Supporting Information

SAR Analysis of Novel Coxsackie Virus A9 Capsid Binders

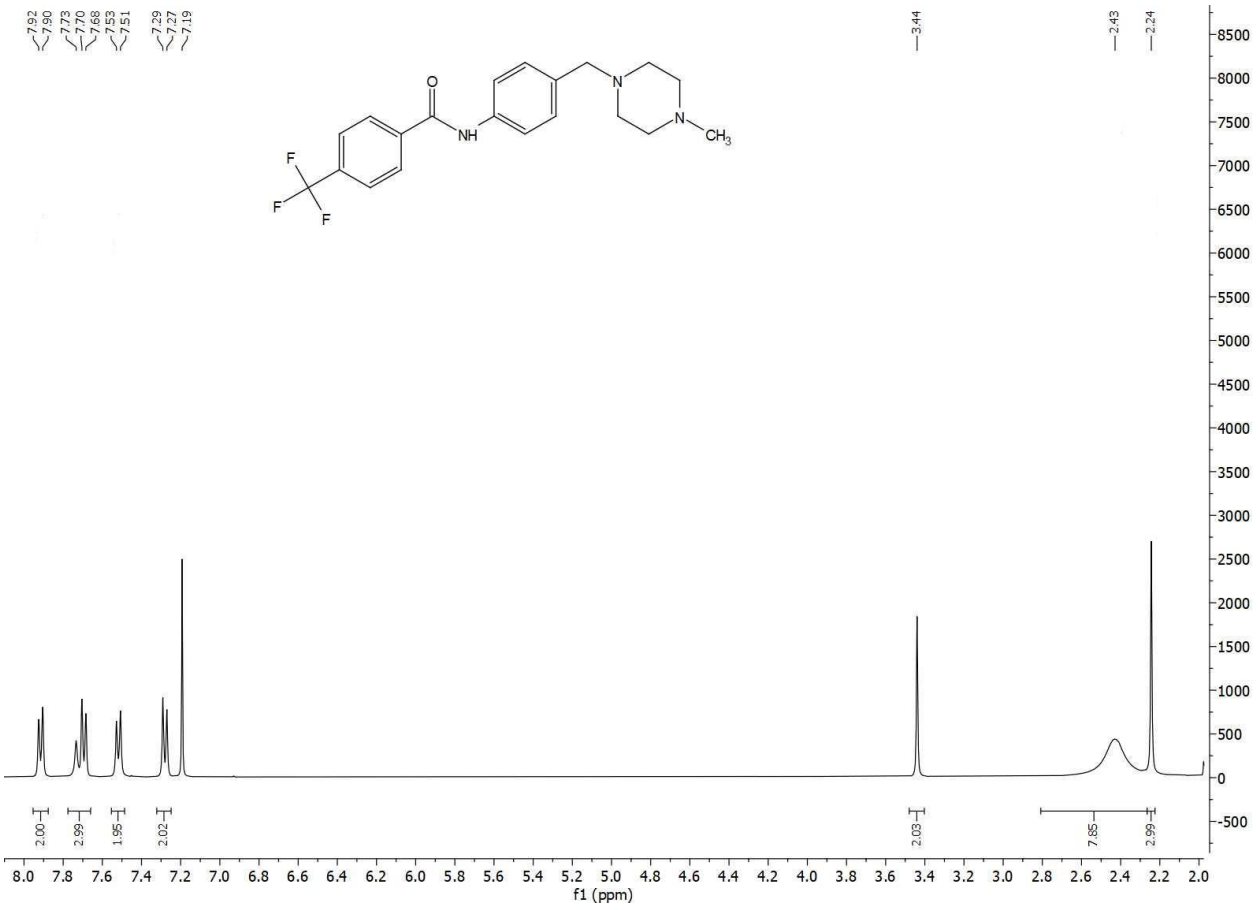
*Chiara Tammaro[a], Zlatka Plavec[b], Laura Myllymäki[c], Cristopher Mitchell[b], Sara Consalvi[a], Mariangela Biava[a], Alessia Ciogli[a], Aušra Domanska[b], Valtteri Leppilampi[b], Cienna Buckner[b,] Simone Manetto[a], Pietro Sciò[a], Antonio Coluccia[a], Mira Laajala[c], Giulio M. Dondio[d], Chiara Bigogno[d],Varpu Marjomäki[c], Sarah J. Butcher[b] and Giovanna Poce[a]**

[a]Department of Drug Chemistry and Technologies, Sapienza University of Rome, 00185 Rome, Italy.
[b]Faculty of Biological and Environmental Sciences, Molecular and Integrative Bioscience Research Programme, & Helsinki Institute of Life Sciences-Institute of Biotechnology, University of Helsinki, FIN-00014 Helsinki, Finland.
[c]Department of Biological and Environmental Science, Nanoscience Center, University of Jyväskylä, 40500 Jyväskylä, Finland.
[d]Aphad, Via della Resistenza, 20090 Buccinasco, Italy.
*Correspondence: Giovanna.poce@uniroma1.it

Table of Contents

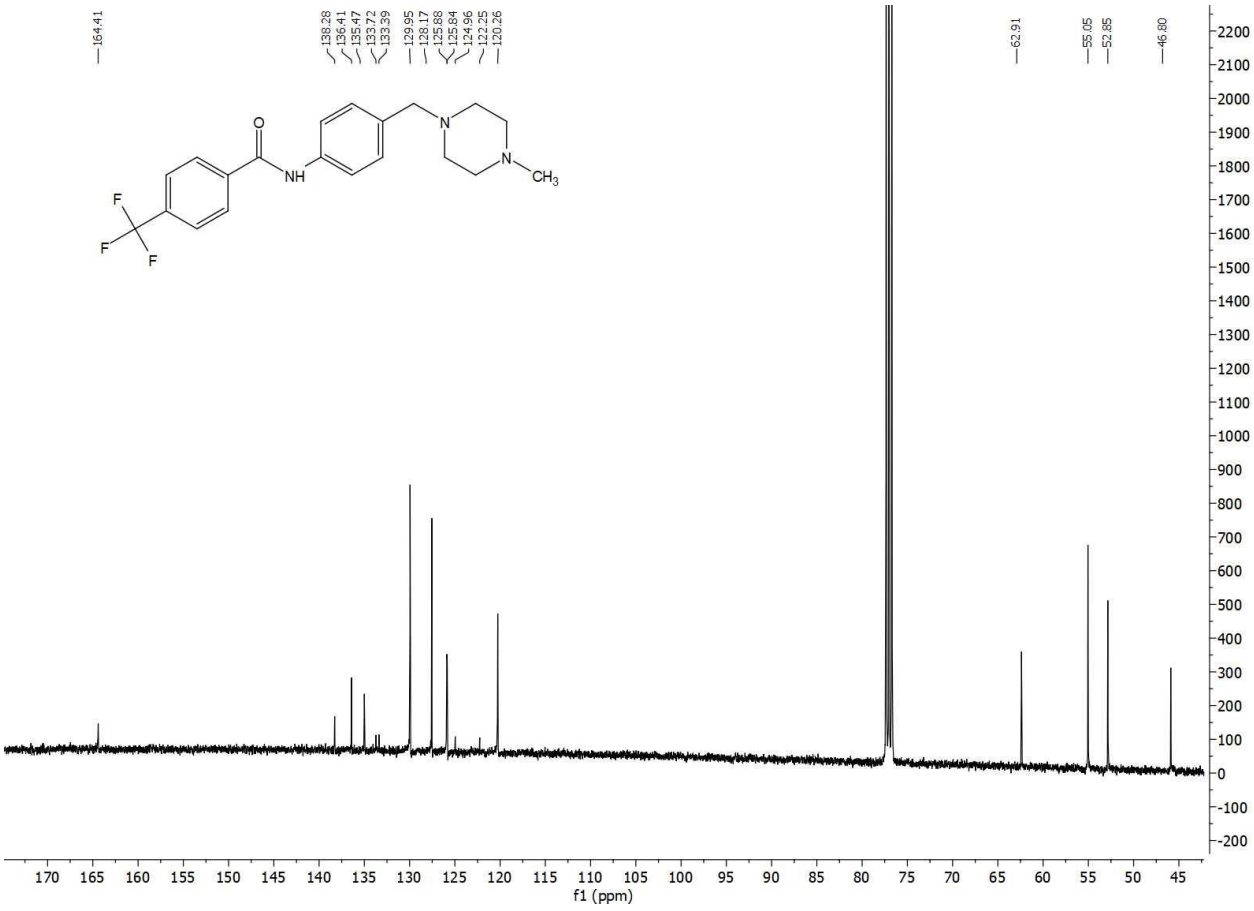
^{1}H NMR, ^{13}C NMR spectra of final compounds 1-29

^{1}H NMR of derivative **1**, ***N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-4-(trifluoromethyl)benzamide**, solvent $CDCl_3$

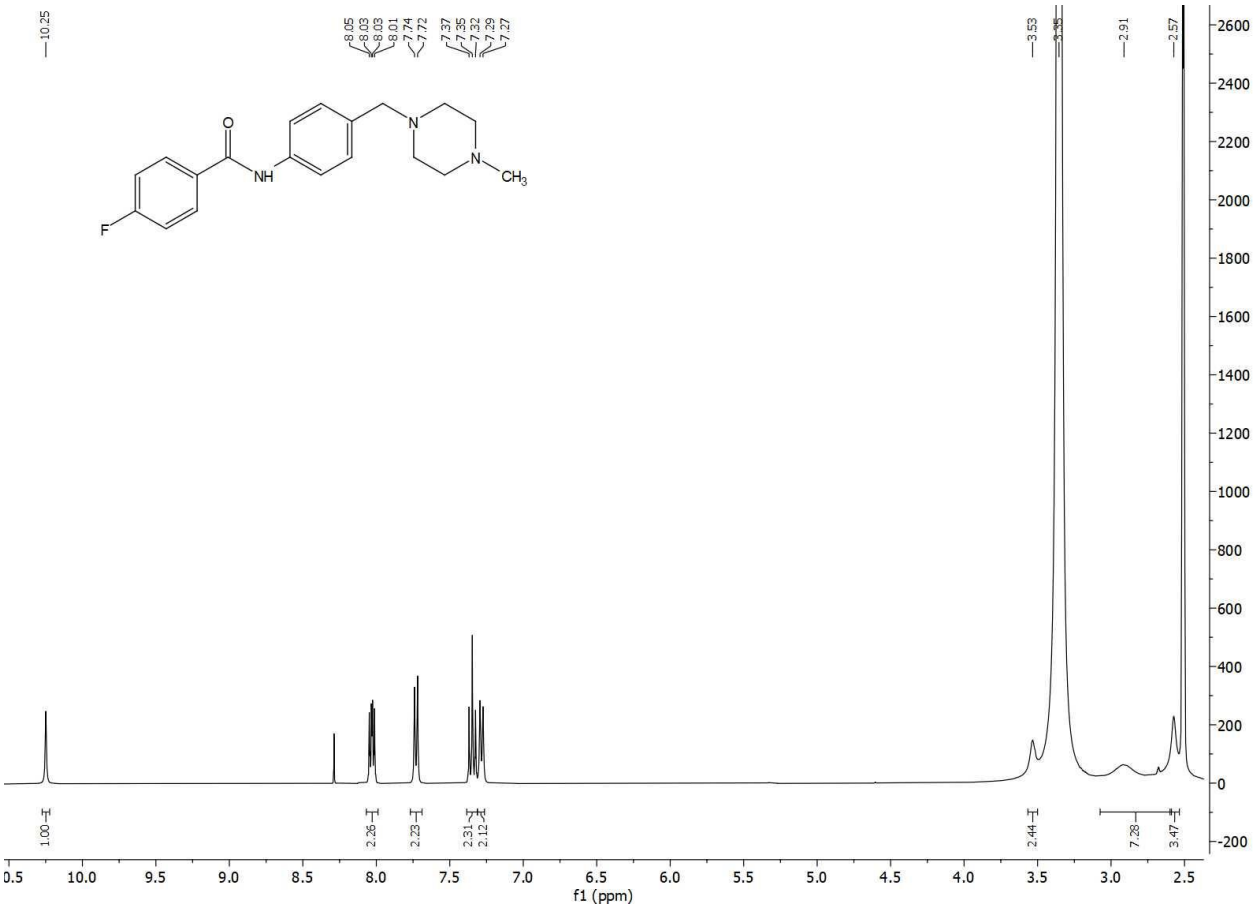


^{1}H NMR of derivative **1**, ***N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-4-(trifluoromethyl)benzamide**, solvent CD_3OD

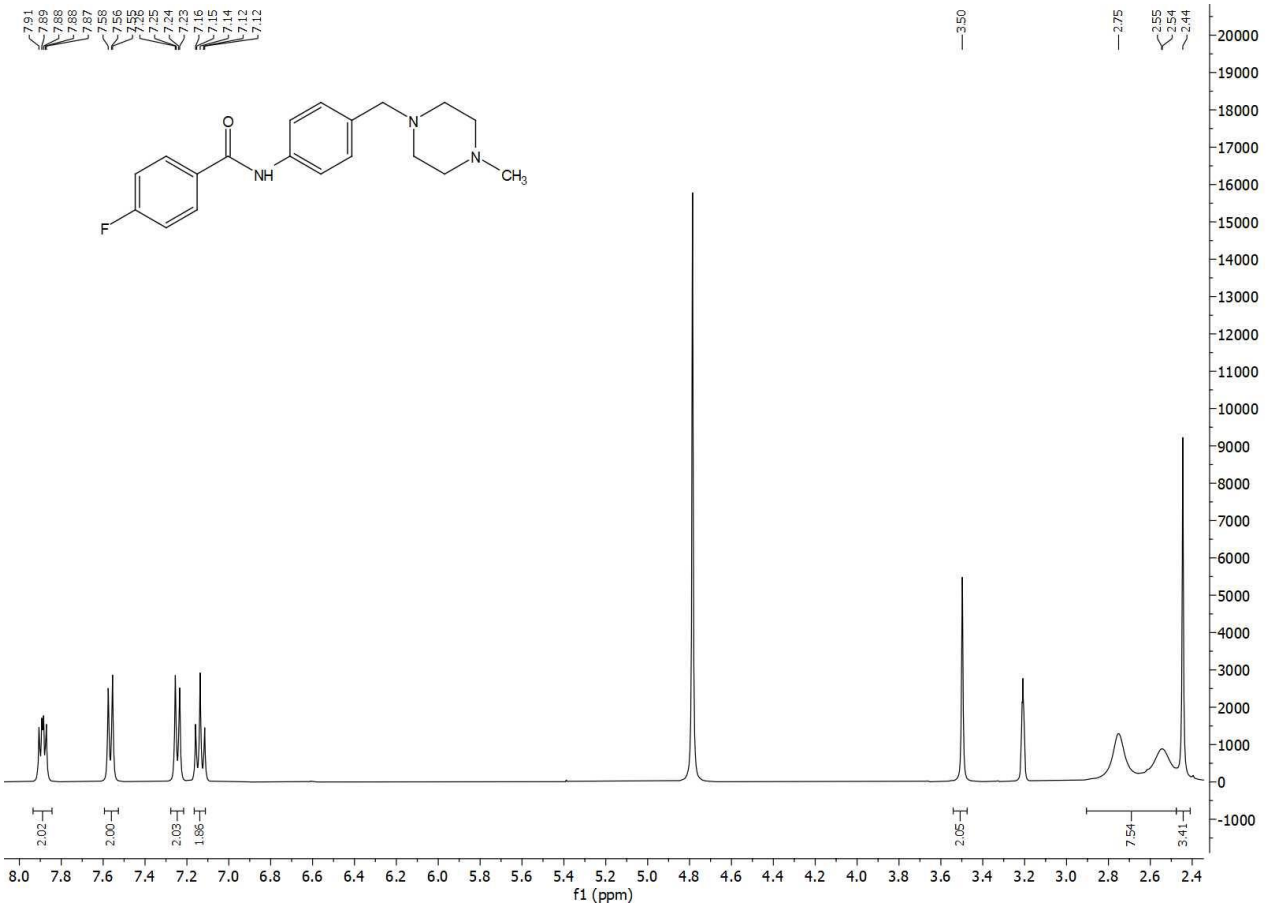
^{13}C NMR of derivative **1**, ***N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-4-(trifluoromethyl)benzamide**, solvent $CDCl_3$



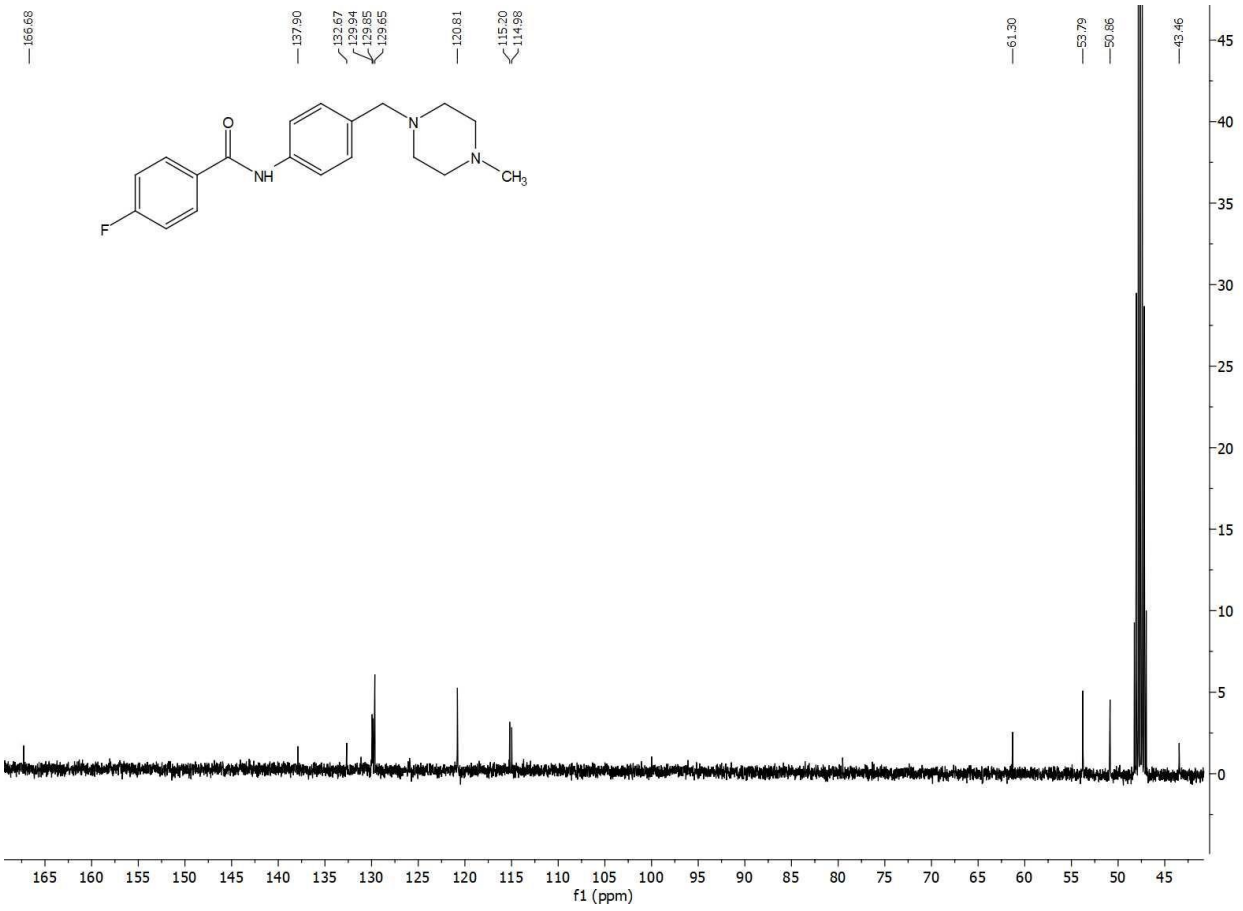
[1]H NMR of derivative **2**, **4-Fluoro-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)benzamide**, solvent DMSO-*d6*



^{1}H NMR of derivative **2**, **4-Fluoro-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)benzamide**, solvent CD_3OD

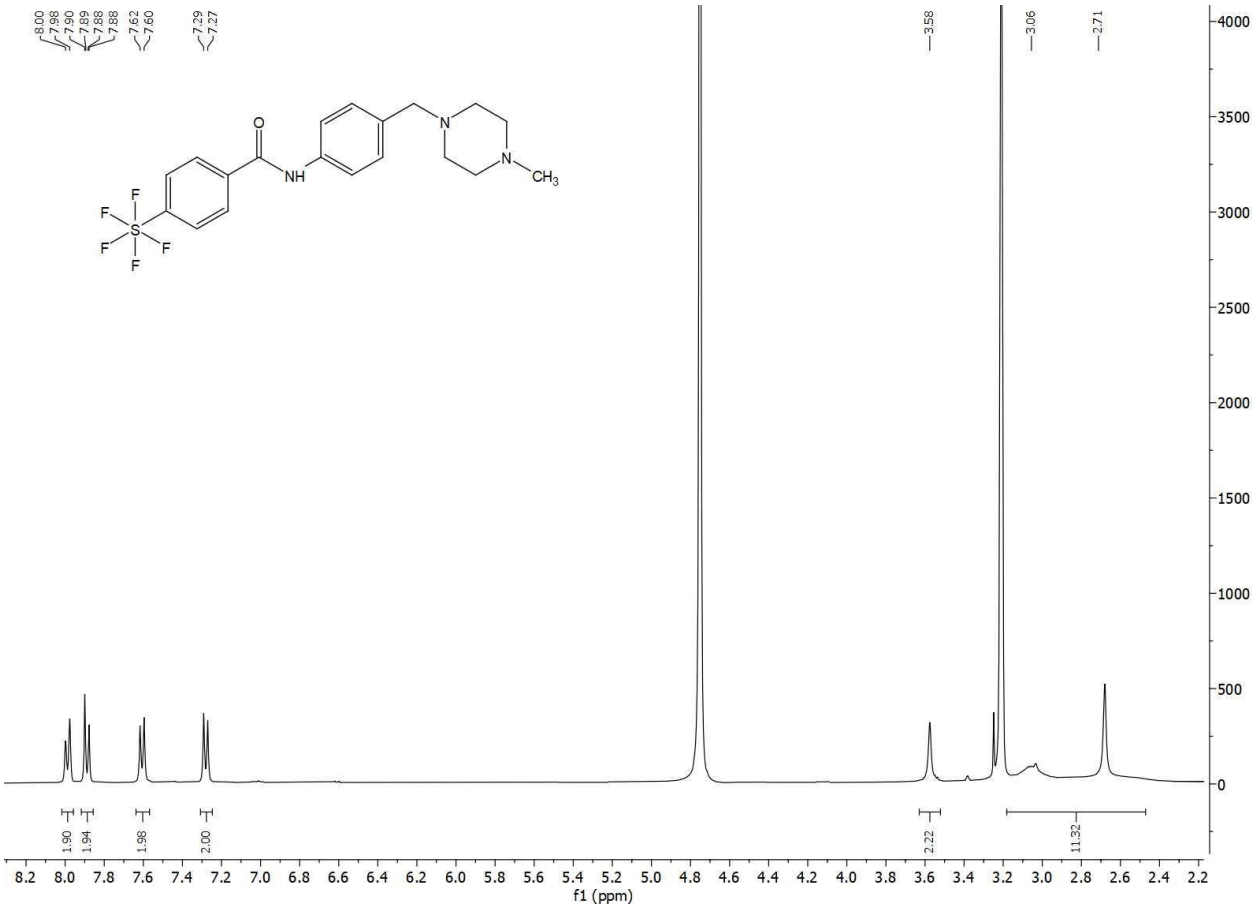


^{13}C NMR of derivative **2**, **4-Fluoro-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)benzamide**, solvent CD_3OD

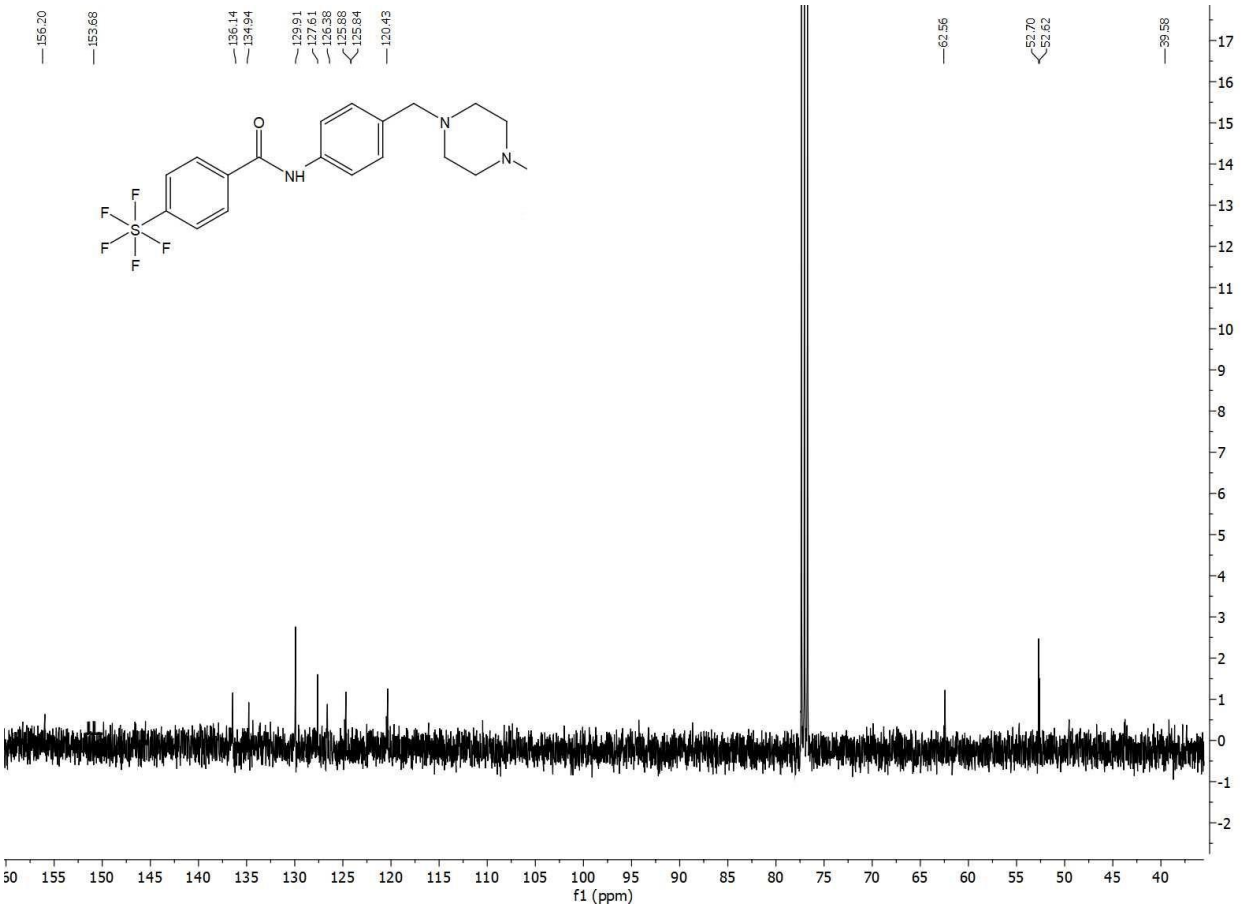


^{1}H NMR of derivative **3**, ***N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-4-(pentafluoro-l6-sulfaneyl)benzamide**, solvent $CDCl_3$

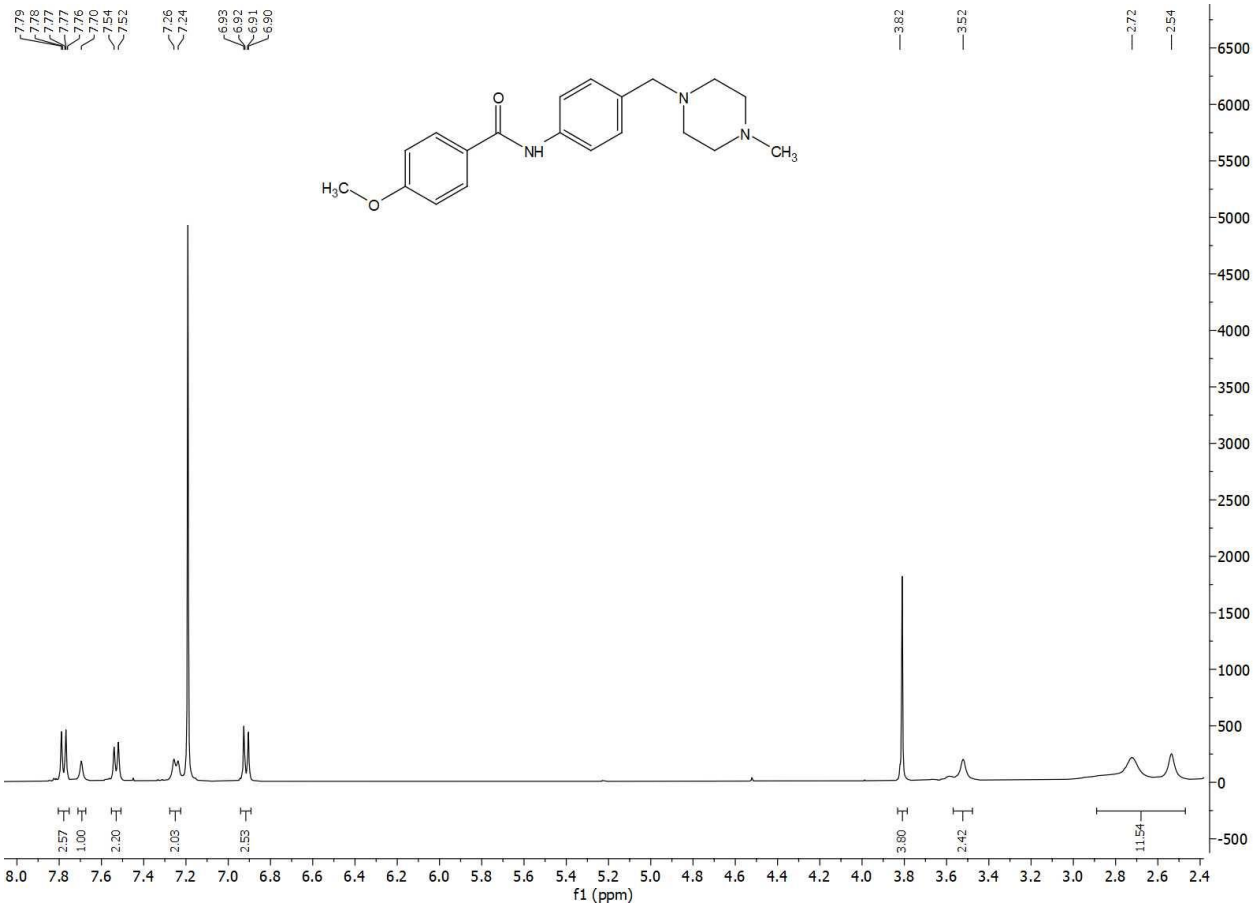
H^1 NMR of derivative **3**, ***N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-4-(pentafluoro-l6-sulfaneyl)benzamide**, solvent CD_3OD



^{13}C NMR of derivative **3**, ***N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-4-(pentafluoro-l6-sulfaneyl)benzamide**, solvent $CDCl_3$

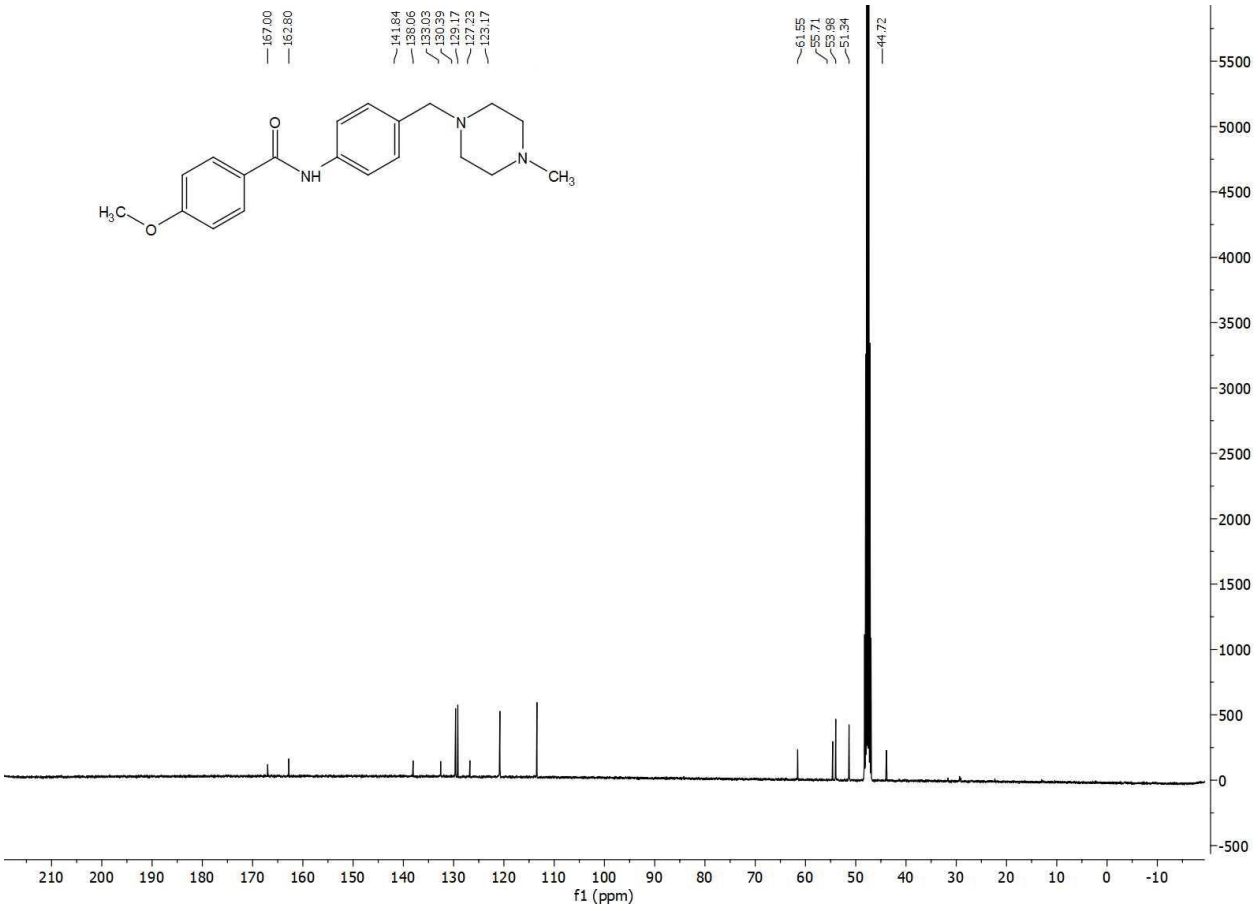


^{1}H NMR of derivative **4**, **4-Methoxy-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)benzamide**, solvent $CDCl_3$

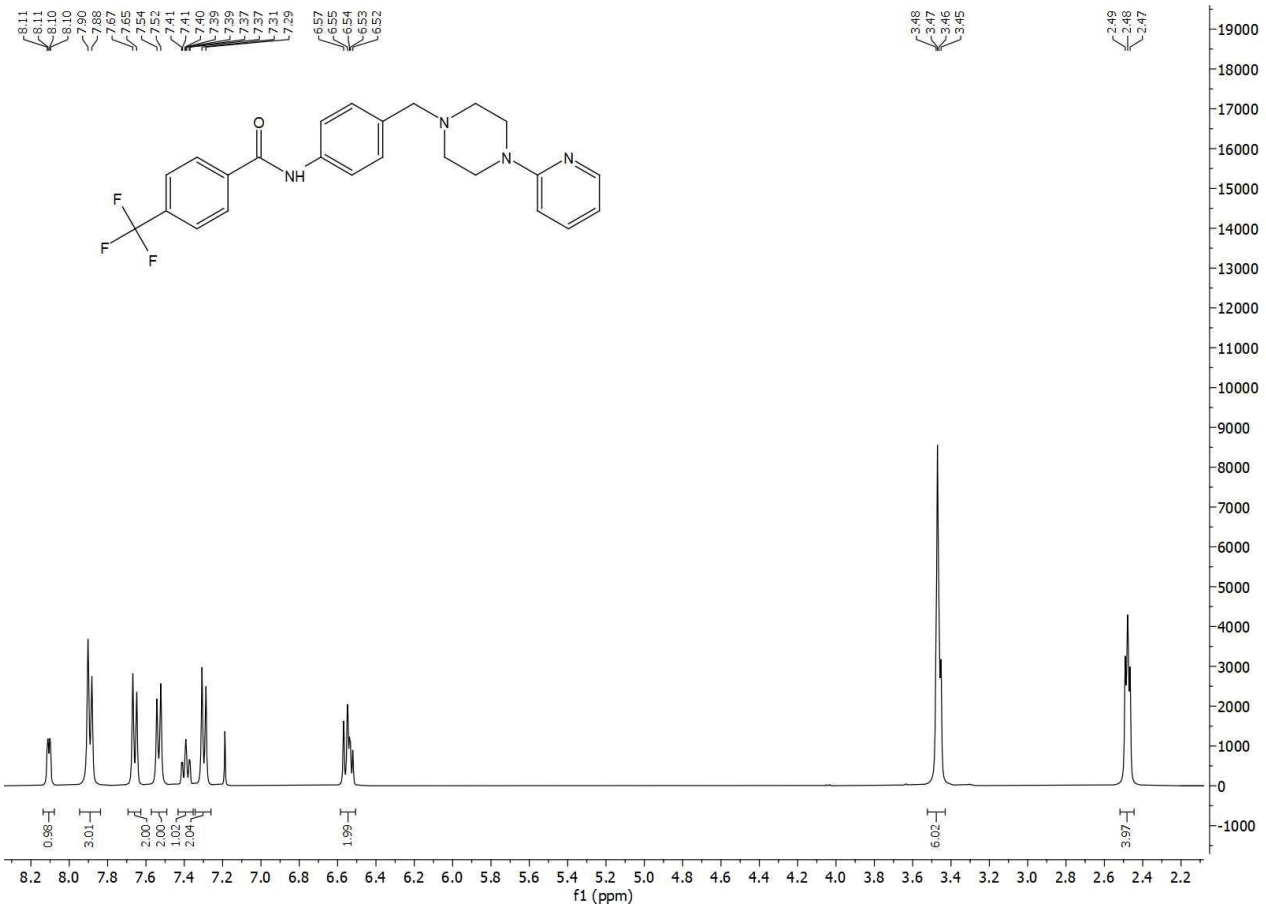


^{1}H NMR of derivative **4**, **4-Methoxy-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)benzamide,** solvent CD_3OD

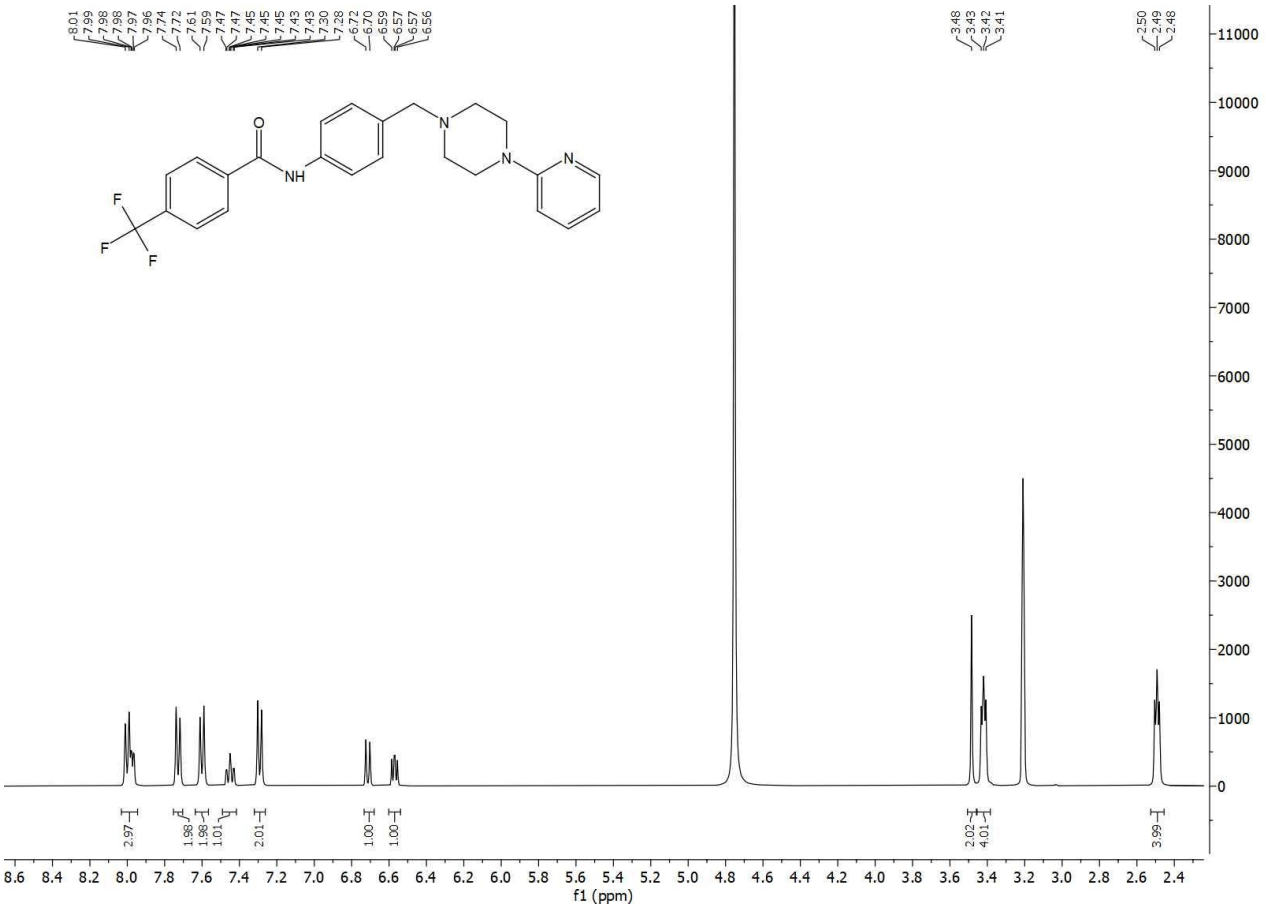
^{13}C NMR of derivative **4**, **4-Methoxy-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)benzamide,** solvent CD_3OD



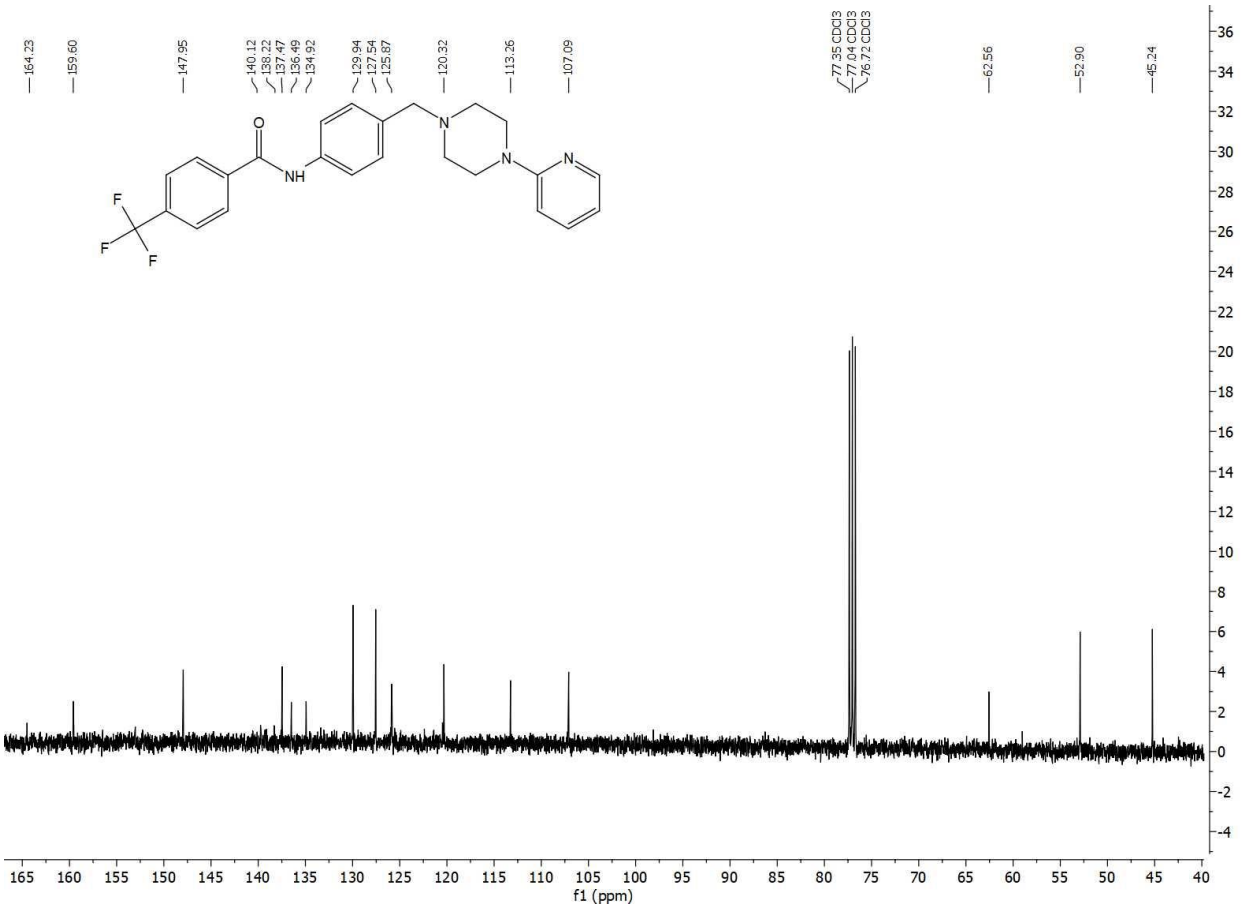
^{1}H NMR of derivative **5**, ***N*-(4-((4-(pyridin-2-yl)piperazin-1-yl)methyl)phenyl)-4-(trifluoromethyl)benzamide**, solvent $CDCl_3$



