Supplementary data

For

Identification of an indole-triazole-amino acid conjugate as highly effective antifungal agent

Kalpana Pawar[a,b#], Anshuman Yadav[a#], Parteek Prasher[c], Sahil Mishra[c], Balwinder Singh[b], Palwinder Singh[c]* and Sneha Sudha Komath[a*]

[a] Jawaharlal Nehru University, New Delhi- 110 067 India

[b] Uttarakhand Technical University, Dehradun- 248 002 India

[c] Department of Chemistry, Guru Nanak Dev University, Amritsar-143005 India

[#] Equal contributing authors

*To whom correspondence may be addressed (palwinder_singh_2000@yahoo.com; sskomath@mail.jnu.ac.in; sskomath@yahoo.com)

1. Experimental

1.1 Chemistry

All reactions were performed in oven-dried glassware with magnetic stirring. Diethyl ether was distilled over activated anhydrous calcium chloride and further dried by passing sodium wire. Acetonitrile was dried by refluxing over anhydrous P_2O_5, anhydrous K_2CO_3 and stored over activated 4 Å molecular sieves. The reactions were monitored by thin-layer chromatography using silica gel GF254; visualization of the developed chromatogram was performed by UV and staining with iodine. Column chromatography was performed with silica gel of 100-200 mesh using hexane and ethyl acetate as eluents.

1.1.1 ^{1}H, ^{13}C NMR and DEPT.

The spectra were recorded at 500 MHz and 125 MHz NMR spectrometer using $CDCl_3$ and DMSO as solvents and TMS as internal standard. Data for ^{1}H NMR spectra are reported as chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd =

double doublet, br s = broad singlet) and coupling constant (*J* in Hz). Data for ^{13}C and DEPT-135 NMR spectra are given in terms of chemical shift; +ve signals correspond to CH_3 and CH carbons, -ve signals correspond to CH_2 carbons while absent means a quaternary carbon. Infrared spectra were examined in KBr pallets using SP 300 PYE UNI CAM Infrared Spectrophotometer.

1.1.2 Mass spectra

Mass spectra were recorded on Bruker MicroTOF QII mass spectrometer (Bruker Daltonik, Bremen, Germany). Machine was calibrated with sodium formate. Using KdScientific automated pump with flow rate 180 µL/h, 50 µM solution in acetonitrile-water-formic acid (7:2.9:0.1) was injected to electrospray ionization source. Desolvation was performed with dry N_2 gas heated at 180 ^oC. Various parameters of the mass spectrometer were optimized for maximum ion abundance. Typically, the capillary voltage was 4500 V and vacuum was maintained at 3-4x10^{-7} mbar. Sodium formate was used as internal calibrant.

[2-(1*H*-Indol-3-yl)-2-oxo-acetylamino]-acetic acid methyl ester (3a).

Cream colored solid, mp 167 ^oC, 95%, IR (KBr, cm^{-1}): 3487 (NH), 2857 (C-H), 1750 (C=O), 1681 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.40 (s, 1H, NH), 8.35 (s, 1H, ArH), 7.82-8.01 (m, 2H, ArH), 7.39-7.43 (m, 2H, ArH), 4.20 (d, *J*=1.5 Hz, 2H, CH_2), 3.83 (s, 3H, OCH_3); δ_C (normal/DEPT- 135) (125 MHz, $CDCl_3$): 180.63 (C=O), 169.30 (C=O), 161.30 (C=O), 138.48 (CH), 134.43 (C), 130.27 (CH), 128.04 (C), 125.98 (CH), 125.17 (CH), 122.79 (CH), 52.74 (OCH_3), 41.09 (-ve, CH_2); ESI-MS (HRMS) calcd for $C_{13}H_{12}N_2O_4Na$ 283.0689. Found *m/z* 283.0670.

3-(1*H*-Indol-3-yl)-2-[2-(1*H*-indol-3-yl)-2-oxo-acetylamino]-propionic acid methyl ester (3b).

White solid, mp 146 ^oC, 89%, IR (KBr, cm^{-1}): 3307 (NH), 2987 (C-H), 1759 (C=O), 1681 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, 1H, NH), 7.32-7.50 (m, 4H, ArH), 7.16-7.28

(m, 5H, ArH), 6.70 (d, 1H, *J*=3.5 Hz), 4.51 (t, 1H, *J*=10 Hz, CH), 3.88 (s, 3H, OCH_3), 3.58-3.63 (dd, *J*=1 Hz, *J*=13.5 Hz, 1H, CH_2), 3.44-3.47 (dd, *J*=7.5 Hz, 16 Hz, 1H, CH_2); δ_C (normal/DEPT-135) (125 MHz, $CDCl_3$): 169.56 (C=O), 161.41 (C=O), 160.72 (C=O), 145.40 (C), 136.53 (C), 134.73 (C), 134.63 (C), 130.72 (C), 129.98 (CH), 126.75 (C), 126.11 (CH), 126.10 (C), 124.74 (CH), 123.53 (CH), 123.23 (CH), 121.51 (CH), 120.45 (CH), 117.84 (CH), 117.61 (CH), ESI-MS (HRMS) calcd for $C_{22}H_{19}N_3O_4$ 390.1448. Found *m/z* 390.1444 ($[M+H]^+$).

