Supporting Information

Optimization of Indazole-Based GSK-3 Inhibitors with Mitigated hERG Issue and *In Vivo* Activity in a Mood Disorder Model

*Federica Prati, Rosa Buonfiglio, Guido Furlotti, Claudia Cavarischia, Giorgina Mangano, Rossella Picollo, Laura Oggianu, Anna di Matteo, Silvana Olivieri, Graziella Bovi, Pier Francesca Porceddu, Angelo Reggiani, Beatrice Garrone, Francesco Paolo Di Giorgio, and Rosella Ombrato**

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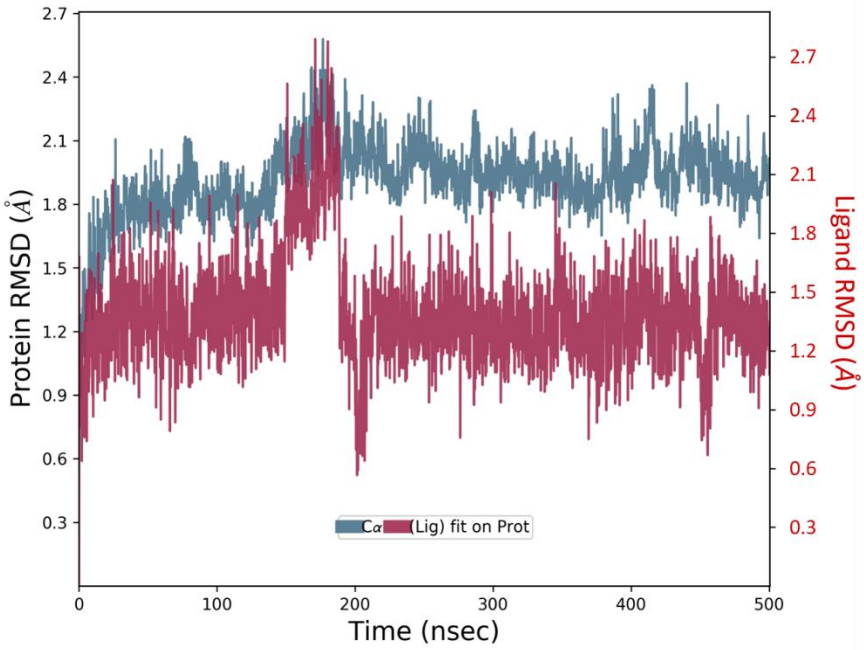


Figure S1. RMSD of the atomic positions for ligand **14** (in red, Lig fit Prot) and the GSK-3β kinase (Cα positions in blue) of the 500 ns molecular dynamics simulations using Desmond package. The complex is stable along the simulation with persistent hydrogen bonds involving the hinge residues. The peak of ligand RMSD is due to the fluctuation of the solvent exposed oxanyl group.

Table S1. CNS-MPO Values Calculated for Compounds 1-16[a]

	T0 value						
Cmpd	**T0_MW**	**T0_cLogP**	**T0_TPSA**	**T0_cLogD**	**T0_HBD**	**T0_pKa**	**CNS_MPO**
1	0.51	0.88	1.00	0.92	0.50	0.80	4.60
2	0.92	0.95	1.00	0.45	0.50	1.00	4.82
3	0.92	0.95	1.00	0.45	0.50	1.00	4.81
4	1.00	1.00	1.00	0.65	0.50	1.00	5.15
5	1.00	1.00	1.00	0.54	0.50	1.00	5.04
6	1.00	1.00	1.00	0.60	0.50	1.00	5.10
7	1.00	1.00	1.00	0.54	0.50	1.00	5.04
8	1.00	1.00	1.00	0.87	0.50	1.00	5.37
9	0.68	1.00	0.66	1.00	0.50	1.00	4.83
10	0.78	1.00	0.66	1.00	0.50	1.00	4.93
11	1.00	1.00	1.00	1.00	0.50	1.00	5.50
12	0.75	1.00	1.00	0.72	0.50	1.00	4.97
13	0.85	1.00	1.00	1.00	0.50	1.00	5.35
14	0.95	1.00	0.66	1.00	0.17	1.00	4.78
15	0.85	1.00	1.00	1.00	0.50	1.00	5.35
16	0.95	1.00	0.66	1.00	0.17	1.00	4.78

[a]Physicochemical properties were calculated using ACD/Percepta 2017.2.

Table S2. GSK-3β IC_{50} and 95% Confidence Intervals

Cmpd	GSK-3β IC_{50} (μM)	95% Lower confidence limit (μM)	95% Upper confidence limit (μM)
1	0.018	0.016	0.020
2	0.014	0.010	0.018
3	0.020	0.016	0.025
4	0.026	0.020	0.035
5	0.055	0.037	0.083
6	0.022	0.021	0.033
7	0.031	0.021	0.045
8	0.130	0.048	0.364
9	0.039	0.020	0.075
10	0.014	0.013	0.017
11	0.022	0.018	0.027
12	0.004	0.0001	0.277
13	0.006	0.005	0.007
14	0.004	0.004	0.005
15	0.028	0.019	0.043
16	0.033	0.026	0.043

Table S3. GSK-3β IC_{50} and 95% Confidence Intervals

Cmpd	hERG IC_{50} (μM)	95% Lower confidence limit (μM)	95% Upper confidence limit (μM)
1	0.044	0.035	0.0557
2	4.1	2.97	5.538
3	22.3	17.64	28.30
4	4.3	3.157	5.846
5	11.0	6.610	18.37
6	1.8	0.825	3.744
7	6.6	4.315	10.01
8	13.3	10.38	17.10
9	2.1	1.537	2.746
10	14.5	8.554	24.68
11	49.3	15.97	152.5
12	0.86	0.617	1.219
13	5.7	2.708	11.85
14	>100	156.8	1383
15	47.1	30.89	71.65
16	41.3	22.86	74.81

Table S4. Percent of Inhibition of 14 at 10 μM and Standard Deviation (S.D.) for a 42 Kinases Panel

Kinase	INH % (10μM)	S.D.
h-ALK	42	11
h-AMPKα	12	2
h-AurA/Aur2 kinase	10	2
h-CDC2/CDK1 (cycB)	97	2
h-CDK2 (cycA)	99	0
h-CDK3 (cycE1)	93	0
h-CDK9 (cycT1)	92	1
h-CHK1	1	10
h-CHK2	18	9
h-CLK1	100	0
h-DLK1 (MAP3K12)	64	2
h-DYRK1a	100	0
h-DYRK2	98	0
h-ERK1	93	1
h-ERK2 (P42mapk)	91	0
h-FLT-3 kinase	32	4
h-GRK2 (ADRBK1)	98	0
h-GSK-3α	100	0
h-HGK (MAP4K4)	96	0
h-HIPK2	58	1
h-IRK (InsR)	-1	3
h-JAK1	3	9
h-JAK2	10	1
h-JAK3	20	4
h-JNK1	75	3
h-JNK3	78	11
h-Lck kinase	-7	14
h-LynA kinase	49	7
h-LynB kinase	99	0
h-MAPKAPK5 (PRAK)	40	4
h-MEK1/MAP2K1	86	0
h-MST3 kinase	31	5
h-p70S6Kβ	-1	7
h-PKA	-2	1
h-PKD1 (PKCμ)	36	15
h-PKN2	9	0
h-ROCK1	4	2
h-ROCK2	10	3
h-smMLCK (MYLK)	37	3
h-Src kinase	41	7
h-TAOK2 (TAO1)	20	2
h-TRKA	10	1

Table S5. IC_{50} Values of 14 for Selected Kinases[a]

Kinase	IC_{50} (μM)
h-CDC2/CDK1 (cycB)	0.410
h-CDK2 (cycA)	0.027
h-CDK3 (cycE1)	0.290
h-CDK9 (cycT1)	0.500
h-CLK1	0.013
h-DLK1 (MAP3K12)	6.10
h-DYRK1a	<0.010
h-DYRK2	0.290
h-ERK1	0.700
h-ERK2 (P42mapk)	0.300
h-GRK2 (ADRBK1)	0.011
h-GSK-3α	<0.010
h-HGK (MAP4K4)	0.350
h-HIPK2	9.00
h-JNK1	1.60
h-JNK3	4.40
h-LynA kinase	54.00
h-LynB kinase	<0.010
h-MEK1/MAP2K1	0.750

[a]percent of inhibition at 10 μM higher than 50%.

CHEMISTRY

Reagents were purchased from Sigma-Aldrich and used as received.
Reaction progress was monitored by TLC or LC-MS. For TLC analysis, Merck silica gel 60 F254 (0.04–0.063 mm) plates with detection by UV (214 or 254 nm) were used. LC-MS analyses were performed on Shimadzu LCMS-2020 Single Quadrupole Liquid Chromatograph Mass Spectrometer and column: Acquity UPLC C18 (2.1 x 50 mm, 1.8 µm).
EM Discover SP focused microwave reactor was used for microwave-mediated reactions.
Final compound purity (always >95%) was determined by HPLC-UV, by comparing the area of the target peak and total peak areas of minor impurities. HPLC-UV analyses was carried out with a pump/autosampler (Waters 2695, Alliance model), a UV photo diode array detector (Waters 2996 model), and a Waters system data management (Empower 2). The column used was generally X-Bridge C18 (150 × 4.6 mm, 5 µm).
UPLC-QTOF analyses for HRMS determination of final compounds were performed by means of a SYNAPT MS-ACQUITY UPLC system (Waters). The system was operated in positive ion mode in the "V-Optics" configuration. Leucine-enkephalin (200 pg/µL) was employed as the lock mass in order to provide authenticated exact mass measurement in MS and MS/MS modes within 5 ppm RMS mass accuracy. The column was an Acquity BEH C18 (50 × 2.1 mm, 1.7 µm).
Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR and ^{13}C NMR) were obtained using Bruker Avance systems, operating at 300, 400 and 500 MHz. Spectra were acquired at 300 K, using DMSO-d_6, CD_3OD, and $CDCl_3$ as solvents. All resonance bands were referenced to tetramethylsilane (internal standard). For ^{1}H NMR spectroscopy: (s) = singlet; (d) = doublet; (t) = triplet; (br) = broad; (dd) = double doublet; (dt) = double triplet; (ddd) = double double doublet; (dtd) = double triple doublet; (m) = multiplet; *J*= coupling constant; and δ = chemical shift (in ppm).
Compounds were purified with one of the following techniques: i) flash chromatography on silica gel (Grace Reveleris flash chromatography system with a 40 µM silica cartridge, flow = 60 mL/min), with an appropriate gradient of mixtures of DCM/MeOH or Cyclohexane/EtOAc as eluents; ii) preparative HPLC-MS system (sample was dissolved at 50 mg/mL in DMSO/CH_3CN in 1:1 ratio), using an appropriate gradient of CH_3CN and H_2O + 0.1% NH_4HCO_3; flow =40 mL/min. The preparative HPLC-MS system consisted of a Waters 2767 Sample manager, a Waters 2478 dual λ absorbance detector, and a Waters Micromass ZQ single quadrupole mass spectrometer with an electrospray ionization (ESI) source. The column used was a X-Bridge Prep C18 5 µm with 19 × 10 mm (Waters) precolumn. Fraction collection was available from the system software MassLynx v. 4.1. Detection wavelength was set to 230 nm and temperature to 25 °C.

General methods for the synthesis of carboxamide intermediates 17 -25

Genaral method (A): HOBt (1.1 eq.) and DCC (1.07 eq.) were added to a solution of 5-bromo-1*H*-indazole-3-carboxylic acid (1.0 eq.) in DMF at 0 ºC. After 1 hour, a solution of the proper amine (1.2 eq.) was added at the same temperature. The mixture was stirred at 0 ºC for 2 hours and left to reach room temperature overnight. The mixture was then concentrated under vacuum, diluted with EtOAc, and washed with aqueous 2N NaOH solution and with brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to give the target compound. Purification by flash chromatography was performed when required.

Genaral method (B): A mixture of 5-bromo-1*H*-indazole-3-carboxylic acid (1.0 eq.), the proper amine (1 - 1.2 eq.), HOBt (1.2 eq.) and EDC.HCl (1.2 eq.) in DMF was stirred at room temperature overnight. The mixture was concentrated under vacuum and then diluted with EtOAc. The solution was washed with aqueous 2N NaOH solution and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to give the target compound. Purification by flash chromatography was performed when required.

General methods for the synthesis of final compounds 2-16

Genaral method (C): A mixture of the suitable carboxamide intermediate (1.0 eq.), the proper boronic acid (1.3 - 4 eq.), and Cs_2CO_3 (1.3 - 4 eq.) in 1,4-dioxane/water (4:1) was placed in a Schlenk tube. The resulting mixture was degassed with N_2 for 10 minutes. Then, $PdCl_2$(dppf) (0.08 – 0.25 eq.) was added and the mixture was heated in the microwave at 130 ºC for 15 minutes. The mixture was diluted with EtOAc/MeOH and the insoluble solids were removed by filtration through Celite. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography to give the target final compound.

General method (D): A mixture of the suitable carboxamide intermediate (1.0 eq.), the proper boronic acid / ester (2.0 eq.) or bromide (1.2 - 2 eq.), and CsF (2 eq.) in 1,4-dioxane and water (4:1) was placed in a Schlenk tube. The resulting mixture was degassed with N_2 for 10 minutes. Then, $PdCl_2$(dppf) (0.05 – 0.1 eq.) was added and the mixture was heated at 100 ºC overnight. The mixture was diluted with MeOH and the insoluble solids were removed by filtration through Celite. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography to give the target final compound.

General method (E): A mixture of the suitable carboxamide intermediate (1.0 eq.), the proper bromide (1.1 eq.) and aqueous 2N Na_2CO_3 solution (1.1 eq.) in DMF was placed in a Schlenk tube. The resulting mixture was degassed with N_2 for 10 minutes.

Then, $Pd(PPh_3)_4$ (0.05 eq.) was added and the mixture was heated at 100 ºC overnight. The mixture was diluted with EtOAc/MeOH and then the insoluble solids were removed by filtration through Celite. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography to give the target final compound.

5-Bromo-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**17**). The title compound was obtained according to general method (A) using 5-bromo-1*H*-indazole-3-carboxylic acid (0.1 g, 0.415 mmol), (tetrahydro-2*H*-pyran-4-yl)methanamine (0.055 g, 0.481 mmol), HOBt (0.062 g, 0.456 mmol), DCC (0.092 g, 0.444 mmol). The crude was purified by flash chromatography (SiO_2, DCM/MeOH), affording 0.11 g of the title product. Yield = 69%. LC-MS (ESI) m/z: found 338.1 $[M+H]^+$; for $C_{14}H_{16}BrN_3O_2$ exact mass calculated 337.0.

5-Bromo-N-((tetrahydro-2H-pyran-3-yl)methyl)-1H-indazole-3-carboxamide (**18**). The title compound was obtained according to general method (A) using 5-bromo-1*H*-indazole-3-carboxylic acid (0.1 g, 0.415 mmol), (tetrahydro-2*H*-pyran-3-yl)methanamine (0.055 g, 0.481 mmol), HOBt (0.062 g, 0.456 mmol), DCC (0.092 g, 0.444 mmol). The crude was purified by flash chromatography (SiO_2, DCM/MeOH), affording 0.10 g of the title product. Yield = 70%. LC-MS (ESI) m/z: found 338.1 $[M+H]^+$; for $C_{14}H_{16}BrN_3O_2$ exact mass calculated 337.0.

5-Bromo-N-((tetrahydrofuran-3-yl)methyl)-1H-indazole-3-carboxamide (**19**). The title compound was obtained according to general method (B) using 5-bromo-1*H*-indazole-3-carboxylic acid (0.2 g, 0.830 mmol), (tetrahydrofuran-3-yl)methanamine (0.084 g, 0.830 mmol), HOBt (0.135 g, 0.996 mmol), EDC.HCl (0.191 g, 0.996 mmol). The crude (0.27 g) was used in the next step without further purification. Yield = 29%. LC-MS (ESI) m/z: found 324.0 $[M+H]^+$; for $C_{13}H_{14}BrN_3O_2$ exact mass calculated 323.0.

5-Bromo-N-((tetrahydrofuran-2-yl)methyl)-1H-indazole-3-carboxamide (**20**). The title compound was obtained according to general method (B) using 5-bromo-1*H*-indazole-3-carboxylic acid (0.2 g, 0.830 mmol), (tetrahydrofuran-2-yl)methanamine (0.084 g, 0.830 mmol), HOBt (0.135 g, 0.996 mmol), EDC.HCl (0.191 g, 0.996 mmol). The crude (0.16 g) was used in the next step without further purification. Yield = 59%. LC-MS (ESI) m/z: found 324.0 $[M+H]^+$; for $C_{13}H_{14}BrN_3O_2$ exact mass calculated 323.0.

5-Bromo-N-(tetrahydro-2H-pyran-4-yl)-1H-indazole-3-carboxamide (**21**). The title compound was obtained according to general method (A) using 5-bromo-1*H*-indazole-3-carboxylic acid (0.1 g, 0.41 mmol), tetrahydro-2*H*-pyran-4-amine (0.049 g, 0.481 mmol), HOBt (0.062 g, 0.456 mmol), DCC (0.092 g, 0.444 mmol). The crude (0.097 mg) was used in the next step without further purification. Yield: 89%. LC-MS (ESI) m/z: found 324.0 $[M+H]^+$; for $C_{13}H_{14}BrN_3O_2$ exact mass calculated 323.0.

5-Bromo-N-(tetrahydro-2H-pyran-3-yl)-1H-indazole-3-carboxamide (**22**). The title compound was obtained according to general method (B) using 5-bromo-1*H*-indazole-3-carboxylic acid (0.4 g, 1.66 mmol), tetrahydro-2*H*-pyran-3-amine hydrochloride (0.274 g, 1.991 mmol), HOBt (0.27 g, 1.991 mmol), EDC.HCl (0.38 g, 1.991 mmol). The crude was purified by flash chromatography (SiO_2, Cyclohexane/EtOAc), affording 0.13 g of the title product. Yield = 21%. LC-MS (ESI) m/z: found 324.0 $[M+H]^+$; for $C_{13}H_{14}BrN_3O_2$ exact mass calculated 323.0.

5-Bromo-N-(tetrahydrofuran-3-yl)-1H-indazole-3-carboxamide (**23**). The title compound was obtained according to general method (B) using 5-bromo-1*H*-indazole-3-carboxylic acid (0.3 g, 1.245 mmol), tetrahydrofuran-3-amine (0.108 g, 1.245 mmol), HOBt (0.202 g, 1.494 mmol), and EDC.HCl (0.286 g, 1.494 mmol). The crude (0.300 g) was used in the next step without further purification. Yield = 78%. LC-MS (ESI) m/z: found 310.0 $[M+H]^+$;for $C_{12}H_{12}BrN_3O_2$ exact mass calculated 309.0.

5-Bromo-N-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-1H-indazole-3-carboxamide (**24**). The title compound was obtained according to general method (B) using 5-bromo-1*H*-indazole-3-carboxylic acid (0.3 g, 1.245 mmol), 4-aminotetrahydro-2H-thiopyrane 1,1-dioxide (0.185 g, 1.245 mmol), HOBt (0.202 g, 1.494 mmol), EDC.HCl (0.286 g, 1.494 mmol). The crude (0.23 g) was used in the next step without further purification. Yield = 50%. LC-MS (ESI) m/z: found 371.2 $[M+H]^+$; for $C_{13}H_{14}BrN_3O_3S$ exact mass calculated 370.9.

5-Bromo-N-(1,1-dioxidotetrahydrothiophen-3-yl)-1H-indazole-3-carboxamide (**25**). The title compound was obtained according to general method (B) using 5-bromo-1*H*-indazole-3-carboxylic acid (0.3 g, 1.245 mmol), 3-aminotetrahydrothiophene 1,1-dioxide (0.168 g, 1.245 mmol), HOBt (0.202 g, 1.494 mmol), EDC.HCl (0.286 g, 1.494 mmol). The crude (0.22 g) was used in the next step without further purification. Yield = 49%. LC-MS (ESI) m/z: found 357.9 $[M+H]^+$; for $C_{12}H_{12}BrN_3O_3S$ exact mass calculated 356.9.

N-((tetrahydro-2H-pyran-4-yl)methyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole-3-carboxamide (**26**). A mixture of 5-bromo-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide **17** (1.5 g, 4.4 mmol), bis(pinacolato)diboron (3.4 g, 13 mmol), and KOAc (0.87 g, 8.9 mmol),in 1,4-dioxane was first degassed with a N_2 stream for 10 minutes and then added with $PdCl_2$(dppf) (0.65 g, 0.89 mmol). After purging again with N_2, the mixture was stirred at 100 ºC overnight, then diluted with MeOH and filtered through Celite to remove the insoluble solids. The solvent was evaporated under reduced pressure and the crude was purified by flash chromatography (SiO_2, DCM/MeOH), affording 0.77 g of the title

product. Yield = 45%. LC-MS (ESI) m/z: found 326.1 [$M_{boronic\ acid}$+Na]$^+$; for boronic ester $C_{20}H_{28}BN_3O_4$ exact mass calculated 385.2, for boronic acid $C_{14}H_{18}BN_3O_4$ exact mass calculated 303.1.

5-(2,3-Difluorophenyl)-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**2**). The title compound was obtained according to general method (C) using 5-bromo-*N*-((tetrahydro-2*H*-pyran-4-yl)methyl)-1*H*-indazole-3-carboxamide (**17**, 0.107 g, 0.316 mmol), 2,3-difluorophenylboronic acid (0.200 g, 1.266 mmol), Cs_2CO_3 (0.412 g, 1.266 mmol), $PdCl_2$(dppf) (0.058 g, 0.079 mmol). The crude was purified by flash chromatography (SiO_2, Cyclohexane/EtOAc) to give 0.016 g of the title product. Yield = 14%. LC-MS (ESI) m/z: found 372.1 [M+H]$^+$. HRMS (ESI) m/z: found 372.1525 [M+H]$^+$; for $C_{20}H_{19}F_2N_3O_2$ exact mass calculated 371.1445 (372.1518 [M+H]$^+$) ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.57 (br s, 1H), 8.36 (br s, 1H), 8.25-8.29 (t, 1H, *J*=6.0Hz), 7.71-7.74 (m, 1H), 7.58-7.62 (m, 1H), 7.28-7.46 (m, 3H), 3.84-3.87 (2H, m), 3.16-3.33 (m, 4H), 1.83-1.91 (m, 1H), 1.60-1.65 (m, 2H), 1.18-1.32 ppm (m, 2H).

5-(2,3-Difluorophenyl)-N-((tetrahydro-2H-pyran-3-yl)methyl)-1H-indazole-3-carboxamide (**3**). The title compound was obtained according to general method (C) using 5-bromo-*N*-((tetrahydro-2*H*-pyran-3-yl)methyl)-1*H*-indazole-3-carboxamide (**18**, 0.107 g, 0.316 mmol), 2,3-difluorophenylboronic acid (0.200 g, 1.266 mmol), Cs_2CO_3 (0.412 g, 1.266 mmol), $PdCl_2$(dppf) (0.058 g, 0.079 mmol). The crude was purified by flash chromatography (SiO_2, Cyclohexane/EtOAc) to give 0.020 g of the title product. Yield = 16%. LC-MS (ESI) m/z: found 372.1 [M+H]$^+$. HRMS (ESI) m/z: , found 372.1537 [M+H]$^+$; for $C_{20}H_{19}F_2N_3O_2$ exact mass calculated 371.1445 (372.1518 [M+H]$^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.67 (br s, 1H). 8.50 (t, 1H, *J*=6.1 Hz), 8.34 (s, 1H), 7.73 (dd, 1H, *J*=0.9, 8.7 Hz), 7.60 (td, 1H, *J*=1.7, 8.6 Hz), 7.28-7.44 (m, 3H), 3.67-3.79 (m, 2H), 3.07-3.33 (m, 4H), 1.81-1.91 (m, 1H), 1.74-1.79 (m,1H), 1.54-1.62 (m, 1H), 1.38-1.49 (m, 1H), 1.19-1.29 ppm (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.8, 151.5, 147.1, 141.1, 139.2, 131.3, 128.3, 128.0, 126.6, 125.6, 122.4, 122.2, 116.7, 111.6, 71.2, 68.0, 41.0, 36.6, 27.5, 25.3 ppm.