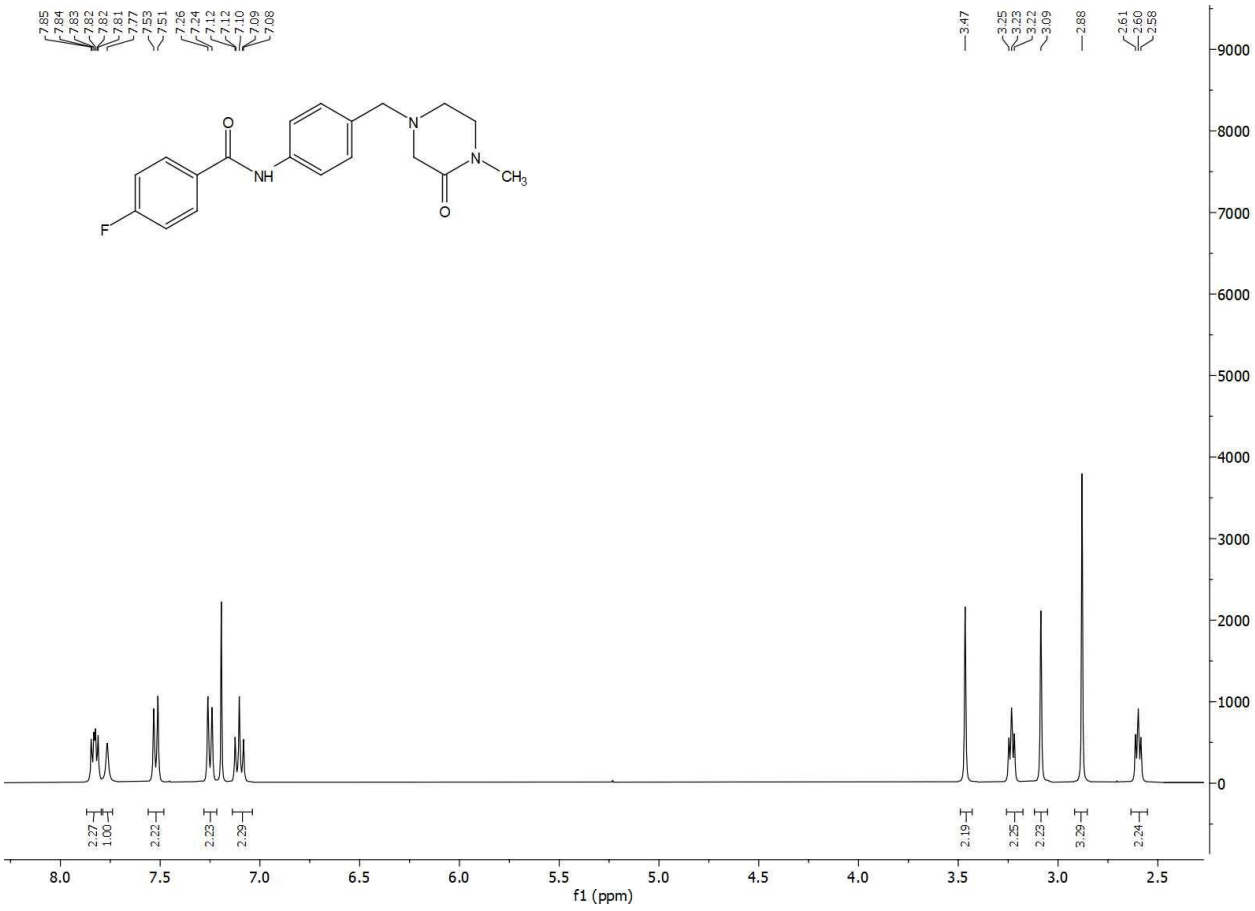
^{1}H NMR of derivative **5**, ***N*-(4-((4-(pyridin-2-yl)piperazin-1-yl)methyl)phenyl)-4-(trifluoromethyl)benzamide,** solvent CD_3OD



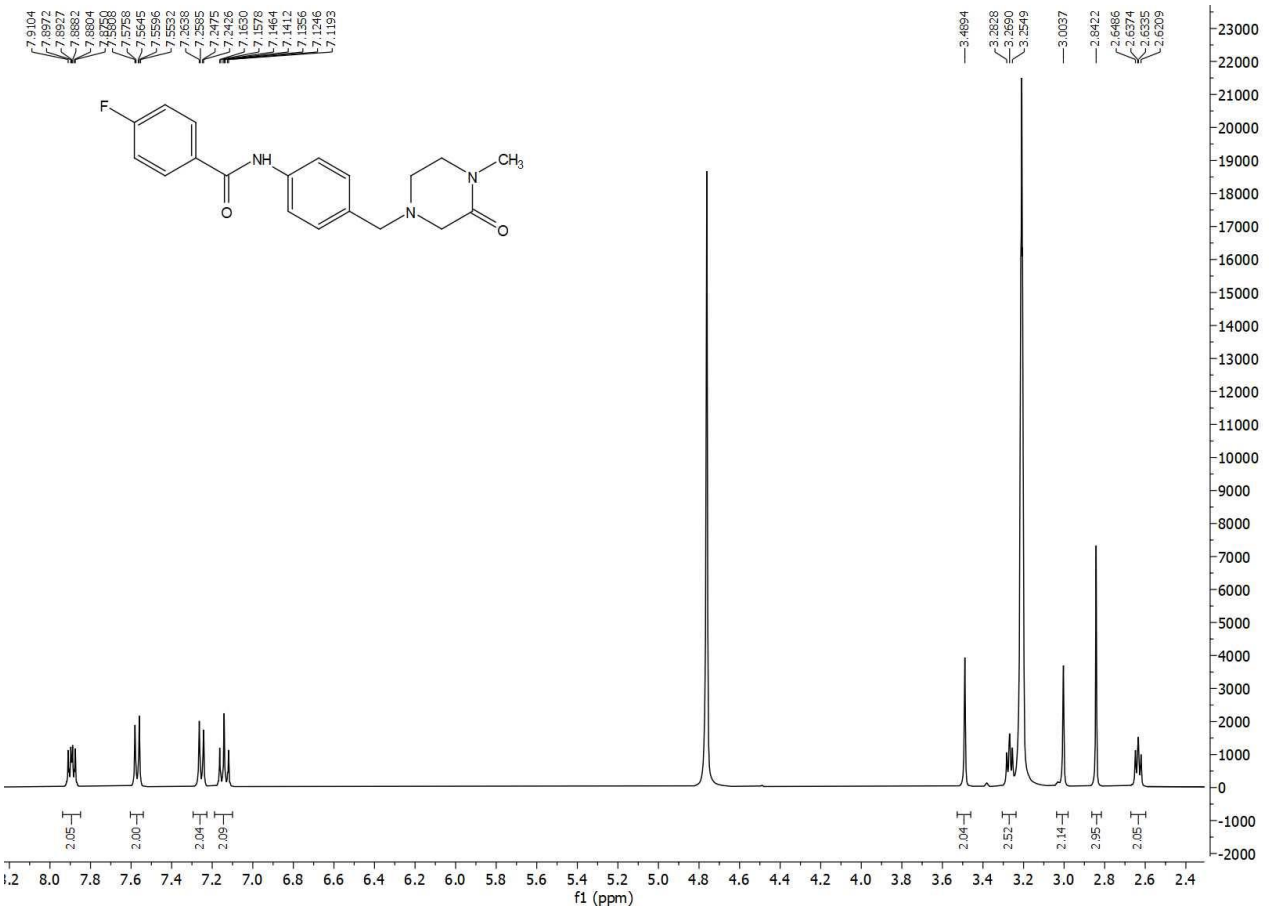
^{13}C NMR of derivative **5**, ***N*-(4-((4-(pyridin-2-yl)piperazin-1-yl)methyl)phenyl)-4-(trifluoromethyl)benzamide,** solvent $CDCl_3$



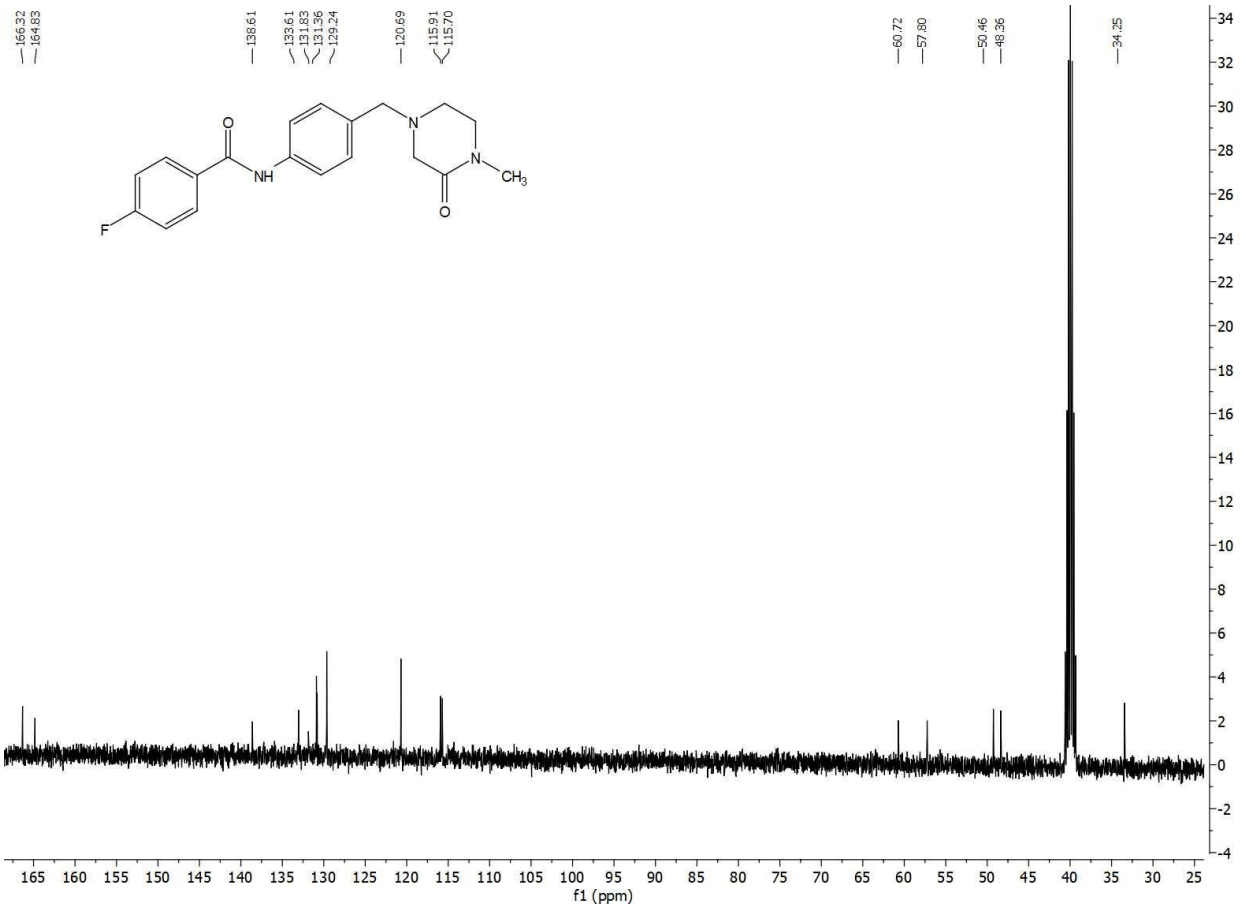
^{1}H NMR of derivative **6**, **4-Fluoro-N-(4-((4-methyl-3-oxopiperazin-1-yl)methyl)phenyl)benzamide,** solvent $CDCl_3$



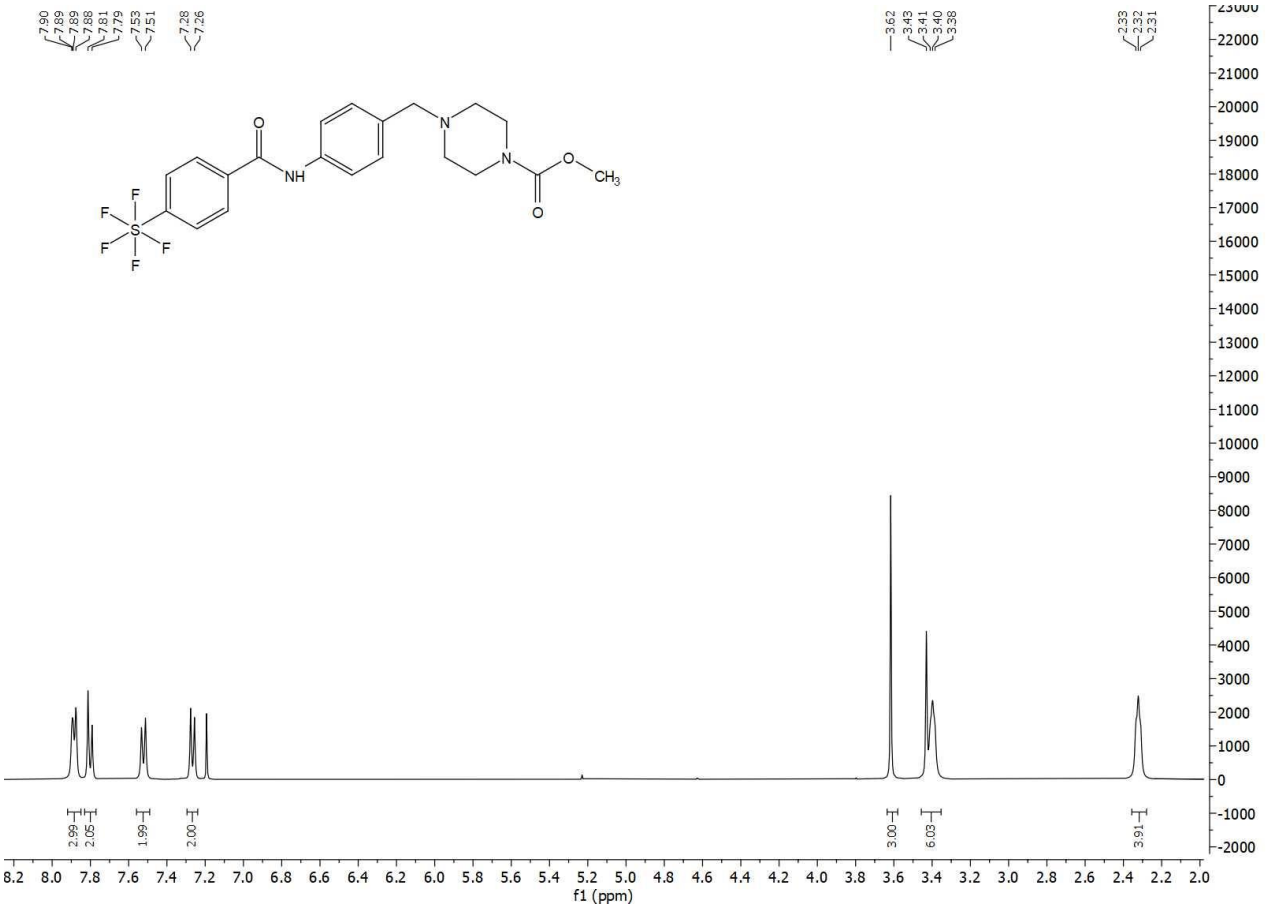
^{1}H NMR of derivative **6**, **4-Fluoro-*N*-(4-((4-methyl-3-oxopiperazin-1-yl)methyl)phenyl)benzamide,** solvent CD_3OD



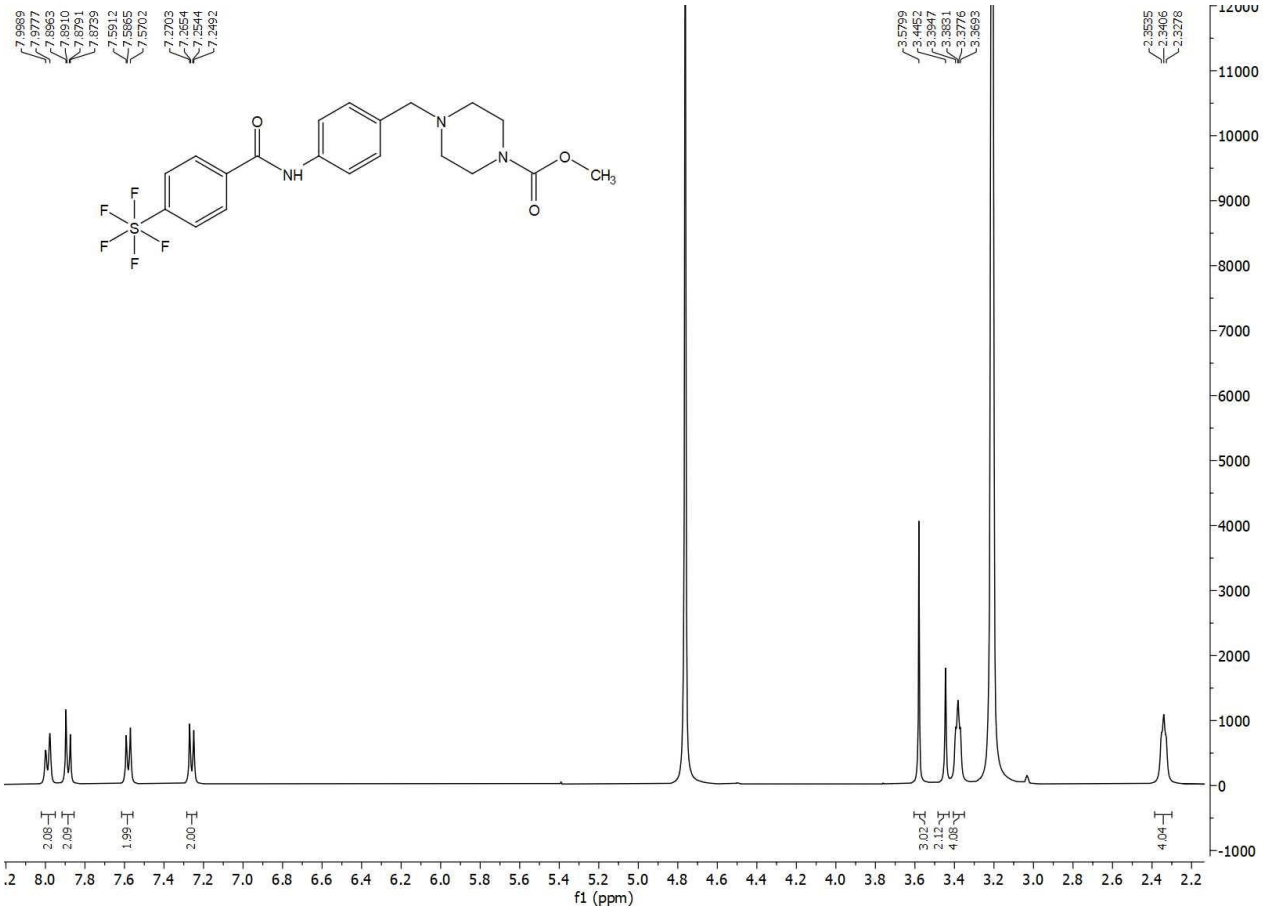
^{13}C NMR of derivative **6**, **4-Fluoro-*N*-(4-((4-methyl-3-oxopiperazin-1-yl)methyl)phenyl)benzamide,** solvent DMSO-*d6*



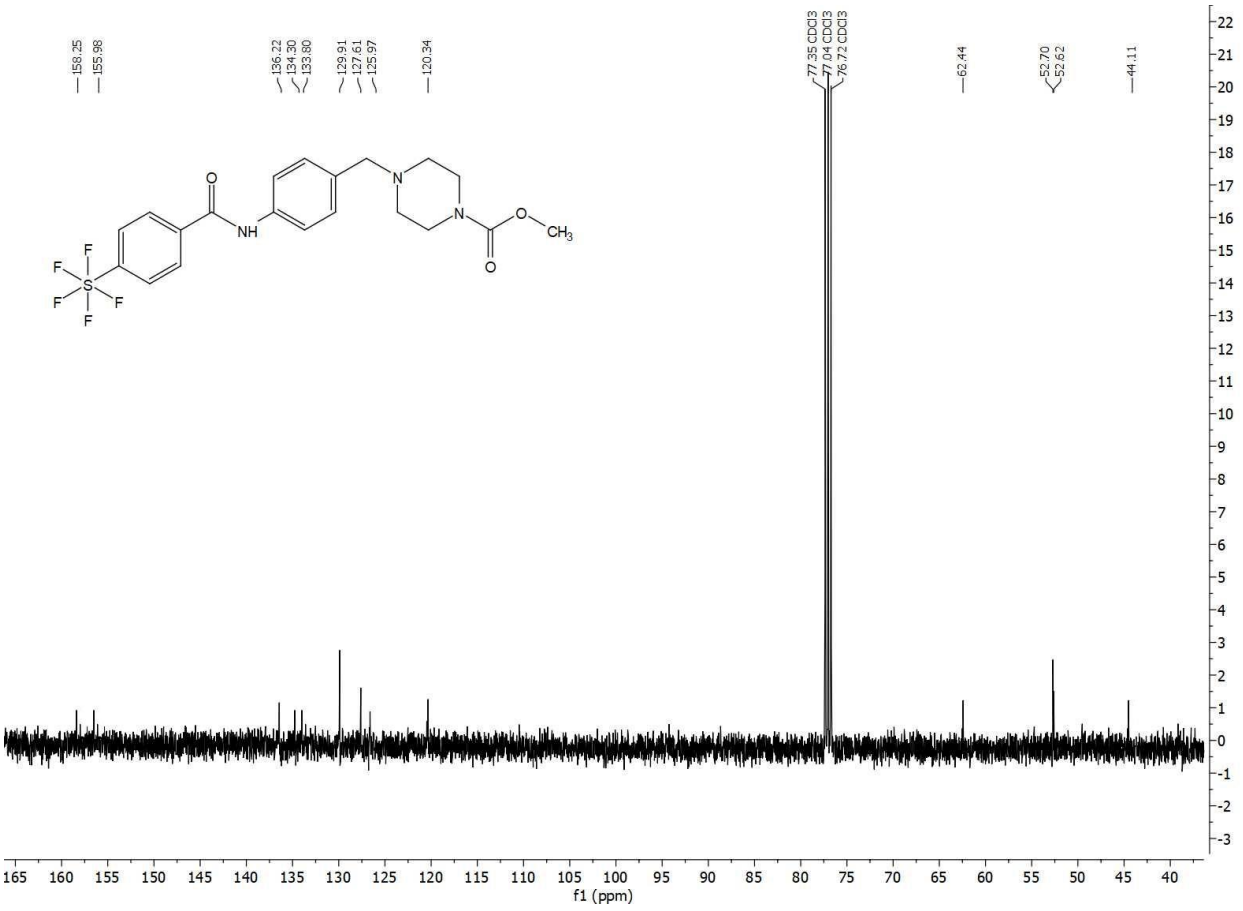
H^1 NMR of derivative **7**, **Methyl 4-(4-(4-(pentafluoro-l6-sulfaneyl)benzamido)benzyl)piperazine-1-carboxylate,** solvent $CDCl_3$



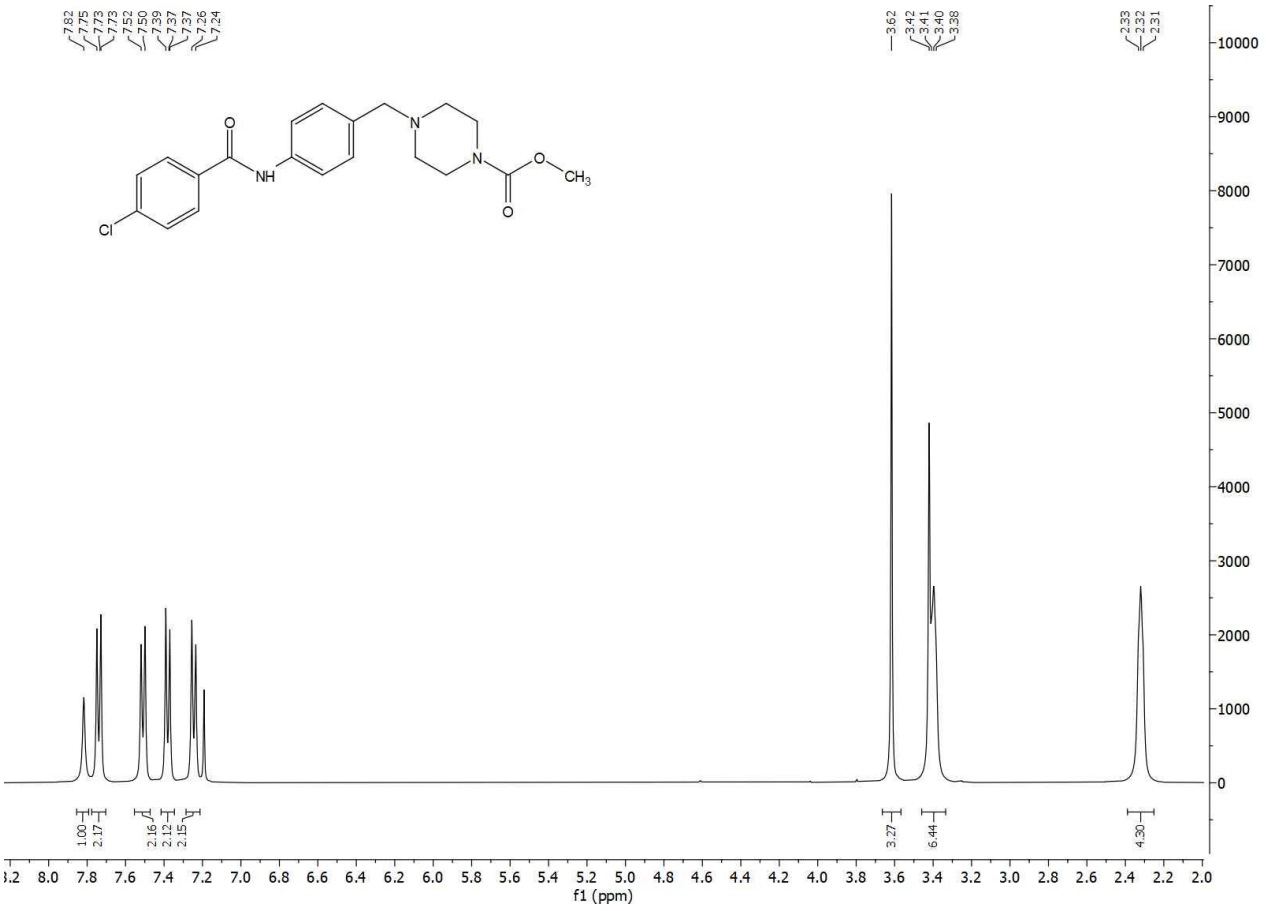
^{1}H NMR of derivative **7**, **Methyl 4-(4-(4-(pentafluoro-l6-sulfaneyl)benzamido)benzyl)piperazine-1-carboxylate,** solvent CD_3OD



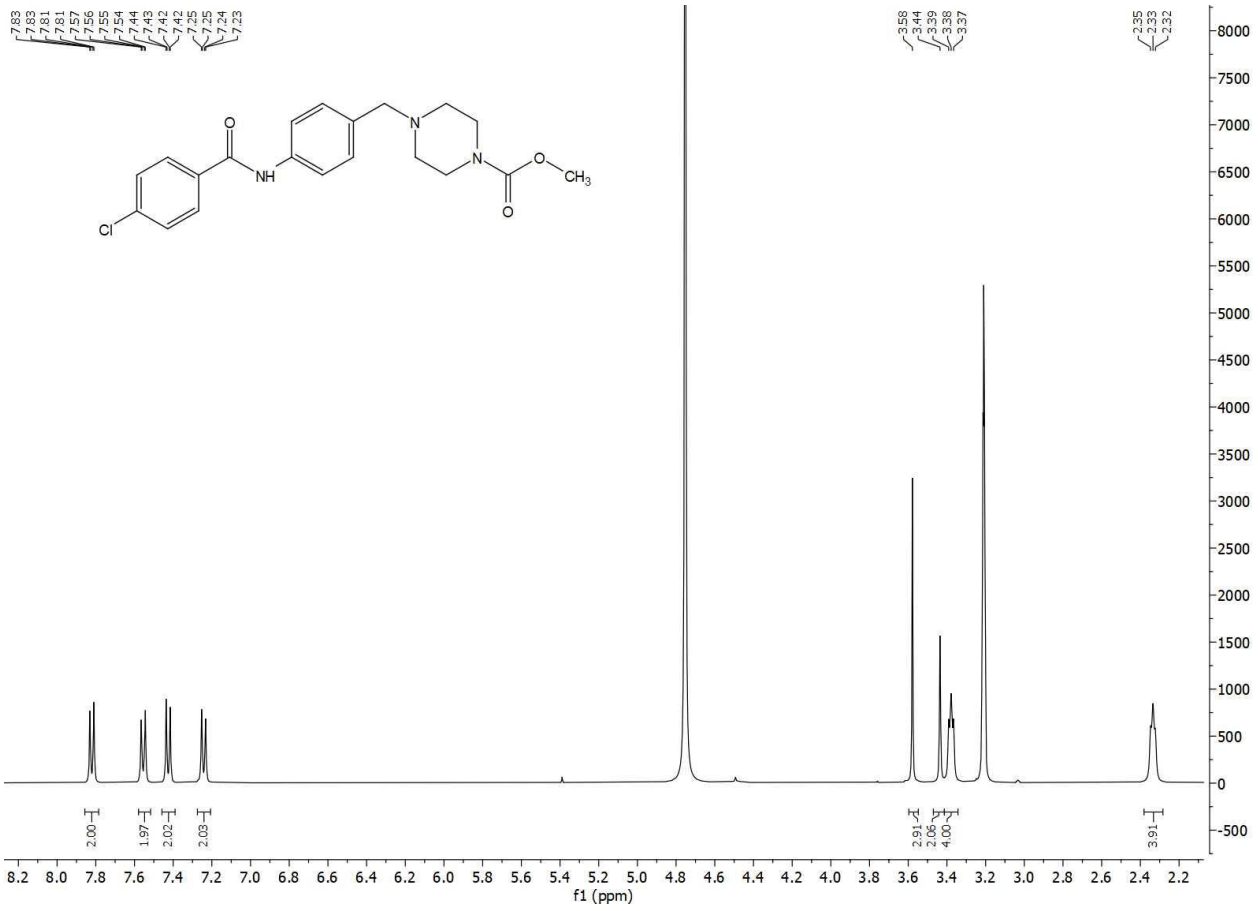
^{13}C NMR of derivative **7**, **Methyl 4-(4-(4-(pentafluoro-l6-sulfaneyl)benzamido)benzyl)piperazine-1-carboxylate,** solvent $CDCl_3$



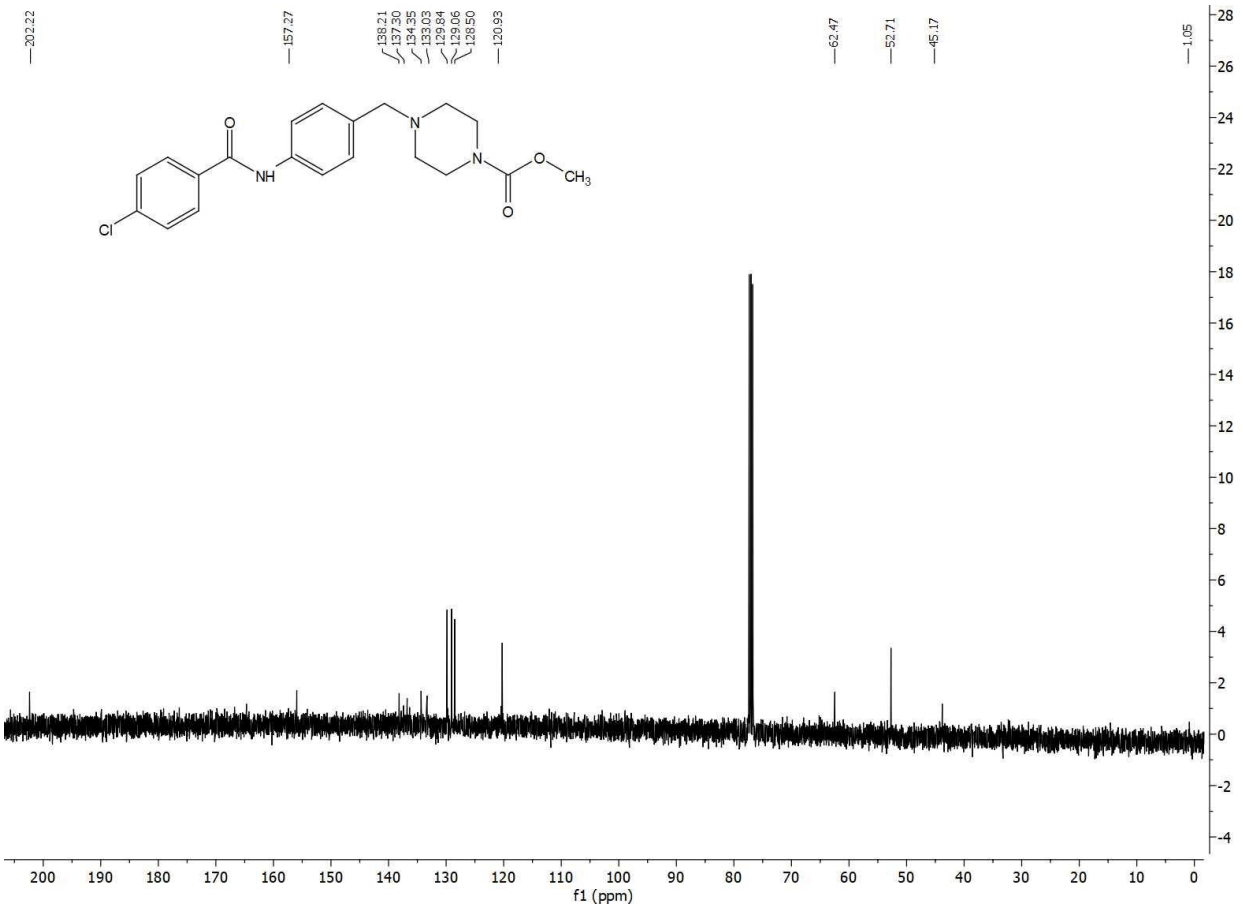
H^1 NMR of derivative **8**, **Methyl 4-(4-(4-chlorobenzamido)benzyl)piperazine-1-carboxylate,** solvent $CDCl_3$



^{1}H NMR of derivative **8**, **Methyl 4-(4-(4-chlorobenzamido)benzyl)piperazine-1-carboxylate,** solvent CD_3OD

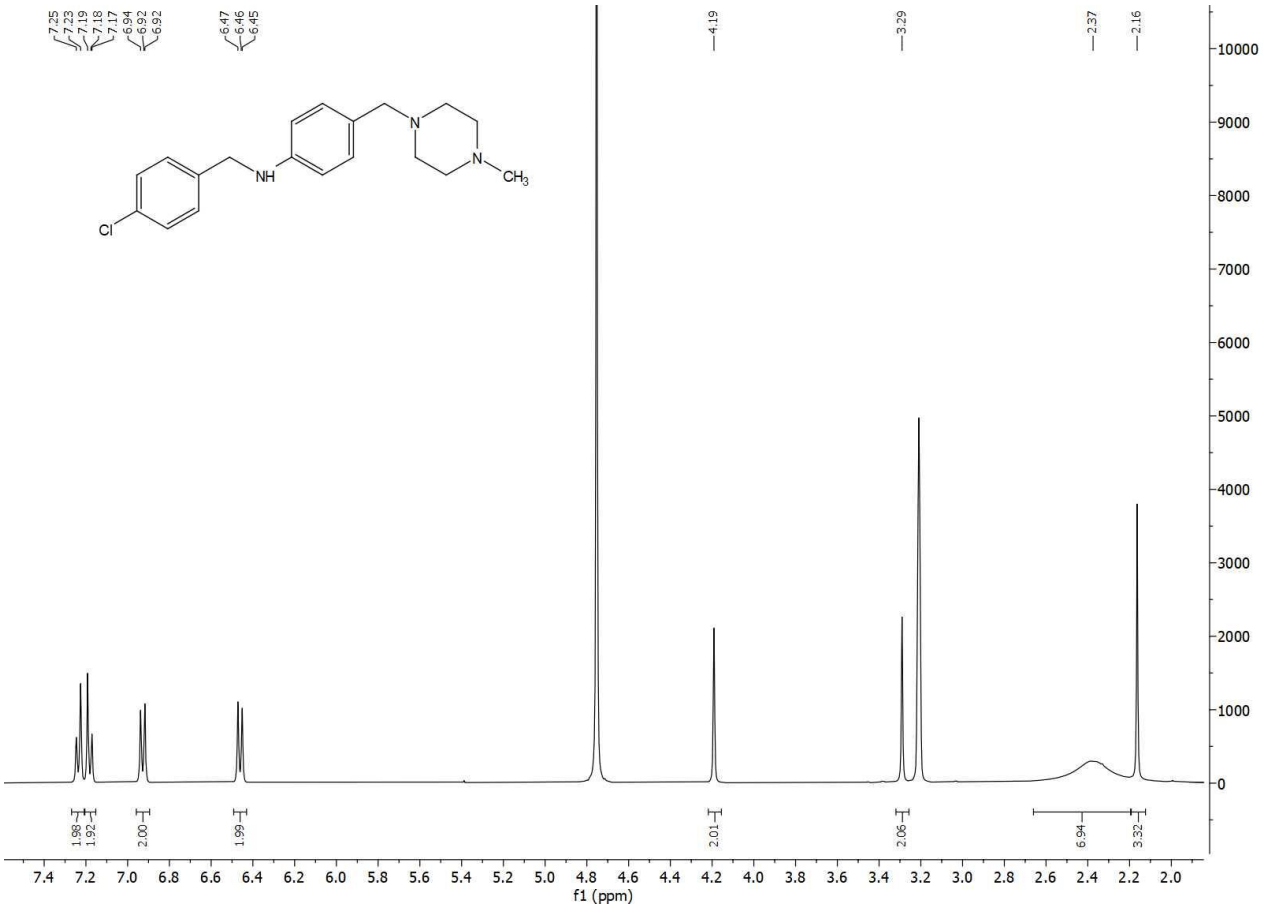


^{13}C NMR of derivative **8**, **Methyl 4-(4-(4-chlorobenzamido)benzyl)piperazine-1-carboxylate,** solvent $CDCl_3$

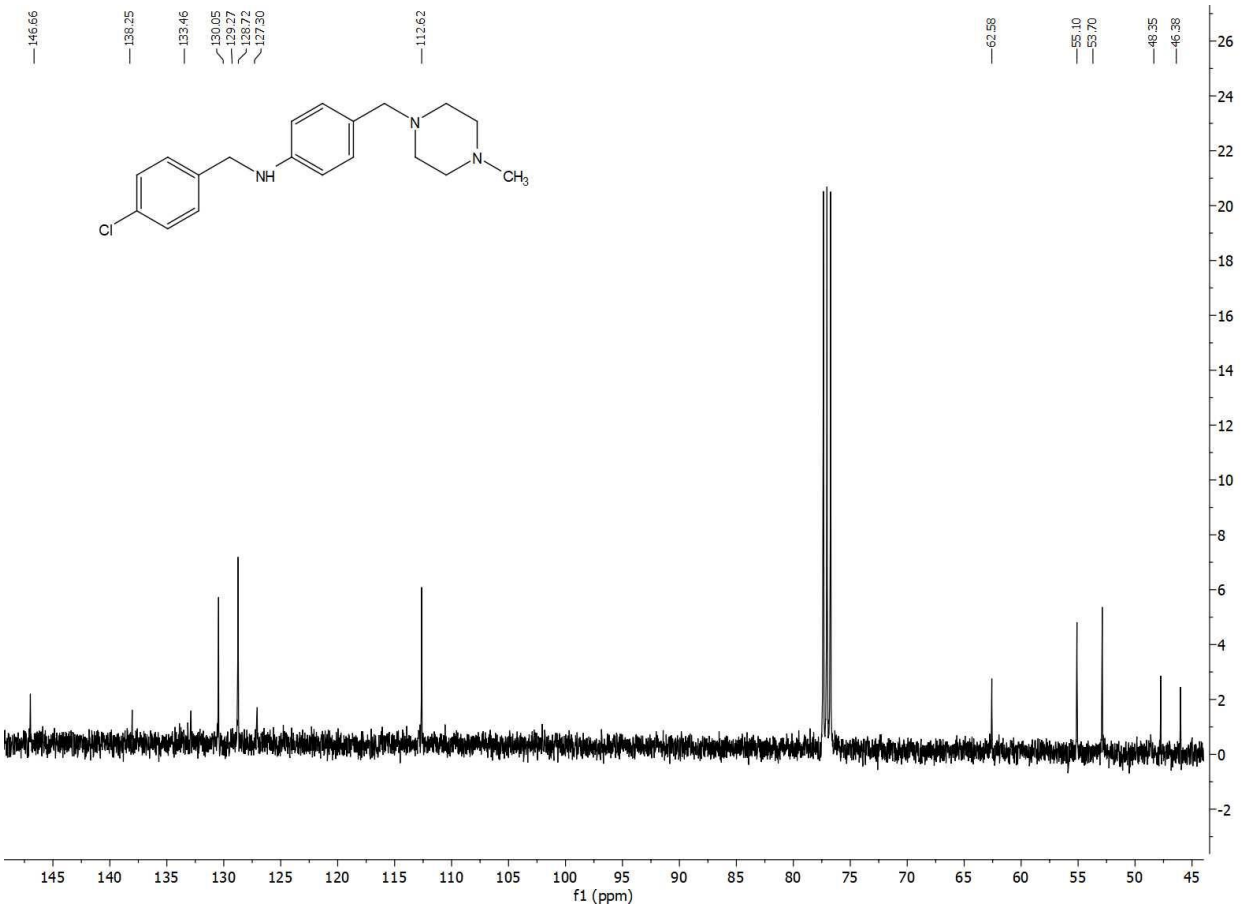


^{1}H NMR of derivative **9**, ***N*-(4-chlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

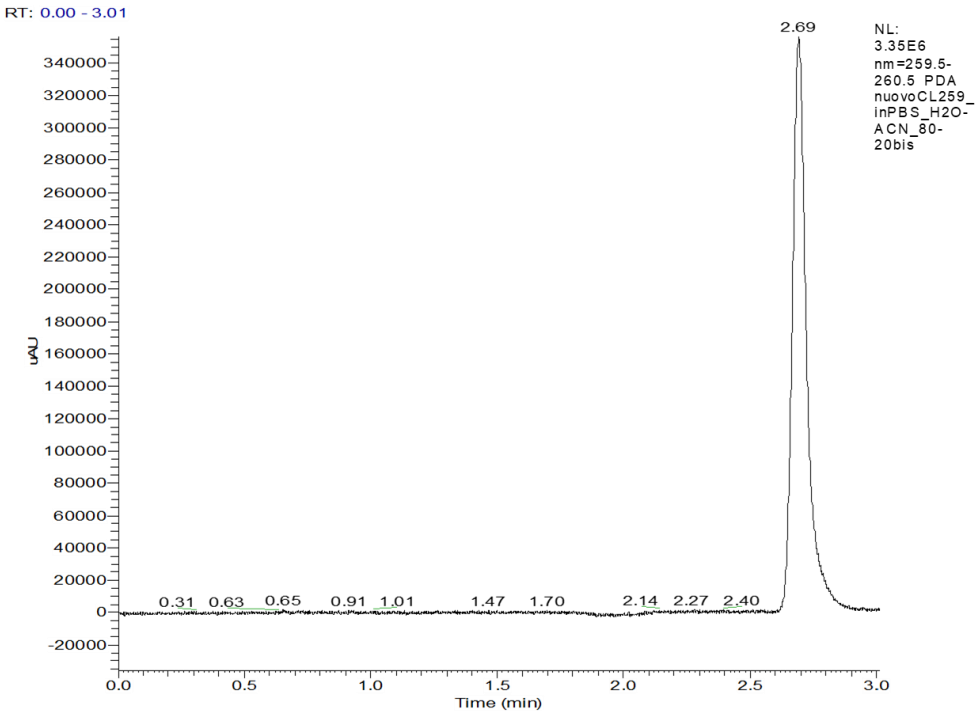
^{1}H NMR of derivative **9**, ***N*-(4-chlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent CD_3OD



