Development of a Specific and Potent IGF2BP1 Inhibitor: A Promising Therapeutic Agent for IGF2BP1-expressing Cancers

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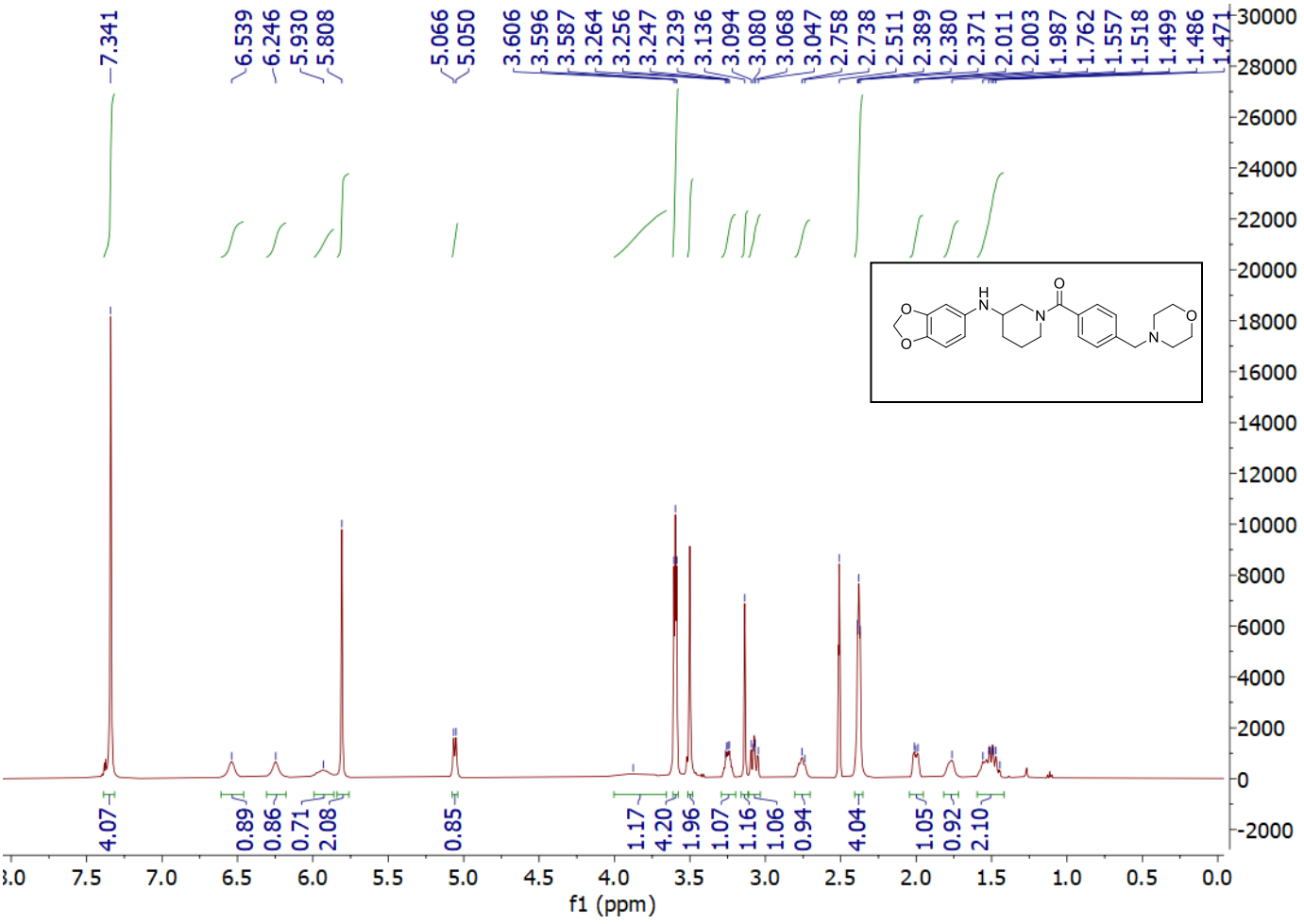
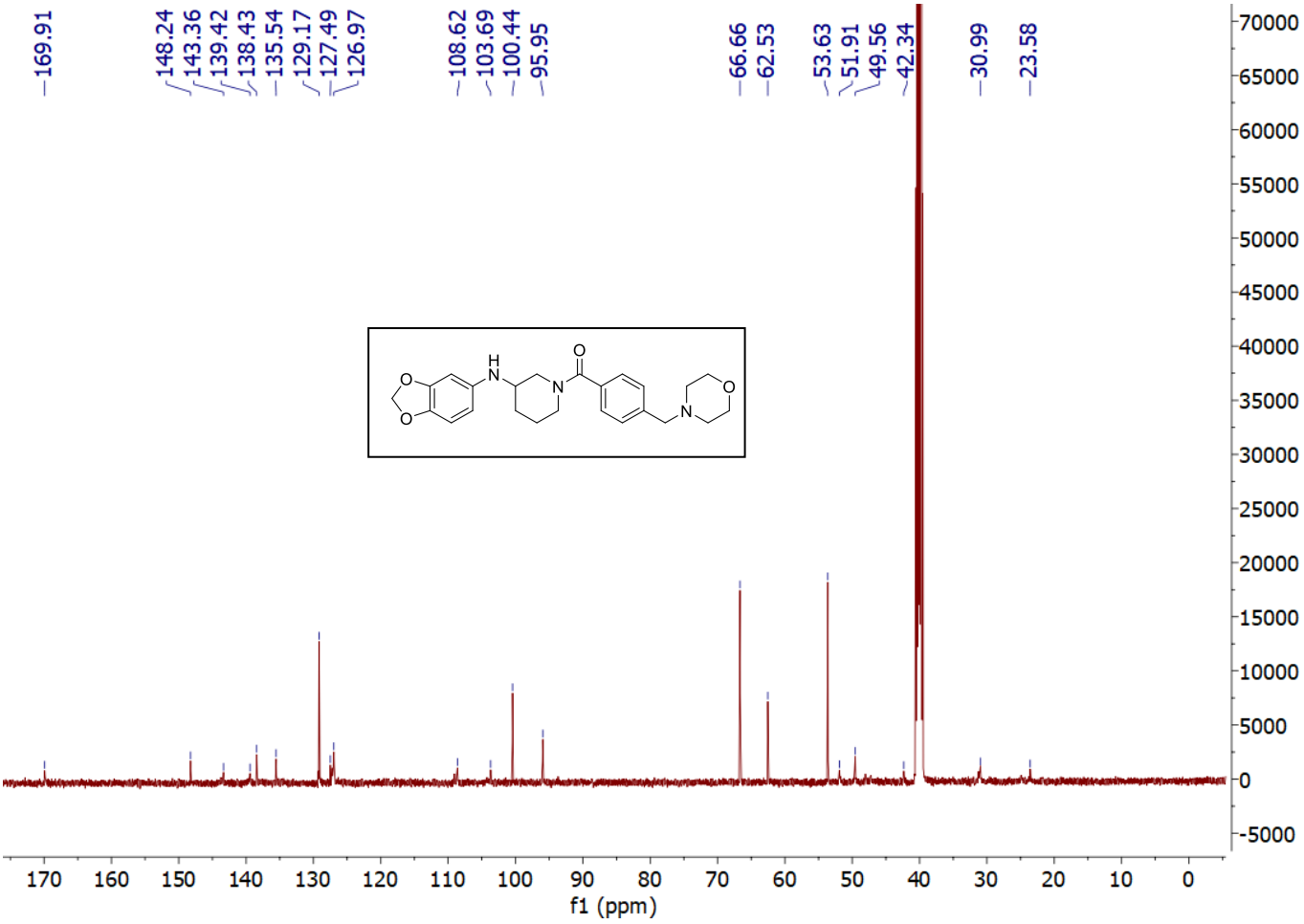
^{1}H NMR, ^{13}C NMR, HRMS and HPLC of compounds 7a-h, 13a-i and 18g-i

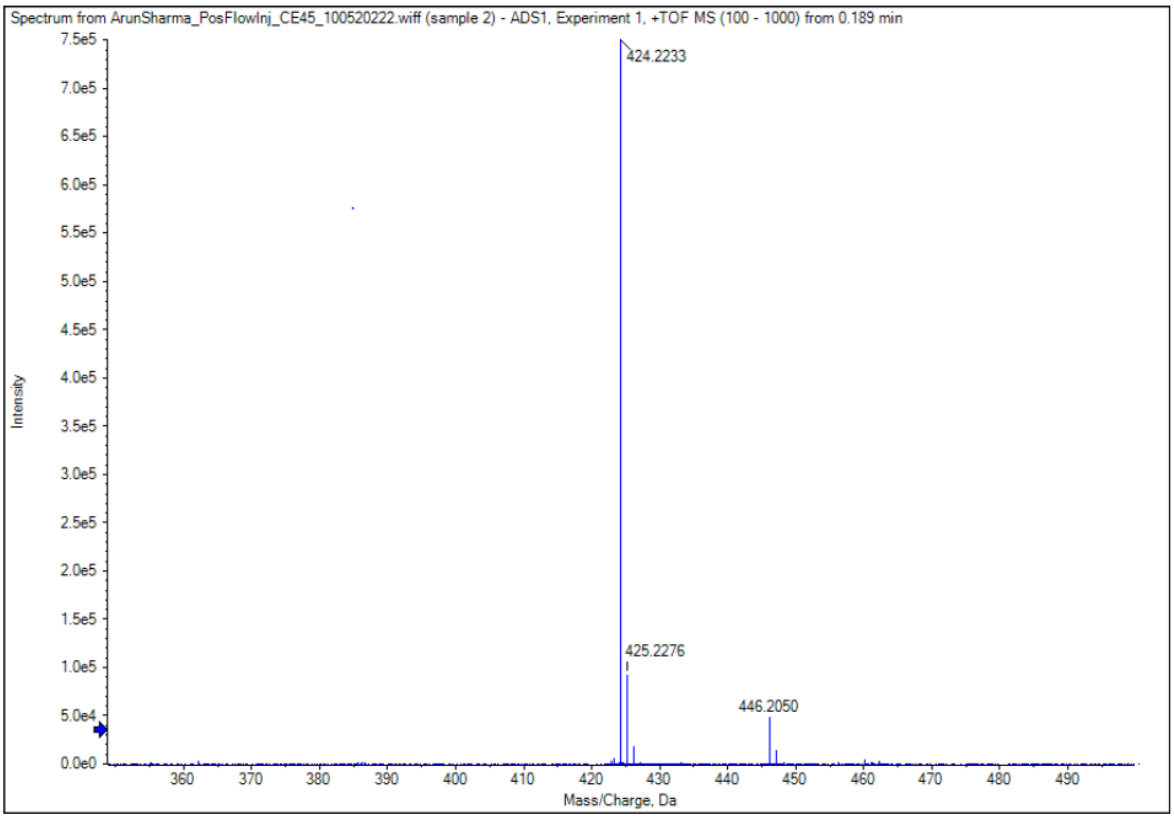
^{1}H NMR of (3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4-(morpholinomethyl) phenyl) methanone (7a):



^{13}C NMR of (3-(Benzo[d] [1,3] ndioxol-5-ylamino) piperidin-1-yl) (4-(morpholinomethyl) phenyl) methanone (7a):



HRMS of (3-(Benzo[d][1,3] dioxol-5-ylamino)piperidin-1-yl)(4-(morpholinomethyl)phenyl)methanone (7a):



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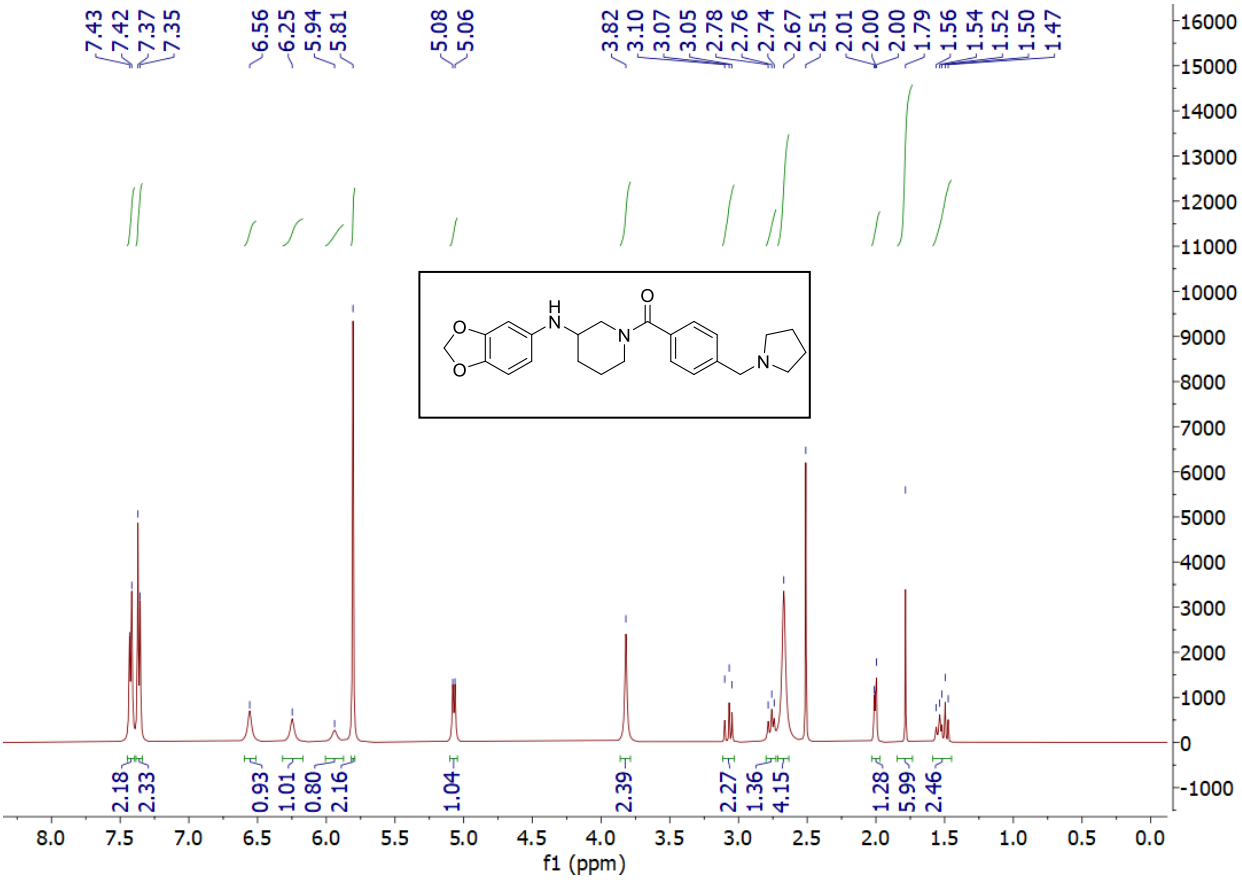
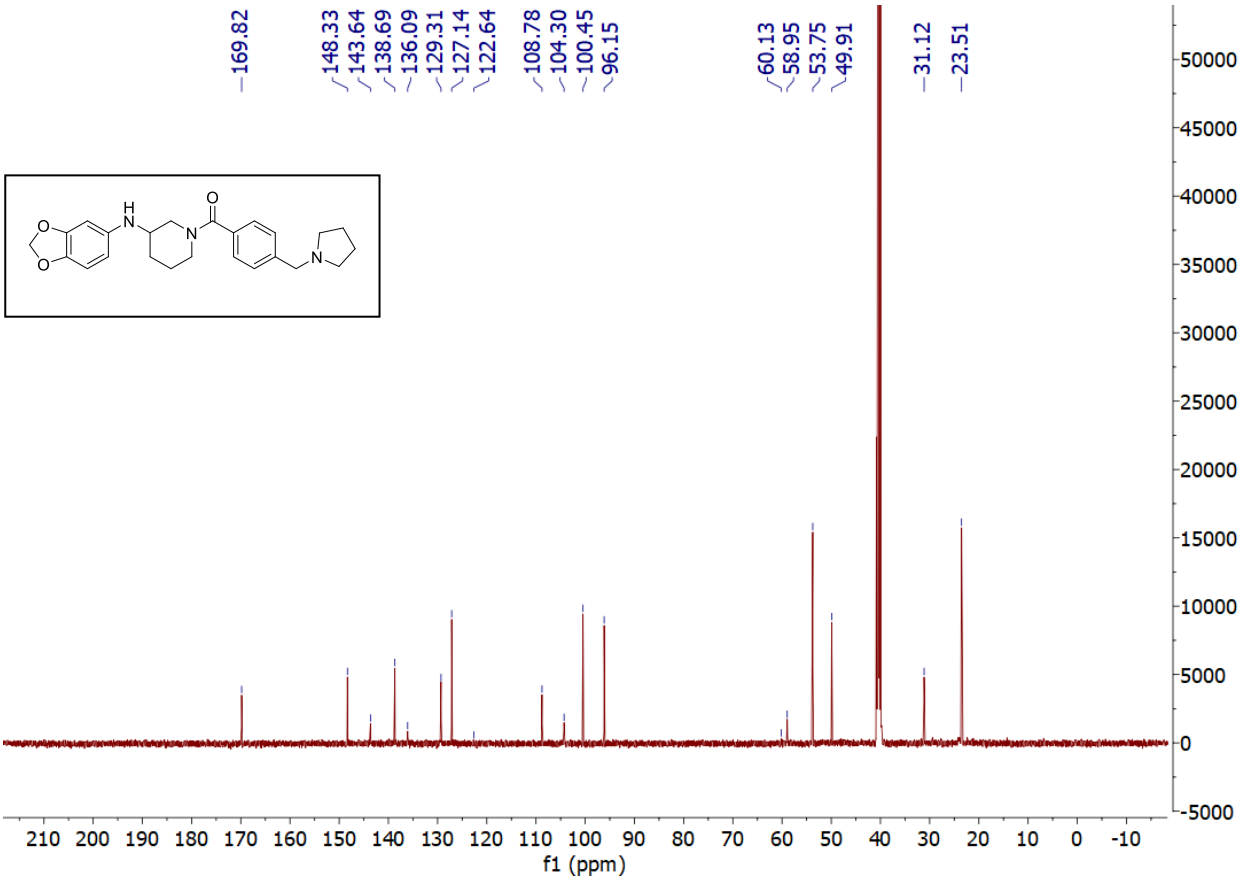
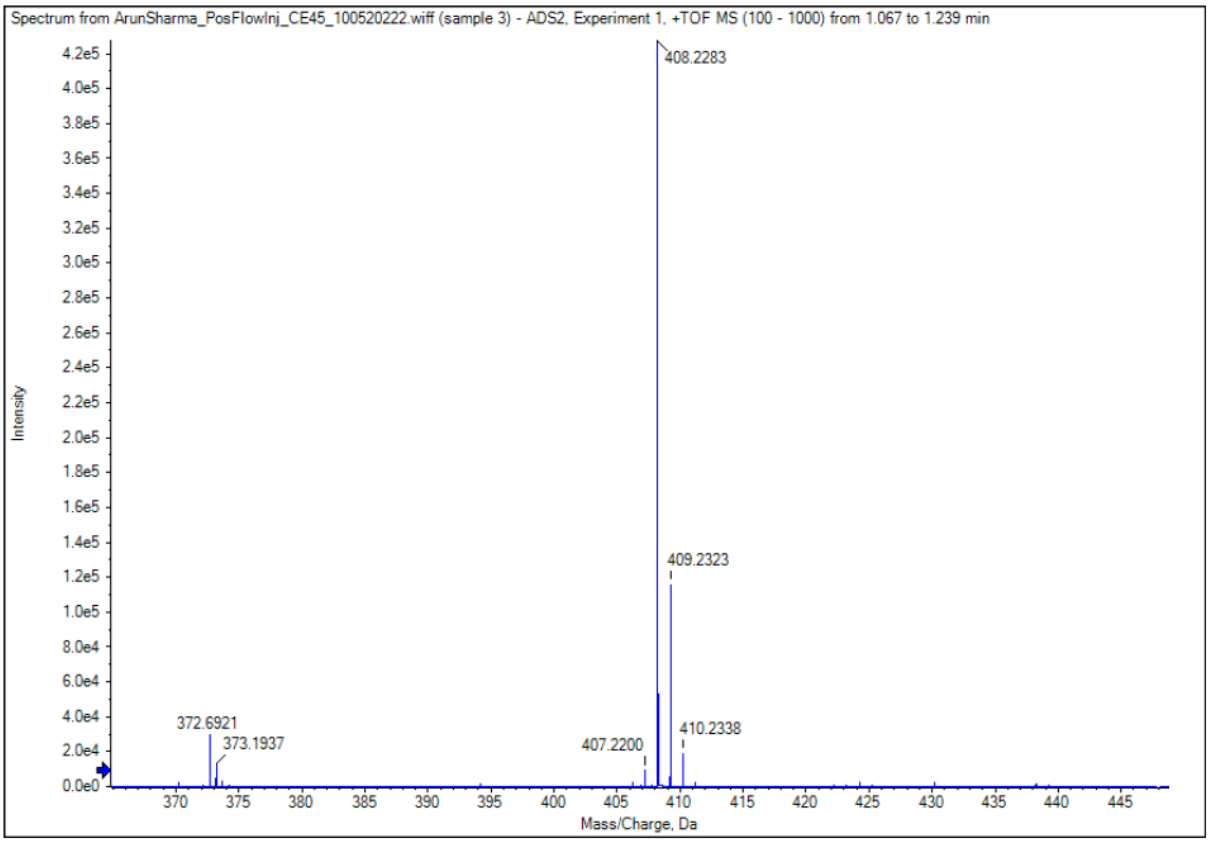
^{1}H NMR of (3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4-(pyrrolidin-1-ylmethyl) phenyl) methanone (7b)



^{13}C NMR of (3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4-(pyrrolidin-1-ylmethyl) phenyl) methanone (7b):



HRMS of (3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4-(pyrrolidin-1-ylmethyl) phenyl) methanone (7b):



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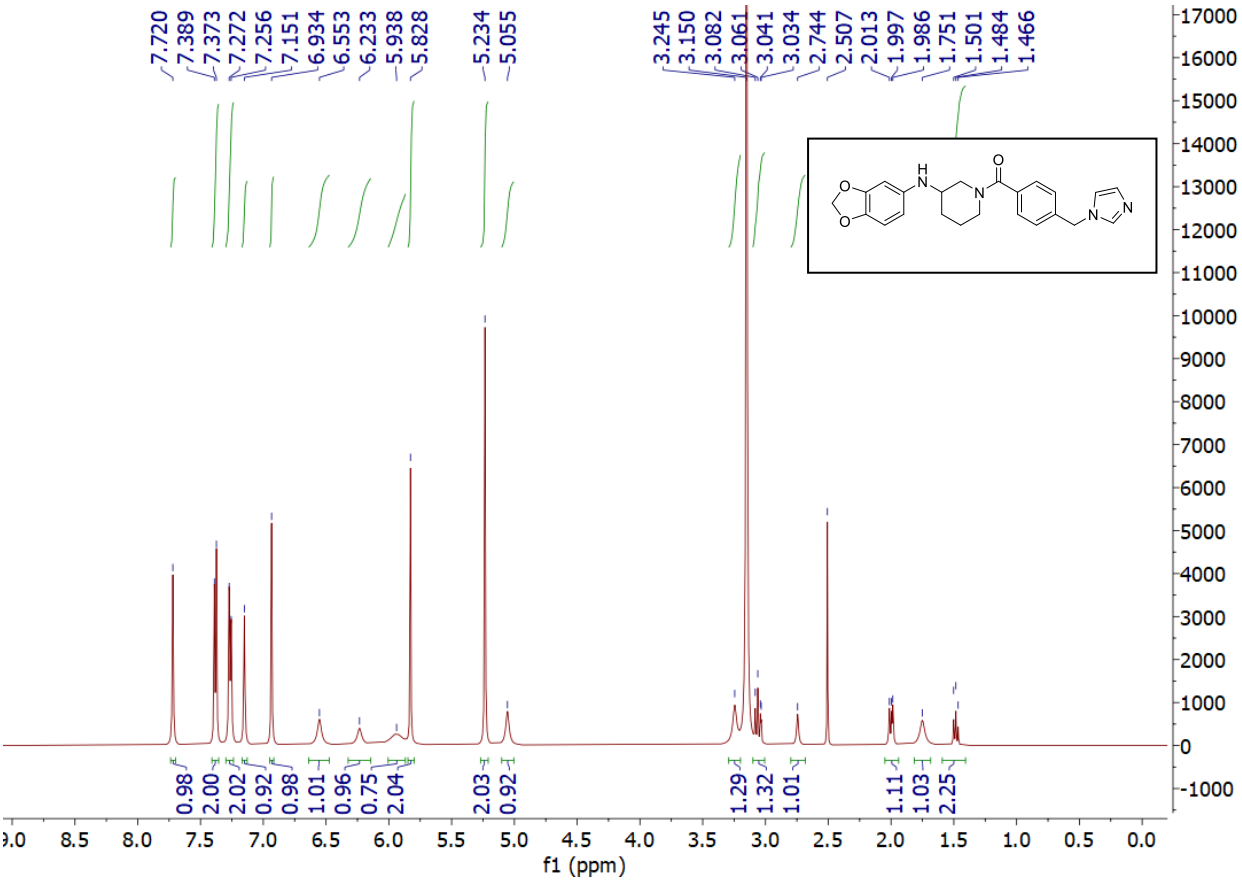
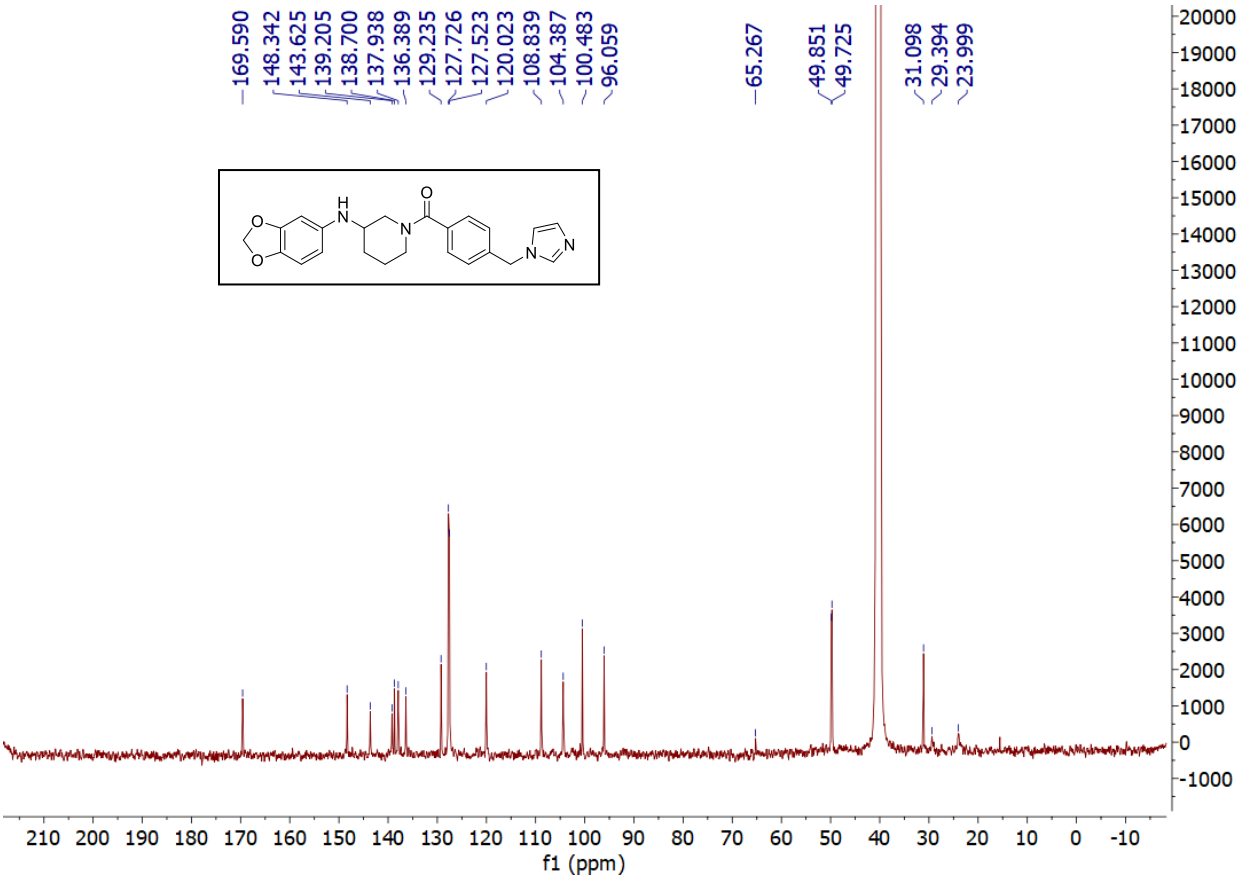
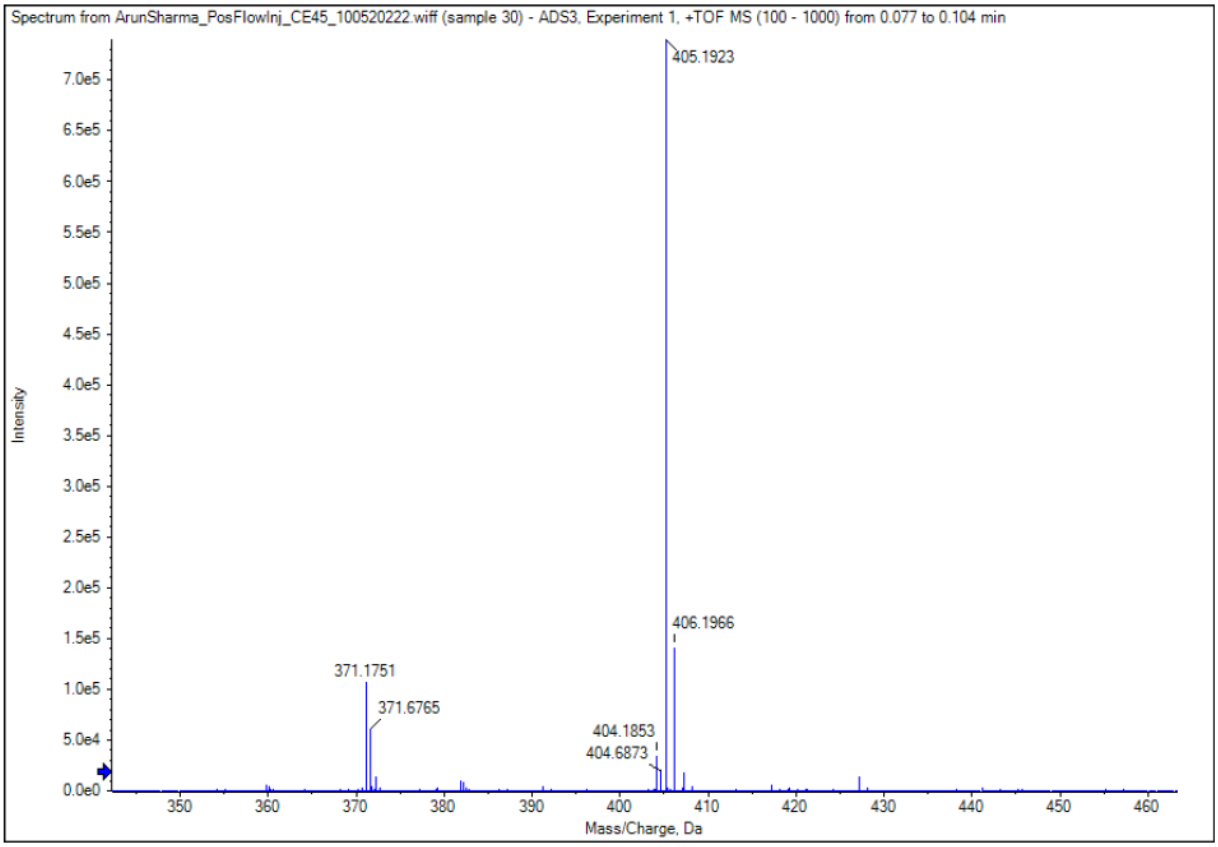
^{1}H NMR of (4-((1H-Imidazol-1-yl) methyl) phenyl) (3-(benzo[d][1,3] dioxol-5-ylamino)piperidin-1-yl)methanone (7c):



