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Supporting Information

Evaluation of 4-(4-Fluorobenzyl)piperazin-1-yl]-Based Compounds as Competitive Tyrosinase Inhibitors Endowed with Antimelanogenic Effects

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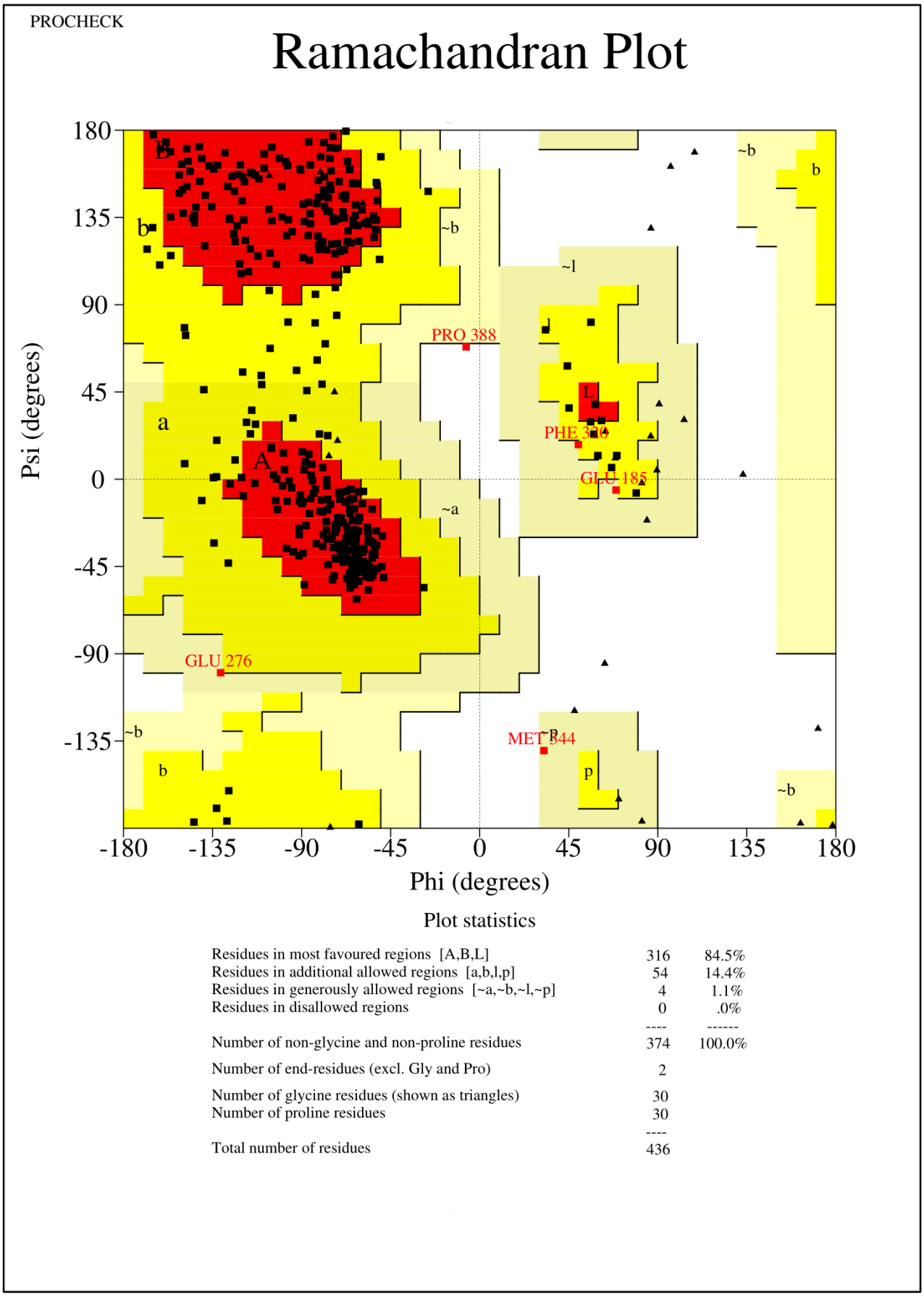
Supporting Information:

Content:

Figure S1. Ramachandran plot of hTYR homology model



[1]H-NMR and selected representative [13]C-NMR spectra

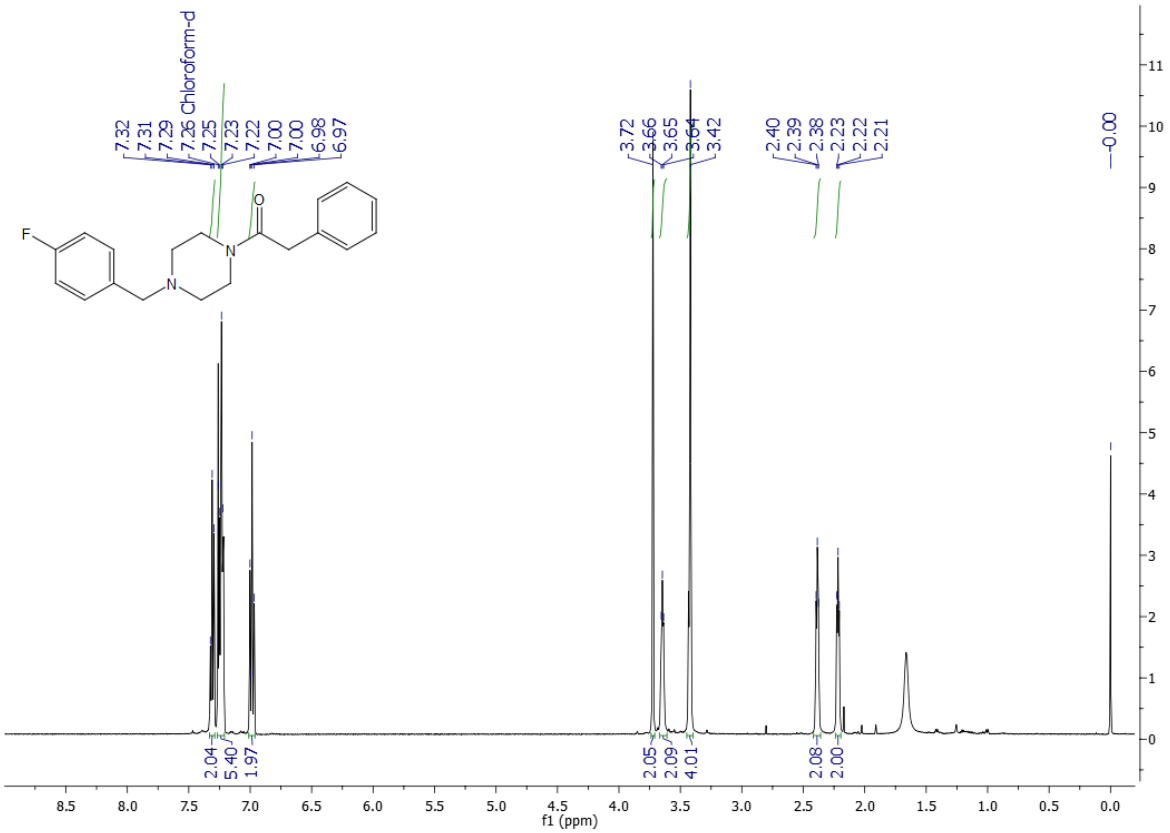


Figure S2: [1]H-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-phenylethan-1-one (**7**)

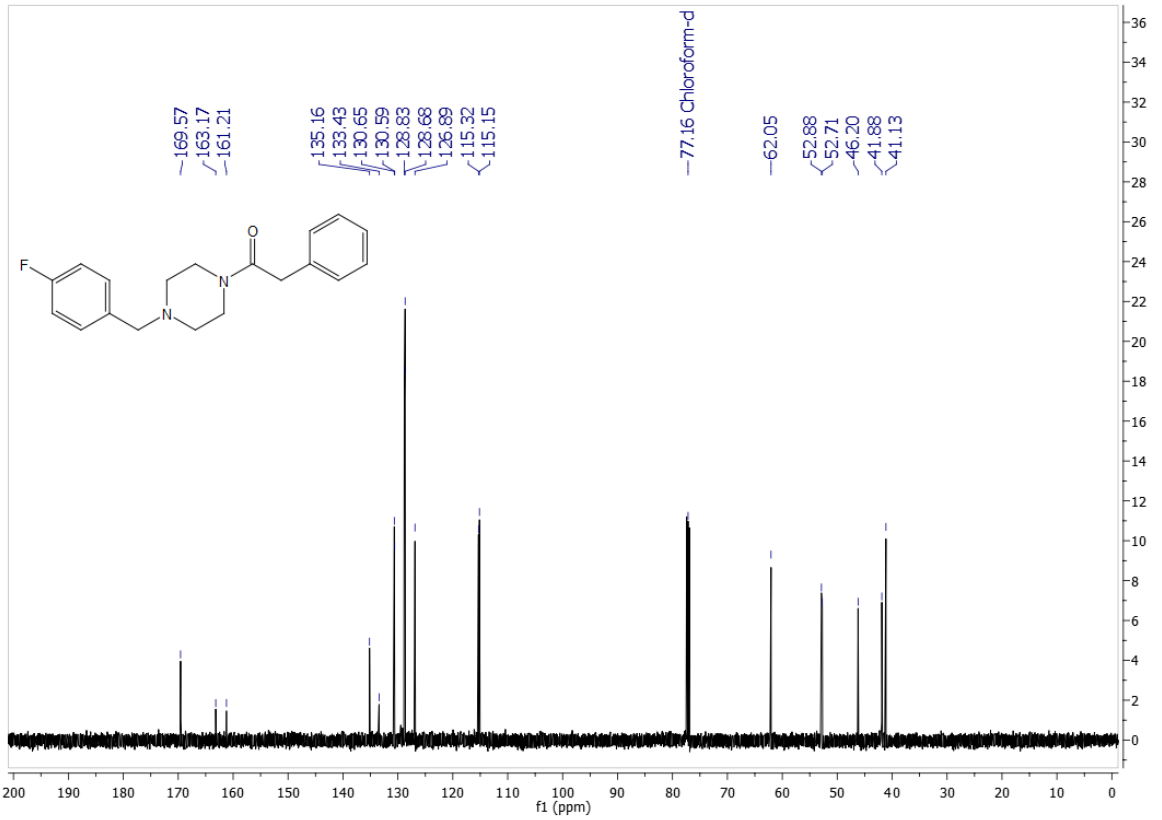


Figure S3: [13]C-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-phenylethan-1-one (**7**)

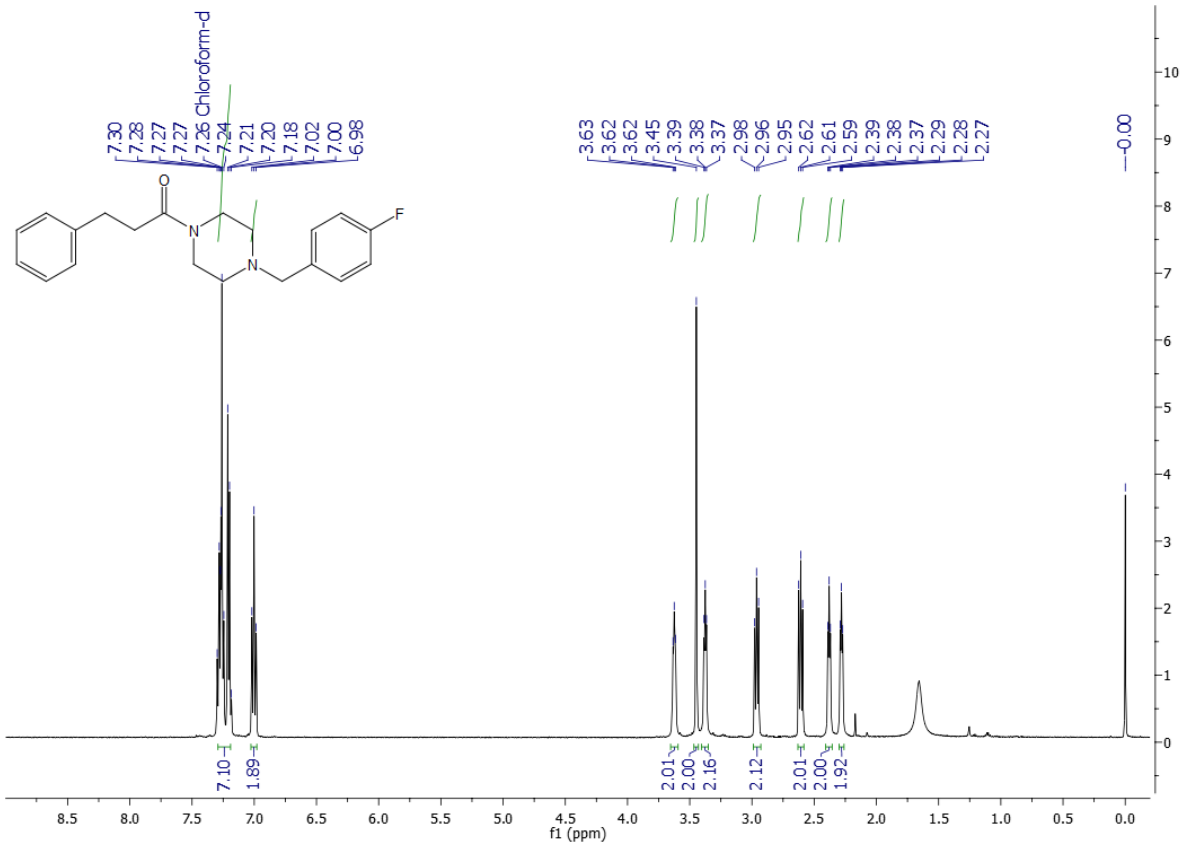


Figure S4: ^{1}H-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-3-phenylpropan-1-one (**8**)

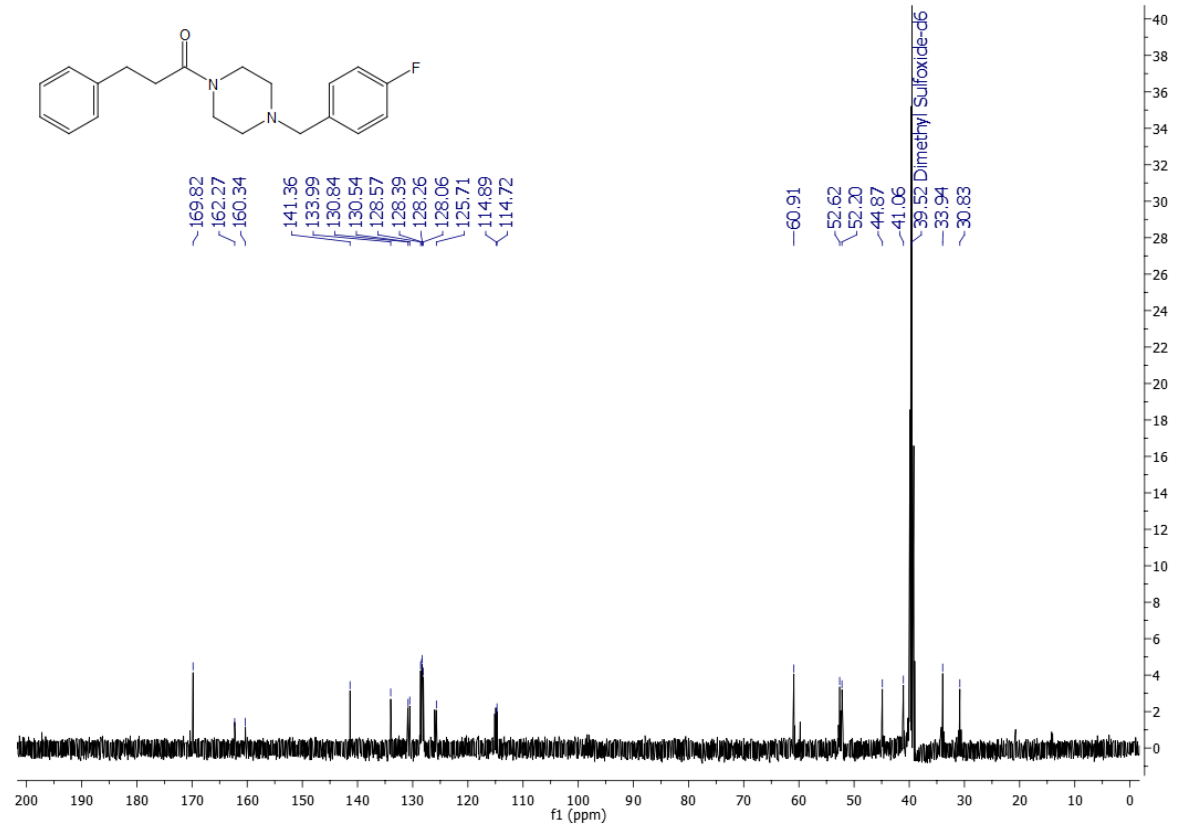


Figure S5: ^{13}C-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-3-phenylpropan-1-one (**8**)

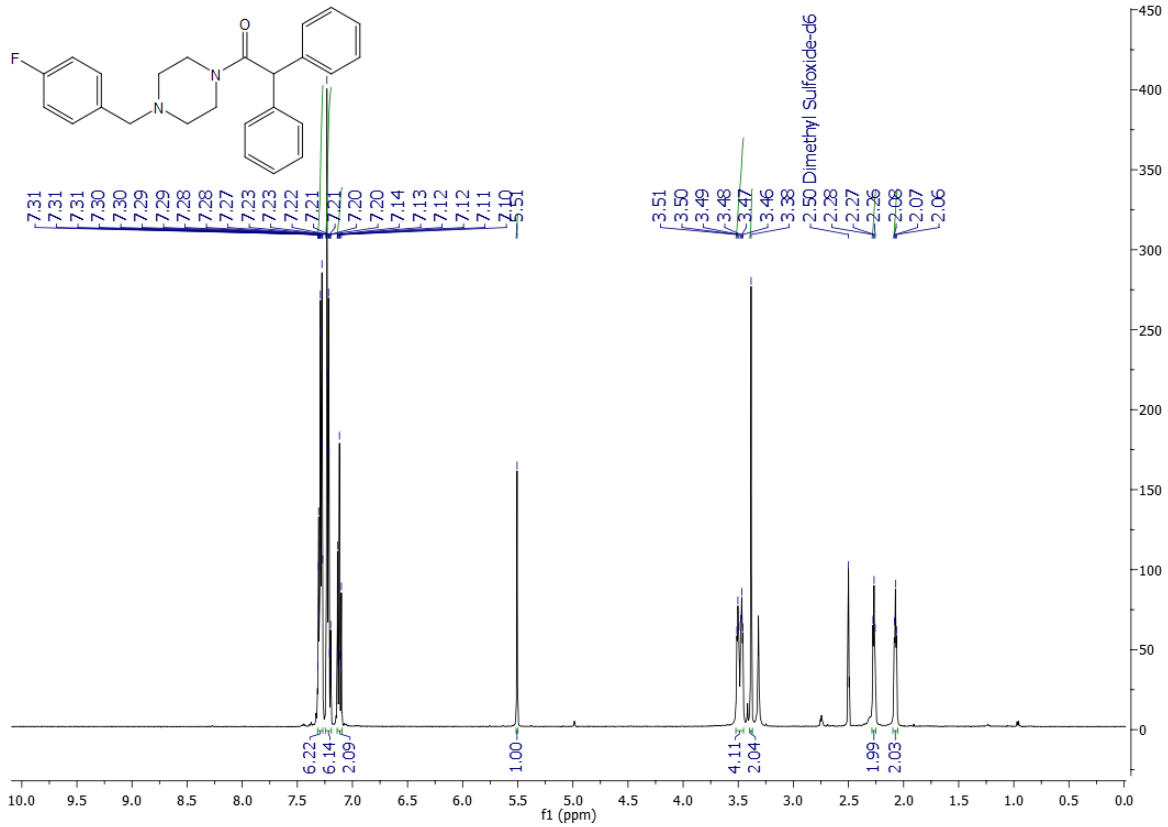


Figure S6: [1]H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2,2-diphenylethan-1-one (**9**)