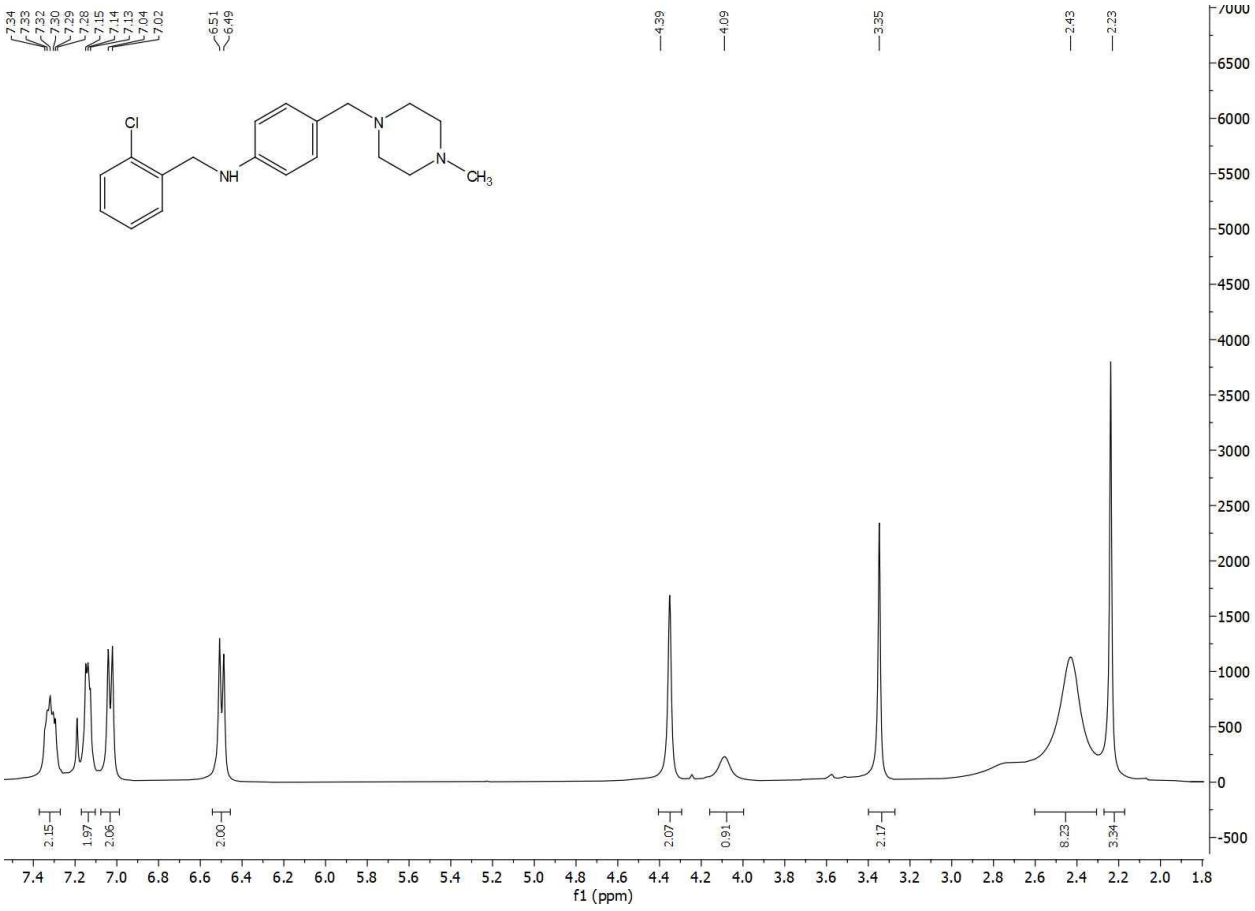
^{13}C NMR of derivative **9**, ***N*-(4-chlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$



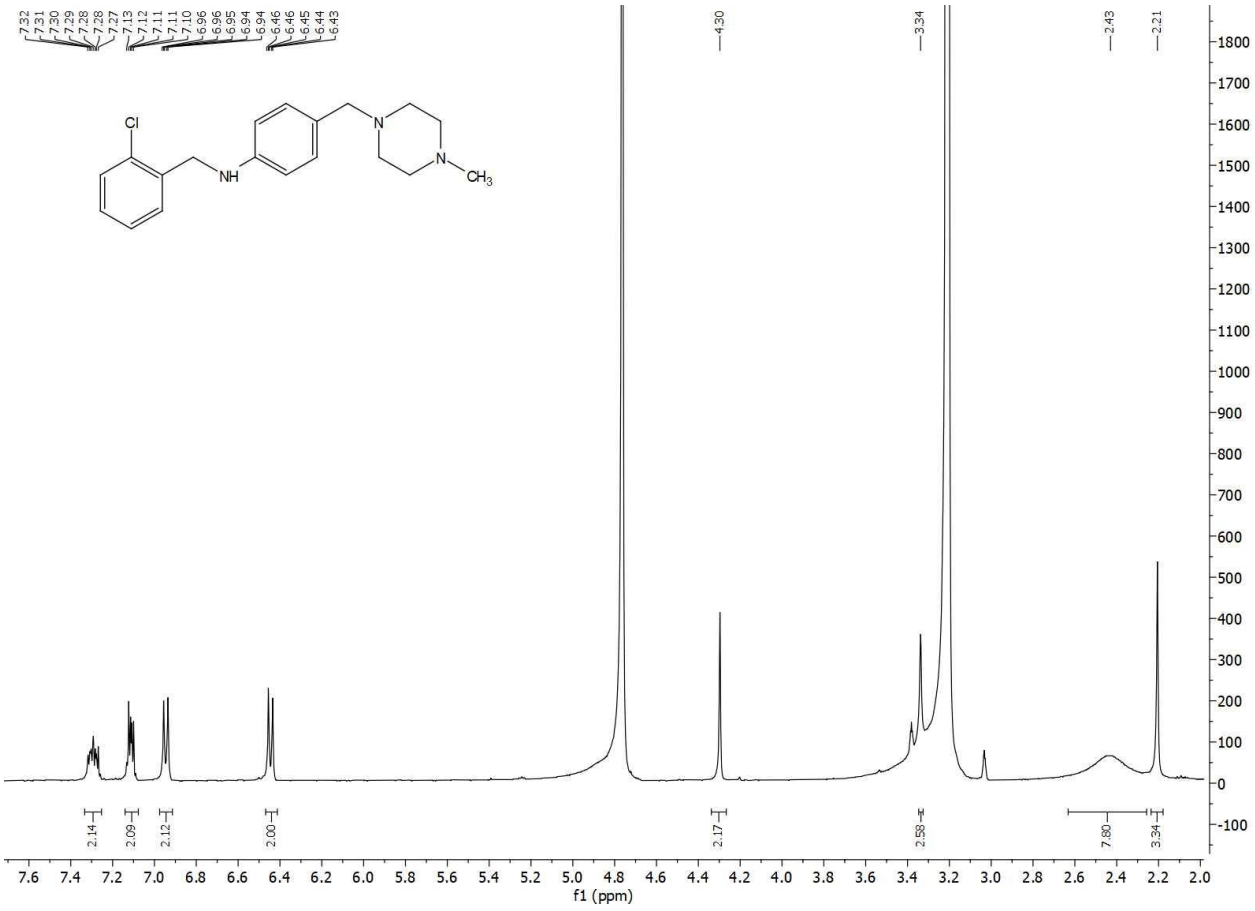
HPLC chart of compound **9**.



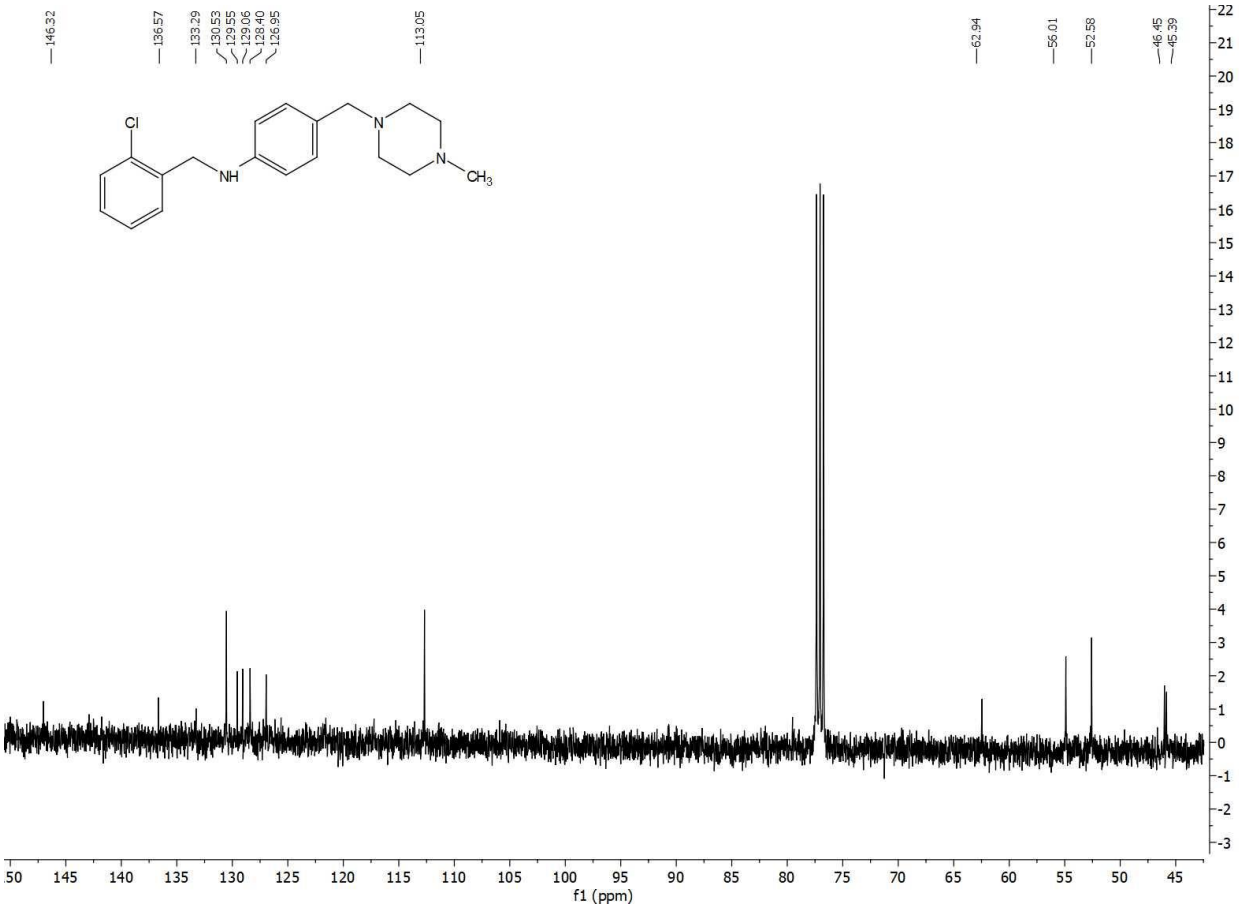
^{1}H NMR of derivative **10**, ***N*-(2-chlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$



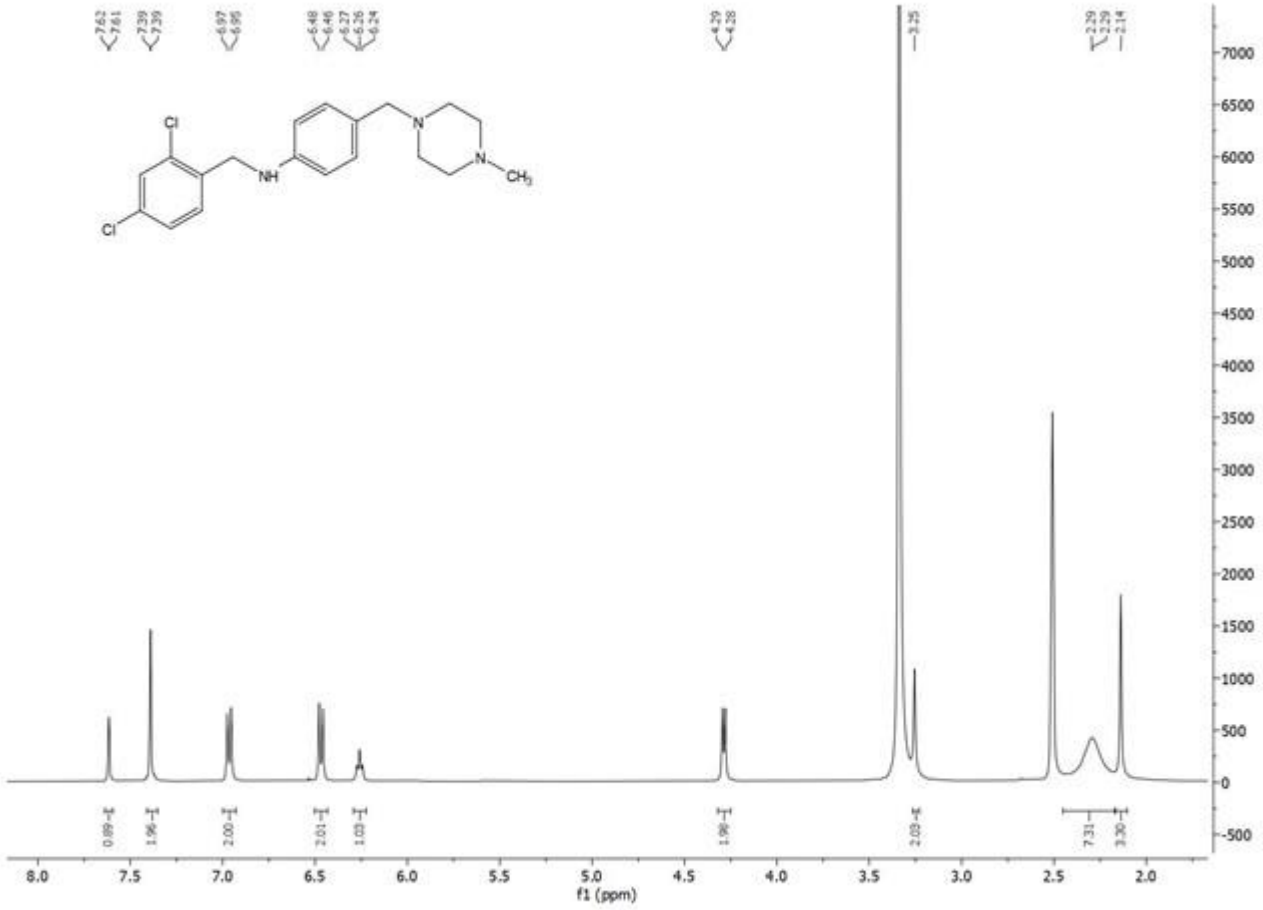
^{1}H NMR of derivative **10**, ***N*-(2-chlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent CD_3OD



C^{13} NMR of derivative **10**, ***N*-(2-chlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

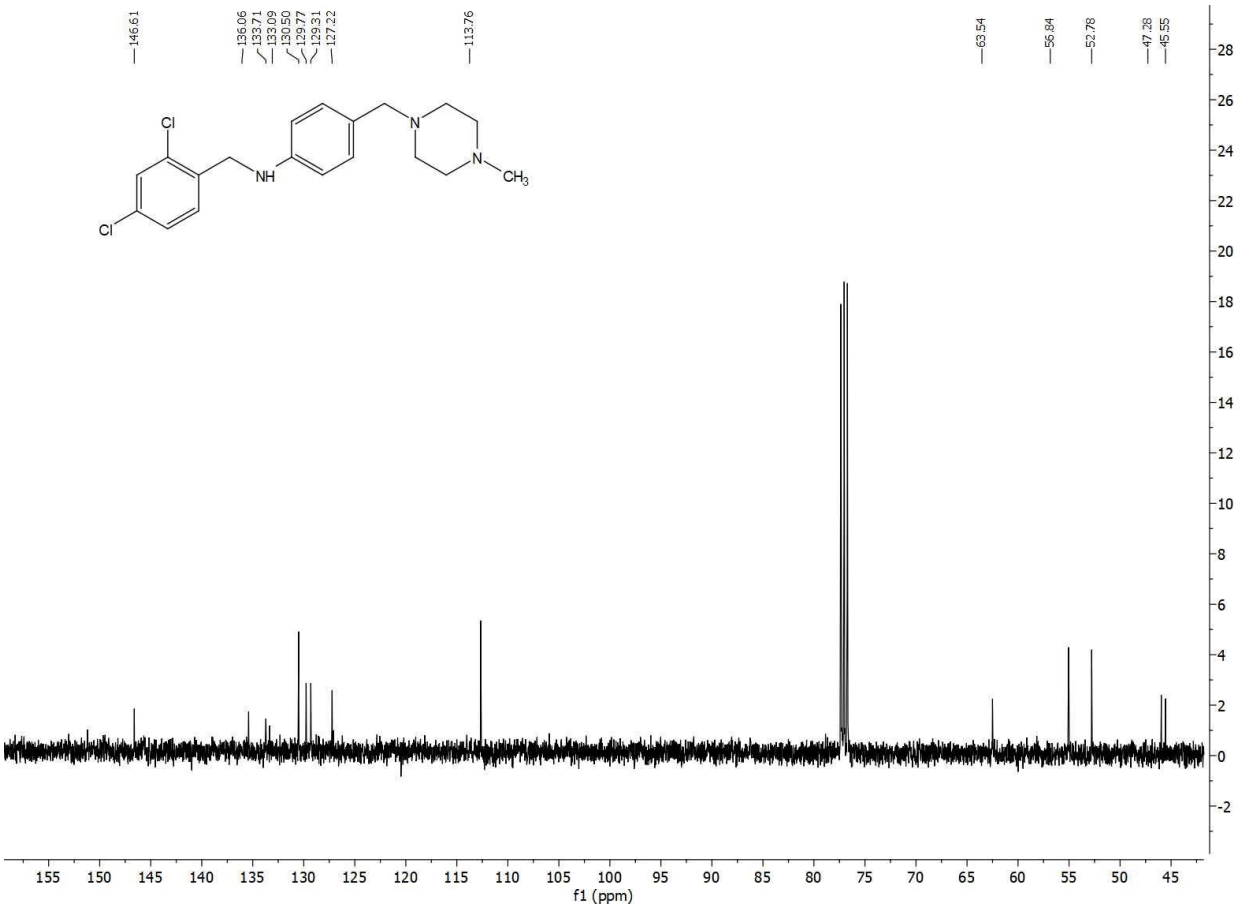


^{1}H NMR of derivative **11**, ***N*-(2,4-dichlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*

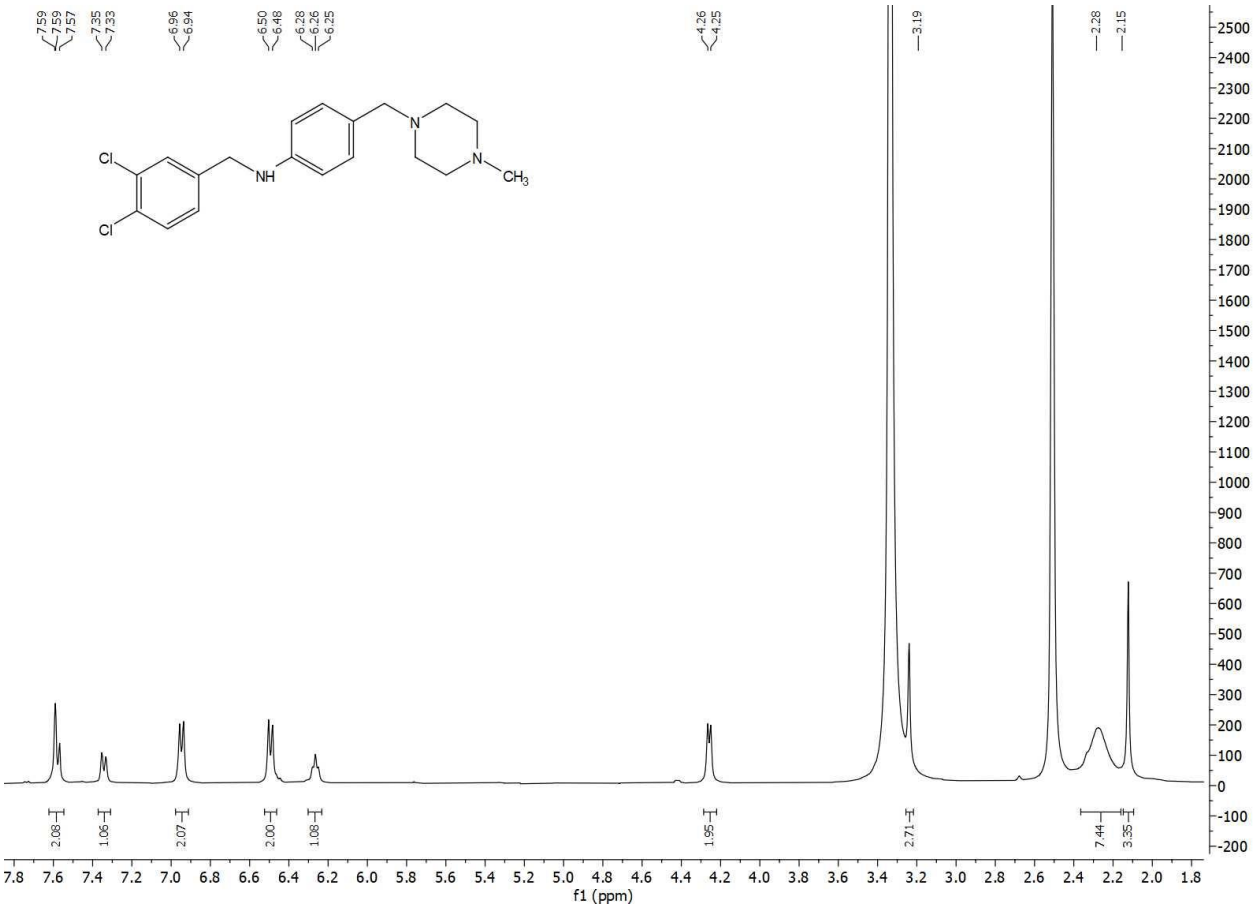


H^1 NMR of derivative **11**, ***N*-(2,4-dichlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO+D_2O

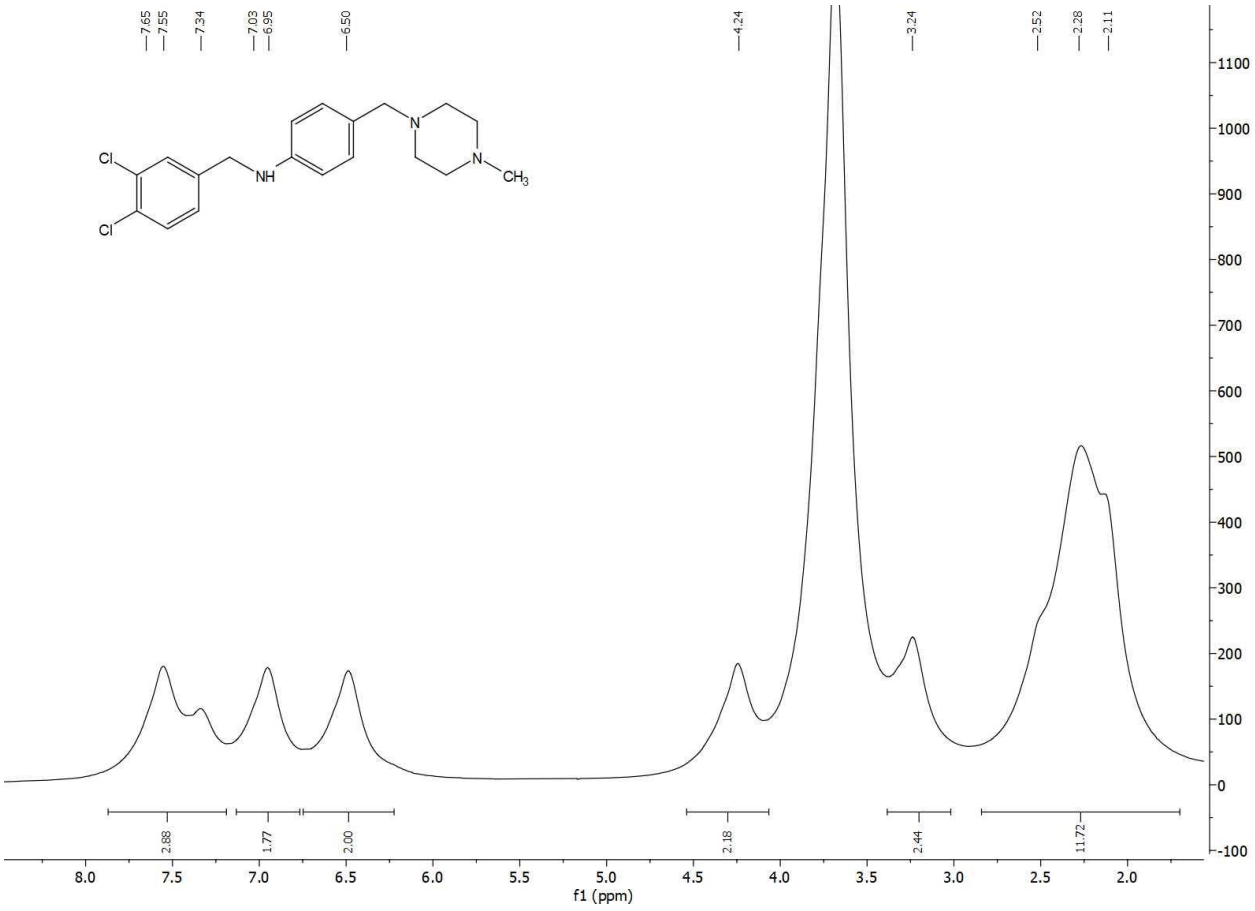
^{13}C NMR of derivative **11**, ***N*-(2,4-dichlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$



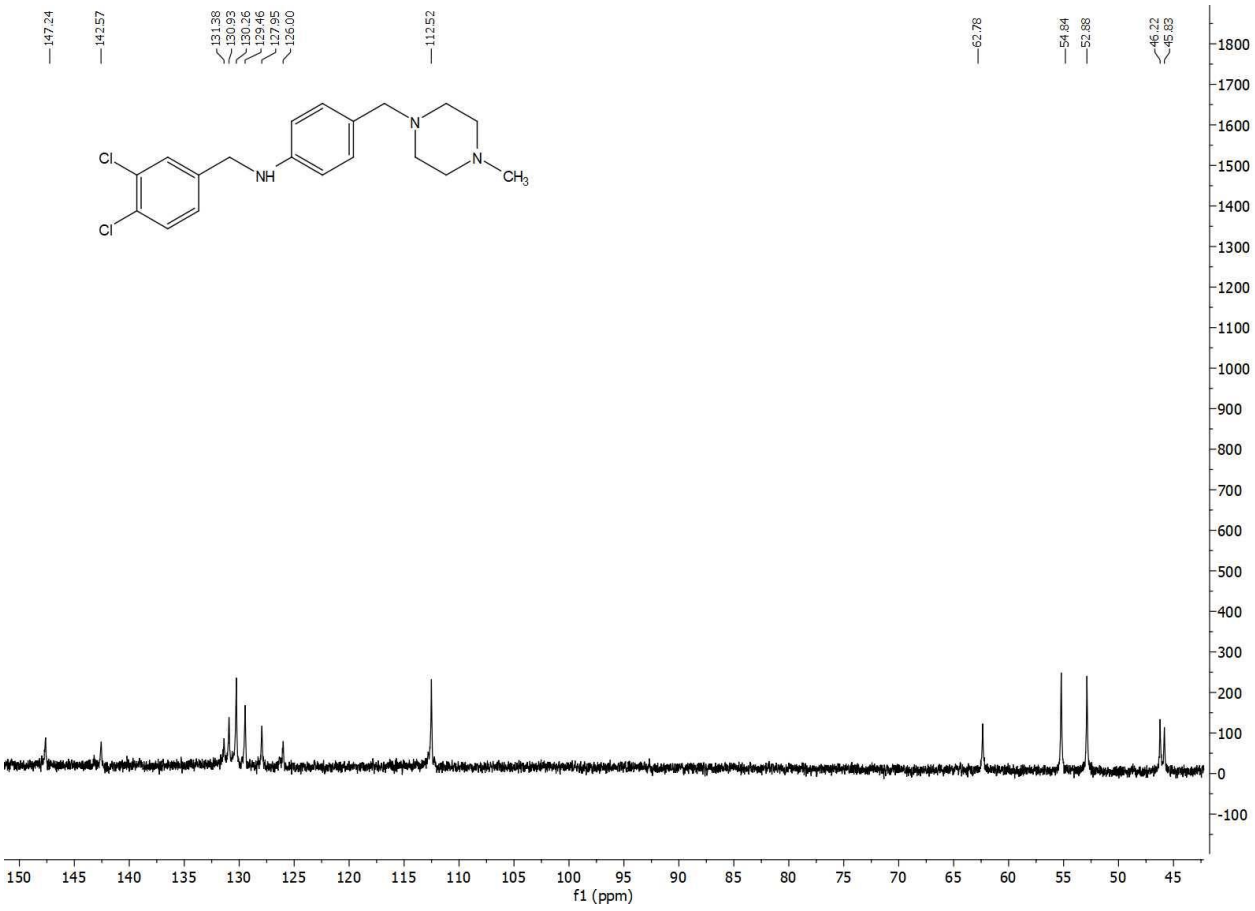
^{1}H NMR of derivative **12**, ***N*-(3,4-dichlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*



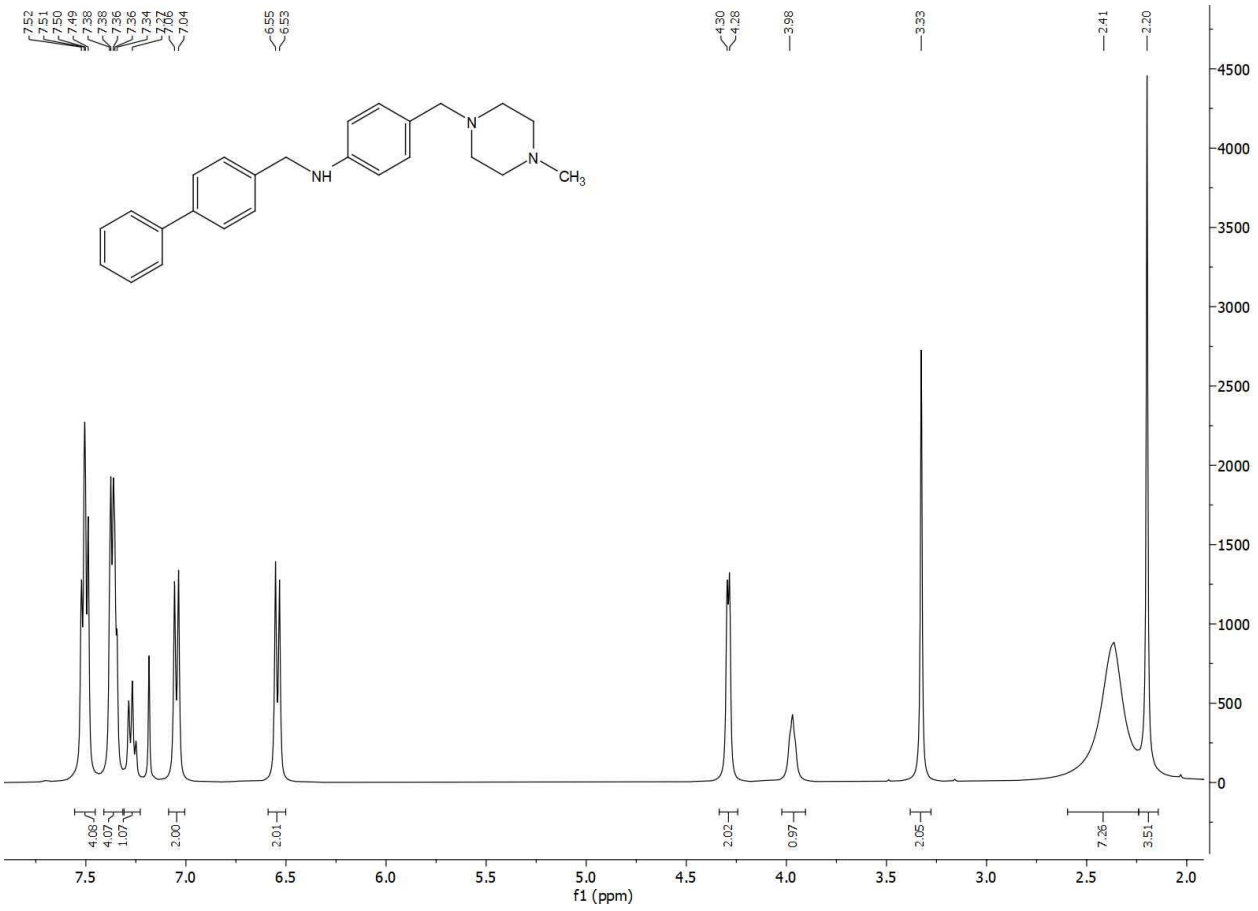
H^1 NMR of derivative **12**, ***N*-(3,4-dichlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*+D_2O



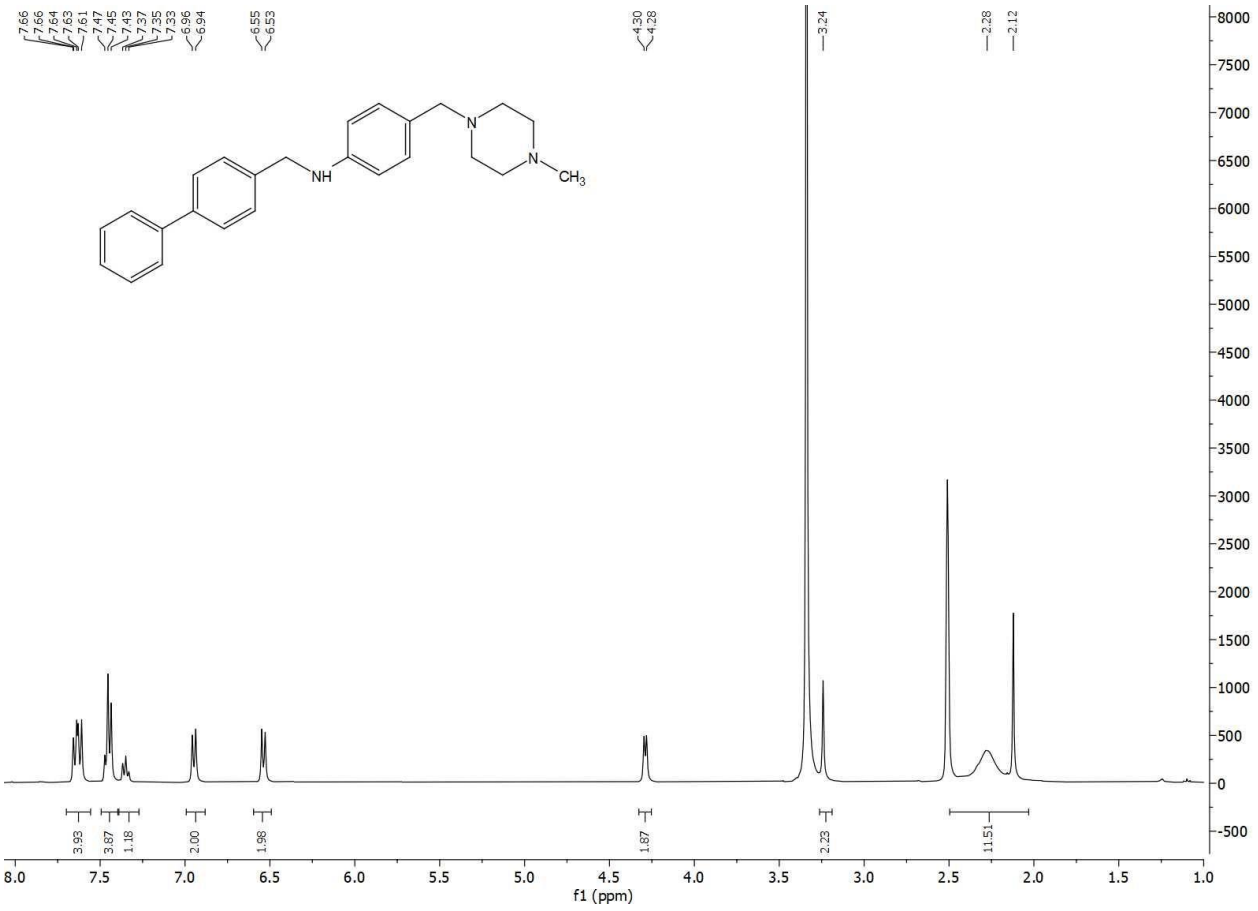
^{13}C NMR of derivative **12**, ***N*-(3,4-dichlorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*



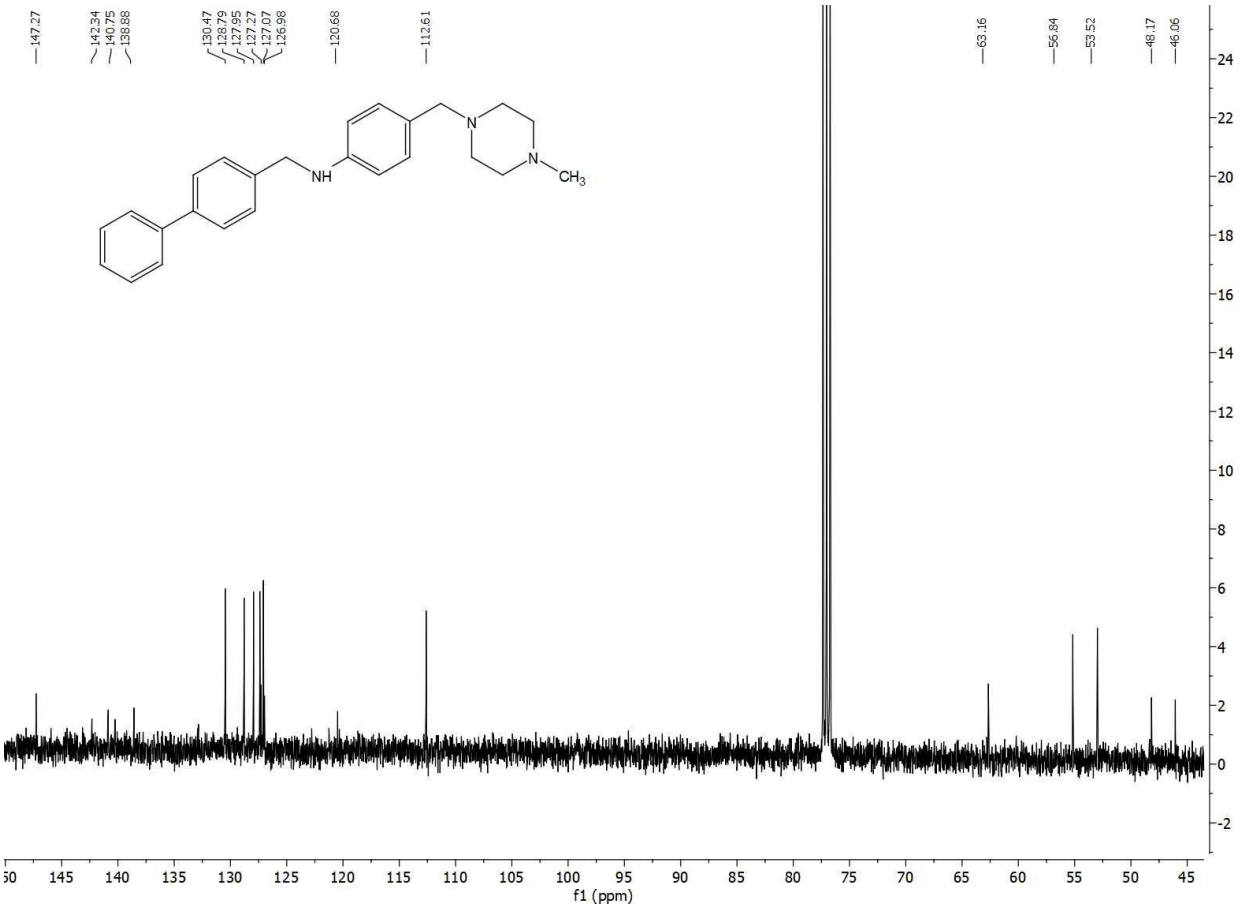
^{1}H NMR of derivative **13**, ***N*-([1,1'-biphenyl]-4-ylmethyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$



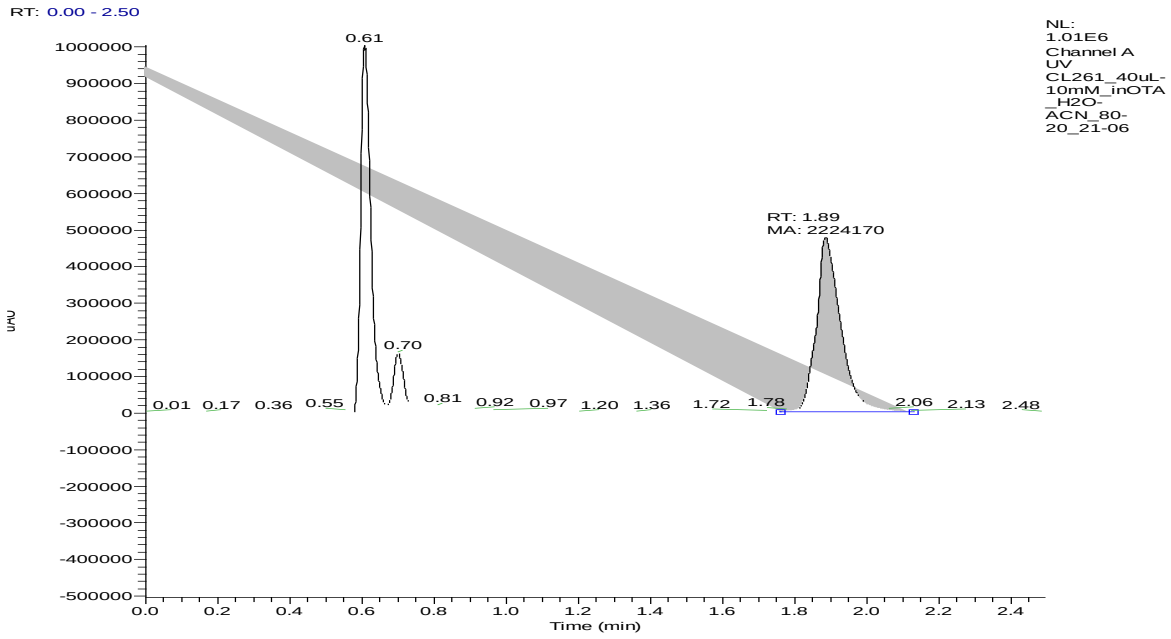
^{1}H NMR of derivative **13**, ***N*-([1,1'-biphenyl]-4-ylmethyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*+D_2O



^{13}C NMR of derivative **13**, ***N*-([1,1'-biphenyl]-4-ylmethyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

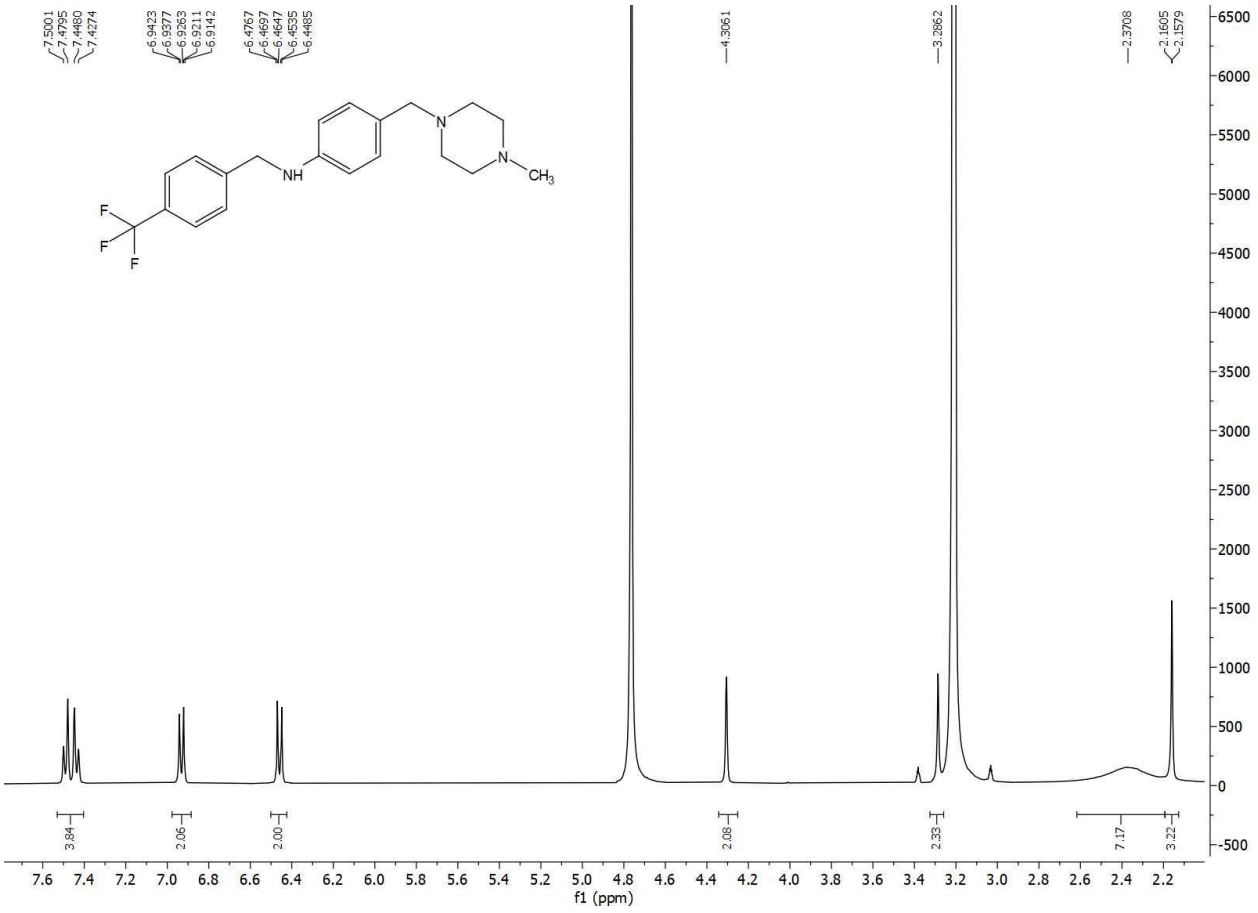


HPLC chart of compound **13**.