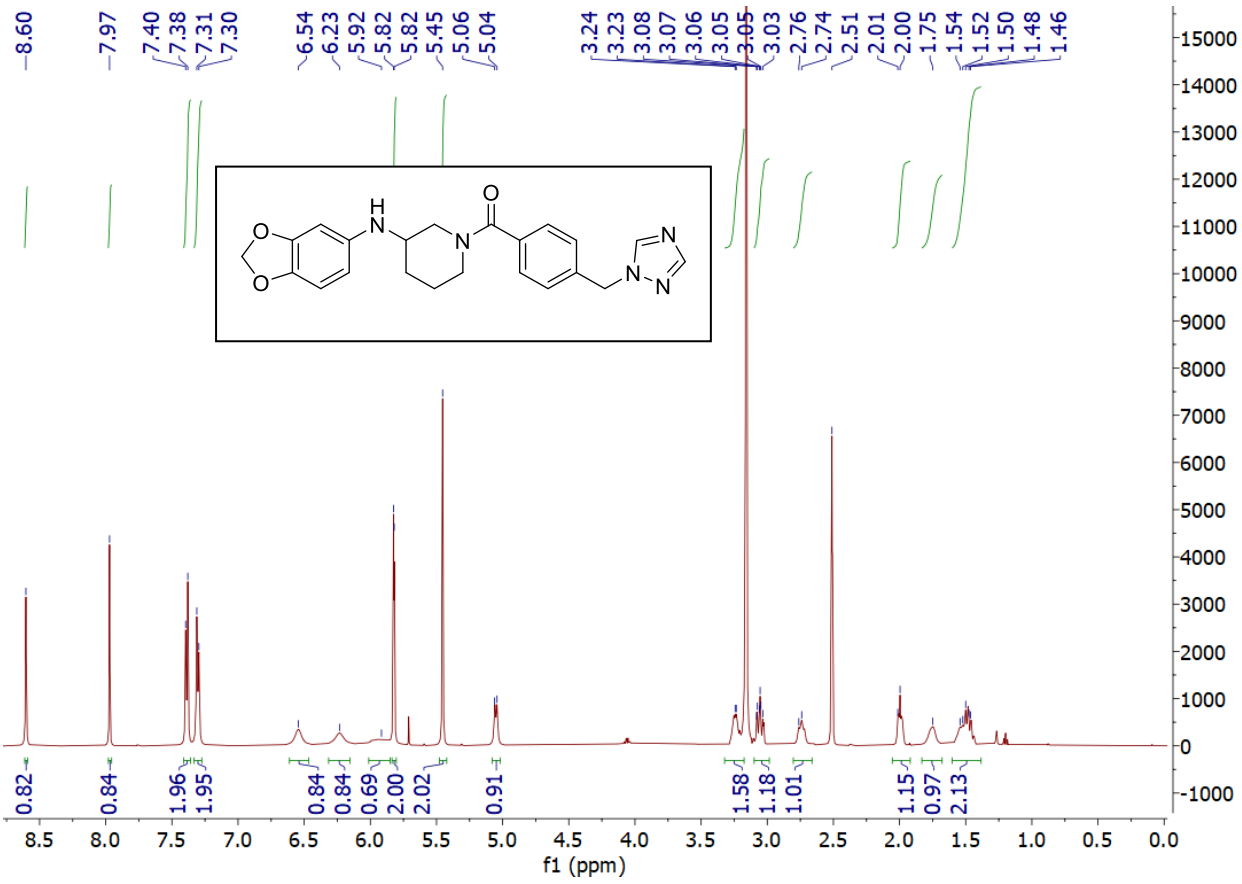
^{13}C NMR of (4-((1H-Imidazol-1-yl) methyl) phenyl) (3-(benzo[d][1,3] dioxol-5-ylamino)piperidin-1-yl)methanone (7c):



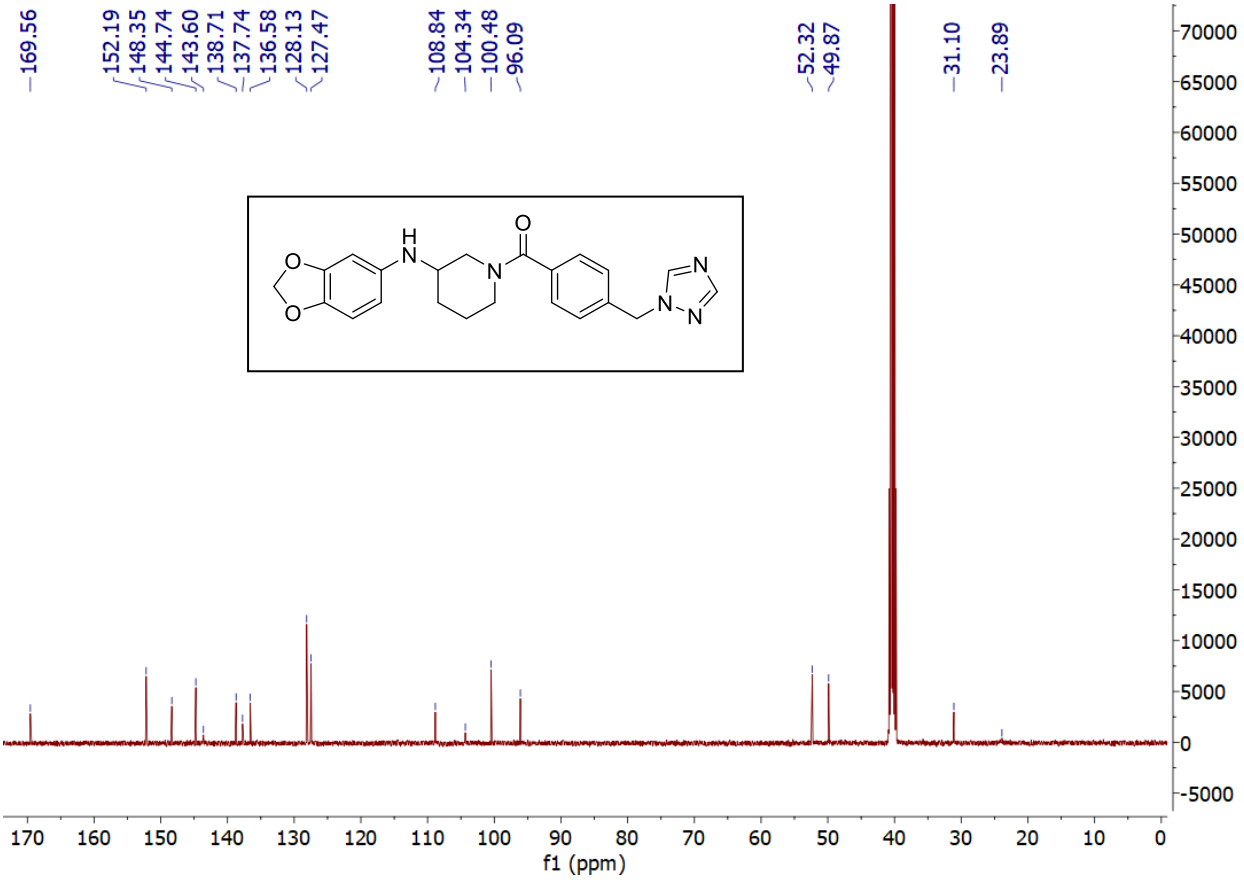
HRMS of (4-((1H-Imidazol-1-yl) methyl) phenyl) (3-(benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) methanone (7c):



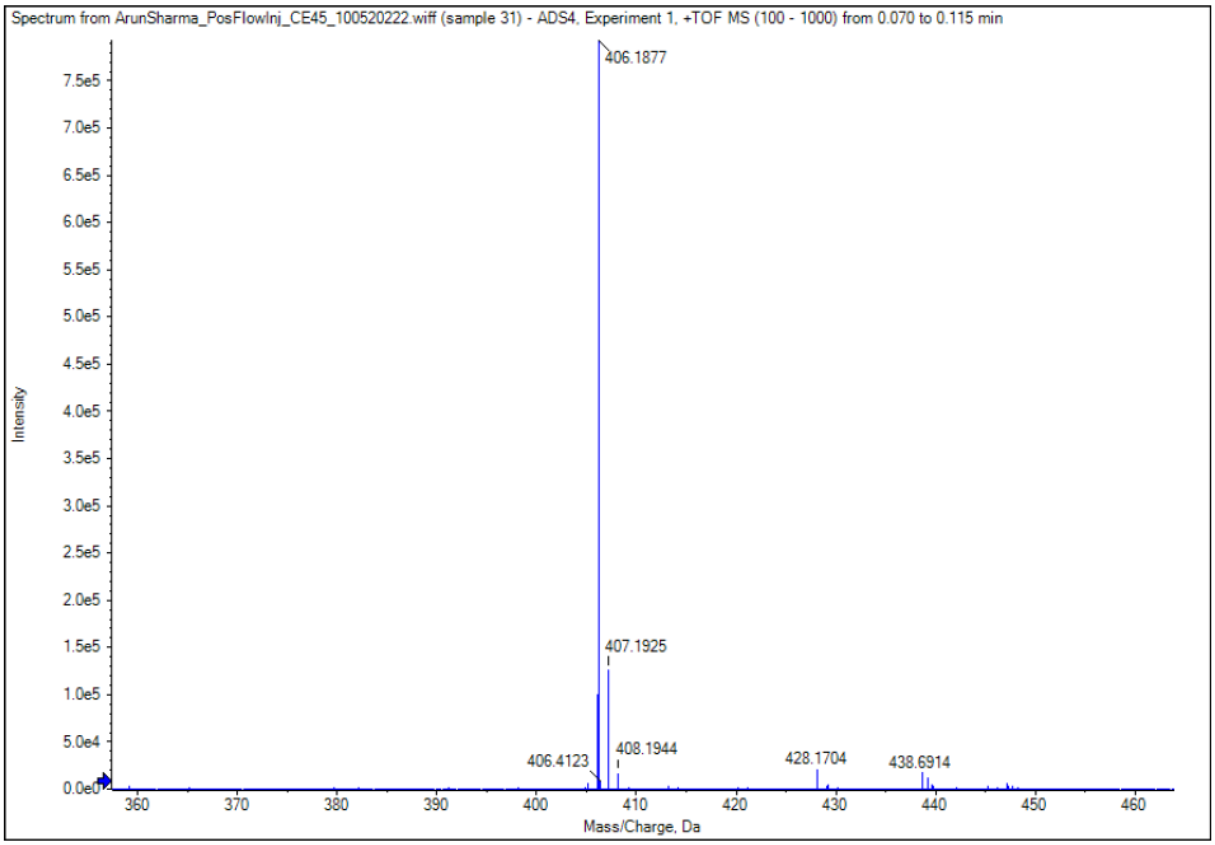
^{1}H of (4-((1H-1,2,4-triazol-1-yl) methyl) phenyl) (3-(benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) methanone (7d):



^{13}C of (4-((1H-1,2,4-triazol-1-yl) methyl) phenyl) (3-(benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) methanone (7d):



HRMS of (4-((1H-1,2,4-triazol-1-yl) methyl) phenyl) (3-(benzo[d][1,3] dioxol-5-ylamino)piperidin-1-yl)methanone (7d):



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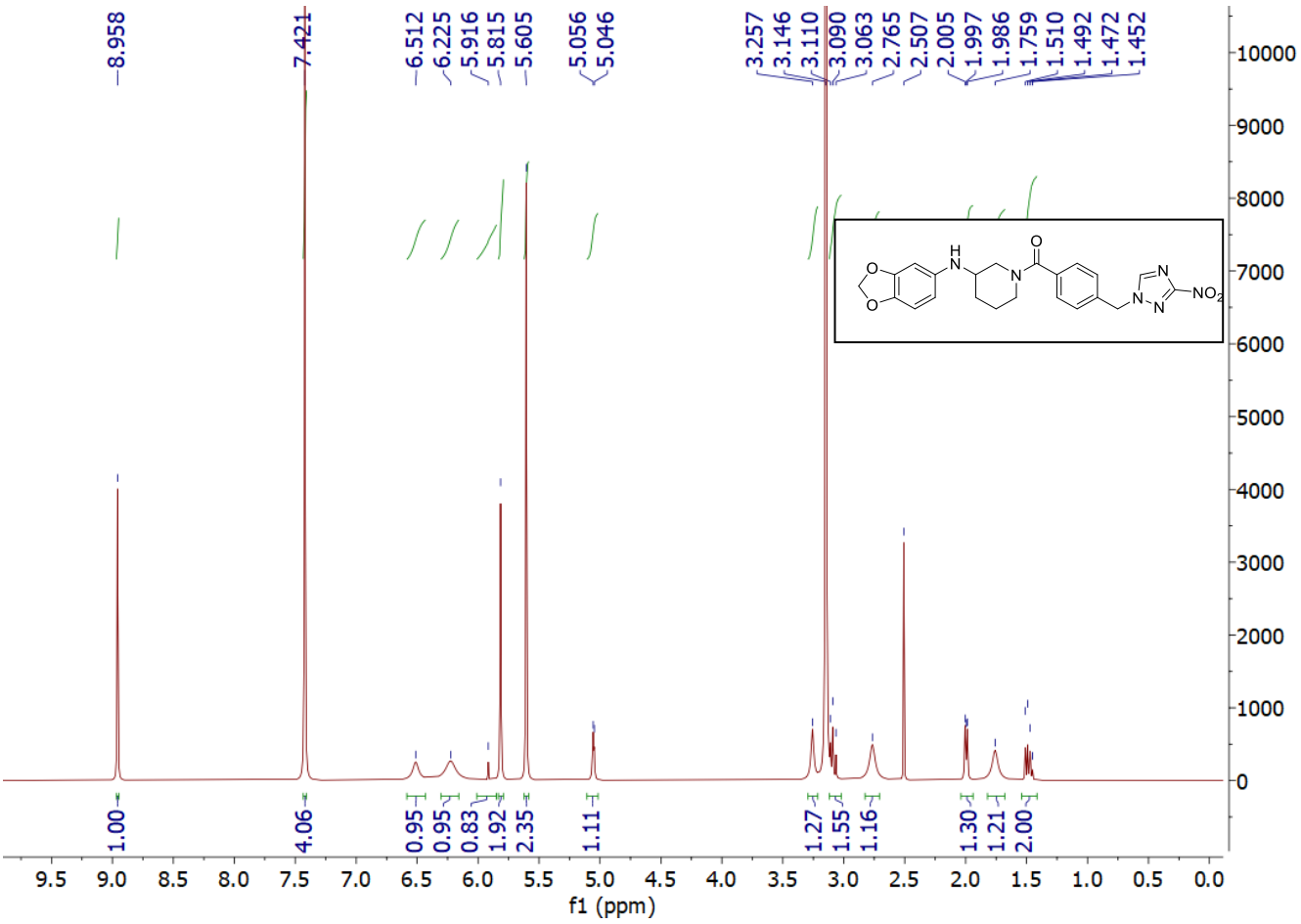
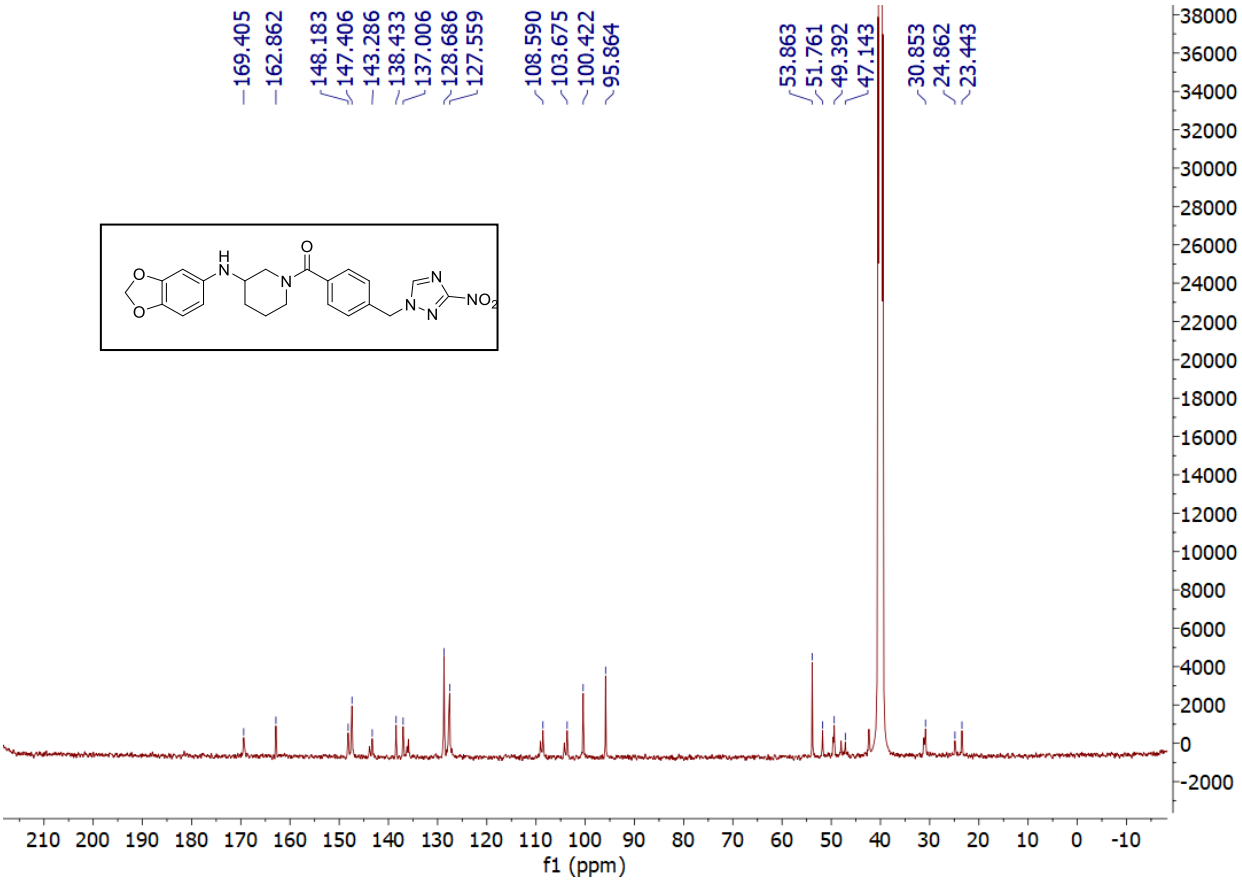
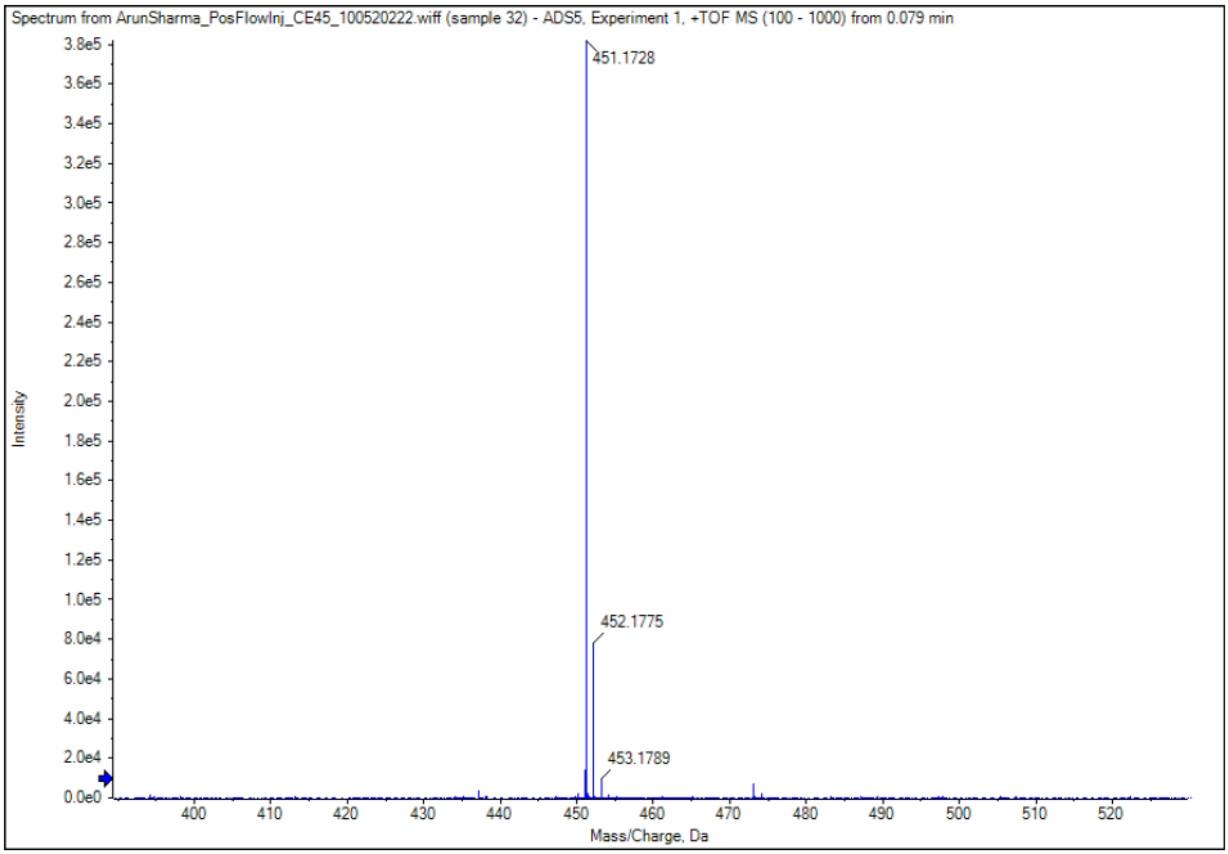
^{1}H NMR of (3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4-((3-nitro-1H-1,2,4-triazol-1-yl) methyl) phenyl) methanone (7e):



^{13}C NMR of (3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4-((3-nitro-1H-1,2,4-triazol-1-yl) methyl) phenyl) methanone (7e):

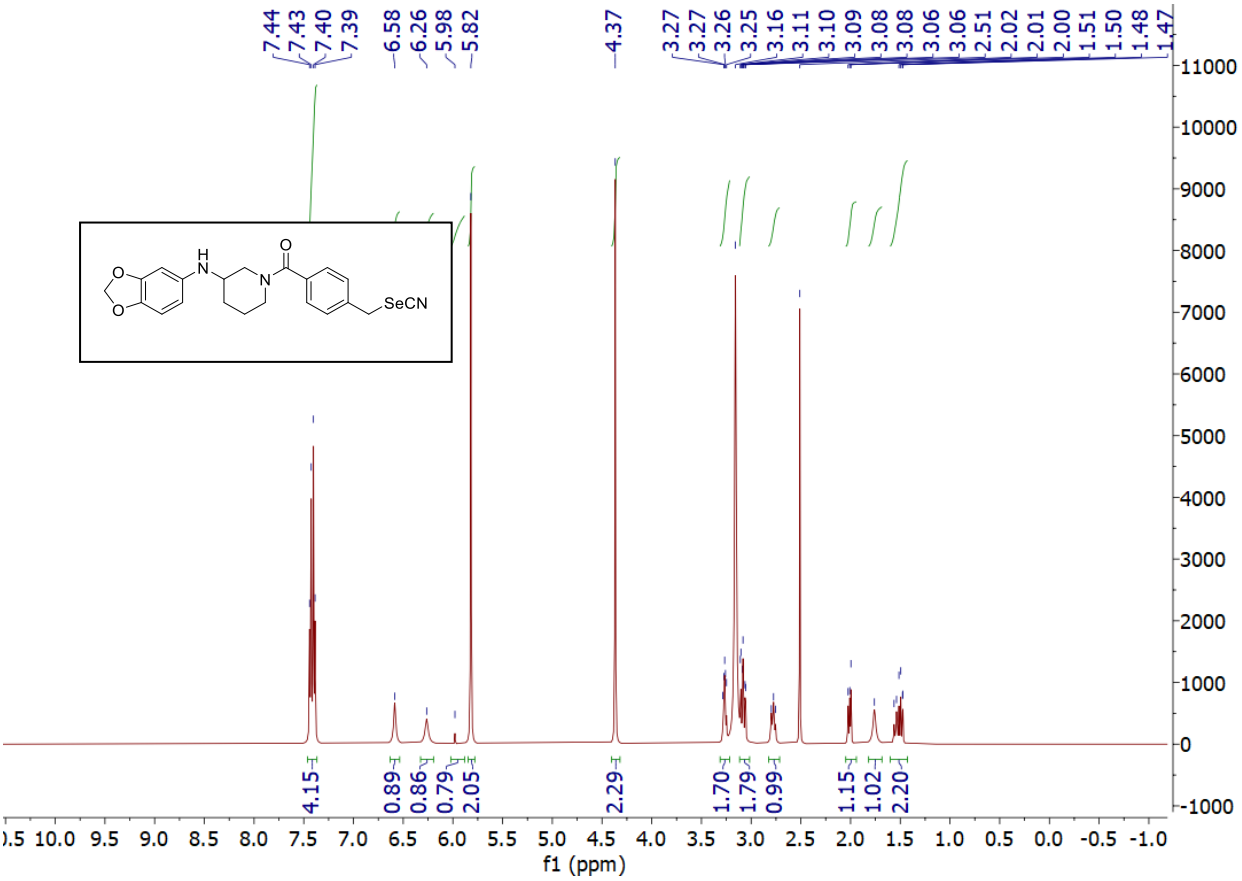


HRMS of of (3-(Benzo[d][1,3] dioxol-5-ylamino)piperidin-1-yl)(4-((3-nitro-1H-1,2,4-triazol-1-yl)methyl)phenyl)methanone (7e):

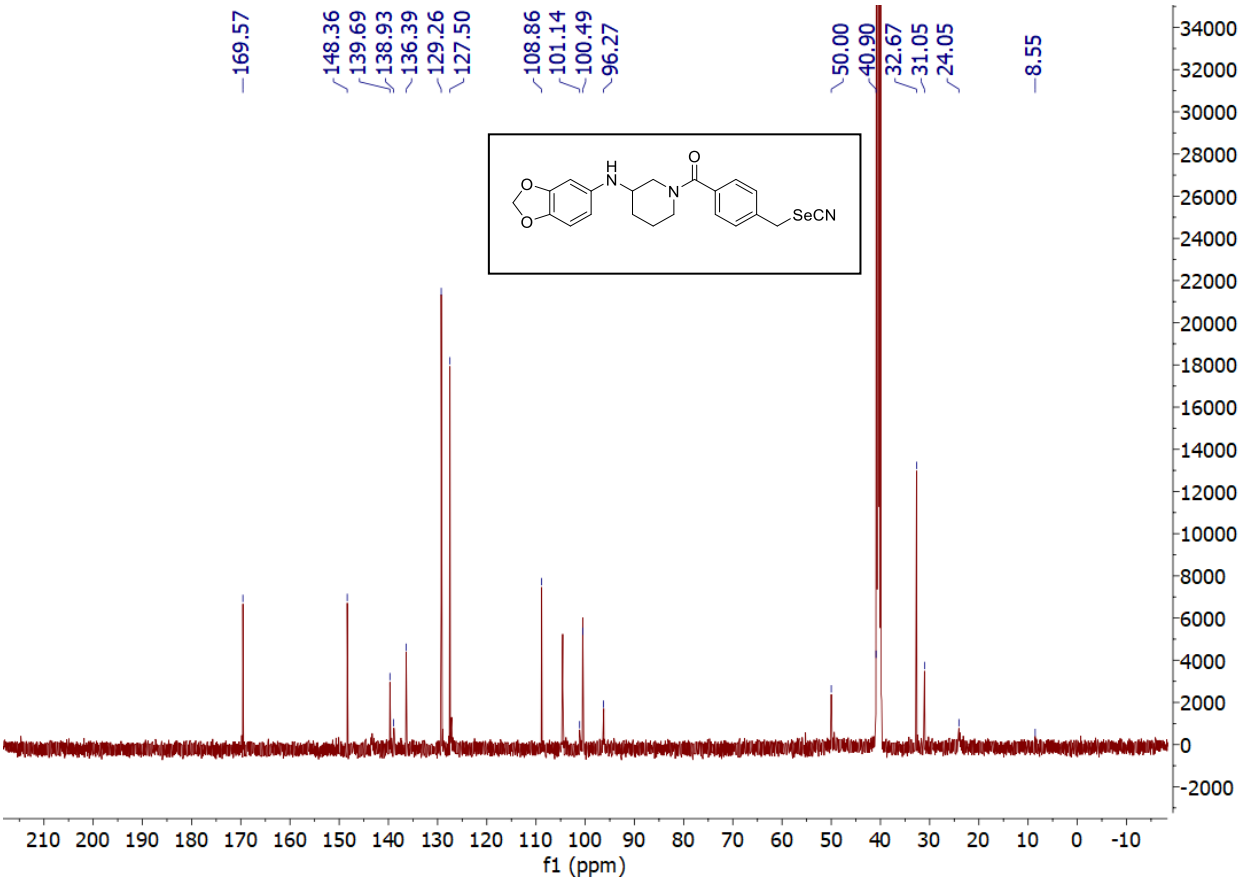


10/11/2022 9:38:03 AM

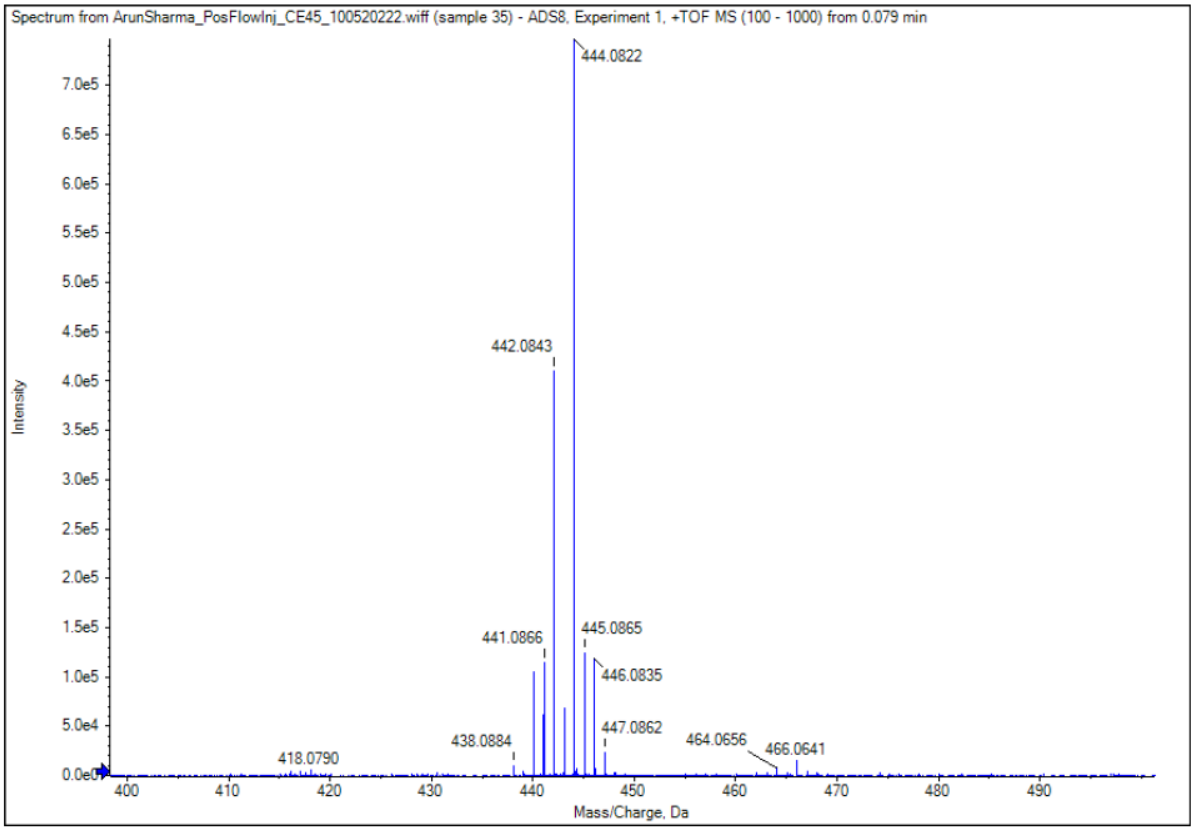
^{1}H NMR of (3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4(selenocyanatomethyl) phenyl) methanone (7g):



^{13}C NMR of (3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4(selenocyanatomethyl) phenyl) methanone (7g):



HRMS of (3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4(selenocyanatomethyl) phenyl) methanone (7g):



