Quinolines and oxazino-quinoline derivatives as small molecule GLI1 inhibitors identified by virtual screening

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COMPUTATIONAL DETAILS

A pharmacophoric-based virtual screening approach and molecular docking simulations were applied following computational protocols already described [1-3] Small molecules were drawn with Maestro (version 11.9, release 2019-1), their structure was optimized within the LigPrep routine (OPLS3e force field), while ionization and tautomerism at pH 7 ± 1 were considered with Epik. A Systematic Pseudo Monte Carlo conformational search was then applied to each compound.

Application of the common feature pharmacophoric hypothesis generation of the software Phase resulted in the five-feature pharmacophoric model for putative GLI1 inhibitors previously reported [3], which was in turn used to perform a virtual screening of the commercially available compounds stored in both the Asinex [4] and AKos [5] databases. In further detail, the Ligand Screening routine of Phase was used to generate a ranked list of putative GLI1 ligands based on the fitness score. Among the best scored entries, structurally different compounds were selected (at maximum, five entries were kept that belonged to the same structural class, based on Tanimoto coefficient comparison), avoiding compounds with undesired features, such as chemically reactive groups, or pan-assay interference compounds. The final list was constituted by 41 entries that were purchased from vendors and submitted to biological evaluation.

The three-dimensional structure of the five-finger GLI/DNA complex (protein data bank pdb code 2gli, 2.6 Å resolution) was prepared with the Protein Preparation Wizard of the Maestro 2019-1 suite, as already described [3]. The SiteMap routine was next applied to the whole structure of the target to find putative binding sites for small molecule ligands. Two regions of the GLI1 structure were identified, located at the zinc-finger 4 and between the zinc-fingers 1 and 3, respectively. Molecular docking simulations were performed on the two putative binding sites, by application of the extra-precision mode of the software Glide. Ligands were considered as flexible structures. At most, ten poses *per* ligand were kept, on which a post-dock minimization was performed with default parameters.

EXPERIMENTAL

Chemistry

All the compounds purchased from either Asinex or Akos had a purity higher than 90%, as declared by vendors.

All reagents were used as purchased from commercial suppliers without further purification. Flash column chromatography was performed in glass columns using Merk silica gel 60 Å, 230-400 mesh particle size or using an automatic LC Isolera Biotage system. For analytical thin-layer chromatography, Merck aluminium-backed plates pre-coated with silica gel 60 (UV254) were used and visualized by staining with a solution of *p*-anisaldehyde in EtOH or a $KMnO_4$ solution. ^1H NMR and ^{13}C NMR spectra were recorded at 25 °C using a 400 or 600 MHz Brucker Advance NMR spectrometer. Deuterated chloroform, or dimethylsulfoxide were used as the solvents. Chemical shift (δ) values are given in parts per million (ppm) and refer to the residual signals of the deuterated solvent (δ 7.26 for ^1H and δ 77.6 for ^{13}C in $CDCl_3$, δ 2.50 for ^1H and δ 39.52 for ^{13}C in d_6-DMSO). The data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, q = quartet, m = multiplet or multiple resonances, bs = broad singlet), coupling constant (J) in hertz and the integration in ppm. Mass spectrometry analyses were recorded by electrospray ionization on an Agilent 1100 Series instrument (flux: 9 L/min, T: 350 °C, P: 40 psi, frag: 70 eV). MW assisted reactions were performed in a CEM Discover MW oven equipped with a 10 mL tube for reactions under pressure and an external IR sensor to record the reaction temperature during irradiation (CEM Corporation).

No unexpected or unusually high safety hazards were encountered.

General procedure for synthesis of open chain 8-hydroxyquinoline derivatives 2-5 and 15-23

To a solution of aldehyde (1.38 mmol, 1 eq.) in MeOH (2 mL), the proper amine (1.38 mmol, 1 eq.) and 8-idrossiquinoline derivative (1.38 mmol, 1 eq.) were added. The reaction mixture was stirred at reflux overnight. After evaporation *in vacuo*, the crude was purified by flash chromatography.

General procedure for synthesis of tricyclic 8-hydroxyquinoline derivatives 26-41

An EtOH (20 mL) solution of the proper amine (2.06 mmol, 1.25 eq.) and paraformaldehyde (191 mg, 6.21 mmol, 3.70 eq.) was refluxed for 6 h. The reaction mixture was cooled down to rt, and the proper 8-hydroxyquinoline (1.66 mmol, 1 eq.) was added. The reaction mixture was stirred at rt overnight. After evaporation *in vacuo*, the crude was purified by flash chromatography.

General procedure for synthesis of tricyclic 8-hydroxyquinoline derivatives 42-43

The proper 8-hydroxyquinoline derivative **2** or **5** (1.66 mmol, 1 eq.) was dissolved in dry THF (20 mL) and carbonyldiimidazole (1.66 mmol, 1 eq.) and Et_3N (3.32, 2 eq.) were added. The reaction mixture was stirred under N_2 at reflux, overnight. The solution was cooled down to rt, diluted with CH_2Cl_2 (30 mL) and washed 3 times with 1N HCl (10 mL x 3). The organic phase was dried over dry Na_2SO_4, filtered and evaporated *in vacuo*. The crude obtained was purified by flash chromatography.

7-((2,4-Dichlorophenyl)(phenethylamino)methyl)quinolin-8-ol (2)

^{1}H NMR (400 MHz, $CDCl_3$, δppm): 8.85 (dd, *J* = 4.2, 1.6 Hz, 1H), 8.05 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.46-7.09 (m, 11H), 5.56 (s, 1H), 3.10-3.00 (m, 2H), 2.98-2.83 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 152.12, 148.37, 138.89, 138.63, 137.14, 135.34, 130.17, 129.11, 128.28, 128.14, 127.13, 126.93, 126.00, 121.29, 120.09, 117.35, 60.75, 49.18, 35.55. ES-MS: 424 $[M+H]^+$. Elemental Analysis for $C_{24}H_{20}Cl_2N_2O$: calcd. C, 68.09, Cl, 16.75, H, 4.76, N, 6.62, O, 3.78; found C, 68.32, Cl, 16.51, H, 4.91, N, 6.78. Yield = 6%.

7-((2,4-Dichlorophenyl)(phenethylamino)methyl)-3-methylquinolin-8-ol (3)

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.69 (d, J = 1.9 Hz, 1H), 7.81 (s, 1H), 7.33-7.06 (m, 12H), 5.52 (s, 1H), 3.09-2.95 (m, 1H), 2.95-2.81 (m, 1H), 2.48 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 152.25, 150.32, 138.66, 137.28, 134.06, 133.77, 133.55, 130.80, 130.22, 129.09, 128.29, 128.15, 127.90, 127.14, 126.98, 126.00, 119.04, 116.77, 60.91, 49.17, 35.55, 18.29. ES-MS: 438 $[M+H]^+$. Elemental Analysis for $C_{25}H_{22}Cl_2N_2O$: calcd. C, 68.65, Cl, 16.21, H, 5.07, N, 6.41, O, 3.66; found C, 68.81, Cl, 16.04, H, 5.00, N, 6.79. Yield = 68%.

5-Chloro-7-[(phenethylamino)methyl]-8-quinolinol (4)

^{1}H NMR: (400 MHz, DMSO-d_6 + MeOH, δ ppm): 8.87 (dd, *J* = 4.3, 1.5 Hz, 1H), 8.39 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.63 (dd, *J* = 8.6, 4.2 Hz, 2H), 7.52 (s, 1H), 7.24–7.02 (m, 4H), 3.90 (s, 2H), 2.87 (t, *J* = 7.6 Hz, 1H), 2.72 (d, *J* = 7.4 Hz, 1H), 2.66-2.55 (m, 1H), 2.32-2.23 (m, 1H). ES-MS: 313 $[M+H]^+$. Column chromatography: CH_2Cl_2/MeOH (90:10). Elemental Analysis for $C_{18}H_{17}ClN_2O$: calcd. C, 69.12, Cl, 11.33, H, 5.48, N, 8.96, O, 5.12; found C, 69.35, Cl, 11.41, H, 5.30, N, 9.03. Yield = 30%.

7-[(Phenethylamino)methyl]-8-quinolinol (5)

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 8.78 (dd, 1H, *J* = 4.3, 1.6 Hz), 8.00 (dd, 1H, *J* = 8.3, 1.7 Hz), 7.38–7.01 (m, 8H), 4.07 (s, 2H), 2.92 (t, 2H, *J* = 6.7 Hz), 2.82 (t, 2H, *J* = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 151.86, 148.10, 139.01, 138.54, 135.34, 128.32, 128.24, 127.62, 125.90, 120.82, 119.45, 116.87, 50.16, 49.59, 35.55. ES-MS: 279 $[M+H]^+$. Column chromatography: CH_2Cl_2/MeOH (90:10). Elemental Analysis for $C_{18}H_{18}N_2O$: calcd. C, 77.67, H, 6.52, N, 10.06, O, 5.75; found C, 77.88, H, 6.76, N, 10.23. Yield = 31%.

7-[(Cyclohexylamino)(2,4-dichlorophenyl)methyl]-8-quinolinol (15)

Column chromatography: PE/EtOAc (from 0 to 50% di EtOAc in 10 min, from 50 to 100% di EtOAc in 3 min, 100% EtOAc 3 min).

^{1}H NMR:(400 MHz, DMSO-d_6, δppm): 8.86 (d, 1H; J =2 Hz); 8.31-8.25 (m, 2H); 7.58- 7.41 (m, 2H); 7.01-6.77 (m, 3H); 3.32 (s, 1H); 1.93-1.49 (m, 2H); 1.52-1.08 (m, 8H). ^{13}C NMR:(100

MHz, DMSO-d_6, δppm) as mixture of rotamers: 147.85; 147.09; 132.72; 131.22; 129.35; 129.17; 127.79; 121.18; 120.58; 117.29; 116.71; 109.60; 108.57; 58.44; 55.06; 32.93; 25.42; 24.57. ES-MS: 401 $[M+H]^+$. Elemental Analysis for $C_{22}H_{22}Cl_2N_2O$: calcd. C, 65.84, Cl, 17.67, H, 5.53, N, 6.98, O, 3.99; found C, 65.70, Cl, 17.83, H, 5.25, N, 7.03. Yield = 12%.

7-((Cyclopentylamino)(2,4-dichlorophenyl)methyl)quinolin-8-ol (16)

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.85 (d, *J* = 3.8 Hz, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.44-7.29 (m, 3H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 5.64 (s, 1H), 3.26-3.14 (m, 1H), 1.97-1.82 (m, 2H), 1.68 (s, 2H), 1.61-1.42 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 152.83, 148.49, 139.21, 135.28, 133.67, 130.62, 129.16, 128.01, 127.30, 126.85, 121.18, 120.41, 117.26, 59.99, 58.15, 32.39, 31.95, 23.20. ES-MS: 388 $[M+H]^+$. Elemental Analysis for $C_{21}H_{20}Cl_2N_2O$: calcd. C, 65.12, Cl, 18.31, H, 5.21, N, 7.23, O, 4.13; found C, 65.02, Cl, 18.39, H, 5.55, N, 7.04. Yield = 11%.

7-[(Butylamino)(2,4-dichlorophenyl)methyl]-8-quinolinol (17)

Column chromatography: PE/EtOAc (from 0 to 50% EtOAc in 10 min, from 50 to 100% EtOAc in 3 min, 100% EtOAc 3 min).

^{1}H NMR:(400 MHz, $CDCl_3$, δppm): 8.86 (d, 1H; *J* =3.2 Hz); 8.05 (d, 1H; *J* =8.4 Hz); 7.39-7.35 (m, 2H); 7.31 (d, 1H; *J* =8.4 Hz); 7.18 (d, 1H; *J* = 4.8 Hz); 7.14-7.07 (m, 2H); 5.53 (s, 1H); 2.76 (t, 2H; *J* = 7.2 Hz); 1.62-1.49 (m, 2H); 1.39-1.30 (m, 2H): 0.87 (t, 3H; *J* = 7.4 Hz). ^{13}C NMR: (100 MHz, $CDCl_3$, δ ppm): 152.73; 148.45; 139.10; 137.13; 135.53; 135.32; 133.72; 130.39; 129.14; 128.05; 127.26; 126.97; 119.77; 117.29; 116.68; 61.57; 48.15; 31.36; 18.95; 13.46. Elemental Analysis for $C_{20}H_{20}Cl_2N_2O$: calcd. C, 64.01, Cl, 18.89, H, 5.37, N, 7.46, O, 4.26; found C, 64.15, Cl, 19.01, H, 5.61, N, 7.22. Yield = 14%.

7-[(Butylamino)methyl]-8-quinolinol (18)

Column chromatography: CH_2Cl_2/MeOH (97:3). ^{1}H NMR:(400 MHz, $CDCl_3$, δ ppm): 8.85 (d, 1H; *J* = 1.6 Hz); 8.03 (d, 1H; *J* = 1.6 Hz); 7.35-7.21 (m, 2H); 7.09 (d, 1H; *J* = 8.4 Hz); 4.10 (s, 2H); 2.80-2.71 (m, 2H); 1.51-1.23 (m, 4H); 0.89-0.77 (m, 3H). ^{13}C NMR: (100 MHz, $CDCl_3$, δppm): 148.86, 148.08, 135.42, 135.29, 127.60, 125.49, 120.89, 120.58, 118.65, 116.82, 80.61, 47.41, 29.59, 29.41. ES-MS: 231 $[M+H]^+$. Elemental Analysis for $C_{14}H_{18}N_2O$: C, 73.01, H, 7.88, N, 12.16, O, 6.95; found C, 73.17, H, 7.61, N, 11.96. Yield = 13%.

7-[(Cyclopentylamino)methyl]-8-quinolinol (19)

Column chromatography: CH_2Cl_2/MeOH (95:5). ^{1}H NMR:(400 MHz, $CDCl_3$, δ ppm): 8.76 (d, 1H; *J* =3.6 Hz); 8.08 (t, 1H; *J* = 8.6 Hz); 7.73 (d, 1H; *J* = 8.2 Hz); 7.42-7.37 (m, 1H); 7.35-7.23 (m, 1H); 4.20 (s, 2H); 2.79-2.74 (m, 1H); 2.20-2.10 (m, 2H); 1.57-1.37 (m, 4H); 1.23-1.08 (m, 4H). ^{13}C NMR:(100 MHz, $CDCl_3$, δ ppm): 147.95; 135.65; 129.14; 128.19; 121.92; 55.53; 55.33; 41.91; 29.26; 29.13; 28.87; 25.16; 24.49; 24.12. ES-MS: 243 $[M+H]^+$. Elemental Analysis for $C_{15}H_{18}N_2O$: calcd. C, 74.35, H, 7.49, N, 11.56, O, 6.60; found C, 74.61, H, 7.53, N, 11.74. Yield = 3%.

7-((Butylamino)(4-chlorophenyl)methyl)quinolin-8-ol (20)

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.79 (d, *J* = 3.1 Hz, 1H), 8.00 (d, *J* = 7.7 Hz, 1H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.31 (dd, *J* = 8.2, 4.2 Hz, 1H), 7.23 (dd, *J* = 7.8, 6.8 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 2H), 5.18 (s, 1H), 2.69 (t, *J* = 7.0 Hz, 2H), 1.54 (dt, *J* = 13.7, 6.1 Hz, 2H), 1.44-1.25 (m, 2H), 0.86 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 151.30, 148.24, 147.61, 140.86, 135.60, 135.29, 132.90, 130.14, 128.48, 128.36, 127.84, 126.57, 122.03, 121.07, 117.30, 116.66, 64.25, 47.82, 31.54, 19.97, 13.49. ES-MS: 341 $[M+H]^+$. Elemental Analysis for $C_{20}H_{21}ClN_2O$: calcd. C, 70.48, Cl, 10.40, H, 6.21, N, 8.22, O, 4.69; found C, 70.76, Cl, 10.66, H, 6.42, N, 8.09. Yield = 12%.

7-((Butylamino)(3,4-dichlorophenyl)methyl)quinolin-8-ol (21)

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.81 (d, *J* = 4.3 Hz, 1H), 8.07 (d, *J* = 9.1 Hz, 1H), 7.74-7.59 (m, 1H), 7.52-7.16 (m, 7H), 5.36 (dd, *J* = 30.6, 20.9 Hz, 1H), 2.79-2.65 (m, 2H), 1.59 (dd, *J* = 8.3, 5.3 Hz, 2H), 1.33 (dt, *J* = 14.4, 7.2 Hz, 2H), 1.06-0.73 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 150.40, 148.10, 142.58, 138.46, 135.45, 132.19, 130.91, 130.19, 128.93, 127.58, 126.41, 126.22, 121.93, 121.27, 117.59, 62.24, 47.64, 31.37, 19.96, 13.48, 0.58. ES-

MS: 376 $[M+H]^+$. Elemental Analysis for $C_{20}H_{20}Cl_2N_2O$: calcd. C, 64.01, Cl, 18.89, H, 5.37, N, 7.46, O, 4.26; found C, 64.17, Cl, 19.01, H, 5.65, N, 7.72. Yield = 14%.

7-((Butylamino)(3,5-dichlorophenyl)methyl)quinolin-8-ol (22)

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.75 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 2H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.32 (dd, *J* = 7.8, 3.5 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.16 (s, 1H), 5.29 (s, 1H), 2.91-2.53 (m, 2H), 1.51 (dd, *J* = 14.2, 7.1 Hz, 2H), 1.33 (dd, *J* = 14.4, 7.2 Hz, 2H), 0.86 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 150.07, 148.00, 146.56, 138.36, 135.48, 134.61, 127.51, 126.94, 126.14, 125.47, 122.67, 121.23, 117.60, 61.75, 47.66, 31.74, 20.00, 13.54. ES-MS: 376 $[M+H]^+$. Elemental Analysis for $C_{20}H_{20}Cl_2N_2O$: calcd. C, 64.01, Cl, 18.89, H, 5.37, N, 7.46, O, 4.26; found C, 64.15, Cl, 18.63, H, 5.59, N, 7.67. Yield = 17%.

7-((Butylamino)(3-chlorophenyl)methyl)quinolin-8-ol (23)

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.69 (d, J = 4.0 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.49 (s, 1H), 7.38-7.30 (m, 2H), 7.27 (d, *J* = 8.5 Hz, 1H), 7.18 (dd, *J* = 8.2, 4.2 Hz, 2H), 7.13-7.06 (m, 3H), 5.20 (s, 1H), 2.61 (t, *J* = 7.1 Hz, 2H), 1.53-1.41 (m, 2H), 1.33-1.21 (m, 2H), 0.80 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 150.81, 148.02, 147.42, 144.82, 138.64, 135.34, 134.00, 129.52, 127.55, 127.08, 126.47, 125.20, 122.67, 121.00, 117.36, 63.32, 47.72, 31.63, 19.99, 13.54. ES-MS: 341 $[M+H]^+$. Elemental Analysis for $C_{20}H_{21}ClN_2O$: calcd. C, 70.48, Cl, 10.40, H, 6.21, N, 8.22, O, 4.69; found C, 70.63, Cl, 10.75, H, 6.05, N, 8.46. Yield =36%.

4-Butoxy-7-[(cyclohexylamino)methyl]-8-quinolinol (24)

Column chromatography: CH_2Cl_2/MeOH (95:5). ^{1}H NMR: (400 MHz, $CDCl_3$, δ ppm): 8.69 (d, 1H; *J* = 5.2 Hz); 7.66 (d, 1H; *J* = 8.8 Hz); 7.06 (d, 1H; *J* = 8.4 Hz); 6.68 (d, 1H; *J* = 5.2 Hz); 5.23 (s, 2H); 4.16 (t, 3H; *J* = 6 Hz); 1.80-1.97 (m, 2H); 1.91-1.86 (m, 4H); 1.61-1.54 (m, 6H); 1.34-1.17 (m, 6H). ES-MS: 351 $[M+Na]^+$. Elemental Analysis for $C_{20}H_{28}N_2O_2$: calcd. C, 73.14, H, 8.59, N, 8.53, O, 9.74; found C, 73.42, H, 8.78, N, 8.81. Yield = 8%.

4-(Benzyloxy)-7-[(cyclohexylamino)methyl]-8-quinolinol (25)

Column chromatography: CH_2Cl_2/MeOH (99:1). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.68 (d, *J* = 5.1 Hz, 1H), 7.70 (d, *J* = 8.6 Hz, 1H),7.50 –7.29 (m, 5H), 7.06 (d, *J* = 8.6 Hz, 1H), 6.75 (d, *J* = 5.2 Hz, 1H), 5.23 (s, 2H), 4.15 (s, 2H), 2.81-2.37 (m, 1H), 2.09-1.85 (m, 2H); 1.72-1.68 (m, 2H), 1.31-1.15 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.15; 148.40; 135.26; 128.11; 127.73; 127.30; 127.04; 126.77; 121.12; 101.40; 100.57; 55.29; 29.24; 28.48; 23.81. ES-MS: 362 $[M+H]^+$. Elemental Analysis for $C_{23}H_{26}N_2O_2$: calcd. C, 76.21, H, 7.23, N, 7.73, O, 8.83; found C, 76.45, H, 7.38, N, 7.92. Yield = 53%.

2-Phenethyl-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (26)

^{1}H NMR: (400 MHz, $CDCl_3$, δ ppm): 8.86 (dd, *J* = 4.2, 1.8 Hz, 1H), 8.03 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.42-7.02 (m, 8H), 5.16 (s, 2H), 4.15 (s, 2H), 3.09 (dd, *J* = 8.9, 6.4 Hz, 2H), 2.90 (dd, *J* = 9.1, 6.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 149.00, 139.29, 135.46, 128.26, 127.93, 127.66, 125.71, 125.60, 120.76, 118.50, 117.68, 82.68, 76.98, 76.67, 76.35, 52.97, 50.39, 34.48. Elemental Analysis for $C_{19}H_{18}N_2O$: calcd. C, 78.59, H, 6.25, N, 9.65, O, 5.51; found C, 78.81, H, 6.48, N, 9.87. Yield = 22%.

