Supporting Information

Synthesis and antifungal activities of miltefosine analogs

Ranga Rao Ravu[a], Ying-Lien Chen[b], Melissa R. Jacob[a], Xuewen Pan[c], Ameeta K. Agarwal[a], Shabana I. Khan[a,d], Joseph Heitman[b], Alice M. Clark[a,d], and Xing-Cong Li[a,d,*]

[a] *National Center for Natural Product Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, MS 38677, USA*

[b] *Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA.*

[c] *Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas, USA.*

[d] *Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, MS 38677, USA.*

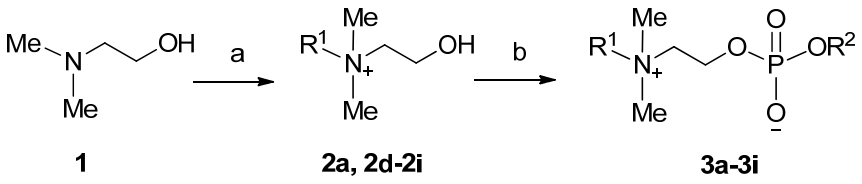
Table of Contents

1. Chemical synthesis procedures **……..……...……..**S2-S8
2. *In vivo* antifungal efficacy studies ……..……...……..S9
3. ^{1}H and ^{13}C NMR spectra of compounds **3a-3i** …..……...…….....S10-S18
4. HRESIMS data of compounds **3a-3i**……S19-S21
5. *In vitro* antifungal activity and cytotoxicity of compounds **3a-3i** with standard deviations.....S22-S23

1. Chemistry

All chemicals used for synthesis were purchased from commercial suppliers.The 1D and 2D NMR spectra using standard pulse programs were recorded at room temperature on a Varian Oxford AS400 spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz. The chemical shift values are relative to the internal standard TMS. HRESIMS data were obtained on an Agilent Series 1100 SL mass spectrometer. Column chromatography was performed using normal phase silica gel (J. T. Baker, 40 μm) and reversed-phase silica gel (RP-18, J. T. Baker, 40 μm). TLC was carried out on silica gel sheets (Alugram® Sil G/UV$_{254}$, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F$_{254S}$, Merck, Germany). Visualization: 10% H_2SO_4 followed by heating. Standard miltefosine was purchased from Cayman chemical company.

Synthesis of alkylphosphocholines 3a-3i.



General procedure for preparation of compounds 2a and **2d-2i.**

The commercially available 2-(*N*,*N*-dimethylamino)ethanol (**1,** 0.011 moles) was reacted with substituted alkyl halides (0.0140 moles) in CH_3CN (50 mL) at room temperature under argon atmosphere for 2-3h. The solvent was evaporated *in vacuo* and the resulting white solid was washed with hexane−$CHCl_3$ (1:1, 100 mL) to afford **2a-2i (**yields 85-97%).

***N*-Benzyl-2-hydroxy-*N*,*N*-dimethylethanaminium bromide (2a):** white solid, yield 95%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.66 (m, 2H, J = 8.0 Hz, aromatic H), 7.59–7.50 (m, 3H, aromatic H), 4.73 (br d,, 2H, *N*-C$\underline{H}_2$-Ph), 4.10 (m, 2H, -C$\underline{H}_2$-OH), 3.56 (m, 2H, *N*-CH_2), 3.17 (s, 6H, 2×*N*-CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 134.4, 131.8, 130.2, 129.0, 70.0, 66.6 (t), 56.9, 51.2 (t, 2C).

2-Hydroxy-*N*,*N*-dimethyl-*N*-(4-nitrobenzyl)ethanaminium bromide (2d): white solid, yield 94%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (d, 2H, J = 8.6 Hz, aromatic H), 7.95 (d, 2H, J = 8.6 Hz, aromatic H), 4.86 (br d, 2H, *N*-C$\underline{H}_2$-Ph), 4.12 (br s, 2H, -C$\underline{H}_2$-OH), 3.58 (m, 2H, *N*-CH_2), 3.21 (s, 6H, 2×*N*-CH_3).

2-Hydroxy-*N*-(4-methoxybenzyl)-*N*,*N*-dimethylethanaminium chloride (2e): white solid, yield 90%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (d, 2H, J = 8.6 Hz, aromatic H), 7.05 (d, 2H, J = 8.6 Hz, aromatic H), 4.60 (br s, 2H, *N*-C$\underline{H}_2$-Ph), 4.06 (m, 2H, -C$\underline{H}_2$-OH), 3.84 (s, 3H, OCH_3), 3.46 (m, 2H, *N*-CH_2), 3.09 (s, 6H, 2×*N*-CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 162.9, 135.8, 120.7, 115.5, 106.4, 70.0, 66.2, 56.9, 55.9, 50.9 (t, 2C).

N-(4-Chlorobenzyl)-2-hydroxy-*N*,*N*-dimethylethanaminium bromide (2f): white solid, yield 97%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.67 (d, 2H, J = 8.6 Hz, aromatic H), 7.53 (d, 2H, J = 8.6 Hz, aromatic H), 4.72 (br s, 2H, *N*-C$\underline{H}_2$-Ph), 4.09 (m, 2H, -C$\underline{H}_2$-OH), 3.54 (m, 2H, *N*-CH_2), 3.16 (s, 6H, 2×*N*-CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 138.0, 136.0, 130.4, 127.7, 69.0, 66.6, 56.9, 51.3 (t, 2C).

***N*-(4-Bromobenzyl)-2-hydroxy-*N*,*N*-dimethylethanaminium bromide (2g):** white solid, yield 95%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.68 (d, 2H, J = 8.6 Hz, aromatic H), 7.59 (d, 2H, J = 8.6

Hz, aromatic H), 4.71 (br s, 2H, *N*-C<u>H</u>$_2$-Ph), 4.08 (br s, 2H, -C<u>H</u>$_2$-OH), 3.54 (m, 2H, *N*-CH$_2$), 3.16 (s, 6H, 2×*N*-CH$_3$); ^{13}C NMR (100 MHz, CD$_3$OD) δ 136.2, 133.4, 128.1, 126.2, 69.0, 66.6 (t), 56.9, 51.3 (t, 2C).

2-Hydroxy-*N*,*N*-dimethyl-*N*-[3-phenylprop-2(*E*)-en-1-yl]ethanaminum bromide (2h): white solid, yield 84%; ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.63 (d, 2H, *J* = 8.0 Hz, aromatic H), 7.40 (m, 3H, aromatic H), 7.09 (d, 1H, *J* = 15.6 Hz, =C<u>H</u>-), 6.53 (dt, 1H, *J* = 15.6, 8.0 Hz, =C<u>H</u>-), 4.31 (d, 2H, *J* = 8.0 Hz, =C-C<u>H</u>$_2$), 4.11 (m, 2H, -CH$_2$-OH), 3.59 (m, 2H, *N*-CH$_2$), 3.20 (s, 6H, 2×*N*-CH$_3$); ^{13}C NMR (100 MHz, CD$_3$OD) δ 144.2, 136.5, 130.3, 129.8, 128.4, 116.3, 68.7 (t), 66.3 (t), 56.9 (d), 51.6 (t, 2C).

2-Hydroxy-*N*,*N*-dimethyl-*N*-(2-propen-1-yl)ethanaminum bromide (2i): white solid, yield 85%; ^{1}H NMR (400 MHz, CD$_3$OD) δ 6.17 (m, 1H, =C<u>H</u>-), 5.79 (d, 1H, *J* = 16.0 Hz, =C<u>H</u>$_2$-*trans*), 5.74 (d, 1H, *J* = 12.0 Hz, =C<u>H</u>$_2$-*cis)*, 4.21 (m, 2H, *N*-C<u>H</u>$_2$-CH=), 4.05 (br s, 2H, -C<u>H</u>$_2$-OH), 3.59 (m, 2H, *N*-C<u>H</u>$_2$CH$_2$OH), 3.24 (s, 6H, 2×*N*-CH$_3$); ^{13}C NMR (100 MHz, CD$_3$OD) δ 129.5, 126.7, 68.4 (t), 66.3 (t), 56.7 (t), 51.8 (t, 2C).

<u>General procedure for preparation of compounds 3a-3i.</u>

A solution of respective aliphatic alcohol (0.0082 moles) in CHCl$_3$ (20 mL) was added dropwise to a stirred solution of POCl$_3$ (0.0090 moles) and triethylamine (0.0181 moles) in CHCl$_3$ (10 mL) at 0°C. The resulting mixture was stirred at room temperature for 2 h. This intermediate was used further without any subsequent purification. To this solution pyridine (15 mL) was added dropwise at 0°C followed by compounds **2a-2i** (0.0113 mmol). The reaction mixture was stirred at room temperature overnight. After cooling, the mixture was hydrolyzed by addition of H$_2$O (2 mL) and stirred for 1 h at room temperature. The solvents were evaporated in

vacuum, and the resulting crude mixture was purified by using normal phase silica gel column chromatography, eluted with an isocratic system of $CHCl_3$−MeOH (70:30). The fraction containing the desired compound was loaded on a reversed-phase silica gel column using gradient eluents of CH_3CN−H_2O (50 to 100%) to afford pure compounds **3a-3i** with yields in 7% to 42%.

***N*-Benzyl-*N*,*N*-dimethyl-2-{[(hexadecyloxy)hydroxyphosphinyl]oxy}ethanaminum inner salt (3a):** white solid, yield 8%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.59 (d, 2H, J = 7.2 Hz, aromatic H), 7.51 (m, 3H, aromatic H), 4.62 (br s, 2H, *N*-C$\underline{H_2}$-Ph), 4.30 (br s, 2H, *N*-$CH_2C\underline{H_2}$-O), 3.85 (q, 2H, J = 6.4 Hz, -OCH_2-), 3.62 (m, 2H, *N*-C$\underline{H_2}CH_2$-O), 3.11 (s, 6H, 2×*N*-CH_3), 1.57 (m, 2H, -CH_2), 1.39–1.20 (m, 26H, 13×CH_2), 0.86 (t, 3H, J = 6.7 Hz, CH_3) (the assignment of the ^{1}H NMR resonances was facilitated by 2D NMR experiments of HMQC and HMBC); ^{13}C NMR (100 MHz, CD_3OD) δ 134.4 (2C), 131.8, 130.2 (2C), 128.8, 70.2, 66.9 (d), 65.3 (m), 60.1 (d), 51.2 (t, 2C), 33.1, 31.9 (d), 30.8 (6C), 30.8 (2C), 30.5 (2C), 26.9, 23.7, 14.6; HRESIMS *m/z* 482.3395 (calcd for $[C_{27}H_{50}NO_4P - H]^-$, 482.3405).