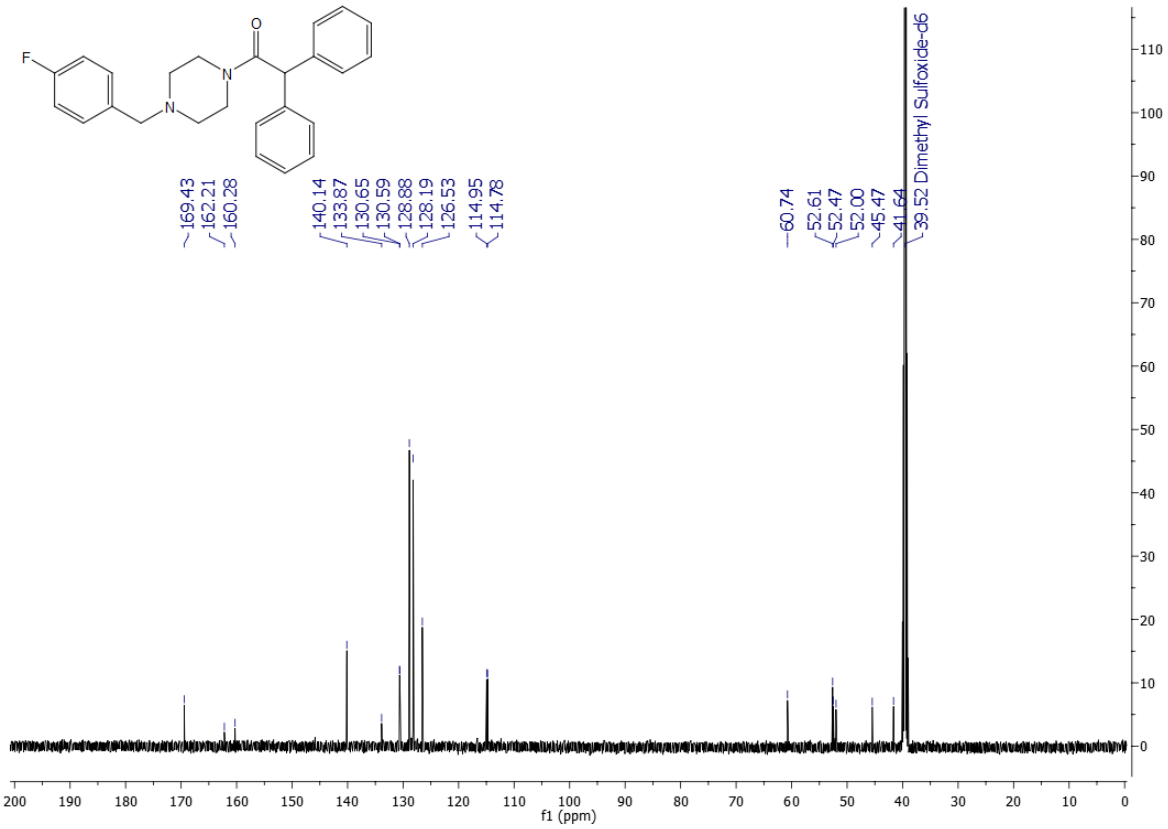


Figure S7: [13]C-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2,2-diphenylethan-1-one (**9**)

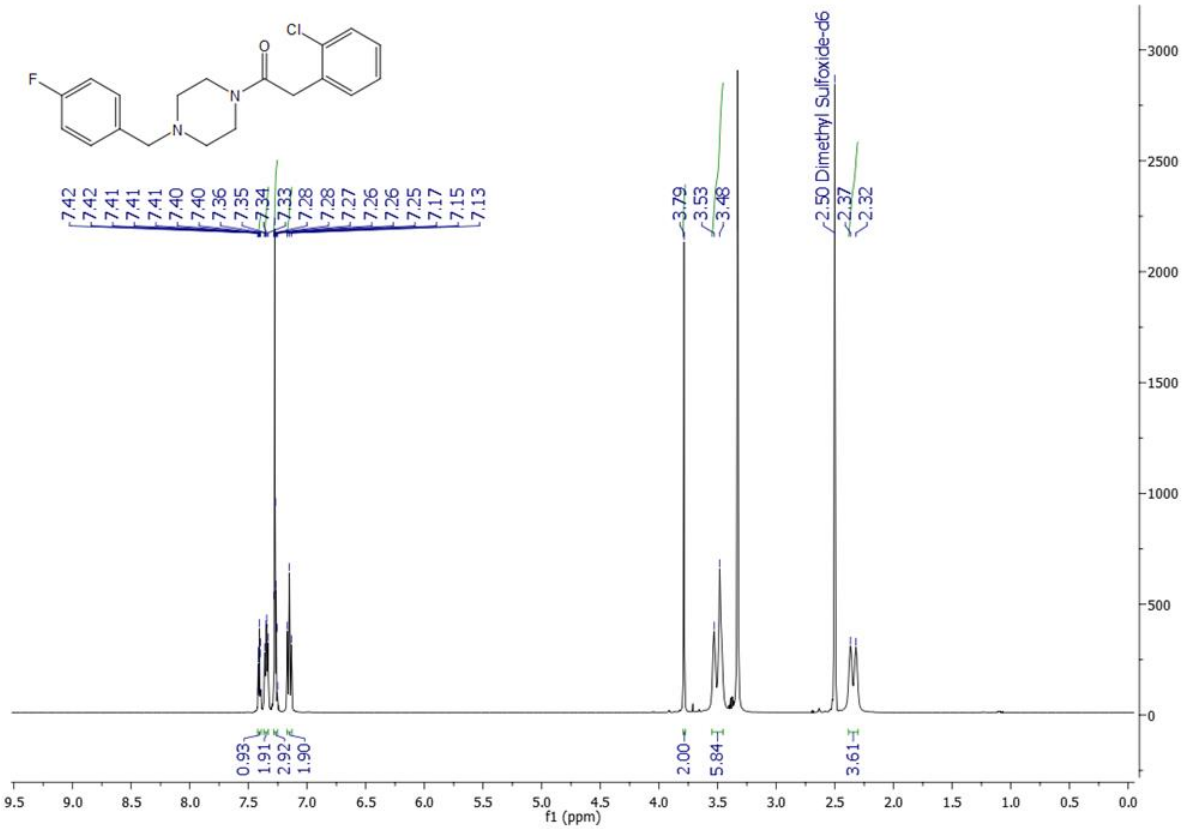


Figure S8: [1]H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(2-chlorophenyl)ethan-1-one (**10**)

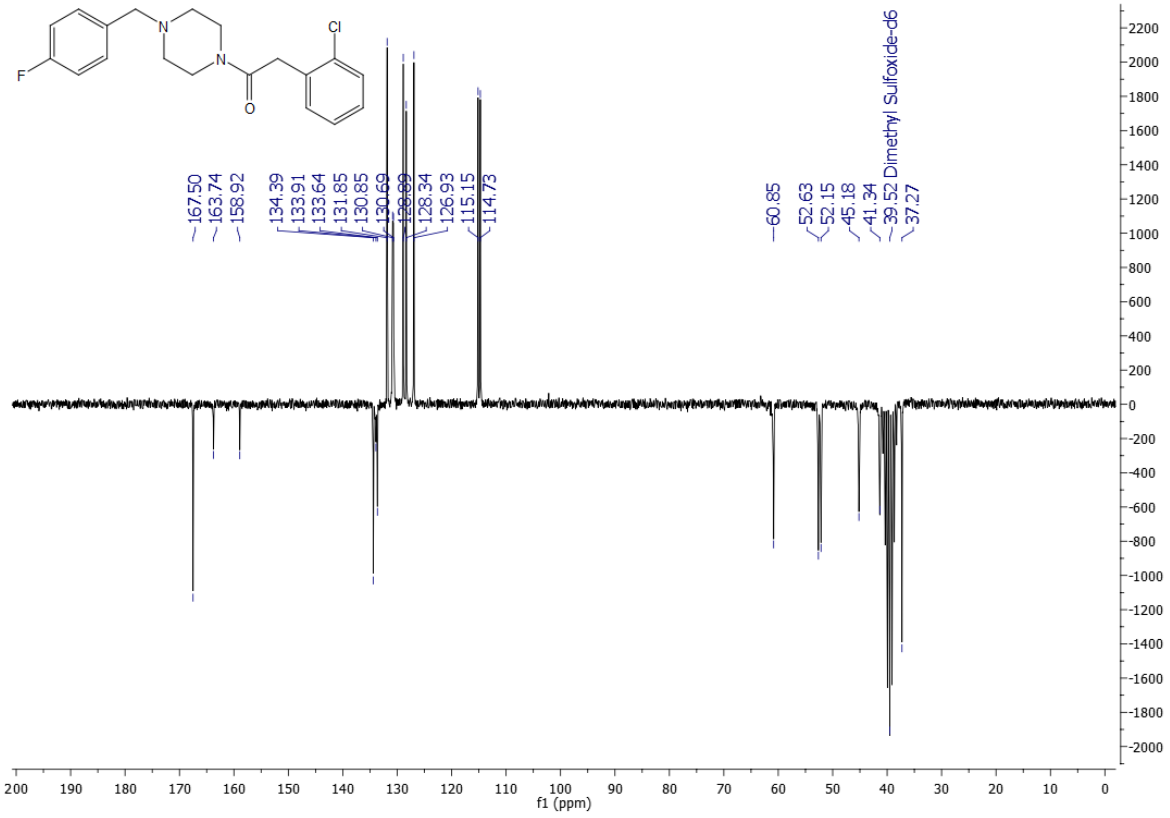


Figure S9: [13]C-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(2-chlorophenyl)ethan-1-one (**10**)

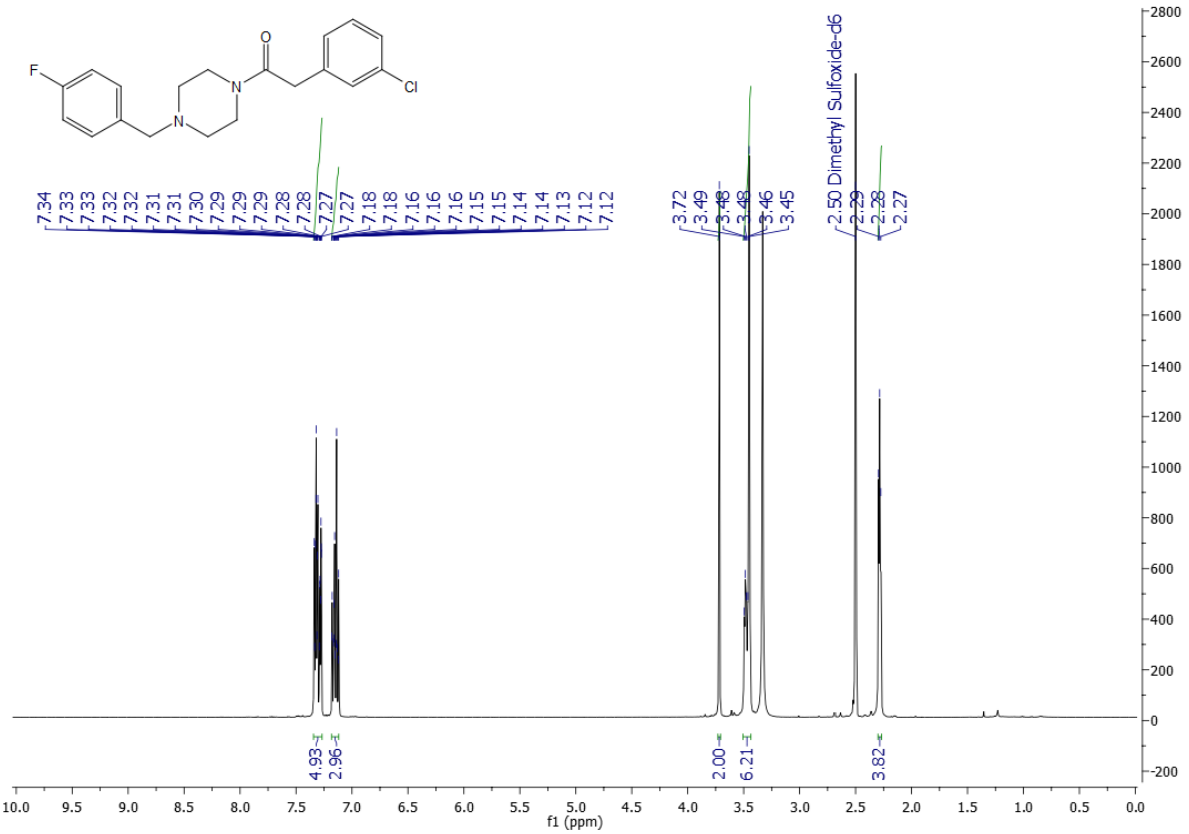


Figure S10: ^{1}H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(3-chlorophenyl)ethan-1-one (**11**)

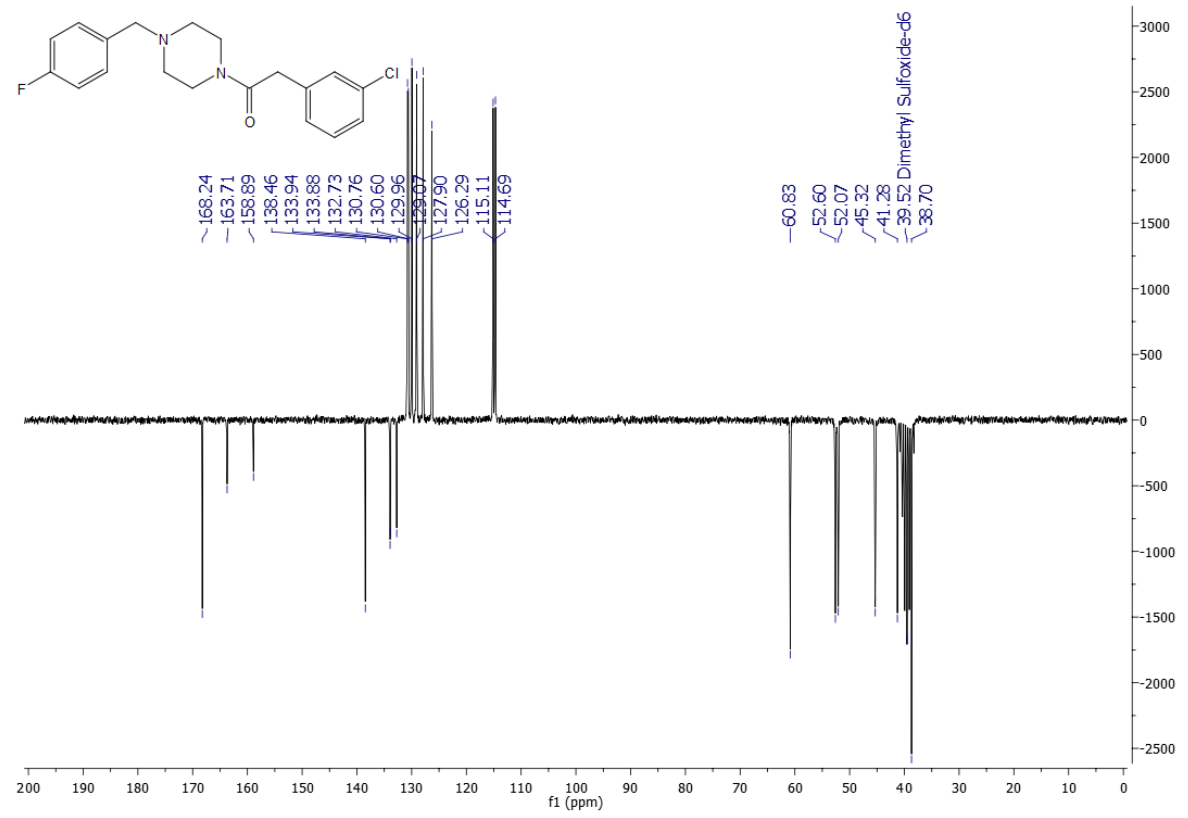


Figure S11: ^{13}C-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(3-chlorophenyl)ethan-1-one (**11**)