3-(3*H*-Imidazol-4-yl)-2-[2-(1*H*-indol-3-yl)-2-oxo-acetylamino]-propionic acid methyl ester (3c).

Yellow solid, mp 118 ^oC, 90%, IR (KBr, cm^{-1}): 3537 (NH), 2857 (C-H), 1750 (C=O), 1688 (C=O) cm^{-1}; ^1H NMR (500 MHz, DMSO d_6): δ 9.10 (s, 1H, NH), 7.58 (m, 1H, ArH), 7.33-7.39 (m, 2H, ArH), 7.28-7.93 (m, 2H, ArH), 6.83-7.28 (m, 3H, ArH), 6.82 (s, 1H, ArH), 4.46 (t, 1H, *J*=3.5 Hz), 3.77 (s, 1H, OCH_3), 3.33 (d, *J*=6.5 Hz, 2H, CH_2); δ_C (normal/DEPT- 135) (125 MHz, DMSO-d_6): 169.07 (C=O), 134.52 (CH), 134.49 (C), 130.98 (C), 130.65 (CH), 127.39 (CH), 127.15 (CH), 127.12 (C), 125.10 (CH), 123.88 (CH), 122.02 (CH), 118.57 (CH), 113.53 (CH), 109.89 (CH), 53.51 (OCH_3), 51.51 (CH), 25.56 (-ve, CH_2); ESI-MS (HRMS) calcd for $C_{17}H_{16}N_4O_4$ 341.1244. Found *m/z* 341.1241 ($[M+H]^+$).

2-[2-(1*H*-Indol-3-yl)-2-oxo-acetylamino]-pentanedioic acid dimethyl ester (3d).

Yellow solid, mp 143 ^oC, 85%, IR (KBr, cm^{-1}): 3477 (NH), 2977 (C-H), 1755 (C=O), 1666 (C=O) cm^{-1}; ^1H NMR (500 MHz $CDCl_3$): δ 7.99 (s, 1H, NH), 7.53-7.84 (m, 1H, ArH), 7.32 (s, 1H, ArH), 7.23-7.28 (m, 2H, ArH), 7.22 (s, 1H, ArH), 4.33 (t, 1H, *J*=5 Hz, CH), 3.78 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 2.69-2.73 (m, 4H, 2xCH_2); δ_C (normal/DEPT-135) (125 MHz, $CDCl_3$): 173.48 (C=O), 169.70 (C=O), 158.75 (C=O), 144.81 (C), 135.30 (CH), 134.82 (C), 130.75 (CH), 129.19 (C), 126.94 (C), 126.33 (CH), 113.53 (CH), 109.04 (CH), 77.24 (CH),

53.30 (OCH_3), 52.27 (OCH_3), 29.65 (-ve, CH_2), 25.04 (-ve, CH_2); ESI-MS (HRMS) calcd for $C_{17}H_{18}N_2O_6$ 347.1238. Found *m/z* 347.1221 ($[M+H]^+$).

[2-Oxo-2-(1-prop-2-ynyl-1*H*-indol-3-yl)-acetylamino]-acetic acid methyl ester (4a).

yellow colored solid, mp 111 ^oC, 97%, IR (KBr, cm^{-1}): 3412 (NH), 2867 (CH), 1732 (C=O), 1689 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.78 (s, 1H, NH), 8.35 (s, 1H, ArH), 7.82-8.01 (m, 2H, ArH), 7.39-7.43 (m, 2H, ArH), 4.83 (d, *J*=3.5 Hz, 2H, CH_2), 4.21 (d, *J*=1.5 Hz, 2H, CH_2), 3.84 (s, 3H, OCH_3), 1.63 (s, 1H, CH); ESI-MS (HRMS) calcd for $C_{16}H_{14}N_2O_4$ 299.1034. Found *m/z* 299.1029 $[M+H]^+$.

3-(1*H*-Indol-3-yl)-2-[2-oxo-2-(1-prop-2-ynyl-1*H*-indol-3-yl)-acetylamino]-propionic acid methyl ester (4b).