***N*-Benzyl-*N*,*N*-dimethyl-2-{[(tetradecyloxy)hydroxyphosphinyl]oxy}ethanaminum inner salt (3b):** white solid, yield 7%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.61 (d, 2H, J = 7.6 Hz, aromatic H), 7.54 (m, 3H, aromatic H), 4.64 (br s, 2H, *N*-C$\underline{H_2}$-Ph), 4.33 (br s, 2H, *N*-$CH_2C\underline{H_2}$-O), 3.88 (q, 2H, J = 6.4 Hz, -OCH_2-), 3.64 (m, 2H, *N*-C$\underline{H_2}CH_2$-O), 3.14 (s, 6H, 2×*N*-CH_3), 1.63 (m, 2H, -CH_2), 1.43–1.20 (m, 22H, 11×CH_2), 0.90 (t, 3H, J = 6.7 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 134.4 (2C), 131.9, 130.3 (2C), 128.8, 70.2, 66.9 (d), 65.3 (m), 60.1 (d), 51.2 (2C, d), 33.0, 31.9 (d), 31.8, 30.8 (6C), 30.5, 26.9, 23.7, 14.5; HRESIMS *m/z* 454.3090 (calcd for $[C_{25}H_{46}NO_4P - H]^-$, 454.3092).

***N*-Benzyl-*N*,*N*-dimethyl-2-{[(octadecyloxy)hydroxyphosphinyl]oxy}ethanaminum inner salt (3c):** white solid, yield 8%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.59 (d, 2H, J = 7.2 Hz, aromatic H), 7.52 (m, 3H, aromatic H), 4.61 (br s, 2H, *N*-C<u>H</u>$_2$-Ph), 4.30 (br s, 2H, *N*-CH_2C<u>H</u>$_2$-O), 3.84 (q, 2H, J = 6.4 Hz, -OCH_2-), 3.59 (m, 2H, *N*-C<u>H</u>$_2$$CH_2$-O), 3.10 (s, 6H, 2×*N*-$CH_3$), 1.59 (m, 2H, -$CH_2$), 1.40–1.20 (m, 30H, 15×CH_2), 0.87 (t, 3H, J = 6.7 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 134.4 (2C), 131.8, 130.3 (2C), 128.8, 70.2, 66.9 (d), 65.3 (d), 60.1 (d), 51.2 (2C), 33.1, 32.0, 31.9 (d), 31.0, 30.9 (9C), 30.5, 27.0, 23.8, 14.6; HRESIMS *m/z* 510.3708 (calcd for $[C_{29}H_{54}NO_4P - H]^-$, 510.3718).

***N*,*N*-Dimethyl-*N*-(4-nitrobenzyl)-2-{[(hexadecyloxy)hydroxyphosphinyl]oxy}ethanaminum inner salt (3d):** white solid, yield 9%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.37 (d, 2H, J = 8.7 Hz, aromatic H), 7.90 (d, 2H, J = 8.7 Hz, aromatic H), 4.79 (br s, 2H, *N*-C<u>H</u>$_2$-Ph), 4.35 (br s, 2H, *N*-CH_2C<u>H</u>$_2$-O), 3.89 (q, 2H, J = 6.7 Hz, -OCH_2-), 3.68 (m, 2H, *N*-C<u>H</u>$_2$$CH_2$-O), 3.19 (s, 6H, 2×*N*-$CH_3$), 1.63 (m, 2H, -$CH_2$), 1.44–1.15 (m, 26H, 13×CH_2), 0.90 (t, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 150.7, 135.8 (2C), 135.5, 125.0 (2C), 68.5, 67.0 (d), 66.0 (m), 60.2 (d), 52.0 (br s, 2C), 33.1, 31.9 (d), 30.9, 30.8 (7C), 30.6, 30.5, 27.0, 23.8, 14.5; HRESIMS *m/z* 527.3253 (calcd for $[C_{27}H_{49}N_2O_6P - H]^-$, 527.3255).

***N*-(4-Methoxybenzyl)-*N*,*N*-dimethyl-2-{[(hexadecyloxy)hydroxyphosphinyl]oxy}-ethanaminum inner salt (3e):** white solid, yield 7%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.50 (d, 2H, J = 8.6 Hz, aromatic H), 7.01 (d, 2H, J = 8.6 Hz, aromatic H), 4.56 (br s, 2H, *N*-C<u>H</u>$_2$-Ph), 4.33 (br s, 2H, *N*-CH_2C<u>H</u>$_2$-O), 3.87 (q, 2H, J = 6.5 Hz, -OCH_2-), 3.81 (s, 3H, OCH_3), 3.60 (m, 2H, *N*-C<u>H</u>$_2$$CH_2$-O), 3.08 (s, 6H, 2×*N*-$CH_3$), 1.59 (m, 2H, -$CH_2$), 1.41–1.15 (m, 26H, 13×CH_2), 0.87 (t, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 162.9, 135.8 (2C), 120.5, 115.5

(br s, 2C), 70.0, 67.3 (d), 64.7 (m), 60.4 (d), 55.9, 50.8 (2C), 33.1, 31.9 (d), 30.9 (6C), 30.8 (2C), 30.5, 26.9, 26.7, 23.8, 14.6; HRESIMS *m/z* 512.3508 (calcd for $[C_{28}H_{52}NO_5P - H]^-$, 512.3510).

***N*-(4-Chlorobenzyl)-*N*,*N*-dimethyl-2{[(hexadecyloxy)hydroxyphosphinyl]oxy}ethanaminum inner salt (3f):** white solid, yield 42%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.62 (d, 2H, J = 8.2 Hz, aromatic H), 7.49 (d, 2H, J = 8.2 Hz, aromatic H), 4.65 (br s, 2H, *N*-C<u>H</u>$_2$-Ph), 4.32 (br s, 2H, *N*-CH_2C<u>H</u>$_2$-O), 3.85 (q, 2H, J = 6.5 Hz, -OCH_2-), 3.64 (m, 2H, *N*-C<u>H</u>$_2$$CH_2$-O), 3.12 (s, 6H, 2×*N*-$CH_3$), 1.59 (m, 2H, -$CH_2$), 1.41–1.20 (m, 26H, 13×CH_2), 0.87 (t, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 137.9, 136.0 (2C), 130.3 (2C), 127.6, 69.0, 66.8 (d), 65.2 (d), 60.1 (d), 51.1 (2C), 33.1, 31.9 (d), 30.9 (4C), 30.8 (3C), 30.5 (2C), 26.9, 26.8, 23.8, 14.7; HRESIMS *m/z* 516.3013 (calcd for $[C_{27}H_{49}ClNO_4P - H]^-$, 516.3015).

***N*-(4-Bromobenzyl)-*N*,*N*-dimethyl-2-{[(hexadecyloxy)hydroxyphosphinyl]oxy}-ethanaminum inner salt (3g):** white solid, yield 12%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.63 (d, 2H, J = 8.1 Hz, aromatic H), 7.51 (d, 2H, J = 8.2 Hz, aromatic H), 4.58 (br s, 2H, *N*-C<u>H</u>$_2$-Ph), 4.27 (br s, 2H, *N*-CH_2C<u>H</u>$_2$-O), 3.78 (q, 2H, J = 6.5 Hz, -OCH_2-), 3.58 (m, 2H, *N*-C<u>H</u>$_2$$CH_2$-O), 3.04 (s, 6H, 2×*N*-$CH_3$), 1.56 (m, 2H, -$CH_2$), 1.41–1.15 (m, 26H, 13×CH_2), 0.84 (t, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 136.2 (2C), 133.5 (2C), 128.0, 126.4, 69.2, 66.9 (d), 65.3 (d), 60.1 (d), 51.2 (2C), 49.8, 49.6, 33.1, 31.9 (d), 30.8 (6C), 30.5 (2C), 27.0, 23.8, 14.6; HRESIMS *m/z* 560.2459 (calcd for $[C_{27}H_{49}BrNO_4P - H]^-$, 560.2510).

***N*,*N*-Dimethyl-*N*-[3-phenylprop-2(*E*)-en-1-yl]-2-{[(hexadecyloxy)hydroxyphosphinyl]oxy}-ethanaminum inner salt (3h):** white solid, yield 7%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (d, 2H, J = 7.0 Hz, aromatic H), 7.33 (m, 3H, aromatic H), 7.00 (d, 1H, J = 16.0 Hz, =C<u>H</u>-), 6.45

(dt, 1H, J = 16.0, 6.4 Hz, =C<u>H</u>-), 4.28 (br s, N-$CH_2C\underline{H}_2$-O), 4.20 (d, 2H, J = 6.4 Hz, =C-$C\underline{H}_2$), 3.86 (q, 2H, J = 6.4 Hz, -OCH_2-), 3.62 (m, 2H, N-$C\underline{H}_2CH_2$-O), 3.17 (s, 6H, 2×N-CH_3), 1.60 (m, 2H, -CH_2), 1.40–1.16 (m, 26H, 13×CH_2), 0.88 (t, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 144.5, 136.6, 130.3, 129.8 (3C), 128.4 (2C), 116.1, 68.8, 66.9 (d), 64.7 (m), 60.1 (d), 51.6 (2C), 33.1, 31.9 (d), 30.8 (7C), 30.5 (2C), 26.9, 23.7, 14.5; HRESIMS m/z 508.3550 (calcd for $[C_{29}H_{52}NO_4P - H]^-$, 508.3561).

***N*,*N*-Dimethyl-*N*-(2-propen-1-yl)-2-{[(hexadecyloxy)hydroxyphosphinyl]oxy}ethanaminum inner salt (3i):** white solid, yield 8%; ^{1}H NMR (400 MHz, CD_3OD) δ 6.09 (m, 1H, -C<u>H</u>=), 5.71 (m, 2H, =$C\underline{H}_2$), 4.30 (br s, N-$CH_2C\underline{H}_2$-O), 4.05 (br s, 2H, =CH-$C\underline{H}_2$), 3.89 (m, 2H, -OCH_2-), 3.61 (m, 2H, N-$C\underline{H}_2CH_2$-O), 3.14 (s, 6H, 2×N-CH_3), 1.62 (m, 2H, -CH_2), 1.40–1.16 (m, 26H, 13×CH_2), 0.87 (t, 3H, J = 7.0 Hz, CH_3);^{13}C NMR (100 MHz, CD_3OD) δ 129.7, 126.3, 68.8, 66.9, 64.9, 60.1, 51.7 (2C), 33.0, 31.9 (d), 31.8, 30.8 (5C), 30.7 (2C), 30.5, 30.4, 26.9, 23.7, 14.5; HRESIMS m/z 434.3312, (calcd for $[C_{23}H_{48}NO_4P + H]^+$, 434.3399).