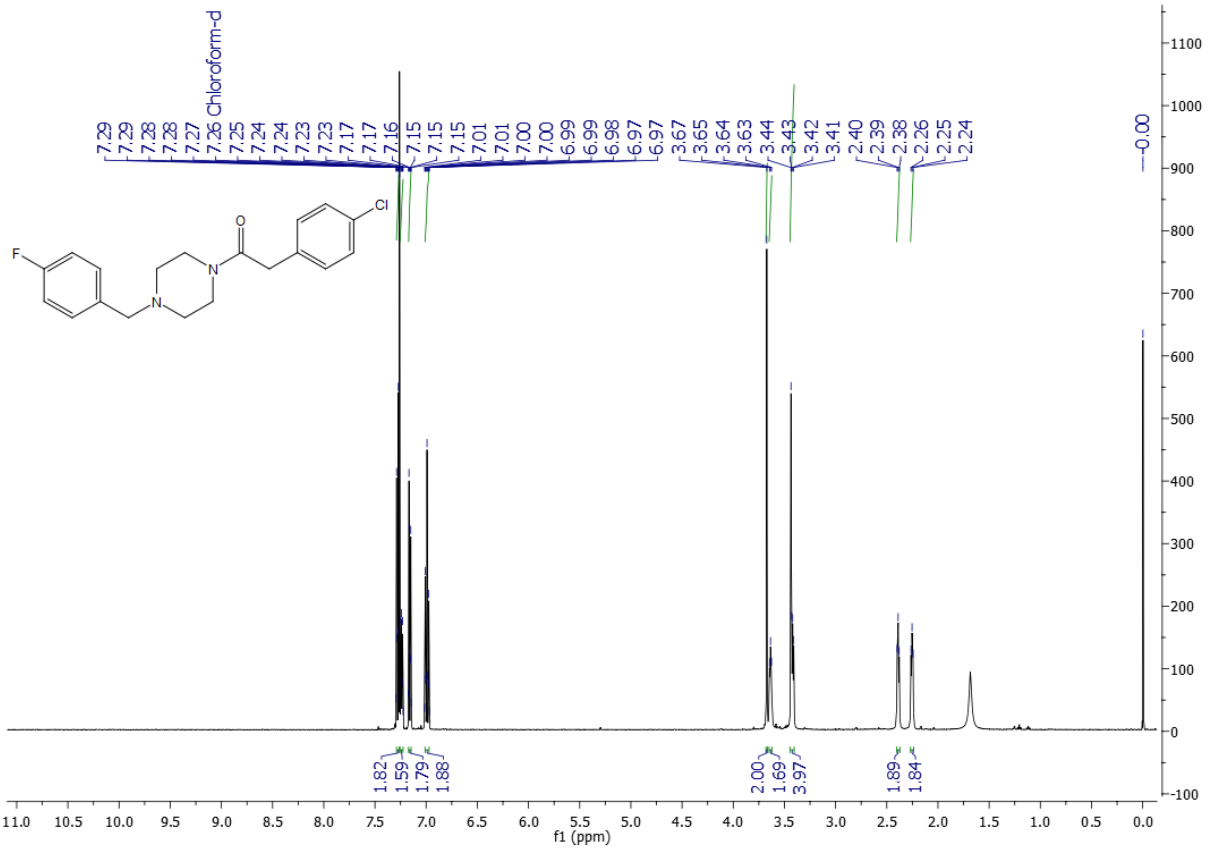


Figure S12: ^{1}H-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(4-chlorophenyl)ethan-1-one (**12**)

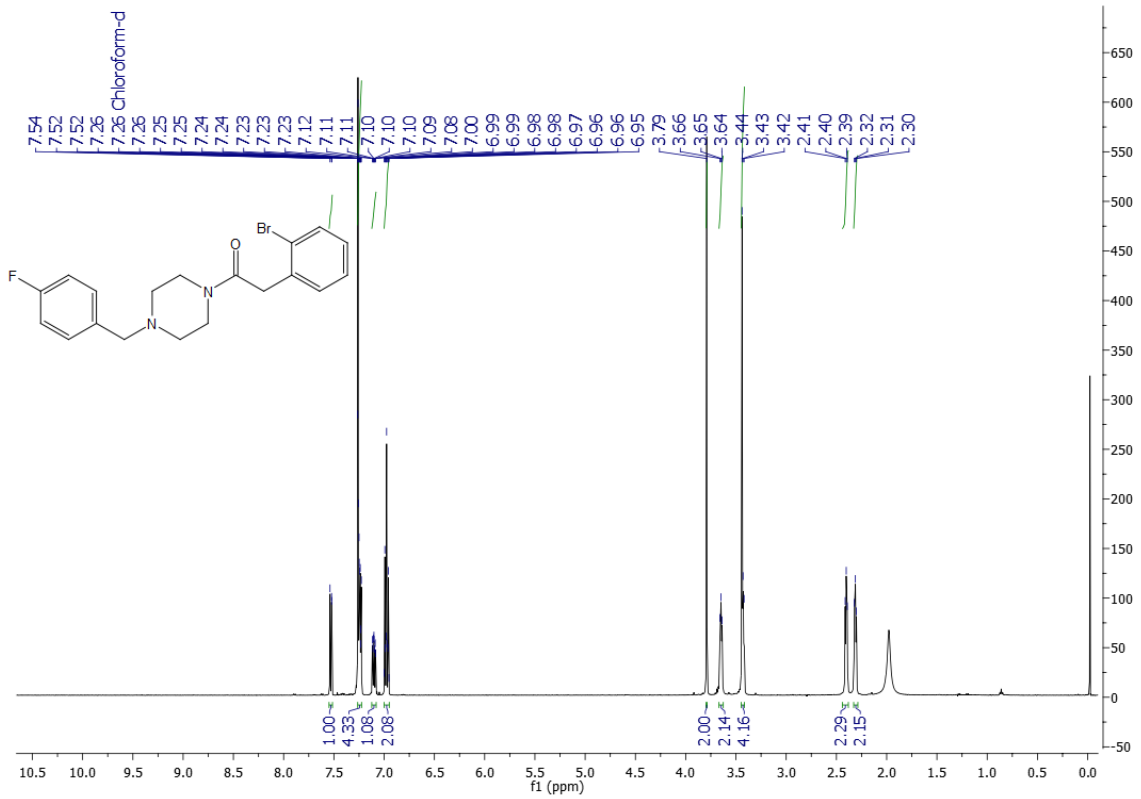


Figure S13: ^{1}H-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(2-bromophenyl)ethan-1-one (**13**)

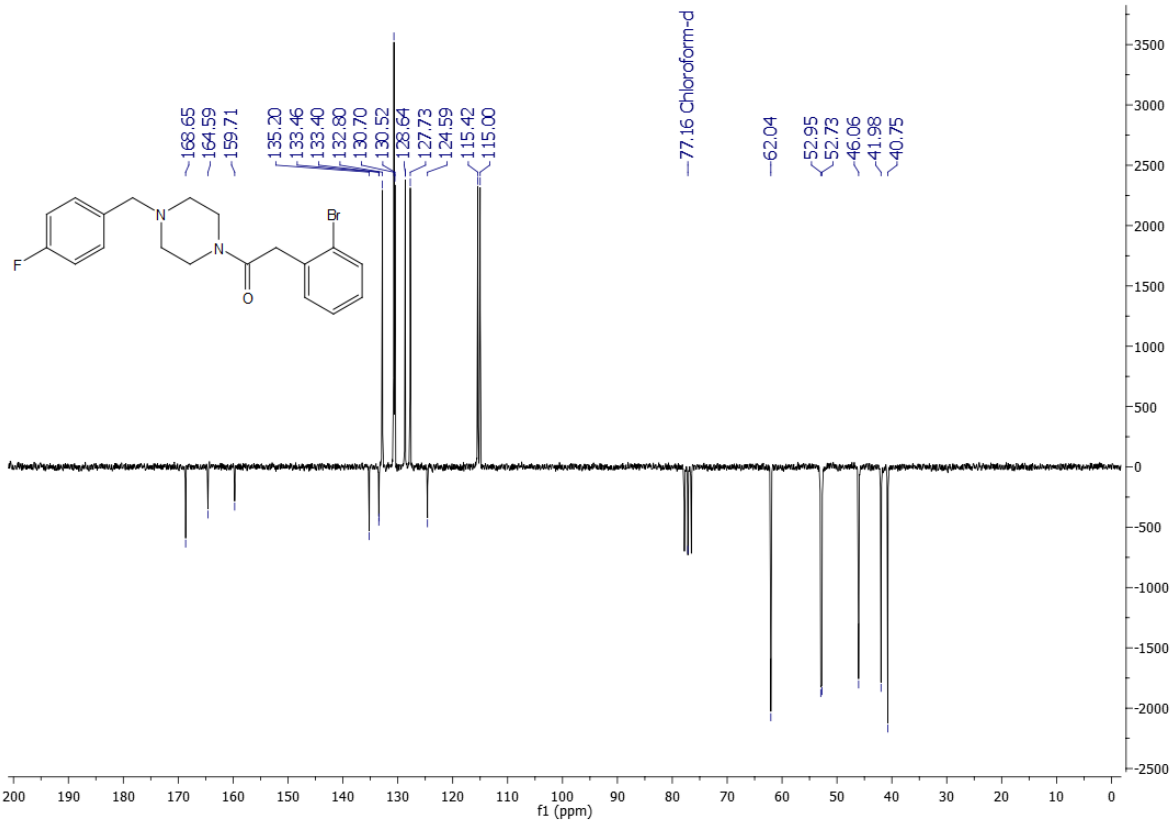


Figure S14: ^{13}C-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(2-bromophenyl)ethan-1-one (**13**)

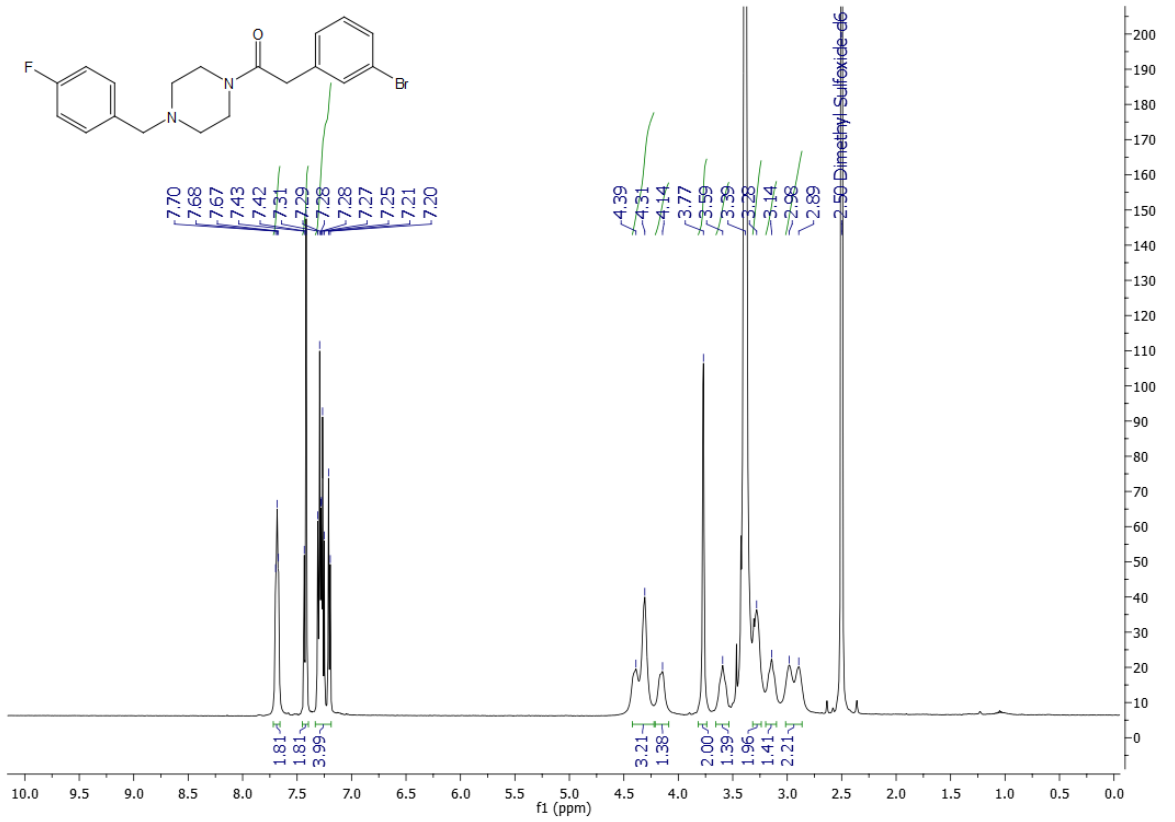


Figure S15: ^{1}H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(3-bromophenyl)ethan-1-one (**14**)

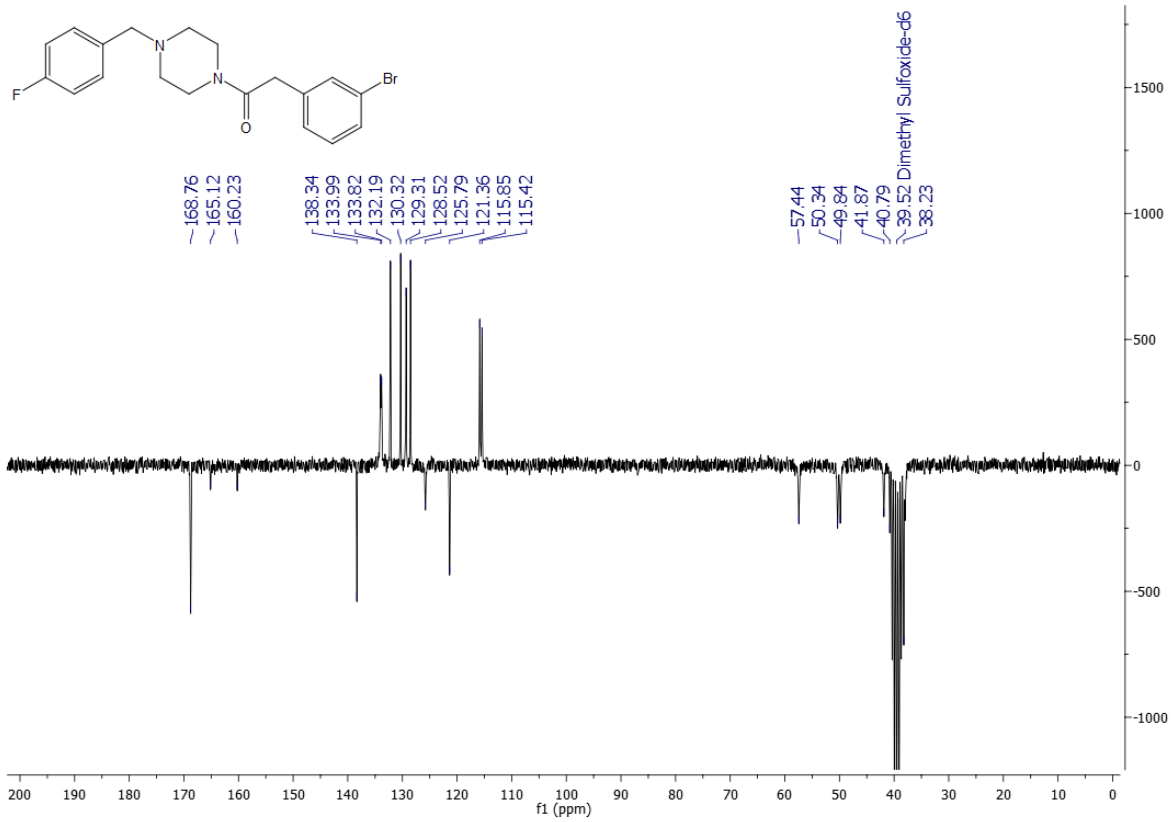


Figure S16: ^{13}C-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(3-bromophenyl)ethan-1-one (**14**)

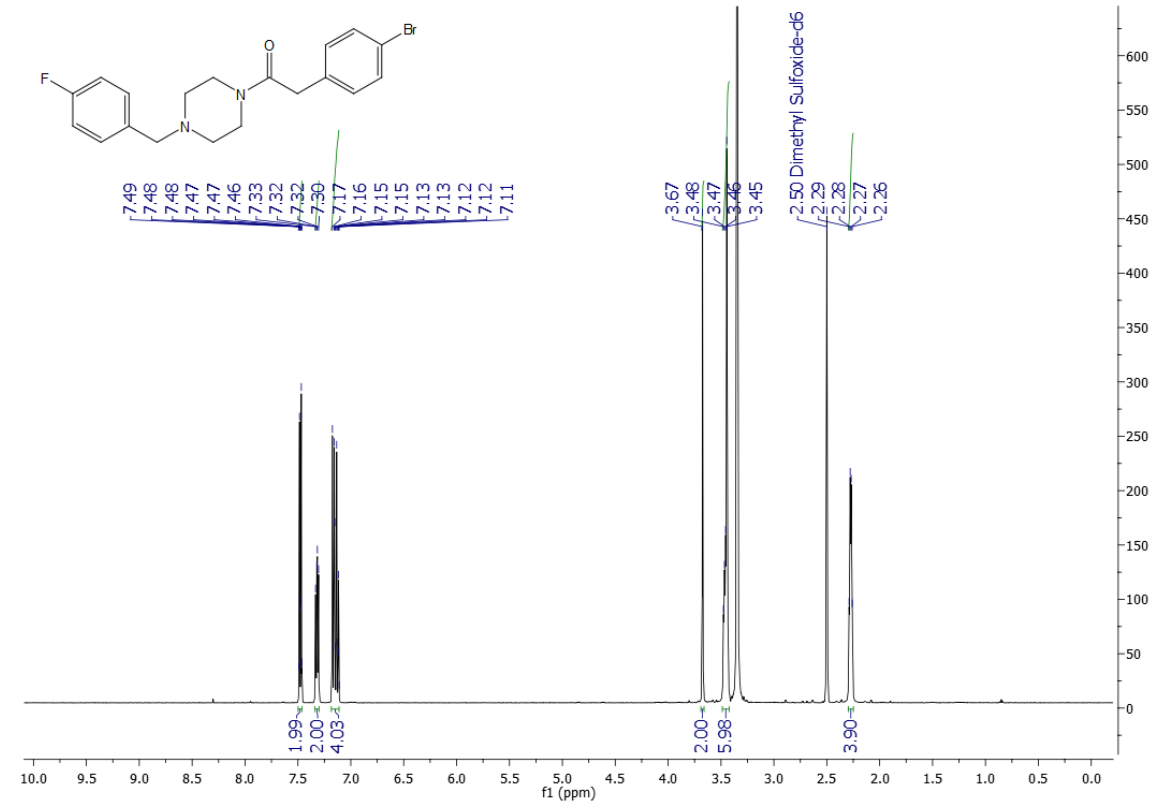


Figure S17: ^{1}H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(4-bromophenyl)ethan-1-one (**15**)