Yellow solid, mp 126 ^oC, 79%, IR (KBr, cm^{-1}): 3297 (NH), 2945 (C-H), 1751 (C=O), 1661 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, 1H, NH), 7.34-7.50 (m, 4H, ArH), 7.16-7.28 (m, 5H, ArH), 6.72 (d, 1H, *J*=3.5 Hz), 4.80 (d, 2H, *J*=3.5 Hz, CH_2), 4.49 (t, 1H, *J*=2 Hz, CH), 3.88 (s, 3H, OCH_3), 3.58-3.63 (dd, *J*= 9 Hz, *J*= 15 Hz, 1H, CH_2), 3.42-3.47 (dd, *J*= 8 Hz, *J*= 15 Hz, 1H, CH_2), 1.60 (s, 1H, CH); ESI-MS (HRMS) calcd for $C_{25}H_{21}N_3O_4$ 428.1603. Found *m/z* 428.1612 $[M+H]^+$.

3-(3*H*-Imidazol-4-yl)-2-[2-oxo-2-(1-prop-2-ynyl-1*H*-indol-3-yl)-acetylamino]-propionic acid methyl ester (4c).

Yellow solid, mp 118 ^oC, 90%, IR (KBr, cm^{-1}): 3535 (NH), 2757 (C-H), 1741 (C=O), 1680 (C=O) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 9.10 (s, 1H, NH), 7.92-7.73 (m, 1H), 7.55-7.63 (m, 2H, ArH), 7.20-7.28 (m, 2H, ArH), 6.83-7.20 (m, 3H, ArH), 6.83 (s, 1H, ArH), 4.81 (d, 2H, *J*=6.5 Hz), 4.37 (t, 1H, *J*=8 Hz), 3.78 (s, 1H, OCH_3), 3.33 (d, *J*=3.5 Hz, 2H, CH_2) 1.63 (s, 1H, CH); ESI-MS (HRMS) calcd for $C_{20}H_{18}N_4O_4$ 379.1408. Found *m/z* 379.1401 $[M+H]^+$.

2-[2-Oxo-2-(1-prop-2-ynyl-1*H*-indol-3-yl)-acetylamino]-pentanedioic acid dimethyl ester (4d).

Yellow solid, mp 123 ^{o}C, 85%, IR (KBr, cm^{-1}): 3467 (NH), 2877 (C-H), 1735 (C=O), 1621 (C=O) cm^{-1}; ^{1}H NMR (500 MHz $CDCl_3$): δ 7.99 (s, 1H, NH), 7.53-7.84 (m, 1H, ArH), 7.32 (s, 1H, *J*=5 Hz), 7.23-7.28 (m, 2H, ArH), 7.22 (s, 1H, ArH), 4.81 (t, 1H, *J*=7.5 Hz, CH_2), 4.31 (t, 1H, *J*=7.5 Hz, CH), 3.78 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 2.36-2.73 (m, 4H, 2xCH_2), 1.63 (s, 1H, CH); ESI-MS (HRMS) calcd for $C_{20}H_{20}N_2O_6$ 385.1400. Found *m/z* 385.1401 $[M+H]^+$.

{2-Oxo-2-[1-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1*H*-indol-3-yl]-acetylamino}-acetic acid methyl ester (5a)

Light yellow solid, mp 180 ^{o}C, 68%, IR (KBr, cm^{-1}): 3407 (NH), 2957 (C-H), 1757 (C=O), 1661 (C=O), 1130 (S=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.39 (s, 1H, NH), 8.35-8.36 (m, 3H, ArH), 7.89-8.01 (m, 2H, ArH), 7.82-7.84 (m, 2H, ArH), 7.28-7.43 (m, 4H, ArH), 5.31 (d, *J* = 1 Hz, 2H, CH_2), 4.20 (d, *J* = 5.5 Hz, 2H, CH_2), 3.83 (s, 3H, OCH_3); δ_C (normal/DEPT- 135) (125 MHz, $CDCl_3$): 180.74 (C=O), 169.34 (C=O), 161.27 (C=O), 146.09 (C), 138.48 (CH), 134.43 (C), 134.23 (C), 130.30 (CH), 128.11 (C), 127.38 (C=O), 125.98 (C=O), 125.17 (CH), 122.81 (CH), 115.93 (C), 113.27 (CH), 52.62 (OCH_3), 41.06 (CH_2 , -ve), 21.66 (CH_2 , -ve); ESI-MS (HRMS) calcd for $C_{22}H_{19}N_5O_4$ 418.1517. Found *m/z* 418.1515 $[M+H]^+$.