5-(2,3-Difluorophenyl)-N-((tetrahydrofuran-3-yl)methyl)-1H-indazole-3-carboxamide (**4**). The title compound was obtained according to general method (C) using 5-bromo-N-((tetrahydrofuran-3-yl)methyl)-1H-indazole-3-carboxamide (**19**, 0.269 g, 0.830 mmol), 2,3-difluorophenylboronic acid (0.262 g, 1.660 mmol), Cs_2CO_3 (0.541 g, 1.660 mmol), and $PdCl_2$(dppf) (0.061 g, 0.083 mmol). The crude was purified by flash chromatography (SiO_2, Cyclohexane/EtOAc) to give 0.015 g of the title product. Yield = 5%. LC-MS (ESI) m/z: found 358.1 [M+H]$^+$. HRMS (ESI) m/z: found 358.1372 [M+H]$^+$; for $C_{19}H_{17}F_2N_3O_2$ exact mass calculated 357.1289 (358.1362 [M+H]$^+$).^{1}H NMR (400 MHz, DMSO-d_6): δ 13.72 (br s, 1H), 8.59-8.62 (m, 1H), 8.33 (br s, 1H), 7.74 (d, 1H, *J*=8.6 Hz), 7.61 (d, 1H, *J*=8.8 Hz), 7.40-7.47 (m, 2H), 7.32-7.38 (m, 1H), 3.57-3.76 (m, 3H), 3.47-3.51 (m, 1H), 3.26-3.31 (m, 2H), 2.52-2.56 (m, 1H), 1.89-1.97 (m, 1H), 1.60-1.67 ppm (m, 1H).

5-(2,3-Difluorophenyl)-N-((tetrahydrofuran-2-yl)methyl)-1H-indazole-3-carboxamide (**5**). The title compound was obtained according to general method (C) using 5-bromo-*N*-((tetrahydrofuran-2-yl)methyl)-1*H*-indazole-3-carboxamide (**20**, 0.158 g, 0.487 mmol), 2,3-difluorophenylboronic acid (0.154 g, 0.975 mmol), Cs_2CO_3 (0.318 g, 0.975 mmol), $PdCl_2$(dppf) (0.036 g, 0.049 mmol). The crude was purified by flash chromatography (SiO_2, Cyclohexane/EtOAc) to give 0.060 g of the title product. Yield = 31%. LC-MS (ESI) m/z: found 358.1 [M+H]$^+$. HRMS (ESI) m/z: found 358.1365 [M+H]$^+$; for $C_{19}H_{17}F_2N_3O_2$ exact mass calculated 357.1289 (358.1362 [M+H]$^+$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.75 (br s, 1H), 8.35 (s., 1H), 8.29 (t, 1H, *J*=10.0 Hz), 7.74 (d, 1H, *J*=8.7 Hz), 7.62 (d, 1H, *J*=8.7 Hz), 7.39-7.48 (m, 2H), 7.31-7.35 (m, 1H), 4.01-4.06 (m, 1H), 3.78-3.82 (m, 1H), 3.62-3.66 (m, 1H), 3.37 (t, 1H, *J*=6.0 Hz), 1.77-1.94 (m, 3H), 1.61-1.67 ppm (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 162.1, 150.3, 146.8, 140.7, 138.6, 130.8, 127.9, 127.6, 126.1, 125.1, 121.9, 121.7, 116.3, 111.2, 77.0, 67.1, 42.4, 28.5, 25.1 ppm.

5-(2,3-Difluorophenyl)-N-(tetrahydro-2H-pyran-4-yl)-1H-indazole-3-carboxamide (**6**). The title compound was obtained according to general method (C) using 5-bromo-*N*-(tetrahydro-2*H*-pyran-4-yl)-1*H*-indazole-3-carboxamide (**21,** 0.097 g, 0.299 mmol), 2,3-difluorophenylboronic acid (0.095 g, 0.598 mmol), Cs_2CO_3 (0.146 g, 0.448 mmol), and $PdCl_2$(dppf) (0.022 g, 0.030 mmol). The crude was purified by flash chromatography (SiO_2, Cyclohexane/EtOAc) to give 0.040 g of the title product. Yield = 37%. LC-MS (ESI) m/z: found 358.1 [M+H]$^+$. HRMS (ESI) m/z: found 358.1367 [M+H]$^+$; for $C_{19}H_{17}F_2N_3O_2$ exact mass calculated 357.1289 (358.1362 [M+H]$^+$). ^{1}H NMR (500 MHz, DMSO-d6): δ 13.74 (br s, 1H), 8.37 (s, 1H), 8.35 (s, 1H), 7.74 (d, 1H, *J*=8.7 Hz), 7.62 (d, 1H, *J*=8.7 Hz), 7.39-7.48 (m, 2H), 7.31-7.35 (m, 1H), 4.05-4.12 (m, 1H), 3.88-3.90 (m, 2H), 3.37-3.42 (m, 2H), 1.66-1.76 ppm (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 161.5, 150.4, 147.2, 140.8, 139.0, 131.0, 127.9, 127.7, 126.2, 125.3, 122.1, 121.9, 116.4, 111.3, 66.4, 45.2, 32.5 ppm.

5-(2,3-Difluorophenyl)-N-(tetrahydro-2H-pyran-3-yl)-1H-indazole-3-carboxamide (**7**). The title compound was obtained according to general method (C) using 5-bromo-*N*-(tetrahydro-2*H*-pyran-3-yl)-1*H*-indazole-3-carboxamide (**22**, 0.130 g, 0.401 mmol), 2,3-difluorophenylboronic acid (0.127 g, 0.802 mmol), Cs_2CO_3 (0.26 g, 0.80 mmol), $PdCl_2$(dppf) (0.03 g, 0.04 mmol). The crude was purified by flash chromatography (SiO_2, DCM/MeOH) to give 0.049 g of the title product. Yield = 34%. LC-MS (ESI) m/z: found 358.1 [M+H]$^+$. HRMS (ESI) m/z: found 358.1345 [M+H]$^+$; for $C_{19}H_{17}F_2N_3O_2$ exact mass calculated 357.1289 (358.1362 [M+H]$^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.76 (br s, 1H), 8.34 (br s, 1H), 8.17 (d, 1H, *J*=8.4 Hz), 7.73-7.76 (m, 1H), 7.59-7.63 (m, 1H), 7.30-7.50 (m, 3H), 3.95-4.04 (m, 1H), 3.70-3.81 (m, 2H), 3.27-3.37 (m, 2H), 1.86-1.94 (m, 1H), 1.56-1.79 ppm (m, 3H).

5-(2,3-Difluorophenyl)-N-(tetrahydrofuran-3-yl)-1H-indazole-3-carboxamide (**8**). The title compound was obtained according to general method (C) using 5-bromo-*N*-(tetrahydrofuran-3- yl)-1*H*-indazole-3-carboxamide (**23**, 0.169 g, 0.545 mmol), 2,3-difluorophenylboronic acid (0.172 g, 1.090 mmol), Cs_2CO_3 (0.26 g, 0.80 mmol), $PdCl_2$(dppf) (0.03 g, 0.04 mmol). The crude was purified by flash chromatography (SiO_2, Cyclohexane/EtOAc) to give 0.027 g of the title product. Yield = 14%. LC-MS (ESI) m/z: found 344.1 $[M+H]^+$. HRMS (ESI) m/z: found 344.1199 $[M+H]^+$; for $C_{18}H_{15}F_2N_3O_2$ exact mass calculated 343.1132 (344.1205 $[M+H]^+$) ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (s, 1H), 7.61-7.65 (m, 1H), 7.55-7.58 (m, 1H), 7.30-7.34 (m, 1H), 7.12-7.17 (m, 2H), 4.75-4.83 (m, 1H), 3.96-4.05 (m, 2H), 3.81-3.90 (m, 2H), 2.33-2.42 (m, 1H), 1.96-1.99 ppm (m, 1H).

5-(2,3-Difluorophenyl)-N-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-1H-indazole-3-carboxamide (**9**). The title compound was obtained according to general method (C) using 5-bromo-N-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-1H-indazole-3-carboxamide (**24**, 0.23 g, 0.614 mmol), 2,3-difluorophenylboronic acid (0.194 g, 1.228 mmol), Cs_2CO_3 (0.400 g, 1.228 mmol), $PdCl_2$(dppf) (0.045 g, 0.061 mmol). The crude was purified by flash chromatography (SiO_2, Cyclohexane/EtOAc) to give 0.075 g of the title product. Yield = 30.2%. LC-MS (ESI) m/z: found 406.1 $[M+H]^+$. HRMS (ESI) m/z: found 406.1024 $[M+H]^+$; for $C_{19}H_{17}F_2N_3O_3S$ exact mass calculated 405.0959 (406.1031 $[M+H]^+$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.77 (br s, 1H), 8.62 (d, 1H, *J*=8.2 Hz), 8.34 (s, 1H), 7.75 (d, 1H, *J*=8.7 Hz), 7.62 (d, 1H, *J*=8.7 Hz), 7.32-7.46 (m, 3H), 4.25- 4.28 (m, 1H), 3.36-3.40 (m, 2H), 3.10-3.13 (m, 2H), 2.11-2.22 ppm (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 162.1, 150.8, 147.6, 141.1, 139.1, 131.3, 128.3, 128.1, 126.6, 125.6, 122.5, 122.4, 116.8, 111.6, 49.6, 44.9, 29.7 ppm.

5-(2,3-Difluorophenyl)-N-(1,1-dioxidotetrahydrothiophen-3-yl)-1H-indazole-3-carboxamide (**10**). The title compound was obtained according to general method (C) using 5-bromo-*N*-(1,1-dioxidotetrahydrothiophen-3-yl)-1*H*-indazole-3-carboxamide (**25**, 0.22 g, 0.614 mmol), 2,3-difluorophenylboronic acid (0.194 g, 1.228 mmol), Cs_2CO_3 (0.400 g, 1.228 mmol), $PdCl_2$(dppf) (0.045 g, 0.061 mmol). The crude was purified by flash chromatography (SiO_2, Cyclohexane/EtOAc) to give 0.072 g of the title product. Yield = 28.5%. LC-MS (ESI) m/z: found 392.0 $[M+H]^+$. HRMS (ESI) m/z: found 392.0876 $[M+H]^+$; for $C_{18}H_{15}F_2N_3O_3S$ exact mass calculated 391.0802 (392.0875 $[M+H]^+$.) ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.84 (br s, 1H), 8.88 (d, 1H, *J*=7.8 Hz), 8.35 (s, 1H), 7.75-7.78 (m, 1H), 7.62-7.65 (m, 1H), 7.28-7.50 (m, 3H), 4.73-4.82 (m, 1H), 3.44-3.52 (m, 1H), 3.34-3.40 (m, 1H), 3.16-3.23 (m, 2H), 2.38-2.44 (m, 1H), 2.26-2.33 ppm (m, 1H).

5-(Pyridin-3-yl)-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**11**). The title compound was obtained according to general method (D) using 5-bromo-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**17**, 0.150 g, 0.44 mmol), 3-pyridylboronic acid (0.11 g, 0.89 mmol), CsF (0.13 g, 0.89 mmol), and $PdCl_2$(dppf) (0.033 g, 0.044 mmol). The crude was purified by flash chromatography (SiO_2, DCM/MeOH) to give 0.053 g of the title product. Yield = 35%. LC-MS (ESI) m/z: found 337.2 $[M+H]^+$. HRMS (ESI) m/z: found 337.1660 $[M+H]^+$; for $C_{19}H_{20}N_4O_2$ exact mass calculated 336.1586 (337.1659 $[M+H]^+$). ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.67 (s, 1H), 8.90 (d, 1H, *J*=2.1 Hz), 8.58 (dd, 1H, *J*=1.4, 4.7 Hz), 8.43-8.47 (m, 2H), 8.08-8.12 (m, 1H), 7.72-7.80 (m, 2H), 7.51 (dd, 1H, *J*=4.7, 7.8 Hz), 3.85 (br dd, 2H, *J*=2.3, 11.3 Hz), 3.20-3.31 (m, 4H), 1.79-1.94 (m, 1H), 1.61 (dd, 2H, *J*=1.7, 12.9 Hz), 1.16-1.29 ppm (m, 2H).

5-(5-Isopropoxypyridin-3-yl)-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**12**). The title compound was obtained according to general method (D) using 5-bromo-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**17**, 0.1 g, 0.296 mmol), (5-isopropoxypyridin-3-yl)boronic acid (0.080 g, 0.44 mmol), CsF (0.09 g, 0.59 mmol), and $PdCl_2$(dppf) (0.024 g, 0.030 mmol). The crude was purified by flash chromatography (SiO_2, DCM/MeOH) to give 0.07 g of the title product. Yield = 63%. LC-MS (ESI) m/z: found 395.2 $[M+H]^+$. HRMS (ESI) m/z: found 395.2082 $[M+H]^+$; for $C_{22}H_{26}N_4O_3$ exact mass calculated 394.2005 (395.2078 $[M+H]^+$). ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.67 (br s, 1H), 8.42-8.49 (m, 3H), 8.26 (d, 1H, *J*=2.7 Hz), 7.71-7.79 (m, 2H), 7.60 (t, 1H, *J*=2.2 Hz), 4.80-4.92 (m, 1H), 3.83-3.87 (m, 2H), 3.21-3.31 (m, 4H), 1.80-1.92 (m, 1H), 1.58-1.63 (m, 2H), 1.34 (d, 6H, *J*=6.0 Hz), 1.11-1.30 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.2, 153.8, 140.8, 139.8, 138.8, 137.4, 137.0, 131.1, 126.3, 122.1, 120.2, 119.9, 111.4, 70.0, 66.7, 44.0, 35.0, 30.5, 21.7 ppm.

5-(5-(Methoxymethyl)pyridin-3-yl)-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**13**). The title compound was obtained according to general method (D) using N-((tetrahydro-2H-pyran-4-yl)methyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole-3-carboxamide (**26**, 0.2 g, 0.29 mmol), 3-bromo-5-(methoxymethyl)pyridine (0.12 g, 0.57 mmol), CsF (0.087 g, 0.57 mmol), and $PdCl_2$(dppf) (0.012 g, 0.014 mmol). The crude was purified by reverse phase chromatography (C18, NH_4HCO_3/CH_3CN) to give 0.015 g of the title product. Yield = 13%. LC-MS (ESI) m/z: found 381.1 $[M+H]^+$. HRMS (ESI) m/z: found 381.1925 $[M+H]^+$; for $C_{21}H_{24}N_4O_3$ exact mass calculated 380.1848 (381.1921 $[M+H]^+$). ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.69 (s, 1H), 8.85 (d, 1H, *J*=2.2 Hz), 8.54 (d, 1H, *J*=1.8 Hz), 8.48-8.51 (m, 1H), 8.45-8.46 (m, 1H), 8.03 (t, 1H, *J*=2.1 Hz), 7.78-7.82 (m, 1H), 7.73-7.76 (m, 1H), 4.57 (s, 2H), 3.85 (dd, 2H, *J*=2.4, 11.4 Hz), 3.38 (s, 3H), 3.17-3.32 (m, 4H), 1.81-1.93 (m, 1H), 1.61 (dd, 2H, *J*=1.9, 12.9 Hz), 1.16-1.30 ppm (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.2, 147.3, 146.9, 140.8, 138.8, 135.7, 133.9, 133.4, 131.1, 126.0, 122.2, 119.8, 111.5, 71.0, 66.7, 57.8, 44.0, 35.0, 30.5 ppm.

5-(5-(Hydroxymethyl)pyridin-3-yl)-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**14**). The title compound was obtained according to general method (D) using N-((tetrahydro-2H-pyran-4-yl)methyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole-3-carboxamide (**26**, 0.1 g, 0.26 mmol), (5-bromopyridin-3-yl)methanol (0.098 g, 0.52

mmol), CsF (0.079 g, 0.52 mmol), $PdCl_2$(dppf) (0.019 g, 0.026 mmol). The crude was purified by reverse phase chromatography (C18, NH_4HCO_3/CH_3CN) to give 0.013 g of the title product. Yield = 13%. LC-MS (ESI) m/z: found 367.1 $[M+H]^+$. HRMS (ESI) m/z: found 367.1767 $[M+H]^+$; for $C_{20}H_{22}N_4O_3$ exact mass calculated 366.1692 (367.1765 $[M+H]^+$). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 13.68 (br s, 1H), 8.79 (d, 1H, *J*=1.6 Hz), 8.46-8.53 (m, 3H), 8.03 (s, 1H), 7.73-7.81 (m, 2H), 5.42 (t, 1H, *J*=5.7 Hz), 4.65 (d, 2H, *J*=5.5 Hz), 3.85 (br dd, 2H, *J*=2.4, 11.3 Hz), 3.21-3.27 (m, 4H), 1.83-1.91 (m, 1H), 1.61 (br d, 2H, *J*=12.6 Hz), 1.23 ppm (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.3, 146.7, 146.2, 140.8, 138.8, 137.8, 135.5, 132.4, 131.3, 125.9, 122.2, 119.7, 111.5, 66.7, 60.6, 44.0, 35.0, 30.5 ppm.

5-(4-(Methoxymethyl)pyridin-3-yl)-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**15**). The title compound was obtained according to general method (D) using N-((tetrahydro-2H-pyran-4-yl)methyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole-3-carboxamide (**26**, 0.2 g, 0.29 mmol), 3-bromo-4-(methoxymethyl)pyridine (0.079 g, 0.39 mmol), CsF (0.079 g, 0.52 mmol), and $PdCl_2$(dppf) (0.021 g, 0.026 mmol). The crude was purified by column reverse phase C18 (NH_4HCO_3/CH_3CN) to give 0.046 g of the title product. Yield = 6%. LC-MS (ESI) m/z: found 381.1 $[M+H]^+$. HRMS (ESI) m/z: found 381.1927 $[M+H]^+$; for $C_{21}H_{24}N_4O_3$ exact mass calculated 380.1848 (381.1921 $[M+H]^+$). ^{1}H NMR (400 MHz, CD_3OD): δ 8.54 (d, *J* = 5.2 Hz, 1H), 8.46 (s, 1H), 8.21-8.23 (m, 1H), 7.69 (dd, *J* = 8.6, 0.8 Hz, 1H), 7.62-7.65 (m, 1H), 7.43 (dd, *J* = 8.6, 1.7 Hz, 1H), 4.45 (s, 2H), 3.93-3.98 (m, 2H), 3.41 (td, *J* = 11.9, 2.1 Hz, 2H), 3.35 (s, 3H), 3.34 (d, *J* = 7.0 Hz, 2H), 1.87-1.98 (m, 1H), 1.68-1.75 (m, 2H), 1.31-1.43 ppm (m, 2H).

5-(4-(Hydroxymethyl)pyridin-3-yl)-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-indazole-3-carboxamide (**16**). The title compound was obtained according to general method (E) using N-((tetrahydro-2H-pyran-4-yl)methyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole-3-carboxamide (**26**, 0.1 g, 0.26 mmol), 3-bromopyridine-4-carbaldehyde (0.053 g, 0.29 mmol), Na_2CO_3 (0.055 g, 0.52 mmol), and $Pd(PPh_3)_4$ (0.015 g, 0.013 mmol). The crude was purified by flash chromatography (SiO_2, DCM/MeOH) to give 0.013 g of the title product. Yield = 14%. LC-MS (ESI) m/z: found 367.1 $[M+H]^+$. HRMS (ESI) m/z: found 367.1760 $[M+H]^+$; for $C_{20}H_{22}N_4O_3$ exact mass calculated 366.1692 (367.1765 $[M+H]^+$). ^{1}H NMR (400 MHz, CD_3OD): δ 8.53 (d, *J* = 5.2 Hz, 1H), 8.40 (bs, 1H), 8.18-8.19 (m,1 H), 7.72-7.73 (m, 1H), 7.68-7.70 (m, 1H), 7.41 (dd, *J* = 8.6, 1.6 Hz, 1H), 4.61 (s, 2H), 3.94 (dd, *J* = 11.4, 2.8 Hz, 2H), 3.38-3.44 (m, 2H), 3.32 (d, *J* = 7.0 Hz, 2H), 1.85-1.99 (m, 1H), 1.65-1.75 (m, 2H), 1.24-1.42 ppm (m, 2H).

X-RAY CRYSTAL STRUCTURE OF GSK-3B KINASE IN COMPLEX WITH COMPOUND 1

Crystals of GSK-3β in complex with ligand **1** were obtained by mixing 0.5 µl protein solution (4 mg/mL in 20 mM MOPS pH 7.0, 98 mM NaCl, 10% (v/v) glycerol, 1 mM DTT, 1x Phosphatase Inhibitor Cocktail preincubated with 5 mM ligand 1) with 0.5 µL reservoir solution (18% (w/v) PEG8000, 0.13 M NaCl, 0.1 M Tris Acetate pH 8.0) using sitting drop vapor diffusion method at 277 K. Before flash freezing in liquid nitrogen crystals were cryo-protected by reservoir solution supplemented with 25% (v/v) glycerol.

Diffraction data were collected at 100 K at the Swiss Light Source (SLS, Villigen, Switzerland). Data were processed to 2.14 Å resolution using the programs XDS and XSCALE.[1] The crystals belong to space group $C222_1$ containing 1 GSK-3β molecule per asymmetric unit. The phase information necessary to determine and analyze the structure of GSK-3β in complex with ligand 1 was obtained by molecular replacement using the program PHASER[2] and a previously solved GSK-3β structure as search model. CORINA[3] was used for ligand parameterization. Subsequent model building and refinement was performed with the software packages CCP4 and COOT.[4-5] For the measure of the free R-factor a measure to cross-validate correctness of the final model, about 5.6% of measured reflections were excluded from the refinement procedure (see table below). Several rounds of manual model building in COOT and bulk solvent correction, positional, B-factor and TLS refinement using REFMAC yielded the final model.[4-5] Data collection and model statistics are shown in the table below. The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 6TCU).

Ligand	**1**
X-ray source	PXI/X06SA (SLS)
Wavelength [Å]	1.0000
Detector	EIGER X 16M
Temperature [K]	100
Space group	C 2 2 2_1
Cell: a; b; c; [Å]	84.78; 107.85; 104.23
a; b; g; [◦]	90.0; 90.0; 90.0
Resolution [Å]	2.14 (2.39-2.14)
Unique reflections	26305 (7373)
Multiplicity	4.3 (4.3)

Completeness [%]	98.5 (99.3)
R_{sym} [%]	3.5 (44.3)
R_{meas} [%]	4.0 (50.7)
CC1/2	1.00 (0.856)
Mean(I)/sd	20.08 (3.52)
Number of reflections (working /test)	24826 / 1478
Rcryst [%]	20.2
Rfree[%]2	24.0
Total number of atoms:	
Protein	2814
Water	87
Ligand 1	31
Acetate	8
Deviation from ideal geometry:	
Bond lengths [Å]	0.011
Bond angles [°]	1.50
Bonded B's [$Å^2$]	3.7
Ramachandran plot:	
Most favoured regions [%]	90.5
Additional allowed regions [%]	8.9
Generously allowed regions [%]	0.3
Disallowed regions [%]	0.3

DOCKING MATERIAL

The coordinates of the X-ray structures of GSK-3β in complex with **1** (PDB ID: 6TCU) were used for ligand docking of **14**. Glide module of the Schrödinger molecular simulation package was used.[6] The protein structure for ligand docking was prepared with Protein Preparation Wizard. The ligand used in this study was converted to 3D structure and prepared in terms of the most probable protonation states and tautomers with Schrödinger's LigPrep tool.[7] A conformational search was also carried out using ConfGen with default setting.[8] The resulting 64 conformers constituted the input dataset for the subsequent ligand docking.

