

Combination of Heme Oxygenase-1 Inhibition and Sigma Receptor Modulation for Anticancer Activity

Giuseppe Romeo ¹, Valeria Ciaffaglione ^{1,*}, Emanuele Amata ¹, Maria Dichiarà ¹, Loredana Calabrese ¹, Luca Vanella ¹, Valeria Sorrenti ¹, Salvo Grosso ¹, Agata Grazia D'Amico ¹, Velia D'Agata ², Sebastiano Intagliata ^{1,*} and Loredana Salerno ¹

¹ Department of Drug and Health Sciences, University of Catania, viale A. Doria 6, 95125 Catania, Italy; gromeo@unict.it (G.R.); eamata@unict.it (E.A.); mariadic.md@gmail.com (M.D.); loredanacalabrese94@gmail.com (L.C.); lvanella@unict.it (L.V.); sorrenti@unict.it (V.S.); salvogrosso@outlook.it (S.G.); agata.damico@unict.it (A.G.D.); l.salerno@unict.it (L.S.)

² Department of Biomedical and Biotechnological Sciences, University of Catania, 95100 Catania, Italy; vdagata@unict.it

* Correspondence: valeria.ciaffaglione@phd.unict.it (V.C.); s.intagliata@unict.it (S.I.); Tel.: +39-095-738-4006 (V.C.); +39-095-738-4053 (S.I.).

Table of contents

Synthesis of intermediates 9–12	S2
Synthesis of intermediates 13–16	S3
Table S1: HO-1 inhibition and binding properties of hybrids 1–4 and reference compounds.	S4
Table S2: Elemental analysis data for compounds 1–4.	S4
Figures S1–S8: ¹H NMR spectra of intermediates 9–16.	S5-S8
Figures S9–S16: ¹H NMR and ¹³C NMR spectra of compounds 1–4.	S9-S12
Figure S17: viability of MDA-MB 231 cell line	S13
References	S13

General procedure for the synthesis of 1-(4-benzylpiperazin-1-yl)hydroxyphenyl derivatives (**9–12**)

4-hydroxybenzoic acid, 2-(4-hydroxyphenyl)acetic acid, 3-(4-hydroxyphenyl)propanoic acid, or 4-(4-hydroxyphenyl)butanoic acid (**5–8**, respectively) (6.02 mmol) was dissolved in THF anhydrous (10 mL). 1,1'-carbonyldiimidazole (CDI) (6.02 mmol) was added and the reaction mixture was left stirring under nitrogen for 10 min. Benzylpiperazine (6.02 mmol) was dissolved in THF anhydrous (5 mL) in a dropping funnel. The reaction mixture was cooled at 0 °C for 10 min. The benzylpiperazine was added dropwise and the reaction occurred at room temperature for 8 h. The solvent was evaporated under vacuum, and for compounds **9–11** the resulting yellow oil was washed with NaHCO₃ 0,5 % m/v aqueous solution (50 mL), and water (2 x 50 mL). Then, ethanol (10 mL) was added and volatiles were again eliminated under vacuum. For compound **12**, ethyl acetate (50 mL) was added to the yellow residue. The organic layer was then washed with saturated solution of NaHCO₃ (3 x 25 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated. The obtained oily residue was purified by flash column chromatography using a mixture of dichloromethane/methanol or ethyl acetate/methanol (9.5/0.5).

(4-Benzylpiperazin-1-yl)(4-hydroxyphenyl)methanone (**9**)

White solid; mp: 170.2–173.5 °C; yield 34.64 %. IR (KBr, selected lines) cm⁻¹: 3196, 2948, 2810, 1734, 1608, 1578, 1434, 1364, 1277, 1169, 1003, 848. ¹H NMR (200 MHz, DMSO-*d*₆): δ 9.89 (s, 1H, OH phenolic), 7.39–7.19 (m, 5H + 2H, aromatic), 6.82–6.74 (m, 2H, aromatic), 3.58–3.25 (m, 2H + 4H, Ar-CH₂-N + piperazine), 2.43–2.32 (m, 4H, piperazine).

1-(4-Benzylpiperazin-1-yl)-2-(4-hydroxyphenyl)ethan-1-one (**10**)

White solid; mp: 156.3–157.4 °C; yield 26.84 %. IR (KBr, selected lines) cm⁻¹: 3319, 2914, 2814, 1631, 1516, 1437, 1410, 1354, 1264, 1208, 1003, 820. ¹H NMR (200 MHz, DMSO-*d*₆): δ 9.28 (s, 1H, OH phenolic), 7.36–7.24 (m, 5H, aromatic), 6.99 (d, *J* = 8.4 Hz, 2H, aromatic), 6.67 (d, *J* = 8.6 Hz, 2H, aromatic), 3.55 (s, 2H, CO-CH₂-Ar), 3.48–3.32 (m, 2H + 4H, Ar-CH₂-N + piperazine), 2.29–2.20 (m, 4H, piperazine).

1-(4-Benzylpiperazin-1-yl)-3-(4-hydroxyphenyl)propan-1-one (**11**)

White solid; mp: 107.8–109.2 °C; yield 28.6 %. IR (KBr, selected lines) cm⁻¹: 3376, 3023, 2805, 1643, 1514, 1454, 1346, 1212, 1026, 999, 812. ¹H NMR (200 MHz, DMSO-*d*₆): δ 9.19 (s, 1H, OH phenolic), 7.38–7.21 (m, 5H, aromatic), 7.00 (d, *J* = 8.2 Hz, 2H, aromatic), 6.64 (d, *J* = 8.2 Hz, 2H, aromatic), 3.42–3.30 (m, 4H, piperazine), 2.67–2.63 (m, 2H, CO-CH₂-CH₂-Ar), 2.54–2.50 (m, 2H, CO-CH₂-CH₂-Ar), 2.39–2.11 (m, 4H, piperazine).

1-(4-Benzylpiperazin-1-yl)-4-(4-hydroxyphenyl)butan-1-one (**12**)

White solid; mp: 145.3–146.9 °C; yield 47 %. IR (KBr, selected lines) cm⁻¹: 3285, 2951, 2910, 2822, 1623, 1595, 1452, 1348, 1257, 1147, 996, 835. ¹H NMR (200 MHz, CDCl₃): δ 7.38–7.35 (m, 5H, aromatic), 6.98 (d, *J* = 8.4 Hz, 2H, aromatic), 6.76 (d, *J* = 8.4 Hz, 2H, aromatic), 3.72–3.69 (m, 2H, piperazine), 3.64 (s, 2H, Ar-CH₂-N), 3.55–3.42 (m, 2H, piperazine), 2.64–2.43 (m, 4H + 2H, piperazine + CO-CH₂-CH₂-CH₂-Ar), 2.29 (t, *J* = 7.5 Hz, 2H, CO-CH₂-CH₂-CH₂-Ar), 2.02–1.80 (m, 2H, CO-CH₂-CH₂-CH₂-Ar).

General procedure for the synthesis of bromobutoxy phenyl derivatives (**13–16**)

The appropriate (4-benzylpiperazin-1-yl)hydroxyphenyl intermediate (**9–12**) (1.34 mmol) was dissolved in acetonitrile (25 mL). K_2CO_3 (5.36 mmol) was added and the reaction mixture was left stirring under reflux for 10 min. Then 1,4-dibromobutane (5.36 mmol) was added and the reaction completed in 6 h. The solvent was evaporated under vacuum, then water (100 mL) was added to the resulting residue and extracted with ethyl acetate (3 x 50 mL). The organic layer was finally washed with brine (50 mL) and dried over anhydrous Na_2SO_4 , filtered, and concentrated. The obtained residue was purified by flash column chromatography using ethyl acetate/methanol (9.5/ 0.5).

(4-Benzylpiperazin-1-yl)(4-(4-bromobutoxy)phenyl)methanone (**13**)

Colorless oil; yield 81.20 %. 1H NMR (200 MHz, $DMSO-d_6$): δ 7.36–7.31 (m, 5H + 2H, aromatic), 6.99–6.94 (m, 2H, aromatic), 4.03 (t, $J = 5.2$ Hz, 2H, O- $CH_2-CH_2-CH_2-CH_2-Br$), 3.61 (t, $J = 6.4$ Hz, 2H, O- $CH_2-CH_2-CH_2-CH_2-Br$), 3.47–3.41 (m, 2H + 4H, Ar- CH_2-N + piperazine), 2.39–2.35 (m, 4H, piperazine), 2.0–1.79 (m, 4H, O- $CH_2-CH_2-CH_2-CH_2-Br$).

