Supporting information for

Synthesis, Cytotoxicity Evaluation and Computational Insights of Novel 1,4-Diazepane-Based Sigma Ligands

Daniele Zampieri[†*], Sara Fortuna[†], Antonella Calabretti[†], Maurizio Romano[¥], Renzo Menegazzi[¥], Dirk Schepmann[‡], Bernhard Wünsch[‡], and Maria Grazia Mamolo[†].
[†]*Department of Chemical and Pharmaceutical Sciences, P.le Europa 1-Via Giorgieri 1, University of Trieste, 34127 Trieste, Italy*.
[¥]*Department of Life Sciences, Via Valerio 28/1, University of Trieste, 34127 Trieste, Italy*.
[‡]*Institute of Pharmaceutical and Medicinal Chemistry, Corrensstraße 48, 48149 Münster, Germany*.
[*]Corresponding author: dzampieri@units.it

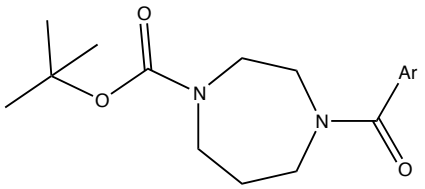
Table of contents

1. Chemistry

1.1 General remarks

Commercially available chemicals were of reagents grade and used as received. Dry column vacuum chromatography (DCVC) was performed using Silica Gel 40 (230-400 mesh, Sigma-Aldrich-Merck) and Celite 545 was used for filtration. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. Infrared spectra were recorded on a Jasco 4700 spectrophotometer in nujol mulls. Nuclear magnetic resonance spectra were determined on a Varian 400 MHz or 500 MHz (400 and 500 MHz for ^{1}H-NMR whereas 101 and 126 MHz for ^{13}C-NMR). Chemical shifts are reported as δ (ppm) in $CDCl_3$ solution (0.05% TMS) related to tetramethylsilane employed as the internal standard ($CDCl_3$, δ = 7.26 ppm for ^{1}H-NMR and δ = 77.2 for ^{13}C-NMR); 10 μL of D_2O was added to assign NH protons. Coupling constants (*J*) are reported in Hz and the splitting abbreviations used are: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; dt, doublet of triplets; td, triplet of doublets; q, quartet; m, multiplet; br, broad. Microanalyses (C, H, N) were carried out with Elementar Vario ELIII apparatus and were in agreement with theoretical values ± 0.4%. ESI-MS spectra (LRMS) were obtained on a Bruker Daltonics Esquire 4000 spectrometer by infusion of a solution of the sample in MeOH (HPLC grade).

1.2 Synthetic procedure



4a-g Ar = 2-furyl, 2-thiophenyl, benzofuran-2-yl, quinolin-2-yl, pyridin-4-yl, 2-naphtyl, phenyl.

1.2.1 General procedure for the synthesis of compounds 4a-g

500 mg (2.50 mmol) of *tert*-butyl 1,4-diazepane-1-carboxylate and an equimolar amount of Et_3N were dissolved in 25 mL of CH_2Cl_2 solution and the mixture was maintained, under stirring, at 0°C on an ice-bath. An equimolar amount of the appropriate aroyl chloride (2.50 mmol) was added and the reaction was monitored by TLC (CH_2Cl_2/EtOH 95:5) until it was completed. 2N KOH solution was added (until pH = 8), and the organic phase was washed with distilled water (3 x 50 mL). The collected organic phase was dried over Na_2SO_4, filtered and evaporated under *vacuum* to afford an oily residue which was used without further purification.

***tert*-butyl 4-(furan-2-carbonyl)-1,4-diazepane-1-carboxylate (4a)**

Yellow oil; Yield: 91%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.45 (s, 9H, $C(CH_3)_3$), 1.95 (m, 2H, diazep.), 3.38-3.88 (m br, 8H, diazep.), 6.47 (m, 1H, fur.), 7.04 (m, 1H, fur.), 7.47 (m, 1H, fur.). LRMS (ESI^+) for $C_{15}H_{22}N_2O_4$: required *m/z* 295.2, found *m/z* 295.2 $[M+H]^+$.

***tert*-butyl 4-(thiophene-2-carbonyl)-1,4-diazepane-1-carboxylate (4b)**
Yellow oil; Yield: 98%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.42 (s, 9H, $C(CH_3)_3$), 1.93 (m br, 2H, diazep.), 3.42 (t, 1H, diazep. J = 5.6 Hz), 3.50 (m, 1H, diazep.), 3.66 (m, 1H, diazep.), 3.70 (t, 2H, diazep. J = 6.0), 3.79 (m, 2H, diazep.), 7.03 (t, 1H, thioph. J = 4.4 Hz), 7.33 (br 1H, thioph.), 7.43 (br, 1H, thioph.). LRMS (ESI^+) for $C_{15}H_{22}N_2O_3S$: required *m/z* 311.1, found *m/z* 311.2 $[M+H]^+$.

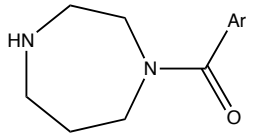
***tert*-butyl 4-(benzofuran-2-carbonyl)-1,4-diazepane-1-carboxylate (4c)**
Pale yellow oil; Yield: 99%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.46 (s, 9H, $C(CH_3)_3$), 2.01 (m br, 2H, diazep.), 3.43-3.93 (m br, 8H, diazep.), 7.28 (t, 1H, arom. J = 7.6 Hz), 7.34 (s, 1H, H_3-arom.), 7.39 (t, 1H, arom. J = 7.6 Hz), 7.50 (d, 1H, arom. J = 8.4 Hz), 7.65 (d, 1H, arom. J = 7.6 Hz). LRMS (ESI^+) for $C_{19}H_{24}N_2O_4$: required *m/z* 345.2, found *m/z* 345.0 $[M+H]^+$.

***tert*-Butyl 4-(quinoline-2-carbonyl)-1,4-diazepane-1-carboxylate (4d)**
Light-violet oil; Yield: 96%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.49 (m, 9H, $C(CH_3)_3$), 1.92 (m, 1H, diazep.), 2.01 (m, 1H, diazep.), 3.55 (m, 2H, diazep.), 3.65 (m, 4H, diazep.), 3.77 (t, 1H, diazep. J = 6.0 Hz), 3.88 (m, 1H, diazep.), 7.60 (m, 1H, arom.), 7.75 (m, 2H, arom.), 7.85 (d, 1H, arom. J = 8.0 Hz), 8.06 (d, 1H, arom. J = 8.4 Hz), 8.25 (d, 1H, arom. J = 8.4 Hz). LRMS (ESI^+) for $C_{20}H_{25}N_3O_3$: required *m/z* 356.2, found *m/z* 356.2 $[M+H]^+$.

***tert*-Butyl 4-isonicotinoyl-1,4-diazepane-1-carboxylate (4e)**
Pale yellow oil; Yield: 98%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.45 (m, 9H, $C(CH_3)_3$), 1.93 (m, 1H, diazep.), 2.87 (m, 1H, diazep.), 3.41 (m, 3H, diazep.), 3.60 (t, 1H, diazep. J = 6.0 Hz), 3.68 (t, 1H, diazep. J = 5.6 Hz), 3.78 (t, 1H, diazep. J = 5.6 Hz), 7.60 (m, 1H, arom.), 7.24 (dd, 2H, arom. pyr. J = 2.0, 8.8 Hz), 8.66 (d, 2H, arom. pyr. J = 1.6, 8.0 Hz). LRMS (ESI^+) for $C_{15}H_{22}N_2O_4$: required *m/z* 306.2, found *m/z* 306.3 $[M+H]^+$.

***tert*-Butyl 4-(1-naphthoyl)-1,4-diazepane-1-carboxylate (4f)**
Yellow oil; Yield: 96%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.48 (m, 9H, $C(CH_3)_3$), 2.11 (m, 2H, diazep.), 3.26 (m, 3H, diazep), 3.52 (m, 3H, diazep.), 3.71 (m, 1H, diazep.), 4.12 (m, 1H, diazep.), 7.40 (dd, 1H, arom. J = 1.6, 7.2 Hz), 7.49 (m, 3H, arom.), 7.79 (m, 1H, arom.), 7.87 (m, 2H, arom.). LRMS (ESI^+) for $C_{21}H_{26}N_2O_3$: required *m/z* 355.2, found *m/z* 355.2 $[M+H]^+$.

***tert*-Butyl 4-benzoyl-1,4-diazepane-1-carboxylate (4g)**
Yellow oil; Yield: 92%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.46 (s, 9H, $C(CH_3)_3$), 1.98 (m, 2H, diazep.), 3.43 (m, 5H, diazep), 3.61-3.79 (m, 3H, diazep.), 7.38 (m, 5H, arom.). LRMS (ESI^+) for $C_{17}H_{24}N_2O_3$: required *m/z* 305.2, found *m/z* 305.1 $[M+H]^+$.



5a-g

Ar = 2-furyl, 2-thiophenyl, benzofuran-2-yl, quinolin-2-yl, pyridin-4-yl, 2-naphtyl, phenyl.

1.2.2 General procedure for the synthesis of compounds 5a-g

300 mg (1.0 mmol) of *tert*-butyl 4-(furan-2-carbonyl)-1,4-diazepane-1-carboxylate **4a** and 2 mL of trifluoroacetic acid were dissolved in few mL of CH_2Cl_2 solution at rt and stirred until the reaction was completed (TLC: CH_2Cl_2/EtOH 90:10). 2N KOH solution was added (until pH = 8) and the organic layer was washed with distilled water (3 x 50 mL). The collected organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford an oily residue which was used without further purification.

(1,4-Diazepan-1-yl)(furan-2-yl)methanone (5a)

Yellow oil; Yield: 76%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.78(s, 1H, NH), 1.90 (m br, 2H, diazep.), 2.91 (t, 2H, diazep. J = 6.0 Hz), 3.04 (m br, 2H, diazep.), 3.79 (m br, 4H, diazep.), 6.46 (dd, 1H, H_4-fur. J = 1.6, 3.2 Hz), 7.04 (d, 1H, H_3-fur. J = 4.0 HZ), 7.46 (br, 1H, H_5-fur.). LRMS (ESI$^+$) for $C_{10}H_{14}N_2O_2$: required *m/z* 195.1, found *m/z* 195.0 $[M+H]^+$.

(1,4-Diazepan-1-yl)(thiophen-2-yl)methanone (5b)

Yellow oil; Yield: 65%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.90 (m br, 3H, 2H diazep. and NH), 2.94 (t, 2H, diazep. J = 6.0 Hz), 3.04 (m br, 2H, diazep.), 3.77 (m, 4H, diazep.), 7.03 (dd, 1H, H_4-thioph. J = 3.6, 5.2 Hz), 7.33 (d br, 1H, H_3-thioph. J = 2.8 Hz), 7.43 (dd, 1H, H_5-thioph. J = 1.2, 5.2 Hz). LRMS (ESI$^+$) for $C_{10}H_{14}N_2OS$: required *m/z* 211.1, found *m/z* 211.1 $[M+H]^+$.

Benzofuran-2-yl(1,4-diazepan-1-yl)methanone (5c)

Pale yellow oil; Yield: 90%; ^{1}HNMR ($CDCl_3$, δ, ppm): 2.03 (m br, 2H, diazep.), 2.79 (m br, 1H, NH), 3.01 (t, 2H, diazep. J = 5.6 Hz), 3.15 (m br, 2H, diazep.), 3.87 (m, 4H, diazep.), 7.28 (m, 1H, arom.), 7.33 (d, 1H, arom. J = 0.8 Hz), 7.39 (td, 1H, arom. J = 1.2, 7.2 Hz), 7.50 (d, 1H, arom. J = 8.4 Hz), 7.64 (d, 1H, arom. J = 8 Hz). LRMS (ESI$^+$) for $C_{14}H_{16}N_2O_2$: required *m/z* 245.1, found *m/z* 245.0 $[M+H]^+$.

(1,4-Diazepan-1-yl)(quinolin-2-yl)methanone (5d)

Pale yellow oil; Yield: 91%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.88 (q, 1H, diazep. J = 6.0 Hz), 2.01 (q, 1H, diazep. J = 6.0 Hz), 2.16 (m br, 1H NH), 3.05 (m, 3H, diazep.), 3.14 (m, 1H, diazep.), 3.68 (m, 2H, diazep.), 3.88 (m, 2H, diazep.), 7.59 (m, 1H, arom.), 7.72 (m, 2H, arom.), 7.84 (dt, 1H, arom. J = 1.6, 8.0 Hz), 8.08 (dd, 1H, arom. J = 4.0, 7.6 Hz), 8.25 (d, 1H, arom. J = 7.6 Hz). LRMS (ESI$^+$) for $C_{15}H_{17}N_3O$: required *m/z* 256.1, found *m/z* 256.1 $[M+H]^+$.

(1,4-Diazepan-1-yl)(pyridin-4-yl)methanone (5e)

Pale yellow oil; Yield: 74%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.63 (q, 1H, diazep. J = 6.0 Hz), 1.86 (m, 2H, 1H diazep. and NH), 2.85 (m, 3H NH), 3.00 (m, 1H, diazep.), 3.32 (m, 2H, diazep.), 3.71 (m, 2H, diazep.),

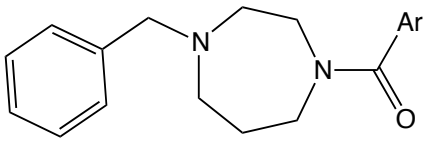
7.59 (m, 1H, arom.), 7.22 (m, 2H, arom.), 8.61 (m, 1H, arom.). LRMS (ESI^+) for $C_{11}H_{15}N_3O$: required *m/z* 206.1, found *m/z* 206.1 $[M+H]^+$.

(1,4-Diazepan-1-yl)(naphthalen-1-yl)methanone (5f)

Yellow oil; Yield: 86%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.55 (q, 1H, diazep. J = 6.0 Hz), 1.78 (s br, 1H NH), 2.02 (m, 1H, diazep.), 2.71 (t, 1H diazep. J = 5.6 Hz), 2.89 (t, 1H, diazep. J = 5.6 Hz), 3.00 (m, 1H, diazep.), 3.15 (td, 1H, diazep. J = 0.8, 4.8 Hz), 3.27 (m, 2H, diazep.), 3.83 (m, 1H, diazep.), 4.04 (m, 1H, diazep.), 7.41 (m, 1H, arom.), 7.45-7.54 (m, 3H, arom.), 7.85 (m, 3H, arom.). LRMS (ESI^+) for $C_{16}H_{18}N_2O$: required *m/z* 255.1, found *m/z* 255.0 $[M+H]^+$.

(1,4-Diazepan-1-yl)(phenyl)methanone (5g)

Pale yellow oil; Yield: 89%; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.68 (q, 1H, diazep. J = 6.0 Hz), 1.72 (s br, 1H NH), 1.92 (q, 1H, diazep. J = 6.0 Hz), 2.82 (t, 1H diazep. J = 5.6 Hz), 2.90 (t, 1H diazep. J = 5.6 Hz), 2.94 (t, 1H diazep. J = 6.0 Hz), 3.06 (t, 1H diazep. J = 5.6 Hz), 3.44 (m, 2H, diazep.), 3.77 (m, 2H, diazep.), 7.37 (br, 5H, arom.). LRMS (ESI^+) for $C_{12}H_{16}N_2O$: required *m/z* 205.1, found *m/z* 205.1 $[M+H]^+$.



2a-g Ar = 2-furyl, 2-thiophenyl, benzofuran-2-yl, quinolin-2-yl, pyridin-4-yl, 2-naphtyl, phenyl.

1.2.3 General synthesis of final compounds 2a-g

A solution of 1-benzyl-1,4-diazepane (200 mg, 1.05 mmol) and Et_3N (110 mg, 1.26 mmol) in 25 mL of CH_2Cl_2 was stirred on an ice-bath at 0°C. The appropriate aroyl chloride (1.05 mmol) was then added dropwise and the reaction was controlled by TLC (CH_2Cl_2/EtOH 9:1). At the end of the reaction the mixture was washed with aqueous 2N KOH solution (1 x 25 mL), water (1 x 25 mL) and brine (1 x 25 mL). The collected organic layer was dried (Na_2SO_4), filtered and concentrated under *vacuum* to afford a chromatographically pure oily residue.

