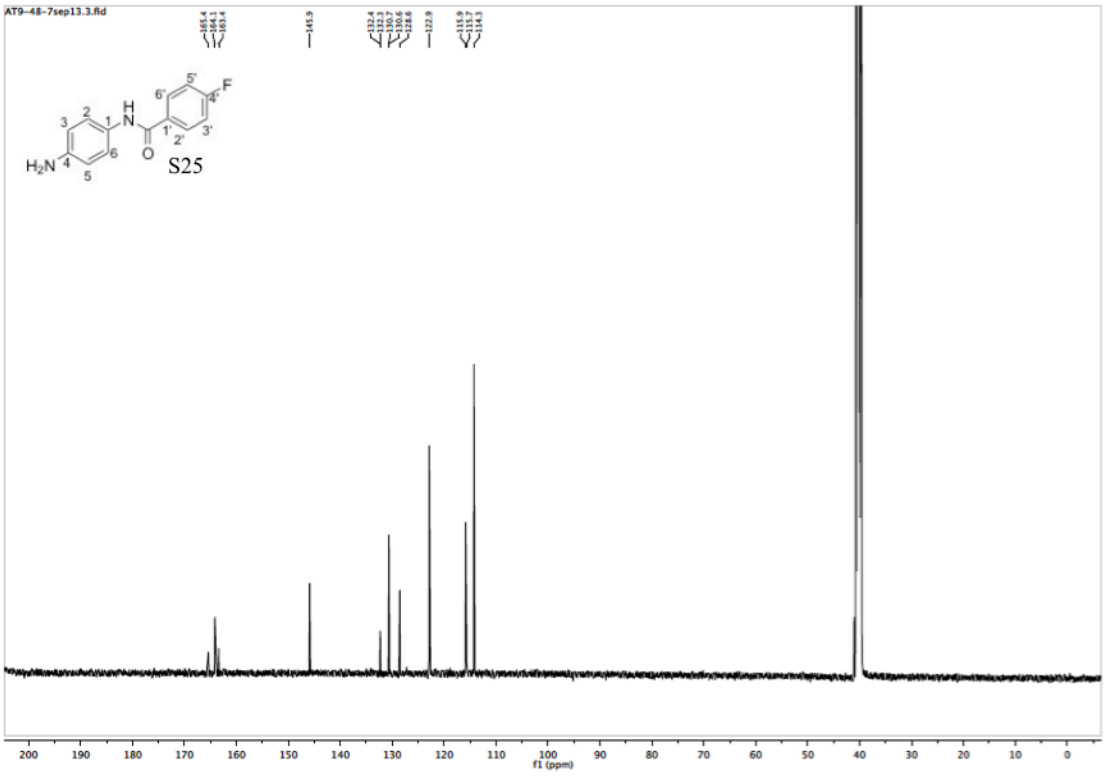


^{13}C NMR (126 MHz, DMSO-d_6)



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