2. *In vivo* antifungal efficacy studies

Ethics statement

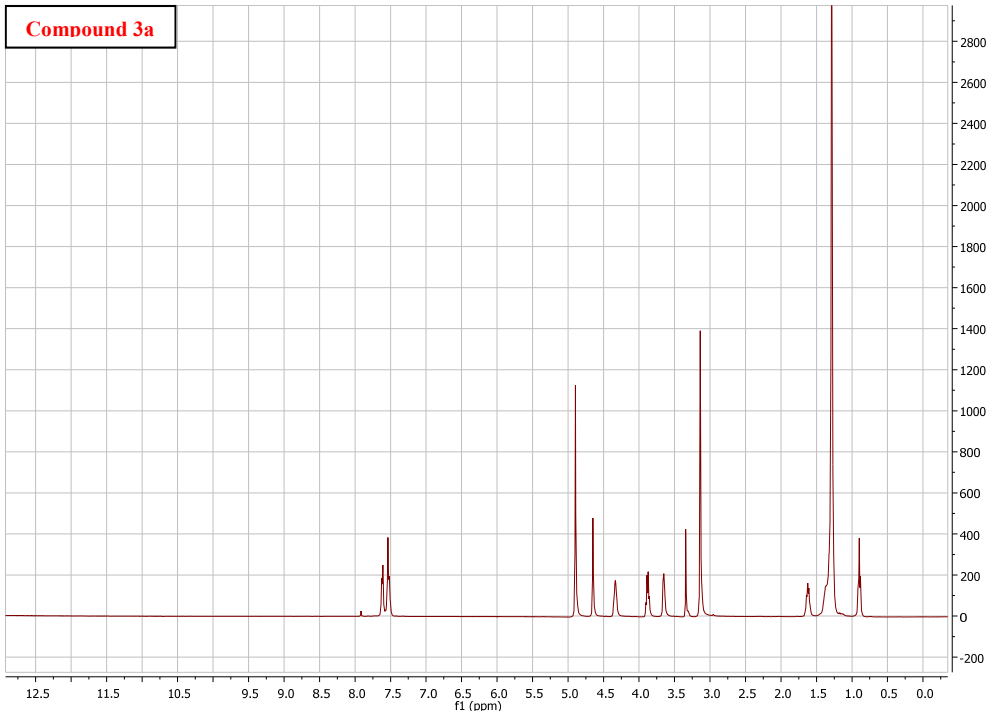
Animals studies were conducted in the Division of Laboratory Animal Resources (DLAR) facilities at Duke University Medical Center (DUMC) in good practice as defined by the United States Animal Welfare Act and in full compliance with the guidelines of the DUMC Institutional Animal Care and Use Committee (IACUC). The vertebrate experiments were reviewed and approved by the DUMC IACUC under protocol number A165-11-06.

Mouse infection and drug treatments

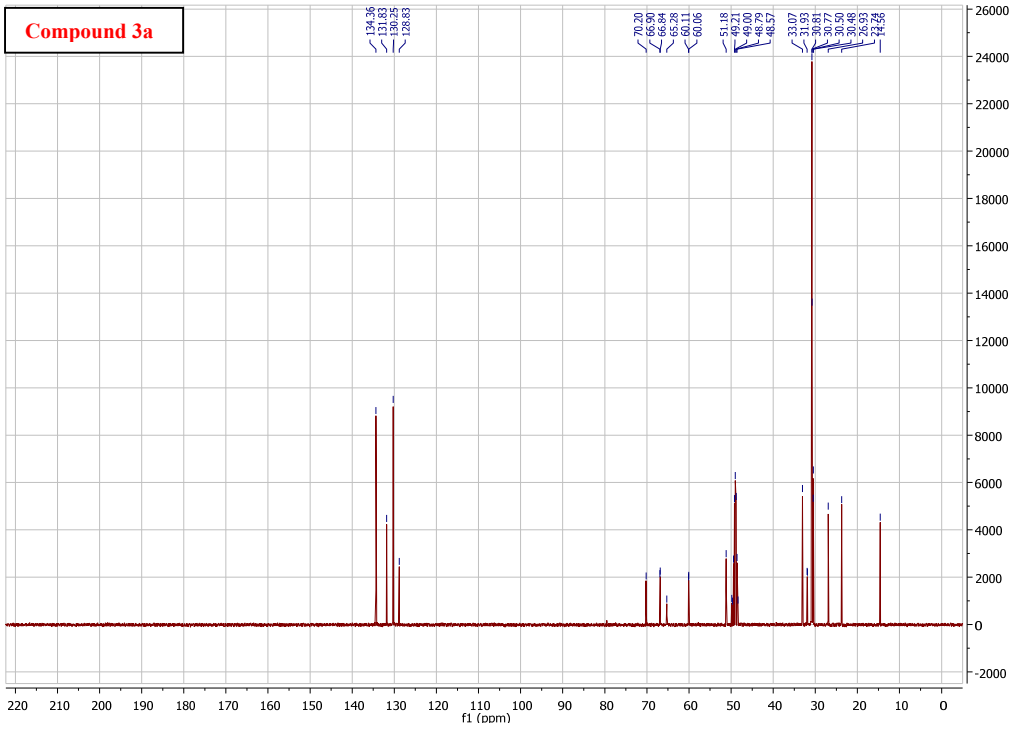
Six- to seven-week-old male CD1 mice (Jackson Laboratory, ~30 g) were used in this study. For infection, *C. albicans* strain SC5314 was cultured in YPD broth overnight at 30°C and washed twice with sterile PBS. Cells were counted with a hemocytometer, and resuspended in sterile PBS at $5x10^6$ cells per ml. Dilutions of the cells were plated onto YPD and incubated at 30°C for 48 h to determine CFU and viability. Groups of five mice were inoculated with *C. albicans* via tail-vein injection of 10^6 cells (in 200 µl). The placebo (PBS) or drug was administered via intraperitoneal or oral route after 4, 24, 48, 72, and 96 h infection. Survival was monitored 1 to 2 times daily, and moribund mice were euthanized with CO_2. Kaplan-Meier survival curves were generated with Prism 5.03 (GraphPad software, La Jolla, CA, USA), and *P* values were evaluated by a Log-rank (Mantel-Cox) test. A *P* value of <0.05 was considered significant.

3. ^{1}H and ^{13}C NMR spectra of compounds 3a-3i

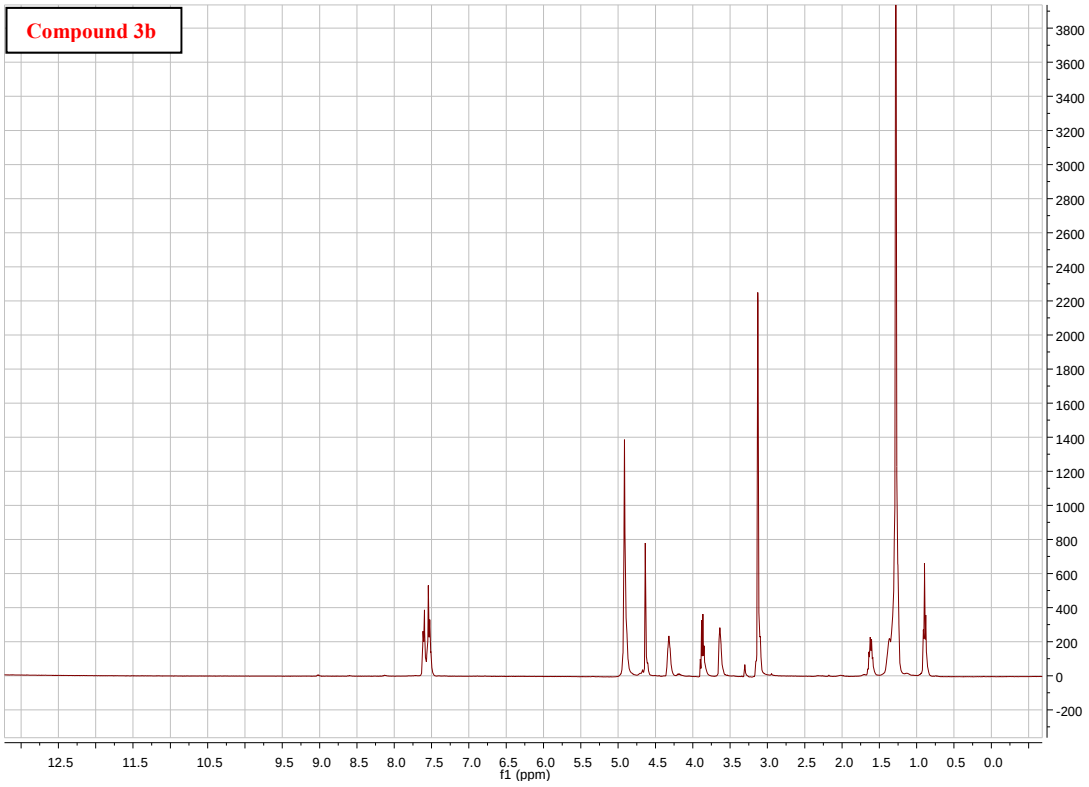
^{1}H NMR Spectrum of Compound 3a (400 MHz, CD_3OD)



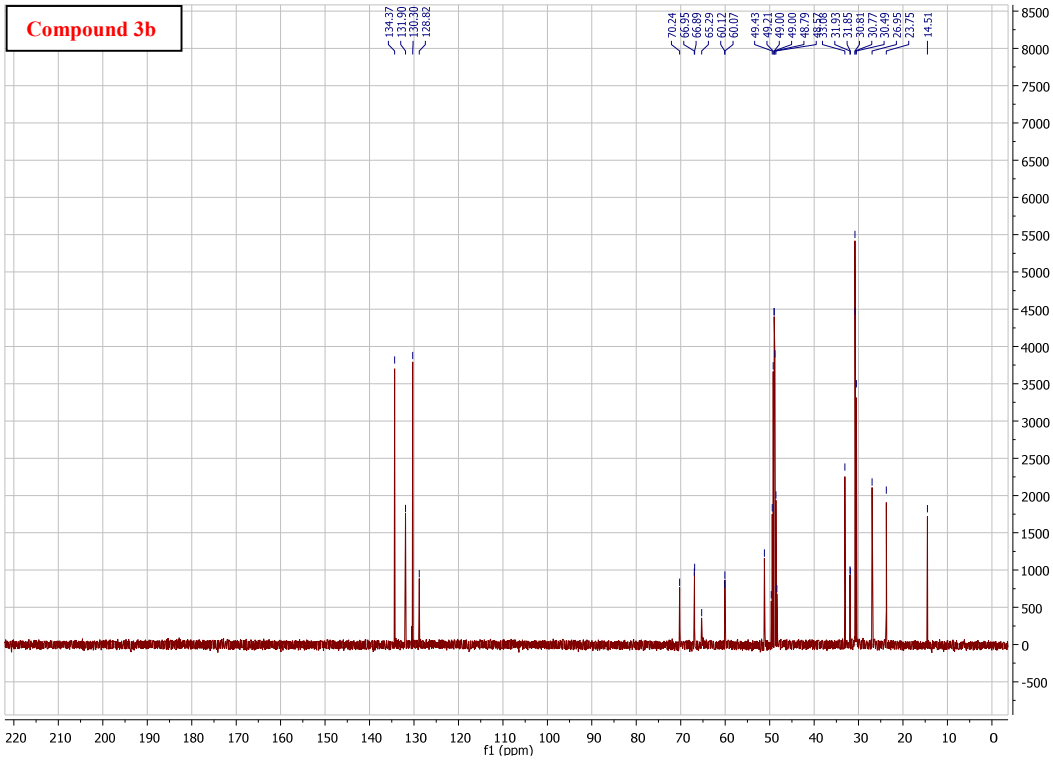
^{13}C NMR Spectrum of Compound 3a (400 MHz, CD_3OD)



