Supporting Information

Synthesis and Evaluation of Potent Novel Inhibitors of Human Sulfide:quinone Oxidoreductase

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Analytical and Compound Purity

LC/MS data for all numbered final compounds were obtained using a Waters Alliance 2695 HPLC/MS and a Waters ZQ mass spectrometer, as previously described[25] (**Fig. S1**). All compounds were >95% purity as indicated by LC analysis using a Waters Symmetry C18 column (4.6 × 75 mm, 3.5 µm) with a 2996 diode array detector and a linear gradient of 5−95% acetonitrile in water (with 0.1% TFA) over nine minutes[25]. Nuclear magnetic resonance spectra (^{1}H NMR) for all final numbered compounds synthesized in this study were recorded with a Varian Mercury 300-MHz NMR (**Fig. S2**). Chemical shifts are reported in parts per million (ppm, δ) using various solvents as internal standards ($CDCl_3$, DMSO-d_6, or methanol-d_4), as indicated. The peak shapes are indicated as follows: s, singlet; d, doublet; t, triplet; dd, double of doublets; m, multiplet; br. s., broad singlet. Coupling constants are reported in hertz (Hz).

General Chemistry Procedures

Compounds 1, 10, 20, and 21 were purchased from Vitas-M Labs, Champaign, IL USA. Compound 7 was purchased from Princeton Bio Molecular Research, Monmouth Junction, NJ,

USA. Each sample was assayed by LCMS to determine both purity and the correct $M+H^+$ mass ion.

Synthesis of 6

4-(4-Chlorophenyl)-2-methoxy-6-phenylpyridine-3-carbonitrile

Step 1. 2-(4-Chlorobenzylidene)malononitrile

To a solution of 4-chlorobenzaldehyde (**25**) (2.27 g 16.00 mmol), and malononitrile (1.06 g, 16.00 mmol) in ethanol (16 mL) was added a 10% aqueous potassium hydroxide solution (0.32 mL). The reaction was stirred for 15 minutes, then was allowed to stand for 30 minutes, followed by cooling to 0 °C. The mixture was filtered, then the solid was washed with ethanol (2 x 5 mL), and hexane (5 mL), to give 2-(4-chlorobenzylidene)malononitrile (**26**) (2.69 g, 89%) as an off-white solid. LC-MS: 189.0 $[M+H]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 7.83-7.94 (m, 2H), 7.72-7.80 (m, 1H), 7.48-7.61 (m, 2H).

Step 2. 4-(4-Chlorophenyl)-2-methoxy-6-phenylpyridine-3-carbonitrile

To a mixture of 2-(4-chlorobenzylidene)malononitrile (**26**) (0.24 g, 1.25 mmol), and acetophenone (150 mg, 1.25 mmol) in methanol (4 mL) was treated with crushed sodium hydroxide (0.10 g, 2.50 mmol). The reaction was stirred for 16 hours. The mixture was filtered, then the solid was washed with cold methanol (2 x 5 mL), and pentane (5 mL). The solid was recrystallized from ethanol, to give 4-(4-chlorophenyl)-2-methoxy-6-phenylpyridine-3-carbonitrile (62 mg, 16%) as a white solid. LC-MS: 321.1 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.24-8.32 (m, 2H), 7.84 (s, 1H), 7.76-7.83 (m, 2H), 7.64-7.71 (m, 2H), 7.51-7.58 (m, 3H), 4.15 (s, 3H). HRMS: Found: 321.0800; Expected: 321.7802.

Synthesis of 18

2-((Pyridin-2-yl)methoxy)-4-(4-fluorophenyl)-6-(3-methylpyridin-2-yl)pyridine-3-carbonitrile bis-hydrochloride

Step 1. 4-(4-Fluorophenyl)-2-hydroxy-6-(3-methylpyridin-2-yl)pyridine-3-carbonitrile

A mixture of 4-fluorobenzaldehyde (**27**) (1.00 g, 0.84 mL, 7.99 mmol), 1-(3-methylpyridin-2-yl)ethan-1-one (**28**) (1.08 g, 7.99 mmol), ethylcyanoacetate (**29**) (0.90 g, 0.85 mL, 7.99 mmol), and ammonium acetate (4.93 g, 63.92 mmol) in 1,4-dioxane (32 mL) was heated at reflux for 16 hours. The mixture was concentrated, then the residue was treated with ethyl acetate (25 mL) and was sonicated for 5 minutes. The mixture was filtered, then the solid was washed with ethyl acetate (2 x 5 mL). The solid was slurried in water (25 mL), then was sonicated for 5 minutes. The mixture was filtered, then the solid was washed with water (5 mL), isopropanol (5 mL), and hexane (5 mL), to give 4-(4-fluorophenyl)-2-hydroxy-6-(3-methylpyridin-2-yl)pyridine-3-carbonitrile (**30**) (833 mg, 34%) as a pale yellow solid. LC-MS: 306.4 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 12.29-12.91 (m, 1H), 8.28-8.70 (m, 1H), 7.64-7.91 (m, 3H), 7.32-7.55 (m, 3H), 6.42-6.89 (m, 1H), 2.35-2.43 (m, 3H).

Step 2. 2-((Pyridin-2-yl)methoxy)-4-(4-fluorophenyl)-6-(3-methylpyridin-2-yl)pyridine-3-carbonitrile bis-hydrochloride

A mixture of 4-(4-fluorophenyl)-2-hydroxy-6-(3-methylpyridin-2-yl)pyridine-3-carbonitrile (**30**) (50 mg, 0.16 mmol), 2-(bromomethyl)pyridine . HBr (81 mg, 0.32 mmol), and silver carbonate (133 mg, 0.48 mmol) in DMF (1 mL) was heated in a sealed vessel at 140 °C for 16 hours. The reaction was treated with water (15 mL), then was extracted with ethyl acetate (2 x 20 mL). The combined organic extracts were washed with water (2 x 10 mL), and brine (1 mL), dried (Na_2SO_4), and concentrated. The crude material was purified by column on silica (0-5% methanol: dichloromethane). The product was dissolved in dichloromethane (4 mL) and methanol (1 mL), then was treated with 4N HCl in 1,4-dioxane (0.25 mL). The mixture was allowed to stand for 5 minutes and was then concentrated to give 2-((pyridin-2-yl)methoxy)-4-(4-fluorophenyl)-6-(3-methylpyridin-2-yl)pyridine-3-carbonitrile as the bis-HCl salt (35 mg, 47%), a

pale yellow solid. LC-MS: 397.5 $[M+H]^+$. ^{1}H NMR (300 MHz, methanol-d_4) δ 8.92 (t, *J*=6.44 Hz, 2H), 8.62-8.79 (m, 2H), 8.35 (d, *J*=8.20 Hz, 1H), 8.04-8.19 (m, 2H), 7.78-8.01 (m, 3H), 7.38 (t, *J*=1.00 Hz, 2H), 6.14 (s, 2H), 2.70 (s, 3H). HRMS: Found: 397.1440; Expected: 397.4243.

Synthesis of 19

4-(4-Aminophenyl)-2-methoxy-6-(3-methylpyridin-2-yl)pyridine-3-carbonitrile bis-hydrochloride monohydrate

Step 1. Tert-butyl 4-((E)-3-(3-methylpyridin-2-yl)-3-oxoprop-1-enyl)phenylcarbamate

A mixture of tert-butyl 4-formylphenylcarbamate (**31**) (1.20 g, 5.42 mmol) in methanol (30 mL) was treated with 1-(3-methylpyridin-2-yl)ethan-1-one (**28**) (1.10 g, 8.14 mmol), and a solution of KOH (457 mg, 8.14 mmol) in water (0.3 mL). The reaction was stirred for 3 days. The mixture was treated with water (50 mL), then was concentrated to remove the methanol. The residue was extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The crude material was purified by column on silica (0-20% ethyl acetate/ hexanes), to give tert-butyl 4-((E)-3-(3-methylpyridin-2-yl)-3-oxoprop-1-enyl)phenylcarbamate (**32**) (1.15 g, 63%) as a yellow solid. LC-MS: 339.6 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48-8.60 (m, 1H), 7.51-7.85 (m, 5H), 7.40 (d, *J*=8.79 Hz, 2H), 7.31 (dd, *J*=4.39, 7.91 Hz, 1H), 6.84 (s, 1H), 2.58 (s, 3H), 1.50 (s, 9H).

Step 2. Tert-butyl 4-(3-cyano-2-methoxy-6-(3-methylpyridin-2-yl)pyridin-4-yl)phenylcarbamate

A mixture of tert-butyl 4-((E)-3-(3-methylpyridin-2-yl)-3-oxoprop-1-enyl)phenylcarbamate (**32**) (429 mg, 1.27 mmol) in methanol (3.4 mL) and 5.4M sodium methoxide in methanol (0.24 mL) was treated with malononitrile (83 mg, 1.27 mmol). The reaction was stirred for 16 hours, then was filtered. The solid was washed with cold methanol (10 mL). The crude solid was purified by column on silica (0-2% methanol: dichloromethane), to give tert-butyl 4-(3-cyano-2-methoxy-6-(3-methylpyridin-2-yl)pyridin-4-yl)phenylcarbamate (**33**) (623 mg, 38%) as a pale yellow solid. LC-MS: 417.6 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50-8.58 (m, 1H), 7.78 (s,

1H), 7.60-7.70 (m, 2H), 7.51 (d, *J*=8.79 Hz, 1H), 7.26-7.32 (m, 1H), 6.56-6.66 (m, 1H), 4.13 (s, 3H), 2.67 (s, 3H), 1.36-1.66 (m, 9H).

Step 3. 4-(4-Aminophenyl)-2-methoxy-6-(3-methylpyridin-2-yl)pyridine-3-carbonitrile bis-hydrochloride monohydrate

To a mixture of tert-butyl 4-(3-cyano-2-methoxy-6-(3-methylpyridin-2-yl)pyridin-4-yl)phenylcarbamate (**33**) (615 mg, 1.48 mmol) in dichloromethane (7 mL) and methanol (3 mL) was added a 4N solution of HCl in 1,4-dioxane (3.75 mL). The reaction was stirred for 3 days. The mixture was filtered, then the solid was washed with dichloromethane (10 mL), to give 4-(4-aminophenyl)-2-methoxy-6-(3-methylpyridin-2-yl)pyridine-3-carbonitrile bis-hydrochloride mono-hydrate (**19**) (572 mg, 99%) as a white solid. LC-MS: 317.4 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62-8.69 (m, 1H), 8.09 (d, *J*=7.62 Hz, 1H), 7.82 (s, 1H), 7.75 (d, *J*=8.20 Hz, 2H), 7.66 (dd, *J*=4.98, 7.91 Hz, 1H), 7.28 (d, *J*=8.20 Hz, 2H), 4.09 (s, 3H), 2.65 (s, 3H). HRMS: Found: 317.1409; Expected: 317.1402. Microanalysis: Found: C, 56.40%, H, 4.92%, N, 13.57%; Expected: C, 56.03%, H, 4.95%, N, 13.76%.

Compound **2**: 4-(4-Fluorophenyl)-2-methoxy-6-(4-methoxyphenyl)pyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 1. LC-MS: 335.1 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.25 (d, *J*=8.79 Hz, 2H), 7.70-7.93 (m, 3H), 7.43 (t, *J*=8.79 Hz, 2H), 7.08 (d, *J*=8.20 Hz, 2H), 4.13 (s, 3H), 3.84 (s, 3H). HRMS: Found: 335.1190; Expected: 335.3516.

Compound **3**: 4-(4-Fluorophenyl)-2-methoxy-6-(3-methoxyphenyl)pyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 1. LC-MS: 335.1 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72-8.06 (m, 6H), 7.35-7.63 (m, 3H), 7.00-7.22 (m, 1H), 4.15 (s, 3H), 3.85 (s, 3H). HRMS: Found: 335.1185; Expected: 335.3516.

Compound **4**. 4-(4-Fluorophenyl)-2-methoxy-6-(2-methoxyphenyl)pyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 1. LC-MS: 335.1 $[M+H]^+$. ^{1}H NMR (300

MHz, $CDCl_3$) δ 8.04 (dd, *J*=1.76, 7.62 Hz, 1H), 7.75 (s, 1H), 7.61-7.70 (m, 2H), 7.40-7.51 (m, 1H), 7.18-7.29 (m, 2H), 7.13 (t, *J*=7.62 Hz, 1H), 7.04 (d, *J*=8.79 Hz, 1H), 4.17 (s, 3H), 3.91 (s, 3H). HRMS: Found: 335.1174; Expected: 335.3516.

Compound **5**. 6-tert-Butyl-4-(4-fluorophenyl)-2-methoxypyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 3. LC-MS: 285.5 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 7.62-7.84 (m, 2H), 7.32-7.52 (m, 2H), 7.18 (s, 1H), 4.03 (s, 3H), 1.34 (s, 9H). HRMS: Found: 285.1410; Expected: 285.3360.

Compound **8**. 4-tert-Butyl-6-methoxy-[2,2']bipyridinyl-5-carbonitrile. Obtained following the procedure reported in Scheme 3. LC-MS: 268.5 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ ppm 8.75 (br. s., 1 H) 8.44 (d, *J*=7.62 Hz, 1 H) 8.17 (s, 1 H) 8.02 (t, *J*=7.03 Hz, 1 H) 7.54 (br. s., 1 H) 4.10 (s, 3 H) 1.48 (s, 9 H). HRMS: Found: 268.1433; Expected: 268.3336.

Compound **9**. 2-(2-Methoxyethoxy)-4-(4-fluorophenyl)-6-phenylpyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 1. LC-MS: 349.1 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.25 (dd, *J*=2.34, 6.44 Hz, 2H), 7.79-7.89 (m, 3H), 7.50-7.58 (m, 3H), 7.44 (t, *J*=8.79 Hz, 2H), 4.64-4.81 (m, 2H), 3.72-3.85 (m, 2H), 3.30 (s, 3H). HRMS: Found: 349.1363; Expected: 349.3782.

Compound **11**. 2-Methoxy-6-phenyl-4-(pyridin-4-yl)pyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 1. LC-MS: 288.2 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.75-9.05 (m, 2H), 8.29 (dd, *J*=2.34, 6.44 Hz, 2H), 7.95 (s, 3H), 7.49-7.68 (m, 3H), 4.10-4.25 (m, 3H). HRMS: Found: 288.1139; Expected: 288.3232.

Compound **12**. 4-(4-Fluorophenyl)-2-methoxy-6-(pyridin-3-yl)pyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 1. LC-MS: 306.1 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.63 (s, 1H), 9.07 (d, *J*=8.20 Hz, 1H), 8.91 (d, *J*=4.69 Hz, 1H), 8.11 (s, 1H), 7.75-

8.05 (m, 3H), 7.48 (t, *J*=9.08 Hz, 2H), 4.11-4.23 (m, 3H). HRMS: Found: 306.1024; Expected: 306.3137.

Compound **13**. 4-(4-Fluorophenyl)-2-methoxy-6-(pyridin-4-yl)pyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 1. LC-MS: 306.1 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 8.84-9.25 (m, 2H), 8.72 (d, *J*=4.10 Hz, 2H), 8.25 (s, 1H), 7.72-8.00 (m, 2H), 7.34-7.62 (m, 2H), 4.15-4.27 (m, 3H). HRMS: Found: 306.1047; Expected: 306.3137.

Compound **14**. 4-(4-Fluorophenyl)-2-methoxy-6-(pyridin-2-yl)pyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 1. LC-MS: 306.6 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.71-8.80 (m, 1H), 8.50 (d, *J*=8.20 Hz, 1H), 8.12-8.18 (m, 1H), 8.01-8.11 (m, 1H), 7.76-7.86 (m, 2H), 7.58 (dd, *J*=5.27, 7.03 Hz, 1H), 7.38-7.51 (m, 2H), 4.18 (s, 3H). HRMS: Found: 306.1031; Expected: 306.3137.

Compound **15**. 4-(4-Fluoro-phenyl)-6-methoxy-3'-methyl-[2,2']bipyridinyl-5-carbonitrile. Obtained following the procedure reported in Scheme 3. LC-MS: 320.5 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.60 (d, *J*=4.10 Hz, 1H), 7.87-7.99 (m, 2H), 7.76-7.87 (m, 3H), 7.38-7.58 (m, 3H), 4.11 (s, 3H), 2.67 (s, 3H). HRMS: Found: 320.1205; Expected: 320.3403.

Compound **16**. 2-(Allyloxy)-4-(4-fluorophenyl)-6-(pyridin-2-yl)pyridine-3-carbonitrile. Obtained following the procedure reported in Scheme 2. LC-MS: 332.5 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.69-8.81 (m, 1H), 8.40-8.55 (m, 1H), 7.98-8.21 (m, 2H), 7.80 (t, *J*=6.15 Hz, 2H), 7.50-7.63 (m, 1H), 7.39-7.49 (m, 2H), 6.09-6.24 (m, 1H), 5.51 (d, *J*=17.57 Hz, 1H), 5.32 (d, *J*=11.13 Hz, 1H), 5.10-5.19 (m, 2H). HRMS: Found: 332.1208; Expected: 332.3510 .

Compound **17**. tert-Butyl 4-(2-(3-cyano-4-(4-fluorophenyl)-6-(3-methylpyridin-2-yl)pyridin-2-yloxy)ethyl)piperazine-1-carboxylate. Obtained following the procedure reported in Scheme 2. LC-MS: 518.7 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.46-8.58 (m, 1H), 7.76 (s, 1H), 7.55-7.71

(m, 3H), 7.10-7.32 (m, 3H), 4.46-4.70 (m, 2H), 3.33-3.49 (m, 4H), 2.77-2.98 (m, 2H), 2.64 (s, 3H), 2.40-2.60 (m, 4H), 1.43 (s, 9H). HRMS: Found: 518.2566; Expected: 518.6024.

Compound **22**. 2-Methoxy-3-methyl-4,6-diphenylpyridine.

(Z)-1,3-Diphenyl-3-(prop-2-ynylamino)prop-2-en-1-one

Step 1: A mixture of 1,3-diphenylprop-2-yn-1-one (200 mg, 0.97 mmol) and propargylamine (64 mg, 74 μL, 1.17 mmol) in methanol (2.6 mL) was heated at 60 °C for 6 hours. The mixture was concentrated, and the residue was partitioned between ethyl acetate (30 mL) and brine (3 mL). The organic layer was dried (Na_2SO_4) and concentrated. The crude material was purified by column on silica (0-20% ethyl acetate: hexane), to give (Z)-1,3-diphenyl-3-(prop-2-ynylamino)prop-2-en-1-one (143 mg, 57%) as a pale yellow solid. LC-MS: 262.4 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 11.05-11.50 (m, 1H), 7.90 (d, *J*=7.03 Hz, 2H), 7.37-7.53 (m, 8H), 5.84 (s, 1H), 3.90-3.98 (m, 2H).

Step 2: A mixture of (Z)-1,3-diphenyl-3-(prop-2-ynylamino)prop-2-en-1-one (50 mg, 0.19 mmol), methanol (12 mg, 15 μL, 0.38 mmol), and NaOH (8 mg, 0.19 mmol) in DMSO (0.l4 mL) was stirred for 1.5 hours. The mixture was treated with water (10 mL) and was extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were dried (Na_2SO_4), and concentrated. The crude material was purified by column on silica (0-5% ethyl acetate: hexane), to give 2-methoxy-3-methyl-4,6-diphenylpyridine (**22**) (19 mg, 36%) as a clear gum. LC-MS: 276.4 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, *J*=7.03 Hz, 2H), 7.34-7.51 (m, 8H), 7.29 (s, 1H), 4.12 (s, 3H), 2.17 (s, 3H). HRMS: Found: 276.1400; Expected: 276.3523.

Compound **23**. 4-(4-Fluorophenyl)-2-methoxy-6-(pyridin-2-yl)pyridine-3-carboxamide.