7-Fluoro-2-phenethyl-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (27)

Column chromatography: EtOAc. ^{1}H NMR:(400 MHz, $CDCl_3$, δ ppm): 8.67 (d, *J* = 2.8 Hz, 1H), 7.54 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.21-6.98 (m, 7H), 5.08 (s, 2H); 4.05 (s, 2H); 2.99 (q, 2H; *J* = 7.6 Hz); 2.80 (q, 2H; *J* = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 157.87, 155.33, 149.90, 140.02, 139.60, 136.36, 136.33, 136.32, 128.74, 128.65, 128.60, 128.54, 128.48, 128.41, 128.32, 127.53, 118.64, 118.47, 118.30, 117.37, 117.35, 83.25, 53.36, 50.51, 34.85. ES-MS: 309 $[M+H]^+$. Elemental Analysis for $C_{19}H_{17}FN_2O$: calcd. C, 74.01, F, 6.16, H, 5.56, N, 9.09, O, 5.19; found C, 74.32, F, 6.23, H, 5.78, N, 9.31. Yield = 55%.

1-(2,4-Dichlorophenyl)-2-phenethyl-2,3-dihydro-1H-4-oxa-2,5-diazaphenanthrene (28)

^{1}H NMR:(400 MHz, $CDCl_3$, δ ppm): 8.93 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.08 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.54-7.34 (m, 3H), 7.31 – 7.00 (m, 6H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 8.3 Hz, 1H), 5.23 (s, 2H), 5.09 (dd, *J* = 10.7, 1.7 Hz, 1H), 4.89 (d, *J* = 10.7 Hz, 1H), 3.46-3.89 (m,

3H), ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): δ 150.05, 149.26, 139.09, 138.49, 135.58, 135.23, 133.80, 131.80, 129.54, 128.32, 128.22, 127.91, 126.79, 126.05, 125.70, 121.39, 118.65, 117.10, 59.70, 54.28, 34.38. ES-MS: 436 $[M+H]^+$. Elemental Analysis for $C_{25}H_{20}Cl_2N_2O$: calcd. C, 68.97, Cl, 16.29, H, 4.63, N, 6.43, O, 3.68; found C, 69.07, Cl, 16.39, H, 4.86, N, 6.77. Yield = 21%.

2-[2-(3-Indolyl)ethyl]-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (29)

Column chromatography: CH_2Cl_2/MeOH (90:10). ^{1}H NMR:(400 MHz, DMSO-d_6, δ ppm): 10.59 (bs, 1H); 8.79 (d, 1H; *J* = 3.6 Hz); 8.26 (d, 1H; *J* = 8.4 Hz); 7.49 (m, 2H); 7.33 (q, 2H; *J* = 26.4 Hz); 7.19 (d, 1H; *J* = 8.4 Hz); 6.91 (m, 2H); 3.95 (s, 2H); 3.63 (s, 2H); 2.85 (t, 2H; *J* = 10.8 Hz); 2.68 (d, 2H; *J* = 4.8 Hz). ES-MS: 330 $[M+H]^+$. Elemental Analysis for $C_{21}H_{19}N_3O$: calcd. C, 76.57, H, 5.81, N, 12.76, O, 4.8613; found C, 76.79, H, 5.94, N, 12.85. Yield = 10%.

9-Chloro-2-[2-(3-indolyl)ethyl]-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (30)

Column chromatography: CH_2Cl_2/MeOH (90:10). ^{1}H NMR: (400 MHz, DMSO-d_6, δ ppm): 10.60 (bs, 1H); 8.90 (s, 1H); 8.44 (d, 1H; *J* = 8.4 Hz); 7.67 (d, 1H; *J* = 8.8 Hz); 7.55 (d, 1H; *J* = 8.4 Hz); 7.31 (d, 1H; *J* = 7.6 Hz); 7.20 (d, 1H; *J* =7.6 Hz); 7.03 (d, 1H; *J* = 8.4 Hz); 6.97-6.87(m, 2H); 3.95 (s, 2H); 3.66 (s, 2H); 3.86 (s, 2H); 2.69 (s, 2H). ES-MS: 364 $[M+H]^+$. Elemental Analysis for $C_{21}H_{18}ClN_3O$: calcd. C, 69.32, Cl, 9.74, H, 4.99, N, 11.55, O, 4.40; found C, 69.54, Cl, 9.92; H, 5.08; N, 11.76. Yield = 16%.

2-[(*p*-Methoxyphenyl)methyl]-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (32)

Column chromatography: PE/EtOAc: (50:50). ^{1}H NMR: (400 MHz, $CDCl_3$, δ ppm): 8.86 (d, 1H; *J* =4.4 Hz); 8.03 (d, 1H; *J* = 8.0 Hz); 7.32 (q, 1H; *J* = 12.4 Hz); 7.25 (q, 3H; *J* = 8.4 Hz); 7.04 (d, 1H; *J* = 8.4 Hz); 6.82 (d, 2H; *J* = 8.4 Hz); 5.12 (s, 2H); 4.05 (s, 2H); 3.89 (s, 2H); 3.75 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 158.98, 149.50, 149.37, 139.32, 135.91, 130.19, 130.08, 128.10, 126.17, 121.15, 119.00, 117.86, 113.81, 83.07, 55.22, 55.17, 49.42. ES-MS: 307 $[M+H]^+$. Elemental Analysis for $C_{19}H_{18}N_2O_2$: calcd. C, 74.49, H, 5.92, N, 9.14, O, 10.44; found C, 74.64, H, 6.02, N, 9.36. Yield = 48%.

2-(1,2,3,4-Tetrahydro-2-naphthyl)-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (33)

Column chromatography: EtOAc. ^{1}H NMR: (400 MHz, $CDCl_3$, δ ppm): 8.84 (d, 1H; *J* = 3.2 Hz); 7.98 (d, 1H; *J* = 8.4 Hz); 7.55 (t, 1H; *J* = 8.8 Hz); 7.28 (q, 1H; *J* = 12.4 Hz); 7.20 (d, 1H; *J* = 8.4 Hz); 7.11 (q, 2H; *J* = 8.8 Hz); 7.02 (d, 1H; *J* = 4.4 Hz); 6.99 (d, 1H; *J* =8.8) 5.25 (t, 2H; *J* = 14.4 Hz); 4.24 (d, 1H; *J* = 8 Hz); 4.15 (d, 1H; *J* = 17.6 Hz) 3.85 (d, 1H; *J* = 17.6 Hz); 2.64 (m, 2H); 2.04 (q, 1H; *J* = 14.8 Hz); 1.78 (t, 1H; *J* = 12.4 Hz); 1.60 (q, 2H; *J* = 12.4 Hz). ES-MS: 317 $[M+H]^+$. Elemental Analysis for $C_{21}H_{20}N_2O$: calcd. C, 79.72, H, 6.37, N, 8.85, O, 5.06; found C, 79.88, H, 6.25, N, 8.64. Yield = 51%.

2-Cyclohexyl-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (34)

Column chromatography: CH_2Cl_2/MeOH (90:10). ^{1}H NMR: (400 MHz, $CDCl_3$, δ ppm): 8.84 (t, 1H; *J* = 4.4 Hz); 8.02 (d, 1H; *J* = 8.4 Hz); 7.36-7.19 (m, 1H); 7.10 (d, 1H; *J* = 8.4 Hz); 5.23 (s, 2H); 4.19 (s, 2H); 2.75 (q, 1H; *J* = 21.6 Hz); 1.99 (t, 2H, *J* = 21.8 Hz); 1.68 (d, 2H; *J* = 12.4 Hz); 1.52 (d, 1H, *J* =11.6 Hz); 1.32-1.18 (m, 6H). ES-MS: 269 $[M+H]^+$. Elemental Analysis for $C_{17}H_{20}N_2O$: calcd. C, 76.09, H, 7.51, N, 10.44, O, 5.96; found C, 76.31, H, 7.80, N, 10.73. Yield = 35%.

2-Cyclohexyl-7-fluoro-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (35)

Column chromatography: CH_2Cl_2/MeOH (9:1). ^{1}H NMR: (400 MHz, $CDCl_3$ δ ppm): 8.67 (d, 1H; *J* = 2.8 Hz); 7.59 (q, 1H; *J* = 11.6 Hz); 7.22 (d, 1H; *J* = 8 Hz) 7.11 (d, 1H *J* = 8.4 Hz); 5.2 (s, 2H); 4.18 (s, 2H); 2.78- 2.71 (m, 1H); 1.94 (d, 2H; *J* = 12.3 Hz) 1.69 (d, 2H; *J* = 12.3 Hz) 1.54 (d,1H; *J* = 12.3 Hz) 1.30-1.06 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 157.89, 155.35, 150.91, 140.13, 139.86, 136.59, 128.40, 128.35, 127.12, 119.07, 119.04, 118.55, 118.38, 117.93, 117.88, 81.46, 59.48, 47.25, 31.63, 25.77, 25.47. ES-MS: 287 $[M+H]^+$. Elemental Analysis for $C_{17}H_{19}FN_2O$: calcd. C, 71.31, F, 6.63, H, 6.69, N, 9.78, O, 5.59; found C, 71.55, F, 6.78, H, 6.54, N, 9.91. Yield = 48%.

2-(4-Methylcyclohexyl)-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (36)

Column chromatography: CH_2Cl_2/toluene/MeOH (90:5:5). ^1H NMR: (400 MHz, $CDCl_3$, δ ppm): 8.81 (d, 1H; *J* = 17.6 Hz); 8.01 (d, 1H; *J* =7.6 Hz); 7.31-7.00 (m, 3H); 5.23 (s, 2H); 4.18 (s, 2H); 2.86-2.81 (m, 1H); 1.77-1.58 (m,4H); 1.40-1.20 (m, 4H); 0.88 (d, 3H; *J* =6.4 Hz). ^{13}C NMR :(100 MHz, $CDCl_3$, δ ppm): 147.98; 135.34; 128.19; 127.71; 120.91; 120.66; 119.10; 117.04; 82.78; 77.05; 51.01; 28.60; 27.98; 20.09; 19.86; 13.46. ES-MS: 283 $[M+H]^+$. Elemental Analysis for $C_{18}H_{22}N_2O$: C, 76.56, H, 7.85, N, 9.92, O, 5.67; found C, 76.65, H, 7.89, N, 9.99. Yield = 18%.

2-(Cyclohexylmethyl)-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (37)

Column chromatography: PE/EtOAc (50:50). ^1H NMR: (400 MHz, $CDCl_3$, δ ppm): 8.75 (t, 1H; *J* = 4.4 Hz); 7.90 (t, 1H; *J* = 8.4 Hz); 7.23-7.15 (m, 1H); 6.99 (d, 1H; *J* = 8 Hz); 5.00 (s, 2H); 3,98 (s, 2H); 2.52 (d, 2H; *J* = 7.2 Hz); 1.48 (m, 9H); 1.14 (q, 2H; *J* = 37.2 Hz). ^{13}C NMR: (100 MHz, $CDCl_3$, δ ppm): 149.70, 149.34, 139.33, 135.88, 128.01, 126.17, 121.10, 118.67, 118.22, 84.05, 58.49, 51.05, 36.34, 31.38, 26.72, 26.01. ES-MS: 283 $[M+H]^+$. Elemental Analysis for $C_{18}H_{22}N_2O$: calcd. C, 76.56, H, 7.85, N, 9.92, O, 5.67; found C, 76.79, H, 7.91, N 9.76. Yield = 48%.

2-Cycloheptyl-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (38)

Column chromatography: PE/EtOAc (50:50). ^1H NMR: (400 MHz, $CDCl_3$, δ ppm): 8.77 (d, 1H; *J* = 3.6 Hz); 7.92 (d, 1H; *J* = 8.4 Hz); 7.22-7.11 (m, 3H); 5.11 (s, 2H); 4.08 (s, 2H); 2.89 (d, 1H; *J* = 8.8 Hz); 1.38 (m, 12H). ^{13}C NMR :(100 MHz, $CDCl_3$, δ ppm): 149.35, 135.85, 127.89, 125.58, 121.05, 118.63, 81.74, 62.56, 47.73, 32.97, 27.86, 24.65. ES-MS: 283 $[M+H]^+$, 305 $[M+Na]^+$. Elemental Analysis for $C_{18}H_{22}N_2O$: calcd. C, 76.56, H, 7.85, N, 9.92, O, 5.67; found C, 76.60, H, 7.88, N, 9.89. Yield = 87%.

2-Cyclooctyl-2,3-dihydro-1*H*-4-oxa-2,5-diazaphenanthrene (39)

Column chromatography: CH_2Cl_2/MeOH (95:5). ^1H NMR:(400 MHz, $CDCl_3$, δ ppm): 8.76 (brs, 1H); 7.80 (d, 1H; *J* = 8.4 Hz); 7.30-7.23 (m, 2H); 7.15 (d, 1H; *J* =8 Hz); 5.24 (s, 2H); 4.15 (s, 2H); 2.79 (brs, 1H); 1.65-1.40 (m, 14H). ^{13}C NMR: (100 MHz, $CDCl_3$, δ ppm): 147.87; 138.77; 134.97; 128.14; 127.94; 127.67; 120.81; 117.05; 116.37; 76.42; 57.76; 47.08; 30.58; 26.60; 23.45. Elemental Analysis for $C_{19}H_{24}N_2O$: calcd. C, 76.99; H, 8.16; N, 9.45; O, 5.40; found C, 76.66; H, 8.28; N, 9.63. Yield = 15%.

2-Phenethyl-1,2-dihydro-3*H*-4-oxa-2,5-diazaphenanthren-3-one (42)

^1H NMR:(400 MHz, $CDCl_3$, δ ppm): 8.97 (dt, *J* = 4.2, 1.9 Hz, 1H), 8.12 – 8.05 (m, 1H), 7.53-7.38 (m, 2H), 7.24 (t, *J* = 2.9 Hz, 4H), 7.18 (tt, *J* = 6.2, 2.6 Hz, 1H), 7.07 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.42 (d, *J* = 2.1 Hz, 2H), 3.76-3.68 (m, 2H), 3.07 – 2.98 (m, 2H). ^{13}C NMR: (100 MHz, $CDCl_3$, δ ppm): 150.46, 135.25, 128.42, 128.25, 126.19, 122.88, 122.55, 121.58, 50.95, 48.39, 32.76. ES-MS: 305 $[M+H]^+$. Elemental Analysis for $C_{19}H_{16}N_2O_2$: calcd. C, 74.98, H, 5.30, N, 9.20, O, 10.51; found C, 75.15, H, 5.51, N, 9.55. Yield = 12%.

1-(2,4-Dichlorophenyl)-2-phenethyl-1,2-dihydro-3*H*-4-oxa-2,5-diazaphenanthren-3-one (43)

^1H NMR:(400 MHz, $CDCl_3$, δ ppm): 9.02 (dd, *J* = 4.3, 1.6 Hz, 1H), 8.08 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.50 – 7.37 (m, 3H), 7.26 – 7.13 (m, 7H), 7.01 (d, *J* = 8.5 Hz, 1H), 5.95 (s, 1H), 3.99 (dq, *J* = 12.1, 6.4 Hz, 1H), 3.04 (dt, *J* = 13.0, 6.5 Hz, 2H), 2.82 (tq, *J* = 13.0, 5.7 Hz, 1H). ^{13}C NMR: (100 MHz, $CDCl_3$, δ ppm): 150.68, 135.30, 130.19, 129.33, 128.45, 128.34, 128.21, 126.20, 123.43, 122.71, 122.01, 58.27, 49.18, 33.07. ES-MS: 450 $[M+H]^+$. Elemental Analysis for $C_{25}H_{18}Cl_2N_2O_2$: calcd. C, 66.83, Cl, 15.78, H, 4.04, N, 6.23, O, 7.12; found C, 66.99, Cl, 15.91, H, 4.26, N, 6.48. Yield = 15%.

Biology

Cell lines and treatments

Human melanoma cells A375 (CRL-1619), medulloblastoma cells Daoy (HTB-186), and murine NIH3T3 cells (CRL-1658) were obtained from ATCC (Manassas, VA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Euroclone, Milan, Italy) (A375 and NIH3T3) or Eagle's Minimum Essential Medium (EMEM) (Euroclone) (Daoy) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, 1% glutamine (Lonza, Basel, Switzerland). Cells were periodically screened for mycoplasma contamination by PCR. A375 and Daoy cells were plated in 24-well or 96-well plates, treated with increasing concentrations of each compound for 72 h in complete medium supplemented with 1% FBS and viable cells counted by trypan blue-exclusion or crystal violet staining. All experimental points were set up in triplicate wells and all experiments were repeated at least three times. IC_{50} were calculated using GraphPad Prism version 6.0 (GraphPad software).

Western blot analysis

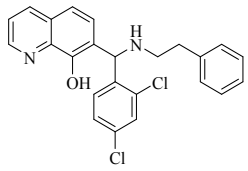
Cells were lysed in ice-cold RIPA buffer supplemented with protease and phosphatase inhibitors, centrifuged at 4 °C, and supernatant was collected, as previously described [6,7]. Equal amounts of protein were resolved by SDS polyacrylamide gel electrophoresis, transferred into nitrocellulose membranes, and incubated in blocking buffer. The following antibodies were used: mouse anti-GLI1 (#2643) (Cell Signaling Technology, Danvers, MA), goat anti-GLI2 (AF-3635, R&D Systems, Minneapolis, MN), mouse anti-HSP90 (sc-13119) (Santa Cruz Biotechnology, Santa Cruz, CA), and the anti-mouse HRP-conjugated (Cell Signaling). Blotted membranes were developed using SuperSignal West Femto (ThermoFisher Scientific, Italy) and imaged with ChemiDoc™ Imaging Systems (Bio-Rad, Italy).

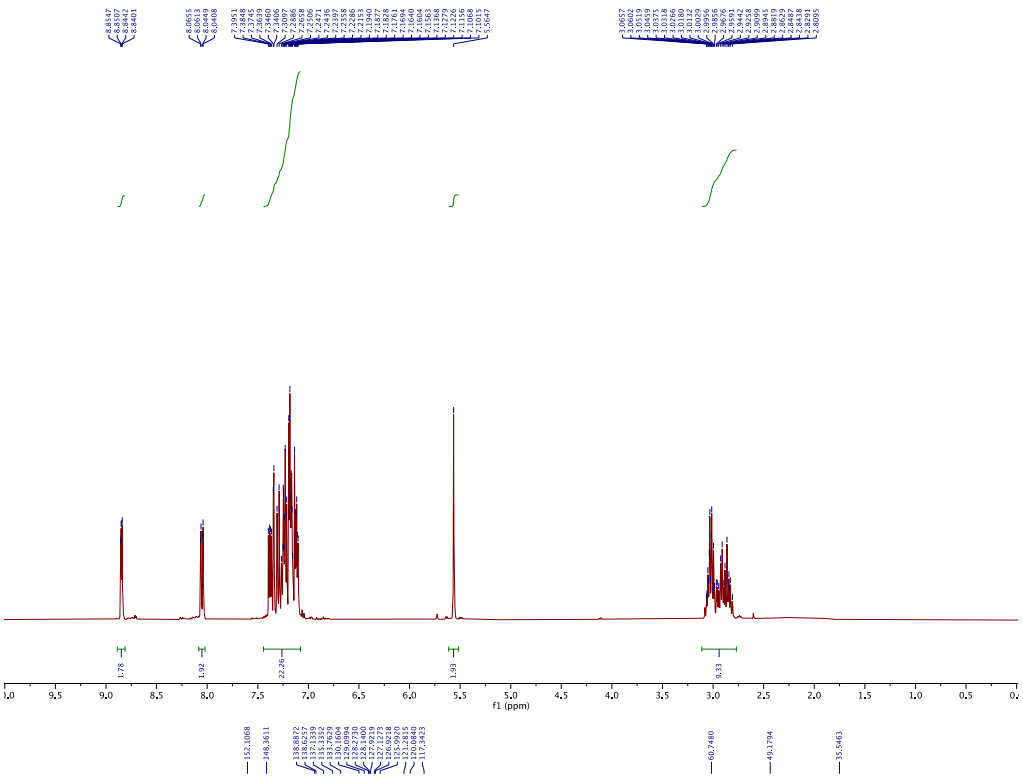
Statistical analysis

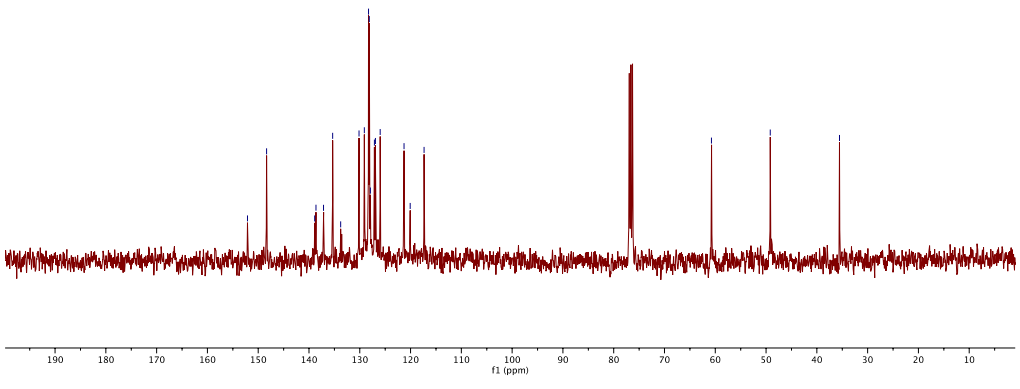
Data represent mean ± SD or mean ± SEM values calculated on at least 3 independent experiments. p Values were calculated using Student's t-test or one-way ANOVA. A two-tailed value of $p<0.05$ was considered statistically significant.