The binding site was defined by a box of 10 Å centered on the centroid of the co-crystalized inhibitor. Default setting was used to prepare the receptor grid. In addition, we treated thiol and hydroxyl groups of Tyr134, Thr138 and Cys199 as rotatable (flexible) groups. Three H-bond constraints were defined being the carbonyl oxygen of the hinge residues Asp133 and Val135, and the hydrogen of the NH- group of Val135. By using this setting, we required that certain receptor-ligand hydrogen bonds crucial for compound activity were formed. Glide SP protocol was used to dock analog **14**. In addition to the default settings, we used the enhanced sampling option to enhance the sampling of conformational space by adding variations on the input structure to the conformational search. We also included aromatic ligand hydrogen atoms as donors. When docking ligands, all H-bond constraints needed to be satisfied.

MD SETUP

The best scored binding pose of **14** within the ATP binding site of GSK-3β (docking score=-9.778 kcal/mol) was processed in a 500 ns MD simulation using the graphics processing unit (GPU) tool of the Desmond MD package as supplied with the Schrödinger 2019-2 suite.[9] Water molecules within the binding site were preserved from the original crystal structure, thus easing the burden of solvent equilibration in critical areas. The Desmond System Builder included in the Schrödinger software suite was used to create a fully solvated system ready for MD simulation. The system was solvated with simple point charge (SPC) waters and a cubic solvation box with a 10 Å buffer around the original complex. The system was electrostatically neutralized by adding Na^+ ions in defined positions by using the ion placement option. Default parameters of OPLS-3e forcefield were used. The prepared systems consisted of approximately 63,300 atoms. The MD simulation protocol followed an extended relaxation schedule consisting of the following steps:

1. 100 ps, constant-volume ensemble (NVT) (T= 10 K); 50 kcal/mol/Å2 restraints on the solute heavy atoms;
2. 120 ps, NVT (T= 10 K); 50 kcal/mol/Å2 restraints on the solute heavy atoms;
3. 120 ps, constant-pressure ensemble (NPT) (T=10K, P= 1 atm); 50 kcal/mol/Å2 restraints on the solute heavy atoms;
4. 120 ps, NPT (T= 300 K, P= 1 atm); 50 kcal/mol/Å2 restraints on the solute heavy atoms;
5. 2400 ps, NPT (T= 300 K,P= 1 atm); unrestrained;
6. 500 ns, NPT (T= 300 K,P= 1 atm); unrestrained.

The production simulation timescale allowed reasonable sampling of solvent and local motions in a reasonable amount of GPU time. The analysis of the MD trajectory was performed with the Simulation Interaction Diagram tool within the Maestro environment of the Schrödinger suite.[9]

ENZYMATIC GSK-3β ASSAY

Human recombinant enzyme (Carna Bioscience, Kobe, Japan) was incubated for 90 minutes at 22°C in the presence of compounds or vehicle in a reaction buffer containing ATP plus unphosphorylated specific substrate peptide. Substrate phosphorylation was measured by LANCE technology (PerkinElmer, CT, USA). Compounds were tested at five concentrations ranging from 1 nM to 10 μM with 10-fold dilutions in duplicate. Staurosporine was used as internal reference compound. The IC_{50} values (concentration causing a half-maximal inhibition of control specific activity), 95% Confidence Intervals (CI) and Hill coefficients (nH) were determined by non-linear regression analysis of the inhibition curves generated with mean replicate values using Hill equation curve fitting.

hERG ASSAY

Using previously described methods,[10] compounds **1-16** were assayed on hERG CHO-K1 cell line by automated whole-cell patch clamp at Eurofins Panlabs, St. Charles, MO, USA. Compounds were tested at five concentrations ranging from 10 nM to 100 μM with 10-fold dilutions in duplicate. E-4031 was used as internal reference compound. The degree of inhibition (%) of tail current was obtained by measuring the tail current amplitude, which was induced by a one second test pulse to - 40 mV after a two second pulse to + 20 mV, before and after drug incubation (the difference current was normalized to control and multiplied by 100 to obtain the percent of inhibition).

Concentration (log) response curves were fitted to a logistic equation (three parameters assuming complete block of the current at very high test compound concentrations) to generate estimates of IC_{50} values. The concentration-response relationship of each compound was constructed from the percentage reductions of current amplitude by sequential concentrations.

CELLULAR GSK-3B ASSAY

Compound **14** was tested in full dose response at eight concentrations ranging from 10 nM to 30 μM with 3-fold dilutions in quadruplicate on recombinant human embryonic kidney (HEK) cells stably transfected with both Tau protein and GSK-3β (T-REx/MAPT+GSK-3β-ISO2_K3) cell line (Axxam S.p.A., Bresso, Italy) to evaluate activity on GSK-3β (GSK-3β Assay Protocol). In parallel the possible toxic effect of compound **14** was also evaluated on the same cells (Cell Viability Protocol). *N*-(4-Methoxybenzyl)-*N′*-(5-nitro-1,3-thiazol-2-yl)urea (AR-A014418), a selective GSK-3β inhibitor was used as reference compound.

GSK-3β Assay Protocol. HEK T-REx/MAPT+GSK-3β-ISO2_K3 cells were seeded at 10,000 cells/well on poly-D-lysine coated black/clear bottom 384 V bottom plate in complete medium. 24 hours after seeding, medium was replaced with complete medium supplemented with 1 μg/mL doxycycline, to induce GSK-3β expression. The cells were incubated with compound **14** at tested concentrations for 18 hours at 37°C, then lysed and processed for the detection of phosphorylated Tau protein (PHERAstar reader).

Cell Viability Protocol. HEK T-REx/MAPT+GSK-3β-ISO2_K3 cells were seeded at 10,000 cells/well on poly-D-lysine coated black/clear bottom 384 V bottom plate in complete medium. 24 hours after seeding, medium was replaced with complete medium supplemented with 1 μg/mL doxycycline, to induce GSK-3β expression. The cells were incubated with compound **14** at tested concentrations for 18 hours at 37°C, then CellTiter-Glo® reagent was added, and the signal of the emitted luminescence was recorded at $FLIPR_{TETRA}$.

The percent values of activity with respect to control (untreated cells) were calculated. IC_{50} curves were generated using Screener 13.0.5 software.

***IN VITRO* ADME METHODS**

Aqueous solubility assay (PBS, pH 7.4). Test compound was dissolved in DMSO as stock solution and then added to isotonic buffer (pH 7.4) at a concentration of 200 μM (2% DMSO final concentration). After a 24 hours incubation under shaking at room temperature, dissolved compound is measured by a chromatographic procedure with photodiode array detection. Metoprolol tartrate, rifampicin, ketoconazole, phenytoin, haloperidol, simvastatin, diethylstilbestrol and tamoxifen were used as reference compounds for different solubility degrees.

Plasma protein binding (human and mouse). Plasma protein binding was assayed by equilibrium dialysis method at Eurofins Panlabs, St. Charles, MO, USA. Briefly, the dialysate compartment was loaded with 0.05 M phosphate buffer and the sample side was loaded with plasma spiked with the test compound (10 μM final concentration). The samples were incubated for 4 hours at 37°C, then they were prepared and analyzed by HPLC-MS/MS. Results were expressed as % plasma bound compound with respect to the initial concentration. The sum of the mass of the test compound in the test buffer and plasma samples compared to the mass in the plasma at the start of the assay is used to calculate percent recovery. Acebutolol, quinidine and warfarin were used as reference compounds.

***In vitro* metabolic stability.** In the metabolic stability assays performed at Eurofins Panlabs, St. Charles, MO, USA, test compound at 0.1 µM was pre-incubated with pooled liver microsomes (human, Sprague-Dawley rat or CD mouse) in phosphate buffer (pH 7.4) for 5 minutes in a 37°C shaking water bath. The reaction was initiated by adding NADPH-generating system and incubated for 0, 15, 30, 45, and 60 minutes. The reaction was stopped by transferring the incubation mixture to CH_3CN/MeOH. Samples were then mixed and centrifuged. Supernatants were used for HPLC-MS/MS analysis. All assays were run with specific positive controls. Imipramine, propranolol, terfenadine and verapamil were used as reference compounds. Metabolic stability, expressed as percent of the parent compound remaining, was calculated by comparing the peak area of the compound at the time point relative to that at time-0. The half-life ($t_{1/2}$) was estimated from the slope of the initial linear range of the logarithmic curve of compound remaining (%) vs. time, assuming the first-order kinetics.

CYP inhibition. CYP inhibition assay was performed at Eurofins Panlabs, St. Charles, MO, USA on human liver microsomes, panel of 6 assays including CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4. Briefly, human liver microsomes were incubated for 20-50 minutes (depending on CYP subtype) at 37°C in the presence of specific substrates, then specific metabolite formation was measured by HPLC-MS/MS method. Inhibition reference compounds were tested concurrently. Peak areas corresponding to the metabolite of each substrate were recorded. The percent of control activity was then calculated by comparing the peak area obtained in the presence of the test compound to that obtained in the absence of the test compound. Subsequently, the percent of inhibition was calculated by subtracting the percent control activity from 100 for each compound.

PK STUDY

Animal treatment. Ten male CD1-CR mice (Charles River, Calco, Italy), 18 - 25 g, were used. The animal house was maintained under artificial lighting (12 hours) between 7:00 and 19:00 in a controlled ambient temperature of 21 ± 3°C, and relative humidity between 20-80%. All animals had free access to food and water.
Compound **14** was administered intraperitoneal (i.p.) route at 10 mg/kg dose. The collection was performed at 0.5, 1, 2, 4, and 7 hours post administration. Compound was prepared in 10 % polyethylene glycol (PEG) 400 and 10% Tween 80 in physiological saline. At each time point, blood and brain samples from two mice were collected.
Animals were placed under isoflurane anaesthesia and approximately 400 µL of blood were collected by cardiac puncture using a sterile disposable syringe for terminal blood sampling. The blood samples were immediately transferred into tubes containing anticoagulant agent (K3 EDTA) for plasma preparation. After sealing each tube, the blood samples were gently agitated and stored on ice until centrifugation (within 30 minutes of sampling). The samples were centrifuged at +4°C, at 2000 g, for 10 minutes. The entire resultant plasma was immediately transferred into 2 tubes (2 aliquots of approximately 100 µL). The tubes were stored at approximately -80°C until analysis.
Immediately after blood collection, animals were euthanized through cervical dislocation. Their brain was quickly removed from the skull, rinsed in physiological saline and placed in pre-labelled tubes and frozen in liquid nitrogen until the storage at -80°C.
The study was conducted in compliance with Animal Health regulations for the ethical treatment of animals. In particular with the Council Directive No. 2010/63/UE on the protection of animals used for scientific purposes and in accordance with the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The study was authorized by Prefet de la Mayenne (France). The investigation of the pharmacokinetic properties of compound **14** was performed by an hybrid approach (snapshot/rapid design) considering the objective of having a robust PK evaluation reducing the number of sacrificed animals.[11]

Bioanalysis. Compound **14** in mouse plasma and brain samples was determined by LC-MS/MS method in the following quantification range 0.488 - 1000 ng/mL and 0.977 - 2000 ng/g, respectively.
Plasma study samples, calibrators and Quality Controls (30 µL) were spiked with a solution (40 ng/mL; 10 µL) of a structurally related substance used as Internal Standard (IS), and successively deproteinized with CH_3CN containing 0.1% formic acid (250 µL). After centrifuging, the supernatant was evaporated under N_2 flow (SPE Dry, Biotage). The residue was dissolved in H_2O/ CH_3CN = 90:10 (% v/v) containing 0.1% formic acid (200 µL).
Brain study samples, calibrators and Quality Controls (100 mg) were added with 1 mL of CH_3CN containing 1% v/v formic acid and then with IS solution (0.5 µg/mL, 10 µL). After vortexing, the sample was submitted to bead beating homogenization procedure (30 s at 6000 rpm for 3 cycles) using Precellys 24 (Bertin Instrument) and successively centrifuged. An aliquot of the clear supernatant (200 µL) was evaporated under N_2 flow (SPE Dry, Biotage). The residue was dissolved in H_2O/CH_3CN 90:10 (%, v/v) (500 µL) containing 1% formic acid.
Analysis was performed by an LC-MS/MS method using Shimadzu Nexera LC30AD/SIL30AC coupled with QTRAP 5500 (SCIEX). Analyte and IS were separated by an Acquity UPLC® BEH C18 1.7µm 2.1 x 50 mm column injecting 3 µL of the final clear solution obtained from plasma and brain samples.
Gradient elution was achieved at a flow rate of 0.8 mL/min using water containing 0.1 % formic acid and 0.1% formic acid in CH_3CN as solvents of the mobile phase. Analysis was conducted by Multiple Reaction Monitoring (MRM) and positive electrospray ionization (ESI). The selected precursor and product ion transition was at *m/z* 367.1 → 252.0 for compound **14**.

PK data analysis. Pharmacokinetic parameters were calculated by non-compartmental analysis (NCA) of sparse plasma and brain concentrations obtained after intraperitoneal administration of 10 mg/kg of compound **14** in ten male mice. Analyses were

performed using the validated software Phoenix™ - WinNonlin® (v. 6.3). Sparse sampling computation was applied because of the few data available. With sparse data analysis, NCA first calculated the mean concentration curve for plasma or brain data at each unique time value for all the animals in the data set and then calculated the relative standard error values.

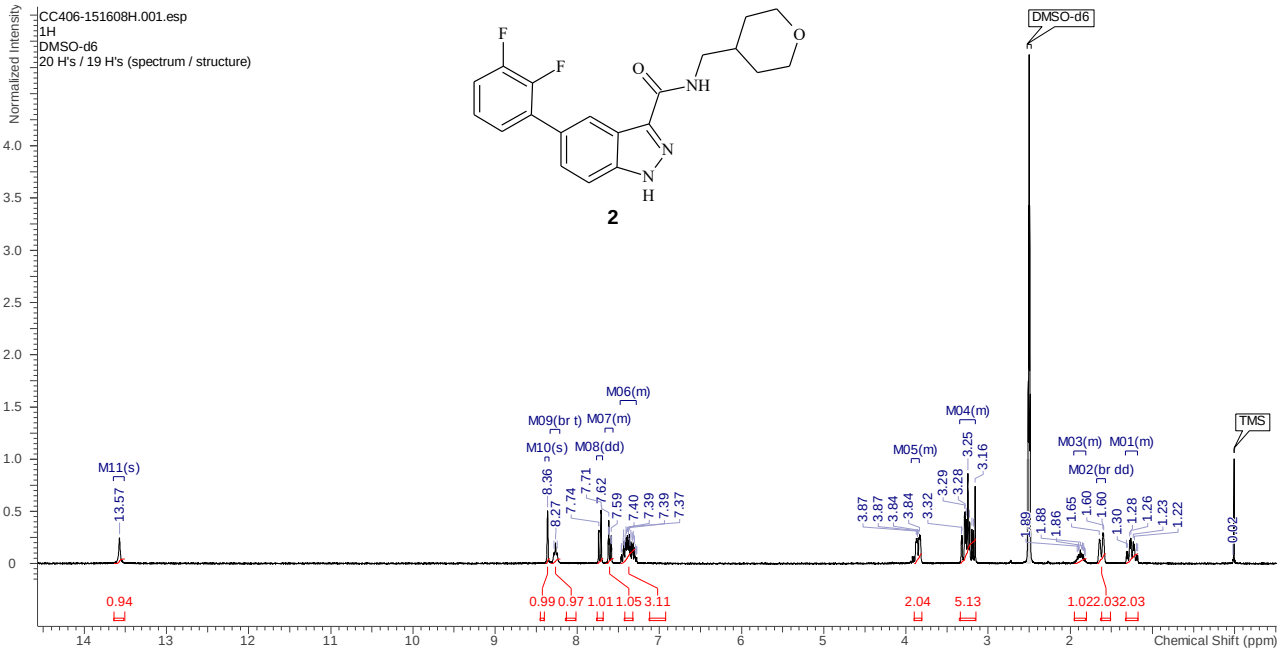
AMPHETAMINE MODEL

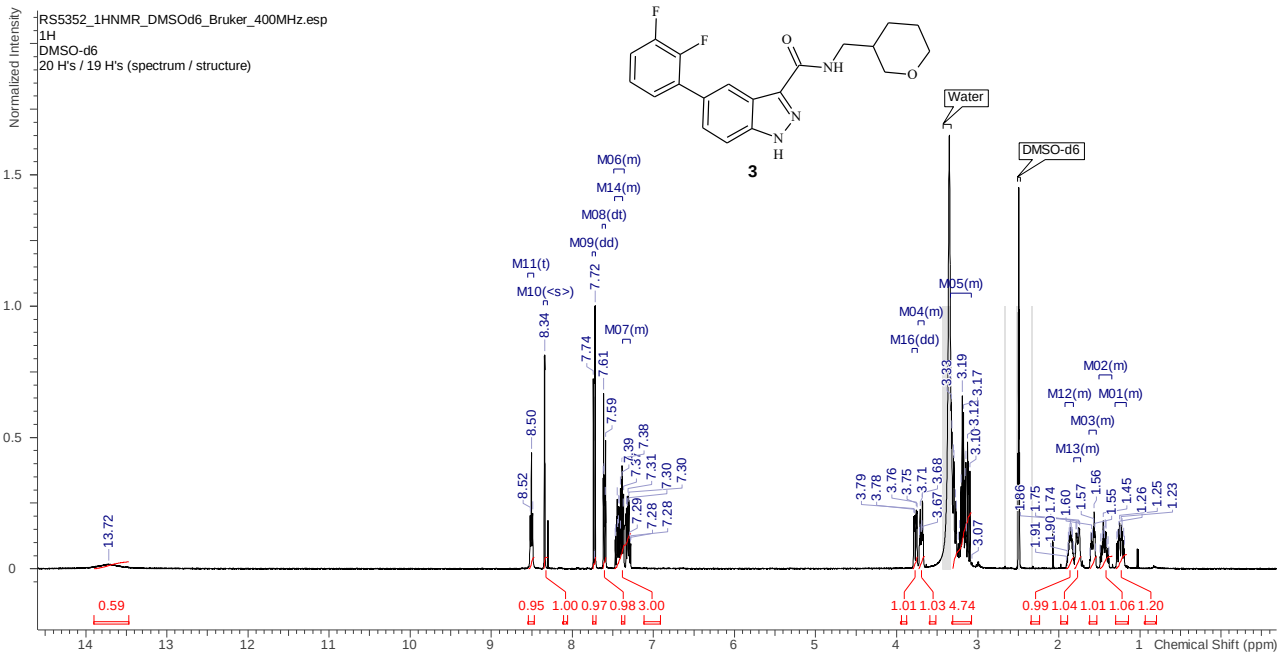
C57BL/6J male mice of 12 weeks old (Charles River, Calco, Italy) were housed in individual cages and allowed for two weeks to habituate in a climate-controlled animal facility (21± 2°C) and maintained on a 12-hour light/dark cycle (light on: 7 a.m. - 7 p.m.) with food and water ad libitum, before beginning experimentation, as previously described.[12] All experiments were carried out in accordance with the guidelines established by the European Communities Council Directive (Directive 2010/63/EU of 22 September 2010) and approved by the National Council on Animal Care of the Italian Ministry of Health. All efforts were made to minimize animal suffering and to use the minimal number of animals required to produce reliable results.

KINOME SELECTIVITY PANEL

Compound **14** was assessed in a 42 kinase assay panel using the LANCE or HTRF technology[12] at Eurofins Cerep, Celle L'Evescault, FRANCE. Compound was firstly tested at 10 μM in duplicate. The results were expressed as a percent of inhibition of control specific activity obtained in the presence of the test compounds. For kinases on which compound showed more than 50% inhibition at this concentration, a further characterization was done, consisting in a concentration-response curve and subsequent IC_{50} determination (Table S3). For 19 kinases, compound **14** was tested in duplicate at five concentrations in the range from 10 nM to 100 μM.