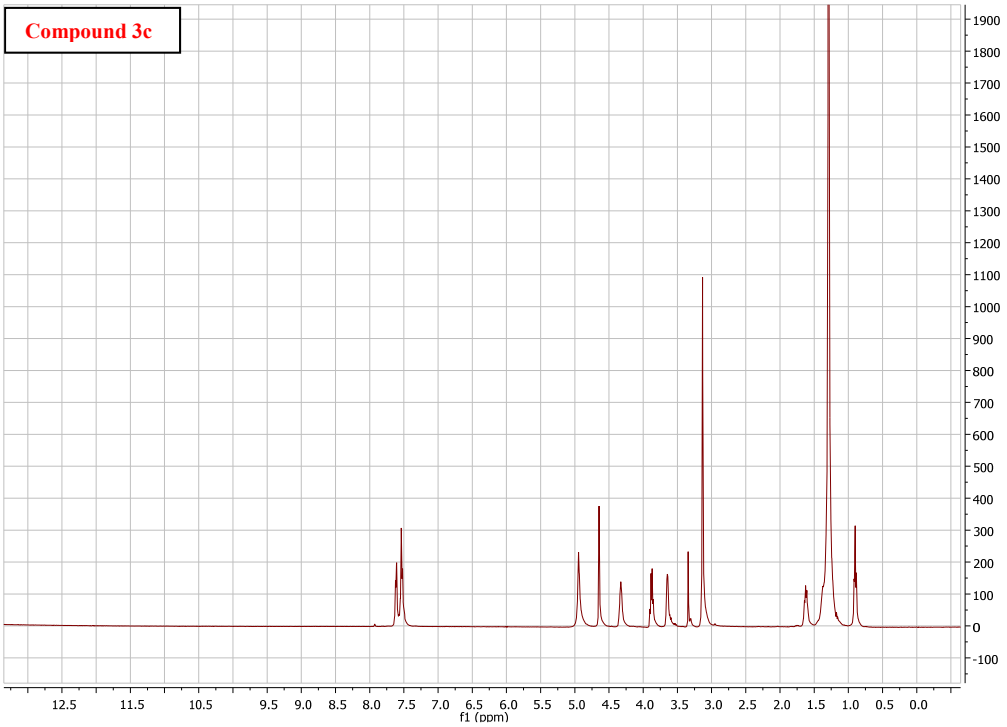
^{1}H NMR Spectrum of Compound 3b (400 MHz, CD_3OD)



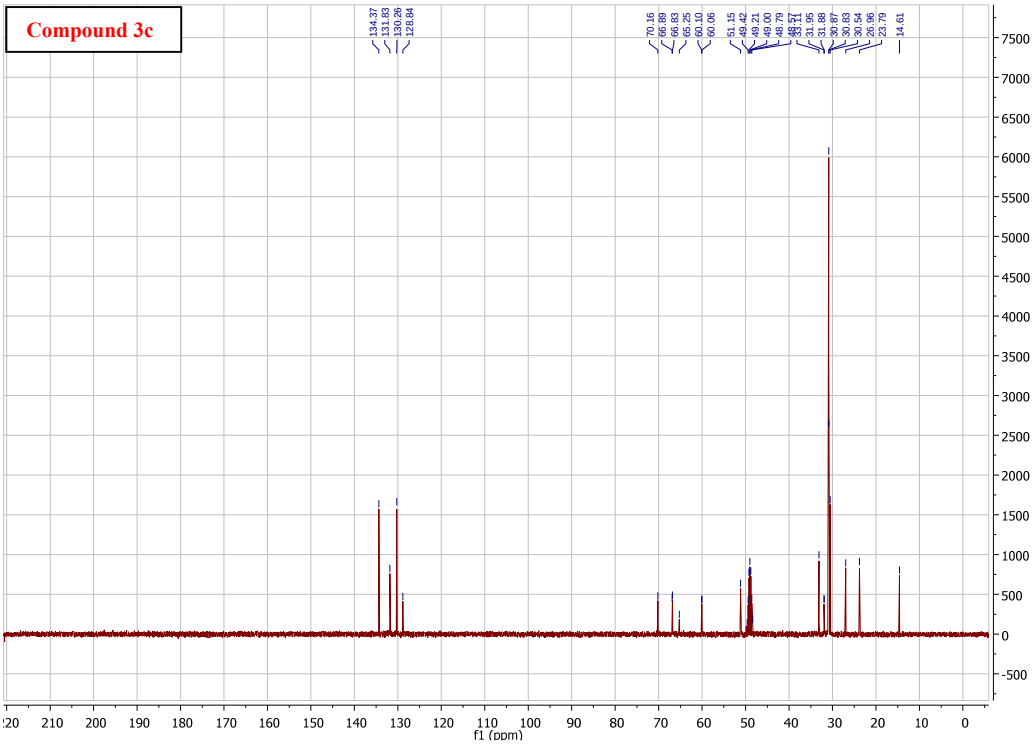
^{13}C NMR Spectrum of Compound 3b (400 MHz, CD_3OD)



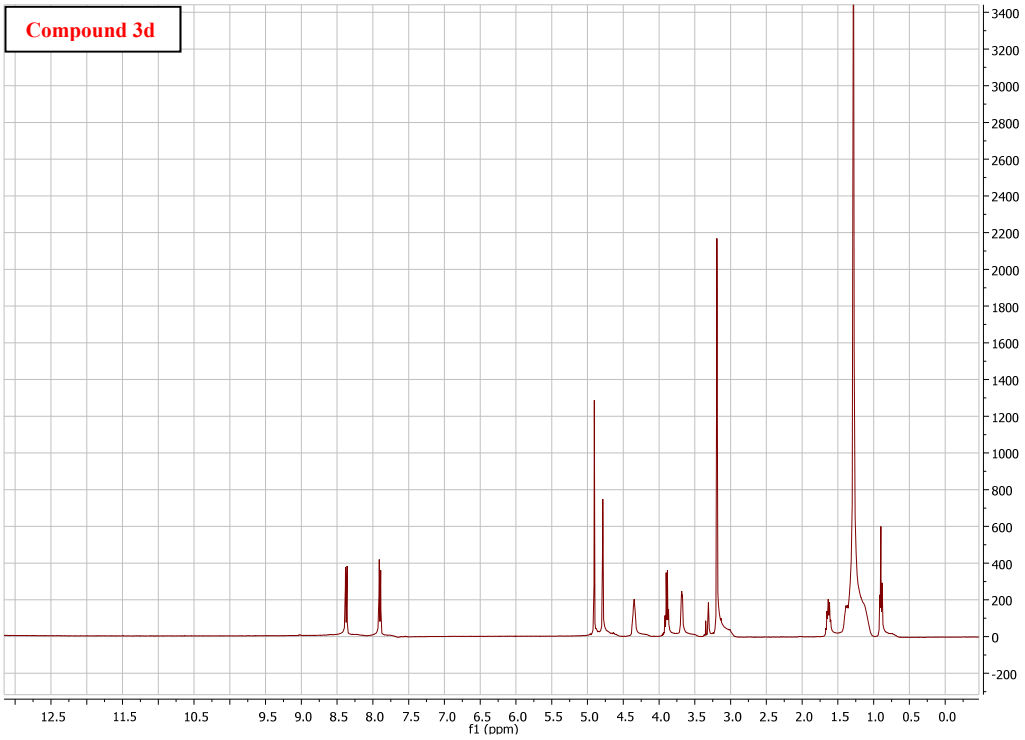
^{1}H NMR Spectrum of Compound 3c (400 MHz, CD_3OD)



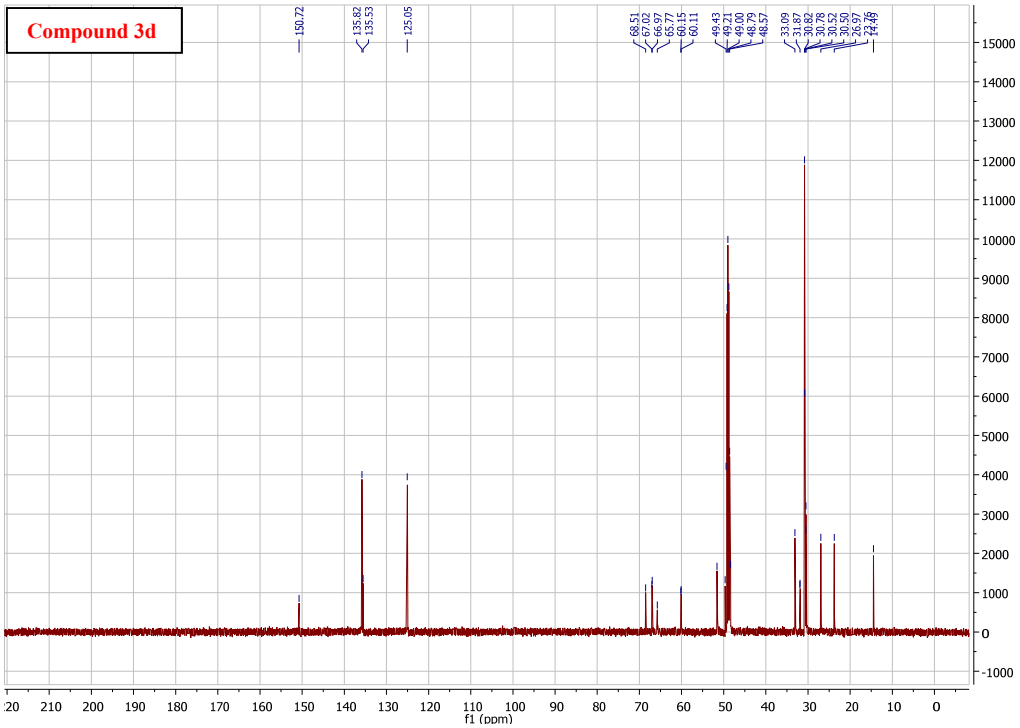
^{13}C NMR Spectrum of Compound 3c (400 MHz, CD_3OD)