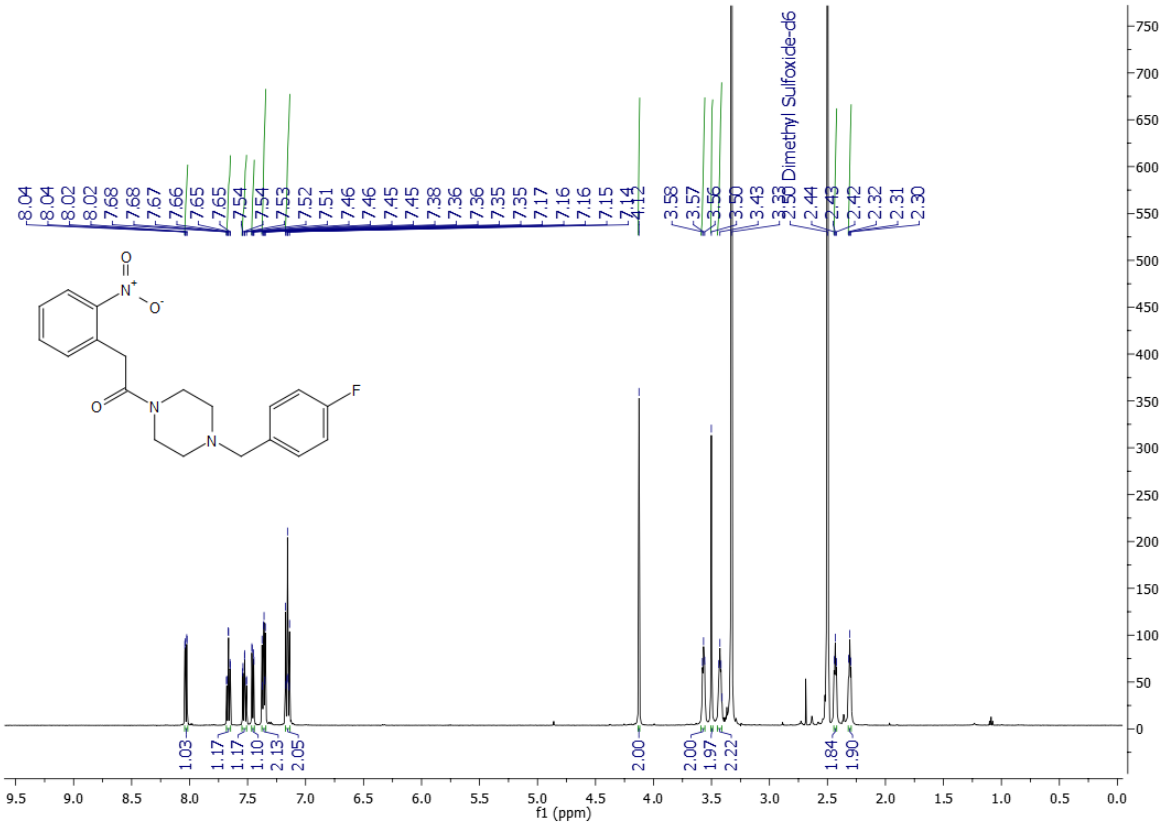


Figure S18: [1]H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(2-nitrophenyl)ethan-1-one (**16**)

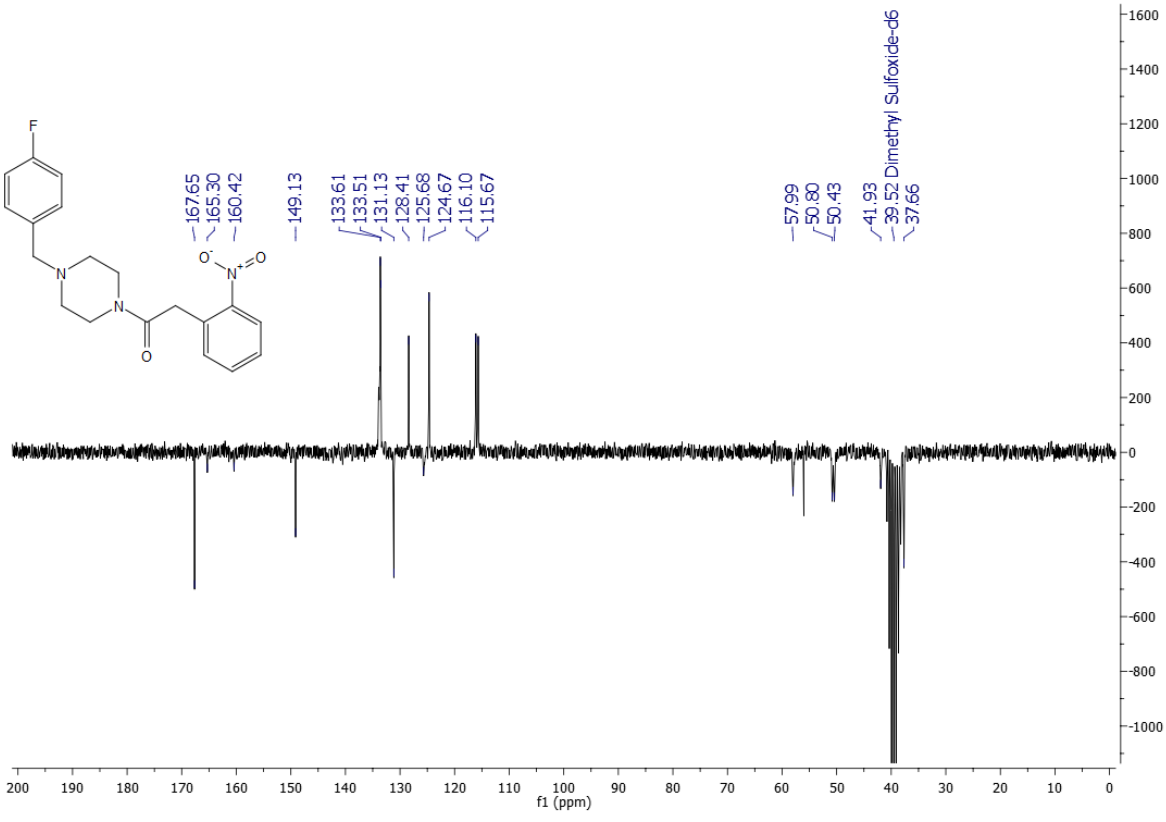


Figure S19: [13]C-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(2-nitrophenyl)ethan-1-one (**16**)

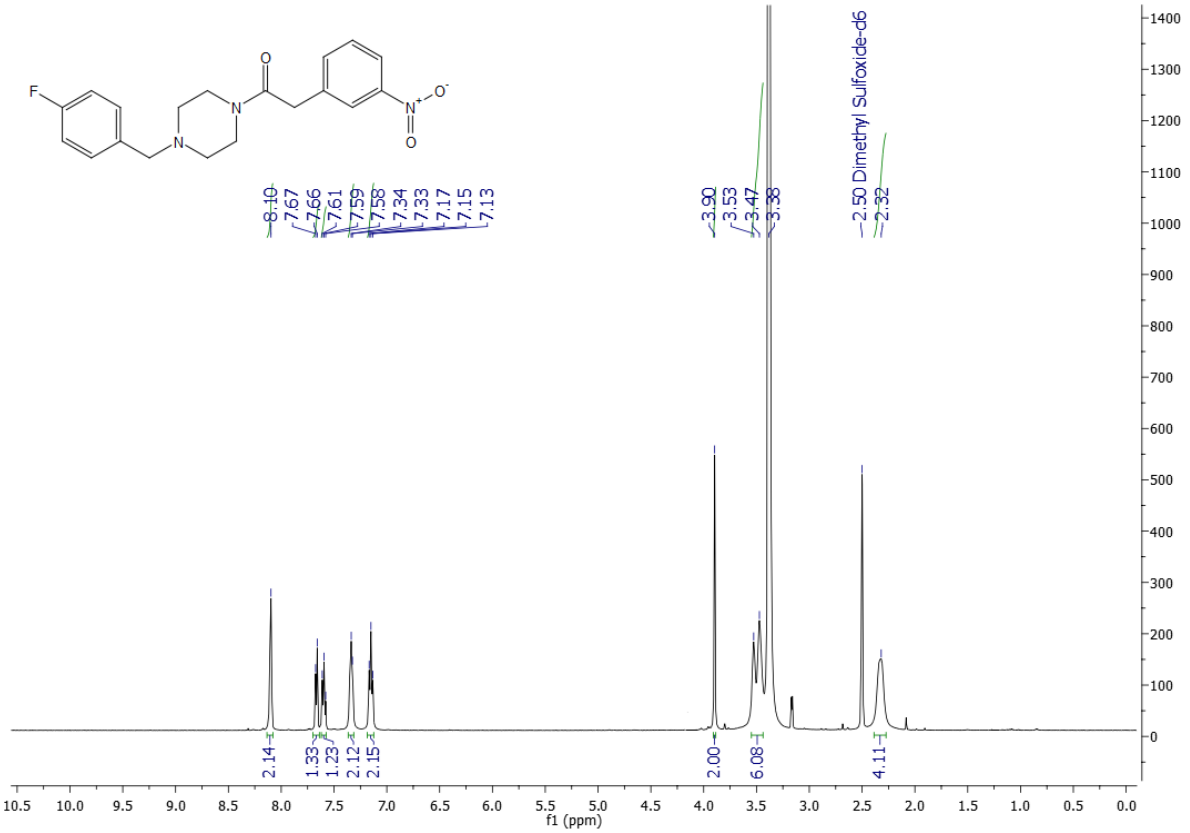


Figure S20: [1]H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(3-nitrophenyl)ethan-1-one (**17**)

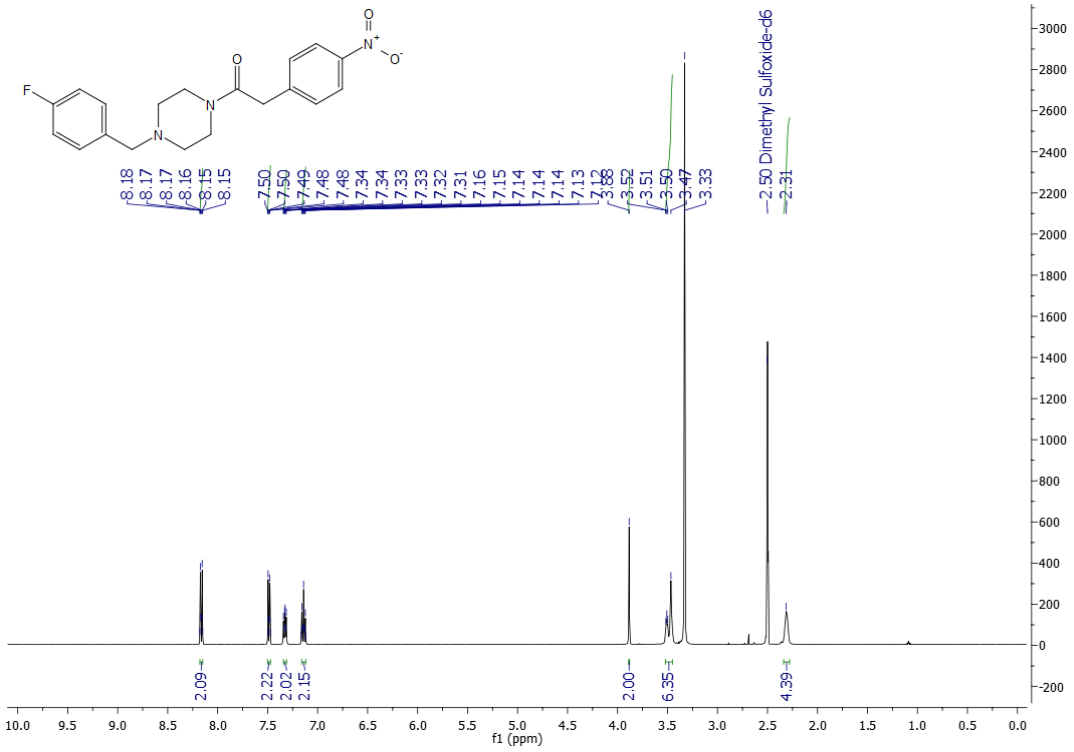


Figure S21: [1]H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(4-nitrophenyl)ethan-1-one (**18**)

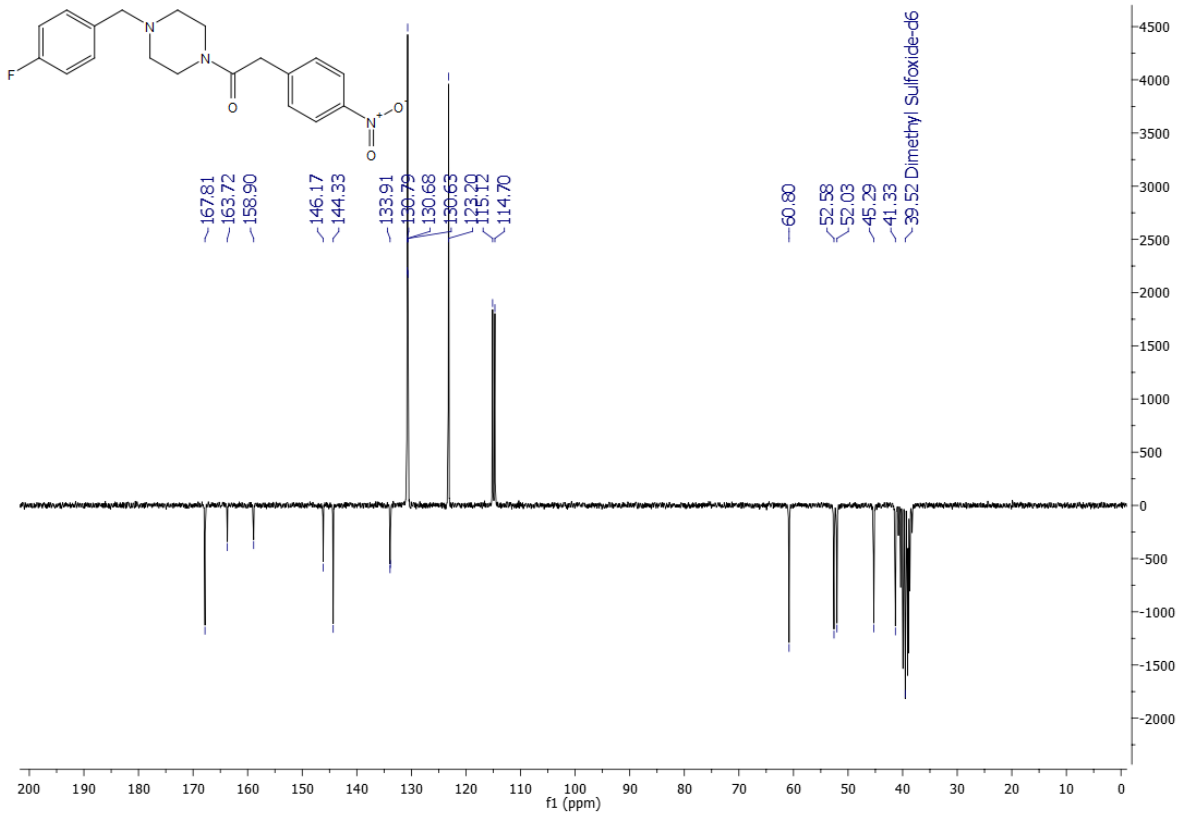


Figure S22: ^{13}C-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(4-nitrophenyl)ethan-1-one (**18**)

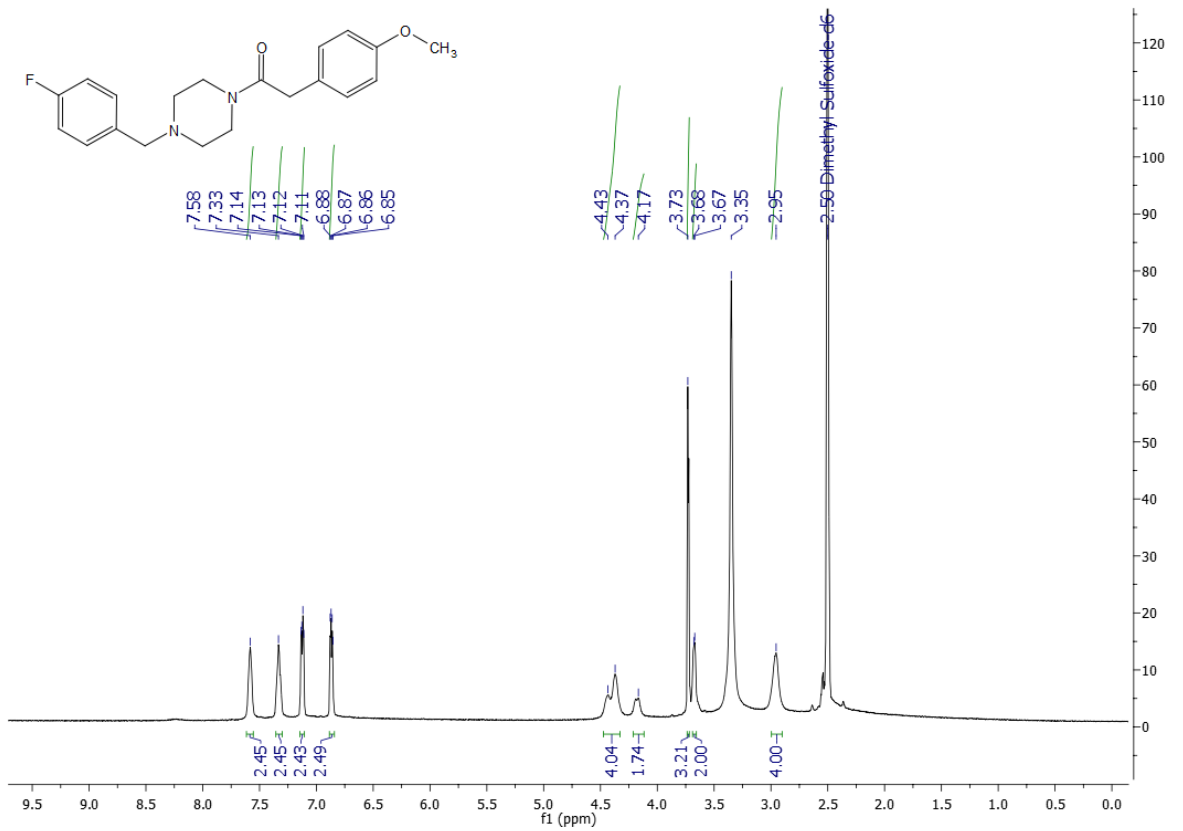


Figure S23: ^{1}H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(4-methoxyphenyl)ethan-1-one (**19**)