[1]HNMR AND [13]CNMR SPECTRA OF FINAL COMPOUNDS

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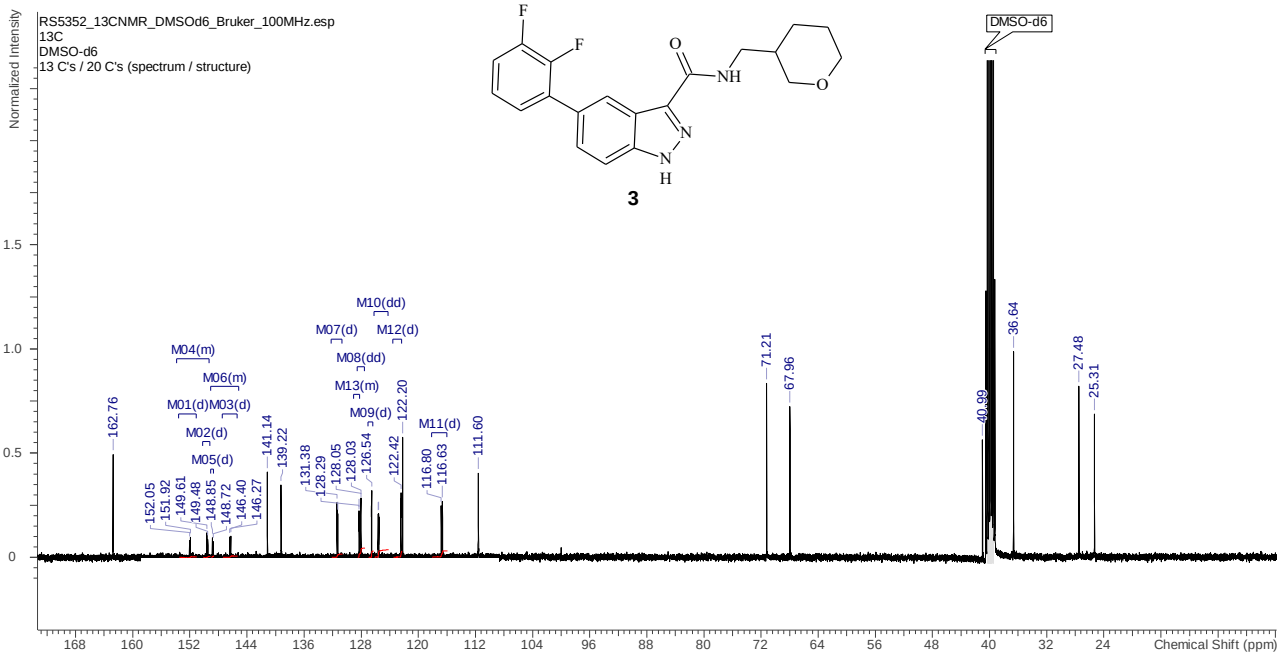
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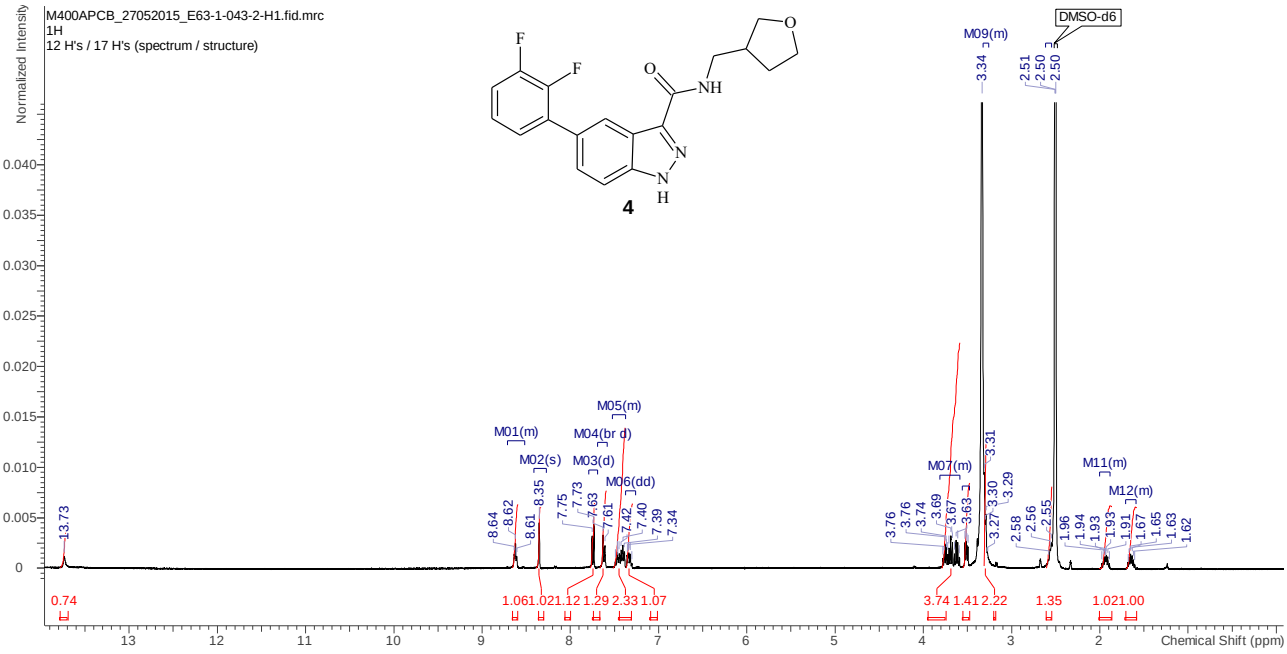
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Normalized Intensity
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F
O
NH
O
N
N
H
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M05(m)
M01(m)
M04(br d)
M02(s)
M03(d)
M06(dd)
M07(m)
M11(m)
M12(m)
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8.64
8.62
8.61
8.35
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7.73
7.63
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7.40
7.39
7.34
3.76
3.76
3.74
3.69
3.67
3.63
3.34
3.31
3.30
3.29
3.27
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1.93
1.91
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1.65
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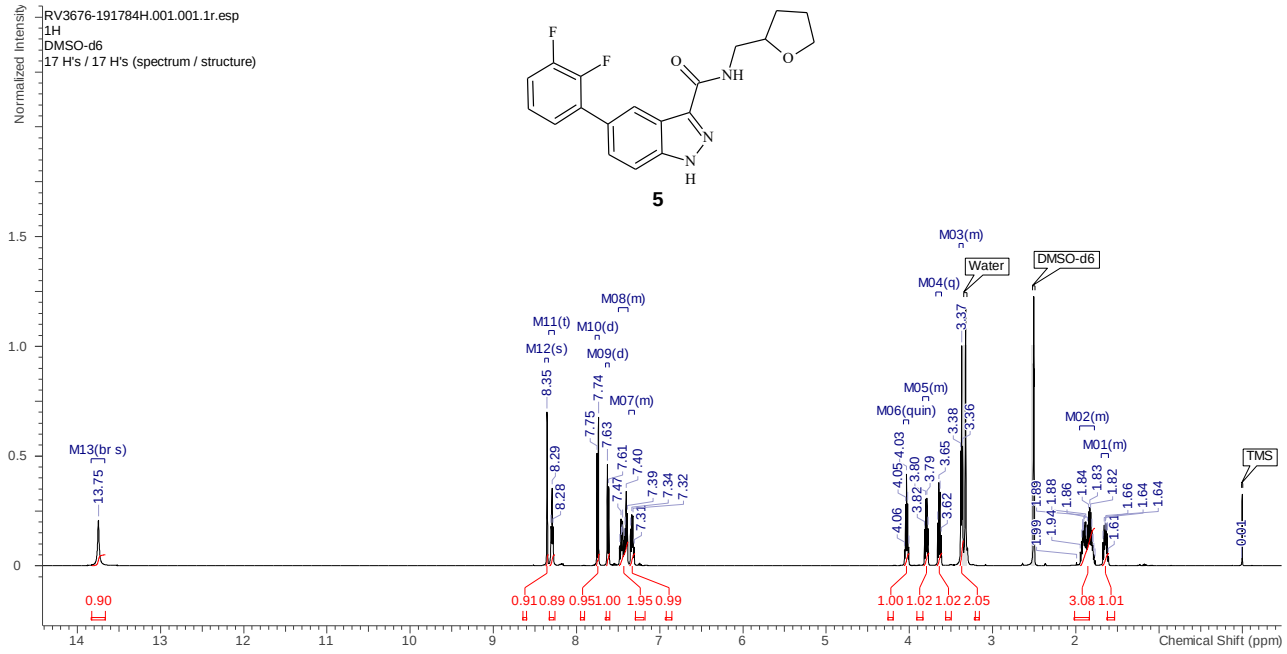
Chemical Shift (ppm)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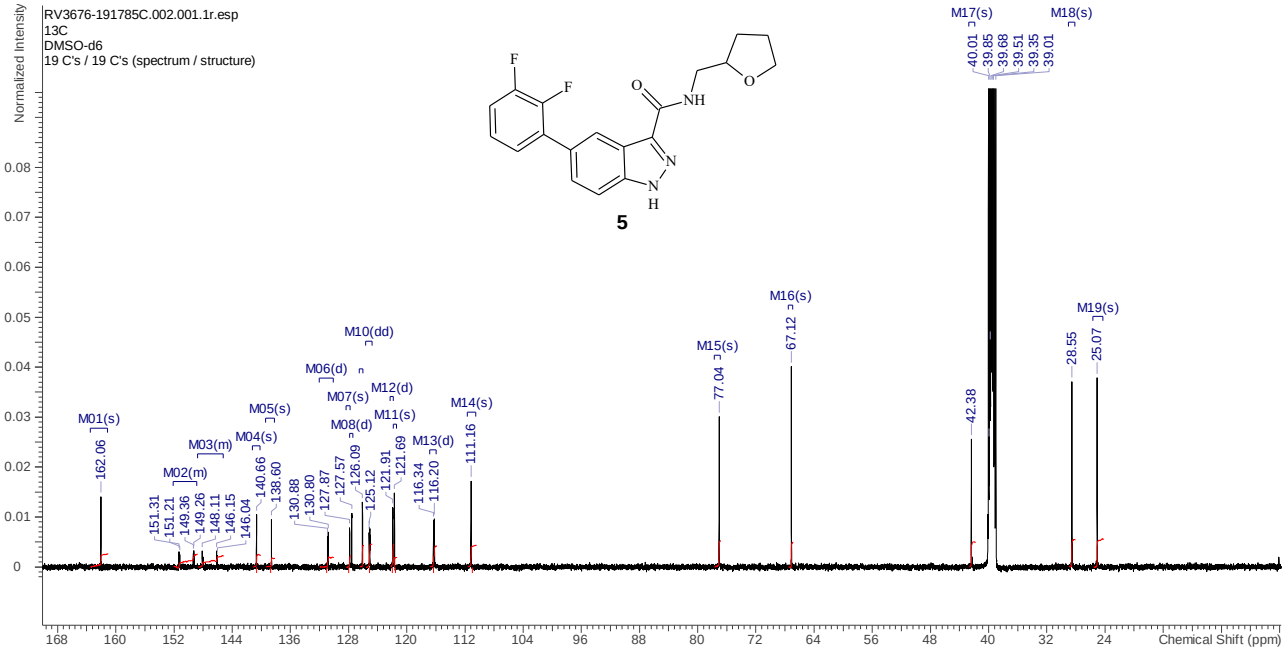
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M11(s)
M14(s)
M03(m)
M04(s)
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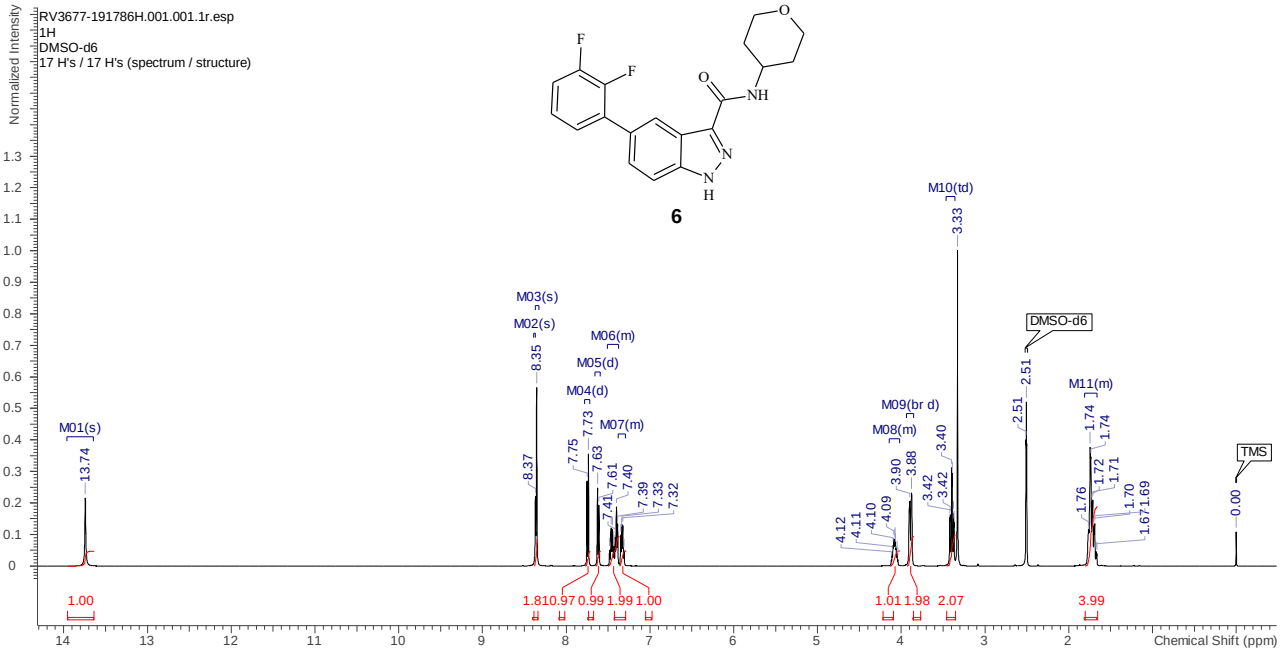
M19(s)
Chemical Shift (ppm)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M04(d)
M05(d)
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M11(m)
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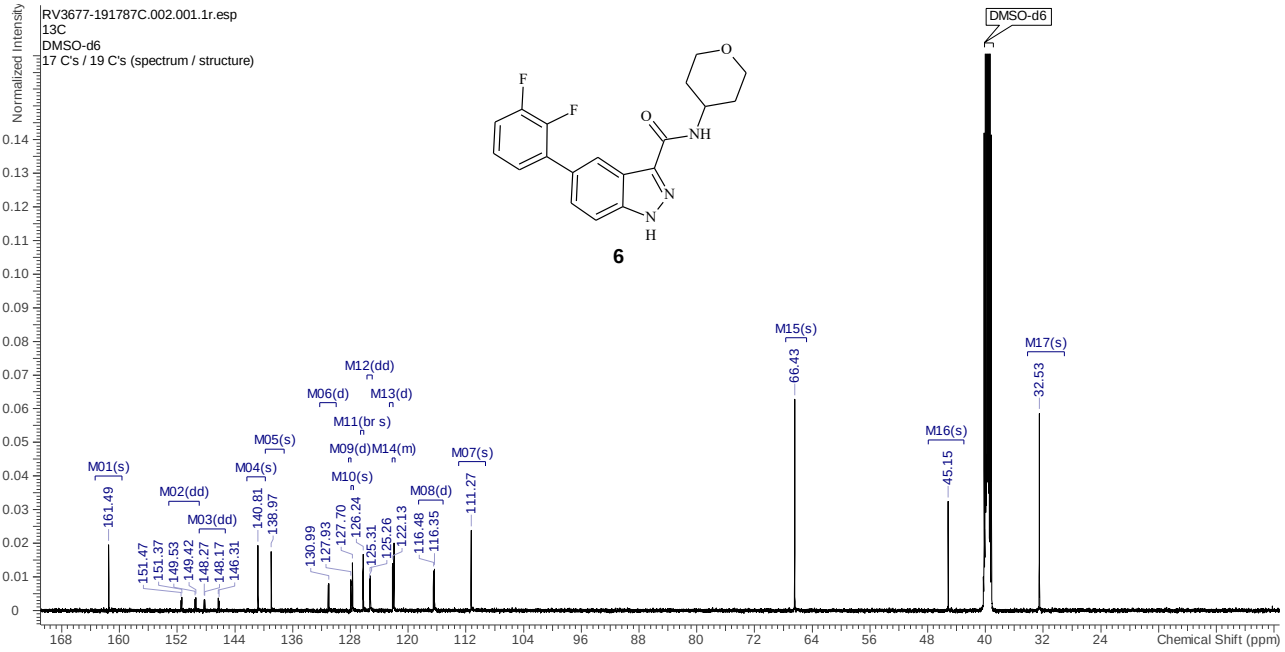
TMS
Chemical Shift (ppm)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M07(s)
M08(d)
M09(d)
M10(s)
M11(br s)
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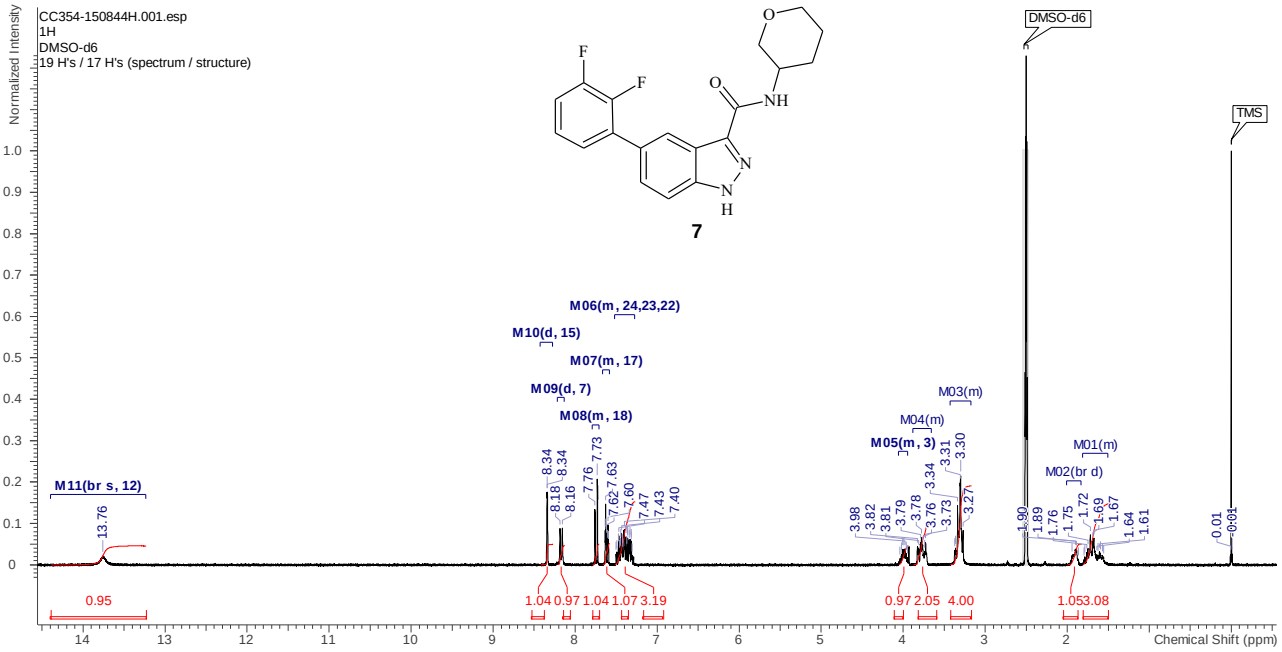
M17(s)
DMSO-d6
Chemical Shift (ppm)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M04(m)
M05(m, 3)
M01(m)
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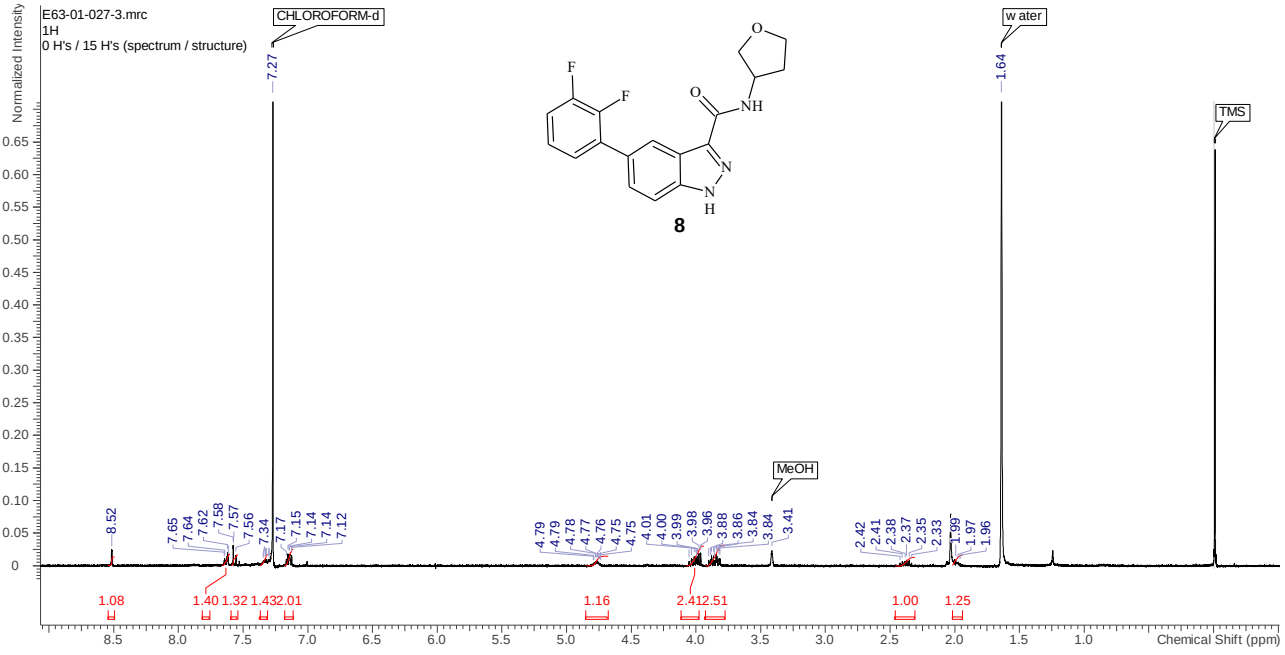
Chemical Shift (ppm)

7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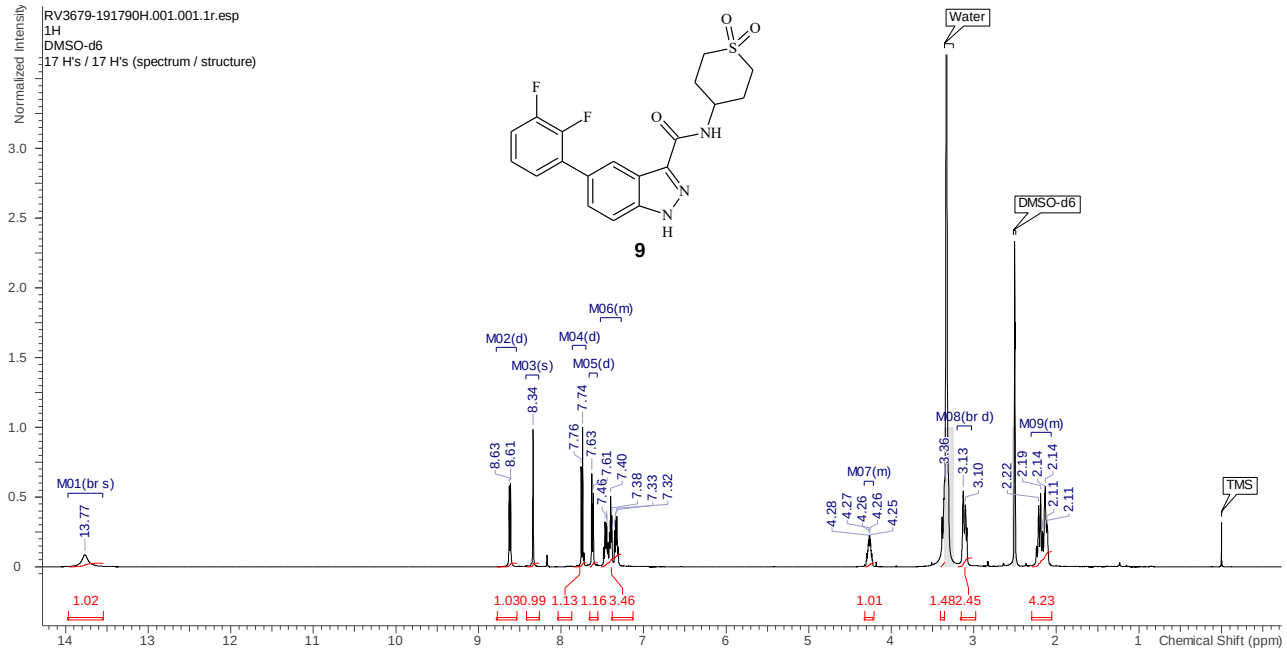
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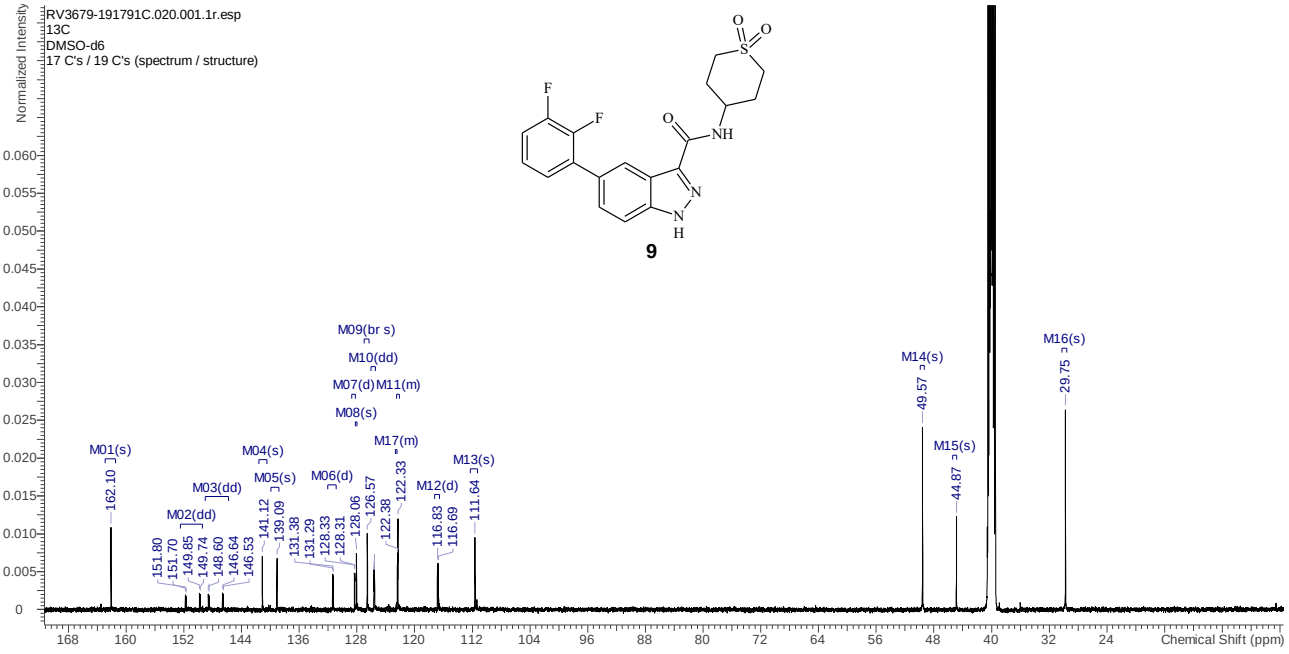
M09(m)
Chemical Shift (ppm)

