Supplementary Information
Discovery of Isoxazolyl-Based Inhibitors of *Plasmodium falciparum* cGMP-Dependent Protein Kinase

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General chemical procedures:
Compound characterization: All reagents and solvents were used as received from commercial suppliers. Compounds were analyzed using a UPLC system with a BEH-C_{18} column (2.1 cm x 50 mm, 0-100% acetonitrile/water gradient over 6 minutes with UV 254 nm detection) and an LCMS 2020 system (LC_{18} 25 cm x 4.6 mm 5 μM column, 0-95% acetonitrile/water gradient over 10 minutes; UV monitor at 220 and 254 nM). Thin layer chromatography was done on silica gel G plates with UV or phosphomolybdic acid detection. Mass spectra were recorded on a CMS system with ESI probe. All of the reported yields are for isolated products and compounds were purified by automated flash chromatography. Proton NMR spectra were obtained at 300 or 400 MHz. All final compounds had purities of at least 95% based on ^{1}H NMR and UPLC analyses.

Synthetic Procedures:

General route for synthesis of 7 and phenyl substituted derivatives:

Synthesis of **12** and **13a-c**: See *ACS Med. Chem. Lett.* 2018, **9**, 210-214.

Oxime formation: 1 equivalent of aldehyde was stirred at room temperature in methanol (~0.3M) in the presence of 1.1 equivalent of hydroxylamine hydrochloride and 1.5 equivalents of sodium acetate until TLC indicated complete consumption of aldehyde. The reaction mixture was poured over ice to precipitate product that was washed with water and dried.

Oxime chlorination: 1 equivalent of oxime was dissolved in DMF (~0.3M) at room temperature in the presence of 1.2 equivalents of recrystallized N-chlorosuccinimde. The reaction was stirred at room temperature until TLC indicated consumption of oxime. The reaction mixture was poured over ice to precipitate a solid that was collected by filtration and dried.

Pyrimidine acylation: 4-methyl-2-thiomethylpyrimidine (1 equivalent) was dissolved at room temperature in THF (~0.1M). 1.1 equivalents of ethyl glyoxylate was added, followed by 3 portions over 30 minutes of 1.2 equivalents of potassium t-butoxide. The resulting suspension was stirred at room temperature for 3 hours, then poured into saturated ammonium chloride solution and extracted with 4 portions of ethyl acetate. The collected organic layers were washed with brine, dried and concentrated to provide a yellow solid ketoester that was used without further purification.

Cycloaddition to form **7** and phenyl substituted derivatives: The ketoester above was dissolved at room temperature in absolute ethanol (~0.4M). To this, 1.5 equivalents of the appropriate chloro-oxime and 4 equivalents of triethylamine were added. The solution was stirred at room temperature overnight, poured into water and extracted with four portions of ethyl acetate. The collected organic extracts were washed twice with brine, dried and concentrated. The crude product was purified by silica gel chromatography eluting with an appropriate ethyl acetate-hexanes mixture to furnish the isoxazole ester product.

Oxidation of methyl sulfide to methyl sulfone (e.g. **7** and **10**): Pyrimdinyl methyl sulfide was dissolved at room temperature in a mixture of methanol/distilled water (2:1 ratio, final concentration ~0.2M). Oxone (potassium peroxymonosulfate, 2.2-2.5 equivalents) was added at room temperature and the reaction was stirred overnight. The reaction mixture was poured into water and extracted with 4 portions of ethyl acetate. The collected organic extracts were washed with brine, dried, concentrated and purified by CombiFlash silica gel chromatography eluting with a mixture of ethyl acetate/hexane to provide the desired sulfone as a white solid.

Reduction of isoxazolyl ester to alcohol (e.g. **9**): The isoxazolyl ester cycloaddition product was dissolved in a 2:1 mixture of THF/methanol at room temperature (~0.2M) and cooled to -10^{o}C in an ice-salt bath. Sodium borohydride (2 equivalents) was added in one portion on small scale (less than 3 mmol), or portionwise in 3 batches every 10 minutes (larger than 3 mmol). The reaction was stirred at -10^{o}C until TLC indicated consumption of starting material. The reaction mixture was poured into saturated ammonium chloride solution and extracted with 4 portions of ethyl acetate. Product was isolated by CombiFlash silica gel chromatography eluting with a mixture of ethyl acetate/hexane to provide the desired alcohol as a white solid.

Amine displacement of sulfone (e.g. **19a-d)**: The appropriate sulfone (e.g. **18**) was dissolved in reagent grade DMSO (~0.3M) at room temperature. Triethylamine (3 equivalents) were added, followed by 2 equivalents of the desired Boc-protected diamine. The reaction was heated under nitrogen to 65^{o}C overnight. After cooling to room temperature the reaction mixture was poured into brine solution and extracted 4 times with ethyl acetate. The collected organic extracts were washed with brine, dried and concentrated. The desired product was isolated by CombiFlash silica gel chromatography eluting with 5-10% methanol in dichloromethane to furnish pure product.

Conversion of isoxazolyl alcohol to corresponding amine: Dissolve isoxazolyl alcohol in dichloromethane (~0.3M) at room temperature then cool in ice bath. Add 2 equivalents each of triphenylphosphine and carbon tetrabromide at 0^{o}C. Stir at 0^{o}C for 10 minutes then warm to room temperature and stir for 3-5 hours, following by TLC for disappearance of alcohol. Concentrate reaction to dryness and purify by CombiFlash silica gel chromatography eluting with a mixture of ethyl acetate/hexane to afford the desired bromomethyl intermediate. This material was then dissolved at room temperature in acetonitrile (~0.3M) to which 1.2 equivalents of trimethylamine was added, followed by 1.2 equivalents of the appropriate amine. The reaction mixture was stirred at room temperature for 4-6 hours until TLC indicated consumption of the starting bromide. The reaction was concentrated on a rotary evaporator. The resulting crude material was dissolved in a mixture of dichloromethane and saturated sodium bicarbonate. The organic phase was washed with two more portions of saturated bicarbonate, dried and concentrated. The product was purified by CombiFlash silica gel chromatography eluting with 5-10% methanol in dichloromethane to furnish the desired amine product.

Boc-group cleavage and amine acylation (**8**, **9**, **11a**-**c**, **16a**-**m**, **19a**-**d**): The appropriate Boc-protected diamine intermediate was dissolved at room temperature in dichloromethane (~0.3M).

Excess trifluoroacetic acid (at least 5 equivalents) was added at room temperature and the reaction stirred at room temperature until TLC indicated consumption of starting material. The reaction mixture was concentrated on a rotary evaporator to afford a thick oily residue. This material was dissolved in reagent grade DMF at room temperature (~0.2M) to which was added at least 5 equivalents of trimethylamine, followed by 1.3 equivalents of HATU and 1.2 equivalents of the desired carboxylic acid. The reaction was stirred at room temperature overnight. In the morning, the reaction mixture was poured into 5 volumes of water and extracted with 4 portions of ethyl acetate. The collected organic extract was washed with two portions of brine, dried and concentrated. The product was purified by CombiFlash silica gel chromatography eluting with 5-10% methanol in dichloromethane to furnish the desired amine product.

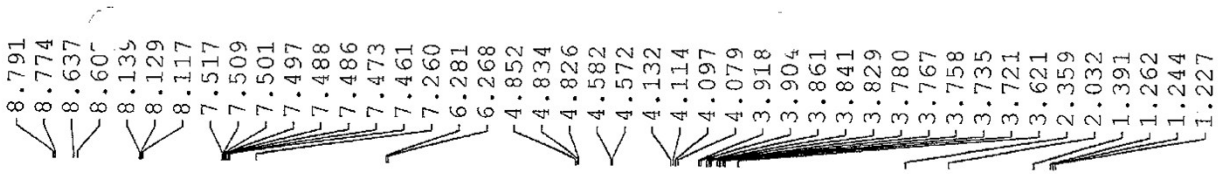
17a/b: Amine **15a** was dissolved in dichloromethane at room temperature (0.1M) to which was added 2 equivalents of each aldehyde, followed by 3 equivalents of sodium triacetoxyborohydride and two drops of acetic acid. After stirring overnight at room temperature, the reaction was concentrated by rotary evaporation. The residue was dissolved in ethyl acetate and washed twice with saturated sodium bicarbonate and brine. The organic layer was dried and concentrated and the desired products isolated by CombiFlash silica gel chromatography eluting with 10% methanol in dichloromethane to furnish the desired amine product.