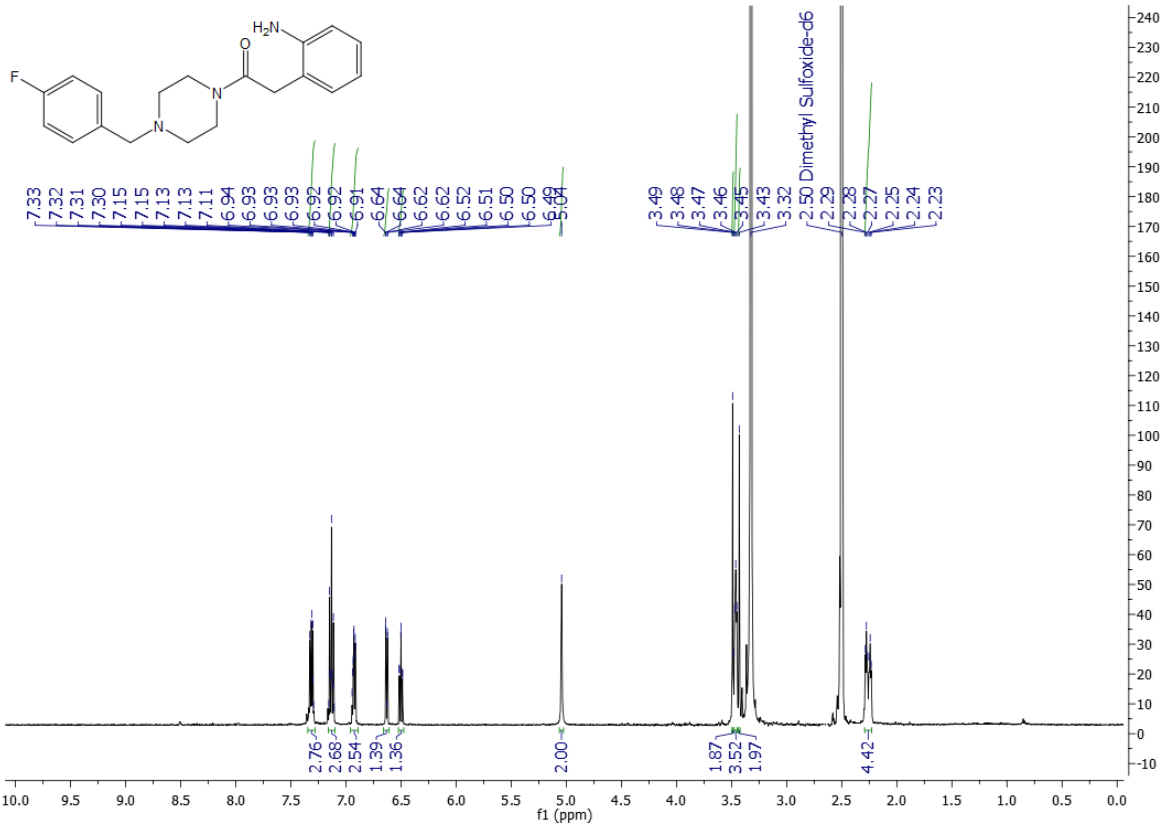


Figure S24: ^{1}H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(2-aminophenyl)ethan-1-one (**20**)

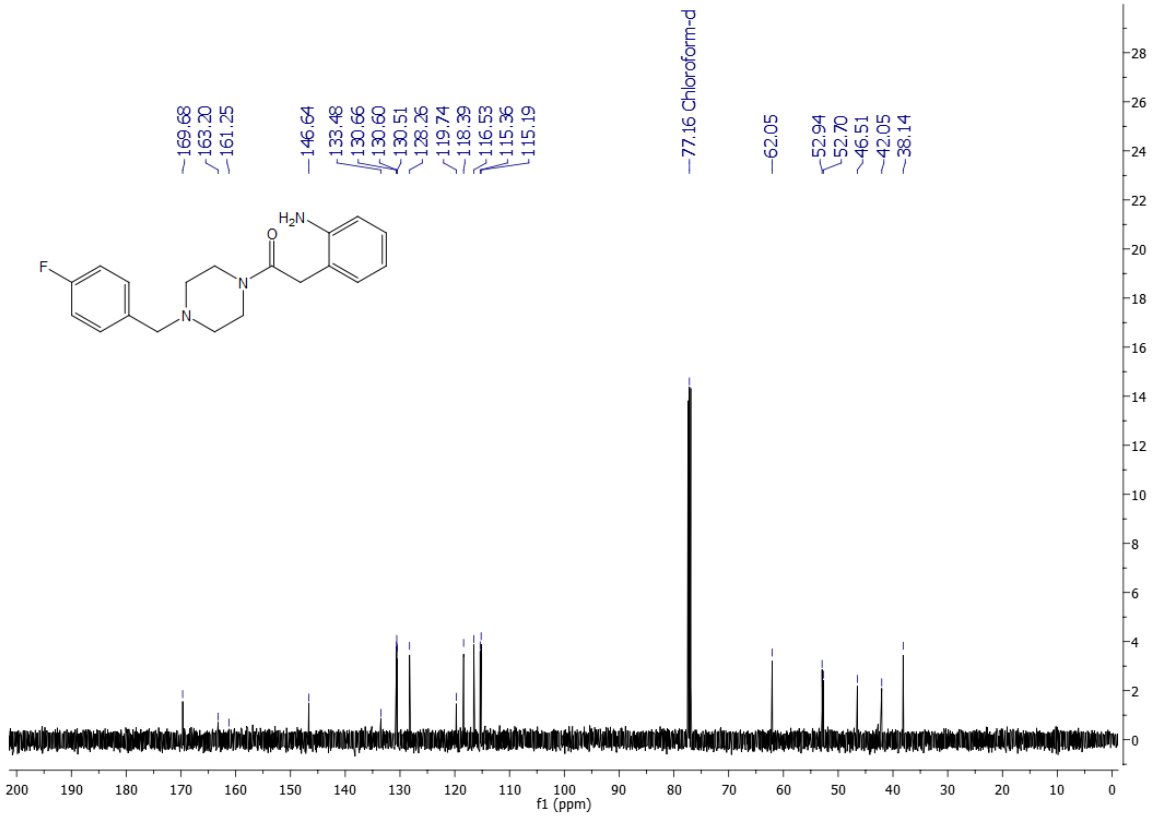


Figure S25: ^{13}C-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(2-aminophenyl)ethan-1-one (**20**)

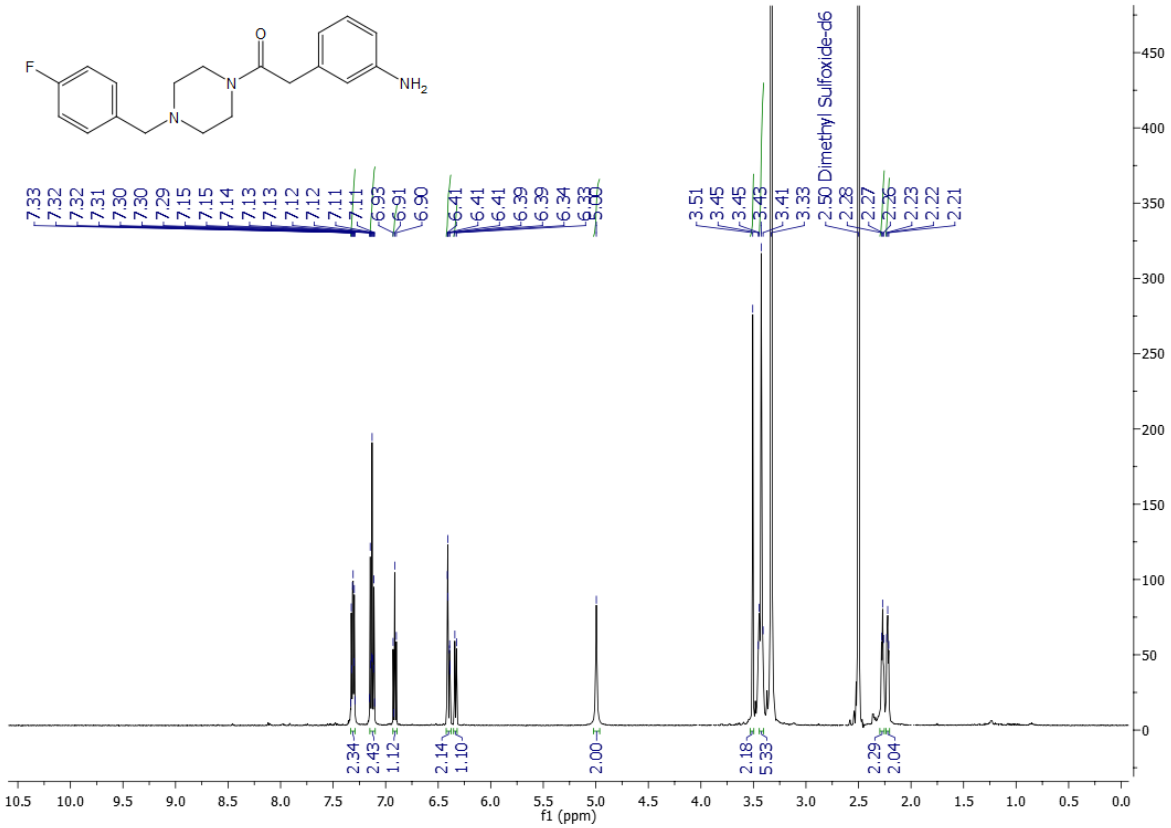


Figure S26: ^{1}H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(3-aminophenyl)ethan-1-one (**21**)

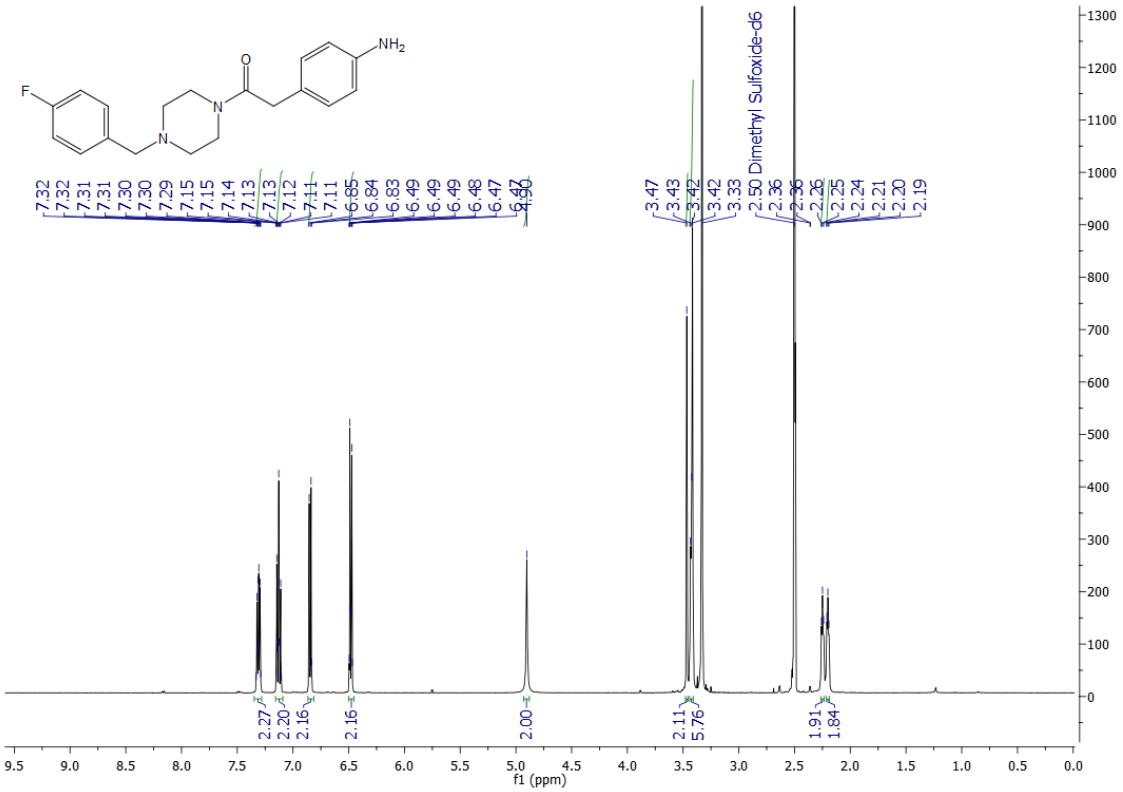


Figure S27: ^{1}H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(4-aminophenyl)ethan-1-one (**22**)

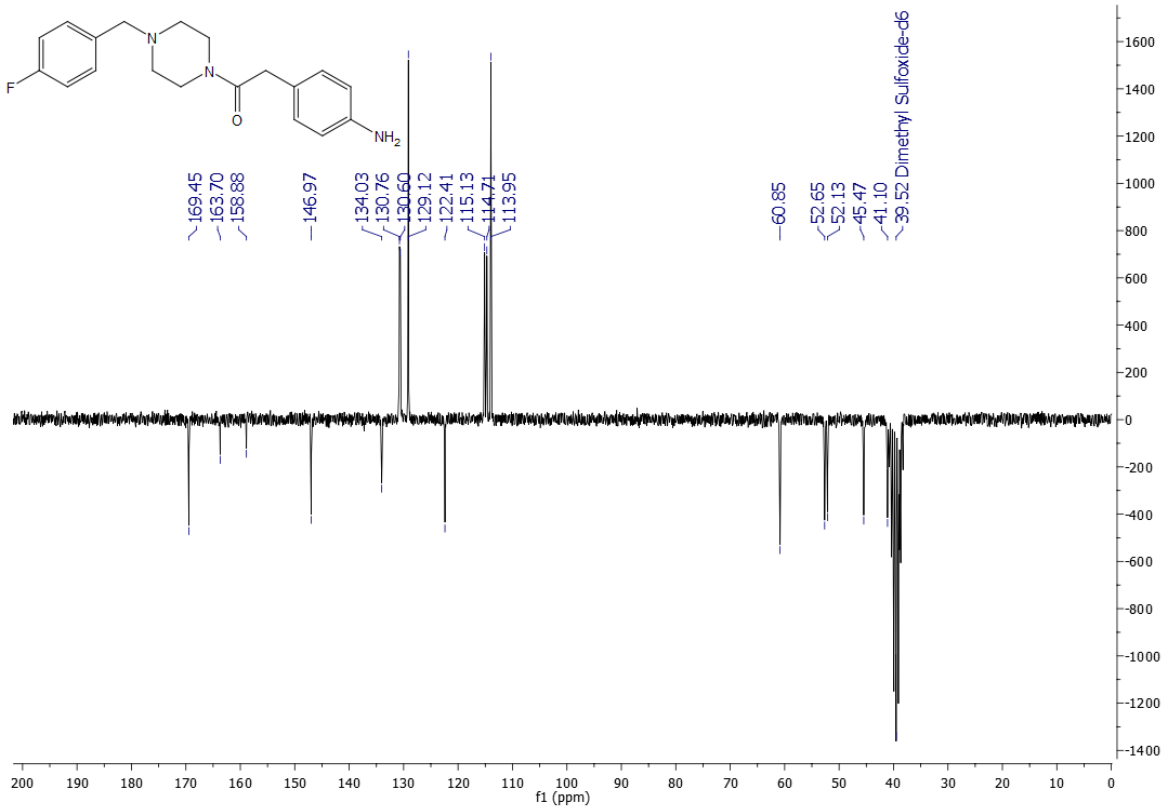


Figure S28: ^{13}C-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(4-aminophenyl)ethan-1-one (**22**)

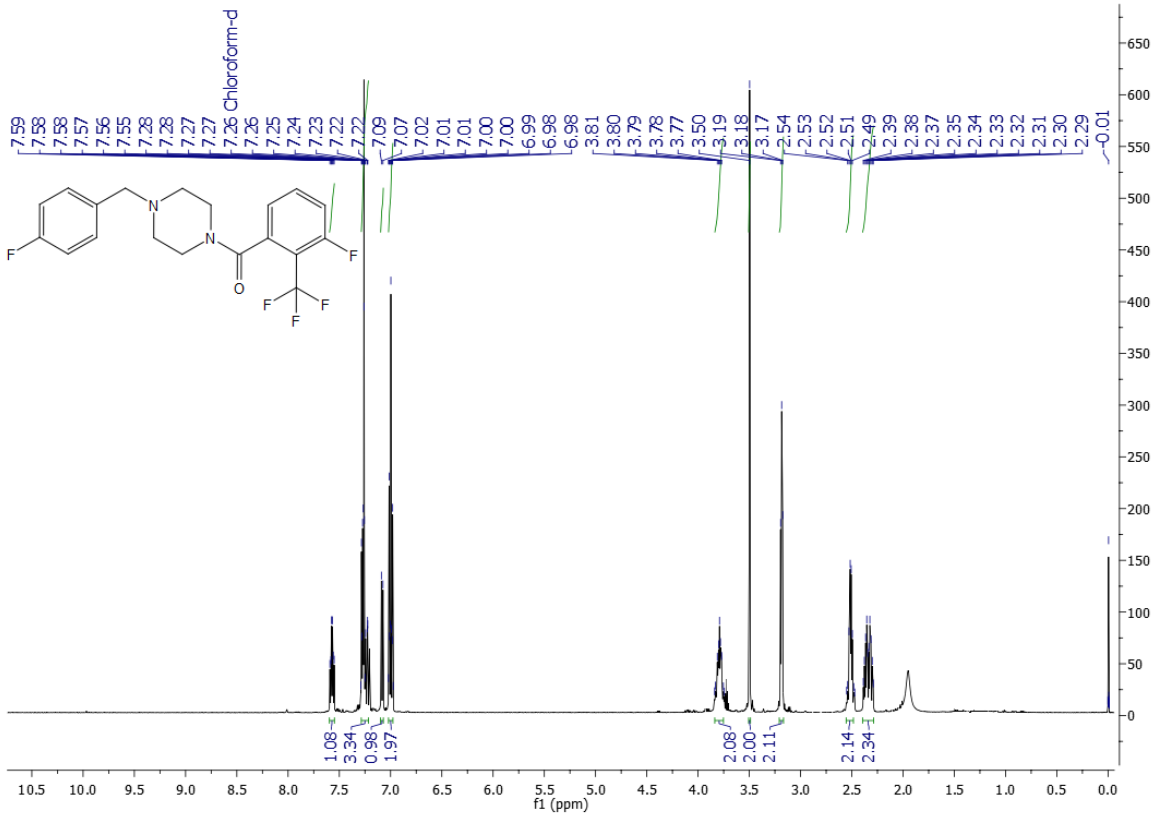


Figure S29: ^{1}H-NMR ($CDCl_3$) spectrum of [4-(4-Fluorobenzyl)piperazin-1-yl]-[3-fluoro-2-(trifluoromethyl)phenyl]methanone (**23**)