(4-Benzyl-1,4-diazepan-1-yl)(furan-2-yl)methanone (2a)

Pale orange oil; yield: 87 %; I.R. (nujol, cm^{-1}): 1621; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.92 (m, 2H, diazep.), 2.64 (m, 2H, diazep.), 2.75 (m, 2H, diazep.), 3.60 (s, 1H, Ph-C*H*H), 3.63 (s, 1H, Ph-CH*H*), 3.64-3.74 (m, 4H, diazep.), 6.70 (dd, 1H, fur. J = 2.0, 7.2 Hz), 7.20-7.30 (m, 6H, arom.), 8.25 (dd, 1H, fur. J = 2.0, 6.4 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 26.87, 28.57, 45.86, 46.19, 54.13, 55.05, 56.65, 62.22, 111.16, 116.05, 127.15, 128.29, 128.78, 128.91, 138.29, 138.59, 143.67, 143.80, 143.87, 148.24, 160.19. LRMS (ESI^+): required *m/z* 285.2, found *m/z* 285.0 $[M+H]^+$; elemental analysis calcd (%) for $C_{17}H_{20}N_2O_2$: C 71.81, H 7.09, N 9.85; found: C 71.95, H 7.30, N 9.92.

(4-Benzyl-1,4-diazepan-1-yl)(thiophen-2-yl)methanone (2b)

Pale yellow oil; yield: 92 %; I.R. (nujol, cm^{-1}): 1609; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.91 (m, 2H, diazep.), 2.73 (m, 4H, diazep.), 3.60 (s, 2H, Ph-CH_2), 3.74 (m, 4H, diazep.), 6.99 (t br, 1H, thioph. J = 4.4 Hz), 7.20-7.30 (m, 6H, Ph e thioph.), 7.38 (d, 1H, thioph. J = 5.2 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 27.17, 29.08, 46.09, 46.66, 48.84, 49.74, 54.19, 55.03, 55.75, 56.28, 62.33, 126.70, 127.08, 128.30, 128.72, 128.76, 138.20, 138.93, 164.31. LRMS (ESI^+): required *m/z* 301.1, found *m/z* 301.1 $[M+H]^+$; elemental analysis calcd (%) for $C_{17}H_{20}N_2OS$: C 67.97, H 6.71, N 9.32; found: C 68.22, H 6.79, N 9.44.

Benzofuran-2-yl(4-benzyl-1,4-diazepan-1-yl)methanone (2c)

Pale yellow oil; yield: 91 %; I.R. (nujol, cm^{-1}): 1633; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.97 (m, 2H, diazep.), 2.67 (m, 2H, diazep.), 2.81 (m, 2H, diazep.) 3.64 (s, 2H, Ph-CH_2), 3.69 (m, 2H, diazep.), 3.87 (m, 2H, diazep.), 7.24-7.38 (m, 8H, arom.), 7.50 (t, 1H, arom. J = 8.8 Hz), 7.63 (dd, 1H, arom. J = 2.8, 8.0 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 27.01, 29.04, 46.17, 46.87, 47.99, 48.90, 54.30, 54.92, 55.28, 56.68, 62.39, 111.51, 111.85, 122.20, 123.51, 126.28, 126.31, 127.03, 127.09, 128.31, 128.72, 128.75, 128.80, 139.00, 149.55, 154.61, 160.94. LRMS (ESI^+): required *m/z* 335.2, found *m/z* 335.0 $[M+H]^+$; elemental analysis calcd (%) for $C_{21}H_{22}N_2O_2$: C 75.42, H 6.63, N 8.38; found: C 75.40, H 6.80, N 8.45.

(4-Benzyl-1,4-diazepan-1-yl)(quinolin-2-yl)methanone (2d)

Reddish oil; yield: 83 %; I.R. (nujol, cm^{-1}): 1630; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.81 (q, 1H, diazep. J = 6.0 Hz), 1.97 (q, 1H, diazep. J = 6.0 Hz), 2.65 (m, 3H, diazep.), 2.82 (m, 1H, diazep.), 3.58 (s, 1H, Ph-C*H*H), 3.61 (m, 2H, diazep.), 3.64 (s, 1H, Ph-CH*H*), 3.84 (m, 2H, diazep.), 7.14-7.33 (m, 5H, arom.), 7.50 (m, 1H, arom.), 7.65 (m, 2H, arom.), 7.75 (dd, 1H, arom. J = 5.2, 8.8 Hz), 8.04 (t, 1H, arom. J = 8.0 Hz), 8.16 (dd, 1H, arom. J = 6.0, 9.2 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 26.92, 28.87, 45.79, 46.62, 48.43, 49.40, 54.20, 54.70, 55.31, 56.49, 62.28, 62.31, 120.36, 120.48, 127.00, 127.02, 127.38, 127.63, 127.65, 127.84, 127.88, 128.15, 128.23, 128.27, 128.68, 128.71, 128.78, 129.60, 129.64129.96, 137.04, 139.03, 139.10, 146.58, 146.63, 154.57, 169.00, 169.07. LRMS (ESI^+): required *m/z* 346.2, found *m/z* 346.2 $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{23}N_3O$: C 76.49, H 6.71, N 12.16; found: C 76.39, H 6.55, N 11.84.

(4-Benzyl-1,4-diazepan-1-yl)(pyridin-4-yl)methanone (2e)

Yellow oil; yield: 74 %; I.R. (nujol, cm^{-1}): 1632; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.76 (q, 1H, diazep. J = 6.0 Hz), 1.96 (q, 1H, diazep. J = 6.0 Hz), 2.56 (t, 1H, diazep. J = 5.2 Hz), 2.62 (t, 1H, diazep. J = 5.6 Hz), 2.72 (t, 1H, diazep. J = 5.6 Hz), 2.79 (t, 1H, diazep. J = 5.2 Hz), 3.38 (m, 2H, diazep.), 3.61 (s, 1H, Ph-C*H*H), 3.66 (s, 1H, Ph-CH*H*), 3.77 (m, 2H, diazep.), 7.24-7.33 (m, 7H, arom.), 8.66 (dd, 2H, arom. J = 6.0, 12.4 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 27.01, 28.96, 45.40, 46.20, 48.54, 49.67, 54.07, 54.65, 55.95, 56.00, 62.61, 120.89, 121.00, 126.82, 127.15, 127.22, 128.16, 128.34, 128.68, 128.75, 128.77, 138.73, 138.82, 144.49, 150.20, 150.23, 168.97. LRMS (ESI^+): required *m/z* 296.2, found *m/z* 296.1 $[M+H]^+$; elemental analysis calcd (%) for $C_{18}H_{21}N_3O$: C 73.19, H 7.17, N 14.23; found: C 73.40, H 7.33, N 14.45.

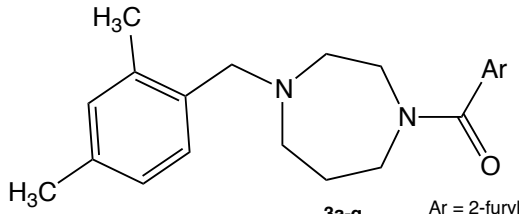
(4-Benzyl-1,4-diazepan-1-yl)(naphthalen-1-yl)methanone (2f)

Yellow oil; yield: 80 %; I.R. (nujol, cm^{-1}): 1630; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.66 (m br, 1H, diazep.), 2.06 (q, 1H, diazep. J = 4.8 Hz), 2.47 (m, 1H, diazep.), 2.62 (t, 1H, diazep. J = 4.4 Hz), 2.73-2.83 (m, 1H, diazep.), 2.92 (t, 1H, diazep. J = 4.4 Hz), 3.29 (m, 2H, diazep.), 3.59 (s, 1H, Ph-C*H*H), 3.71 (s, 1H, Ph-CH*H*), 3.97 (m, 2H, diazep.), 7.20-7.55 (m, 9H, arom.), 7.88 (m, 3H, arom.).

^{13}CNMR ($CDCl_3$, δ, ppm): 27.45, 28.80, 44.95, 45.59, 48.47, 48.60, 53.96, 55.30, 55.97, 56.06, 62.45, 62.63, 123.47, 123.64, 125.00, 06, 125.13, 125.15, 126.38, 126.90, 127.03, 127.09, 128.22, 128.31, 128.33, 128.35, 128.61, 128.83, 128.84, 129.52, 133.49, 133.51, 135.15, 135.18, 138.93, 138.95, 170.70, 170.80. LRMS (ESI^+): required *m/z* 345.2, found *m/z* 345.3 $[M+H]^+$; elemental analysis calcd (%) for $C_{23}H_{24}N_2O$: C 80.20, H 7.02, N 8.13; found: C 80.55, H 7.30, N 8.25.

(4-Benzyl-1,4-diazepan-1-yl)(phenyl)methanone (2g)

Yellow oil; yield: 97 %; I.R. (nujol, cm^{-1}): 1629; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.79 (m br 1H, diazep.), 1.99 (q, 1H, diazep. J = 4.8 Hz), 2.59 (t, 1H, diazep. J = 4.0 Hz), 2.63 (t, 1H, diazep. J = 4.8 Hz), 2.74 (t, 1H, diazep. J = 4.8 Hz), 2.82 (t, 1H, diazep. J = 4.0 Hz), 3.46 (t, 2H, diazep. J = 4.8 Hz), 3.62 (s, 1H, Ph-C*H*H), 3.68 (s, 1H, Ph-CH*H*), 3.80 (t, 2H, diazep. J = 4.8 Hz), 7.20-7.40 (m, 10H, arom.).
^{13}CNMR ($CDCl_3$, δ, ppm): 27.04, 28.93, 45.47, 48.78, 49.86, 54.05, 54.95, 55.95, 56.21, 62.36, 62.56, 126.61, 126.66, 127.10, 128.28, 128.31, 128.39, 128.70, 128.84, 128.88, 129.17, 129.25, 130.56, 134.53, 137.06, 171.61. LRMS (ESI^+): required *m/z* 295.2, found *m/z* 295.0 $[M+H]^+$; elemental analysis calcd (%) for $C_{19}H_{22}N_2O$: C 77.52, H 7.53, N 9.52; found: C 77.13, H 7.40, N 9.21.



3a-g Ar = 2-furyl, 2-thiophenyl, benzofuran-2-yl, quinolin-2-yl, pyridin-4-yl, 2-naphtyl, phenyl.

1.2.4 General synthesis of final compounds 3a-g

A solution of (1,4-diazepan-1-yl)(furan-2-yl)methanone **5a** (150 mg, 0.77 mmol), 2,4-dimetylbenzaldehyde (100 mg, 0.77 mmol) in 20 mL of CH_2Cl_2 was stirred for 2h at rt. A slight excess of $NaCNBH_3$ (70 mg, 1.16 mmol) was added and the mixture was left to stir overnight. The organic solution was then extracted with distilled water (2 x 25 mL) and brine (1 x 25 mL). The collected organic layer was dried (Na_2SO_4), filtered and concentrated under *vacuum* and the residue was purified by DCVC.

(4-(2,4-Dimethylbenzyl)-1,4-diazepan-1-yl)(furan-2-yl)methanone (3a)

Pale yellow oil; yield: 35 % (DCVC, CH_2Cl_2 100 then CH_2Cl_2/EtOH 95:5); I.R. (nujol, cm^{-1}): 1607; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.93 (q, 2H, diazep. J = 5.6, 6.0 Hz), 2.29 (s, 3H, CH_3.), 2.31 (s, 3H, CH_3.), 2.64 (m br 2H, diazep.), 2.76 (t, 2H, diazep. J = 4.8 Hz), 3.54 (s, 2H, Ph-CH_2), 3.72 (t, 2H, diazep. J = 6.0 Hz), 3.81 (m, 2H, diazep.), 6.46 (br, 1H, fur.), 6.97 (m, 3H, arom. Ph), 7.13 (1H, fur. J = 6.0 Hz), 7.45 (d br, 1H, fur. J = 13.6 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 19.13, 20.97, 27.03, 28.45, 28.92, 46.01, 46.16, 47.85, 48.59, 54.10, 54.20, 56.60, 60.08, 63.31, 111.13, 116.03, 126.12, 126.54, 127.82, 129.71, 131.13, 131.18, 133.84, 135.63, 137.34, 143.60, 143.72, 148.37, 160.27. LRMS (ESI^+): required *m/z* 313.2, found *m/z* 313.2 $[M+H]^+$; elemental analysis calcd (%) for $C_{19}H_{24}N_2O_2$: C 73.05, H 7.74, N 8.97; found: C 72.88, H 7.55, N 8.76.

(4-(2,4-Dimethylbenzyl)-1,4-diazepan-1-yl)(thiophen-2-yl)methanone (3b)
Pale yellow oil; yield: 37 % (DCVC, CH_2Cl_2 100 then CH_2Cl_2/EtOH 95:5); I.R. (nujol, cm^{-1}): 1606; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.92 (m br 2H, diazep.), 2.30 (s, 3H, CH_3.), 2.32 (s, 3H, CH_3.), 2.72 (br 4H, diazep.), 3.54 (s, 2H, Ph-CH_2), 3.75 (m, 4H, diazep.), 6.97 (m, 3H, arom. Ph), 7.12 (d br, 1H, thioph. J = 7.2 Hz), 7.30 (br, 1H, thioph.), 7.42 (d, 1H, fur. J = 5.2 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 19.15, 20.98, 25.59, 27.17, 29.11, 29.69, 46.22, 46.72, 49.00, 49.08, 53.99, 55.75, 60.13, 126.13, 126.63, 128.53, 128.66, 128.70, 129.74, 131.21, 133.82, 136.67, 137.36, 164.39. LRMS (ESI^+): required *m/z* 329.2, found *m/z* 329.1 $[M+H]^+$; elemental analysis calcd (%) for $C_{19}H_{24}N_2OS$: C 69.47, H 7.36, N 8.53; found: C 69.70, H 7.65, N 8.23.

Benzofuran-2-yl(4-(2,4-dimethylbenzyl)-1,4-diazepan-1-yl)methanone (3c)
Pale yellow oil; yield: 34 % (DCVC, CH_2Cl_2 100 then CH_2Cl_2/EtOH 95:5); I.R. (nujol, cm^{-1}): 1630; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.97 (q br, 2H, diazep. J = 5.2, 6.0 Hz), 2.30 (s, 3H, CH_3.), 2.33 (s, 3H, CH_3.), 2.67 (m, 2H, diazep.), 2.80 (t, 2H, diazep. J = 5.2 Hz), 3.56 (s, 2H, Ph-CH_2), 3.84 (m, 4H, diazep.), 6.95 (m, 2H, arom.), 7.14 (t, 1H, arom. J = 8.0 Hz), 7.28 (d, 2H, arom. J = 7.6 Hz), 7.38 (m, 1H, arom.), 7.50 (dd, 1H, arom. J = 4.4, 12.8 Hz), 7.64 (t, 1H, arom. J = 7.2 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 19.15, 20.99, 27.01, 29.09, 46.30, 46.97, 48.10, 48.96, 54.12, 54.84, 55.28, 56.48, 60.19, 111.47, 111.82, 122.19, 122.44, 123.48, 123.83, 126.13, 126.26, 127.01, 129.71, 131.22, 133.88, 136.67, 137.37, 149.46, 154.59, 154.70, 161.01. LRMS (ESI^+): required *m/z* 363.2, found *m/z* 363.2 $[M+H]^+$; elemental analysis calcd (%) for $C_{23}H_{26}N_2O_2$: C 76.21, H 7.23, N 7.73; found: C 75.90, H 7.15, N 7.50.

