**Supporting Information**

# Novel fluconazole derivatives with promising antifungal activity

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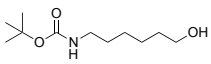
## 1. Chemistry:

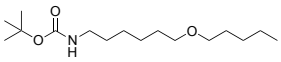
**1.1. Materials and instrumentation.** All the chemicals used in this study (including compounds **1** and **9**) were purchased from Sigma-Aldrich (St. Louis, MO) or AK Scientific (Union City, CA)

and used without any further purification. DMF and THF were freshly distilled prior to use. Chemical reactions were monitored by TLC (Merck, Silica gel 60 F254). Visualization was achieved using one of the following methods: Iodine stain ($I_2$ in $SiO_2$ gel) or UV light. Compounds were purified by $SiO_2$ flash chromatography (Dynamic Adsorbents Inc., Flash $SiO_2$ gel 32-63μ). $^1H$ and $^{13}C$ NMR spectra were recorded on a Varian 400 MHz spectrometer. Mass spectra were recorded using an Agilent 1200 series Quaternary LC system equipped with a diode array detector, and Eclipse XDB-$C_{18}$ column (250 mm × 4.6 mm, 5 μm), and an Agilent 6120 Quadrupole MSD mass spectrometer. For compounds **1** and **2** $[M\text{-}Boc+H]^+$ are reported, which correspond to the mass of the molecules that have lost their Boc protecting group during the mass spectrometry experiments. All reactions were carried out under nitrogen atmosphere and all yields reported represent isolated yields. Known compounds were characterized by $^1H$ NMR and are in complete agreement with samples reported in the literature.

**1.2. Experimental protocols for the preparation of compounds 1-16.**

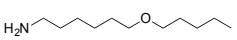


**Synthesis of compound 1.** To a solution of 6-amino-1-hexanol (700 mg, 5.97 mmol) in $CH_2Cl_2$ (8 mL), di-*tert*-butyldicarbonate (1.95 g, 8.96 mmol) and $Et_3N$ (1.2 mL, 8.96 mmol) were added. The reaction mixture was stirred at room temperature for 12 h and progress of the reaction was monitored by TLC (1:19/MeOH:$CH_2Cl_2$, $R_f$ 0.45). The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 1:19/MeOH:$CH_2Cl_2$) to afford compound **1** (1.02 g, 79%) as a colorless liquid: $^1H$ NMR (400 MHz, $CD_3OD$, Fig. S1) δ 3.52 (t, $J$ = 6.6 Hz, 2H), 3.00 (t, $J$ = 7.0 Hz, 2H), 1.58-1.46 (m, 2H), 1.44-1.42 (m, 2H), 1.41 (s, 9H), 1.38-1.26 (m, 4H); $^{13}C$ NMR (100 MHz, $CD_3OD$, Fig. S2) δ 157.1, 78.3, 61.4, 39.9, 32.1, 29.5, 27.4, 26.2, 25.2; LRMS *m/z* calcd for $C_{11}H_{23}NO_3$: 217.2; found 117.2 $[M\text{-}Boc+H]^+$.

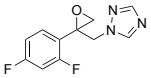


**Synthesis of compound 2.** A solution of compound **1** (500 mg, 2.30 mmol) in DMF (5 mL) was cooled to 0 °C followed by the addition of sodium hydride (72 mg, 2.99 mmol) and 1-iodopentane (0.4 mL, 2.99 mmol). The reaction mixture was stirred at room temperature for 24 h and progress of the reaction was monitored by TLC (1:19/EtOAc:Hexanes, $R_f$ 0.65). The reaction mixture was quenched with $H_2O$ (20 mL) and extracted with EtOAc (60 mL). The organic layer was washed with $H_2O$ (60

mL) and brine (20 mL), and dried over $MgSO_4$. The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 1:19/EtOAc:Hexanes) to afford compound **2** (241 mg, 36%) as a colorless liquid: $^1$H NMR (400 MHz, $CD_3OD$, Fig. S3) δ 3.39 (td, $J_1$ = 6.6 Hz, $J_2$= 1.8 Hz, 4H), 3.00 (t, $J$ = 7.0 Hz, 2H), 1.56-1.50 (m, 4H), 1.46-1.42 (m, 2H), 1.41 (s, 9H), 1.38-1.26 (m, 8H), 0.90 (m, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$, Fig. S4) δ 157.1, 78.3, 70.5, 70.4, 39.9, 29.5, 29.3, 29.0, 28.1, 27.4, 26.3, 25.5, 22.1, 13.0; LRMS *m/z* calcd for $C_{16}H_{33}NO_3$: 287.3; found 187.2 $[M\text{-Boc}+H]^+$.

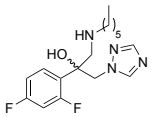


**Synthesis of compound 3.** To a solution of compound **2** (200 mg, 0.69 mmol) in $CH_2Cl_2$ (2 mL), trifluoroacetic acid (1.5 mL) was added. The reaction mixture was stirred at room temperature for 2 h and progress of the reaction was monitored by TLC (1:19/MeOH:$CH_2Cl_2$, $R_f$ 0.25). The organic layer was removed under reduced pressure to afford compound **3** (118 mg, 90%) as a colorless liquid: $^1$H NMR (400 MHz, $CD_3OD$, Fig. S5) δ 3.40 (q, $J$ = 6.4 Hz, 4H), 2.89 (t, $J$ = 7.7 Hz, 2H), 1.65-1.61 (m, 2H), 1.59-1.50 (m, 4H), 1.40 (p, $J$ = 3.7 Hz, 4H), 1.31 (p, $J$ = 3.7 Hz, 4H), 0.90 (m, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$, Fig. S6) δ 70.5, 70.2, 39.2, 29.0 (2C), 28.1, 27.1, 25.8, 25.3, 22.1, 12.9; LRMS *m/z* calcd for $C_{11}H_{25}NO$: 187.2; found 188.2 $[M+H]^+$.

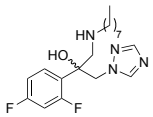


**Synthesis of compound 4.** To a solution of 2,4-difuoro-2-(1H-1,2,4-triazo-1-yl)acetophenone (1 g, 4.48 mmol) in toluene (10 mL), trimethylsulfoxonium iodide (1.08 g, 4.93 mmol), hexadecyltrimethylammonium bromide (163 mg, 0.45 mmol) and 1.5 mL 20% (*v*/*v*) sodium hydroxide was added. The reaction mixture was stirred at 60 °C for 1 h and progress of the reaction was monitored by TLC (3:2/EtOAc:Hexanes, $R_f$ 0.35). The reaction mixture was diluted with EtOAc (20 mL) and washed with $H_2O$ (20 mL). The organic layer was removed under reduced pressure and the residue was purified by flash column chromatography ($SiO_2$, 3:2/EtOAc:Hexanes) to afford compound (720 mg, 68%) as a yellow gummy liquid: $^1$H NMR (400 MHz, $CDCl_3$, Fig. S7) δ 8.08 (s, 1H), 7.86 (s, 1H), 7.20-7.11 (m, 1H), 6.86-6.75 (m, 2H), 4.82 (d, $J$ = 14.8 Hz, 1H), 4.49 (d, $J$ = 14.8 Hz, 1H), 2.93 (d, $J$ = 4.6 Hz, 1H), 2.87 (d, $J$ = 4.6 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$, Fig. S8) δ 164.4, 164.3, 161.9, 161.8, 161.7, 159.4, 159.2, 151.8, 144.1, 129.64, 129.59, 129.55, 129.49, 111.90, 111.87, 111.69, 111.65, 104.3,

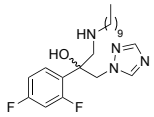
104.1, 103.8, 56.3, 53.59, 53.55, 52.2; LRMS *m/z* calcd for $C_{11}H_9F_2N_3O$: 237.1; found 238.1 $[M+H]^+$.



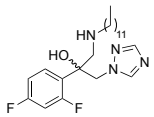
**Synthesis of compound 5.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), hexylamine (0.07 mL, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at 80 °C for 12 h and progress of the reaction was monitored by TLC (3:2/EtOAc:Hexanes, $R_f$ 0.09). The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 3:2/EtOAc:Hexanes) to afford compound **5** (11 mg, 14%) as a colorless liquid: $^1$H NMR (400 MHz, $CDCl_3$, Fig. S9) δ 8.10 (s, 1H), 7.81 (s, 1H), 7.60-7.46 (m, 1H), 6.85-6.75 (m, 2H), 4.65 (d, *J* = 14.2 Hz, 1H), 4.52 (d, *J* = 14.2 Hz, 1H), 3.20 (d, *J* = 12.6 Hz, 1H), 3.00 (d, *J* = 12.6 Hz, 1H), 2.56 (t, *J* = 7.2 Hz, 2H), 1.42-1.38 (p, *J* = 7.6 Hz, 2H), 1.28-1.16 (m, 6H), 0.85 (t, *J* = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$, Fig. S10) δ 166.5, 164.1, 153.2, 153.1, 151.8, 151.4, 144.5, 124.93, 124.92, 124.0, 123.9, 110.0, 104.1, 103.8, 95.6, 95.4, 78.1, 63.4, 56.0, 49.8, 47.9, 31.5, 31.4, 29.7, 26.8, 26.7, 26.5, 22.6, 22.5, 14.1, 14.0, 13.9; LRMS *m/z* calcd for $C_{17}H_{24}F_2N_4O$: 338.2; found 338.3 $[M+H]^+$.



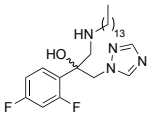
**Synthesis of compound 6.** To a solution of compound **4** (80 mg, 0.33 mmol) in EtOH (3 mL), *n*-octylamine (0.12 mL, 0.51 mmol) and $Et_3N$ (0.07 mL, 0.51 mmol) were added. The reaction mixture was stirred at 80 °C for 12 h and progress of the reaction was monitored by TLC (3:2/EtOAc:Hexanes, $R_f$ 0.20). The organic layer was removed under reduced pressure and the residue was purified by flash column chromatography ($SiO_2$, 3:2/EtOAc:Hexanes) to afford compound **6** (85 mg, 70%) as a colorless liquid: $^1$H NMR (400 MHz, $CDCl_3$, Fig. S11) δ 8.10 (s, 1H), 7.81 (s, 1H), 7.60-7.49 (m, 1H), 6.85-6.75 (m, 2H), 4.63 (d, *J* = 14.3 Hz, 1H), 4.51 (d, *J* = 14.3 Hz, 1H), 3.18 (d, *J* = 12.6 Hz, 1H), 2.95 (d, *J* = 12.6 Hz, 1H), 2.52 (t, *J* = 7.2 Hz, 2H), 1.42-1.36 (m, 2H), 1.29-1.18 (m, 10H), 0.85 (t, *J* = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$, Fig. S12) δ 164.0, 163.9, 161.6, 161.4, 160.2, 160.1, 157.8, 157.7, 151.3, 144.7, 129.9, 129.83, 129.79, 129.7, 124.99, 124.86, 111.7, 111.6, 111.44, 111.41, 104.5, 104.22, 104.20, 104.0, 73.0, 72.9, 56.1, 56.0, 54.13, 54.09, 50.0, 31.7, 29.5, 29.3, 29.1, 26.9, 22.6, 14.1; LRMS *m/z* calcd for $C_{19}H_{28}F_2N_4O$: 366.2; found 367.2 $[M+H]^+$.



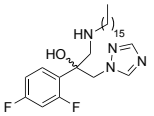
**Synthesis of compound 7.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), *n*-decylamine (0.06 mL, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at 80 °C for 12 h and progress of the reaction was monitored by TLC (3:2/EtOAc:Hexanes, $R_f$ 0.20). The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 3:2/EtOAc:Hexanes) to afford compound **7** (75 mg, 91%) as a yellow liquid: $^1$H NMR (400 MHz, $CD_3OD$, Fig. S13) δ 8.32 (s, 1H), 7.76 (s, 1H), 7.48-7.42 (m, 1H), 6.96-6.82 (m, 2H), 4.69 (d, $J$ = 14.3 Hz, 1H), 4.61 (d, $J$ = 14.3 Hz, 1H), 3.15 (dd, $J_1$ = 12.6 Hz, $J_2$ = 1.4 Hz 1H), 2.98 (dd, $J_1$ = 12.6 Hz, $J_2$ = 1.4 Hz 1H), 2.56-2.44 (m, 2H), 1.42 (p, $J$ = 7.2 Hz, 2H), 1.34-1.25 (m, 14H), 0.90 (t, $J$ = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$, Fig. S14) δ 164.0, 163.9, 161.6, 161.5, 160.6, 160.5, 158.2, 158.1, 149.8, 144.6, 129.8, 129.73, 129.69, 129.6, 125.02, 124.98, 124.8, 110.71, 110.68, 110.50, 110.47, 103.8, 103.6, 103.5, 103.3, 73.83, 73.78, 55.92, 55.87, 54.92, 54.87, 49.8, 31.6, 29.30, 29.26, 29.25, 29.1, 29.0, 26.8, 22.3, 13.0; LRMS *m/z* calcd for $C_{21}H_{32}F_2N_4O$: 394.3; found 395.2 $[M+H]^+$.