A mixture of 4-(4-fluorophenyl)-2-methoxy-6-(pyridin-2-yl)pyridine-3-carbonitrile (**14)** (50 mg, 0.16 mmol) in tert-butanol (0.5 mL) was treated with crushed potassium hydroxide (33 mg, 0.59 mmol). The mixture was heated in a sealed vessel at 95 ºC for 2 hours. The reaction was

treated with brine (2 mL), and water (10 mL). The mixture was extracted with 5% methanol in dichloromethane (3 x 15 mL). The combined organic extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The crude material was purified by column on silica (0-5% methanol: dichloromethane). The product was dissolved in CH_2Cl_2 (3 mL) and methanol (1 mL), then was treated with 4N HCl in 1,4-dioxane (0.25 mL). The mixture was allowed to stand for 5 minutes, and then was concentrated to give 4-(4-fluorophenyl)-2-methoxy-6-(pyridin-2-yl)pyridine-3-carboxamide (**23)** as the HCl salt (16 mg, 28%) as a pale yellow solid. LC-MS: 324.6 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.66-8.80 (m, 1H), 8.49 (d, *J*=7.62 Hz, 1H), 8.04-8.18 (m, 1H), 7.95-8.04 (m, 1H), 7.88 (br. s., 1H), 7.59-7.72 (m, 2H), 7.50-7.59 (m, 1H), 7.28-7.41 (m, 1H). HRMS: Found: 324.1131; Expected: 324.3289.

Compound **24**. 4-Ethoxy-2,6-diphenylpyrimidine-5-carbonitrile.

Step 1. A mixture of ethyl benzimidate.HCl (1.00 g, 5.39 mmol) in toluene (15 mL) was treated with trimethylamine (1.20 g, 1.64 mL, 11.86 mmol). The reaction was stirred for 5 minutes, then a solution of benzoyl chloride (0.76 g, 0.63 mL, 5.39 mmol) in toluene (4 mL) was added dropwise. The reaction was stirred for 40 hours, then was filtered. The solid was washed with toluene (10 mL), then the combined filtrates were concentrated, to give (Z)-N-(ethoxy(phenyl) methylene)benzamide (1.25 g, 92%) as a viscous liquid, which was used without further purification. ^1H NMR (300 MHz, $CDCl_3$) δ 8.11-8.25 (m, 1H), 8.01 (d, *J*=7.03 Hz, 2H), 7.36-7.76 (m, 8H), 7.17-7.36 (m, 2H), 4.31-4.57 (m, 2H), 1.36-1.60 (m, 3H).

Step 2. To a mixture of 0.5 M sodium methoxide in methanol (9.87 mL, 4.94 mmol) and methanol (7 mL) was added in one portion cyanoacetamide (0.42 g, 4.94 mmol). The mixture was stirred for 5 minutes, then a solution of (Z)-N-(ethoxy(phenyl)methylene)benzamide (1.25 g, 4.94 mmol) in methanol (3 mL) was added dropwise. The reaction was stirred for 4 days, then was treated with sulfuric acid (0.15 mL). The mixture was stirred for 10 minutes, then was filtered. The solid was washed with cold methanol (2 x 5 mL), and hexane (5 mL). The solid

was treated with ethanol (50 mL), then was heated to boiling, and then was allowed to cool to 20 °C. The mixture was filtered, then the solid was washed with cold ethanol (2 x 5 mL), and hexane (5 mL), to give 4-hydroxy-2,6-diphenylpyrimidine-5-carbonitrile (672 mg, 50%) as a white solid. LC-MS: 274.5 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16-8.33 (m, 2H), 7.89-8.09 (m, 2H), 7.42-7.68 (m, 6H).

Step 3. 4-Ethoxy-2,6-diphenylpyrimidine-5-carbonitrile (**24**) was obtained following the procedure reported in Scheme 2. LC-MS: 302.5 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42-8.59 (m, 2H), 7.95-8.15 (m, 2H), 7.44-7.76 (m, 6H), 4.63-4.82 (m, 2H), 1.35-1.56 (m, 3H). HRMS: Found: 302.1273; Expected: 302.3498.

SQOR Activity Assays

Recombinant human SQOR was expressed in *E. coli* BL21(DE3) cells and purified as previously described[16,24]. Enzyme activity was measured using a 2,6-dichlorophenolindophenol (DCIP) endpoint assay developed for high-throughput screening, as we recently reported[21,22]. Briefly, reaction mixtures containing enzyme, substrates (H_2S, sulfite, CoQ_1) and inhibitors are incubated in 96-well plates. The reactions are quenched after 60 s by addition of formaldehyde to denature SQOR and N-ethylmaleimide to consume unreacted H_2S, a step that prevents the nonenzymic reduction of DCIP by H_2S. DCIP is added after a 10 min incubation. The absorbance at 600 nm is read 2 min after DCIP addition. For IC_{50} determinations, different concentrations of inhibitor are added to columns 3-10. Columns 1-2 and 11-12 are reserved for negative and positive control reactions, respectively. SQOR is not added to negative control wells. For each inhibitor concentration, fractional activity values were calculated and IC_{50} values and Hill slopes were determined using SigmaPlot 10 (Systat Software) and a four-parameter logistic equation with top or bottom values of the sigmoidal curve anchored to 1 or 0 fractional activity, respectively.

Docking Studies

Docking of inhibitors into the previously identified CoQ-binding site in human SQOR[24] was conducted using GLIDE (Schrödinger Suite 2019-3; Schrödinger, LLC). The protein-preparation wizard in Maestro 12.1.013 (Schrödinger Suite 2019-3) was used to prepare the ligand-free protein (PDB:ID 6M06) for docking. The Builder and LigPrep tools in Maestro 12.1.013 were used to prepare compounds for docking, which was conducted using the extra precision mode of Glide-XP[26]. Refinement of ligand-protein complexes was performed using Prime refine mode[27,28].

Docking studies were also conducted using the GOLD Suite of Programs (CSDC-2019.1, CCDC Software Ltd., Cambridge, UK). The protein was prepared for docking and the CoQ-binding cavity (delimited by atoms within 6 Å of the binding site of DCQ) was defined using the Gold Setup Wizard. Mercury was used to prepare compounds for docking. Docking was conducted using Hermes and the GOLD High Accuracy Sampling and Scoring Protocol with settings intended to produce diverse solutions with highly flexible ligands. Docking and rescore were performed using GoldScore and CHEMPLP scoring functions, respectively. Docking solutions were ranked, in part, based on Goldscore Fitness and PLP Fitness scores. In each case, solution 1 was designated as the top-scoring pose and, in tests with 6 inhibitors, was found to be very similar to that obtained with Glide.

Structural figures of models of SQOR•inhibitor complexes were prepared using Pymol 2.3.4.

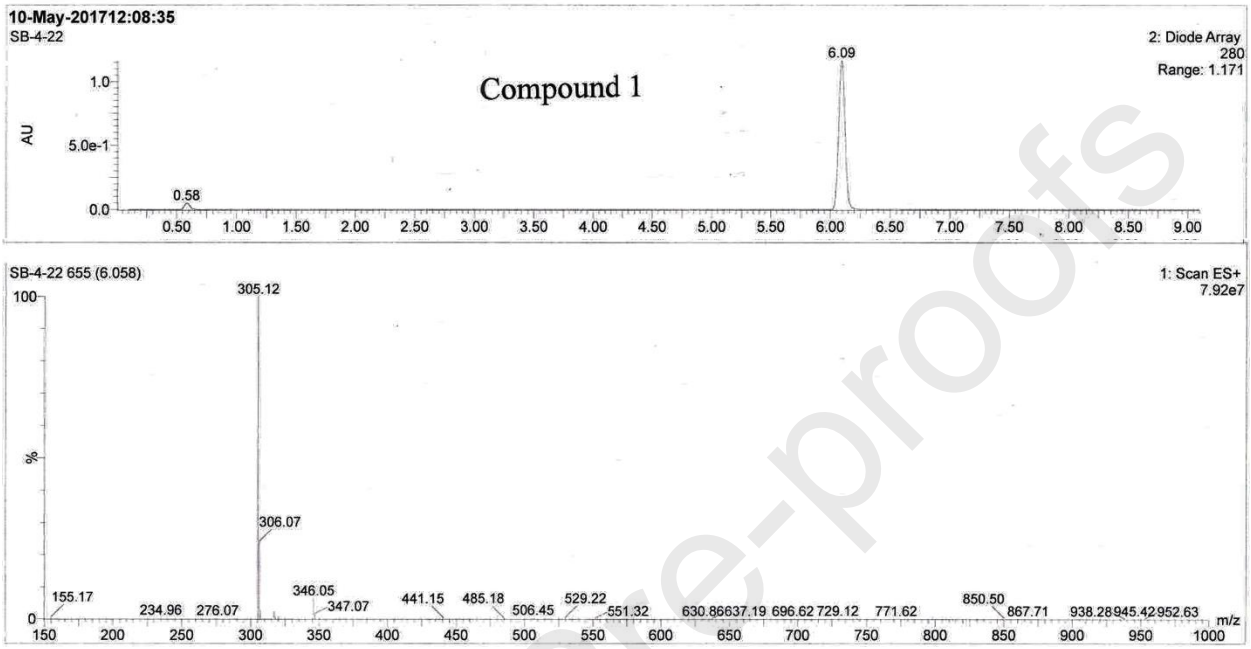
Physicochemical and ADME/PK Properties of SAR Class A' Lead Compounds

The aqueous solubility, mouse plasma protein binding, and Caco-2 permeability of **15** were determined by Alliance Pharma Inc. (Malvern, PA). The aqueous solubility, mouse plasma protein binding, and Caco-2 permeability of **19** were determined by Pharmaron, Inc. (Beijing,

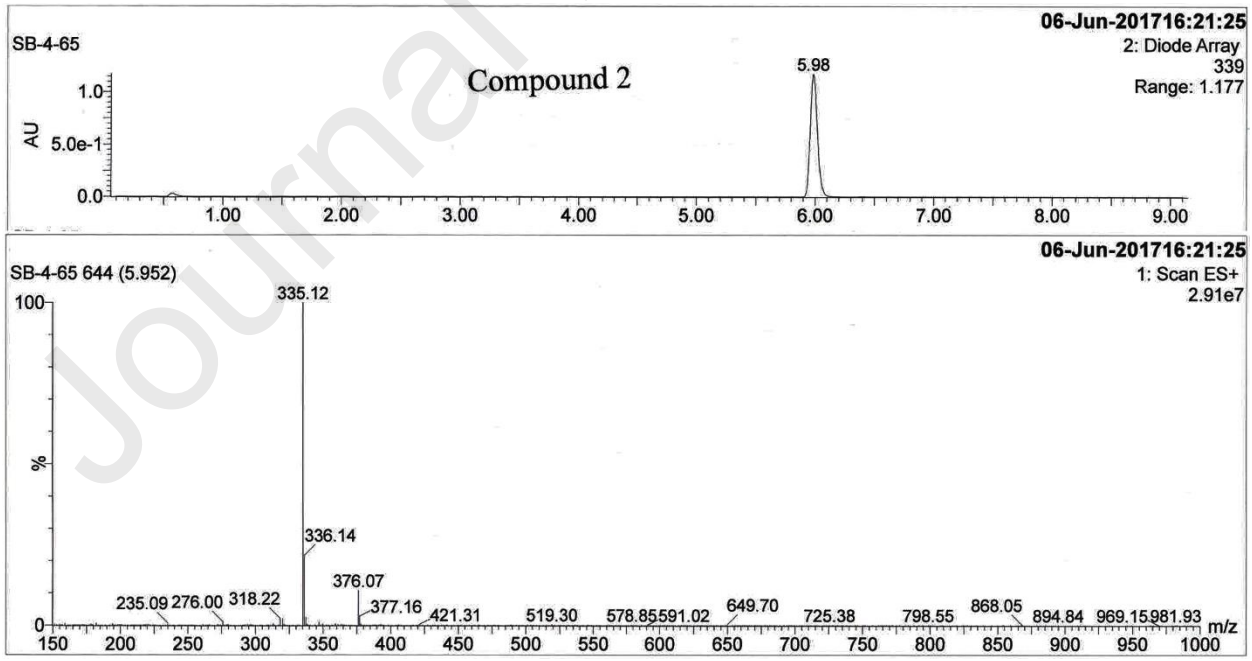
China). Mouse PK studies with **15** were conducted by Pharmaron, Inc. Values for tPSA and clogP were calculated using ChemBioDraw Ultra Version 14.0.0.117.