3-(1*H*-Indol-3-yl)-2-{2-oxo-2-[1-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1*H*-indol-3-yl]-acetylamino}-propionic acid methyl ester (5b)

Light brown solid, mp 131 ^{o}C, 79 %, IR (KBr, cm^{-1}): 2927 (CH), 3411 (N-H), 1741 (C=O), 1651 (C=O), 1135 (S=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.97 (s, 1H, NH), 7.77 (d, *J* = 10 Hz, 1H, ArH), 7.56 (d, *J* = 10 Hz, 1H, ArH), 7.49 (d, *J* = 10 Hz, 1H, ArH), 7.41 (d, *J* = 10 Hz, 1H, ArH), 7.32-7.35 (m, 2H, ArH), 7.22-7.28 (m, 6H, ArH), 7.16-7.18 (m, 2H, ArH), 6.70 (d, *J* = 3

Hz, 1H, ArH), 5.16 (d, *J* = 1 Hz, 2H, CH_2), 4.51 (t, *J* = 1 Hz, 1H, CH), 3.88 (s, 3H, OCH_3), 3.59-3.63 (dd, *J*=4.5, *J*=15.5 Hz 1H, CH_2), 3.44-3.49 (dd, *J*=7.5, *J*=15.5 Hz 1H, CH_2); (normal/DEPT- 135) (125 MHz, $CDCl_3$): 169.61 (C=O), 145.41 (C), 136.56 (C), 134.76 (C), 134.63 (C), 130.84 (C), 129.98 (CH), 126.75 (CH), 126.16 (CH), 126.10 (C), 124.74 (CH), 124.71 (CH), 123.53 (CH), 123.23(CH), 121.51 (CH), 120.45 (CH), 117.72 (C), 117.63 (CH), 115.46 (C), 113.43 (CH), 113.19 (C), 111.92 (CH), 110.93 (C), 109.56 (CH), 105.46 (C), 54.11 (CH), 54.09 (CH), 26.11 (CH_2, -ve), 21.47 (CH_2, -ve); ESI-MS (HRMS) calcd for $C_{31}H_{26}N_6O_4$ 547.2096. Found *m/z* 547.2091 $[M+H]^+$.

3-(3*H*-Imidazol-4-yl)-2-{2-oxo-2-[1-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1*H*-indol-3-yl]-acetylamino}-propionic acid methyl ester (5c)

Grey solid, mp 121 ^oC, 85%. IR (KBr, cm^{-1}): 3401 (N-H), 1744 (C=O), 2925 (CH), 1628 (C=O), 1087.19 (S=0) cm^{-1}; ^1H NMR (500 MHz, DMSO d_6): ^1H NMR (500 MHz, DMSO d_6): δ 9.10 (s, 1H, NH), 7.92 (d, *J* = 10 Hz, 1H, ArH), 7.84 (d, *J* = 10 Hz, 1H, ArH), 7.77 (d, *J* = 5 Hz, 1H, ArH), 7.59 (d, *J* = 10 Hz, 1H, ArH), 7.54 (s, 1H, ArH), 7.35 (t, *J* = 10 Hz, 3H, ArH), 7.31 (m, 2H, ArH), 7.23 (t, *J* = 5 Hz, 1H, ArH), 6.82 (d, *J*= 5 Hz, 1H, ArH), 5.13 (d, *J* = 3.5 Hz, 2H, CH_2), 4.49 (t, *J* = 7 Hz, 1H, CH), 3.72 (s, 3H, OCH_3), 3.34 (d, *J*=7 Hz, 2H, CH_2); (normal/DEPT- 135) (125 MHz, $CDCl_3$): 169.02 (C=O), 145.92 (C), 134.63 (C), 134.56 (C), 134.52 (CH), 130.93 (C), 130.68 (CH), 127.39 (C), 127.38 (CH), 127.15 (CH), 127.12 (C), 125.10 (CH), 123.89 (CH), 122.07 (CH), 118.57 (CH), 113.53 (CH), 109.88 (CH), 53.51 (OCH_3), 51.49 (CH), 25.51 (CH_2, -ve), 21.46 (CH_2, -ve); ESI-MS (HRMS) calcd for $C_{26}H_{23}N_7O_4$ 498.1882. Found *m/z* 498.1882 $[M+H]^+$.