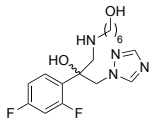
**Synthesis of compound 8.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), dodecylamine (0.07mL, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at 80 °C for 2 h and progress of the reaction was monitored by TLC (1:10/MeOH:$CH_2Cl_2$, $R_f$ 0.19). The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 1:10/MeOH:$CH_2Cl_2$) to afford compound **8** (49 mg, 59%) as a yellow solid: $^1$H NMR (400 MHz, $CDCl_3$, Fig. S15) δ 8.09 (s, 1H), 7.82 (s, 1H), 7.59-7.53 (m, 1H), 6.85-6.76 (m, 2H), 4.67 (d, $J$ = 14.2 Hz, 1H), 4.54 (d, $J$ = 14.2 Hz, 1H), 3.23 (d, $J$ = 12.6 Hz, 1H), 3.08 (d, $J$ = 12.6 Hz, 1H), 2.60 (t, $J$ = 7.4 Hz, 2H), 1.49-1.43 (m, 2H), 1.29-1.21 (m, 18H), 0.86 (t, $J$ = 6.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$, Fig. S16) δ 164.0, 161.5, 161.4, 160.2, 160.1, 157.8, 157.7, 151.2, 144.7, 129.9, 129.84, 129.80, 129.7, 125.04, 125.00, 124.9, 111.62, 111.58, 111.41, 111.38, 104.5, 104.20, 104.19, 103.9, 73.0, 72.9, 56.1, 56.0, 54.2, 54.2, 54.1, 50.0, 31.9, 29.7, 29.60, 29.59, 29.54, 29.5, 29.4, 29.34, 29.30, 26.9, 22.7, 14.1; LRMS *m/z* calcd for $C_{23}H_{36}F_2N_4O$: 422.3; found 423.2 $[M+H]^+$.



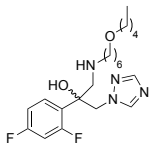
**Synthesis of compound 9.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), tetradecylamine (68 mg, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at room temperature for 3 h and progress of the reaction was monitored by TLC (1:10/MeOH:$CH_2Cl_2$, $R_f$ 0.31). The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 1:10/MeOH:$CH_2Cl_2$) to afford compound **9** (90 mg, 94%) as a yellow liquid: $^1$H NMR (400 MHz, $CD_3OD$, Fig. S17) δ 8.29 (s, 1H), 7.74 (s, 1H), 7.46-7.40 (m, 1H), 6.93-6.80 (m, 2H), 4.67 (d, $J$ = 14.3 Hz, 1H), 4.59 (d, $J$ = 14.3 Hz, 1H), 3.13 (dd, $J_1$ = 12.6 Hz, $J_2$ = 1.3 Hz, 1H), 2.96 (dd, $J_1$ = 12.6 Hz, $J_2$ = 1.3 Hz, 1H), 2.54-2.42 (m, 2H), 1.42-1.36 (p, $J$ = 7.1 Hz, 2H), 1.32-1.23 (m, 22H), 0.88 (t, $J$ = 6.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$, Fig. S18) δ 164.0, 163.9, 161.6, 160.6, 160.5, 158.2, 158.1, 149.7, 144.6, 129.8, 129.72, 129.69, 129.6, 125.0, 124.8, 110.71, 110.68, 110.50, 110.47, 103.8, 103.6, 103.5, 103.3, 73.82, 73.76, 55.9, 55.85, 54.91, 54.86, 49.8, 31.7, 29.37, 29.35, 29.34, 29.28, 29.27, 29.14, 29.10, 29.06, 26.8, 22.3, 13.0; LRMS *m/z* calcd for $C_{25}H_{40}F_2N_4O$: 450.3; found 451.3 $[M+H]^+$.



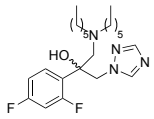
**Synthesis of compound 10.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), hexadecylamine (76 mg, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at room temperature for 2 h and progress of the reaction was monitored by TLC (1:10/MeOH:$CH_2Cl_2$, $R_f$ 0.29). The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 1:10/MeOH:$CH_2Cl_2$) to afford compound **10** (92 mg, 91%) as a yellow liquid: $^1$H NMR (400 MHz, $CD_3OD$, Fig. S19) δ 8.29 (s, 1H), 7.74 (s, 1H), 7.47-7.40 (m, 1H), 6.93-6.80 (m, 2H), 4.67 (d, $J$ = 14.3 Hz, 1H), 4.59 (d, $J$ = 14.3 Hz, 1H), 3.13 (dd, $J_1$ = 12.6 Hz, $J_2$ = 1.3 Hz, 1H), 2.96 (dd, $J_1$ = 12.6 Hz, $J_2$ = 1.3 Hz, 1H), 2.54-2.41 (m, 2H), 1.40 (p, $J$ = 7.1 Hz, 2H), 1.32-1.23 (m, 26H), 0.88 (t, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$, Fig. S20) δ 164.0, 163.9, 161.6, 161.4, 160.6, 160.5, 158.2, 158.1, 150.6, 149.7, 144.6, 129.8, 129.72, 129.69, 129.63, 125.02, 124.98, 124.89, 124.85, 110.71, 110.68, 110.50, 110.47, 103.8, 103.6, 103.5, 103.3, 73.83, 73.78, 55.91, 55.86, 54.94, 54.90, 49.8, 31.7, 29.39, 29.37, 29.35, 29.30, 29.28, 29.2, 29.1, 26.8, 22.3, 13.0; LRMS *m/z* calcd for $C_{27}H_{44}F_2N_4O$: 478.4; found 479.3 $[M+H]^+$.



**Synthesis of compound 11.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), 6-amino-1-hexanol (37 mg, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at room temperature for 12 h and progress of the reaction was monitored by TLC (1:20/MeOH:$CH_2Cl_2$, $R_f$ 0.66). The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 1:20/MeOH:$CH_2Cl_2$) to afford compound **11** (35 mg, 51%) as a colorless liquid: $^1$H NMR (400 MHz, $CD_3OD$, Fig. S21) δ 8.31 (s, 1H), 7.80 (s, 1H), 7.52-7.45 (m, 1H), 7.00-6.95 (m, 1H), 6.93-6.87 (m, 1H), 4.71 (d, *J* = 14.5 Hz, 1H), 4.66 (d, *J* = 14.5 Hz, 1H), 3.51 (t, *J* = 6.5 Hz, 2H), 3.40 (d, *J* = 12.8 Hz, 1H), 3.20 (d, *J* = 12.8 Hz, 1H), 2.77-2.65 (m, 2H), 1.56-1.45 (m, 4H), 1.38-1.25 (m, 4H); $^{13}$C NMR (100 MHz, $CD_3OD$, Fig. S22) δ 164.5, 164.4, 162.0, 161.9, 160.7, 160.6, 158.3, 158.2, 150.2, 144.8, 129.92, 129.86, 129.82, 129.76, 123.2, 111.14, 111.11, 110.93, 110.90, 104.2, 103.9, 103.6, 73.1, 73.0, 61.3, 55.52, 55.47, 53.8, 53.7, 49.1, 32.0, 27.1, 26.3, 25.2; LRMS *m/z* calcd for $C_{17}H_{24}F_2N_4O_2$: 354.2; found 355.1 $[M+H]^+$.

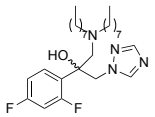


**Synthesis of compound 12.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), compound **3** (60 mg, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at 80 °C for 12 h and progress of the reaction was monitored by TLC (3:2/EtOAc:Hexanes, $R_f$ 0.30). The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 3:2/EtOAc:Hexanes) to afford compound **12** (34 mg, 39%) as a yellow liquid: $^1$H NMR (400 MHz, $CD_3OD$, Fig. S23) δ 8.29 (s, 1H), 7.75 (s, 1H), 7.47-7.40 (m, 1H), 6.95-6.80 (m, 2H), 4.67 (d, *J* = 14.3 Hz, 1H), 4.60 (d, *J* = 14.3 Hz, 1H), 3.39 (t, *J* = 6.6 Hz, 2H), 3.38 (t, *J* = 6.6 Hz, 2H), 3.16 (d, *J* = 12.6 Hz, 1H), 2.99 (d, *J* = 12.6 Hz, 1H), 2.59-2.43 (m, 2H), 1.58-1.46 (m, 4H), 1.43 (p, *J* = 6.9 Hz, 2H), 1.36-1.22 (m, 8H), 0.89 (t, *J* = 6.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$, Fig. S24) δ 164.1, 161.6, 158.2, 149.8, 144.6, 129.8, 129.7, 129.6, 124.8, 110.7, 110.6, 103.9, 103.6, 103.3, 73.73, 73.67, 70.5, 70.3, 55.9, 55.8, 54.8, 54.7, 49.7, 29.2, 29.0, 28.8, 28.1, 26.6, 25.6, 22.1, 13.0; LRMS *m/z* calcd for $C_{22}H_{34}F_2N_4O_2$: 424.3; found 425.2 $[M+H]^+$.

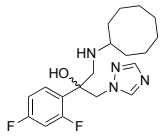


**Synthesis of compound 13.** To a solution of compound **4** (80 mg, 0.33 mmol) in EtOH (3 mL), di-*n*-hexylamine (0.12 mL, 0.51 mmol) and $Et_3N$ (0.07 mL,

0.51 mmol) were added. The reaction mixture was stirred at 80 °C for 12 h and progress of the reaction was monitored by TLC (3:2/EtOAc:Hexanes, $R_f$ 0.82). The organic layer was removed under reduced pressure and the residue was purified by flash column chromatography ($SiO_2$, 1:4/EtOAc:Hexanes) to afford compound **13** (82 mg, 57%) as a colorless liquid: $^1$H NMR (400 MHz, $CDCl_3$, Fig. S25) δ 8.17 (s, 1H), 7.77 (s, 1H), 7.55 (m, 1H), 6.85-6.74 (m, 2H), 5.66 (br s, 1H), 4.50 (d, $J$ = 14.1 Hz, 1H), 4.41 (d, $J$ = 14.1 Hz, 1H), 3.05 (d, $J$ = 13.7 Hz, 1H), 2.69 (d, $J$ = 13.7 Hz, 1H), 2.26-2.04 (m, 4H), 1.25-0.98 (m, 16H), 0.83 (t, $J$ = 7.2 Hz, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$, Fig. S26) δ 160.2, 160.1, 157.7, 157.6, 150.8. 144.8, 129.5, 127.1, 111.4, 111.2, 104.4, 104.1, 103.9, 71.0, 58.9, 56.52, 56.47, 55.0, 31.5, 26.8, 26.7, 22.5, 13.9; LRMS *m/z* calcd for $C_{23}H_{36}F_2N_4O$: 422.3; found 423.2 $[M+H]^+$.

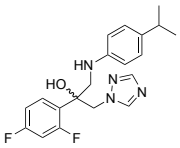


**Synthesis of compound 14.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), di-*n*-octylamine (0.09 mL, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at 80 °C for 12 h and progress of the reaction was monitored by TLC (3:2/EtOAc:Hexanes, $R_f$ 0.60). The organic layer was removed under reduced pressure and the residue was purified by flash column chromatography ($SiO_2$, 1:4/EtOAc:Hexanes) to afford compound **14** (65 mg, 65%) as a yellow liquid: $^1$H NMR (400 MHz, $CD_3OD$, Fig. S27) δ 8.39 (s, 1H), 7.79 (s, 1H), 7.60-7.54 (m, 1H), 6.98-6.85 (m, 2H), 4.59 (d, $J$ = 14.3 Hz, 1H), 4.55 (d, $J$ = 14.3 Hz, 1H), 3.09 (dd, $J_1$ = 13.9 Hz, $J_2$ = 1.8 Hz, 1H), 2.81 (d, $J$ = 13.9 Hz, 1H), 2.34-2.20 (m, 4H), 1.34-1.05 (m, 24H), 0.90 (t, $J$ = 6.7 Hz, 6H); $^{13}$C NMR (100 MHz, $CD_3OD$, Fig. S28) δ 164.1, 163.9, 161.6, 161.5, 160.5, 160.4, 158.0, 157.9, 149.6, 144.9, 129.61, 129.55, 129.52, 129.46, 126.95, 126.91, 126.82, 126.78, 110.8, 110.7, 110.6, 110.5, 103.8, 103.57, 103.56, 103.3, 71.7, 71.6, 59.23, 59.19, 56.1, 56.0, 54.9, 31.5, 29.0, 28.9, 26.8, 26.6, 22.3, 13.0; LRMS *m/z* calcd for $C_{27}H_{44}F_2N_4O$: 478.4; found 479.3 $[M+H]^+$.