17c: 30 mg (0.07 mmol) of amine **15a** was dissolved in 2 ml dichloromethane at 0^{o}C. 1.2 equivalents (15.3 mg) of pyrazole sulfonyl chloride was added, followed by 29 μl (21 mg, 0.21 mmol, 3 equivalents) trimethylamine. The reaction was stirred in an ice bath for 10 minutes, then diluted with 10 ml dichloromethane. The mixture was extracted with two portions of 1N aqueous HCl and brine, dried and concentrated. The desired product was isolated by CombiFlash silica gel chromatography eluting with 5% methanol in dichloromethane.

PfPKG assay procedure:

PfPKG kinase activity was assayed as described[7] using the commercial immobilized metal ion affinity-based fluorescence polarization (IMAP) assay (Molecular Devices, San Jose, CA.). Briefly, at room temperature the kinase solution was preincubated with inhibitor at various concentrations for 15 minutes. The kinase assays (20 μl) contained 10 mM Tris-HCl, pH 7.2, 10 mM MgCl2, 0.05% NaN3, 0.01% Tween®20 (RB-T) and 17 ng of recombinant PfPKG. Reactions were initiated with addition of 24 nM fluorescent substrate (FAM-PKAtide) with 10 μM ATP, 1 μM cGMP, 1.0 mM DTT prepared in RB-T. Following a 1 hour incubation, 60 μL of the PBR mixture (Progressive Binding Reagent (PBR) mixture was made according to the commercial protocol for FAM-PKAtide substrate. 1X IMAP Progressive Binding Buffer A, Progressive Binding Reagent diluted 400-fold) was added and incubated for 30 minutes. Fluorescent polarization was read in parallel and perpendicular with an excitation wavelength of 485 nm and an emission wavelength of 528 nm using a Synergy 2 Microplate reader (BioTek, Winooski, VT). The data was analyzed using four parameter logistic curve using Microsoft Excel Solver and dose response curves were generated using Microsoft Excel.

Compound characterization data



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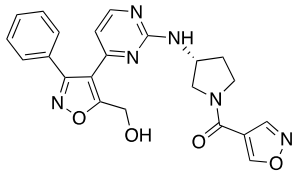

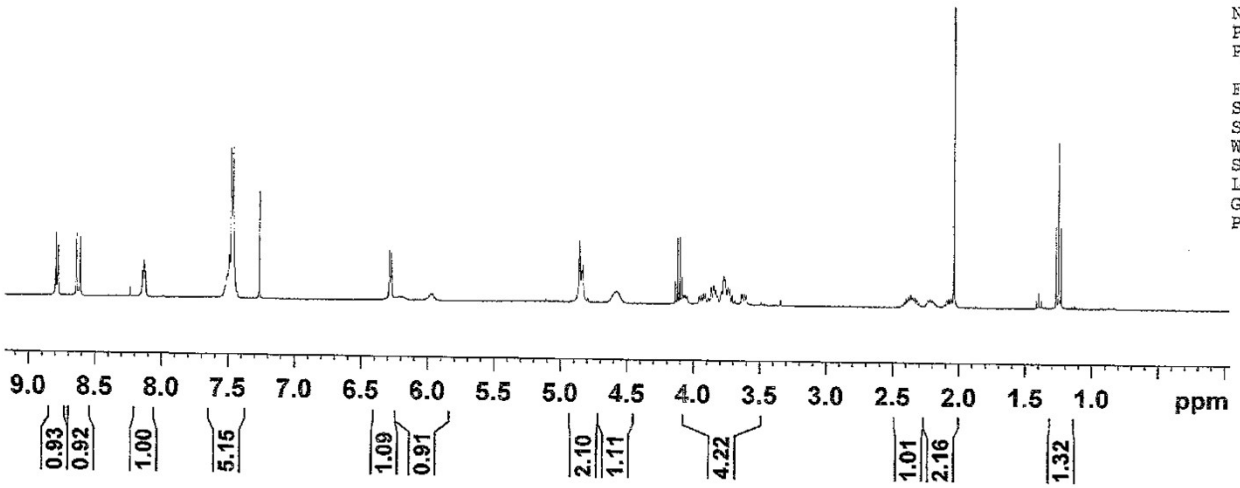

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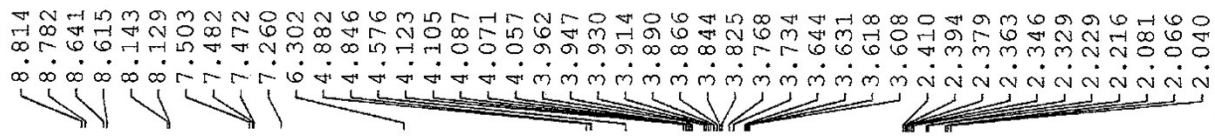

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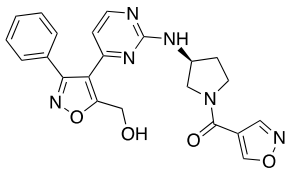

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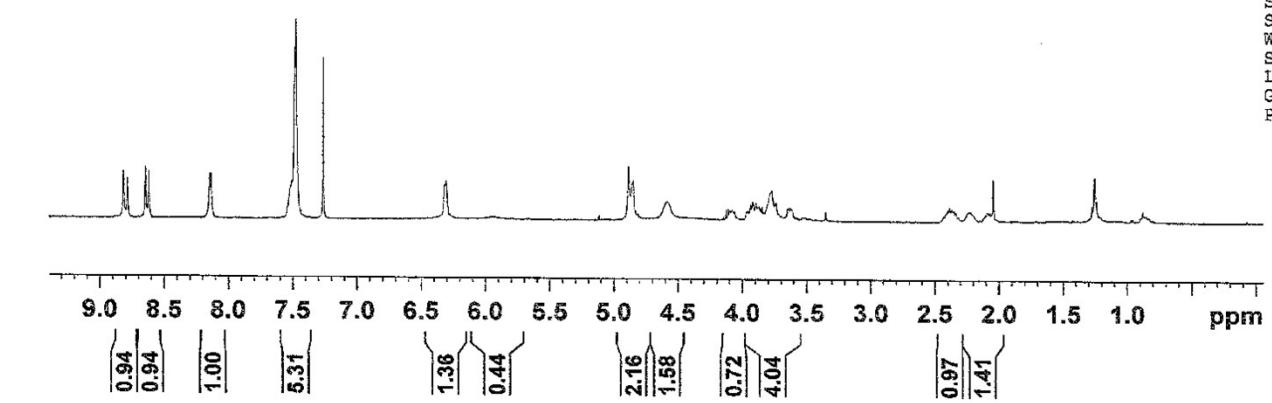

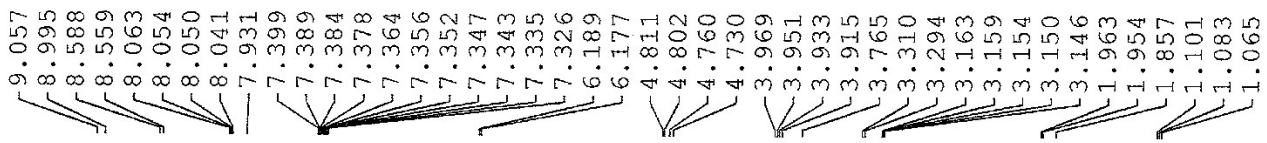

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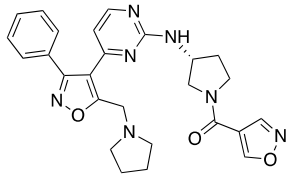