(4-(2,4-dimethylbenzyl)-1,4-diazepan-1-yl)(quinolin-2-yl)methanone (3d)
Pale yellow oil; yield: 36 % (DCVC, CH_2Cl_2 100 then CH_2Cl_2/EtOH 95:5); I.R. (nujol, cm^{-1}): 1626; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.83 (q, 1H, diazep. J = 6.0 Hz), 2.00 (q, 1H, diazep. J = 6.0 Hz), 2.27-2.35 (m, 6H, 2 x CH_3.), 2.68 (m, 2H, diazep.), 2.75 (t, 1H, diazep. J= 5.2 Hz), 2.85 (t, 1H, diazep. J = 5.2 Hz), 3.54 (s, 1H, Ph-C*H*H), 3.59 (s, 1H, Ph-CH*H*), 3.65 (m, 2H, diazep.), 3.85 (m, 2H, diazep.), 6.99 (m, 2H, arom.), 7.14 (dd, 1H, arom. J = 8.0, 31.2 Hz), 7.58 (m, 1H, arom.), 7.67 (dd, 1H, arom. J = 6.8, 8.4 Hz), 7.74 (m, 1H, arom.), 7.84 (m, 1H, arom.), 8.08 (td, 1H, arom. J = 1.2, 8.8 Hz), 8.23 (t, 1H, arom. J = 8.8 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 19.15, 19.18, 20.95, 20.99, 26.95, 28.94, 45.89, 46.70, 54.08, 54.73, 55.32, 56.40, 60.10, 60.16, 63.37, 120.38, 120.52, 126.08, 126.13, 126.56, 127.37, 127.60, 127.62, 127.87, 127.91, 129.66, 129.70, 129.74, 129.95, 131.17, 131.19, 136.59, 137.03, 137.33, 137.36, 137.44, 146.61, 146.67, 154.58, 169.03, 169.11. LRMS (ESI^+): required *m/z* 374.2, found *m/z* 374.3 $[M+H]^+$; elemental analysis calcd (%) for $C_{24}H_{27}N_3O$: C 77.18, H 7.29, N 11.25; found: C 77.20, H 7.29, N 11.35.

(4-(2,4-dimethylbenzyl)-1,4-diazepan-1-yl)(pyridin-4-yl)methanone (3e)
Pale yellow oil; yield: 29 % (DCVC, CH_2Cl_2 100 then CH_2Cl_2/EtOH 95:5); I.R. (nujol, cm^{-1}): 1629; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.67 (m, 1H, diazep.), 1.87 (m, 1H, diazep. J = 6.0 Hz), 2.22-2.27 (m, 6H, 2xCH_3.), 2.48 (t, 1H, diazep. J = 5.2 Hz), 2.53 (t, 1H, diazep. J = 5.6 Hz), 2.64 (t, 1H, diazep. J = 5.6 Hz), 2.71 (t, 1H, diazep. J = 5.68 Hz), 3.27 (t, 1H, diazep. J = 5.6 Hz), 3.31 (t, 1H, diazep. J = 6.0 Hz), 3.45 (s, 1H, Ph-C*H*H), 3.49 (s, 1H, Ph-CH*H*), 3.69 (m, 2H, diazep.), 6.86 (t, 1H, arom. J = 7.6 Hz), 6.90 (d, 1H, arom. J = 7.2 Hz), 7.03 (m, 1H, arom.) 7.14 (d, 1H, arom. pyr. J = 6.0 Hz), 7.20 (d, 1H, arom. pyr. J = 6.0 Hz), 8.54 (d, 1H, arom. pyr. J = 6.0 Hz), 8.61 (d, 1H, arom. pyr. J = 6.0 Hz). ^{13}CNMR ($CDCl_3$, δ, ppm): 18.11, 18.15, 19.95, 19.98, 26.05, 27.97, 28.67, 44.43, 45.17, 47.59, 48.65, 52.83, 53.57, 54.63,

54.92, 59.36, 59.40, 108.98, 119.89, 119.97, 152.14, 128.70, 128.74, 130.23, 130.26, 135.84, 136.29, 143.52, 149.13, 149.19, 167.83, 167.90. LRMS (ESI^+): required *m/z* 324.2, found *m/z* 324.2 $[M+H]^+$; elemental analysis calcd (%) for $C_{20}H_{25}N_3O$: C 74.27, H 7.79, N 12.99; found: C 74.10, H 7.92, N 12.75.

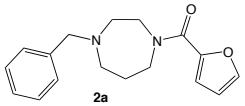
(4-(2,4-Dimethylbenzyl)-1,4-diazepan-1-yl)(naphthalen-1-yl)methanone (3f)

Pale yellow oil; yield: 40 % (DCVC, CH_2Cl_2 100 then CH_2Cl_2/EtOH 95:5); I.R. (nujol, cm^{-1}): 1626; ^{1}HNMR ($CDCl_3$, δ, ppm): 2.03 (m, 1H, diazep.), 2.25-2.36 (m, 7H, 2 x CH_3 and diazep.), 2.44 (m, 1H, diazep.), 2.58 (m, 1H, diazep.), 2.77 (m, 1H, diazep.), 2.88 (t, 1H, diazep. J = 4.4 Hz), 3.26 (t, 2H, diazep. J = 5.6 Hz), 3.48 (s, 1H, Ph-C*H*H), 3.59 (s, 1H, Ph-CH*H*), 3.95 (m, 2H, diazep.), 6.99 (m, 2H, arom.), 7.19 (dd, 1H, arom. J = 7.6, 16.8 Hz), 7.48 (m, 2H, arom.), 7.52 (m, 2H, arom.), 7.74 (m, 1H, arom.), 7.85 (m, 2H, arom.). ^{13}CNMR ($CDCl_3$, δ, ppm): 19.26, 19.35, 21.08, 21.14, 27.54, 29.00, 45.17, 45.81, 48.62, 49.82, 54.08, 54.46, 56.02, 56.16, 60.34, 60.54, 123.63, 123.80, 125.12, 125.26, 125.30, 126.20, 126.29, 126.50, 126.73, 127.05, 128.00, 128.46, 128.50, 129.63, 129.72, 129.90, 131.29, 131.34, 131.35, 133.64, 133.96, 134.12, 135.31, 136.23, 136.76, 136.79, 137.36, 137.48, 170.84. LRMS (ESI^+): required *m/z* 373.2, found *m/z* 373.1 $[M+H]^+$; elemental analysis calcd (%) for $C_{25}H_{28}N_2O$: C 80.61, H 7.58, N 7.52; found: C 80.65, H 7.65, N 7.77.

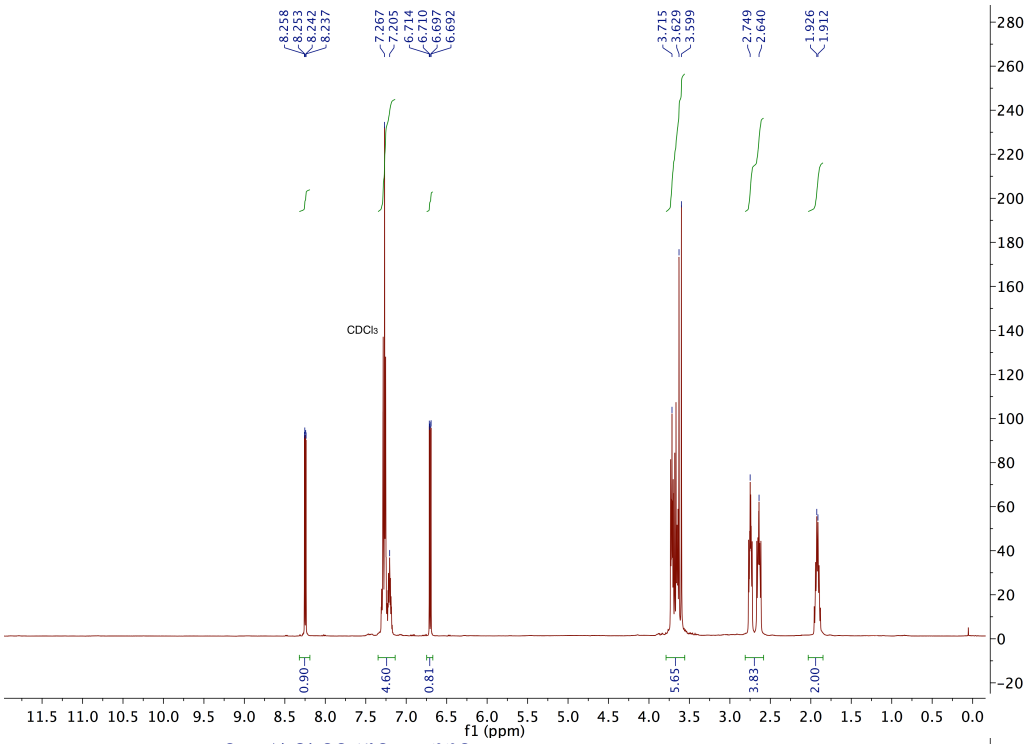
(4-(2,4-dimethylbenzyl)-1,4-diazepan-1-yl)(phenyl)methanone (3g)

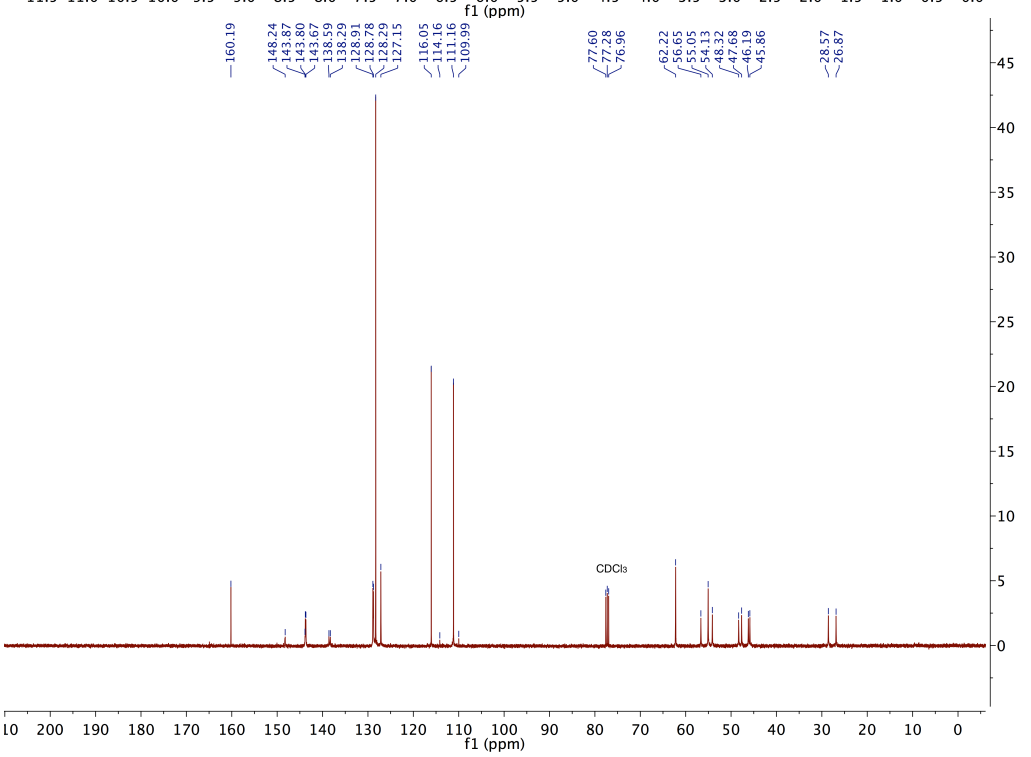
Pale yellow oil; yield: 30 % (DCVC, CH_2Cl_2 100 then CH_2Cl_2/EtOH 95:5); I.R. (nujol, cm^{-1}): 1625; ^{1}HNMR ($CDCl_3$, δ, ppm): 1.74 (m, 1H, diazep.), 1.95 (q, 1H, diazep. J = 6.0 Hz), 2.28-2.38 (m, 6H, 2xCH_3.), 2.55 (t, 1H, diazep. J = 4.8 Hz), 2.60 (m, 1H, diazep.), 2.71 (t, 1H, diazep. J = 5.6 Hz), 2.79 (m, 1H diazep.), 3.43 (m, 2H, diazep.), 3.51 (s, 1H, Ph-C*H*H), 3.56 (s, 1H, Ph-CH*H*), 3.78 (m, 2H, diazep.), 6.93 (m, 2H, arom.), 7.11 (dd, 1H, arom. J = 7.2, 32.8 Hz), 7.37 (m, 5H, arom.). ^{13}CNMR ($CDCl_3$, δ, ppm): 19.26, 19.31, 21.10, 21.13, 27.23, 29.18, 45.64, 46.32, 49.00, 50.08, 54.02, 55.03, 56.02, 56.14, 60.29, 60.48, 126.23, 126.27, 126.74, 126.79, 128.49, 128.57, 129.27, 129.36, 129.80, 129.91, 131.34, 136.81, 137.19, 137.48, 171.72. LRLRMS (ESI^+): required *m/z* 323.2, found *m/z* 323.1 $[M+H]^+$; elemental analysis calcd (%) for $C_{21}H_{26}N_2O$: C 78.22, H 8.13, N 8.69; found: C 77.95, H 8.02, N 8.35.

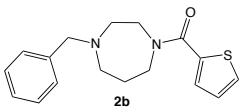
1.2.5 NMR spectral data of the final compounds 2a-g and 3a-g.