2-{2-Oxo-2-[1-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1*H*-indol-3-yl]-acetylamino}-pentanedioic acid dimethyl ester (5d)

Cream colored solid, mp 141 ^{o}C, 72%, IR (KBr, cm^{-1}): 3435 (NH), 2926 (C-H), 1736 (C=O), 1634 (C=O), 1088 (S=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.46 (b, 1H, NH), 8.00 (d, *J* = 10 Hz, 1H, ArH), 7.77 (d, *J* = 5 Hz, 1H, ArH), 7.53-7.58 (m, 1H, ArH), 7.32 (t, *J* = 5 Hz, 1H, ArH), 7.23 (t, *J* = 5 Hz, 2H, ArH), 6.73 (s, 2H, ArH), 6.66 (d, *J* = 5 Hz, 1H, ArH), 5.22 (d, *J* = 3 Hz, 2H, CH_2), 4.36 (t, *J* = 1 Hz, 1H, CH), 3.84 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 2.63-2.72 (m, 2H, CH_2), 2.41 (d, *J* = 5 Hz, 2H, CH_2); (normal/DEPT- 135) (125 MHz, $CDCl_3$ + TFA): 173.30 (C=O), 169.70 (C=O), 144.94 (C), 135.30 (C), 134.82 (C), 130.75 (C), 129.87 (CH), 126.81 (CH), 126.33 (CH), 124.55 (CH), 123.27 (CH), 121.37 (CH), 115.95 (C), 113.67 (C), 113.53 (CH), 111.40 (C), 109.04 (CH), 53.48 (OCH_3), 52.76 (OCH_3), 52.14 (CH), 29.75 (CH_2, -ve), 25.19 (CH_2, -ve) 21.54 (CH_2, -ve); ESI-MS (HRMS) calcd for $C_{26}H_{25}N_5O_6$ 504.5144. Found *m/z* 504.5146 $[M+H]^+$.

{2-Oxo-2-[1-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1*H*-indol-3-yl]-acetylamino}-acetic acid (6a)

Dark brown solid, mp 140 ^{o}C, 80%, IR (KBr, cm^{-1}): 3412 (NH), 1764 (C=O), 1630 (C=O), 1120 (S=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.26 (s, 1H, OH), 8.37 (m, 1H, NH), 7.98 – 8.01 (m, 2H, ArH), 7.82 – 7.98 (m, 3H, ArH), 7.40 – 7.43 (m, 2H, ArH), 7.26-7.28 (t, *J* = 5 Hz, 2H, ArH), 7.13-7.15 (d, *J* = 10 Hz, 2H, ArH), 5.31 (d, *J* = 0.5 Hz, 2H, CH_2), 4.21 (d, *J*= 4.5 Hz, 2H, CH_2); δ_C (normal/DEPT- 135) (125 MHz, $CDCl_3$): 180.66 (C=O), 169.35 (C=O), 161.27 (C=O), 146.09 (C), 138.48 (CH), 134.43 (C), 134.24 (C), 128.11 (CH), 127.38 (CH), 125.74 (CH), 125.17 (CH), 122.81 (CH), 115.79 (C), 113.30 (CH), 41.07 (CH, -ve), 21.98 (CH_2, -ve); ESI-MS (HRMS) calcd for $C_{19}H16N_2O_6S$ 423.0621. Found *m/z* 423.0944 ($[M+Na]^+$). ESI-MS (HRMS) calcd for $C_{21}H_{17}N_5O_4$ 404.1361. Found *m/z* 404.1361 $[M+H]^+$.

3-(1*H*-Indol-3-yl)-2-{2-oxo-2-[1-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1*H*-indol-3-yl]-acetylamino}-propionic acid (6b). Cream colored solid, mp 110 ^{0}C, 69%, IR (KBr, cm^{-1}): 3421 (NH), 1735 (C=O), 1632 (C=O), 1110 (S=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.058 (s, 1H, OH), 8.19 (s, 2H, NH), 7.93 (d, *J* = 10 Hz, 1H, ArH), 7.85 (d, *J* = 10 Hz, 1H, ArH), 7.78 (d, *J* = 5 Hz, 1H, ArH), 7.56-7.60 (m, 4H, ArH), 7.32-7.39 (m, 4H, ArH), 7.24 (t, *J* = 5 Hz, 1H, ArH), 7.10 (t, *J* = 5 Hz, 1H, ArH), 7.01 (t, *J* = 5 Hz, 1H, ArH), 6.82 (d, *J* = 5 Hz, 1H, ArH), 5.17 (d, *J* = 1 Hz, 2H, CH_2), 4.17 (d, *J* = 1 Hz, 1H, CH), 3.26 (s, 2H, CH_2); δ_C (normal/DEPT- 135) (125 MHz, DMSO d_6): 171.35 (C=O), 145.92 (C), 136.76 (C), 134.66 (C), 134,57 (C), 130.93 (C), 130.67 (CH), 127.47 (C), 127.38 (CH), 127.15 (CH), 125.41 (CH), 125.08 (CH), 123.88 (CH), 122.05 (CH), 121.65 (CH), 119.20 (C), 119.09 (CH), 118.66 (CH), 116.90 (C), 114.60 (C), 113.53 (CH), 112.29 (C), 111.98 (CH), 109.85 (CH), 107.07 (C), 53.05 (CH), 26.61 (CH_2, -ve), 21.44 (CH_2, -ve); ESI-MS (HRMS) calcd for $C_{30}H_{24}N_6O_4$ 533.1939. Found *m/z* 533.1931 $[M+H]^+$.