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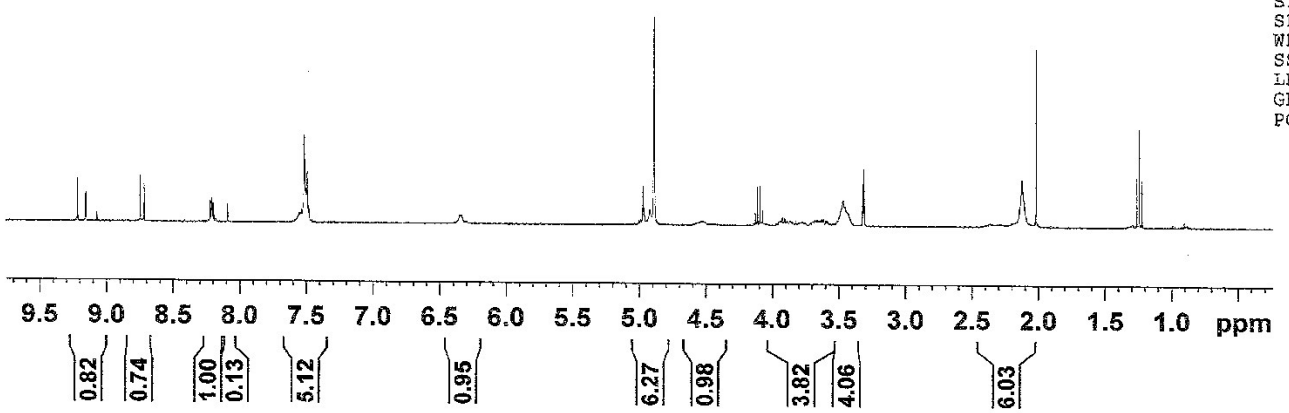

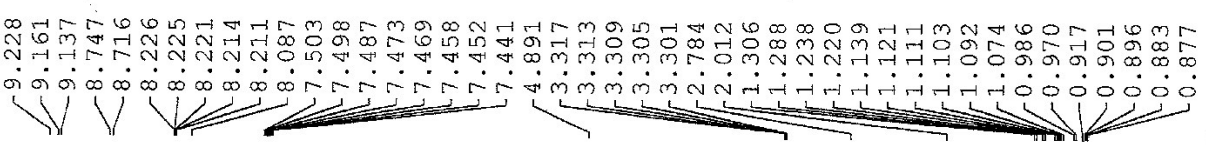

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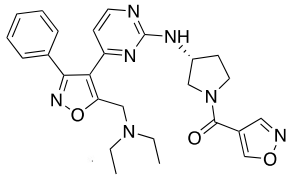

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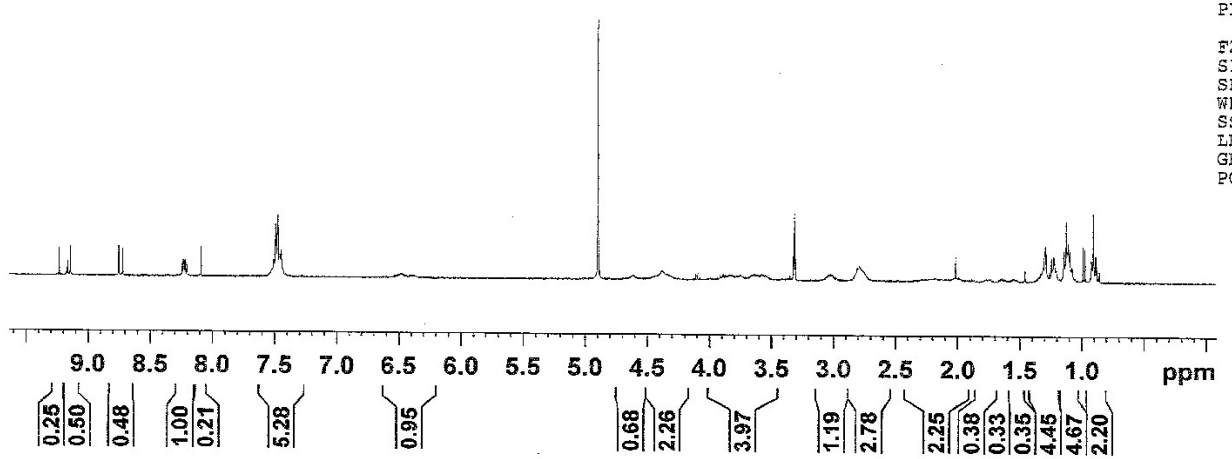

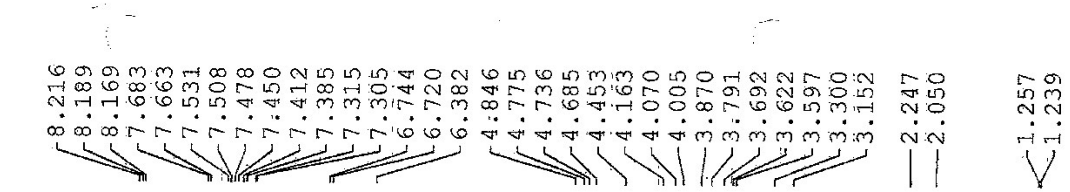

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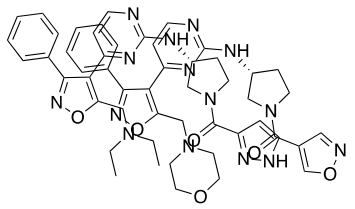