9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M09(br s)
M10(dd)
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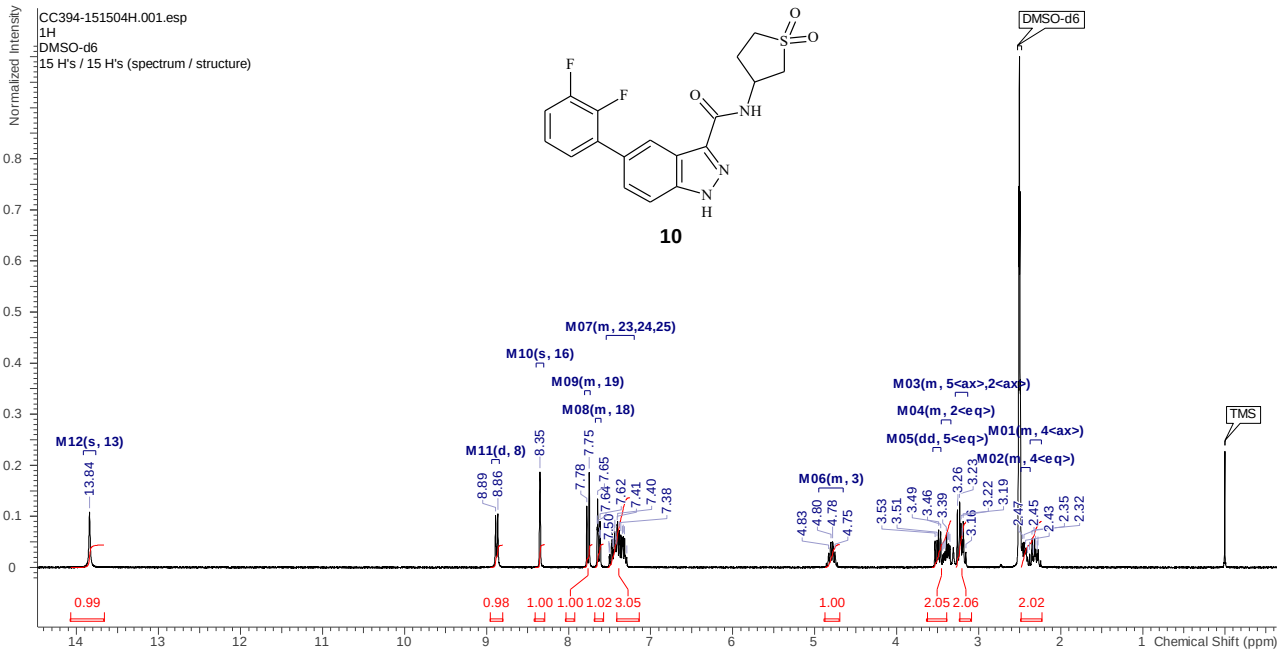
M17(m)
Chemical Shift (ppm)

9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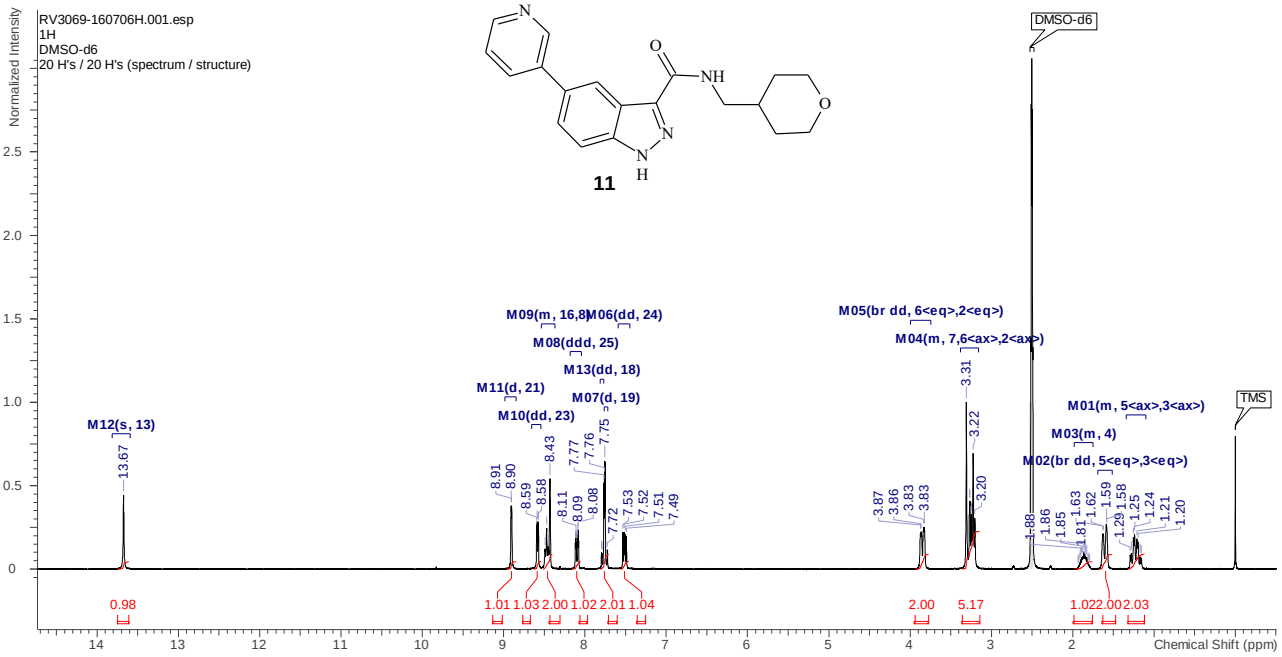
Chemical Shift (ppm)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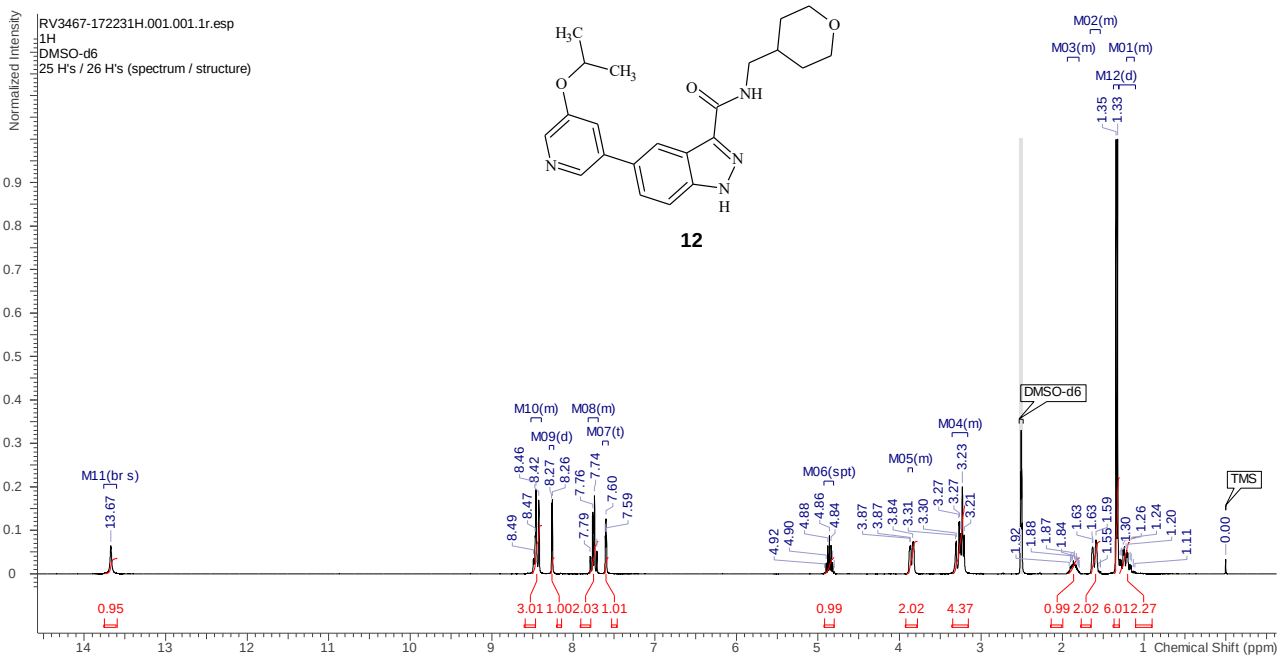
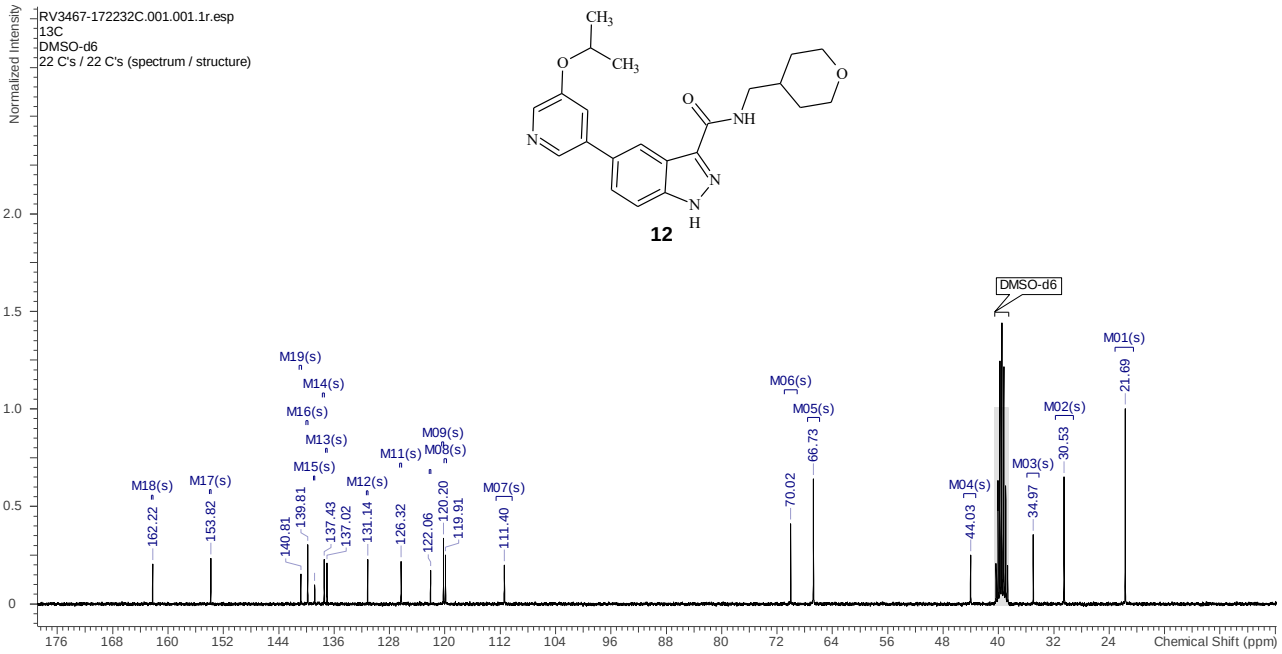
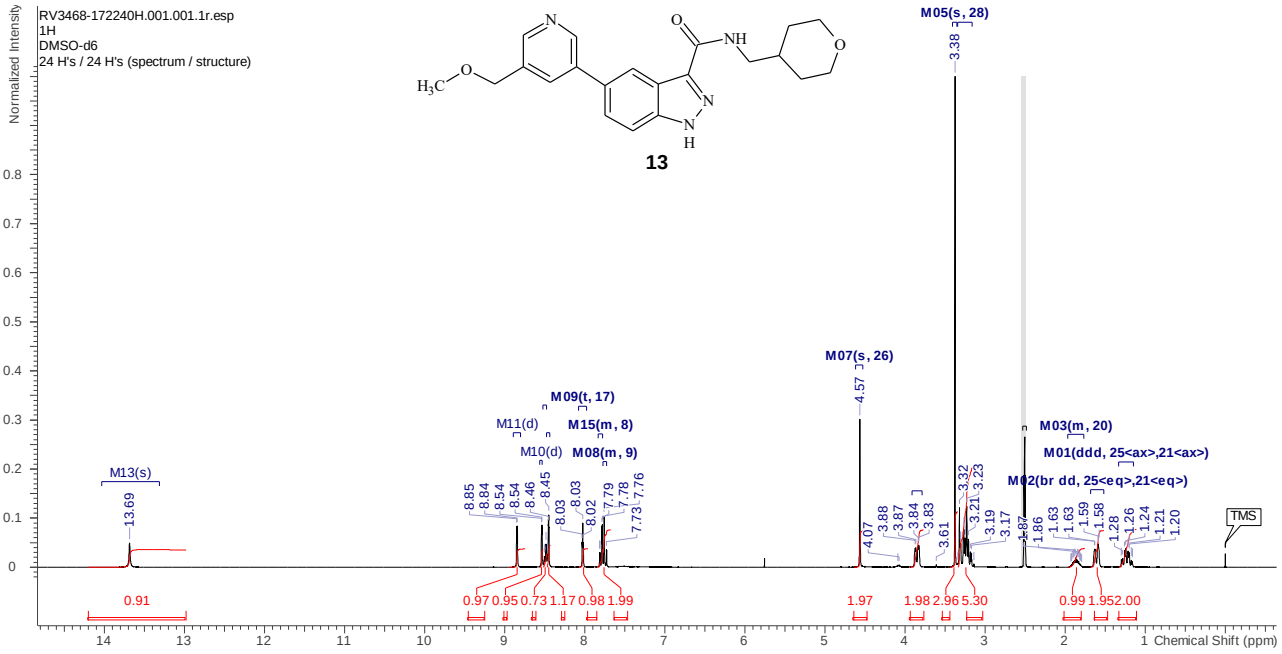
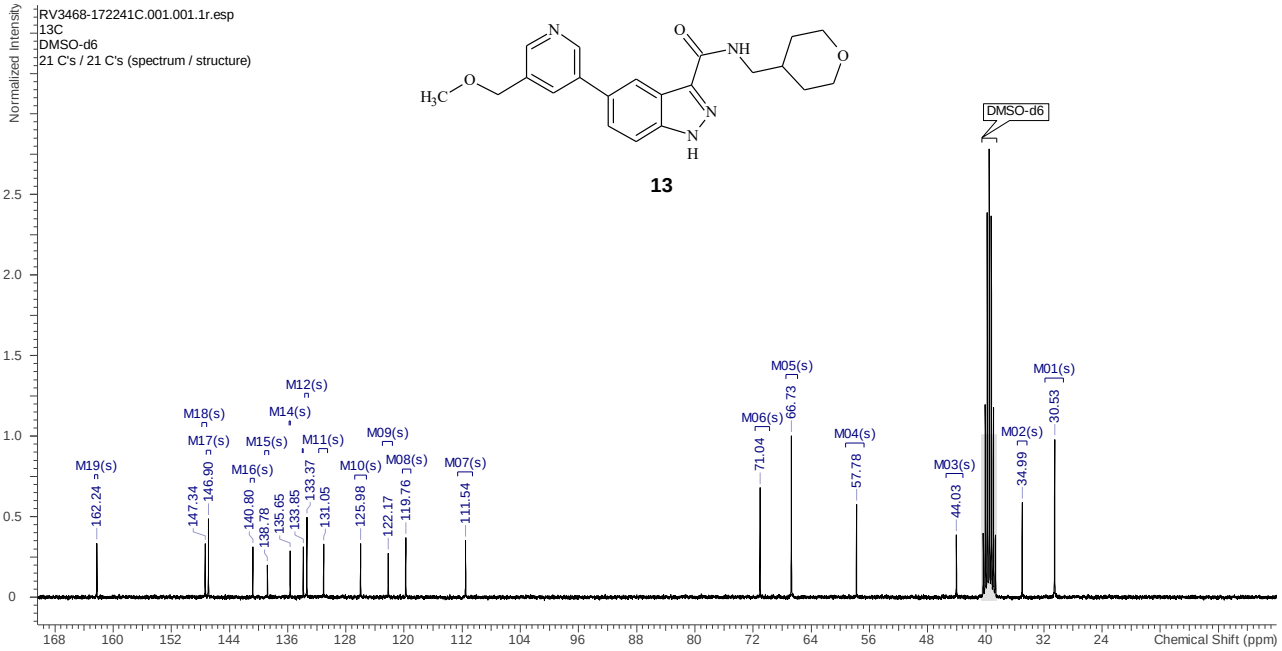
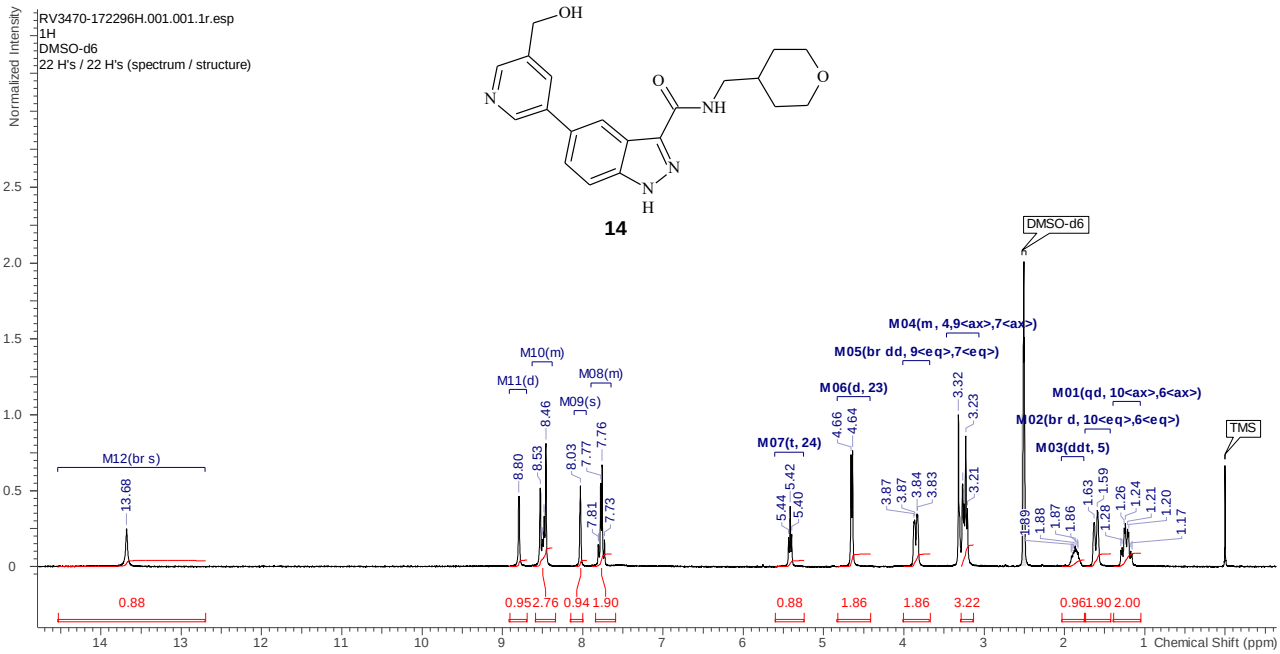
1.04
2.00
5.17
1.02
2.00
2.03
Chemical Shift (ppm)

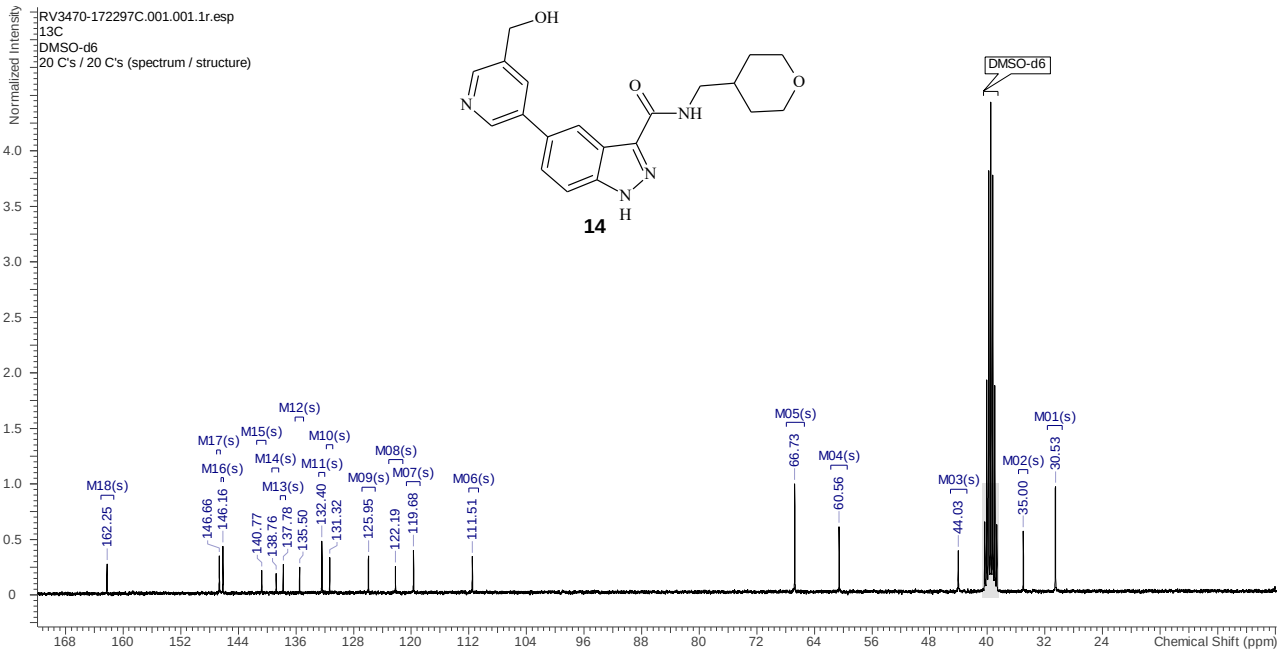

RV3467-172231H.001.001.1r.esp
1H
DMSO-d6
25 H's / 26 H's (spectrum / structure)
Normalized Intensity
Chemical Shift (ppm)
12
M11(br s)
M10(m)
M08(m)
M09(d)
M07(t)
M06(spt)
M05(m)
M04(m)
M02(m)
M03(m)
M01(m)
M12(d)
DMSO-d6
TMS
0.95
3.01
1.00
2.03
1.01
0.99
2.02
4.37
0.99
2.02
6.01
2.27

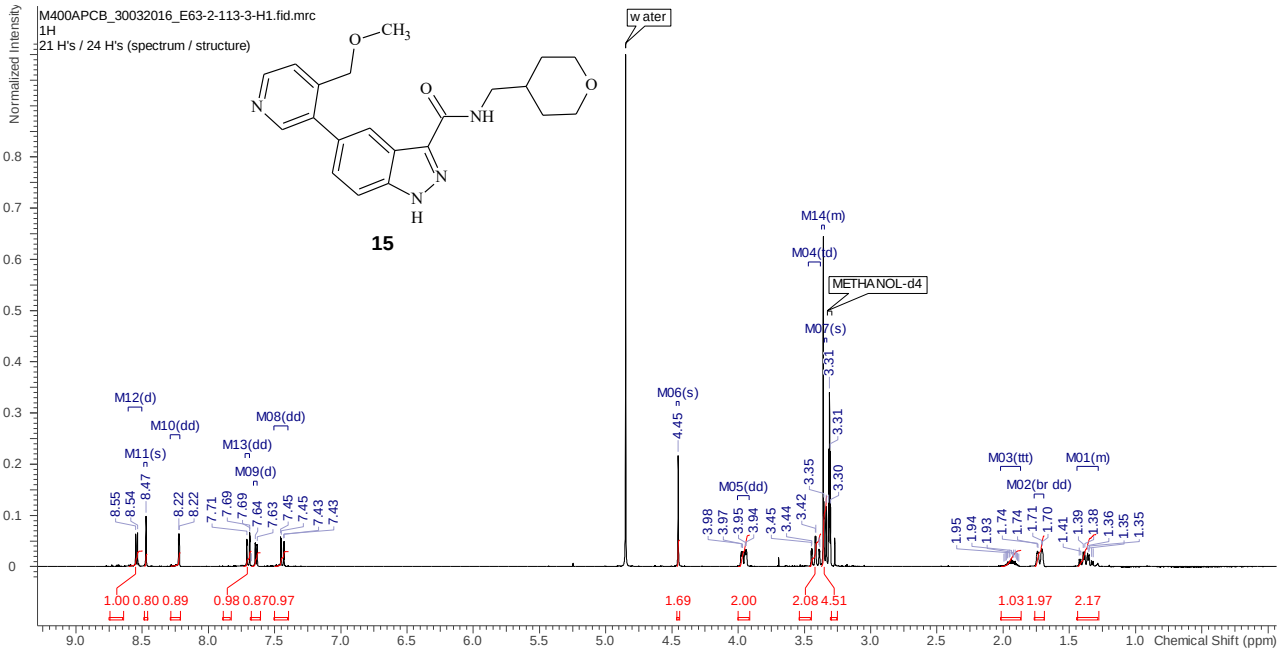

RV3467-172232C.001.001.1r.esp
13C
DMSO-d6
22 C's / 22 C's (spectrum / structure)
Normalized Intensity
Chemical Shift (ppm)
12
M19(s)
M14(s)
M16(s)
M13(s)
M15(s)
M18(s)
M17(s)
M12(s)
M11(s)
M09(s)
M08(s)
M07(s)
M06(s)
M05(s)
M04(s)
M03(s)
M02(s)
M01(s)
DMSO-d6
162.22
153.82
140.81
139.81
137.43
137.02
131.14
126.32
122.06
120.20
119.91
111.40
70.02
66.73
44.03
34.97
30.53
21.69