1-(4-Benzylpiperazin-1-yl)-2-(4-(4-bromobutoxy)phenyl)ethan-1-one (**14**)

Yellow oil; yield 69.84 %. 1H NMR (200 MHz, $DMSO-d_6$): δ 7.37–7.19 (m, 5H, aromatic), 7.10 (d, $J = 8.4$ Hz, 2H, aromatic), 6.85 (d, $J = 8.6$ Hz, 2H, aromatic), 3.98 (t, $J = 6.2$ Hz, 2H, O- $CH_2-CH_2-CH_2-CH_2-Br$), 3.72–3.56 (m, 2H + 2H, O- $CH_2-CH_2-CH_2-CH_2-Br$ + CO- CH_2-Ar), 3.45 (s, 2H, Ar- CH_2-N), 3.40–3.25 (m, 4H, piperazine), 2.27–2.25 (m, 4H, piperazine), 1.99–1.78 (m, 4H, O- $CH_2-CH_2-CH_2-CH_2-Br$).

1-(4-Benzylpiperazin-1-yl)-3-(4-(4-bromobutoxy)phenyl)propan-1-one (**15**)

Colorless oil; yield 86.80 %. 1H NMR (200 MHz, $DMSO-d_6$): δ 7.39–7.20 (m, 5H, aromatic), 7.12 (d, $J = 8.6$ Hz, 2H, aromatic), 6.82 (d, $J = 8.6$ Hz, 2H, aromatic), 3.98 (t, $J = 6.0$ Hz, 2H, O- $CH_2-CH_2-CH_2-CH_2-Br$), 3.60 (t, $J = 6.4$ Hz, 2H, O- $CH_2-CH_2-CH_2-CH_2-Br$), 3.45–3.36 (m, 2H + 4H, Ar- CH_2-N + piperazine), 2.80–2.67 (m, 2H, CO- CH_2-CH_2-Ar), 2.57–2.48 (m, 2H, CO- CH_2-CH_2-Ar), 2.27–2.17 (m, 4H, piperazine), 2.10–1.76 (m, 4H, O- $CH_2-CH_2-CH_2-CH_2-Br$).

1-(4-Benzylpiperazin-1-yl)-4-(4-(4-bromobutoxy)phenyl)butan-1-one (**16**)

Yellow oil; yield 60 %. IR (KBr, selected lines) cm^{-1} : 3430, 2927, 1645, 1510, 1433, 1242, 1177, 1030, 746. 1H NMR (200 MHz, $CDCl_3$): δ 7.48–7.30 (m, 5H, aromatic), 7.08 (d, $J = 8.6$ Hz, 2H, aromatic), 6.80 (d, $J = 8.6$ Hz, 2H, aromatic), 3.97 (t, $J = 5.8$ Hz, 2H, O- $CH_2-CH_2-CH_2-CH_2-Br$), 3.73 (s, 2H + 2H, Ar- CH_2-N + piperazine), 3.57–3.46 (m, 2H + 2H, O- $CH_2-CH_2-CH_2-CH_2-Br$, piperazine), 2.60 (t, $J = 7.3$ Hz, 2H + 4H, CO- $CH_2-CH_2-CH_2-Ar$ + piperazine), 2.28 (t, $J = 7.4$ Hz, 2H, CO- $CH_2-CH_2-CH_2-Ar$), 2.15–1.81 (m, 2H + 4H, CO- $CH_2-CH_2-CH_2-Ar$, O- $CH_2-CH_2-CH_2-CH_2-Br$).

Table S1. HO-1 inhibition and binding properties of hybrids **1–4** and reference compounds.

Compd	n	IC ₅₀ HO-1 (μM)	σ ₁ R K _i (nM) (%) ¹	σ ₂ R K _i (nM) (%) ¹
1	0	229.91 ± 5.1	> 10,000 (90)	> 10,000 (54)
2	1	215.59 ± 3.6	> 10,000 (73)	> 10,000 (67)
3	2	161.92 ± 1.8	> 10,000 (78)	> 10,000 (66)
4	3	87.72 ± 1.3	> 10,000 (55)	4,624 ± 663
LS0 ²		2.1 ± 0.3 ³	--	--
LS4/28 ²		0.9 ± 0.08 ³	--	--
LS6/42 ²		0.95 ± 0.02 ³	--	--
Azalanstat		5.30 ± 0.4 ³	--	--
SI1/13 ²		--	1.6 ± 0.05 ⁴	1,418 ± 18 ⁴
RFB/13 ²		--	8.8 ± 0.22 ⁴	3,253 ± 40 ⁴
(+)-Pentazocine		--	4.3 ± 0.5	1,465 ± 224
DTG		--	124 ± 19	18 ± 1
Haloperidol		--	2.6 ± 0.4	77 ± 18

¹ % of inhibition at 10 μM² Figure 1 main text.³ Data taken from [1-3].⁴ Data taken from [4, 5].**Table S2.** Elemental analysis data for compounds **1–4**.

Compd	Formula	Mw	Calcd			Found		
			C	H	N	C	H	N
1	C ₂₅ H ₃₀ N ₄ O ₂	418.4	71.74	7.23	13.39	71.56	7.21	13.42
2	C ₂₆ H ₃₂ N ₄ O ₂	432.7	72.19	7.46	12.95	71.98	7.44	12.99
3	C ₂₇ H ₃₄ N ₄ O ₂	446.0	72.62	7.67	12.55	72.53	7.66	12.58
4	C ₂₈ H ₃₆ N ₄ O ₂	460.2	73.01	7.88	12.16	72.91	7.87	12.20

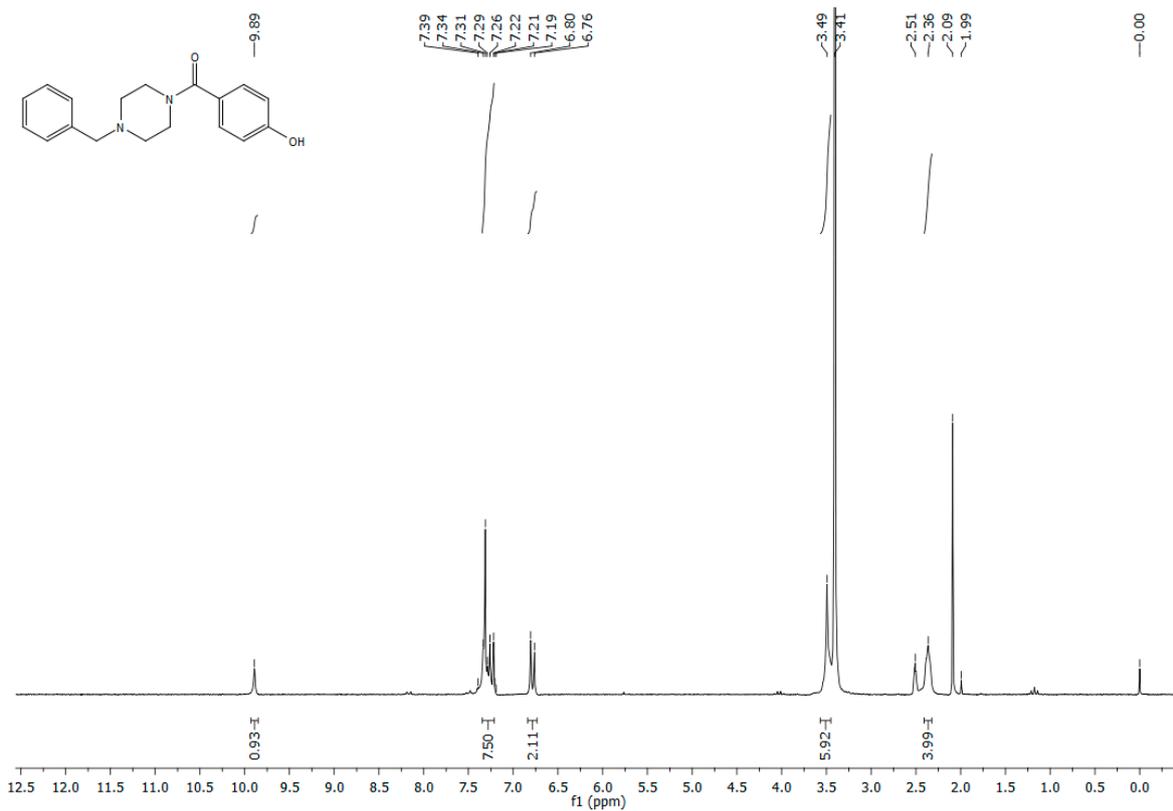


Figure S1. ¹H NMR of compound 9.