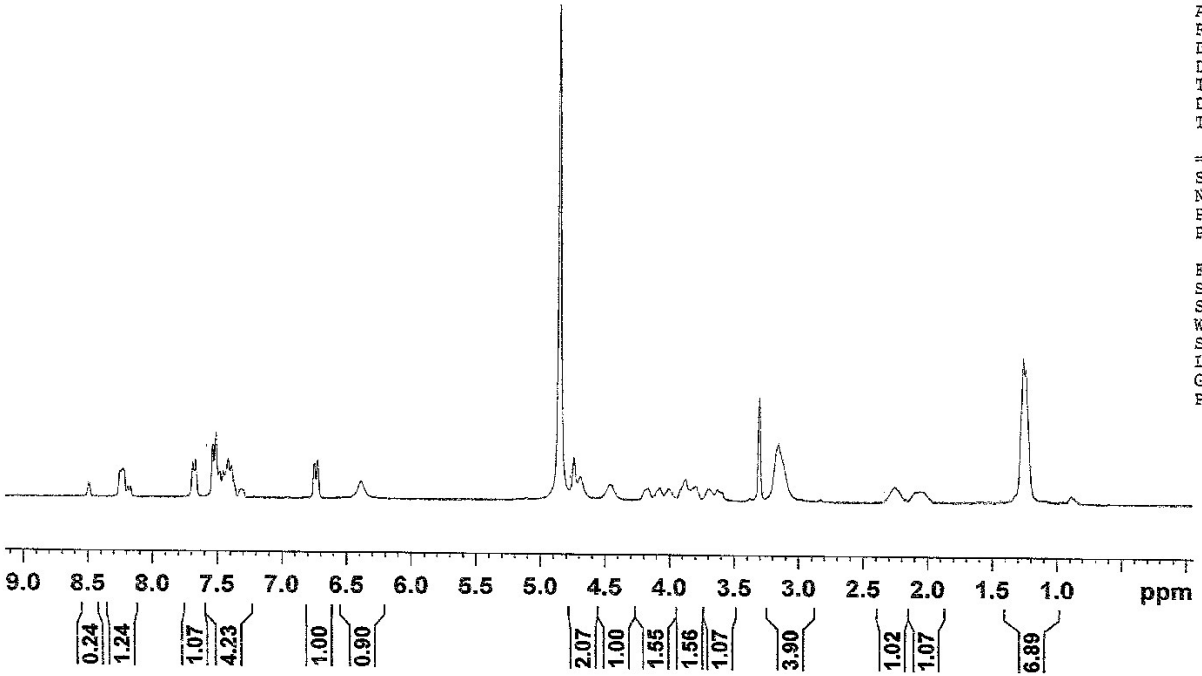

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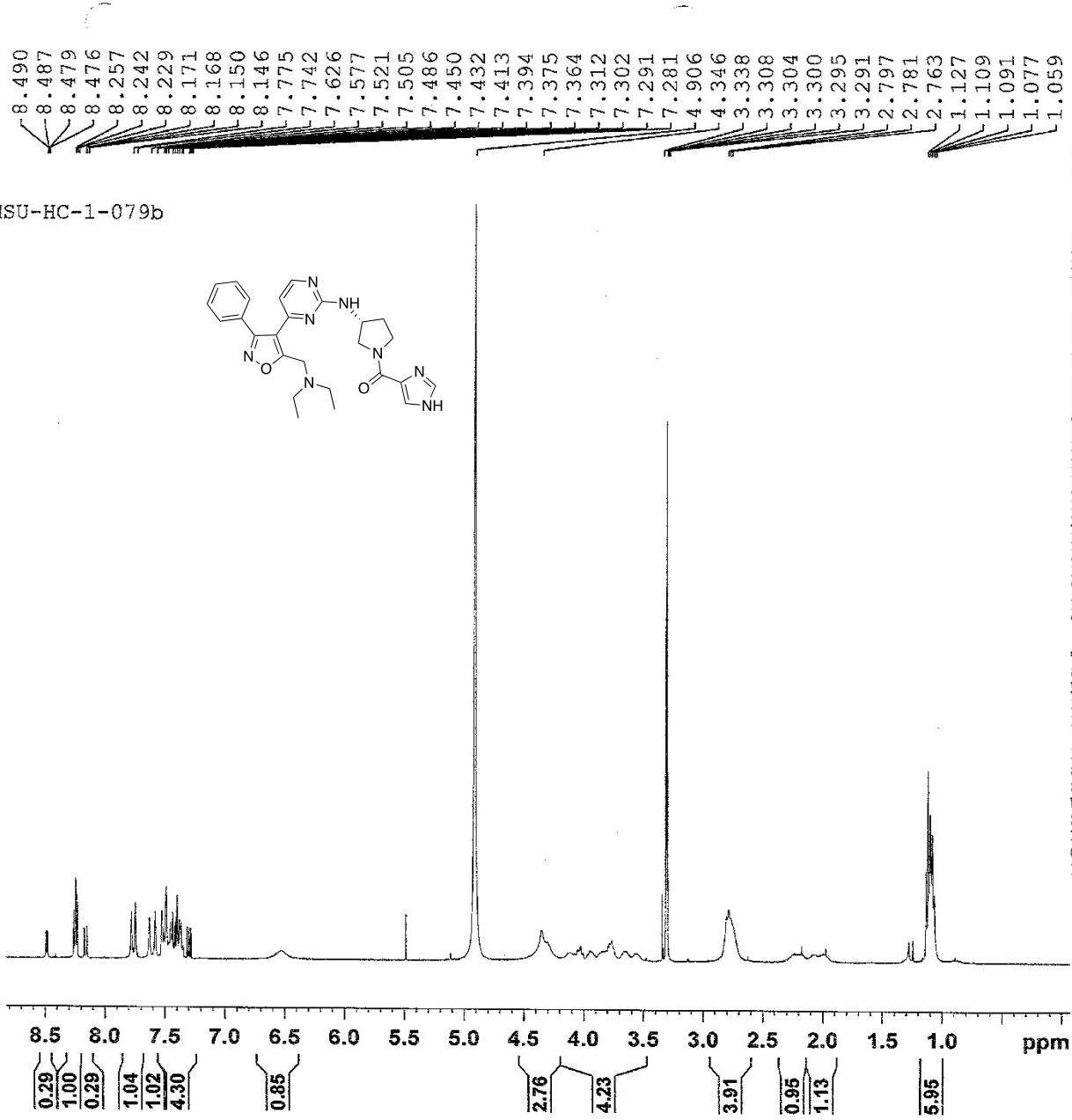

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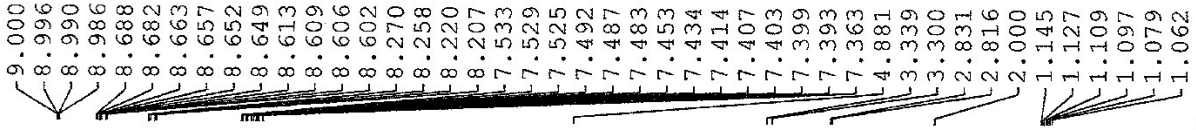

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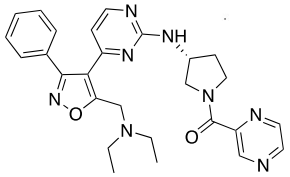