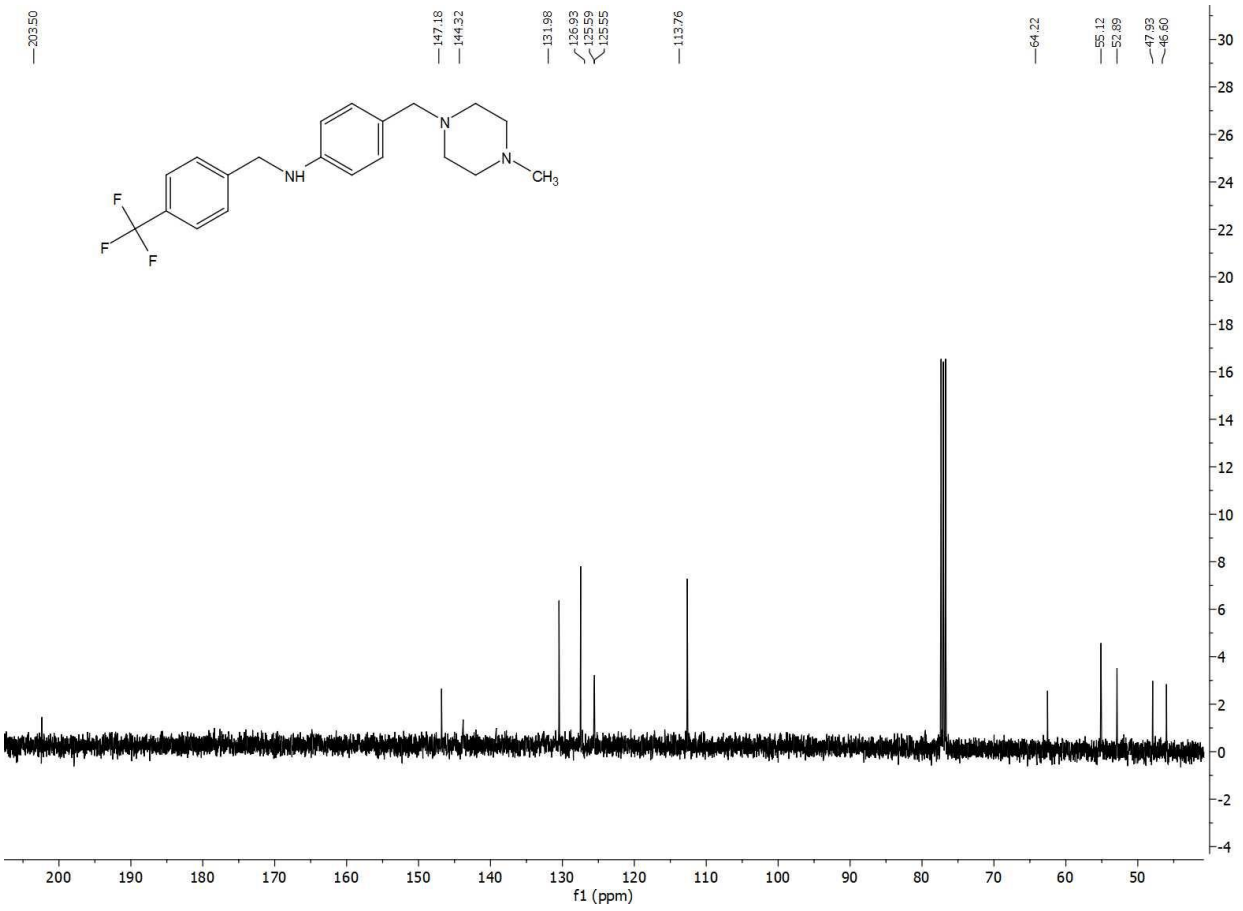


H^1 NMR of derivative **14**, **4-((4-Methylpiperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)benzyl)aniline,** solvent $CDCl_3$

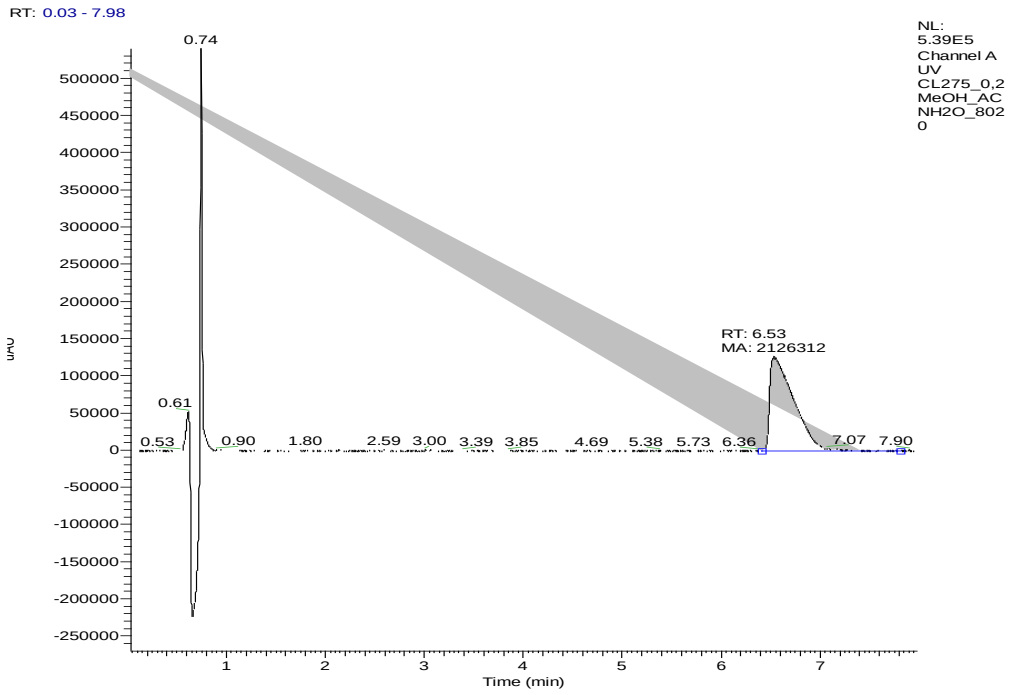
^{1}H NMR of derivative **14**, **4-((4-Methylpiperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)benzyl)aniline,** solvent CD_3OD



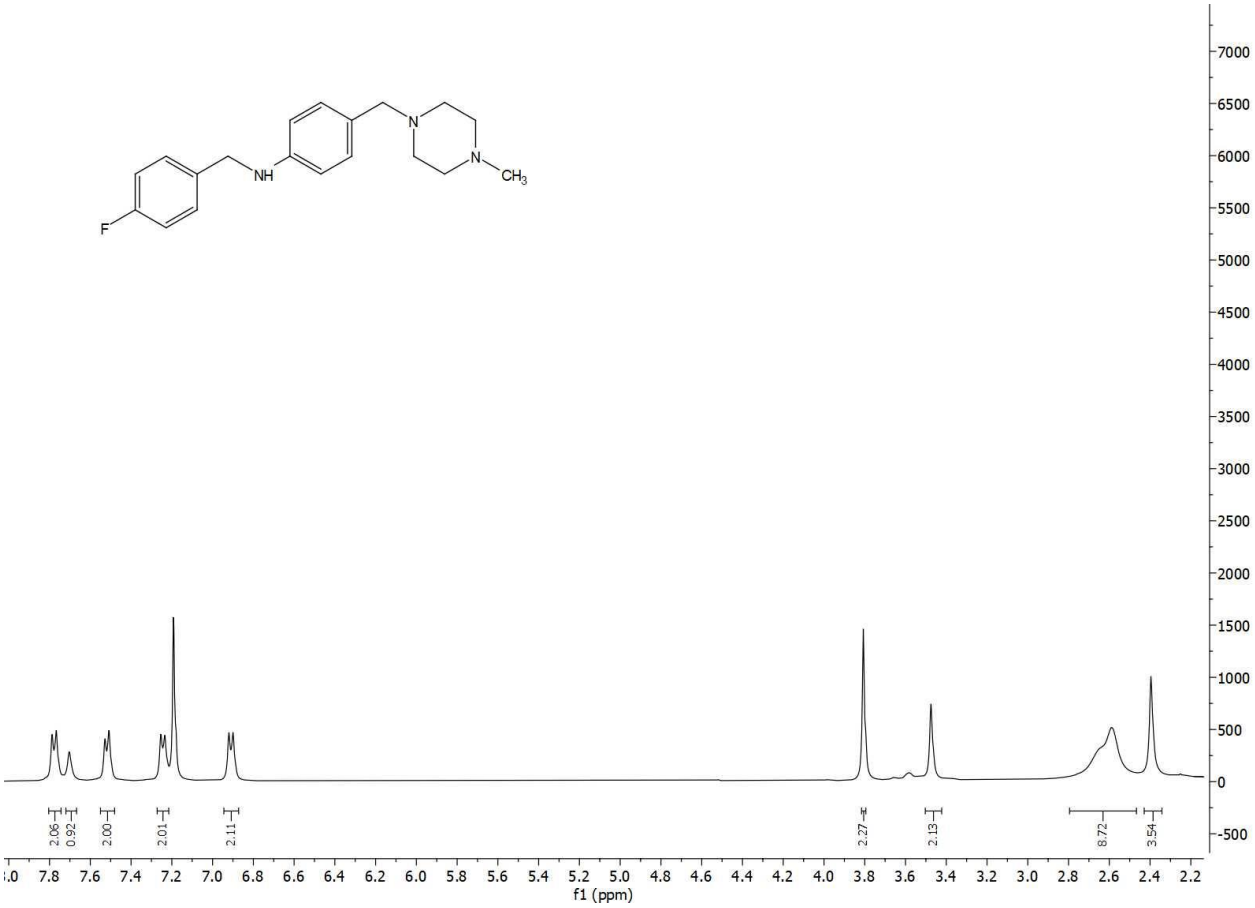
^{13}C NMR of derivative **14**, **4-((4-Methylpiperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)benzyl)aniline,** solvent $CDCl_3$



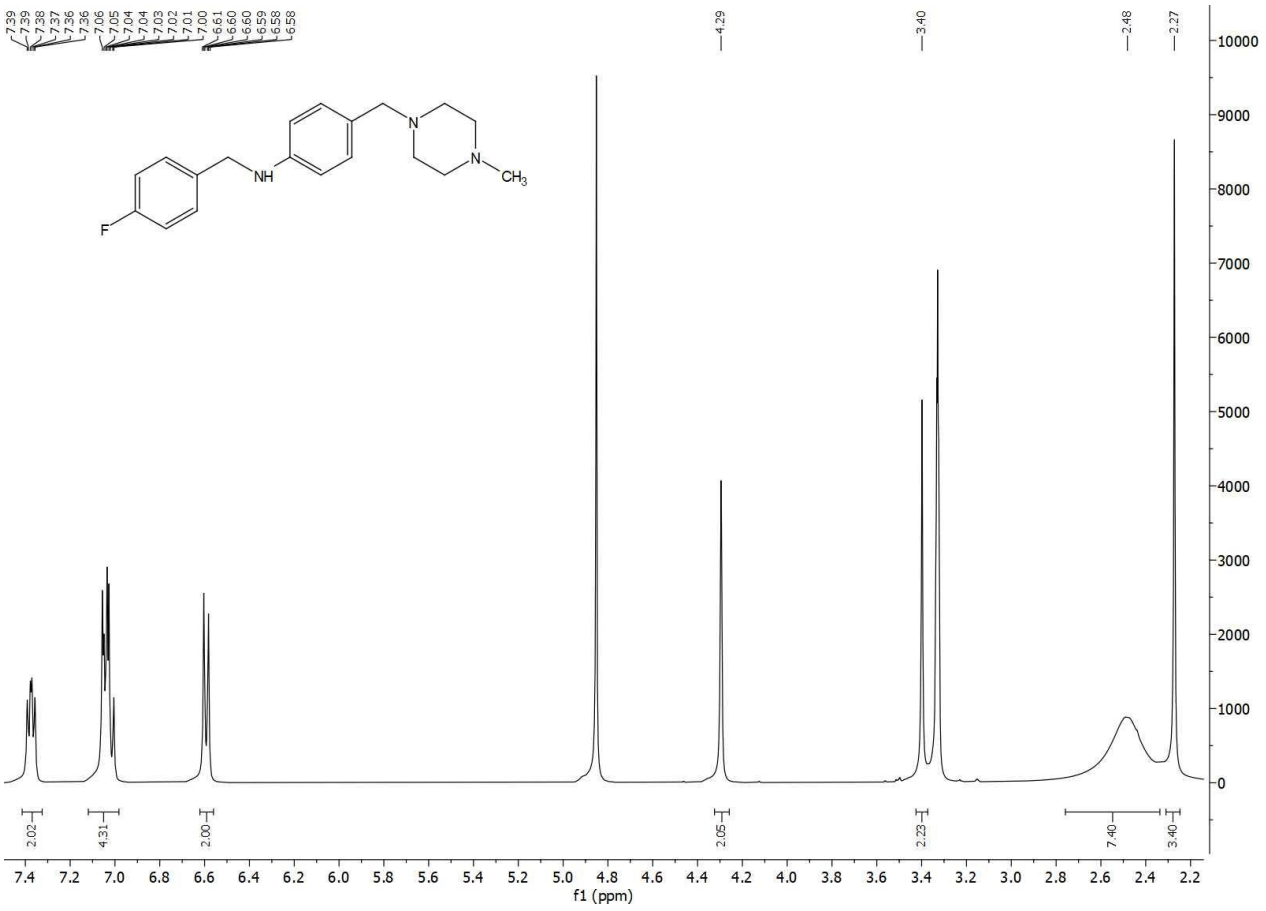
HPLC chart of compound **14**.



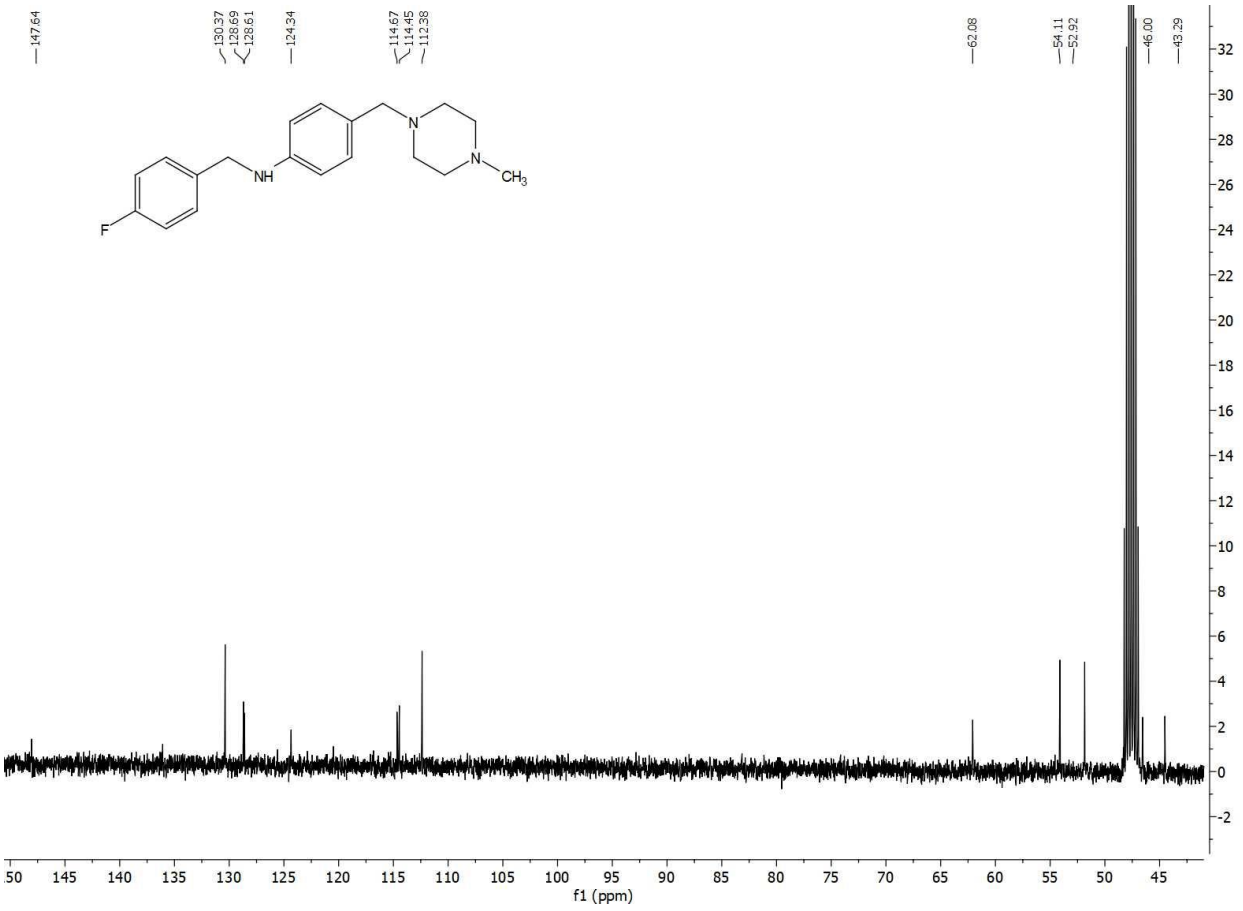
^{1}H NMR of derivative **15**, ***N*-(4-fluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

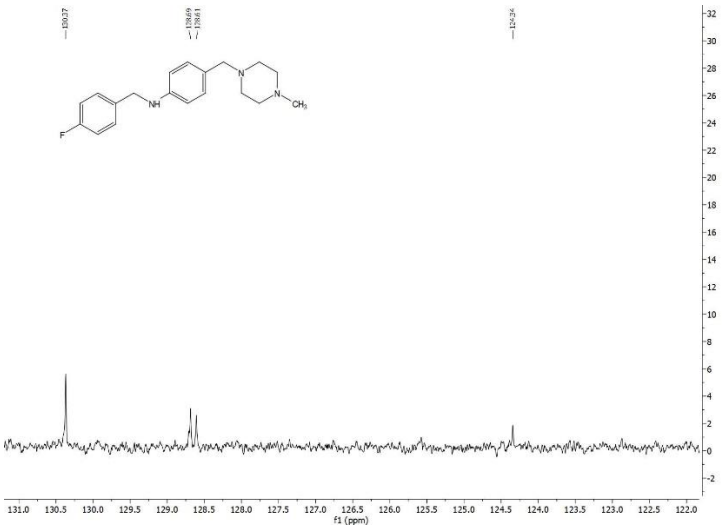


^{1}H NMR of derivative **15**, ***N*-(4-fluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent CD_3OD

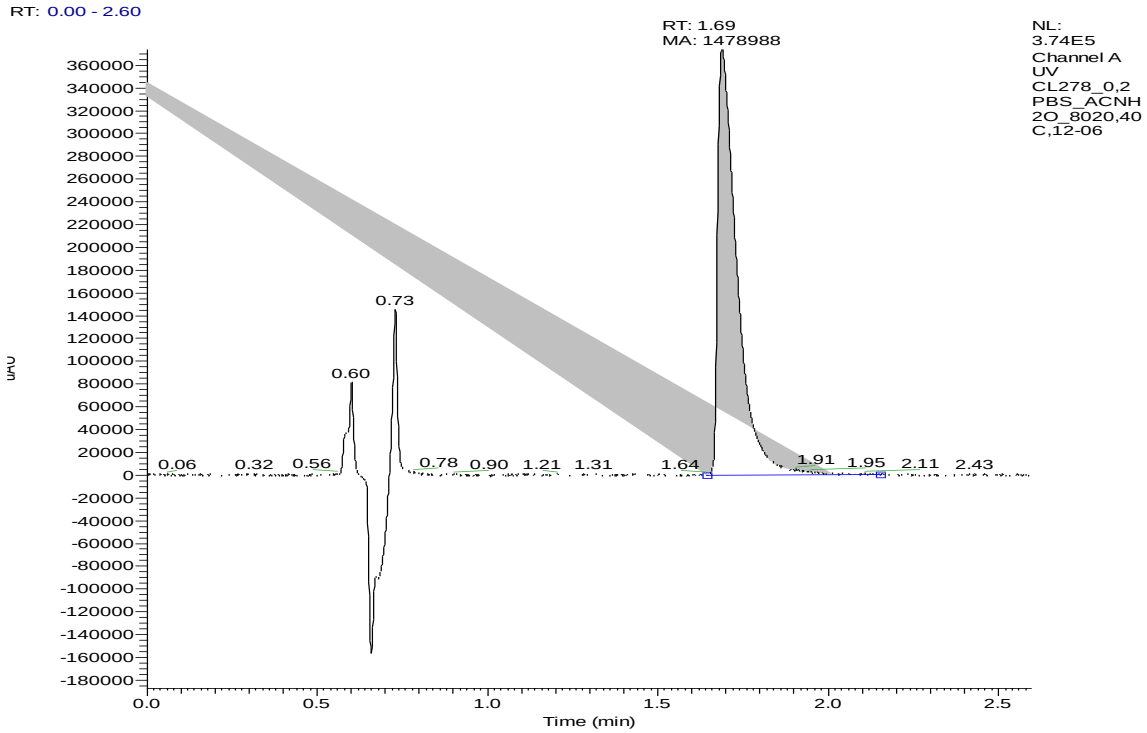


^{13}C NMR of derivative **15**, ***N*-(4-fluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent CD_3OD

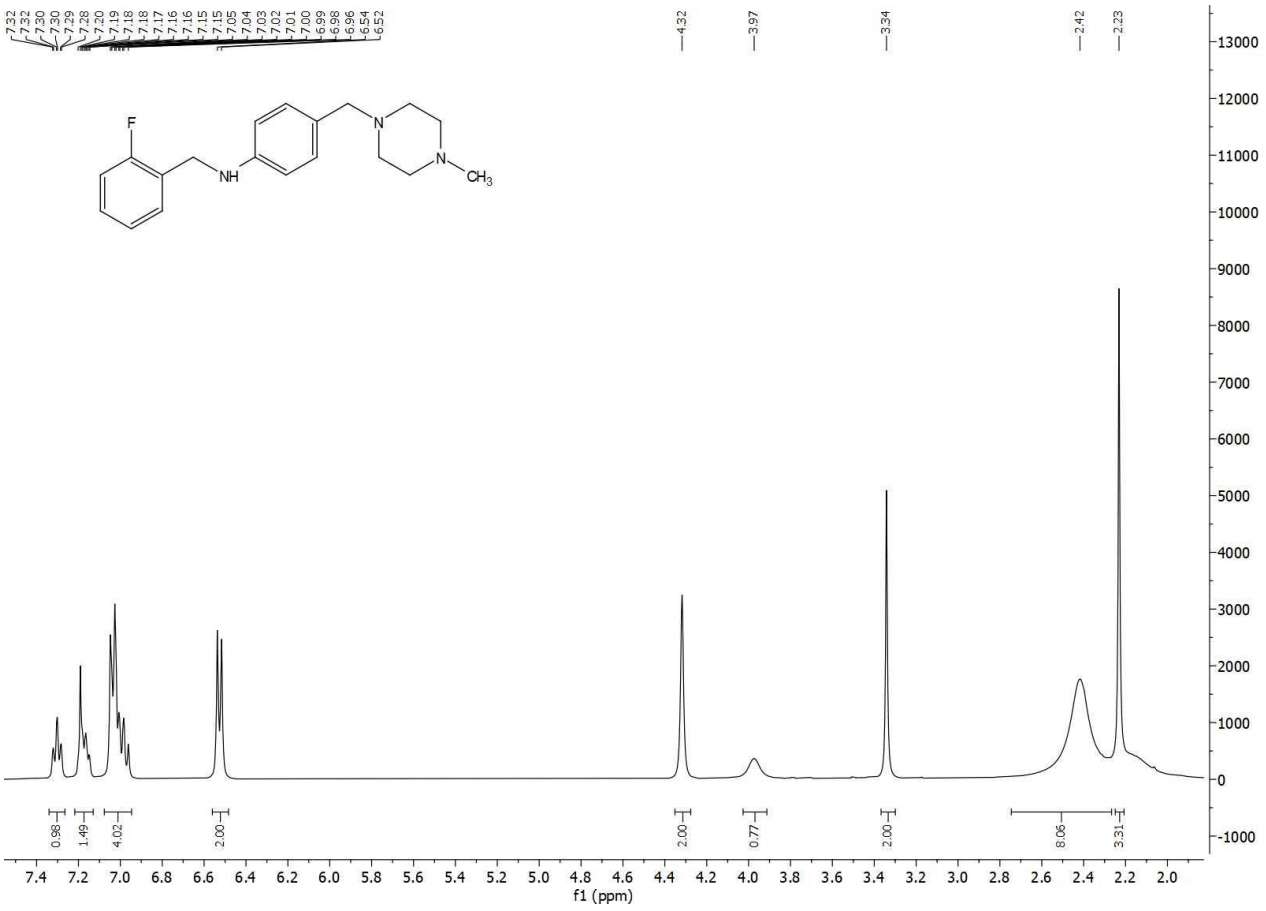




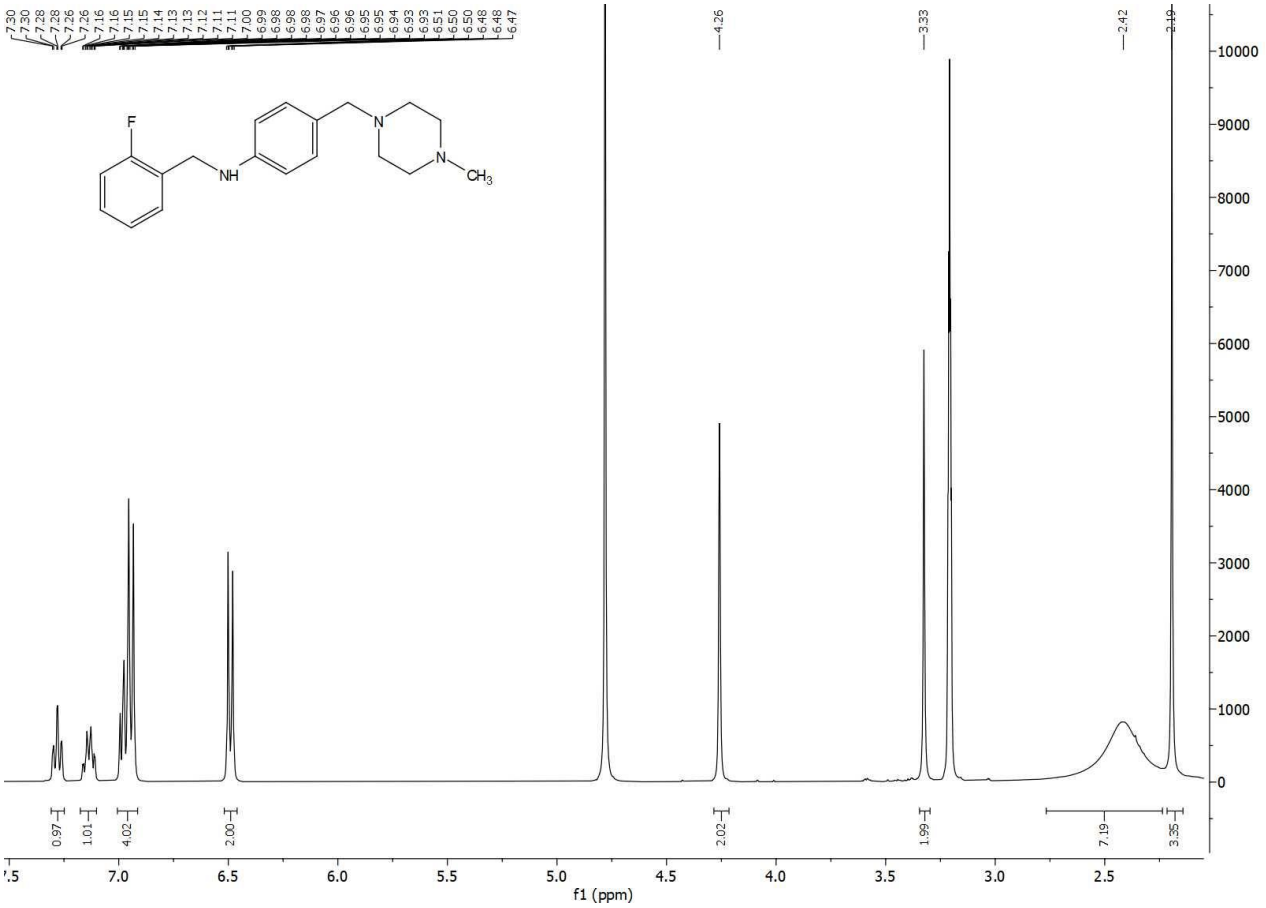
HPLC chart of compound **15**.



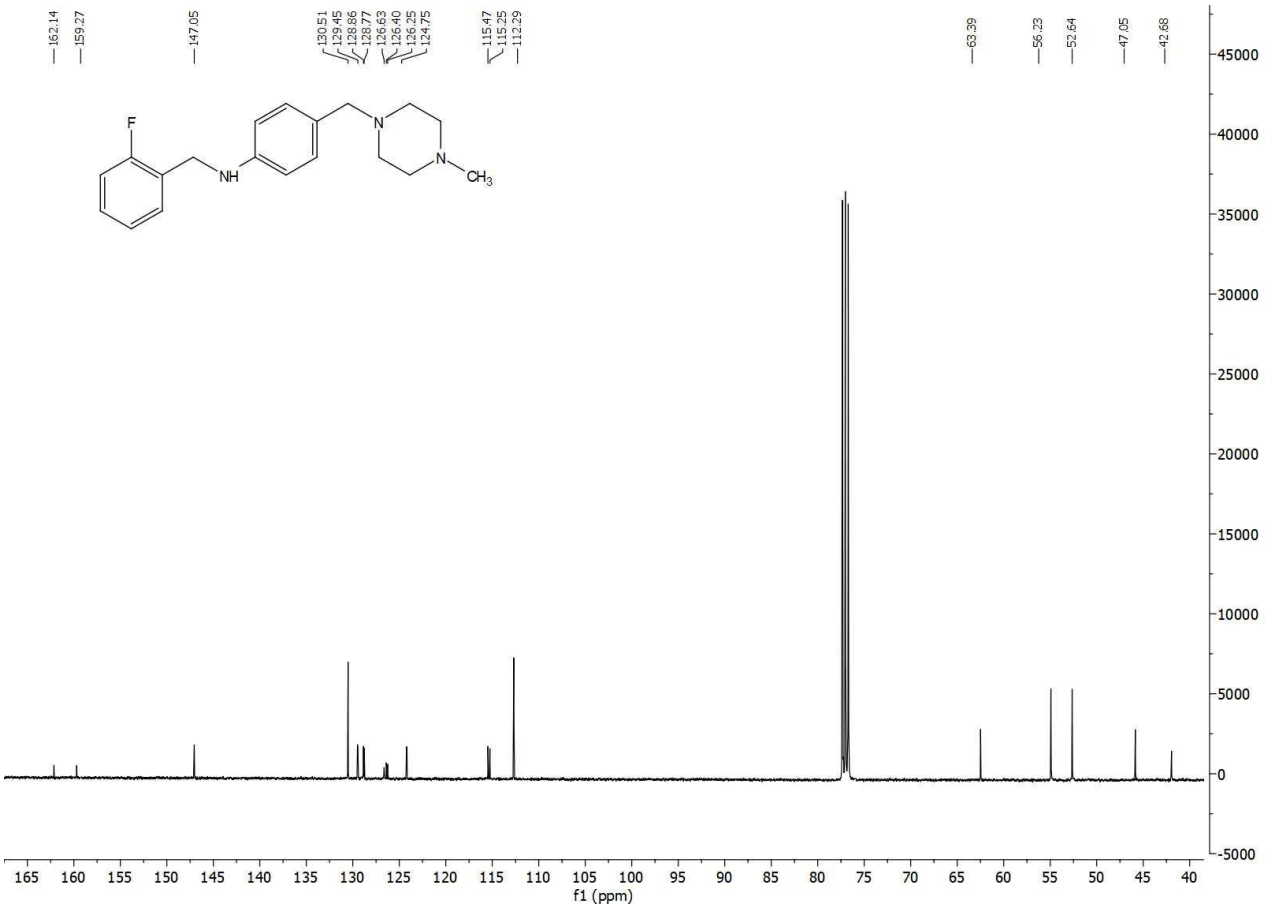
^{1}H NMR of derivative **16**, ***N*-(2-fluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

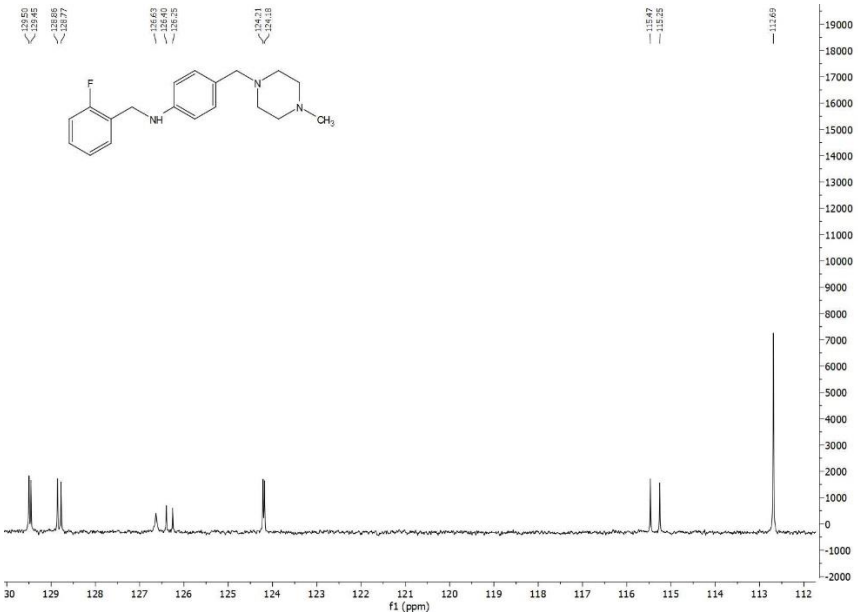


^{1}H NMR of derivative **16**, ***N*-(2-fluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent CD_3OD

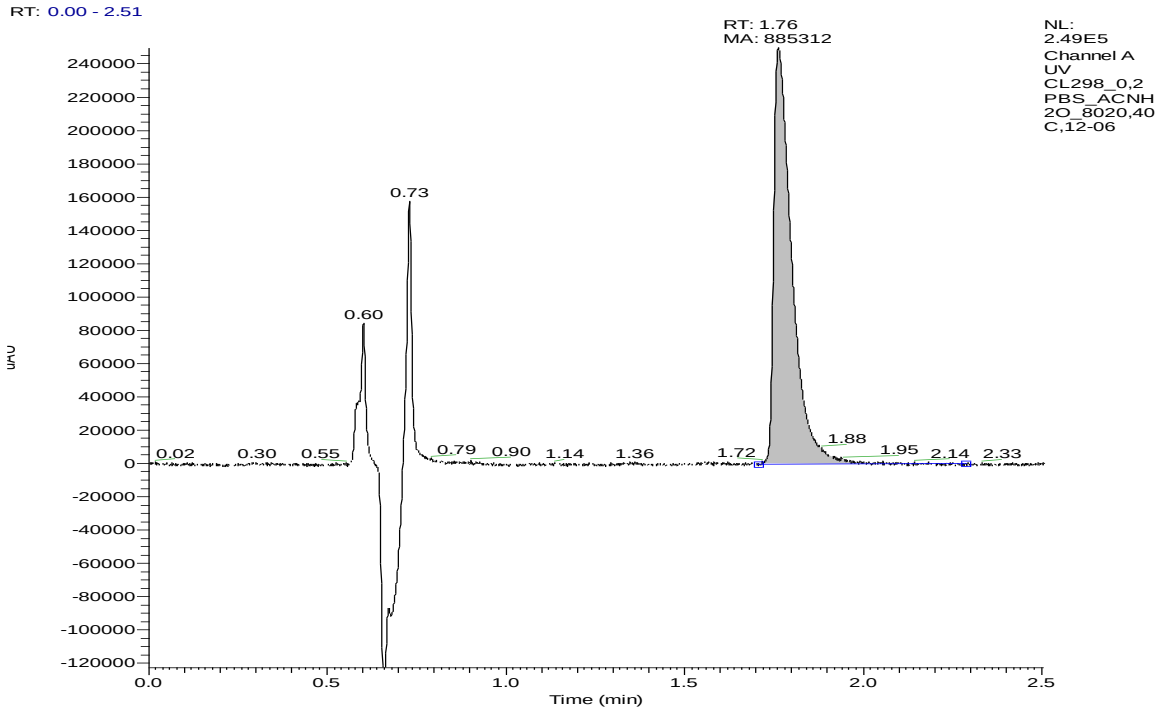


^{13}C NMR of derivative **16**, ***N*-(2-fluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

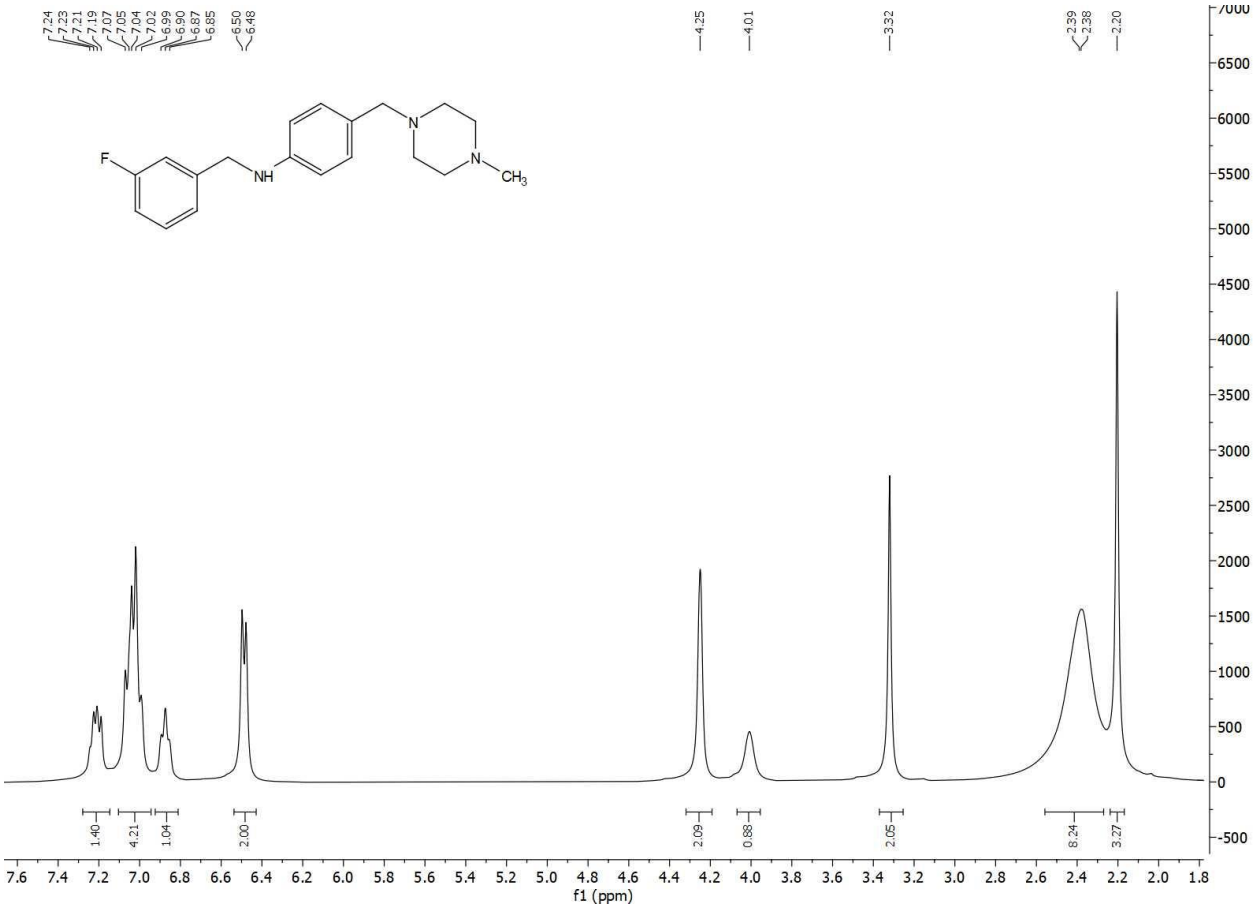




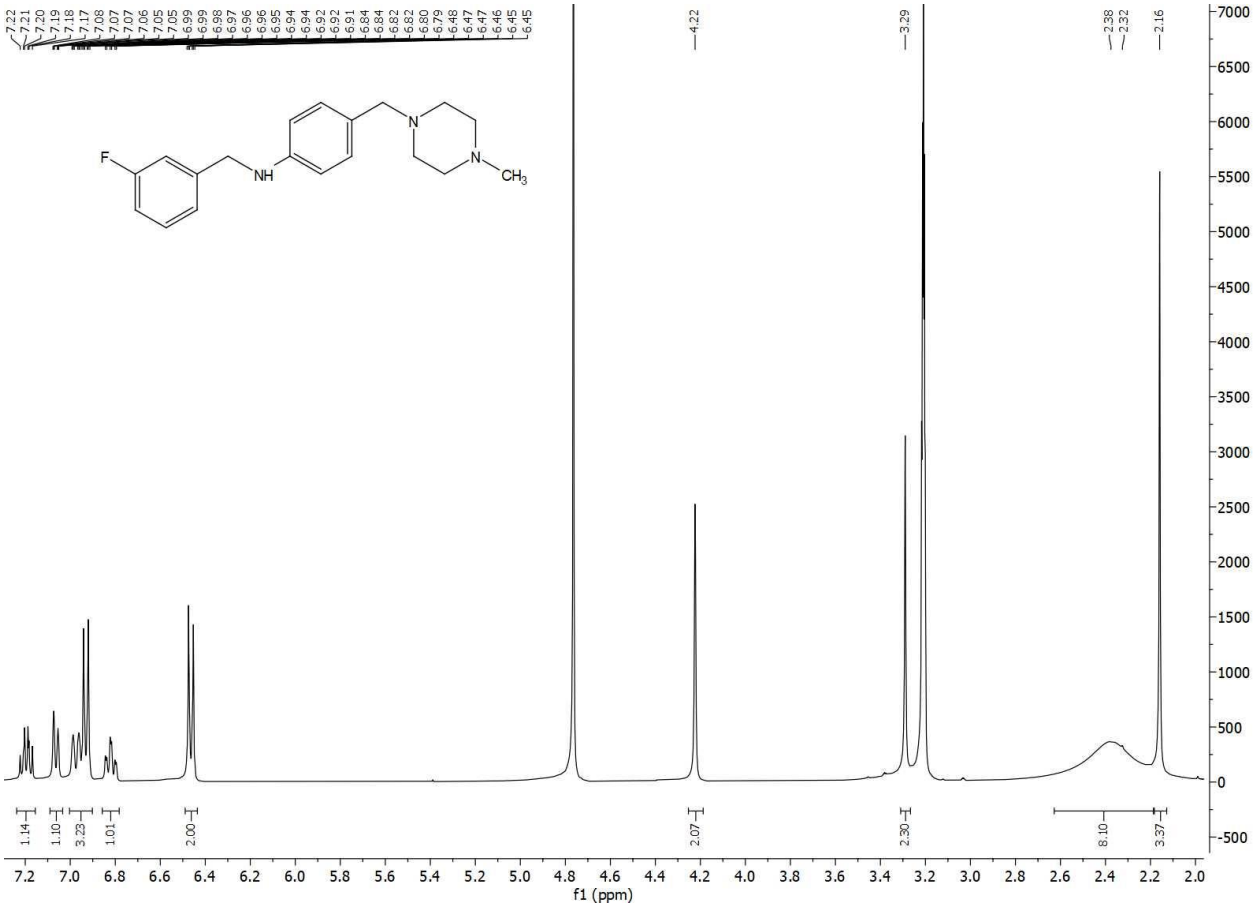
HPLC chart of compound **16**.