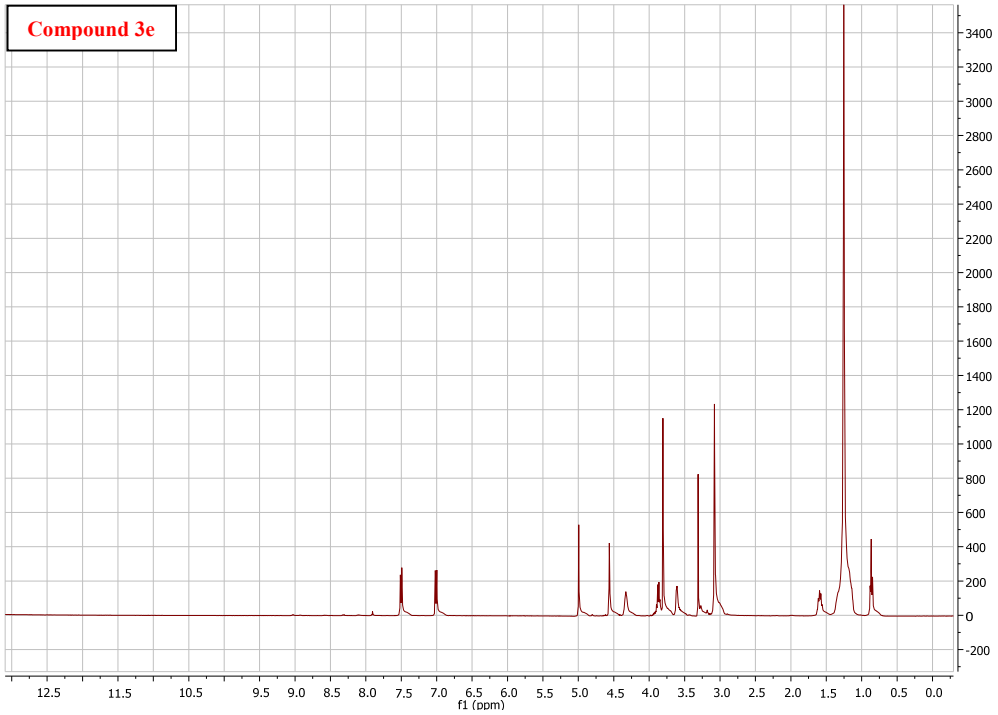
^{1}H NMR Spectrum of Compound 3d (400 MHz, CD_3OD)



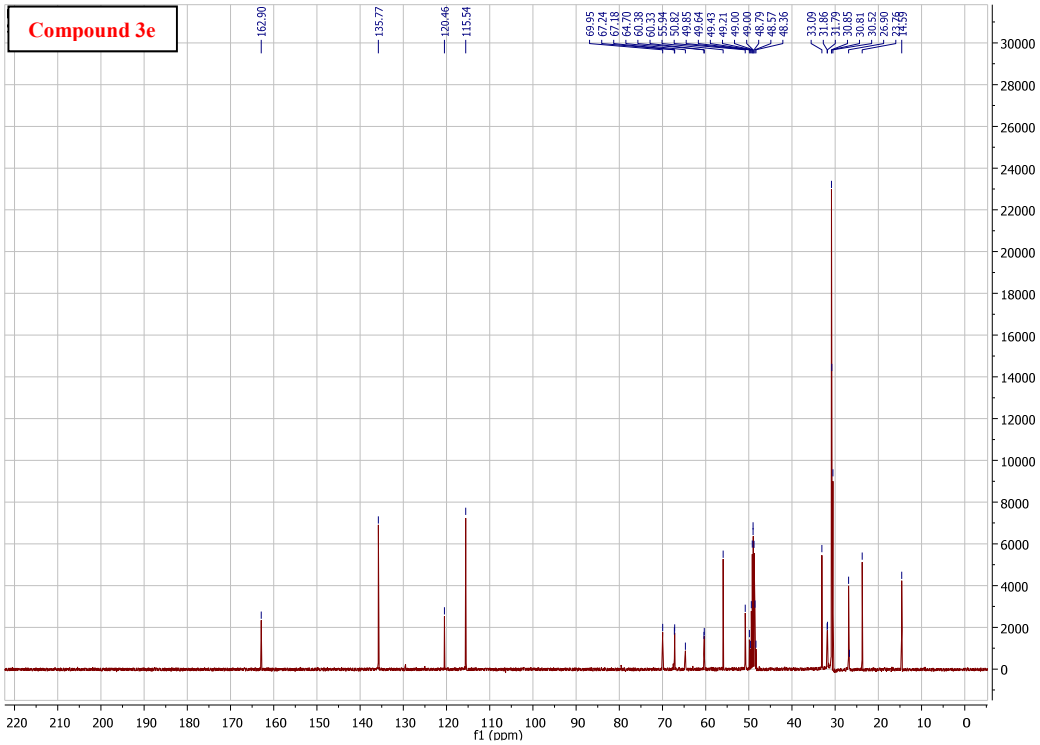
^{13}C NMR Spectrum of Compound 3d (400 MHz, CD_3OD)



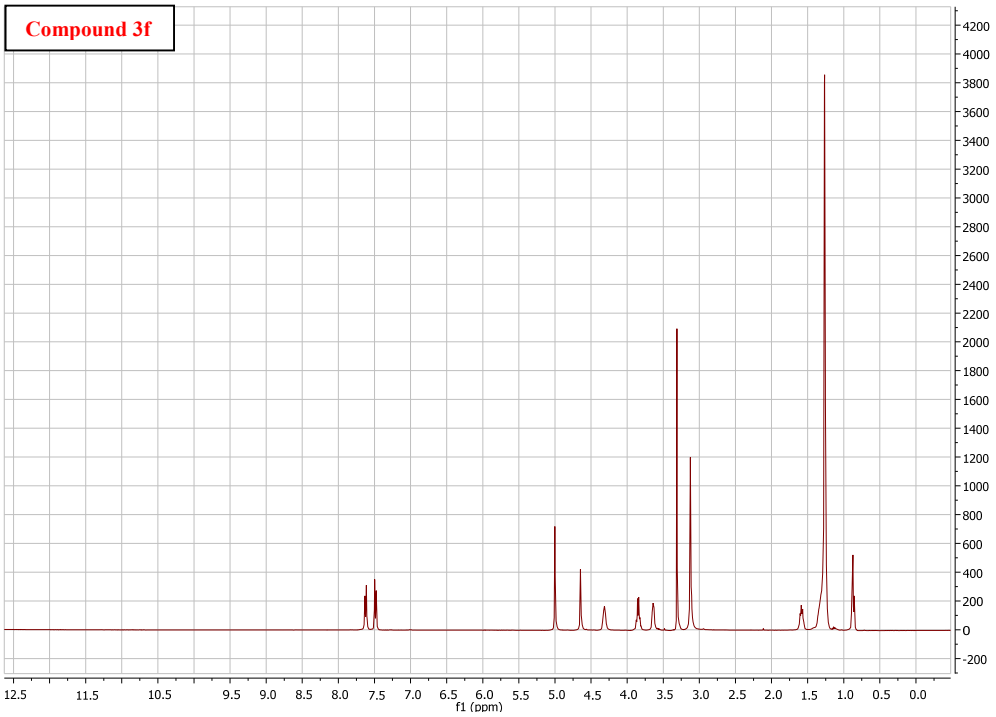
^{1}H NMR Spectrum of Compound 3e (400 MHz, CD_3OD)



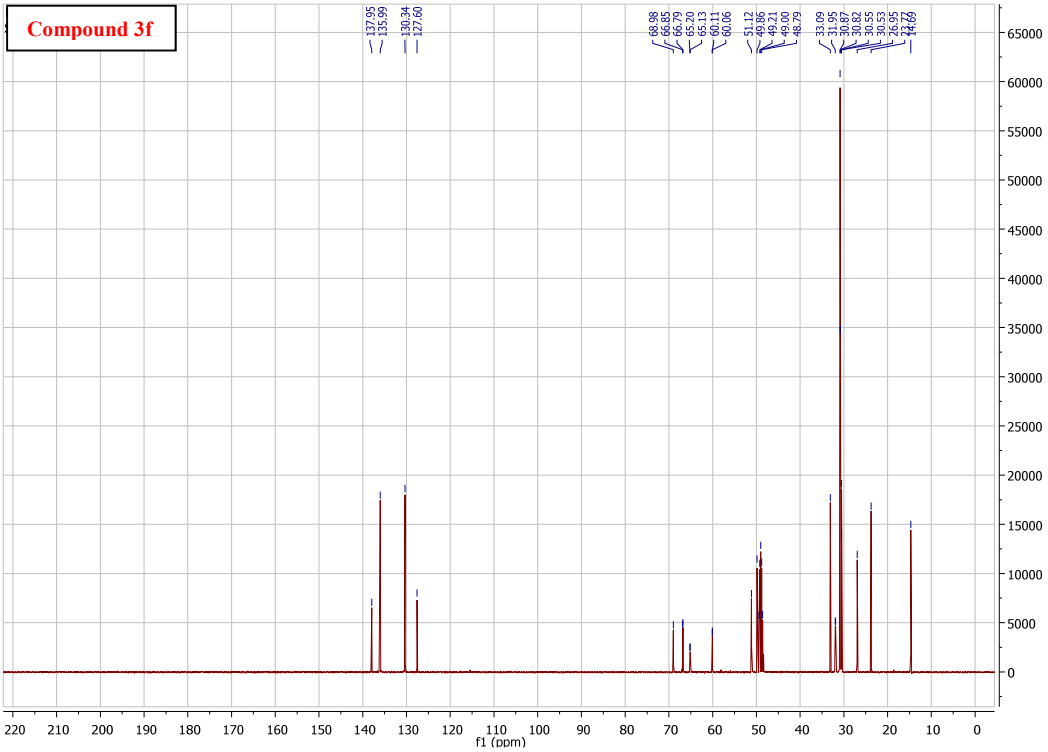
^{13}C NMR Spectrum of Compound 3e (400 MHz, CD_3OD)