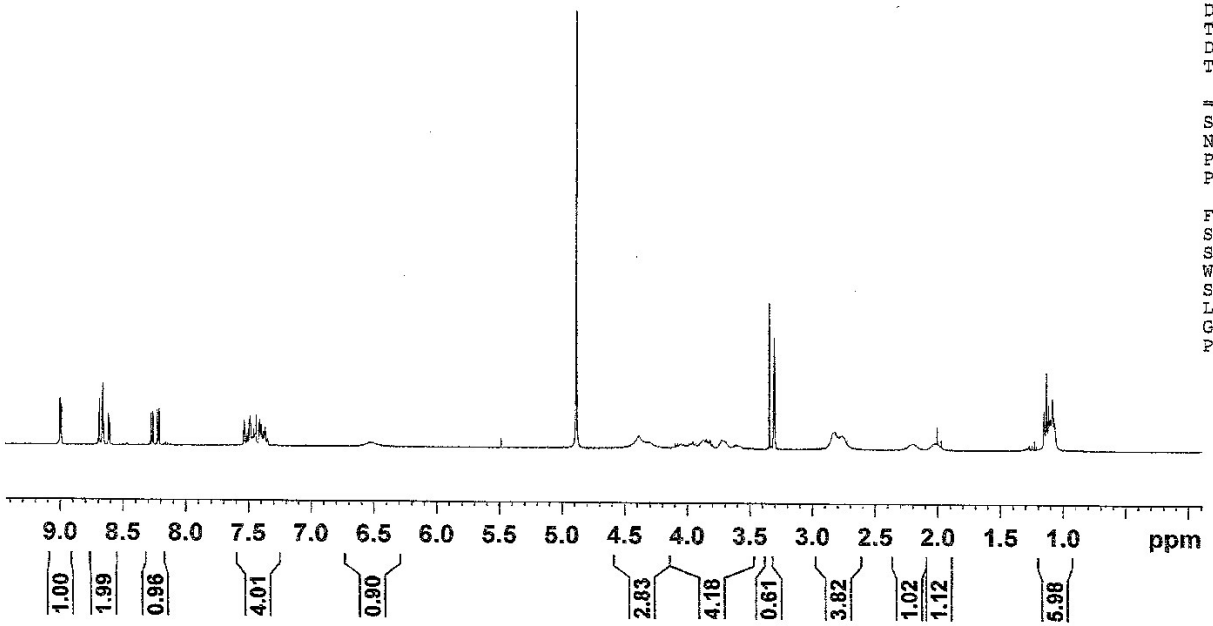

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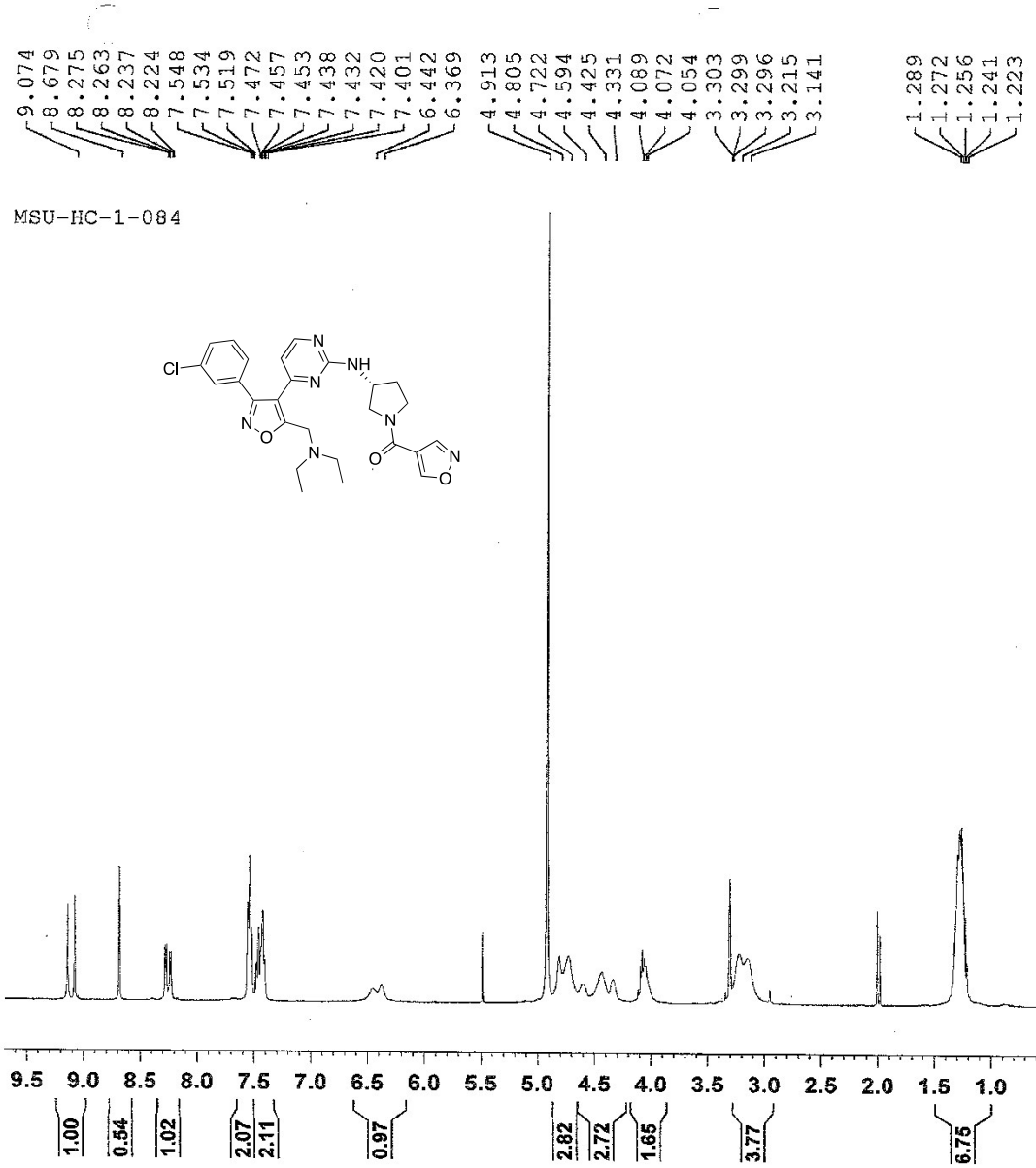