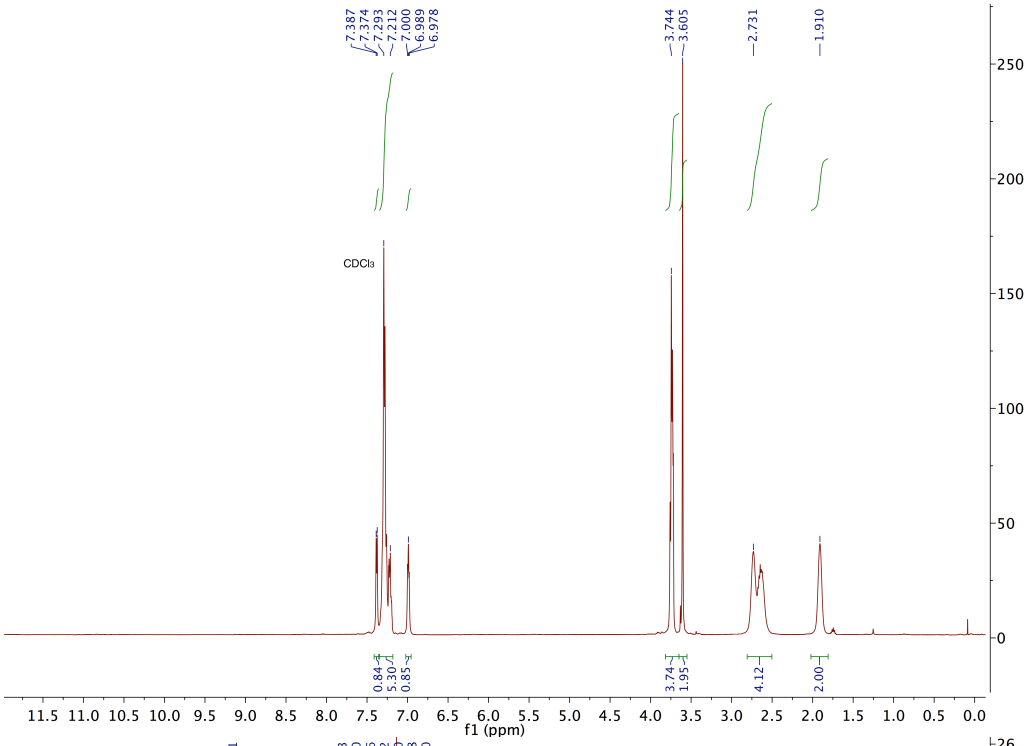
2a

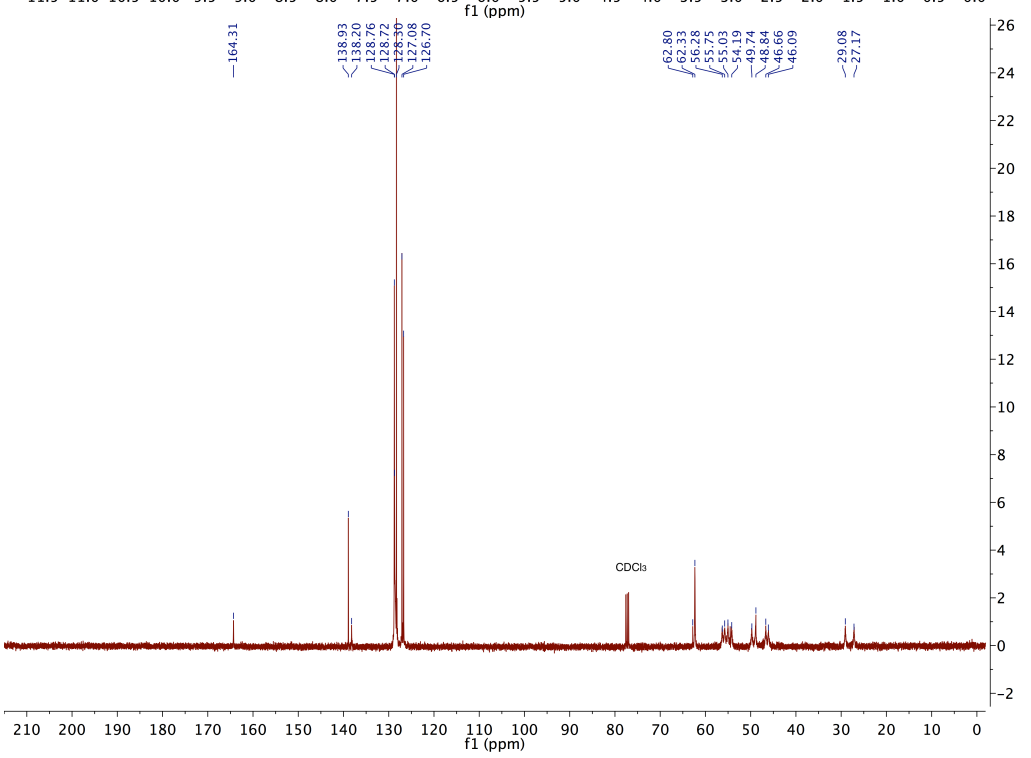


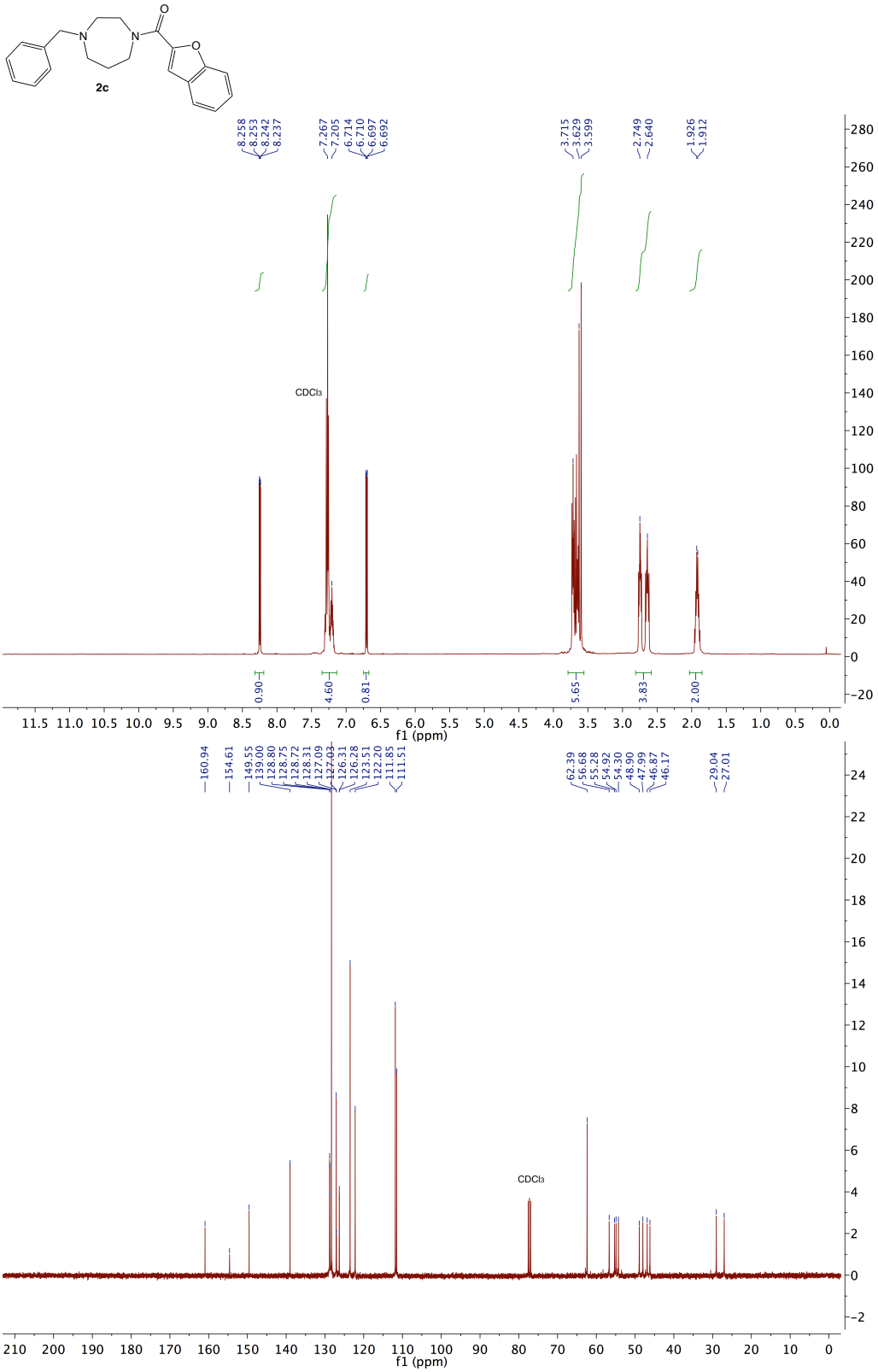


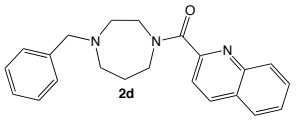

O
N
N
S

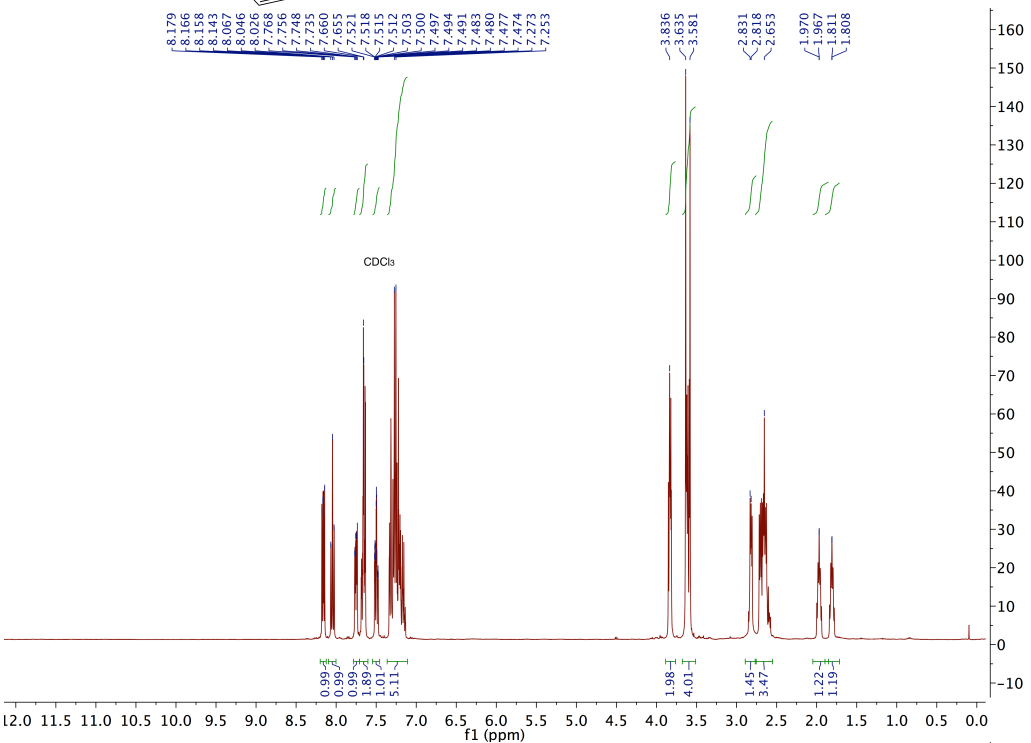
2b

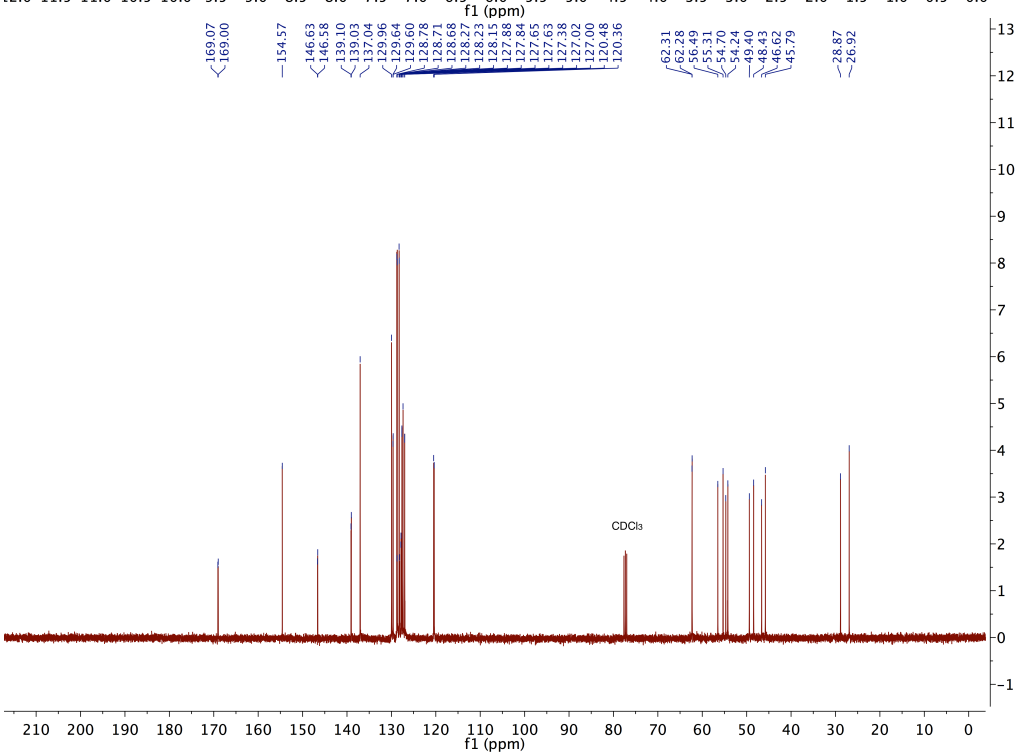

7.387
7.374
7.293
7.212
7.000
6.989
6.978
3.744
3.605
2.731
1.910
CDCl3
0.84
5.30
0.85
3.74
1.95
4.12
2.00
f1 (ppm)

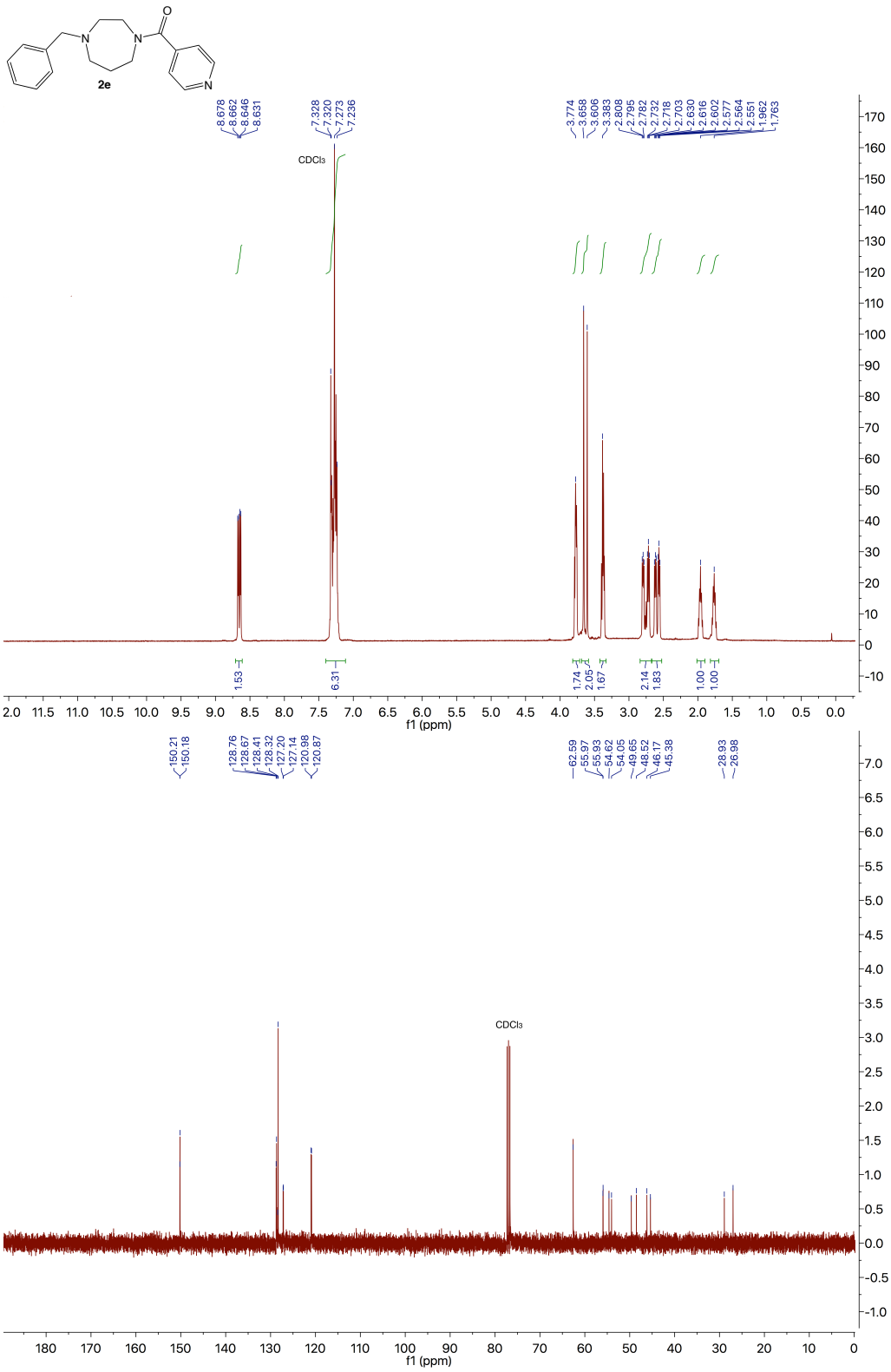

164.31
138.93
138.20
128.76
128.72
128.30
127.08
126.70
62.80
62.33
56.28
55.75
55.03
54.19
49.74
48.84
46.66
46.09
29.08
27.17
CDCl3
f1 (ppm)