^{1}H and ^{13}C NMR of synthesized compounds.

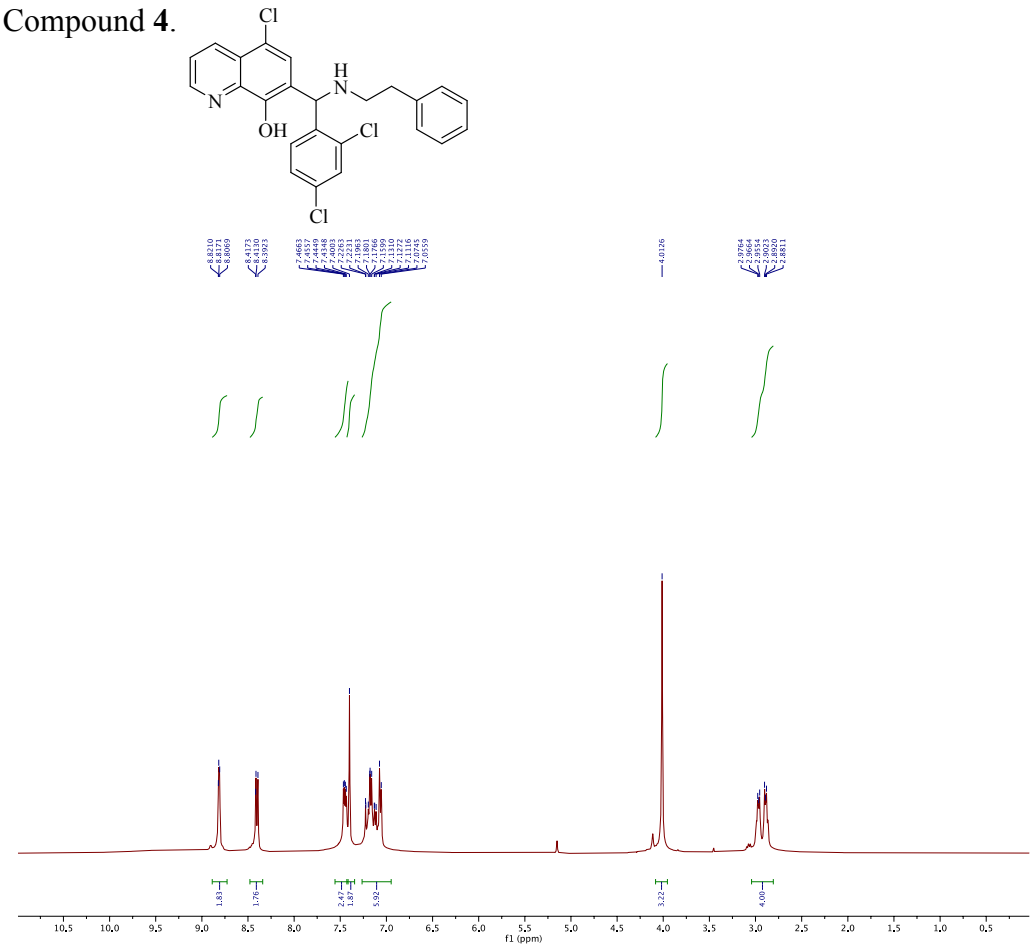
Compound **2**.



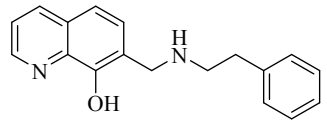


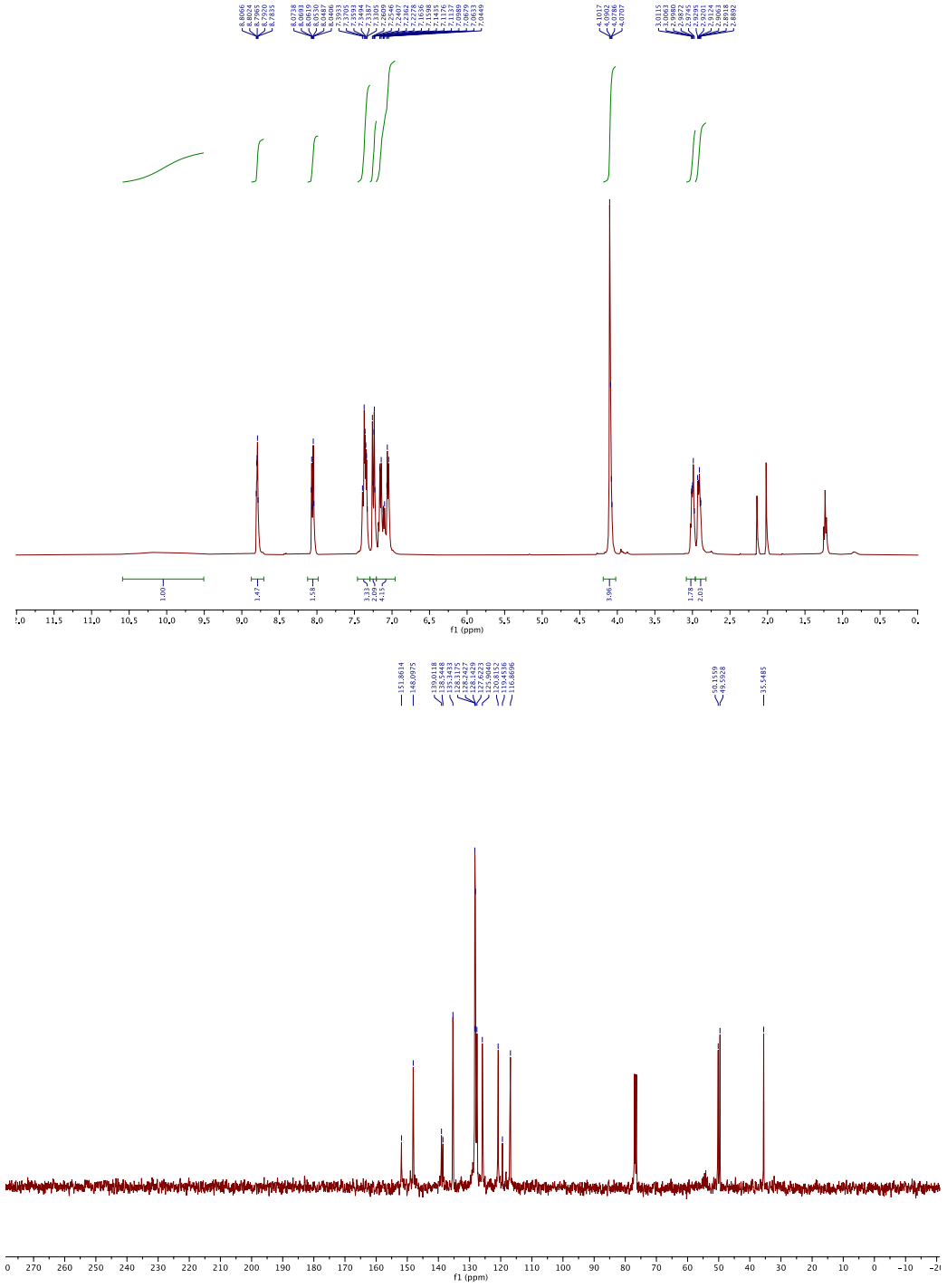


Compound **4**.



Compound **5**.





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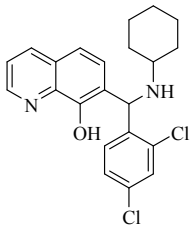
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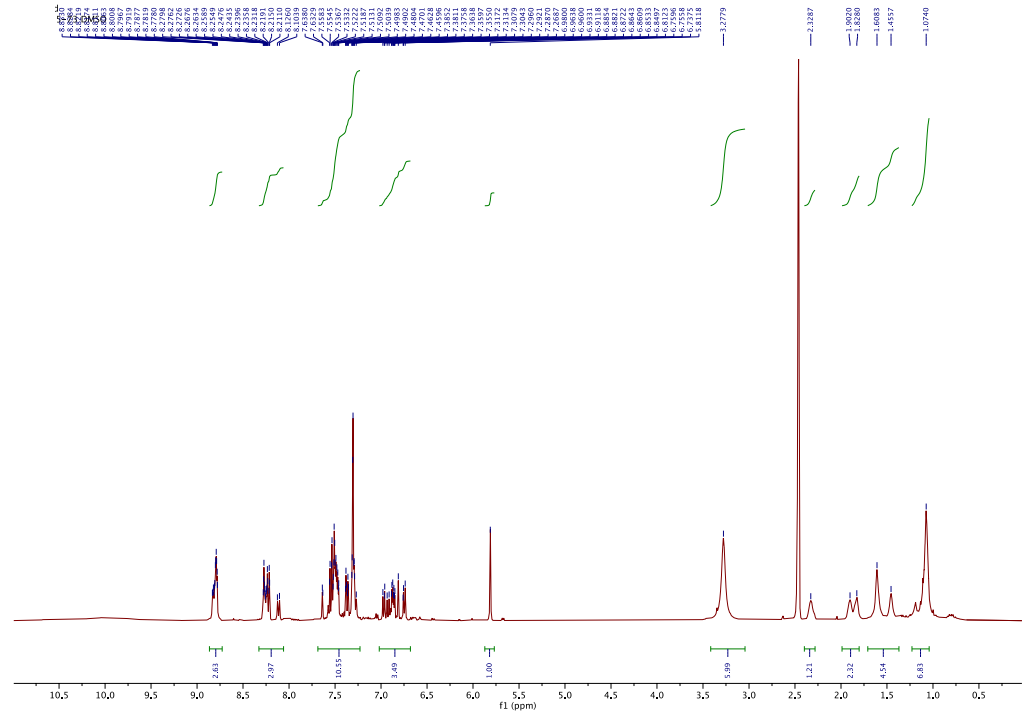
N
OH
Cl
Cl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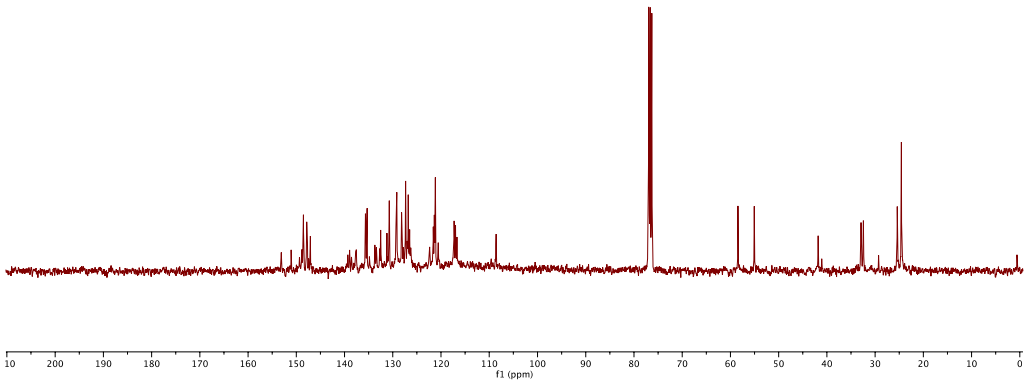
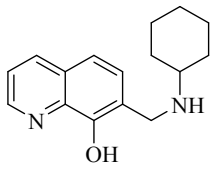
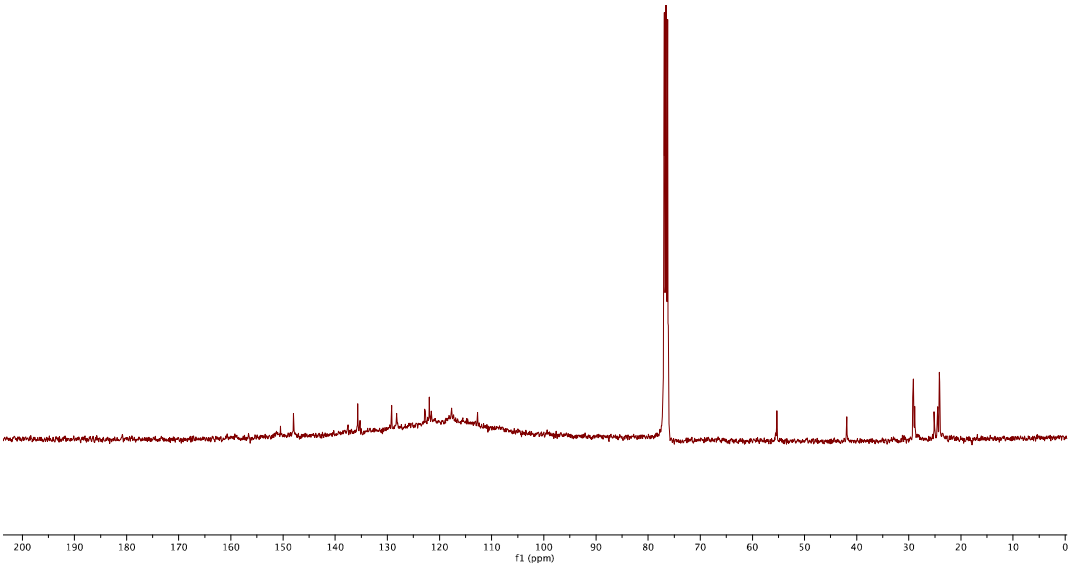
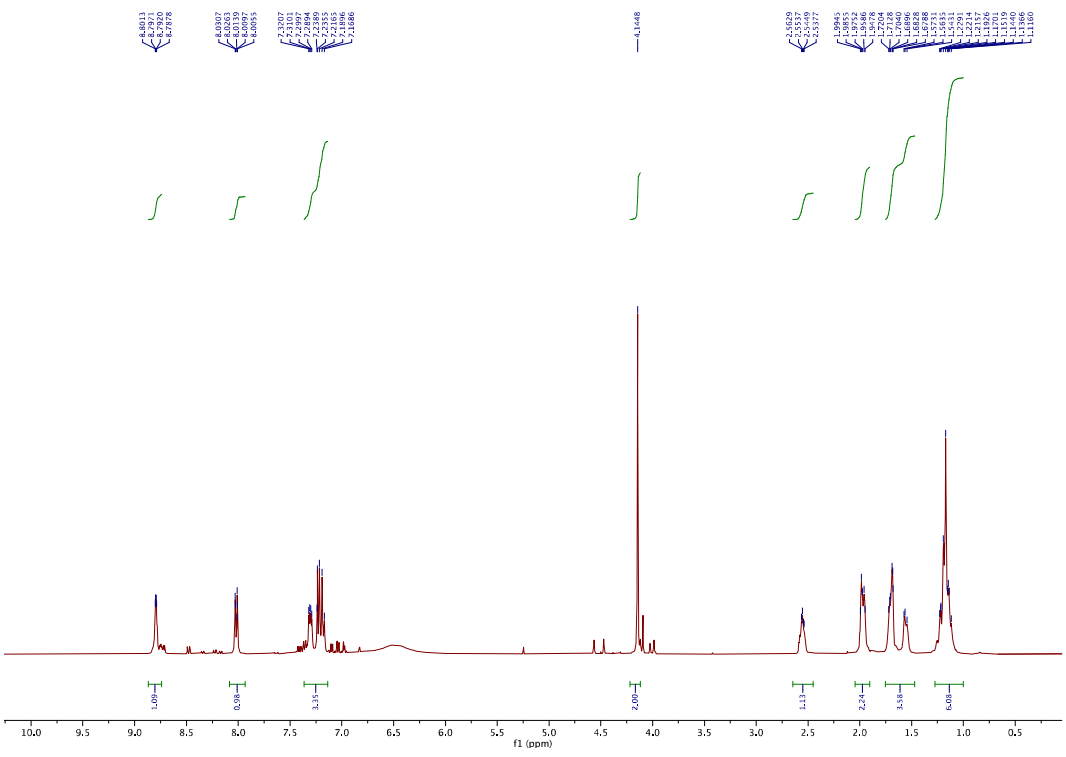

f1 (ppm)

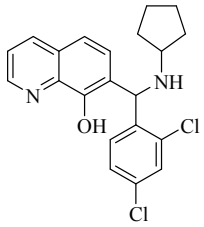
Compound **19**.

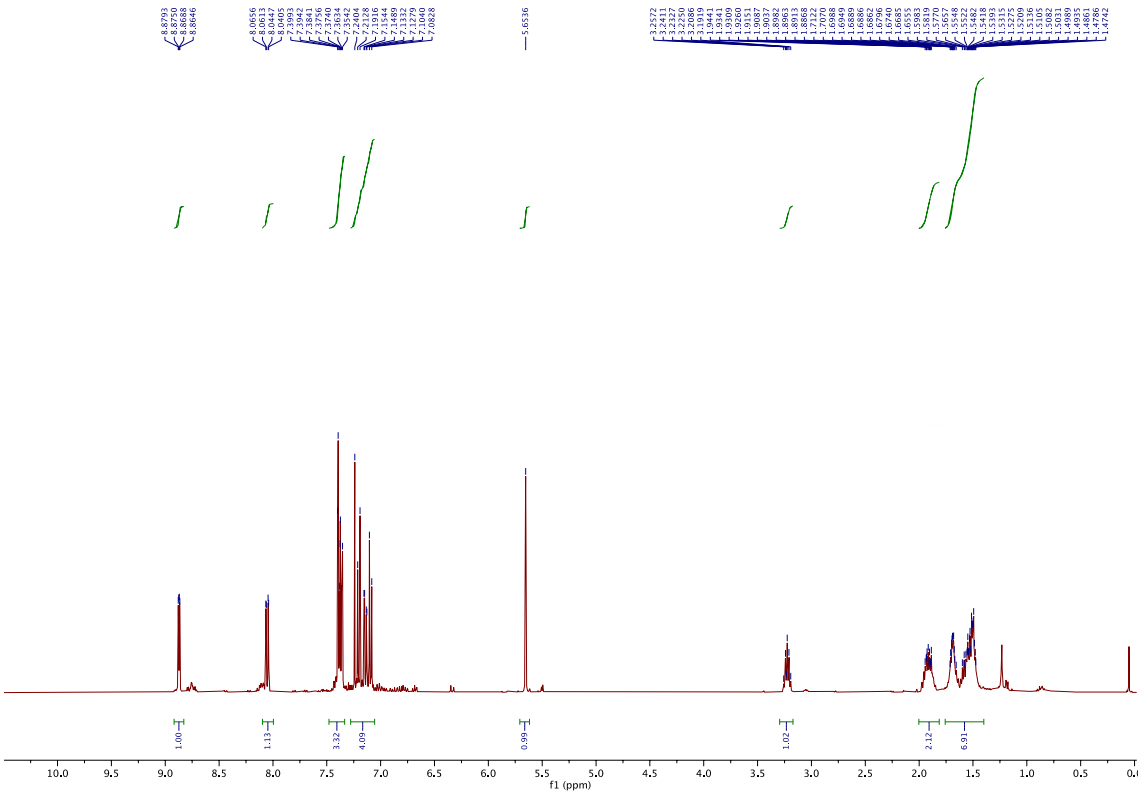


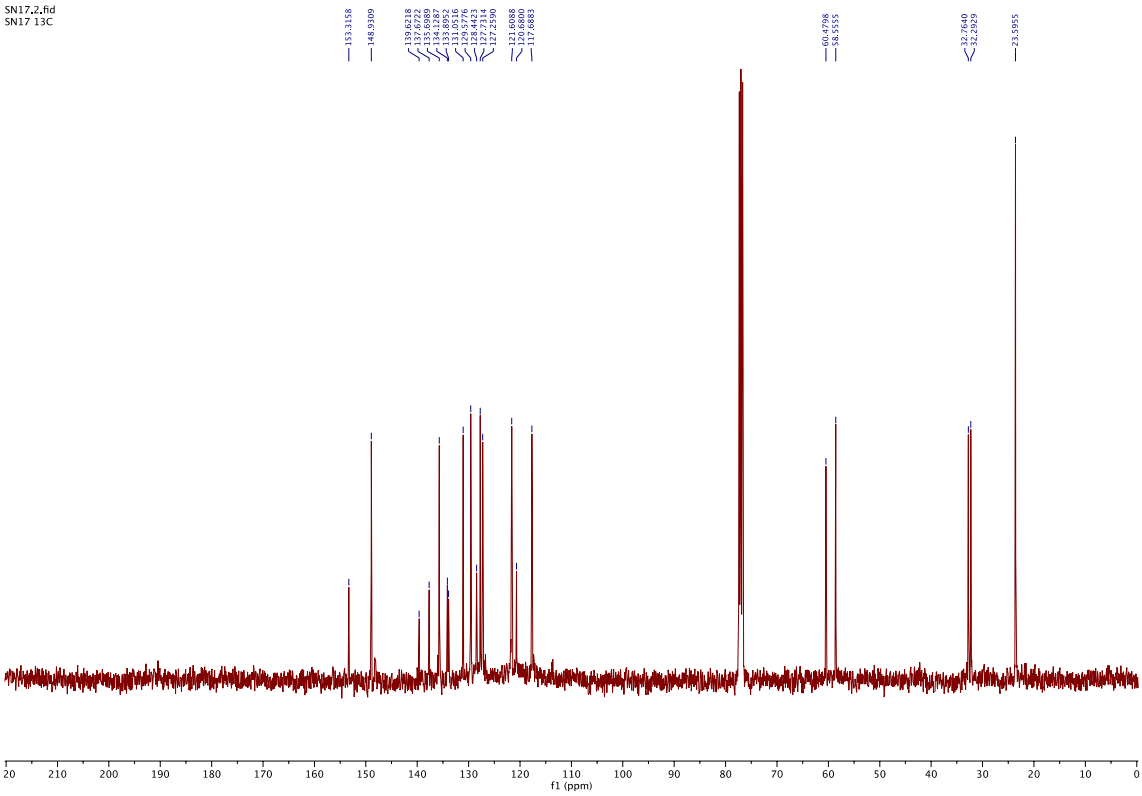


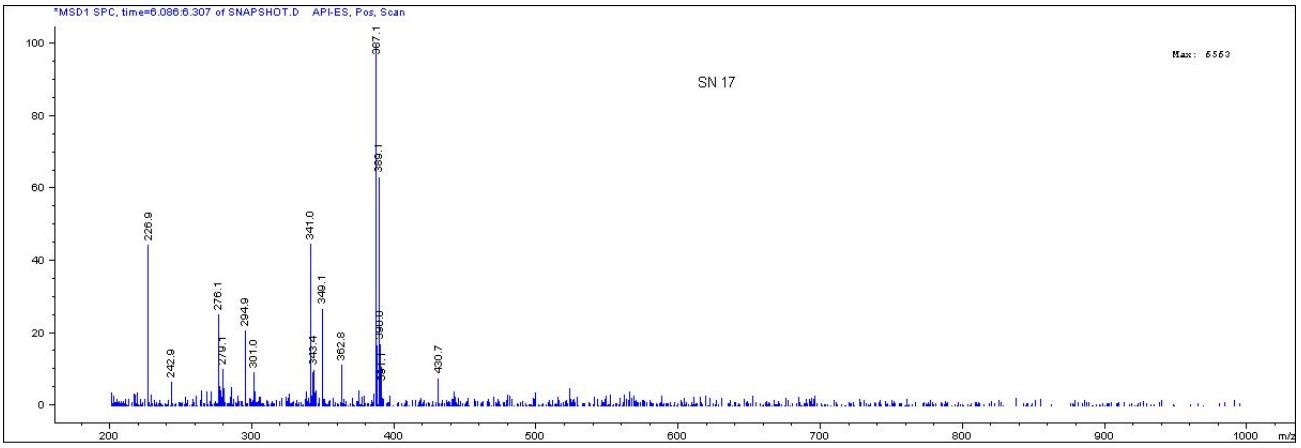


Compound **16**.