Figure S1 LC/MS Analysis

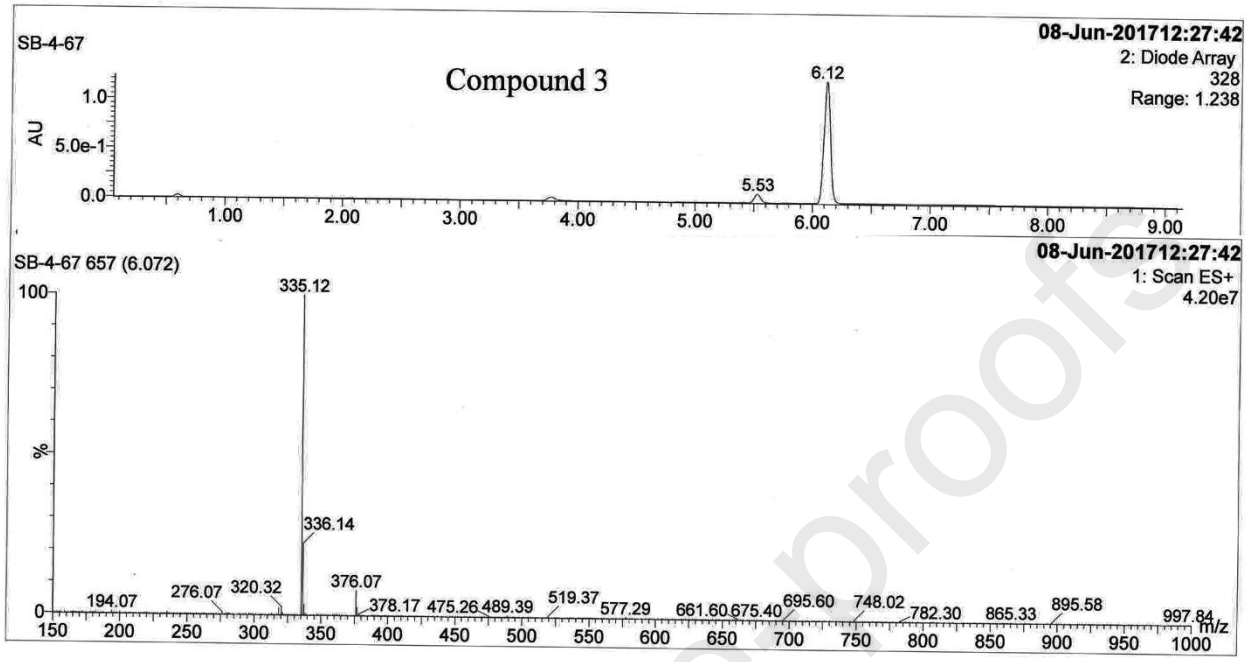
LC/MS analysis of compound 1



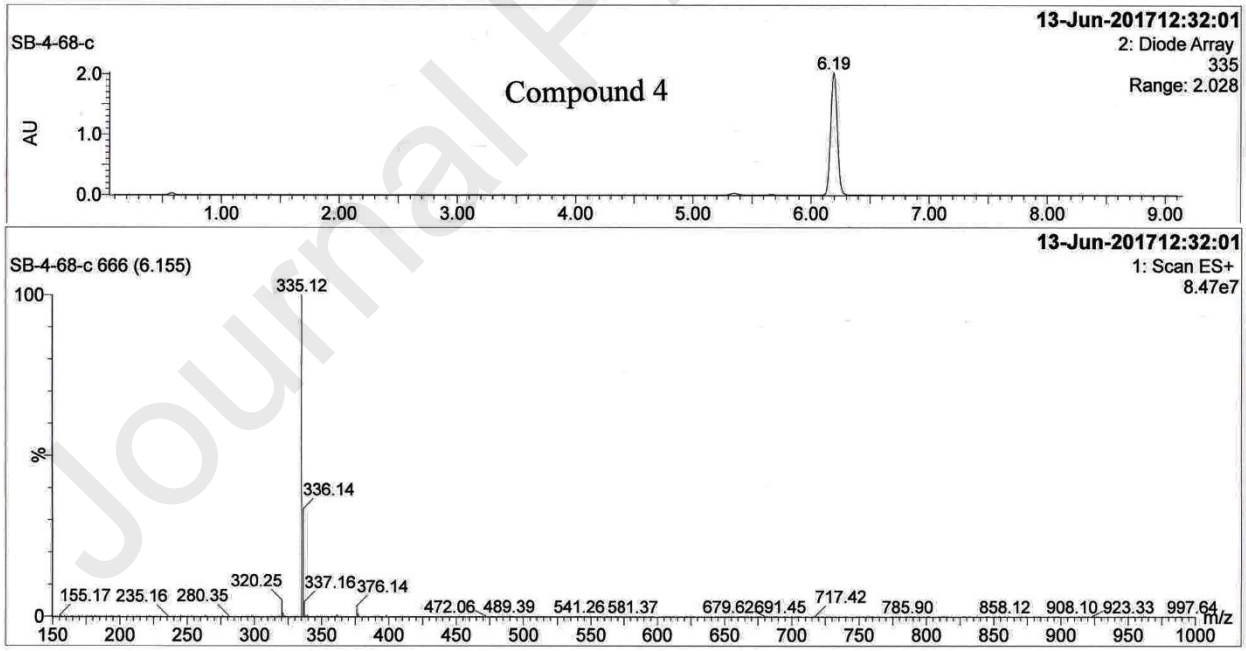
LC/MS analysis of compound 2



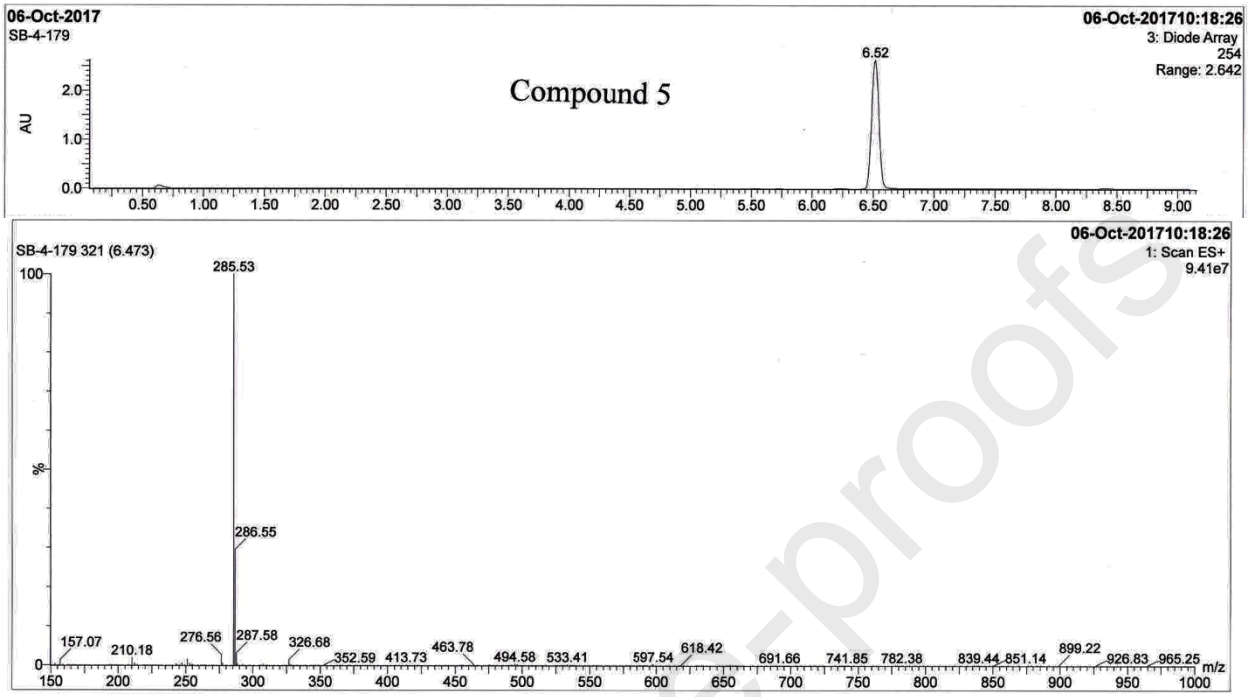
LC/MS analysis of compound 3



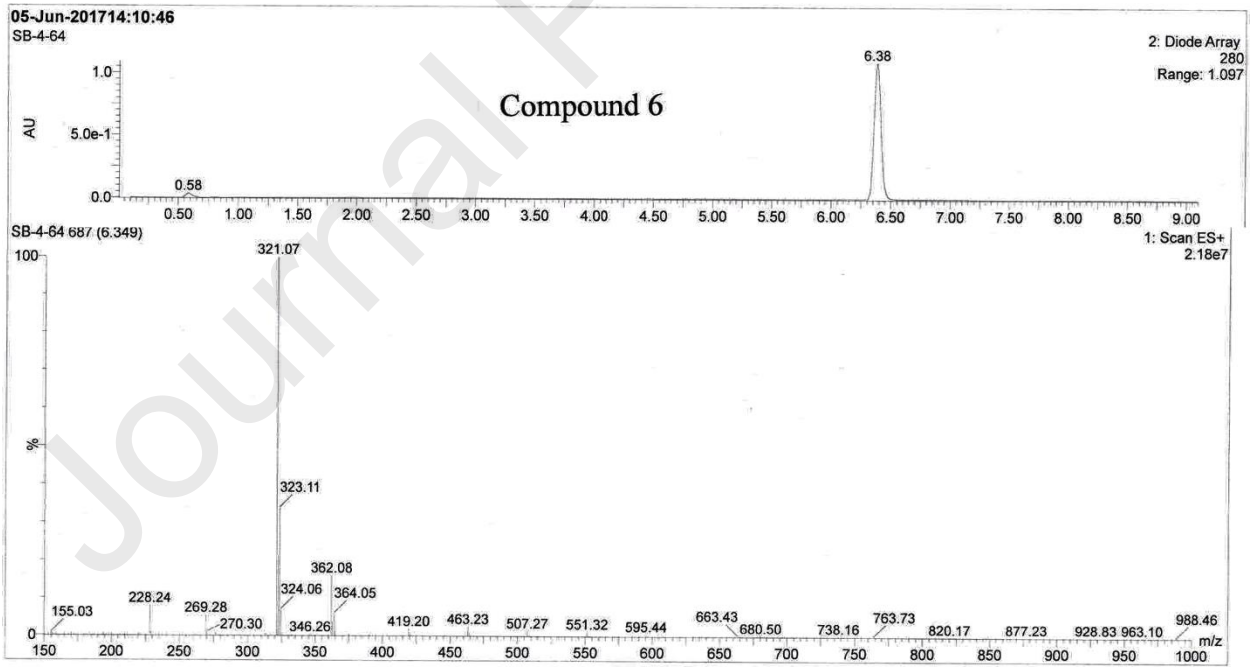
LC/MS analysis of compound 4



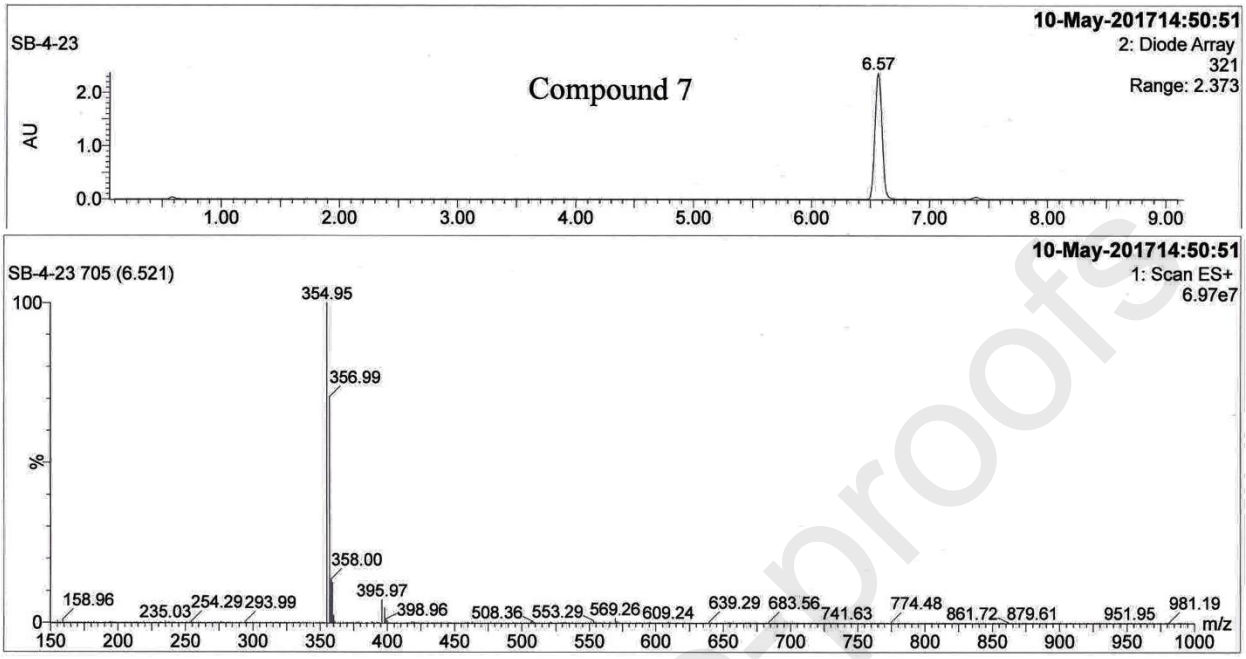
LC/MS analysis of compound 5



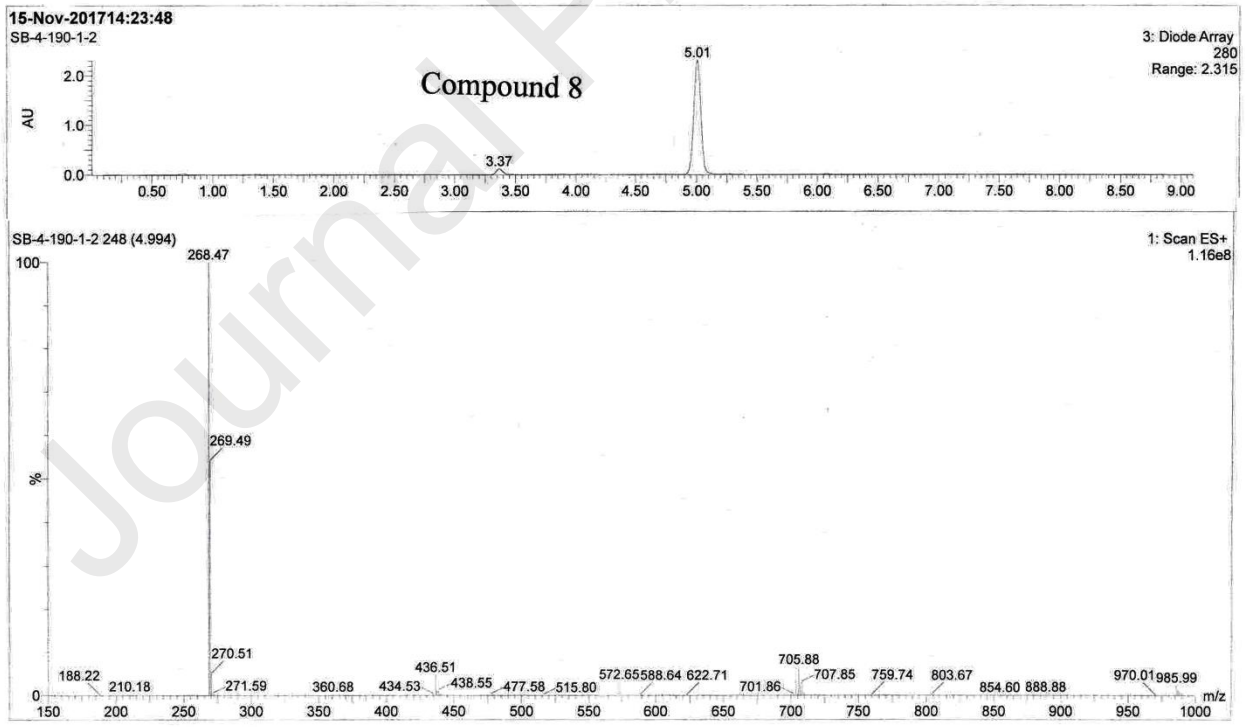
LC/MS analysis of compound 6



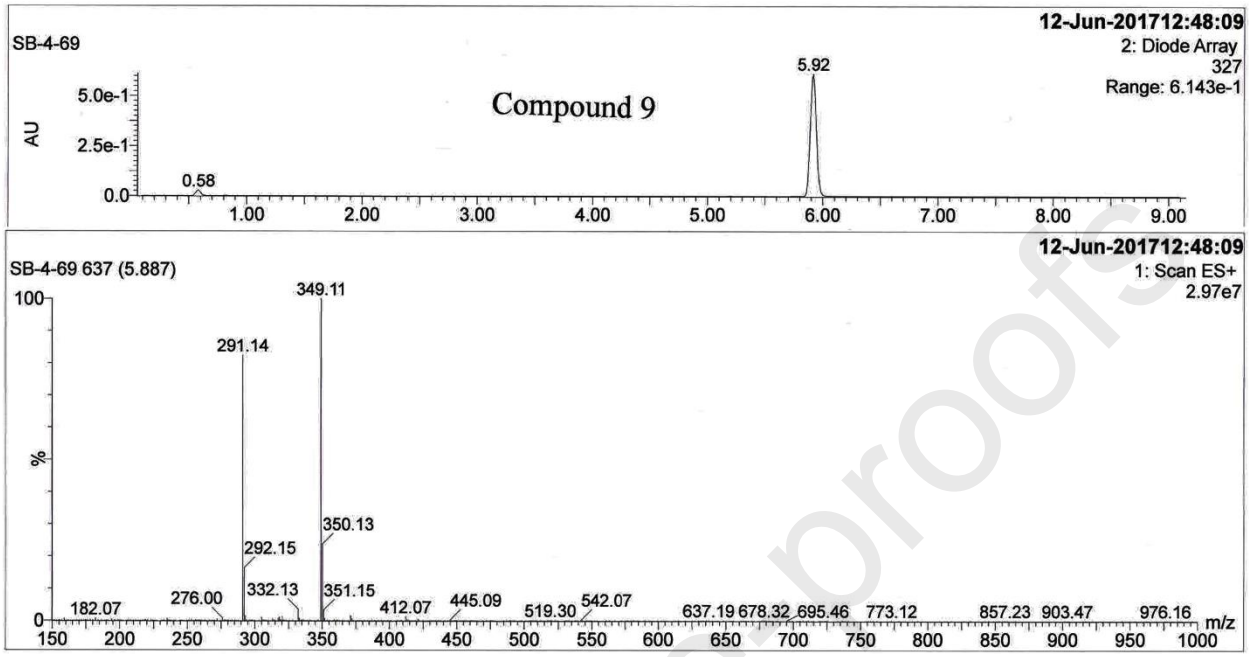
LC/MS analysis of compound 7



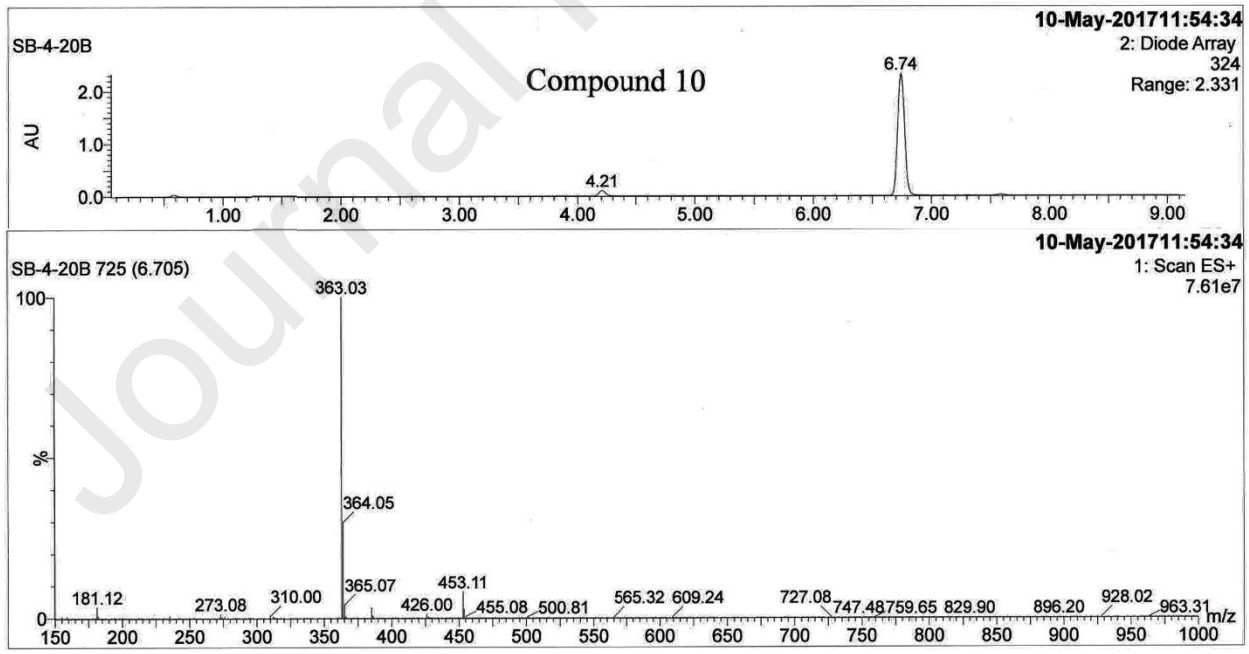