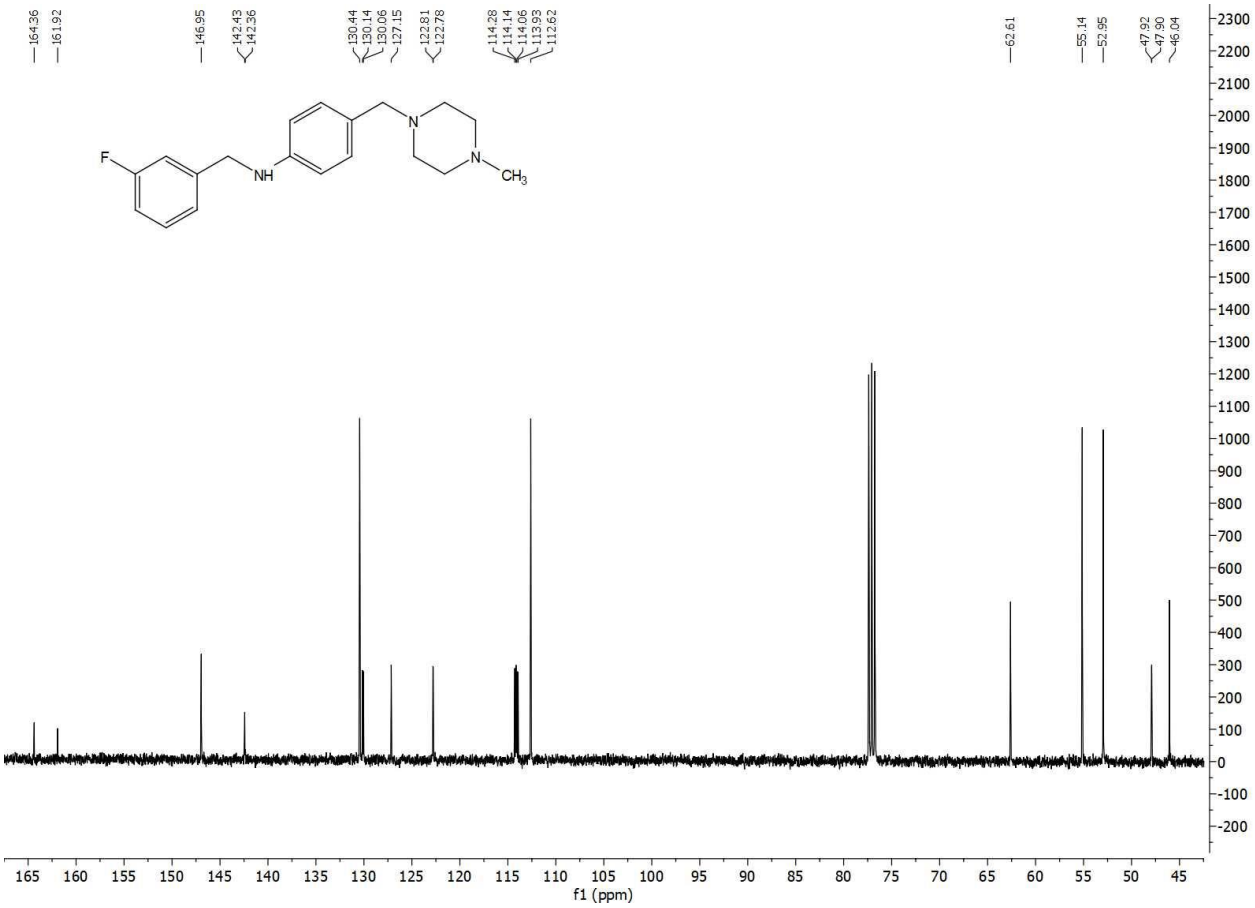
^{1}H NMR of derivative **17**, ***N*-(3-fluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

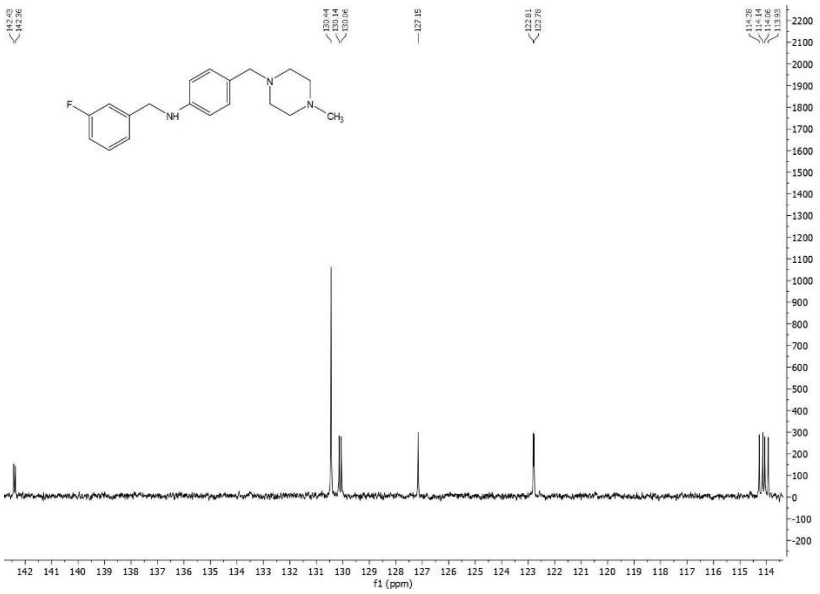


H^1 NMR of derivative **17**, ***N*-(3-fluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent CD_3OD

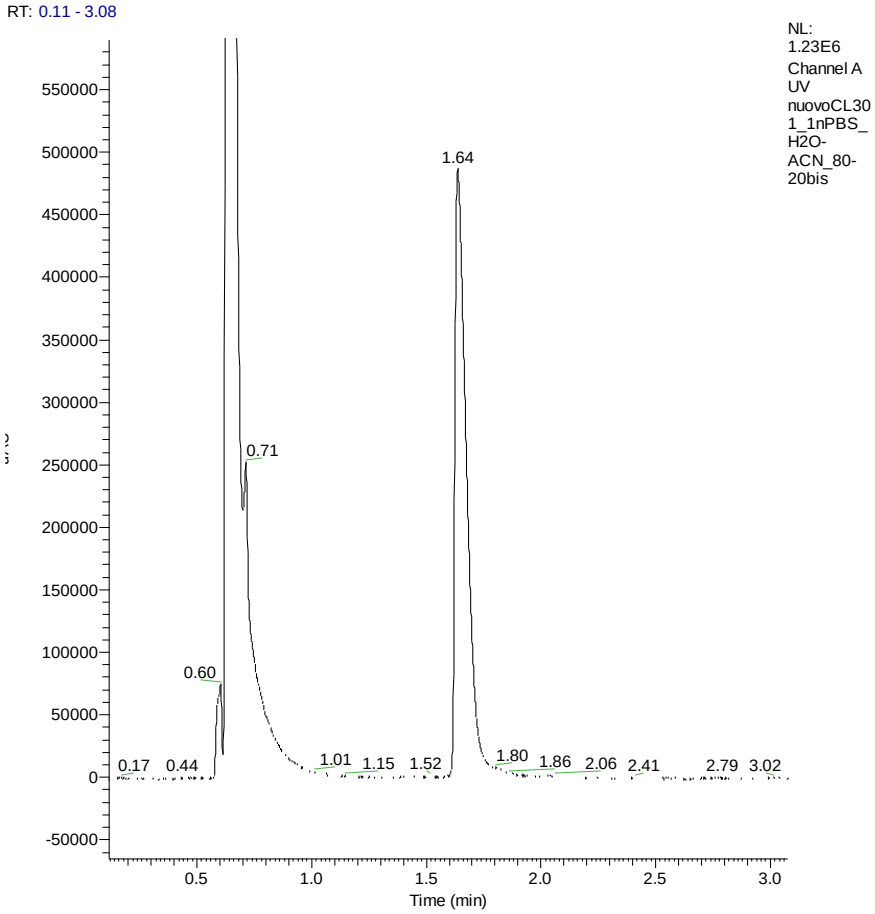


C^{13} NMR of derivative **17**, ***N*-(3-fluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

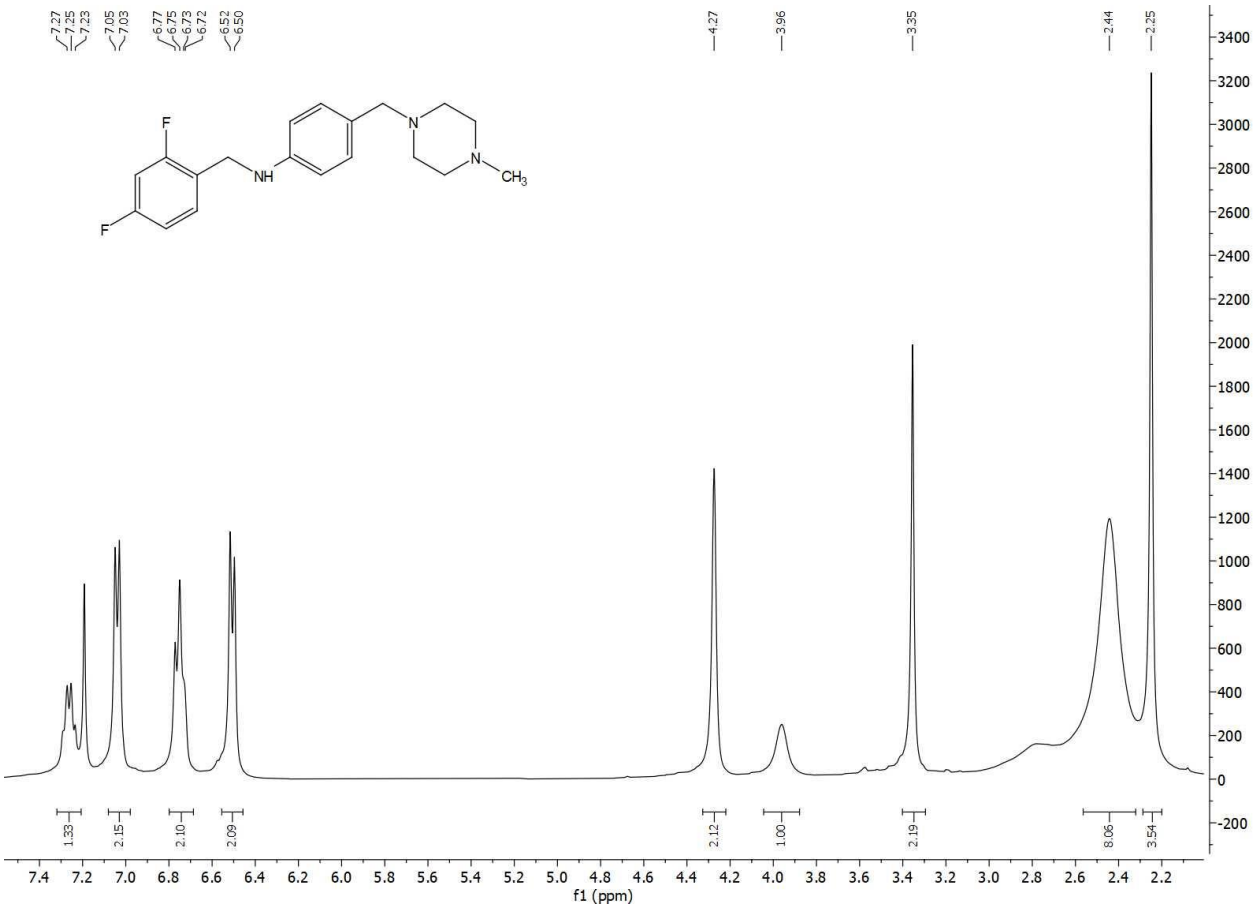




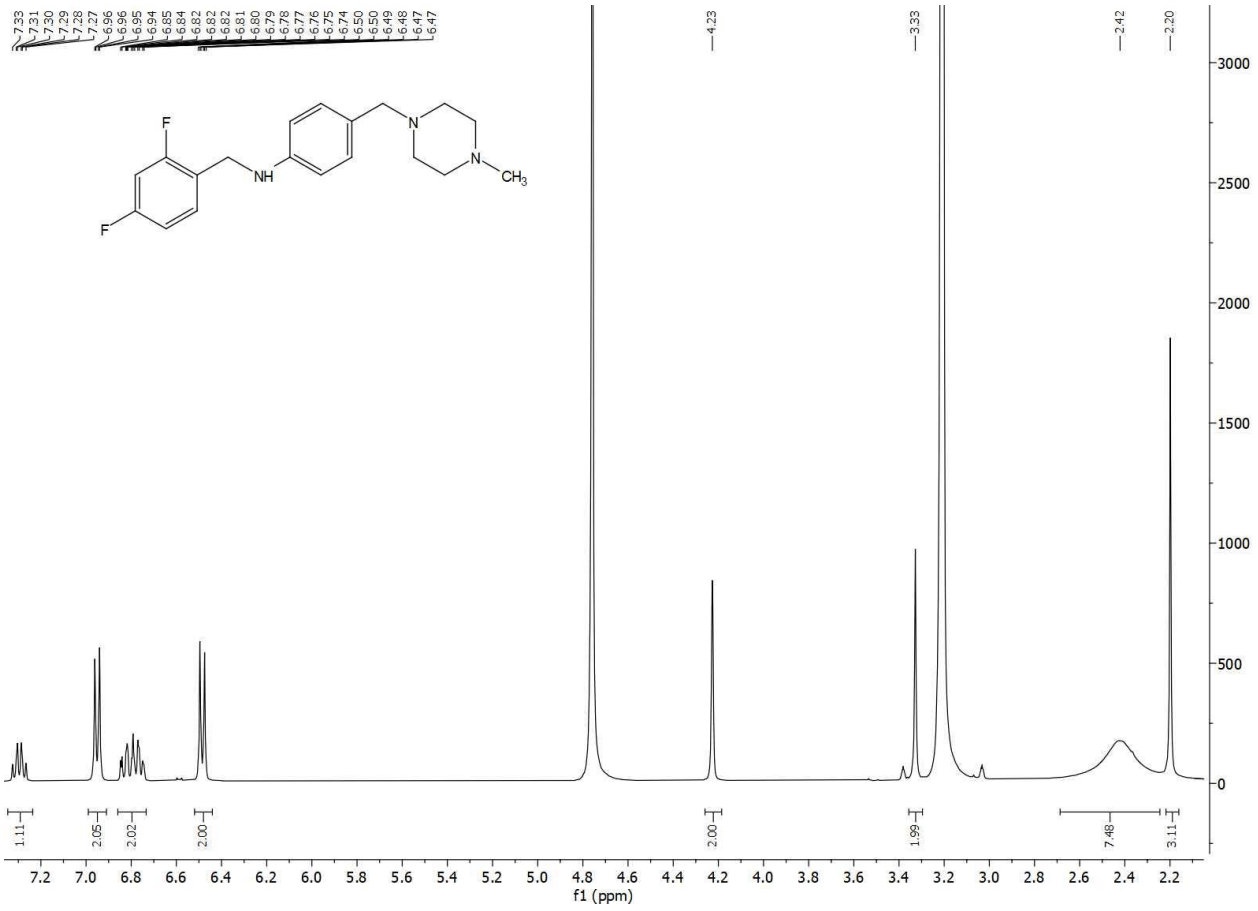
HPLC chart of compound **17**.



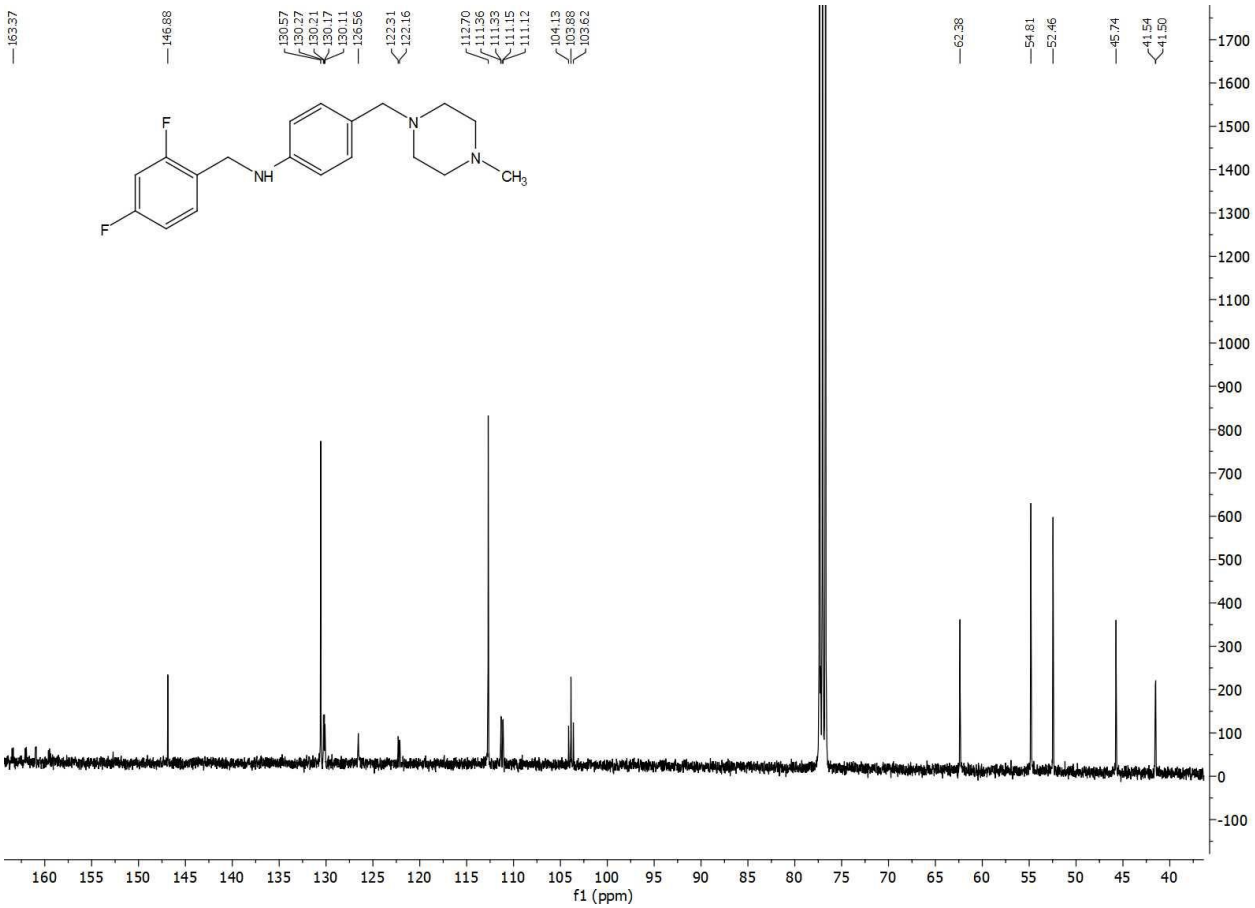
^{1}H NMR of derivative **18**, ***N*-(2,4-difluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

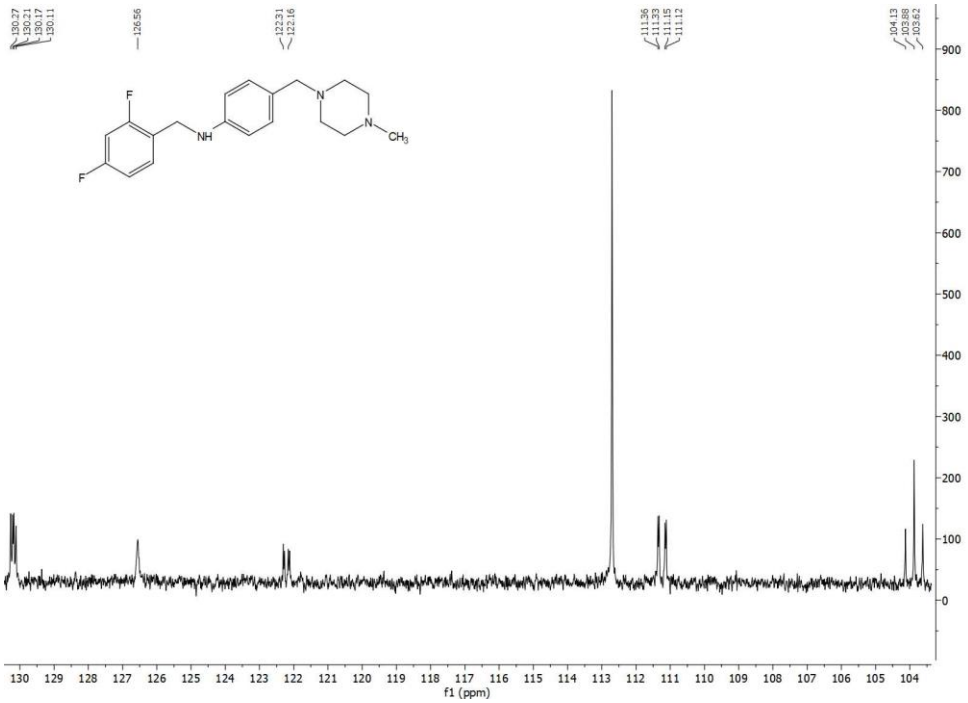


^{1}H NMR of derivative **18**, ***N*-(2,4-difluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent CD_3OD

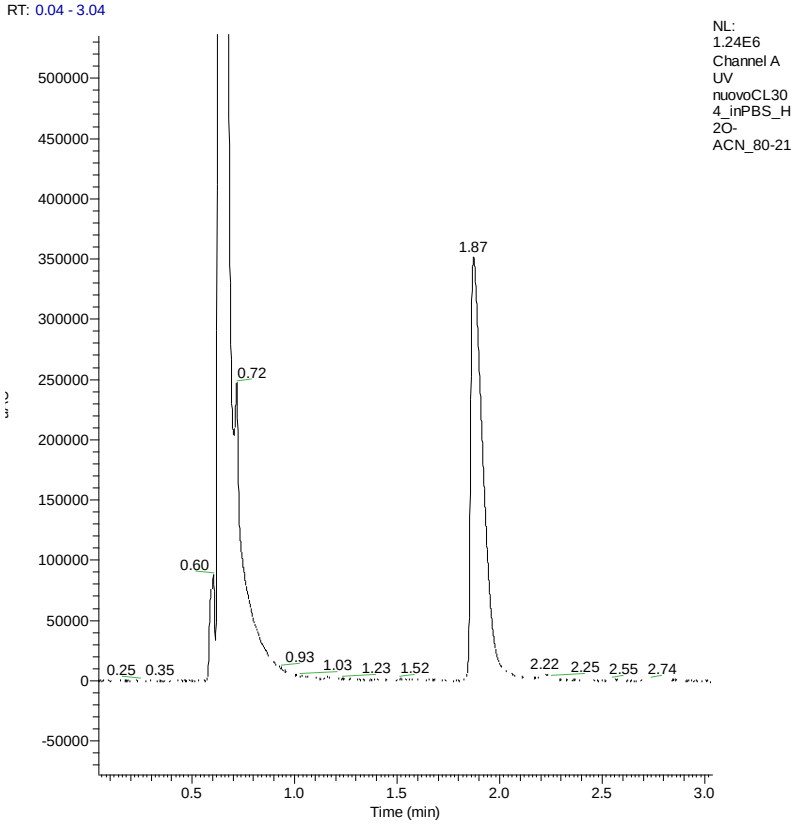


^{13}C NMR of derivative **18**, ***N*-(2,4-difluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$

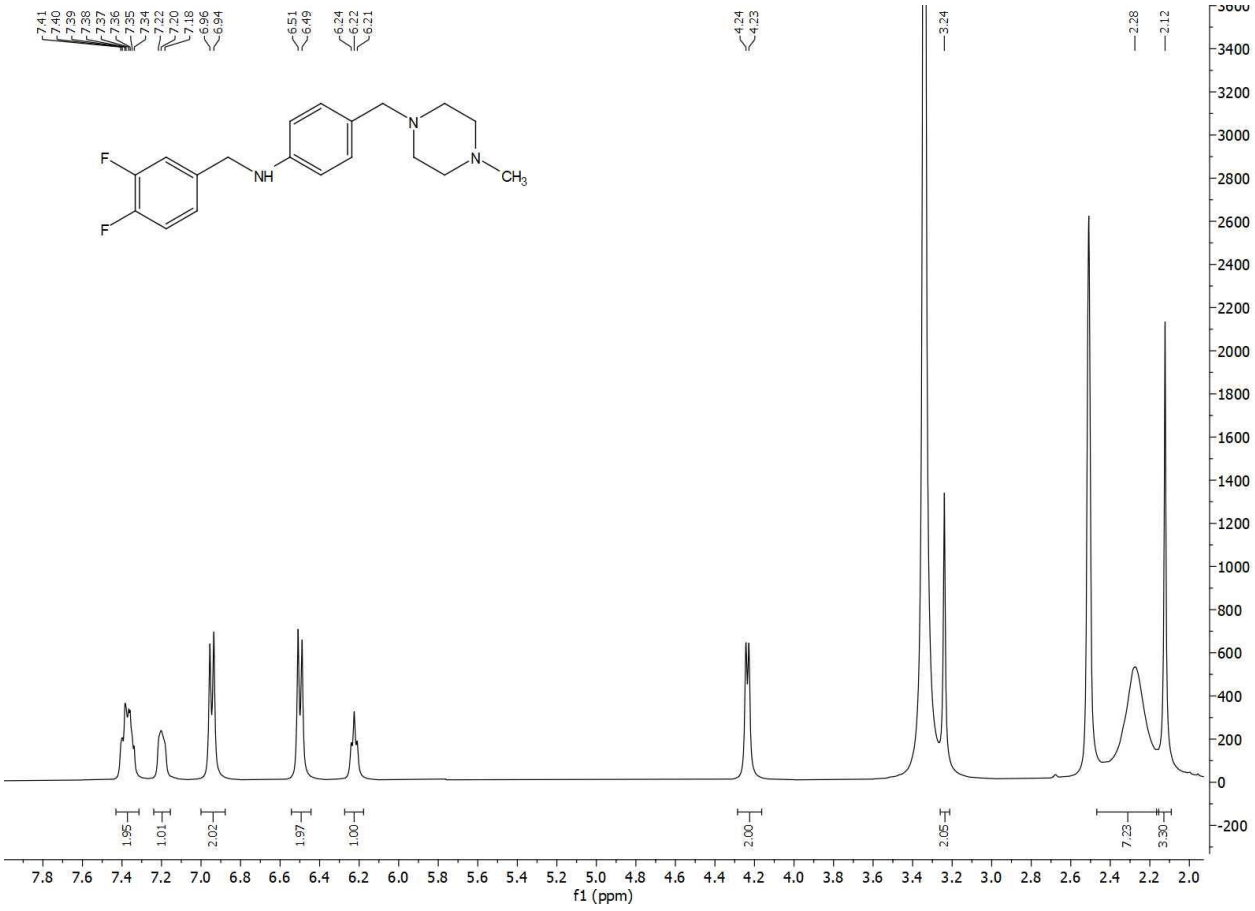




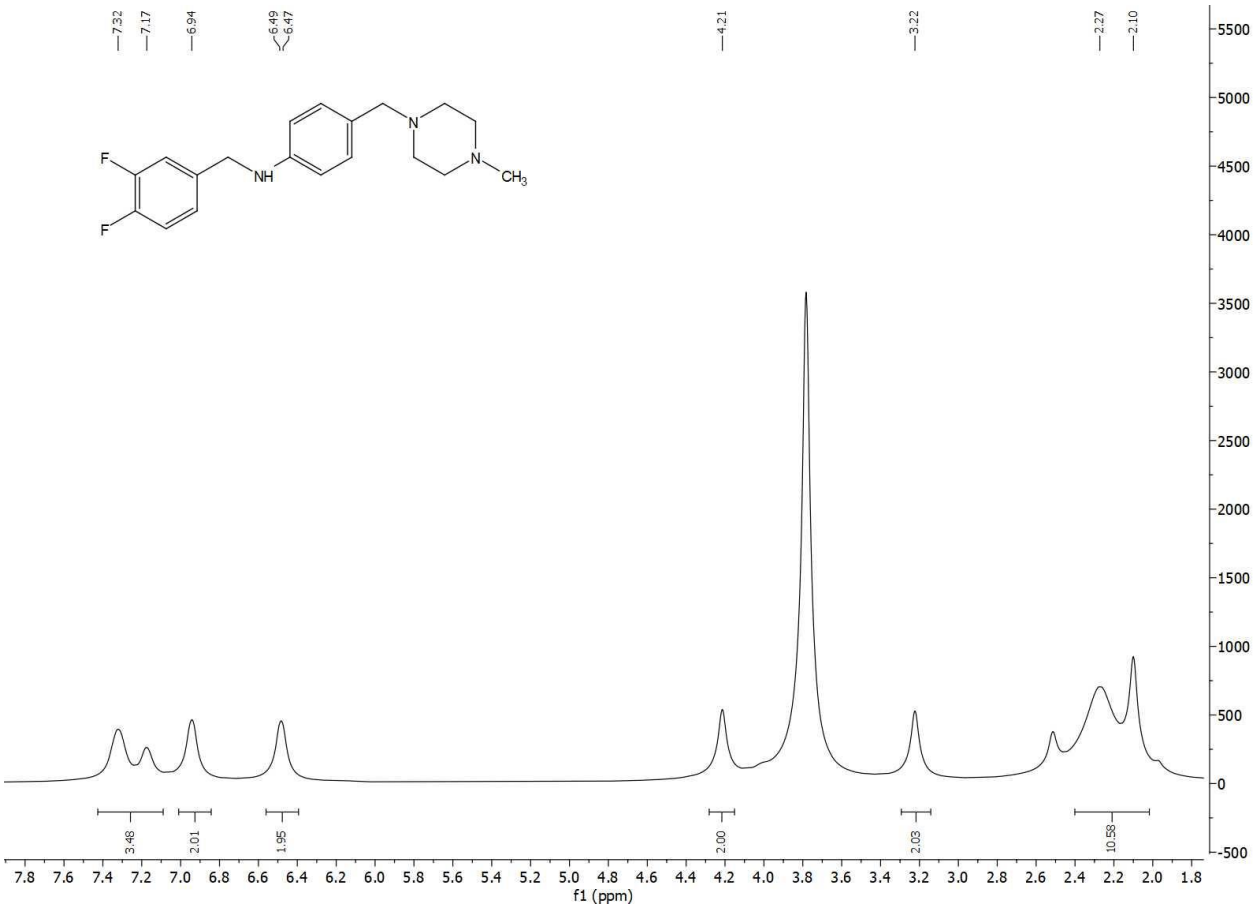
HPLC chart of compound **18** (main peak at 1.87 min, impurity at 2.22 min= 1.83%).



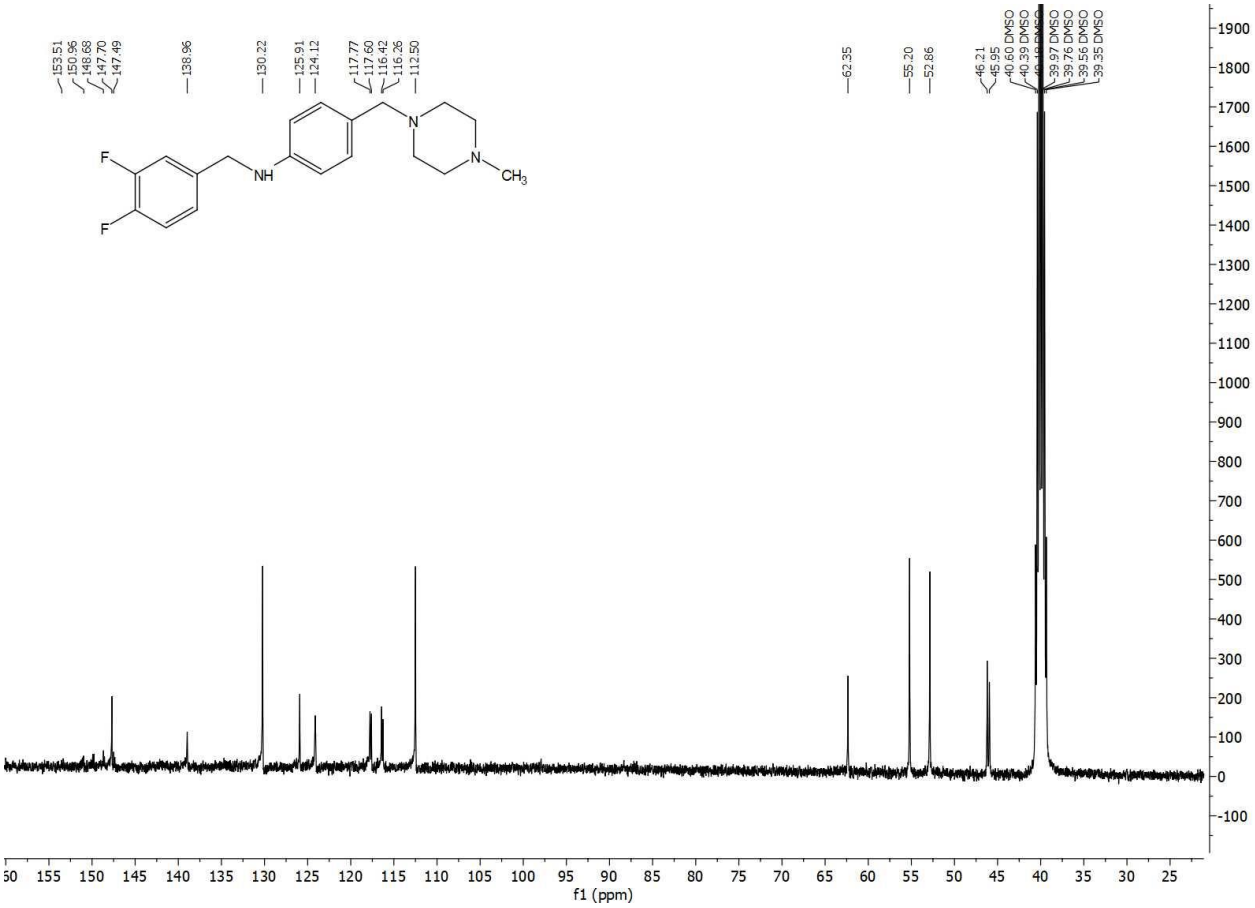
^{1}H NMR of derivative **19**, ***N*-(3,4-difluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*

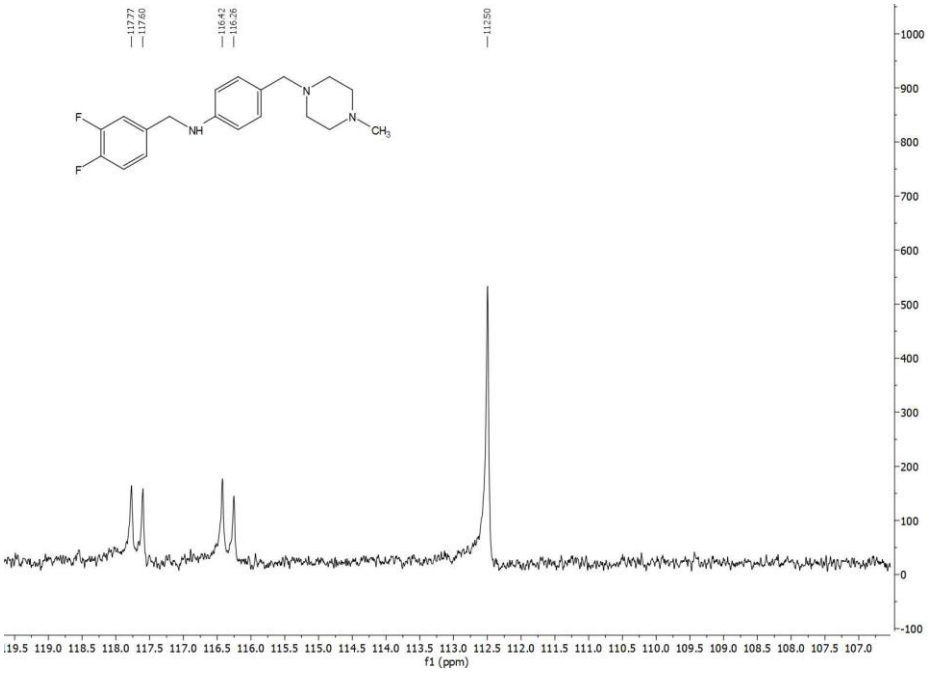


^{1}H NMR of derivative **19**, ***N*-(3,4-difluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*+D_2O

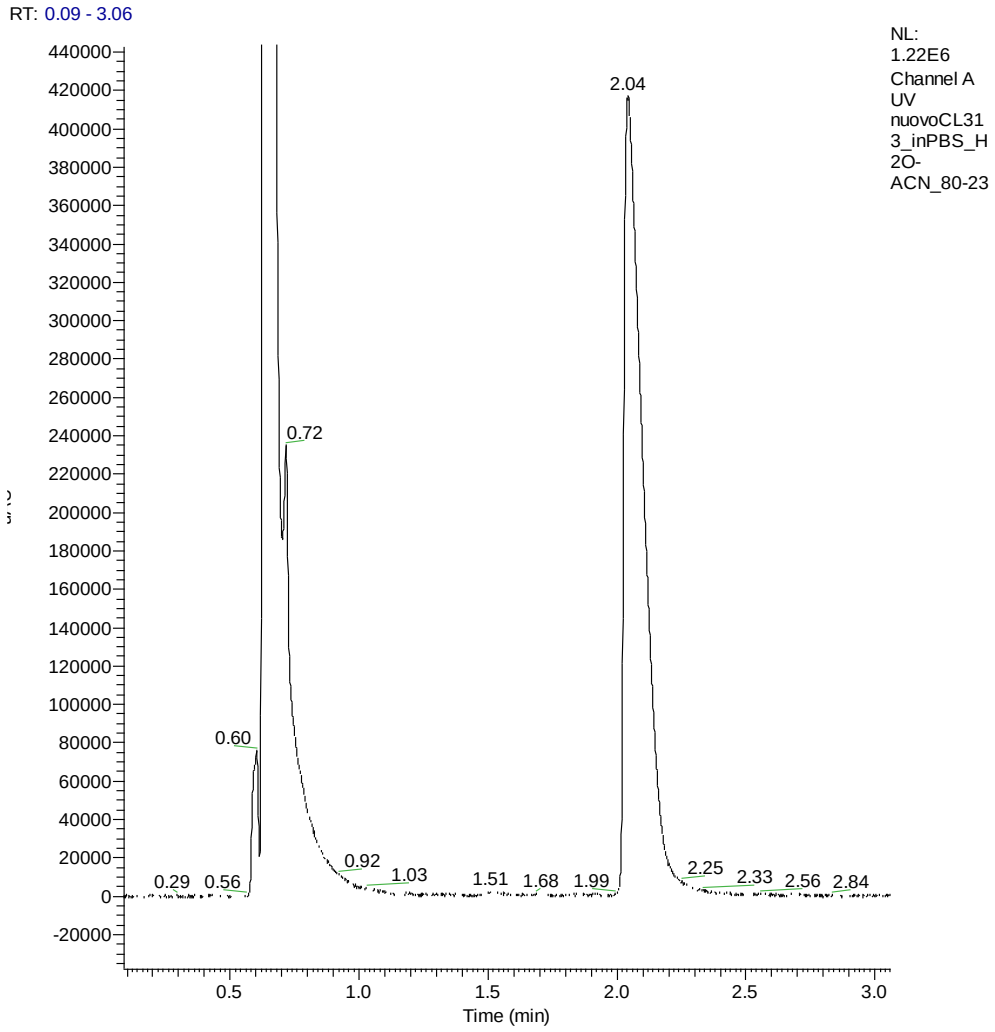


^{13}C NMR of derivative **19**, ***N*-(3,4-difluorobenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*

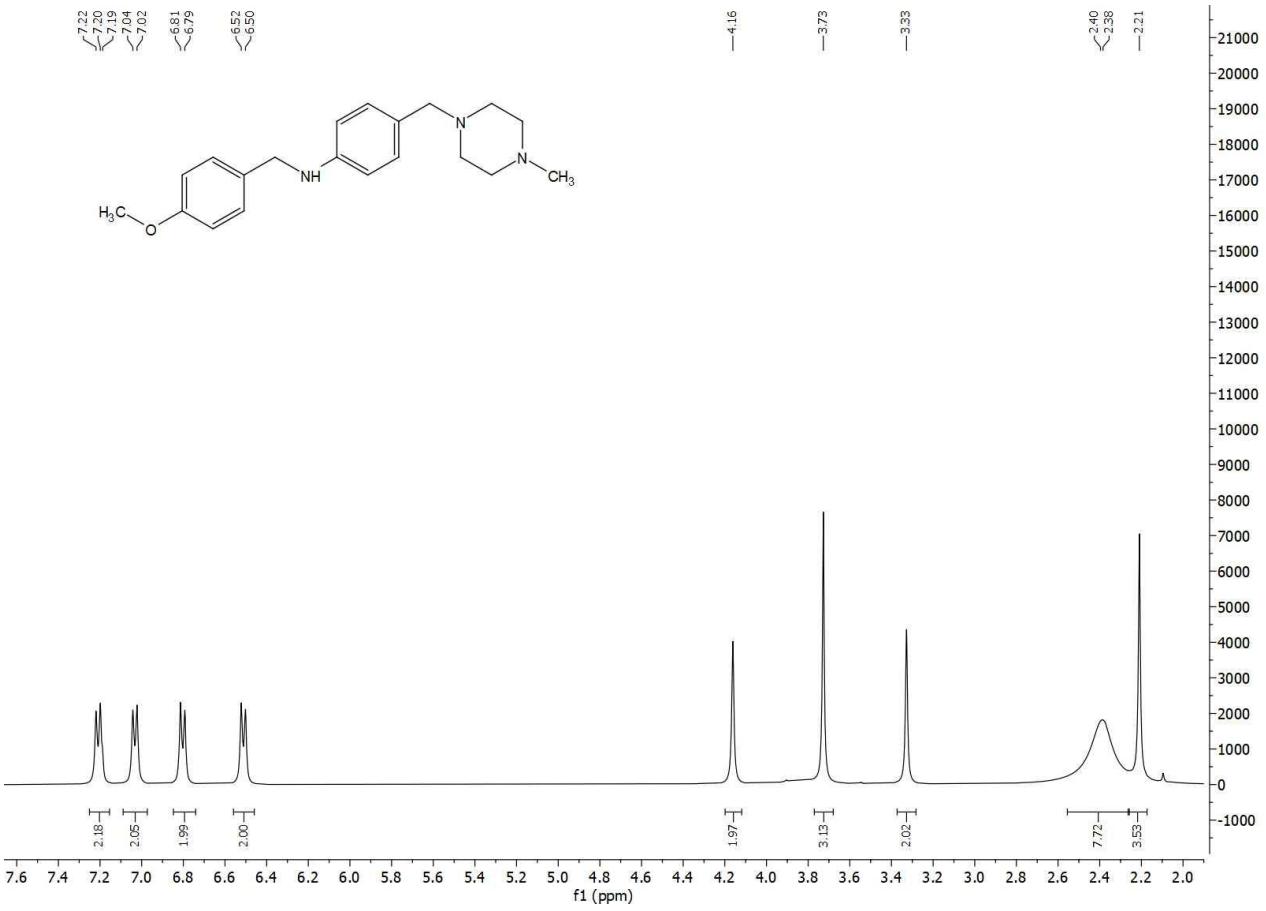




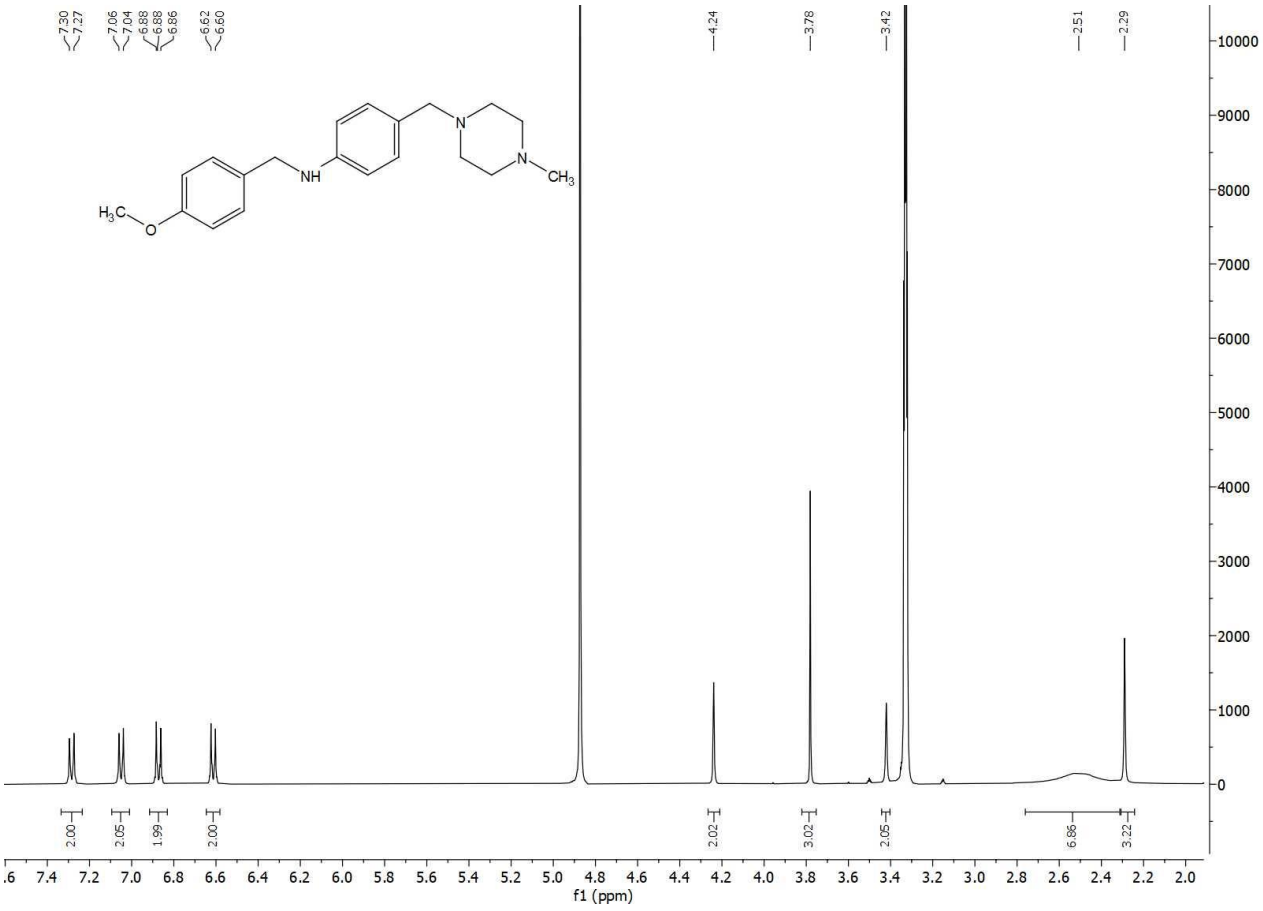
HPLC chart of compound **19** (main peak at 2.04 min, impurity at 1.51 min= 0.14%).