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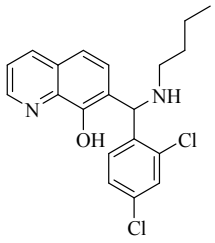
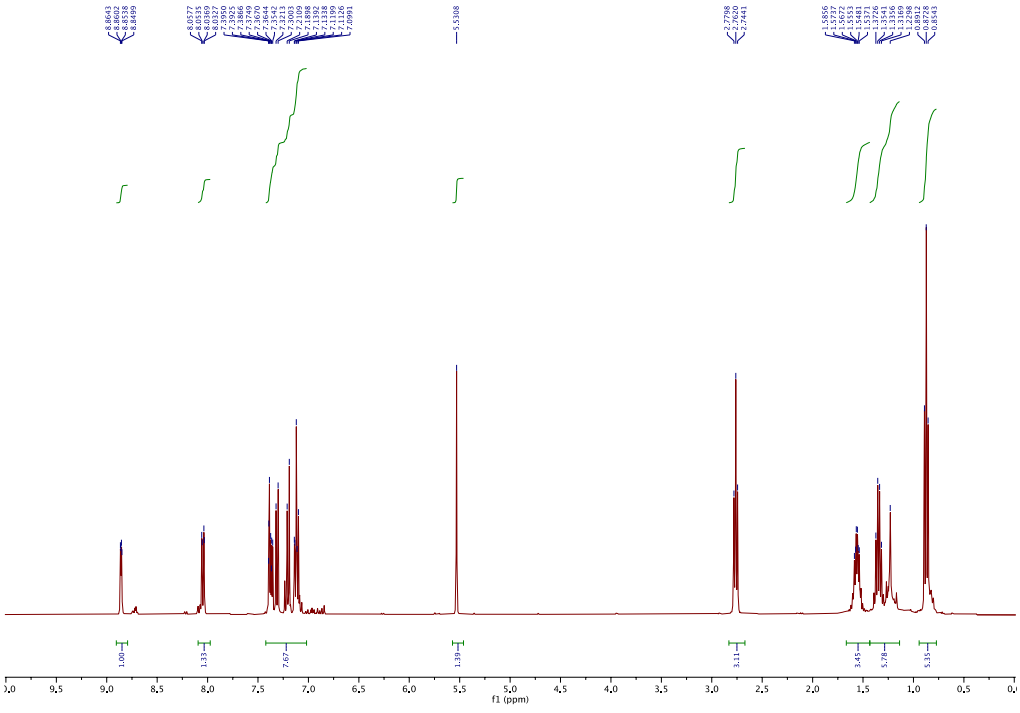
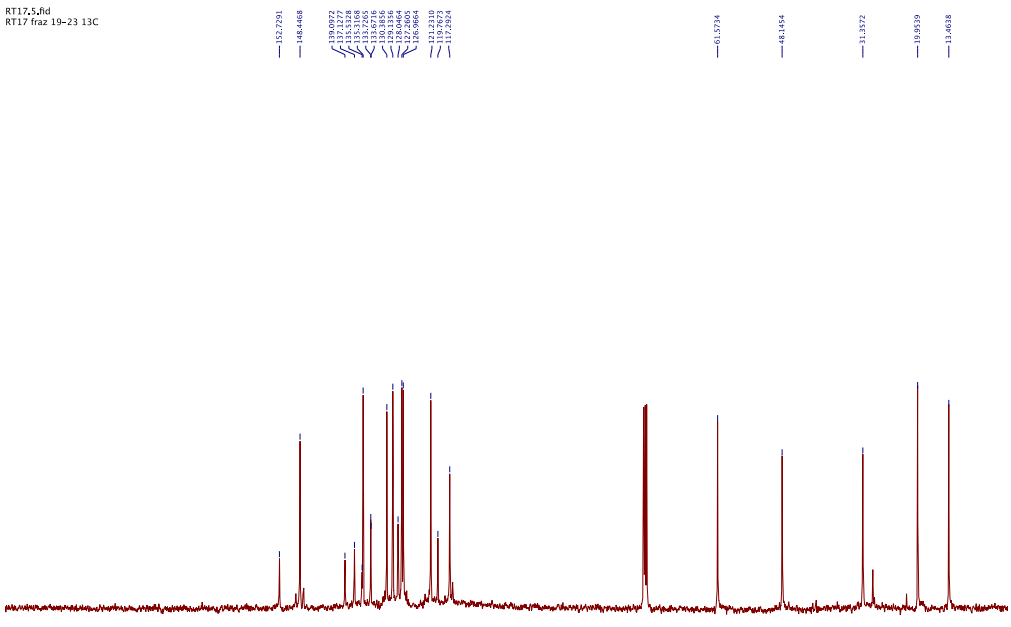
242.9
279.1
343.4
362.8
430.7
m/z

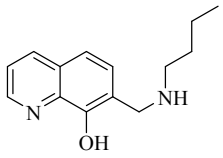
Compound **17**.

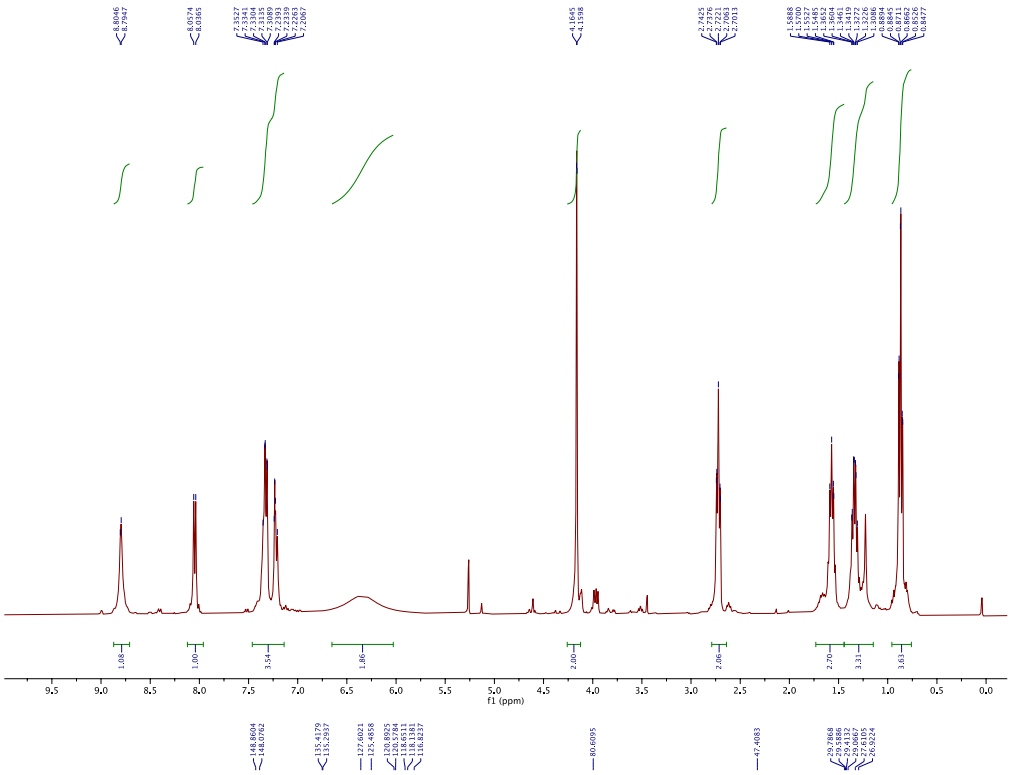


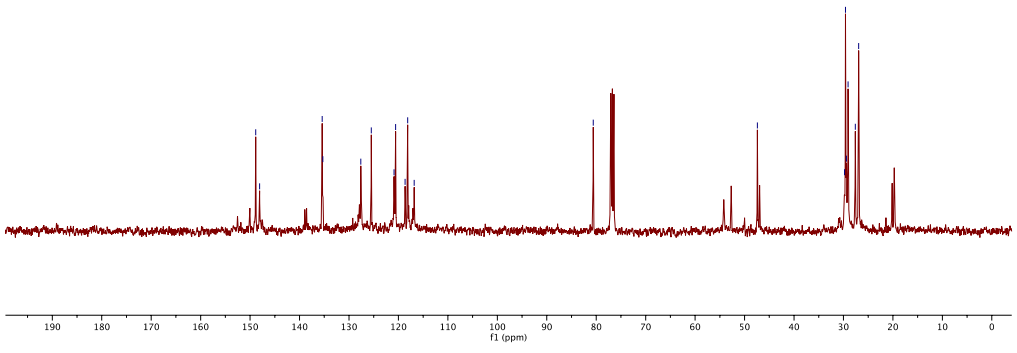




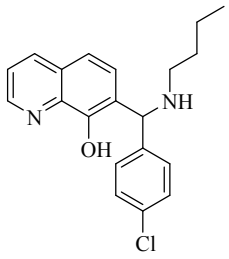
Compound **18**.

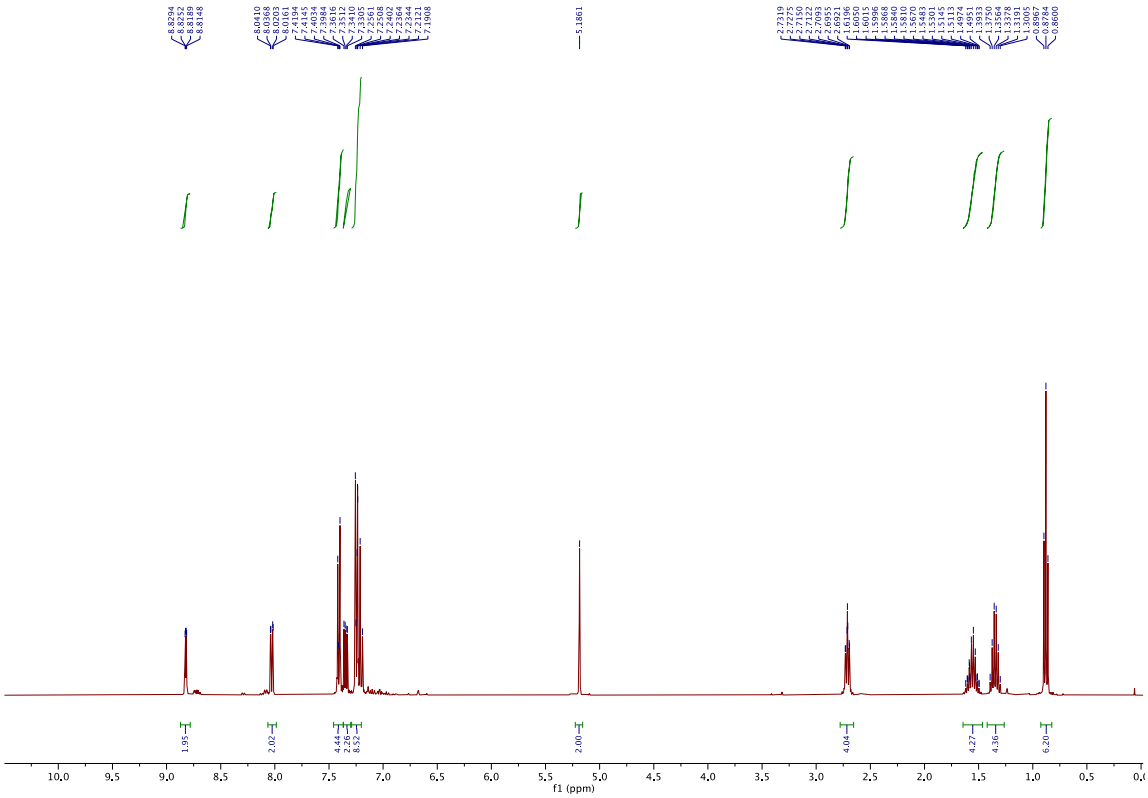


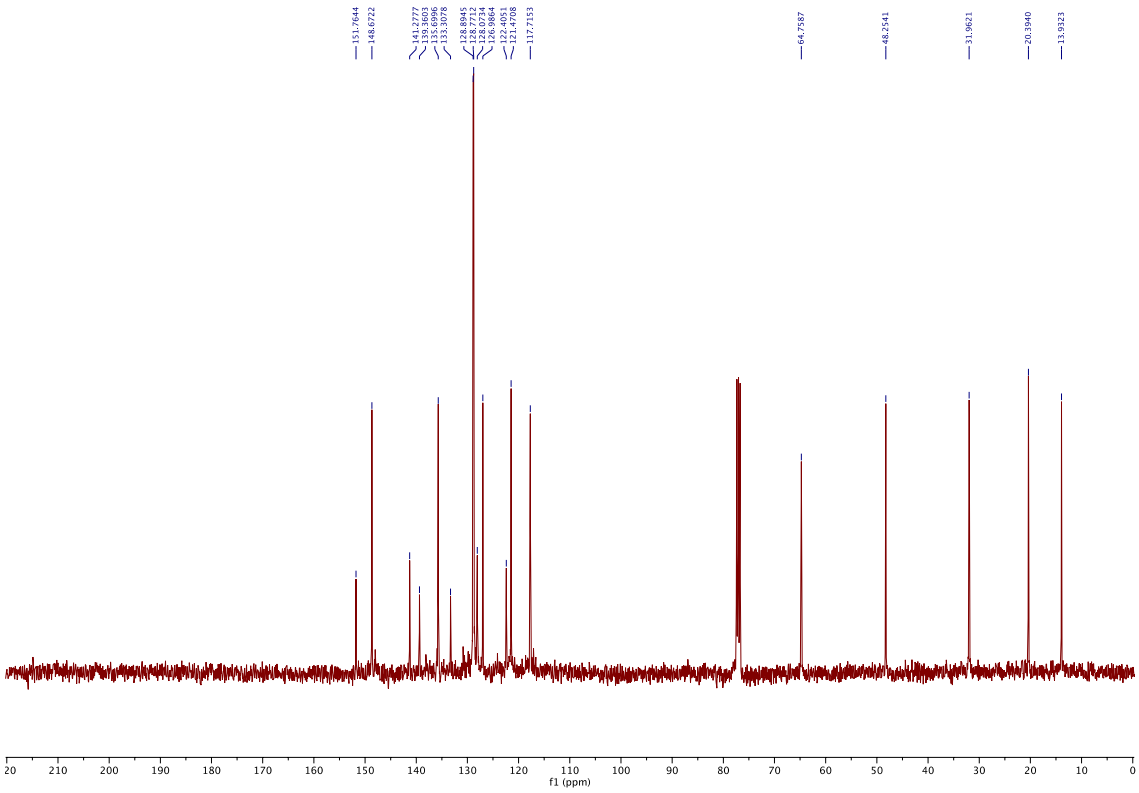


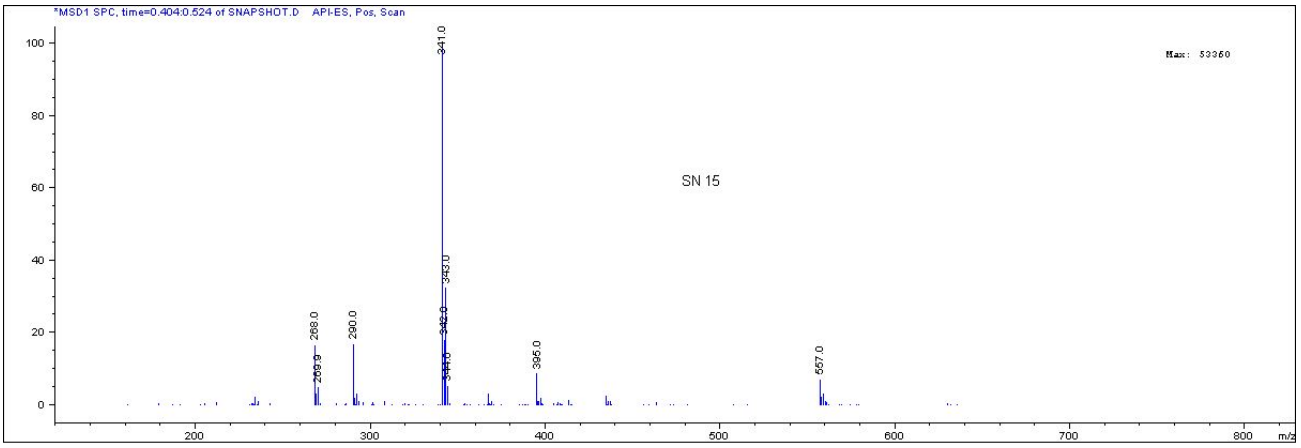


Compound **20**.

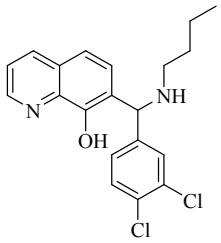


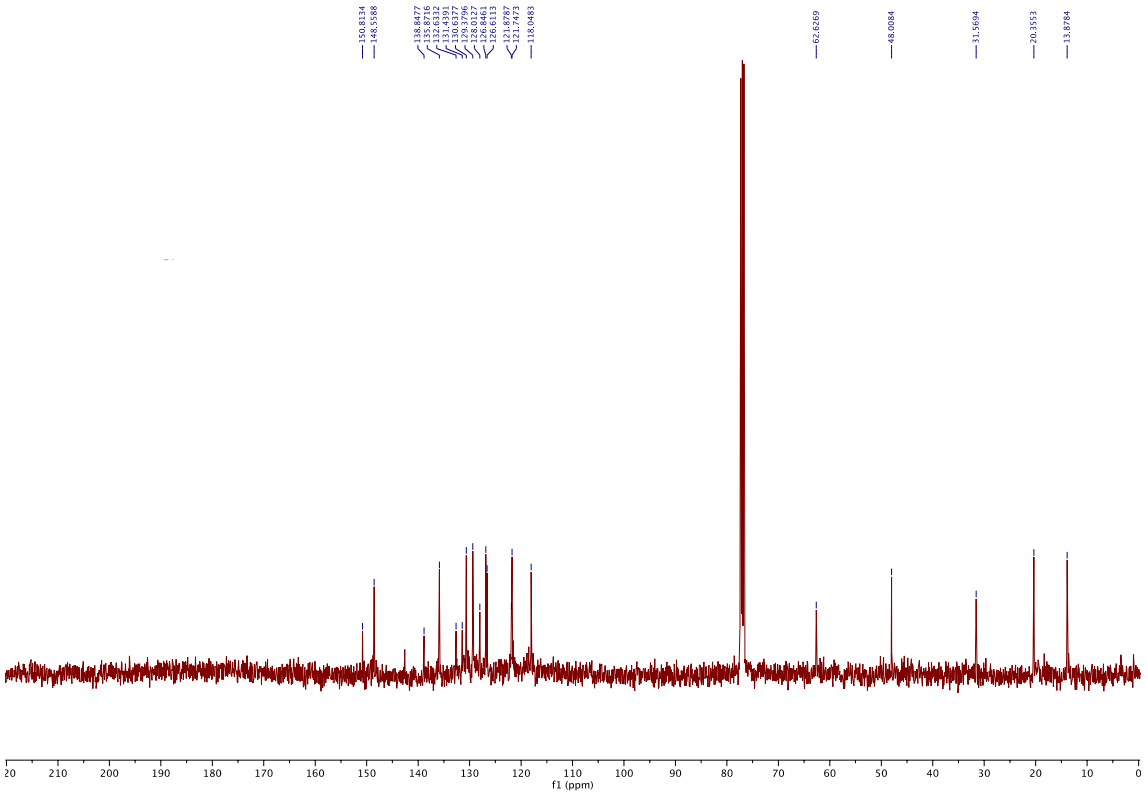


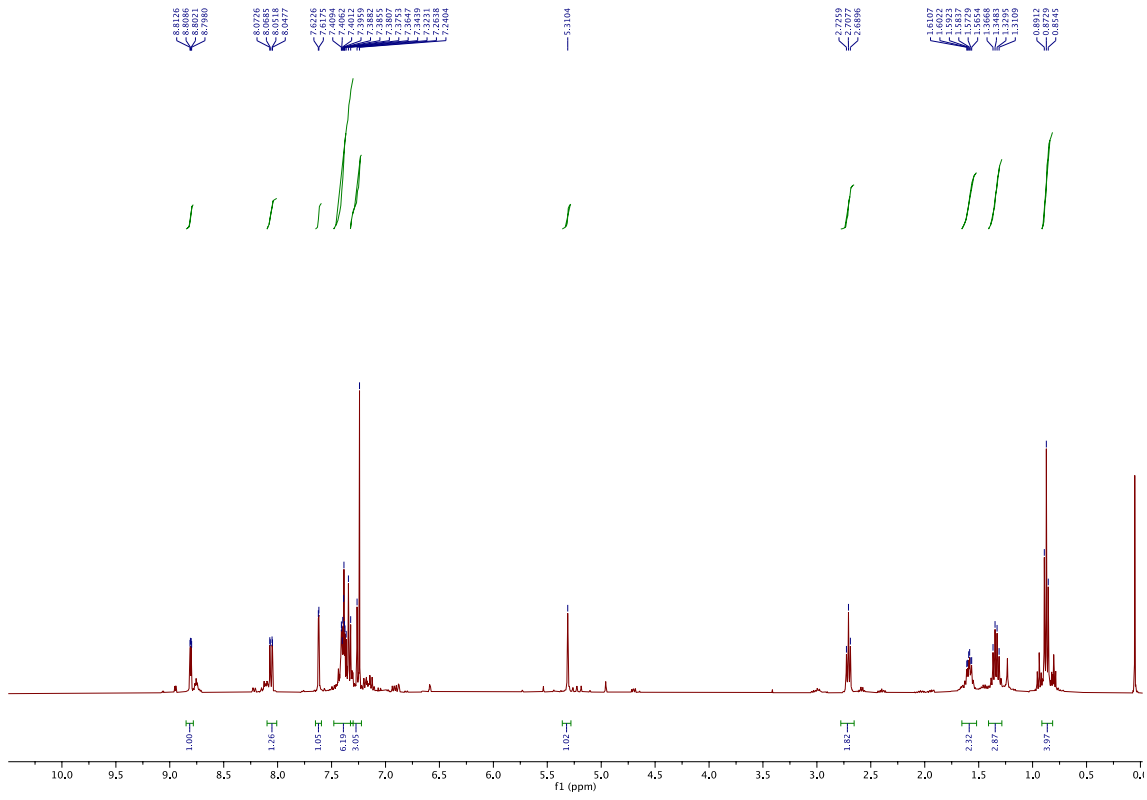

151.7644
148.6722
141.2277
139.3603
135.6996
133.3078
128.8945
128.7712
128.6704
126.9864
122.4051
121.4708
117.7153
64.7587
48.2541
31.9621
20.3940
13.9323
f1 (ppm)