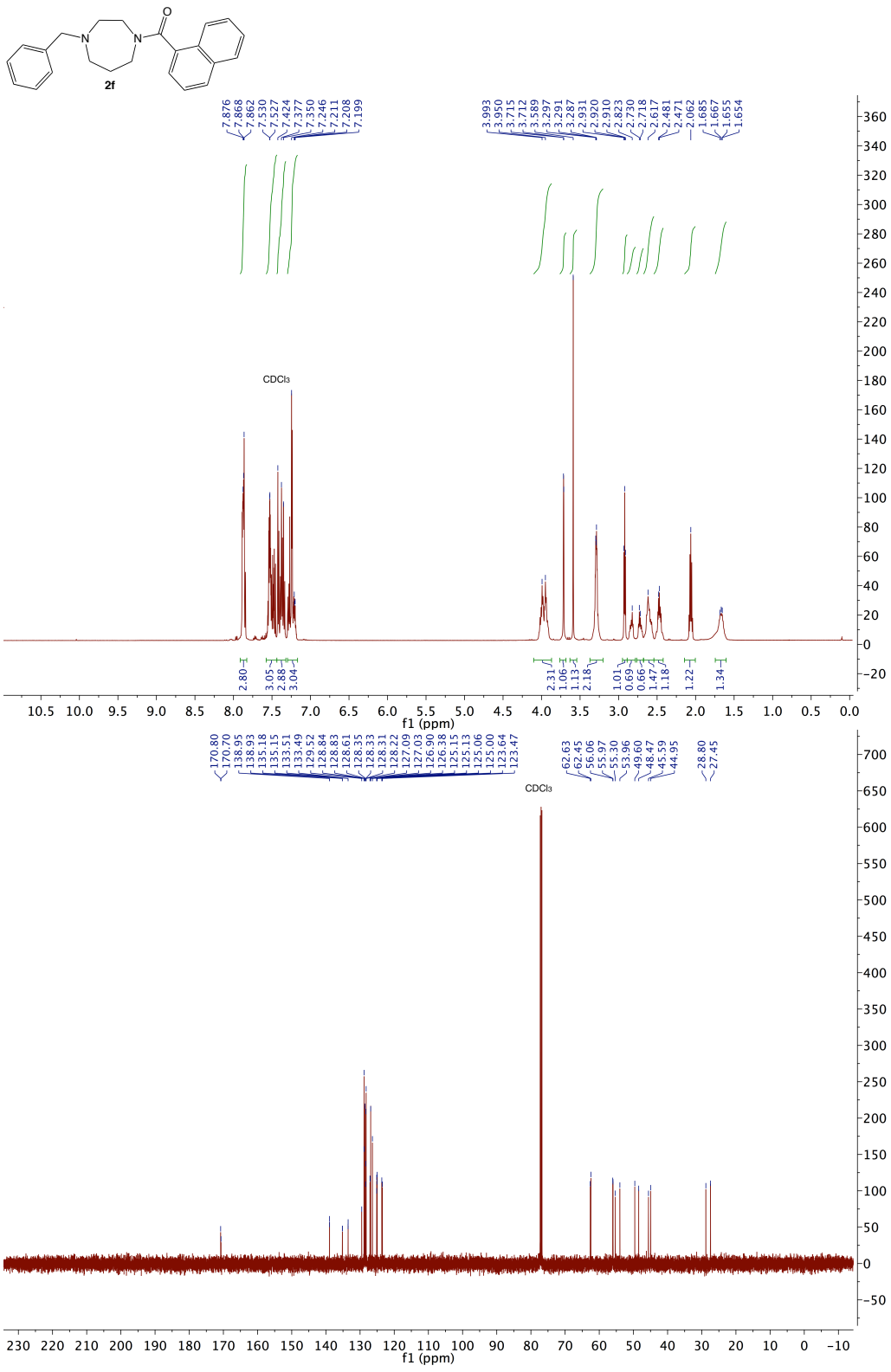

2c
8.258
8.253
8.242
8.237
7.267
7.205
6.714
6.710
6.697
6.692
3.715
3.629
3.599
2.749
2.640
1.926
1.912
CDCl3
0.90
4.60
0.81
5.65
3.83
2.00
f1 (ppm)
160.94
154.61
149.55
139.00
128.80
128.75
128.72
128.31
127.09
126.31
126.28
123.51
122.20
111.65
111.51
62.39
56.68
55.28
54.92
54.30
48.90
47.99
46.87
46.17
29.04
27.01
CDCl3
f1 (ppm)

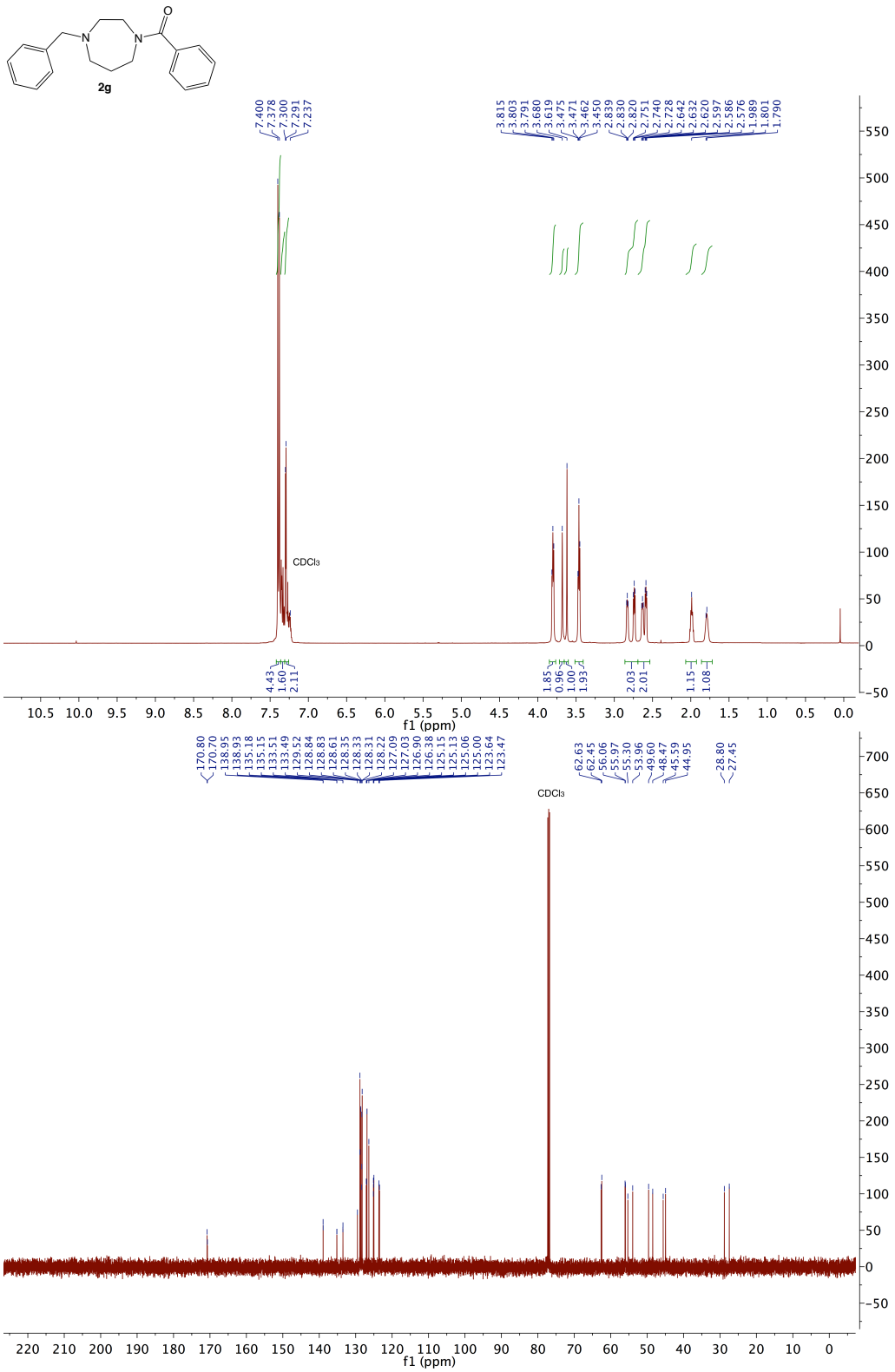

O
N
N
N
2d

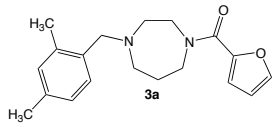

8.179
8.166
8.158
8.143
8.067
8.046
8.026
7.768
7.756
7.748
7.735
7.660
7.655
7.521
7.518
7.515
7.512
7.503
7.500
7.497
7.494
7.491
7.483
7.480
7.477
7.474
7.273
7.253
3.836
3.635
3.581
2.831
2.818
2.653
1.970
1.967
1.811
1.808
CDCl3
0.99
0.99
0.99
1.89
1.01
5.11
1.98
4.01
1.45
3.47
1.22
1.19
f1 (ppm)

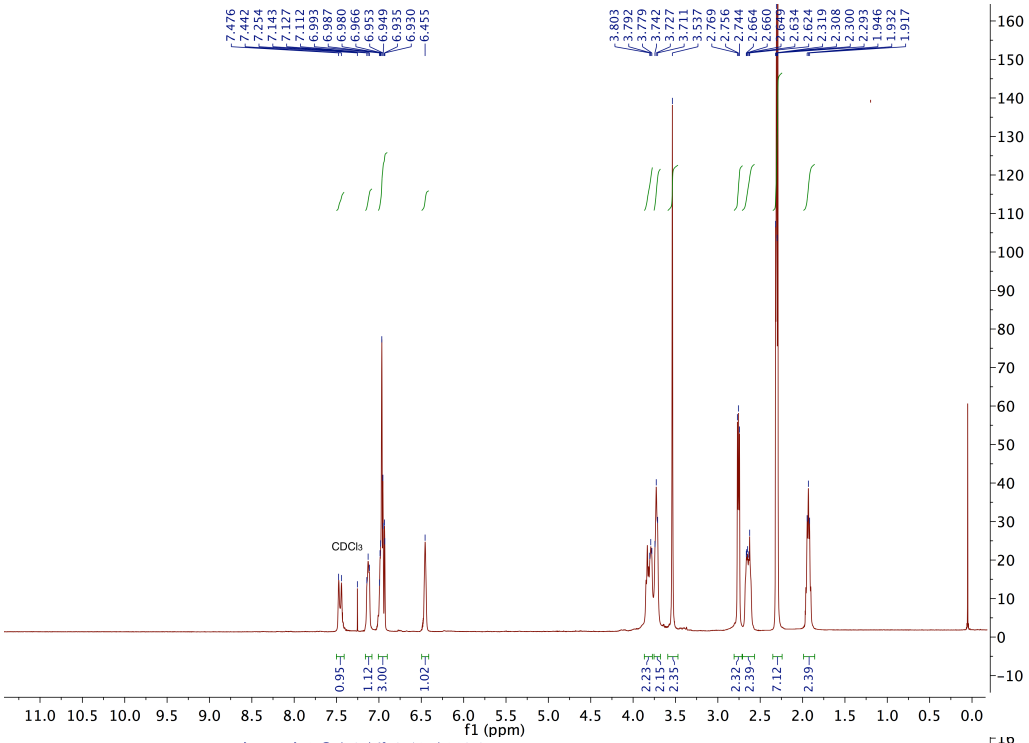

169.07
169.00
154.57
146.63
146.58
139.10
139.03
137.04
129.96
129.64
129.60
128.78
128.71
128.68
128.27
128.23
128.15
127.88
127.84
127.65
127.63
127.38
127.02
127.00
120.48
120.36
62.31
62.28
55.49
55.31
54.70
54.24
49.40
48.43
46.62
45.79
28.87
26.92
CDCl3
f1 (ppm)

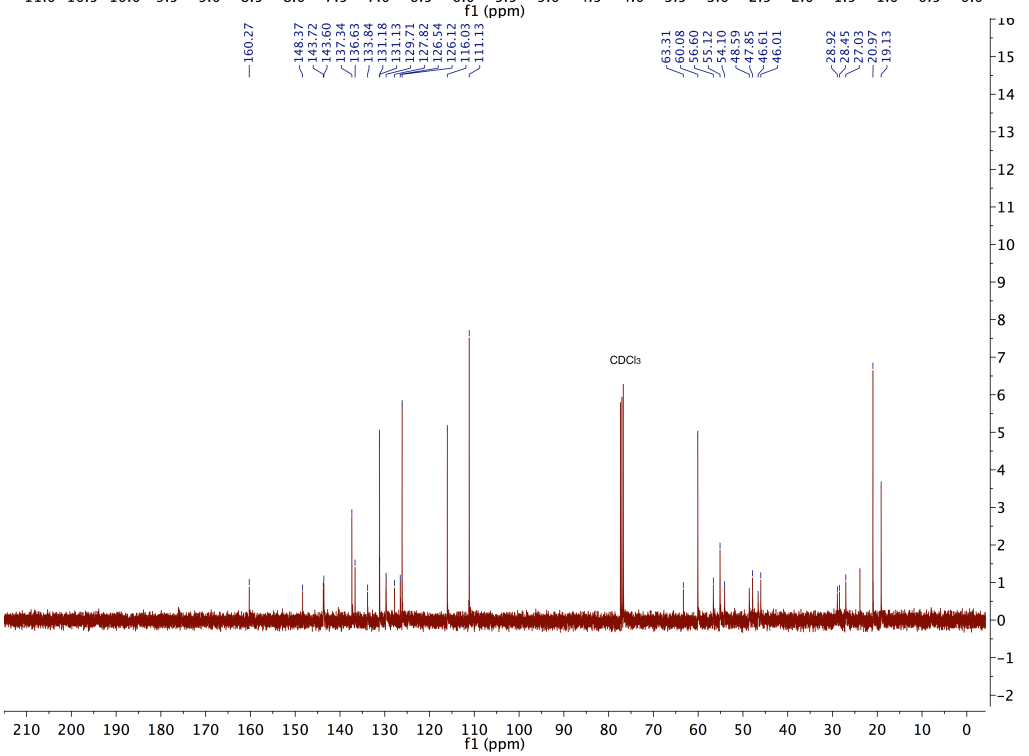

2e
8.678
8.662
8.646
8.631
7.328
7.320
7.273
7.236
CDCl3
3.774
3.658
3.606
3.383
2.808
2.795
2.782
2.732
2.718
2.703
2.630
2.616
2.602
2.577
2.564
2.551
1.962
1.763
1.53
6.31
1.74
2.05
1.67
2.14
1.83
1.00
1.00
f1 (ppm)
150.21
150.18
128.76
128.67
128.41
128.32
127.20
127.14
120.98
120.87
62.59
55.97
55.93
54.62
54.05
49.65
48.52
46.17
45.38
28.93
26.98
CDCl3
f1 (ppm)

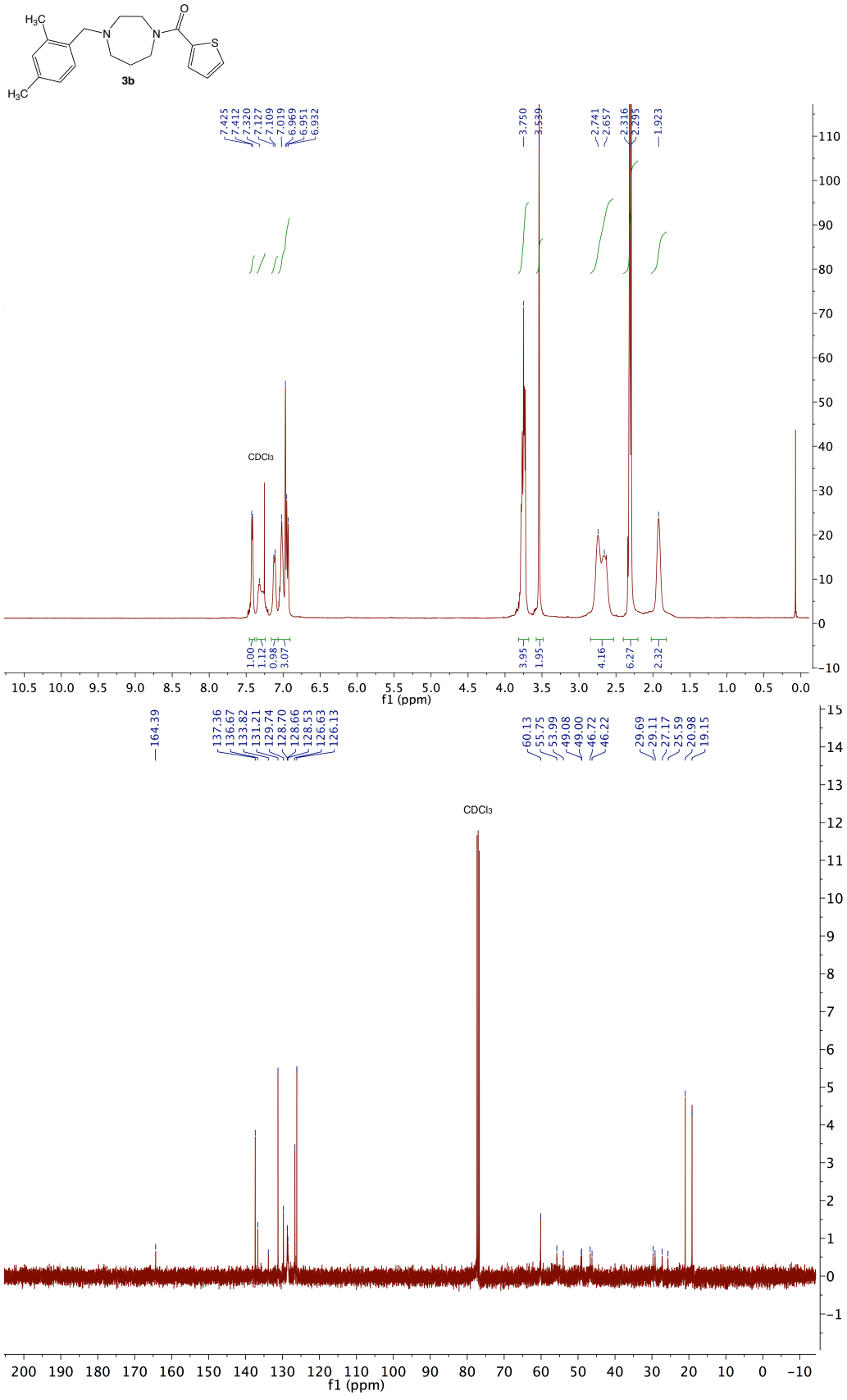

2f
CDCl3
f1 (ppm)
CDCl3
f1 (ppm)