LC/MS analysis of compound 8



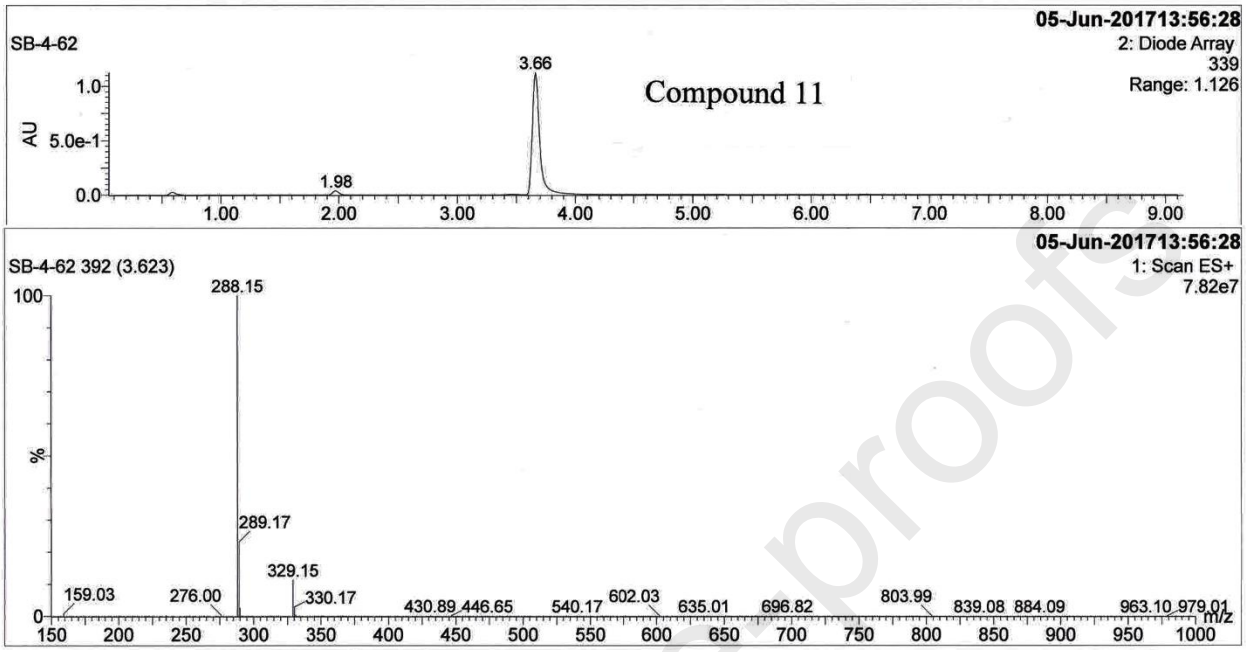
LC/MS analysis of compound 9



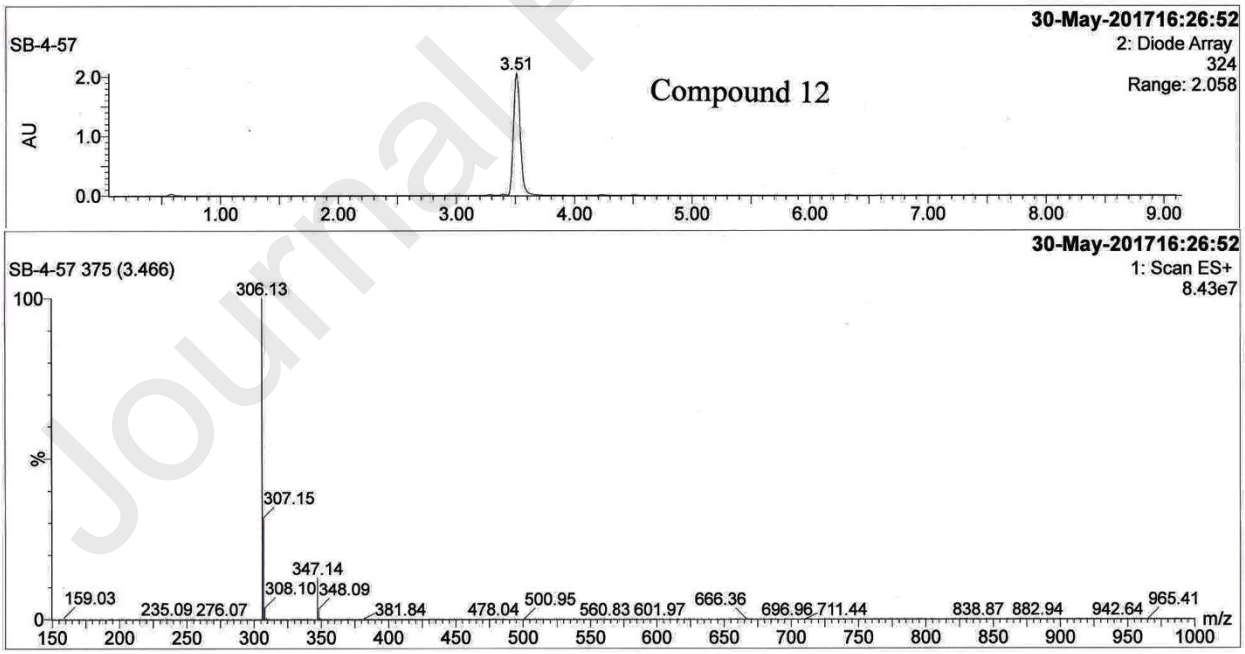
LC/MS analysis of compound 10



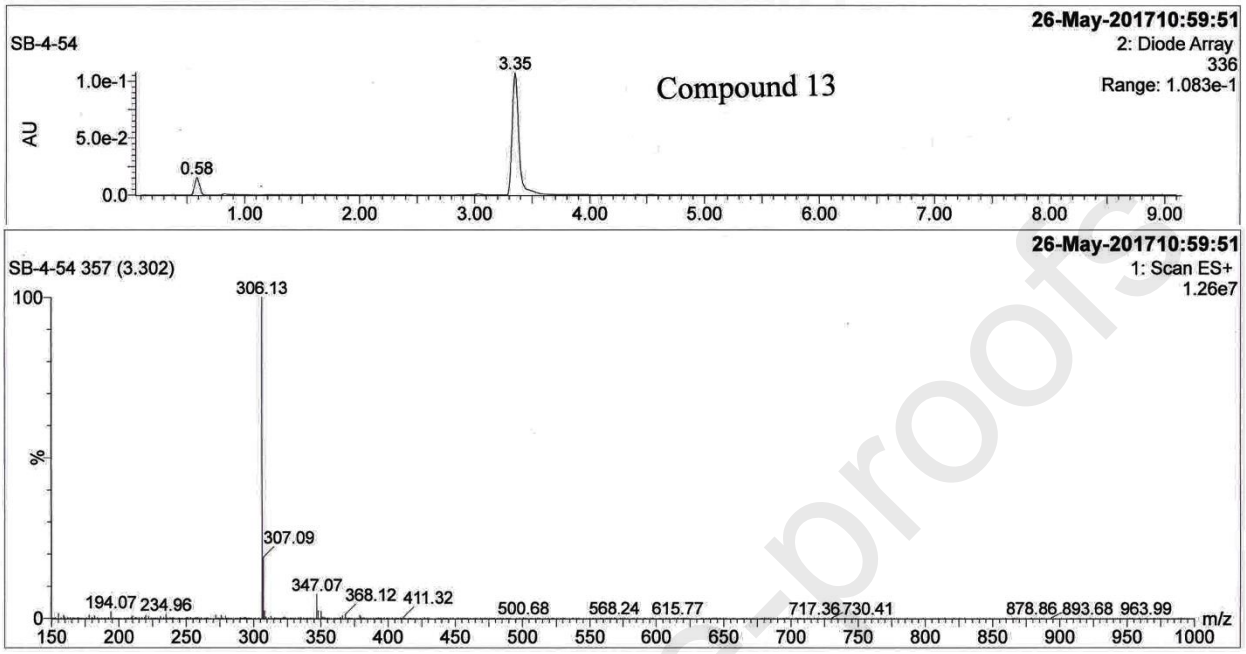
LC/MS analysis of compound 11



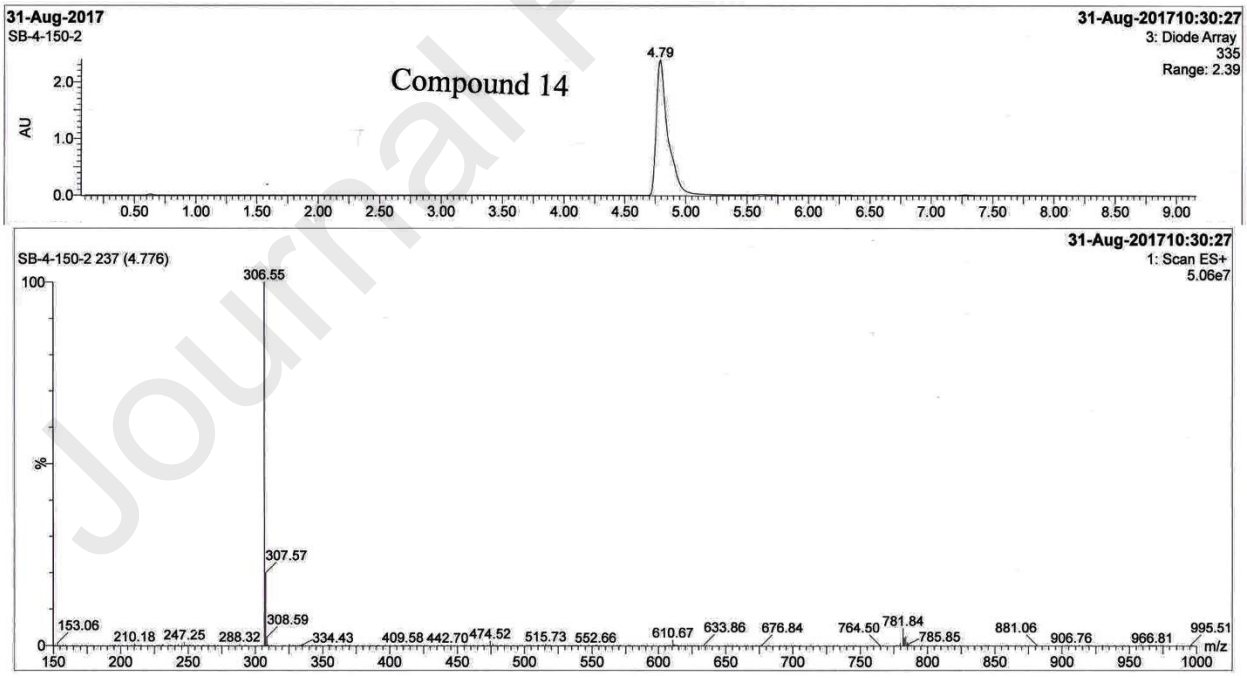
LC/MS analysis of compound 12



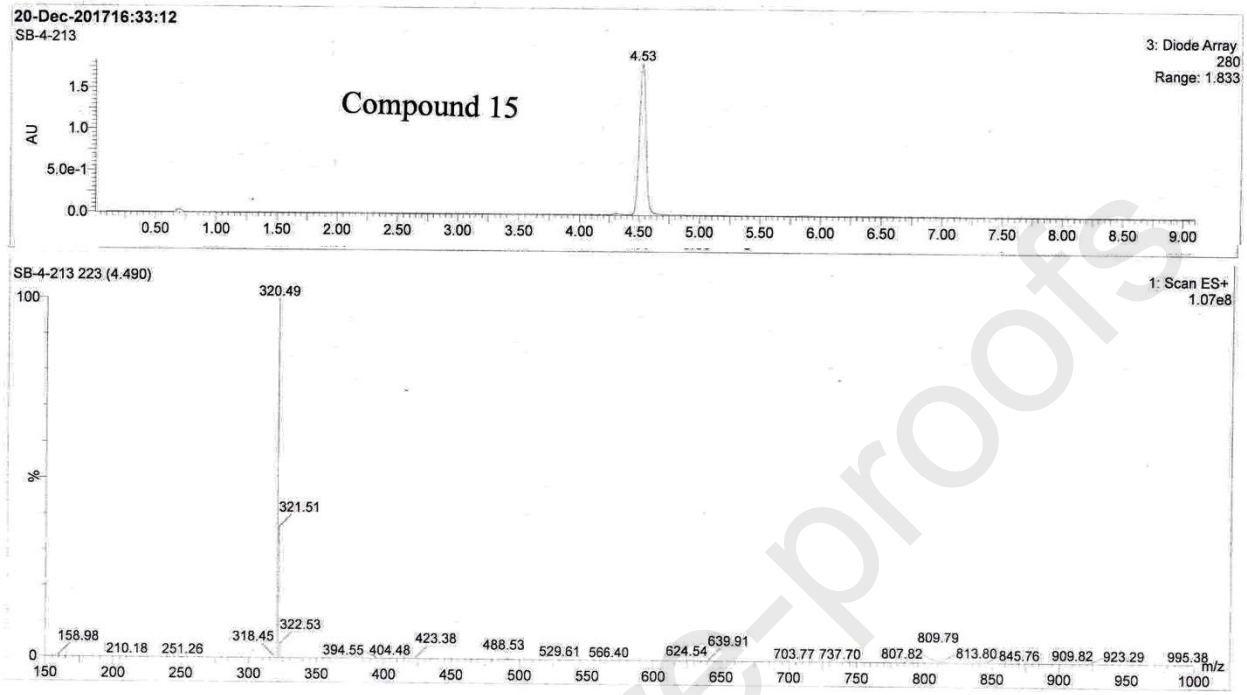
LC/MS analysis of compound 13



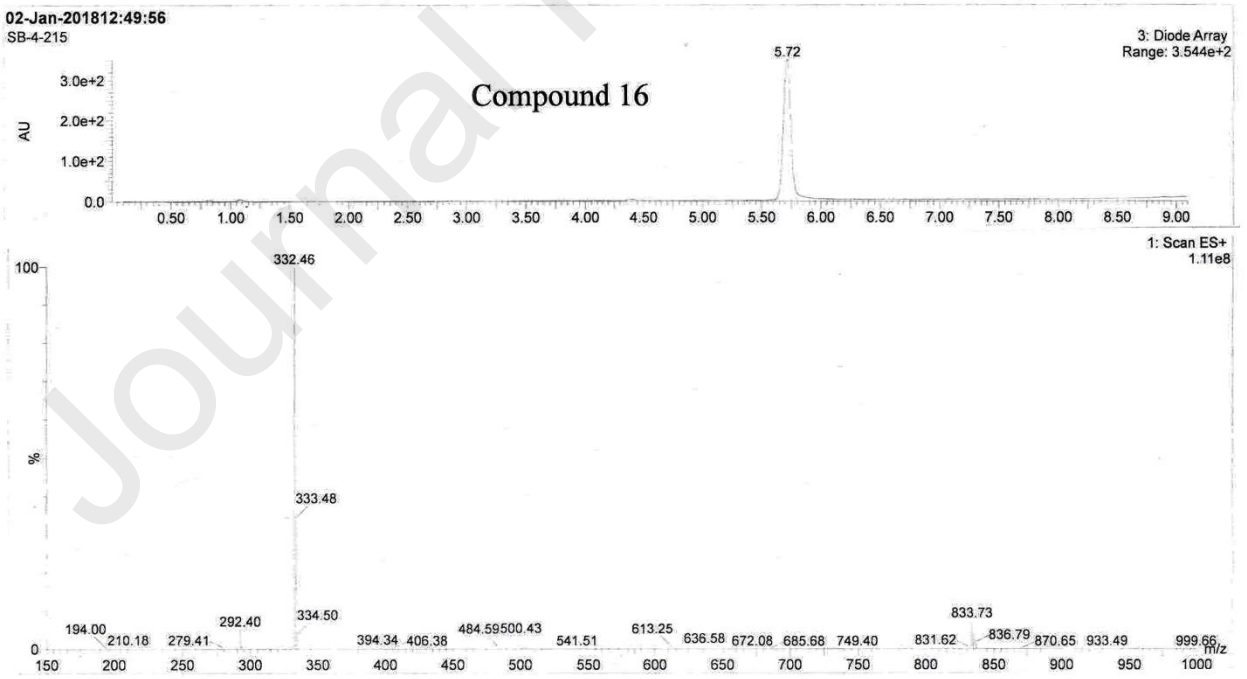
LC/MS analysis of compound 14



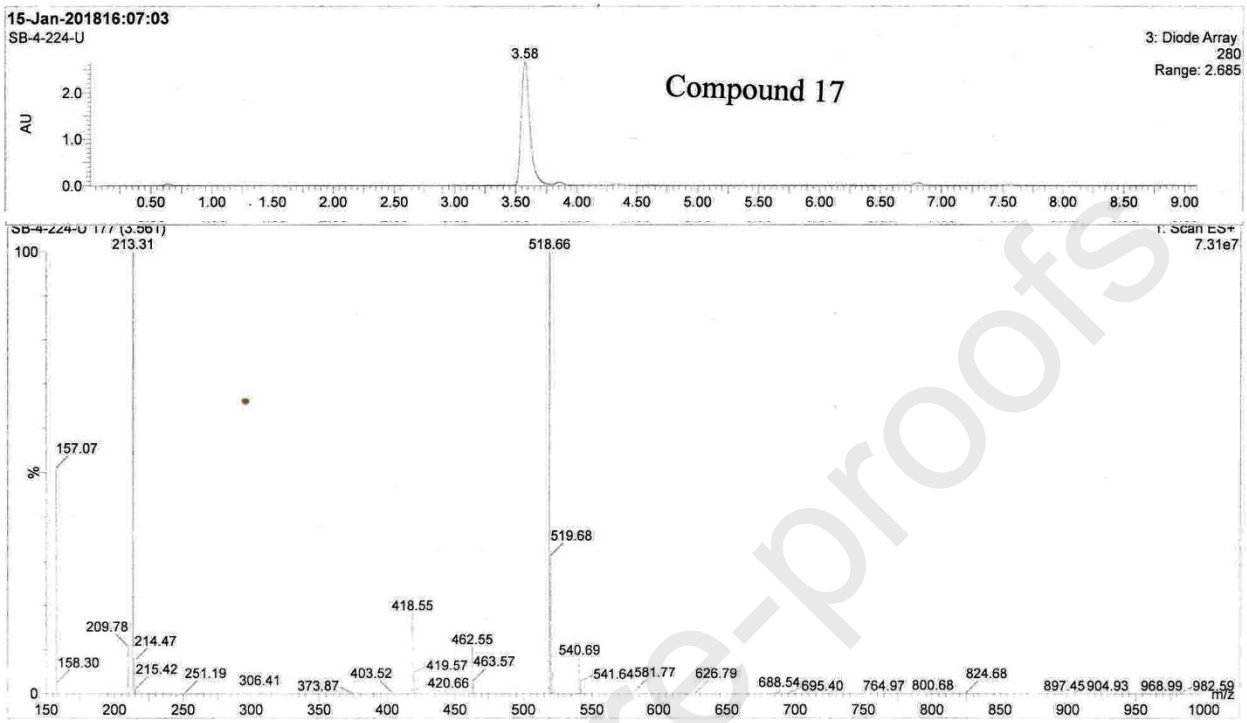
LC/MS analysis of compound 15



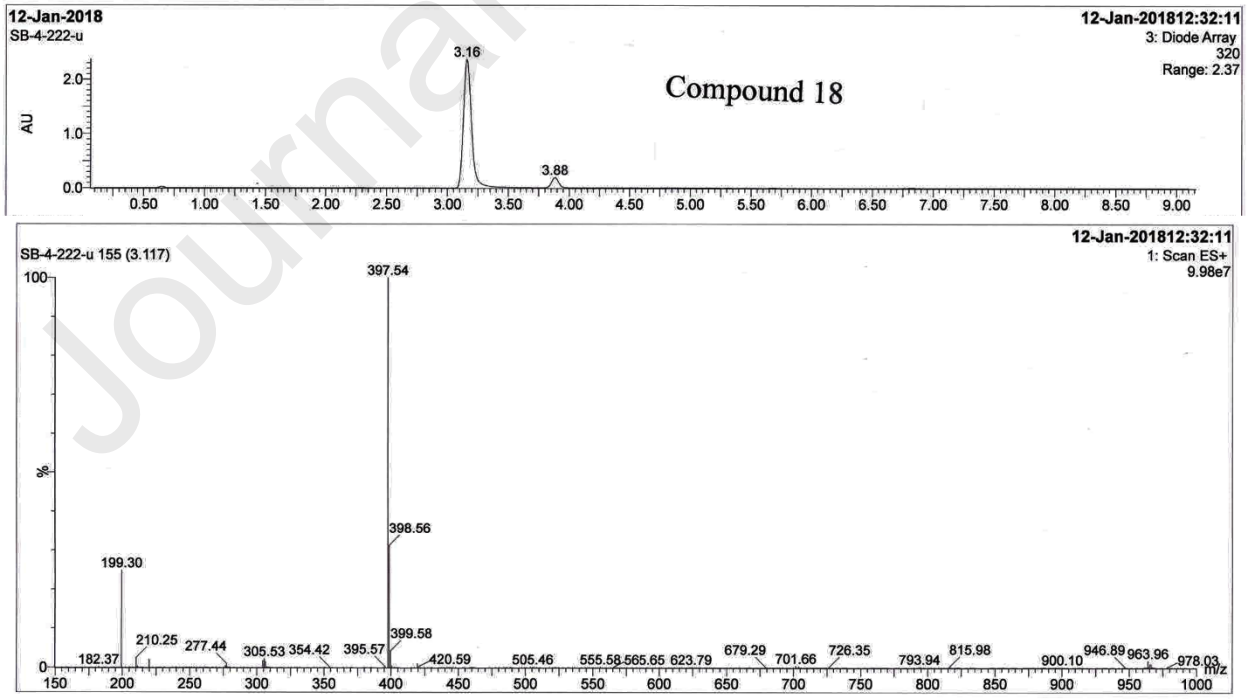