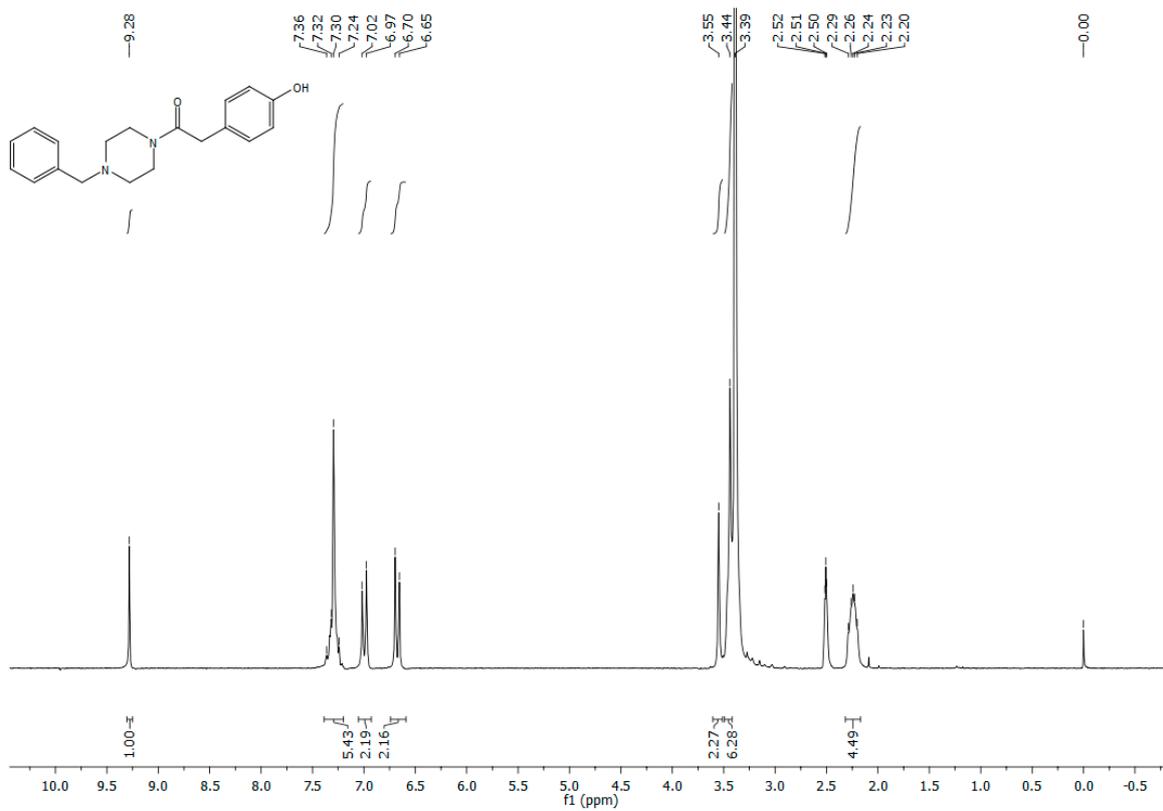


Figure S2. ¹H NMR of compound 10.

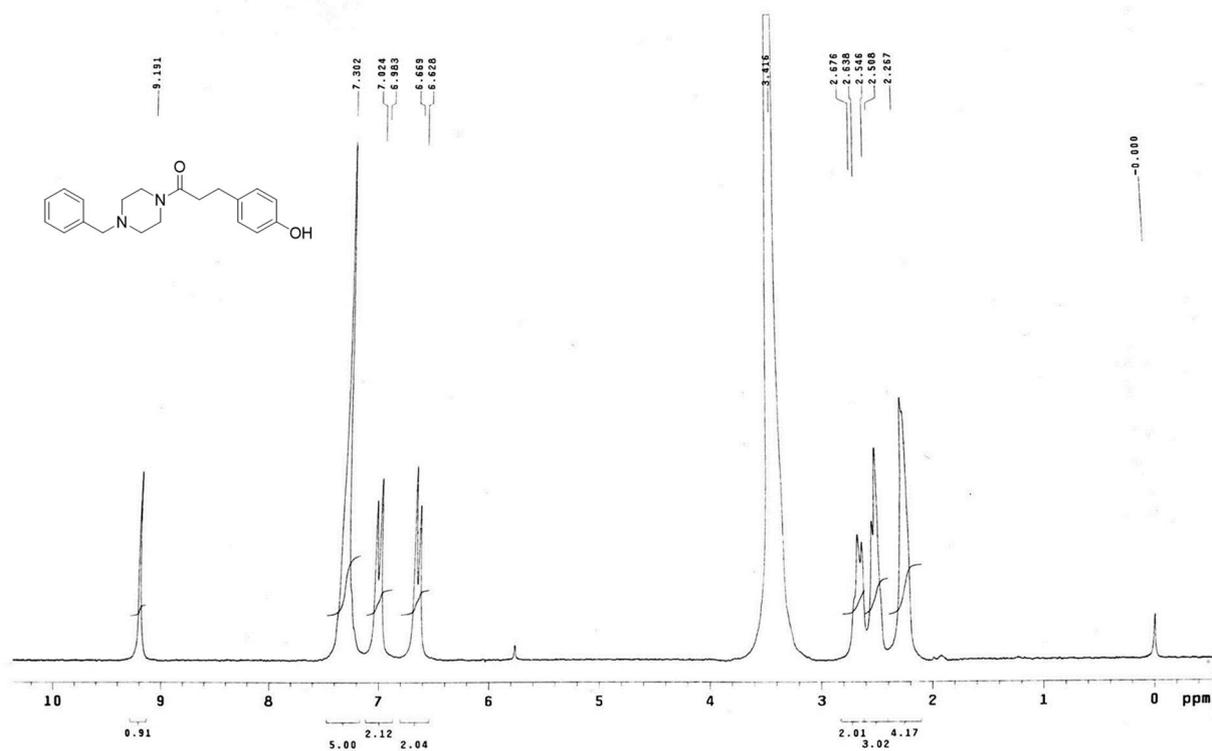


Figure S3. ¹H NMR of compound 11.

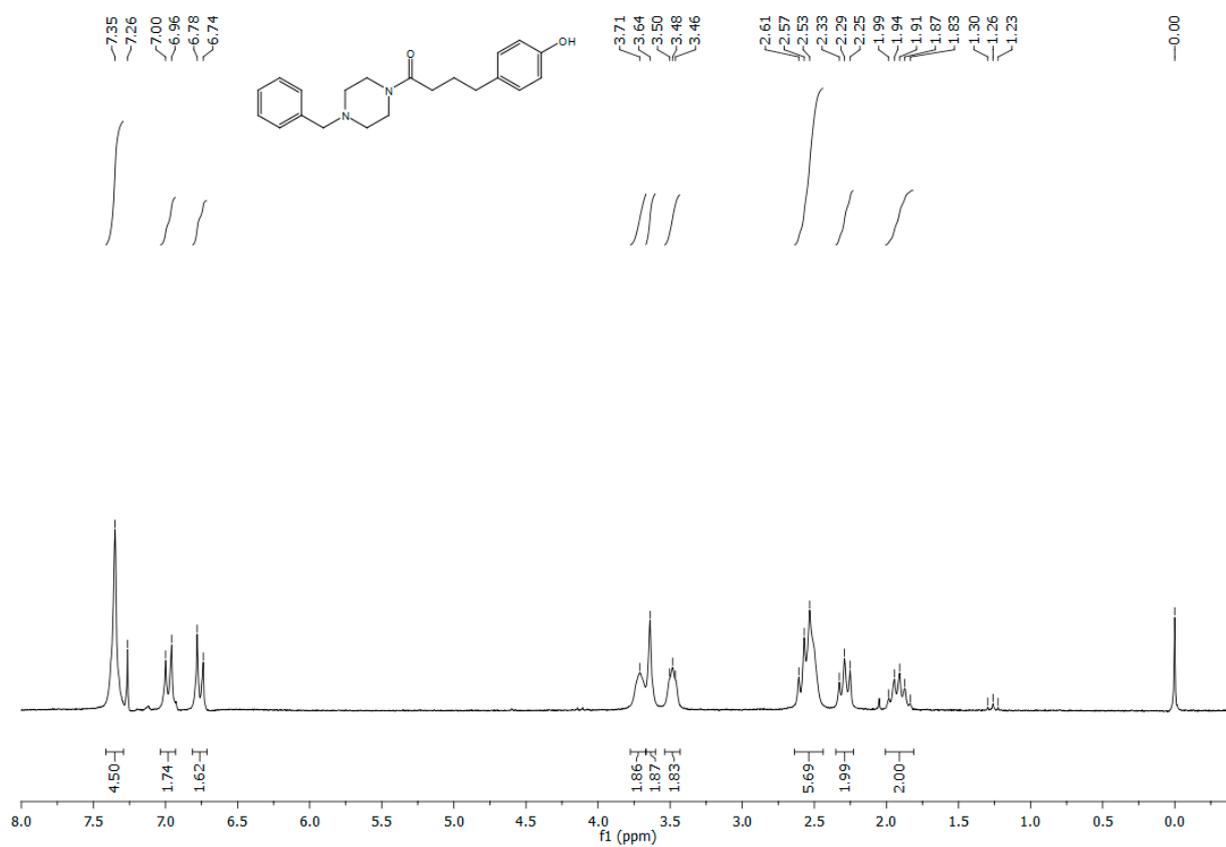


Figure S4. ¹H NMR of compound 12.