**Synthesis of compound 15.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), cyclooctylamine (0.04 mL, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at 80 °C for 2 h and progress of the reaction was monitored by TLC (1:20/MeOH:$CH_2Cl_2$, $R_f$ 0.52). The organic layer was removed under reduced pressure and the residue was purified by

column chromatography ($SiO_2$, 1:20/MeOH:$CH_2Cl_2$) to afford compound **15** (43 mg, 57%) as a colorless liquid: $^1$H NMR (400 MHz, $CDCl_3$, Fig. S29) δ 8.11 (s, 1H), 7.81 (s, 1H), 7.59-7.53 (m, 1H), 6.85-6.75 (m, 2H), 4.62 (d, *J* = 14.2 Hz, 1H), 4.48 (d, *J* = 14.2 Hz, 1H), 3.14 (d, *J* = 12.6 Hz, 1H), 2.96 (d, *J* = 12.6 Hz, 1H), 2.53 (br s, 1H), 2.02-1.22 (m, 15H); $^{13}$C NMR (100 MHz, $CDCl_3$, Fig. S30) δ 164.0, 163.9, 161.5, 161.4, 160.3, 160.2, 157.8, 157.7, 151.2, 144.8, 129.9, 129.82, 129.78, 129.7, 125.3, 125.2, 111.6, 111.5, 111.4, 111.3, 104.4, 104.2, 103.9, 72.63, 72.58, 58.0, 56.24, 56.19, 51.73, 51.69, 32.6, 32.1, 27.1, 27.0, 25.6, 23.8, 23.6; LRMS *m/z* calcd for $C_{19}H_{26}F_2N_4O$: 364.2; found 365.1 $[M+H]^+$.




**Synthesis of compound 16.** To a solution of compound **4** (50 mg, 0.21 mmol) in EtOH (3 mL), 4-isopropylaniline (0.04mL, 0.32 mmol) and $Et_3N$ (0.04 mL, 0.32 mmol) were added. The reaction mixture was stirred at 80 °C for 2 h and progress of the reaction was monitored by TLC (1:10/MeOH:$CH_2Cl_2$, $R_f$ 0.88). The organic layer was removed under reduced pressure and the residue was purified by column chromatography ($SiO_2$, 1:20/MeOH:$CH_2Cl_2$) to afford compound **16** (45 mg, 57%) as a yellow solid: $^1$H NMR (400 MHz, $CDCl_3$, Fig. S31) δ 7.96 (s, 1H), 7.83 (s, 1H), 7.53-7.47 (m, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.81-6.73 (m, 2H), 6.63 (d, *J* = 8.4 Hz, 2H), 4.76 (d, *J* = 14.2 Hz, 1H), 4.70 (d, *J* = 14.2 Hz, 1H), 3.64 (dd, $J_1$ = 13.1 Hz, $J_2$ = 1.3 Hz, 1H), 3.51 (dd, $J_1$ = 13.1 Hz, $J_2$ = 1.3 Hz, 1H), 2.79 (septet, *J* = 6.9 Hz, 1H), 1.17 (d, *J* = 6.9 Hz, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$, Fig. S32) δ 164.1, 164.0, 161.6, 161.5, 159.9, 159.8, 157.5, 157.4, 151.9, 145.4, 144.4, 139.6, 130.1, 130.04, 130.00, 129.9, 127.2, 124.04, 124.00, 123.91, 123.87, 114.0, 111.91, 111.88, 111.71, 111.68, 104.5, 104.27, 104.26, 104.0, 75.7, 75.6, 55.2, 55.1, 51.24, 51.20, 33.2, 24.2; LRMS *m/z* calcd for $C_{20}H_{22}F_2N_4O$: 372.2; found 373.1 $[M+H]^+$.

**2. Biological studies:**

**2.1. Antifungal agents.** Azole derivatives **5-16** were chemically synthesized as described in section 1.2 above. A 5 mg/mL stock solution of compounds **5-16** was prepared in DMSO and stored at -20 °C. The antifungal agents amphotericin B (AmB), fluconazole (FLC), and voriconazole (VOR) were obtained from AK Scientific Inc. (Mountain View, CA). The antifungal agent caspofungin (CAS) was purchased from Sigma-Aldrich (St. Louis, MO). AmB,

FLC, VOR, and CAS were dissolved in DMSO at a final concentration of 5 mg/mL and were stored at -20 °C.

**2.2. Organisms and culture conditions.** *Candida albicans* ATCC 10231 (**A**), *C. albicans* ATCC 64124 (**B**), and *C. albicans* ATCC MYA-2876 (**C**) were kindly provided by Dr. Jon Y. Takemoto (Utah State University, Logan, UT, USA). *C. albicans* ATCC MYA-90819 (**D**), *C. albicans* ATCC MYA-2310 (**E**), *C. albicans* ATCC MYA-1237 (**F**), *C. albicans* ATCC MYA-1003 (**G**), *Candida glabrata* ATCC 2001 (**H**), *Candida krusei* ATCC 6258 (**I**), *Candida parapsilosis* ATCC 22019 (**J**), *Aspergillus flavus* ATCC MYA-3631 (**K**), and *Aspergillus terreus* ATCC MYA-3633 (**M**) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). *Aspergillus nidulans* ATCC 38163 (**L**) was received from Dr. Jon S. Thorson (University of Kentucky, Lexington, KY, USA). All clinical fungal isolates, *C. glabrata* (**CG1**, **CG2**, and **CG3**), *C. parapsilosis* (**CP1**, **CP2**, **CP3**), and *Cryptococcus neoformans* (**CN1**, **CN2**, and **CN3**) were obtained from Dr. Nathan P. Wiederhold, University of Texas Health Science Center, San Antonio, USA). Filamentous fungi and yeasts were cultivated at 35 °C in RPMI 1640 medium (with L-glutamine, without sodium bicarbonate, Sigma-Aldrich, St. Louis, MO, USA) buffered to a pH of 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma-Aldrich).

The human embryonic kidney cell line HEK-293 (ATCC CRL-1573), the human bronchus normal cell line BEAS-2B (ATCC CRL-9609), and the human lung carcinoma cell line A549 (ATCC CRL-185) were kind gifts from the laboratories of Dr. Matthew S. Gentry (University of Kentucky, Lexington, KY, USA) and Dr. David K. Orren (University of Kentucky, Lexington, KY, USA). Mammalian cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (from ATCC) with 10% fetal bovine serum (FBS) (from ATCC) and 1% Pen/Strep (from ATCC). Cell lines were incubated at 37 °C and 5% $CO_2$ and passaged by trypsinization with 0.05% trypsin:0.53 mM EDTA (from ATCC). Cell confluency was determined by using a Nikon Eclipse TS100 microscope (Minato, Tokyo, Japan).

**2.3. Antifungal susceptibility testing.** The MIC values of compounds **5-16** against yeasts (strains **A-J** (Table 1), and **CG1-3**, **CP1-3**, and **CN1-3** (Table 2)) were evaluated in 96-well

microtiter plates as described in the CLSI document M27-A3[1] with minor modifications. The final concentrations of antifungal agents studied ranged from 0.03-31.3 μg/mL for compounds **5-16**, 0.48-31.3 μg/mL for AmB (only presented in Table 1), 0.03-31.3 μg/mL for CAS, 0.975-62.5 μg/mL for FLC, and 0.03-31.3 μg/mL for VOR. Briefly, overnight yeast cultures were grown in yeast peptone dextrose (YPD) broth and the cell density was adjusted to an $OD_{600}$ of 0.12 (~$1\times10^6$ CFU/mL) by using a spectrophotometer. Yeast cell suspensions were further diluted to achieve 1-$5\times10^3$ CFU/mL in RPMI 1640 medium, and 100 μL of these yeast cells was added to 96-well microtiter plates containing RPMI 1640 medium and titrated compounds. Each test was performed in triplicate. The plates were incubated at 35 °C for 48 h. The MIC values for compounds **5-16**, AmB, and CAS were defined as the lowest drug concentration that prevented visible growth (also known as MIC-0) when compared to the growth control. For FLC and VOR, the minimum drug concentration that yielded at least 50% growth inhibition (MIC-2) when compared with the growth control well was reported. One exception for the reporting of the MIC of the azoles was that of VOR against *C. albicans* ATCC 10231 (strain **A**), where the MIC-0 (indicating optically clear well) was reported. These data are presented in Tables 1 (MIC values in μg/mL) and S1 (MIC values in μM) (for strains **A-J**) and 2 (MIC values in μg/mL) and S2 (MIC values in μM) (for strains **CG1-3**, **CP1-3**, and **CN1-3**).

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Similarly, the MIC values of compounds **5-16**, as well as that of all control drugs against filamentous fungi (strains **K-M**) were determined as previously described in CLSI document M38-A2.[2] Spores were harvested from sporulating cultures growing on potato dextrose agar (PDA) by filtration through sterile glass wool and enumerated by using a hemocytometer (Hausser Scientific, PA, USA) to obtain the desired inoculum size. Two-fold serial dilutions of compounds **5-16**, as well as VOR and CAS were made in sterile 96-well microtiter plates in the range of 0.03-31.3 μg/mL in RPMI 1640 medium. The concentration range for FLC and AmB were 0.975-62.5 μg/mL and 0.48-31.3 μg/mL, respectively. Spore suspensions were added to the wells to afford a final concentration of $5\times10^5$ spores/mL. The plates were incubated at 35 °C for 48 h. The MIC values of all compounds, including compounds **5-16**, azoles, AmB, and CAS against filamentous fungi were based on complete inhibition of growth (optically clear well) when compared to the growth control (MIC-0). Each test was performed in triplicate. These data are also presented in Table 1 (strains **K-M**).

**Table S1:** MIC values[a] (in μM) (the corresponding values in μg/mL are presented in Table 1) determined for compounds **5-16** and for four control antifungal agents (AmB, CAS, FLC, and VOR) against various yeast strains and filamentous fungi.

| | Yeast strains | | | | | | | | | | Filamentous fungi | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cpd #** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** | **I** | **J** | **K** | **L** | **M** |
| **5** | 23.0 | >92.3 | 46.1 | 92.3 | 92.3 | >92.3 | 92.3 | 46.1 | 23.0 | 2.9 | >92.3 | >92.3 | >92.3 |
| **6** | 2.7 | >85.4 | 85.4 | 21.3 | 85.4 | 42.6 | 85.4 | 21.3 | 2.7 | 0.16 | >85.4 | 5.3 | 42.6 |
| **7** | 1.2 | 39.5 | 9.9 | 9.9 | 19.8 | 19.8 | 19.8 | 2.5 | 1.2 | 0.08 | 19.8 | 2.5 | 9.9 |
| **8** | 2.3 | 76.0 | 9.2 | 4.6 | 76.0 | 18.5 | 76.0 | 2.3 | 2.3 | 1.1 | 18.5 | 4.6 | 9.2 |
| **9** | 4.3 | >69.5 | >69.5 | 17.3 | 69.5 | >69.5 | 69.5 | 2.2 | 8.7 | 1.1 | >69.5 | 8.7 | 17.3 |
| **10** | >65.4 | >65.4 | 65.4 | 65.4 | 8.1-65.4 | >65.4 | >65.4 | 2.0 | 8.1 | 1.0 | >65.4 | 8.1 | >65.4 |
| **11** | >88.3 | >88.3 | 88.3 | 88.3 | >88.3 | >88.3 | >88.3 | >88.3 | >88.3 | >88.3 | >88.3 | >88.3 | >88.3 |
| **12** | 9.2 | >73.7 | 73.7 | 9.2 | 36.7 | 9.2 | 18.4 | 36.7 | 4.6 | 0.57 | >73.7 | 18.4 | 73.7 |
| **13** | >74.1 | >74.1 | >74.1 | >74.1 | >74.1 | >74.1 | >74.1 | >74.1 | >74.1 | 74.1 | >74.1 | >74.1 | >74.1 |
| **14** | 65.4 | >65.4 | 65.4 | 65.4 | >65.4 | 65.4 | >65.4 | 65.4 | 65.4 | 2.0 | >65.4 | 32.7 | >65.4 |
| **15** | 85.8 | 85.8 | >85.8 | 85.8 | 10.7-85.8 | 85.8 | >85.8 | 85.8 | 85.8 | 10.7 | 85.8 | >85.8 | >85.8 |
| **16** | >84.0 | >84.0 | >84.0 | 84.0 | 84.0 | 84.0 | 84.0 | 20.9 | >84.0 | 20.9 | 84.0 | 20.9 | 41.9 |
| **AmB** | 4.2 | 4.2 | 2.1 | 1.1 | 2.1 | 4.2 | 4.2 | 2.1 | 4.2 | 2.1 | 15.6 | 4.2 | 4.2 |
| **CAS** | 0.8 | 0.2 | 0.05 | 0.1 | 0.1 | 0.2 | 0.4 | 0.05 | 0.4 | 1.6 | >25.8 | >25.8 | >25.8 |
| **FLC** | 204.1 | >408.1 | 50.9 | >408.1 | >408.1 | 204.1 | 204.1 | >102.2 | >102.2 | 6.4 | 204.1 | 204.1 | 204.1 |
| **VOR** | 0.69 | 11.2 | 5.6 | 5.6 | 2.8) | 22.3 | 5.6 | 0.17 | 0.34 | <0.06 | 0.69 | 0.34 | 0.34 |