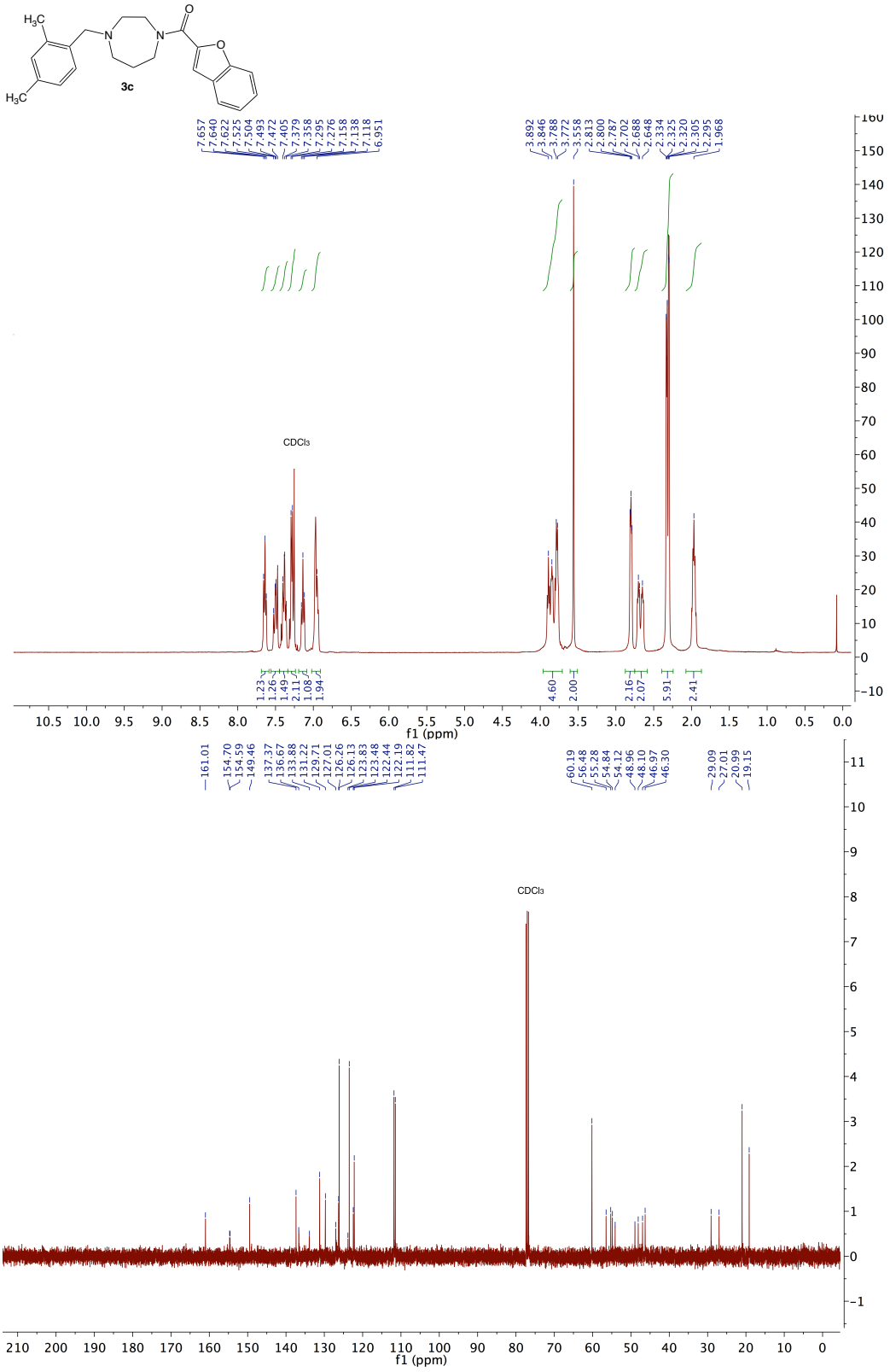

2g
7.400
7.378
7.300
7.291
7.237
3.815
3.803
3.791
3.680
3.619
3.475
3.471
3.462
3.450
2.839
2.830
2.820
2.751
2.740
2.728
2.642
2.632
2.620
2.597
2.586
2.576
1.989
1.801
1.790
CDCl3
4.43
1.60
2.11
1.85
0.96
1.00
1.93
2.03
2.01
1.15
1.08
f1 (ppm)
170.80
170.70
138.93
138.93
135.18
135.15
133.51
133.49
129.52
128.84
128.83
128.61
128.35
128.33
128.31
128.22
127.09
127.03
126.90
126.38
125.15
125.13
125.06
125.00
123.64
123.47
62.63
62.45
56.06
55.37
55.30
53.86
49.60
48.47
44.59
44.95
28.80
27.45
CDCl3
f1 (ppm)

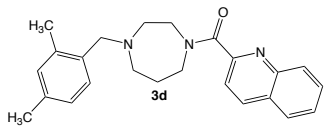

H3C
N
N
O
O
H3C
3a

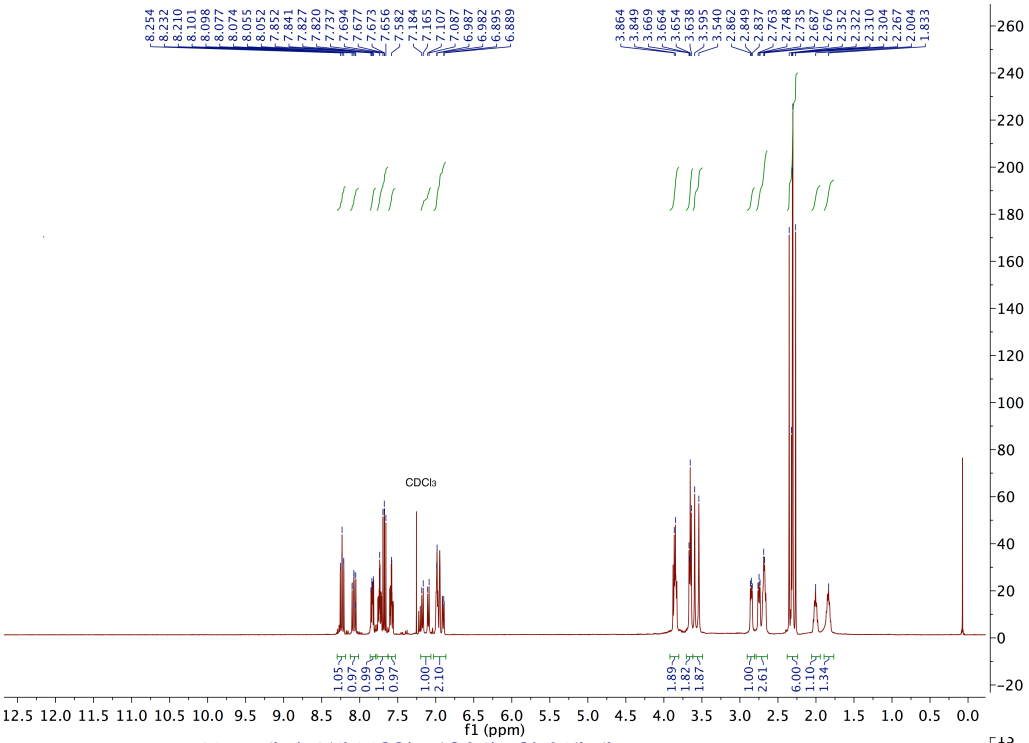

7.476
7.442
7.254
7.143
7.127
7.112
6.993
6.987
6.980
6.966
6.953
6.949
6.935
6.930
6.455
3.803
3.792
3.779
3.742
3.727
3.711
3.537
2.769
2.756
2.744
2.664
2.660
2.649
2.634
2.624
2.319
2.308
2.300
2.293
1.946
1.932
1.917
CDCl3
0.95
1.12
3.00
1.02
2.23
2.15
2.35
2.32
2.39
7.12
2.39
f1 (ppm)

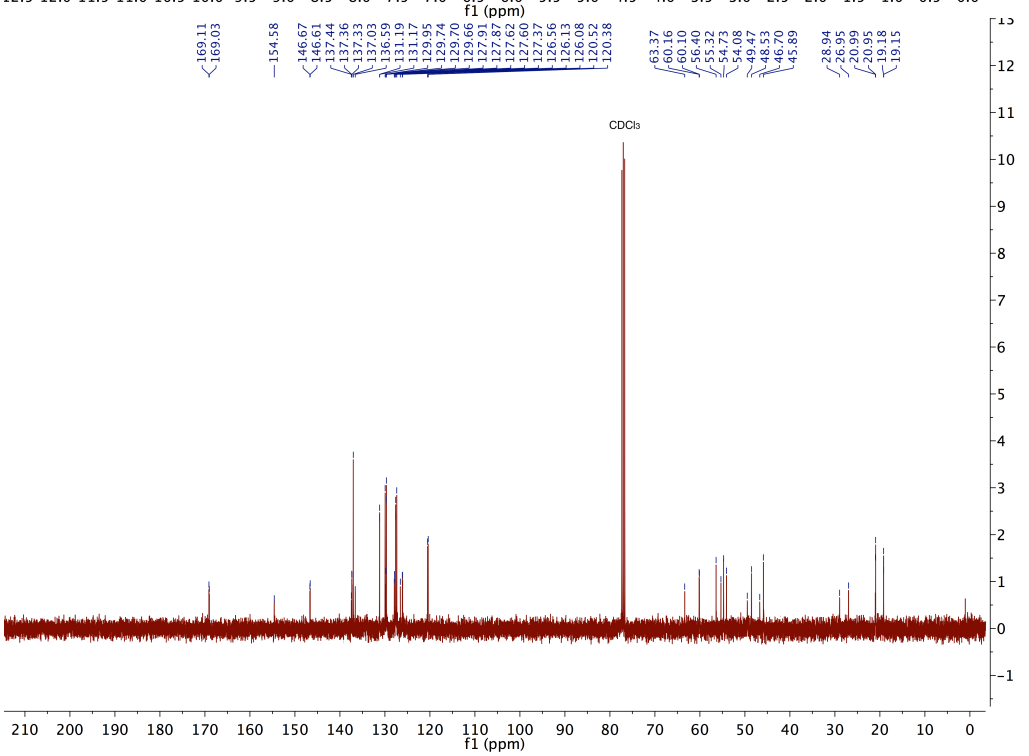

160.27
148.37
143.72
143.60
137.34
136.63
133.84
131.18
131.13
129.71
127.82
126.54
126.12
116.03
111.13
63.31
60.08
56.60
55.12
54.10
48.59
47.85
46.61
46.01
28.92
28.45
27.03
20.97
19.13
CDCl3
f1 (ppm)

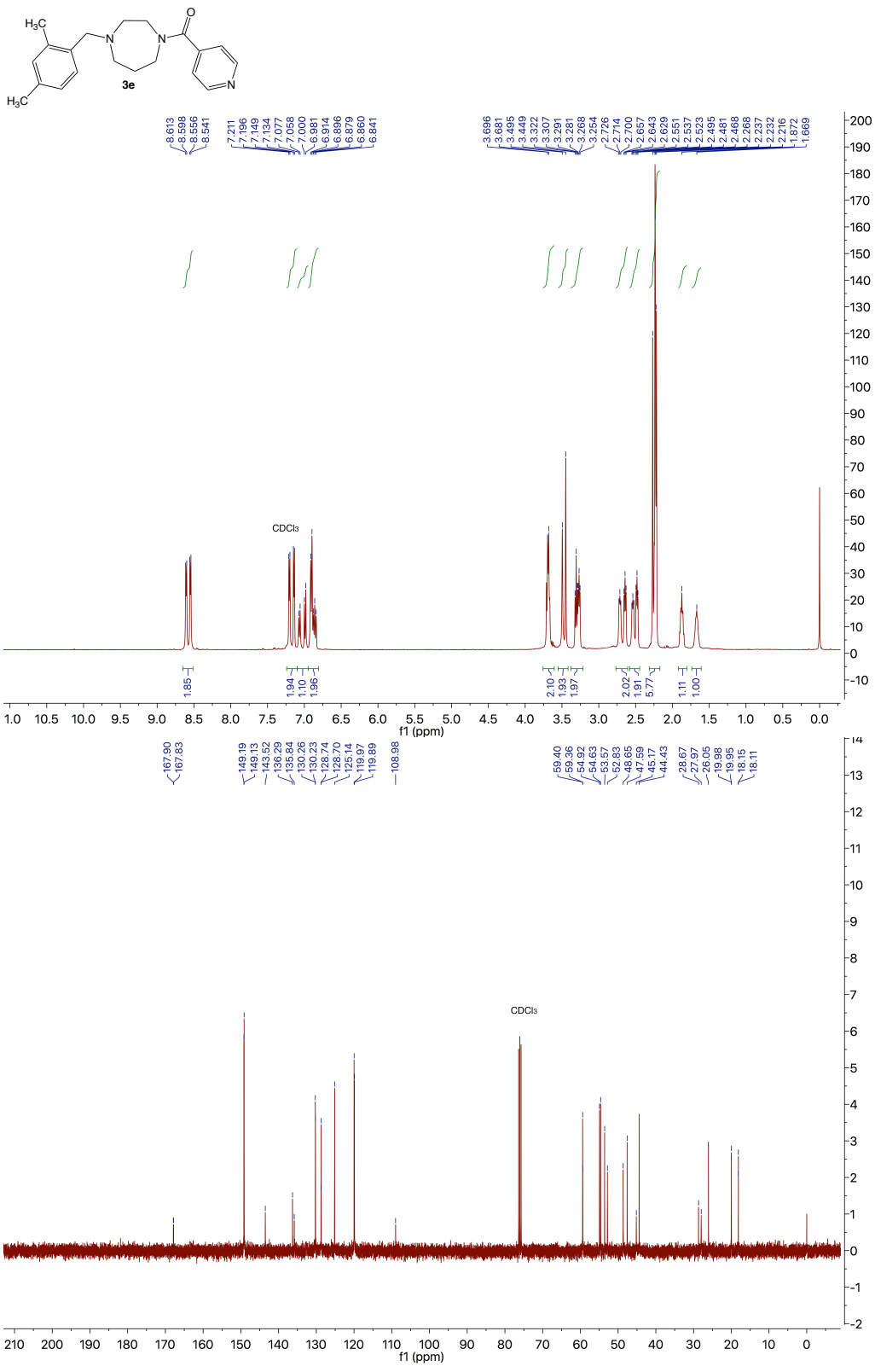

3b
7.425
7.412
7.320
7.127
7.109
7.019
6.969
6.951
6.932
3.750
3.539
2.741
2.657
2.316
2.295
1.923
CDCl3
1.00
1.12
0.98
3.07
3.95
1.95
4.16
6.27
2.32
f1 (ppm)
164.39
137.36
136.67
133.82
131.21
129.74
128.70
128.66
128.53
126.63
126.13
60.13
55.75
53.99
49.08
49.00
46.72
46.22
29.69
29.11
27.17
25.59
20.98
19.15
CDCl3
f1 (ppm)

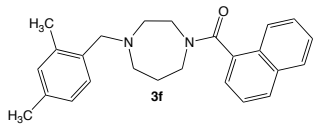

3c
7.657
7.640
7.622
7.525
7.504
7.493
7.472
7.405
7.379
7.358
7.295
7.276
7.158
7.138
7.118
6.951
3.892
3.846
3.788
3.772
3.558
2.813
2.800
2.787
2.702
2.688
2.648
2.334
2.325
2.320
2.305
2.295
1.968
CDCl3
1.23
1.26
1.49
2.11
1.08
1.94
4.60
2.00
2.16
2.07
5.91
2.41
f1 (ppm)
161.01
154.70
154.59
149.46
137.37
136.67
133.88
131.22
129.71
127.01
126.26
126.13
123.83
123.48
122.44
122.19
111.82
111.47
60.19
56.48
55.28
54.84
54.12
48.96
48.10
46.97
46.30
29.09
27.01
20.99
19.15
CDCl3
f1 (ppm)