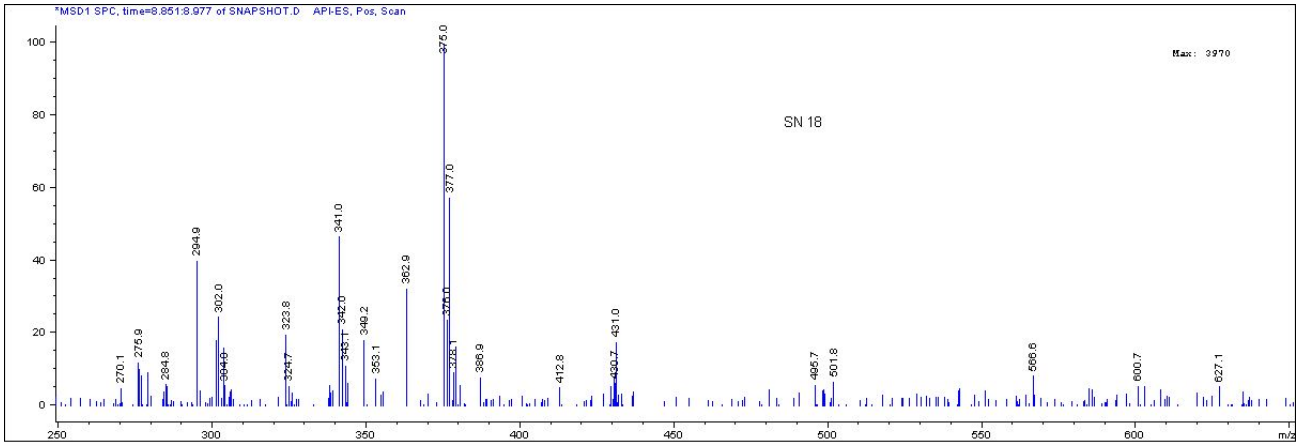

*MSD1 SPC, time=0.404:0.524 of SNAPSHOT.D API-ES, Pos, Scan
Max: 53360
SN 15
341.0
343.0
342.0
344.0
268.0
269.9
290.0
395.0
557.0
m/z

Compound **21**.

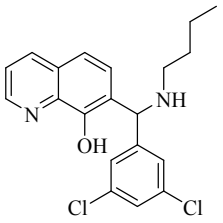

NH
N
OH
Cl
Cl

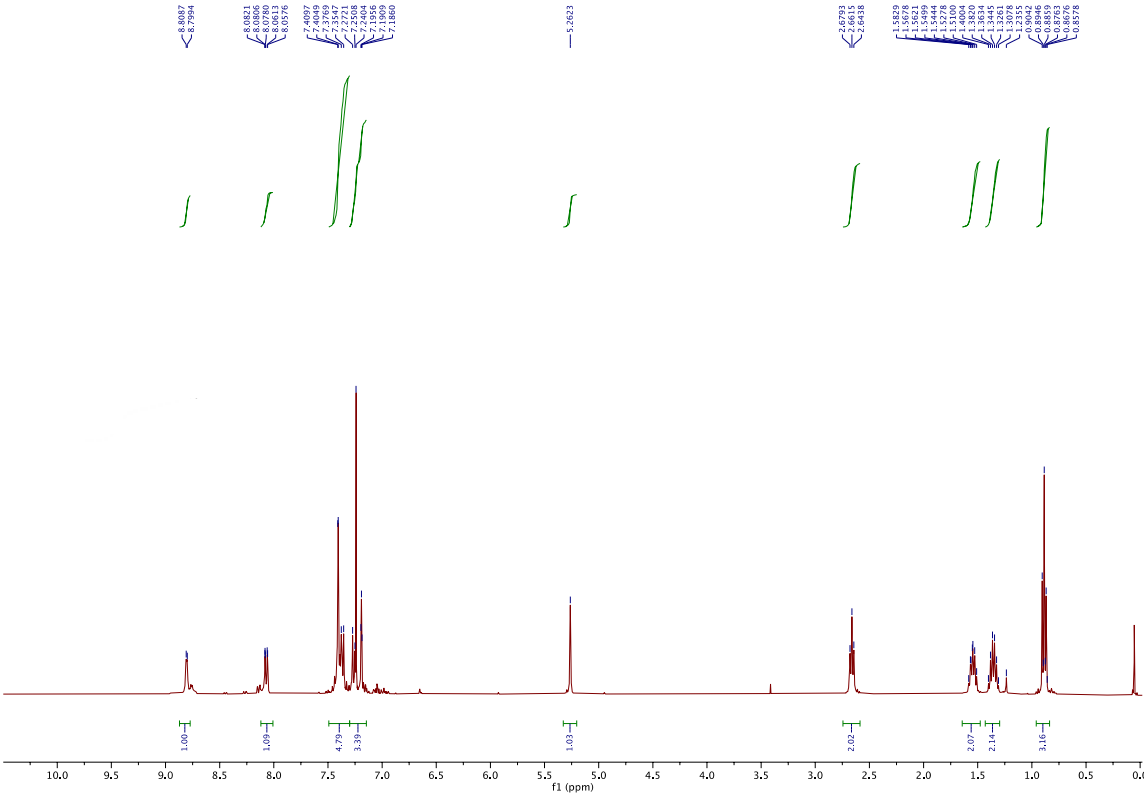

150.8134
148.5588
138.8477
135.8716
132.6332
131.4301
130.6377
129.3796
128.8027
126.8461
126.6113
121.8787
121.7425
118.0483
62.6269
48.0084
31.5694
20.1553
13.8784
f1 (ppm)

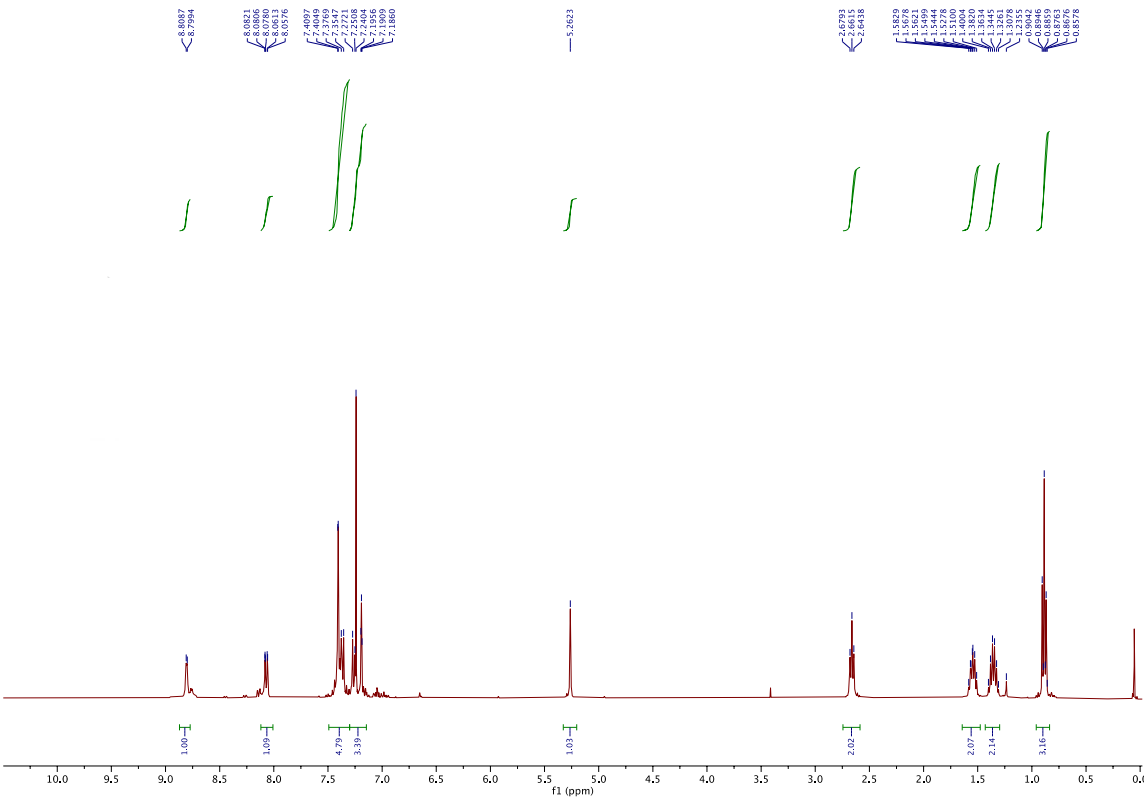

8.8126
8.8086
8.8021
8.7980
8.0726
8.0685
8.0518
8.0477
7.6226
7.6175
7.4094
7.4062
7.3959
7.3882
7.3855
7.3800
7.3753
7.3447
7.3349
7.3231
7.2838
7.2644
5.3104
2.7259
2.7077
2.6896
1.6107
1.5922
1.5837
1.5679
1.5564
1.3668
1.3483
1.3305
1.3109
0.8912
0.8729
0.8545
1.00
1.26
1.05
6.19
3.05
1.02
1.82
2.32
2.87
3.97
f1 (ppm)

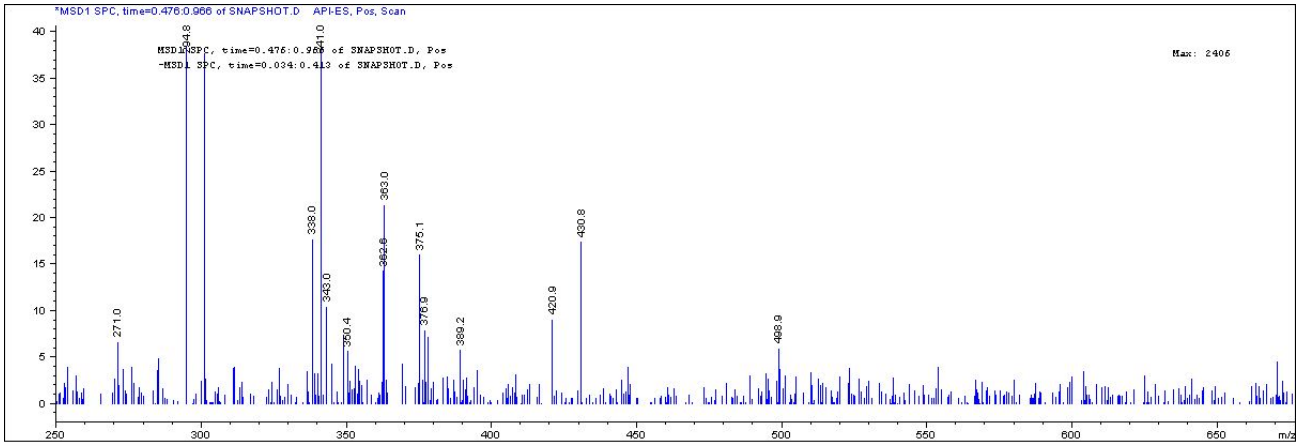

*MSD1 SPC, time=8.851:8.977 of SNAPSHOT.D API-ES, Pos, Scan
Max: 3970
SN 18
375.0
377.0
341.0
294.9
362.9
302.0
323.8
342.0
349.2
343.1
353.1
324.7
304.0
270.1
275.9
284.8
376.0
378.1
386.9
412.8
430.7
431.0
495.7
501.8
566.6
600.7
627.1
m/z

Compound **22**.

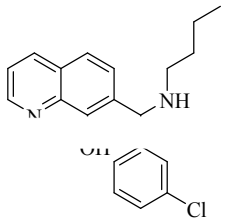


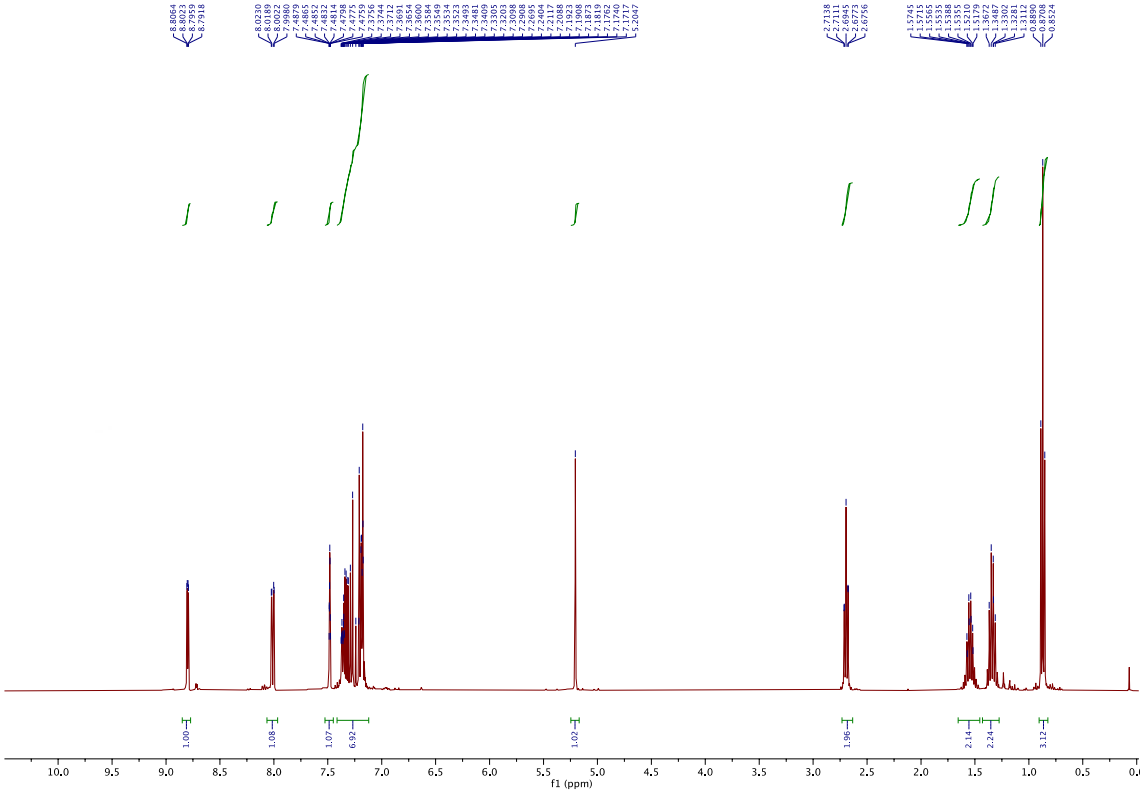


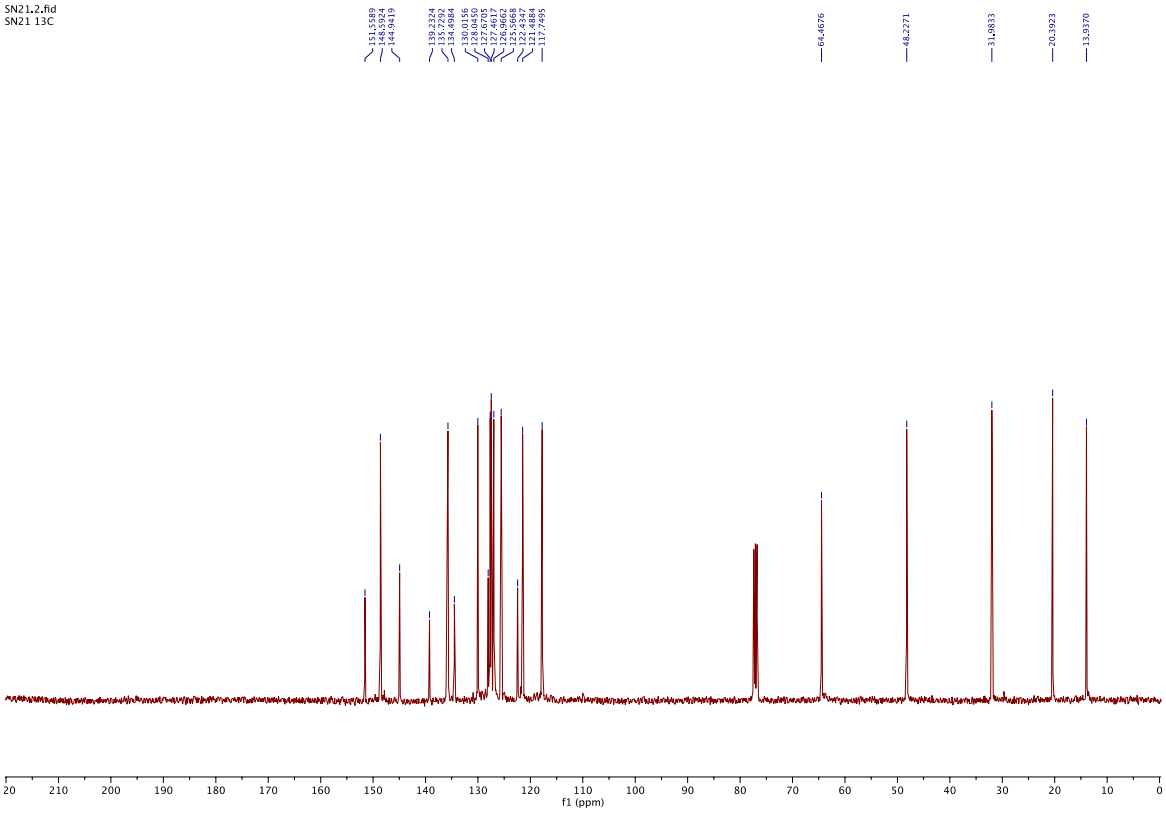


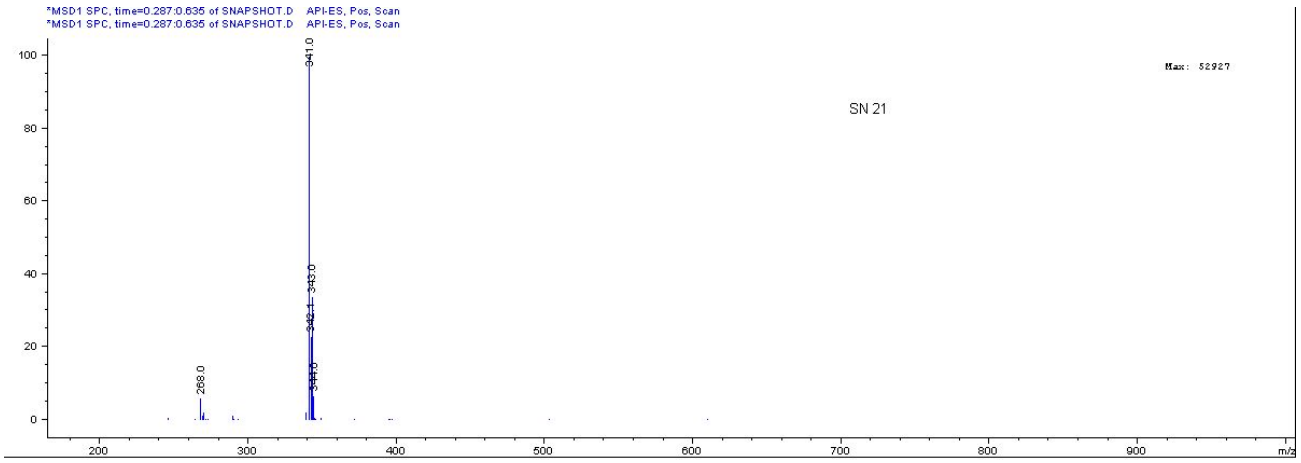

*MSD1 SPC, time=0.476:0.966 of SNAPSHOT.D API-ES, Pos, Scan
-MSD1 SPC, time=0.034:0.413 of SNAPSHOT.D, Pos
Max: 2406
294.8
341.0
338.0
363.0
375.1
343.0
350.4
376.9
389.2
420.9
430.8
271.0
498.9
m/z