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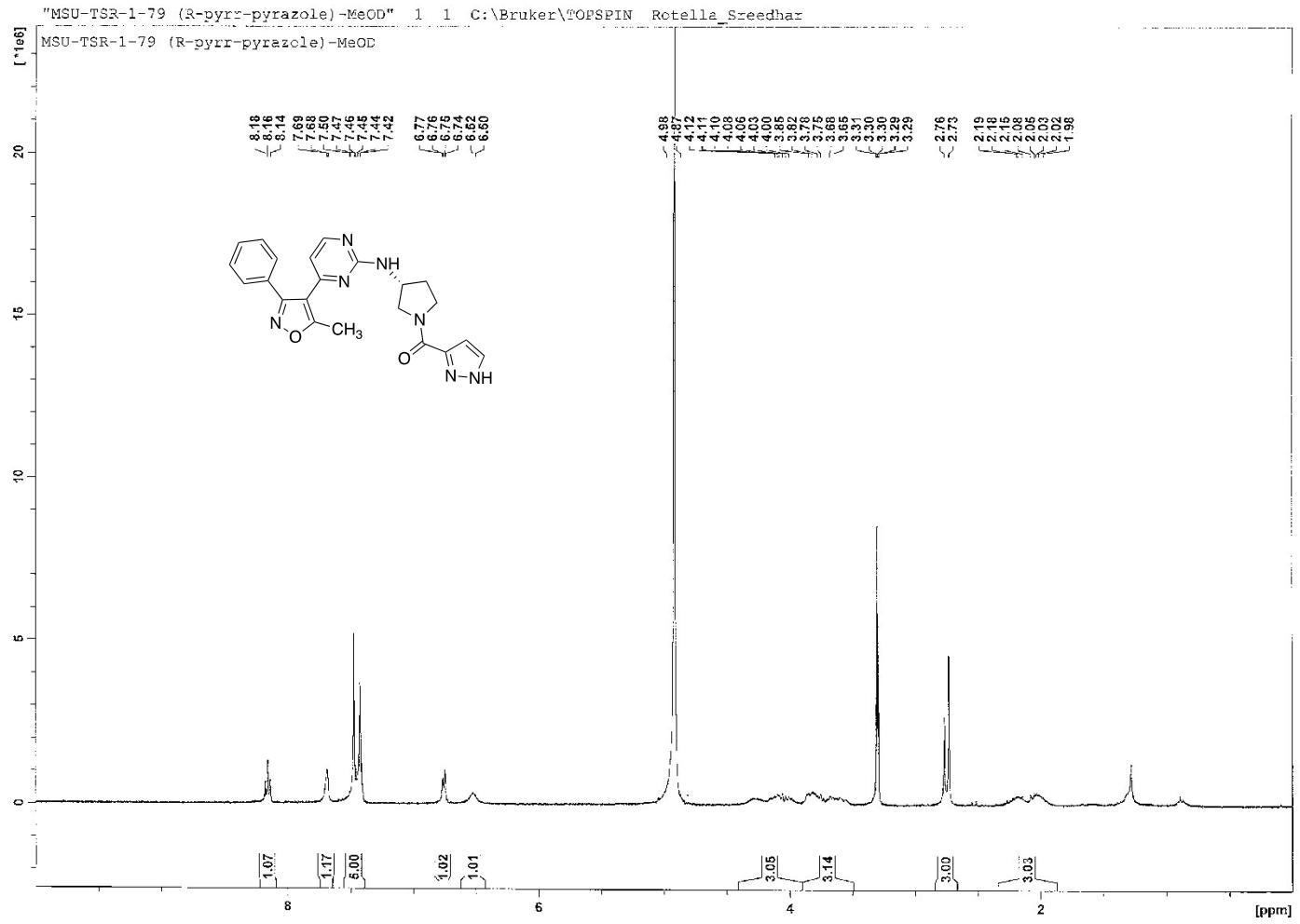
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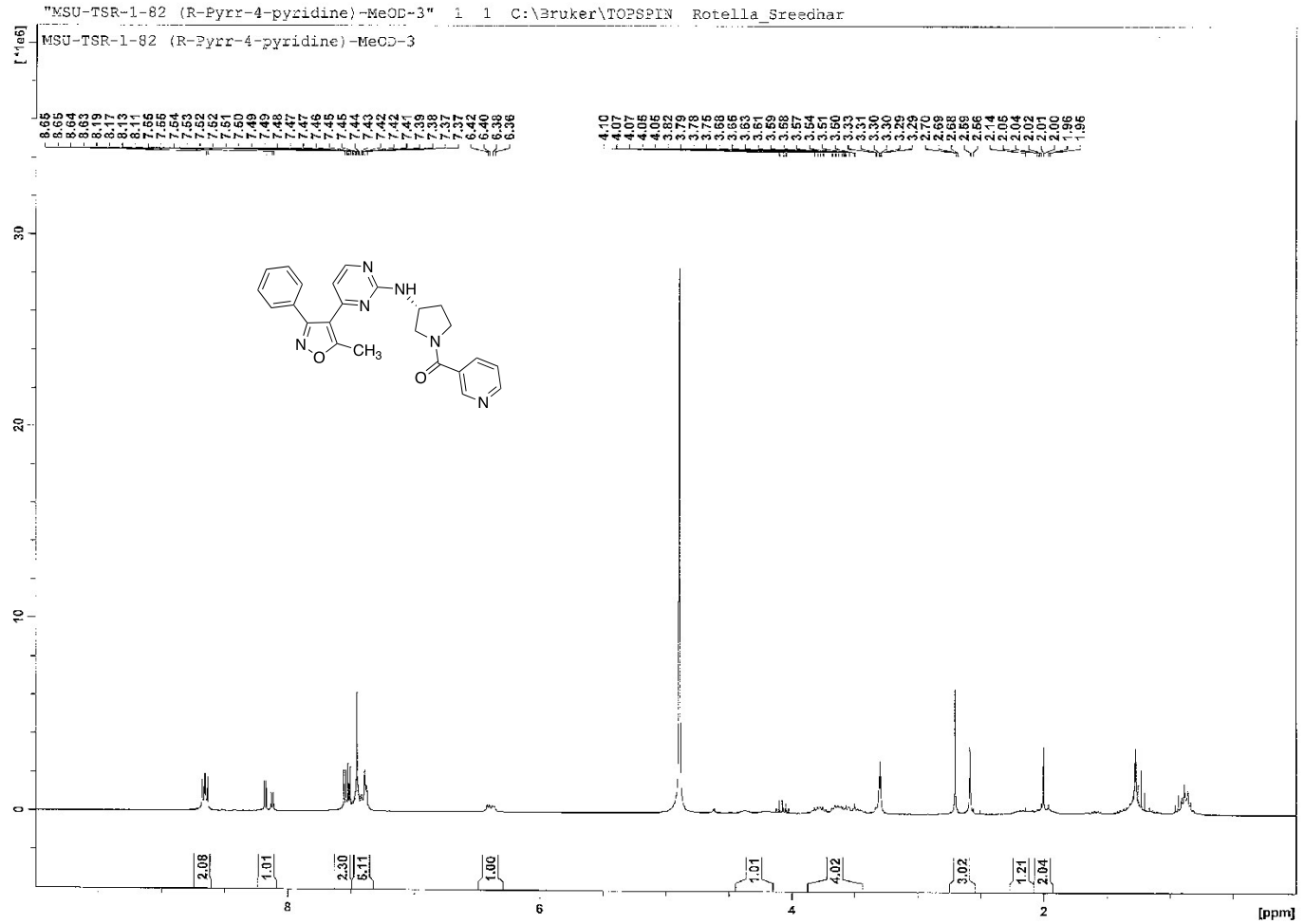
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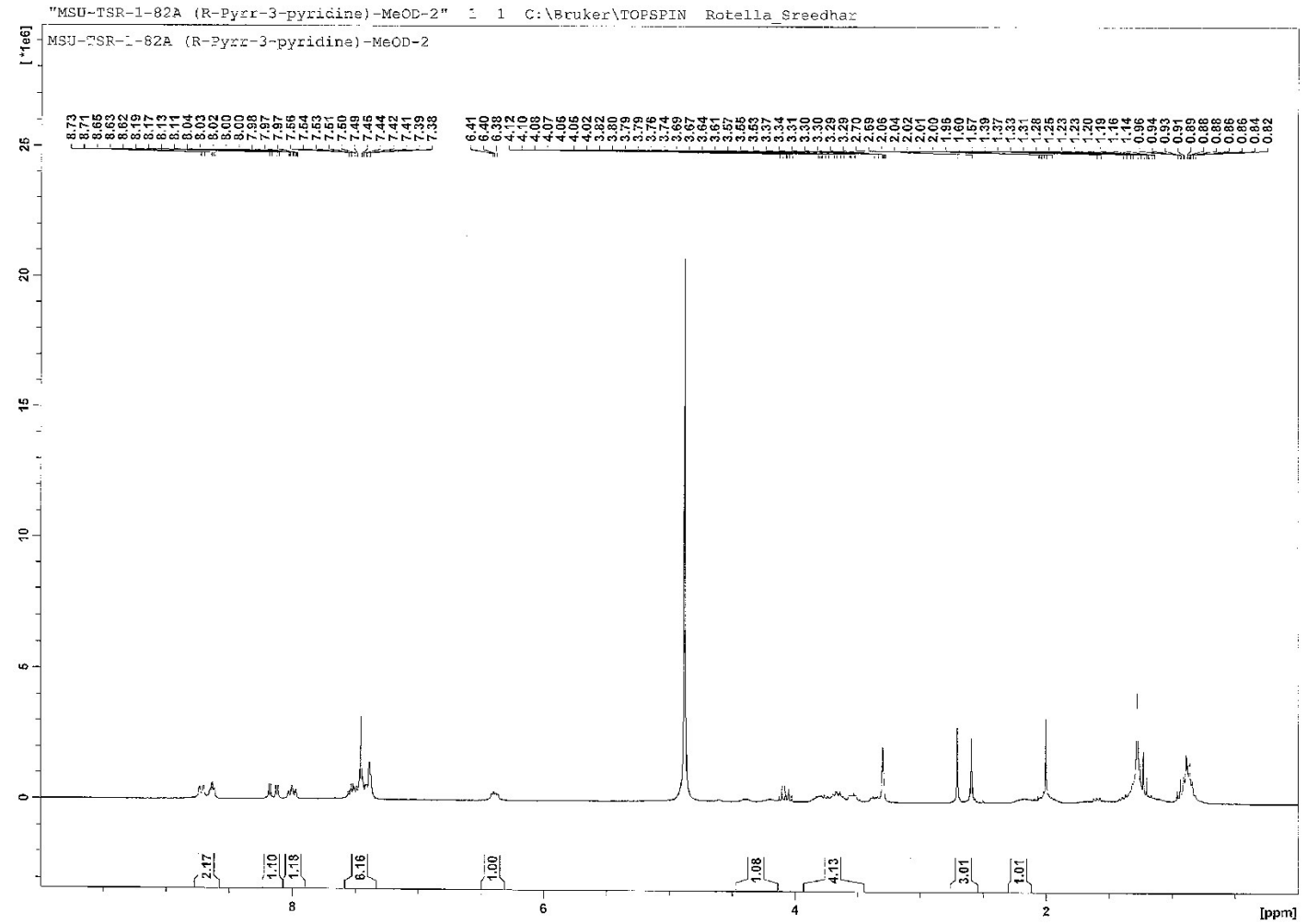
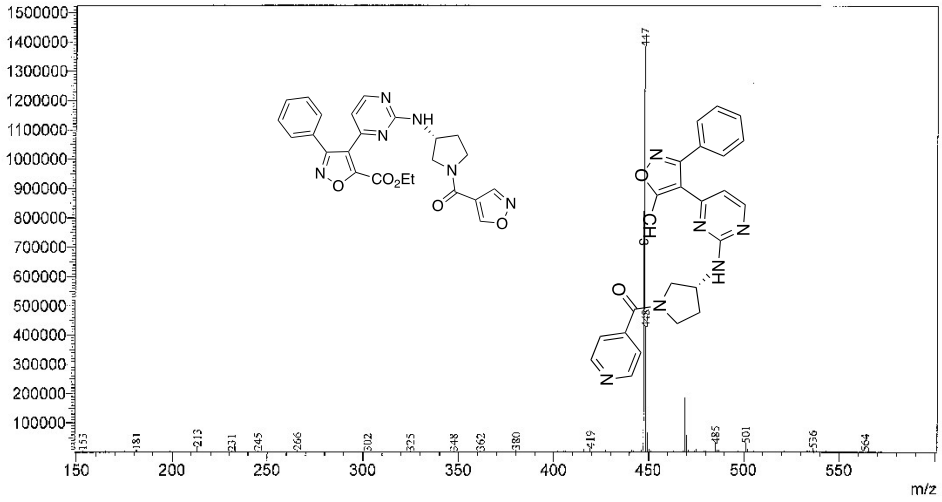
MSU-TSR-1-82 (R-Pyrr-4-pyridine)-MeOD-3
[*1e6]
8.65 8.65 8.64 8.63 8.19 8.17 8.13 8.11 7.55 7.55 7.54 7.53 7.52 7.52 7.51 7.50 7.49 7.49 7.48 7.47 7.47 7.46 7.45 7.45 7.44 7.43 7.42 7.42 7.41 7.39 7.38 7.37 7.37 6.42 6.40 6.38 6.38
4.10 4.07 4.07 4.06 4.06 3.82 3.79 3.78 3.75 3.68 3.66 3.63 3.61 3.59 3.58 3.57 3.54 3.51 3.50 3.33 3.31 3.30 3.30 3.29 3.29 2.70 2.69 2.68 2.58 2.56 2.14 2.05 2.04 2.02 2.01 2.00 1.96 1.96
30
20
10
0
N
NH
N
N
O
CH3
O
N
2.08
1.01
2.30
5.11
1.00
1.01
4.02
3.02
1.21
2.04
8
6
4
2
[ppm]