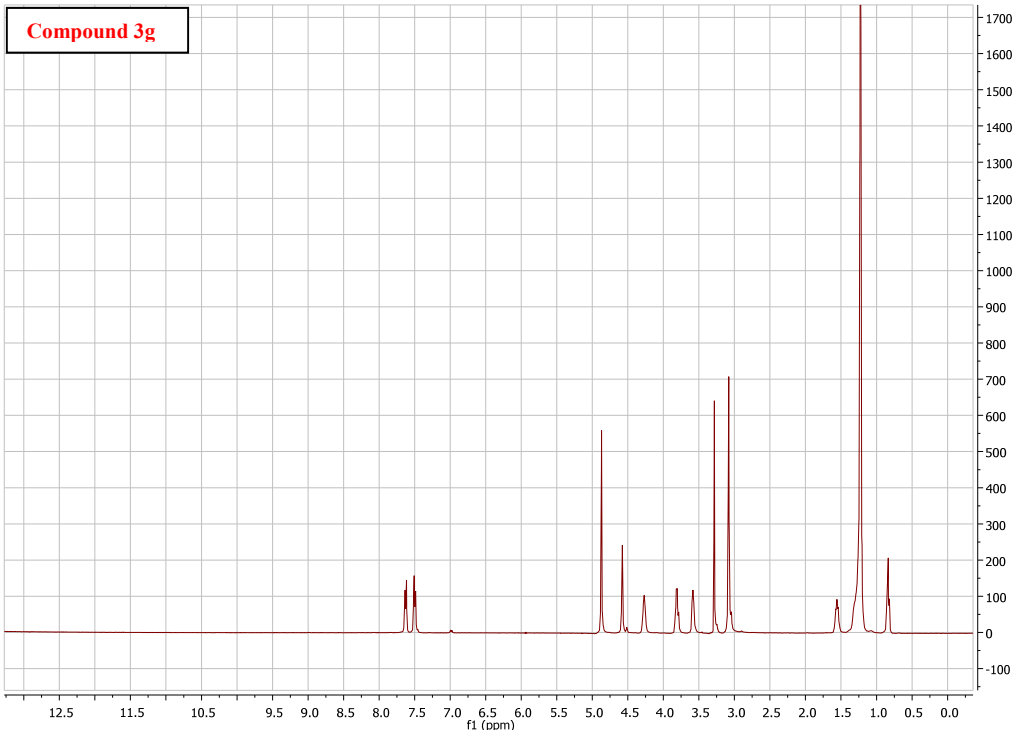
^{1}H NMR Spectrum of Compound 3f (400 MHz, CD_3OD)



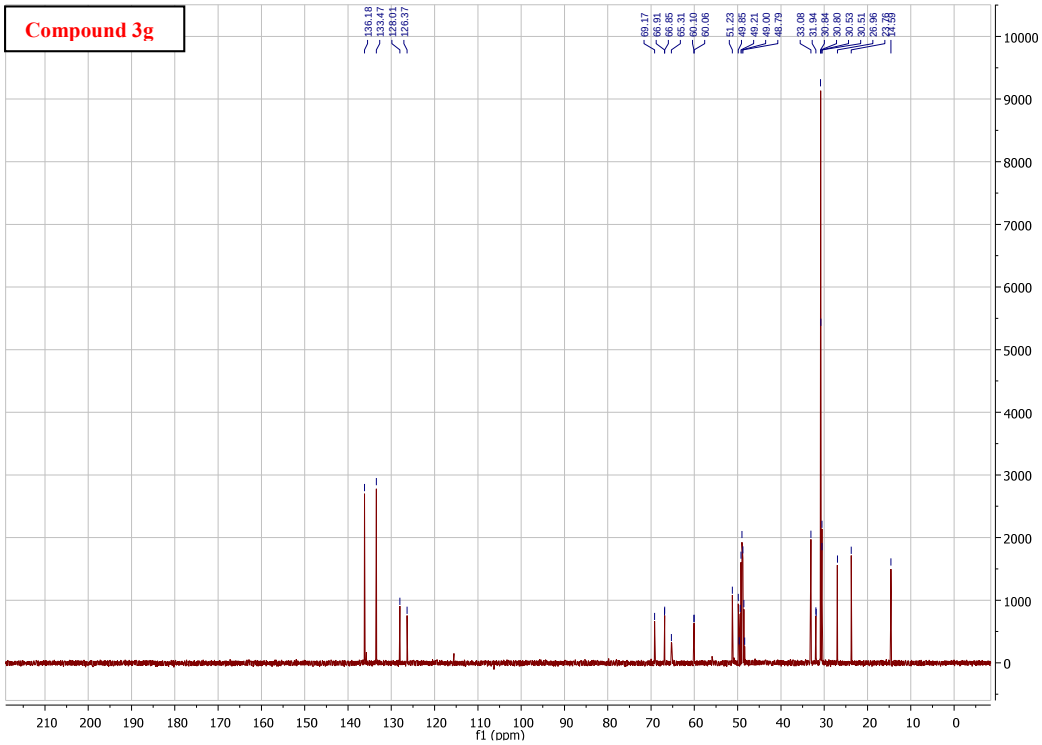
^{13}C NMR Spectrum of Compound 3f (400 MHz, CD_3OD)



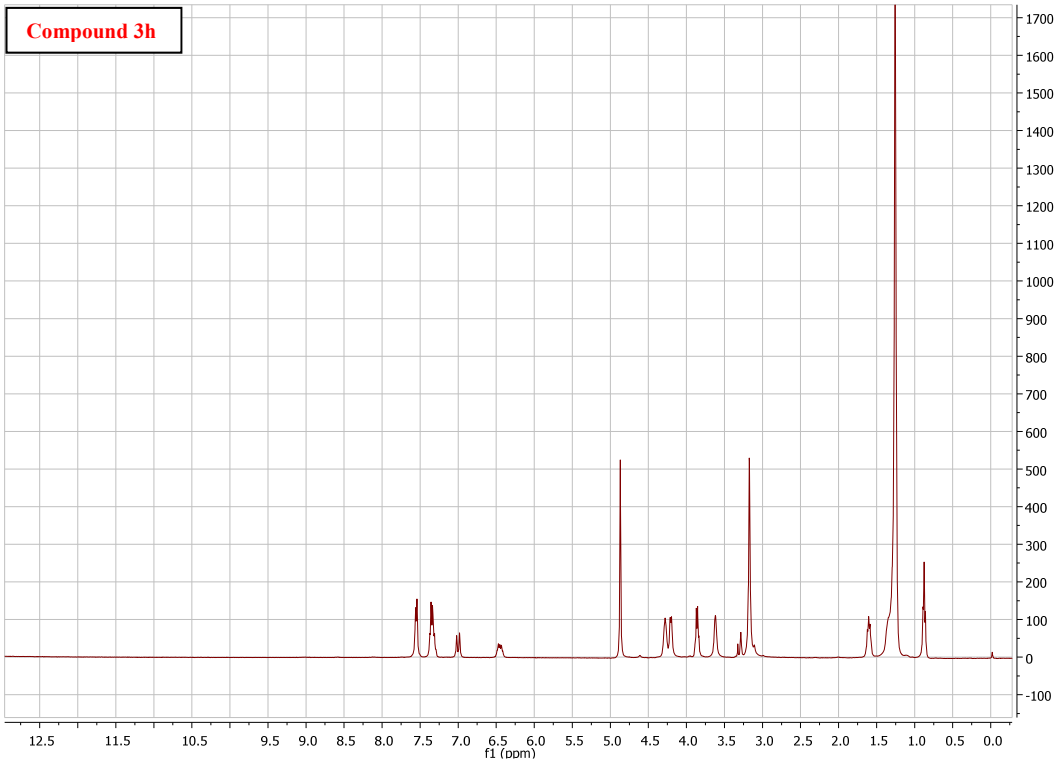
^{1}H NMR Spectrum of Compound 3g (400 MHz, CD_3OD)



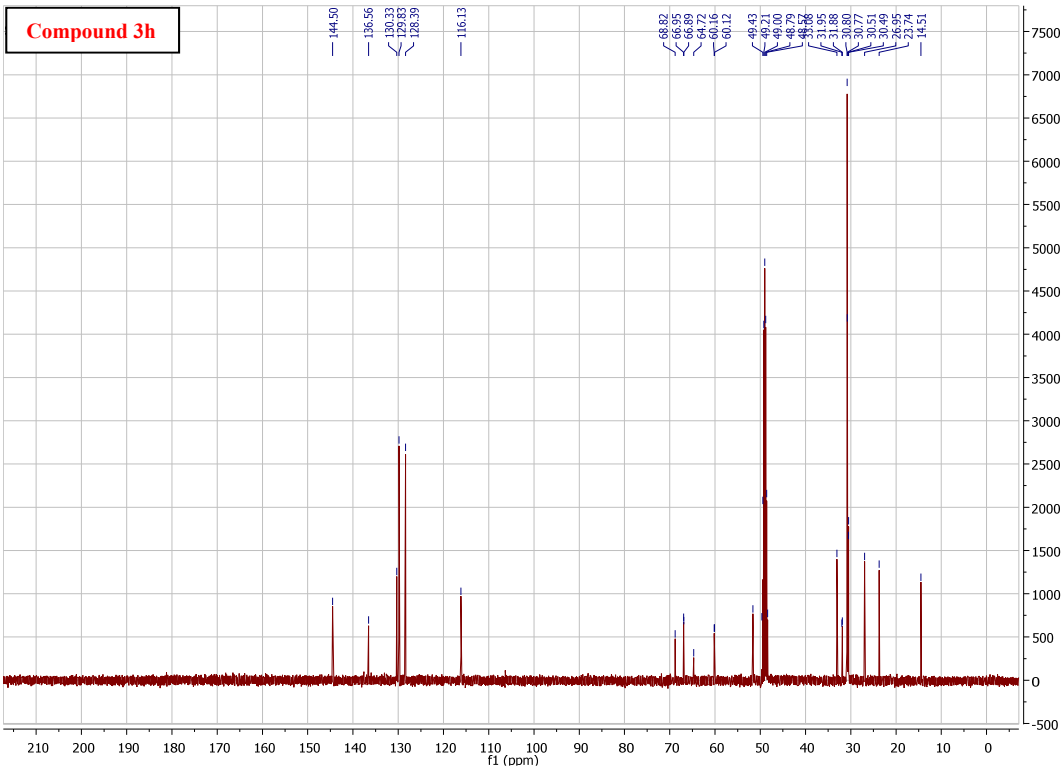
^{13}C NMR Spectrum of Compound 3g (400 MHz, CD_3OD)