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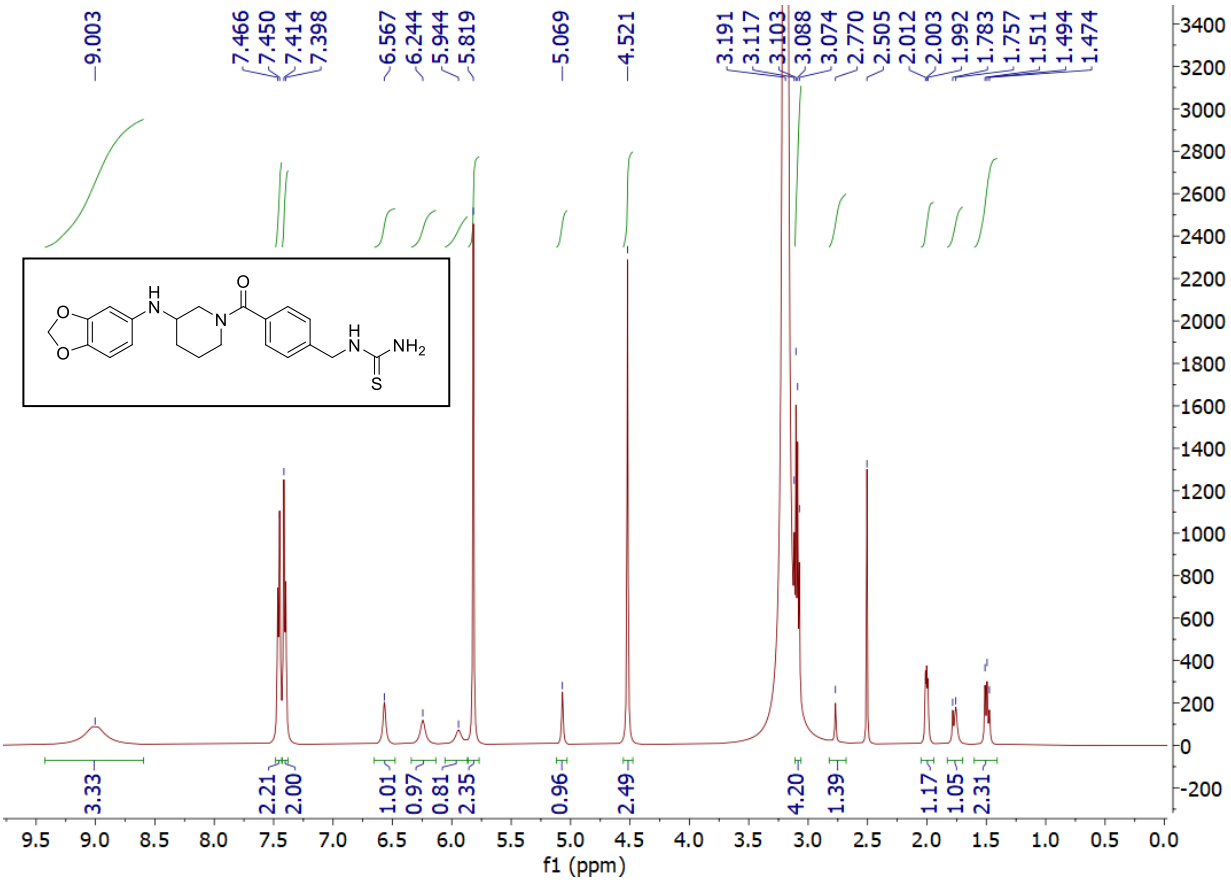
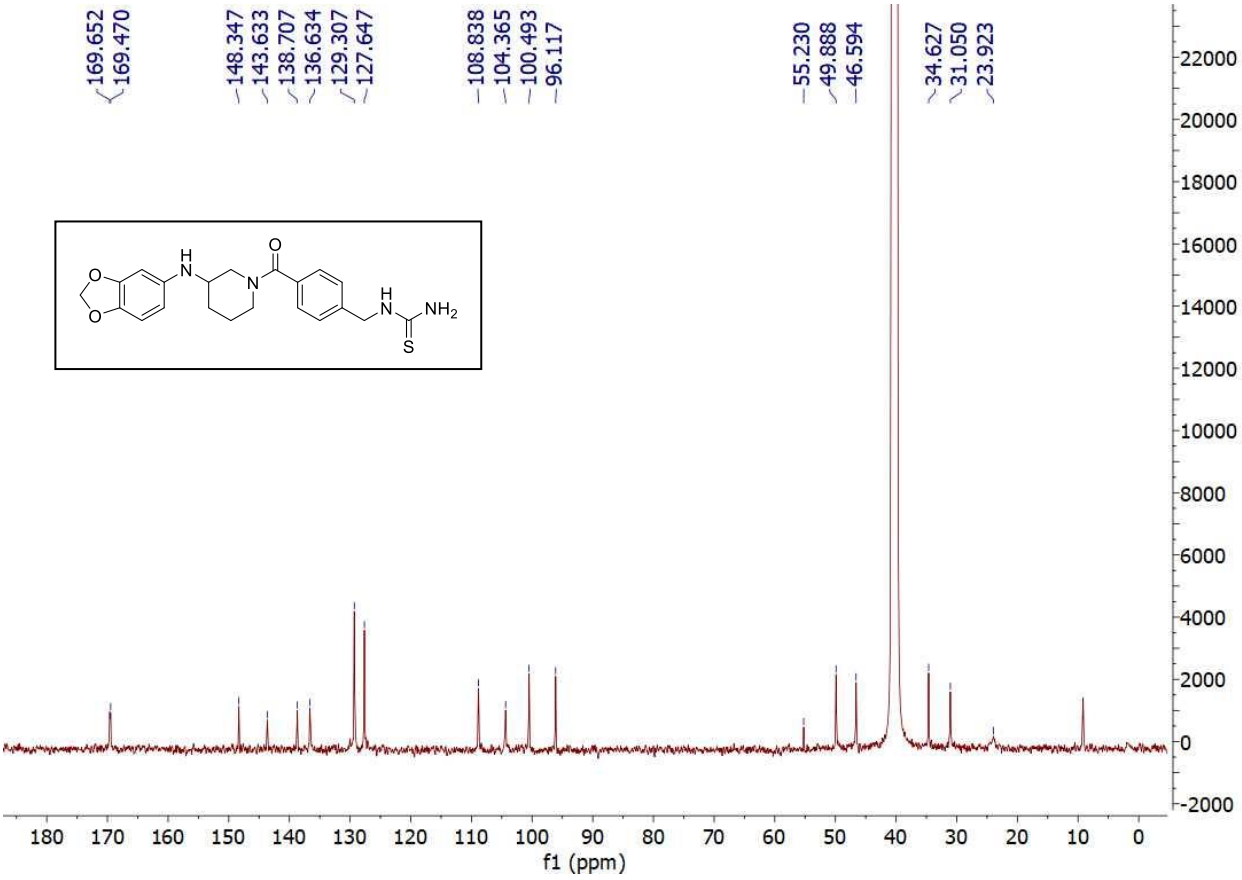
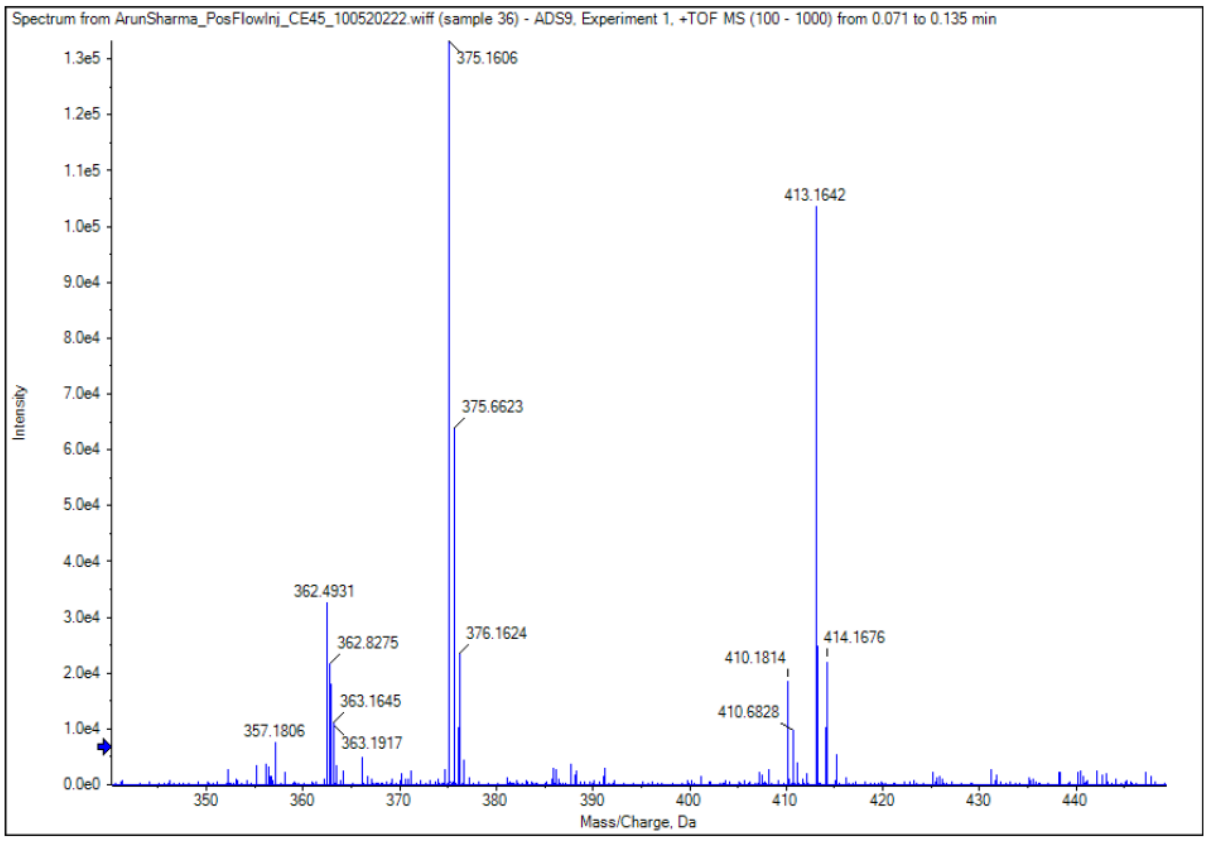
^{1}H NMR of 1-(4-(3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidine-1-carbonyl) benzyl) thiourea(7h):



^{13}C NMR of 1-(4-(3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidine-1-carbonyl) benzyl) thiourea(7h):



HRMS of 1-(4-(3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidine-1-carbonyl) benzyl) thiourea(7h):



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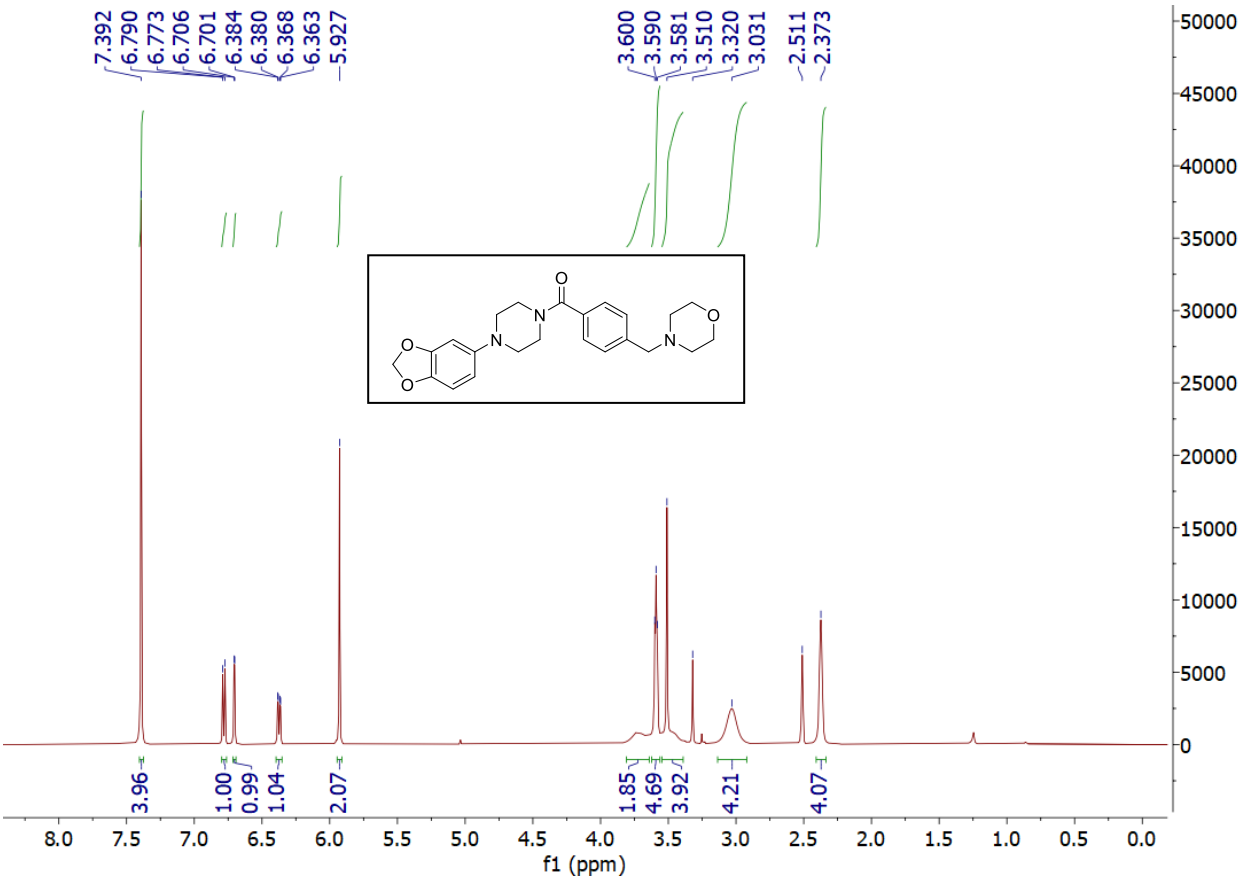
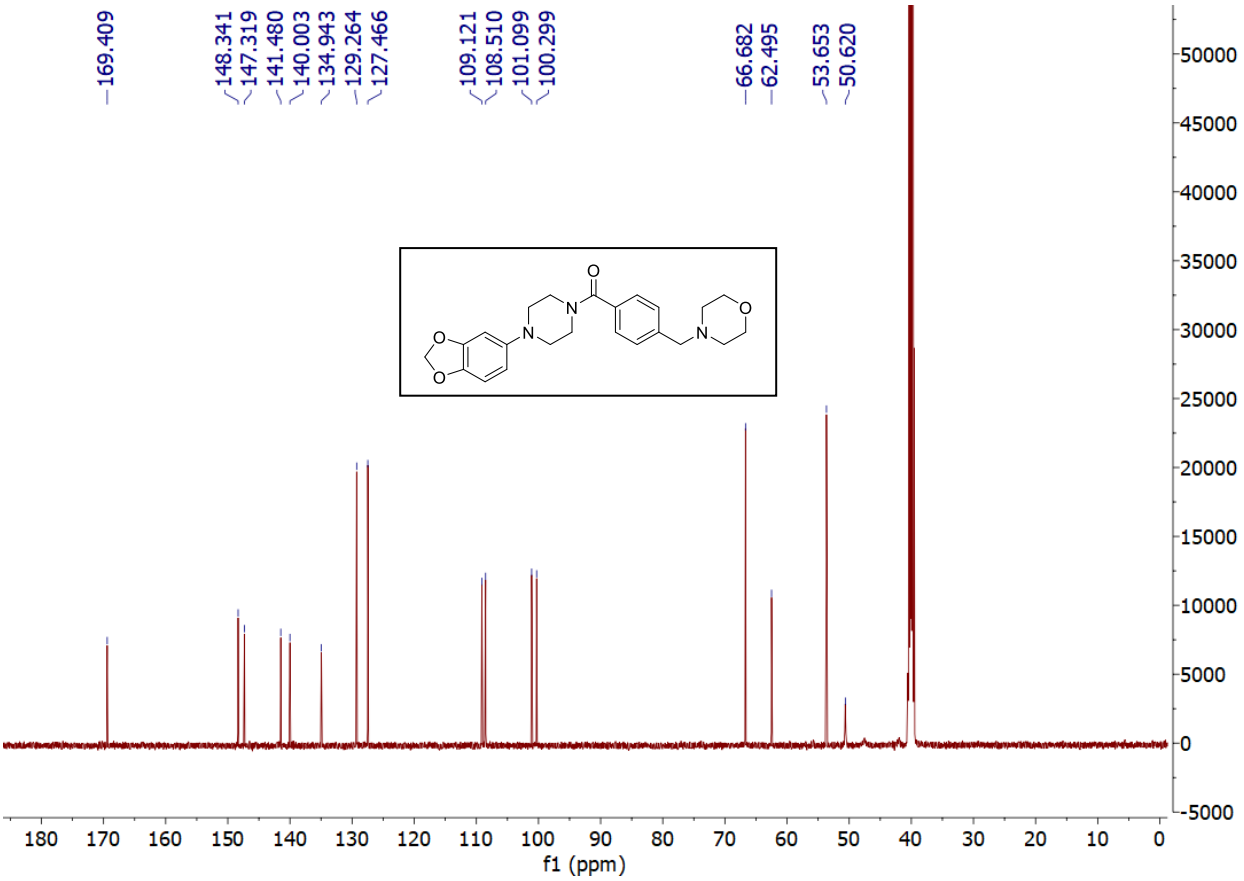
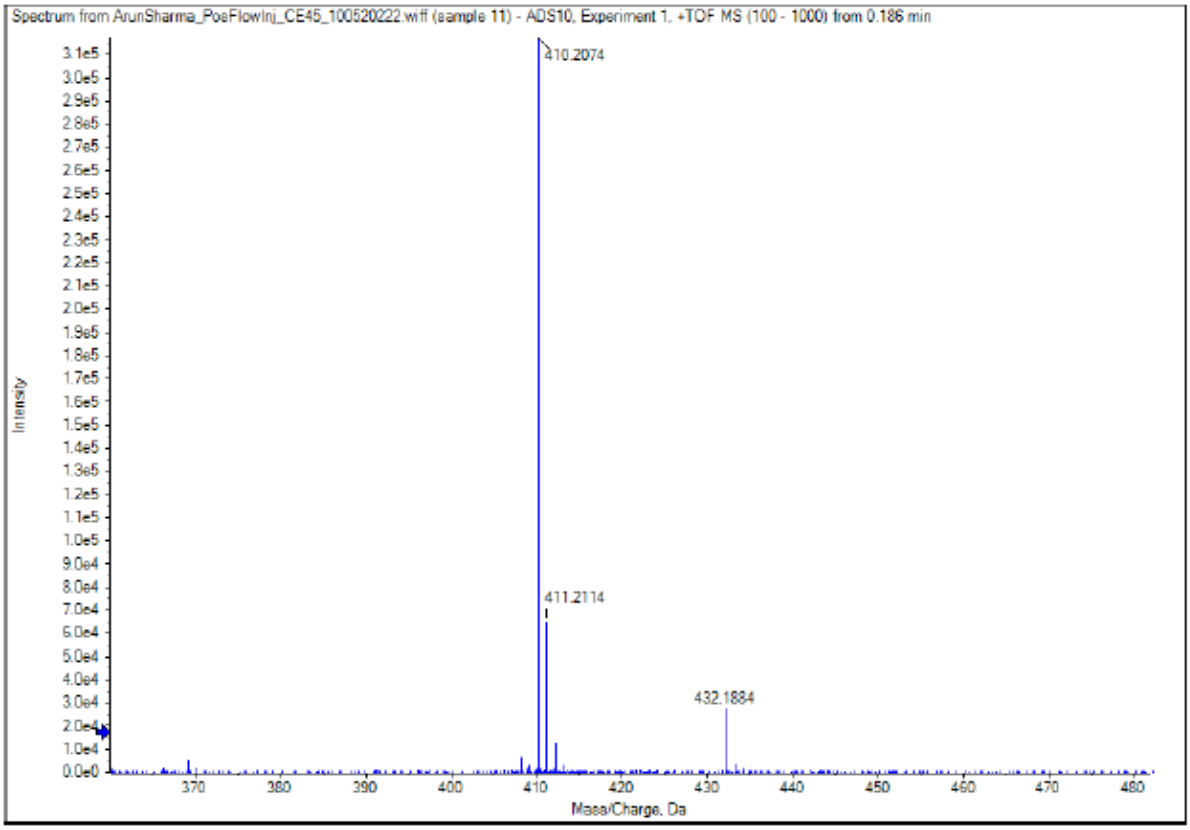
^{1}H NMR of (4-(benzo[d][1,3]dioxol-5-yl)piperazin-1-yl)(4-(morpholinomethyl) phenyl) methanone (13a):



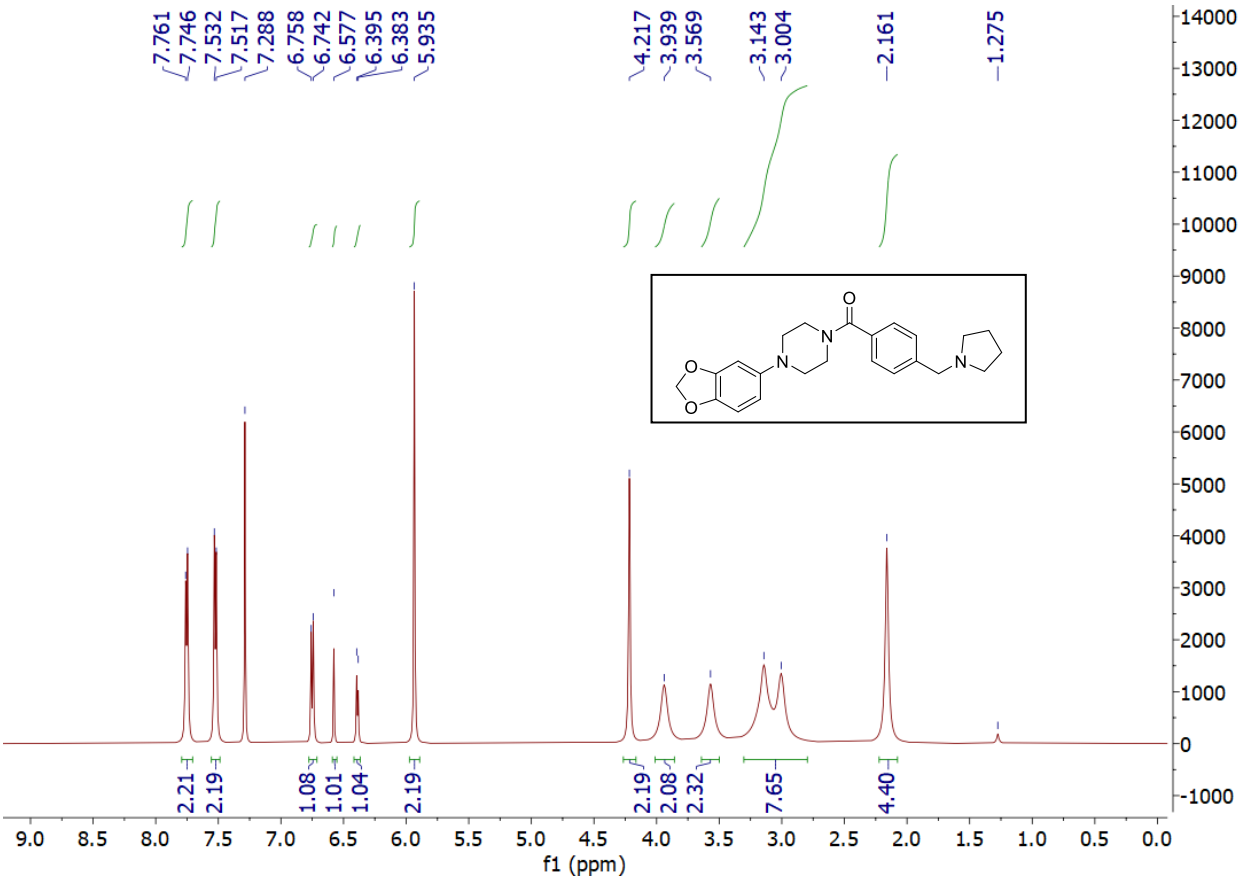
^{13}C NMR of (4-(benzo[d][1,3]dioxol-5-yl)piperazin-1-yl)(4-(morpholinomethyl) phenyl) methanone (13a):



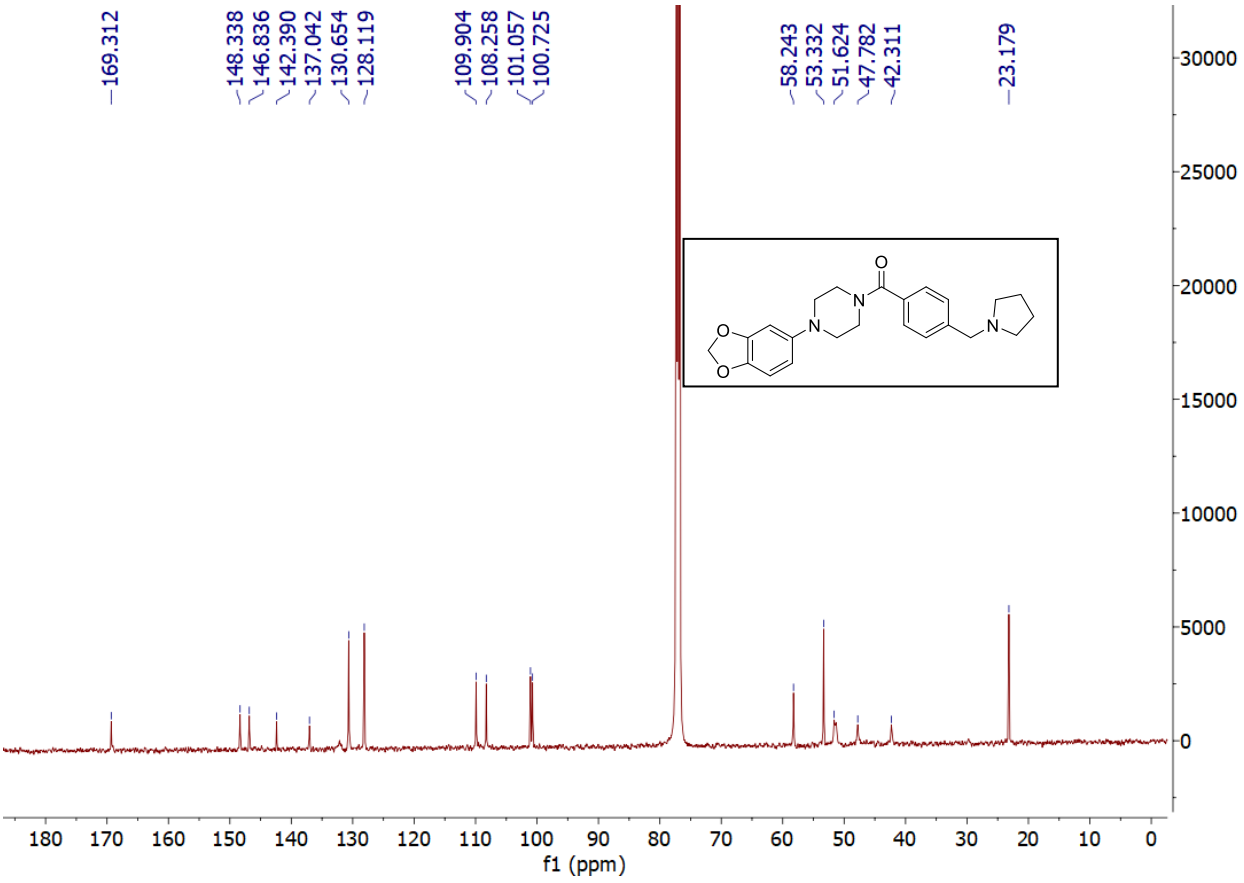
HRMS of (4-(benzo[d][1,3]dioxol-5-yl)piperazin-1-yl)(4-(morpholinomethyl) phenyl) methanone (13a):



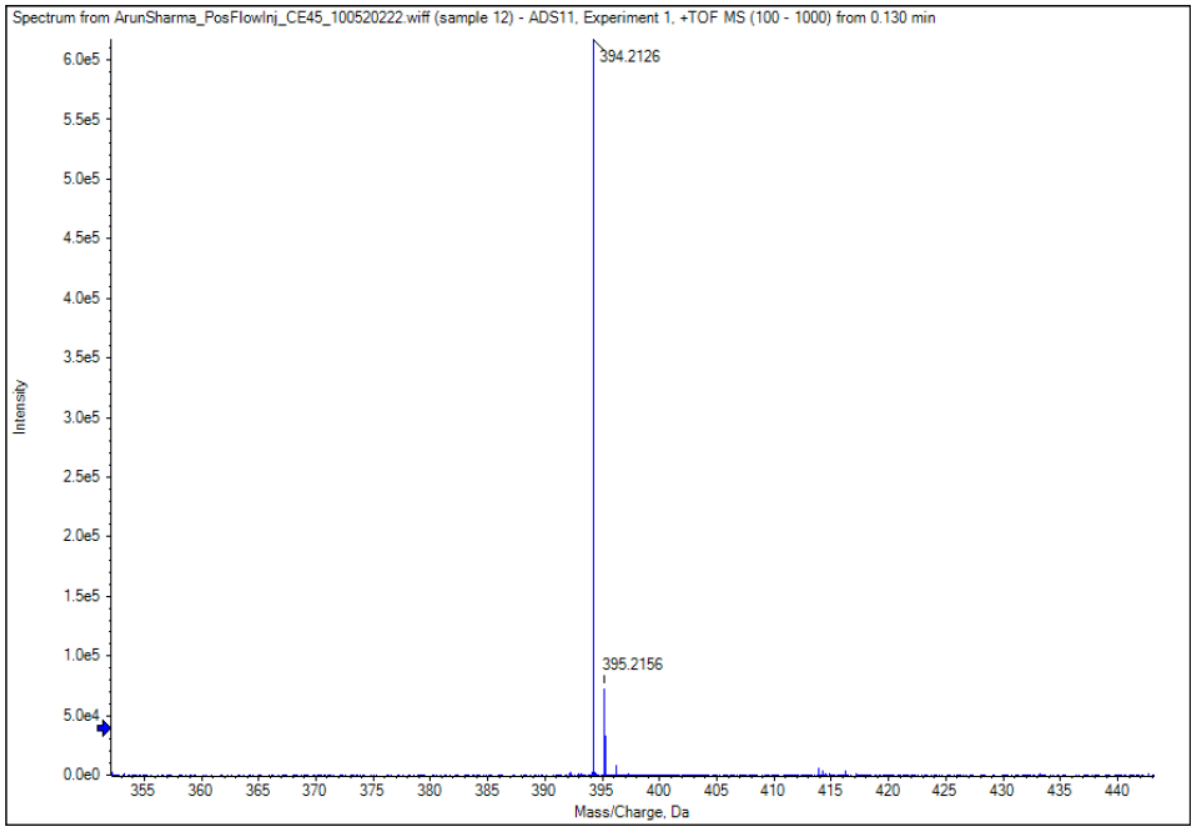
^{1}H NMR of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-(pyrrolidin-1-ylmethyl) phenyl) methanone (13b):



^{13}C NMR of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-(pyrrolidin-1-ylmethyl) phenyl) methanone(13b):



HRMS of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-(pyrrolidin-1-ylmethyl) phenyl) methanone (13b):