"MSU-TSR-1-82A (R-Pyrr-3-pyridine)-MeOD-2" 1 1 C:\Bruker\TOPSPIN Rotella_Sreedhar
MSU-TSR-1-82A (R-Pyrr-3-pyridine)-MeOD-2
[*1e6]
8.73
8.71
8.65
8.63
8.62
8.19
8.17
8.13
8.11
8.04
8.03
8.02
8.00
8.00
7.98
7.97
7.97
7.56
7.54
7.53
7.51
7.50
7.49
7.45
7.44
7.42
7.41
7.39
7.38
6.41
6.40
6.38
4.12
4.10
4.08
4.07
4.06
4.05
4.02
3.82
3.80
3.79
3.79
3.76
3.74
3.69
3.67
3.64
3.61
3.57
3.55
3.53
3.37
3.34
3.31
3.30
3.30
3.29
3.29
2.70
2.69
2.06
2.04
2.02
2.01
2.00
1.96
1.60
1.57
1.39
1.37
1.33
1.31
1.28
1.25
1.23
1.23
1.20
1.19
1.16
1.14
0.96
0.94
0.93
0.91
0.89
0.88
0.86
0.86
0.84
0.82
2.17
1.10
1.18
6.16
1.00
1.08
4.13
3.01
1.01
[ppm]

==== Shimadzu LabSolutions Data Report ====

<Spectrum>

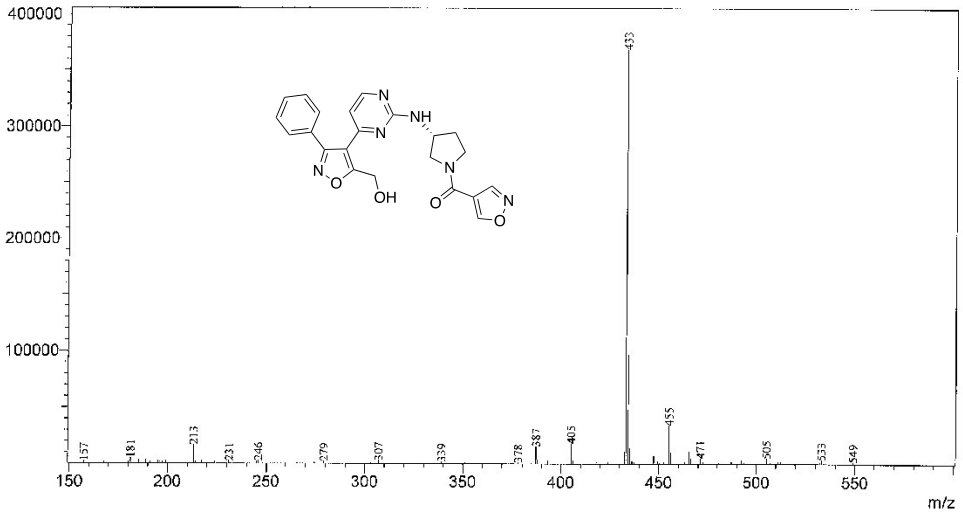
Line#:1 R.Time:8.408(Scan#:5026)
MassPeaks:85
RawMode:Single 8.408(5026) BasePeak:447(1383896)
BG Mode:None Segment 1 - Event 1



==== Shimadzu LabSolutions Data Report ====

<Spectrum>

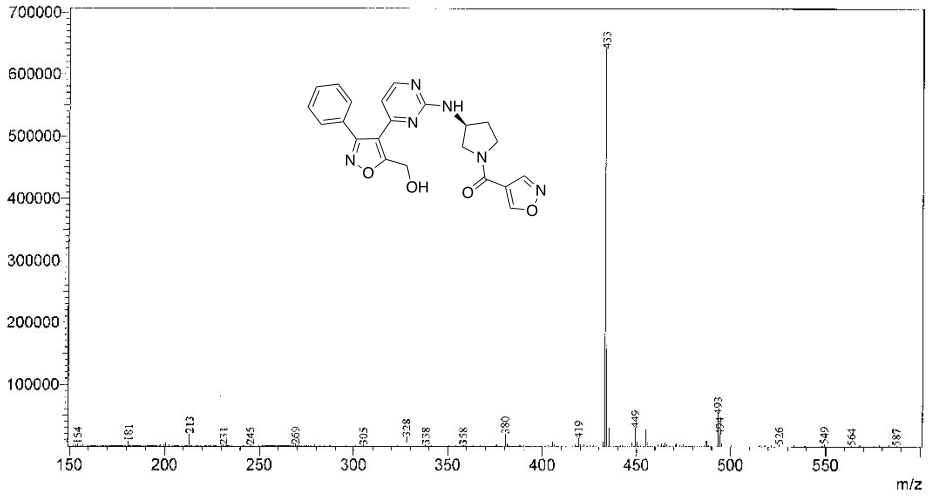
Line#:1 R.Time:6.638(Scan#:3964)
MassPeaks:63
RawMode:Single 6.638(3964) BasePeak:433(368663)
BG Mode:None Segment 1 - Event 1



==== Shimadzu LabSolutions Data Report ====

<Spectrum>

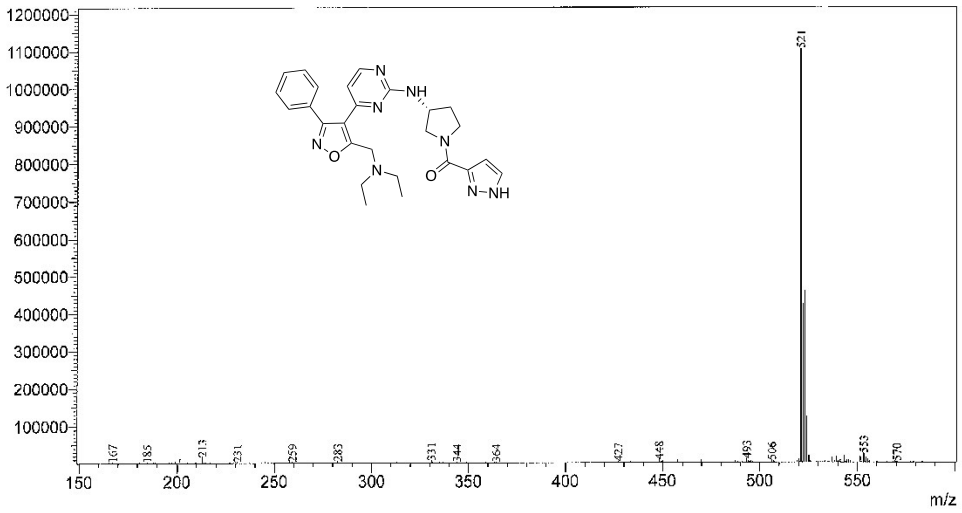
Line#:1 R.Time:8.028(Scan#:4798)
MassPeaks:105
RawMode:Single 8.028(4798) BasePeak:433(641222)
BG Mode:None Segment 1 - Event 1