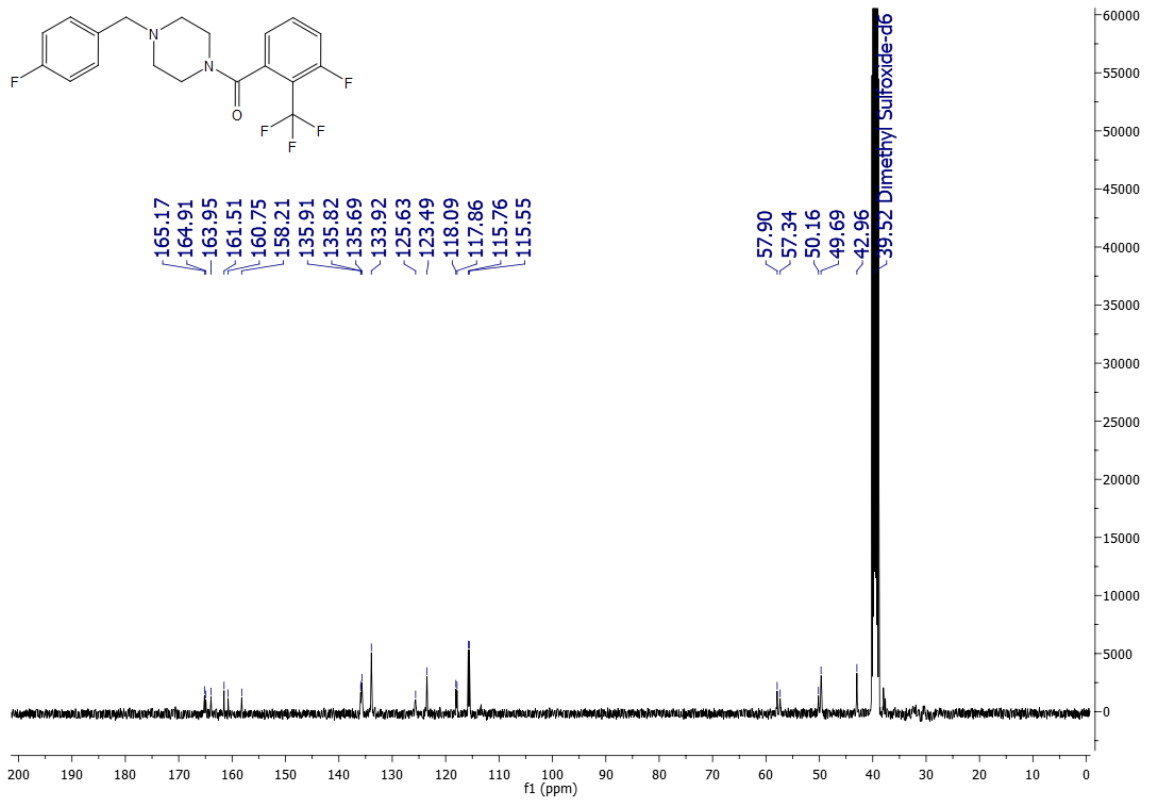


Figure S30: ^{13}C-NMR (DMSO-d6) spectrum of [4-(4-Fluorobenzyl)piperazin-1-yl]-[3-fluoro-2-(trifluoromethyl)phenyl]methanone (**23**)

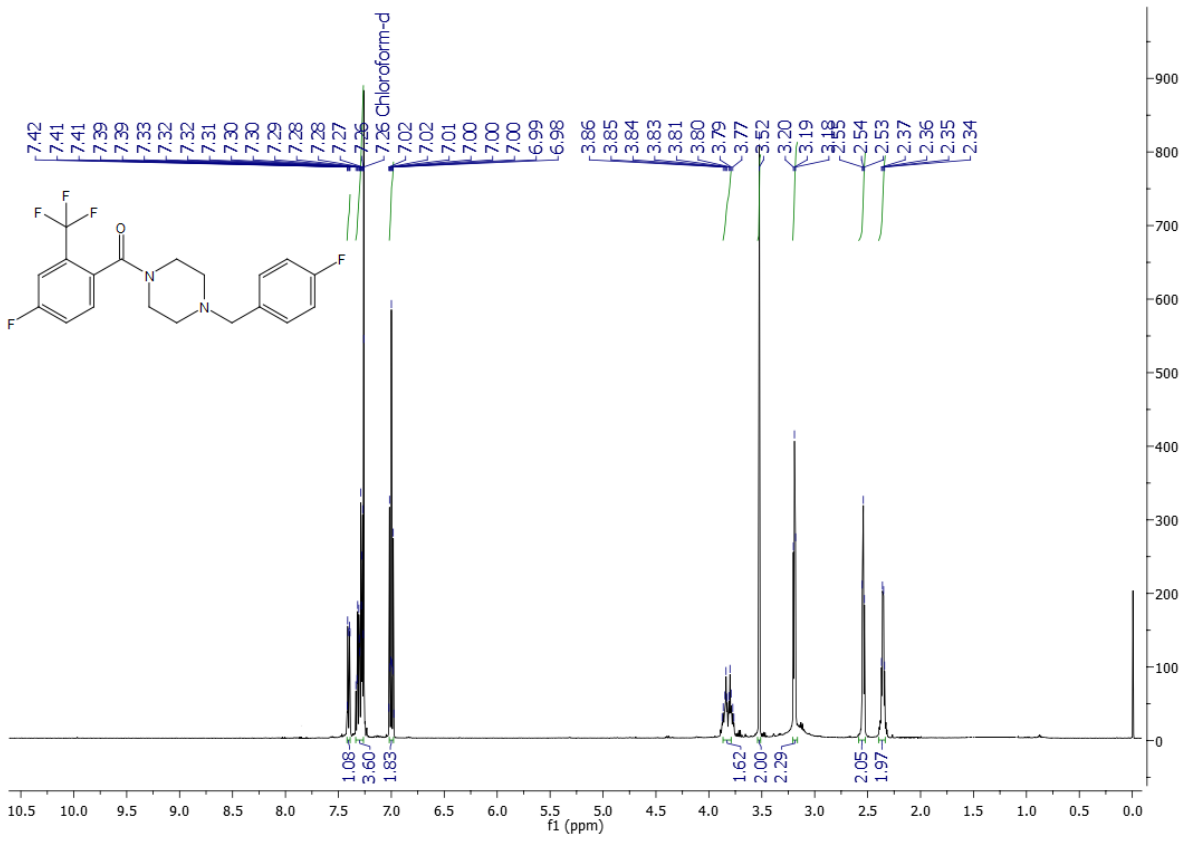


Figure S31: ^{1}H-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-[4-fluoro-2-(trifluoromethyl)phenyl]methanone (**24**)

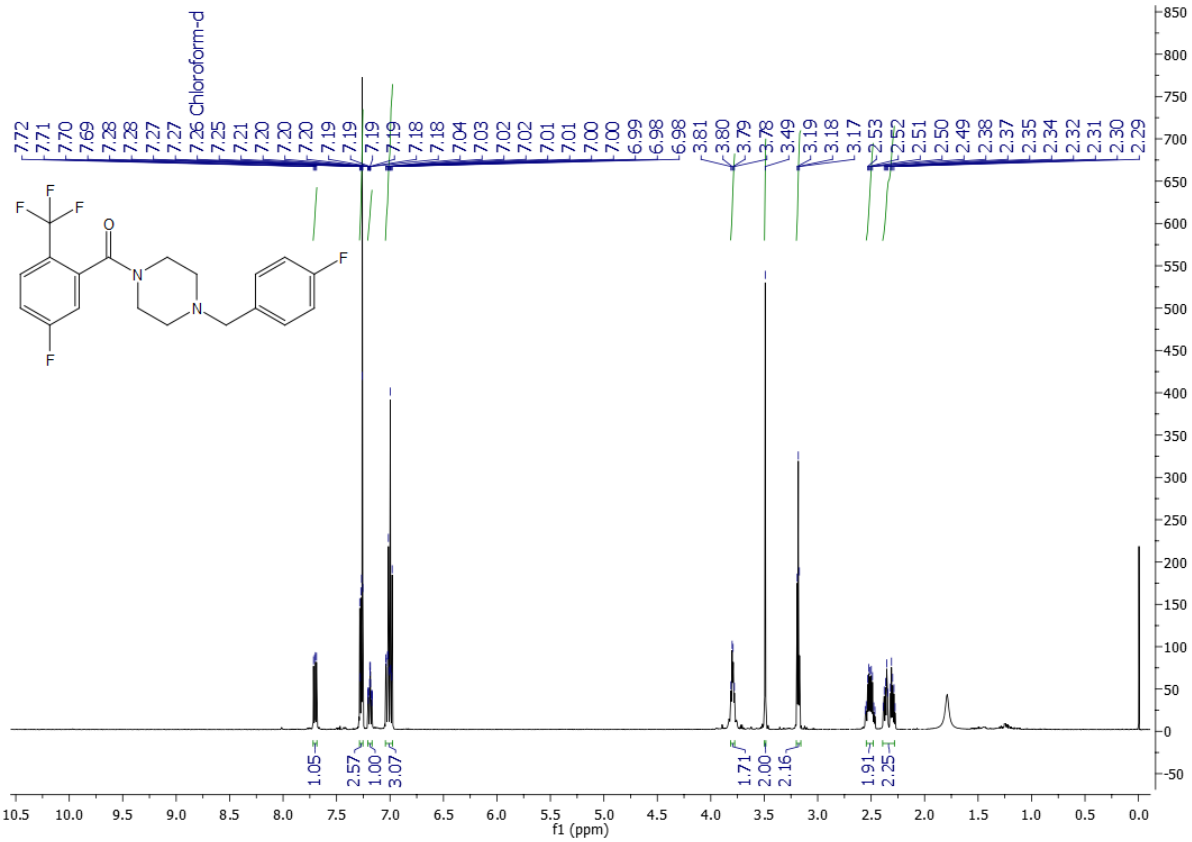


Figure S32: ^{1}H-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-[5-fluoro-2-(trifluoromethyl)phenyl]methanone (**25**)

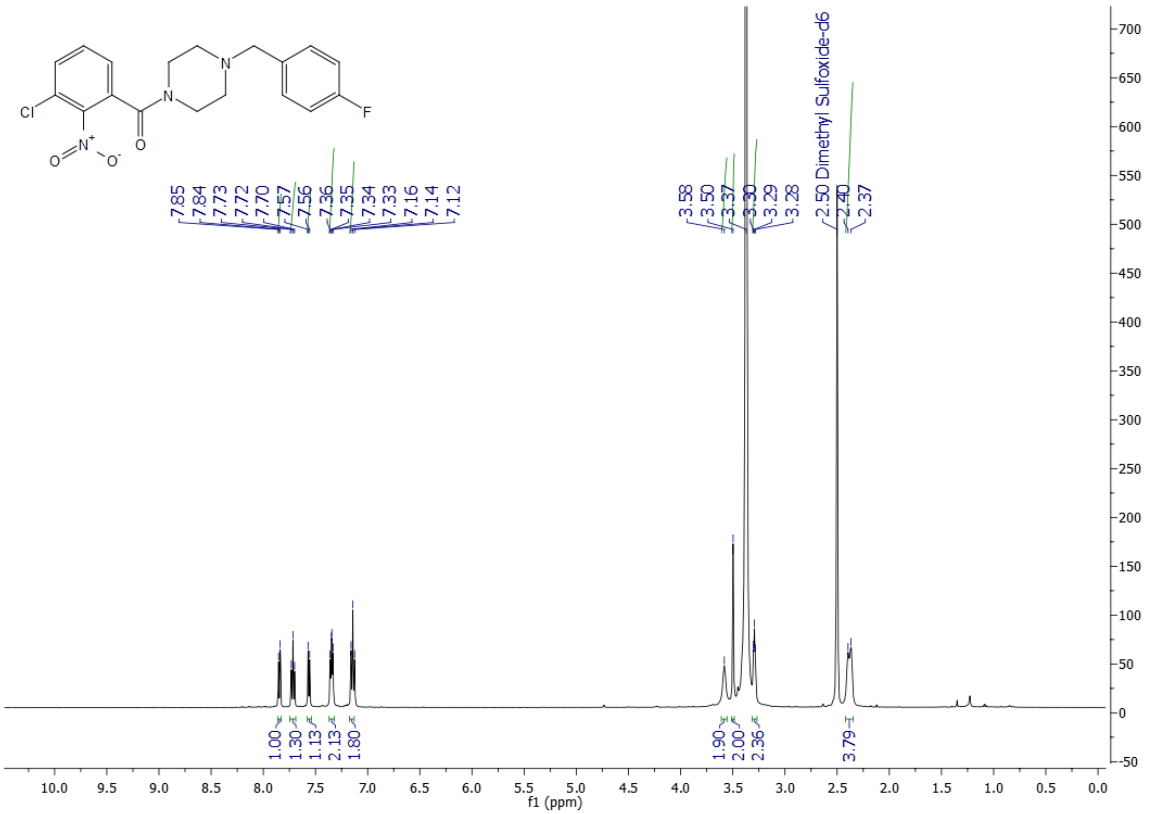


Figure S33: ^{1}H-NMR (DMSO-d6) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-(3-chloro-2-nitro-phenyl)methanone (**26**)

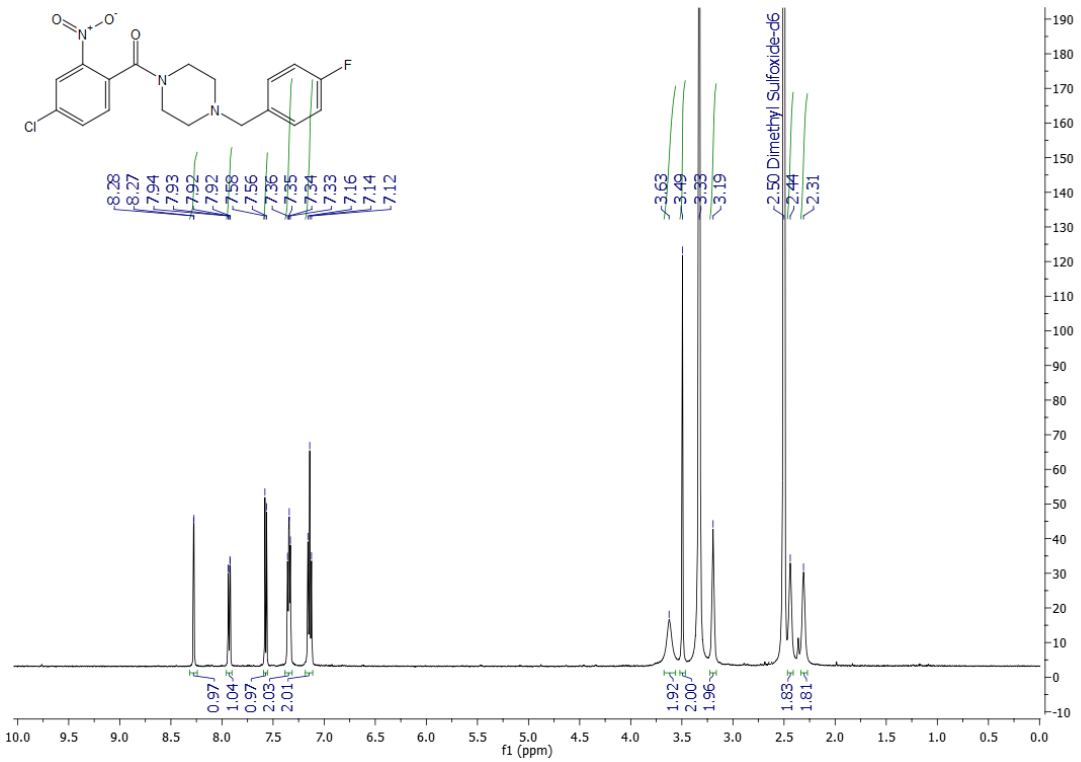


Figure S34: [1]H-NMR (DMSO-d6) spectrum of [4-(4-Fluorobenzyl)piperazin-1-yl]-(4-chloro-2-nitro-phenyl)methanone (**27**)

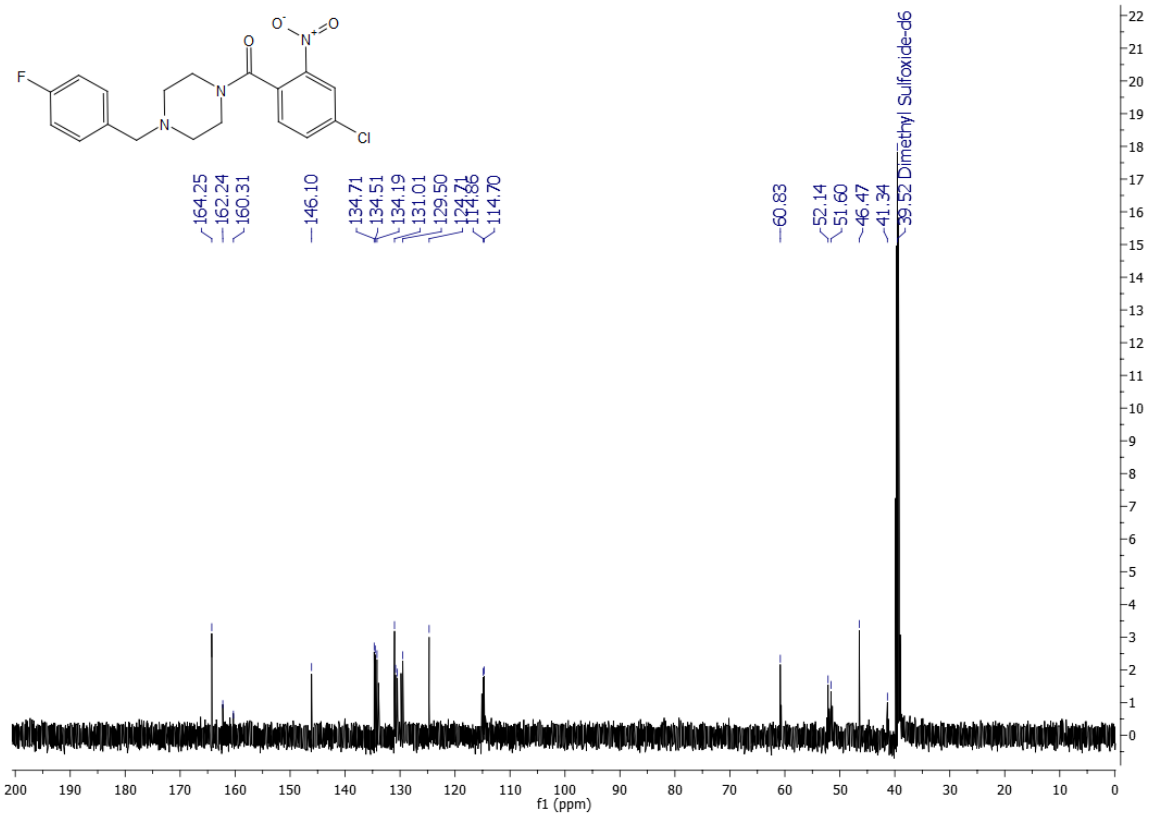


Figure S35: [13]C-NMR (DMSO-d6) spectrum of [4-(4-Fluorobenzyl)piperazin-1-yl]-(4-chloro-2-nitro-phenyl)methanone (**27**)