**Yeast strains: A** = *Candida albicans* ATCC 10231, **B** = *C. albicans* ATCC 64124, **C** = *C. albicans* ATCC MYA-2876(S), **D** = *C. albicans* ATCC 90819(R), **E** = *C. albicans* ATCC MYA-2310(S), **F** = *C. albicans* ATCC MYA-1237(R), **G** = *C. albicans* ATCC MYA-1003(R), **H** = *Candida glabrata* ATCC 2001, **I** = *Candida krusei* ATCC 6258, **J** = *Candida parapsilosis* ATCC 22019. NOTE: Here, the (S) and (R) indicate that ATCC reports these strains to be susceptible (S) and resistant (R) to ITC and FLC.
**Filamentous fungi: K** = *Aspergillus flavus* ATCC MYA-3631, **L** = *Aspergillus nidulans* ATCC 38163, **M** = *Aspergillus terreus* ATCC MYA-3633.
**Known antifungal agents: AmB** = amphotericin B, **CAS** = caspofungin, **FLC** = fluconazole, **VOR** = voriconazole.
[a] For yeast strains: MIC-0 values are reported for FLC analogues **5-16**, AmB, and CAS, whereas MIC-2 values are reported for azoles. For filamentous fungi, MIC-0 values are reported for all compounds.

**Table S2:** MIC values[a] (in μM) (the corresponding values in μg/mL are presented in Table 2) determined for compounds **5-16** and for two control antifungal agents (FLC and VOR) against various non-*albicans Candida* and *Cryptococcus neoformans* clinical isolates.

| | Yeast strains | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cpd #** | **H** | **CG1** | **CG2** | **CG3** | **J** | **CP1** | **CP2** | **CP3** | **CN1** | **CN2** | **CN3** |
| **5** | 23.0 | 92.3 | 23.0 | 92.3 | 2.9 | 2.9 | 2.9 | 2.9 | 23.0 | >92.3 | 92.3 |
| **6** | 2.7 | 21.3 | 2.7 | 42.6 | 0.16 | 1.3 | 0.33 | 0.65 | 5.3 | 10.6 | 10.6 |
| **7** | 1.2 | 4.9 | 1.2 | 9.9 | 0.08 | 0.15 | 0.30 | 0.61 | 0.61 | 0.61 | 1.2 |
| **8** | 2.3 | 4.6 | 2.3 | 4.6 | 1.1 | 0.14 | 0.57 | 0.28 | 0.57 | 0.57 | 1.1 |
| **9** | 4.3 | 4.3 | 4.3 | 8.7 | 1.1 | 1.1 | 2.2 | 1.1 | 0.27 | 0.53 | 1.1 |
| **10** | >65.4 | 4.1 | 2.0 | 16.3 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| **11** | >88.3 | >88.3 | >88.3 | >88.3 | >88.3 | 88.3 | 88.3 | 88.3 | >88.3 | >88.3 | >88.3 |
| **12** | 9.2 | 36.7 | 4.6 | 73.7 | 0.57 | 0.28 | 0.28 | 0.28 | 1.1 | 4.6 | 4.6 |
| **13** | >74.1 | >74.1 | >74.1 | >74.1 | 74.1 | >74.1 | >74.1 | >74.1 | >74.1 | >74.1 | 37.1 |
| **14** | 65.4 | 32.6 | 16.3 | >65.4 | 2.0 | 2.0 | 4.1 | 4.1 | >65.4 | >65.4 | >65.4 |
| **15** | 85.8 | 85.8 | 42.8 | 85.8 | 10.7 | 2.7 | 2.7 | 5.4 | 21.4 | >85.8 | 42.8 |
| **16** | >84.0 | 20.9 | 20.9 | 20.9 | 20.9 | 10.5 | 10.5 | 10.5 | 20.9 | 84.0 | >84.0 |
| **CAS** | 0.05 | 0.16 | 0.2 | 0.8 | 1.6 | 0.4 | 0.4 | 0.4 | 12.9 | 25.8 | 12.9 |
| **FLC** | 204.1 | >102.1 | >102.1 | >102.1 | 6.4 | 3.2 | 3.2 | 3.2 | 25.6 | >102.1 | >102.1 |
| **VOR** | 0.69 | 2.76 | 11.2 | >89.9 | <0.06 | <0.06 | <0.06 | <0.06 | 0.69 | 2.76 | 0.35 |

**Yeast strains: H** = *Candida glabrata* ATCC 2001, **CG1-CG3** = *C. glabrata* clinical isolates, **J** = *Candida parapsilosis* ATCC 22019, **CP1-CP3** = *C. parapsilosis* clinical isolates, **CN1-CN3** = *Cryptococcus neoformans* clinical isolates.
**Known antifungal agents: CAS** = caspofungin, **FLC** = fluconazole, **VOR** = voriconazole.
[a] For these yeast strains: MIC-0 values are reported for FLC analogues **5-16**, whereas MIC-2 values are reported for azoles.

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**2.4. Time-kill studies.** The time-kill curve analyses were done using a previously published protocol.[3] Fungal cells (*C. albicans* ATCC 10231 (strain **A**) and *C. parapsilosis* ATCC 22019 (strain **J**)) were cultured in YPD medium (3 mL) overnight. Cells (~10-15 μL) were then added

to Eppendorf tubes and diluted (to 1 mL) with RPMI 1640 medium to achieve the proper concentration of working stocks with $OD_{600}$ of 0.12-0.13. Prior to performing the time-kill studies, cells (300 µL) were aliquoted into 15 mL conical tubes along with 3.7 mL of RPMI 1640 medium (total of 4 mL) to establish that 300 µL from working stock of $OD_{600}$ of 0.12-0.13 would be sufficient to achieve the initial inoculum (time 0 h) of 1-4×$10^5$ CFU/mL on PDA plates after at least 24 h incubation. Once established, 300 µL of cells and appropriate volumes of RPMI 1640 medium and compounds (**6**, **7**, and VOR) were then added to make up solutions of cells and drugs with concentrations of 1×, 4×, and 8× the respective MIC values of each compound. The final volume for each tube was 4 mL. The tubes were then incubated at 35 °C and agitated at 200 rpm for 24 h. At 0, 3, 6, 9, 12, and 24 h time points, 100 µL of cells from each tube was serially diluted with RPMI 1640 medium to the appropriate dilutions ($10^2$-$10^7$ times) depending on turbidity. Afterwards, the cells were plated on PDA plates. Please note that proper dilution comes with experience and the dilutions were done to make sure that each plate contains 30-300 colonies, which is considered statistically significant. The plates were then incubated for 24-48 h and the number of colonies were counted. The experiments were performed in duplicate. These data are presented in Fig. 3.

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**2.5. Hemolytic activity assay.** The hemolytic activity of compounds **6-10** was determined by using previously described methods with minor modifications.[4] Murine whole blood was suspended in 4 mL of PBS and centrifuged at 1,000 rpm for 10 min at room temperature to obtain the mRBCs. The mRBCs were washed four times in PBS and resuspended in the same buffer to a final concentration of $10^7$ erythrocytes/mL. Two-fold serial dilutions of compounds **6-10** were prepared using 100 µL of PBS buffer in Eppendorf tubes followed by the addition of 100 µL of mRBC suspension that made the final concentration of compounds and mRBCs to be 0.48-62.5 µg/mL and 5×$10^6$ erythrocytes/mL, respectively. The tubes were incubated at 37 °C for 1 h. Tubes with PBS buffer (200 µL) and Triton™ X-100 (1% *v*/*v*, 2 µL) served as negative (blank) and positive controls, respectively. The percentage of hemolysis was calculated using the following equation: % hemolysis = [(absorbance of sample) – (absorbance of blank)] × 100/(absorbance of positive control). These data are presented in Table S3 and Fig. 4.

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**Table S3:** The % hemolysis caused by azole derivatives and VOR against mRBCs with the error bars (± SDEV).

| | Concentration (μg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cpd # | **0.48** | **0.975** | **1.95** | **3.9** | **7.8** | **15.6** | **31.3** | **62.5** |
| **6** | 2.0 ± 0.3 | 2.0 ± 1.0 | 0 | 2.0 ± 0.8 | 0 | 3.0 ± 0.3 | 9.0 ± 0.3 | 78.0 ± 1.0 |
| **7** | 0 | 0 | 2.0 ± 0.1 | 3.0 ± 3.0 | 8.0 ± 2.0 | 33.0 ± 7.0 | 92.0 ± 7.0 | 100.0 ± 3.0 |
| **8** | 2.0 ± 0.9 | 2.0 ± 0.8 | 2.0 ± 1.4 | 8.0 ± 4.0 | 21.0 ± 5.0 | 86.0 ± 11.0 | 100.0 ± 5.0 | 98.0 ± 2.0 |
| **9** | 2.0 ± 0.9 | 6.0 ± 3.0 | 3.0 ± 2.0 | 10.0 ± 2.0 | 17.0 ± 2.0 | 29.0 ± 0.3 | 57.0 ± 6.0 | 77.0 ± 8.0 |
| **10** | 4.0 ± 0.2 | 5.0 ± 4.2 | 6.0 ± 4.0 | 3.0 ± 2.0 | 4.0 ± 0.2 | 4.0 ± 1.0 | 4.0 ± 0.5 | 8.0 ± 3.0 |
| **VOR** | 9.0 ± 5.0 | 10.0 ± 2.0 | 6.0 ± 2.0 | 7.0 ± 0.5 | 11.0 ± 11.0 | 3.0 ± 1.2 | 83.0 ± 5.0 | 100.0 ± 2.3 |

**2.6. *In vitro* cytotoxicity assay.** Mammalian cytotoxicity assays were performed as previously described with minor modifications.[5] HEK-293, BEAS-2B, and A549 were cultured as described in section 2.2 and were counted by a hemocytometer when cells were about 80% confluent in flasks. Cells were plated in 96-well microtiter plates at concentrations of 10,000 cells per well for HEK-293 and 3,000 cells per well for BEAS-2B and A549. The 96-well microtiter plates were incubated at 37 °C and 5% $CO_2$ for 16 h to allow time for adherence. The medium was then removed and fresh medium with compounds **6-10** or VOR at 0.13-31 μg/mL and 0.1% DMSO vehicle were added. The stock solutions of compounds **6-10** were previously prepared at 1000× the intended tested concentrations. The positive control contained 20% Triton™ X-100. The negative control contained 0.1% DMSO and no drugs. A blank control was also prepared to have only medium and no cells. After 24 h of incubation, cell survival was assessed *via* addition of resazurin (10 μL of 10 mM solution) for 6-10 h. Live cells were detected by a color change from purple to pink *via* conversion of the compound to resorufin, which could be quantified at $\lambda_{560}$ absorption and $\lambda_{590}$ emission by a SpectraMax M5 plate reader. The percentage survival rates were calculated by using the following formula: % cell survival = [(fluorescence of sample) – (fluorescence of blank)] × 100/[(fluorescence of negative control)-(fluorescence of blank)]. The experiments were performed in duplicate. These data are presented in Fig. 5.