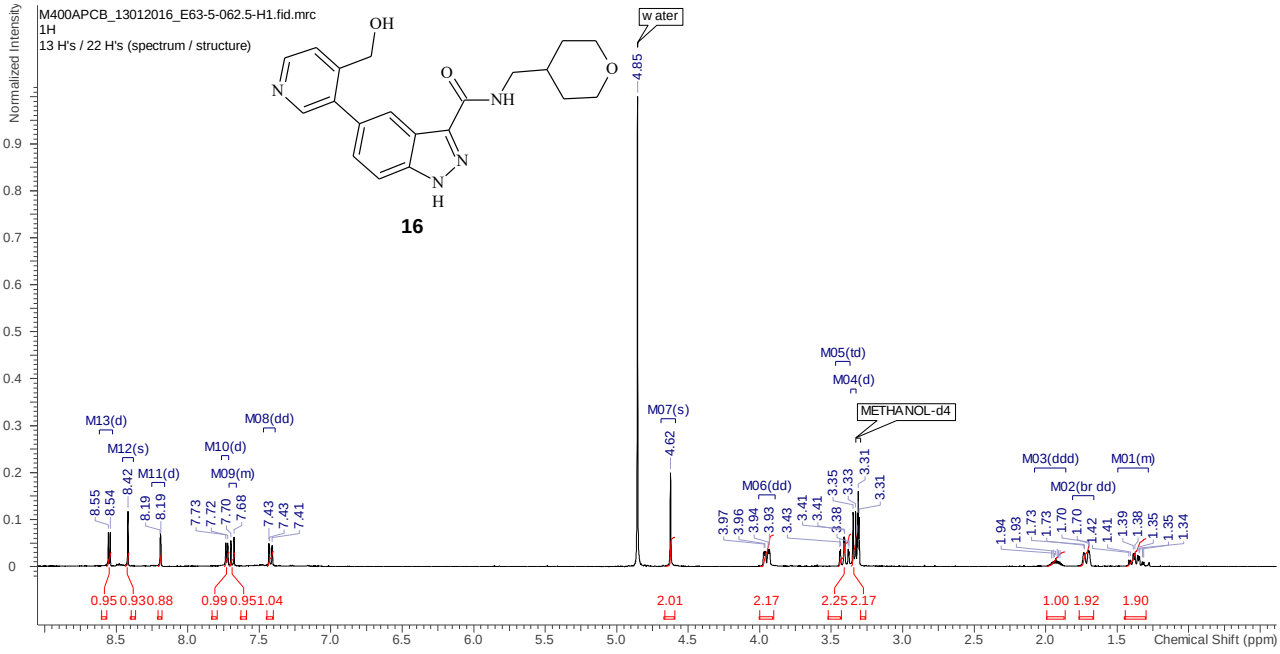

RV3468-172240H.001.001.1r.esp
1H
DMSO-d6
24 H's / 24 H's (spectrum / structure)
Normalized Intensity
13
M05(s, 28)
M07(s, 26)
M09(t, 17)
M11(d)
M15(m, 8)
M10(d)
M08(m, 9)
M03(m, 20)
M01(ddd, 25<ax>,21<ax>)
M02(br dd, 25<eq>,21<eq>)
M13(s)
TMS
Chemical Shift (ppm)


RV3468-172241C.001.001.1r.esp
13C
DMSO-d6
21 C's / 21 C's (spectrum / structure)
Normalized Intensity
13
DMSO-d6
M19(s)
M18(s)
M17(s)
M16(s)
M15(s)
M14(s)
M12(s)
M11(s)
M10(s)
M09(s)
M08(s)
M07(s)
M06(s)
M05(s)
M04(s)
M03(s)
M02(s)
M01(s)
Chemical Shift (ppm)


RV3470-172296H.001.001.1r.esp
1H
DMSO-d6
22 H's / 22 H's (spectrum / structure)
Normalized Intensity
14
DMSO-d6
TMS
M12(br s)
M11(d)
M10(m)
M08(m)
M09(s)
M07(t, 24)
M06(d, 23)
M05(br dd, 9<eq>,7<eq>)
M04(m, 4,9<ax>,7<ax>)
M02(br d, 10<eq>,6<eq>)
M03(ddt, 5)
M01(qd, 10<ax>,6<ax>)
Chemical Shift (ppm)

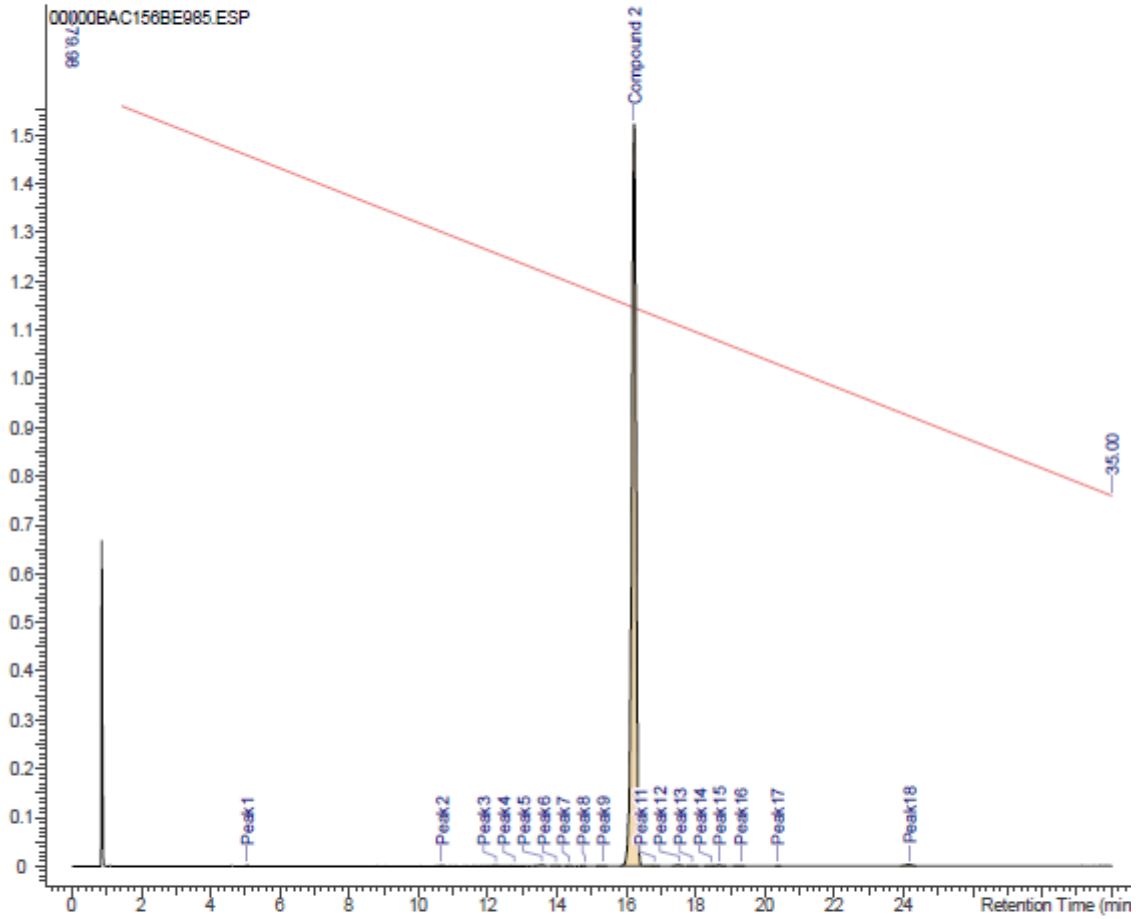

RV3470-172297C.001.001.1r.esp
13C
DMSO-d6
20 C's / 20 C's (spectrum / structure)
Normalized Intensity
14
DMSO-d6
M18(s)
M17(s)
M16(s)
M15(s)
M14(s)
M13(s)
M12(s)
M11(s)
M10(s)
M09(s)
M08(s)
M07(s)
M06(s)
M05(s)
M04(s)
M03(s)
M02(s)
M01(s)
Chemical Shift (ppm)


M400APCB_30032016_E63-2-113-3-H1.fid.mrc
1H
21 H's / 24 H's (spectrum / structure)
Normalized Intensity
Chemical Shift (ppm)
w ater
METHANOL-d4
M14(m)
M04(td)
M07(s)
M12(d)
M10(dd)
M11(s)
M13(dd)
M08(dd)
M09(d)
M06(s)
M05(dd)
M03(ttt)
M02(br dd)
M01(m)
15


M400APCB_13012016_E63-5-062.5-H1.fid.mrc
1H
13 H's / 22 H's (spectrum / structure)
Normalized Intensity
Chemical Shift (ppm)
w ater
METHANOL-d4
M05(td)
M04(d)
M07(s)
M13(d)
M12(s)
M10(d)
M11(d)
M09(m)
M08(dd)
M06(dd)
M03(ddd)
M02(br dd)
M01(m)
16

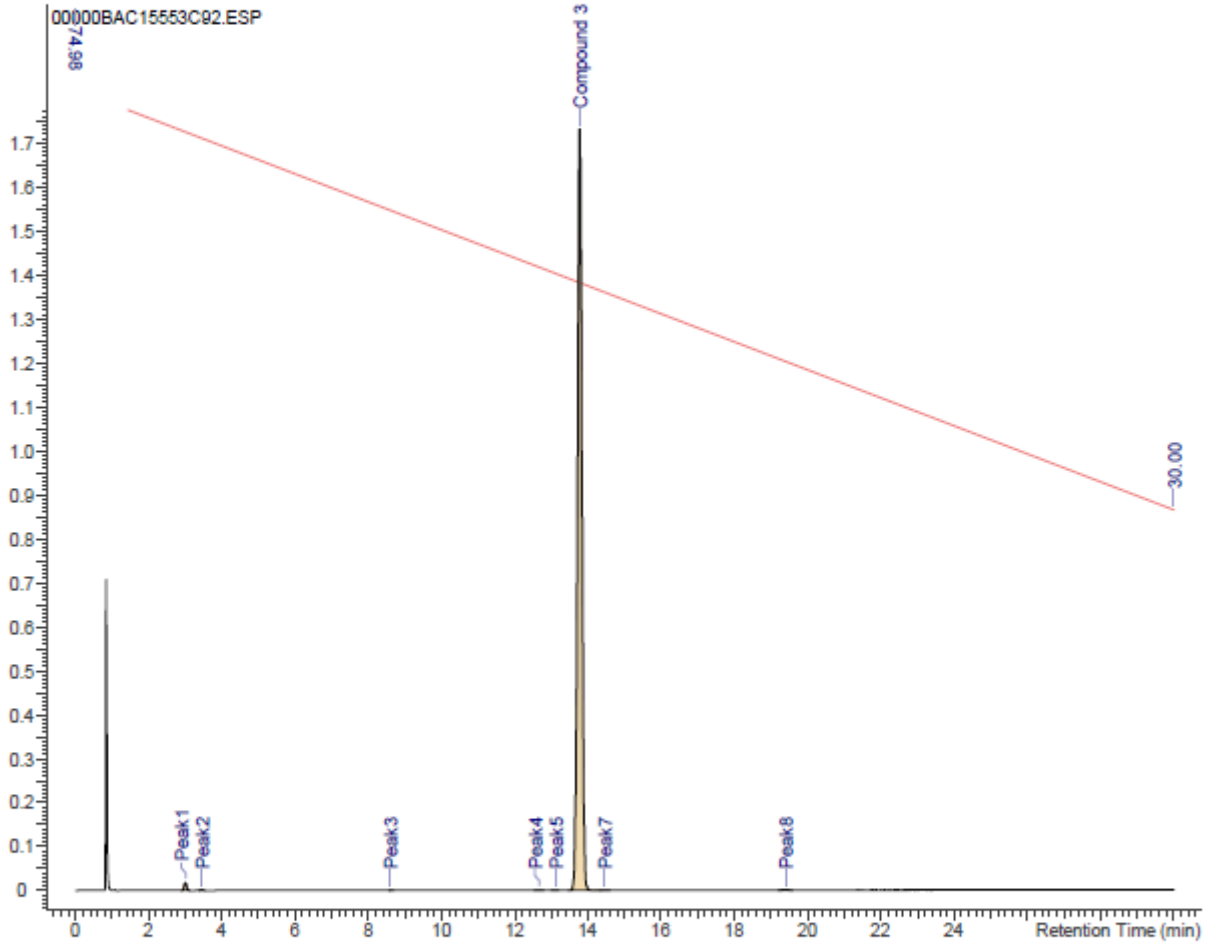
HPLC ANALYSIS OF FINALCOMPOUNDS

Injection Volume	5 uL
Detector	UV (237 nm)
Mobile Phase A	ACN+0.01%TFA
Mobile Phase B	water+0.01%TFA
Gradient	80-35B (30 min)
Temperature	25°C
Column Name	Xbridge C19
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



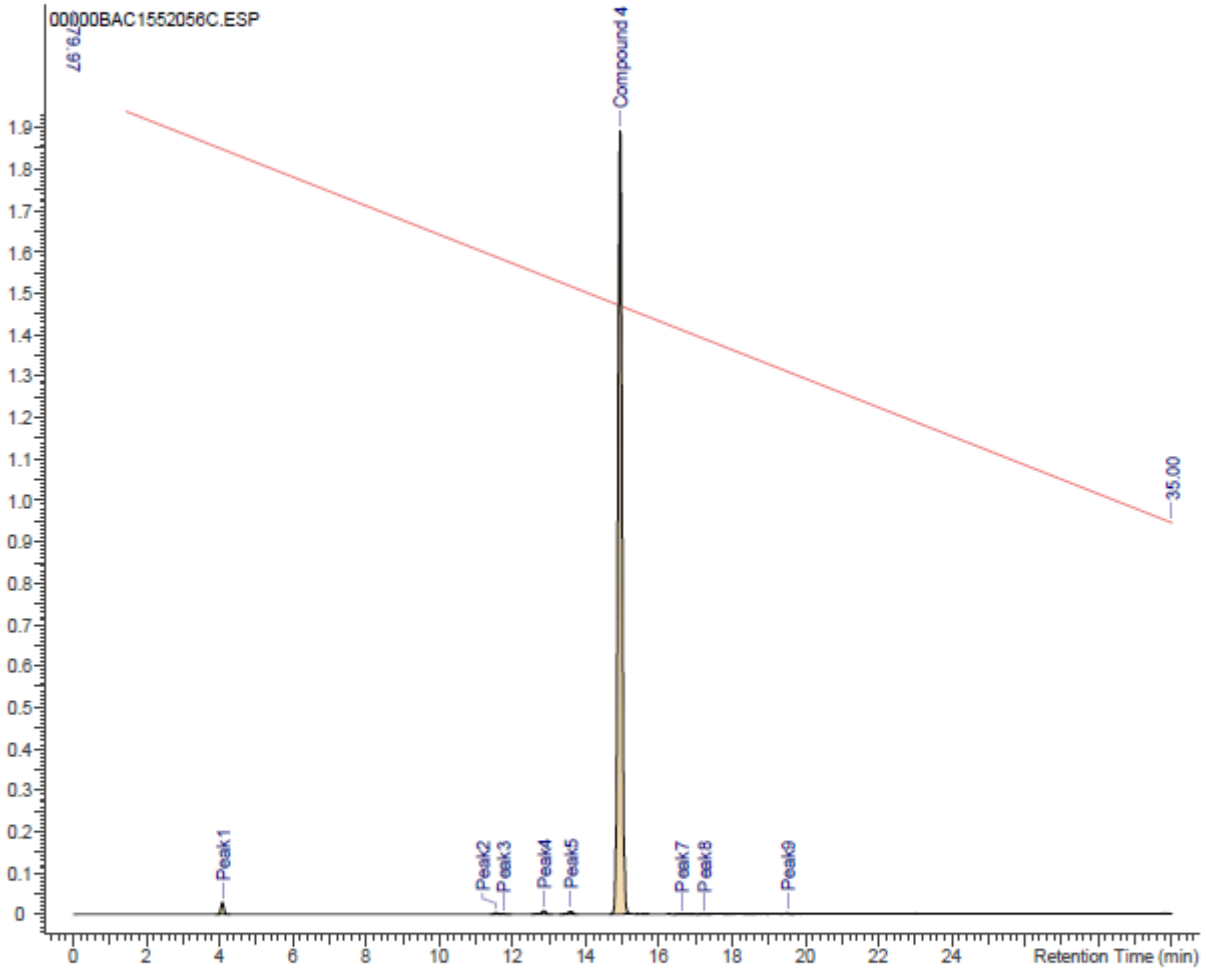
No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	5.05	3.065	0.023	0.208	490323.031
2	Peak2	10.67	4.425	0.033	0.225	625034.625
3	Peak3	12.24	3.415	0.026	0.208	393321.375
4	Peak4	12.76	1.195	0.009	0.167	239651.797
5	Peak5	13.56	17.432	0.131	0.333	2007282.000
6	Peak6	13.97	5.389	0.040	0.275	611553.875
7	Peak7	14.34	1.320	0.010	0.167	225614.281
8	Peak8	14.81	1.172	0.009	0.150	143490.953
9	Peak9	15.30	7.430	0.056	0.283	786867.625
10	Compound 2	16.22	13219.035	99.194	0.917	1521110528.000
11	Peak11	16.82	1.232	0.009	0.208	253796.984
12	Peak12	17.51	14.724	0.110	0.342	1477789.500
13	Peak13	17.89	2.966	0.022	0.317	291230.969
14	Peak14	18.45	2.361	0.018	0.225	236173.391
15	Peak15	18.68	11.634	0.087	0.375	1125883.250
16	Peak16	19.30	4.213	0.032	0.308	471650.625
17	Peak17	20.35	1.075	0.008	0.142	127037.344
18	Peak18	24.16	24.370	0.183	0.442	2183629.000

Sampling Rate	120.002
Injection Volume	5 uL
Detector	UV (237 nm)
Mobile Phase A	ACN + 0.01% TFA
Mobile Phase B	water + 0.01% TFA
Gradient	75-30B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



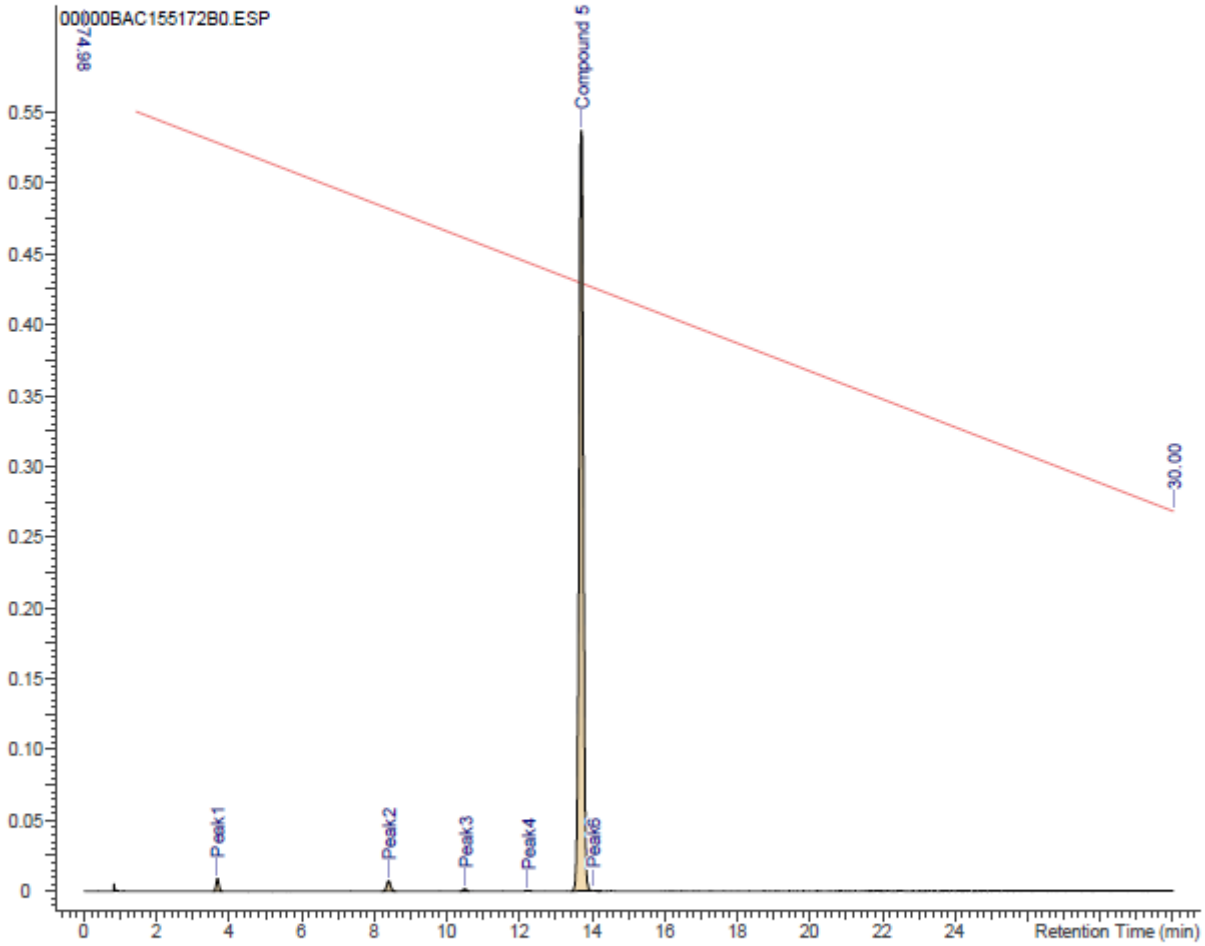
No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	3.00	83.163	0.594	0.233	17478178.000
2	Peak2	3.45	6.834	0.049	0.158	1593834.625
3	Peak3	8.59	0.766	0.005	0.117	215923.234
4	Peak4	12.67	4.526	0.032	0.258	473215.500
5	Peak5	13.12	2.512	0.018	0.200	422335.656
6	Compound 3	13.78	13899.097	99.198	0.875	1734281856.000
7	Peak7	14.44	2.831	0.020	0.258	296548.438
8	Peak8	19.39	11.744	0.084	0.383	1221446.625

Injection Volume	5 uL
Detector	UV (237 nm)
Mobile Phase A	ACN + 0.01% TFA
Mobile Phase B	water + 0.01% TFA
Gradient	80-35B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