==== Shimadzu LabSolutions Data Report ====

<Spectrum>

Line#:1 R.Time:3.327(Scan#:1977)
MassPeaks:93
RawMode:Single 3.327(1977) BasePeak:521(1104647)
BG Mode:None Segment 1 - Event 1

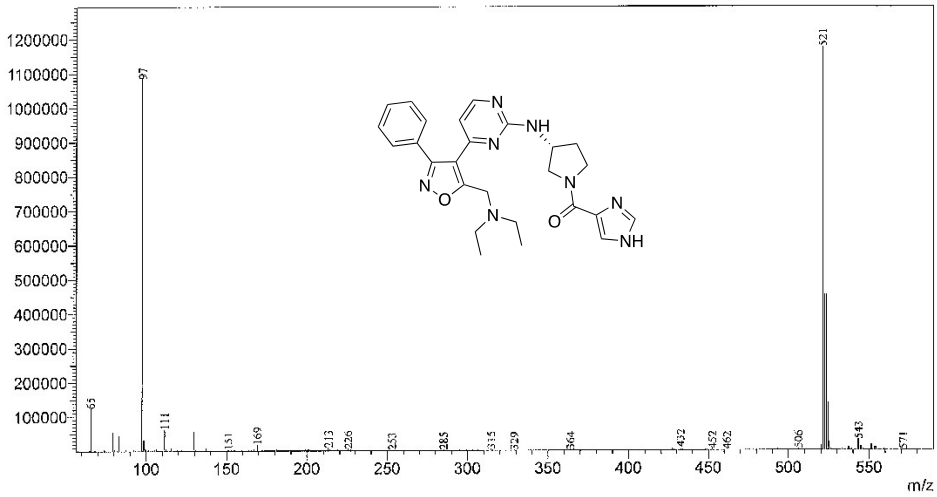


MSU-HC-1-079b

==== Shimadzu LabSolutions Data Report ====

<Spectrum>

Line#:1 R.Time:4.402(Scan#:2622)
MassPeaks:125
RawMode:Single 4.402(2622) BasePeak:521(1174467)
BG Mode:None Segment 1 - Event 1

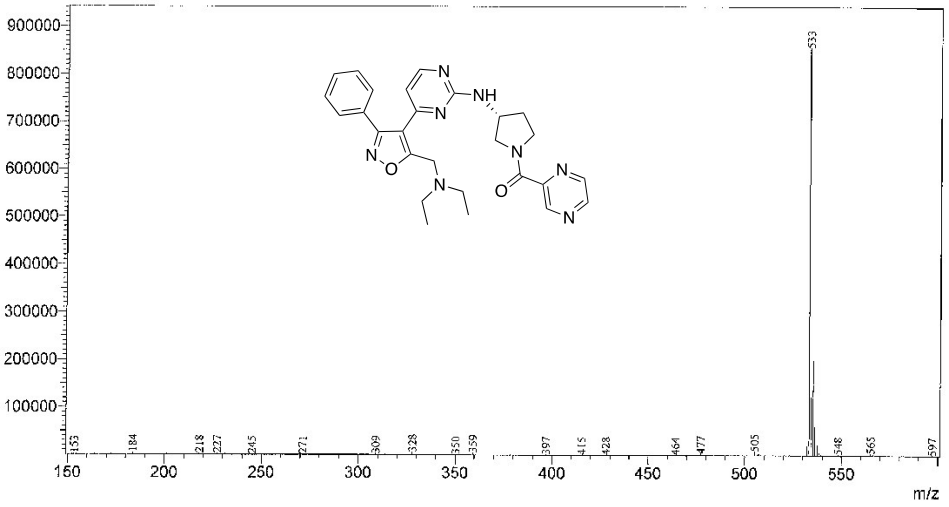


MSU-HC-1-79d

==== Shimadzu LabSolutions Data Report ====

<Spectrum>

Line#:1 R.Time:4.580(Scan#:----)
MassPeaks:82
RawMode:Averaged 4.578-4.582(2728-2730) BasePeak:533(855968)
BG Mode:Calc Segment 1 - Event 1

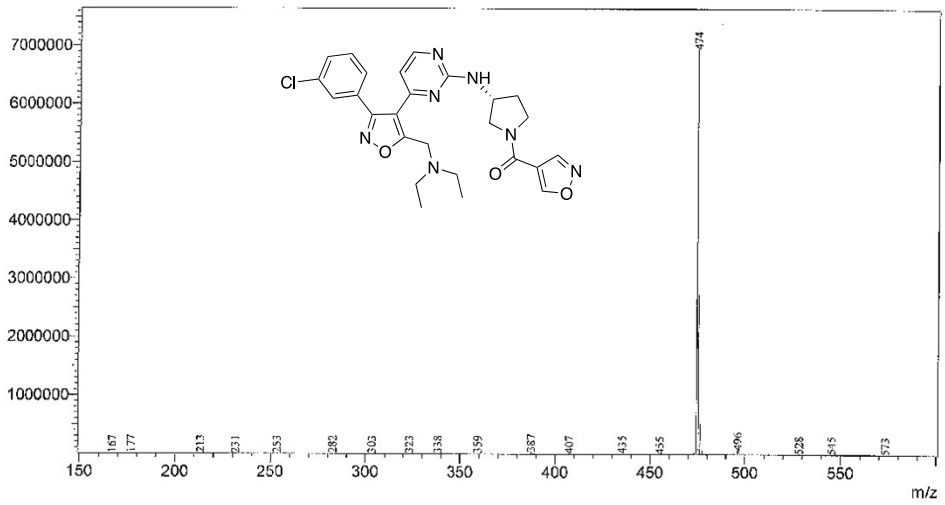


MSU-MC-1-084

==== Shimadzu LabSolutions Data Report ====

<Spectrum>

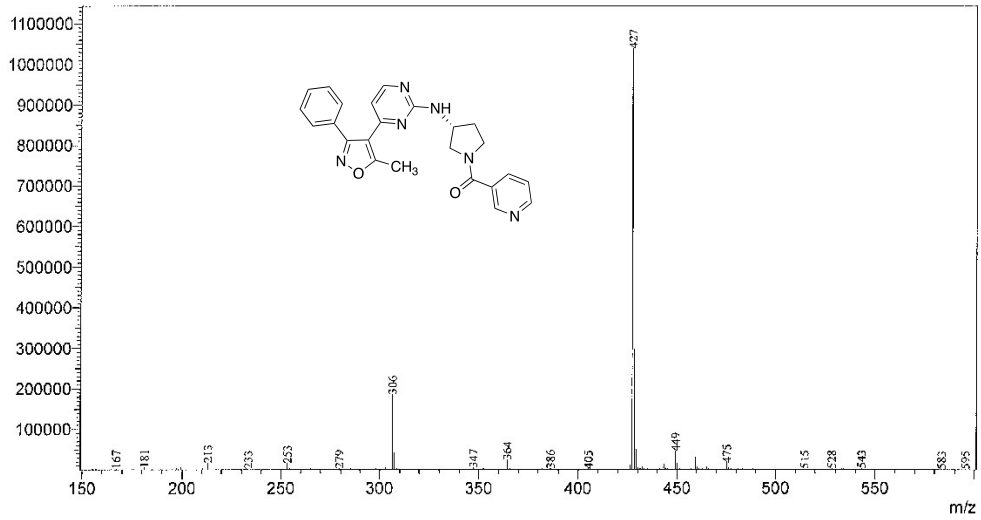
Line#:1 R.Time:5.572(Scan#:3324)
MassPeaks:117
RawMode:Single 5.572(3324) BasePeak:474(6950175)
BG Mode:None Segment 1 - Event 1



==== Shimadzu LabSolutions Data Report ====

<Spectrum>

Line#:1 R.Time:6.430(Scan#:3839)
MassPeaks:92
RawMode:Single 6.430(3839) BasePeak:427(1038474)
BG Mode:None Segment 1 - Event 1



==== Shimadzu LabSolutions Data Report ====

<Spectrum>

Line#:1 R.Time:7.010(Scan#:4187)
MassPeaks:68
RawMode:Single 7.010(4187) BasePeak:213(42264)
BG Mode:None Segment 1 - Event 1