3-(3*H*-Imidazol-4-yl)-2-{2-oxo-2-[1-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1*H*-indol-3-yl]-acetylamino}-propionic acid (6c). Yellow colored solid, mp 135 ^{o}C, 65%, IR (KBr, cm^{-1}): 3395 (NH), 1630 (C=O), 1735 (C=O), 1065 (S=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.083 (s, 1H, OH), 8.30-8.33 (b, 2H, NH), 7.59 (d, *J* = 10 Hz, 1H, ArH), 7.57 (d, *J* = 5 Hz, 1H, ArH), 7.50 (d, *J* = 10 Hz, 2H, ArH), 7.37 (d, *J* = 10 Hz, 1H, ArH), 7.24 (d, *J* = 5 Hz, 1H, ArH), 7.12 – 7.14 (m, 2H, ArH), 7.09-7.12 (m, 4H, ArH), 7.00 (t, *J*= 5 Hz, 1H, ArH), 5.13 (d, *J* = 7.5 Hz, 2H, CH_2), 4.12 (t, *J* = 5.5 Hz, 1H, CH), 3.28 (d, *J* = 6 Hz, 2H, CH_2); δ_C (normal/DEPT- 135) (125 MHz, DMSO d_6); 171.27 (C=O), 158.95 (C=O), 158.64 (C=O), 145.77 (C), 138.36 (C), 136.73 (C), 128.61 (CH), 127.49 (C), 125.96 (CH), 125.46 (CH), 121.59 (CH), 119.05 (CH), 118.71 (CH),

116.64 (C), 111.97 (CH), 107.08 (C), 53.04 (CH), 26.54 (CH_2, -ve), 21.24 (CH_2, -ve); ESI-MS (HRMS) calcd for $C_{25}H_{21}N_7O_4$ 484.1735. Found *m/z* 484.1733 $[M+H]^+$.

2-{2-Oxo-2-[1-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethyl)-1*H*-indol-3-yl]-acetylamino}-pentanedioic acid (6d). White solid, mp 175 ^oC, 85%; IR (KBr, cm^{-1}): 3450 (NH), 1717 (C=O), 1613 (C=O), 1077 (S=O); ^1H NMR (500 MHz, DMSO-d_6): δ 12.74 (b, 2H, OH), 8.26 (s, 1H, NH), 7.92 (d, *J* = 5 Hz, 1H, ArH), 7.84 – 7.92 (m, 4H, ArH), 7.77 (d, *J* = 5 Hz, 1H, ArH), 7.59 (d, *J* = 5 Hz , 1H, ArH), 7.31-7.37 (m, 2H, ArH), 7.23 (t, *J* = 5 Hz, 1H, ArH), 6.82 (d, *J* = 3.5 Hz, 1H, ArH), 5.22 (d, *J* = 2.5 Hz, 2H, CH_2), 3.96 (d, *J* = 1Hz, 1H, CH), 2.34-2.47 (m, 2H, CH_2), 1.96-2.05 (m, 2H, CH_2); δ_C (normal/DEPT- 135) (125 MHz, DMSO d_6); 173.70 (C=O), 171.23 (C=O), 145.90 (C), 134.66 (C), 134.57 (C), 130.93 (C), 130.65 (CH), 127.35 (CH), 127.14 (CH), 125.06 (CH), 123.86 (CH), 122.03 (CH), 119.15 (C), 116.85 (C), 114.55 (C), 113.53 (CH), 112.25 (C), 109.83 (CH), 51.78 (CH), 29.66 (CH_2, -ve), 25.78 (CH_2, -ve), 21.40 (CH_2, -ve); ESI-MS (HRMS) calcd for $C_{24}H_{21}N_5O_6$ 476.1574. Found *m/z* 474.1572 $[M+H]^+$.