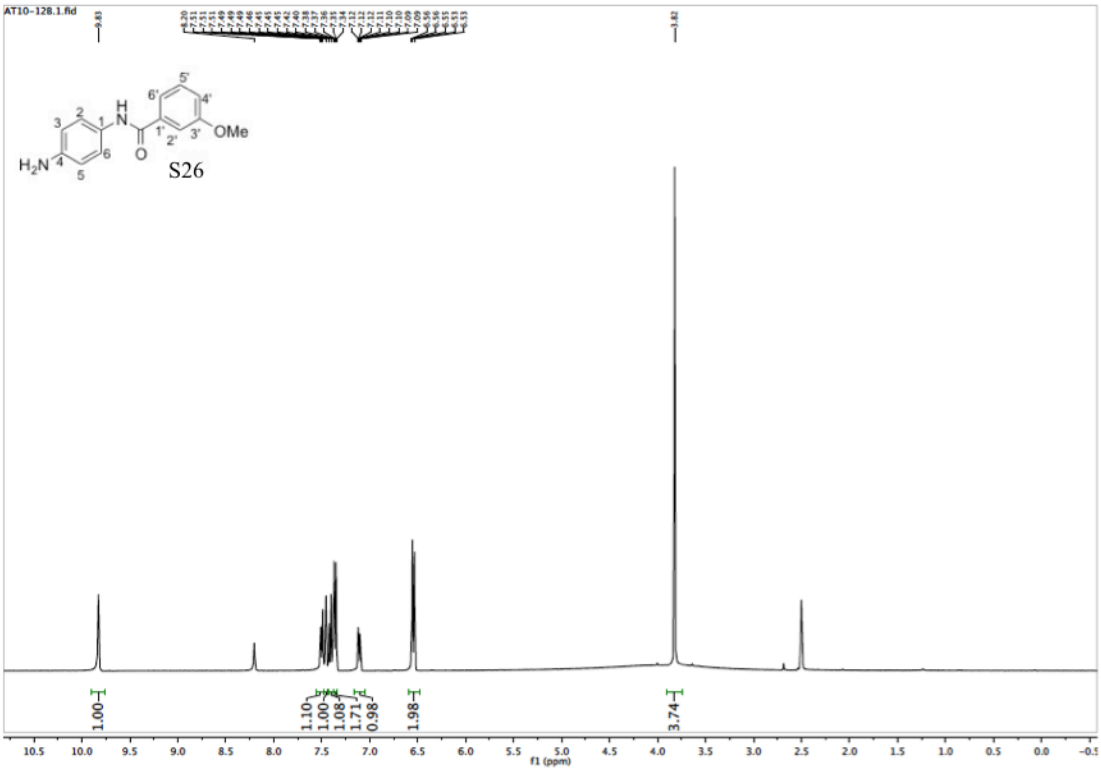
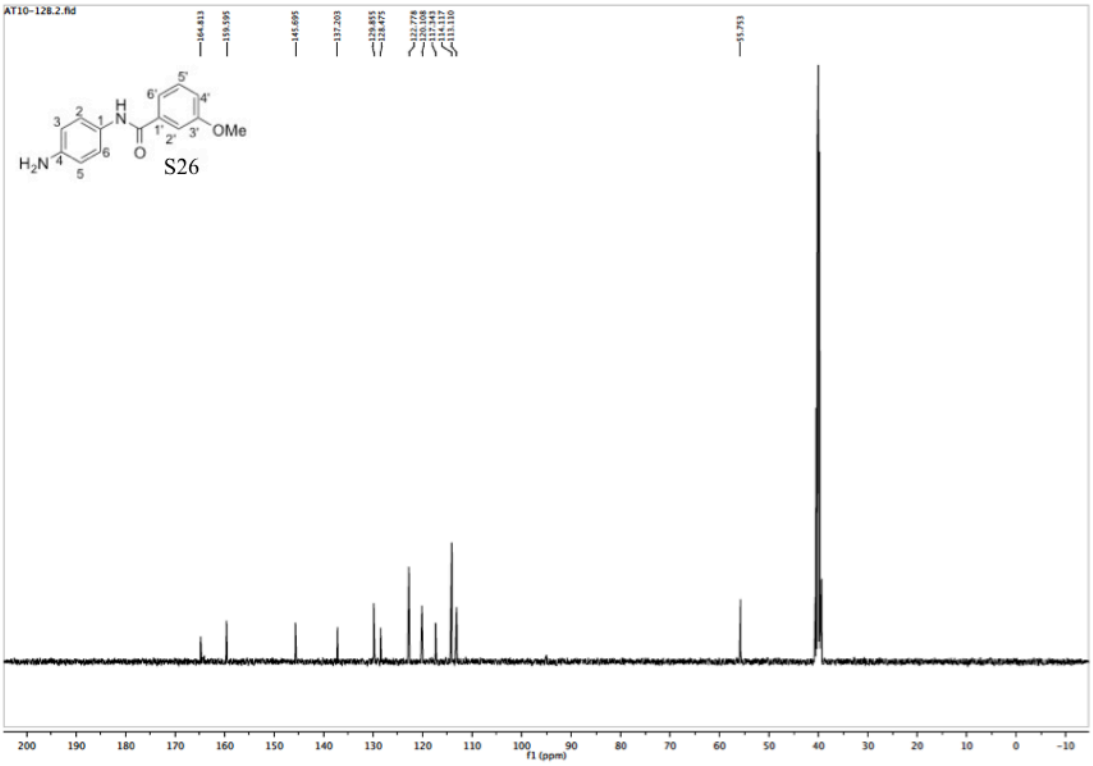
^{1}H NMR (400 MHz, DMSO-d_6)



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