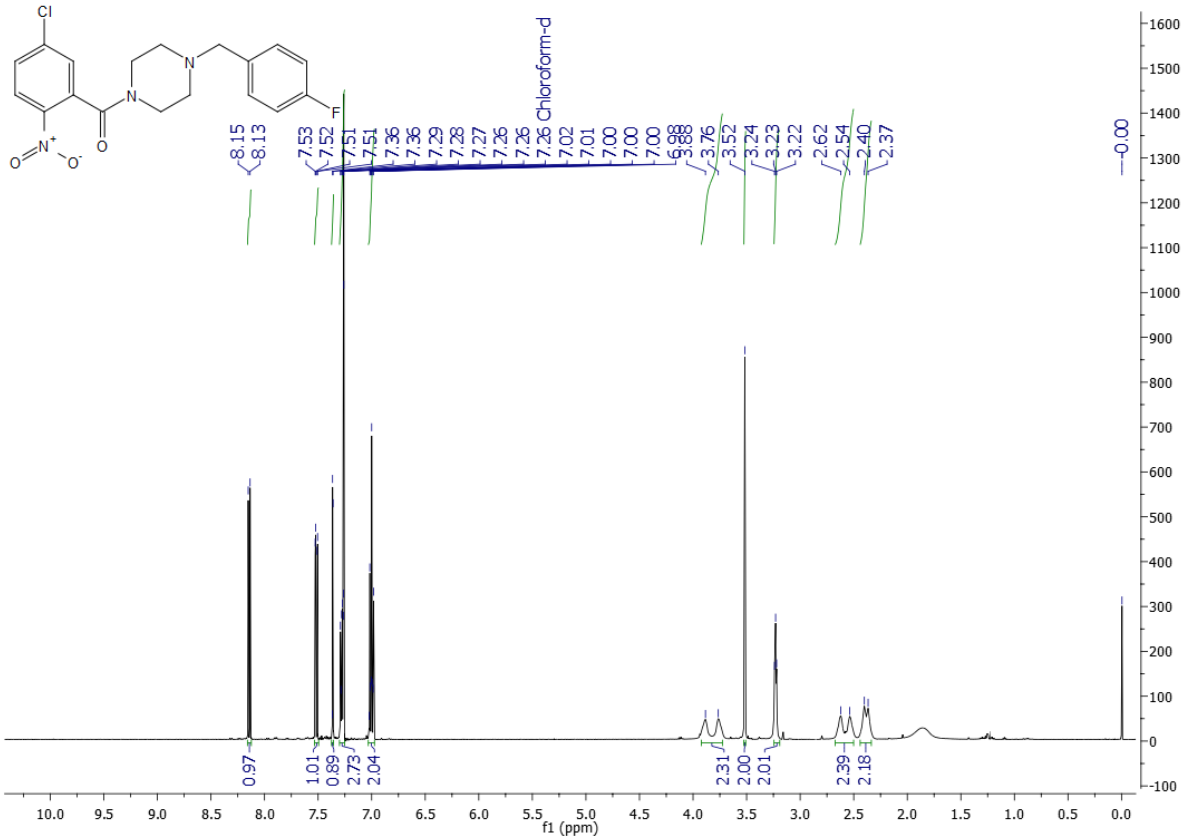


Figure S36: ^{1}H-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-(5-chloro-2-nitro-phenyl)methanone (**28**)

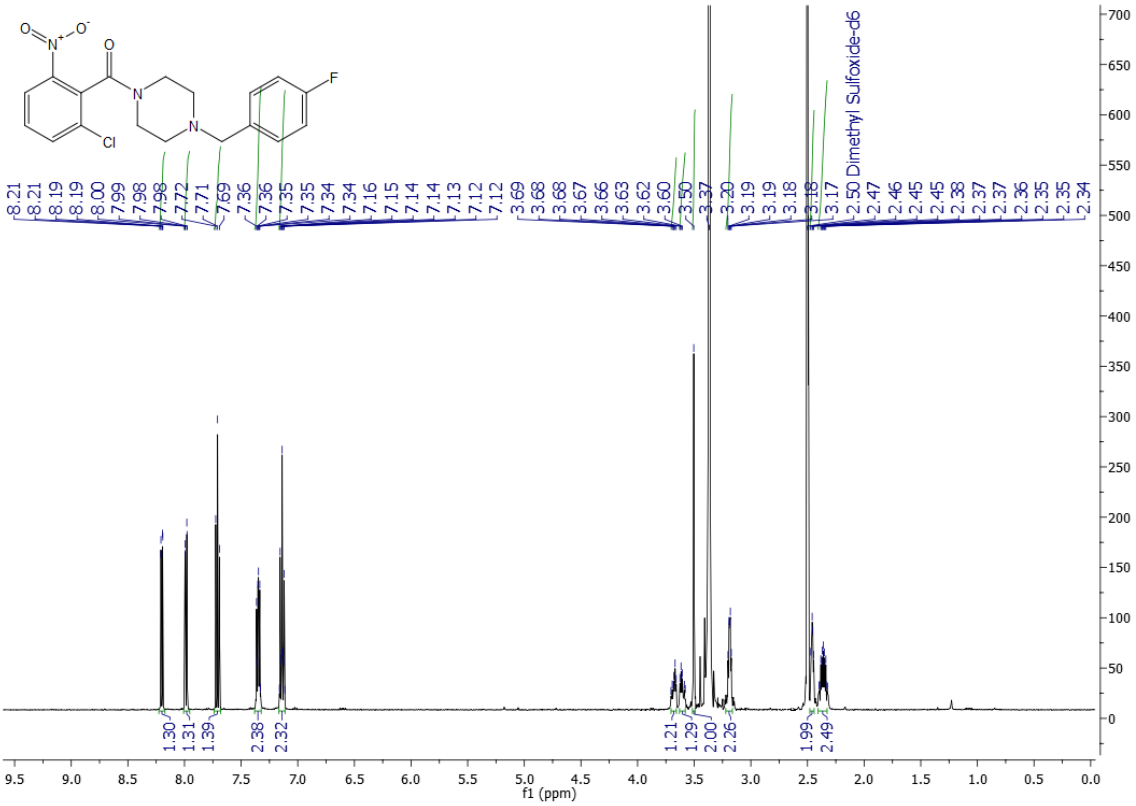


Figure S37: ^{1}H-NMR (DMSO-d6) spectrum of [4-(4-Fluorobenzyl)piperazin-1-yl]-(2-chloro-6-nitro-phenyl)methanone (**29**)

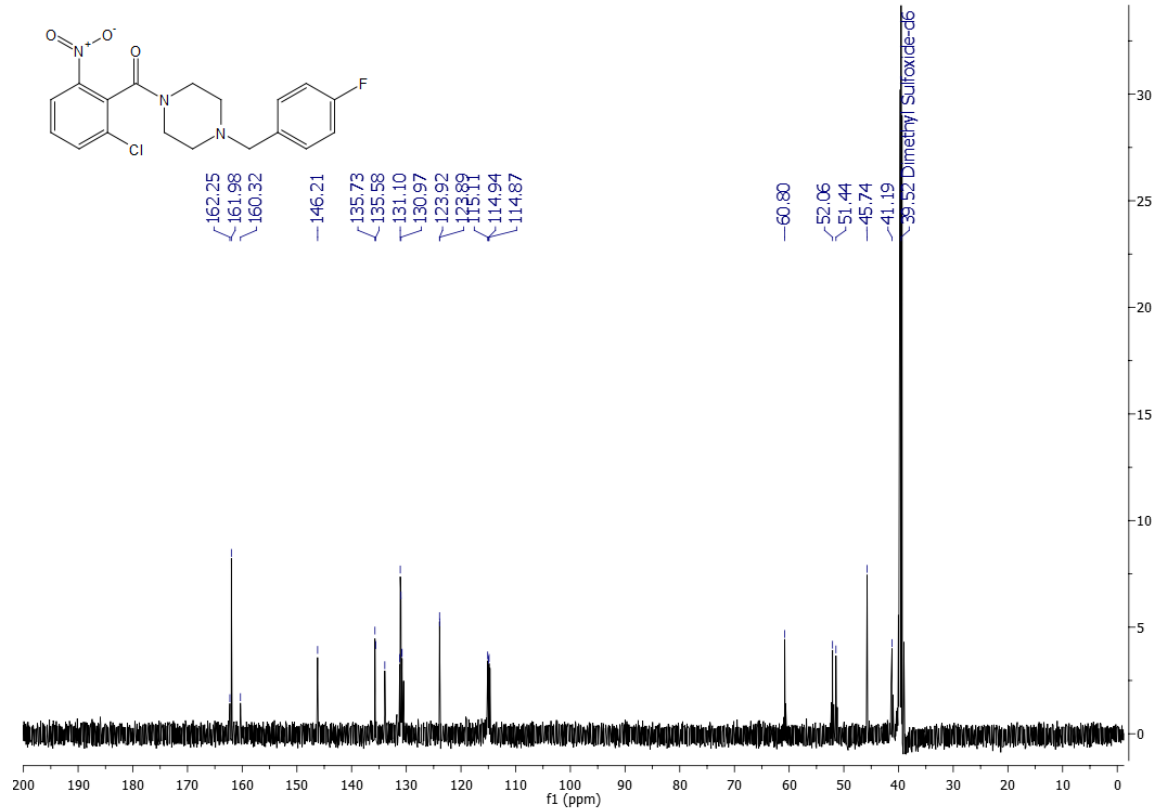


Figure S38: ^{13}C-NMR (DMSO-d6) spectrum of [4-(4-Fluorobenzyl)piperazin-1-yl]-(2-chloro-6-nitro-phenyl)methanone (**29**)

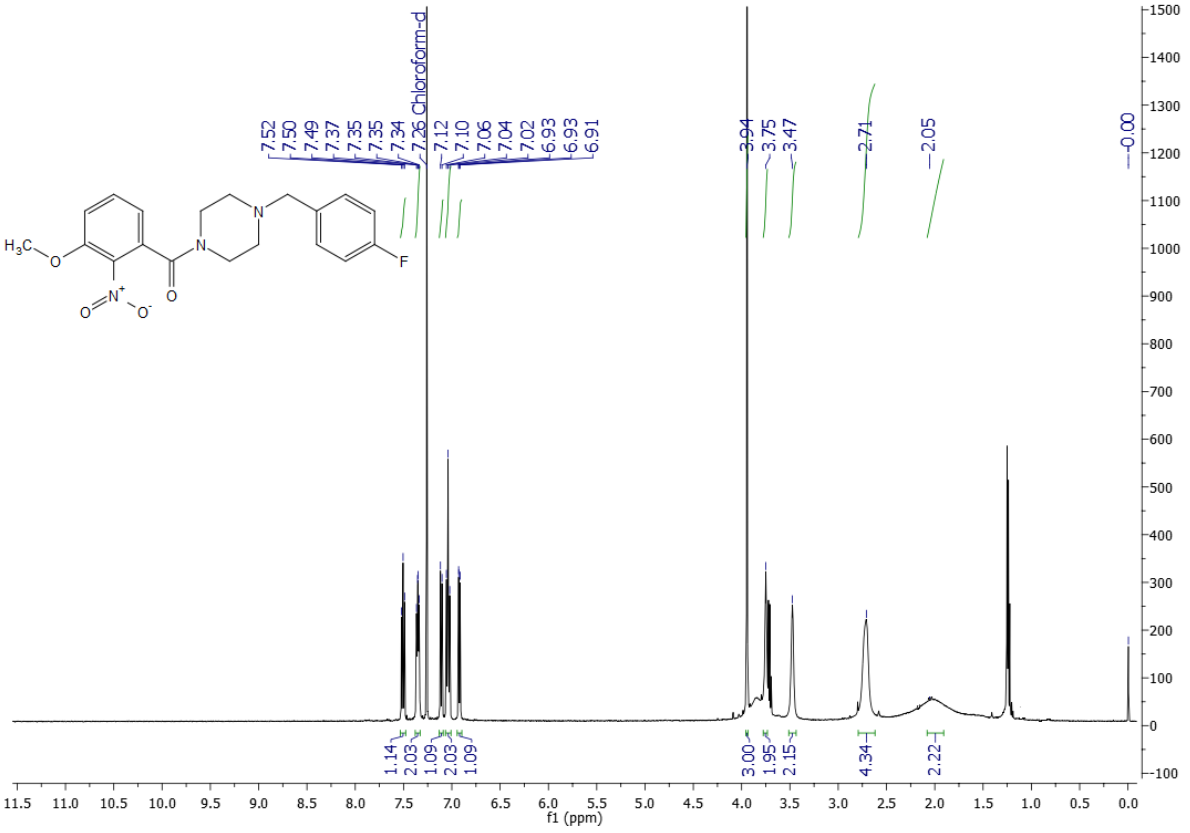


Figure S39: ^{1}H-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-(3-methoxy-2-nitro-phenyl)methanone (**30**)

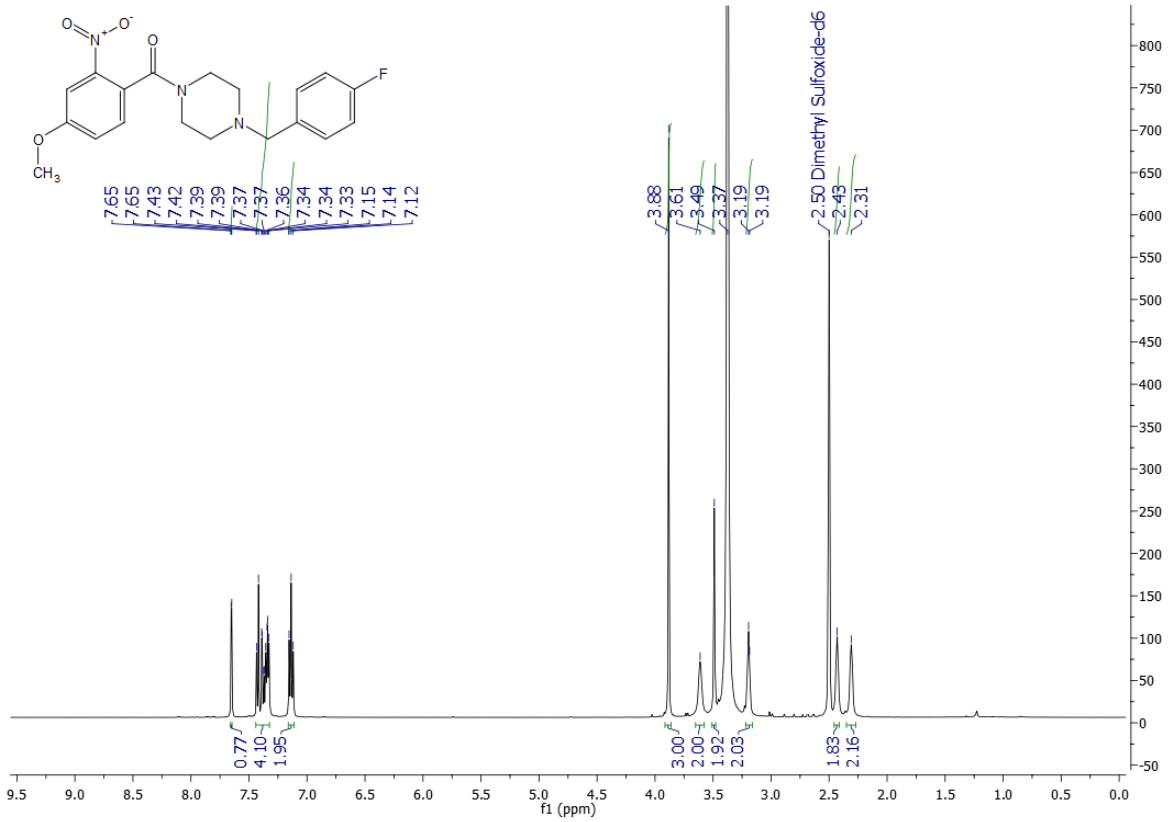


Figure S40: ^{1}H-NMR (DMSO-d6) spectrum of [4-(4-Fluorobenzyl)piperazin-1-yl]-(4-methoxy-2-nitro-phenyl)methanone (**31**)

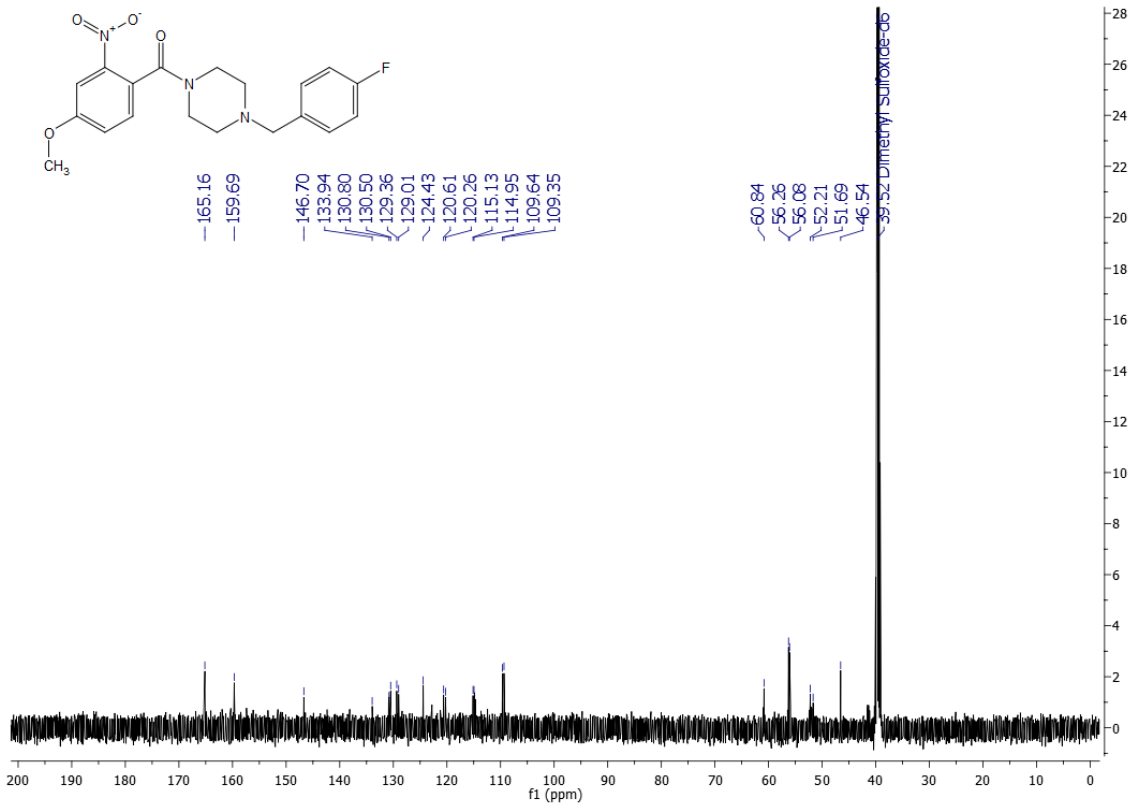


Figure S41: ^{13}C-NMR (DMSO-d6) spectrum of [4-(4-Fluorobenzyl)piperazin-1-yl]-(4-methoxy-2-nitro-phenyl)methanone (**31**)