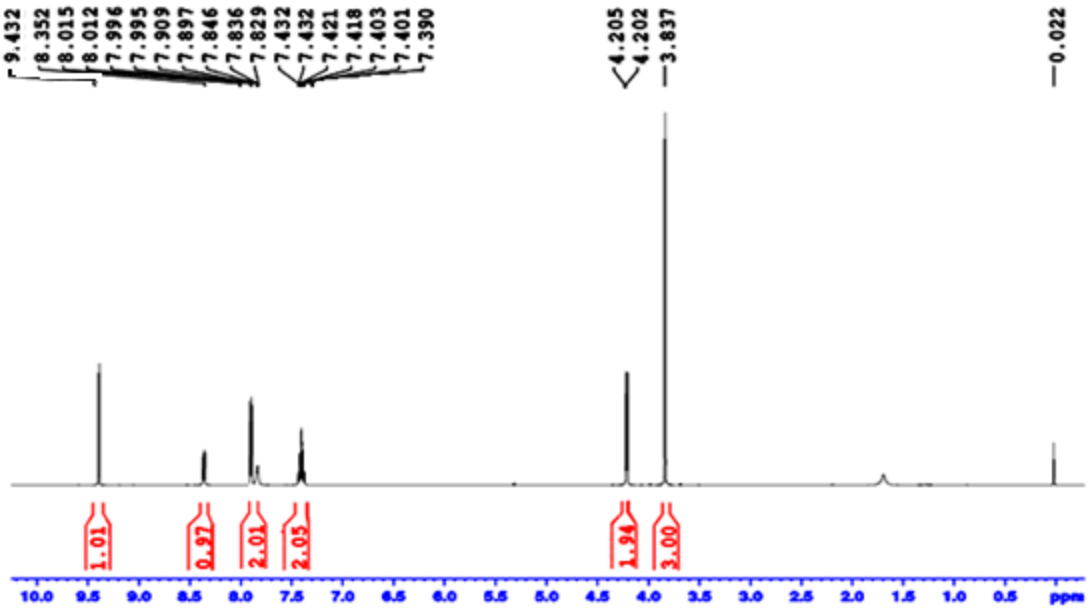

Figure S1. ^{1}H NMR spectrum of compound 3a

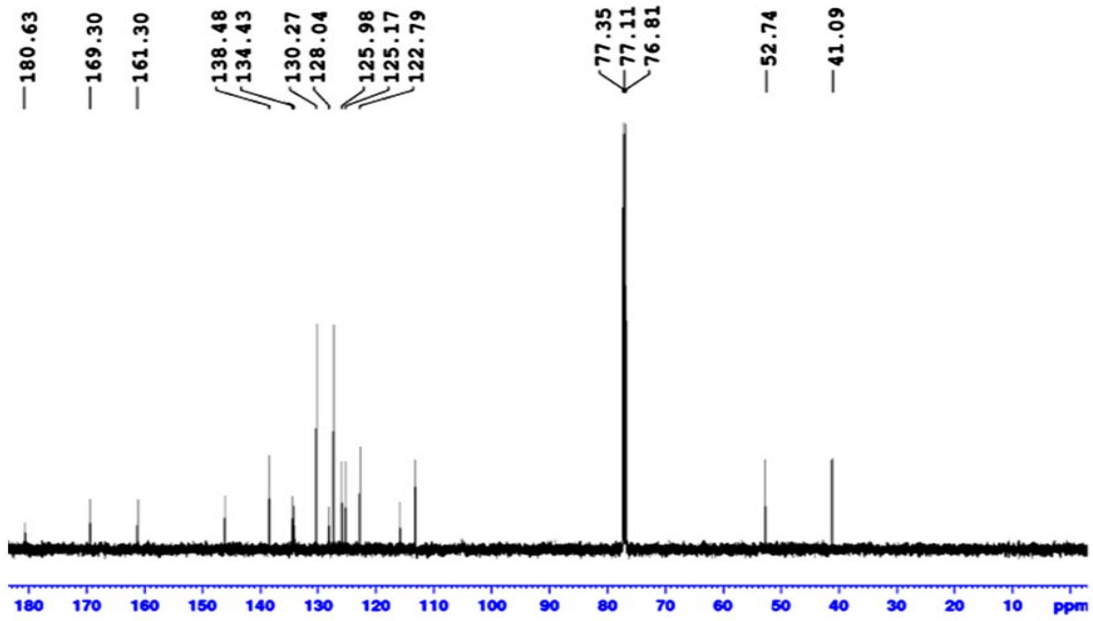

Figure S2. ^{13}C NMR spectrum of compound 3a