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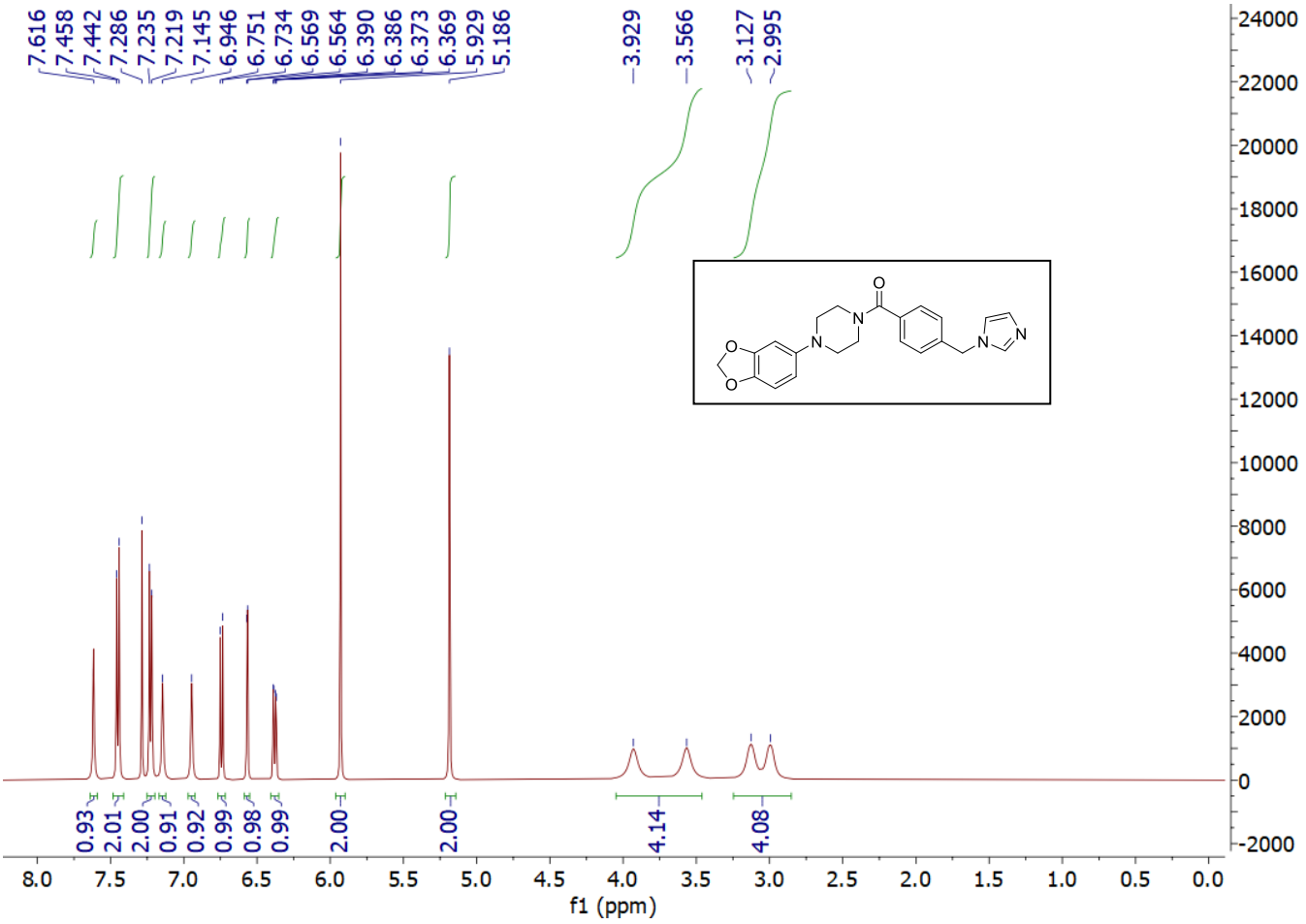
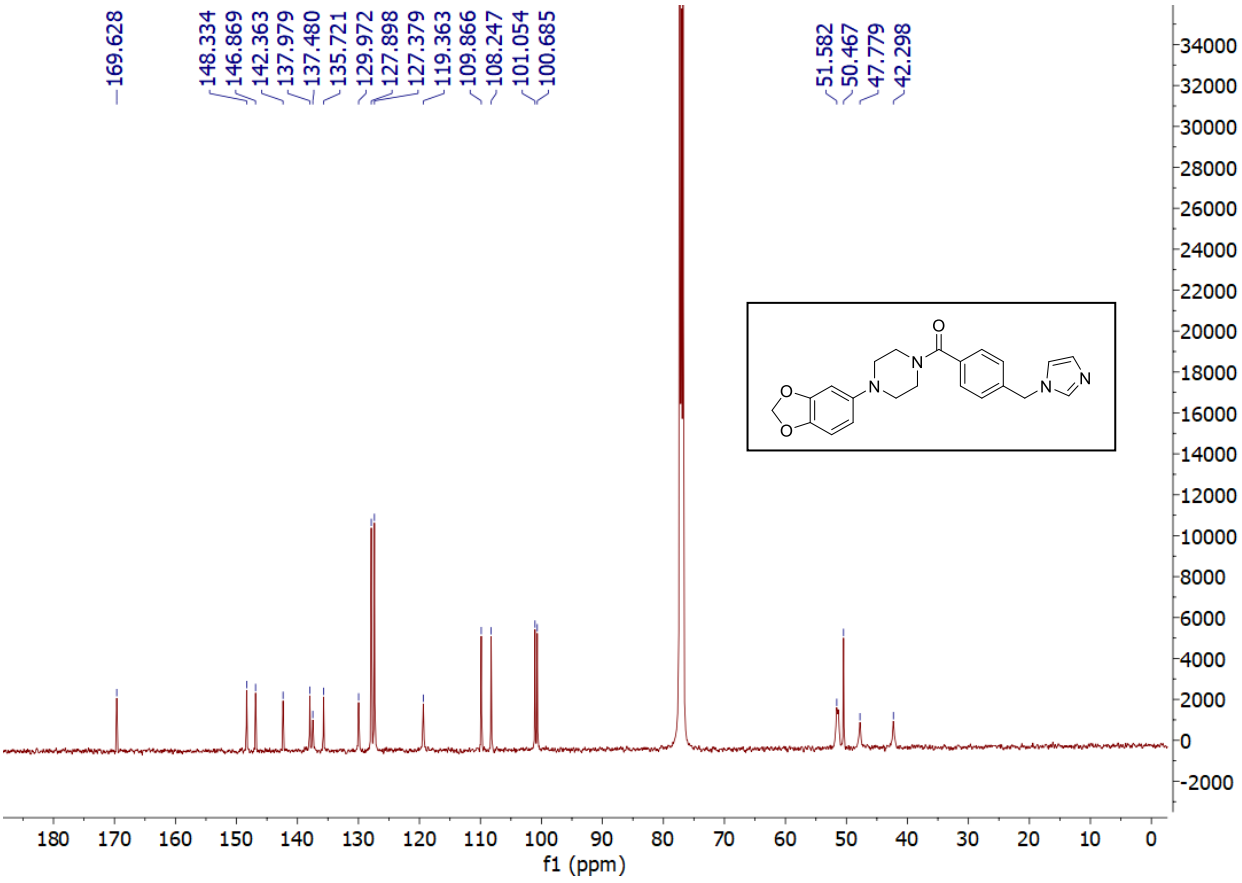
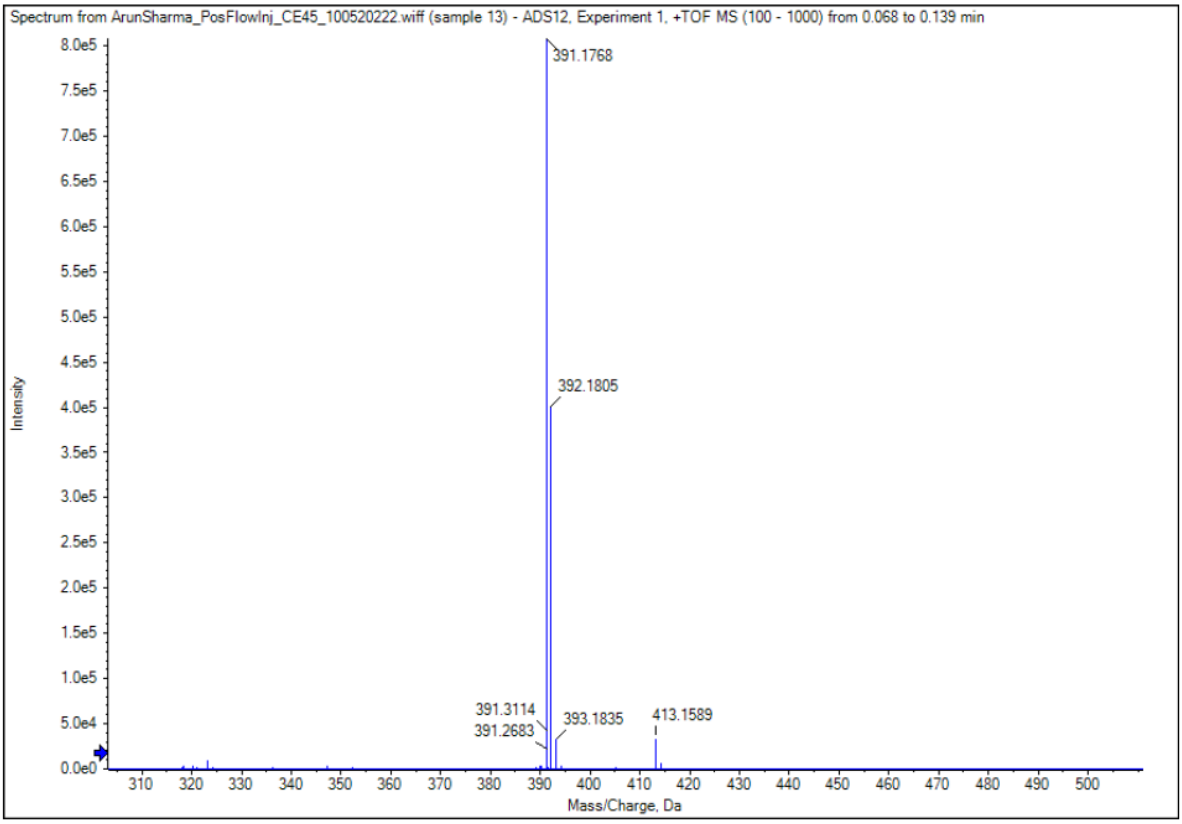
^{1}H NMR of (4-((1H-Imidazol-1-yl) methyl) phenyl) (4-(benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) methanone (13c):



^{13}C NMR of (4-((1H-Imidazol-1-yl) methyl) phenyl) (4-(benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) methanone (13c):



HRMS of (4-((1H-Imidazol-1-yl) methyl) phenyl) (4-(benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) methanone (13c):



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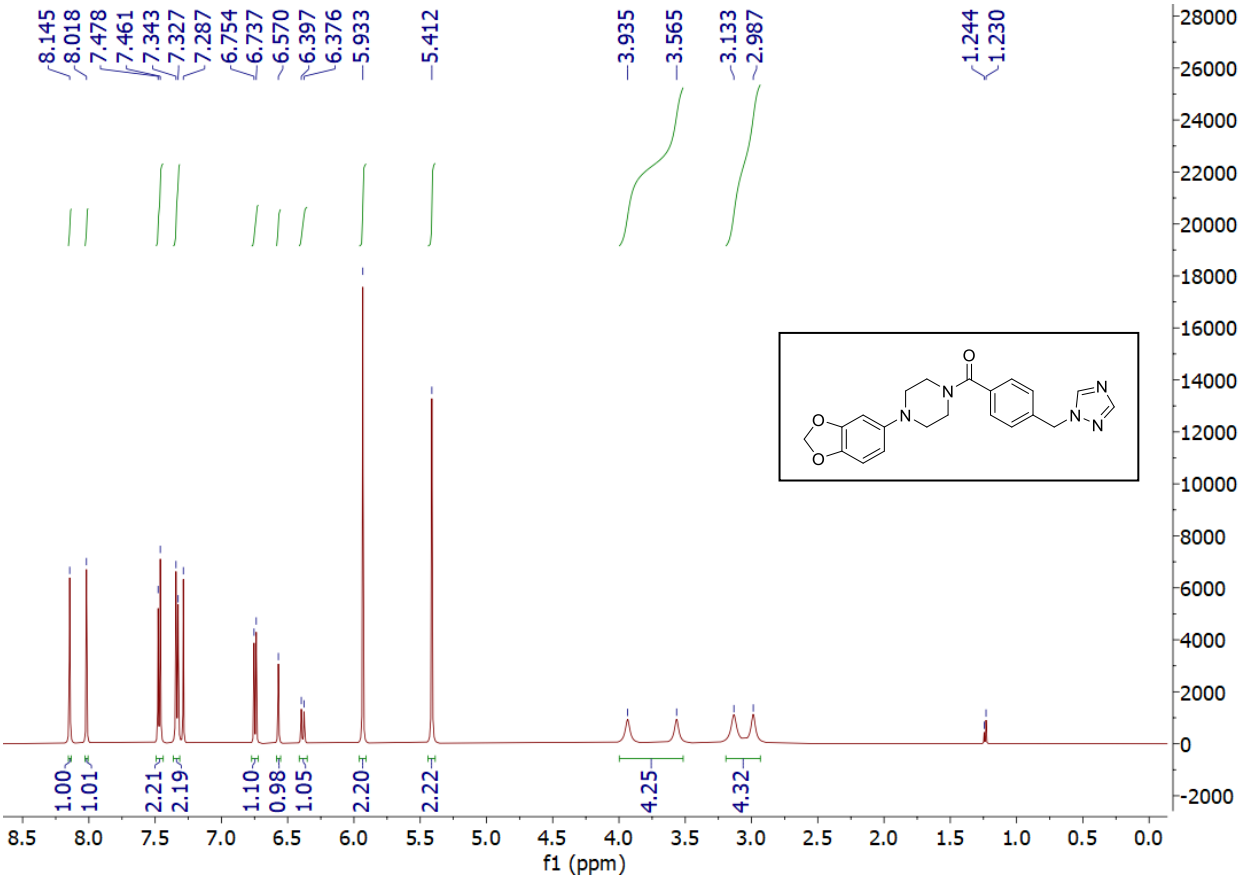
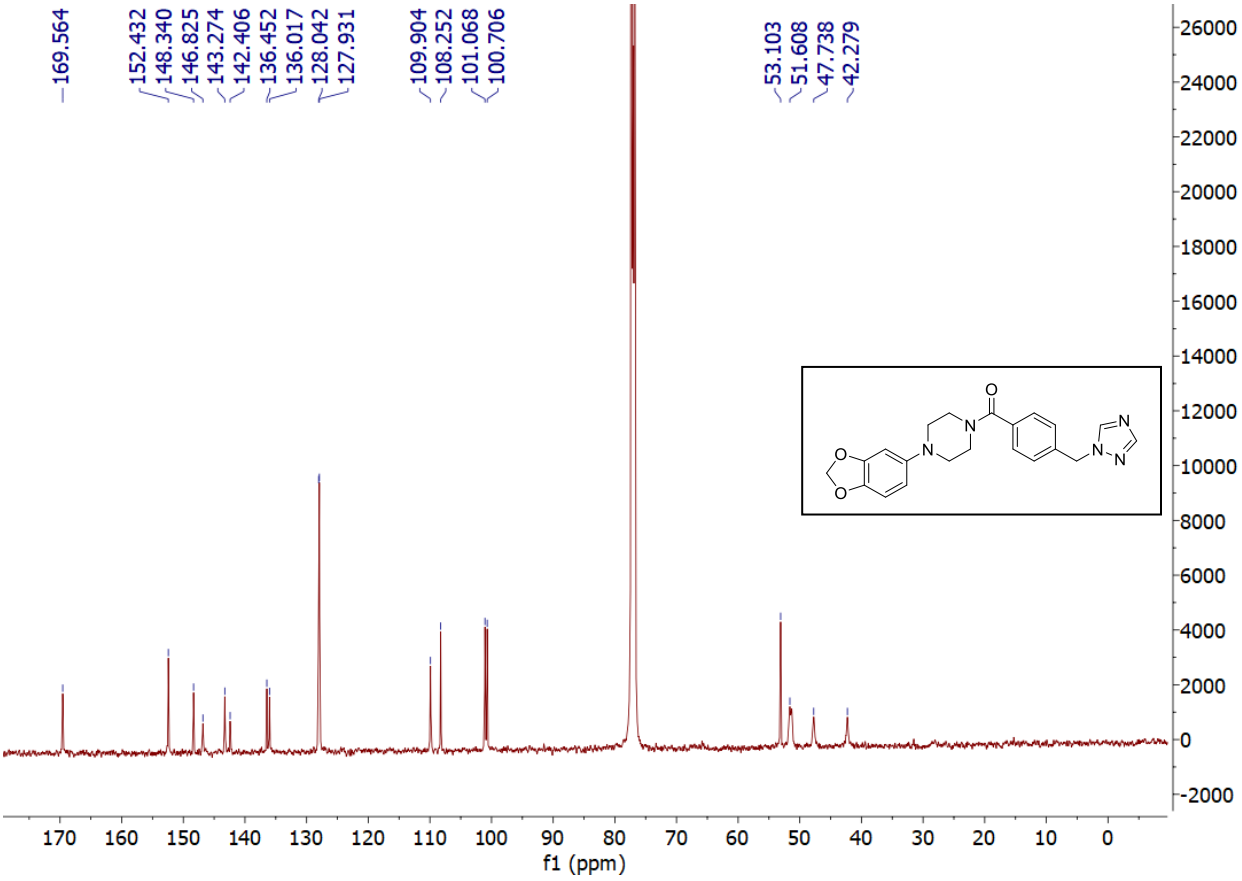
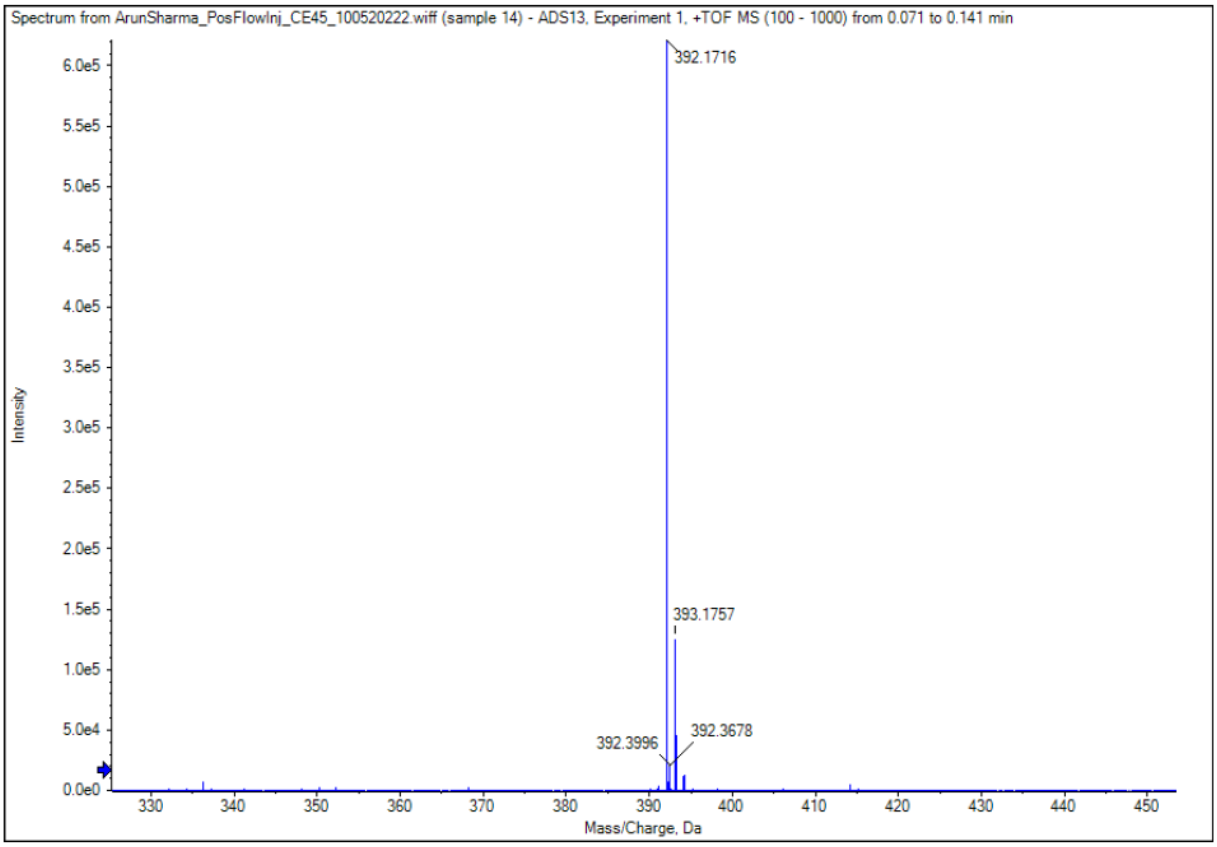
^{1}H NMR of (4-((1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) methanone (13d):



^{13}C NMR of (4-((1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) methanone (13d):



HRMS of (4-((1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) methanone (13d):



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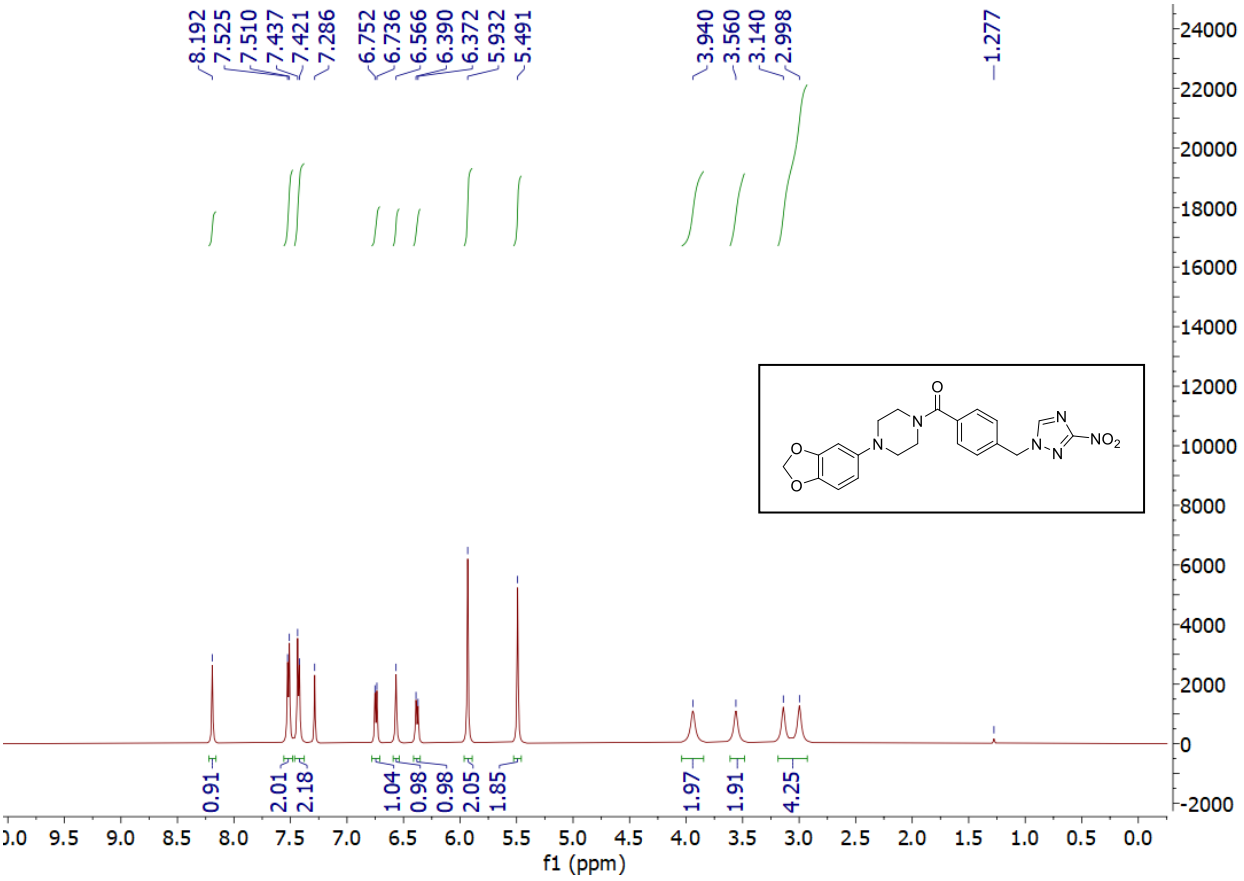
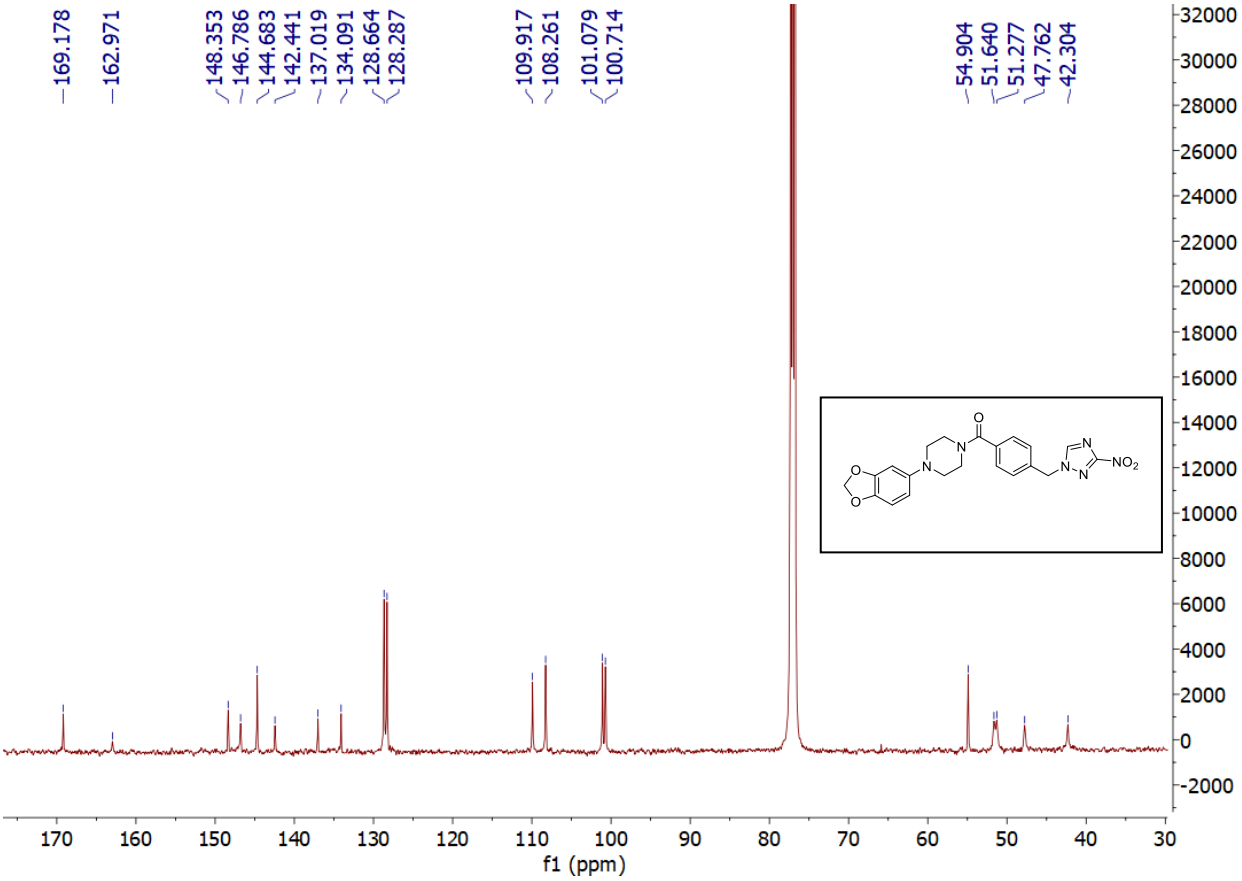
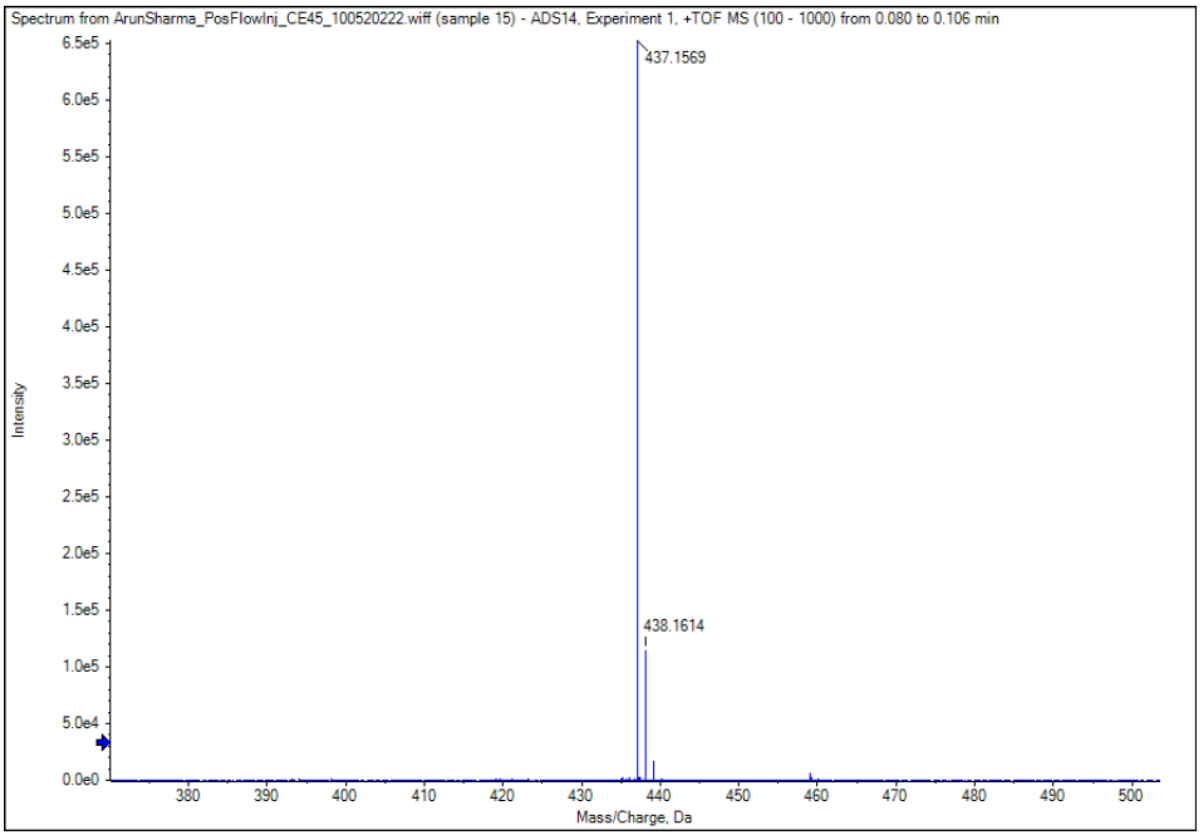
^{1}H NMR of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-((3-nitro-1H-1,2,4-triazol-1-yl) methyl) phenyl) methanone (13e):



^{13}C NMR of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-((3-nitro-1H-1,2,4-triazol-1-yl) methyl) phenyl) methanone (13e):



HRMS of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-((3-nitro-1H-1,2,4-triazol-1-yl) methyl) phenyl) methanone (13e):



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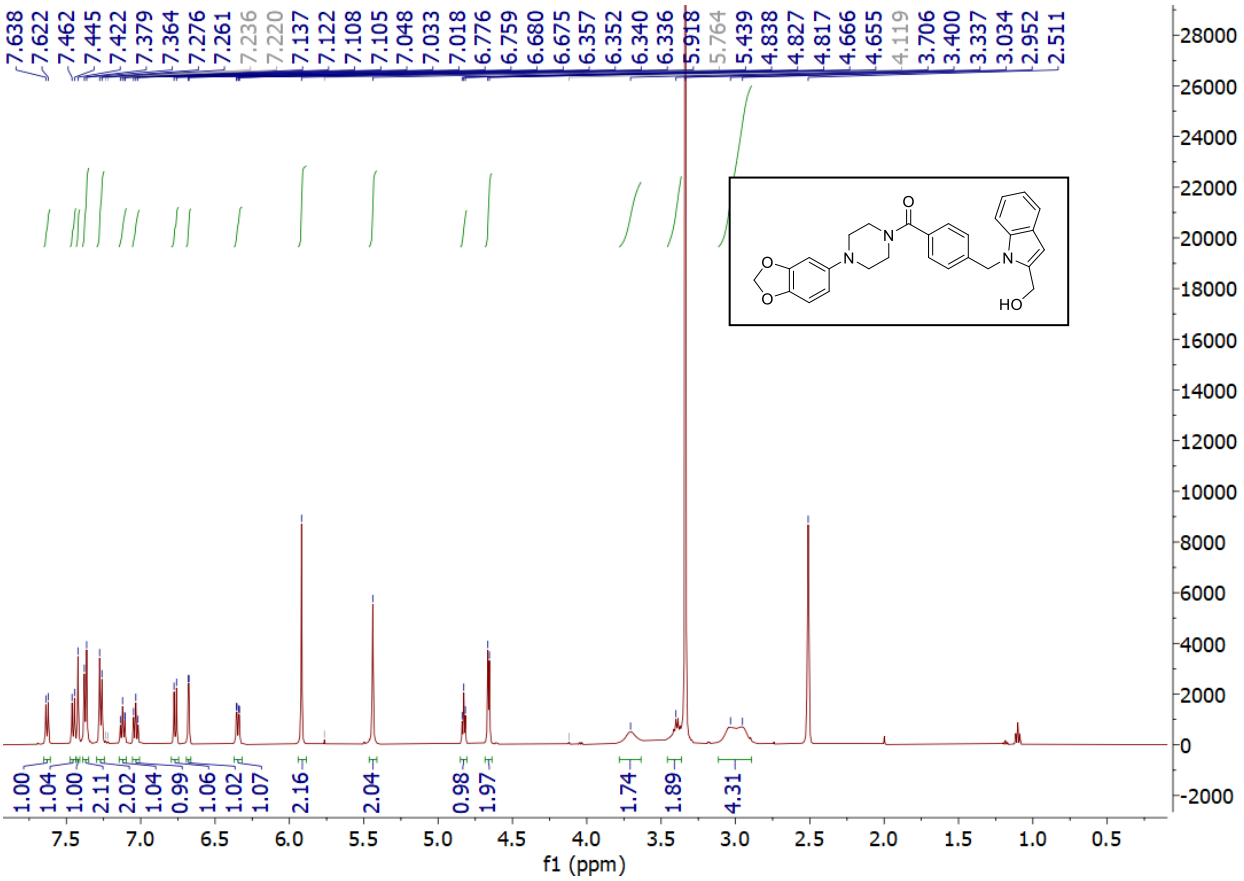
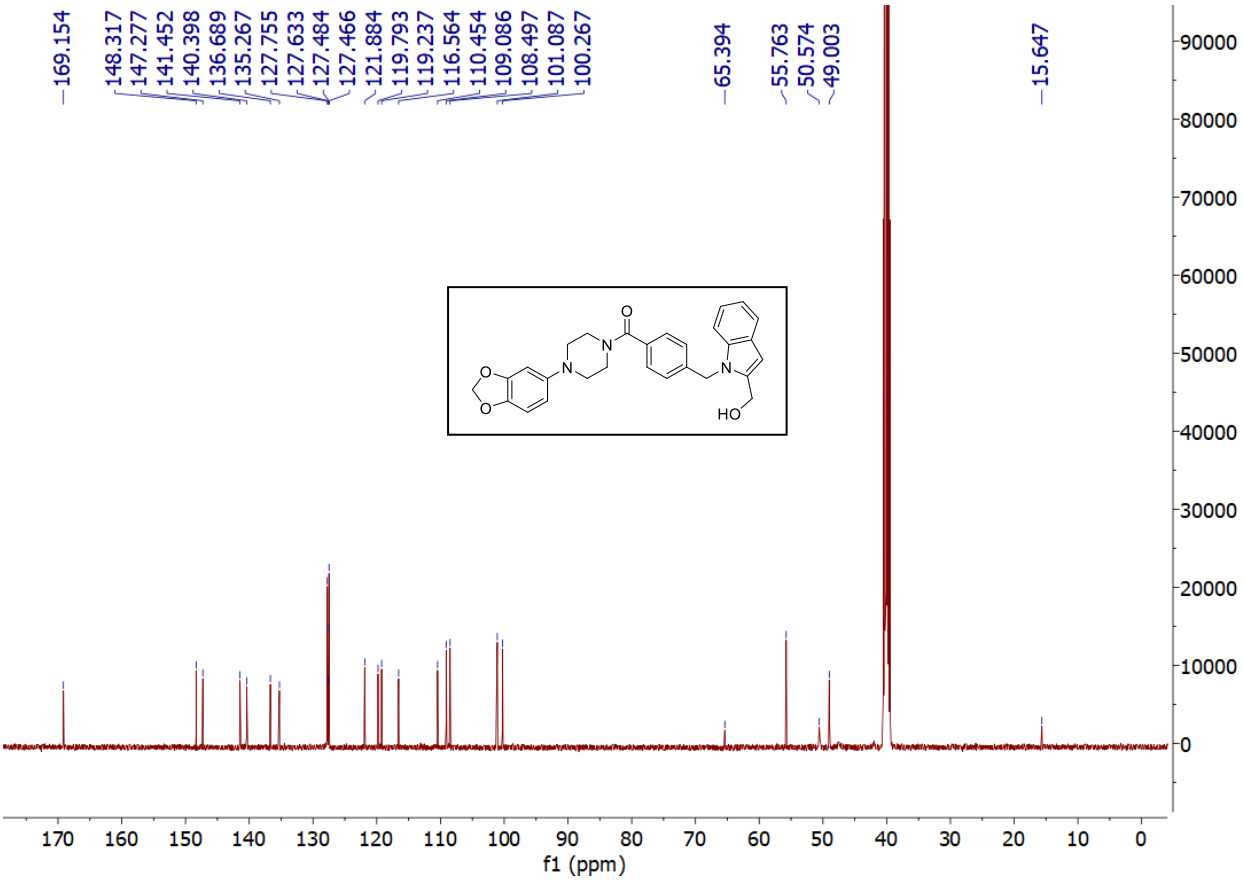
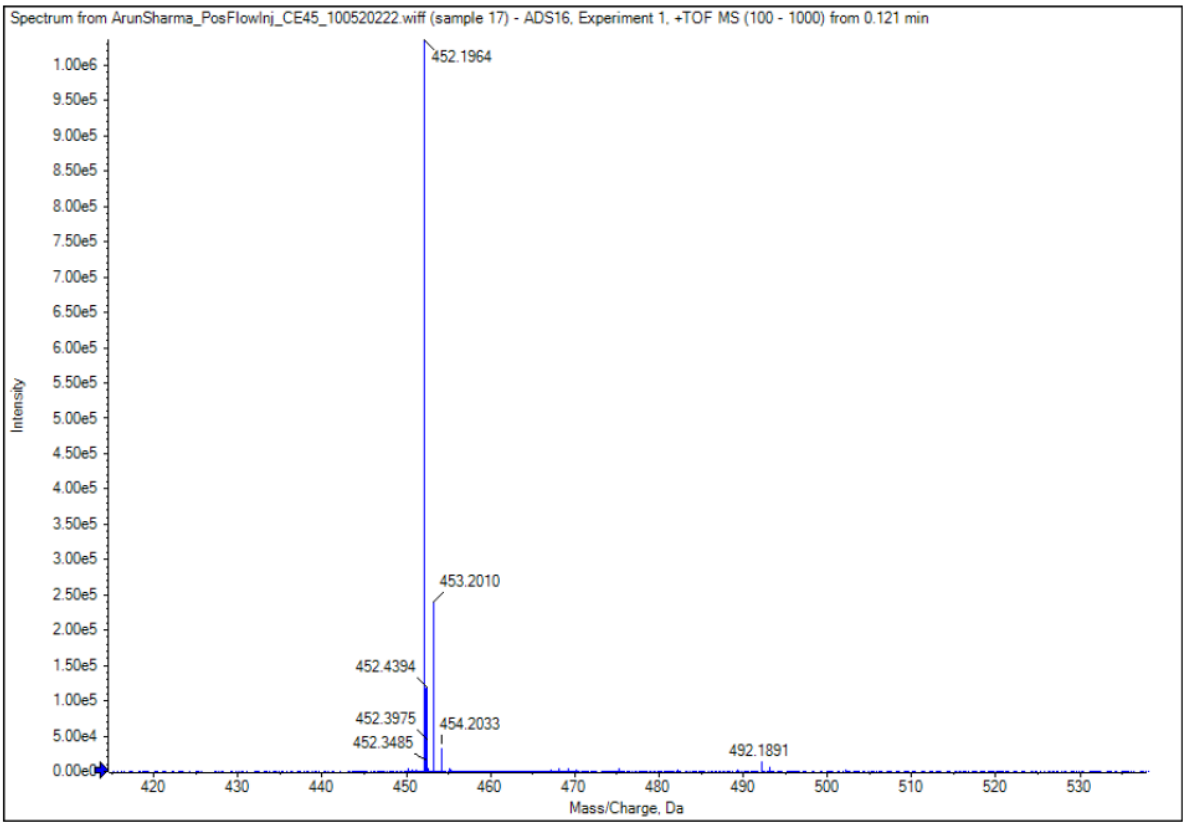
^{1}H NMR of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-((3-(hydroxymethyl)-1H-indol-1-yl) methyl) phenyl) methanone (13g):