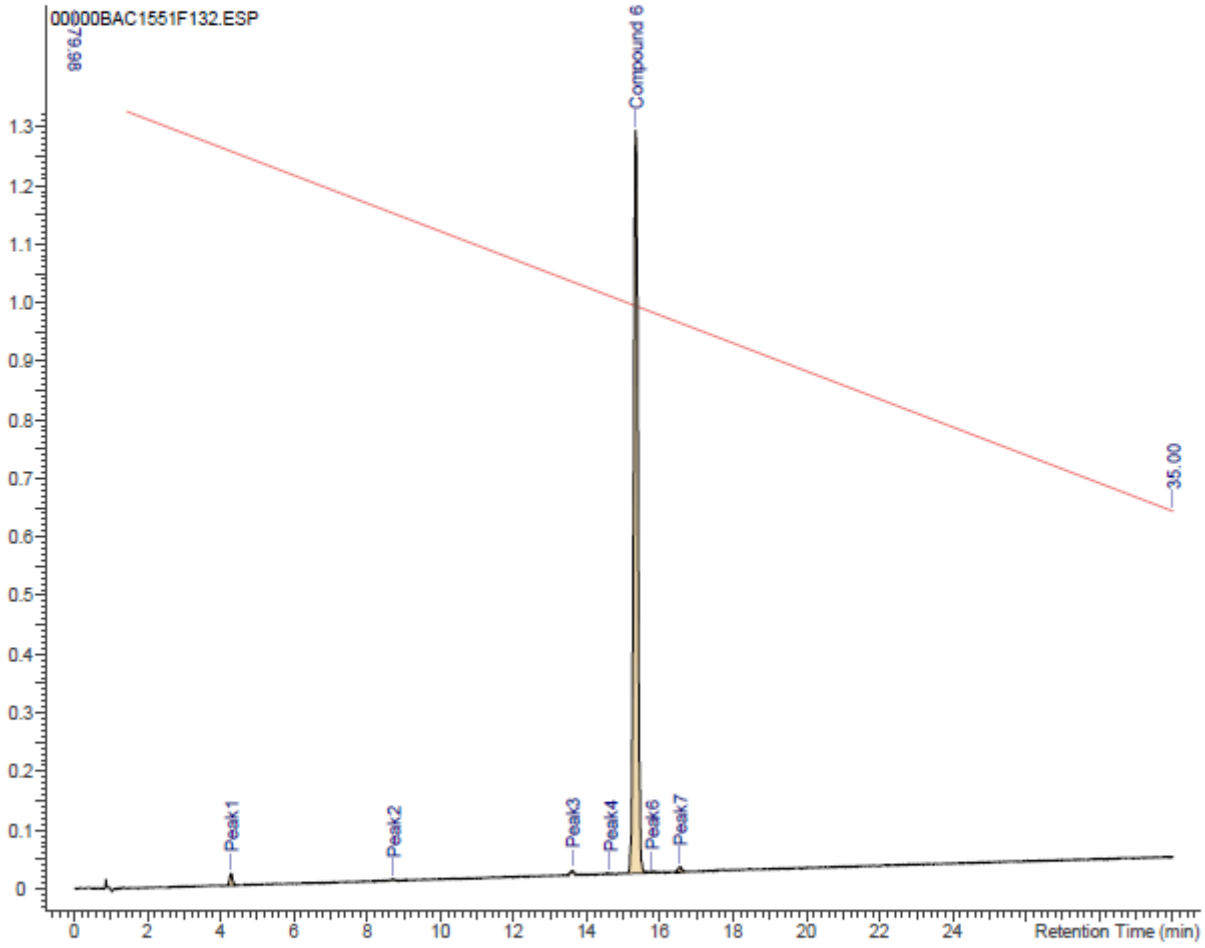
No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	4.07	156.699	1.072	0.142	28.777
2	Peak2	11.54	19.277	0.132	0.183	2.745
3	Peak3	11.74	10.706	0.073	0.201	1.445
4	Peak4	12.86	67.137	0.459	0.187	7.580
5	Peak5	13.57	54.089	0.370	0.200	6.569
6	Compound 4	14.93	14278.296	97.635	0.198	1891.492
7	Peak7	16.61	15.581	0.107	0.224	1.361
8	Peak8	17.22	9.470	0.065	0.217	1.132
9	Peak9	19.51	12.842	0.088	0.221	1.407

Sampling Rate	120.002
Injection Volume	5 uL
Detector	UV (302 nm)
Mobile Phase A	ACN + 0.01% TFA
Mobile Phase B	water + 0.01% TFA
Gradient	75-30B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



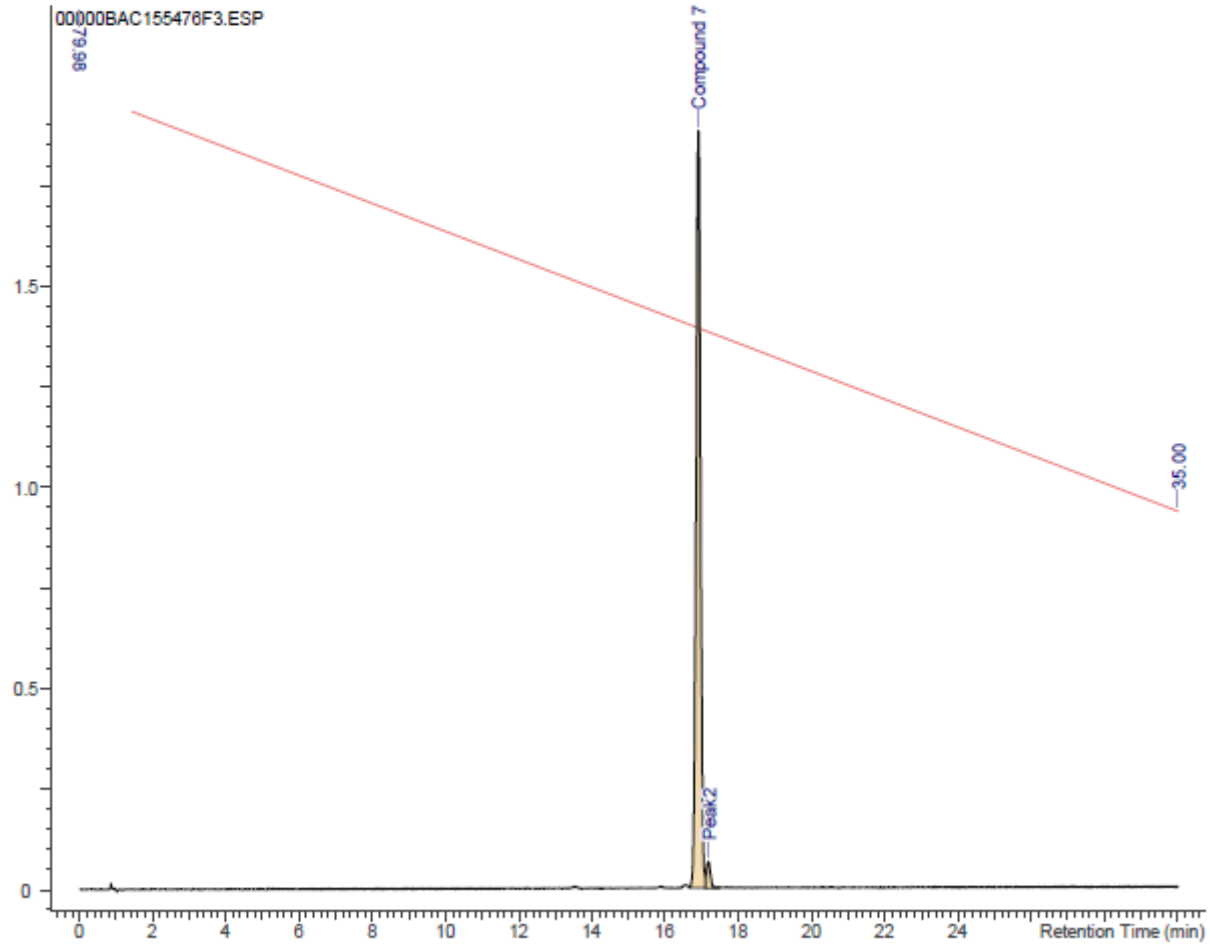
No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	3.68	44.219	1.016	0.250	9042178.000
2	Peak2	8.39	51.704	1.188	0.383	7058270.500
3	Peak3	10.49	10.108	0.232	0.250	1410121.750
4	Peak4	12.23	2.804	0.064	0.183	374293.750
5	Compound 5	13.70	4241.393	97.437	0.575	536880448.000
6	Peak6	14.01	2.738	0.063	0.183	452684.188

Sampling Rate	120.0017
Injection Volume	5 uL
Detector	UV (208 nm)
Mobile Phase A	ACN + 0.01% TFA
Mobile Phase B	water + 0.01% TFA
Gradient	80-35B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



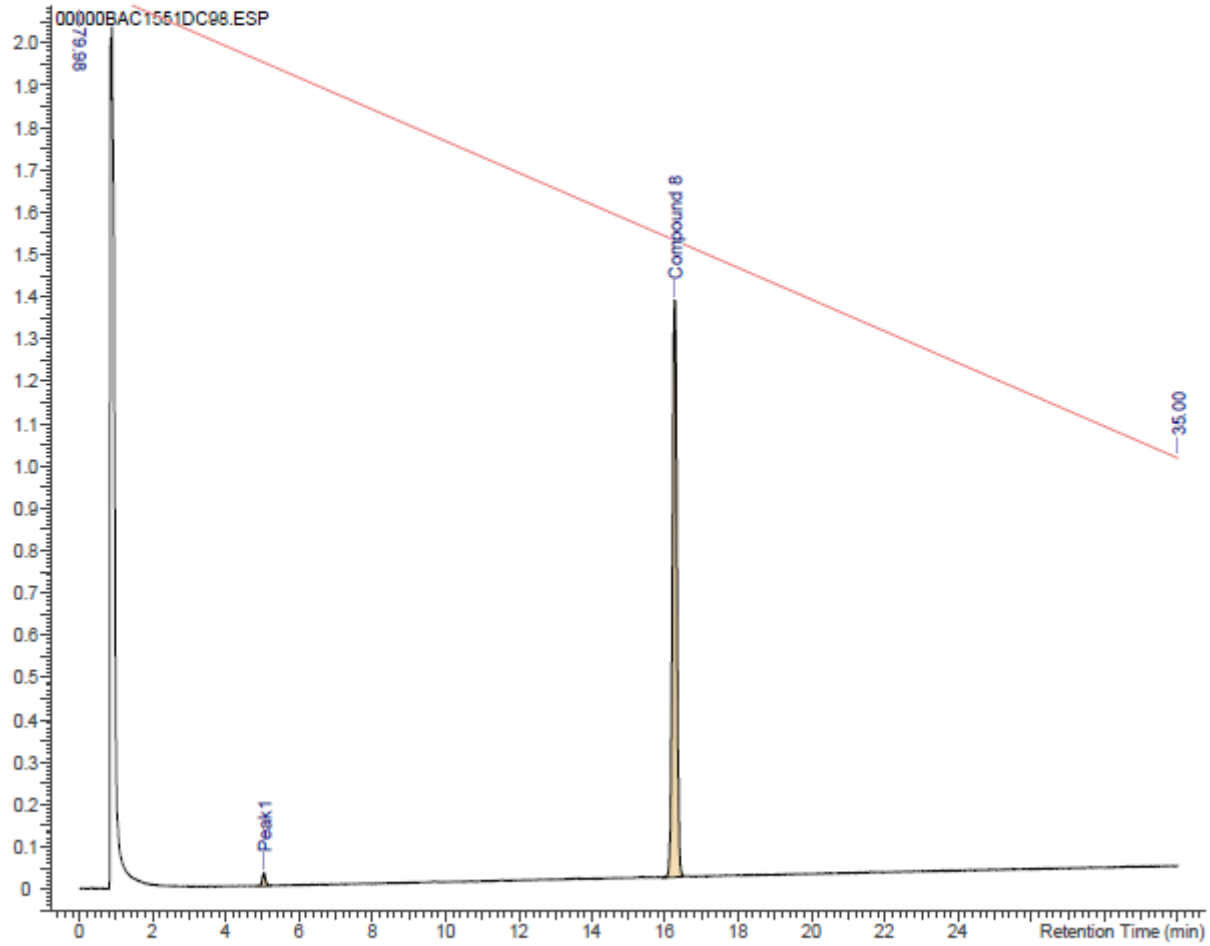
No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	4.28	103.507	1.018	0.200	18774478.000
2	Peak2	8.72	14.979	0.147	0.167	2111837.250
3	Peak3	13.60	49.630	0.488	0.200	7510794.500
4	Peak4	14.63	6.657	0.065	0.117	1665834.500
5	Compound 6	15.33	9905.073	97.460	0.575	1257106176.000
6	Peak6	15.78	19.432	0.191	0.208	1747031.875
7	Peak7	16.54	63.913	0.629	0.267	8419971.000

Sampling Rate	120.002
Injection Volume	5 uL
Detector	UV (206 nm)
Mobile Phase A	ACN + 0.01% TFA
Mobile Phase B	water + 0.01% TFA
Gradient	80-35B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



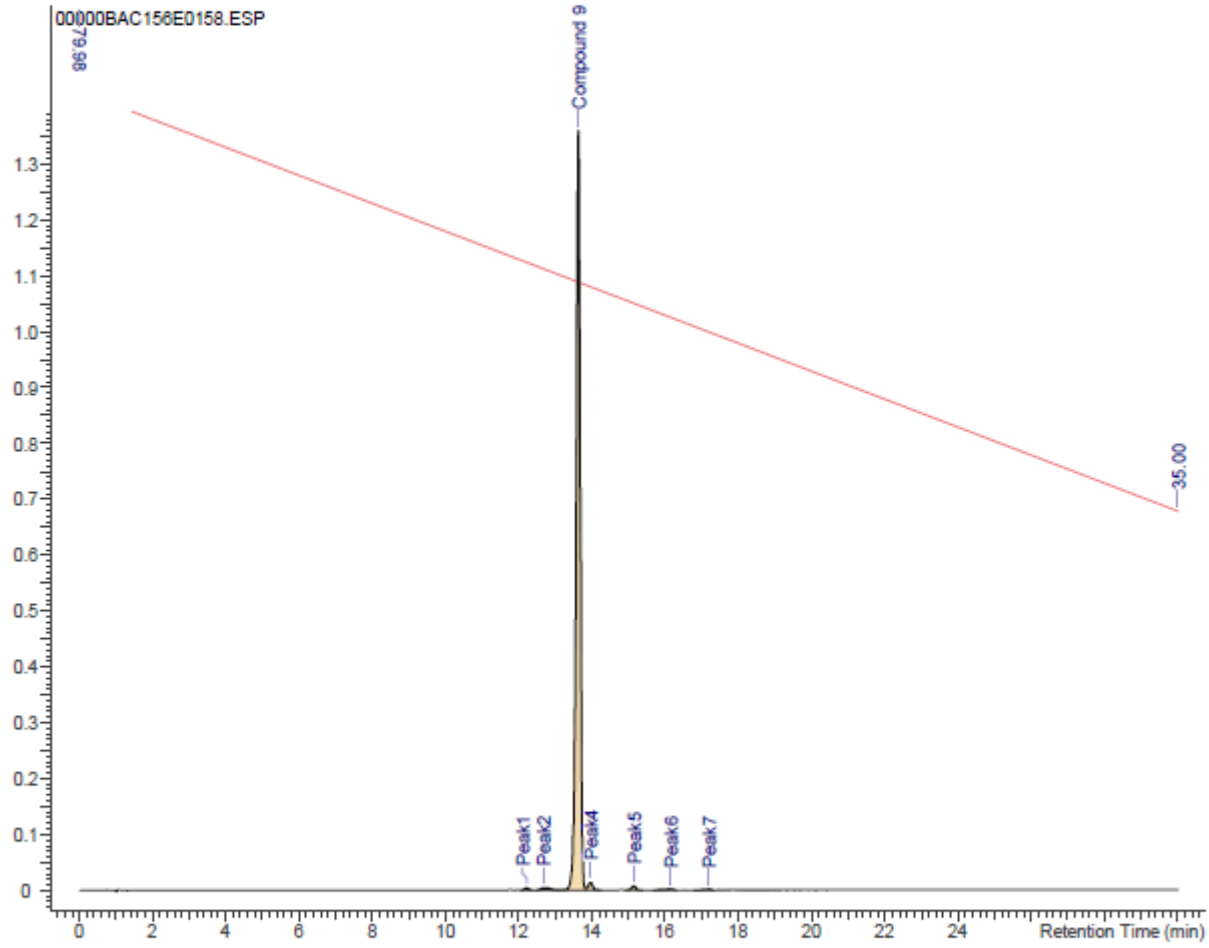
No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Compound 7	16.90	15588.066	96.842	0.222	1881.541
2	Peak2	17.18	508.255	3.158	0.217	65.299

Sampling Rate	120.0017
Injection Volume	5 uL
Detector	UV (208 nm)
Mobile Phase A	ACN + 0.01% TFA
Mobile Phase B	water + 0.01% TFA
Gradient	80-35B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



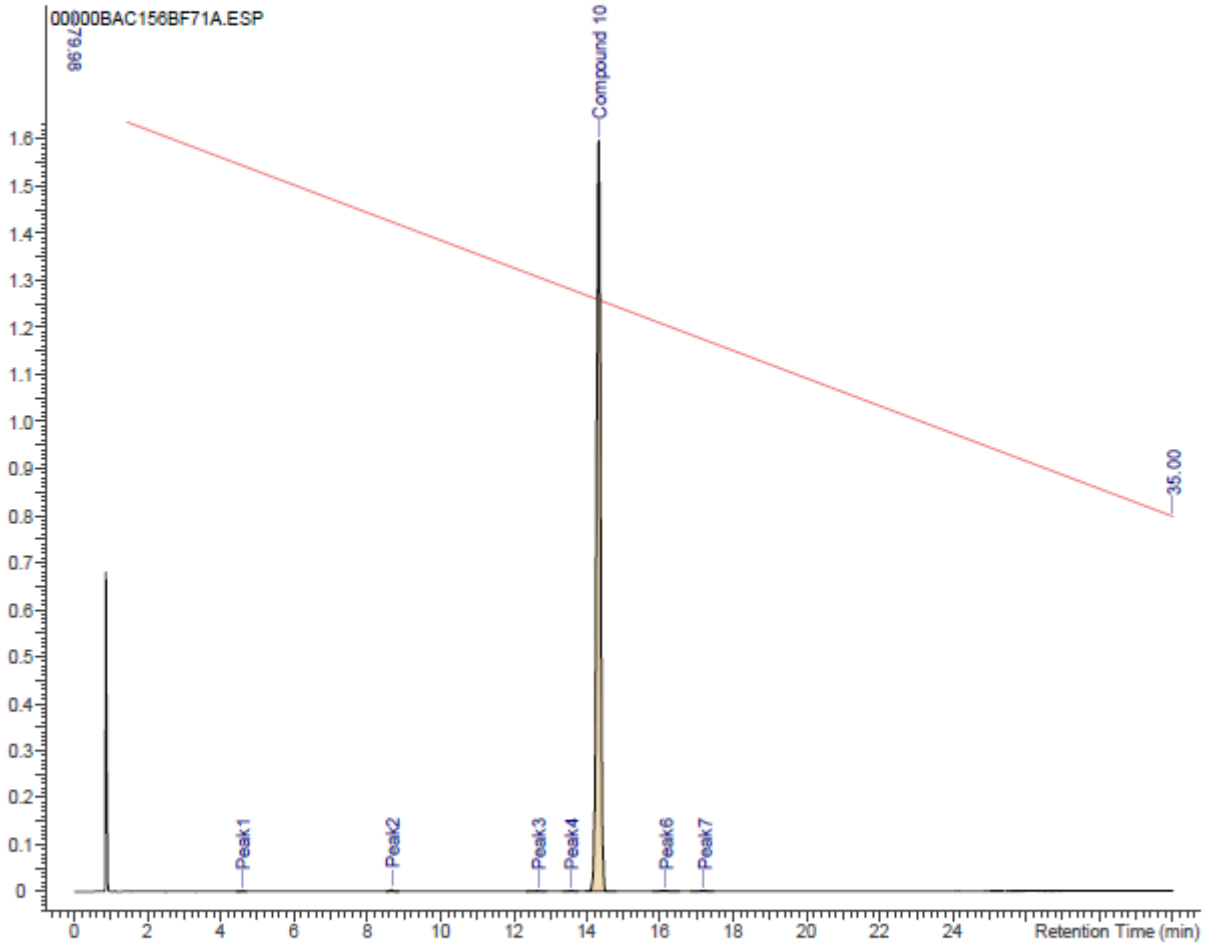
No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	5.04	195.423	1.739	0.148	30.894
2	Compound 8	16.26	11042.730	98.261	0.212	1362.278

Sampling Rate	120.0017
Injection Volume	5 uL
Detector	UV (237 nm)
Mobile Phase A	ACN+0.01%TFA
Mobile Phase B	water+0.01%TFA
Gradient	80-35B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



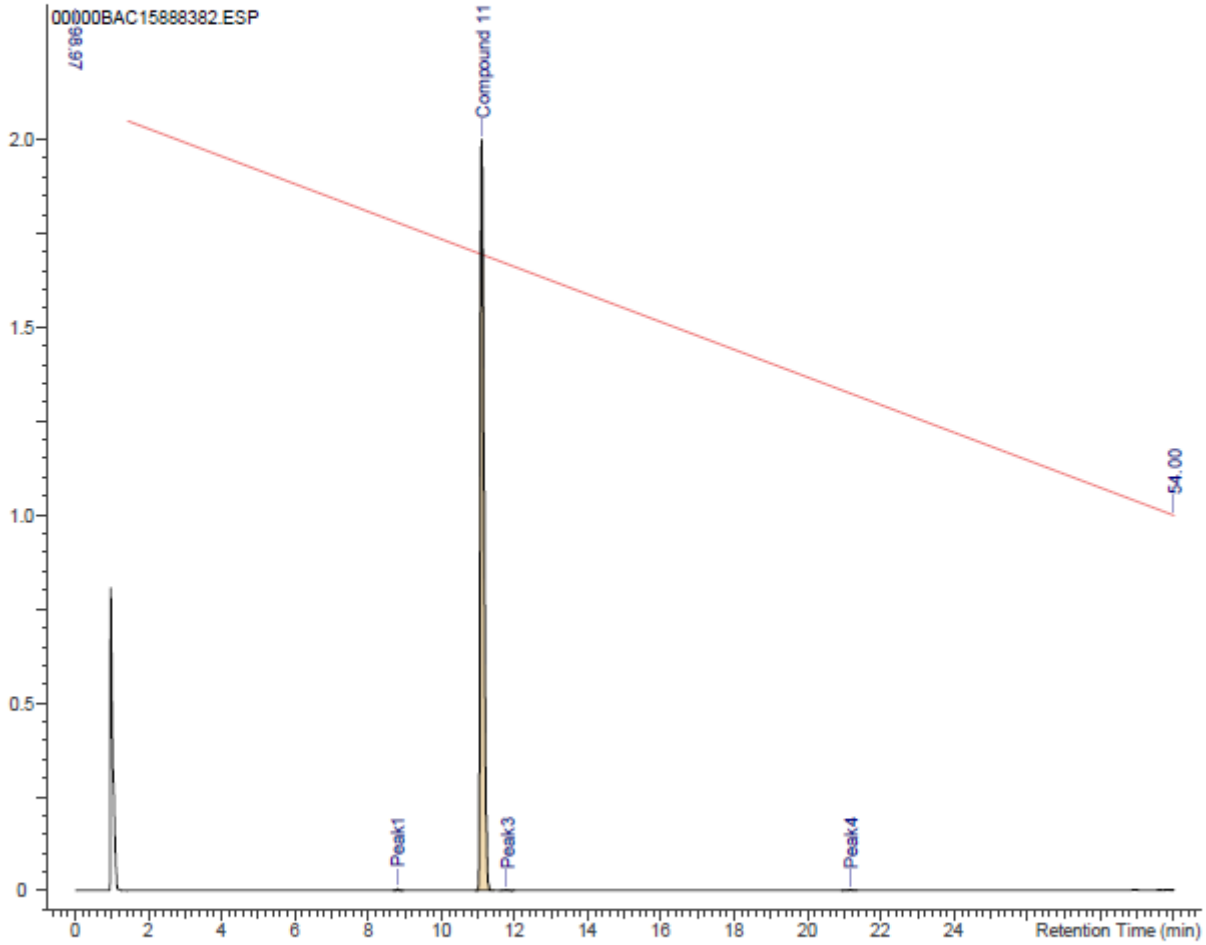
No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	12.21	28.092	0.264	0.189	3.927
2	Peak2	12.67	67.416	0.634	0.481	3.855
3	Compound 9	13.63	10325.489	97.145	0.194	1359.348
4	Peak4	13.96	116.846	1.099	0.225	13.456
5	Peak5	15.15	56.264	0.529	0.200	7.274
6	Peak6	16.15	20.218	0.190	0.242	1.925
7	Peak7	17.19	14.639	0.138	0.240	1.408