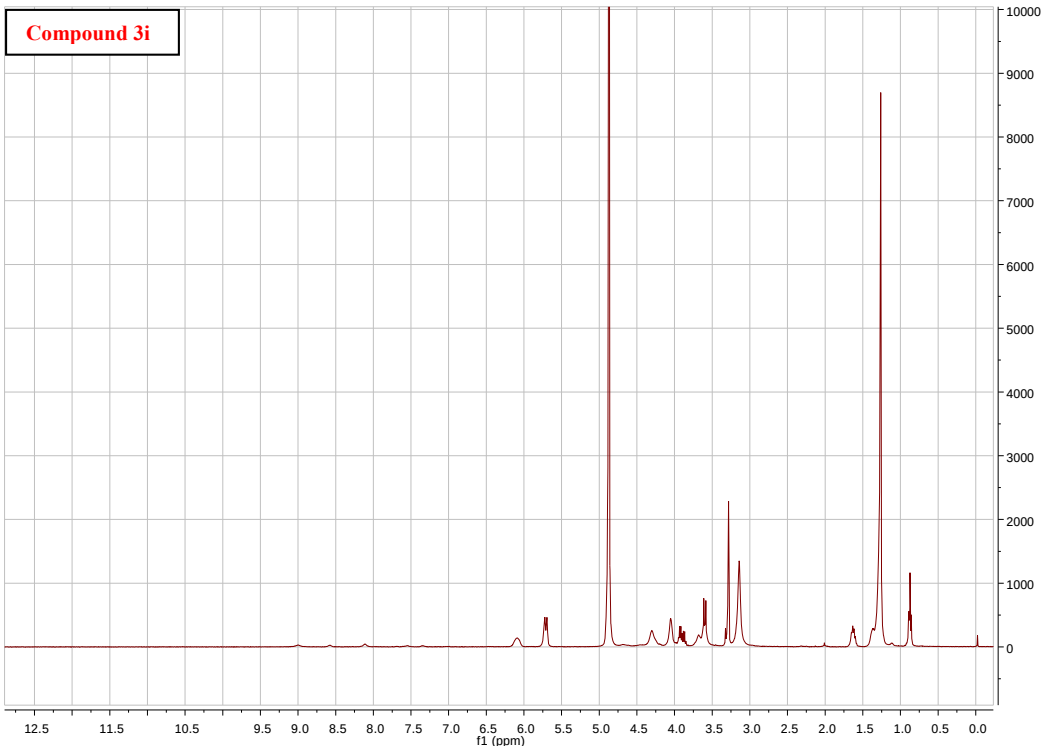
^{1}H NMR Spectrum of Compound 3h (400 MHz, CD_3OD)



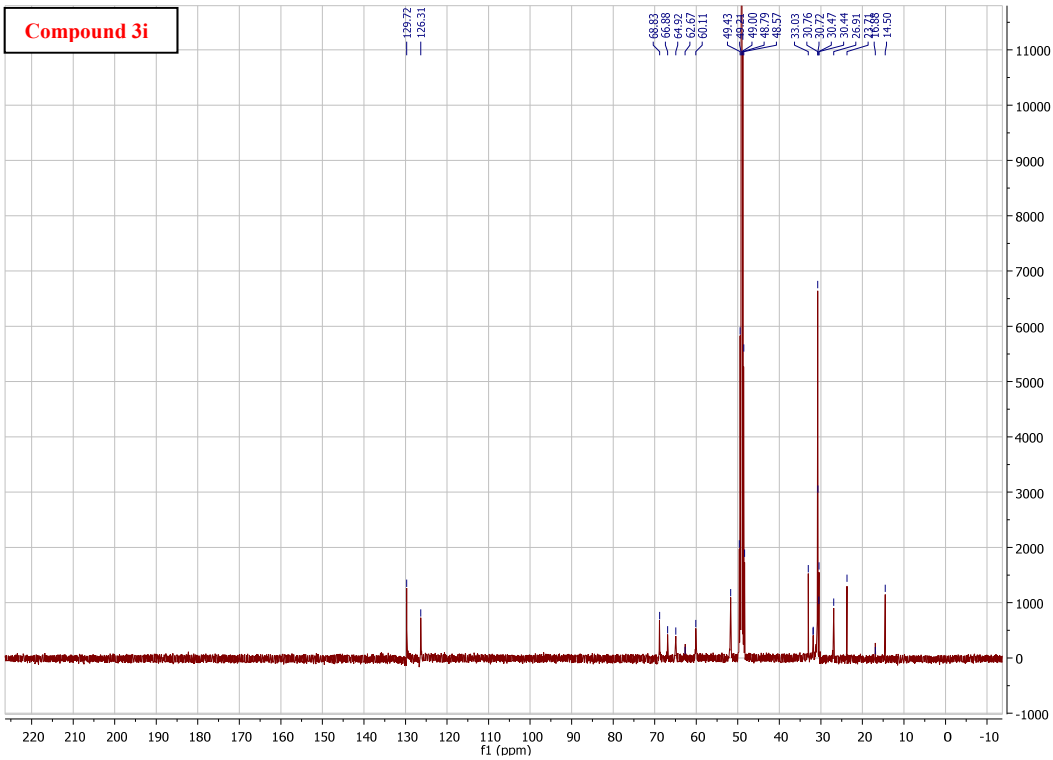
^{13}C NMR Spectrum of Compound 3h (400 MHz, CD_3OD)



^{1}H NMR Spectrum of Compound 3i (400 MHz, CD_3OD)

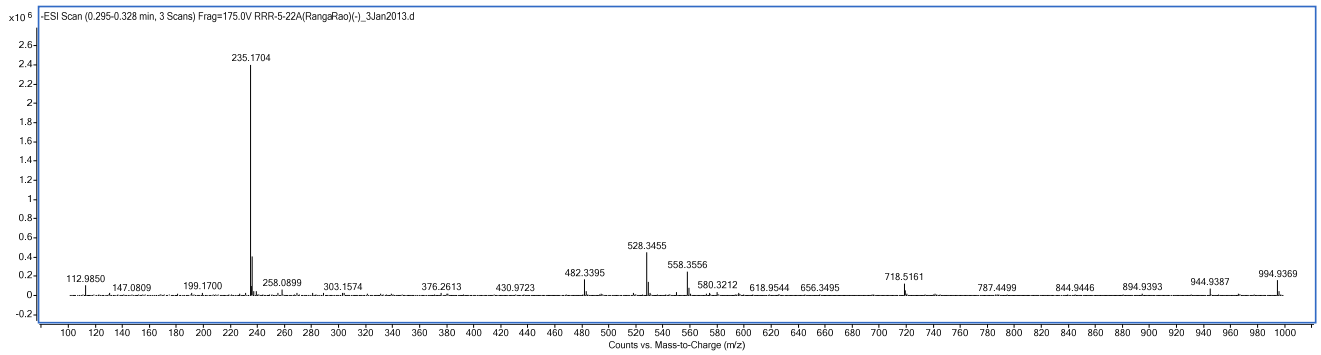


^{13}C NMR Spectrum of Compound 3i (400 MHz, CD_3OD)