Com

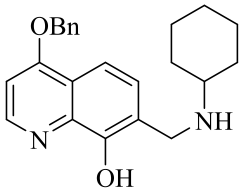


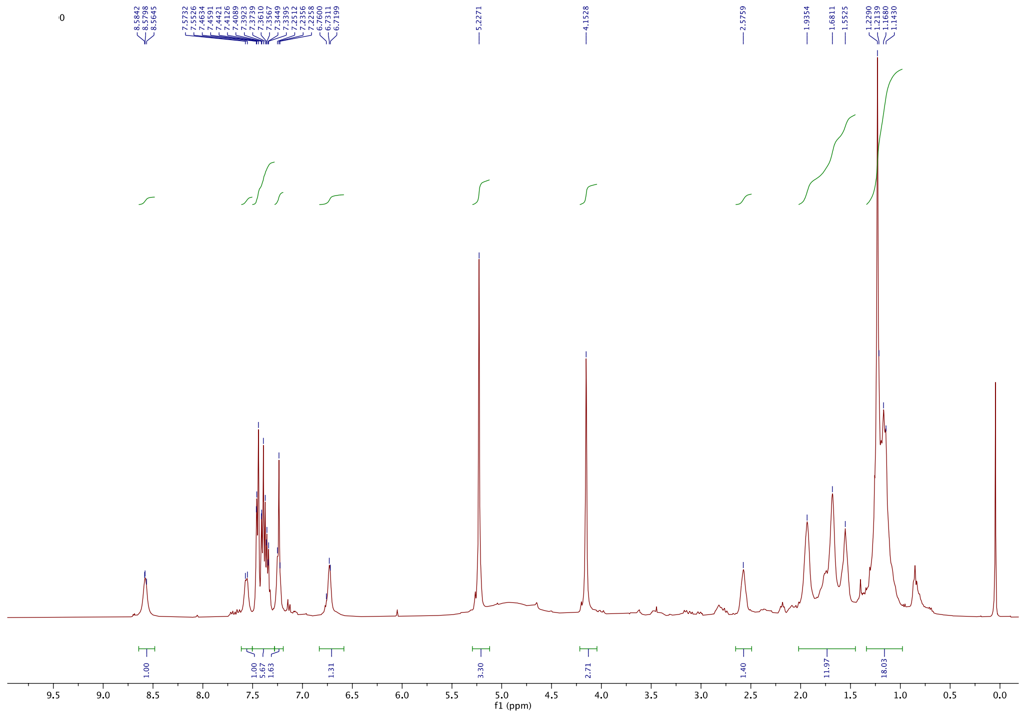


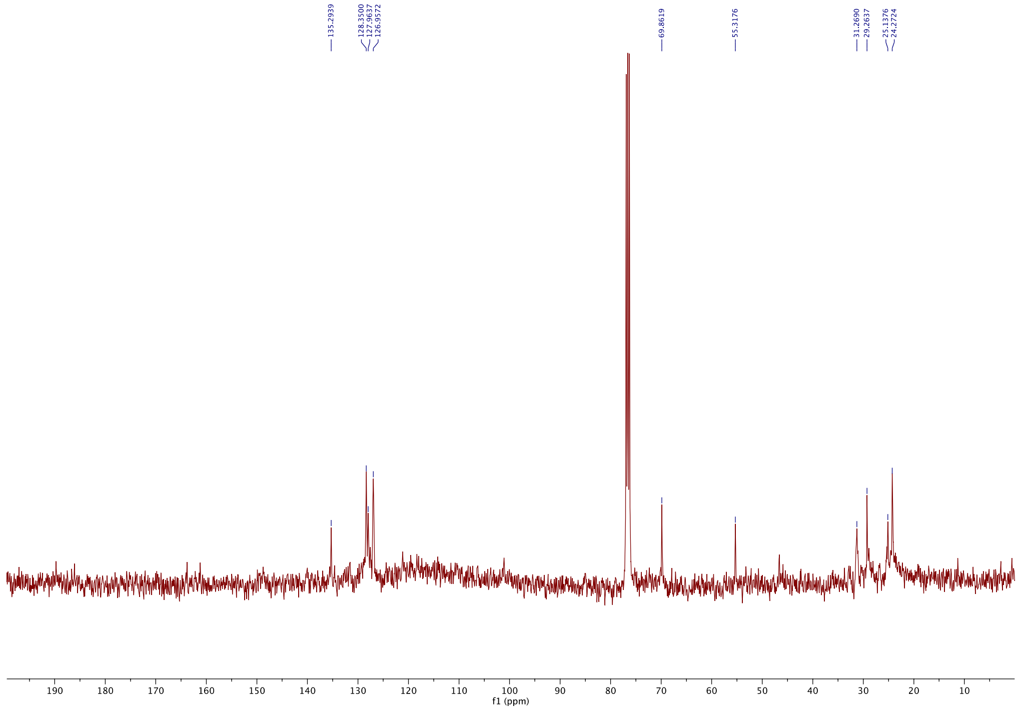



*MSD1 SPC, time=0.287:0.635 of SNAPSHOT.D API-ES, Pos, Scan
*MSD1 SPC, time=0.287:0.635 of SNAPSHOT.D API-ES, Pos, Scan
Max: 52927
SN 21
341.0
343.0
342.1
344.0
268.0
100
80
60
40
20
0
200
300
400
500
600
700
800
900
m/z

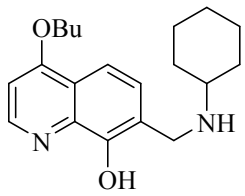
Compound **25**.

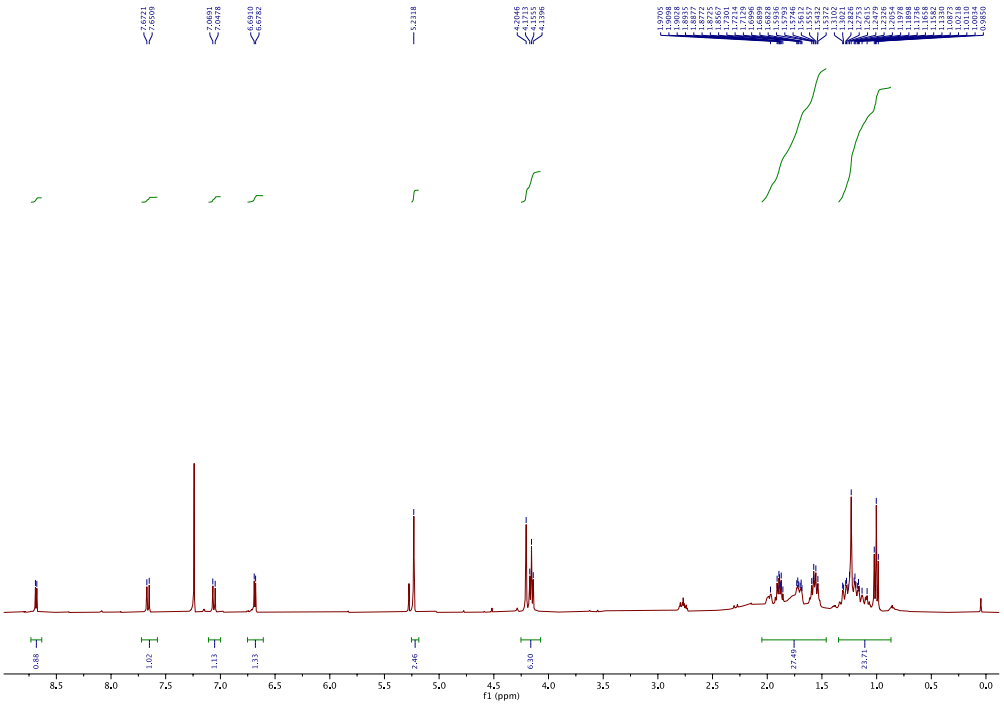




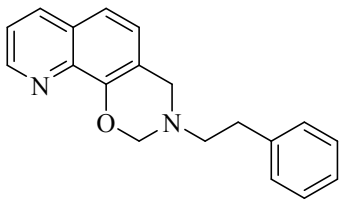


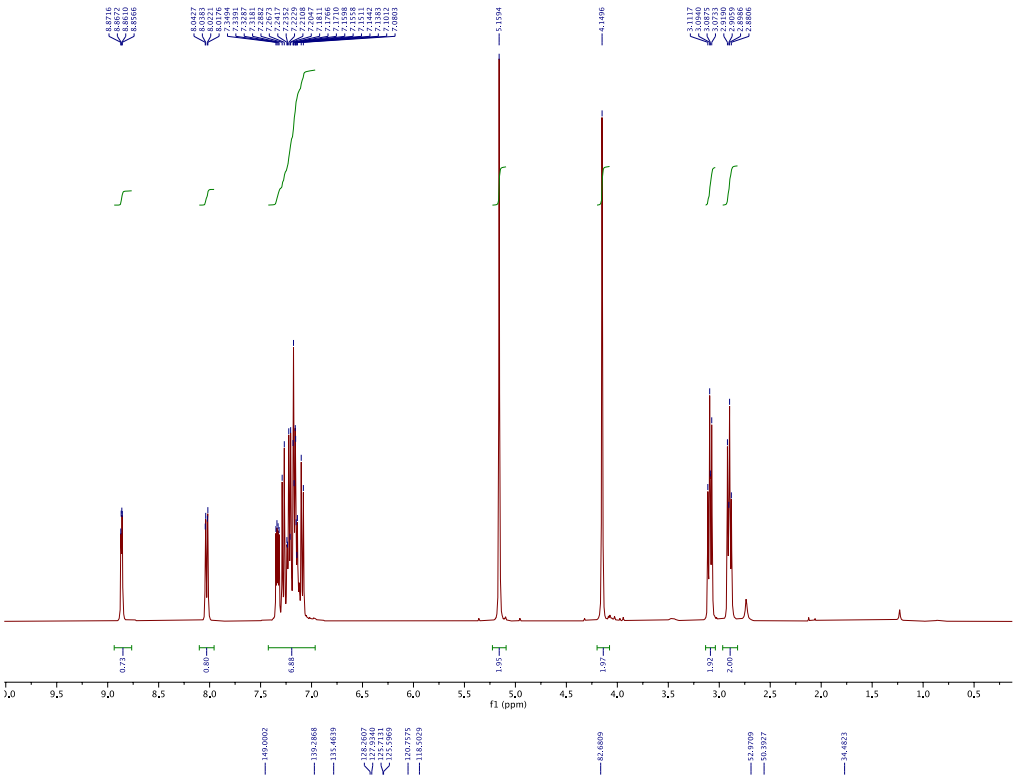
Compound **24**.

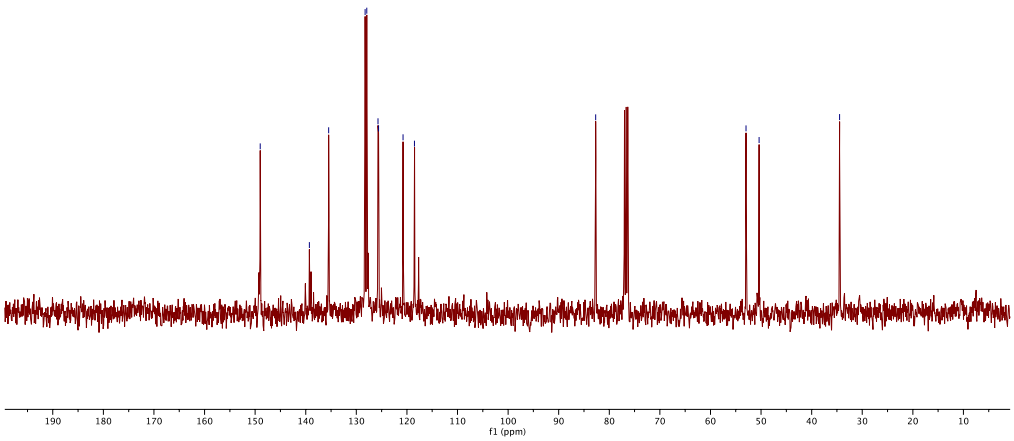




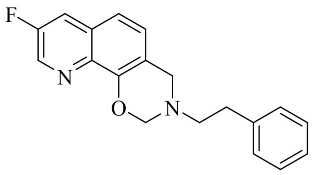
Compound **26**.

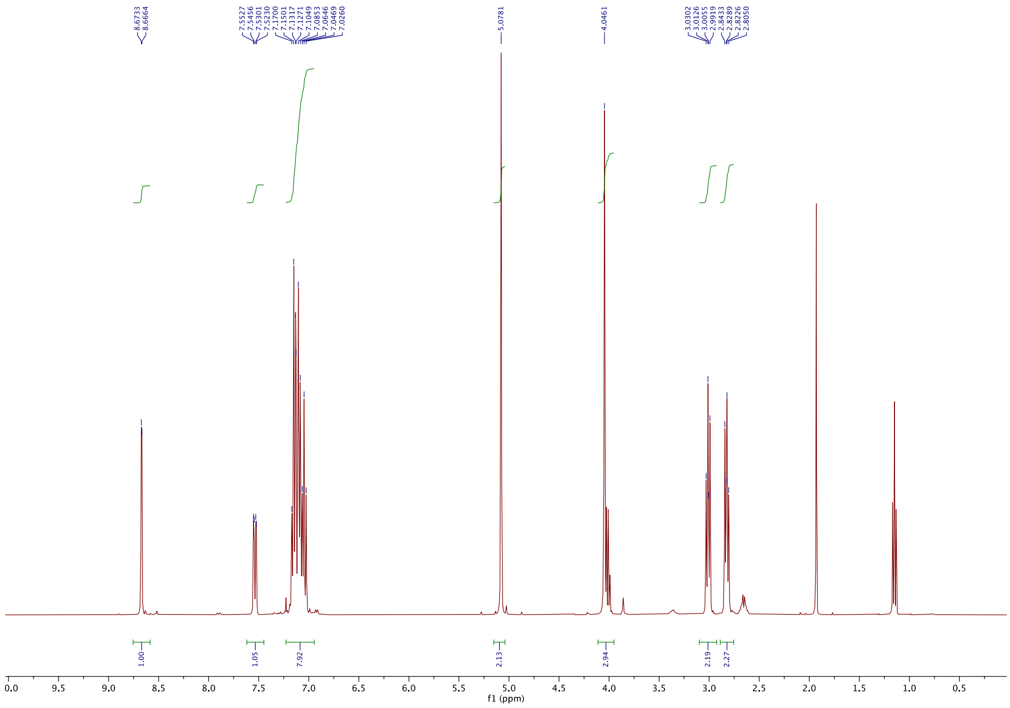


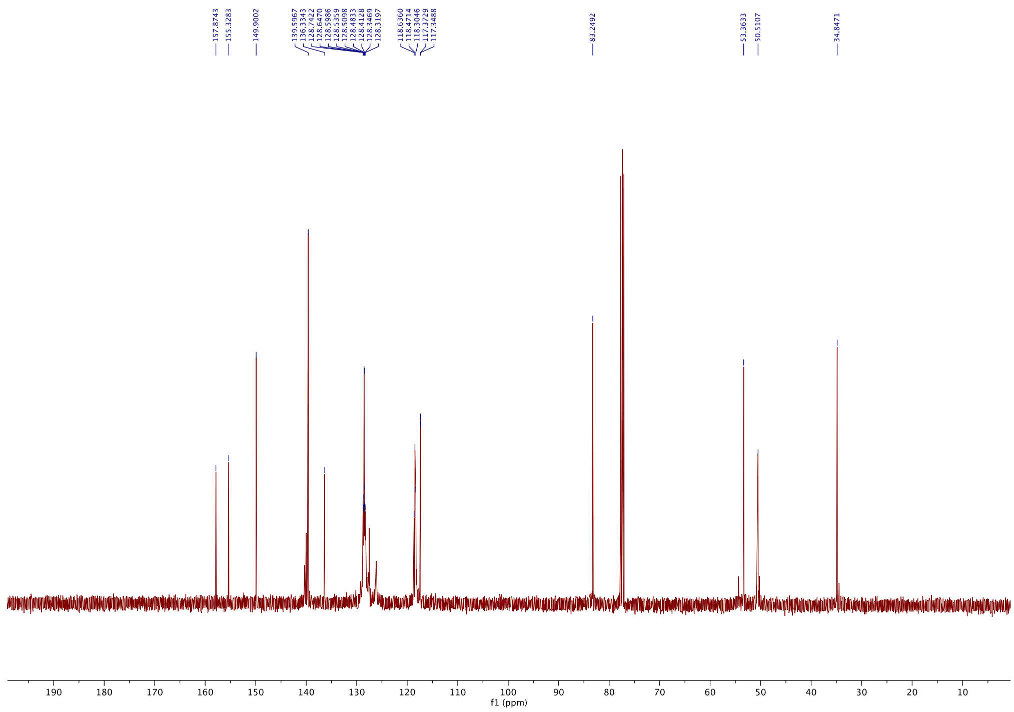




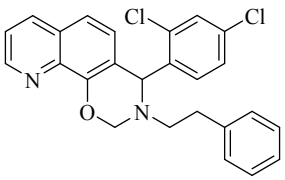
Compound **27**.

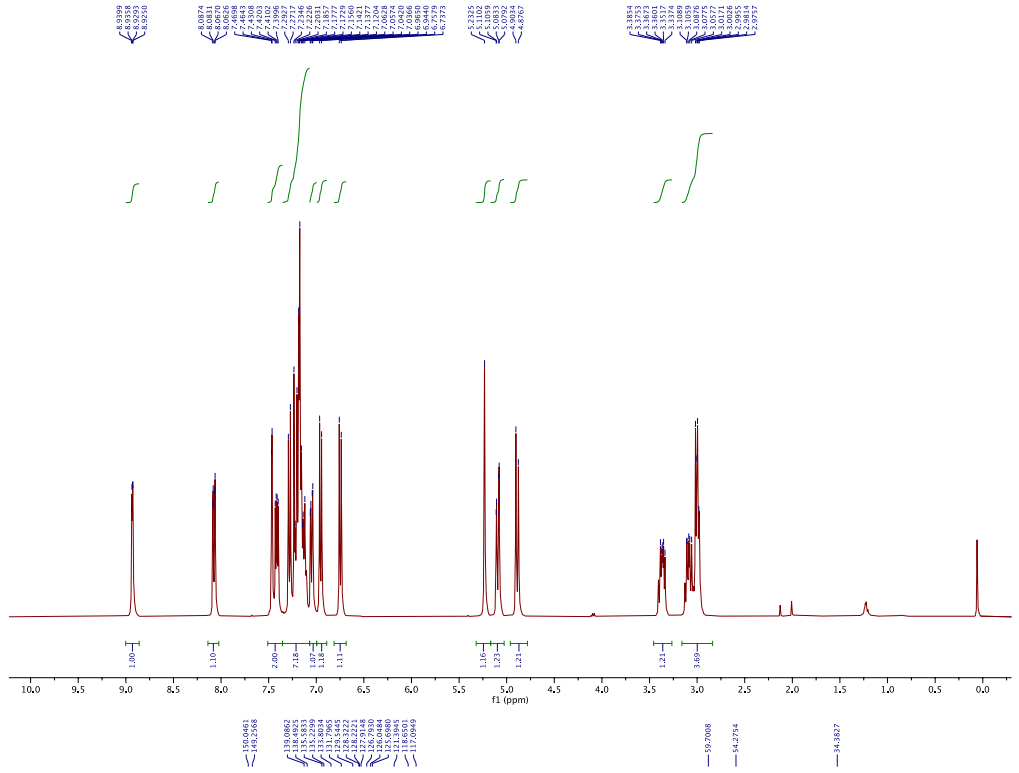


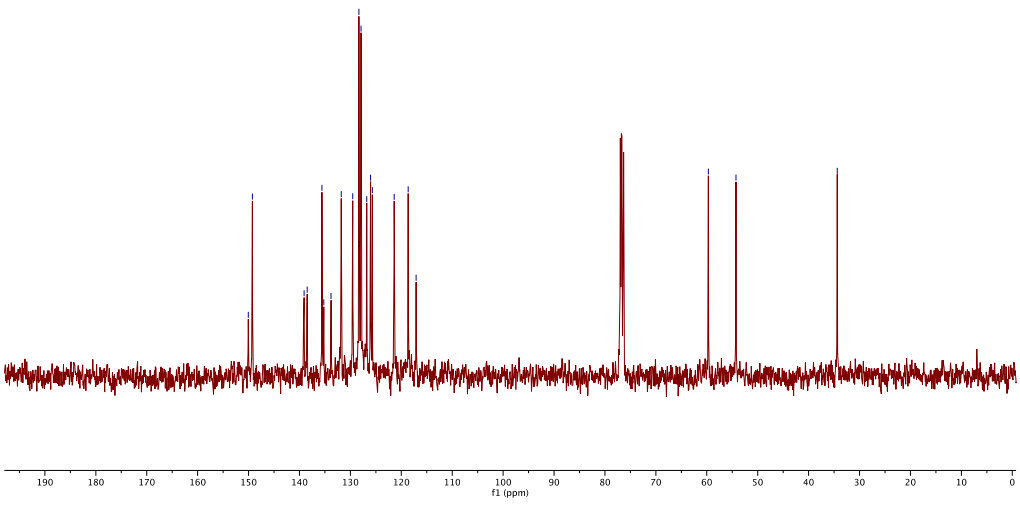




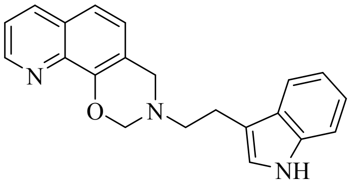
Composto **28**.