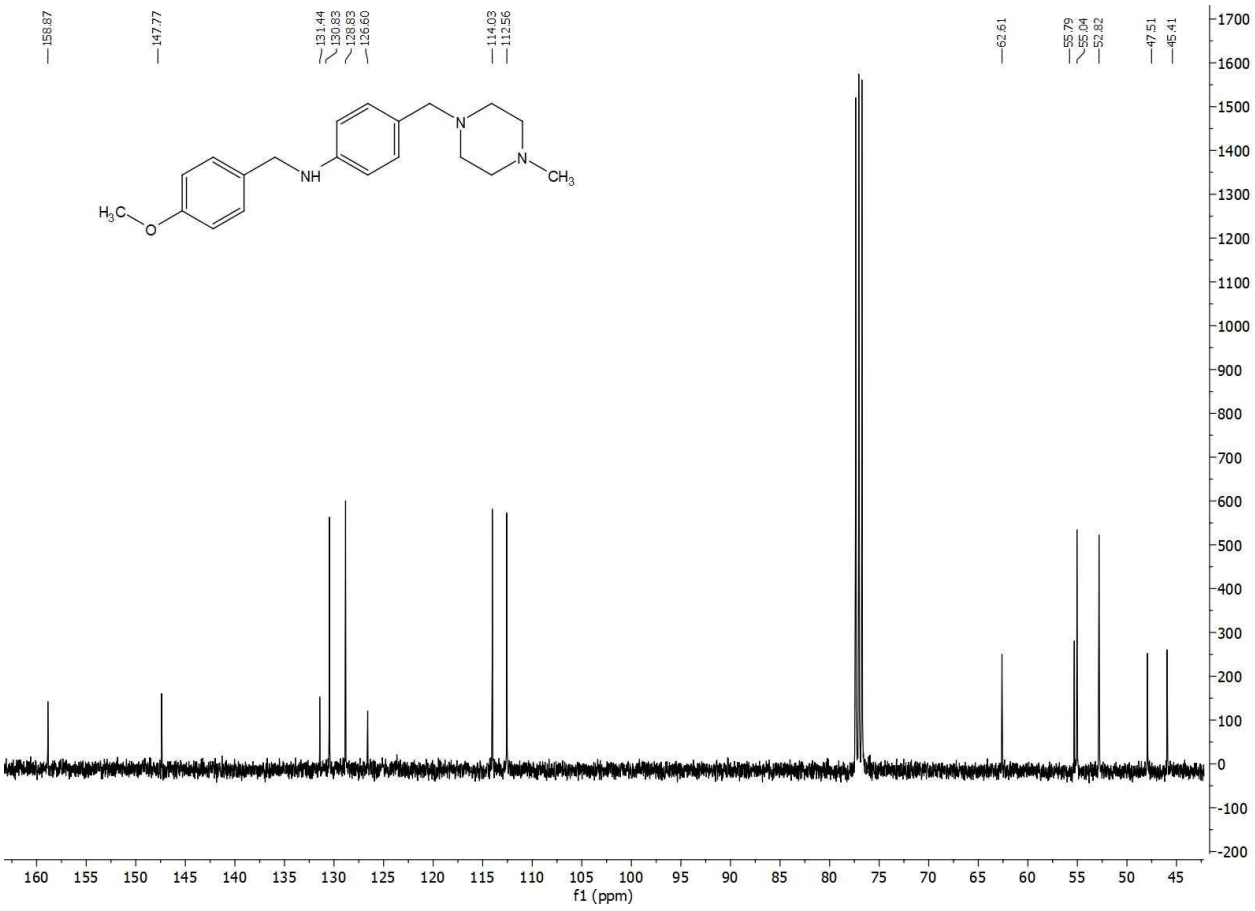
[1] H NMR of derivative **20**, ***N*-(4-methoxybenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$



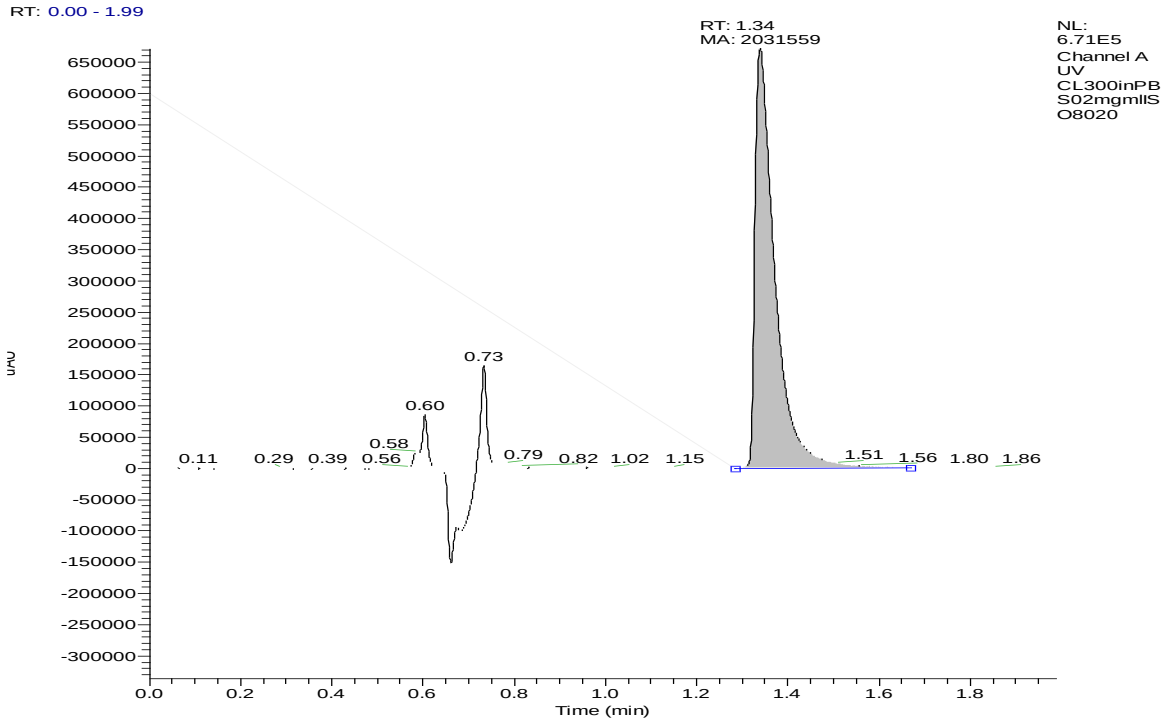
^{1}H NMR of derivative **20**, ***N*-(4-methoxybenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent CD_3OD



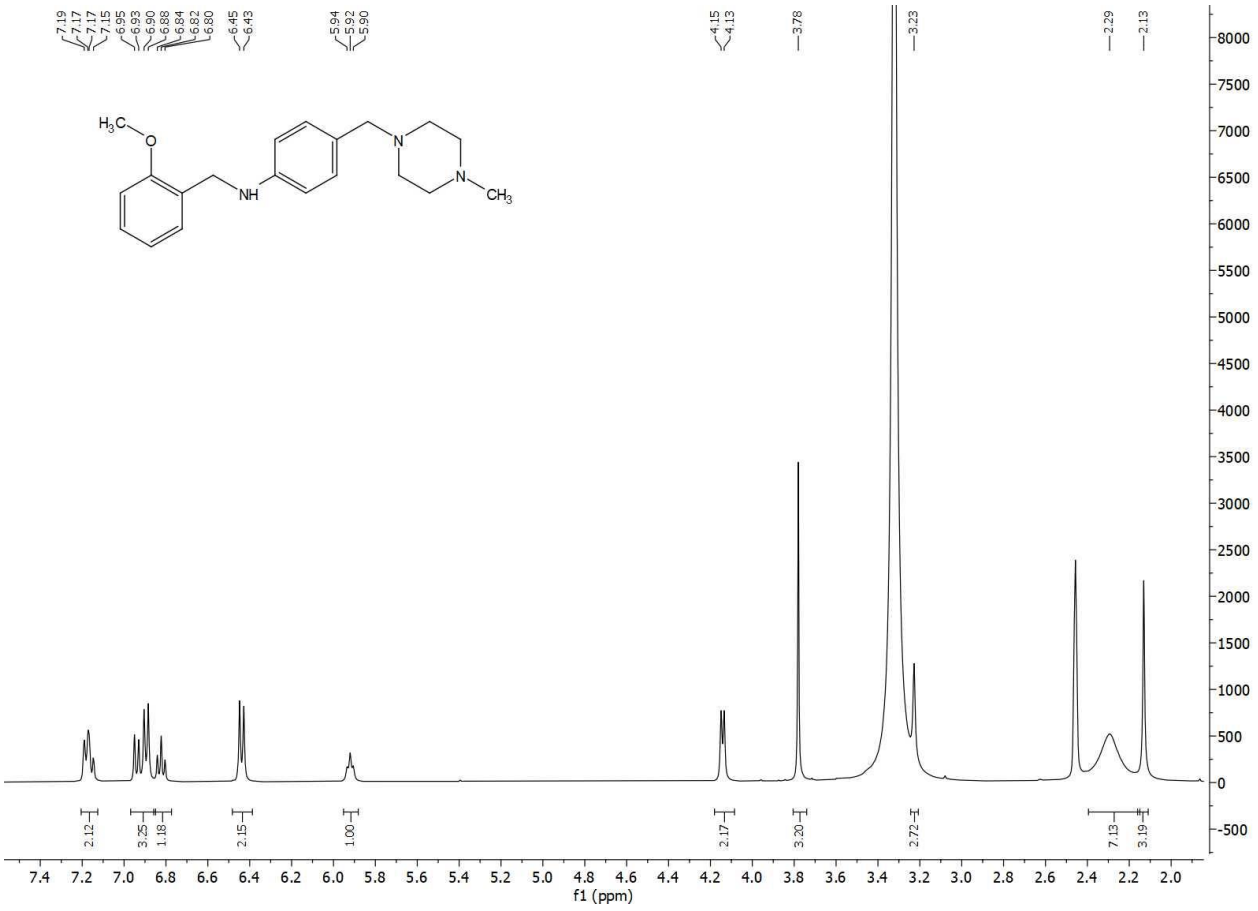
^{13}C NMR of derivative **20**, ***N*-(4-methoxybenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent $CDCl_3$



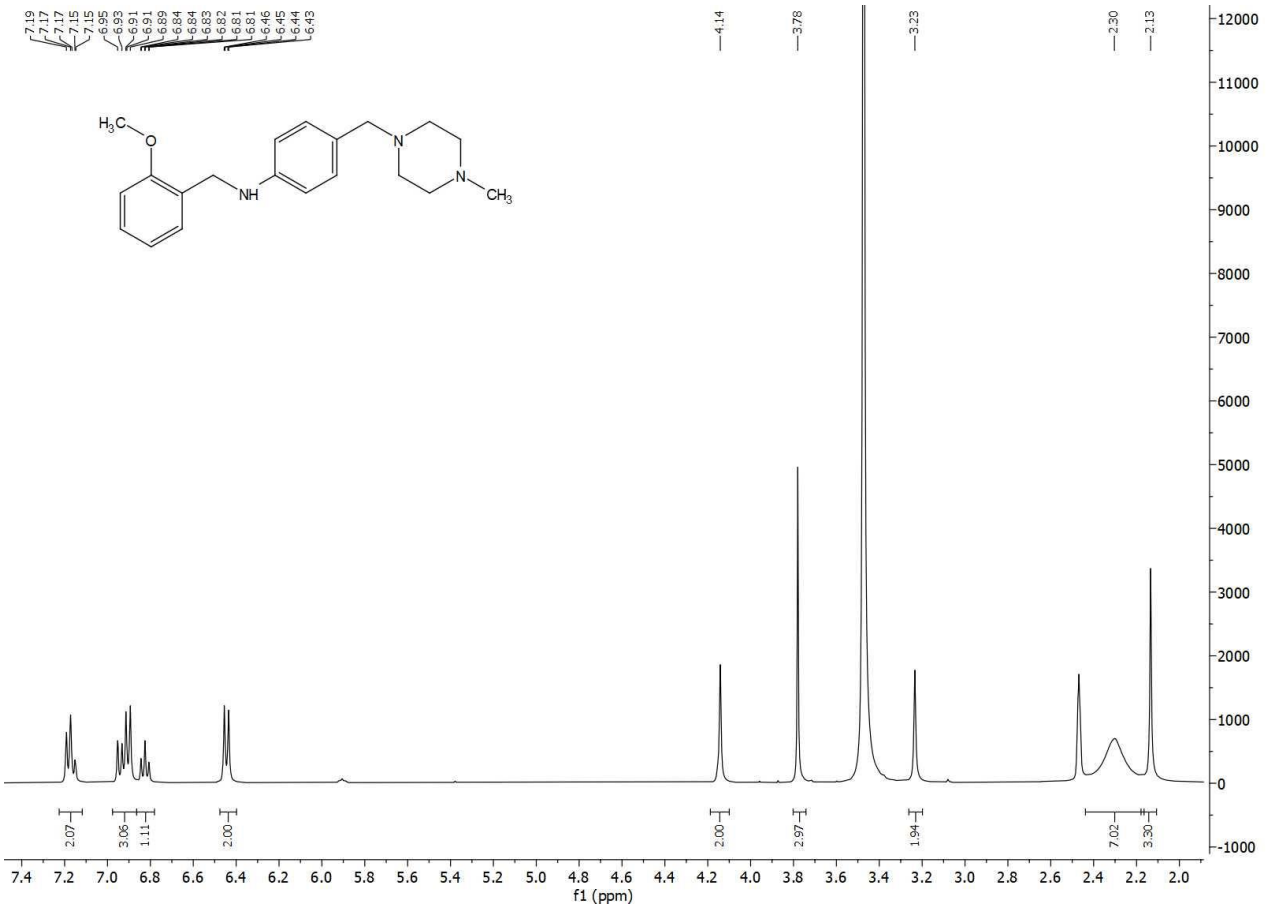
HPLC chart of compound **20**.



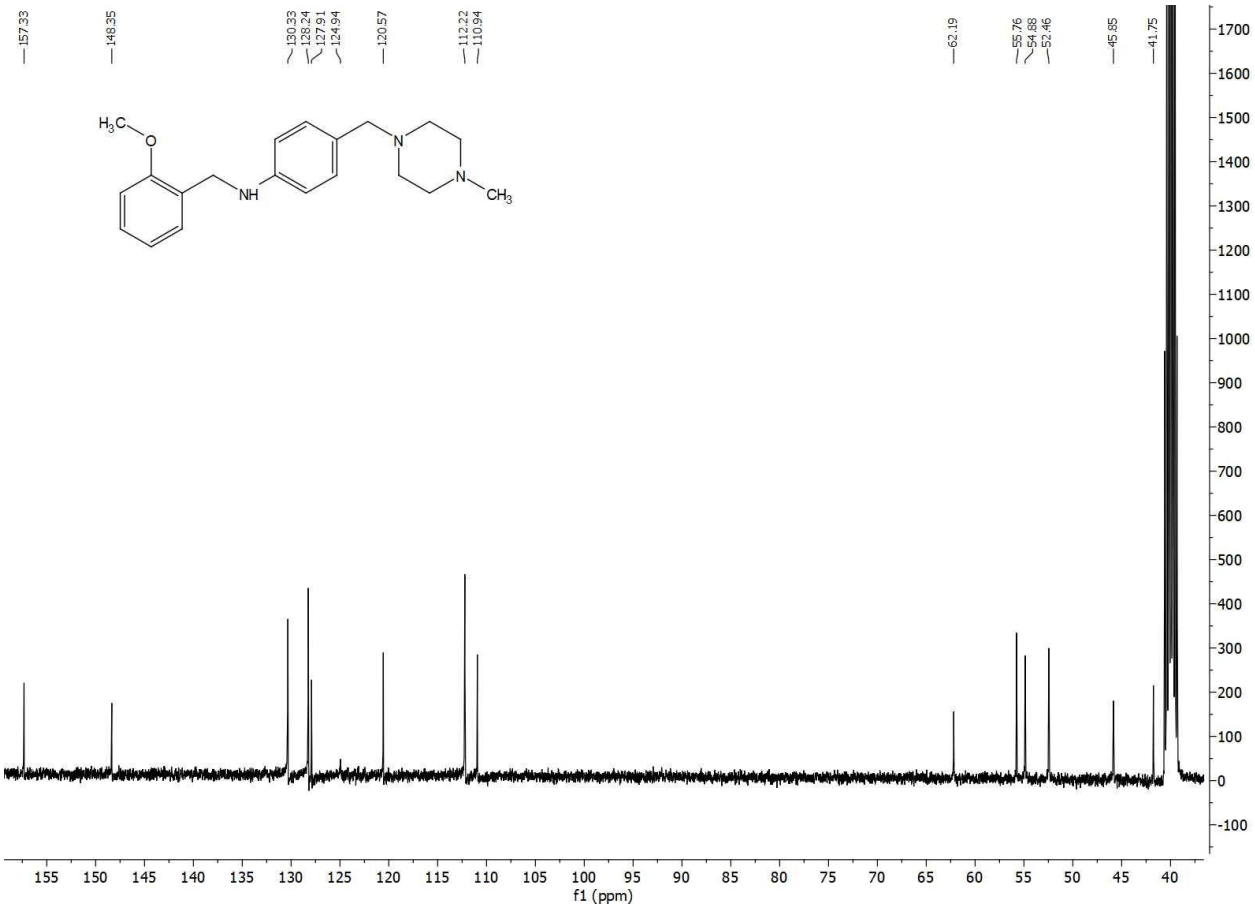
^{1}H NMR of derivative **21**, ***N*-(2-methoxybenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*



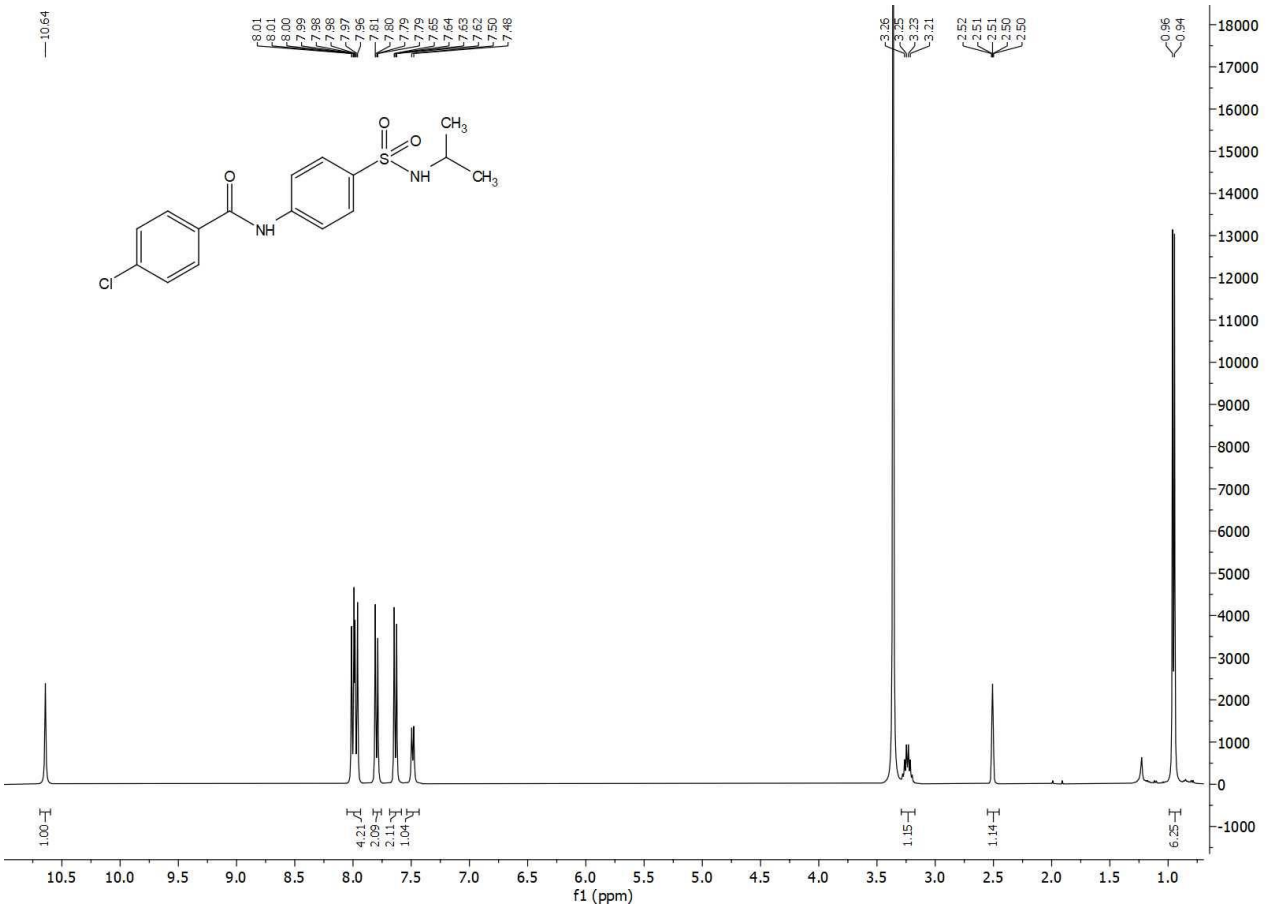
^{1}H NMR of derivative **21**, ***N*-(2-methoxybenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*+D_2O



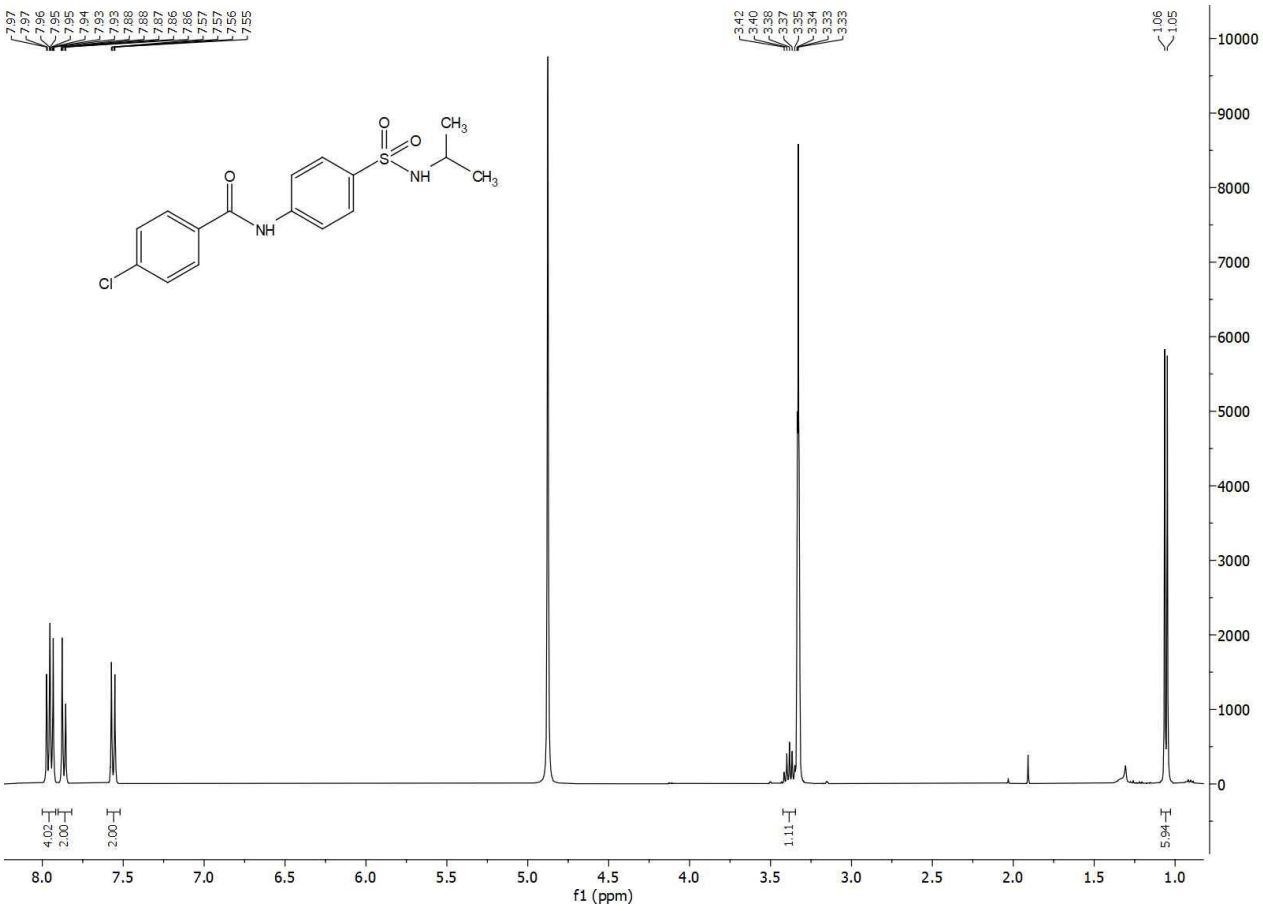
^{13}C NMR of derivative **21**, ***N*-(2-methoxybenzyl)-4-((4-methylpiperazin-1-yl)methyl)aniline,** solvent DMSO-*d6*



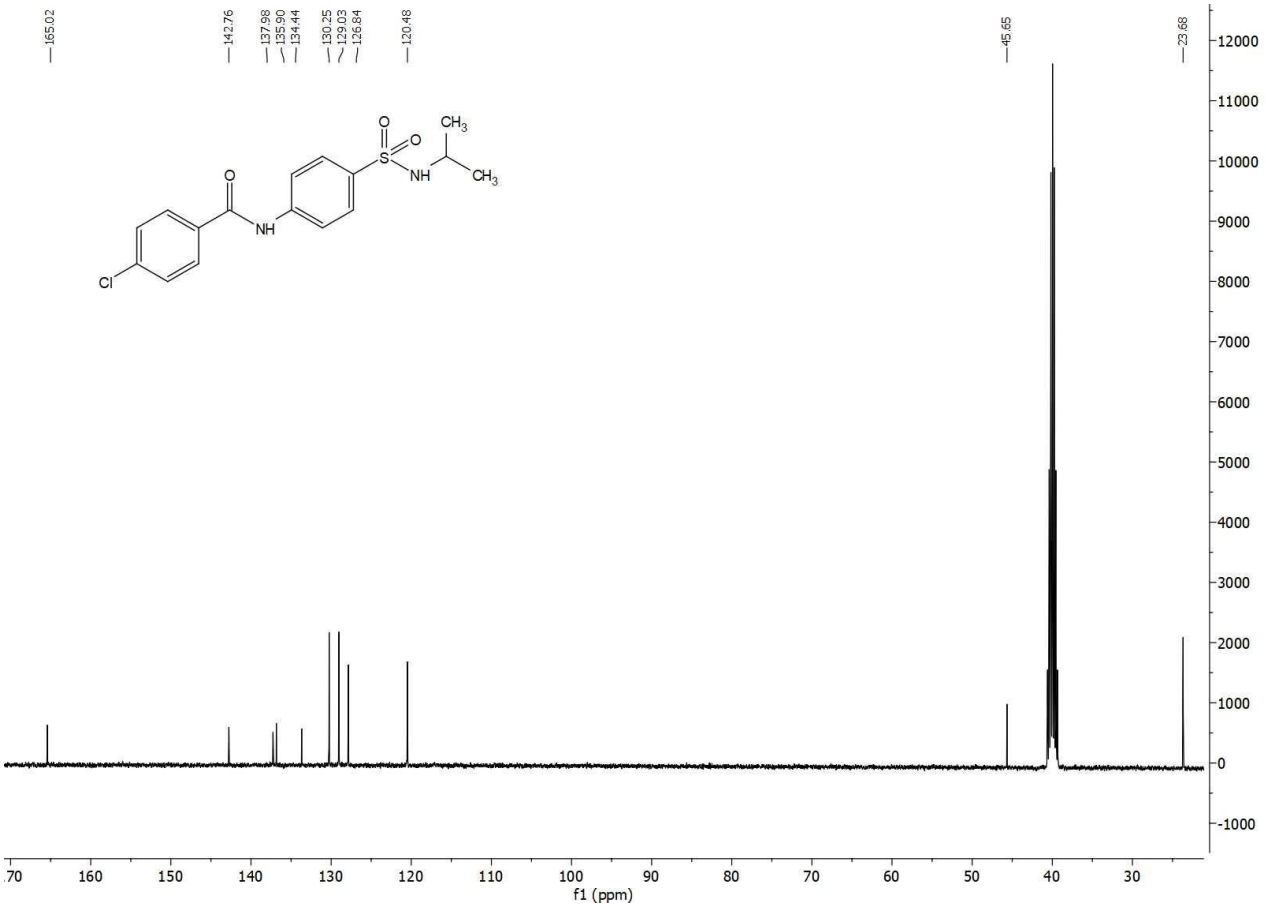
^{1}H NMR of derivative **22**, **4-Chloro-*N*-(4-(N-isopropylsulfamoyl)phenyl)benzamide,** solvent DMSO-*d6*



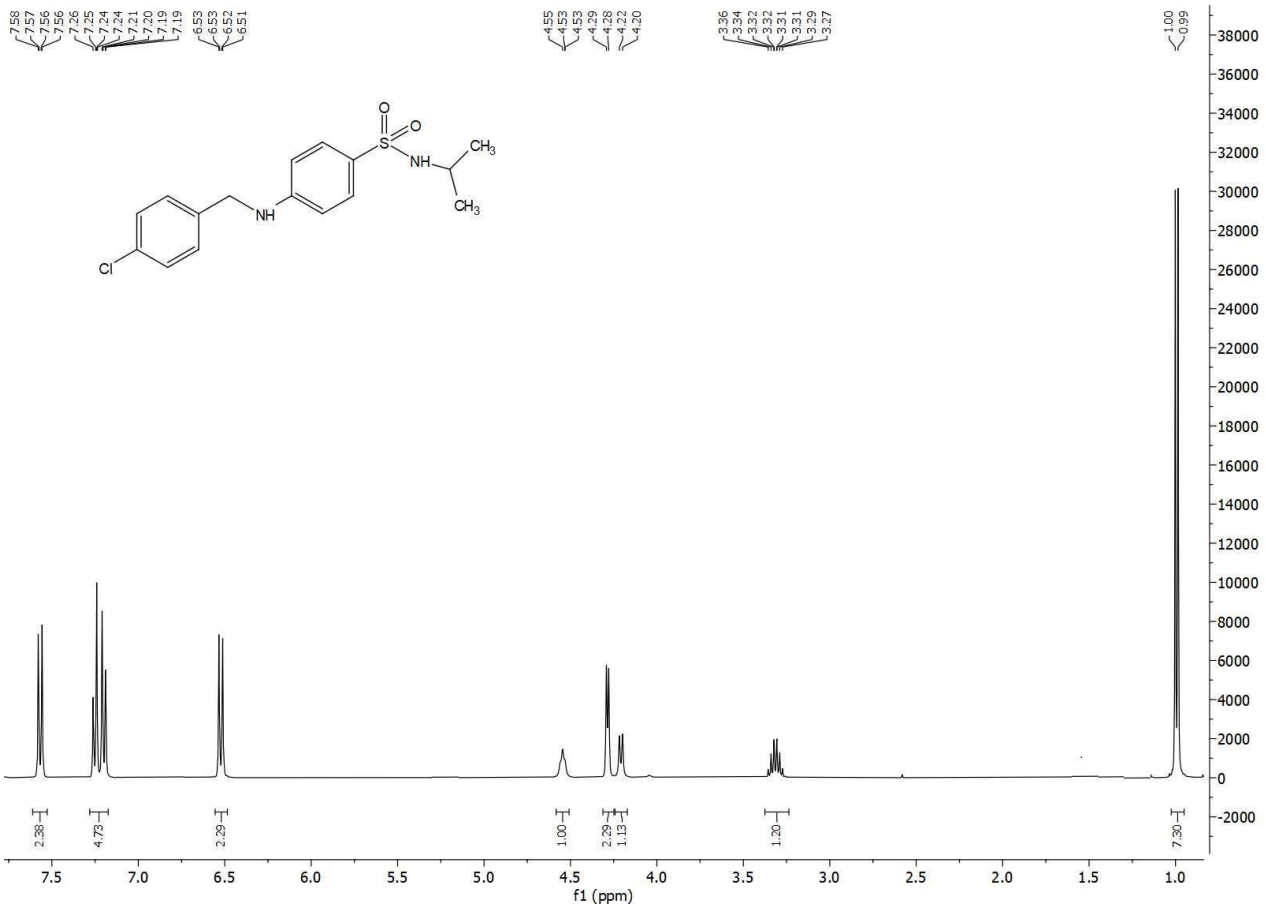
^{1}H NMR of derivative **22**, **4-Chloro-*N*-(4-(N-isopropylsulfamoyl)phenyl)benzamide,** solvent CD_3OD



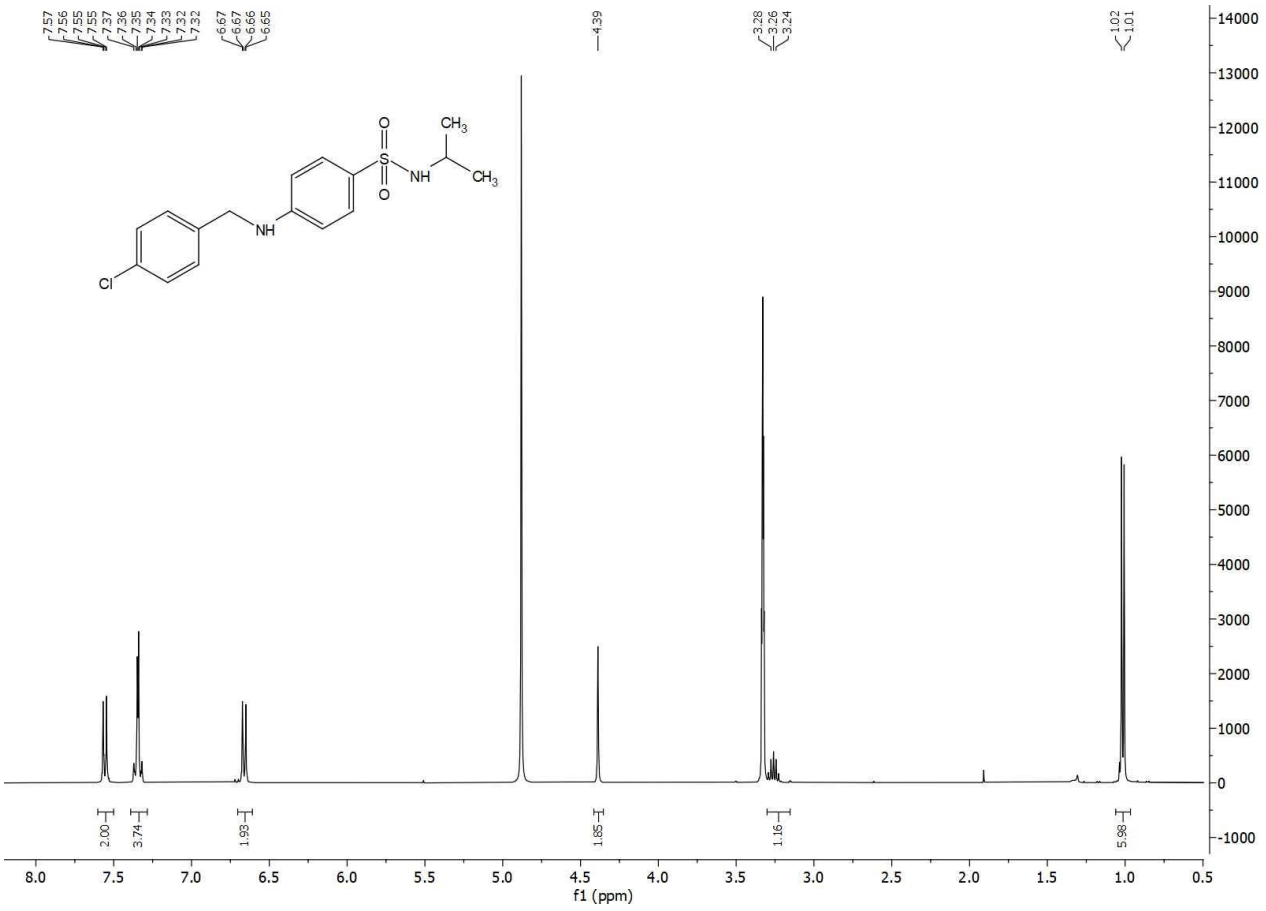
^{13}C NMR of derivative **22**, **4-Chloro-*N*-(4-(N-isopropylsulfamoyl)phenyl)benzamide,** solvent DMSO-*d6*



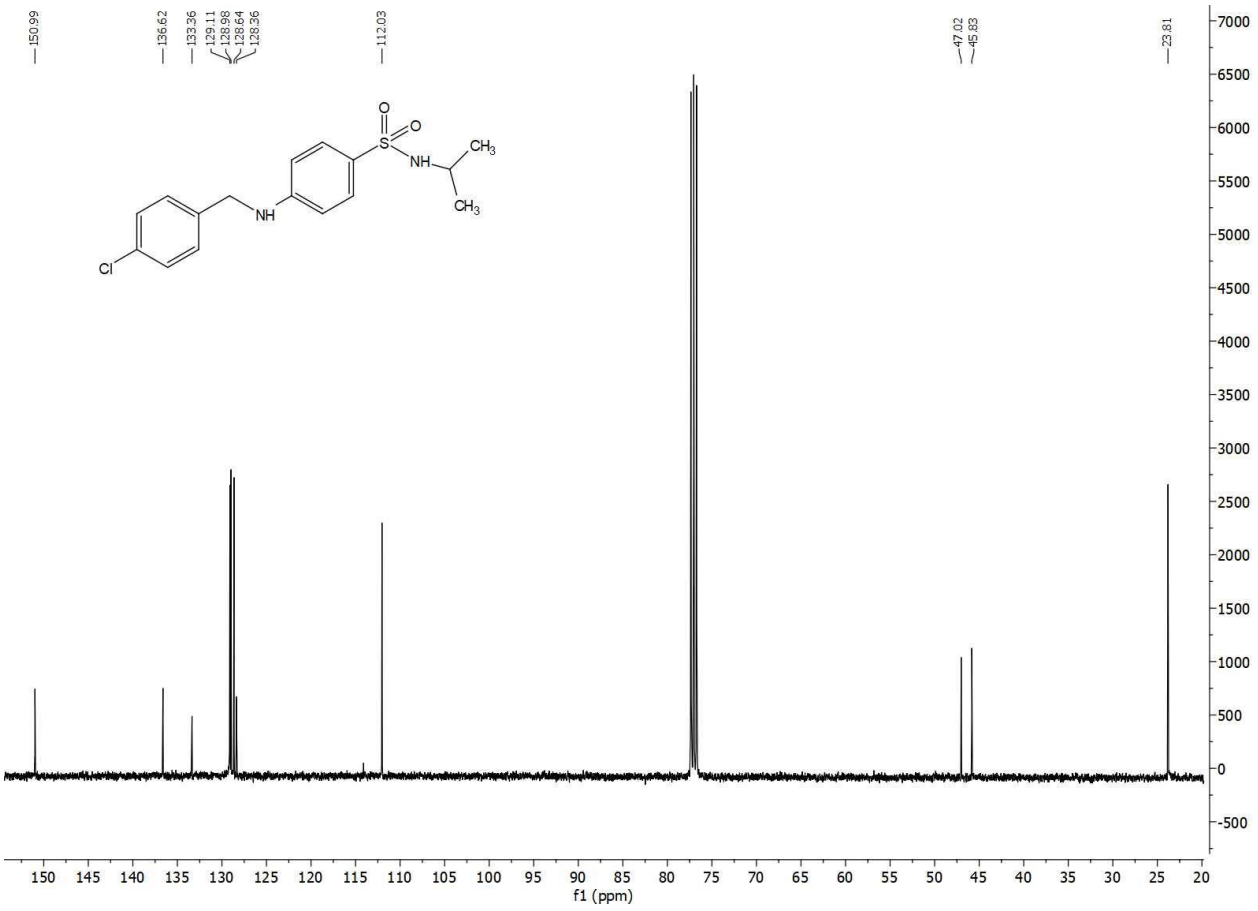
^{1}H NMR of derivative **23**, **4-((4-Chlorobenzyl)amino)-*N*-isopropylbenzenesulfonamide,** solvent $CDCl_3$



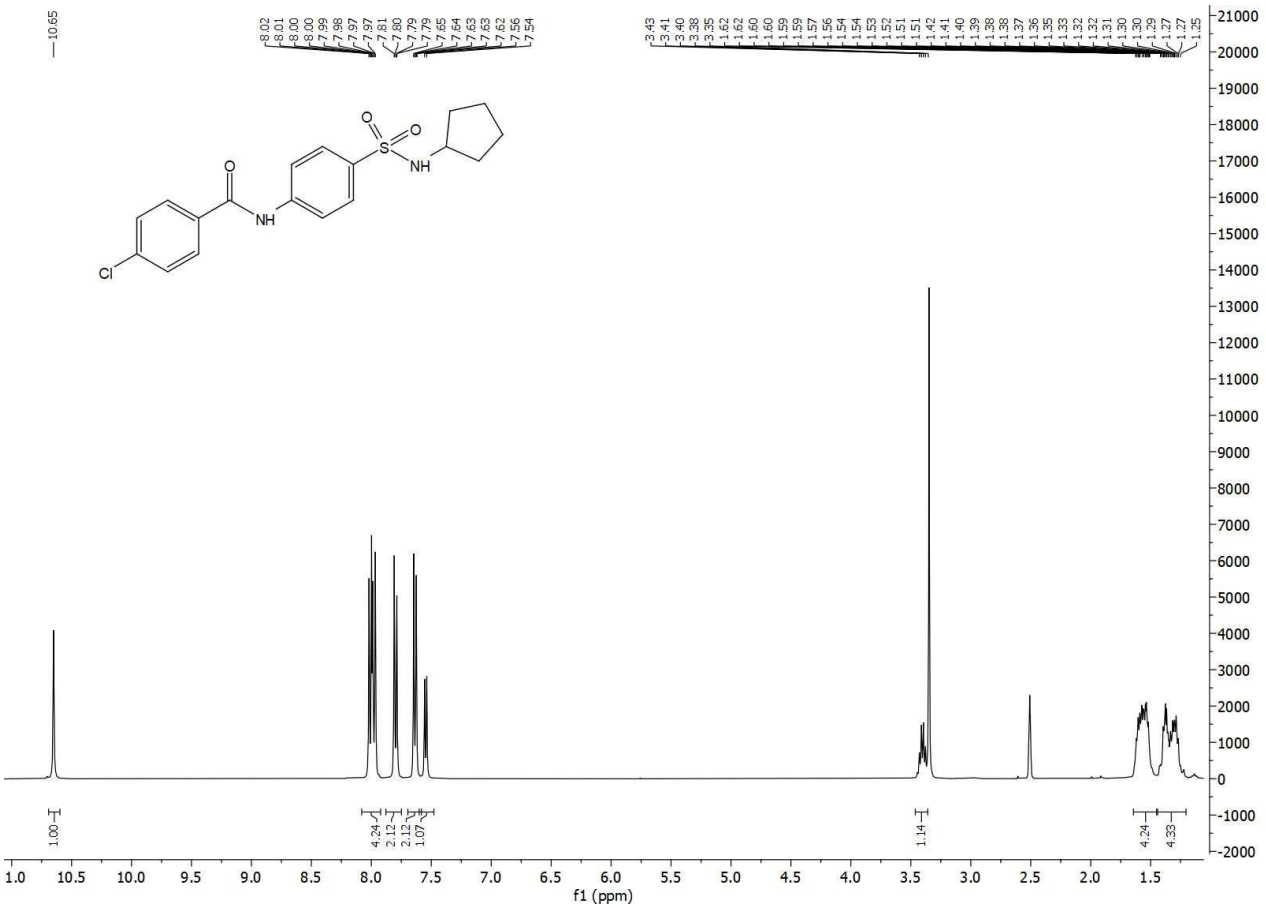
^{1}H NMR of derivative 23, **4-((4-Chlorobenzyl)amino)-*N*-isopropylbenzenesulfonamide,** solvent CD_3OD