Sampling Rate	120.0017
Injection Volume	5 uL
Detector	UV (237 nm)
Mobile Phase A	ACN+0.01%TFA
Mobile Phase B	water+0.01%TFA
Gradient	80-35B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



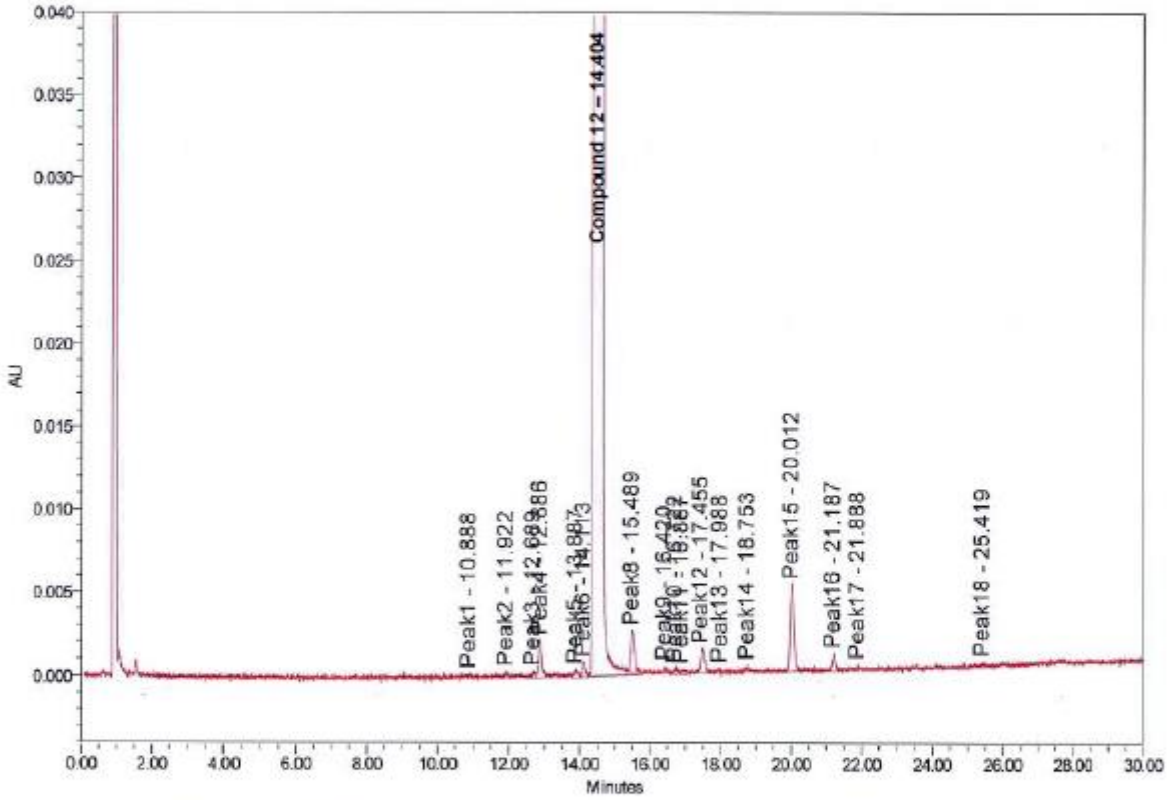
No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	4.60	8.757	0.074	0.250	1178717.250
2	Peak2	8.66	19.446	0.165	0.333	2709650.000
3	Peak3	12.66	11.197	0.095	0.230	1.087
4	Peak4	13.55	12.198	0.103	0.223	1.340
5	Compound 10	14.32	11711.702	99.211	0.775	1597104640.000
6	Peak6	16.14	25.827	0.219	0.388	1.711
7	Peak7	17.20	15.750	0.133	0.271	1.317

Sampling Rate	120.002
Injection Volume	5 uL
Detector	UV (234 nm)
Mobile Phase A	Acetonitrile
Mobile Phase B	10mM KH2PO4 pH3HClO4
Gradient	99-54B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	8.79	17.589	0.134	0.127	3.470
2	Compound 11	11.10	13109.971	99.656	0.176	1998.378
3	Peak3	11.78	12.426	0.094	0.191	1.615
4	Peak4	21.17	15.199	0.116	0.170	2.110

Injection Volume	5 uL
Detector	UV (234 nm)
Mobile Phase A	ACN+0.01%TFA
Mobile Phase B	water+0.01%TFA
Gradient	95-50B (30 min)
Temperature	22°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2ml/min

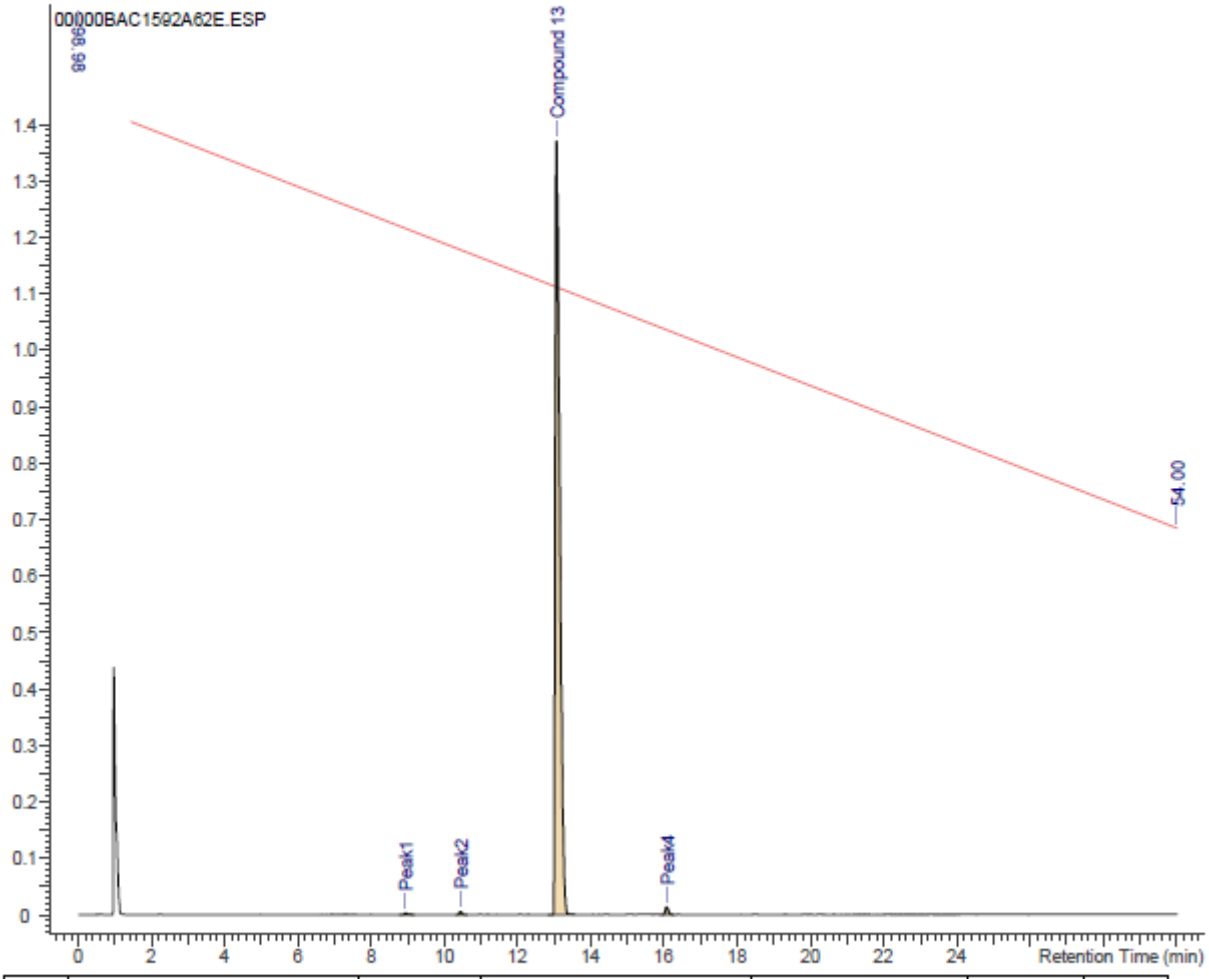


Peak Results

	Peak Name	RT	Area (µV*sec)	% Area	RT Ratio
1	Peak1	10.888	604	0.004	0.756
2	Peak2	11.922	1535	0.011	0.838
3	Peak3	12.689	2100	0.015	0.881
4	Peak4	12.886	12737	0.094	0.895
5	Peak5	13.887	3085	0.023	0.964
6	Peak6	14.113	5248	0.039	0.980
7	Compd12	14.404	13425632	99.214	1.000
8	Peak8	15.489	15286	0.113	1.075
9	Peak9	16.420	1561	0.012	1.140
10	Peak10	16.722	3047	0.023	1.161
11	Peak11	16.887	2087	0.015	1.172
12	Peak12	17.455	11799	0.087	1.212
13	Peak13	17.988	1484	0.011	1.248
14	Peak14	18.753	1280	0.009	1.302

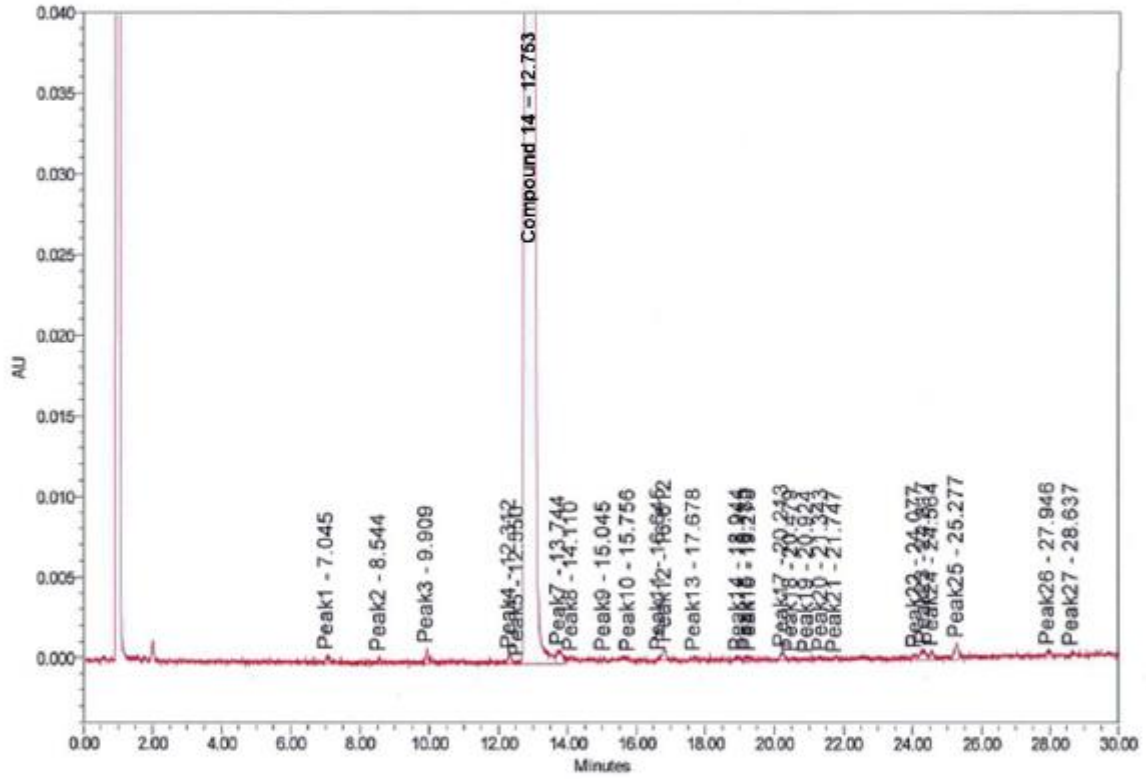
	Peak Name	RT	Area (µV*sec)	% Area	RT Ratio
15	Peak15	20.012	35549	0.263	1.389
16	Peak16	21.187	6381	0.047	1.471
17	Peak17	21.888	1078	0.008	1.520
18	Peak18	25.419	1478	0.011	1.765

Injection Volume	5 uL
Detector	UV (236 nm)
Mobile Phase A	ACN+0.01%TFA
Mobile Phase B	water+0.01%TFA
Gradient	99-54B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	8.93	15.585	0.134	0.128	2.473
2	Peak2	10.44	25.834	0.222	0.123	5.306
3	Compound 13	13.06	11493.586	98.960	0.227	1370.273
4	Peak4	16.06	79.407	0.684	0.149	13.430

Injection Volume	5 uL
Detector	UV (236 nm)
Mobile Phase A	ACN+0.01%TFA
Mobile Phase B	water+0.01%TFA
Gradient	98-68B (30 min)
Temperature	22°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2ml/min

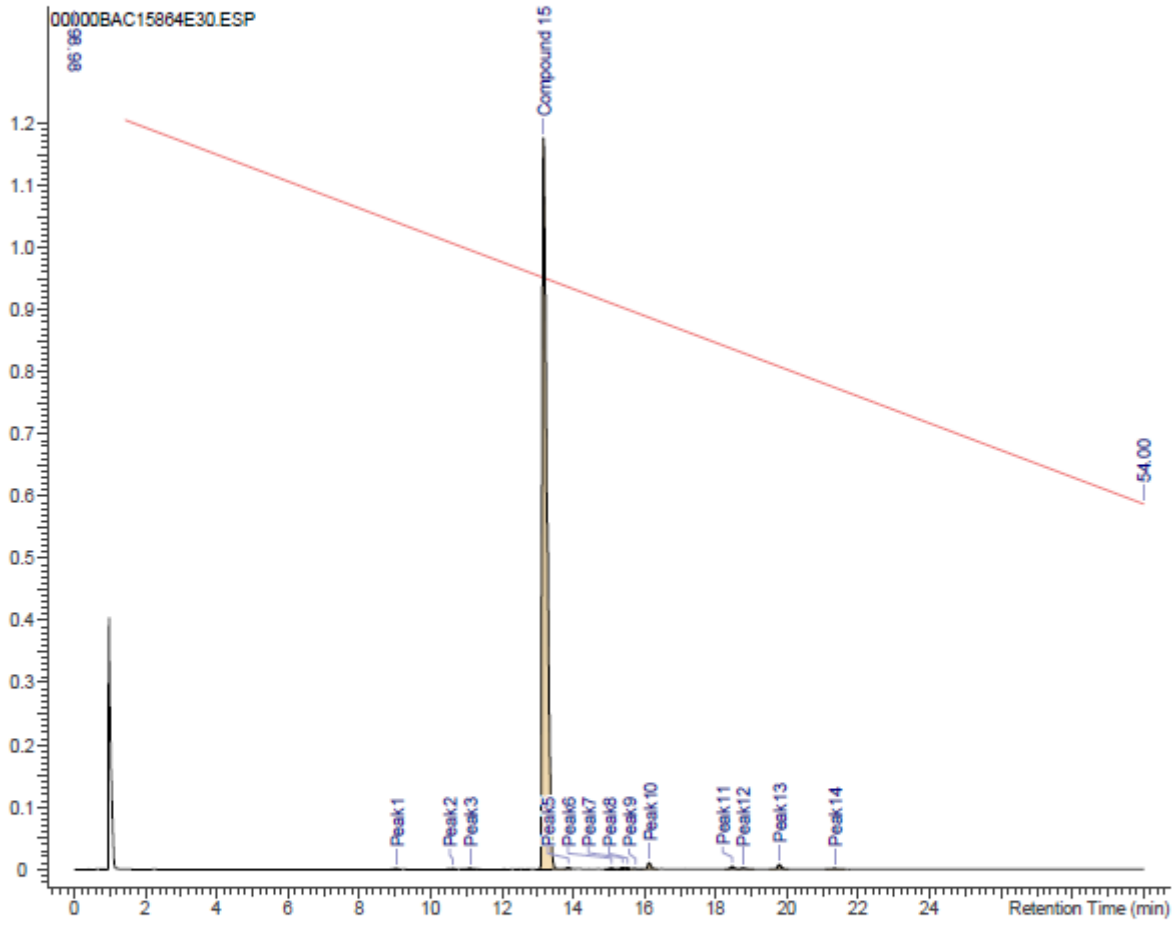


Peak Results

	Peak Name	RT	Area (µV*sec)	% Area	RT Ratio
1	Peak1	7.045	3085	0.030	0.552
2	Peak2	8.544	1654	0.016	0.670
3	Peak3	9.909	5159	0.050	0.777
4	Peak4	12.312	3490	0.034	0.965
5	Peak5	12.550	713	0.007	0.984
6	Compd14	12.753	10329222	99.313	1.000
7	Peak7	13.744	9659	0.093	1.078
8	Peak8	14.110	1269	0.012	1.106
9	Peak9	15.045	1143	0.011	1.180
10	Peak10	15.756	1945	0.019	1.235
11	Peak11	16.645	1161	0.011	1.305
12	Peak12	16.812	5310	0.051	1.318
13	Peak13	17.678	1323	0.013	1.386
14	Peak14	18.944	1206	0.012	1.485

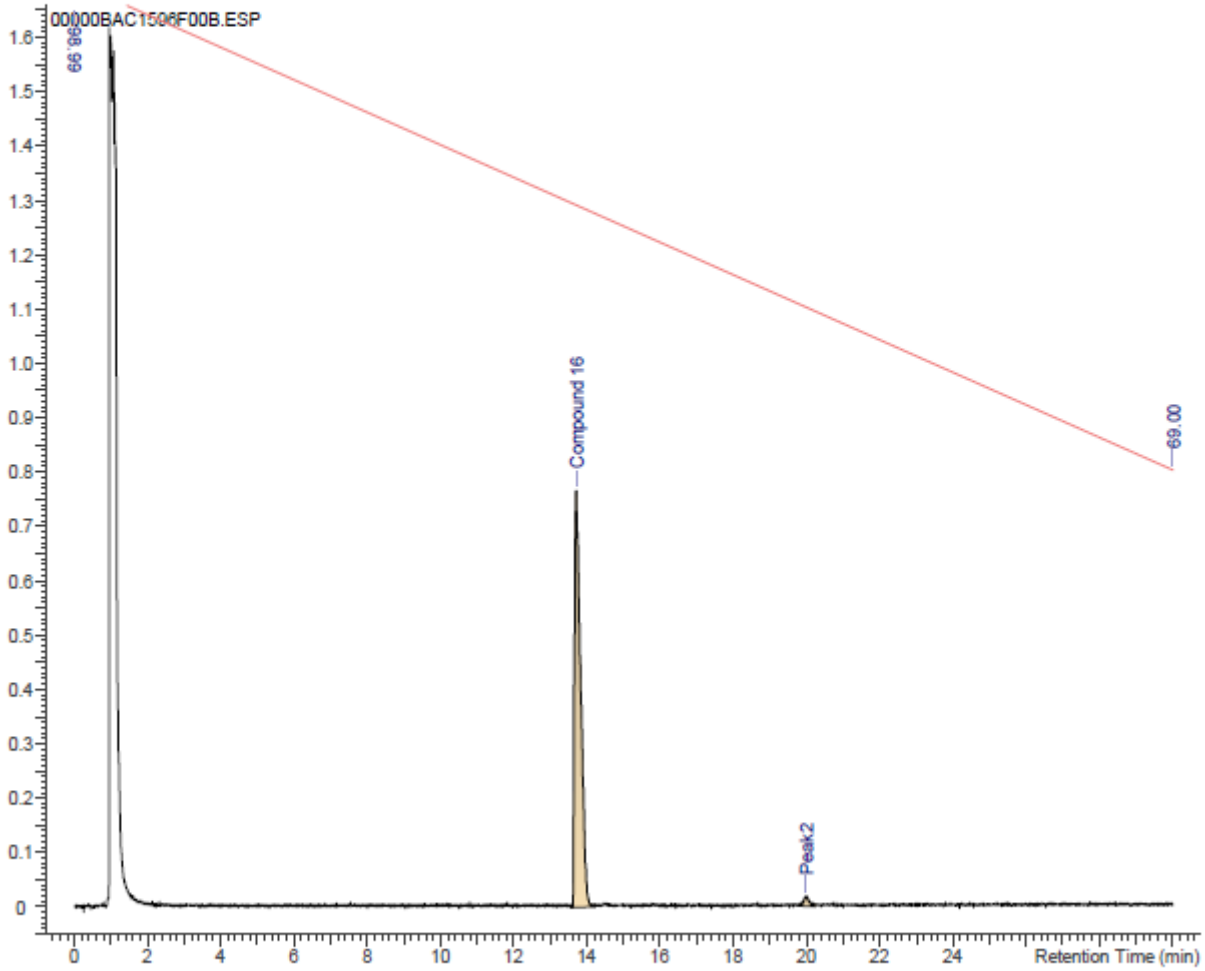
	Peak Name	RT	Area (µV*sec)	% Area	RT Ratio
15	Peak15	19.180	3460	0.024	1.504
16	Peak16	19.278	1396	0.013	1.512
17	Peak17	20.213	3742	0.036	1.585
18	Peak18	20.479	587	0.006	1.606
19	Peak19	20.924	568	0.005	1.641
20	Peak20	21.343	711	0.007	1.674
21	Peak21	21.747	1267	0.012	1.705
22	Peak22	24.077	2712	0.026	1.888
23	Peak23	24.317	6940	0.063	1.907
24	Peak24	24.564	3418	0.033	1.926
25	Peak25	25.277	8134	0.079	1.982
26	Peak26	27.946	2297	0.022	2.191
27	Peak27	28.637	1502	0.014	2.245

Sampling Rate	120.0017
Injection Volume	5 uL
Detector	UV (236 nm)
Mobile Phase A	ACN+0.01%TFA
Mobile Phase B	water+0.01%TFA
Gradient	99-54B (30 min)
Temperature	25°C
Column Name	Xbridge C18
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Peak1	9.04	6.130	0.058	0.137	1.076
2	Peak2	10.62	5.031	0.047	0.129	0.849
3	Peak3	11.10	9.333	0.088	0.163	1.242
4	Compound 15	13.17	10408.501	97.729	0.240	1175.252
5	Peak5	13.87	12.038	0.113	0.164	1.922
6	Peak6	15.08	13.482	0.127	0.169	1.838
7	Peak7	15.39	19.433	0.182	0.181	2.853
8	Peak8	15.52	14.687	0.138	0.086	2.337
9	Peak9	15.75	5.563	0.052	0.161	0.858
10	Peak10	16.14	60.756	0.570	0.157	9.685
11	Peak11	18.45	26.057	0.245	0.177	3.763
12	Peak12	18.76	13.215	0.124	0.183	1.630
13	Peak13	19.79	48.019	0.451	0.170	6.944

Injection Volume	5 uL
Detector	UV (205 nm)
Mobile Phase A	ACN+0.01%TFA
Mobile Phase B	water+0.01%TFA
Gradient	99-69B (30 min)
Temperature	25°C
Column Name	Xbridge
Length	15 cm
Diameter	0.46 cm
Particle Size	5 um
Column Flow Rate	2 ml/min



No.	Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Height
1	Compound 16	13.70	8611.731	97.789	0.306	766.720
2	Peak2	19.99	194.703	2.211	0.219	18.578

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