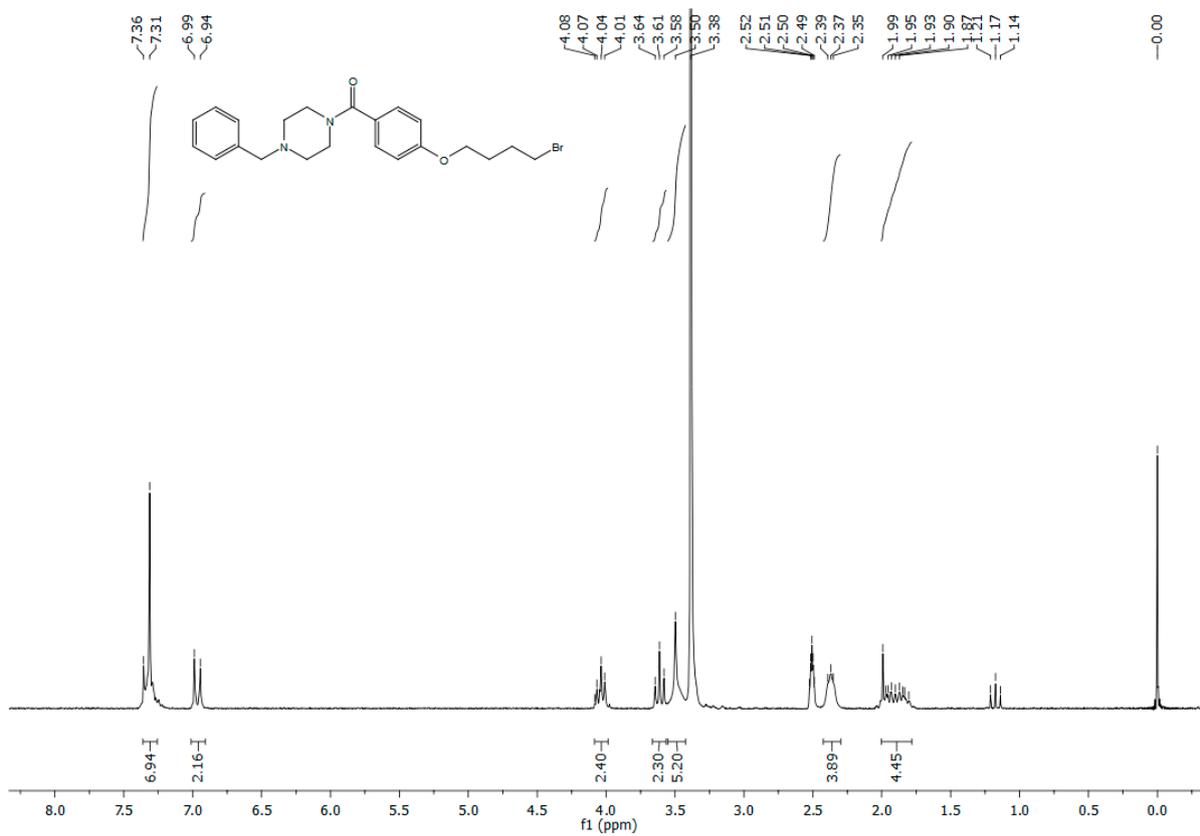


Figure S5. ¹H NMR of compound 13.

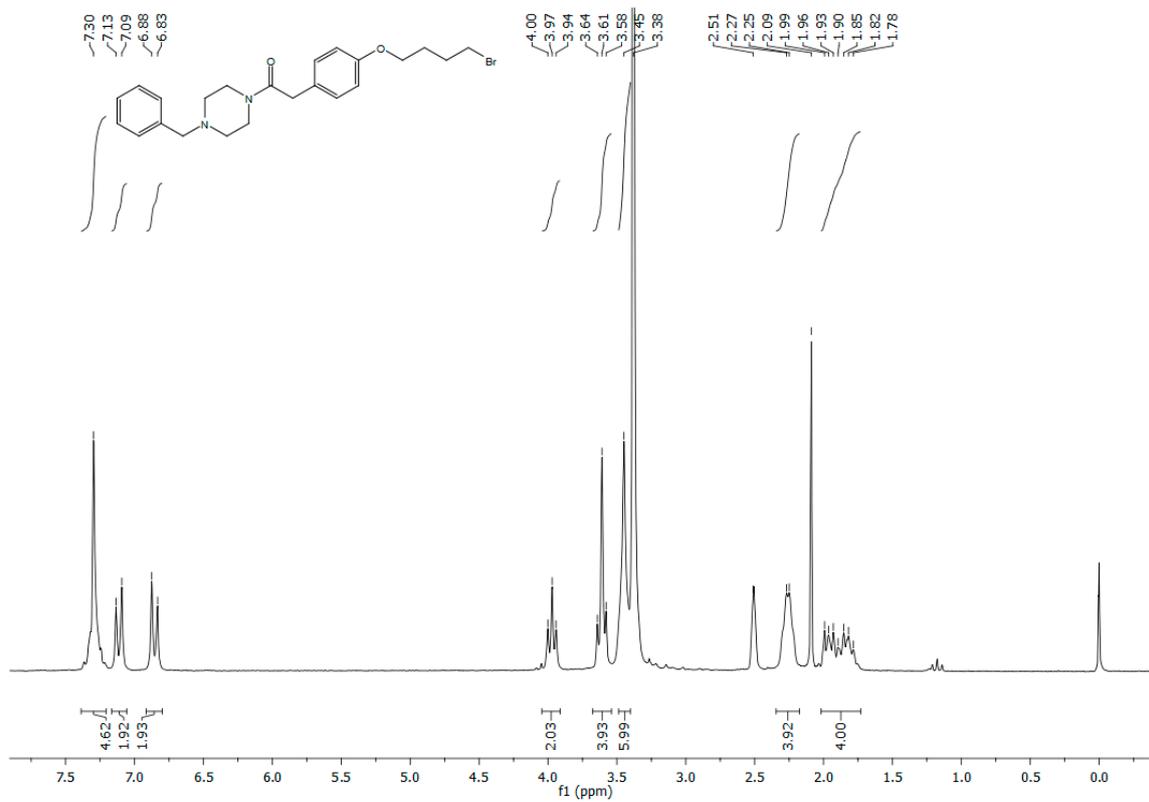


Figure S6. ¹H NMR of compound 14.