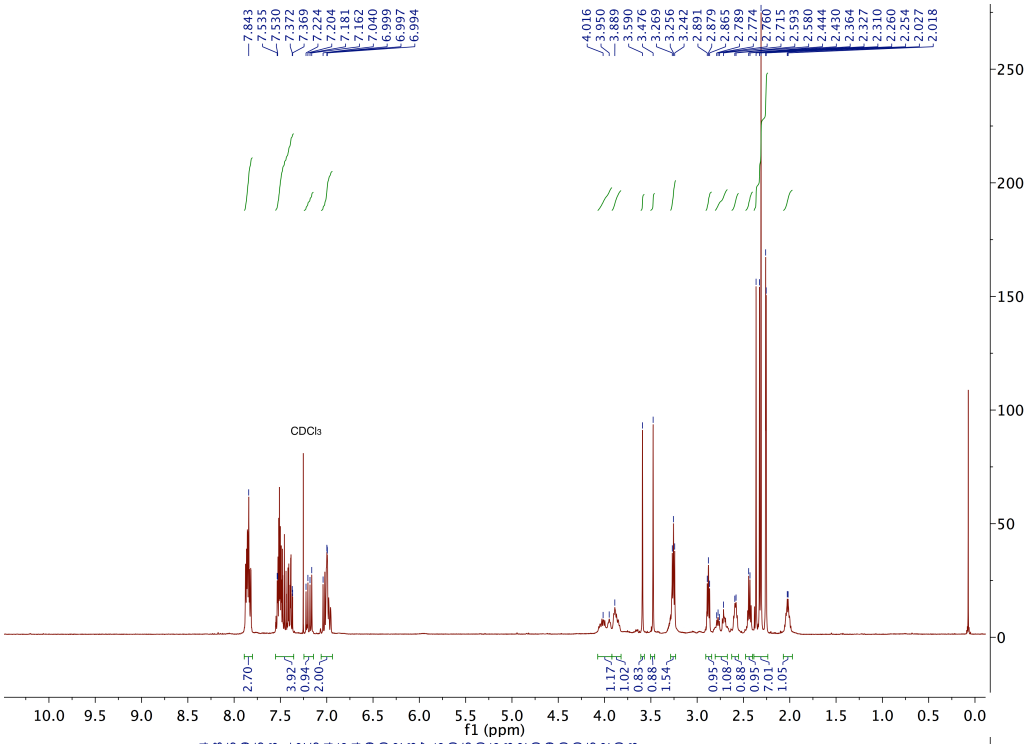

H3C
O
N
N
N
3d
H3C

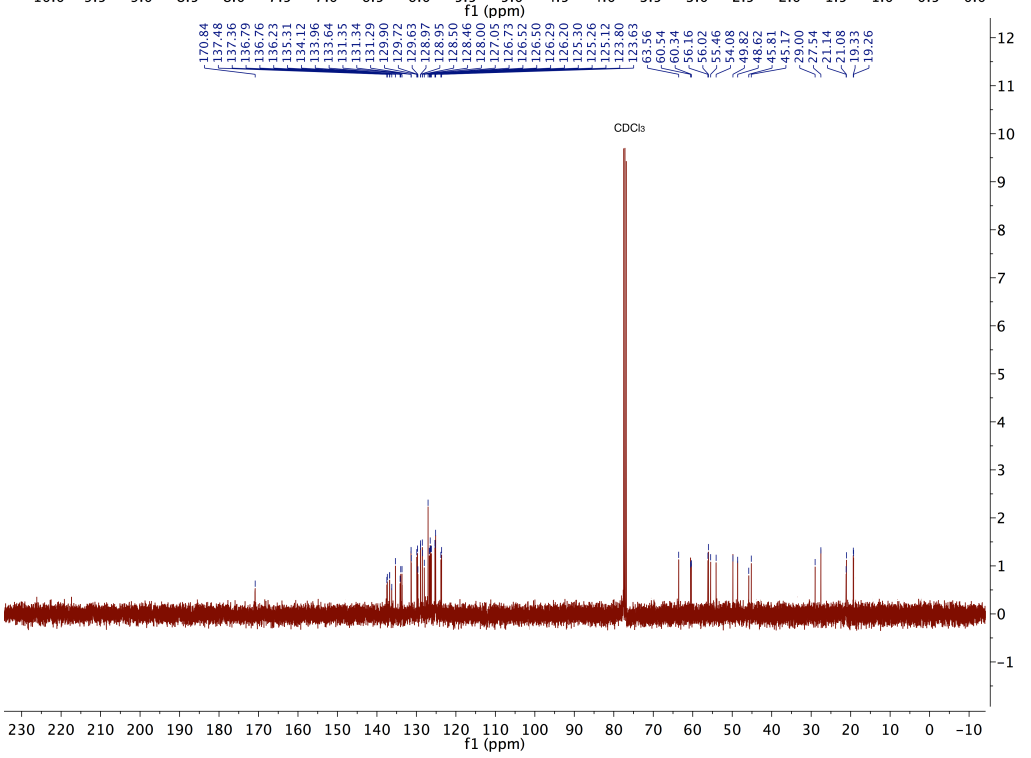

CDCl3
f1 (ppm)

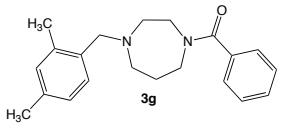

CDCl3
f1 (ppm)

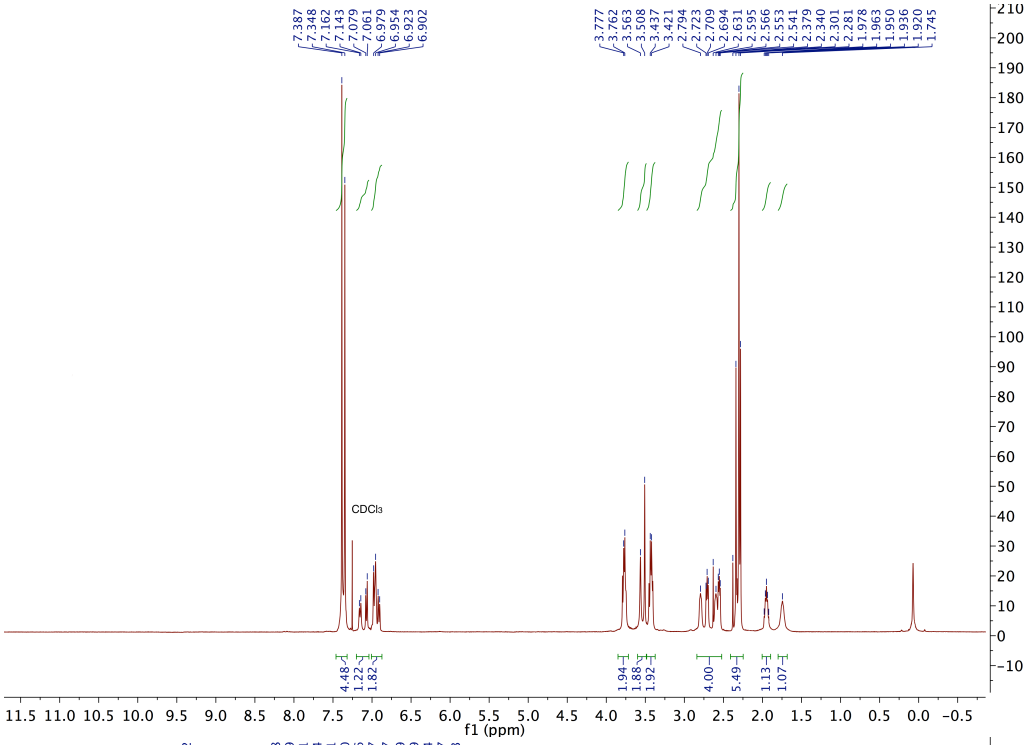

3e
CDCl3
f1 (ppm)
CDCl3
f1 (ppm)

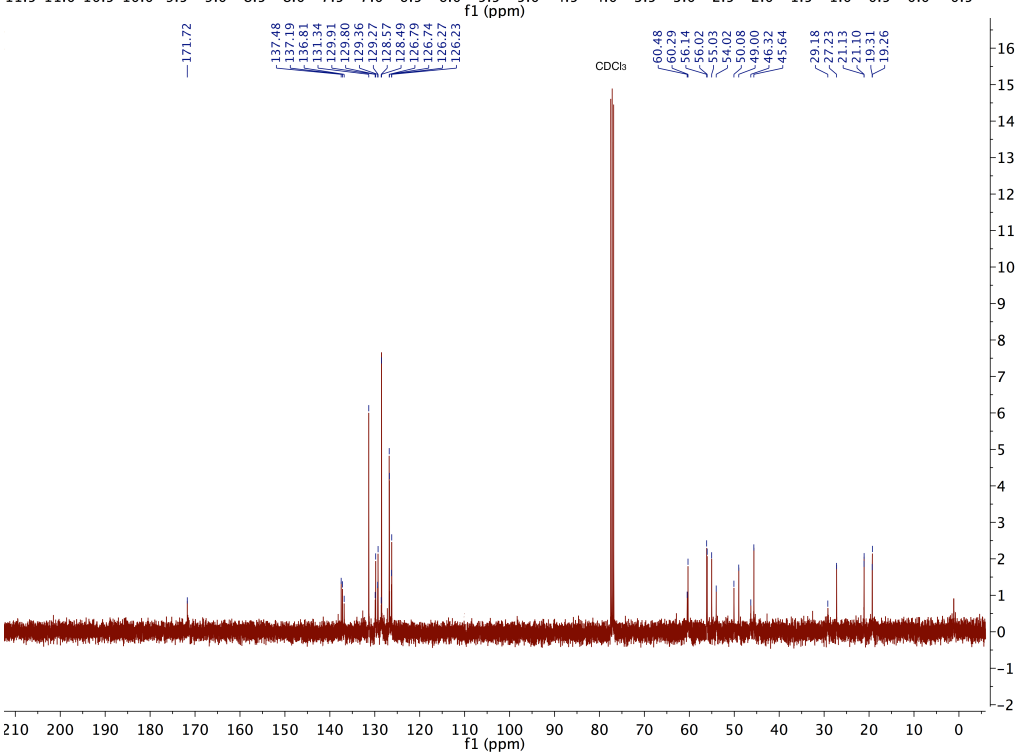

H3C
O
N
N
H3C
3f


7.843
7.535
7.530
7.372
7.369
7.224
7.204
7.181
7.162
7.040
6.999
6.997
6.994
4.016
3.950
3.889
3.590
3.476
3.269
3.256
3.242
2.891
2.879
2.865
2.789
2.774
2.760
2.715
2.593
2.580
2.444
2.430
2.364
2.327
2.310
2.260
2.254
2.027
2.018
CDCl3
2.70
3.92
0.94
2.00
1.17
1.02
0.83
0.88
1.54
0.95
1.08
0.88
0.95
7.01
1.05
f1 (ppm)


170.84
137.48
137.36
136.79
136.78
136.23
135.31
134.12
133.96
133.64
131.35
131.34
131.29
129.90
129.72
129.63
128.97
128.95
128.50
128.46
128.00
127.05
126.73
126.52
126.50
126.29
126.20
125.30
125.26
125.12
123.80
123.63
63.56
60.54
60.34
56.16
56.02
55.46
54.08
49.82
48.62
45.81
45.17
29.00
27.54
21.14
21.08
19.33
19.26
CDCl3
f1 (ppm)


H3C
N
N
O
H3C
3g


7.387
7.348
7.162
7.143
7.079
7.061
6.979
6.954
6.923
6.902
3.777
3.762
3.563
3.508
3.437
3.421
2.794
2.723
2.709
2.694
2.631
2.595
2.566
2.553
2.541
2.379
2.340
2.301
2.281
1.978
1.963
1.950
1.936
1.920
1.745
CDCl3
4.48
1.22
1.82
1.94
1.88
1.92
4.00
5.49
1.13
1.07
f1 (ppm)


171.72
137.48
137.19
136.81
131.34
129.91
129.80
129.36
129.27
128.57
128.49
126.79
126.74
126.27
126.23
CDCl3
60.48
60.29
56.14
56.02
55.03
54.02
50.08
49.00
46.32
45.64
29.18
27.23
21.13
21.10
19.31
19.26
f1 (ppm)

2. Computational details

2.1 Protein preparation. We chose chain A of structure 5HK2.[1] We added missing atoms and residues with Swiss-PDB Viewer.[2] We performed a steepest descent minimization of the protein chain to be stopped either when the maximum force was lower than 1000.0 kJ/mol/nm or when 50000 minimisation steps were performed with 0.005 kJ/mol energy step size, with Verlet cutoff scheme, short-range electrostatic cut-off and Van der Waals cut-off of 1.0nm. We used GROMOS 54a7 force field[3] and ran the minimisation as implemented in the Gromacs package v. 2016.1.[4]

2.2 Molecule preparation and docking. Molecule initial conformations were protonated, and minimized with AM1 method as implemented in MOPAC.[5] The system was prepared with AutoDock tools, and docked with AutoDock.[6] We used Lamarckian Genetic Algorithm with docking box (15.000x15.750x15.00)Å centered on the hydroxyl oxygen of TYR 103. The docking was performed with 100 runs and 2,500,000 maximum numbers of evaluations and standard parameters. We chose as representative conformation the representative conformation of the most populated cluster. 2D ligand-protein interaction diagrams were generated with LigPlot+.[7]

2.3 Molecular Dynamics Simulations. We minimized the complex by first minimizing the protein side chains alone, then whole protein and finally the whole system by constraining selected portions of the system. We placed the complex in a cubic box with a water layer of 0.7 nm and performed a second minimization. We used GROMOS 54a7 force field[3] and Simple Point Charge water. Ligand topologies were built with ATB.[8] For the protein we consider only the extracellular domain (residues 33-209). We performed NVT and NPT equilibrations for 100 ps, followed by 250 ns NPT production run at 300 K. The iteration time step was set to 2 fs with the Verlet integrator and LINCS[9] constraint. We used periodic boundary conditions. All the simulations and their analysis were run as implemented in the Gromacs package v. 2016.1.[4] RMSDs and RMSF have been calculated from configurations sampled every 10 ps and as running averages over 100 sampled points. Autodock Vina scorings[10] were calculated over configurations sampled every 100 ps and as running averages over 10 points. 2D ligand-protein interaction diagrams were generated with LigPlot+.[7] The binding free energy was estimated with the MM/PBSA method, with the apolar solvation energy calculated as solvent accessible surface area (SASA) and default parameters, as implemented in the g_mmpbsa tool.[11] Simulations were run on Marconi (CINECA, Italy).

3. Binding studies

3.1 Materials

Guinea pig brains, rat brains and rat livers were commercially available (Harlan-Winkelmann, Borchen, Germany). Pig brains were a donation of the local slaughterhouse (Coesfeld, Germany). The recombinant L(tk-) cells stably expressing the GluN2B receptor were obtained from Prof. Dr. Dieter Steinhilber (Frankfurt, Germany). Homogenizers: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany) and Soniprep® 150 (MSE, London, UK). Centrifuges: Cooling centrifuge model Eppendorf 5427R (Eppendorf, Hamburg, Germany) and High-speed cooling centrifuge model Sorvall® RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Multiplates: standard 96 well multiplates (Diagonal, Muenster, Germany). Shaker: self-made device with adjustable temperature and tumbling speed (scientific workshop of the institute). Harvester: MicroBeta® FilterMate 96 Harvester. Filter: Printed Filtermat Typ A and B. Scintillator: Meltilex® (Typ A or B) solid state scintillator. Scintillation analyzer: MicroBeta® Trilux (all Perkin Elmer LAS, Rodgau-Jügesheim, Germany).

3.2 Preparation of membrane homogenates from pig brain cortex

Fresh pig brain cortex was homogenized with the potter (500-800 rpm, 10 up and down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000 x g for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of TRIS/EDTA buffer (5 mM TRIS/1 mM EDTA, pH 7.5) and centrifuged again at 31,000 x g (20 min, 4 °C). The final pellet was resuspended in 5-6 volumes of buffer and frozen (-80 °C) in 1.5 mL portions containing about 0.8 mg protein/mL.