**2.7. Membrane permeabilization assay.** 2 mL of YPD broth was first inoculated using a fresh colony of *C. albicans* ATCC 10231 (strain **A**) in a sterile culture tube and was grown overnight at 35 °C at 200 rpm. 50 μL of an overnight culture was transferred to RPMI 1640 medium (0.5 mL) containing no drug (negative control) or compound **8** at 1× MIC (1.95 μg/mL), 2× MIC (3.9 μg/mL), and 4× MIC (7.8 μg/mL) or compound **9** at 1× MIC (3.9 μg/mL), 2× MIC (7.8 μg/mL), and 4× MIC (15.6 μg/mL). VOR at 4× MIC (0.975 μg/mL), and KANB $(C_{14})^4$ at 4× MIC (15.6 μg/mL) were also used as negative and positive controls, respectively. After establishing that

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using 4× MIC was required to display membrane permeabilization, compounds **5**, **6**, and **7** were also tested at their respective 4× MIC (31.2, 3.9, and 1.95 µg/mL, respectively). The cell suspensions were then treated for 1 h at 35 °C with continuous agitation (200 rpm). The cells were then centrifuged and resuspended in 500 µL of PBS buffer (pH 7.2). Subsequently, cells were treated with propidium iodide (9 µM, final concentration) and incubated for 20 min at room temperature in the dark. Glass slides prepared with 10 µL of each mixture were observed in bright field and fluorescence modes (using Texas red filter set, excitation and emission wavelengths of 535 and 617 nm, respectively) using a Zeiss Axiovert 200M fluorescence microscope. A magnification lens of 63X was used. Data were obtained from at least two independent experiments. The images were also post-processed utilizing automatic contrast and brightness setting in Microsoft PowerPoint 2013 to eliminate background noise. These images are presented in Fig. 6.

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**2.8. Sterol profile by GC-MS.** A single colony of *C. albicans* ATCC 10231 (strain **A**) was picked from a fresh culture plate to inoculate 3 mL of yeast peptone dextrose broth (YPDB) and was incubated at 35 °C for ~18 h with continuous agitation (180 rpm). The overnight yeast culture was used to inoculate RPMI 1640 medium (15 mL) and the final inoculum concentration was adjusted to $1\times10^6$ CFU/mL (~$OD_{600}$ = 0.12) by using spectrophotometric method. Afterwards, the yeast cells were treated with compounds **8** (0.48 µg/mL), compound **9** (0.975 µg/mL), or VOR (0.12 µg/mL) at their sub-MIC values. An equivalent amount of DMSO without drug (untreated control) was also prepared. Cells were harvested by centrifugation (5,000 rpm) for 10 min at room temperature, and cell pellets were saponified at 80 °C for 2 h with 3 mL of MeOH, 2 mL of pyrogallol dissolved in MeOH (0.5%, *wt*/*v*) (CAS # 87-66-1, Sigma-Aldrich, St. Louis, MO.), and 2 mL of KOH (60%, *wt*/*v*). The non-saponifiable sterols were then extracted with 3×5 mL of heptane. The extracts were evaporated under a stream of nitrogen to dryness and resuspended in 500 µL of heptane. The sterol suspension was then transferred to a GC-MS vial and derivatized with 250 µL of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA, CAS # 24589-78-4, Sigma-Aldrich, St. Louis, MO) at 70 °C for 20 min. GC-MS analyses were performed on an Agilent 7890A gas chromatograph with splitless injection, coupled to an Agilent 5970C inert XL mass spectrometer with a triple-axis detector and an Agilent 19091S-433 capillary column (30 m x 250 µm). The oven temperature was

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programmed to hold at 70 °C for 2 min and then ramped to 270 °C at a rate of 20 °C/min. Helium (10 psi) was used as the carrier gas, the electron ionization energy was 70 eV, and the inlet temperature 250 °C. Identification of sterols was achieved using the NIST (the National Institute of Standards and Technology) reference database. These data are presented in Fig. 7.

**3. References:**


1. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts - Approved standard. CLSI document M27-A3. Wayne, PA. **2008**.
2. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi - 2nd Edition: CLSI document M38-A2. Wayne, PA. **2008**.
3. Shrestha, S. K.; Fosso, M. Y.; Green, K. D.; Garneau-Tsodikova, S. Amphiphilic tobramycin analogues as antibacterial and antifungal agents. *Antimicrob. Agents Chemother.* **2015**, *59*, 4861-4869.
4. Fosso, M. Y.; Shrestha, S. K.; Green, K. D.; Garneau-Tsodikova, S. Synthesis and bioactivities of kanamycin B-derived cationic amphiphiles. *J. Med. Chem.* **2015**, *58*, 9124-9132.
5. Thamban Chandrika, N.; Shrestha, S. K.; Ngo, H. X.; Garneau-Tsodikova, S. Synthesis and investigation of novel benzimidazole derivatives as antifungal agents. *Bioorg. Med. Chem.* **2016**, *24*, 3680-3686.