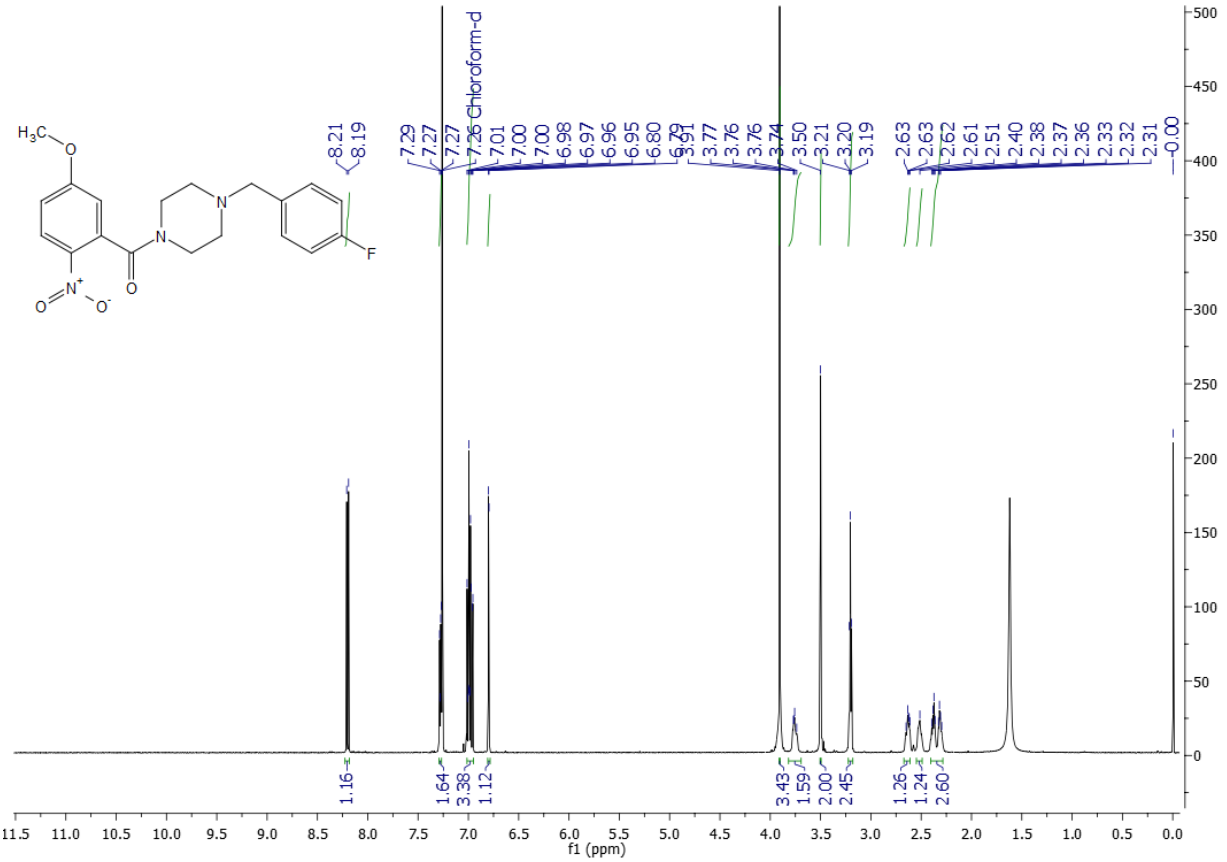


Figure S42: ^{1}H-NMR ($CDCl_3$) spectrum of 1-[4-(4-Fluorobenzyl)piperazin-1-yl]-(5-methoxy-2-nitro-phenyl)methanone (**32**)

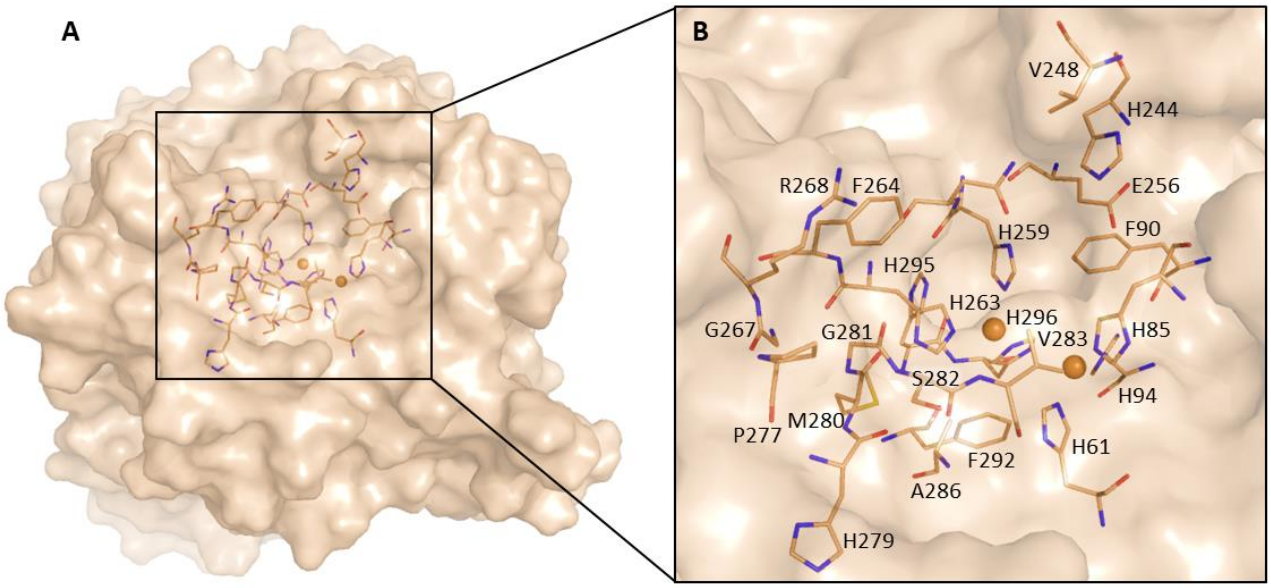


Figure S43. Representation of AbTYR binding site. A) AbTYR surface. The binding site defined for the docking calculation is lined by a black square. B) Close view of AbTYR binding site. The residues of the pocket are represented as wheat sticks.

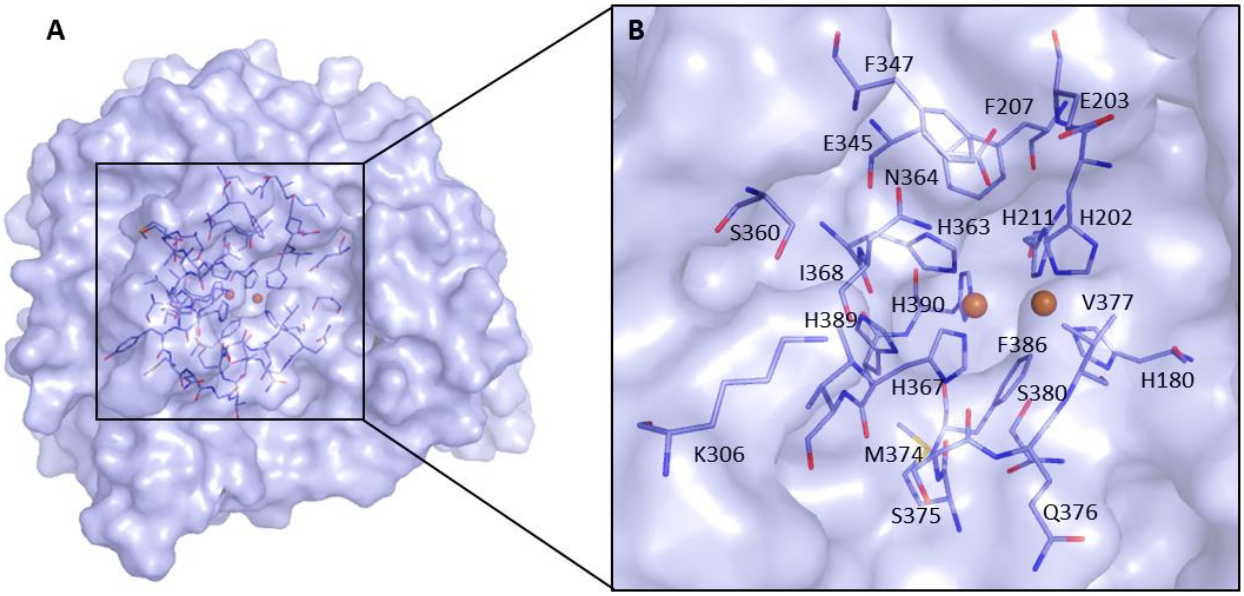


Figure S44. Representation of hTYR binding site. A) hTYR surface. The binding site defined for the docking calculation is lined by a black square. B) Close view of hTYR binding site. The residues of the pocket are represented as lightblue sticks.

Figure S45: Sequence alignment of hTYR (target) and (TYRP-1) mutant (T391V-R374S-Y362F) 5m8q.

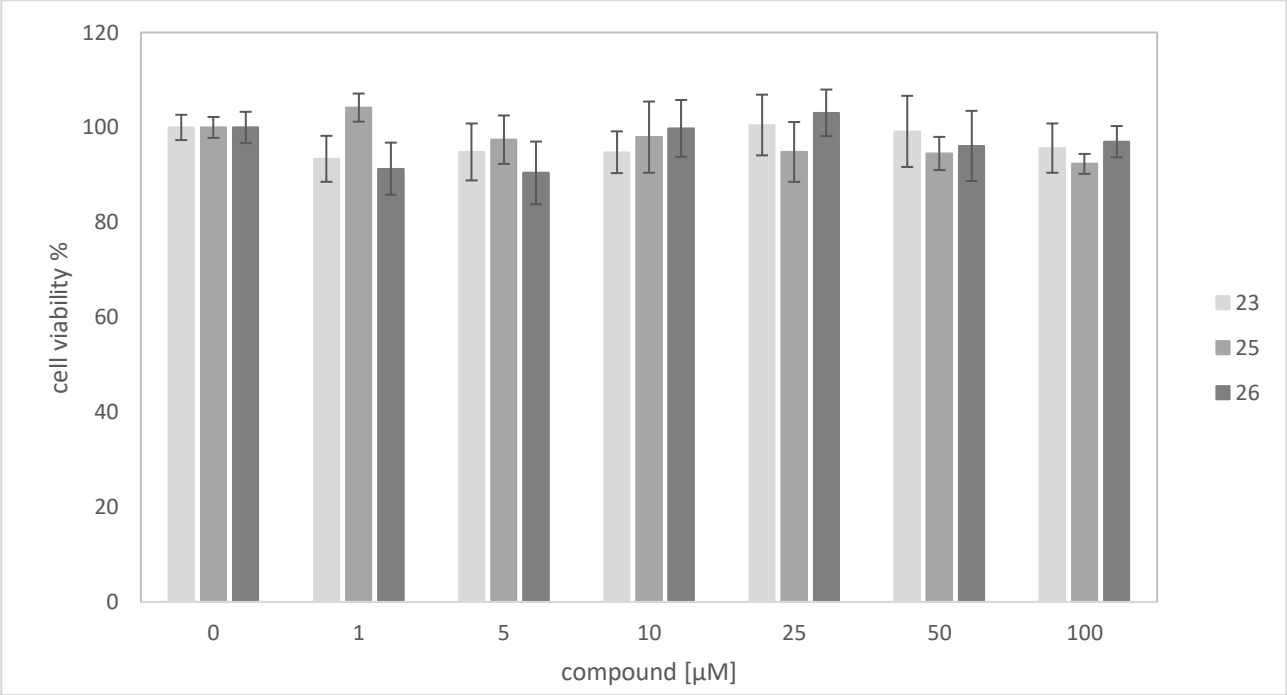


Figure S46: B16F10 melanoma cell viability after treatment with compounds **23**, **25** and **26**. The mean value and standard deviation were calculated from triplicate experiments.

Table S1: CAS numbers and smiles for compounds **7-32**

COMPOUND	CAS Number	SMILES
7.	**423743-29-7**	Fc1ccc(CN2CCN(C(Cc3ccccc3)=O)CC2)cc1
8.	**439848-20-1**	Fc1ccc(CN2CCN(CC2)C(=O)CCc3ccccc3)cc1
9.	**423739-67-7**	Fc1ccc(CN2CCN(CC2)C(=O)C(c3ccccc3)c4ccccc4)cc1
10.	**1387739-82-3**	Fc1ccc(CN2CCN(CC2)C(=O)Cc3ccccc3Cl)cc1
11.	**-**	Fc1ccc(CN2CCN(CC2)C(=O)Cc3cccc(Cl)c3)cc1
12.	**1329294-83-8**	Fc1ccc(CN2CCN(CC2)C(=O)Cc3ccc(Cl)cc3)cc1
13.	**1988172-38-8**	Fc1ccc(CN2CCN(CC2)C(=O)Cc3ccccc3Br)cc1
14.	**1985981-75-6**	Fc1ccc(CN2CCN(CC2)C(=O)Cc3cccc(Br)c3)cc1
15.	**1146917-07-8**	Fc1ccc(CN2CCN(CC2)C(=O)Cc3ccc(Br)cc3)cc1
16.	**-**	[O-][N+](=O)c1ccccc1CC(=O)N2CCN(Cc3ccc(F)cc3)CC2
17.	**-**	[O-][N+](=O)c1cccc(CC(=O)N2CCN(Cc3ccc(F)cc3)CC2)c1
18.	**-**	[O-][N+](=O)c1ccc(CC(=O)N2CCN(Cc3ccc(F)cc3)CC2)cc1
19.	**1796840-99-7**	COc1ccc(CC(=O)N2CCN(Cc3ccc(F)cc3)CC2)cc1
20.	**-**	Nc1ccccc1CC(=O)N2CCN(Cc3ccc(F)cc3)CC2
21.	**-**	Nc1cccc(CC(=O)N2CCN(Cc3ccc(F)cc3)CC2)c1
22.	**-**	Nc1ccc(CC(=O)N2CCN(Cc3ccc(F)cc3)CC2)cc1
23.	**-**	Fc1ccc(CN2CCN(CC2)C(=O)c3cccc(F)c3C(F)(F)F)cc1
24.	**-**	Fc1ccc(CN2CCN(CC2)C(=O)c3ccc(F)cc3C(F)(F)F)cc1
25.	**-**	Fc1ccc(CN2CCN(CC2)C(=O)c3cc(F)ccc3C(F)(F)F)cc1
26.	**-**	[O-][N+](=O)c1c(Cl)cccc1C(=O)N2CCN(Cc3ccc(F)cc3)CC2
27.	**2344352-87-8**	[O-][N+](=O)c1cc(Cl)ccc1C(=O)N2CCN(Cc3ccc(F)cc3)CC2
28.	**-**	[O-][N+](=O)c1ccc(Cl)cc1C(=O)N2CCN(Cc3ccc(F)cc3)CC2
29.	**-**	[O-][N+](=O)c1cccc(Cl)c1C(=O)N2CCN(Cc3ccc(F)cc3)CC2
30.	**-**	COc1cccc(C(=O)N2CCN(Cc3ccc(F)cc3)CC2)c1[N+](=O)[O-]
31.	**-**	COc1ccc(C(=O)N2CCN(Cc3ccc(F)cc3)CC2)c(c1)[N+](=O)[O-]
32.	**2345032-69-9**	COc1ccc(c(c1)C(=O)N2CCN(Cc3ccc(F)cc3)CC2)[N+](=O)[O-]

Table S2. Selected physicochemical parameters, lipophilicity, solubility and drug-likeness for compound **7-32** predicted by SwissADME(http://swissadme.ch/), and Molinspiration tools(https://molinspiration.com).

entry	MW (g/mol)*	TPSA*	TPSA**	iLogP*	miLogP**	Water solubility*	Lipinski*	Ghose*	PAINS*
7	312.38	23.55	23.55	3.21	2.92	Soluble	Yes 0 violation	Yes	0 alert
8	326.41	23.55	23.55	3.54	3.44	Soluble	Yes 0 violation	Yes	0 alert
9	388.48	23.55	23.55	3.79	4.31	Moderately soluble	Yes 0 violation	Yes	0 alert
10	346.83	23.55	23.55	3.44	3.55	Soluble	Yes 0 violation	Yes	0 alert
11	346.83	23.55	23.55	3.55	3.58	Soluble	Yes 0 violation	Yes	0 alert
12	346.83	23.55	23.55	3.52	3.60	Soluble	Yes 0 violation	Yes	0 alert
13	391.28	23.55	23.55	3.51	3.68	Soluble	Yes 0 violation	Yes	0 alert
14	391.28	23.55	23.55	3.64	3.71	Soluble	Yes 0 violation	Yes	0 alert
15	391.28	23.55	23.55	3.63	3.73	Soluble	Yes 0 violation	Yes	0 alert
16	357.38	69.37	69.37	2.83	2.83	Soluble	Yes 0 violation	Yes	0 alert
17	357.38	69.37	69.37	2.95	2.86	Soluble	Yes 0 violation	Yes	0 alert
18	357.38	69.37	69.37	2.92	2.88	Soluble	Yes 0 violation	Yes	0 alert
19	342.41	32.78	32.78	3.46	2.98	Soluble	Yes 0 violation	Yes	0 alert
20	327.40	49.57	49.57	2.92	2.36	Soluble	Yes 0 violation	Yes	0 alert
21	327.40	49.57	49.57	2.88	1.97	Soluble	Yes 0 violation	Yes	0 alert
22	327.40	49.57	49.57	2.78	2.00	Soluble	Yes 0 violation	Yes	1 alert: anil_no_alk
23	384.34	23.55	23.55	3.27	3.32	Soluble	Yes 1 violation: MLogP>4.15	Yes	0 alert
24	384.34	23.55	23.55	3.36	3.35	Soluble	Yes 1 violation: MLogP>4.15	Yes	0 alert
25	384.34	23.55	23.55	3.34	3.35	Soluble	Yes 1 violation: MLogP>4.15	Yes	0 alert

26	377.80	69.37	69.37	2.84	2.90	Moderately soluble	Yes 0 violation	Yes	0 alert
27	377.80	69.37	69.37	2.89	2.92	Moderately soluble	Yes 0 violation	Yes	0 alert
28	377.80	69.37	69.37	2.89	2.92	Moderately soluble	Yes 0 violation	Yes	0 alert
29	377.80	69.37	69.37	2.76	2.90	Moderately soluble	Yes 0 violation	Yes	0 alert
30	373.38	78.60	78.61	2.66	2.28	Soluble	Yes 0 violation	Yes	0 alert
31	373.38	78.60	78.61	2.65	2.30	Soluble	Yes 0 violation	Yes	0 alert
32	373.38	78.60	78.61	2.72	2.30	Soluble	Yes 0 violation	Yes	0 alert

***SwissADME** *Water solubility:* Soluble= *Log S (Ali) values between -4 and -2;* Moderately soluble= *Log S (Ali) values between -6 and -4.*

****Molinspiration**