^{13}C NMR of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-((3-(hydroxymethyl)-1H-indol-1-yl) methyl) phenyl) methanone (13g):

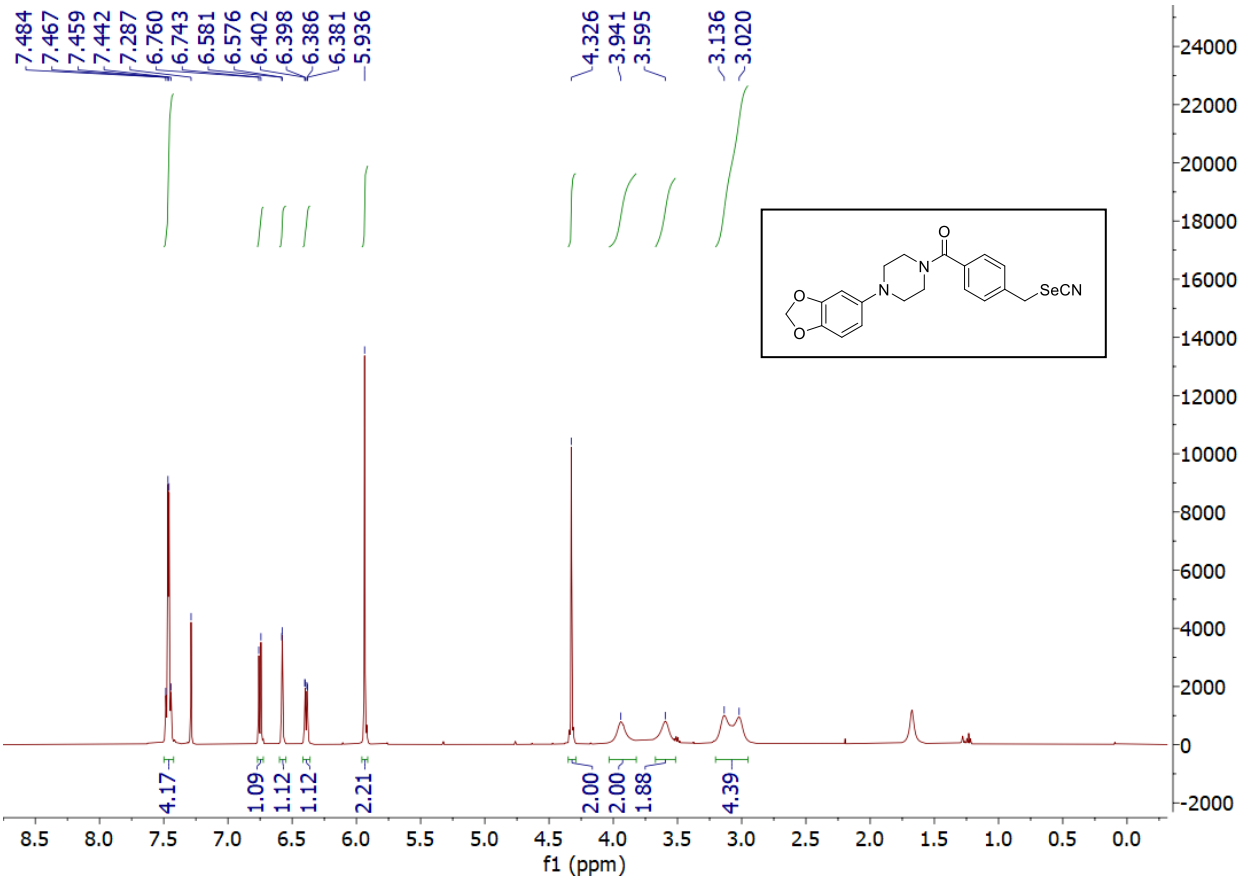


HRMS of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-((3-(hydroxymethyl)-1H-indol-1-yl) methyl) phenyl) methanone (13g):

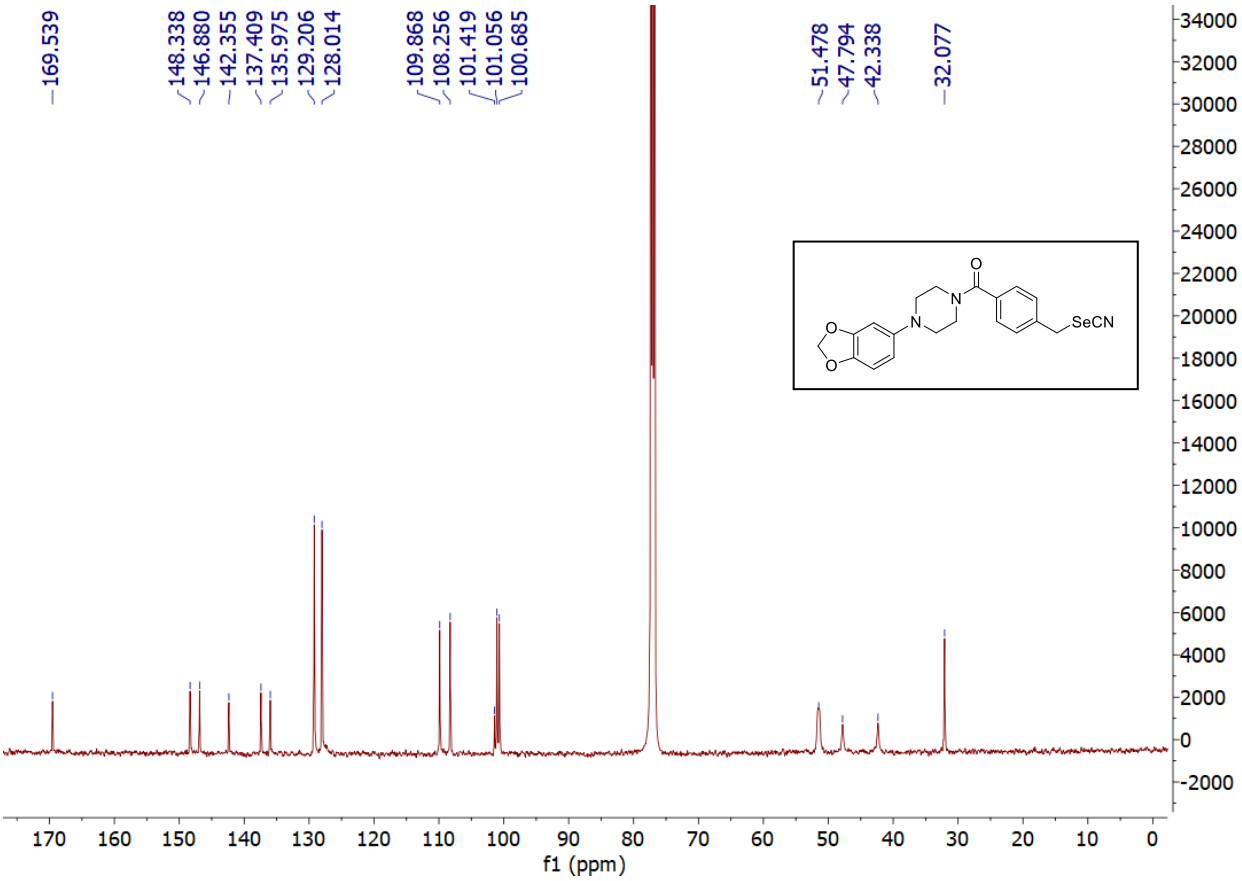


10/11/2022 11:05:48 AM

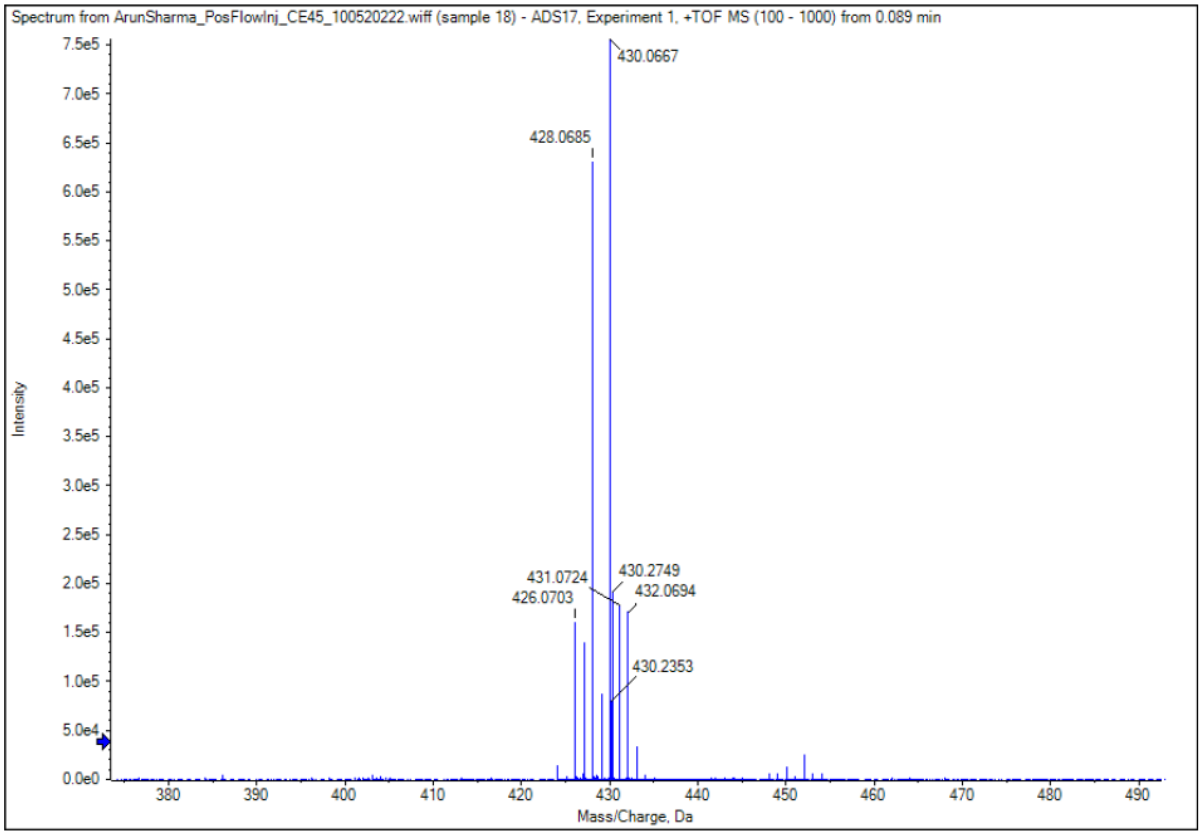
[1]H NMR of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-(selenocyanatomethyl) phenyl) methanone (13h):



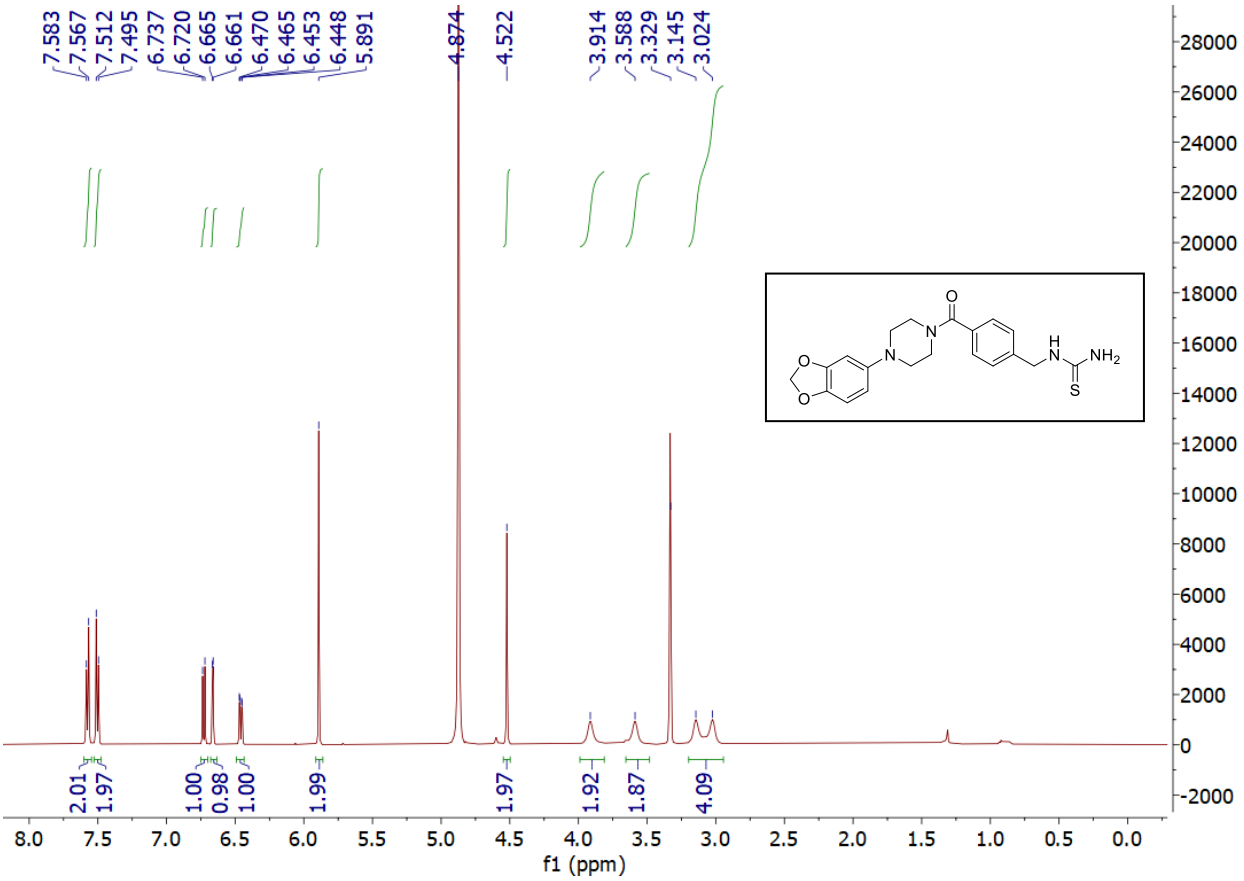
^{13}C NMR of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-(selenocyanatomethyl) phenyl) methanone (13h):



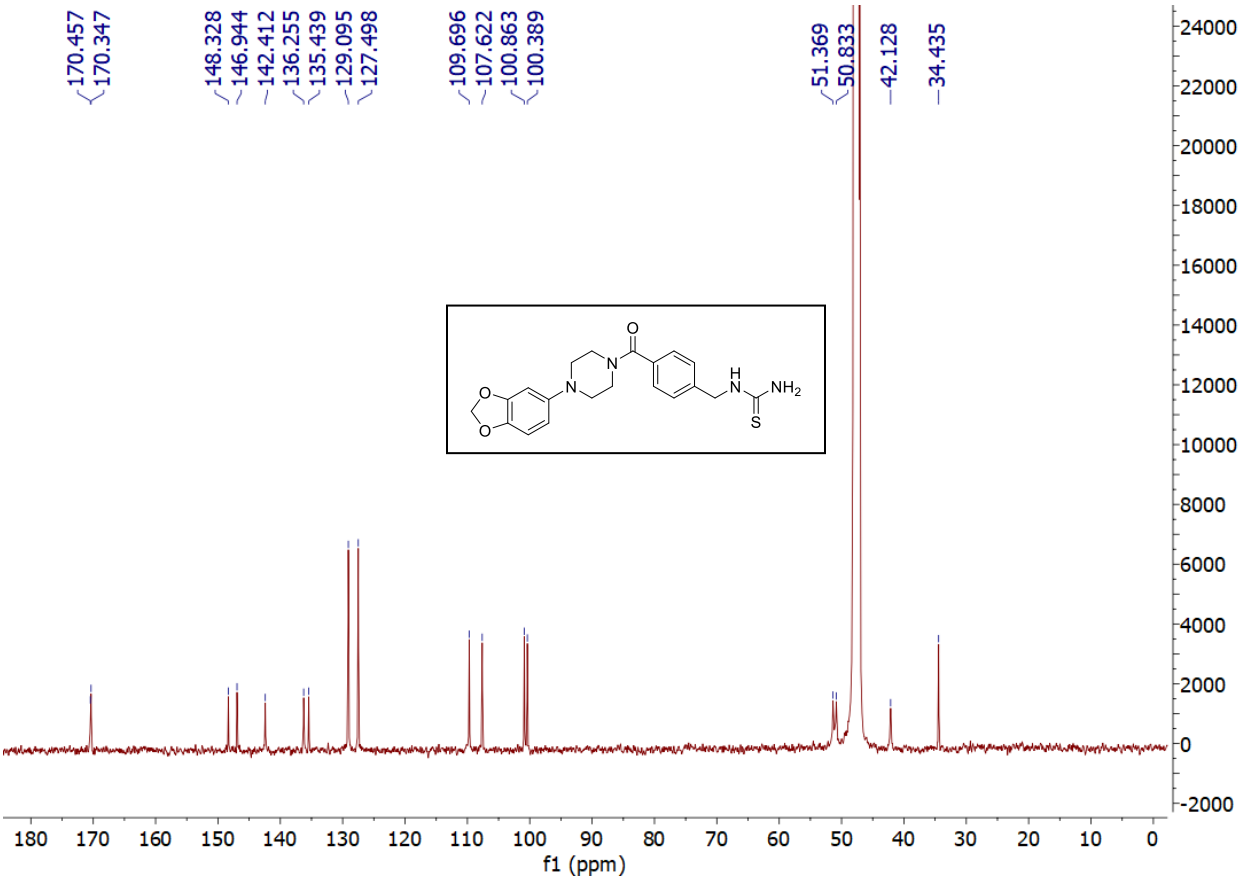
HRMS of (4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-(selenocyanatomethyl) phenyl) methanone (13h):



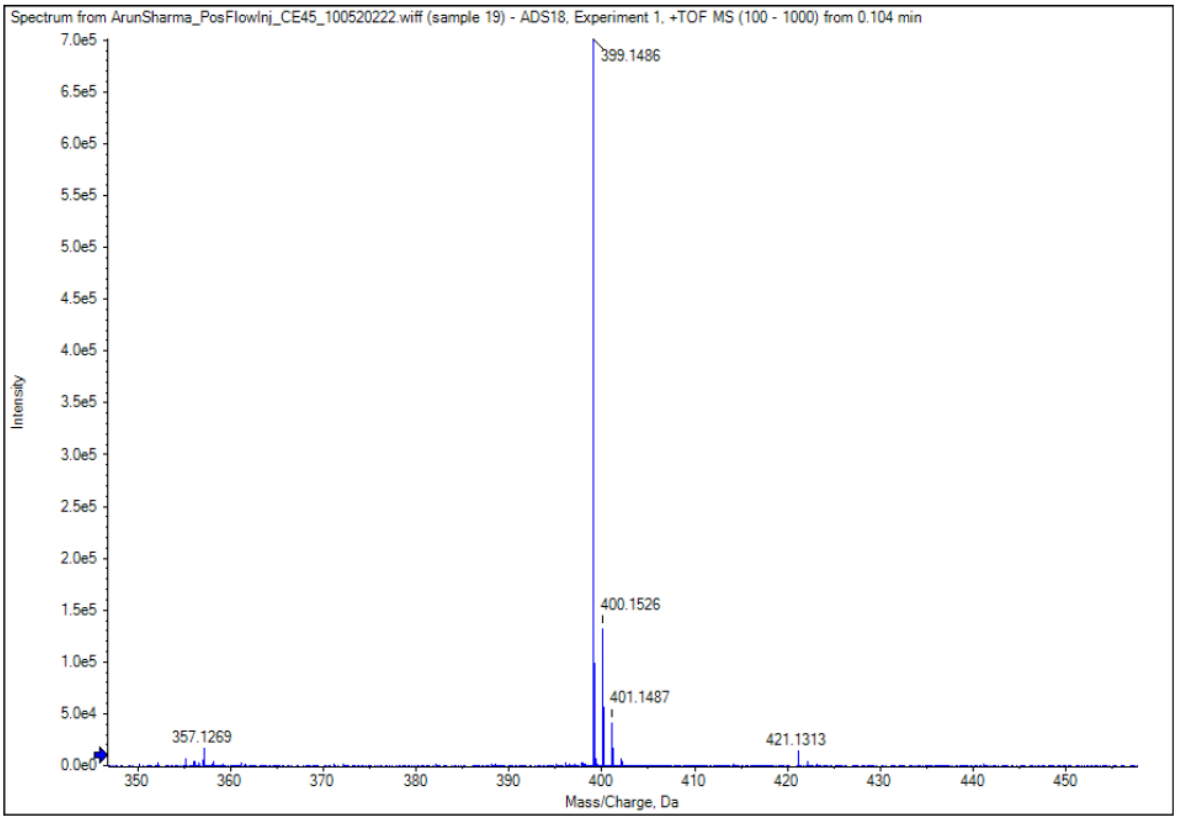
^{1}H NMR of 1-(4-(4-(Benzo[d] [1,3] dioxol-5-yl) piperazine-1-carbonyl) benzyl) thiourea(13i):



^{13}C NMR of 1-(4-(4-(Benzo[d] [1,3] dioxol-5-yl) piperazine-1-carbonyl) benzyl) thiourea(13i):



HRMS of 1-(4-(4-(Benzo[d] [1,3] dioxol-5-yl) piperazine-1-carbonyl) benzyl) thiourea(13i):



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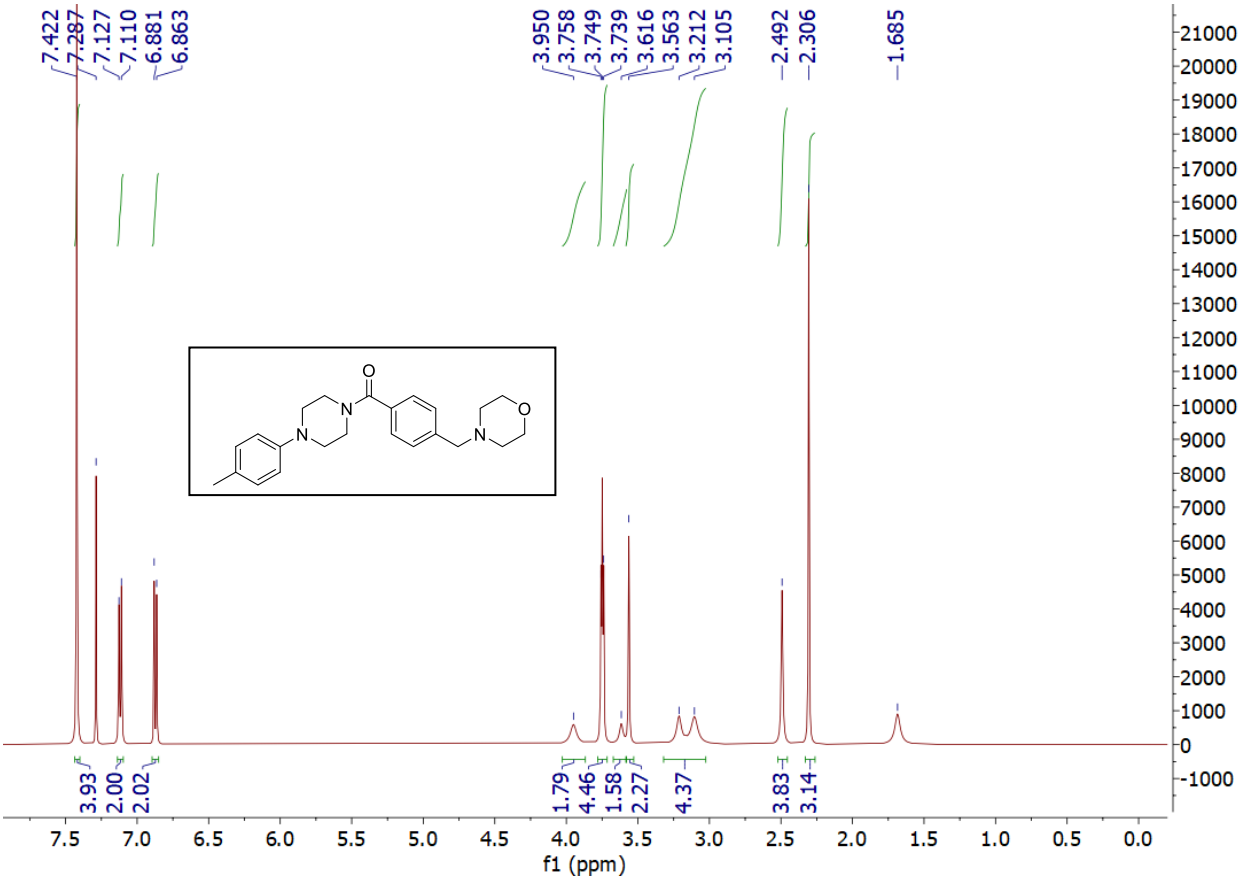
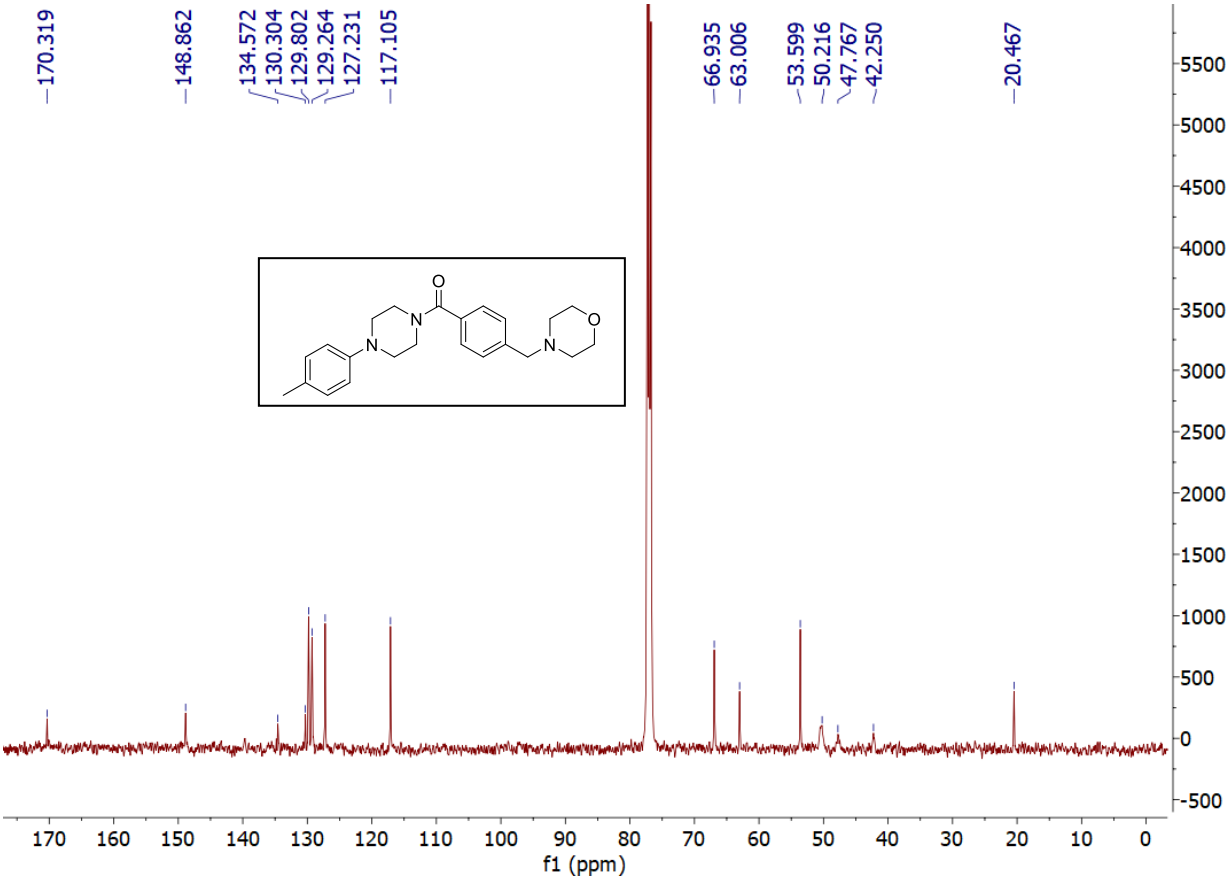
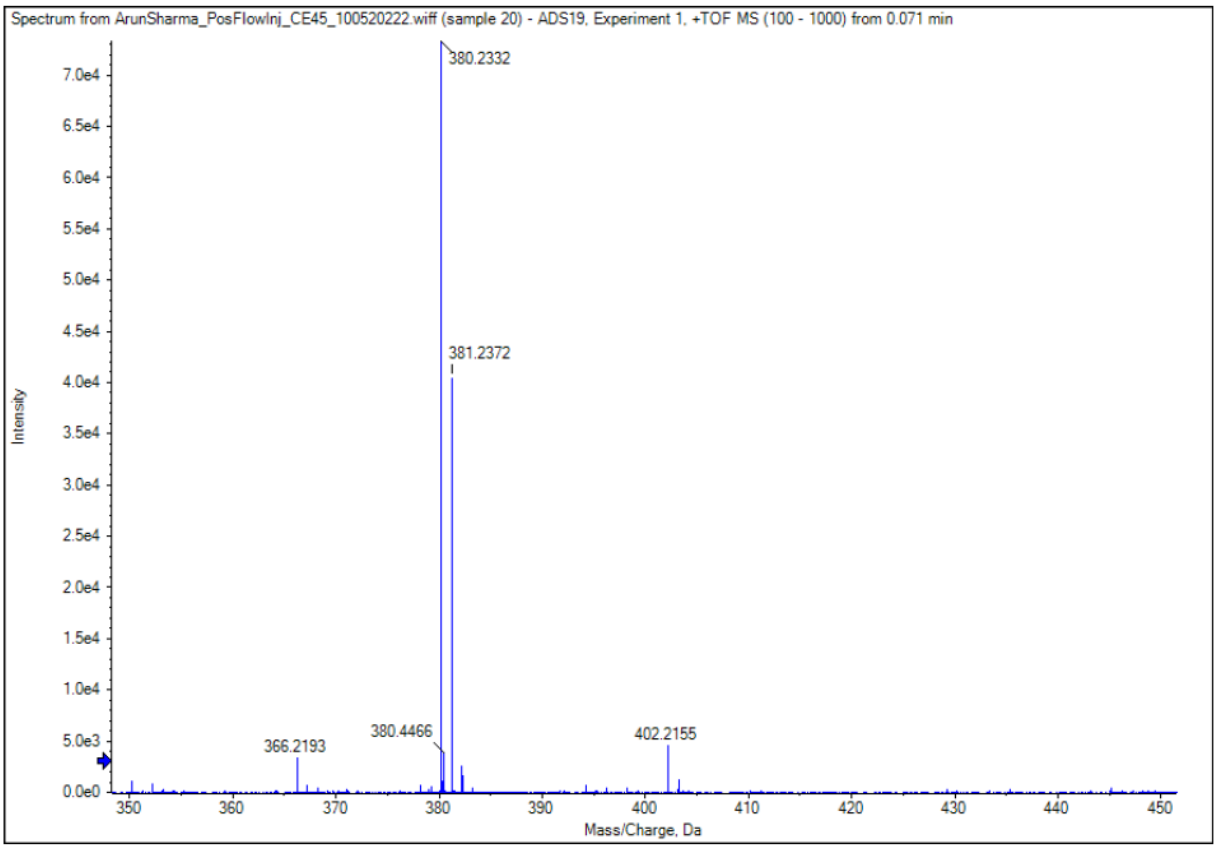
^{1}H NMR of (4-(Morpholinomethyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18a):