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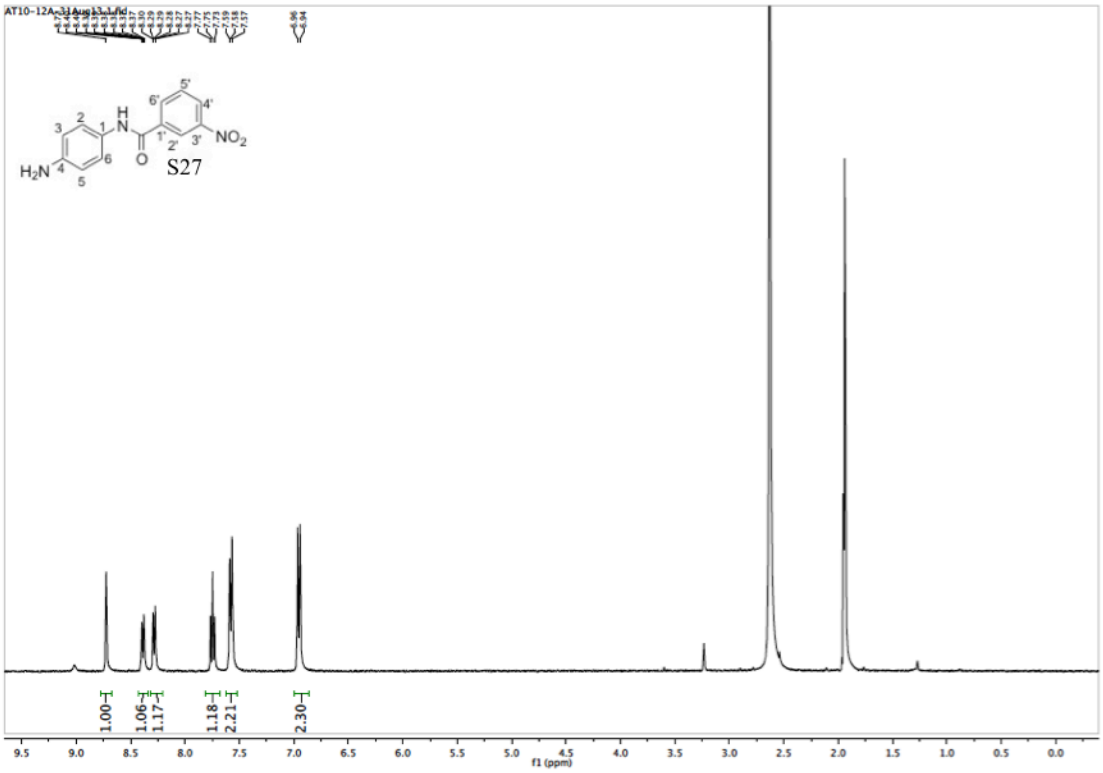
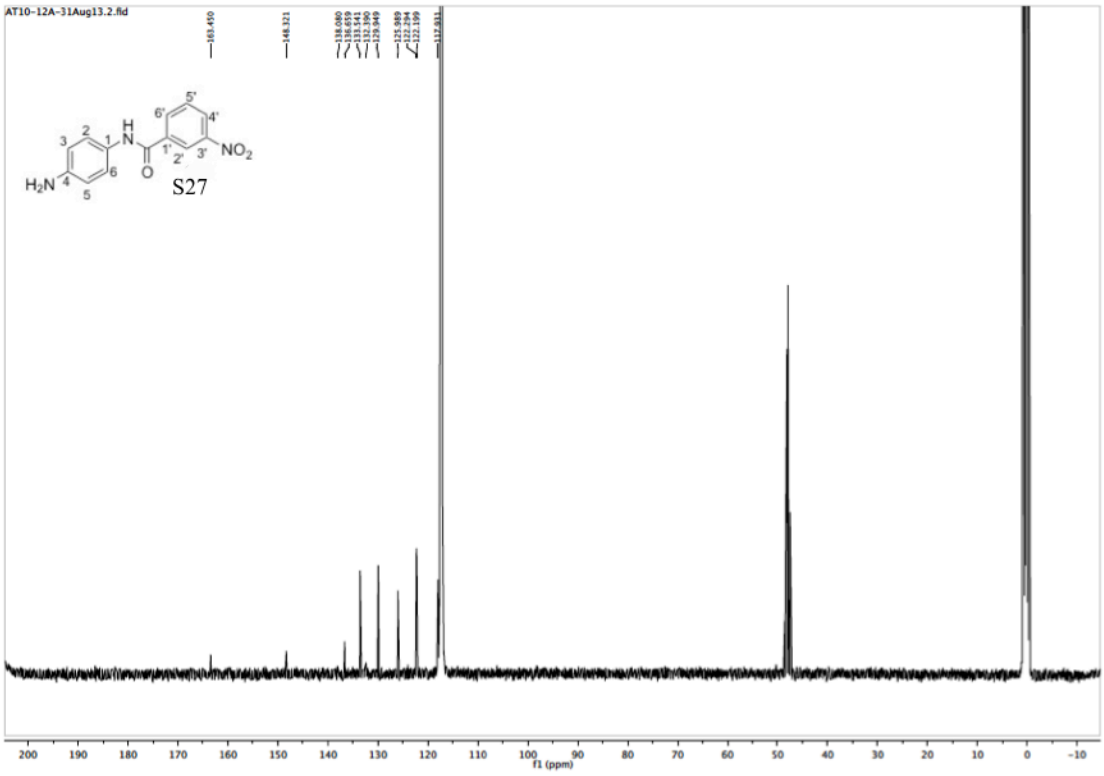