LC/MS analysis of compound 16



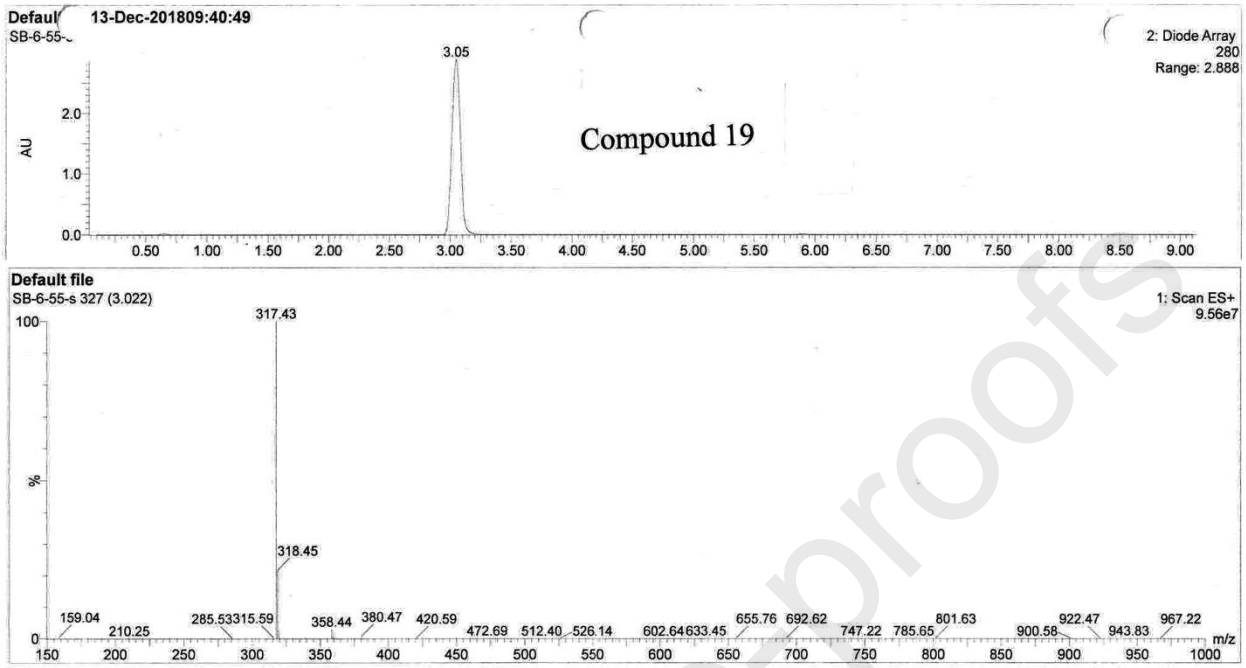
LC/MS analysis of compound 17



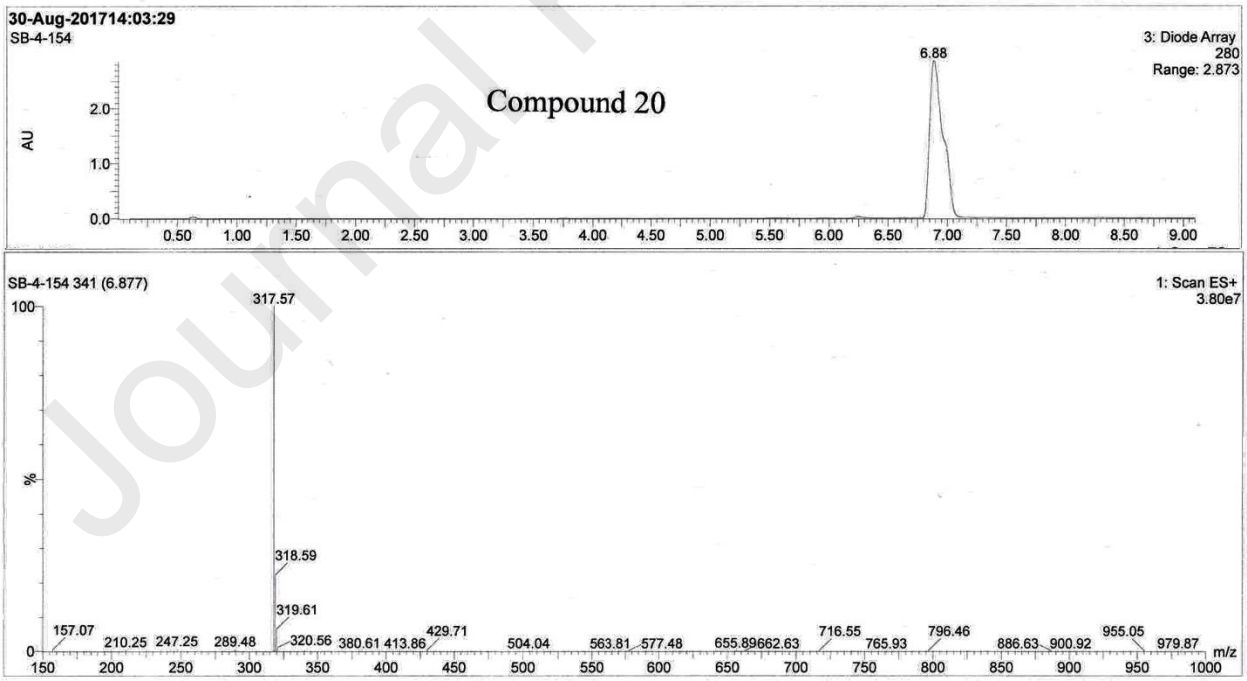
LC/MS analysis of compound 18



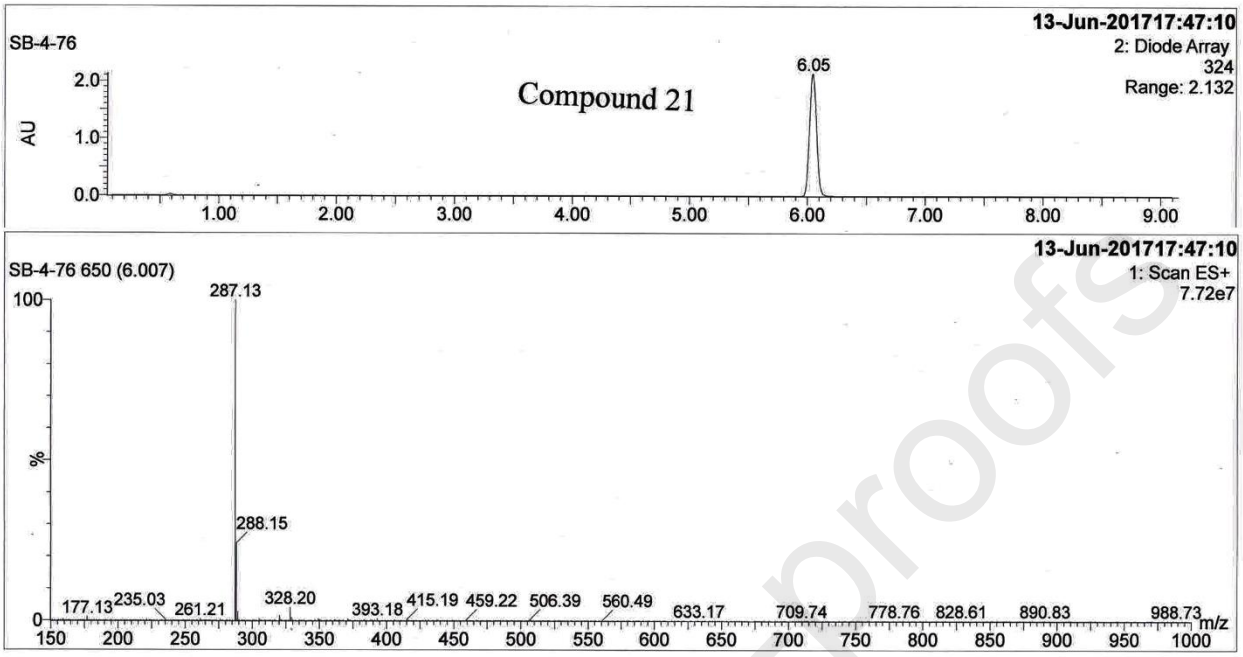
LC/MS analysis of compound 19



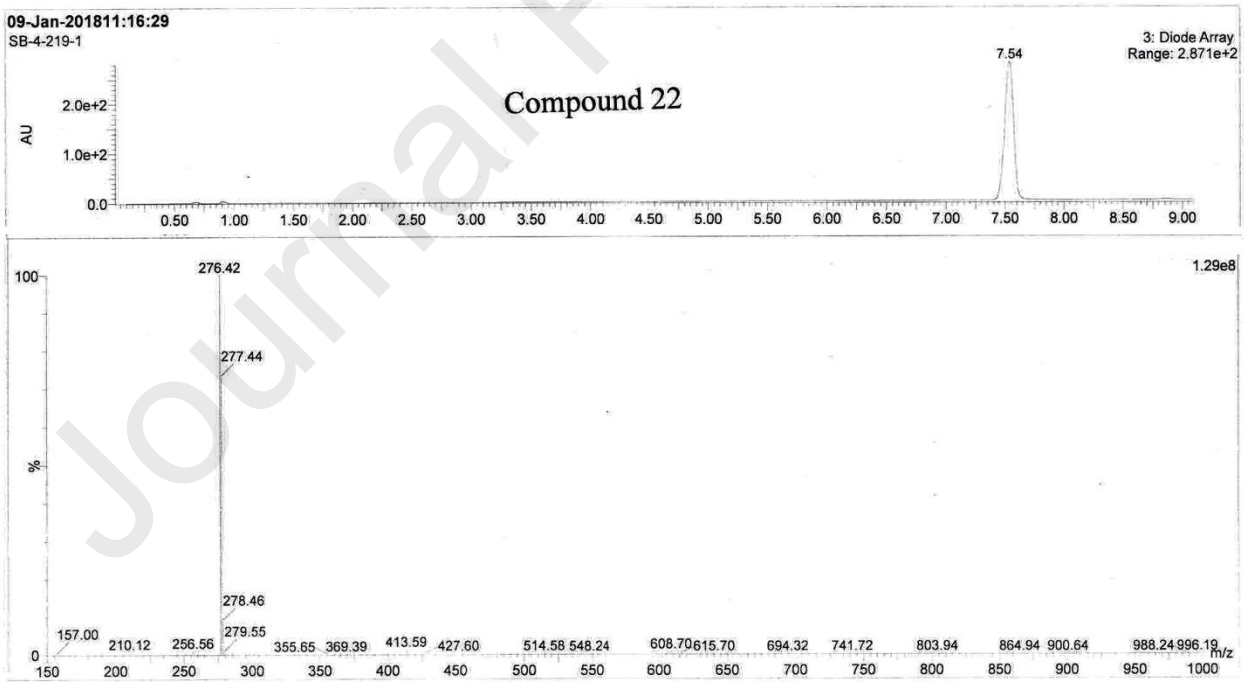
LC/MS analysis of compound 20



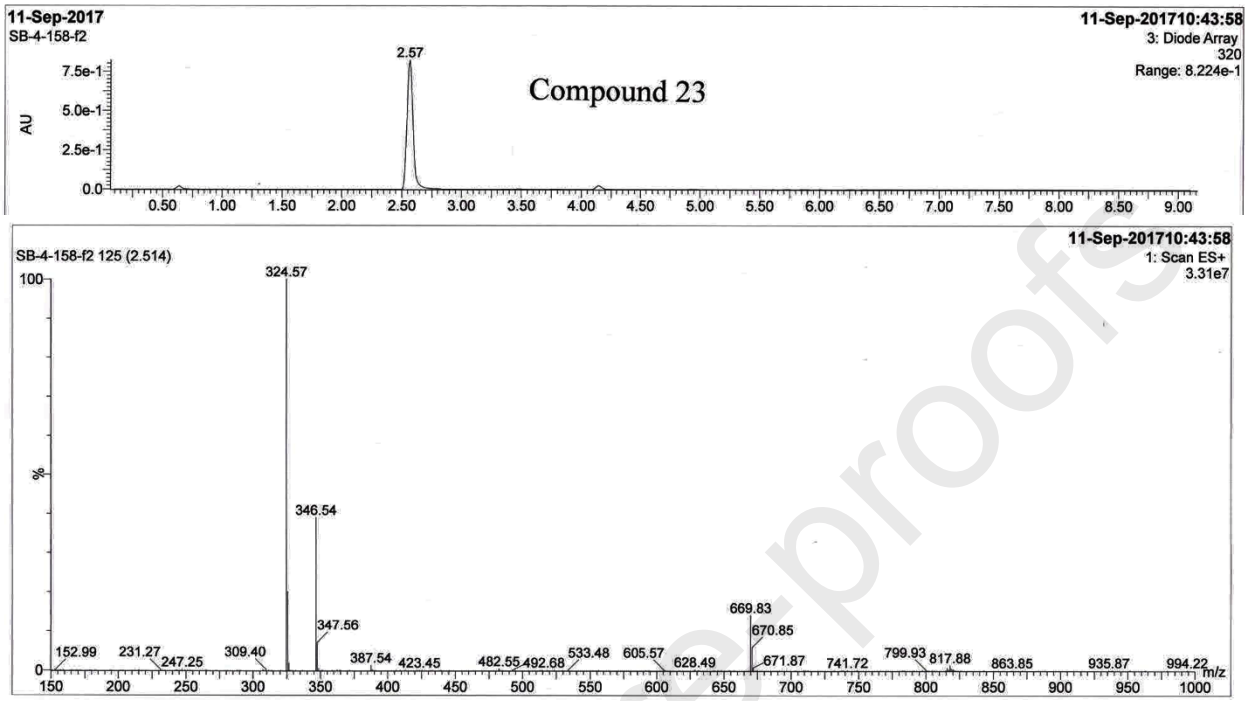
LC/MS analysis of compound 21



LC/MS analysis of compound 22



LC/MS analysis of compound 23



LC/MS analysis of compound 24

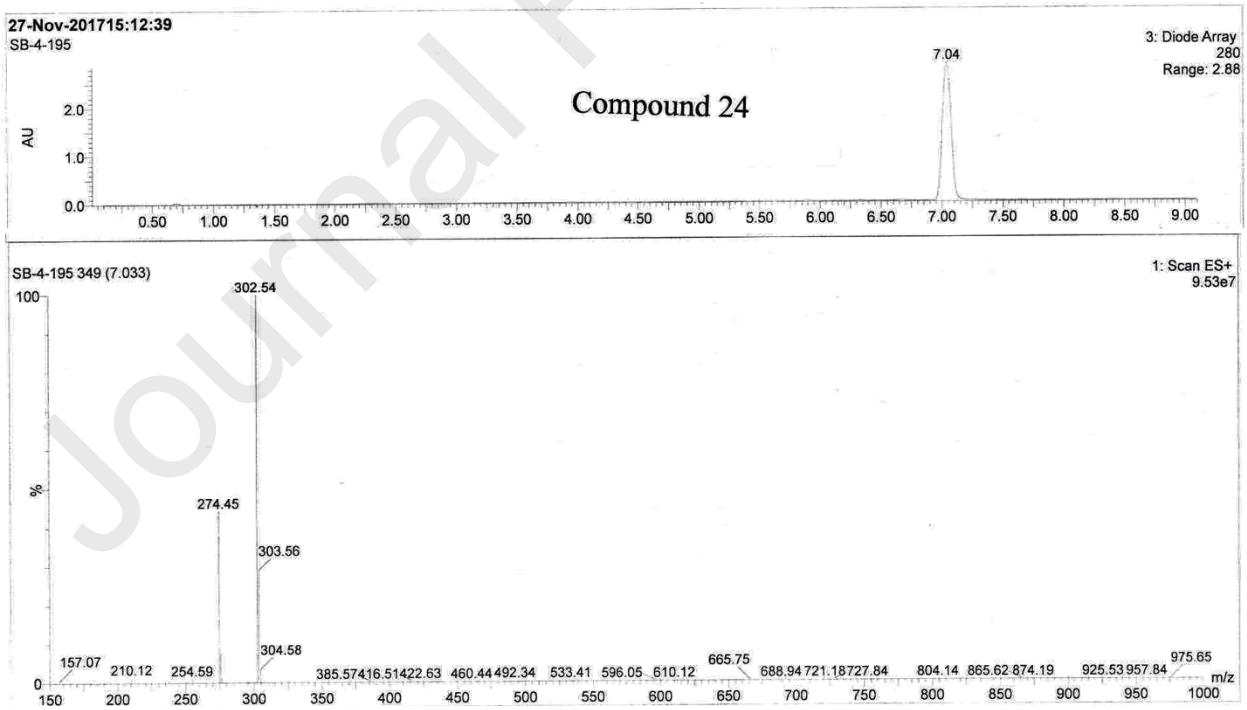
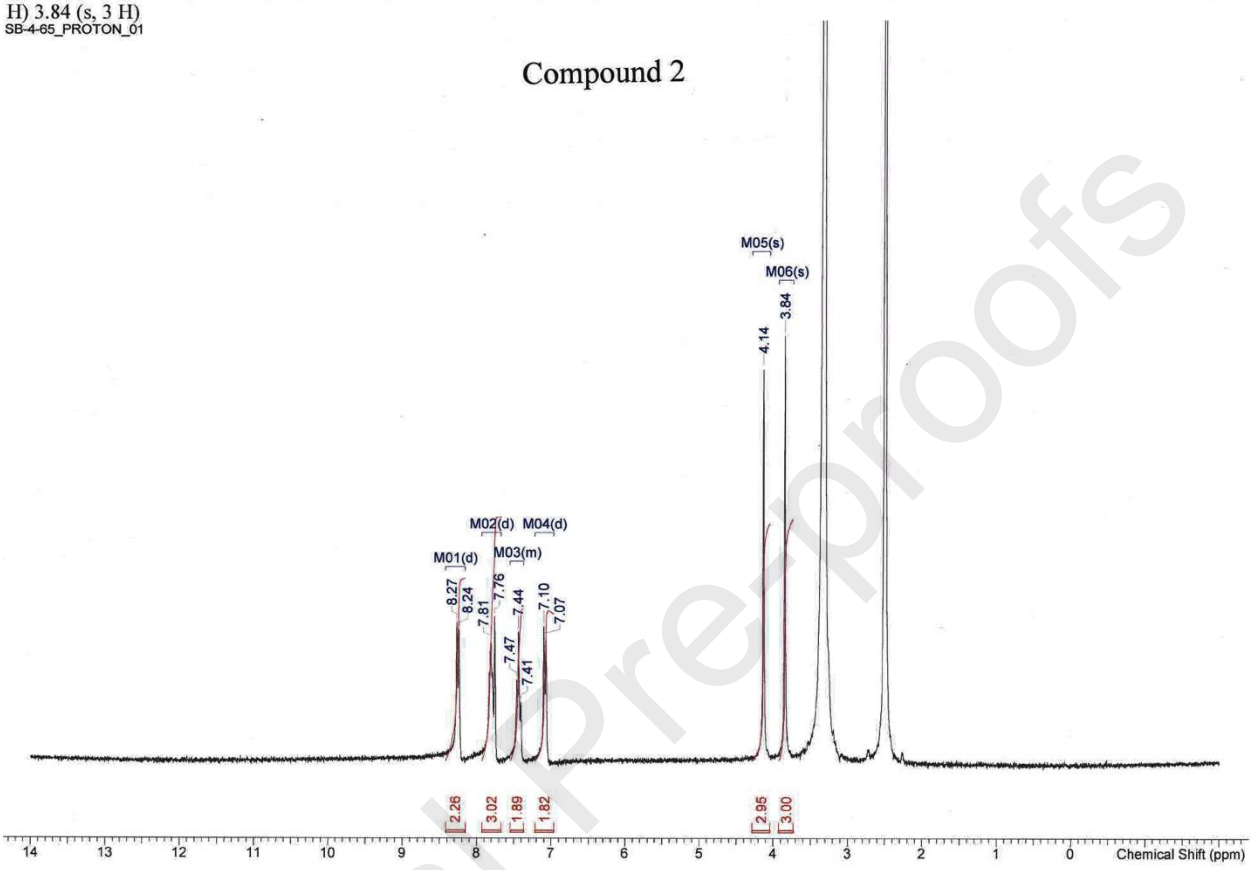