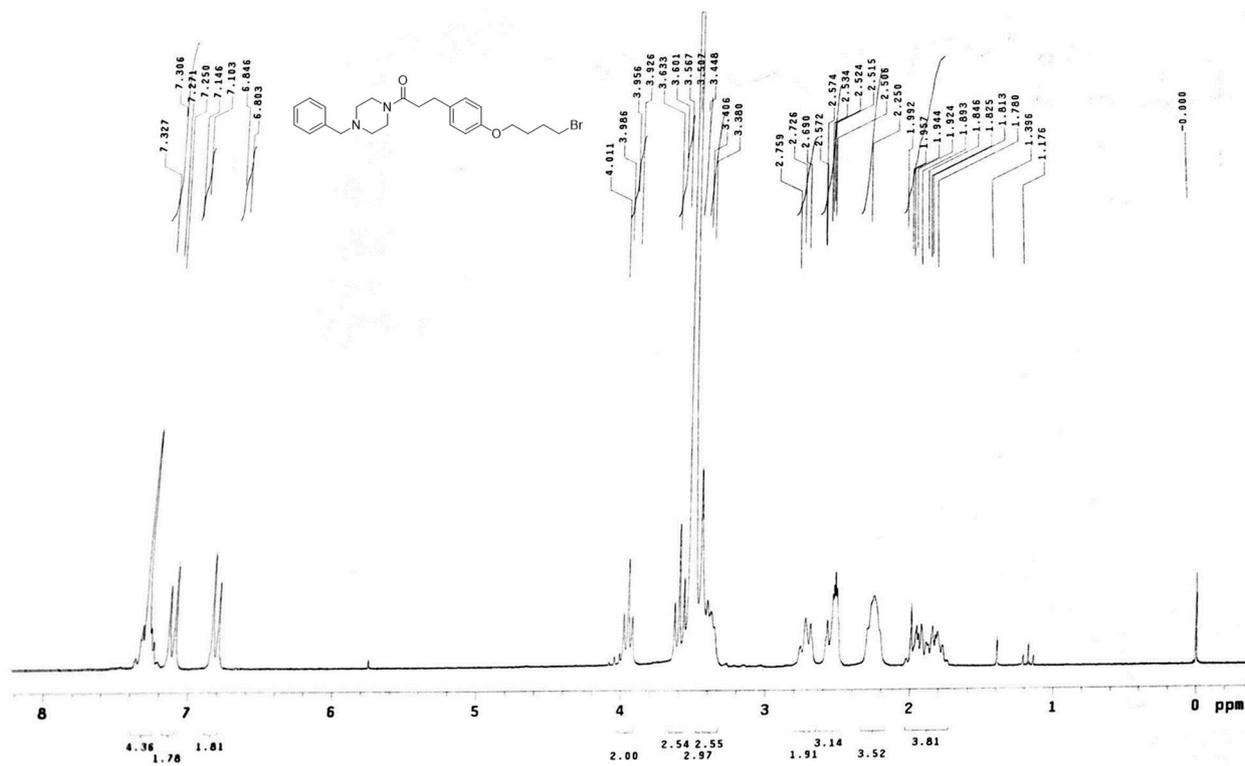


Figure S7. ¹H NMR of compound 15.

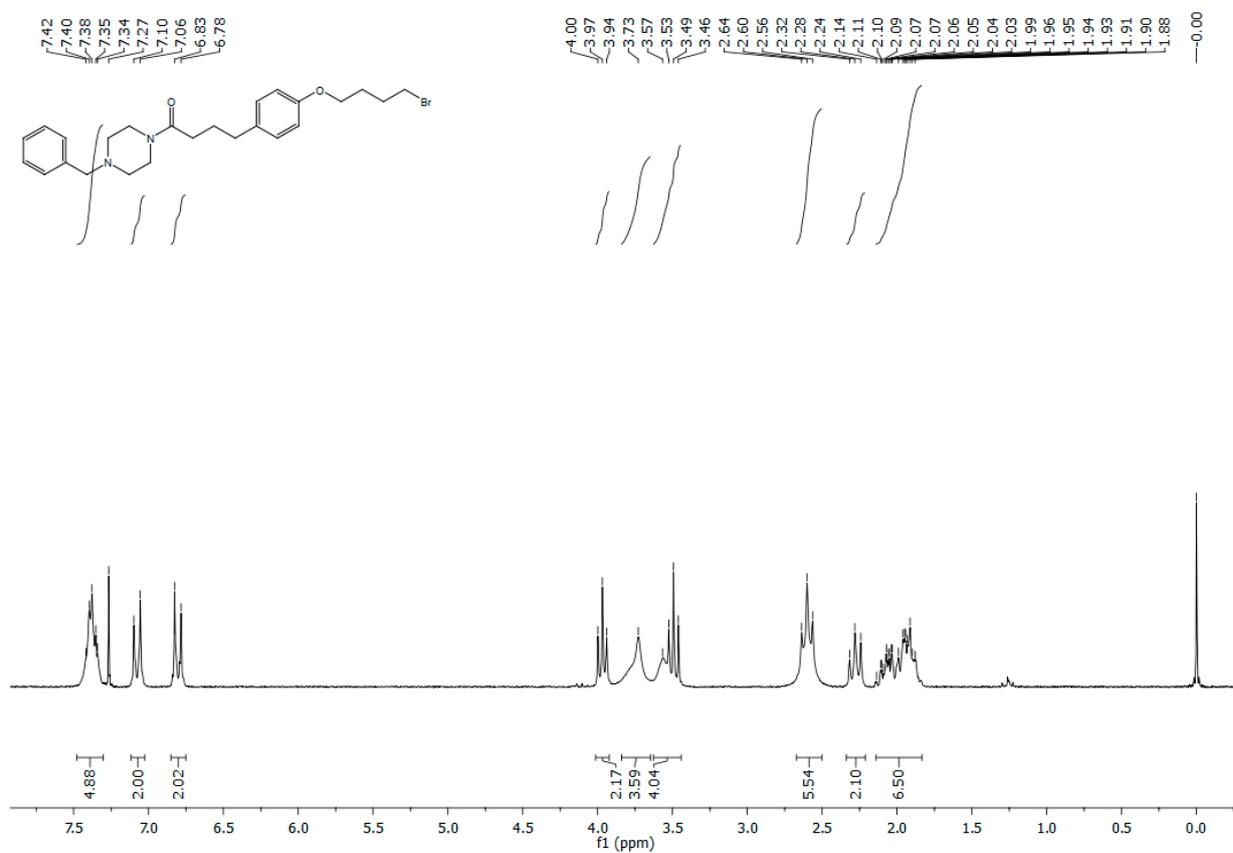


Figure S8. ¹H NMR of compound 16.

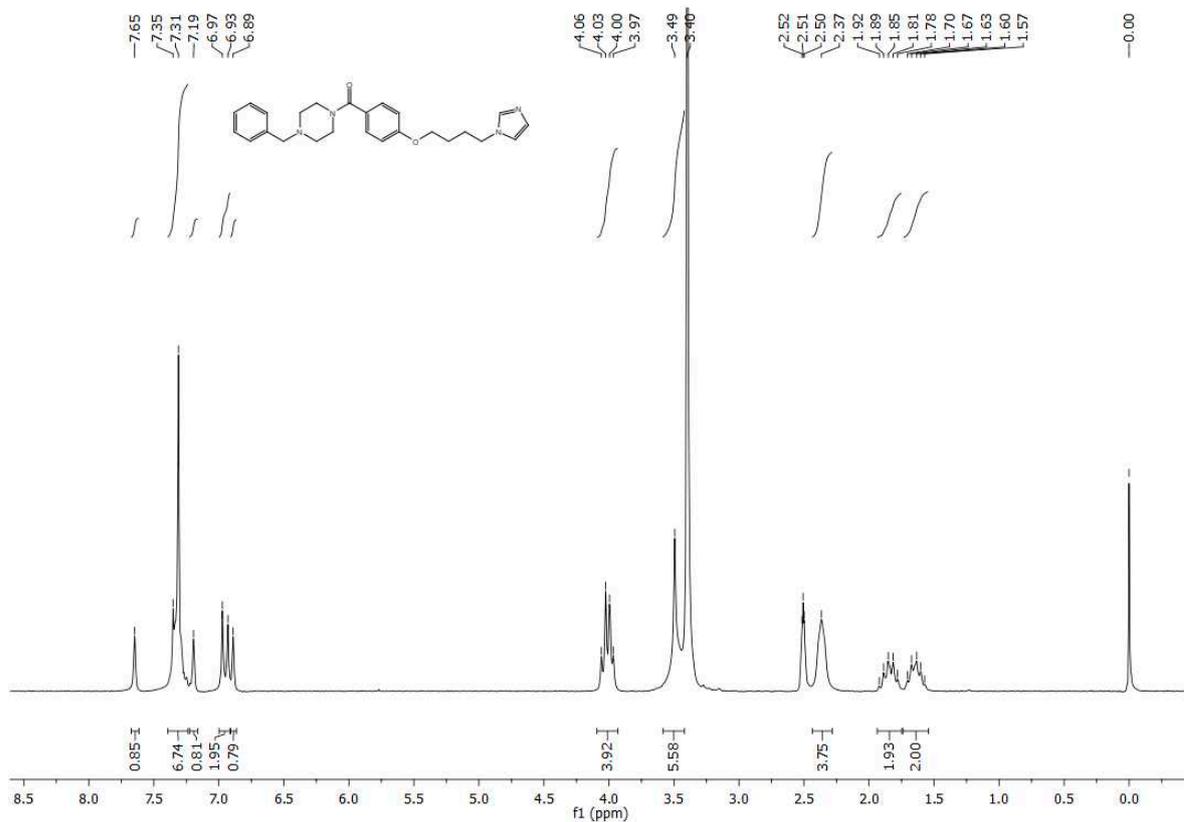


Figure S9. ¹H NMR of compound 1.