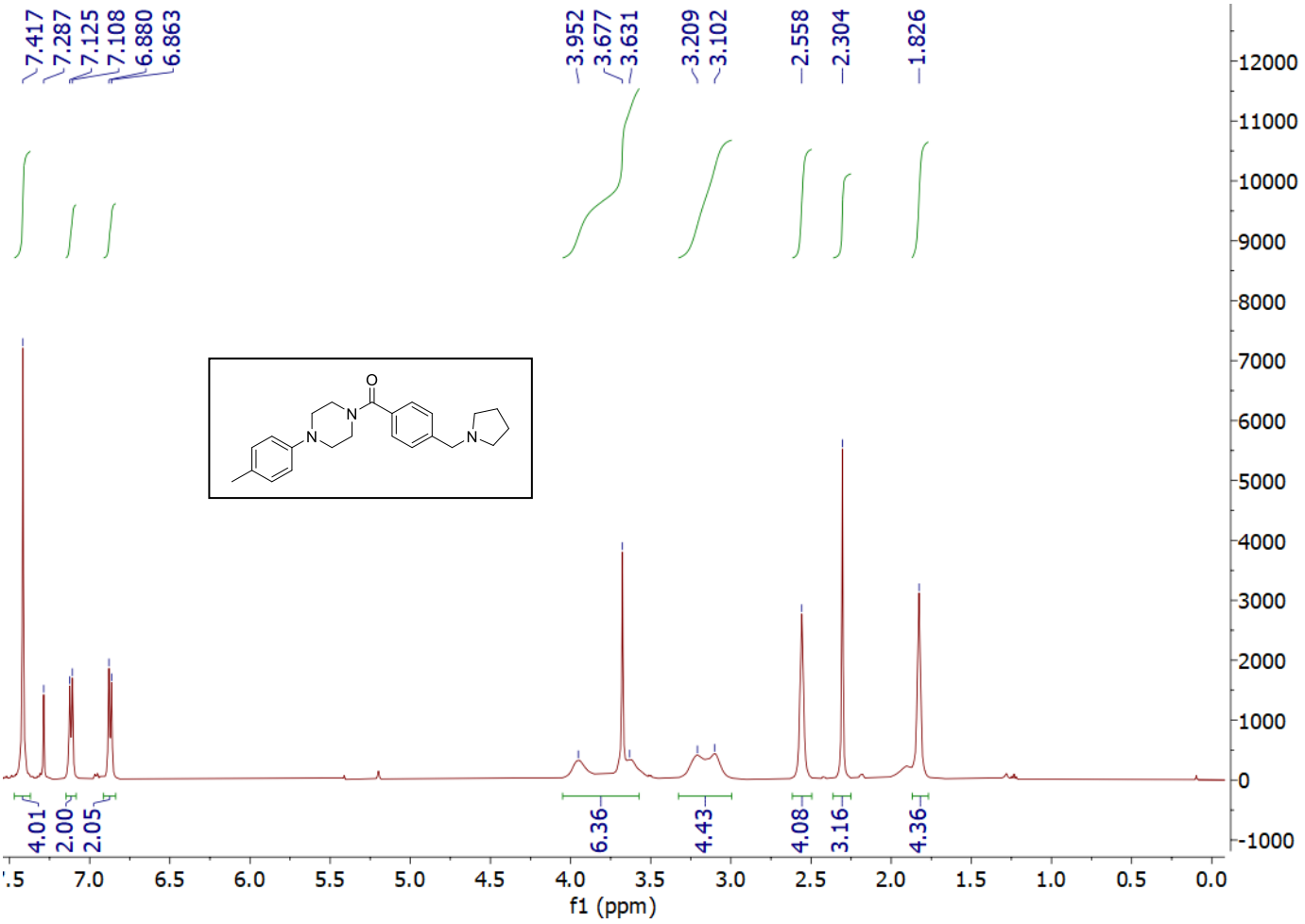
^{13}C NMR of (4-(Morpholinomethyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18a):



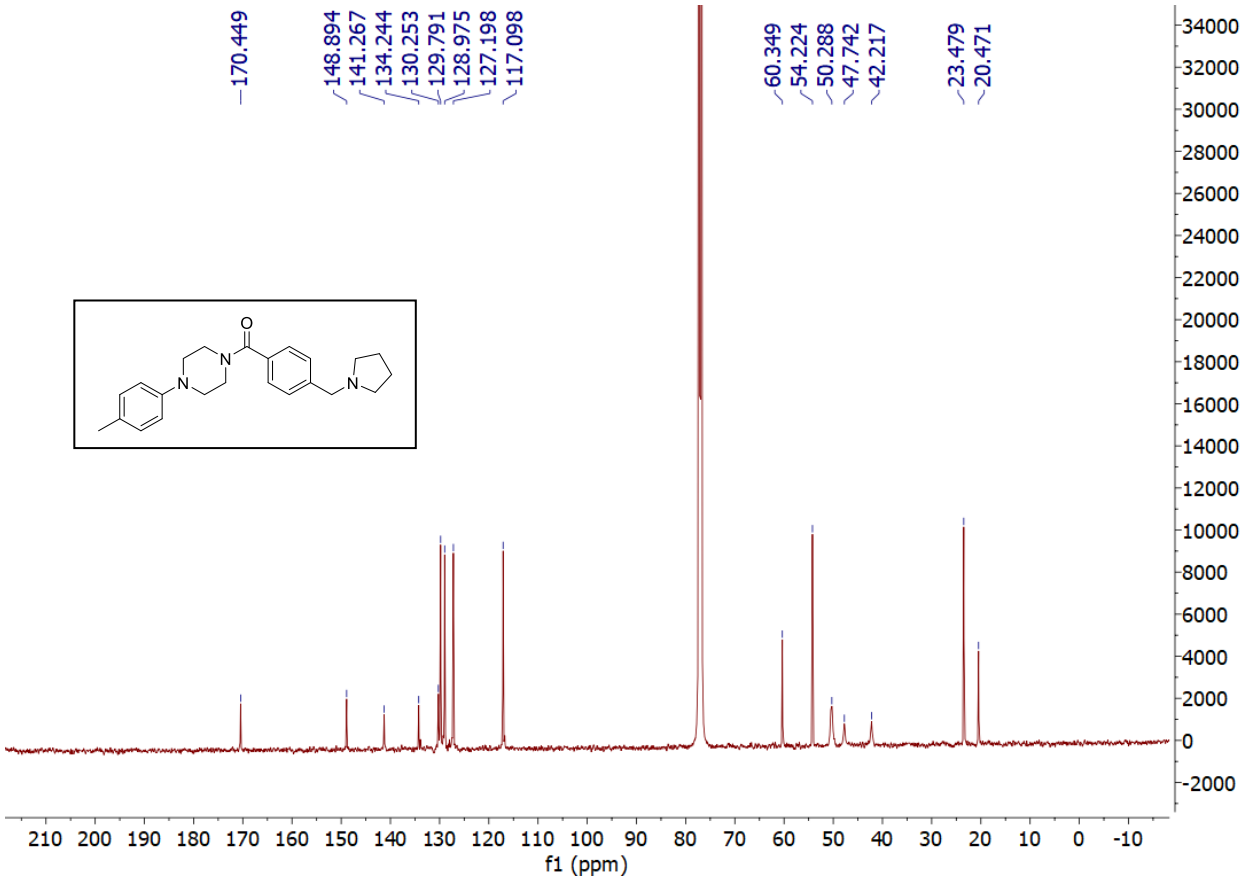
HRMS of (4-(Morpholinomethyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18a):



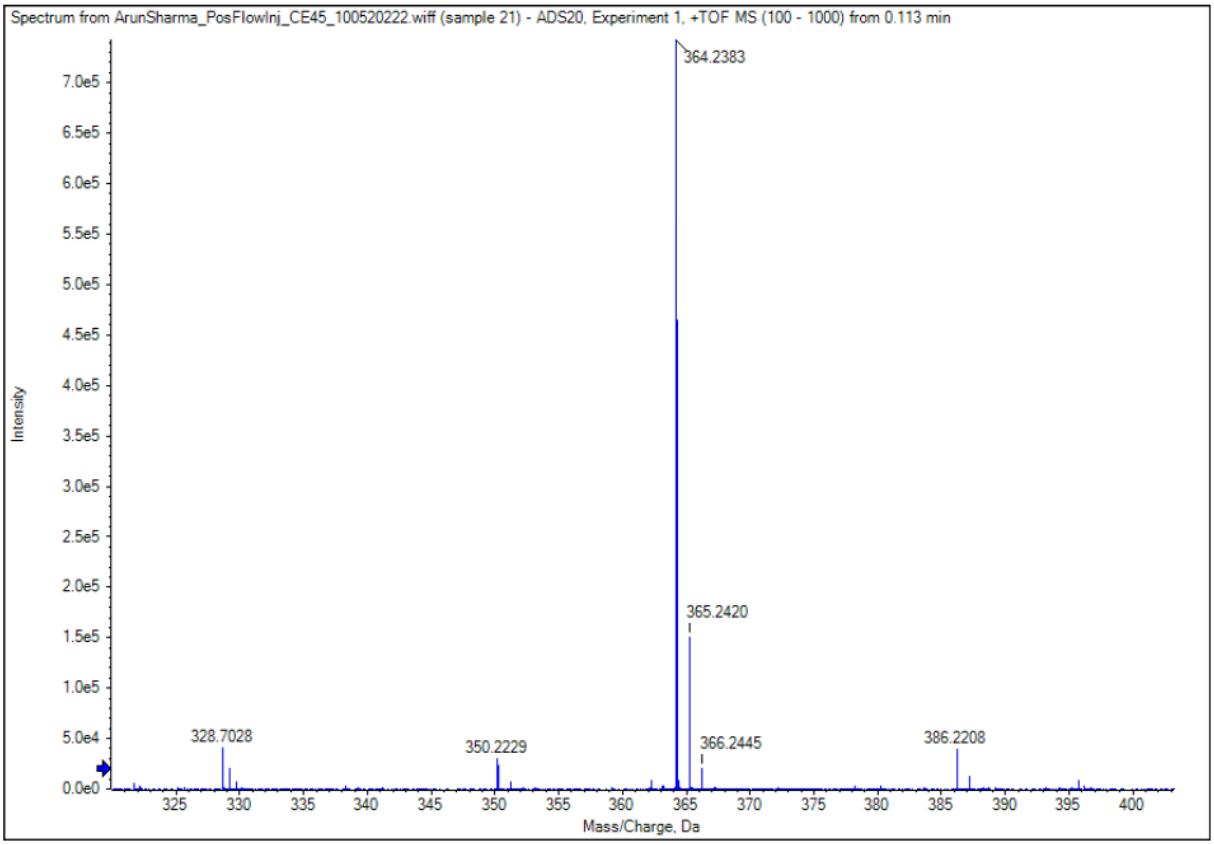
^{1}H NMR of (4-(Pyrrolidin-1-ylmethyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18b):



^{13}C NMR of (4-(Pyrrolidin-1-ylmethyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18b):



HRMS of (4-(Pyrrolidin-1-ylmethyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18b):



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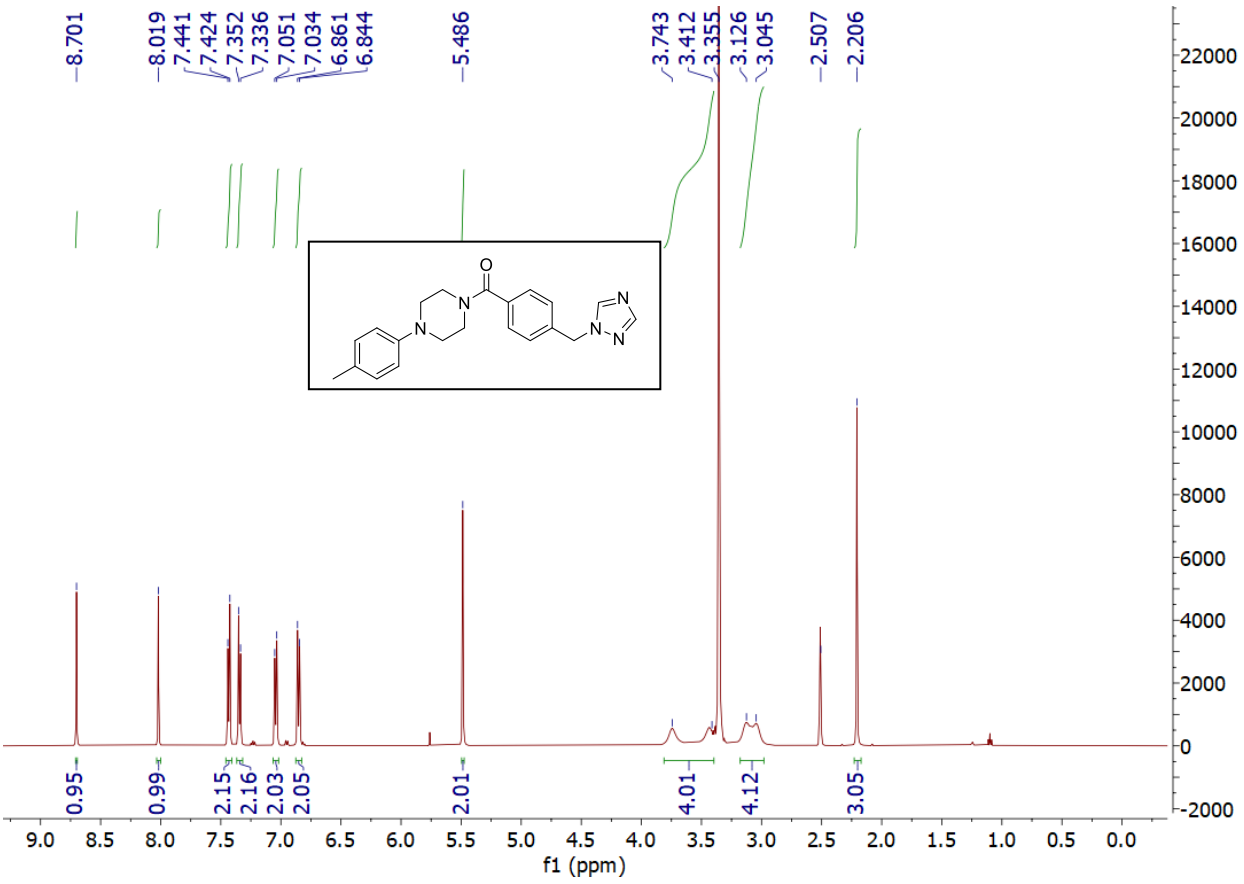
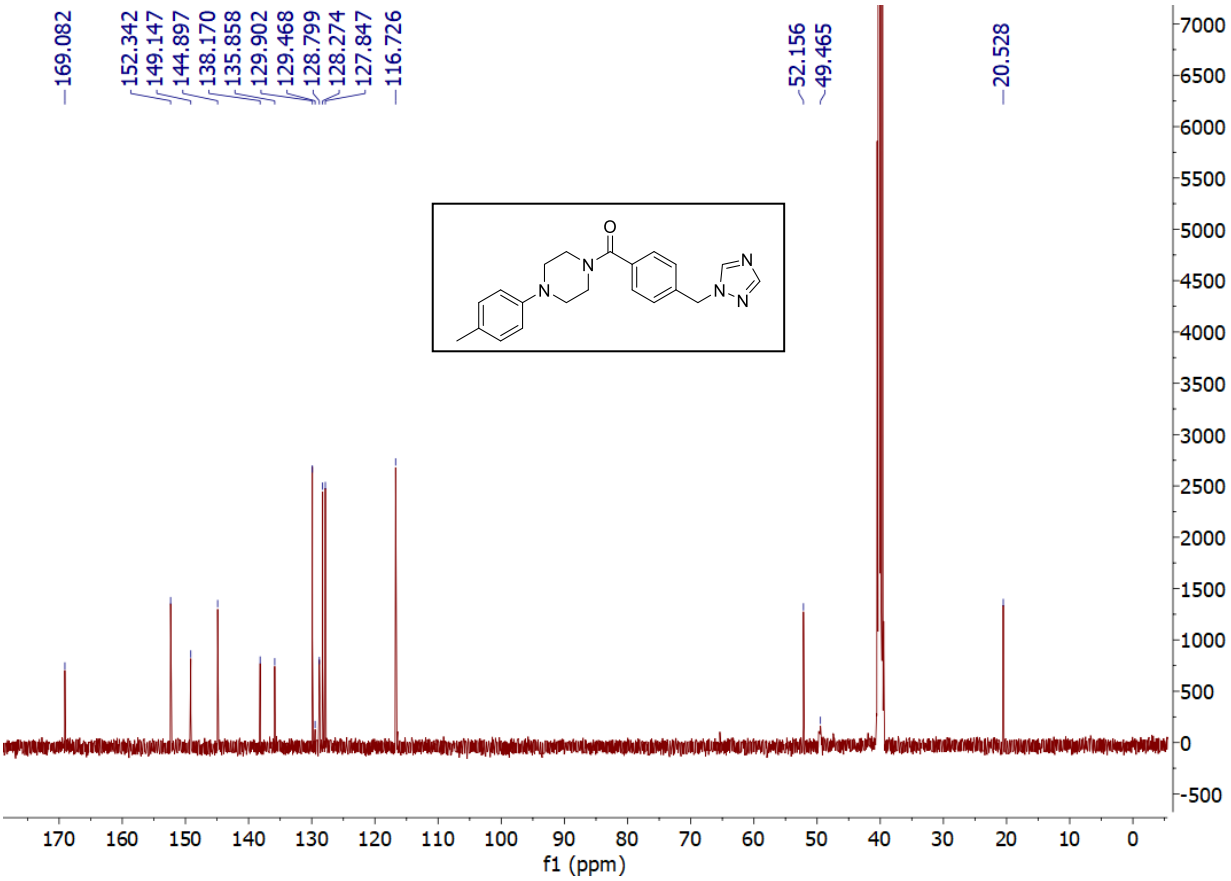
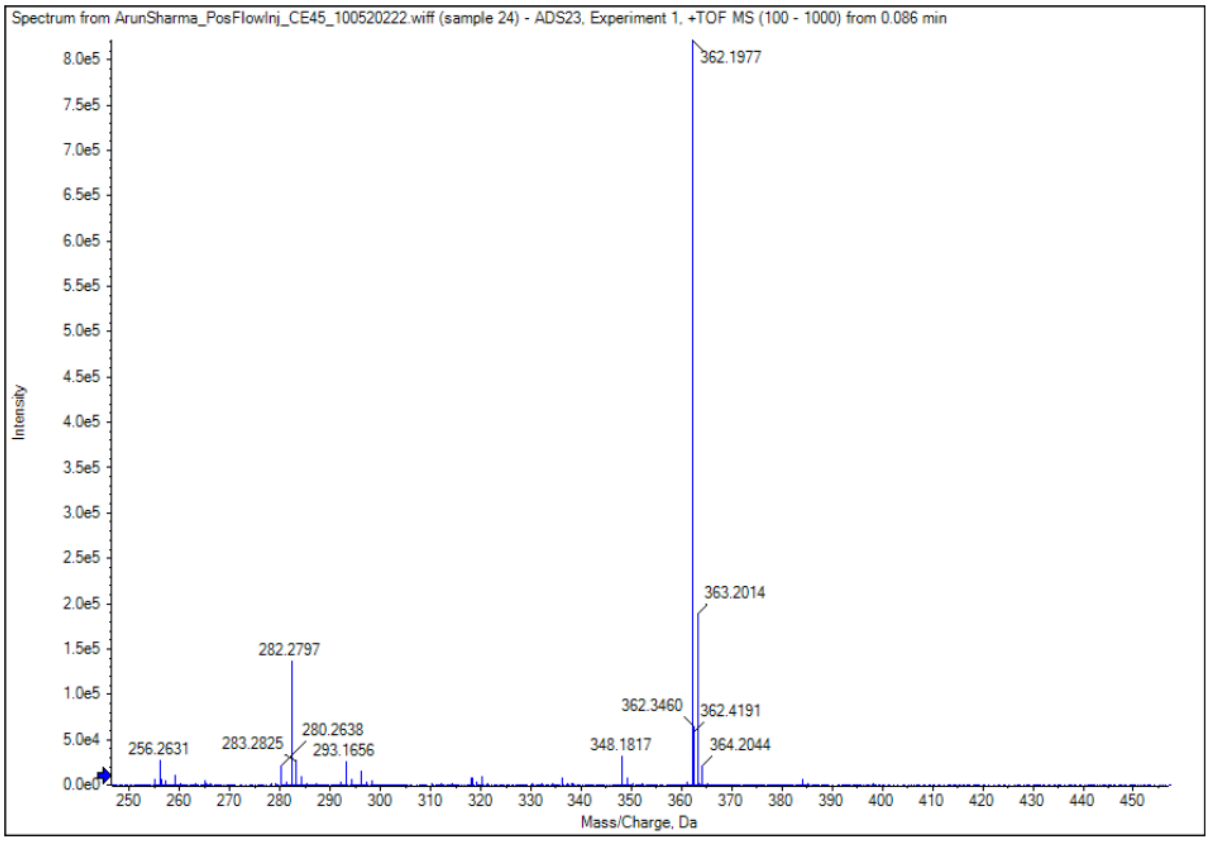
^{1}H NMR of (4-((1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18d):



^{13}C NMR of (4-((1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18d):



HRMS of (4-((1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18d):



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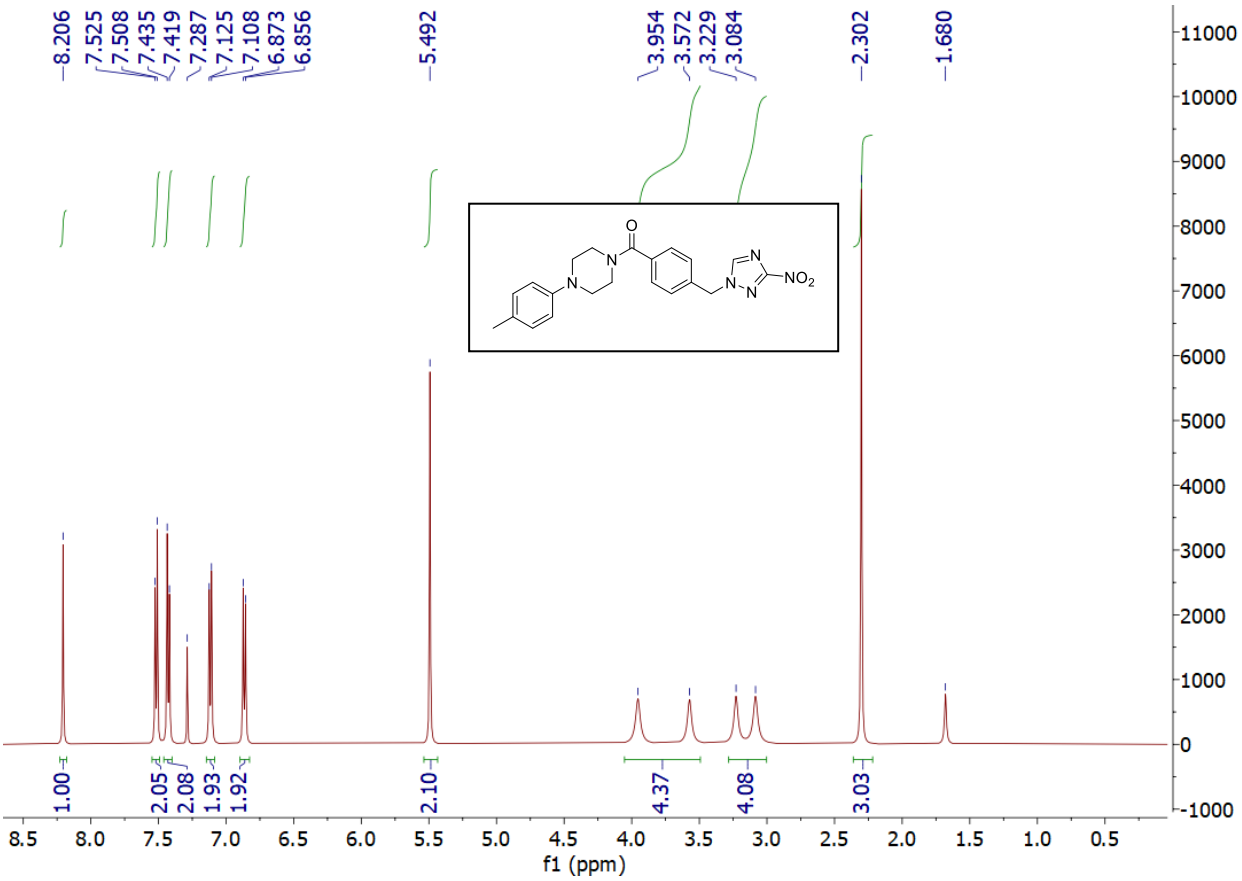
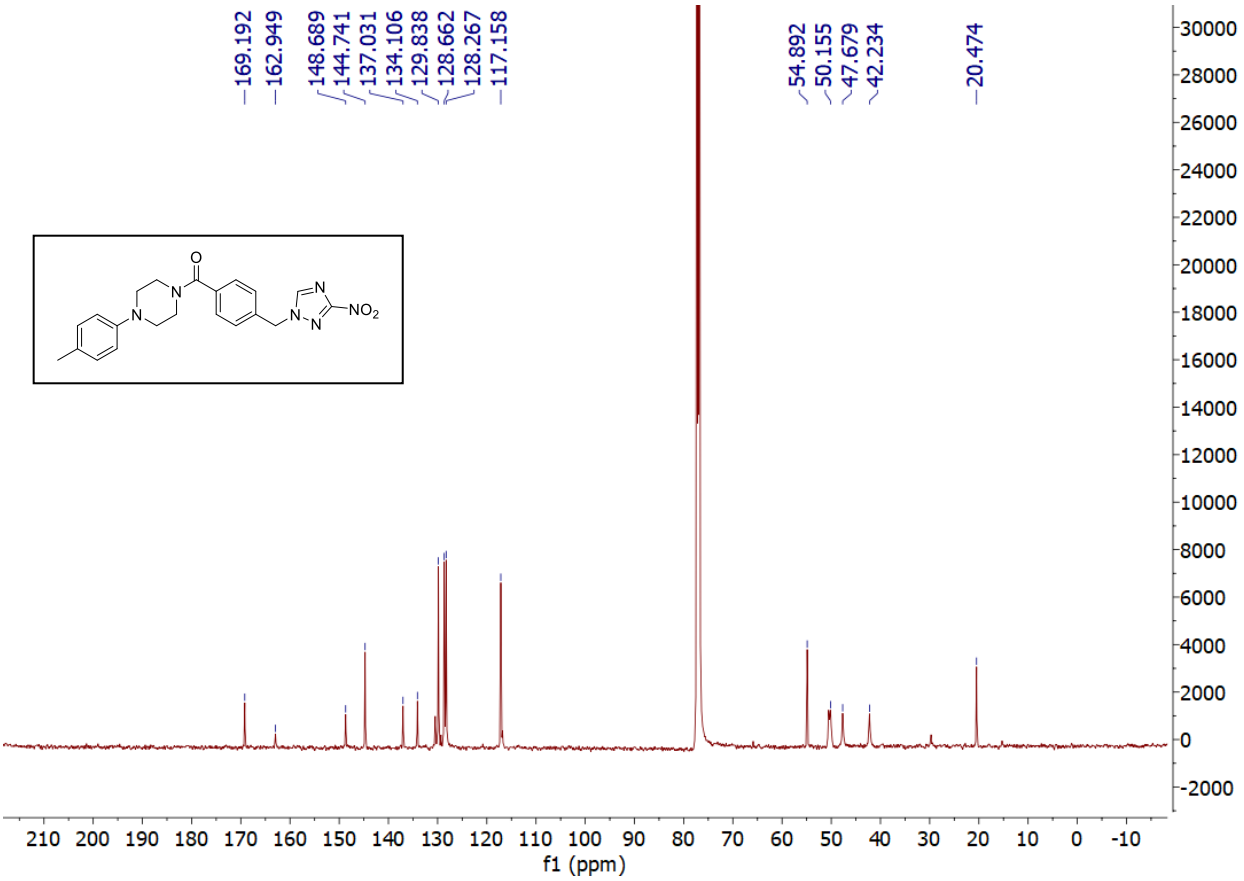
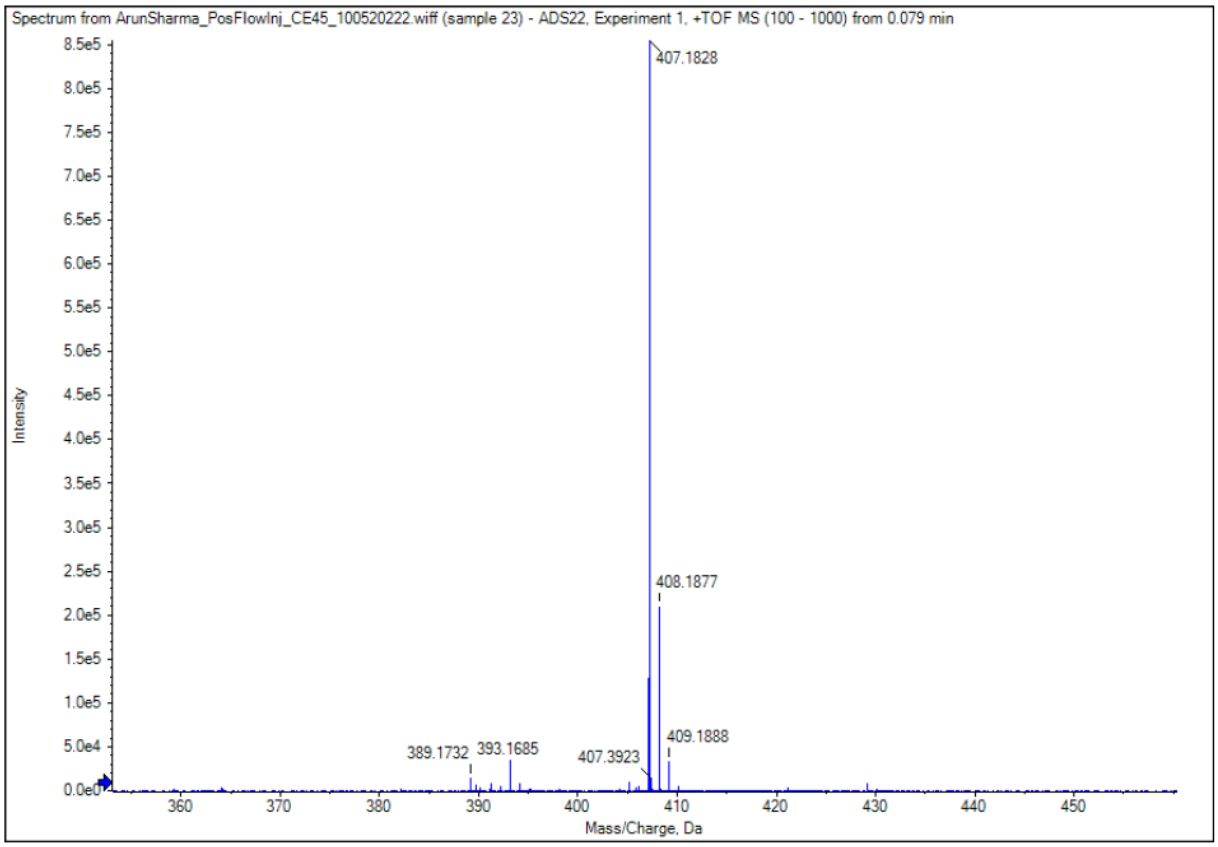
^{1}H NMR of (4-((3-nitro-1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone(18e):



^{13}C NMR of (4-((3-nitro-1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone(18e):



HRMS of (4-((3-nitro-1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone(18e):