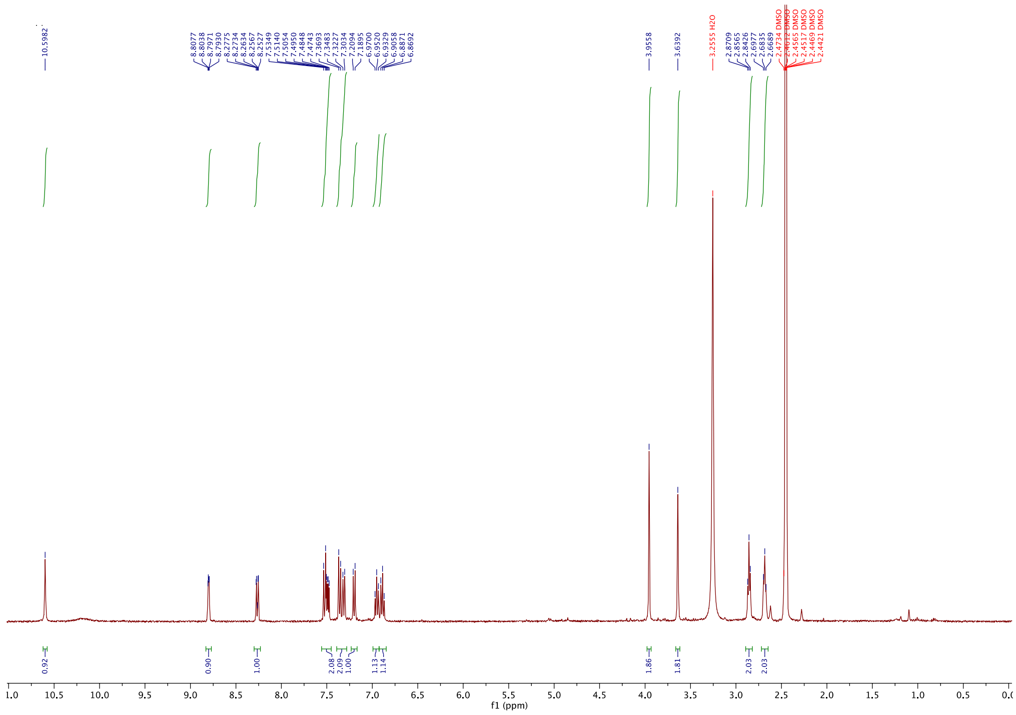




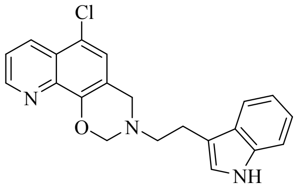


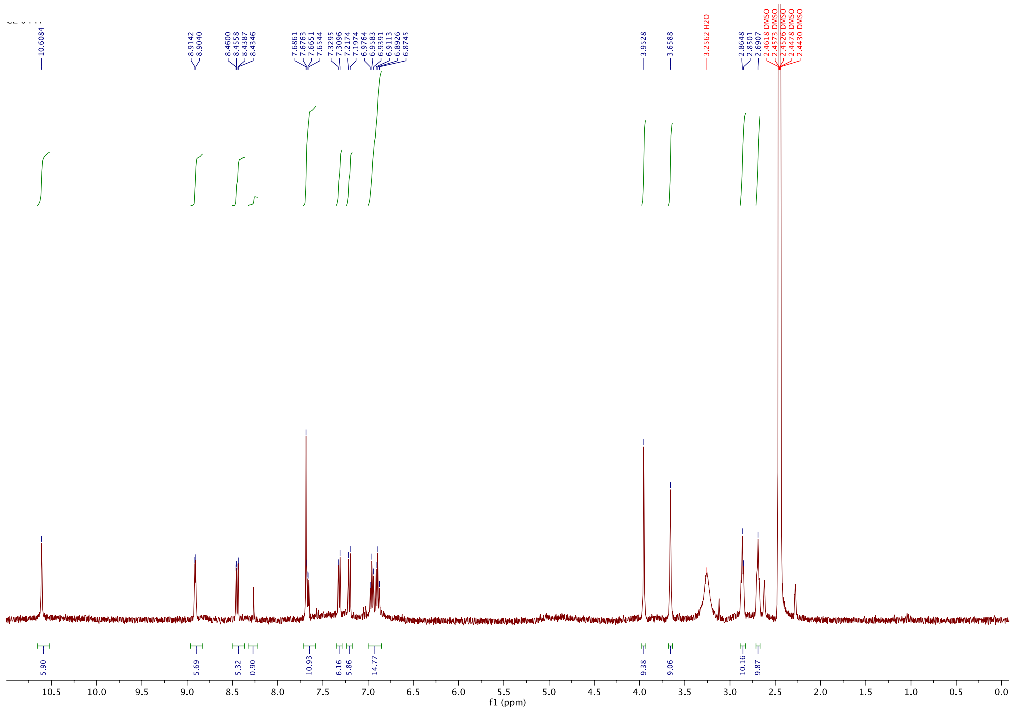
Composto **29**.



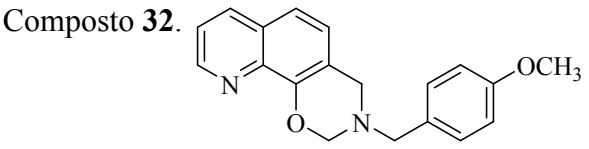


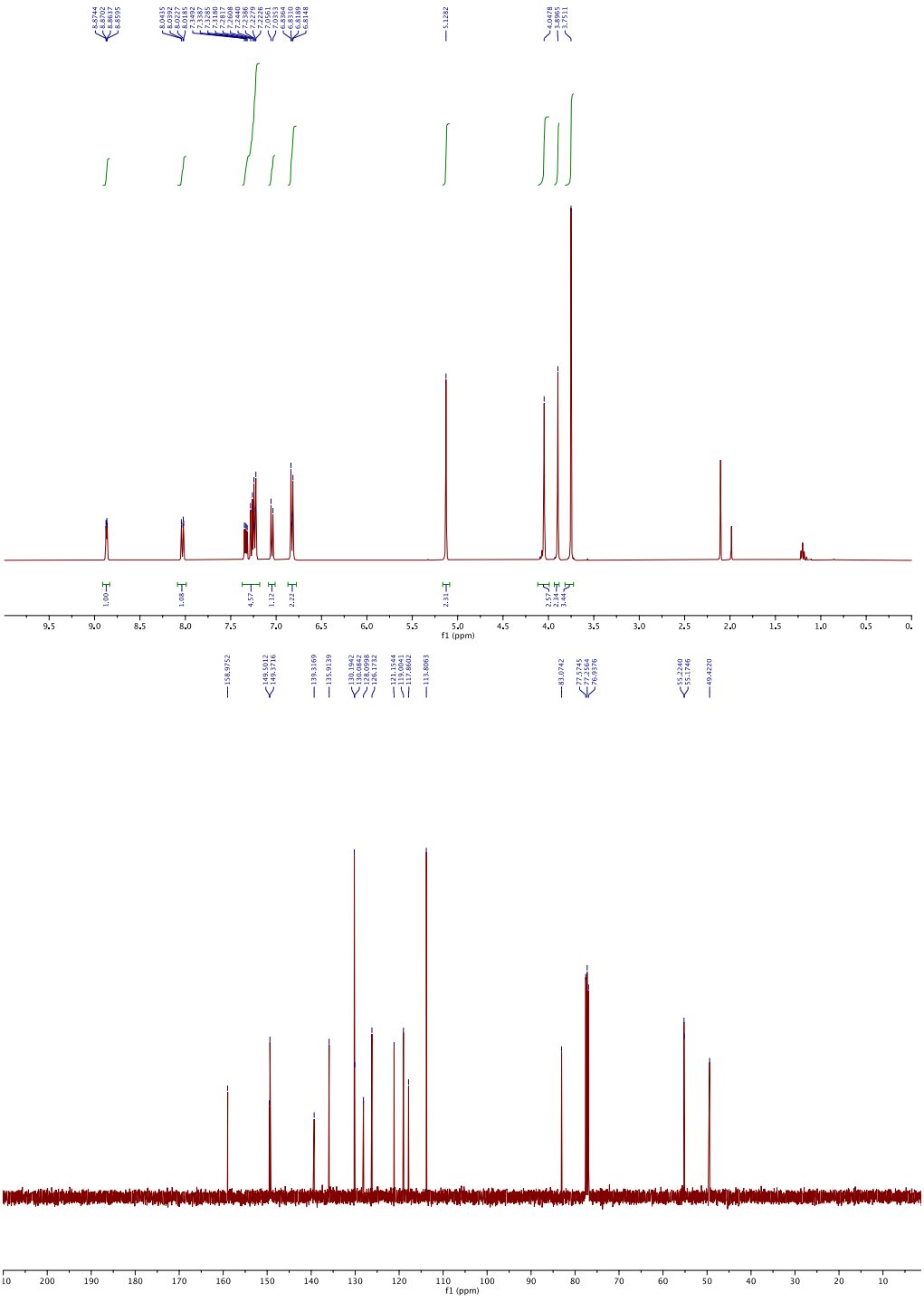
Composto **30**.



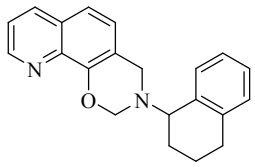


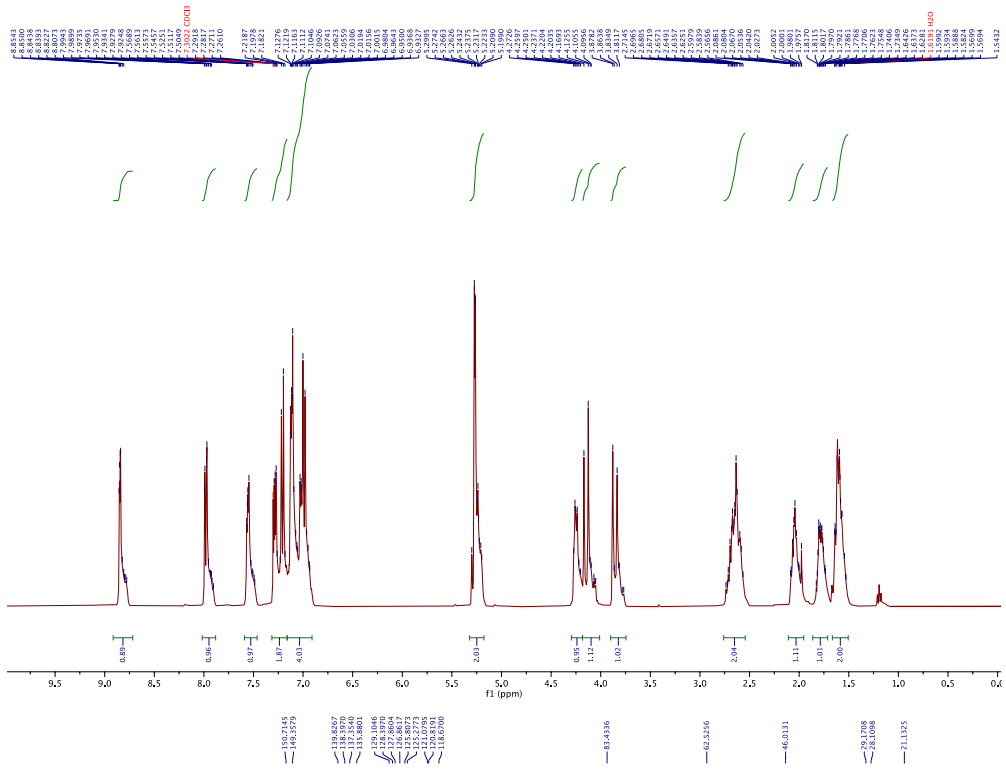
Composto **32**.

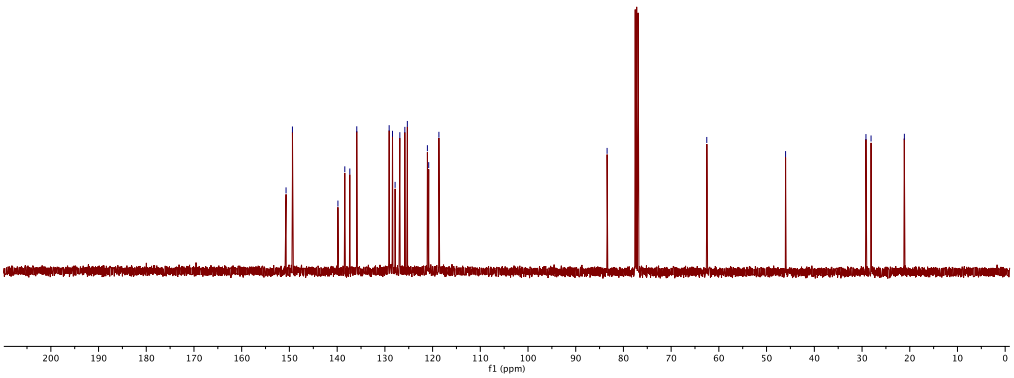




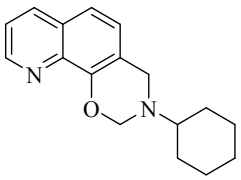
Composto **33**.

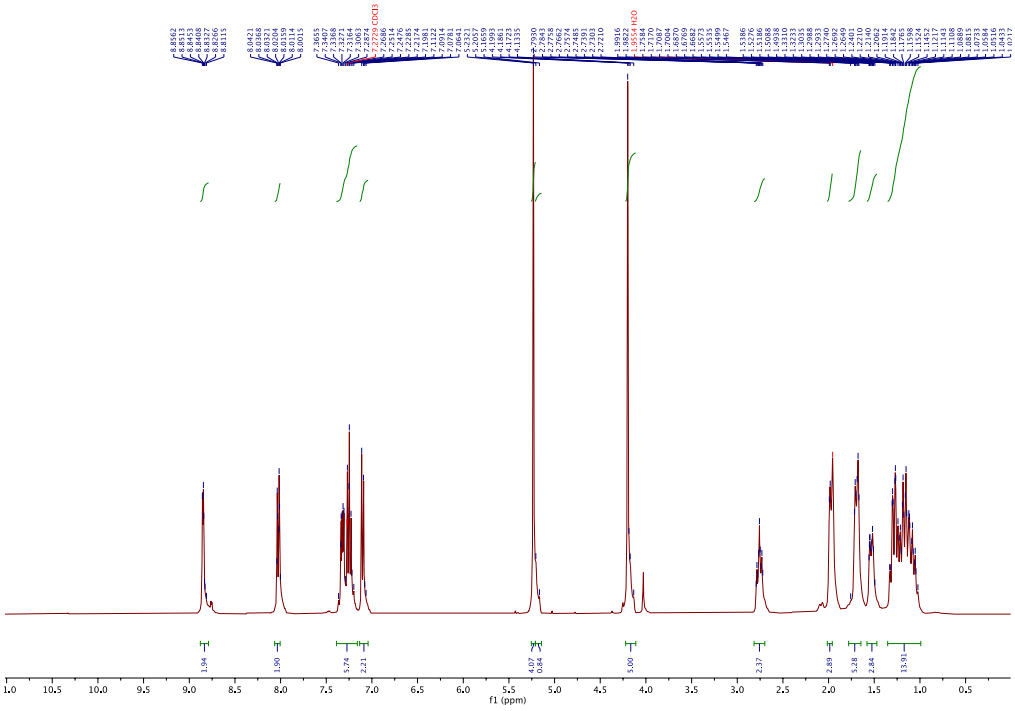




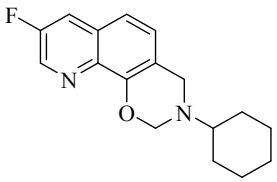


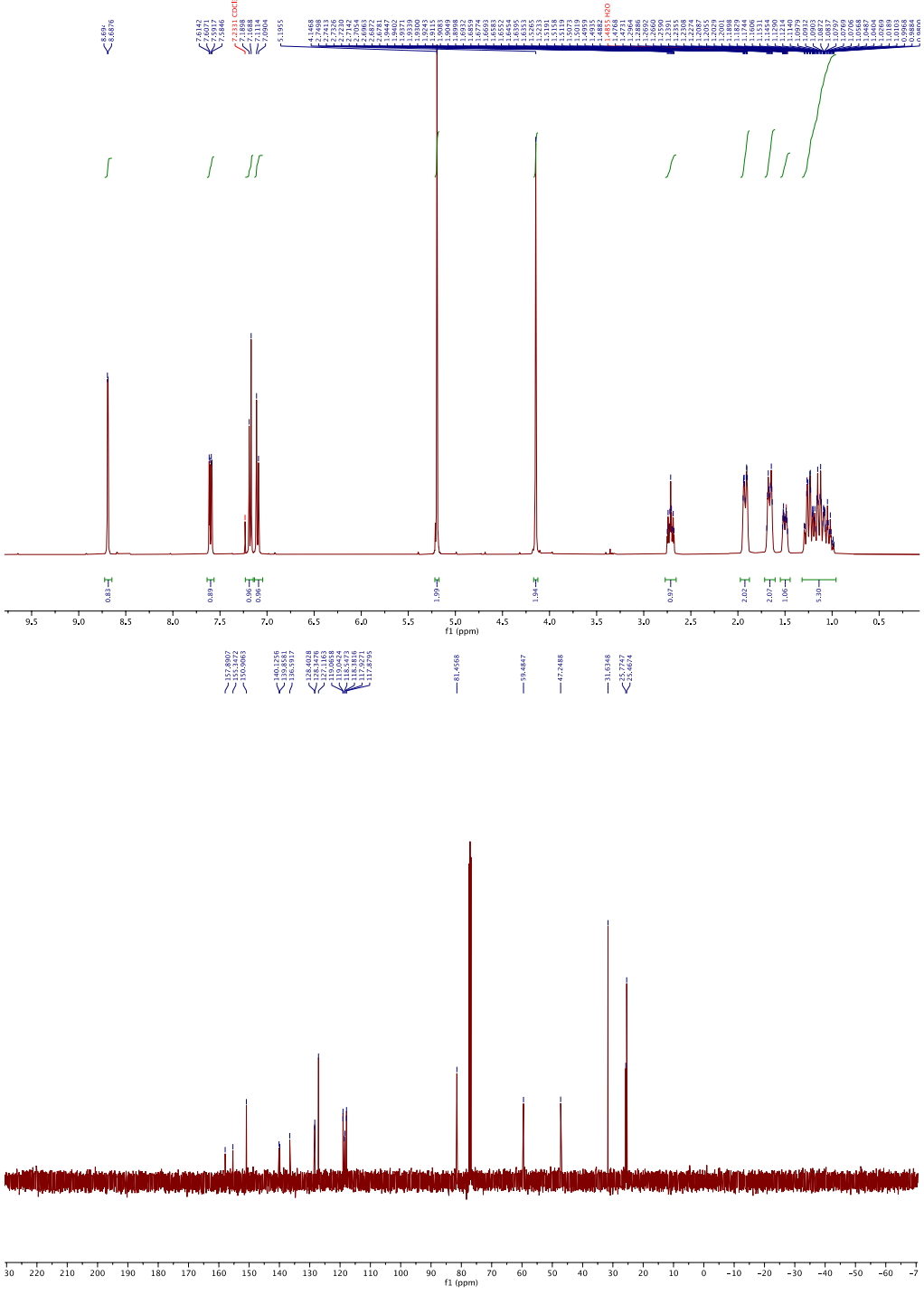
Composto **34**.



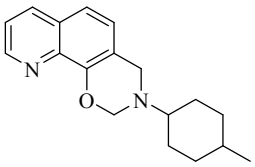


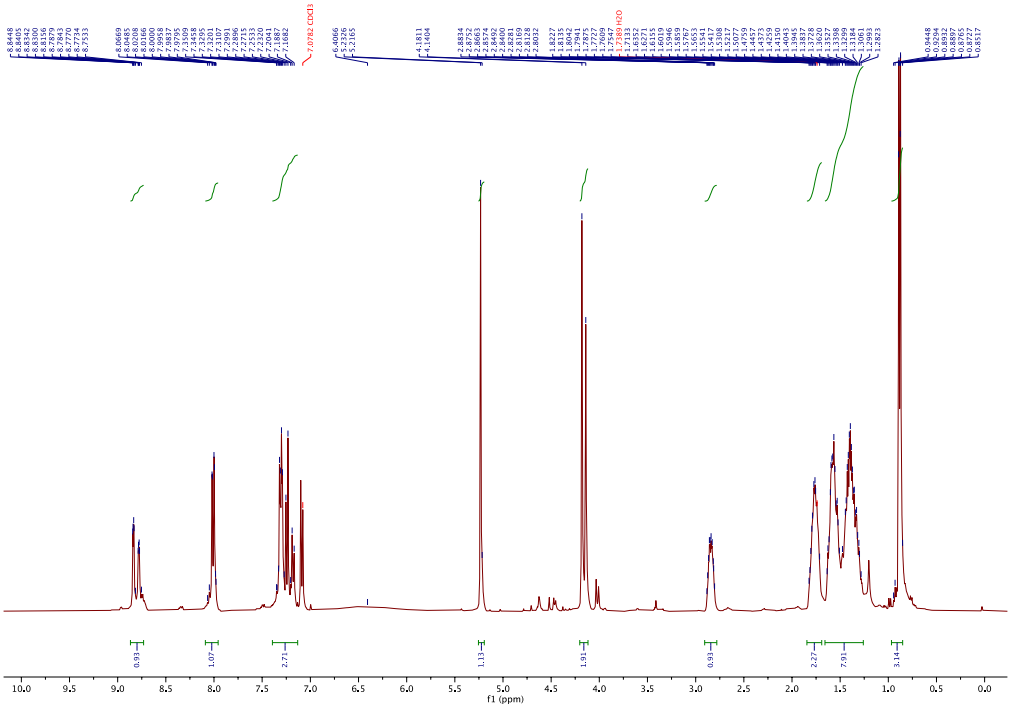
Composto **35**.