## 4. Supplementary Figures (S1-S32):

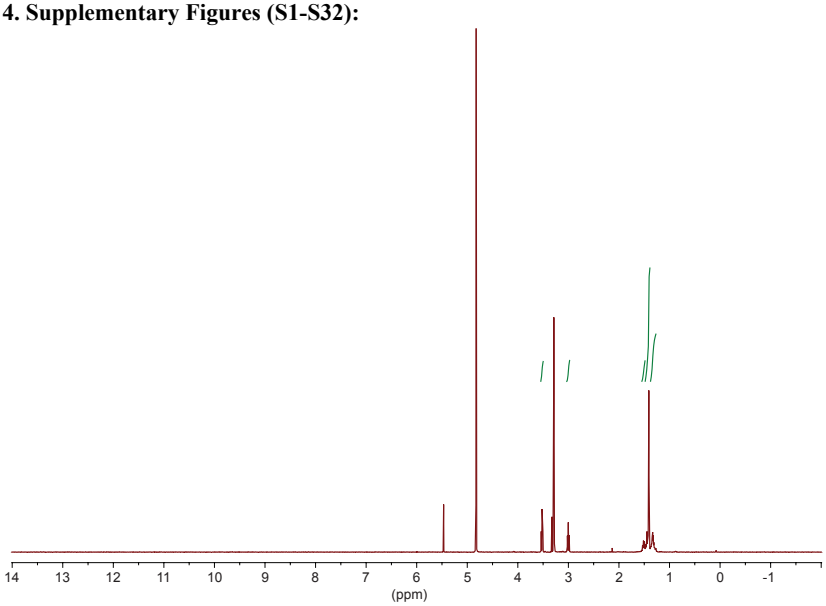


**Fig. S1:** $^{1}$H NMR spectrum for compound **1** in $CD_3OD$ (400 MHz).

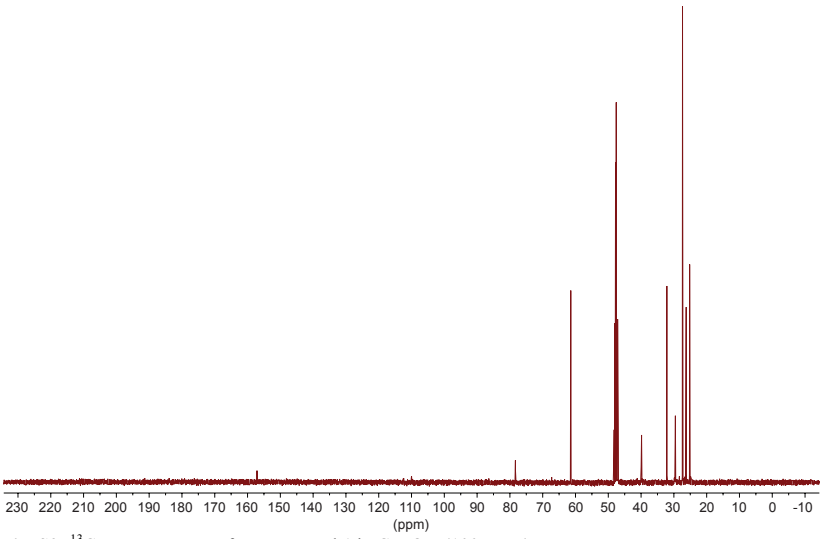


**Fig. S2:** $^{13}$C NMR spectrum for compound **1** in $CD_3OD$ (100 MHz).

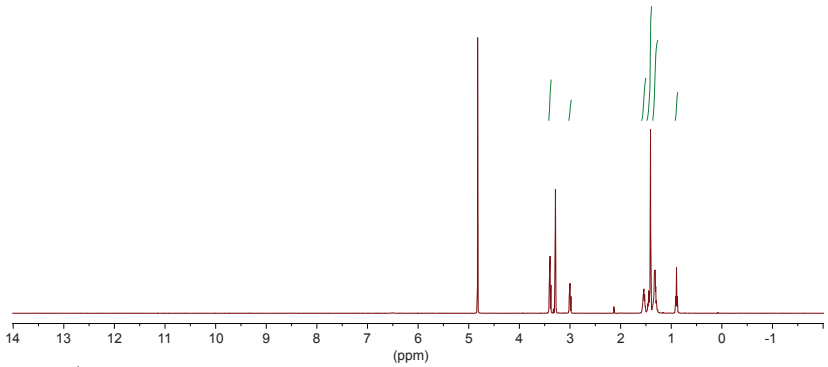


**Fig. S3:** $^{1}$H NMR spectrum for compound **2** in $CD_3OD$ (400 MHz).

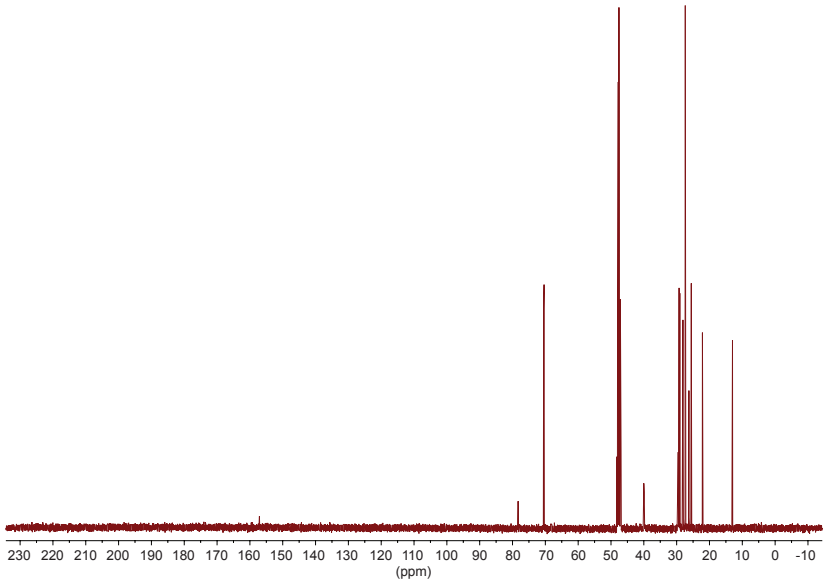


**Fig. S4:** $^{13}$C NMR spectrum for compound **2** in $CD_3OD$ (100 MHz).

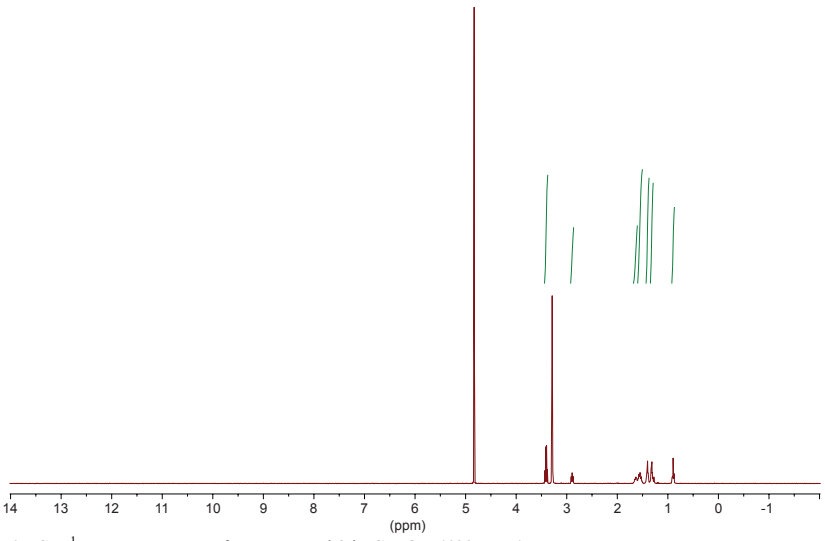


**Fig. S5:** $^{1}$H NMR spectrum for compound **3** in $CD_3OD$ (400 MHz).

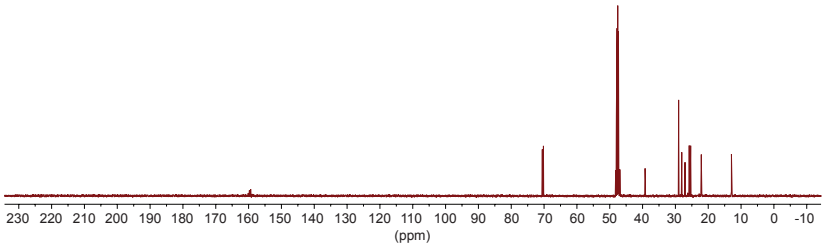


**Fig. S6:** $^{13}$C NMR spectrum for compound **3** in $CD_3OD$ (100 MHz).

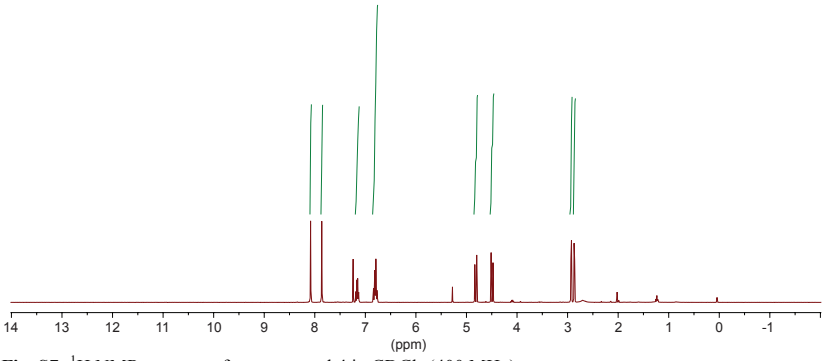


**Fig. S7:** $^{1}$H NMR spectrum for compound **4** in $CDCl_3$ (400 MHz).

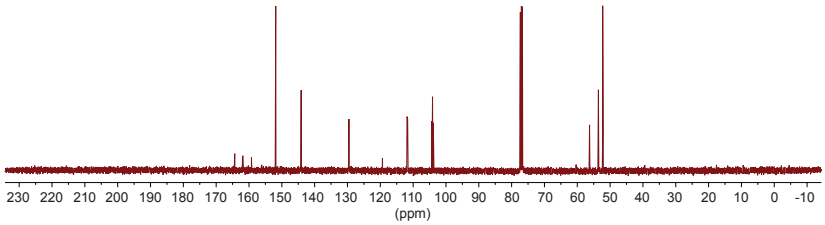


**Fig. S8:** $^{13}C$ NMR spectrum for compound **4** in $CDCl_3$ (100 MHz).

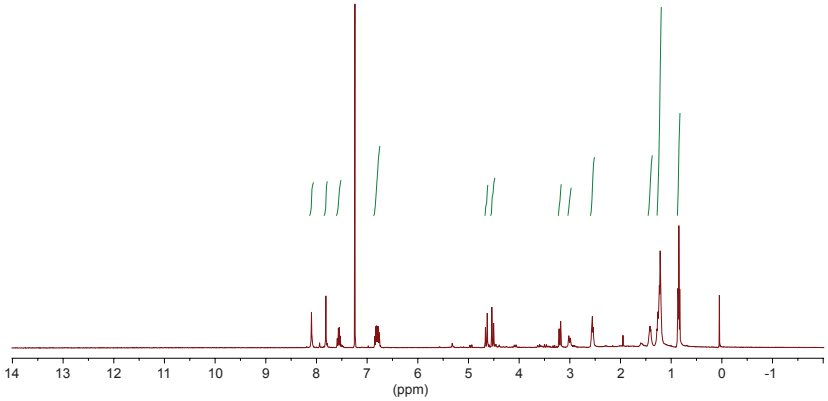


**Fig. S9:** $^{1}H$ NMR spectrum for compound **5** in $CDCl_3$ (400 MHz).

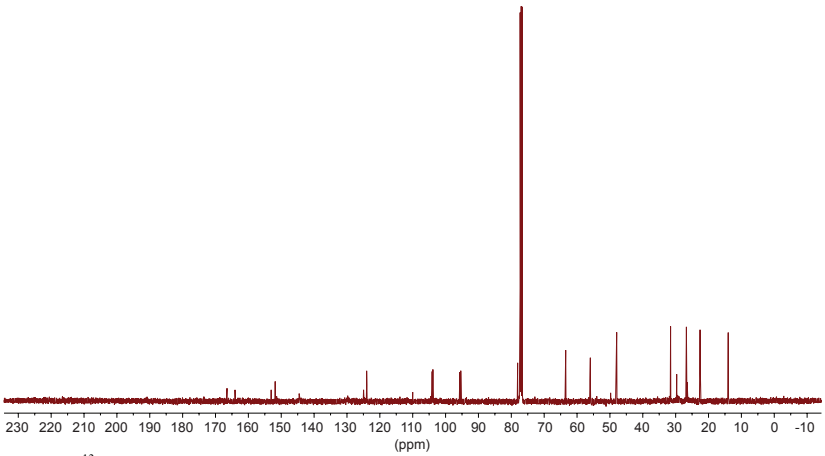


**Fig. S10:** $^{13}C$ NMR spectrum for compound **5** in $CDCl_3$ (100 MHz).

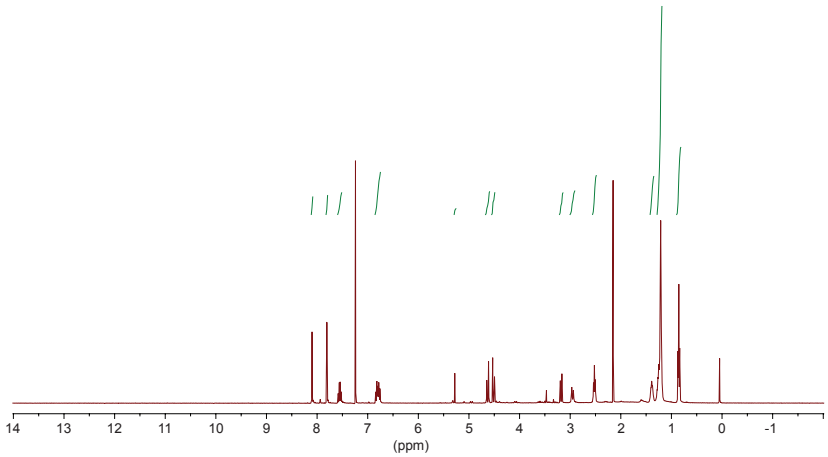