^{13}C NMR (101 MHz, CD_3CN)



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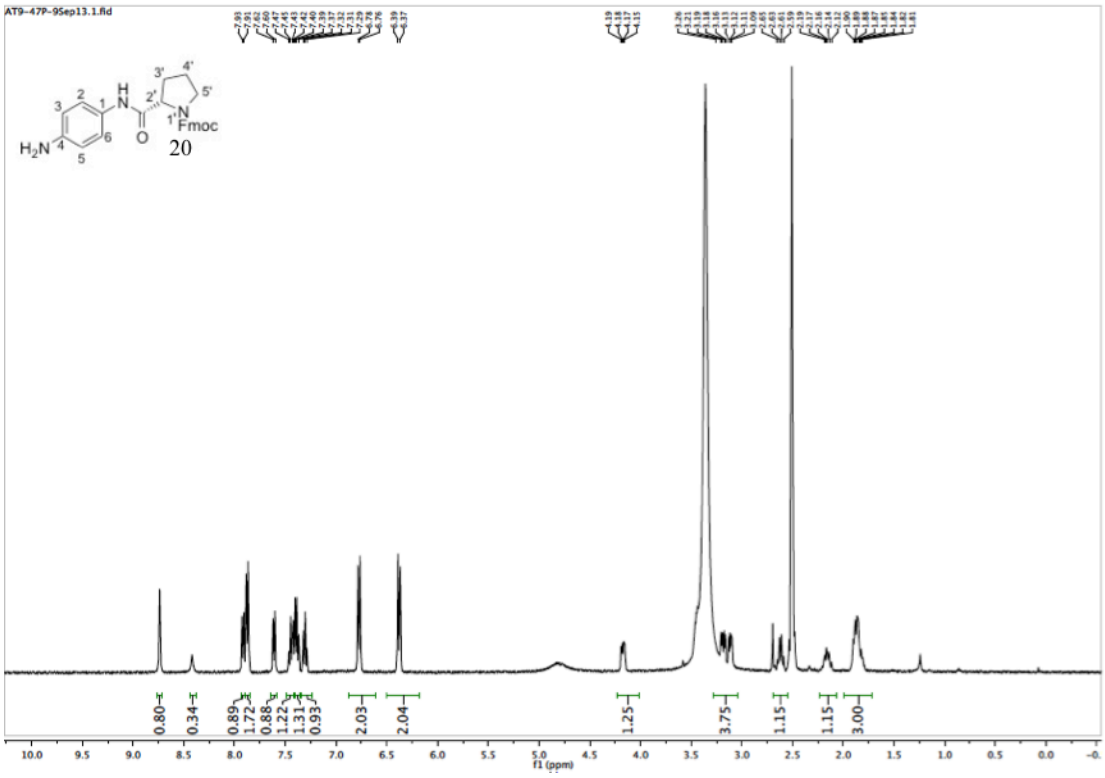