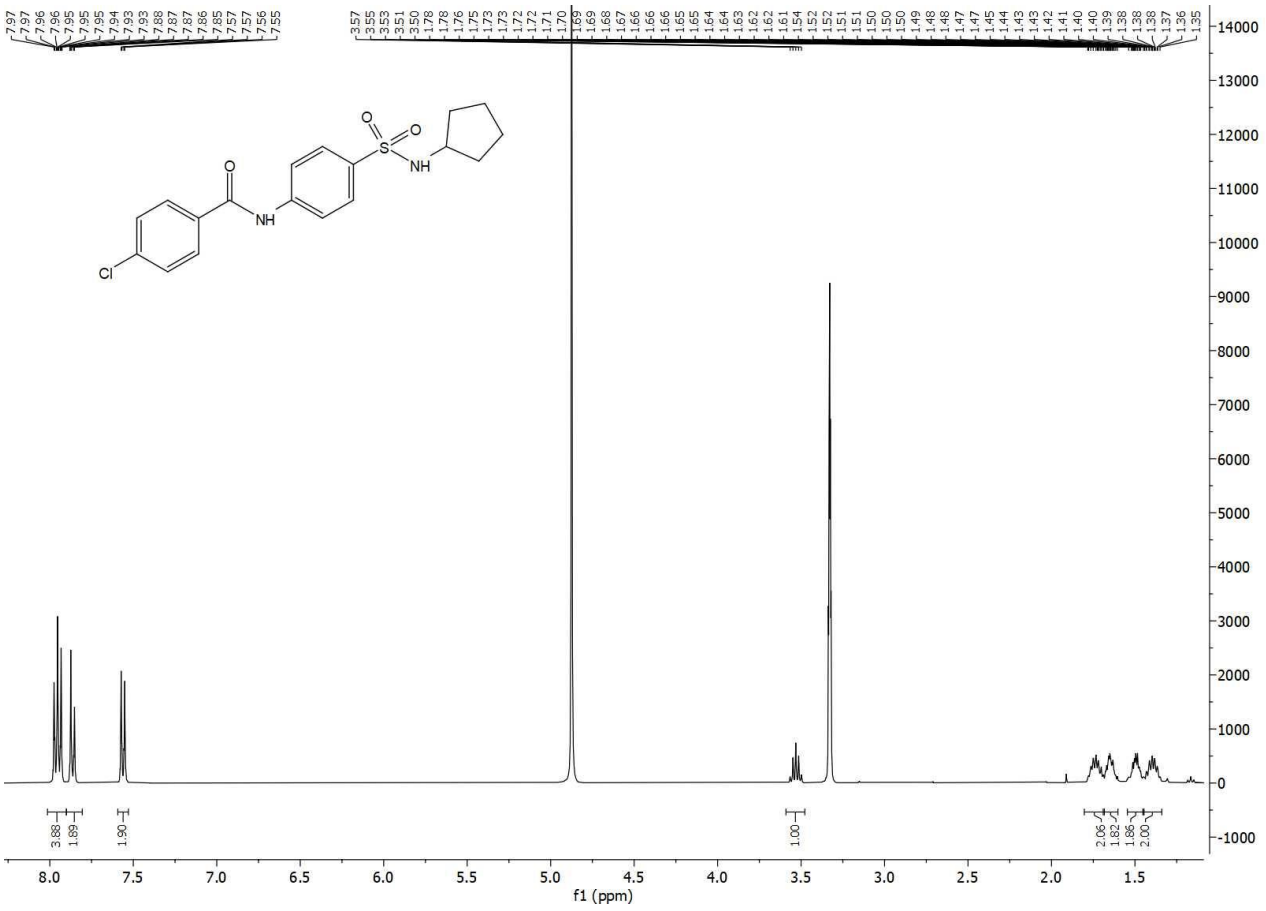
^{13}C NMR of derivative **23**, **4-((4-Chlorobenzyl)amino)-*N*-isopropylbenzenesulfonamide,** solvent $CDCl_3$



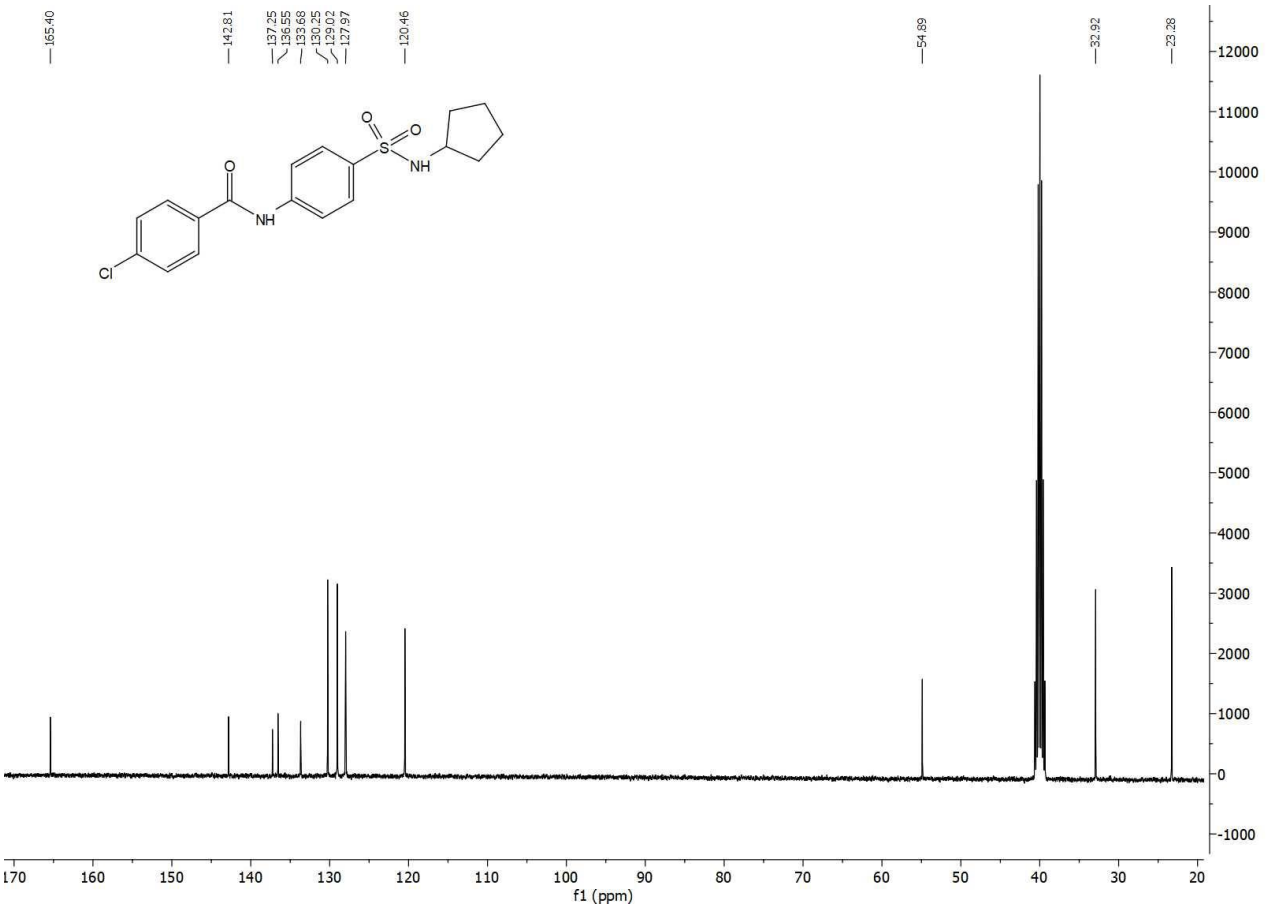
^{1}H NMR of derivative **24**, **4-Chloro-*N*-(4-(N-cyclopentylsulfamoyl)phenyl)benzamide,** solvent DMSO-*d6*



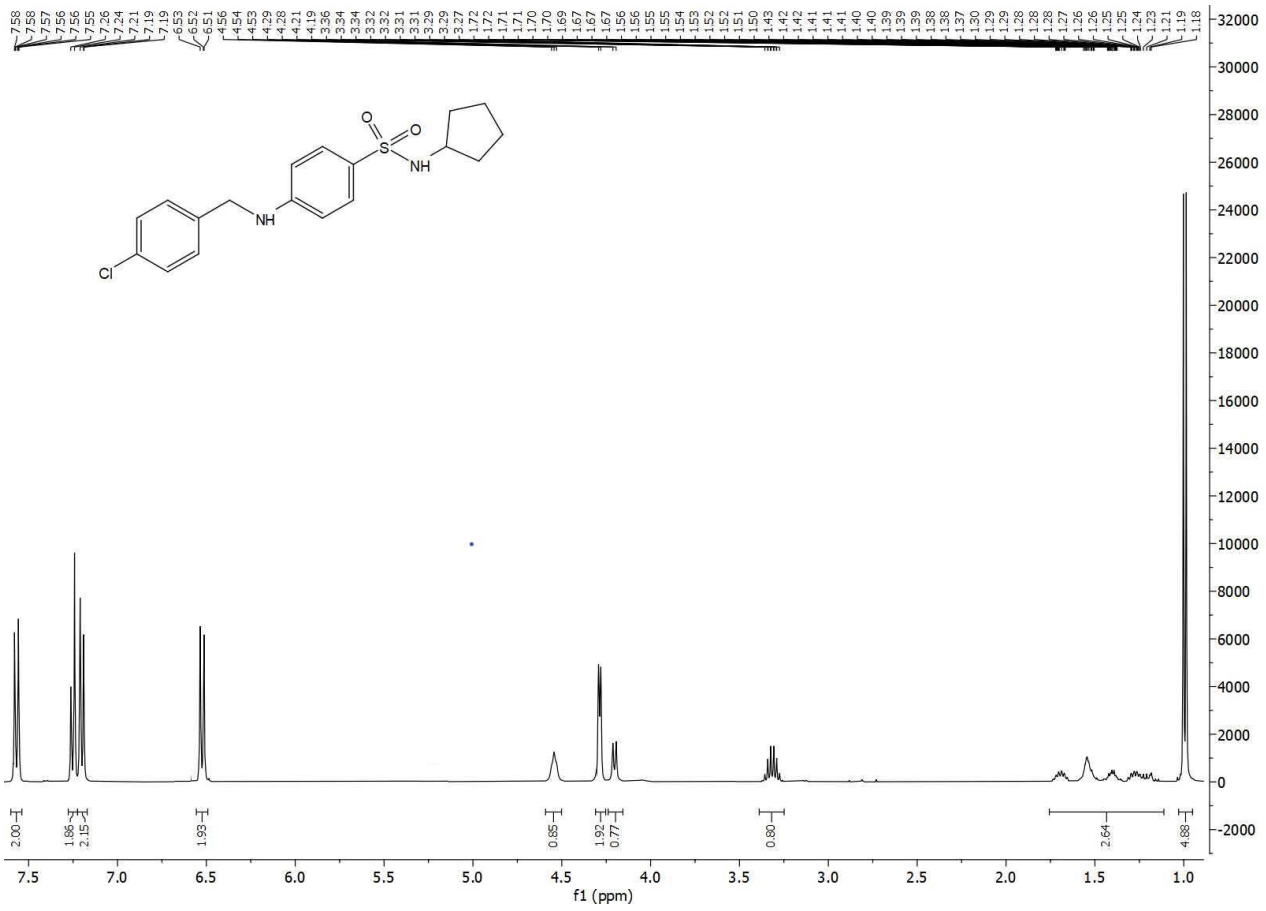
^{1}H NMR of derivative **24**, **4-Chloro-*N*-(4-(N-cyclopentylsulfamoyl)phenyl)benzamide,** solvent CD_3OD



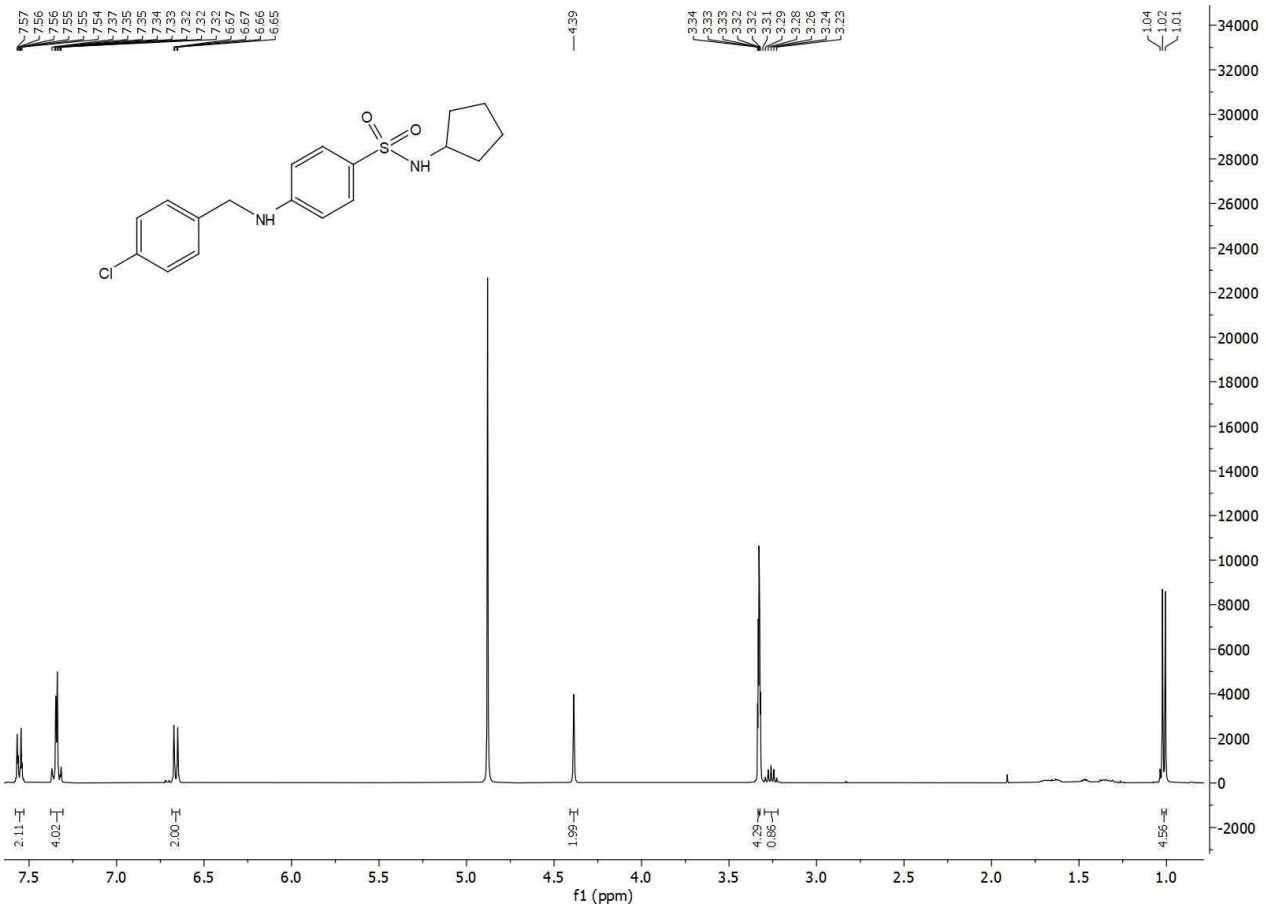
^{13}C NMR of derivative **24**, **4-Chloro-*N*-(4-(N-cyclopentylsulfamoyl)phenyl)benzamide,** solvent DMSO-*d6*



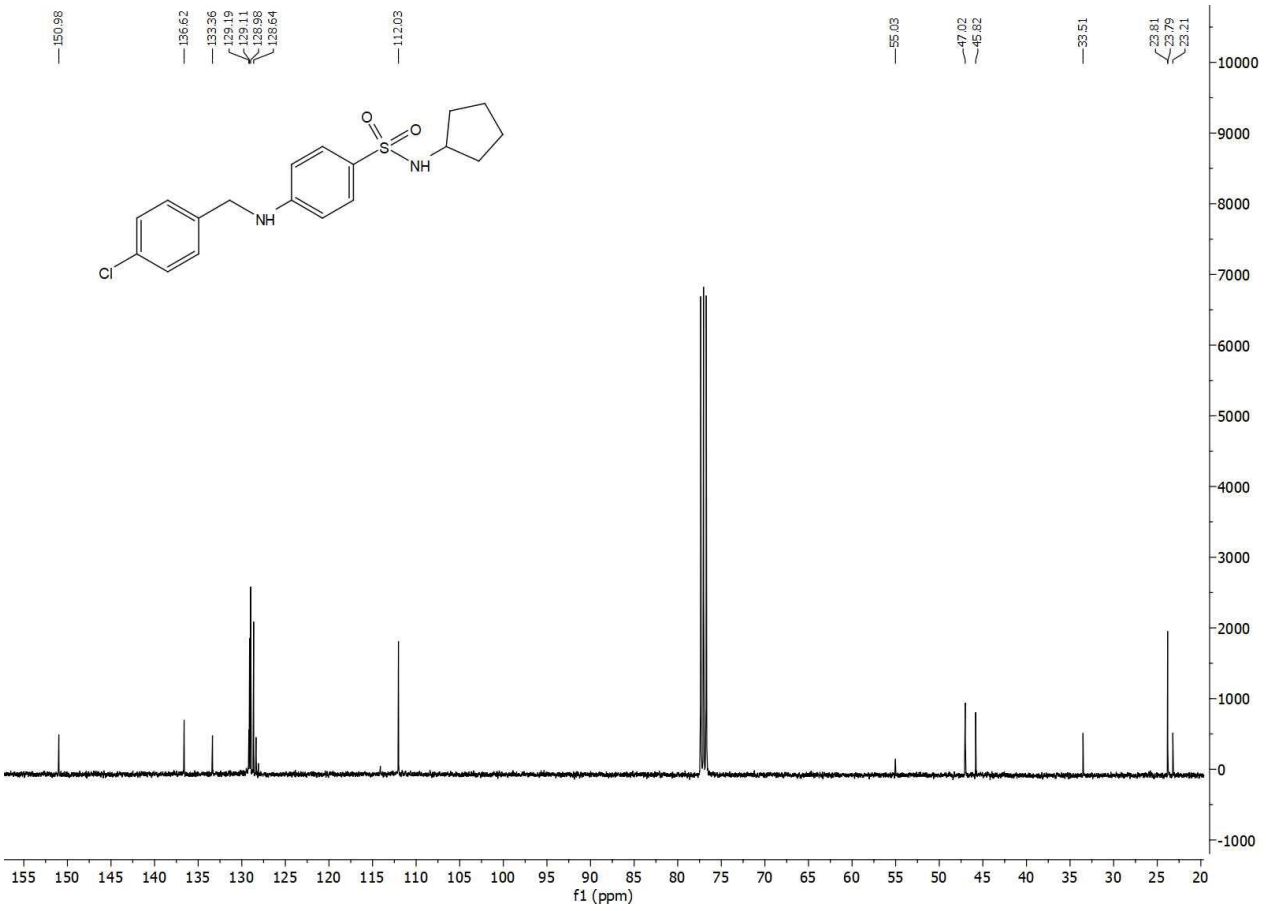
^{1}H NMR of derivative **25**, **4-((4-Chlorobenzyl)amino)-*N*-cyclopentylbenzenesulfonamide,** solvent $CDCl_3$



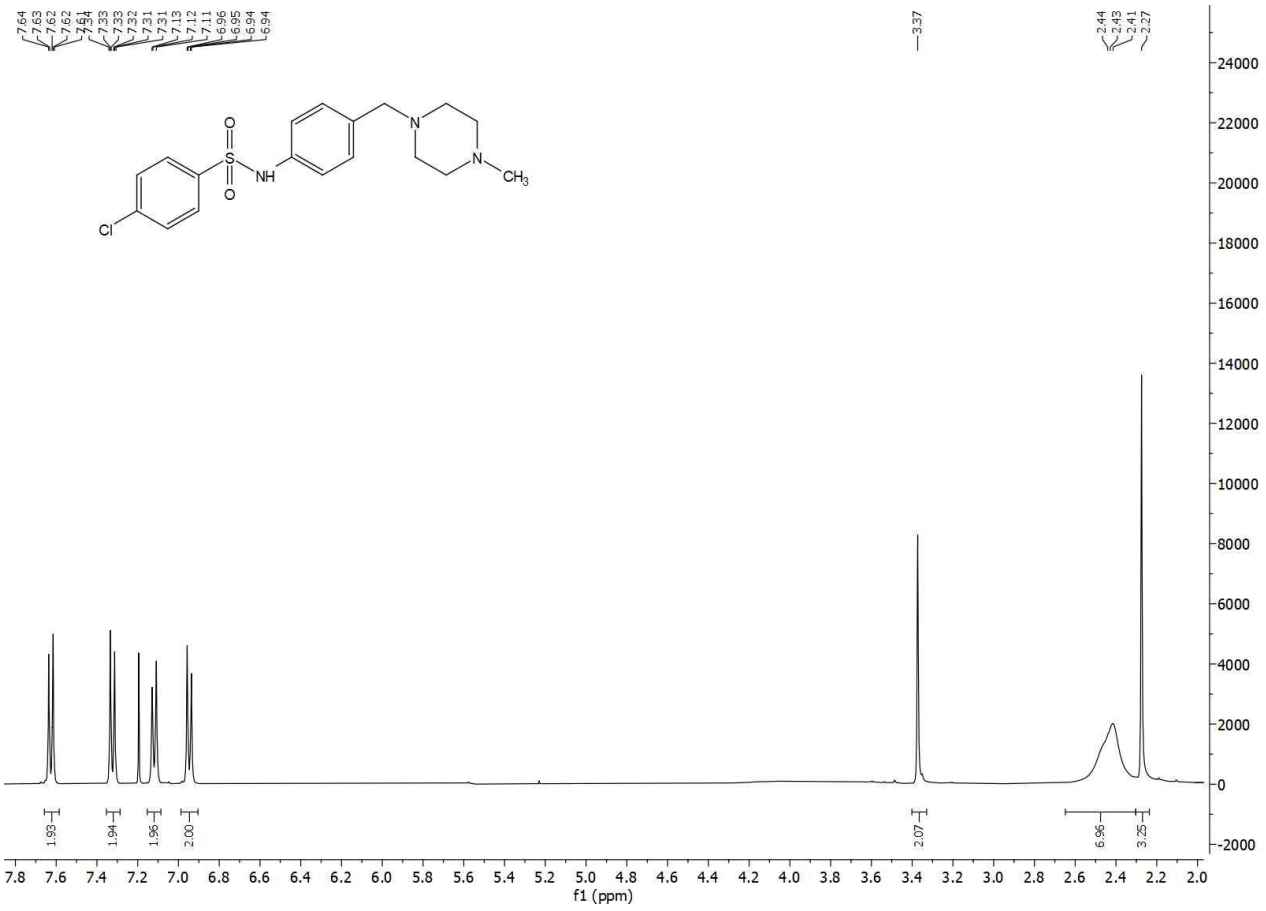
^{1}H NMR of derivative **25**, **4-((4-Chlorobenzyl)amino)-*N*-cyclopentylbenzenesulfonamide,** solvent CD_3OD



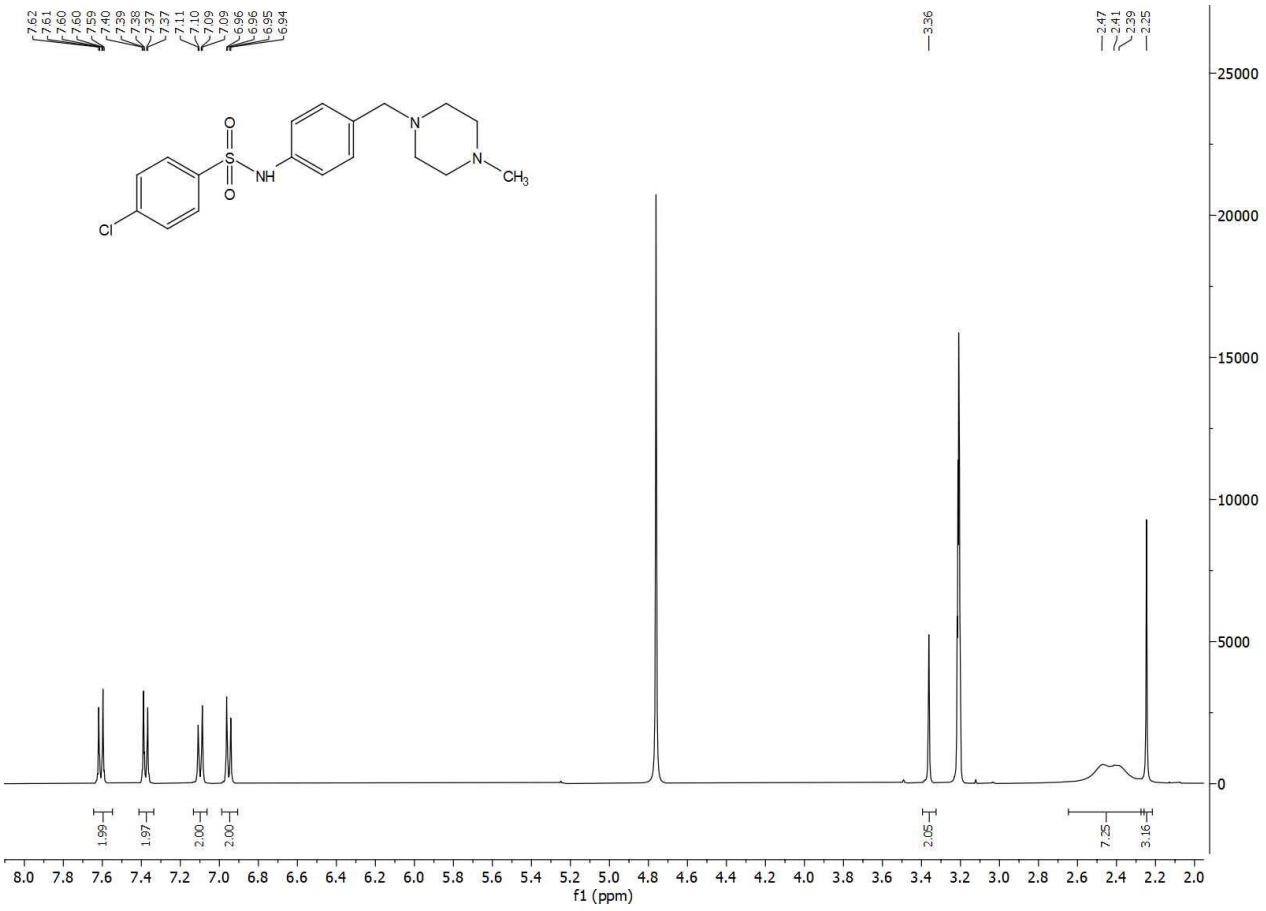
^{13}C NMR of derivative **25**, **4-((4-Chlorobenzyl)amino)-*N*-cyclopentylbenzenesulfonamide,** solvent $CDCl_3$



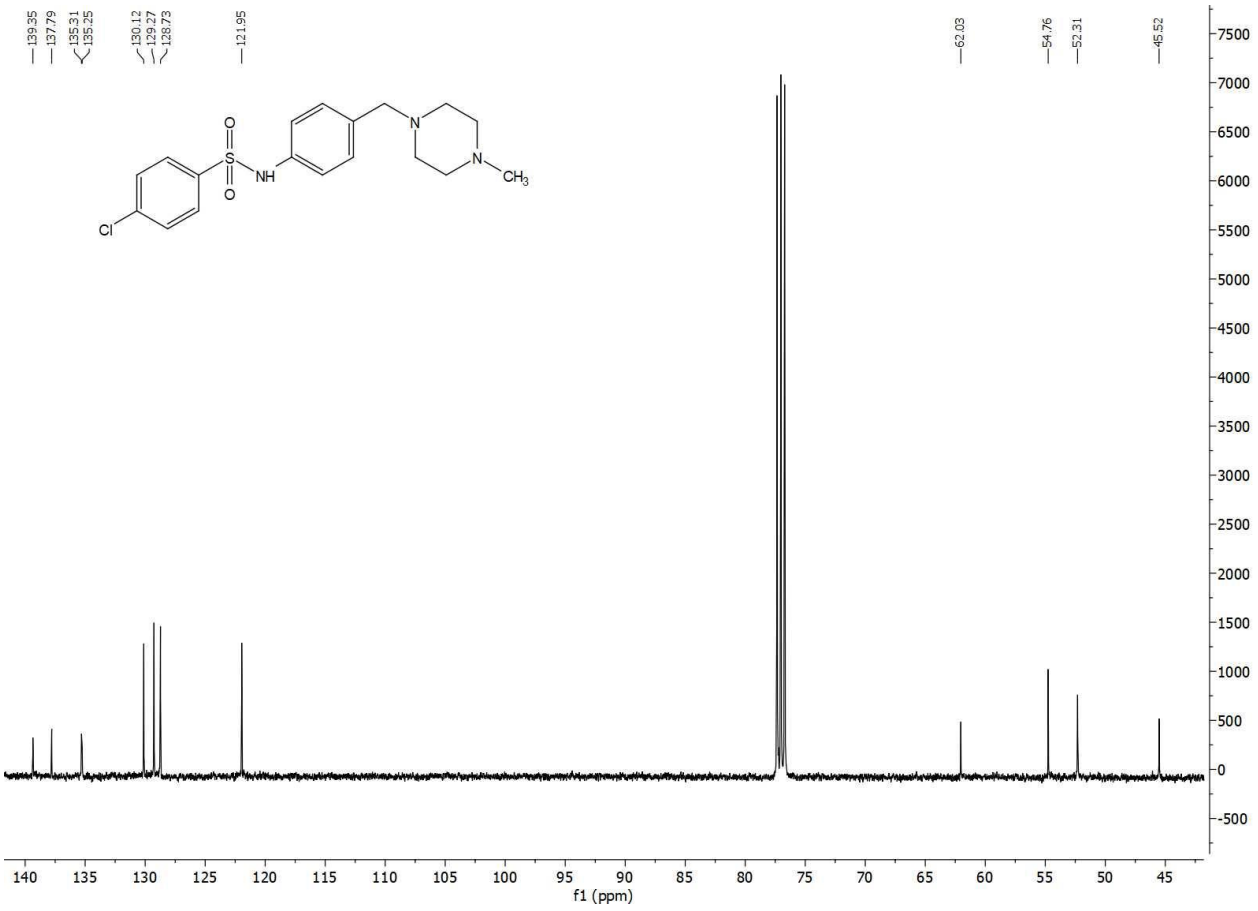
^{1}H NMR of derivative **26**, **4-Chloro-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)benzenesulfonamide,** solvent $CDCl_3$



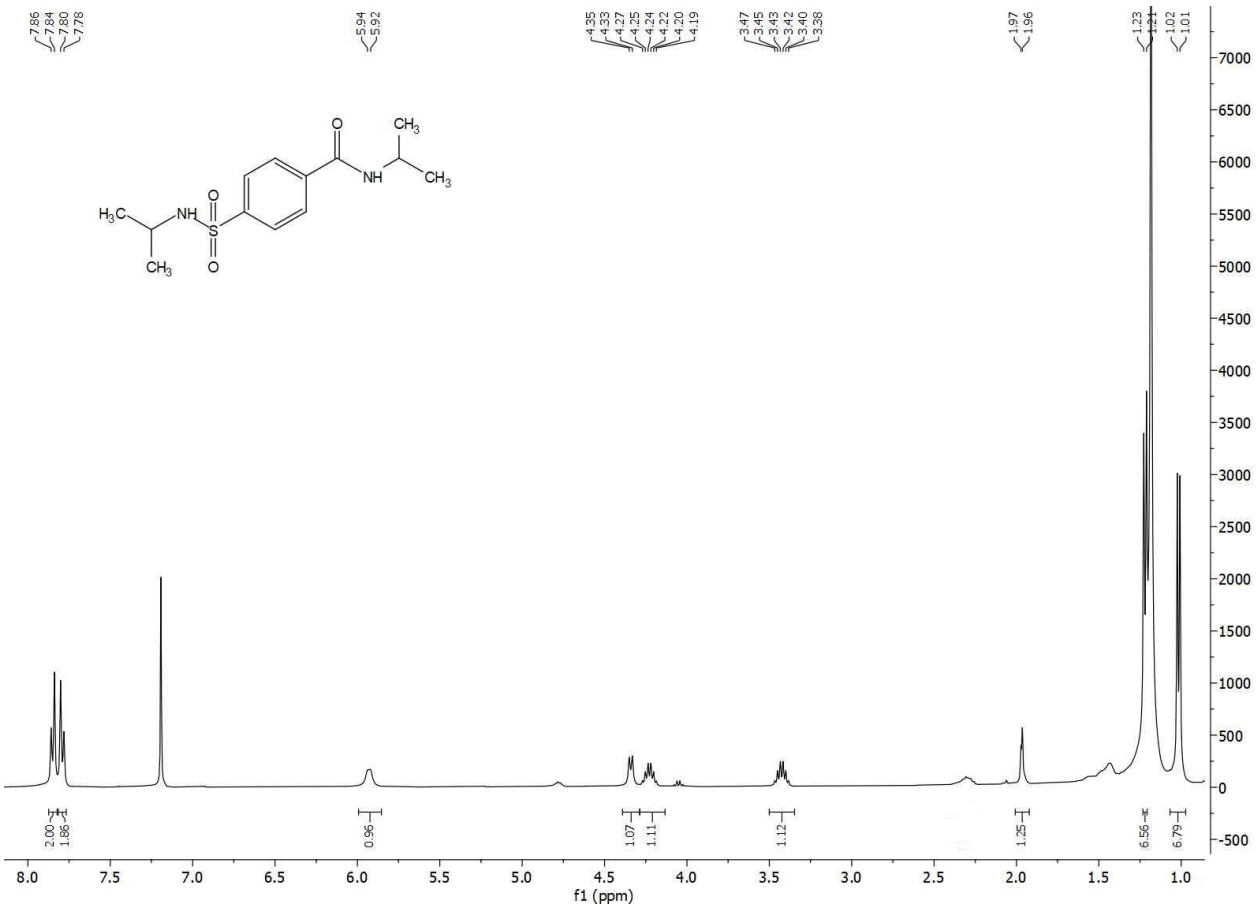
^{1}H NMR of derivative **26**, **4-Chloro-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)benzenesulfonamide,** solvent CD_3OD



^{13}C NMR of derivative **26**, **4-Chloro-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)benzenesulfonamide,** solvent $CDCl_3$

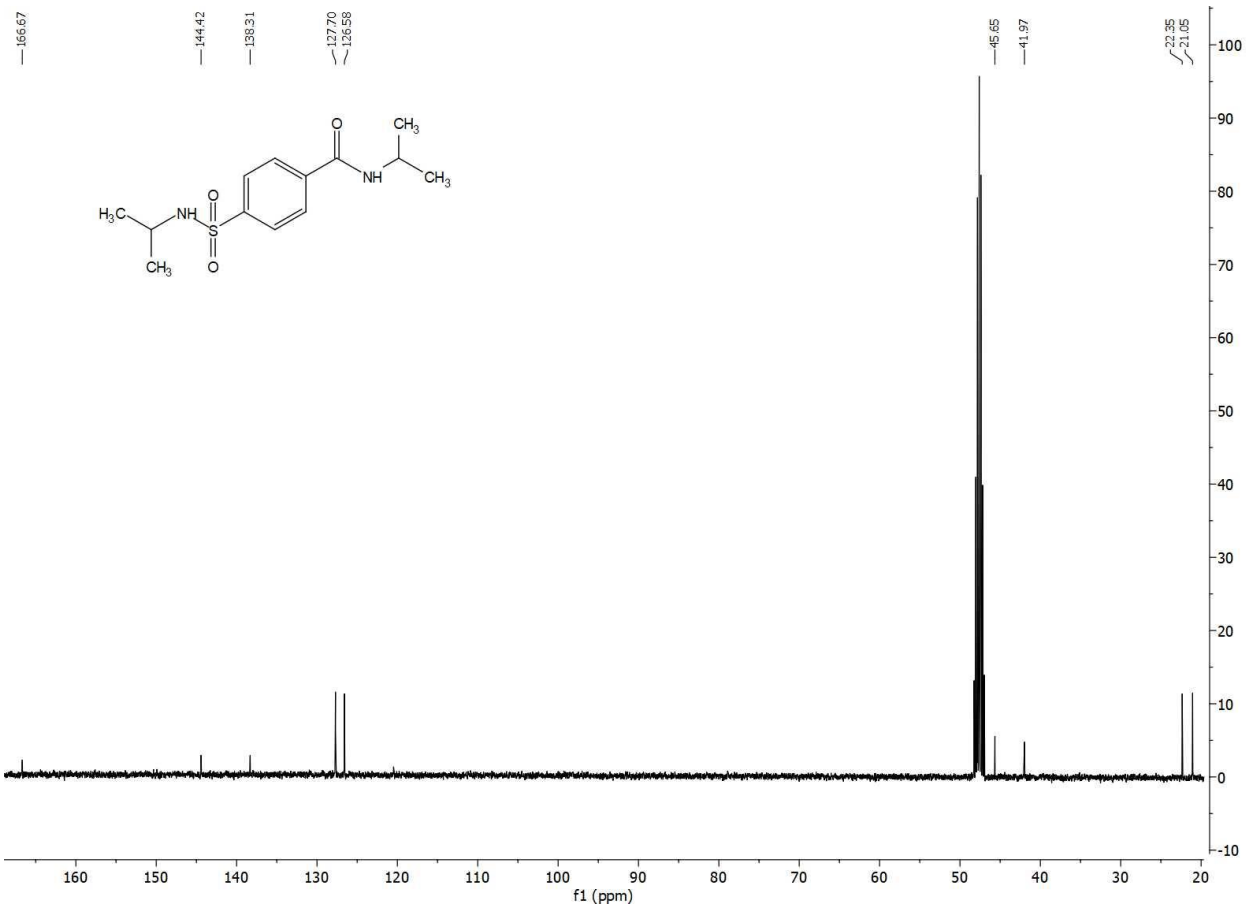


^{1}H NMR of derivative **27**, ***N*-isopropyl-4-(*N*-isopropylsulfamoyl)benzamide,** solvent $CDCl_3$

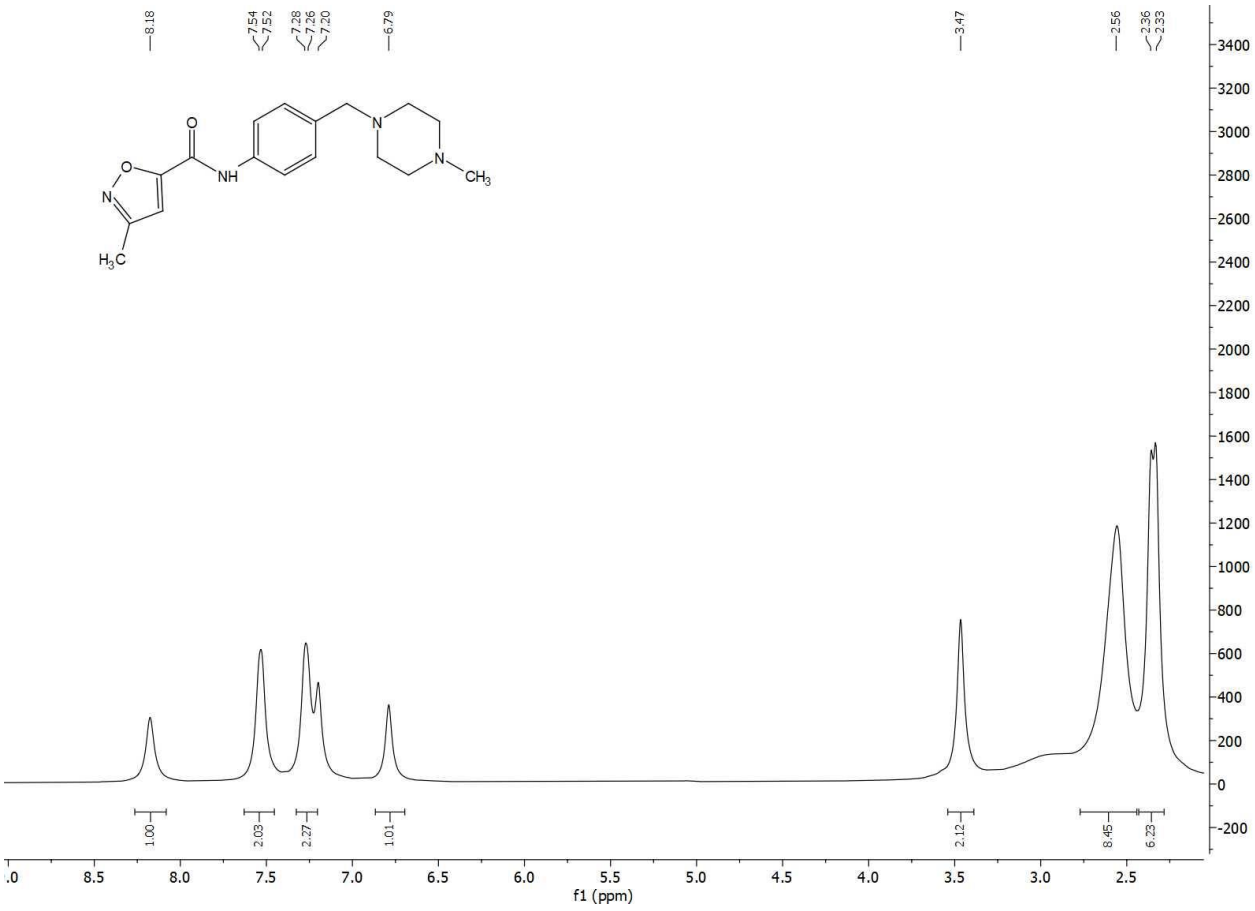


^{1}H NMR of derivative **27**, ***N*-isopropyl-4-(*N*-isopropylsulfamoyl)benzamide,** solvent CD_3OD

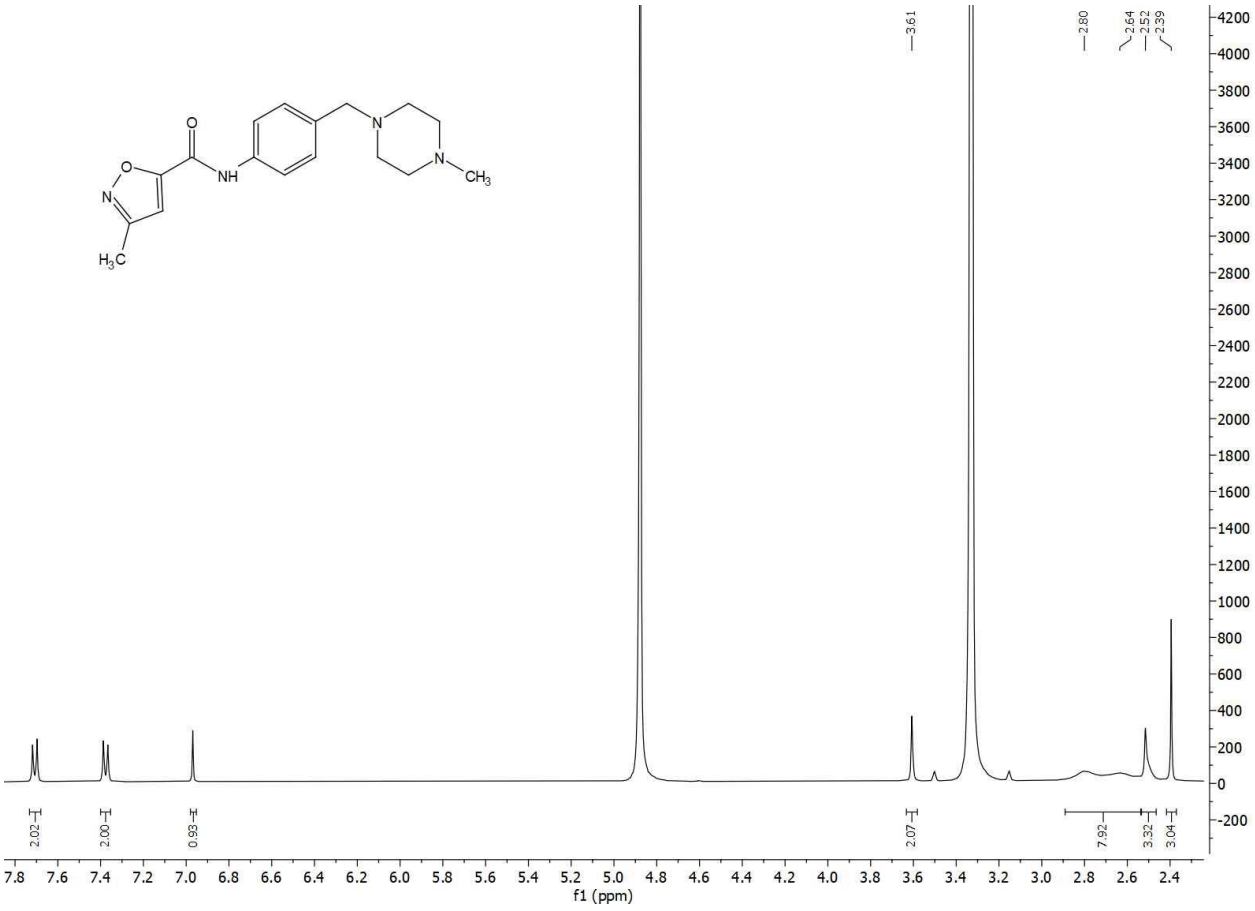
^{13}C NMR of derivative **27**, ***N*-isopropyl-4-(*N*-isopropylsulfamoyl)benzamide,** solvent CD_3OD