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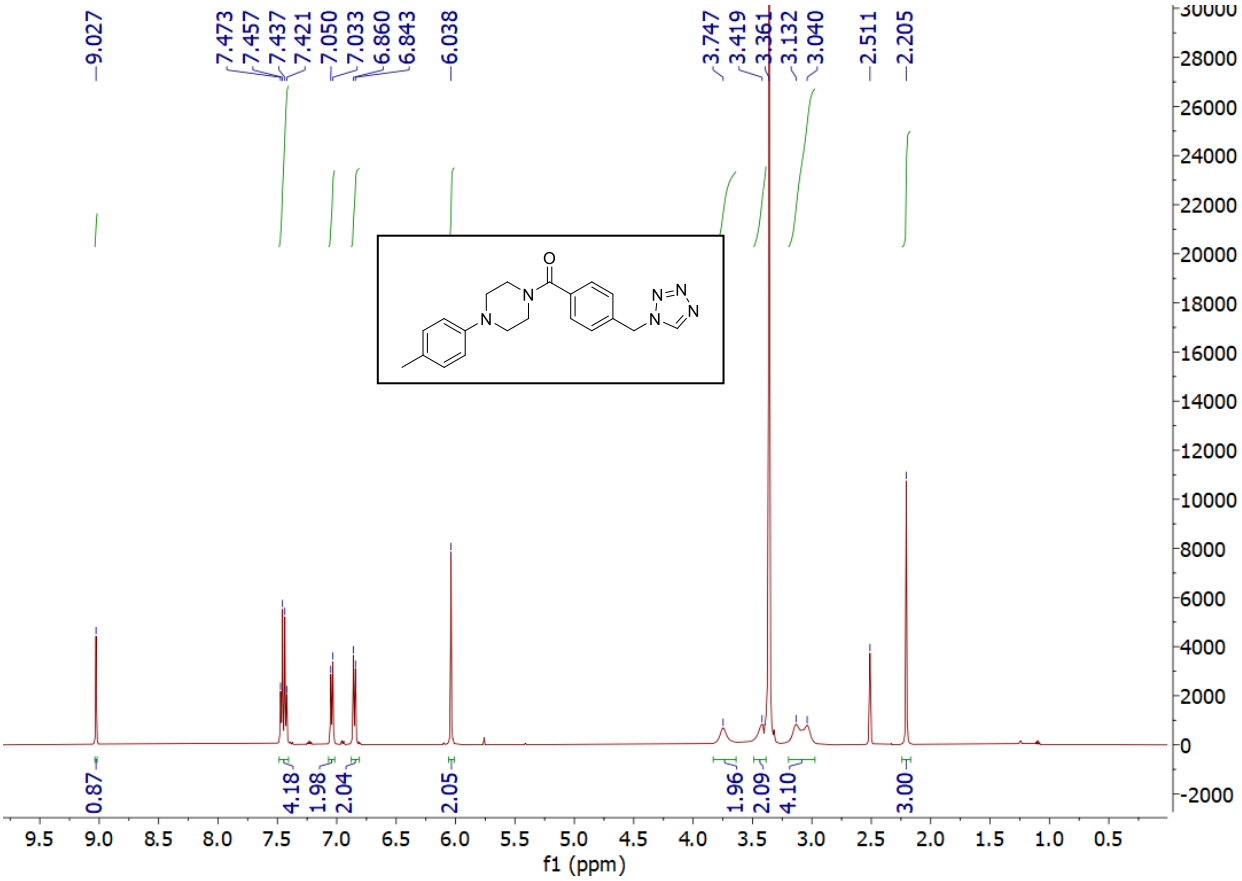
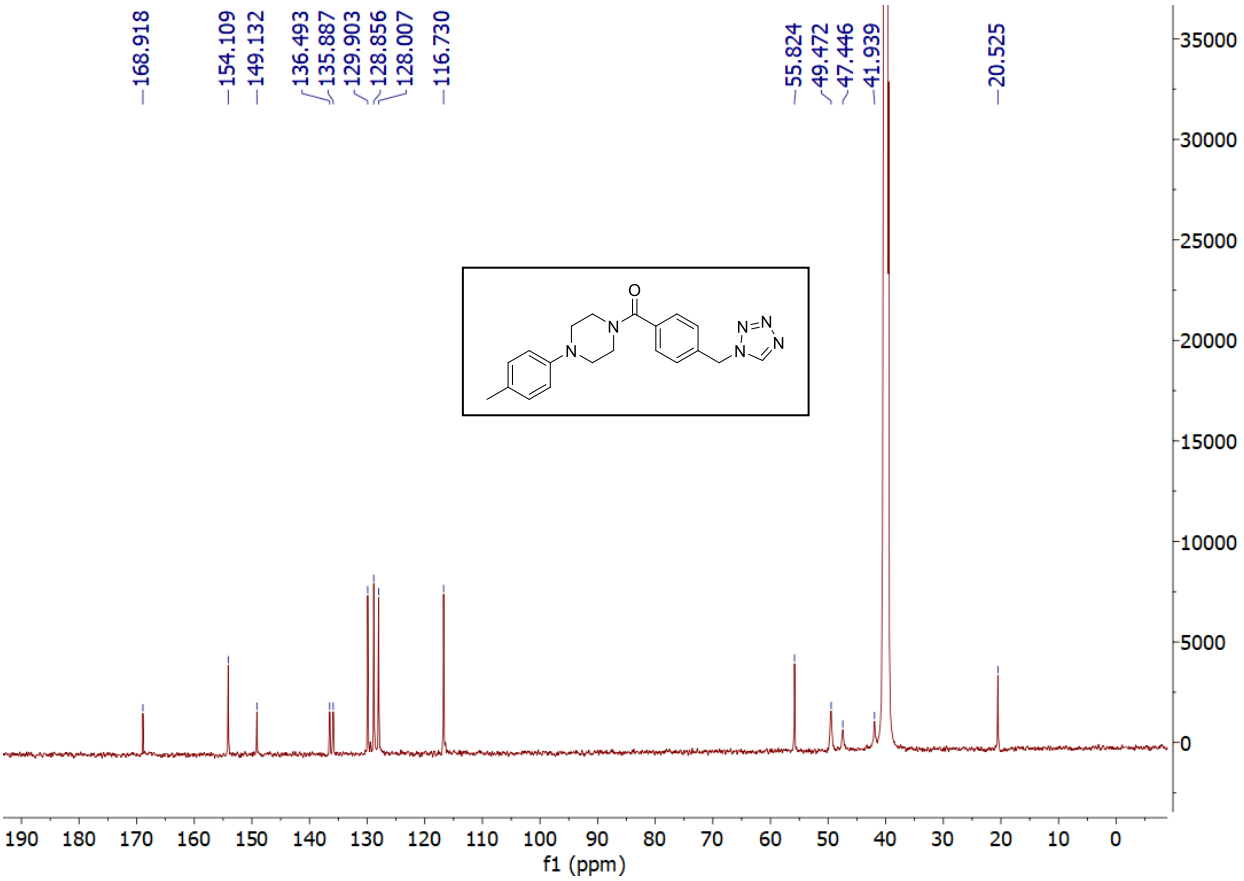
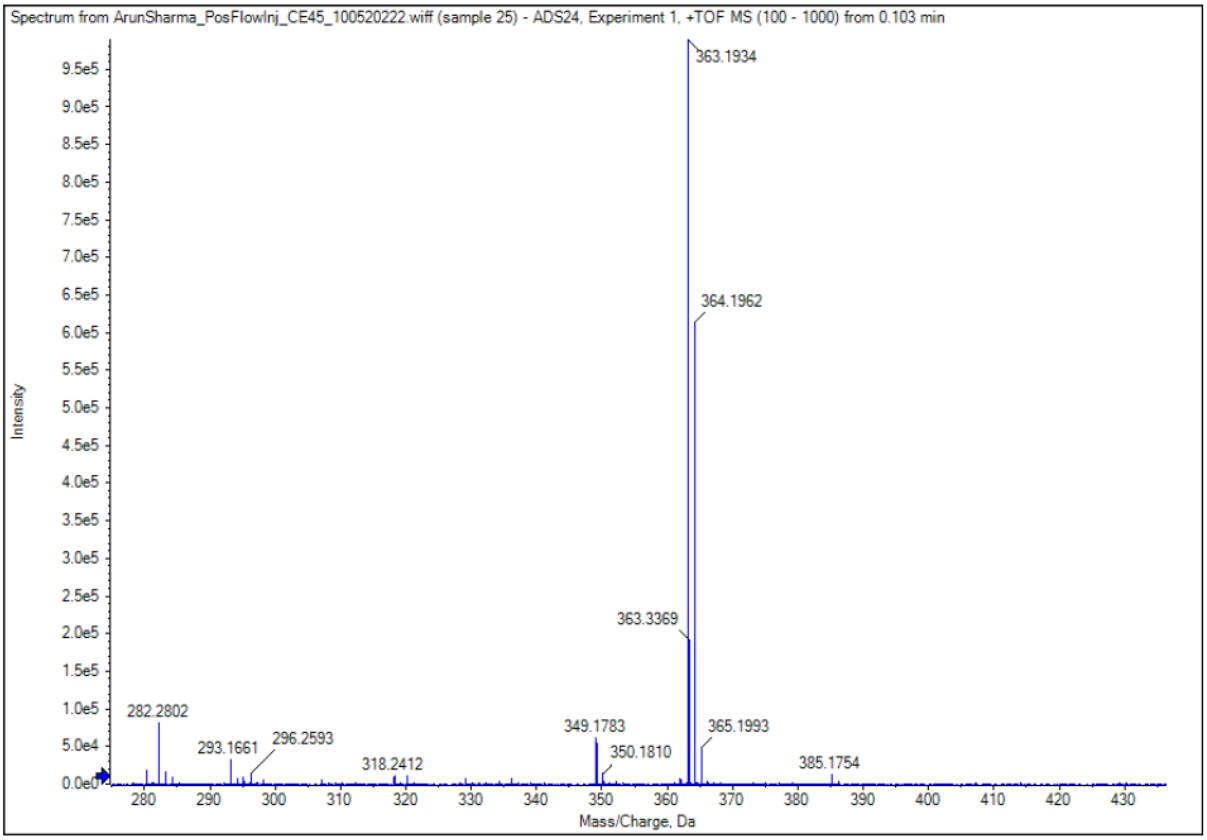
^{1}H NMR of (4-((1H-Tetrazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18f):



^{13}C NMR of (4-((1H-Tetrazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18f):



HRMS of (4-((1H-Tetrazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18f):



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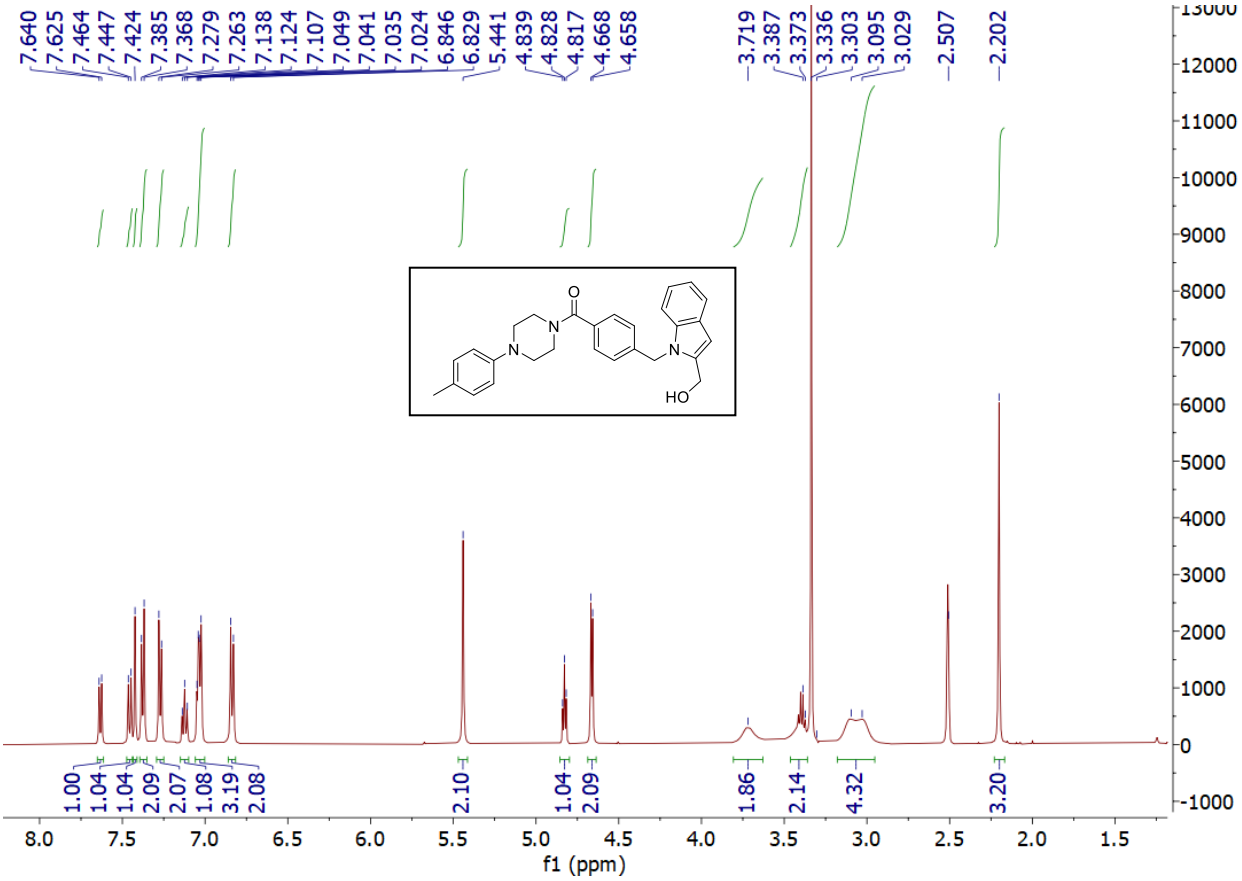
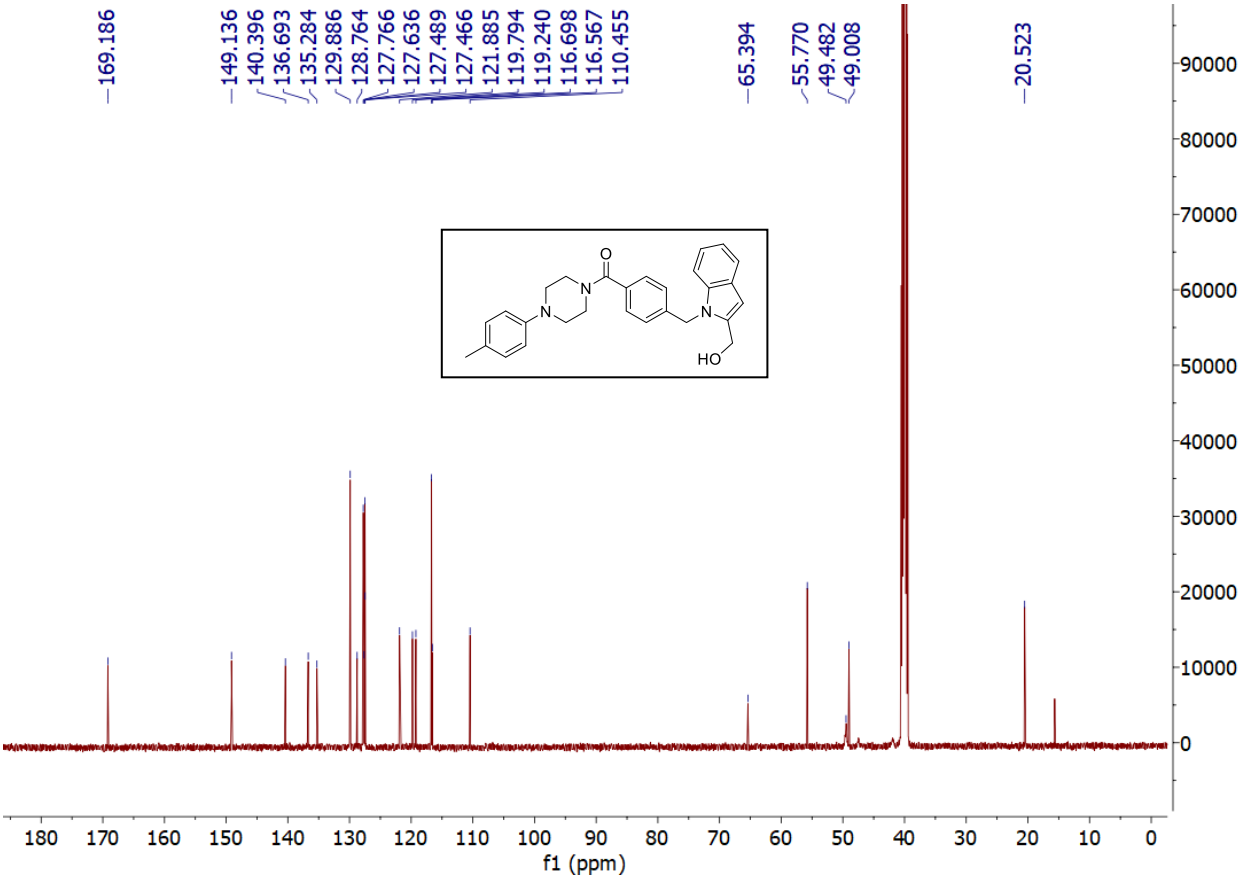
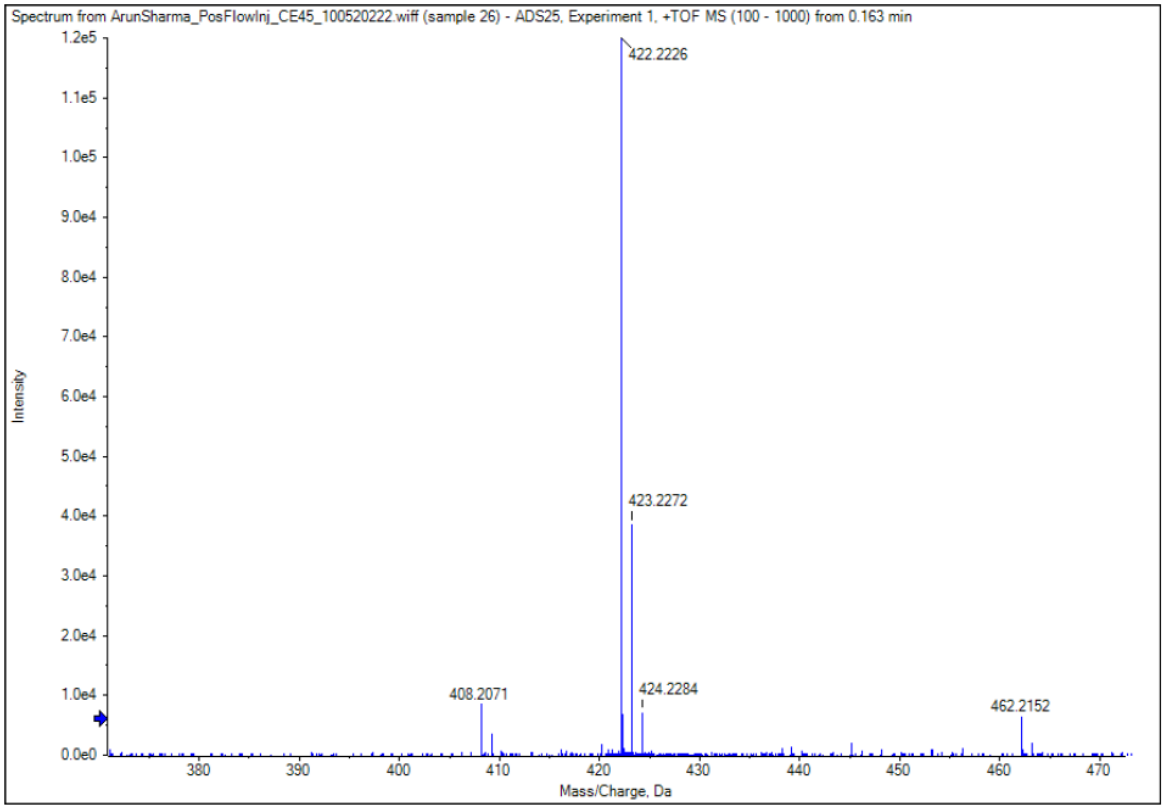
^{1}H NMR of (4-((3-(Hydroxymethyl)-1H-indol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18g):



^{13}C NMR of (4-((3-(Hydroxymethyl)-1H-indol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18g):



HRMS of (4-((3-(Hydroxymethyl)-1H-indol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18g):



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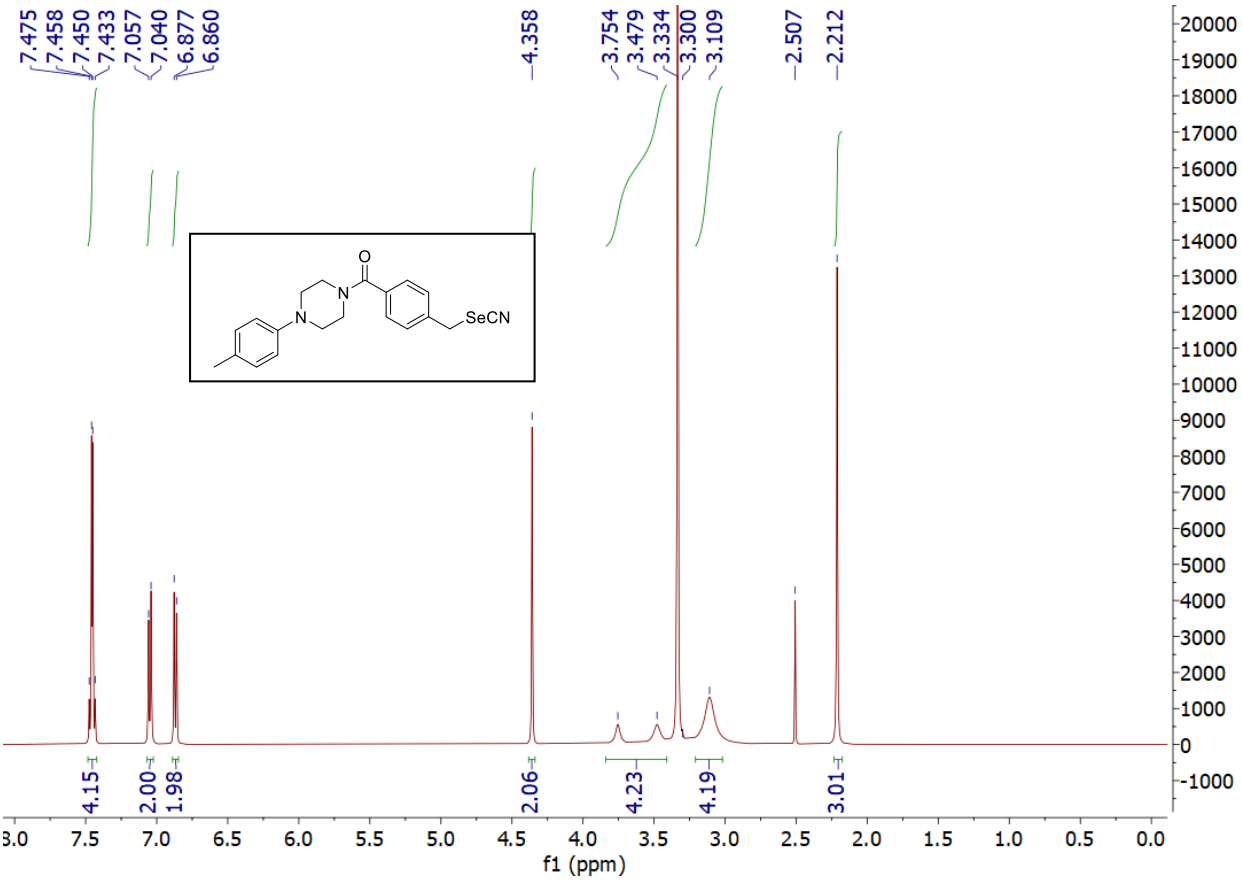
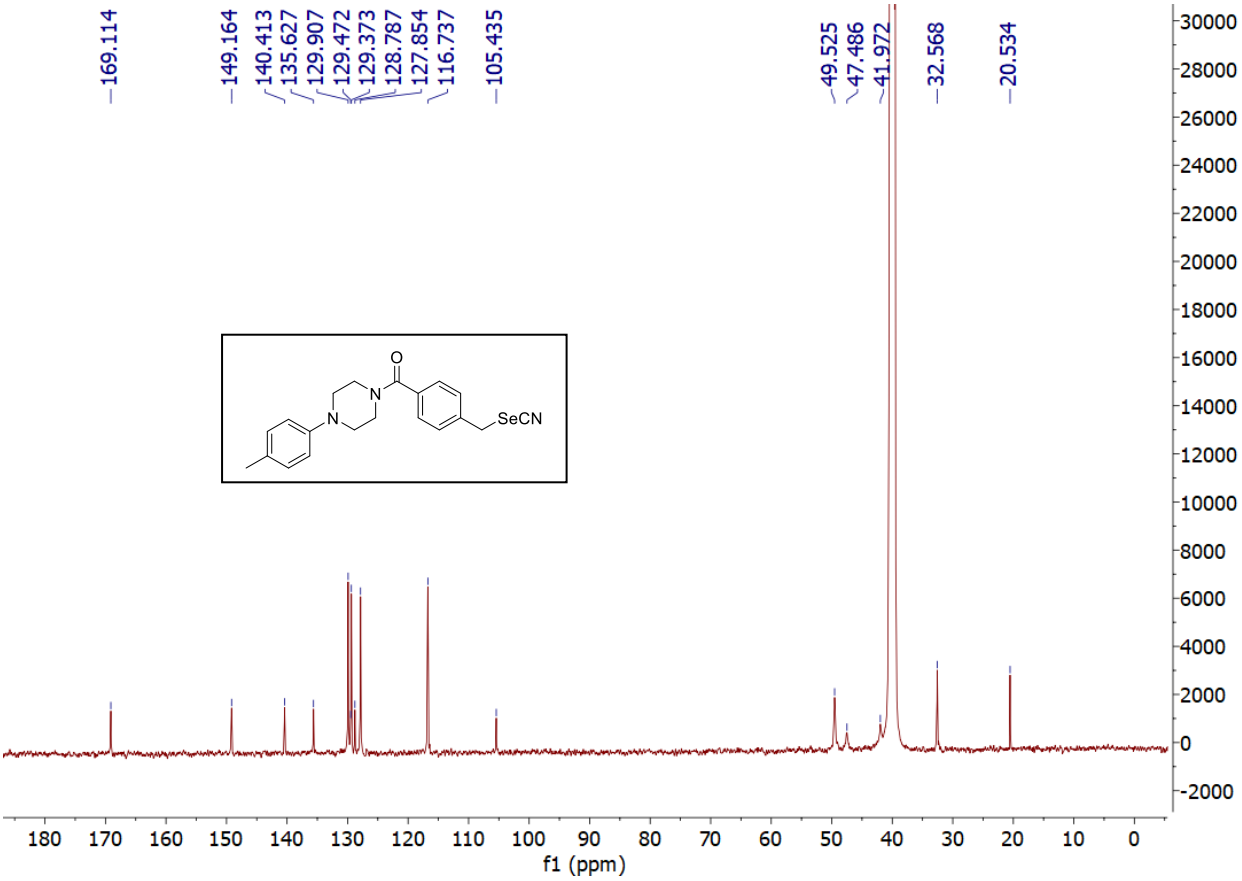
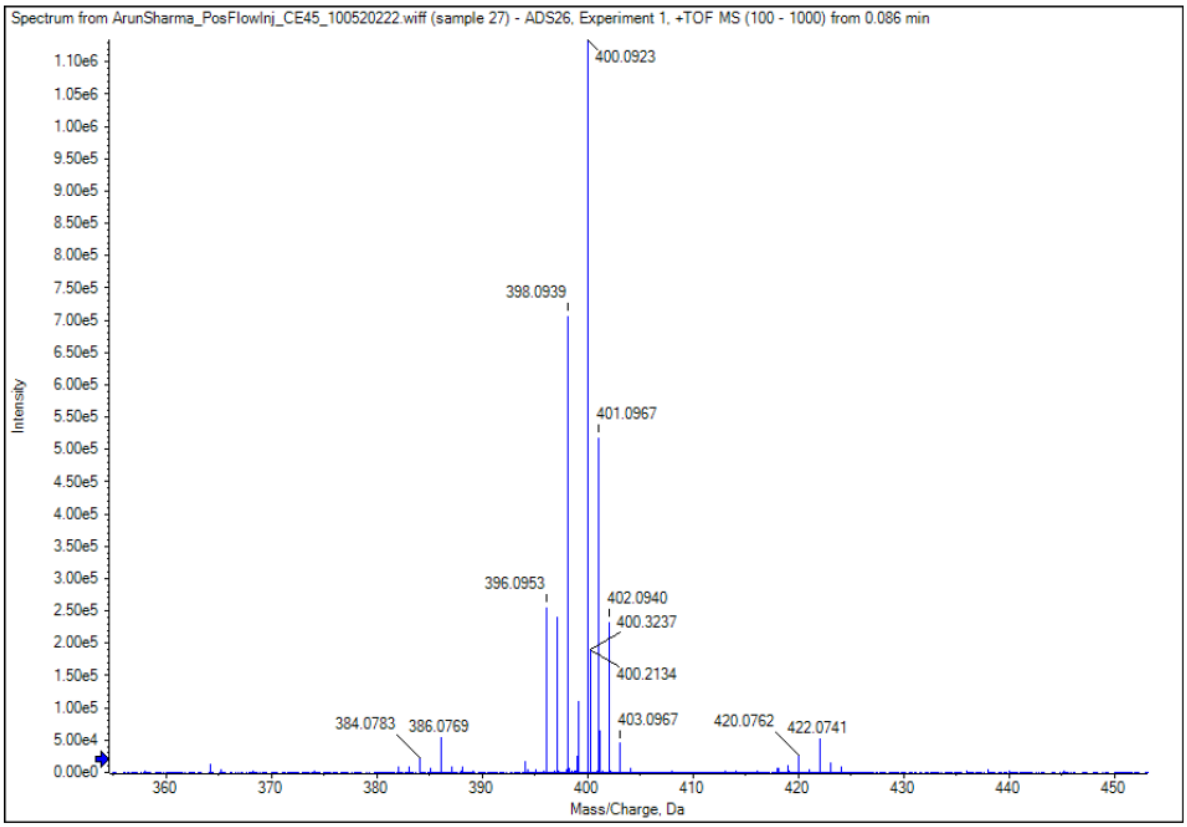
^{1}H NMR of (4-(Selenocyanatomethyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18h):



^{13}C NMR of (4-(Selenocyanatomethyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18h):



HRMS of (4-(Selenocyanatomethyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone (18h):