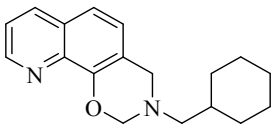


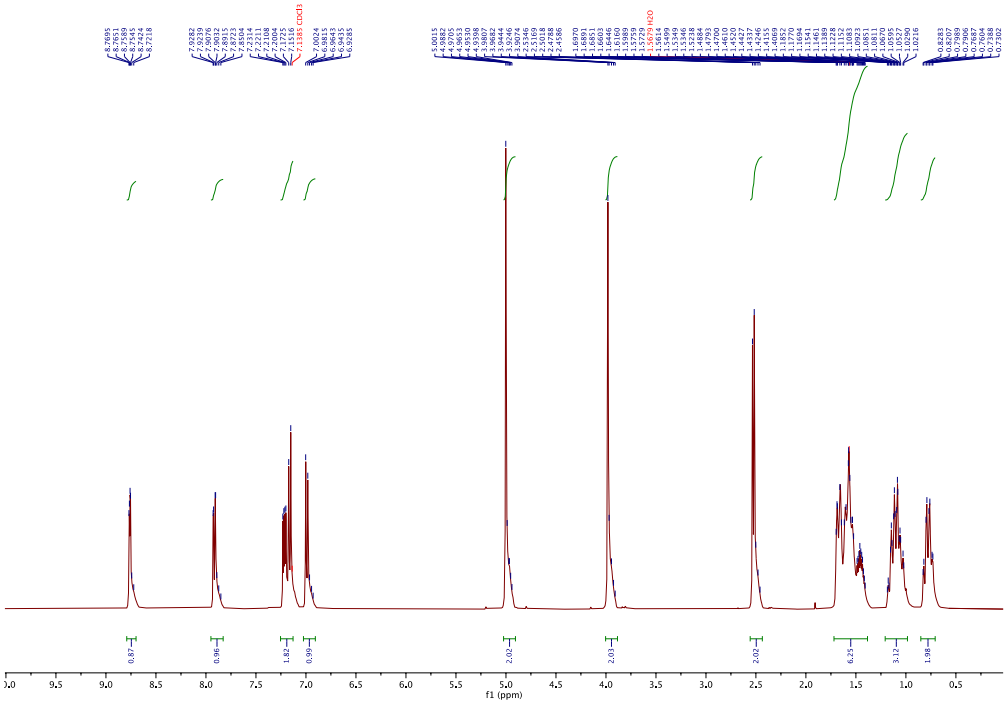
Composto **36**.

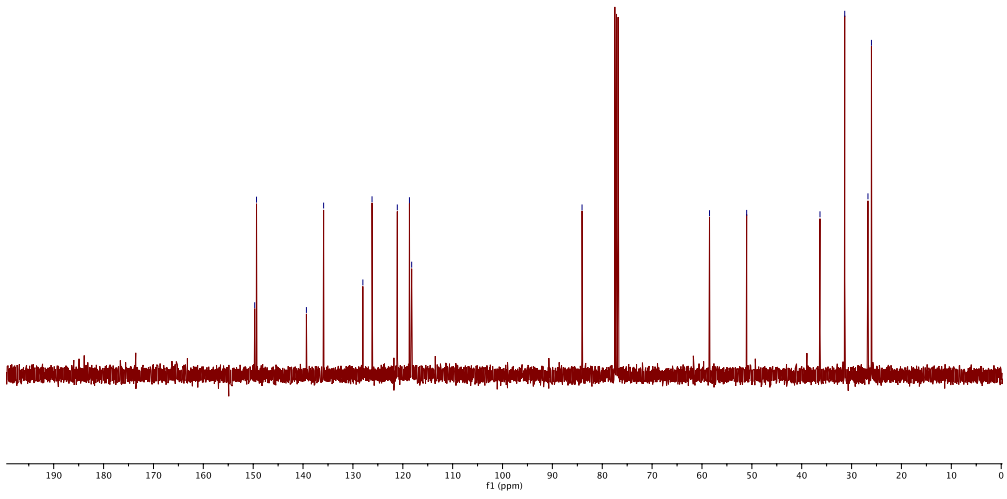




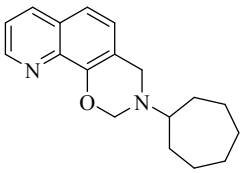
Composto **37**.

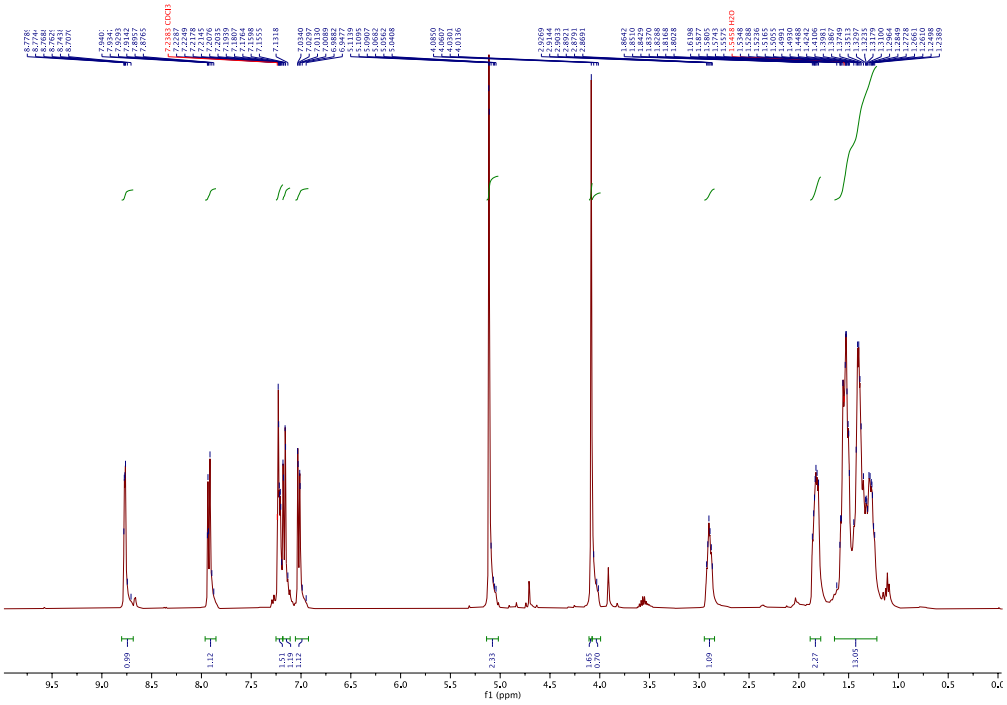


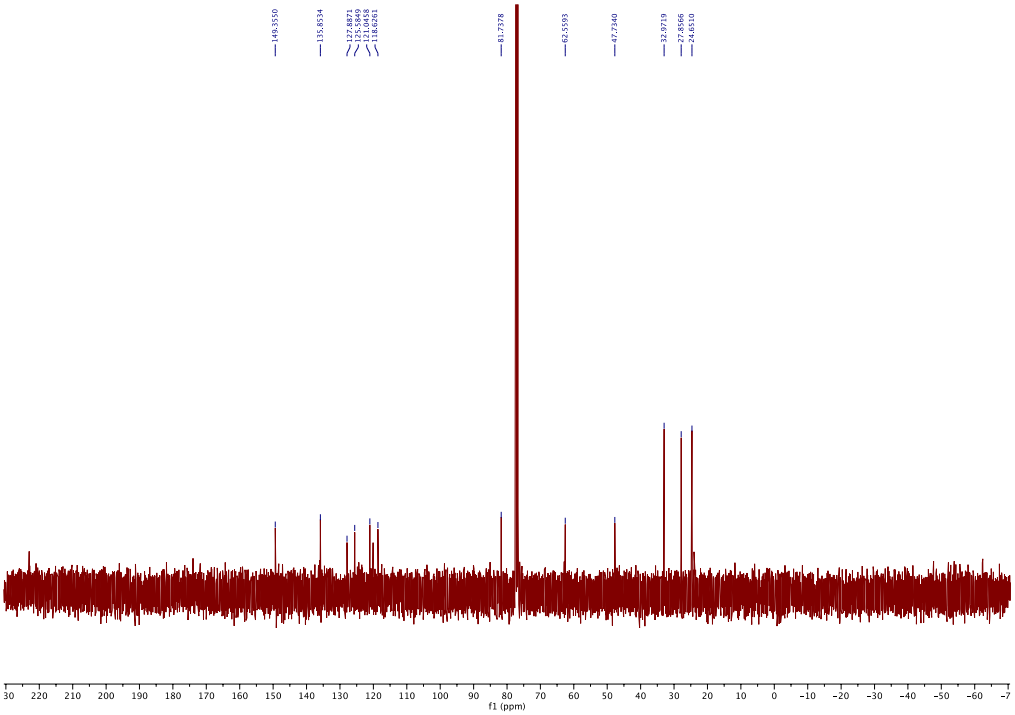




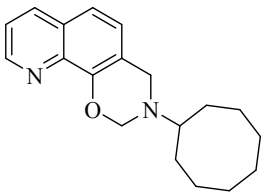
Composto **38**.

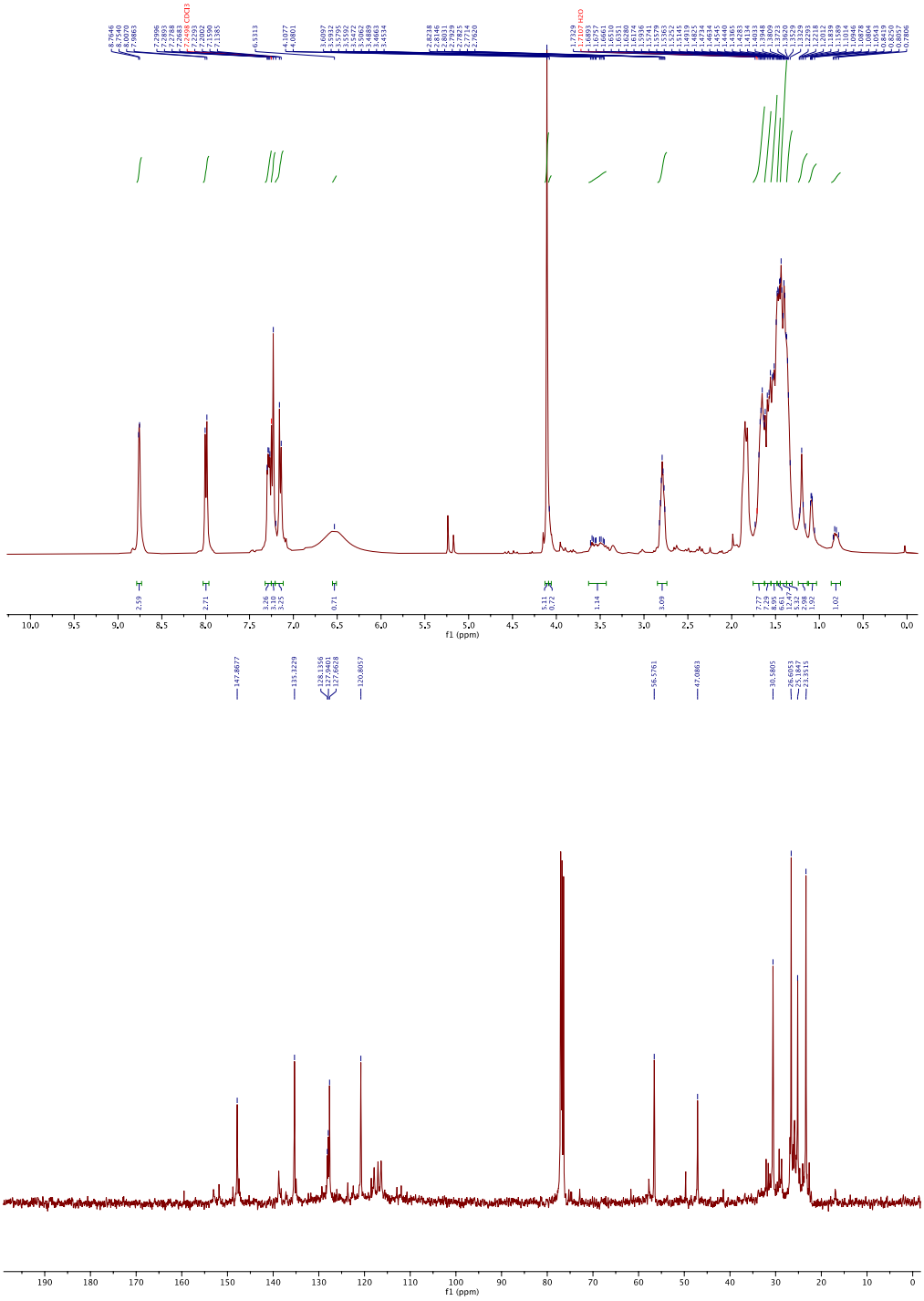




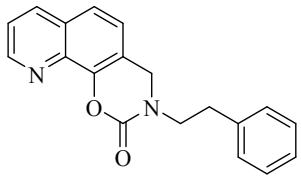


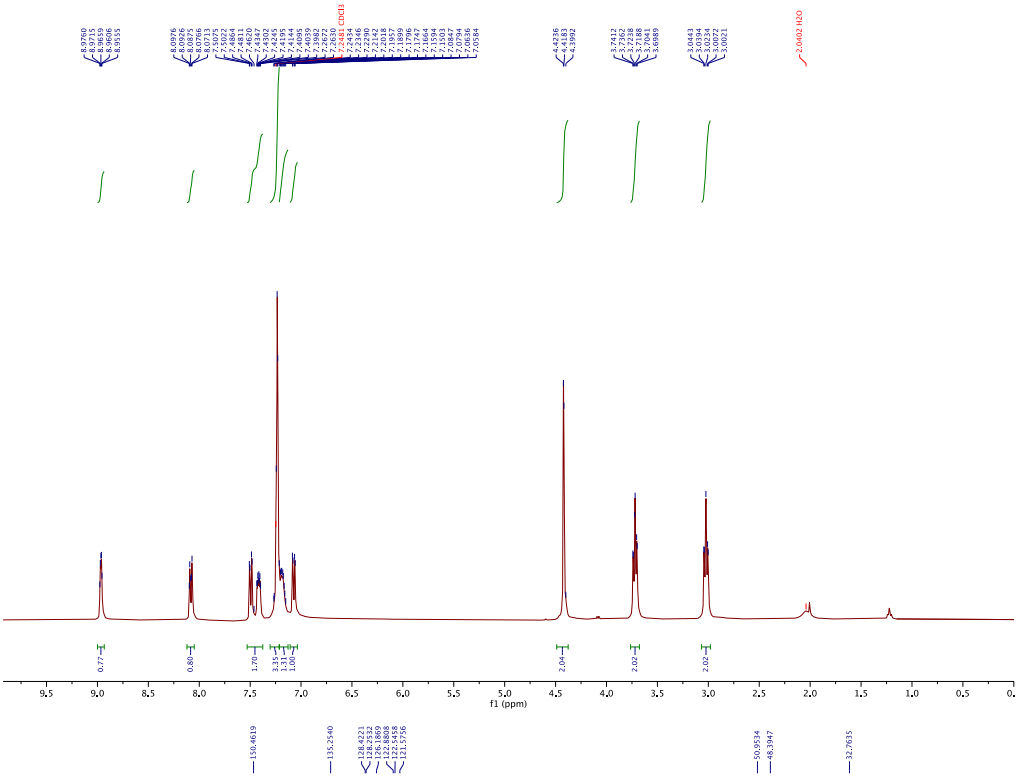
Composto **39**.

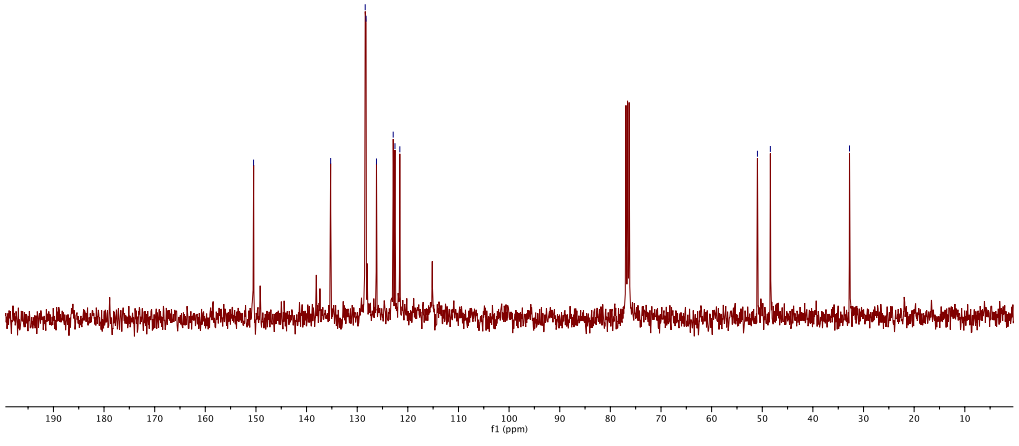




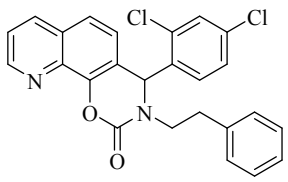
Composto **42**.

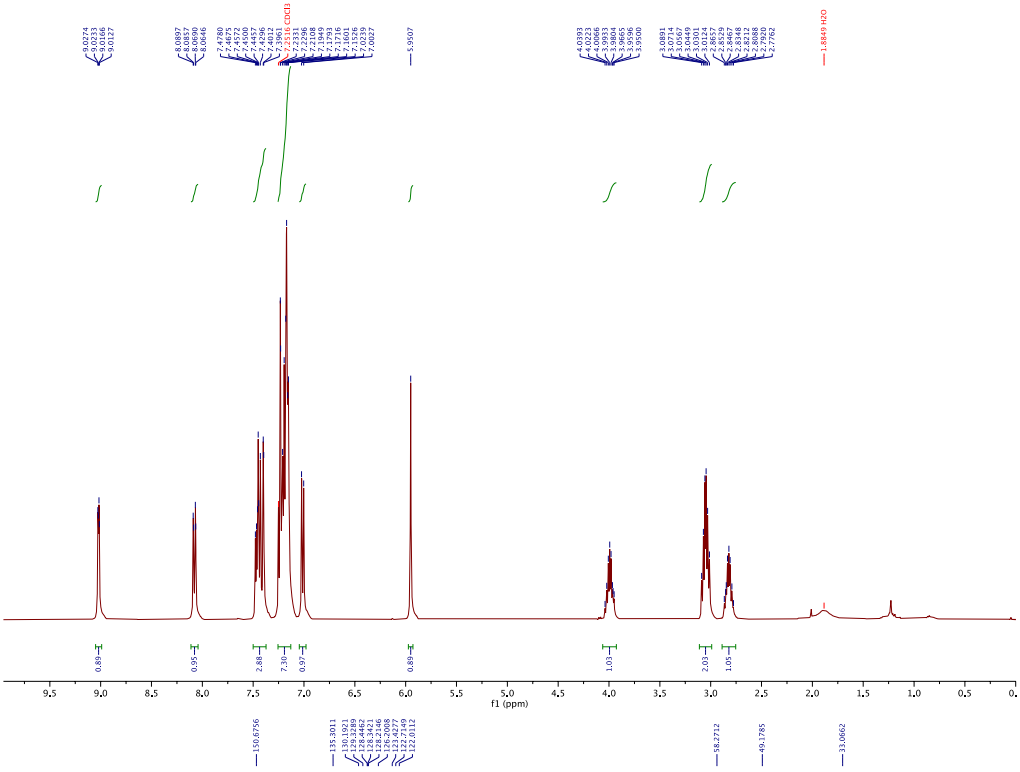


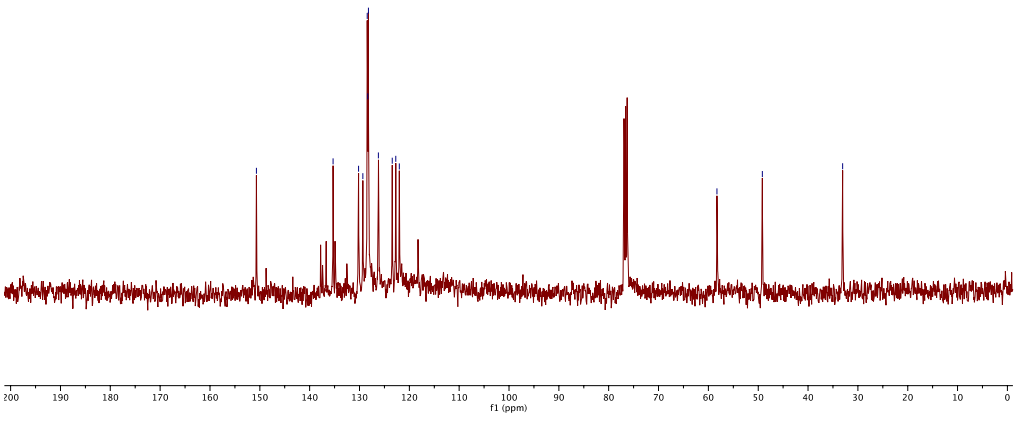




Composto **43**.







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