3.3 Preparation of membrane homogenates from rat liver

Two rat livers were cut into small pieces and homogenized with the potter (500-800 rpm, 10 up and down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000 x g for 20 min at 4°C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 8.0) and incubated at rt for 30 min. After the incubation, the suspension was centrifuged again at 31,000 x g for 20 min at 4 °C. The final pellet was resuspended in 5-6 volumes of buffer and stored at -80 °C in 1.5 mL portions containing about 2 mg protein/mL.

3.4 Cell culture and preparation of membrane homogenates for the GluN2B[12]

Mouse L(tk-) cells stably transfected with the dexamethasone-inducible eukaryotic expression vectors pMSG GluN1a, pMSG GluN2B (1:5 ratio) were grown in Modified Earl's Medium (MEM) containing 10 % of standardized FCS (Biochrom AG, Berlin, Germany). The expression of the NMDA receptor at the cell surface was induced after the cell density of the adherent growing cells had reached approximately 90 % of confluency. For the induction, the original growth medium was replaced by growth medium containing 4µM dexamethasone and 4µM ketamine (final concentration). After 24 h, the cells were rinsed with phosphate buffered saline solution (PBS, Biochrom AG, Berlin, Germany), harvested by mechanical detachment and pelleted (10 min, 5,000xg).

For the binding assay, the cell pellet was resuspended in PBS solution and the number of cells was determined using a Scepter® cell counter (MERCK Millipore, Darmstadt, Germany). Subsequently, the cells were lysed by sonication (4 C, 6x10s cycles with breaks of 10 s). The resulting cell fragments were

centrifuged with a high performance cool centrifuge (23,500xg, 4 C). The supernatant was discarded and the pellet was resuspended in a defined volume of PBS yielding cell fragments of approximately 500,000 cells/mL. The suspension of membrane homogenates was sonicated again (4 °C, 2 x 10 s cycles with a break of 10 s) and stored at -80 °C.

3.5 Protein determination

The protein concentration was determined by the method of Bradford[13] modified by Stoscheck.[14] The Bradford solution was prepared by dissolving 5 mg of Coomassie Brilliant Blue G 250 in 2.5 mL of EtOH (95 %, v/v). 10 mL deionized H_2O and 5 mL phosphoric acid (85%, m/v) were added to this solution, the mixture was stirred and filled to a total volume of 50 mL with deionized water. The calibration was carried out using bovine serum albumin as a standard in 9 concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 and 4.0 mg /mL). In a 96 well standard multiplate, 10 µL of the calibration solution or 10 µL of the membrane receptor preparation were mixed with 190 µL of the Bradford solution, respectively. After 5 min, the UV absorption of the protein-dye complex at λ = 595 nm was measured with a plate reader (Tecan Genios®, Tecan, Crailsheim, Germany).

3.6 General procedures for the binding assays

The test compound solutions were prepared by dissolving approximately 10 µmol (usually 2-4 mg) of test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution was diluted with the respective assay buffer. The filtermats were presoaked in 0.5 % aqueous polyethylenimine solution for 2h at rt before use. All binding experiments were carried out in duplicates in the 96 well multiplates. The concentrations given are the final concentration in the assay. Generally, the assays were performed by addition of 50 µL of the respective assay buffer, 50 µL of test compound solution in various concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} mol/L), 50 µL of the corresponding radioligand solution and 50 µL of the respective receptor preparation into each well of the multiplate (total volume 200 µL). The receptor preparation was always added last. During the incubation, the multiplates were shaken at a speed of 500-600 rpm at the specified temperature. Unless otherwise noted, the assays were terminated after 120 min by rapid filtration using the harvester. During the filtration, each well was washed five times with 300 µL of water. Subsequently, the filtermats were dried at 95 °C. The solid scintillator was melted on the dried filtermats at a temperature of 95 °C for 5 min. After solidifying of the scintillator at rt, the trapped radioactivity in the filtermats was measured with the scintillation analyzer. Each position on the filtermat corresponding to one well of the multiplate was measured for 5 min with the [^{3}H]-counting protocol. The overall counting efficiency was 20 %. The IC_{50} values were calculated with the program GraphPad Prism® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. Subsequently, the IC_{50} values were transformed into K_i values using the equation of Cheng and Prusoff.[15] The K_i values are given as mean value ± SEM from three independent experiments.

3.7 Performance of the binding assays

3.7.1 Ifenprodil binding site of GluN2B subunit containing NMDA receptors

The competitive binding assay was performed with the radioligand [^{3}H]ifenprodil (60 Ci/mmol; BIOTREND, Cologne, Germany). The thawed cell membrane preparation from the transfected L(tk-) cells (about 20 µg protein) was incubated with various concentrations of test compounds, 5 nM [^{3}H]-ifenprodil, and TRIS/EDTA-buffer (5 mM TRIS/1 mM EDTA, pH 7.5) at 37 °C. The non-specific binding was determined with 10 µM unlabeled ifenprodil. The K_d value of ifenprodil is 7.6 nM.[12]

3.7.2 σ_1 receptor assay

The assay was performed with the radioligand [^{3}H]-(+)-pentazocine (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation of guinea pig brain cortex (about 100 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and TRIS buffer (50 mM, pH 7.4) at 37 °C. The non-specific binding was determined with 10 μM unlabeled (+)-pentazocine. The K_d value of (+)-pentazocine is 2.9 nM.[16]

3.7.3 σ_2 receptor assay

The assays were performed with the radioligand [^{3}H]di-*o*-tolylguanidine (specific activity 50 Ci/mmol; ARC, St. Louis, MO, USA). The thawed rat liver membrane preparation (about 100 μg protein) was incubated with various concentrations of the test compound, 3 nM [^{3}H]di-*o*-tolylguanidine and buffer containing (+)-pentazocine (500 nM (+)-pentazocine in TRIS buffer (50 mM TRIS, pH 8.0)) at rt. The non-specific binding was determined with 10 μM non-labeled di-*o*-tolylguanidine. The K_d value of di-*o*-tolylguanidine is 17.9 nM.[17]

4. Cytotoxicity assay

The human SH-SY5Y (neuroblastoma), and PANC1 (pancreatic carcinoma) cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) Glutamax (Life Technologies) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1x Antibiotic Antimycotic Solution (Sigma-Aldrich, 100 U penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B) at 37°C in a humidified incubator with a 5%CO_2/95% air atmosphere. The cytotoxic effects of the compounds were evaluated by MTT test as previously described[18]. Briefly, SH-SY5Y cells or PANC1 cells were plated ($5x10^3$ cells/well) in 96-well plates 24 h prior to treatment with the compounds. The compounds, dissolved in DMSO, were serially diluted in culture medium to achieve the desired final concentrations. The final concentration of DMSO in the culture medium was always = 1.0 %. After 48 h, the medium was replaced with 100μl/well of MTT in DMEM (0.75 mg/mL, Sigma-Aldrich, St. Louis, MO, USA), and plates were incubated for 4 h at 37°C. After incubation, the medium was carefully removed and plates were incubated at -20°C for 1h. Subsequently, 100 μL of isopropanol 100% was added into each well and the plates were shaked on an orbital shaker for 15 minutes. Absorbances were read in an EnVision Multilabel Plate Reader (PerkinElmer, Waltham, MA, USA) at 590 nm with a reference filter of 650nm. All compounds were assayed in triplicates, and the results are the average of three independent experiments. Results are shown as mean absorbance values (A590 subtracted by A650) ± SEM. Cell viability is expressed as a percentage of the control (DMSO). The statistical analysis was performed with GraphPad Prism 6 (GraphPad Software, Inc, La Jolla, CA, USA) software by using an unpaired t-test. P values < 0.05 were considered statistically significant.

Table S1. Percentage viability ± SEM of the SH-SY5Y cells treated with four different concentrations of the title compounds **2c, 2d, 3c** and **3d**.

Cmpd	SH-SY5Y			
	12.5 μM	25 μM	50 μM	100 μM
2c	134 ± 20	143 ± 21	127 ± 19	98 ± 15
2d	136 ± 20	144 ± 22	131 ± 20	122 ± 18
3c	137 ± 21	121 ± 18	109 ± 16	77 ± 11
3d	123 ± 18	120 ± 18	127 ± 19	53 ± 8

Table S2. % Percentage viability ± SEM of the PANC1 cells treated with four different concentrations of the title compounds **2c, 2d, 3c** and **3d**.

Cmpd	PANC1			
	12.5 μM	25 μM	50 μM	100 μM
2c	258 ± 36	278 ± 39	196 ± 27	127 ± 18
2d	185 ± 26	188 ± 26	130 ± 18	107 ± 15
3c	224 ± 31	214 ± 30	201 ± 28	88 ± 12
3d	121 ± 17	119 ± 17	93 ± 13	51 ± 7

5. Antioxidant activity

5.1 ABTS radical scavenging activity

The antioxidant activity of the compounds was tested from the bleaching of the green colored ethanolic solution of ABTS.[19] To 1.8 mL of ethanolic solution of ABTS 7mM 200 μL of test compounds, each one diluted according the following concentration 0.05, 0.1, 0.2 and 0.4 mg/mL. These mixtures were incubated for 40 min at room temperature, then the absorbances were recorded at 735 nm against ABTS solution. The results were measured as the percent of inhibition (IC%) of ABTS radical, calculated by the following formula.

% IC = [(Abs ABTS - Abs Sample) / Abs ABTS] x 1

Tests were performed in triplicate and data were expressed ad mean value ± SEM.

The IC % was used to determine the IC_{50} values.

The ABTS method was applied also to measure the IC_{50} of Ascorbic acid, used as antioxidant compound comparing value.

5.2 Hydrogen peroxide radical scavenging activity

Four different concentrations of test compounds (0.05, 0.1, 0.2 and 0.4 mg/mL) were diluted in phosphate buffer (pH 7.4).[20] Also, a solution of Hydrogen peroxide 4 mM was prepared in phosphate buffer and 0.353 mL of this solution were added to 2.0 mL of each solution of test compounds. The mixtures were measured at 239 nm. The percent of inhibition of free radical production from hydrogen peroxide was calculated using the above formula.

Tests were performed in triplicate and data were expressed as mean value ± SEM.

Table S3. Antioxidant activity of compounds **2c, 2d, 3c** and **3d.**

Cmpd	IC_{50} (μg/mL)[a]	
	ABTS	H_2O_2
2c	12.71 ± 0.25	15.89 ± 0.18
2d	14.26 ± 0.15	20.35 ± 0.27
3c	10.05 ± 0.09	18.56 ± 0.31
3d	9.43 ± 0.11	17.44 ± 0.18
Ascorbic Acid	12.75 ± 0.12	19.27 ± 0.54
Trolox	18.73 ± 0.26	20.38 ± 0.19

[a]All measurements were performed in triplicate

6. Drug likeness prediction

Table SI 4. *In silico* predicted main pharmacokinetic parameters[21] (www.swissadme.ch) of the title compounds **2a-g** and **3a-g**. Data for reference compound **Haloperidol** (σ1 ligand) and **Siramesine** (σ2 ligand) are reported for comparison.

Cmpd	MW[b]	HBA[c]	HBD[d]	clogP[e]	clogS[f] (mol/L)	TPSA[g] (Å<140)	RO5 violation	BBB permeant	GI abs.
RO5[a]	<500	≤10	≤5	≤5	≤5	-	≤1	-	-
2a	284.35	3	0	2.22	-3.26	36.69	0	Yes	High
2b	300.42	2	0	2.89	-3.75	51.79	0	Yes	High
2c	334.41	3	0	3.25	-4.47	36.69	0	Yes	High
2d	345.44	3	0	3.22	-4.47	36.44	0	Yes	High
2e	295.38	3	0	2.10	-3.05	36.44	0	Yes	High
2f	344.45	2	0	3.68	-4.87	23.55	0	Yes	High
2g	294.39	2	0	2.84	-3.72	23.55	0	Yes	High
3a	312.41	3	0	2.86	-3.86	36.69	0	Yes	High
3b	328.47	2	0	3.56	-4.35	51.79	0	Yes	High
3c	362.46	3	0	3.84	-5.08	36.69	0	Yes	High
3d	373.49	3	0	3.77	-5.07	36.44	0	Yes	High
3e	323.43	3	0	2.73	-3.65	36.44	0	Yes	High
3f	372.50	2	0	4.36	-5.47	23.55	0	Yes	High
3g	322.44	2	0	3.51	-4.32	23.55	0	Yes	High
Halop.	375.86	4	1	4.22	-4.82	40.54	0	Yes	High
SRMS	454.59	3	0	5.85	-6.52	17.40	1	No	Low

[a] Lipinski's rule of five; [b] Molecular weight; [c] # of hydrogen bond acceptors; [d] # of hydrogen bond donors; [e] calculated log partition coefficient; [f] calculated log of water solubility; [g] topological polar surface area.

References

1. Schmidt, H.R. et al., Crystal structure of the human σ 1 receptor. *Nature*, **2016**, *532*, 527-530.
2. Guex, N. and M.C. Peitsch, Swiss model and the Swiss-Pdb Viewer: an environment for comparative protein modeling. *Electrophoresis*, **1997**, *18*, 2714-2723.
3. Schmid, N. et al., Definition and testing of the GROMOS force-field versions 54A7 and 54B7. *Eur. Biophys. J.* **2011**, *40*, 843-856.
4. Pronk, S. et al., GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinf.* **2013**, *29*, 845-854.
5. Stewart, J.J.P., Special issue - MOPAC – A semiempirical molecular-orbital program. *J. Comput. Aided Mol. Des.* **1990**, *4*, 1-45.
6. Morris, G.M. et al., AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30*, 2785-2791.
7. Laskowski, R.A. and M.B. Swindells, LigPlot+: multiple ligand–protein interaction diagrams for drug discovery, *J. Chem. Inf. Model.* **2011**, *24*, 2778-2786.
8. Malde, A.K. et al., An Automated Force Field Topology Builder (ATB) and Repository: Version 1.0. *J. Chem. Theory Comput.*, **2011**, *7,* 4026-4037.
9. Hess, B. et al., LINCS: a linear constraint solver for molecular simulations. *J. Comput. Chem.* **1997**, *18*,1463-1472.
10. Trott, O. and A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455-461.
11. Kumari, R. et al., g_mmpbsa A GROMACS tool for high-throughput MM-PBSA calculations. *J. Chem. Inf. Model.* **2014**, *54*, 1951-1962.
12. Schepmann, D. et al., Development of a selective competitive receptor binding assay for the determination of the affinity to NR2B containing NMDA receptors, *J. Pharm. Biomed. Anal.* **2010**, *53*, 603–608.
13. Bradford, M.M. A Rapid and sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, *Anal. Biochem.* **1976**, *72,* 248–254.
14. Stoscheck, C. Quantification of Protein, *Methods in Enzymology*, **1990**, *182*, 50–68.
15. Cheng, Y.C. and Prusoff, W.H. Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction, *Biochem. Pharmacol.* **1973**, *22*, 3099–3108
16. DeHaven-Hudkins, D.L. et al., Characterization of the binding of [3H](+)-pentazocine to sigma recognition sites in guinea pig brain, *Eur. J. Pharmacol.: Molecular Pharmacology* **1992**, *1227*, 371–378.
17. Mach, R.H. et al. Ibogaine possesses a selective affinity for sigma 2 receptors, Life *Sciences* **1995**, *57*, PL57–PL62.
18. Zampieri, D. et al., Computer-assisted design, synthesis, binding and cytotoxicity assessments of new 1-(4-(aryl(methyl)amino)butyl)-heterocyclic σ1 ligands, *Eur. J. Med Chem.* **2016**, *121*, 712–726.
19. Re, R. et al, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free radical biology & medicine* **1999**, *26*, 1231-1237.
20. Ashok, D. et al., Conventional and microwave-assisted synthesis of new indole-tethered benzimidazole-based 1,2,3-triazoles and evaluation of their antimycobacterial, antioxidant and antimicrobial activities, *Mol. Divers.* 2018, *22,* 769-778.
21. Daina, A et al., SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, *Sci. Report* **2017**, *7*, 42717.