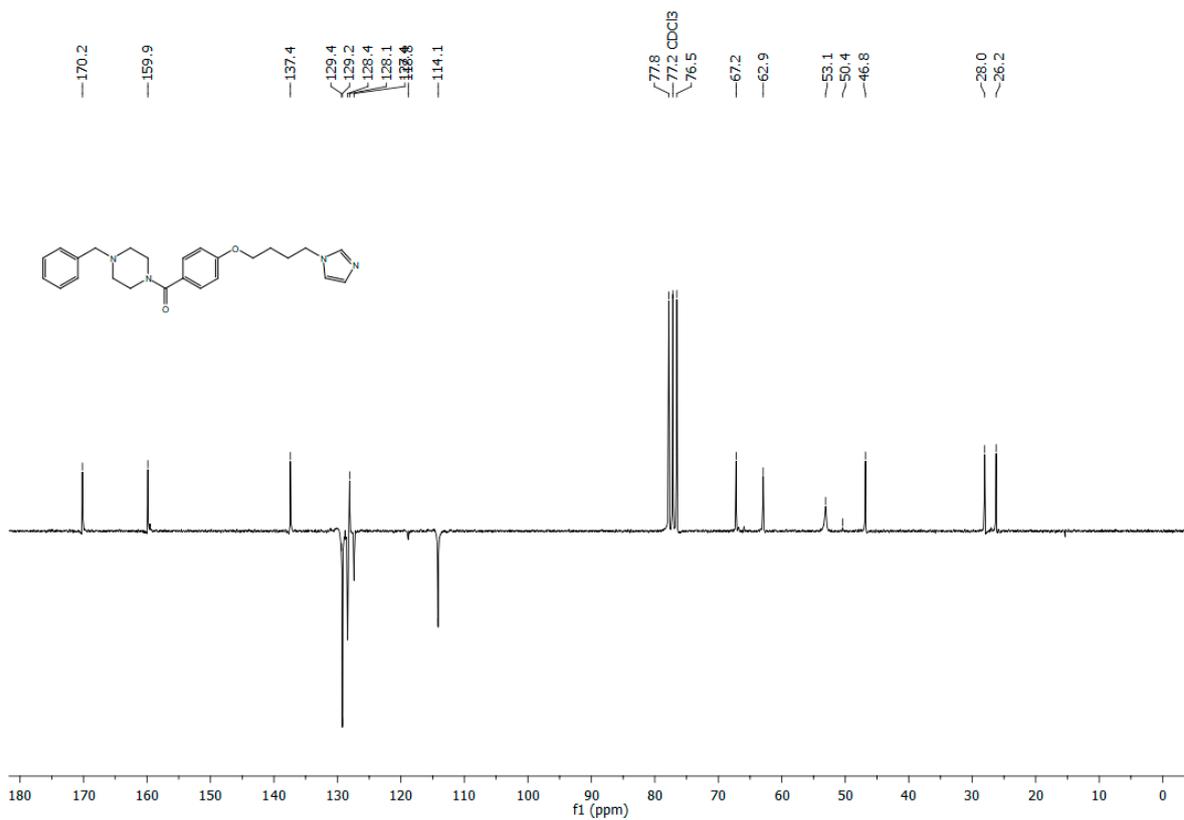


Figure S10. ¹³C NMR of compound 1.

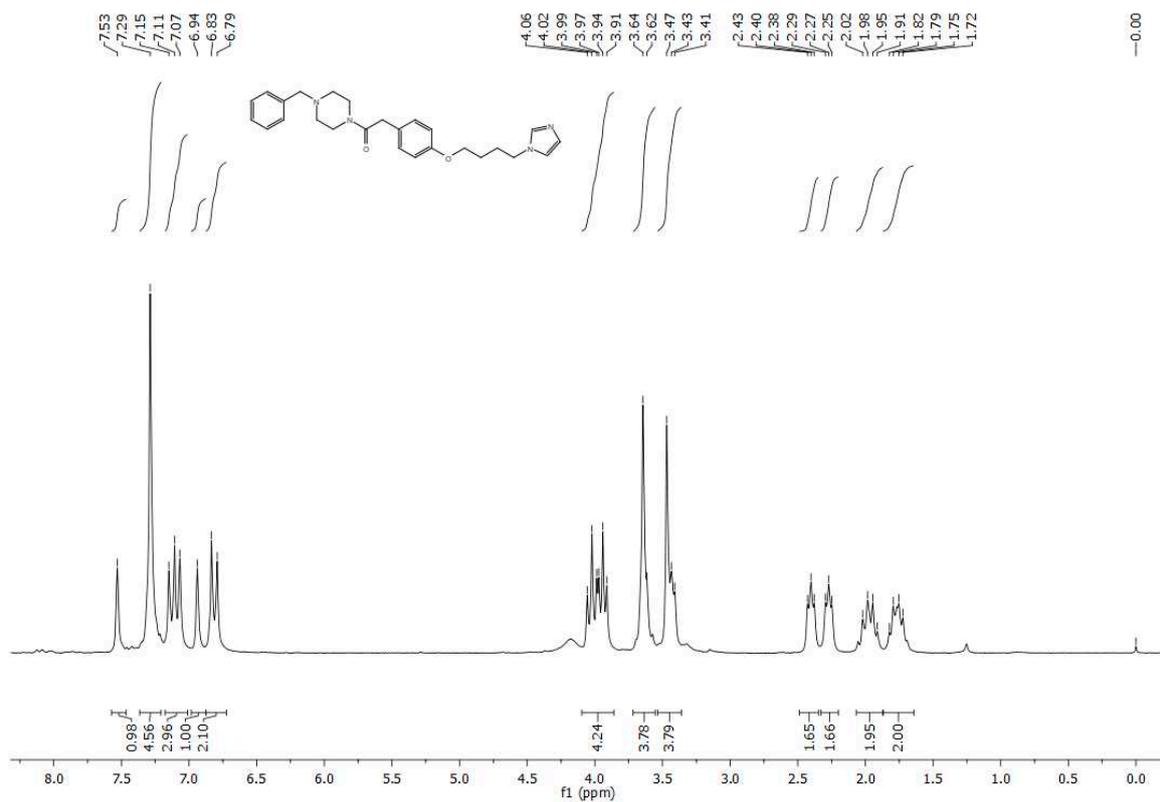


Figure S11. ¹H NMR of compound 2.

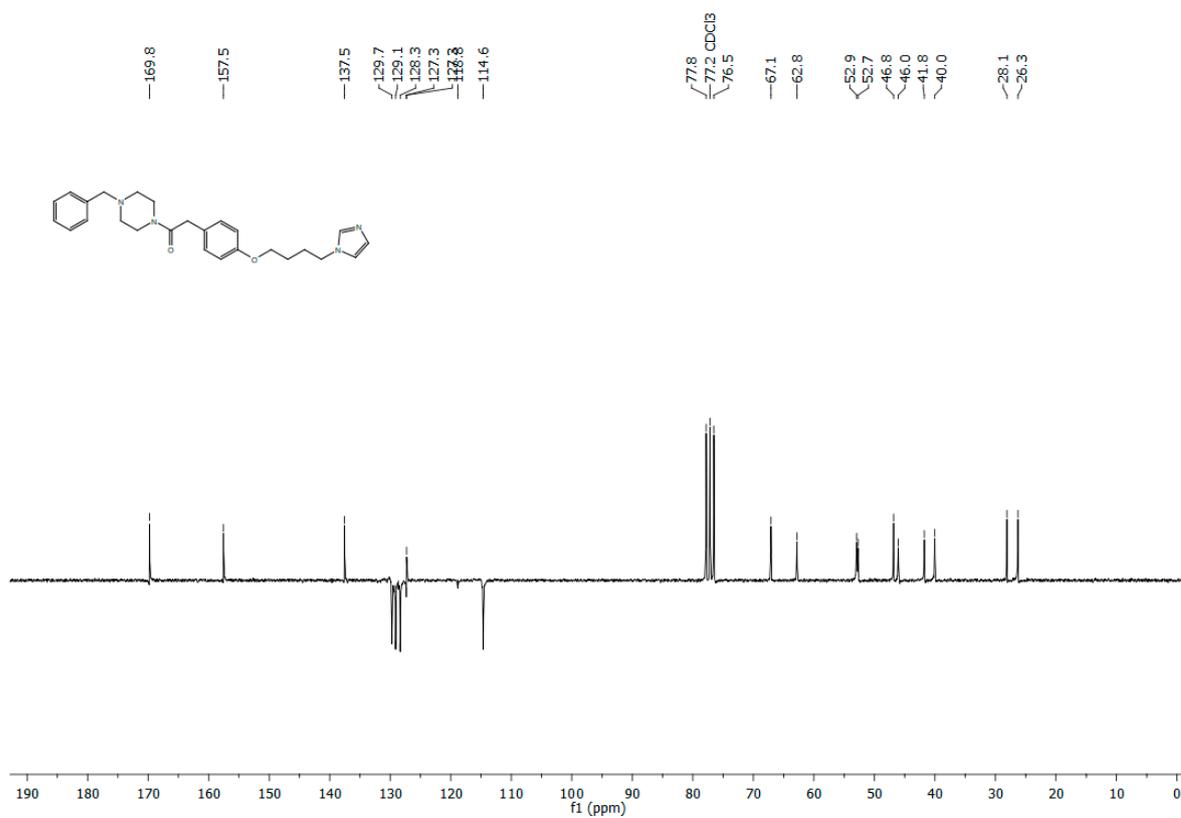


Figure S12. ¹³C NMR of compound 2.