**Fig. S11:** $^{1}$H NMR spectrum for compound **6** in $CDCl_3$ (400 MHz).

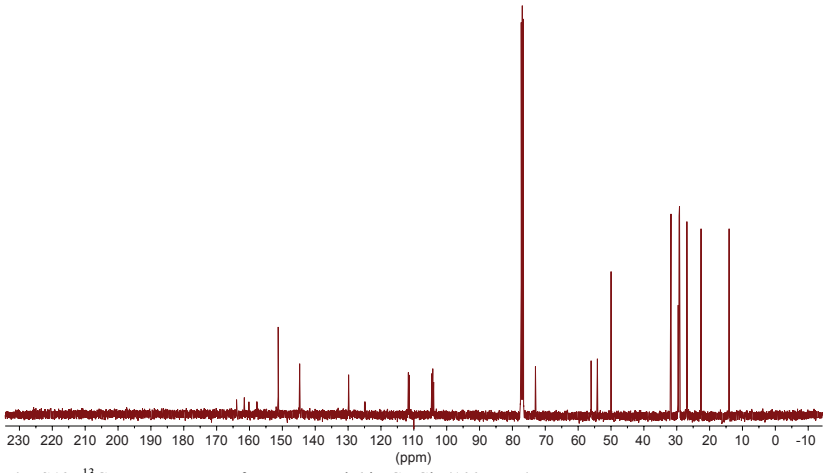


**Fig. S12:** $^{13}$C NMR spectrum for compound **6** in $CDCl_3$ (100 MHz).

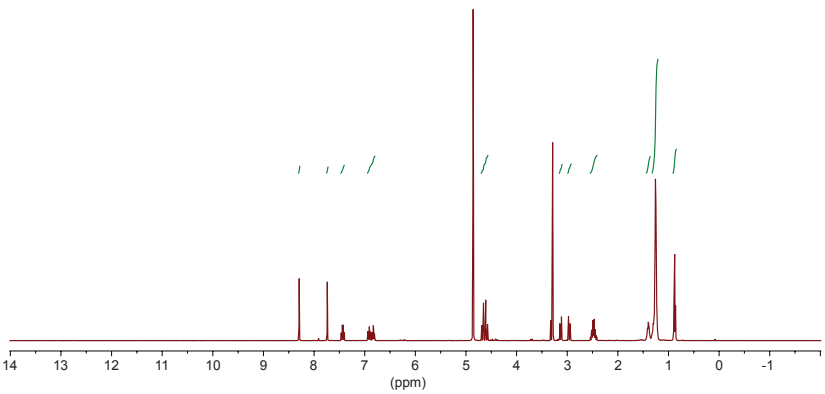


**Fig. S13:** $^{1}$H NMR spectrum for compound **7** in $CD_3OD$ (400 MHz).

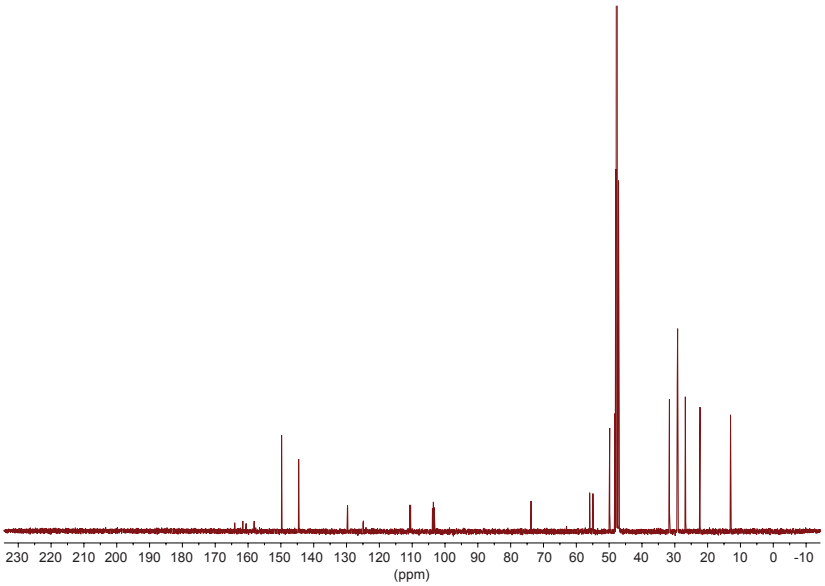


**Fig. S14:** $^{13}$C NMR spectrum for compound **7** in $CD_3OD$ (100 MHz).

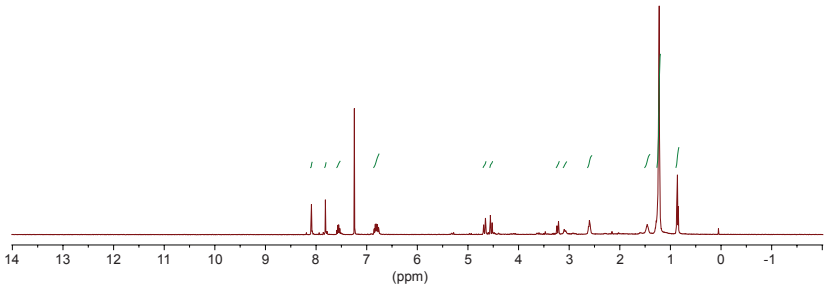


**Fig. S15:** $^{1}$H NMR spectrum for compound **8** in $CDCl_3$ (400 MHz).

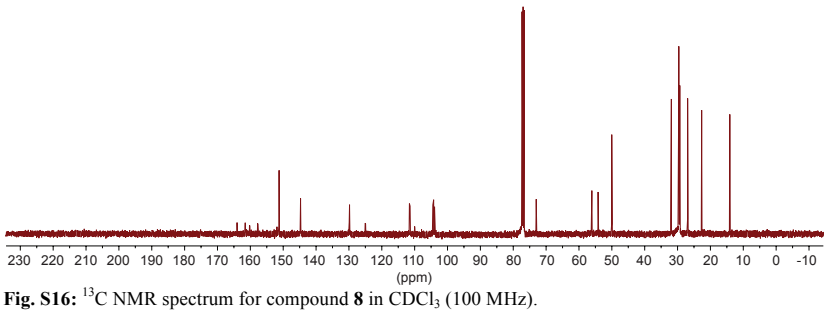


**Fig. S16:** $^{13}$C NMR spectrum for compound **8** in $CDCl_3$ (100 MHz).

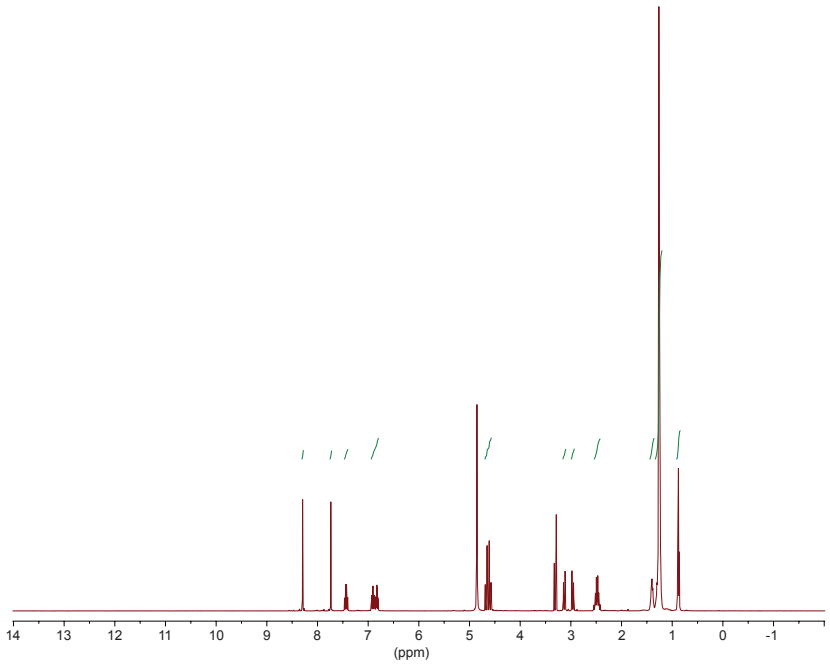


**Fig. S17:** $^{1}$H NMR spectrum for compound **9** in $CD_3OD$ (400 MHz).

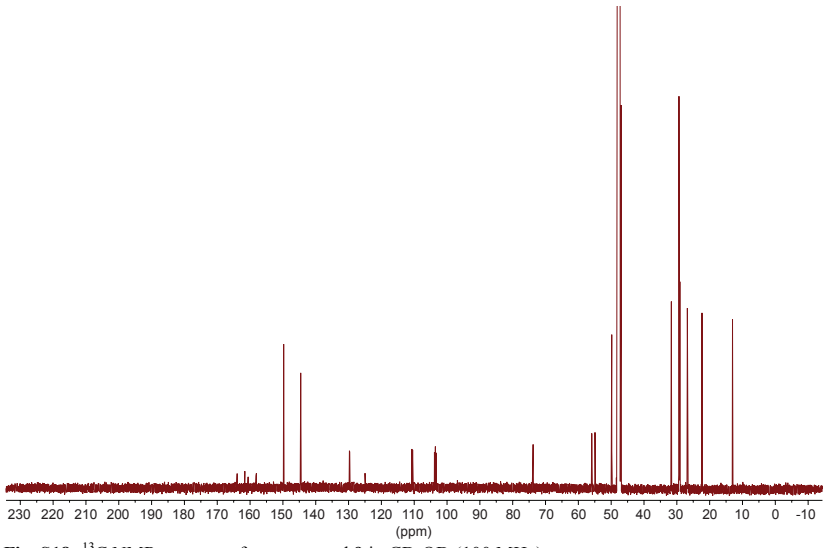


**Fig. S18:** $^{13}$C NMR spectrum for compound **9** in $CD_3OD$ (100 MHz).

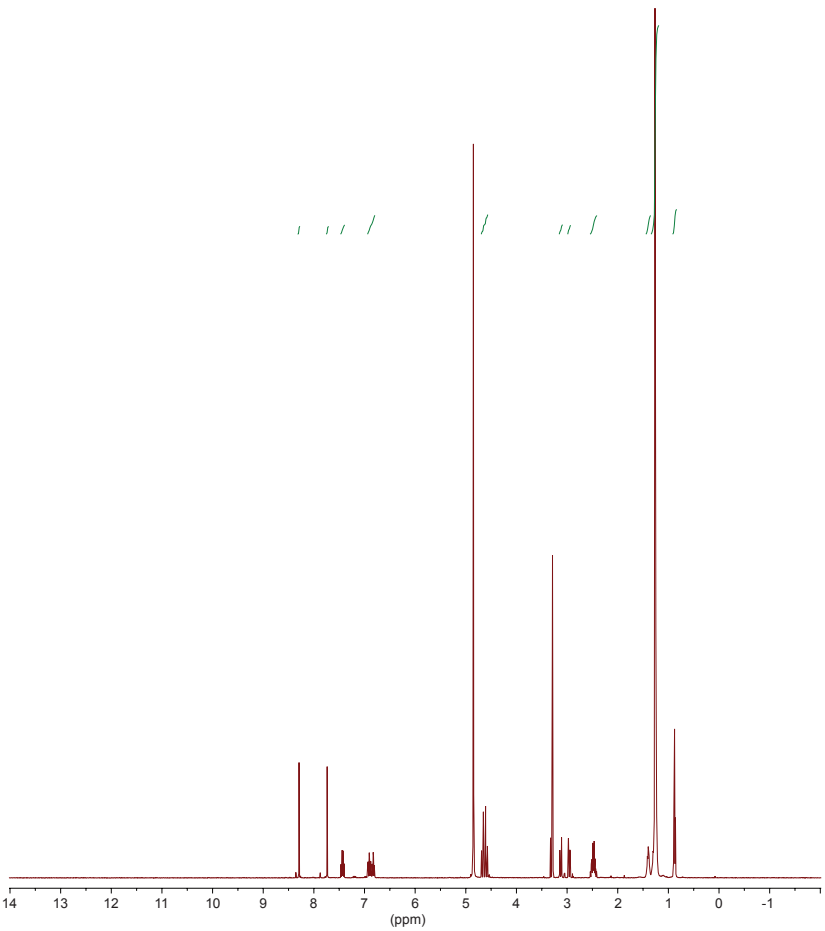


**Fig. S19:** $^{1}$H NMR spectrum for compound **10** in $CD_3OD$ (400 MHz).

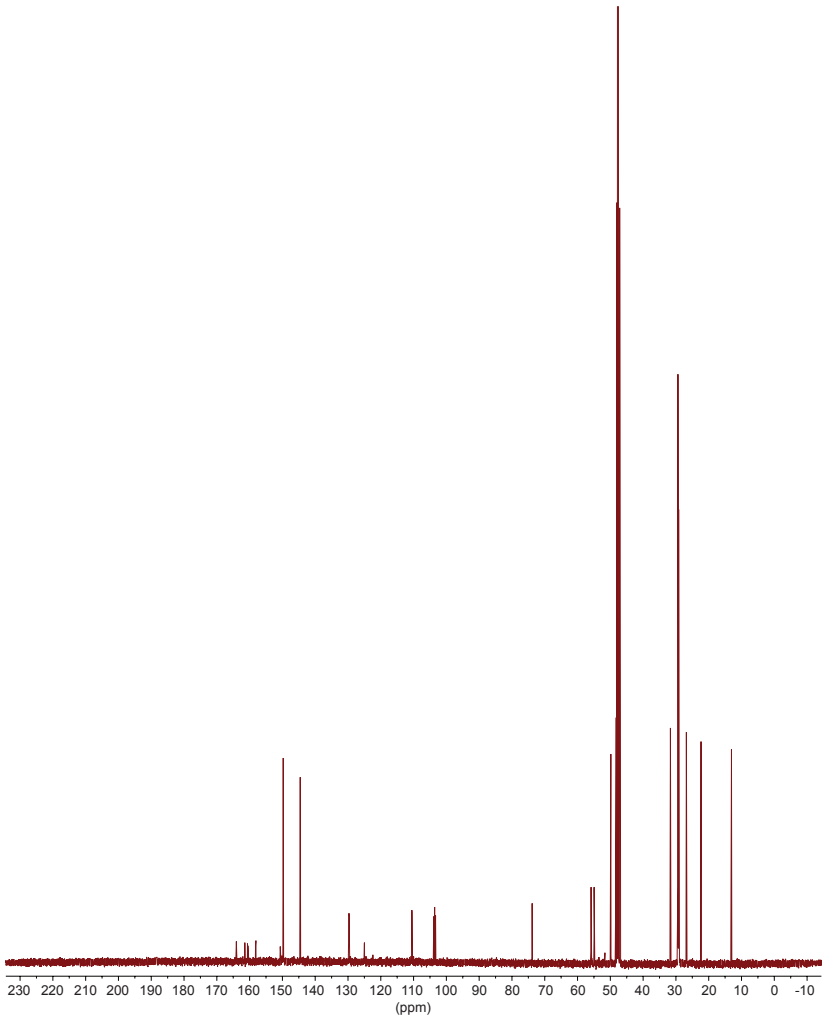


**Fig. S20:** $^{13}C$ NMR spectrum for compound **10** in $CD_3OD$ (100 MHz).

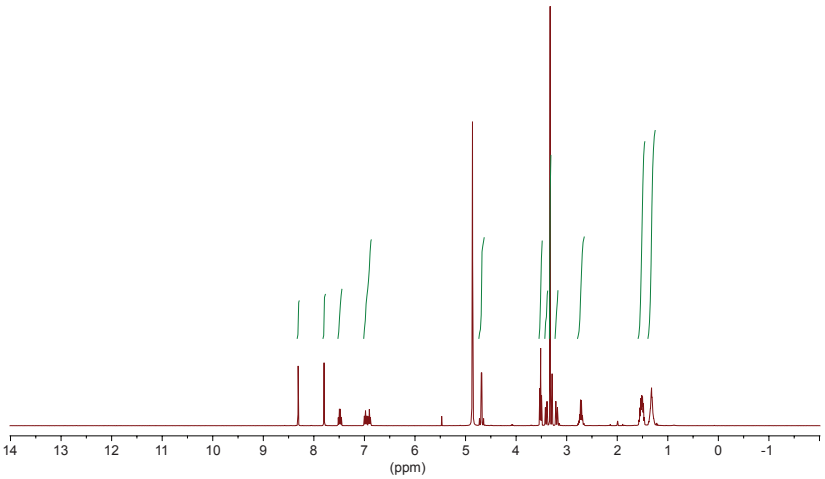


**Fig. S21:** $^{1}$H NMR spectrum for compound **11** in $CD_3OD$ (400 MHz).

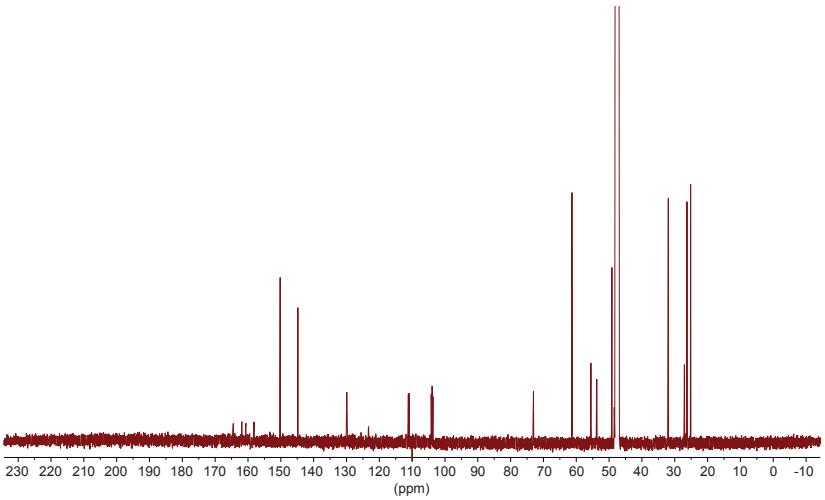