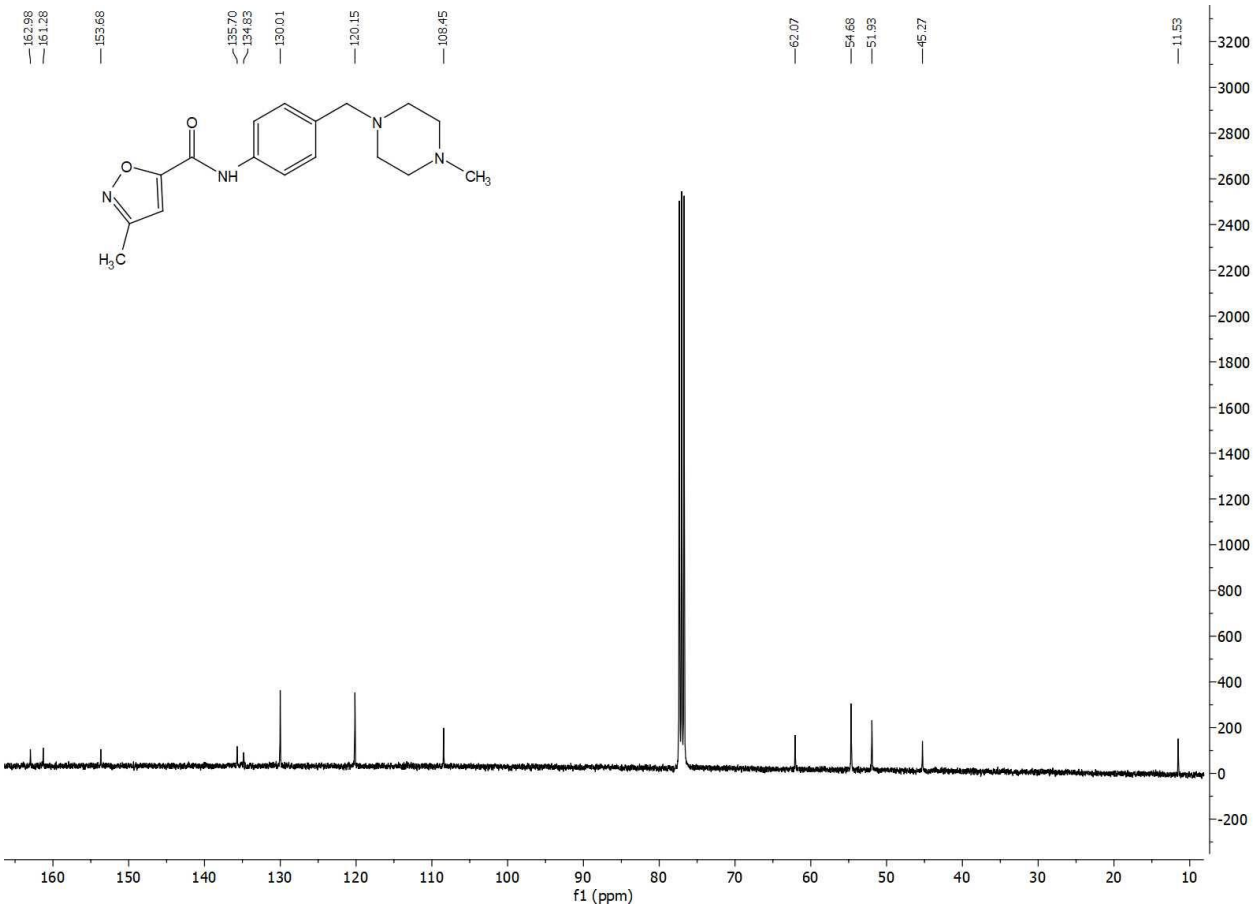
^{1}H NMR of derivative **28**, **3-Methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)isoxazole-5-carboxamide,** solvent $CDCl_3$



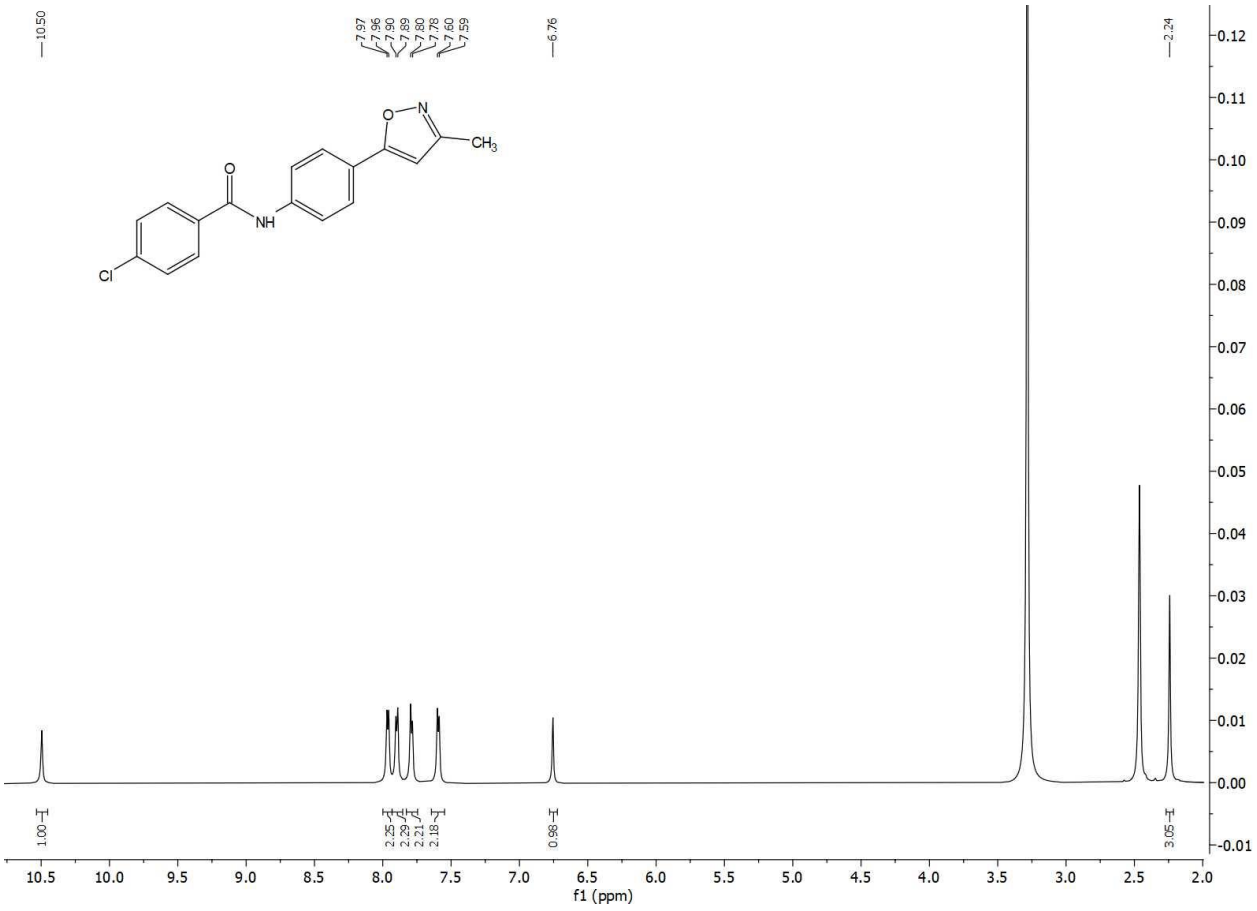
^{1}H NMR of derivative **28**, **3-Methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)isoxazole-5-carboxamide,** solvent CD_3OD



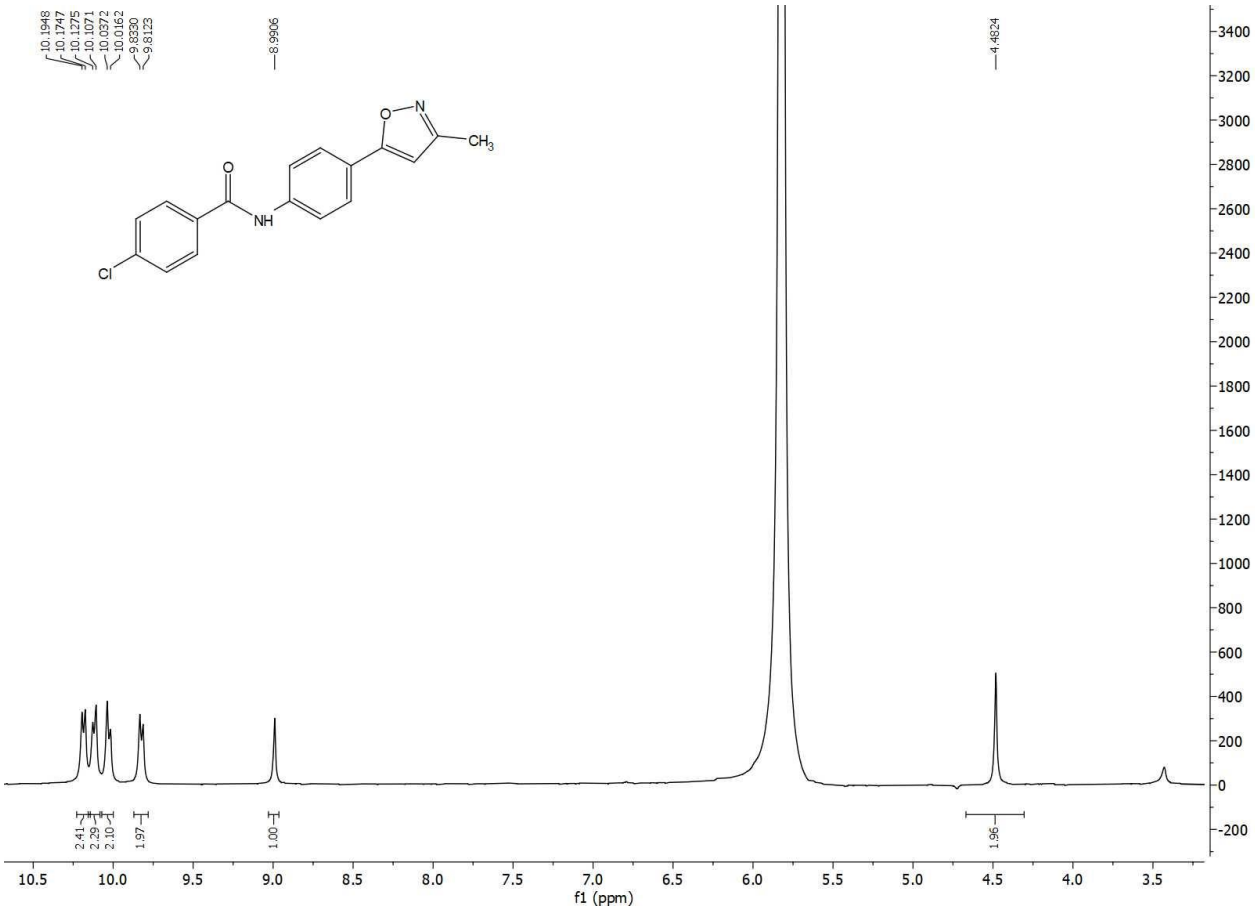
^{13}C NMR of derivative **28**, **3-Methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)phenyl)isoxazole-5-carboxamide,** solvent $CDCl_3$



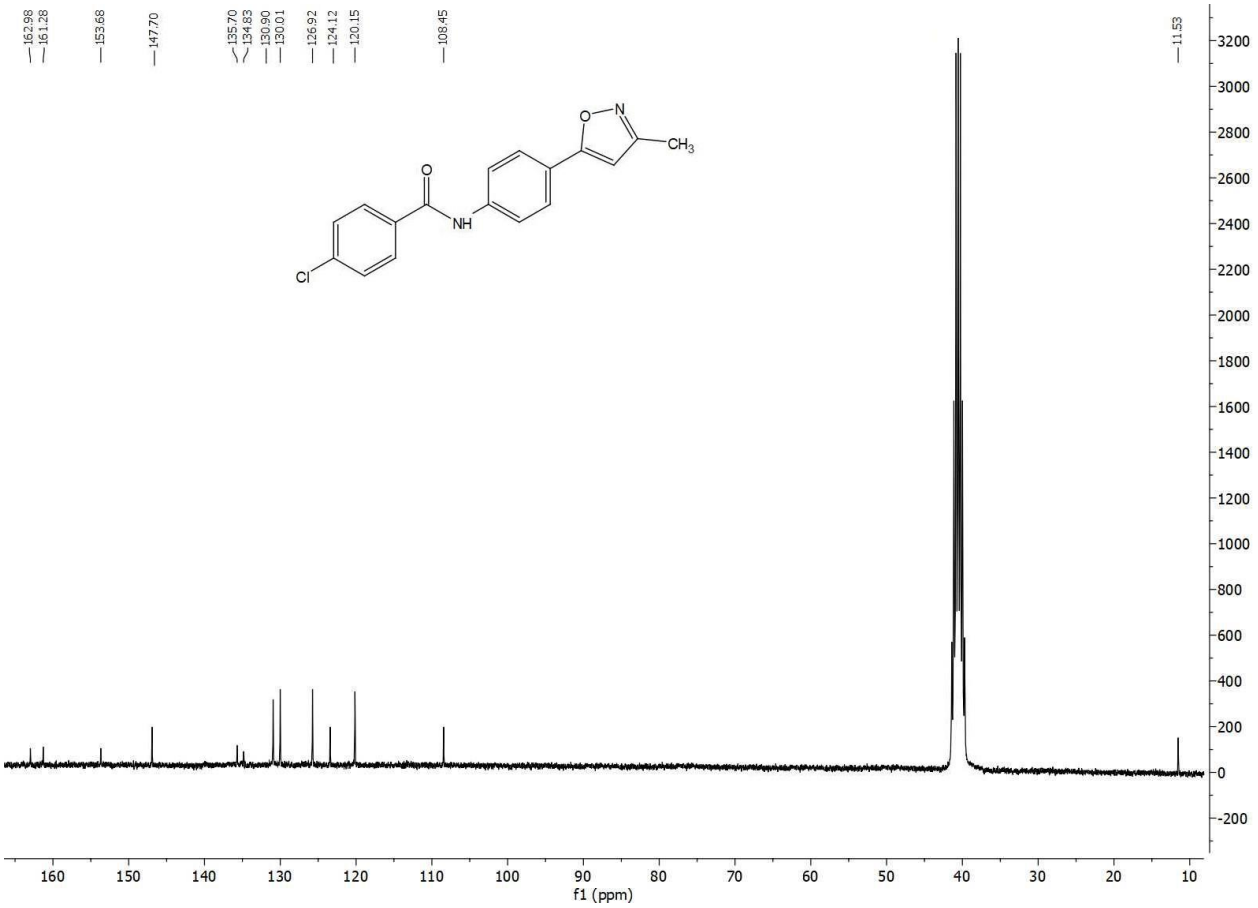
^{1}H NMR of derivative **29**, **4-Chloro-*N*-(4-(3-methylisoxazol-5-yl)phenyl)benzamide,** solvent DMSO-*d6*

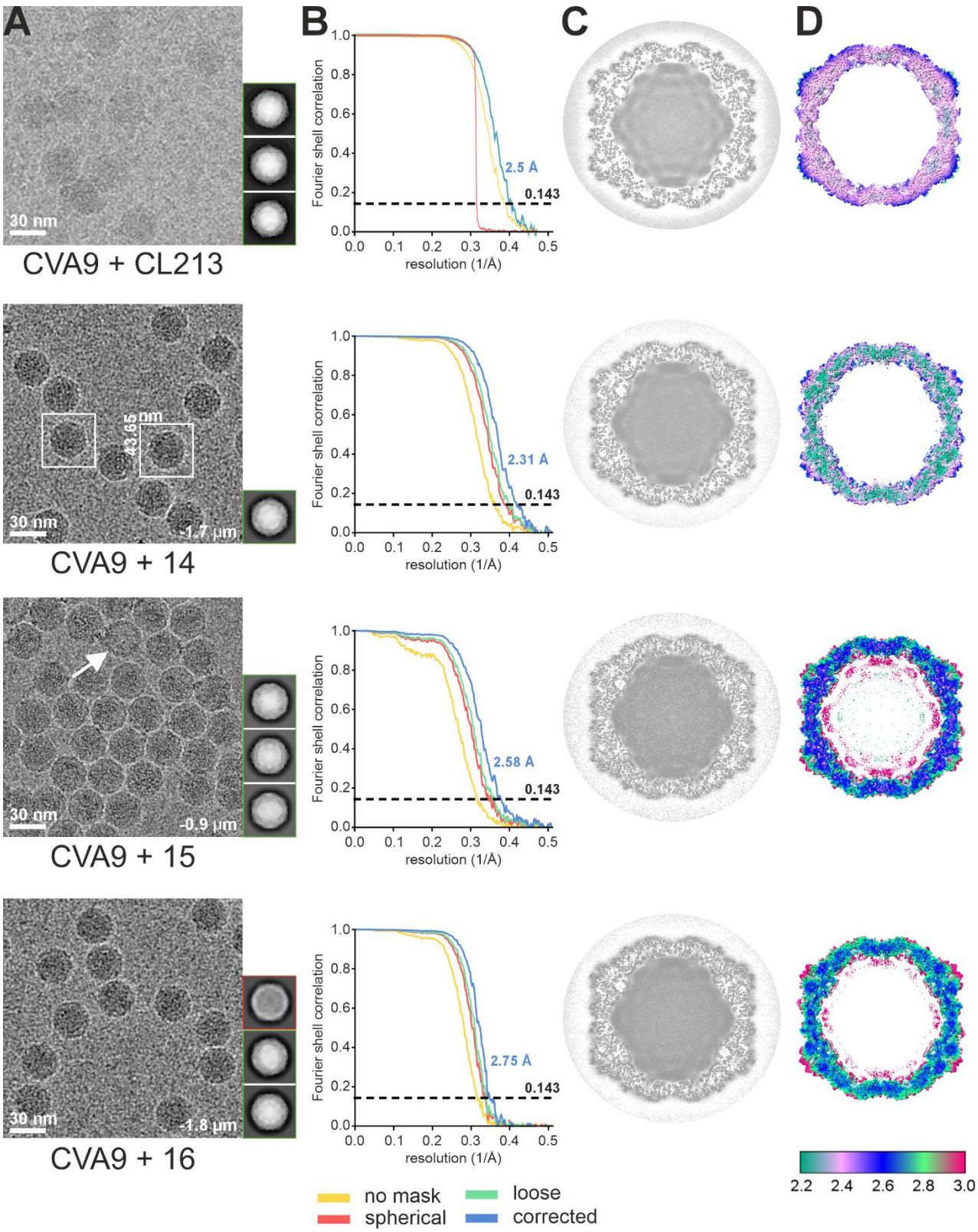


^{1}H NMR of derivative **29**, **4-Chloro-*N*-(4-(3-methylisoxazol-5-yl)phenyl)benzamide,** solvent DMSO-*d6*+D_2O



^{13}C NMR of derivative **29**, **4-Chloro-*N*-(4-(3-methylisoxazol-5-yl)phenyl)benzamide,** solvent DMSO-*d6*




A
B
C
D
30 nm
CVA9 + CL213
43.65 nm
-1.7 µm
CVA9 + 14
-0.9 µm
CVA9 + 15
-1.8 µm
CVA9 + 16
Fourier shell correlation
resolution (1/Å)
2.5 Å
2.31 Å
2.58 Å
2.75 Å
0.143
2.2 2.4 2.6 2.8 3.0
no mask
spherical
loose
corrected

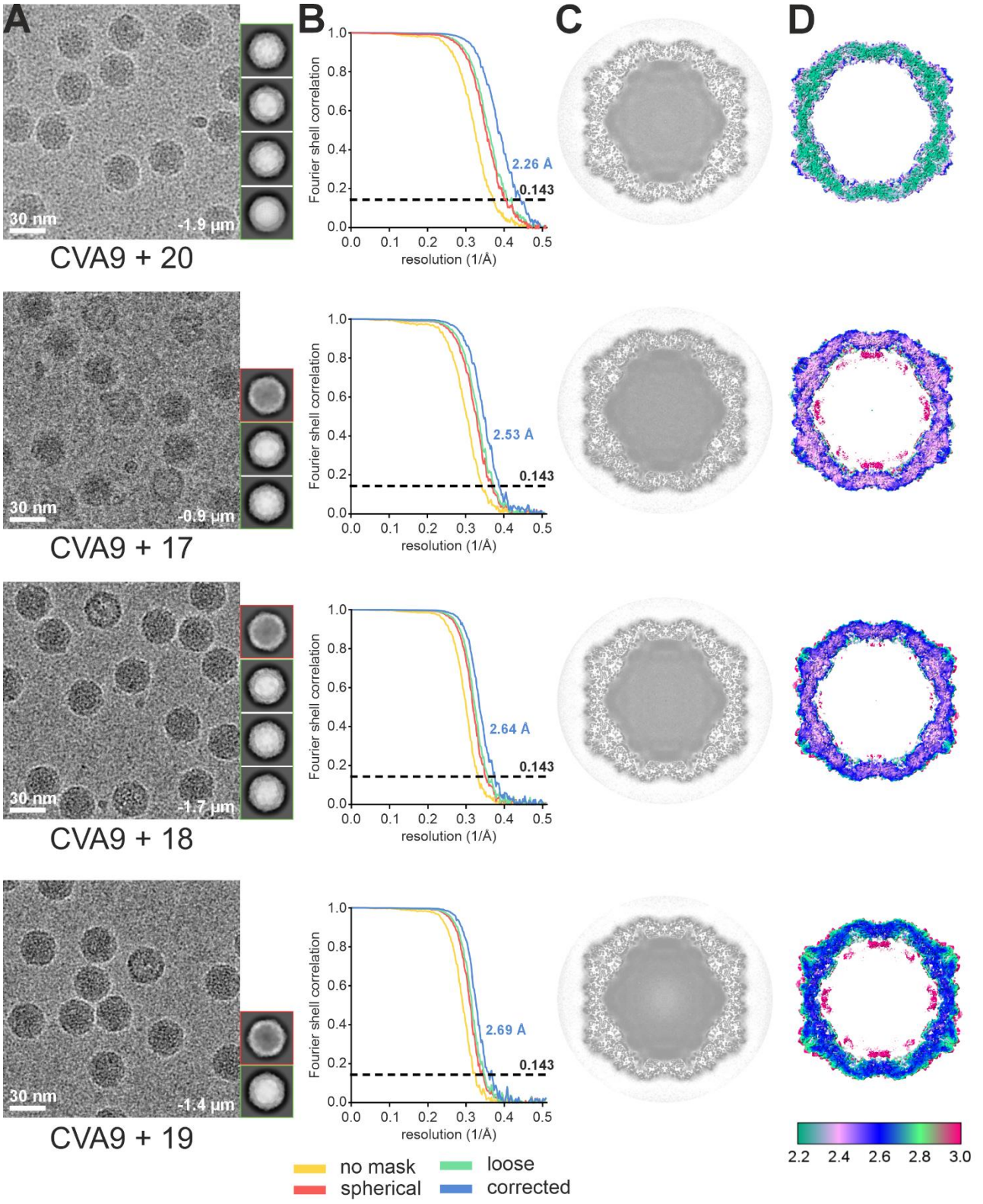


Figure S1. CryoEM data collection and image processing steps. **A.** A representative section of a micrograph from **CVA9 + CL213**, **14** – **20** datasets. Scale bar of 30 nm is in the bottom left corner of each micrograph and the defocus value in µm is in the bottom right corner for compounds 14-20.

Representative picked particles are boxed in white in a box corresponding to 450 × 450 pixels or 43.65 nm which was used for particle extraction. A representative empty particle is marked with a white arrow. On the right of the micrograph are 2D classes representatives which were selected for further processing (in green) and empty particle classes (in red) for those datasets which had empty particles. **B**. Fourier shell correlation graph showing curves for resolution estimation with no mask (yellow), spherical mask (red), loose mask (green) and corrected mask (blue). Resolution obtained using the corrected mask is used as the final global resolution and is shown on the right of the curve in blue. **C**. Central plane view of the final reconstructions. **D**. Central section of the final density map at threshold value 2σ above mean colored to represent local resolution as shown in color key below the structures with value in Å.

Table S1. Cryo-EM data collection, refinement and validation statistics.

	CVA9 + CL213	CVA9 + 14	CVA9 + 15	CVA9 + 16	CVA9 + 20	CVA9 + 17	CVA9 + 18	CVA9 + 19
Data collection and processing								
Magnification	81000	150000	150000	150000	150000	150000	150000	150000
Voltage (kV)	300	200	200	200	200	200	200	200
Electron exposure (e–/Å^2)	36.606	40	40	40	40	40	40	40
Defocus range in final reconstruction (μm)	-0.4 to -0.2	-0.7 to -3	-0.4 to -2.9	-0.8 to -2.8	-0.8 to -2.7	-0.6 to -2.3	-0.6 to -2.3	-0.5 to -2.4
Pixel size (Å)	1.06	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Symmetry imposed	I2	I2	I2	I2	I2	I2	I2	I2
Micrographs (no.)	180308	567	618	530	569	561	625	630
Initial particle images (no.)	216957	16479	14445	19317	31050	27450	26019	27004
Good particles		16479	10752	15691	24361	24208	23455	24360
Final particle images (no.)	90678	16479	2558	15691	21230	24208	22815	22525
Map resolution (Å) FSC threshold	2.5 0.143	2.31 0.143	2.58 0.143	2.75 0.143	2.26 0.143	2.53 0.143	2.64 0.143	2.69 0.143
Map resolution range (Å)	999-1.94	999-1.94	999-1.94	999-1.94	999-1.94	999-1.94	999-1.94	999-1.94
Refinement								
Map sharpening *B* factor (Å^2)	-70	-92.8	-82	-119.5	-89.7	-105	-132.1	-131.7
Model composition								

Non-hydrogen atoms Protein residues								
VP1	2-7, 11-283	1-6, 11-282	1-6, 11-282	1-6, 11-282	1-283	1-7, 11-283, 301	1-6, 11-282	1-282
VP2	10-260	10-259	10-259	10-259	10-260	10-260	10-259	10-259
VP3	1-238	1-238	1-237	2-237	1-238	1-238	3-237	2-237
VP4	MYR-2-14, 23-68	MYR-2-14, 24-69	MYR-2-14, 24-69	MYR-2-10, 12-14, 25-68	MYR-2-14, 24-69	MYR-2-14, 24-69	MYR-2-10, 12-14, 25-68	MYR-2-10, 12-14, 25-68
Ligands	PLM	CL275	CL278	CL298	CL300	CL301	CL304	CL313
R.m.s. deviations								
Bond lengths (Å)	0.013	0.004	0.005	0.005	0.006	0.006	0.005	0.005
Bond angles (°)	1.87	0.715	1.114	0.732	1	1.008	0.574	0.574
Validation								
MolProbity score	1.35	1.64	1.58	1.64	1.35	1.61	1.54	1.72
Clashscore	2.34	7.2	6.97	5.6	2.26	5.15	4.1	5.81
Poor rotamers (%)	0.14	0.56	0.84	0.98	0	1.11	0.42	1.25
Ramachandran plot								
Favored (%)	95.21	96.31	96.8	95.18	95.01	95.71	95.29	95.32
Allowed (%)	4.79	3.69	3.2	4.82	4.99	3.79	4.46	4.56
Disallowed (%)	0	0	0	0	0	0.25	0.25	0.12
EMDB accession number	50636	50039	50614	50269	19774	50324	50414	50617
PDB ID	9FP5	9EXI	9FO2	9FA9	8S7J	9FCZ	9FGN	9FO5

Table S2. Computed docking score and experimental IC_{50} values

Compound name	Score (Kcal/mol)	IC_{50} (μM)
1	-89.53	>30
2	-89.04	>30
3	-84.32	>30
4	-97.75	>30
5	-115.00	>30
6	-92.41	>30
7	-91.03	>30
8	-101.97	>30
9	-95.56	1.35
10	-98.73	>30
11	-96.56	>30
12	-94.13	>30
13	-121.18	0.68
14	-100.09	0.97
15	-96.06	2.13
16	-100.66	4.58
17	-98.71	7.77
18	-95.07	8.32
19	-94.49	5.99

20	-96.85	10
21	-91.58	14.06
22	-73.34	>30
23	-93.21	>30
24	-78.92	>30
25	-83.64	>30
26	-84.76	>30
27	-69.44	>30
28	-86.42	>30
29	-79.74	>30
CL212	-92.58	5.92
CL213	-94.11	1.09

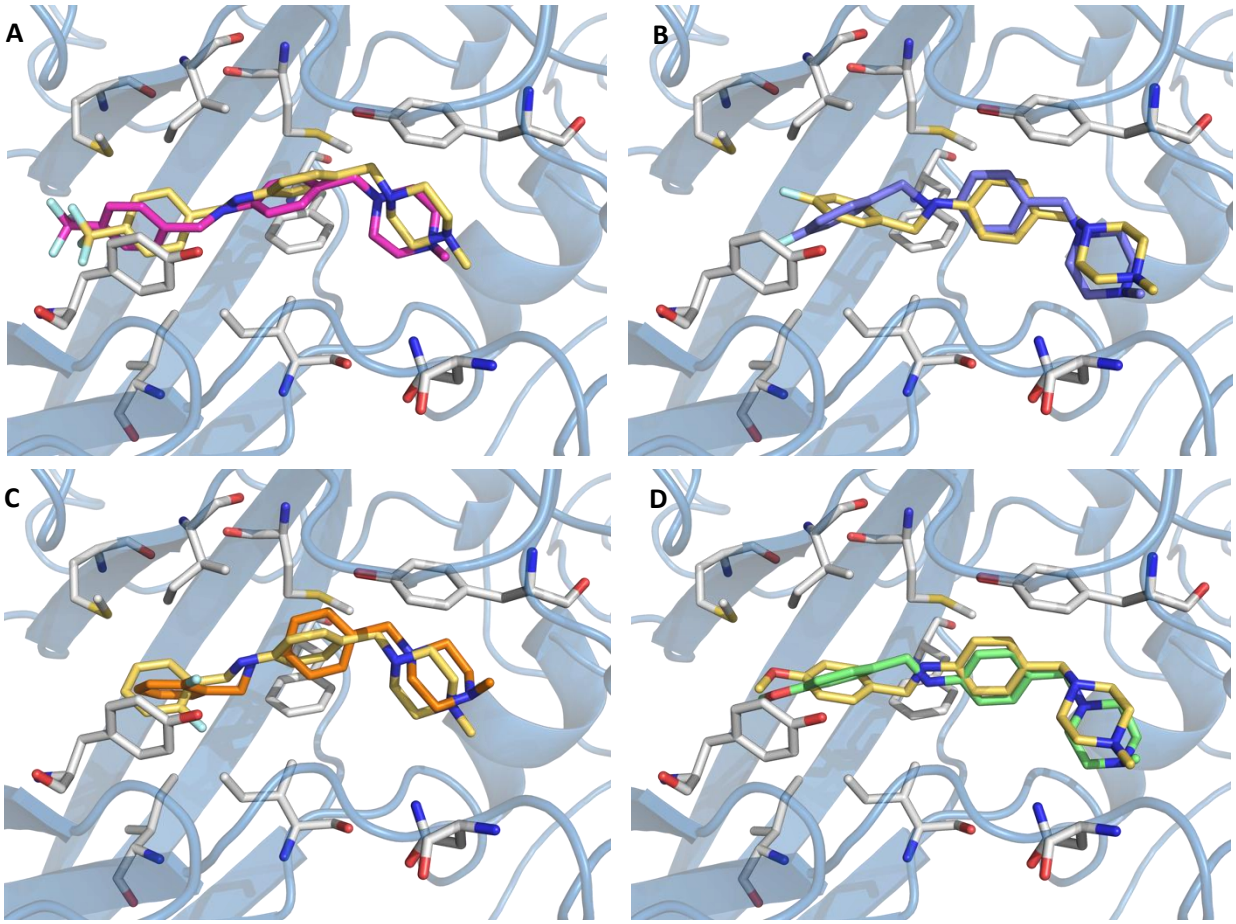


Figure S2. Superimposition of Cryo-EM (yellow) and docking proposed binding mode. All derivatives showed an extended superimposition. Derivatives **14** (magenta), **15** (purple), **16** (orange) and **20** (green) had an RMSD value of 1.33, 1.25,1.32 and 1.29 Å respectively.

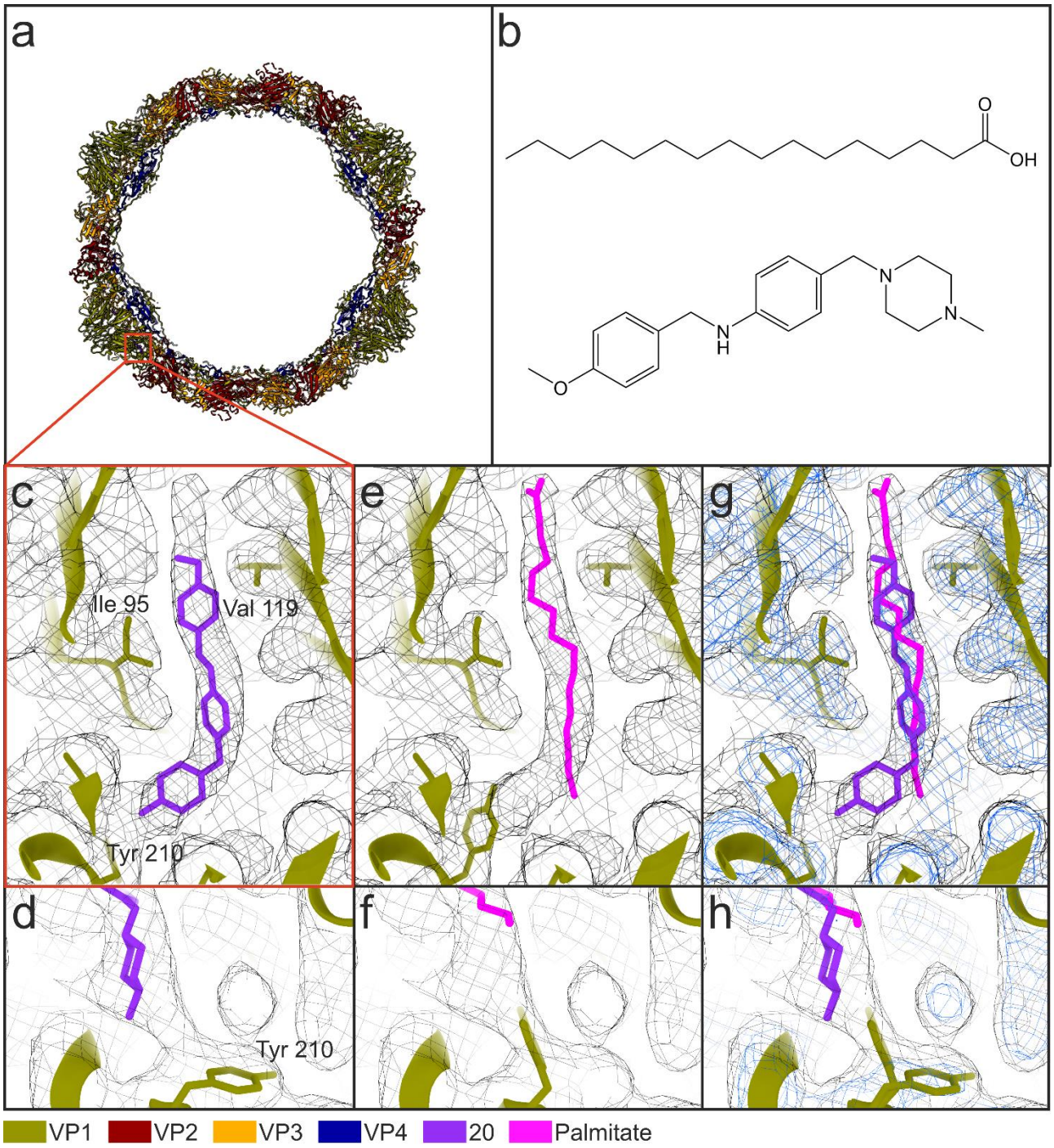


Figure S3. Hydrophobic pocket density in the CVA9 + **20** map. **A.** A cross-section of the capsid model with the position of compound **20** highlighted with a red box. **B.** Chemical models of a palmitate fatty acid (above) and compound **20** (below). **C.** Compound **20** modeled in the ligand density with a close-up of the Tyr 210 rotamer shown in **D**. **E.** A palmitate molecule modeled in the ligand density with a close-up of the Tyr 210 rotamer shown in **F**. **G.** Compound **20** and palmitate models superimposed to represent an averaged density, with a close-up of the Tyr 210 rotamers shown

in **H**. The map is depicted as a black mesh at a 1 σ above the mean threshold. The map is depicted as blue mesh at a 3 σ above the mean threshold. The molecular models were created in ChemDraw v22.2.0. The other maps and models were visualized in UCSF ChimeraX v1.5.

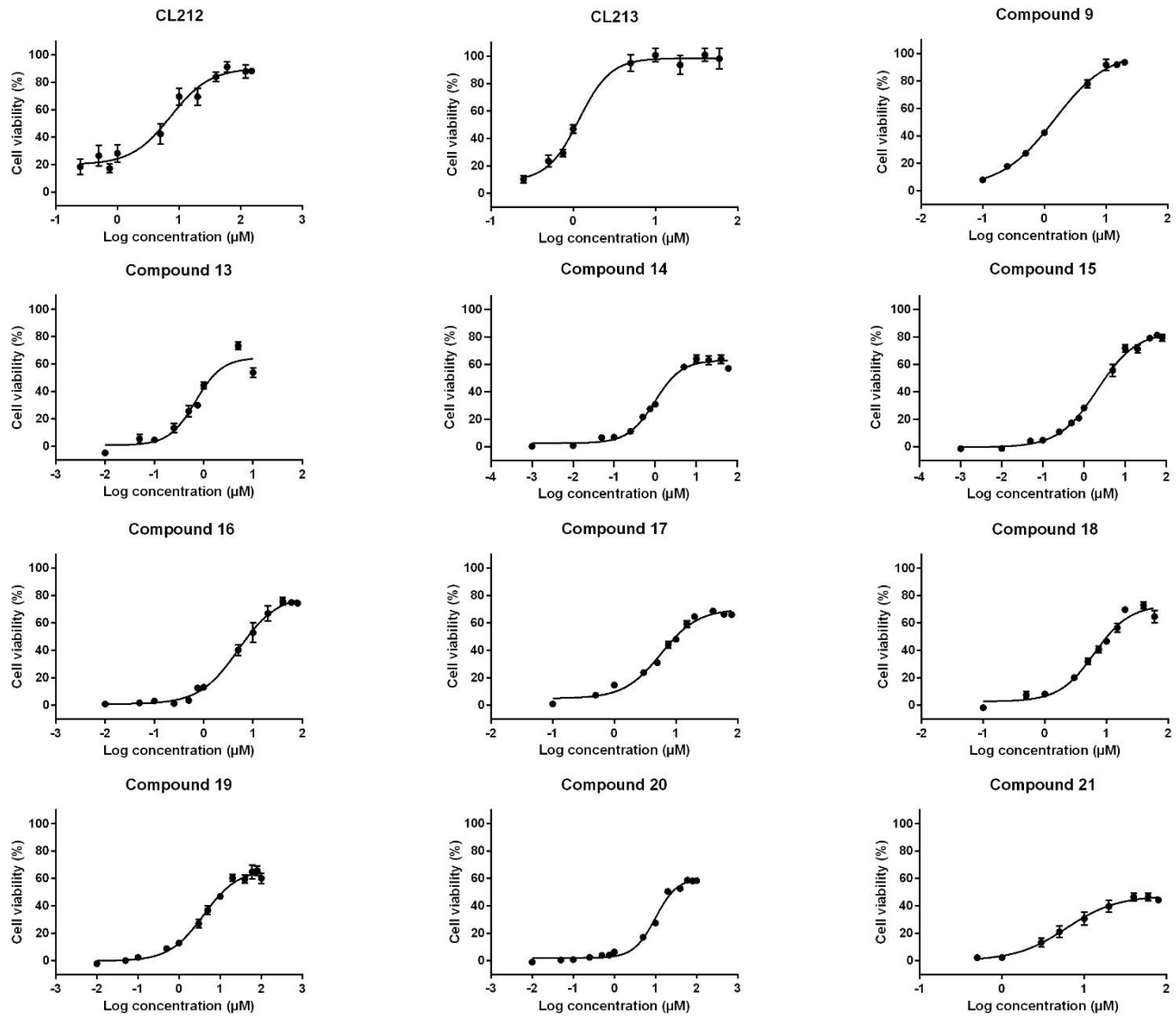


Figure S4. EC_{50} curves for compounds **CL212, CL213, 9** and **13-21.**

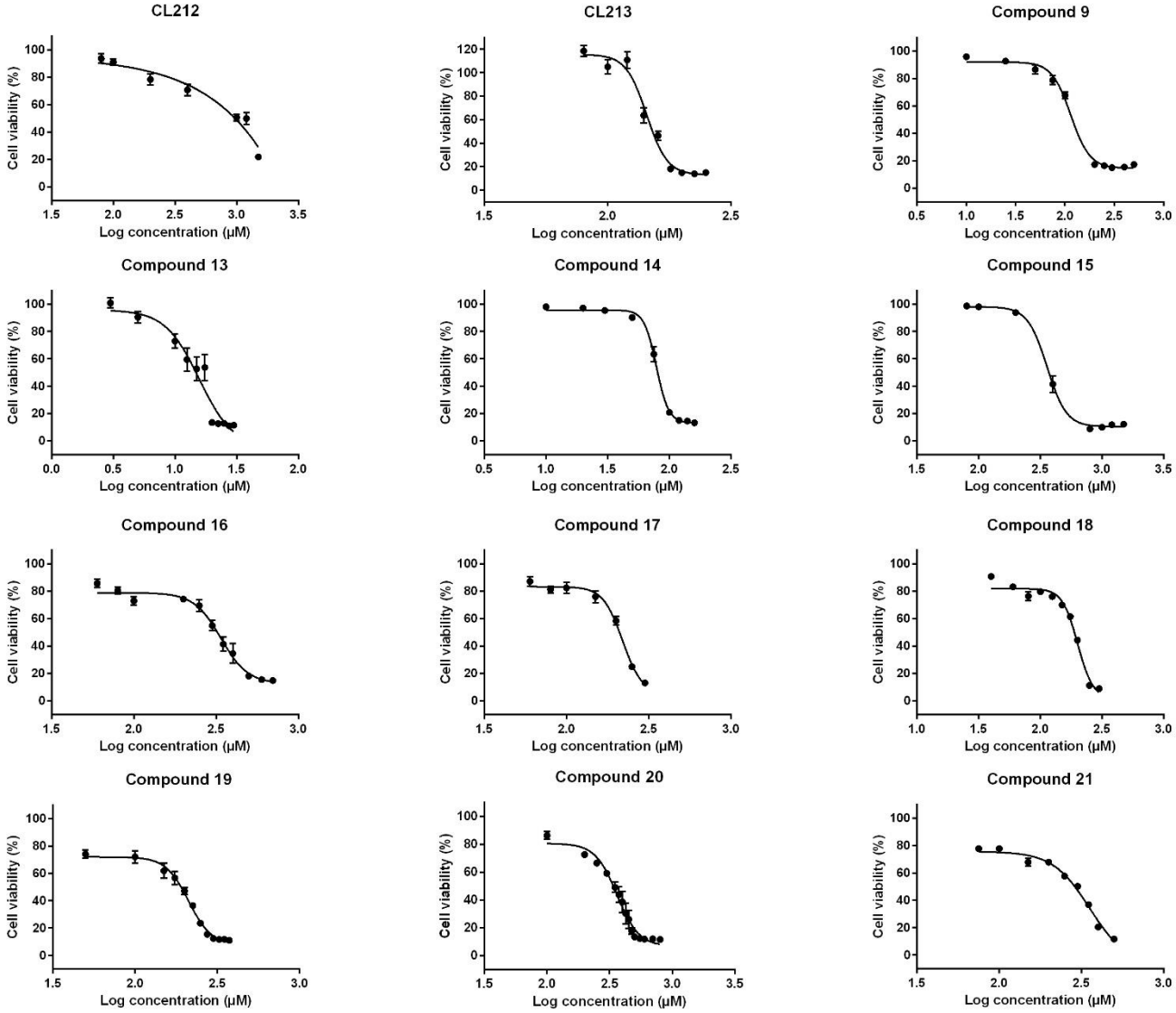


Figure S5. CC_{50} curves for compounds **CL212, CL213, 9** and **13-21.**