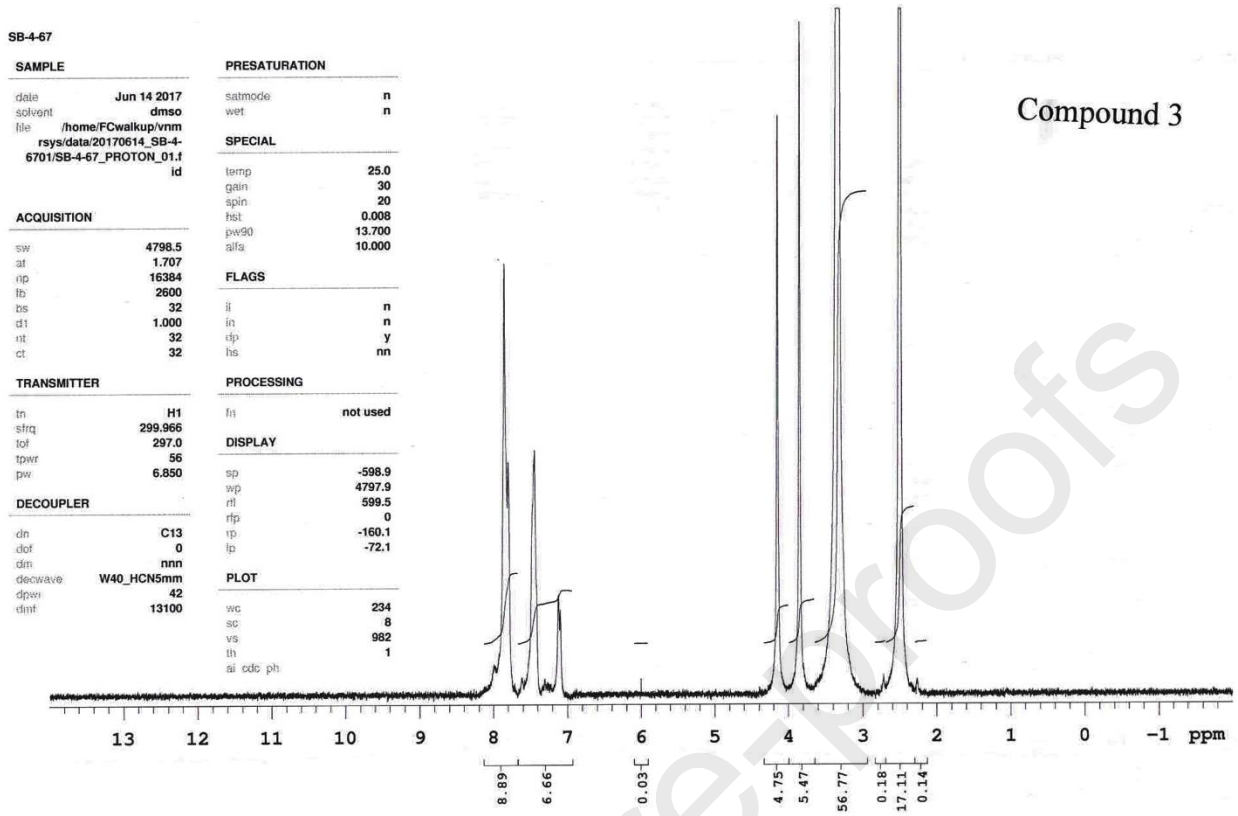
Figure S2 NMR Analysis

NMR analysis of compound 2

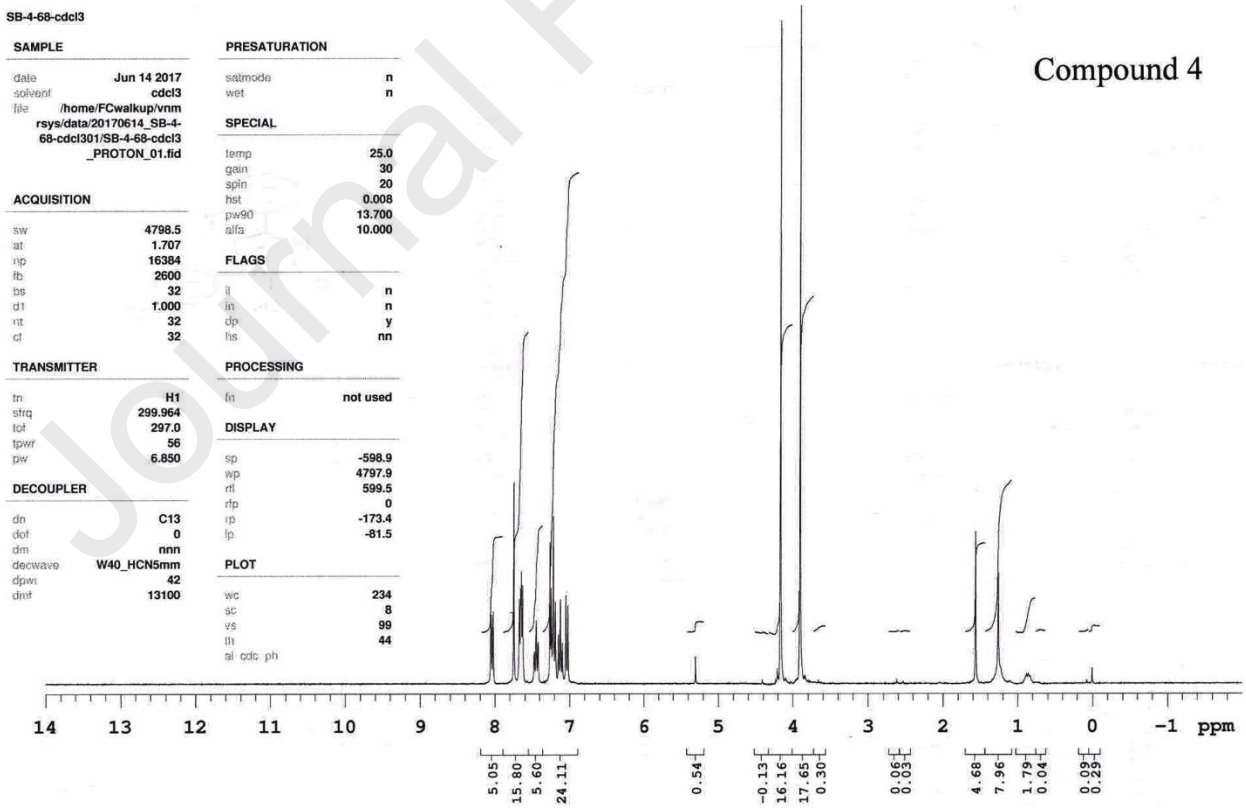
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.25 (d, J=8.20 Hz, 2 H) 7.78 (d, J=14.65 Hz, 3 H) 7.37 - 7.55 (m, 2 H) 7.08 (d, J=8.20 Hz, 2 H) 4.14 (s, 3 H) 3.84 (s, 3 H)