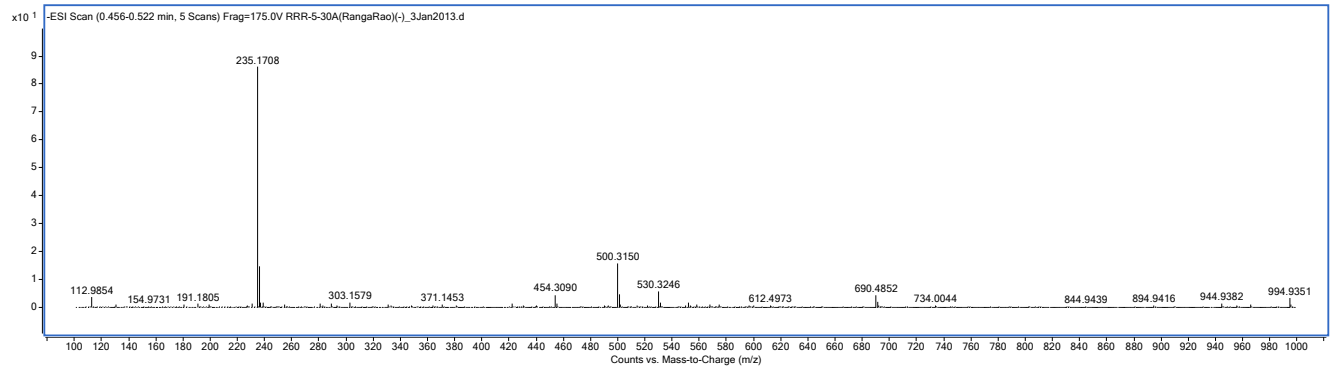


4. HRESIMS data of compounds 3a-3h.

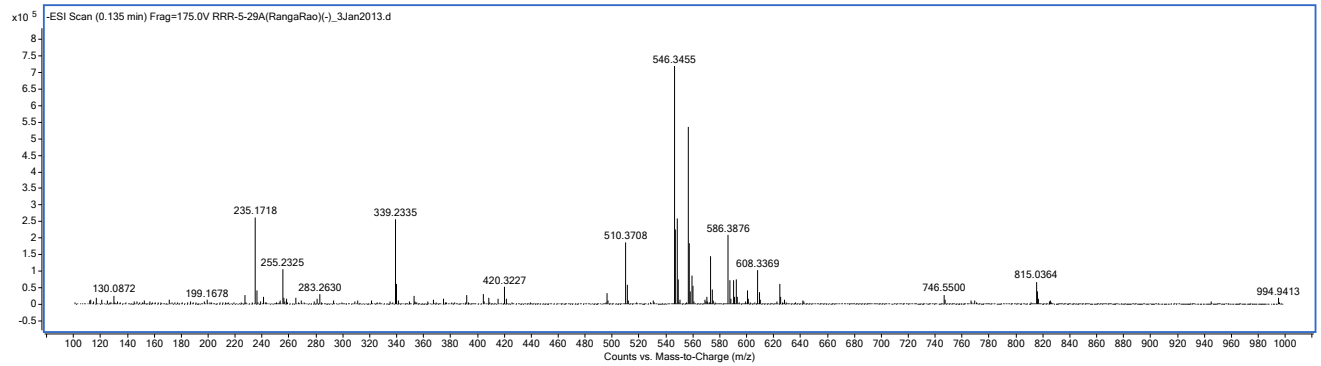
(–Ve) HRESIMS Spectrum of Compound 3a



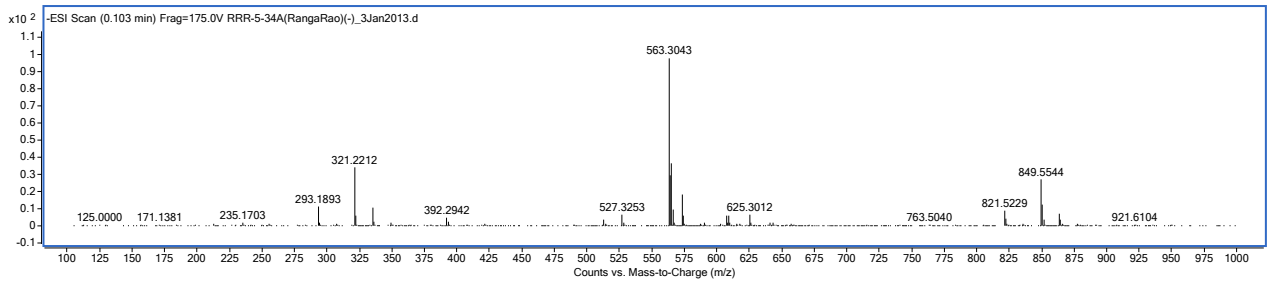
(–Ve) HRESIMS Spectrum of Compound 3b



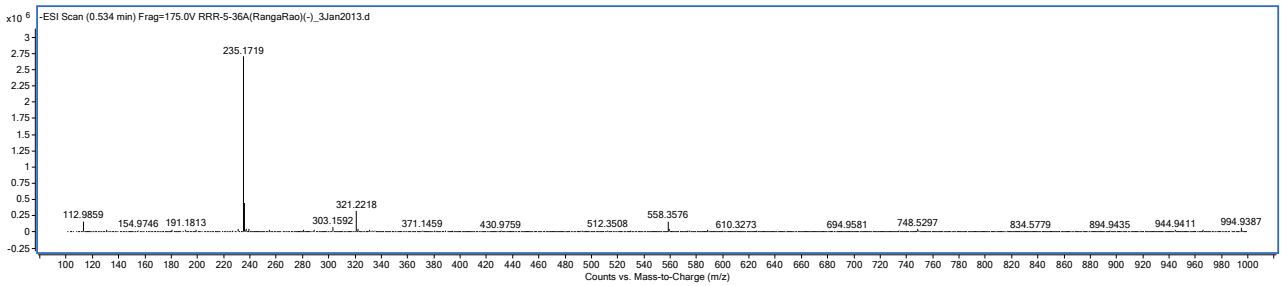
(–Ve) HRESIMS Spectrum of Compound 3c



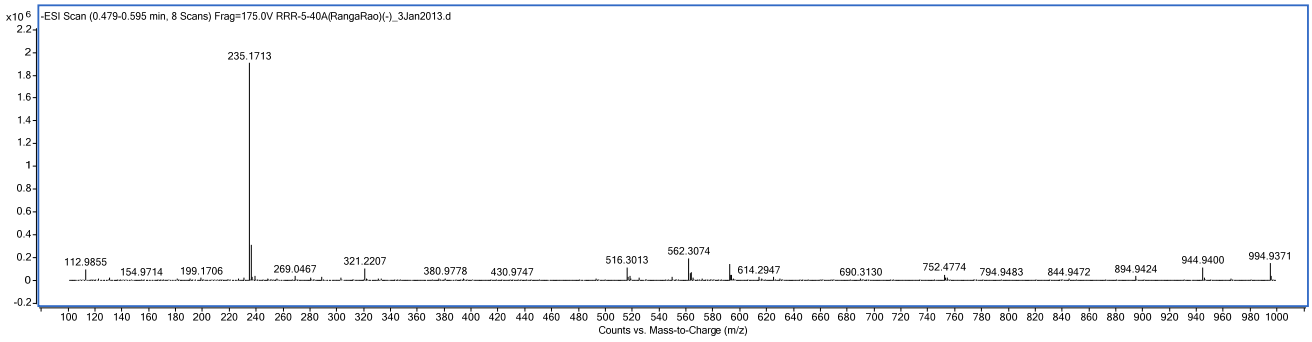
(–Ve) HRESIMS Spectrum of Compound 3d



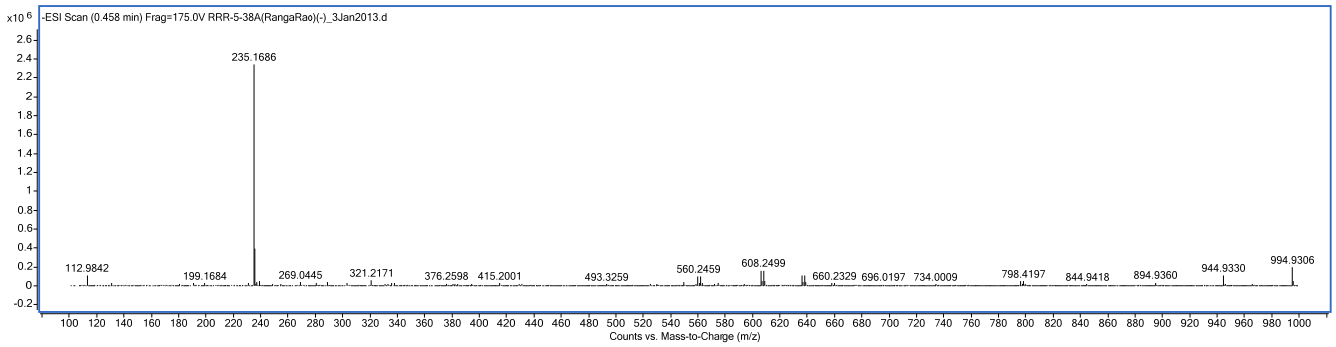
(–Ve) HRESIMS Spectrum of Compound 3e



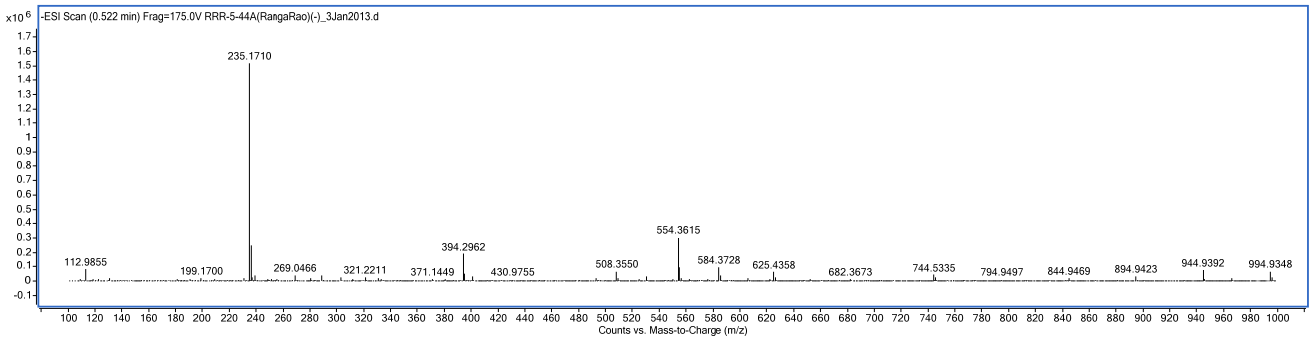
(–Ve) HRESIMS Spectrum of Compound 3f



(–Ve) HRESIMS Spectrum of Compound 3g



(–Ve) HRESIMS Spectrum of Compound 3h



(+Ve) HRESIMS Spectrum of Compound 3i

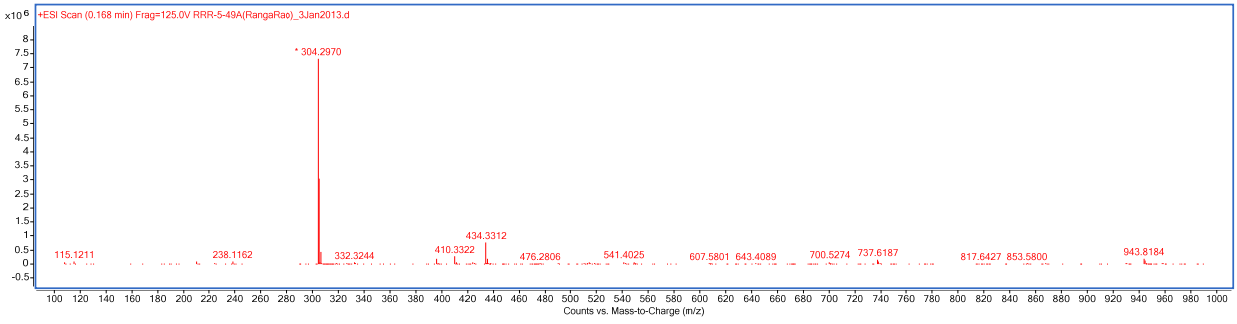


Table S1

In vitro antifungal activity of compounds **3a-3i** (MIC/MFC, μg/mL) [a]

	C. albicans ATCC 90028	*C. glabrata* ATCC 90030	*C. krusei* ATCC 6258	*A. fumigatus* ATCC 90906	*C. neoformans* ATCC 90113
Miltefosine	2.5±0/2.5±0	3.3±1.4/4.2±1.4	2.5±0/3.3±1.4	2.9±1.9/9.2±9.5	2.1±0.7/2.1±0.7
3a	2.5±0/2.5±0	3.3±1.4/3.3±1.4	5.0±0/15.0±8.7	2.5±0/2.5±0	3.3±1.4/3.3±1.4
3b	6.7±2.9/8.3±2.9	8.3±2.9/8.3±2.9	10.0±0/10.0±0	10.0±0/–	3.3±1.4/3.3±1.4
3c	–/–[b]	4.2±1.4/4.2±1.4	–/–	–/–	–/–
3d	2.5±0/2.5±0	2.5±0/2.5±0	2.5±0/3.3±1.4	2.5±0/7.5±3.5	2.5±0/2.5±0
3e	4.2±1.4/4.2±1.4	2.5±0/3.3±1.4	2.0±0.7/3.3	2.5±0/7.5±3.5	4.2±1.4/4.2±1.4
3f	–/–	11.2/11.2	–/–	12.5/12.5	–/–
3g	–/–	10.8±8.8/10.8±8.8	–/–	20.0±0/20.0±0	–/–
3h	–/–	2.5±0/3.3±1.4	3.3±1.4/3.3±1.4	2.5±0/5.0±4.3	–/–
3i	–/–	16.6±5.8/16.6±5.8	–/–	–/–	–/–
Amphotericin B	0.9±0.5/1.0±0.4	1.3±1.1/1.5±1.0	1.7±0.7/1.7±0.7	2.5±0/5.0±0	0.5±0.2/0.5±0.2

[a] Mean values with standard deviations based on three independent experiments except for compound **3f** with mean values from two independent experiments. MIC: minimum inhibitory concentration (lowest concentration that allows no detectable growth). MFC: minimum fungicidal concentration (the lowest concentration that kills the fungus), which was determined by removing 5 μL from each assay well with no visible growth, transferring to fresh media and incubating at the appropriate temperature for 2-3 days. The highest test concentration for compounds **3a-3i** and miltefosine was 20 μg/mL; the highest test concentration for amphotericin B was 5 μg/mL. [b] Not active at 20 μg/mL.

Table S2

In vitro cytotoxicity of compounds **3a**-**3i** (IC_{50}, μg/mL) [a]

	Vero	HepG2	LLC-PK_{11}
Miltefosine	>25	>25	2.7±1.3
3a	>25	>25	4.8±0.7
3b	>25	>25	10.8±2.4
3c	>25	13±4.1	4.7±0.4
3d	>25	11.5±3.3	1.6±0.1
3e	>25	12.9±0.9	1.9±0.4
3f	>25	21±3.2	3.2±0.5
3g	>25	21.3±3.6	5.5±1.0
3h	>25	19.6±1.8	2.1±0.6
3i	>25	13.2±2.9	4.1±0.1
Doxorubicin	>5	0.9±0.1	0.7±0.1

[a] Mean values with standard deviations based on three independent experiments. IC_{50}: 50% growth inhibition. The highest test concentration for compounds **3a-3i** and miltefosine was 25 μg/mL; the highest test concentration for doxorubicin was 5 μg/mL.