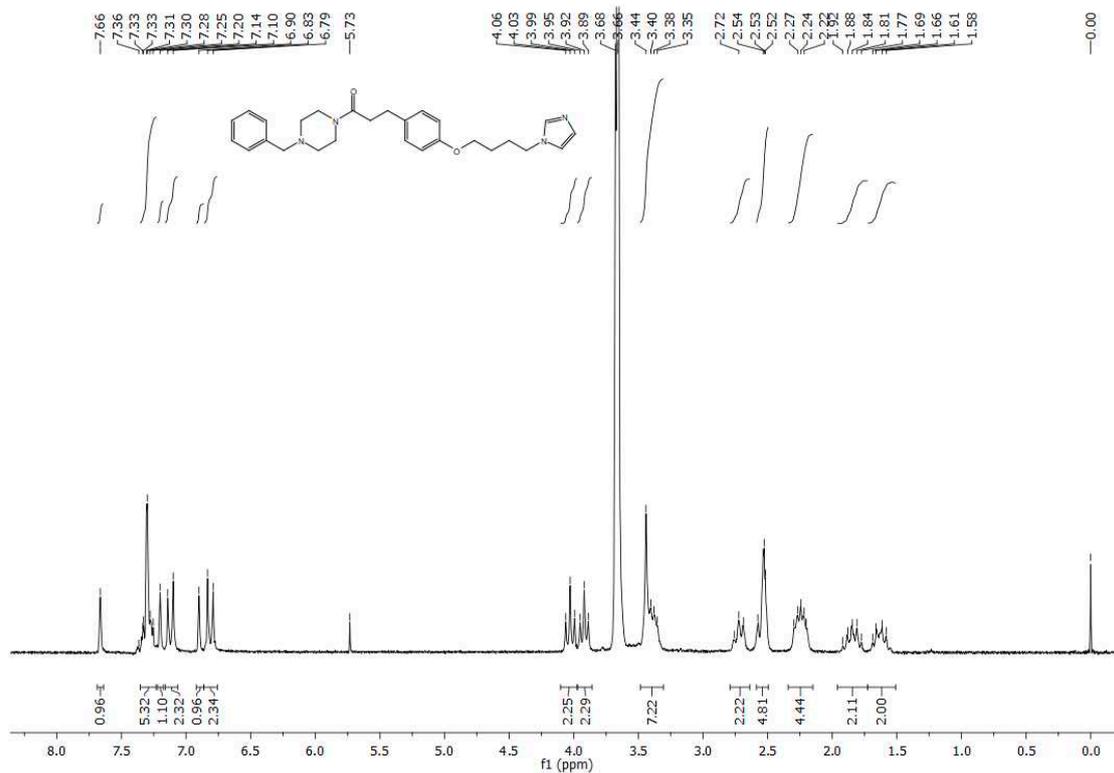


Figure S13. ^1H NMR of compound 3.

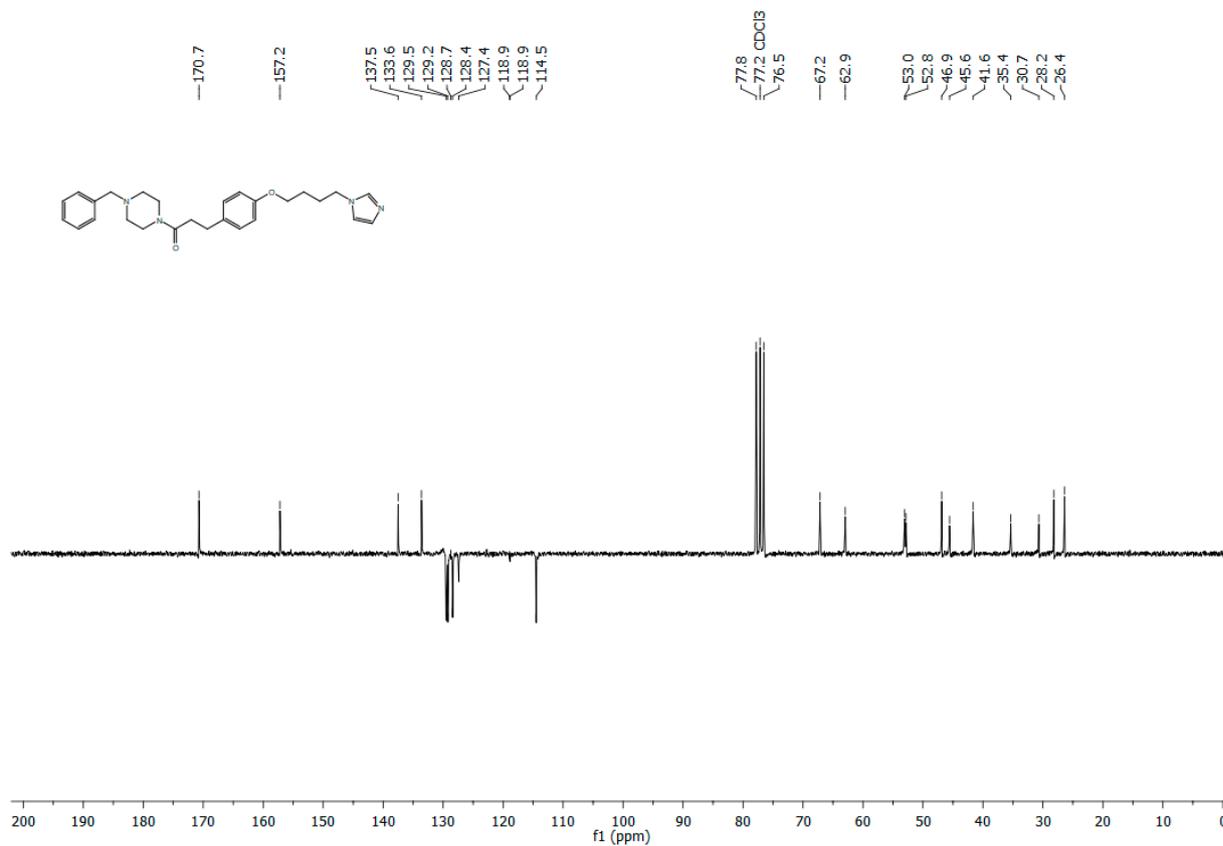


Figure S14. ^{13}C NMR of compound 3.