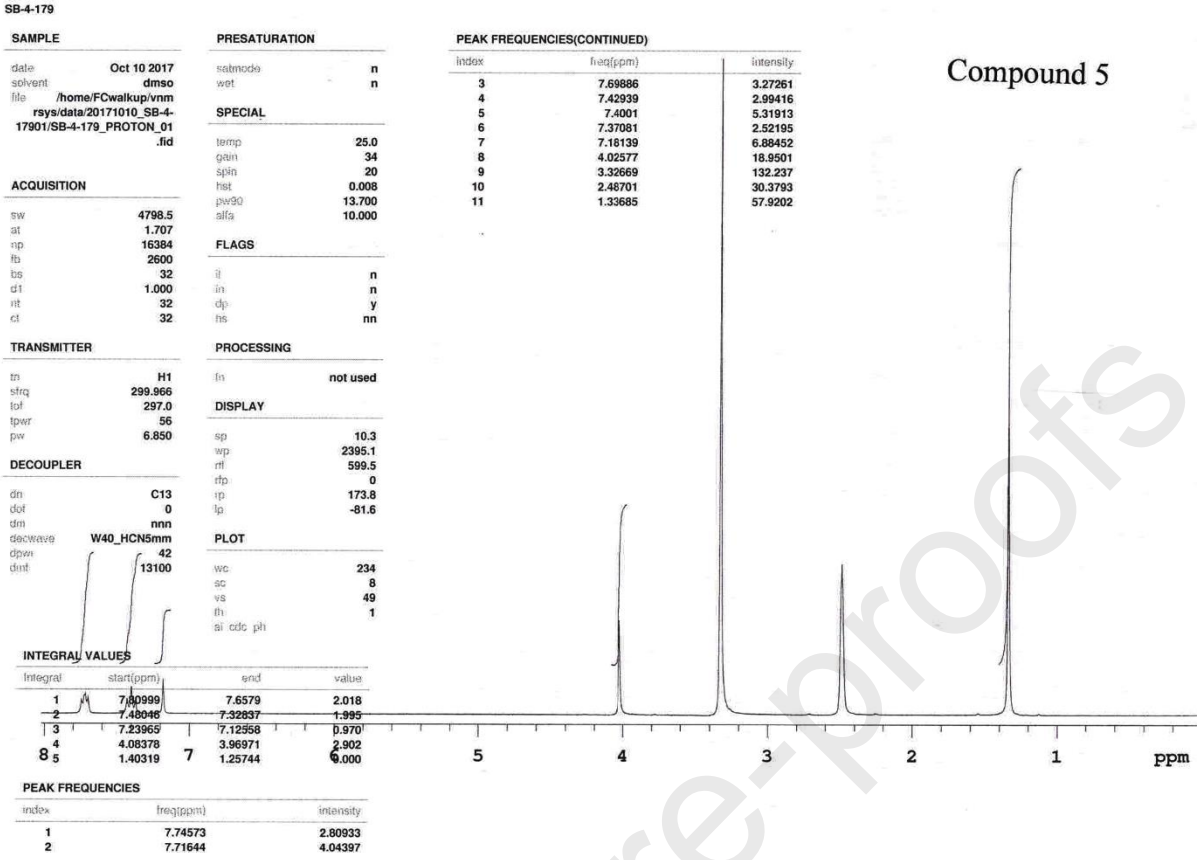
NMR analysis of compound 3



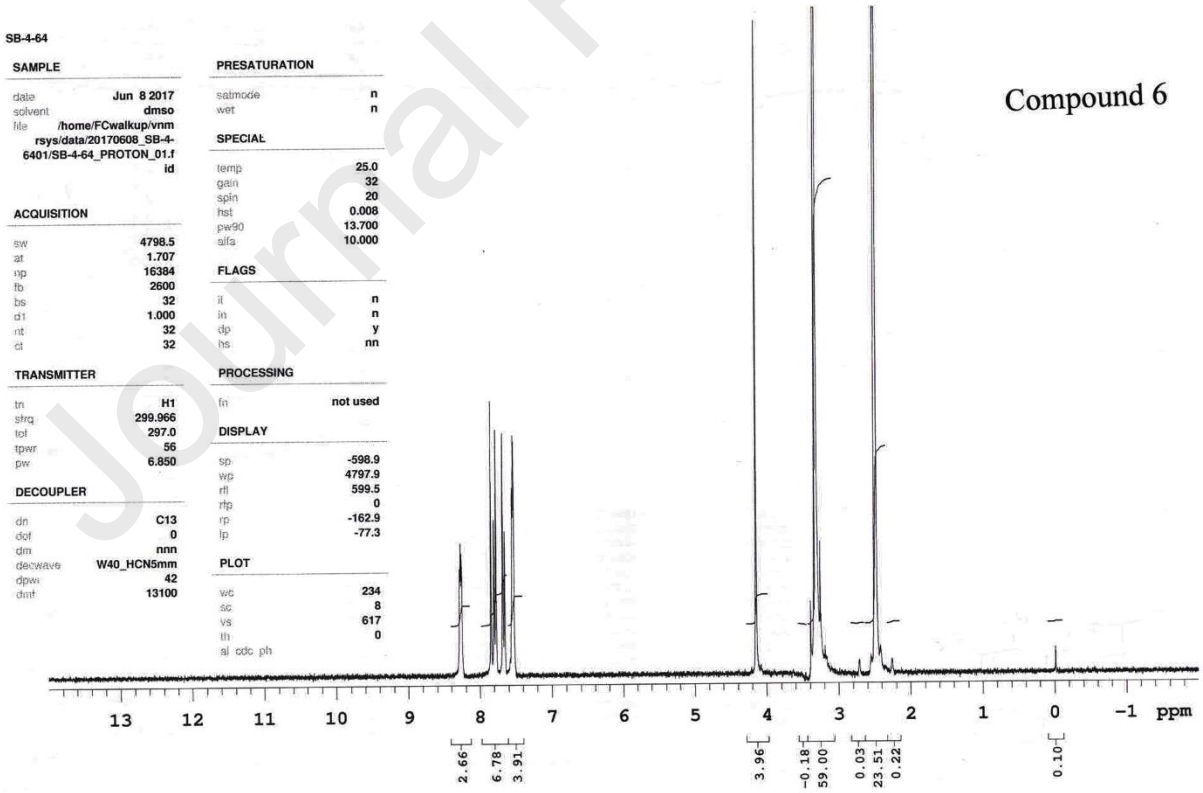
NMR analysis of compound 4



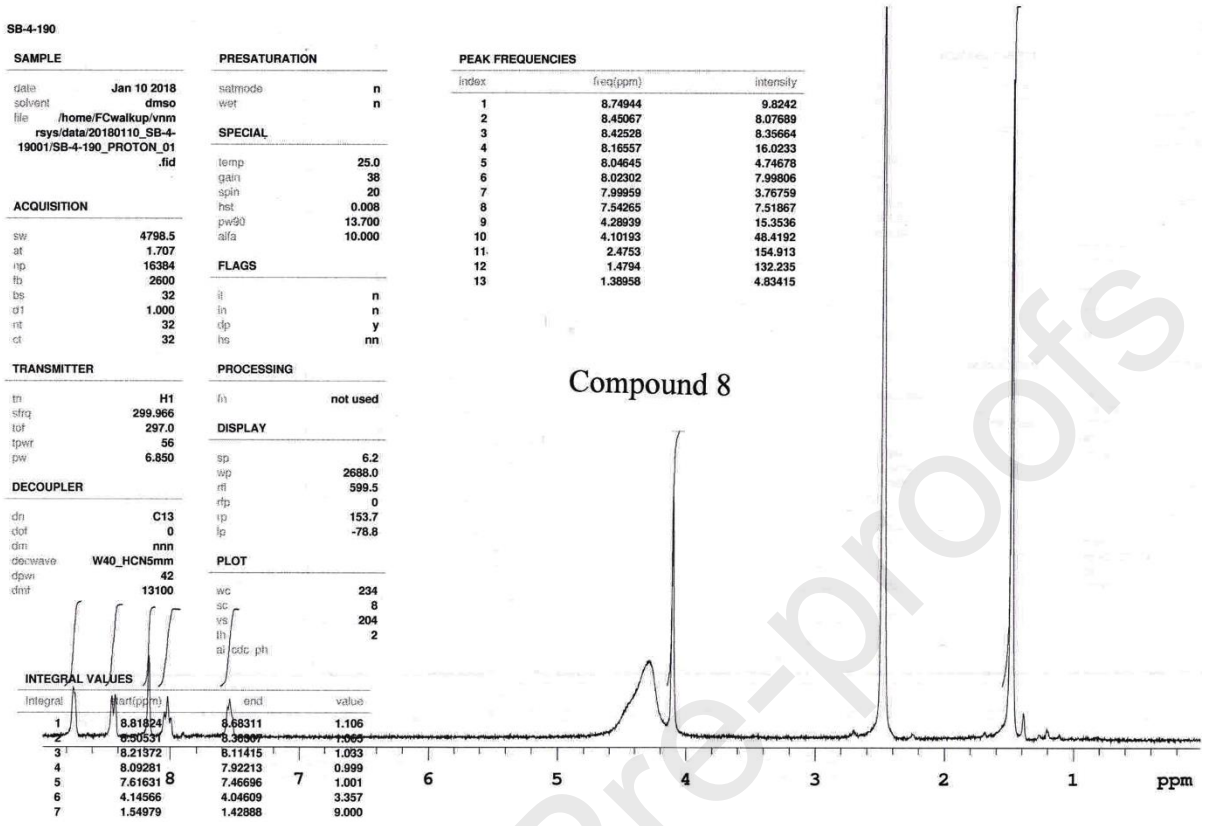
NMR analysis of compound 5



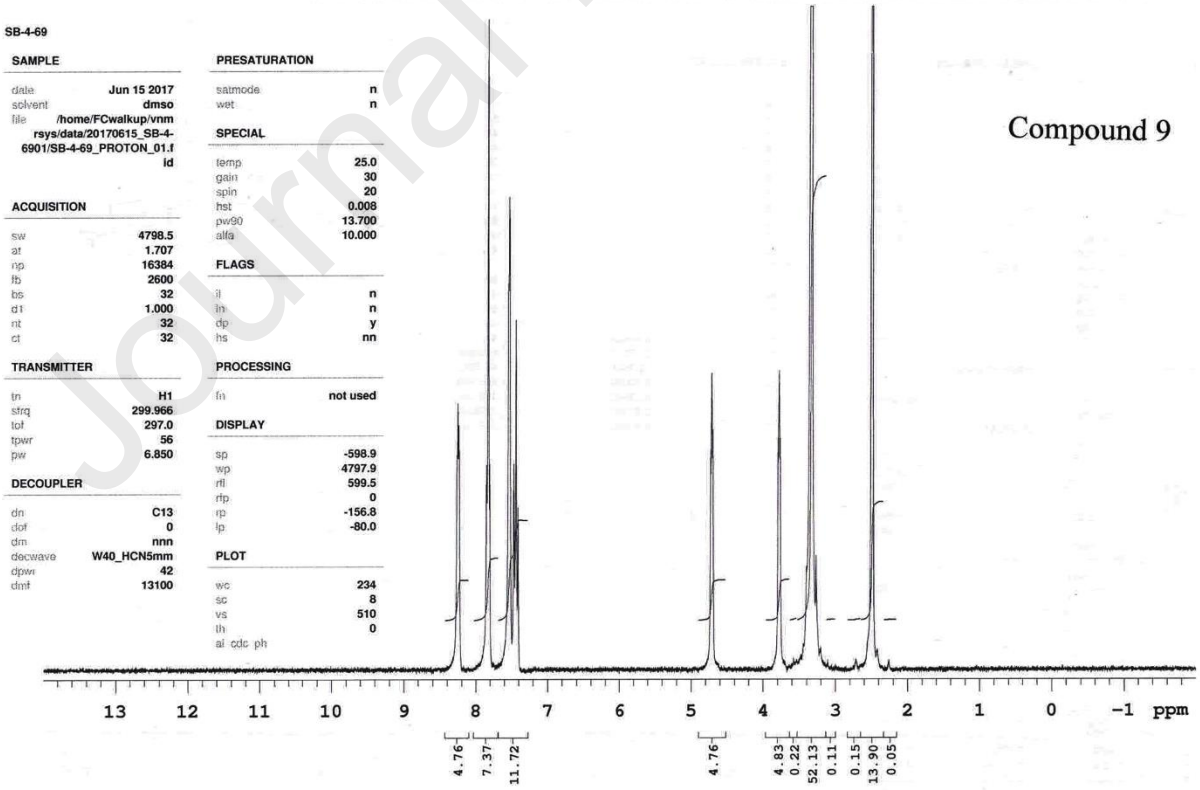
NMR analysis of compound 6



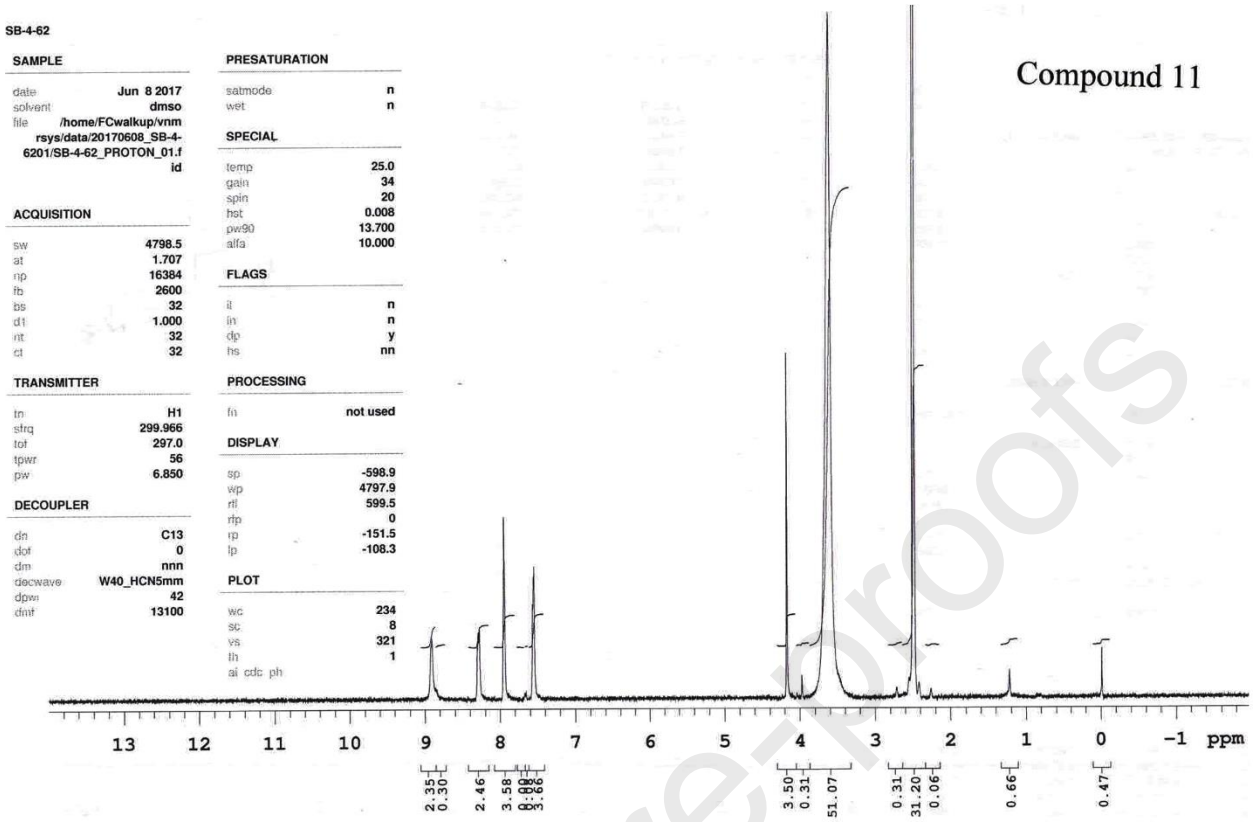
NMR analysis of compound 8



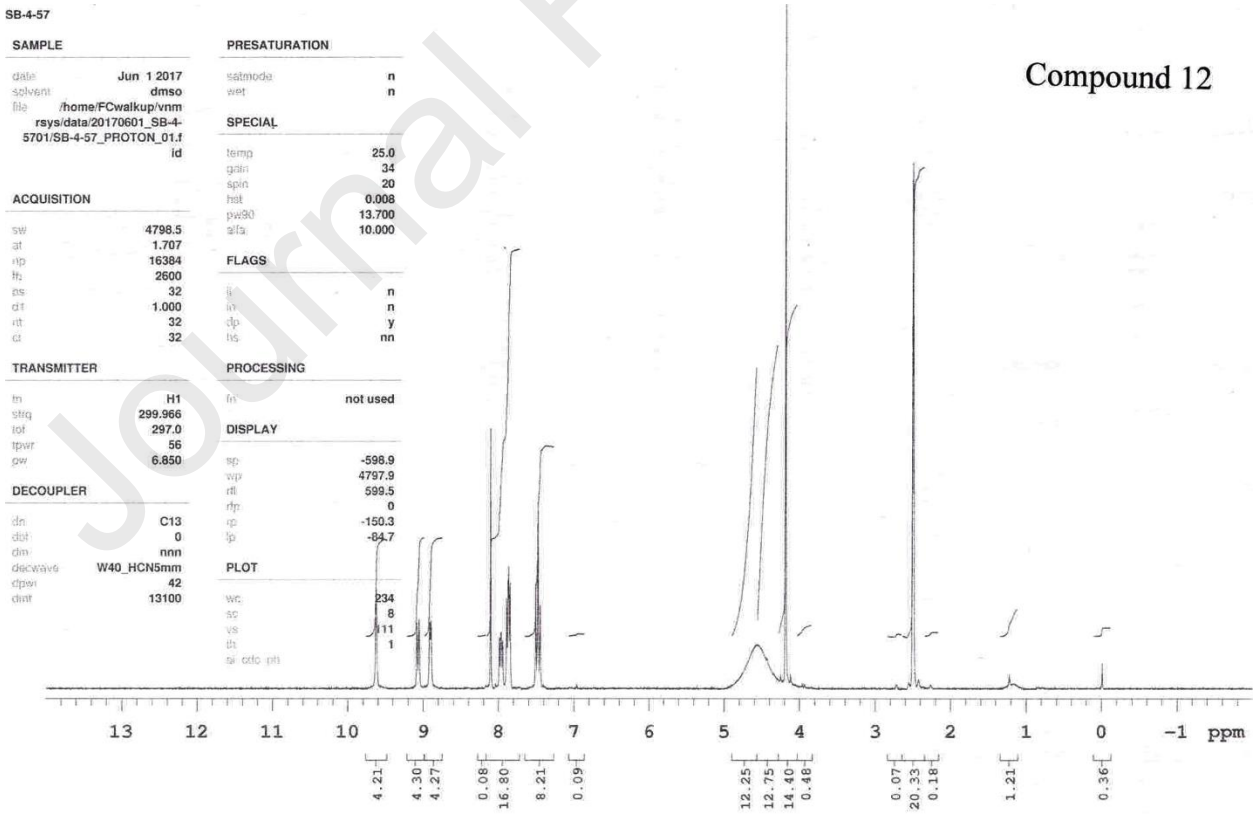
NMR analysis of compound 9



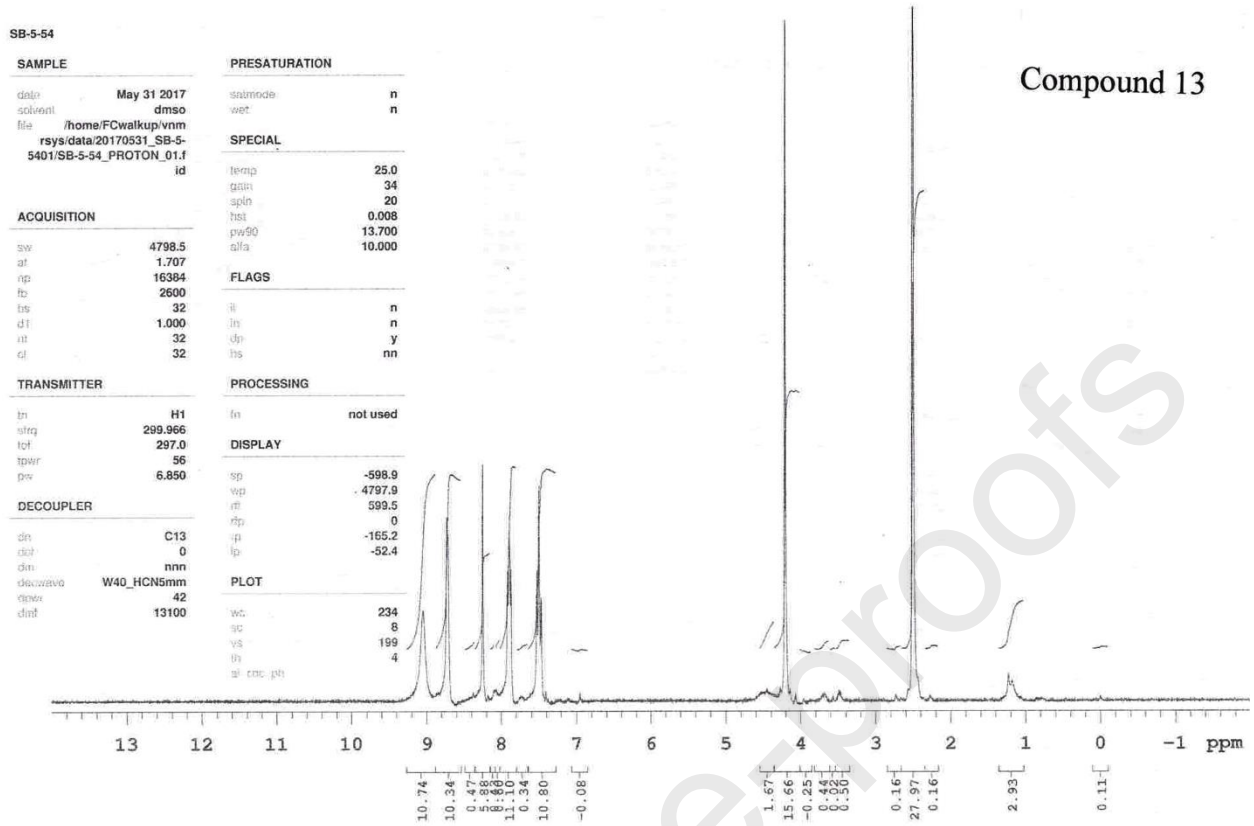
NMR analysis of compound 11



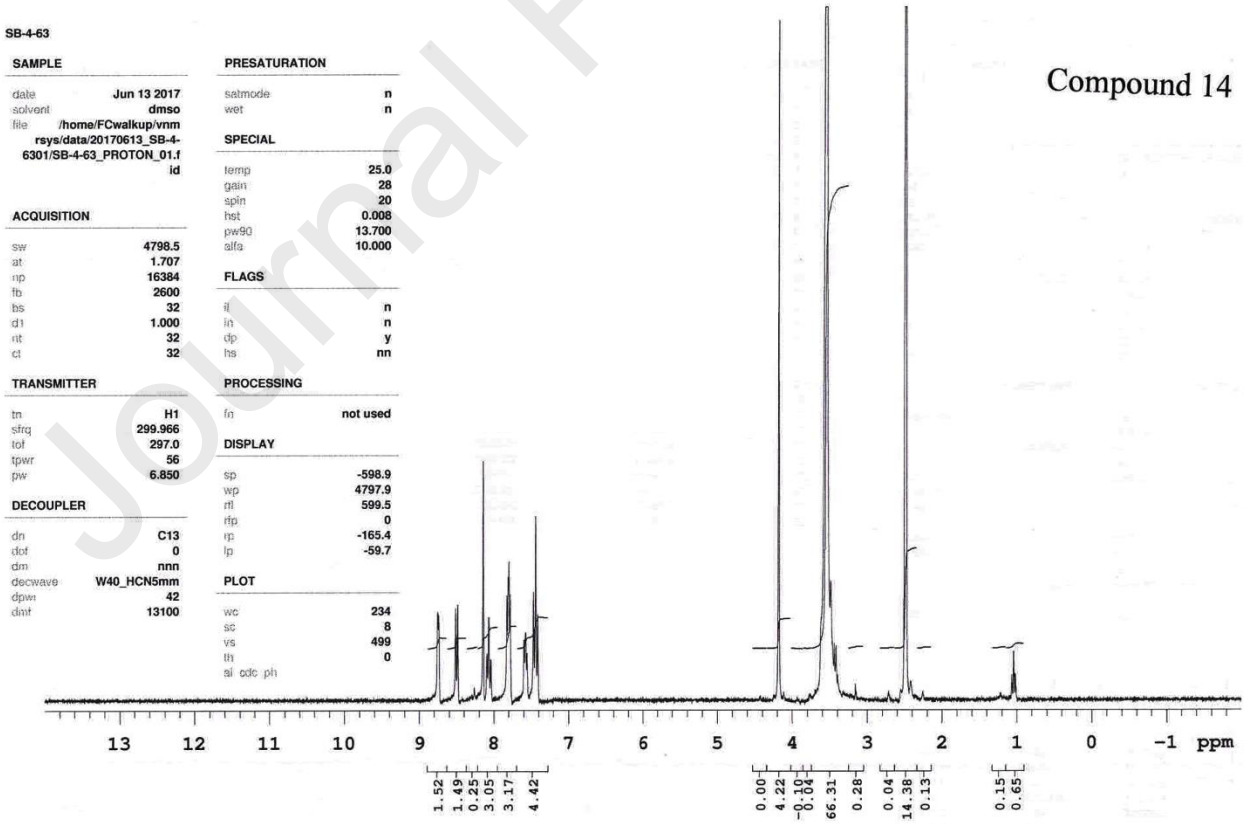
NMR analysis of compound 12



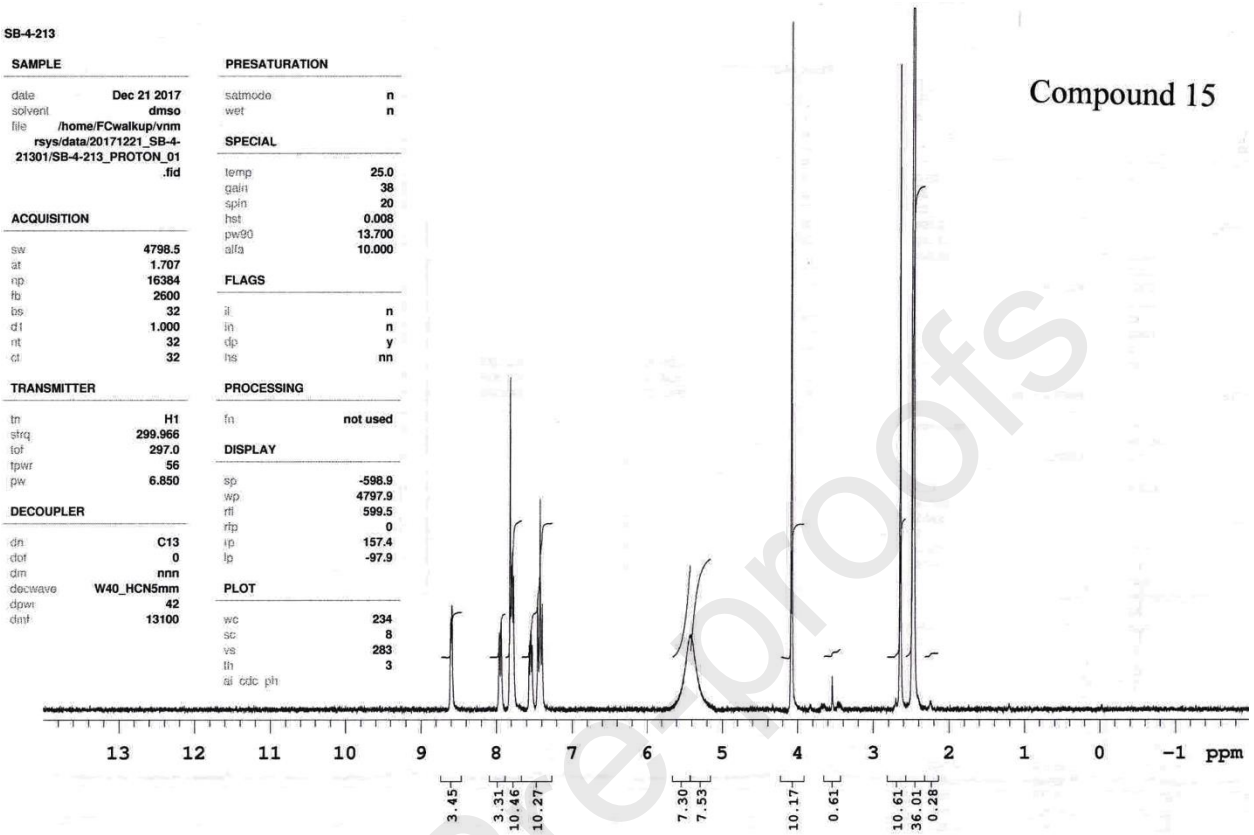
NMR analysis of compound 13



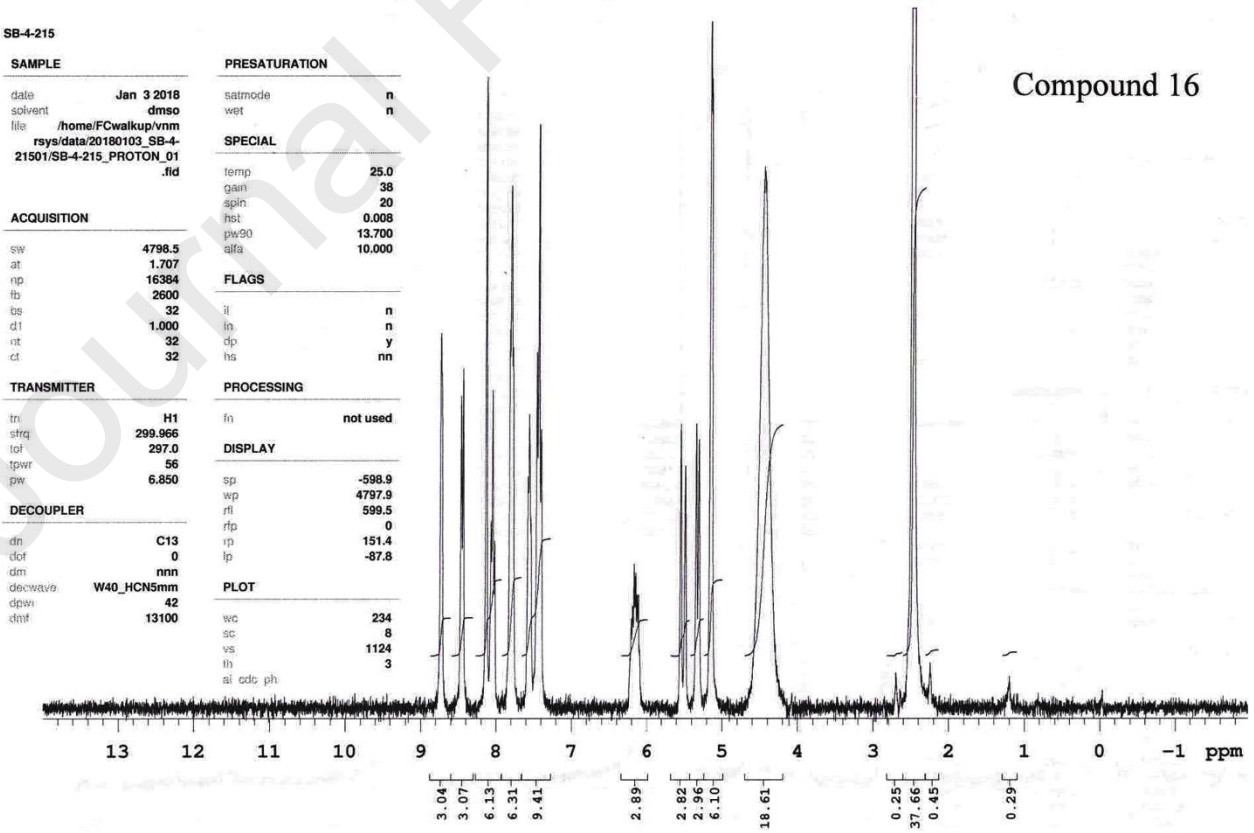
NMR analysis of compound 14



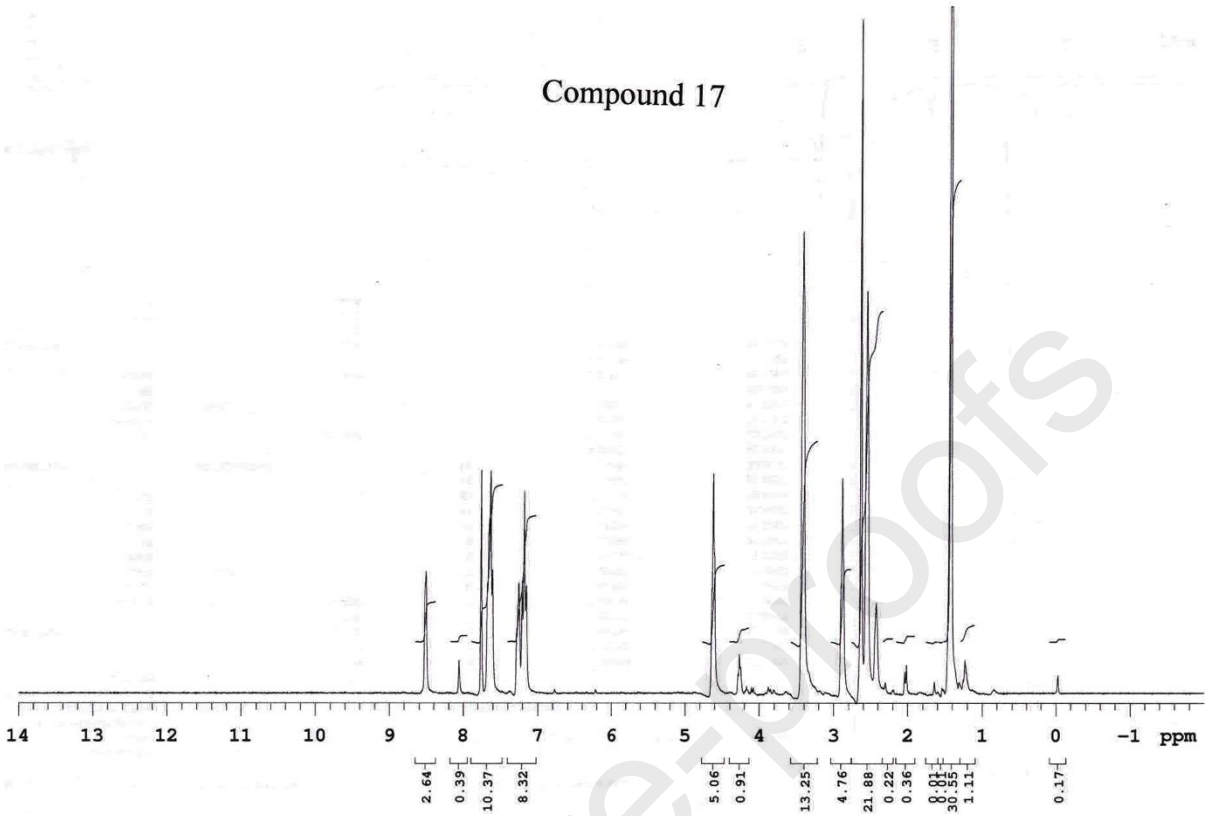
NMR analysis of compound 15



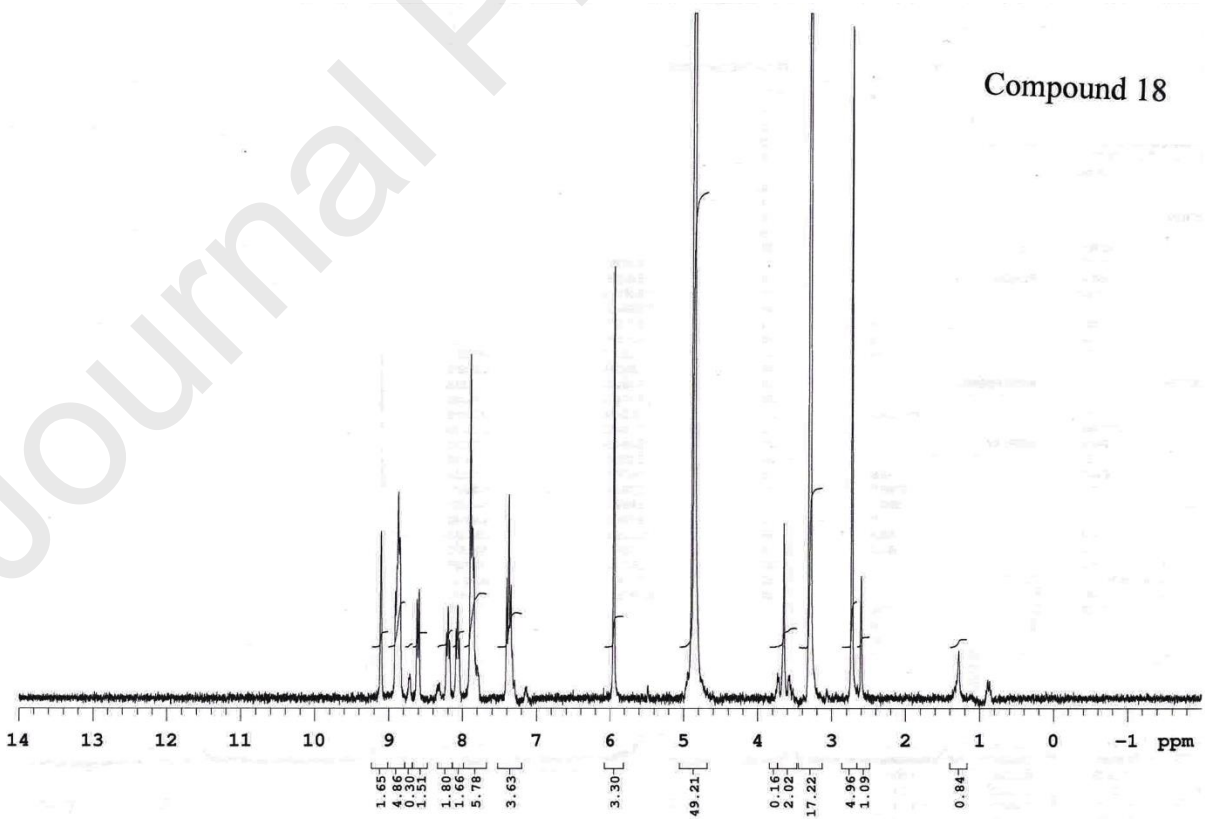
NMR analysis of compound 16



NMR analysis of compound 17

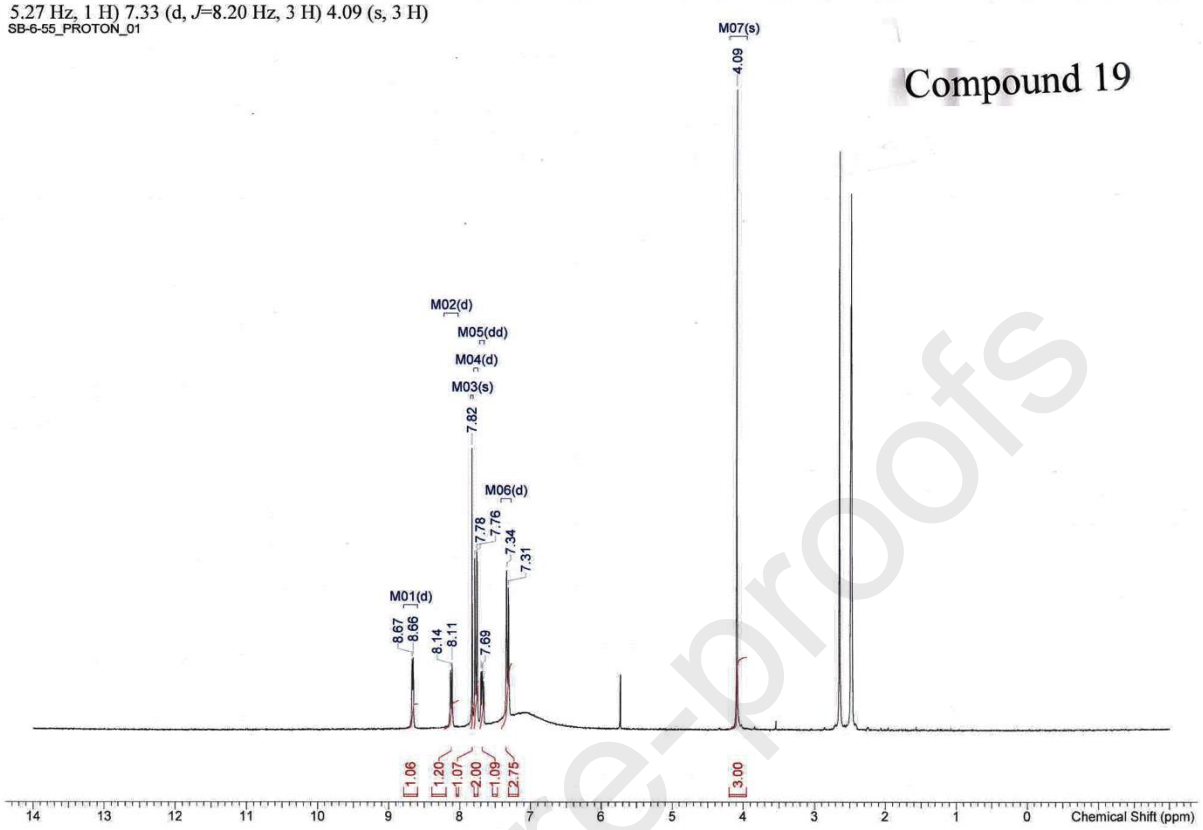


NMR analysis of compound 18

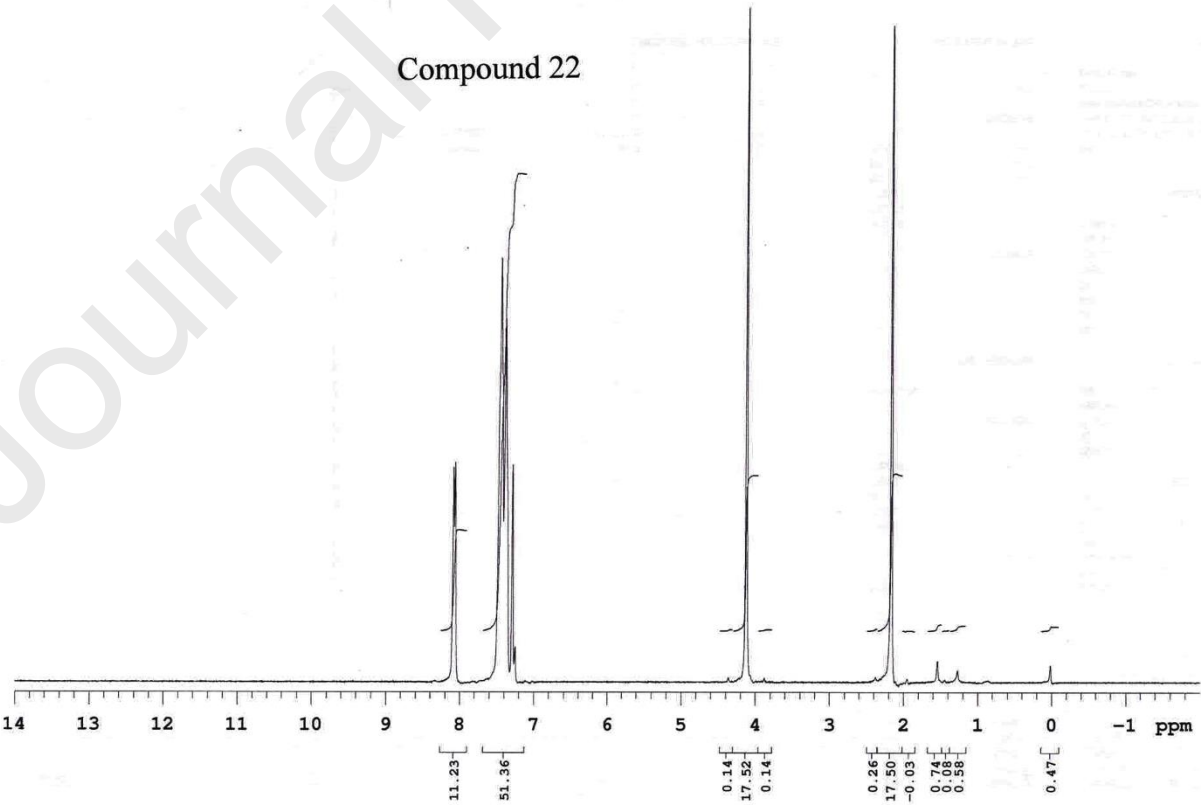