**Fig. S22:** $^{13}$C NMR spectrum for compound **11** in $CD_3OD$ (100 MHz).

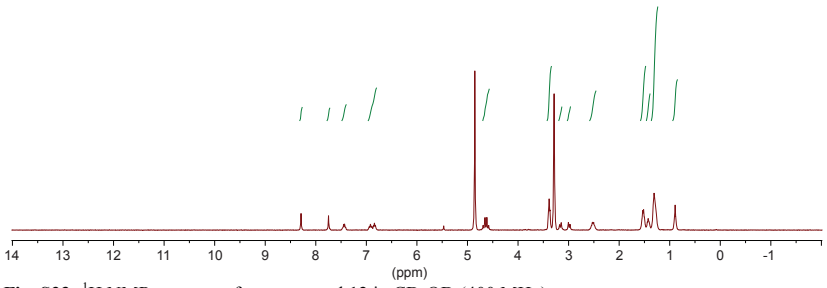


**Fig. S23:** $^{1}$H NMR spectrum for compound **12** in $CD_3OD$ (400 MHz).

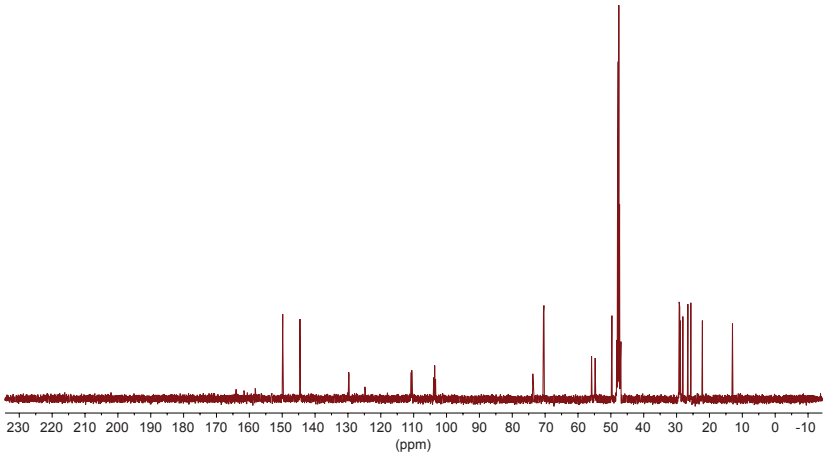


**Fig. S24:** $^{13}$C NMR spectrum for compound **12** in $CD_3OD$ (100 MHz).

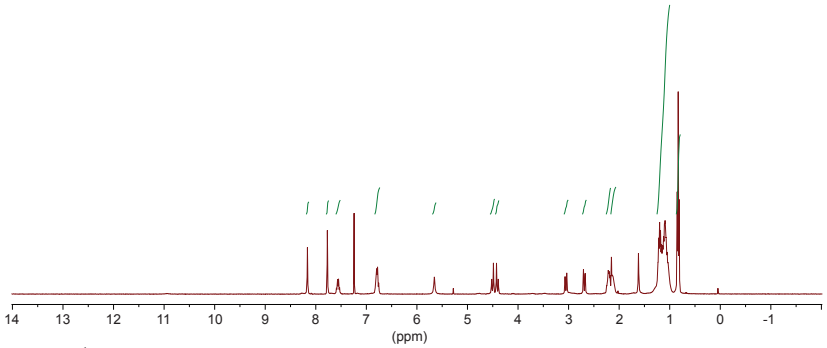


**Fig. S25:** $^{1}$H NMR spectrum for compound **13** in $CDCl_3$ (400 MHz).

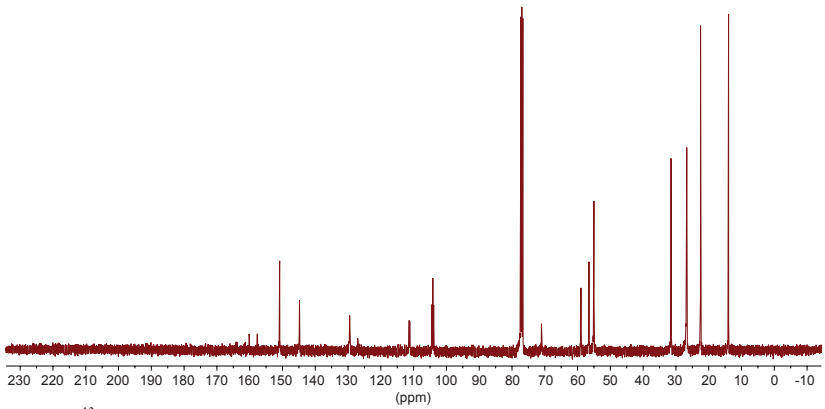


**Fig. S26:** $^{13}C$ NMR spectrum for compound **13** in $CDCl_3$ (100 MHz).

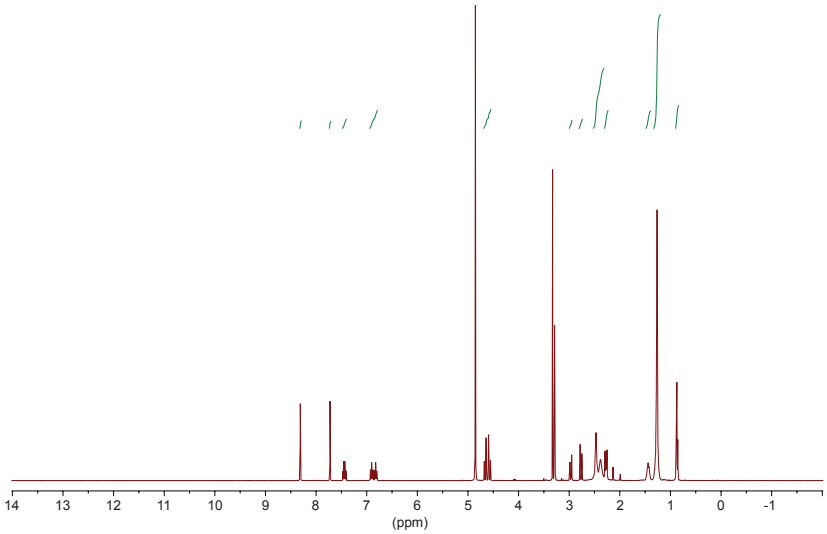


**Fig. S27:** $^{1}H$ NMR spectrum for compound **14** in $CD_3OD$ (400 MHz).

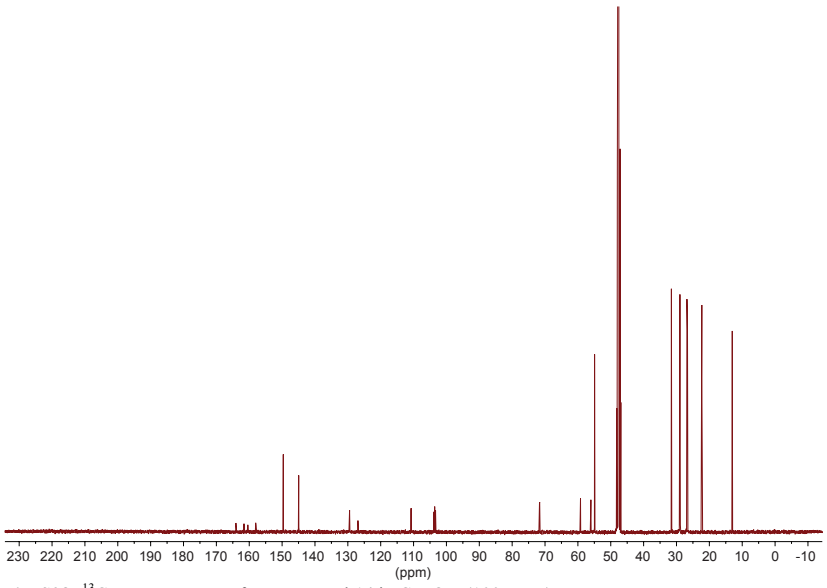


**Fig. S28:** $^{13}C$ NMR spectrum for compound **14** in $CD_3OD$ (100 MHz).

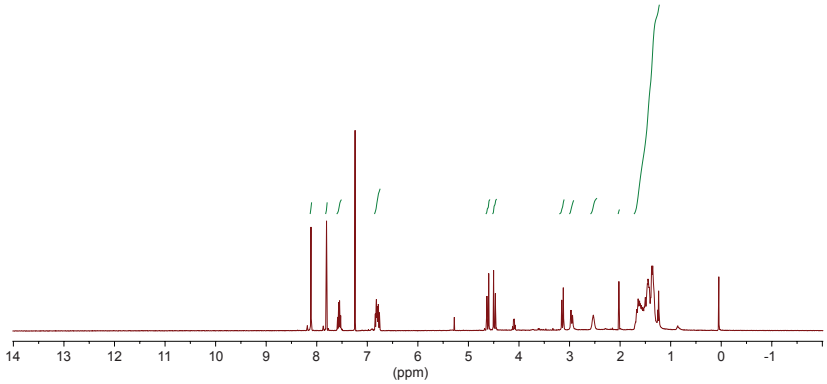


**Fig. S29:** $^{1}H$ NMR spectrum for compound **15** in $CDCl_3$ (400 MHz).

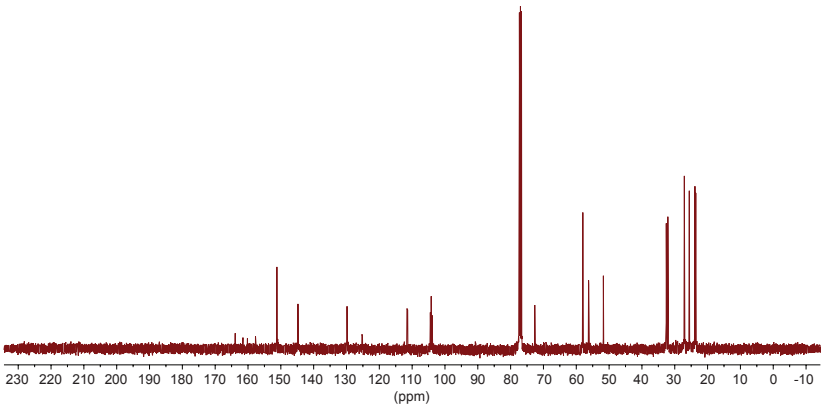


**Fig. S30:** $^{13}C$ NMR spectrum for compound **15** in $CDCl_3$ (100 MHz).

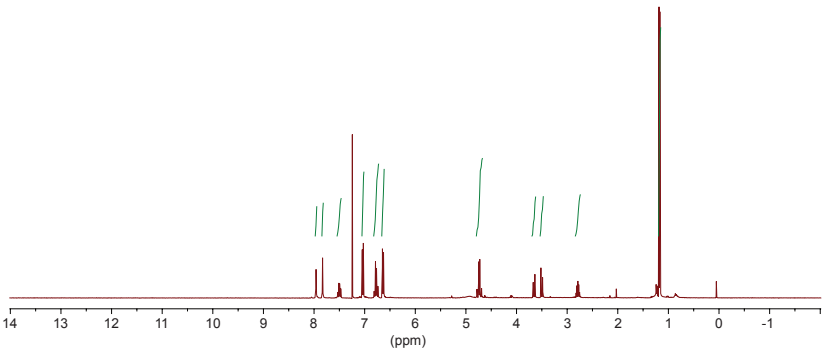


**Fig. S31:** $^{1}H$ NMR spectrum for compound **16** in $CDCl_3$ (400 MHz).

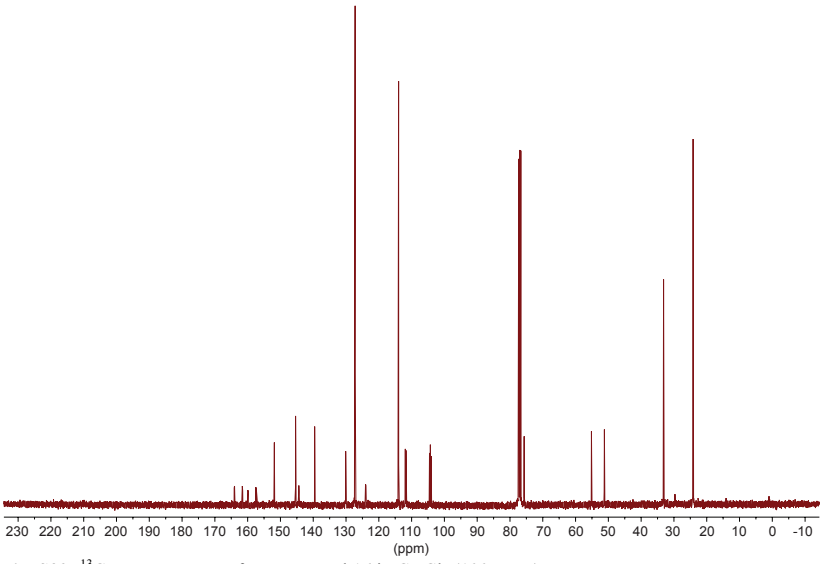


**Fig. S32:** $^{13}C$ NMR spectrum for compound **16** in $CDCl_3$ (100 MHz).