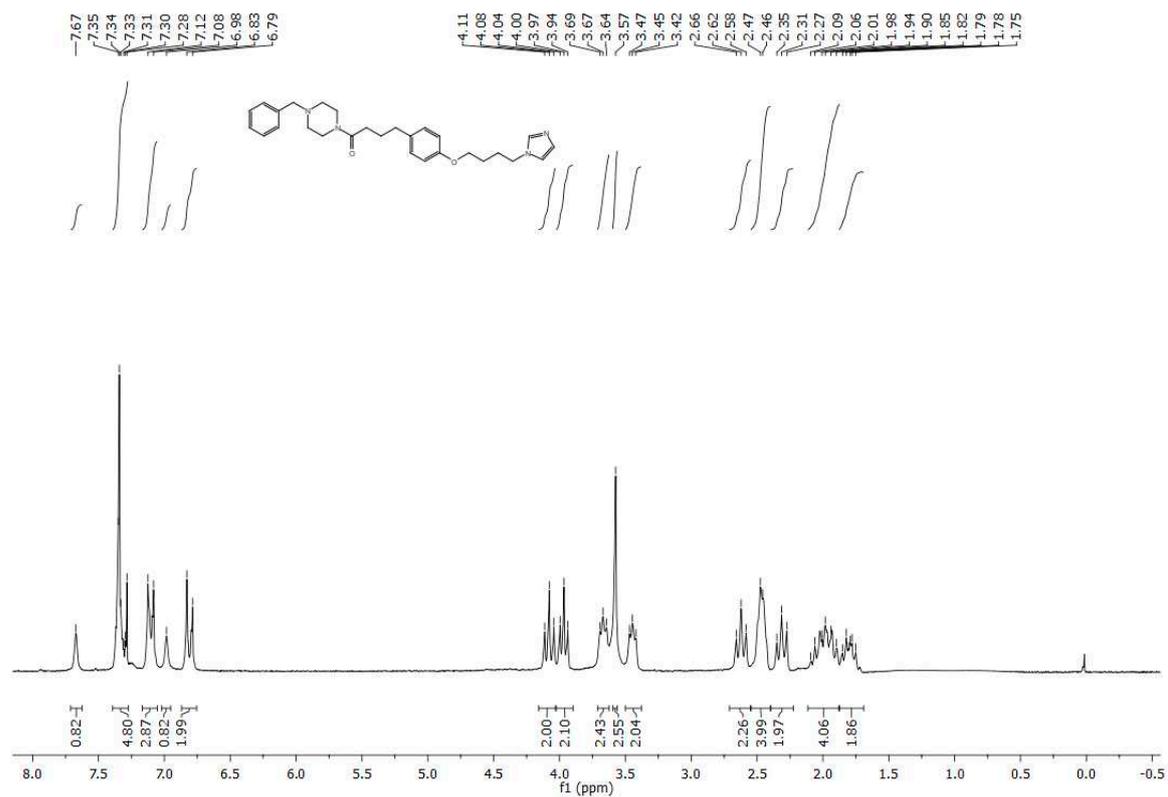


Figure S15. ¹H NMR of compound 4.

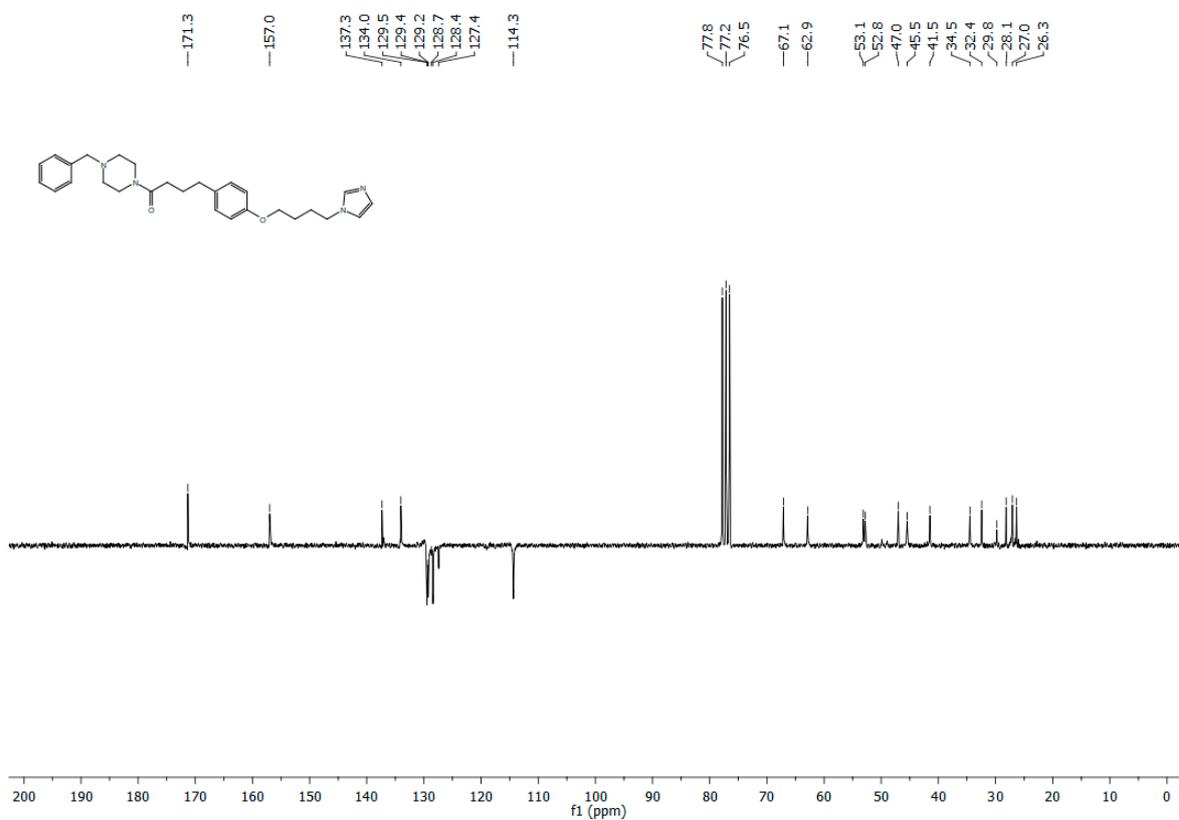


Figure S16. ¹³C NMR of compound 4.

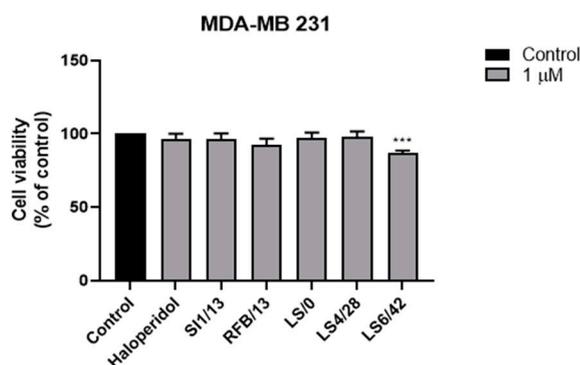


Figure S17. Effect of σ R ligands haloperidol, SI1/13 and RFB/13 and of HO-1 inhibitors LS/0, LS4/28 and LS6/42 treatments on cell viability of MDA-MB 231 cell line, assessed by MTT assay at the doses of 1 μ M. Results are representative of at least three independent experiments and values are expressed as percentage of control (% of control). Data represent means \pm SEM. *** p <0.001 vs control as determined by One-way ANOVA followed by Tukey's multiple comparison test.

References

1. Salerno, L.; Pittala, V.; Romeo, G.; Modica, M. N.; Siracusa, M. A.; Di Giacomo, C.; Acquaviva, R.; Barbagallo, I.; Tibullo, D.; Sorrenti, V., Evaluation of novel aryloxyalkyl derivatives of imidazole and 1,2,4-triazole as heme oxygenase-1 (HO-1) inhibitors and their antitumor properties. *Bioorg Med Chem.* **2013**, *21*, 5145-5153.
2. Salerno, L.; Amata, E.; Romeo, G.; Marrazzo, A.; Prezzavento, O.; Floresta, G.; Sorrenti, V.; Barbagallo, I.; Rescifina, A.; Pittala, V., Potholing of the hydrophobic heme oxygenase-1 western region for the search of potent and selective imidazole-based inhibitors. *Eur J Med Chem.* **2018**, *148*, 54-62.
3. Greish, K. F.; Salerno, L.; Al Zahrani, R.; Amata, E.; Modica, M. N.; Romeo, G.; Marrazzo, A.; Prezzavento, O.; Sorrenti, V.; Rescifina, A.; Floresta, G.; Intagliata, S.; Pittala, V., Novel Structural Insight into Inhibitors of Heme Oxygenase-1 (HO-1) by New Imidazole-Based Compounds: Biochemical and In Vitro Anticancer Activity Evaluation. *Molecules.* **2018**, *23*.
4. Romeo, G. S., L.; Bonanno, F.; Pittalà, V.; Modica, M.N.; Siracusa, M.A.; Marrazzo, A.; Amata, E.; Arena, E.; Prezzavento, O., Synthesis of Novel Benzylpiperazine Derivatives as Ligands for the σ 1 Receptor, Synthesis of Novel Benzylpiperazine Derivatives as Ligands for the σ 1 Receptor. XXVI Congresso Nazionale della Società Chimica Italiana, Paestum (SA) **2017**.
5. Intagliata, S. S., L.; Pittalà, V.; Modica, M.N.; Arena, E.; Wilson, L.S; Mc Laughlin, J.P.; Prezzavento, O.; Romeo, G., Synthesis, binding, and functional properties of new benzylpiperazine derivatives as selective σ 1 receptor ligands. Autumn Meeting for Young Chemists in Biomedical Sciences - Virtual conference. **2020**.