NMR analysis of compound 19

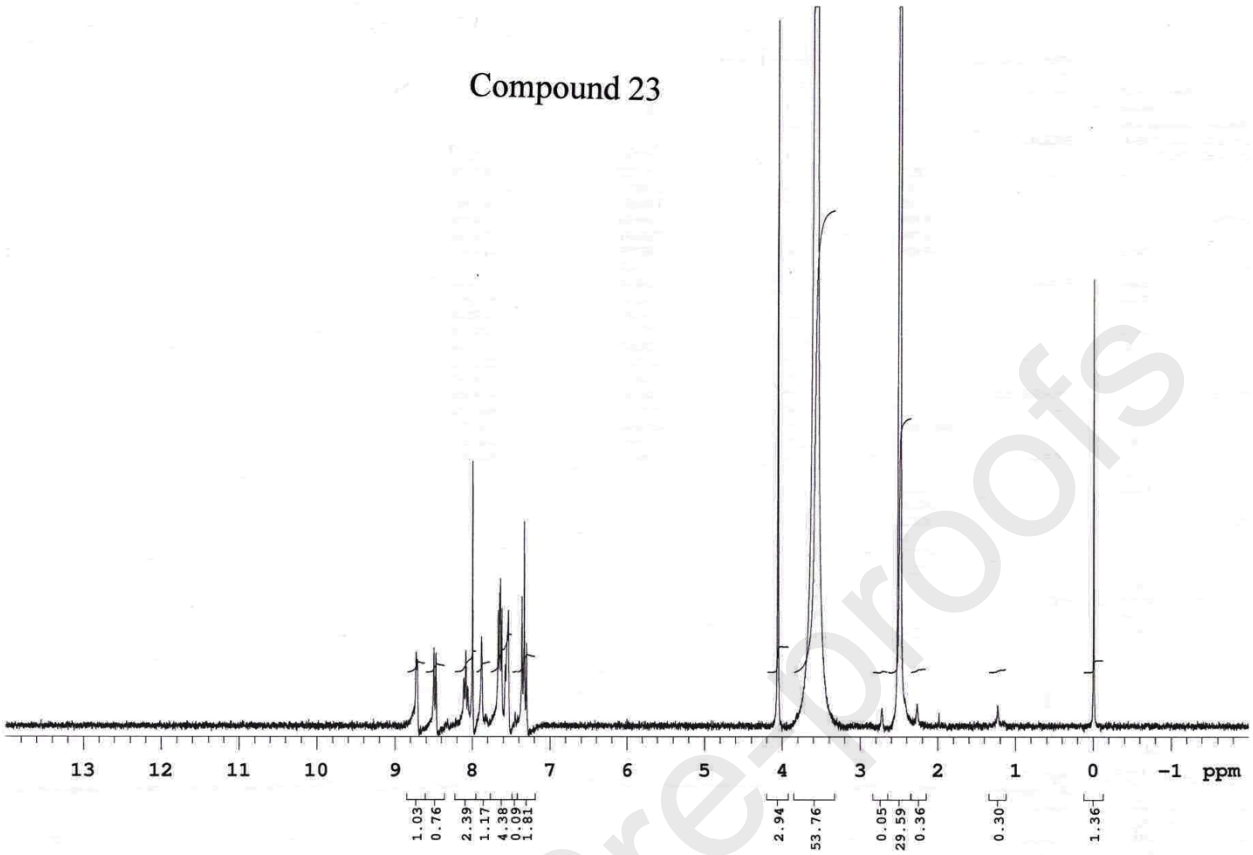
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.67 (d, *J*=4.10 Hz, 1 H) 8.12 (d, *J*=7.62 Hz, 1 H) 7.82 (s, 1 H) 7.77 (d, *J*=8.20 Hz, 2 H) 7.68 (dd, *J*=7.62, 5.27 Hz, 1 H) 7.33 (d, *J*=8.20 Hz, 3 H) 4.09 (s, 3 H)



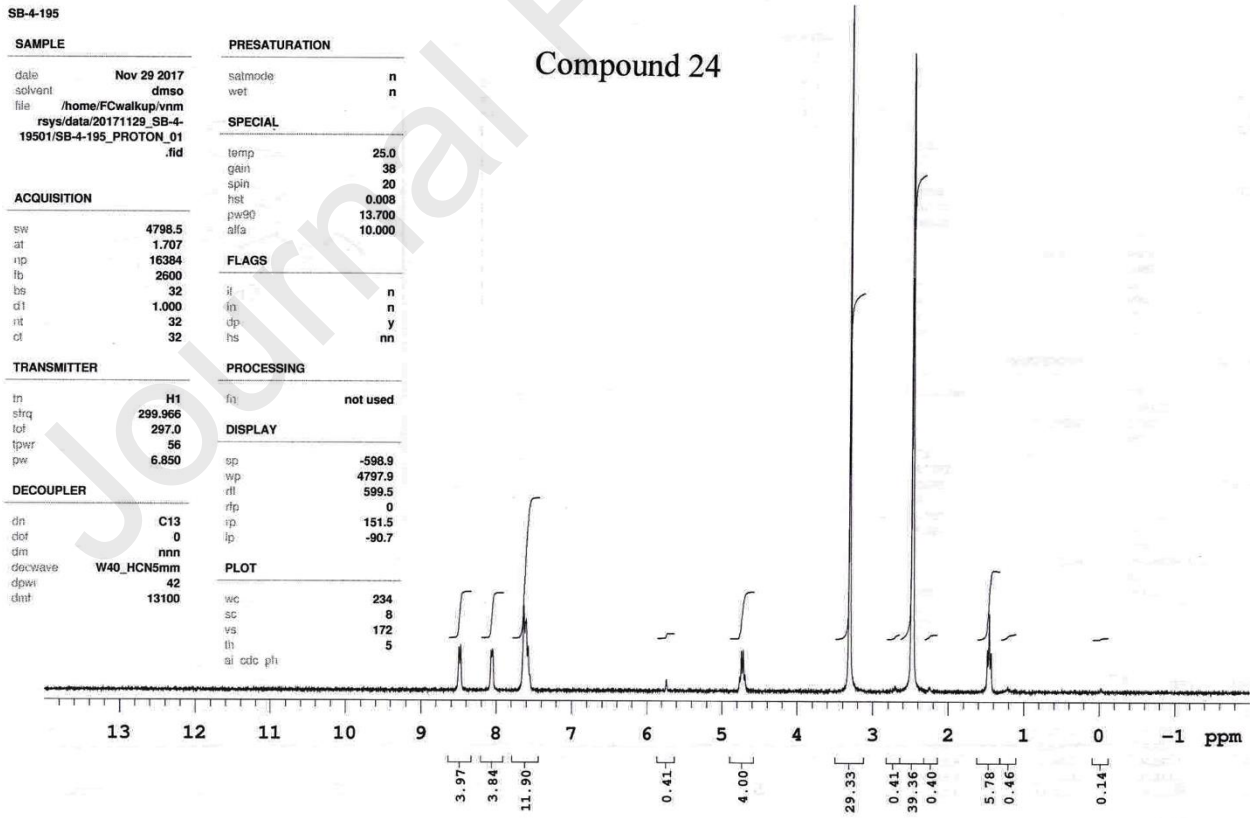
NMR analysis of compound 22

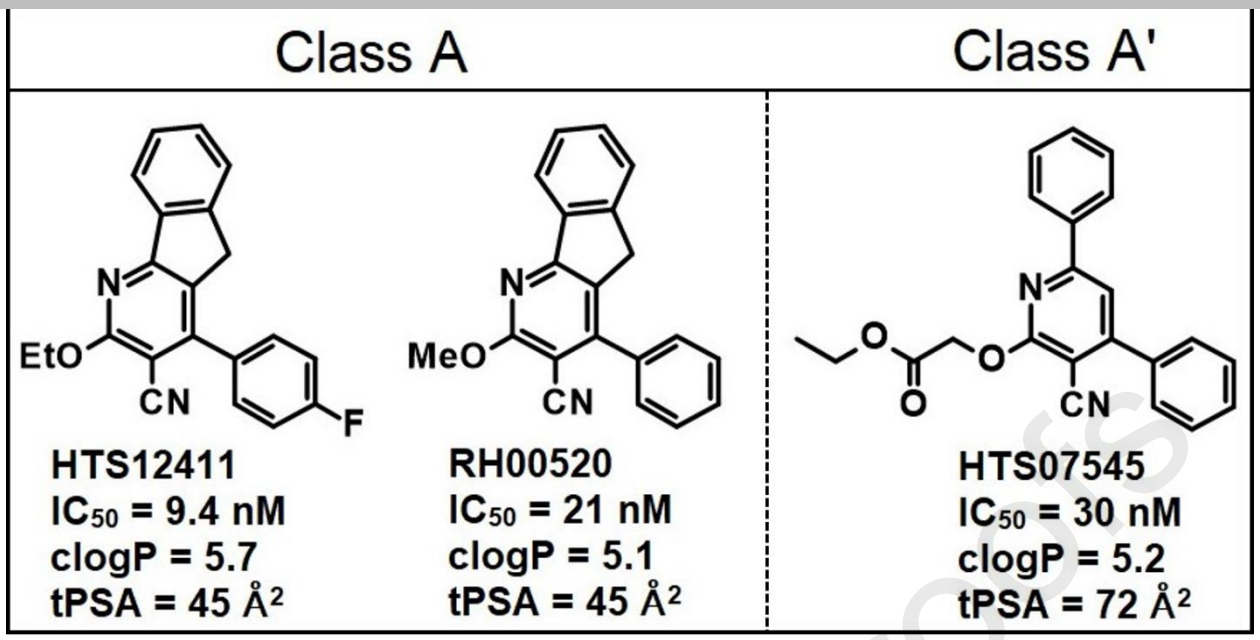


NMR analysis of compound 23



NMR analysis of compound 24

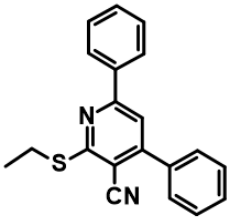




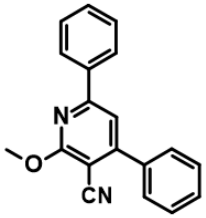
HTS12411
IC_{50} = 9.4 nM
clogP = 5.7
tPSA = 45 Å^2

RH00520
IC_{50} = 21 nM
clogP = 5.1
tPSA = 45 Å^2

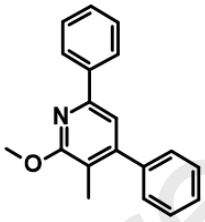
HTS07545
IC_{50} = 30 nM
clogP = 5.2
tPSA = 72 Å^2



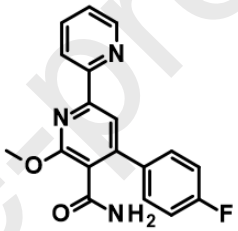
20
IC_{50}: 132 ± 6.4 nM



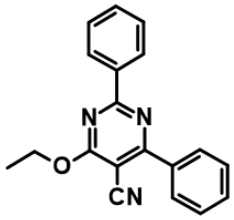
21
IC_{50}: 53 ± 4.3 nM



22
IC_{50}: 437 ± 62 nM



23
IC_{50}: 16200 ± 6400 nM



24
IC_{50}: 121 ± 6.1 nM