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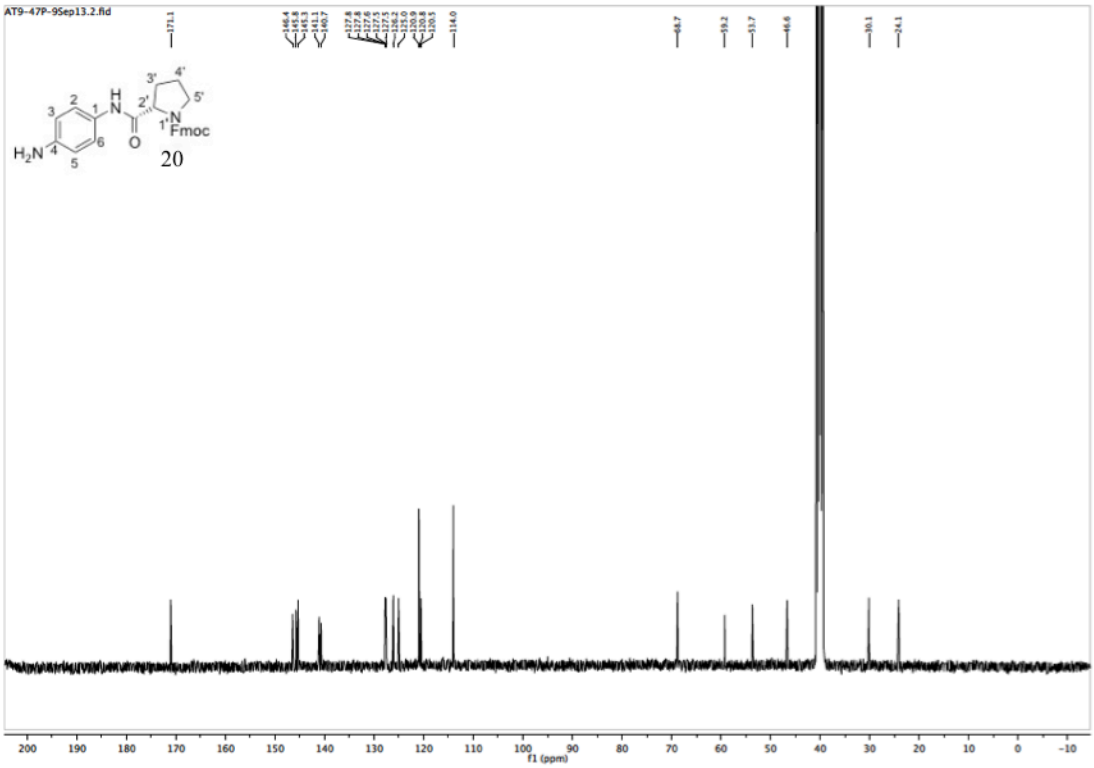

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