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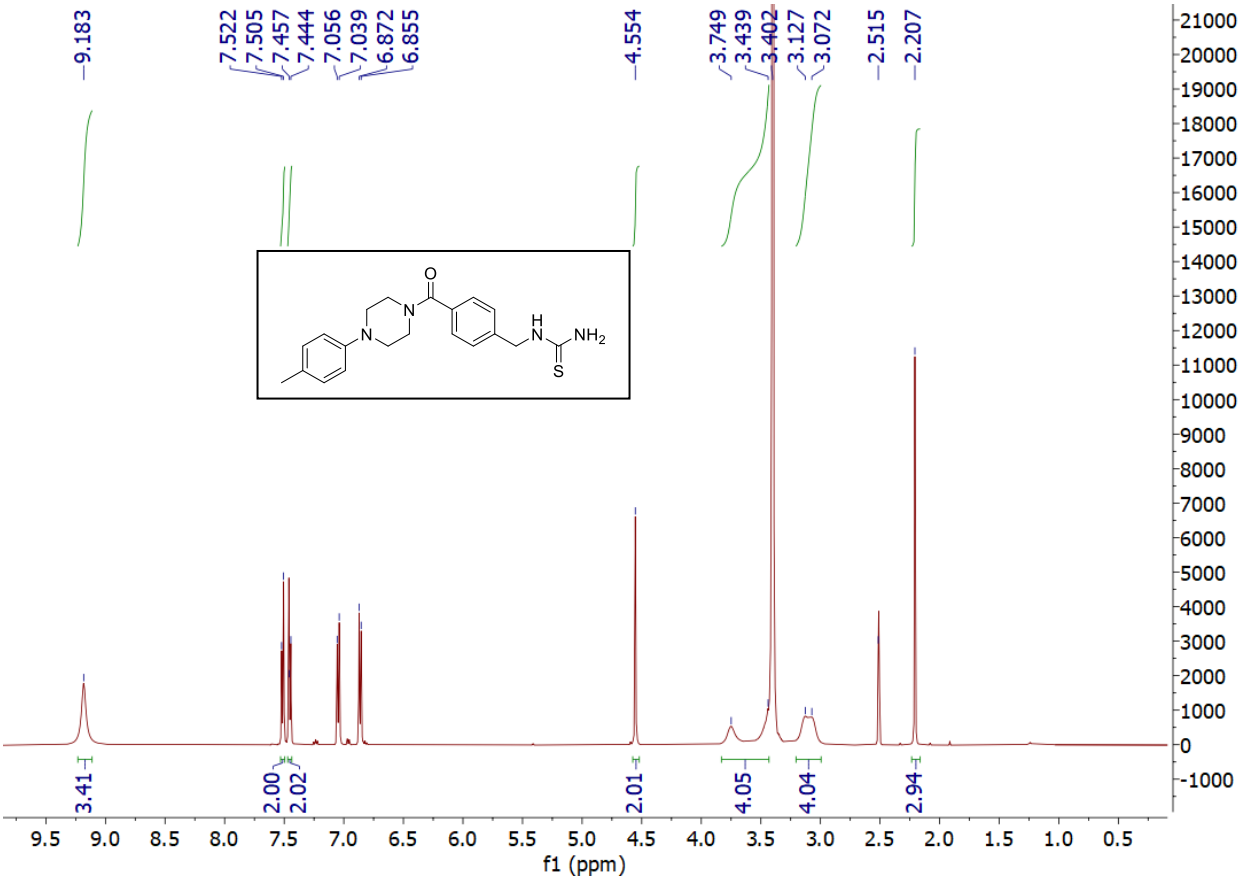
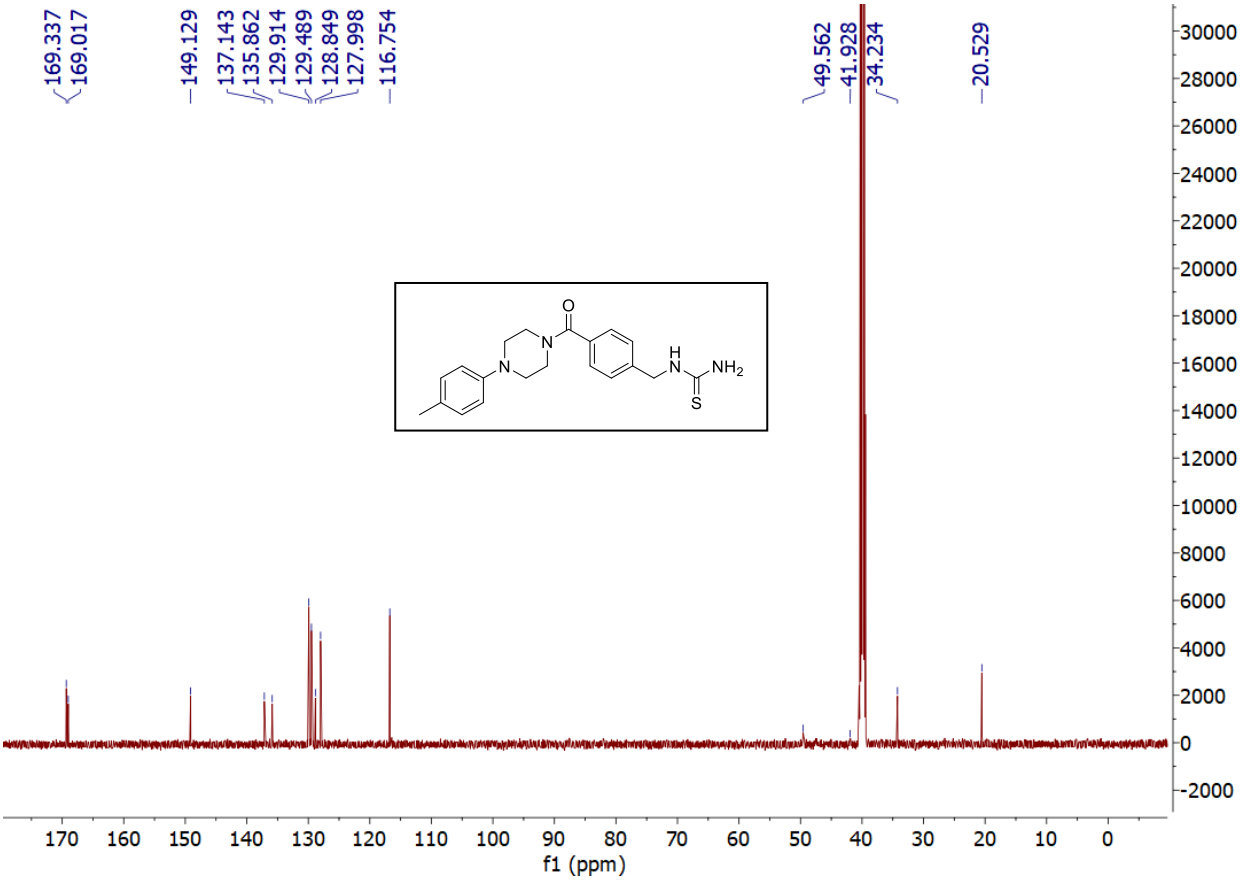
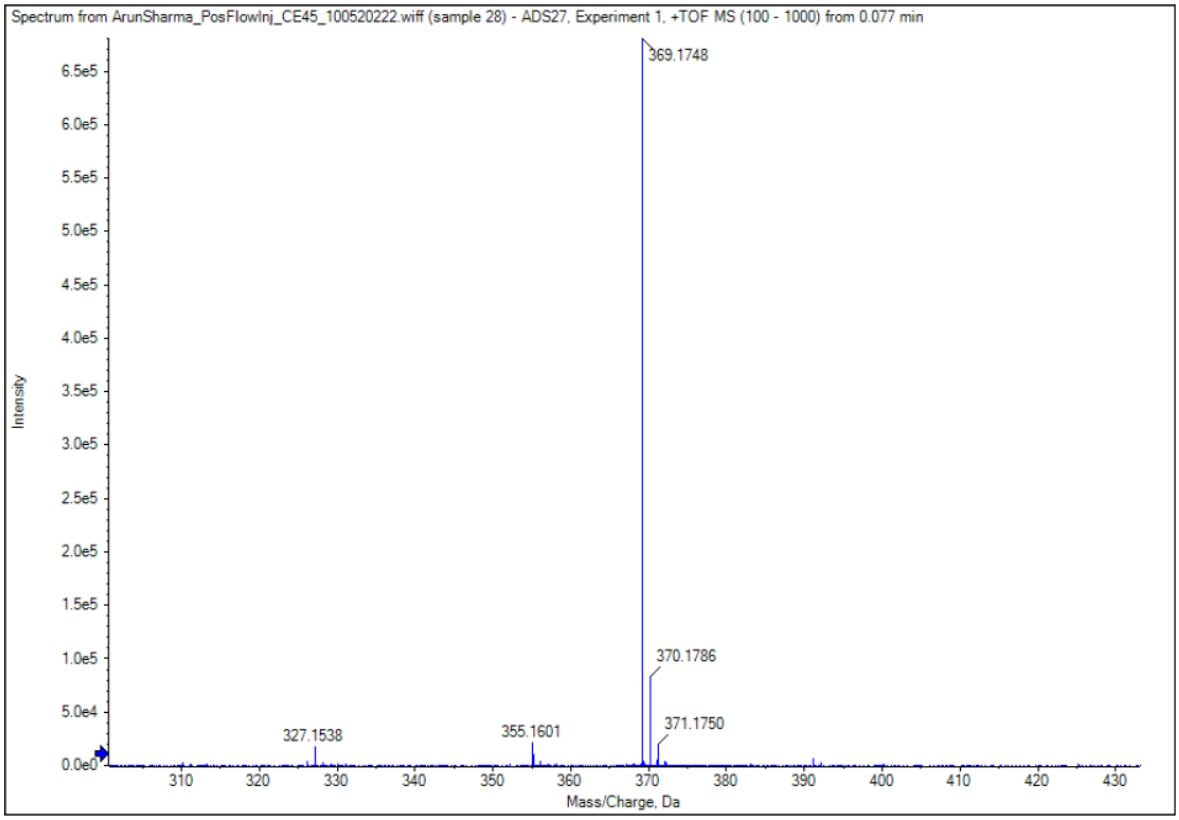
^{1}H NMR of 1-(4-(4-(p-Tolyl) piperazine-1-carbonyl) benzyl) thiourea(18i):



^{13}C NMR of 1-(4-(4-(p-Tolyl) piperazine-1-carbonyl) benzyl) thiourea(18i):



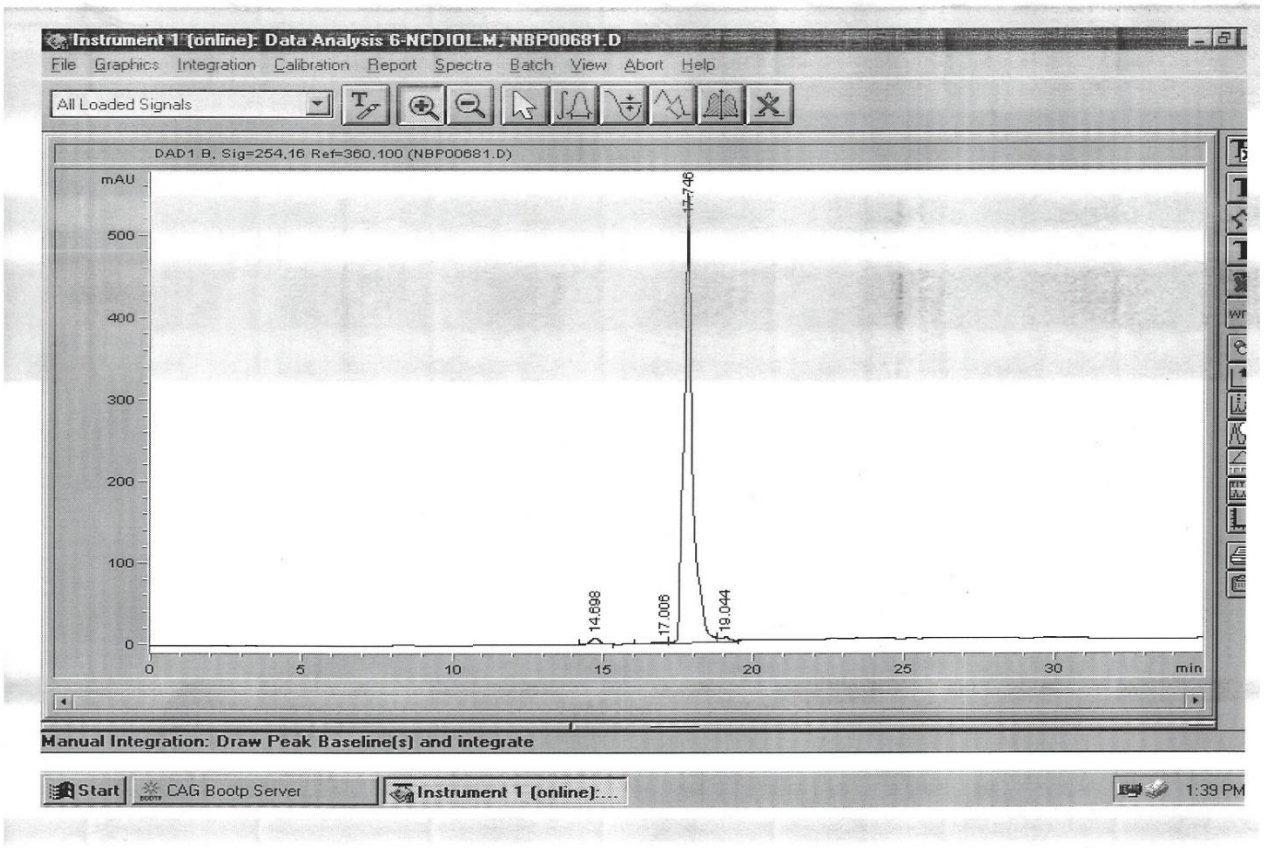
HRMS of 1-(4-(4-(p-Tolyl) piperazine-1-carbonyl) benzyl) thiourea(18i):



HPLC analysis of selected compounds

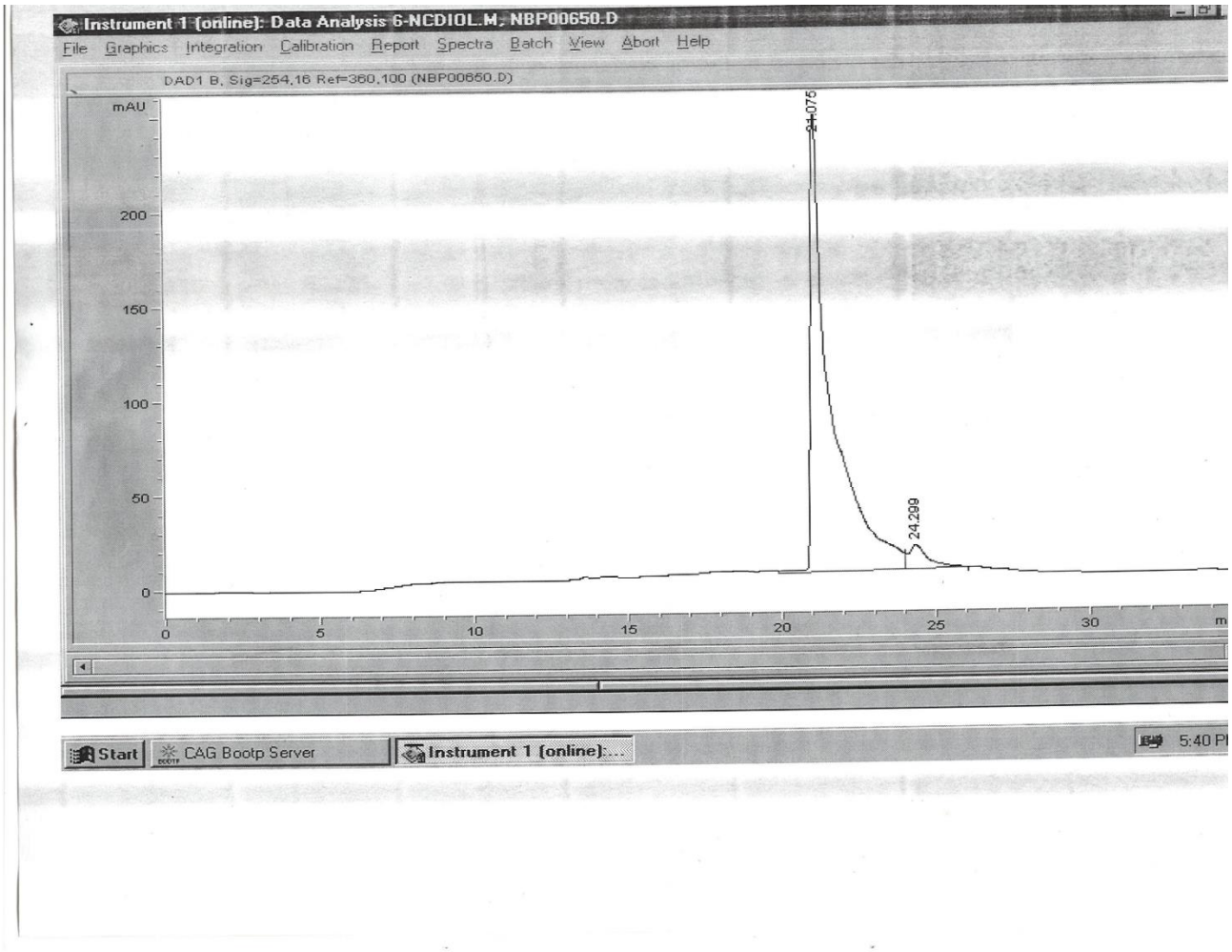
The purity of the compounds was determined using analytical high-performance liquid chromatography (HPLC) using Phenomenex (Synergi 4 μ, 250 × 4.60 mm) column and DAD1B detector at wavelength 254 λ with a linear gradient of 5–100% methanol/water in 20 min and held at 100% methanol for 10.0 min at a flow rate of 1 mL/mL.

(3-(Benzo[d] [1,3] dioxol-5-ylamino) piperidin-1-yl) (4-((3-nitro-1H-1,2,4-triazol-1-yl) methyl) phenyl) methanone (7e):



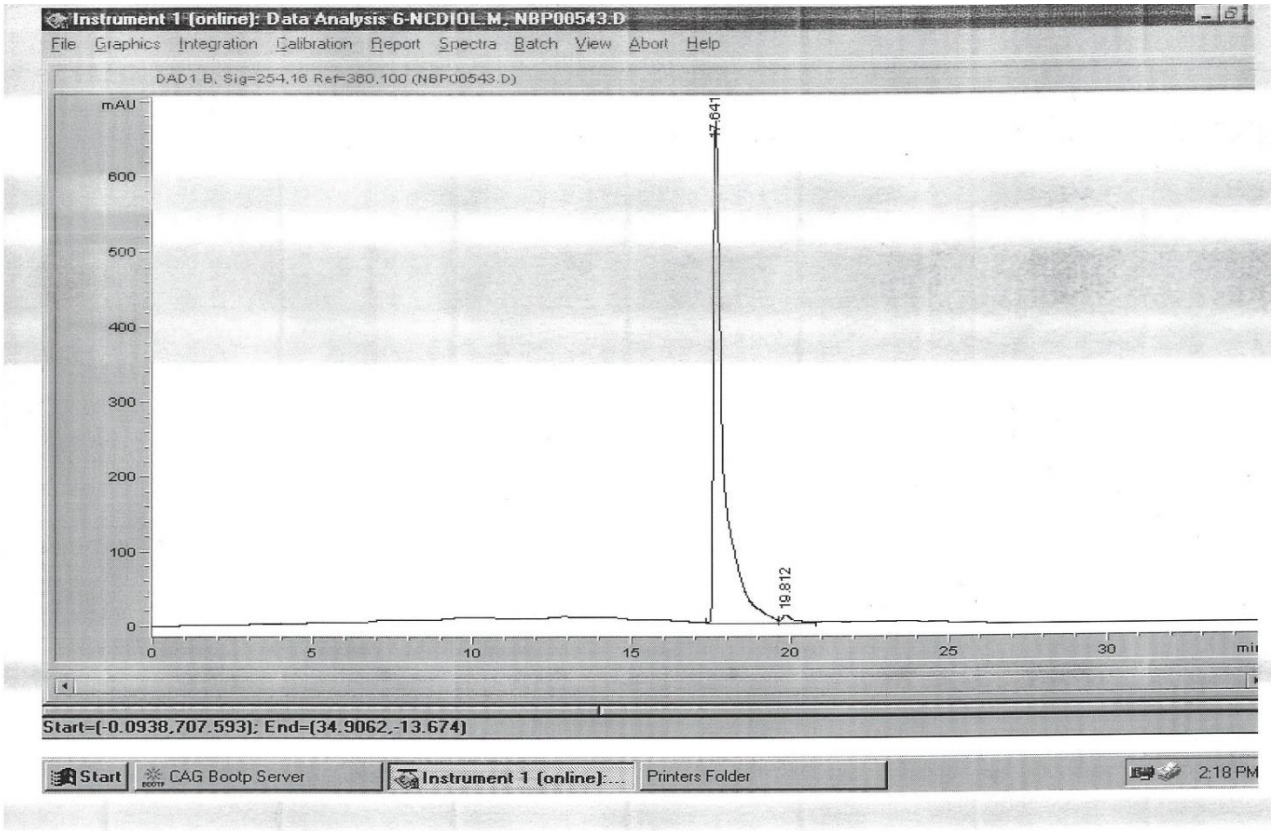
Peak	Ret Time (min)	Width (min)	Area (mAU*s)	Height (mAU)	Area %
1	14.698	0.3249	164.92667	8.12259	1.4557
2	17.006	0.4899	84.58064	2.43086	0.7465
3	17.746	0.2717	1.08768e4	547.74548	95.9994
4	19.044	0.4006	203.76184	6.66660	1.7984

(4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-(morpholinomethyl) phenyl) methanone (13a):



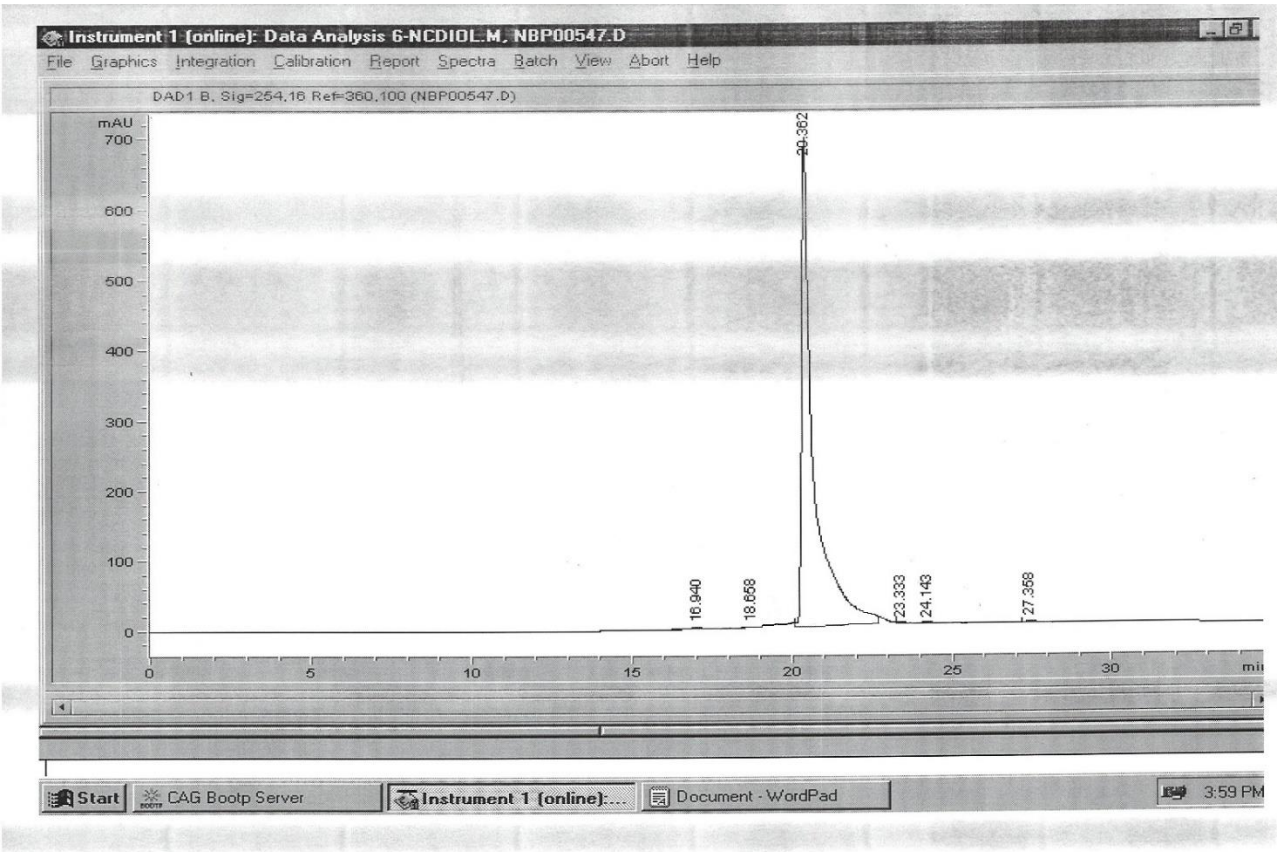
Peak	Ret Time (min)	Width (min)	Area (mAU*s)	Height (mAU)	Area %
1	21.075	0.6238	1.12792e4	241.98361	95.1666
2	24.299	0.6068	572.85364	12.88244	4.8334

(4-(Benzo[d] [1,3] dioxol-5-yl) piperazin-1-yl) (4-((3-(hydroxymethyl)-1H-indol-1-yl) methyl) phenyl) methanone (13g) (AVJ16):



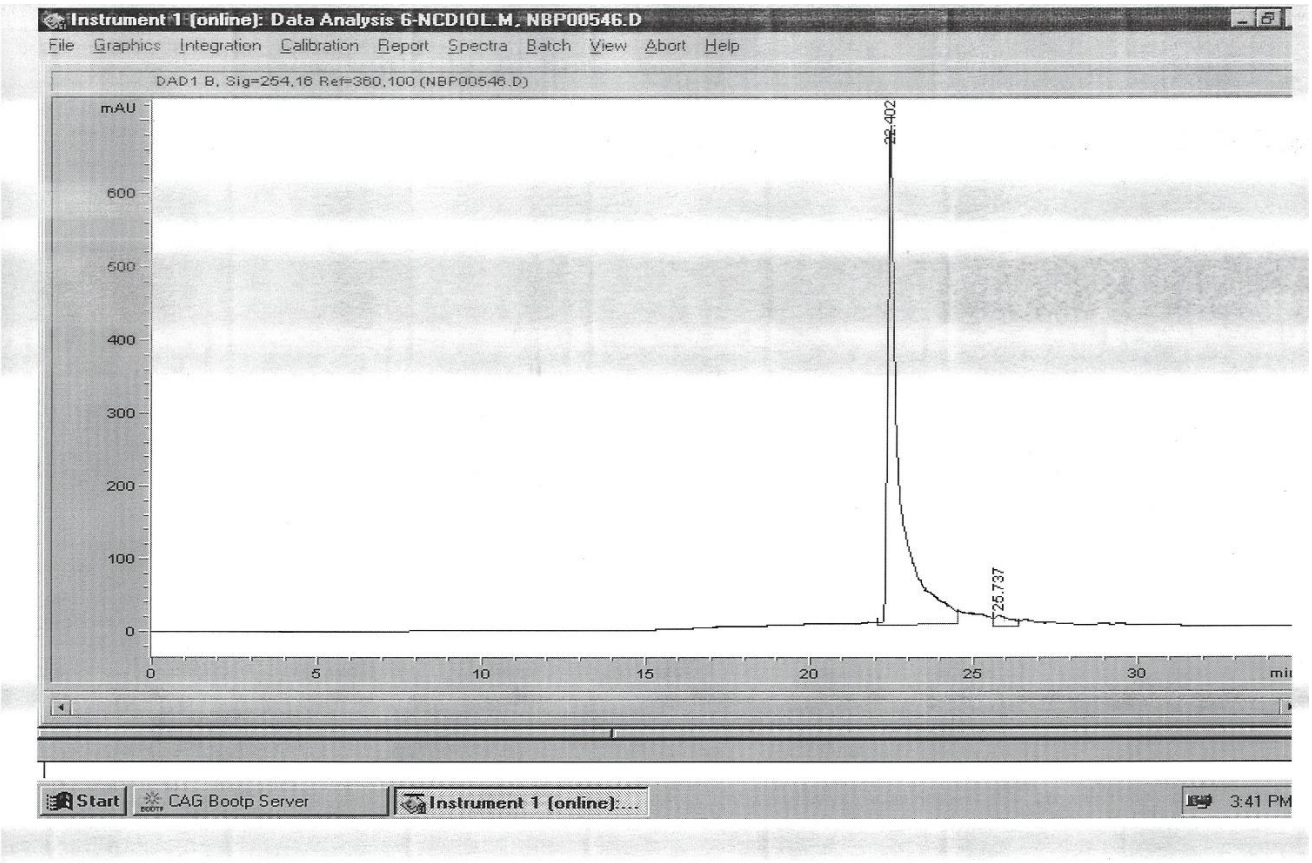
Peak	Ret Time (min)	Width (min)	Area (mAU*s)	Height (mAU)	Area %
1	17.641	0.3175	1.50217e4	670.28412	97.8007
2	19.812	0.4031	337.79855	11.76068	2.1993

(4-((3-nitro-1H-1,2,4-Triazol-1-yl) methyl) phenyl) (4-(p-tolyl) piperazin-1-yl) methanone(18e):



Peak	Ret Time (min)	Width (min)	Area (mAU*s)	Height (mAU)	Area %
1	16.940	0.4571	109.60283	3.06595	0.6209
2	18.658	0.4634	54.04274	1.48931	0.3061
3	20.362	0.3367	1.72378e4	696.58929	97.6446
4	23.333	0.2910	70.51685	3.17685	0.3994
5	24.143	0.6768	129.30013	2.35391	0.7324
6	27.358	0.3007	52.34805	2.30681	0.2965

(4-((3-(Hydroxymethyl)-1H-indol-1-yl) methyl) phenyl)(4-(p-tolyl) piperazin-1-yl)methanone (18g):



Peak	Ret Time (min)	Width (min)	Area (mAU*s)	Height (mAU)	Area %
1	22.402	0.4172	1.72108e4	687.62366	96.8431
2	25.737	0.4659	561.04083	15.51322	3.1569

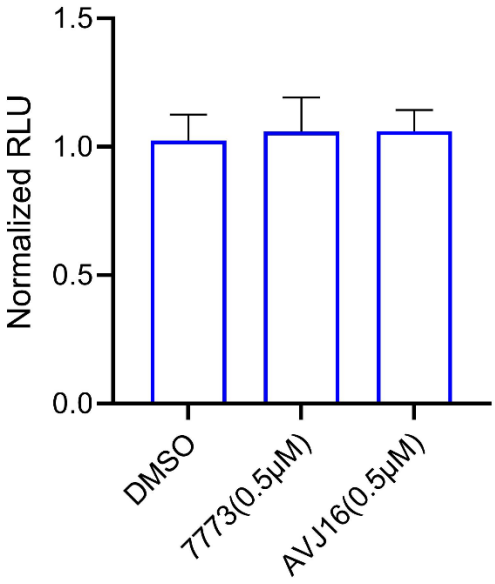


Figure 1S. This bar chart illustrates the results of the control luciferase experiment conducted in RKO cells subjected to two different treatments: DMSO or 0.5 μM of compounds **7773** and **AVJ16**. The RKO cells were co-transfected with two vectors: pcDNA3-FLAG-Luciferase, which expresses Firefly luciferase, and pRL-TK-Renilla, serving as an internal control reporter vector to express Renilla luciferase.

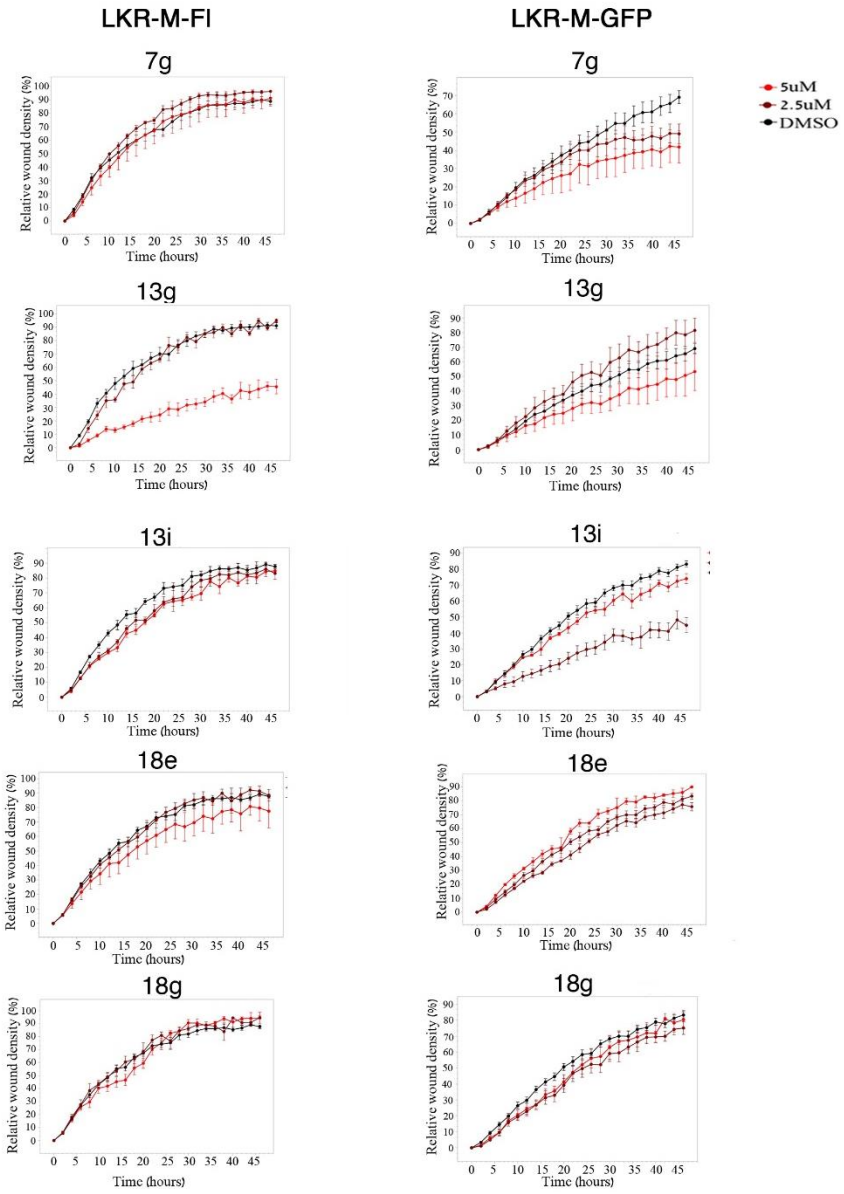


Figure 2S: Analogs screened for specificity to IGF2BP1

The 5 analogs that demonstrated the highest inhibition of wound healing in H1299 cells were tested for their specificity for IGF2BP1 by comparing their ability to inhibit wound healing in LKR-M cells expressing either full-length exogenous human IGF2BP1 (LKR-M-Fl) or control GFP (LKR-M-GFP). In each case, 2 concentrations of the inhibitor (5 µM or 2.5 µM) were compared to DMSO (vehicle). For purposes of comparison, the inhibition curves that showed the strongest effect on LKR-M-Fl cells (13 g, 5 µM vs. DMSO) were plotted on the same graph as their controls (LKR-M-GFP, 5 µM vs. DMSO) and presented as Figure 3B in the text.

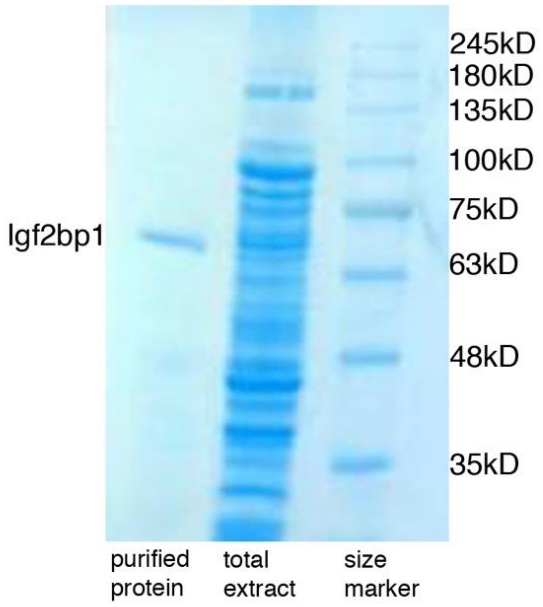


Figure 3S. Commasse blue stained gel picture showing recombinant IGF2BP1 purification profile.