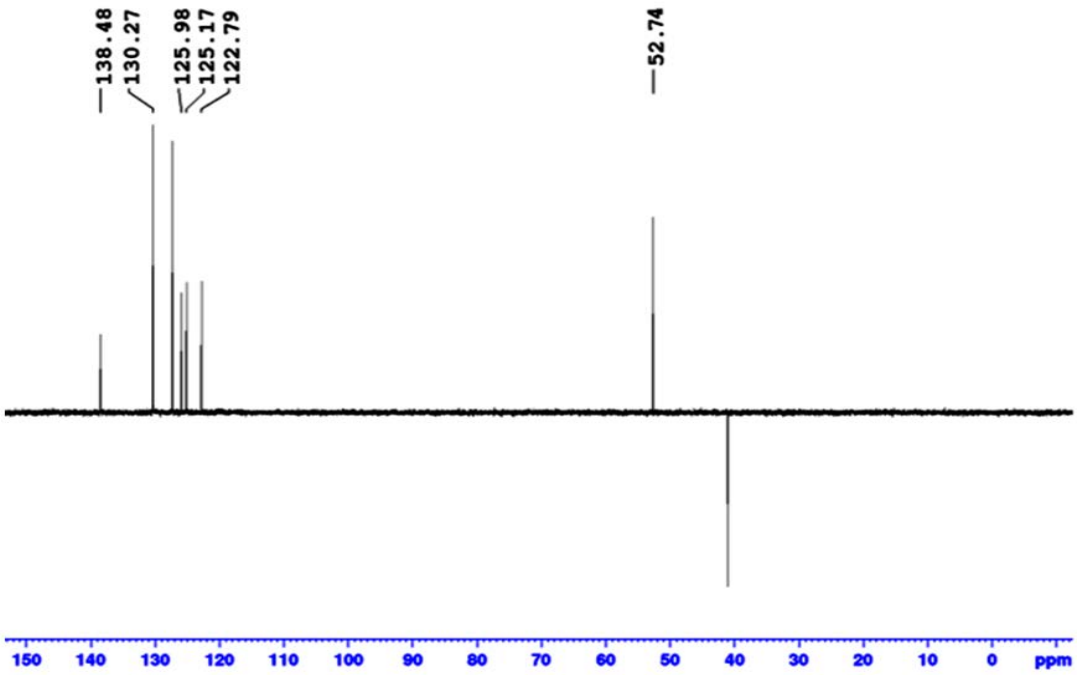

Figure S3. DEPT-135 NMR spectrum of compound 3a

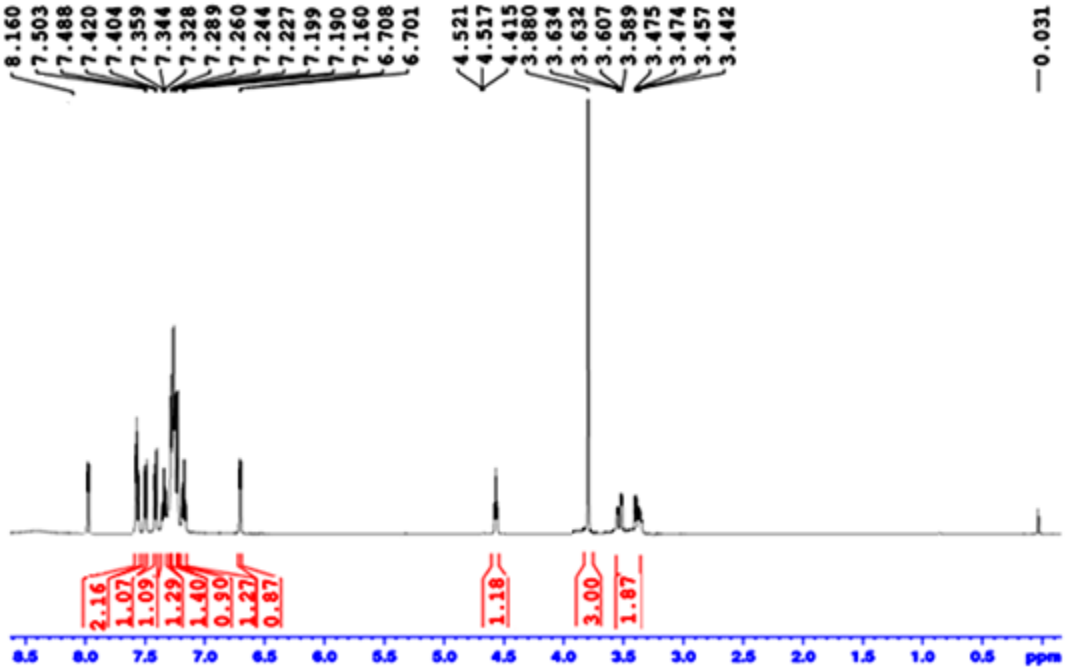

Figure S4. ^{1}H NMR spectrum of compound 3b

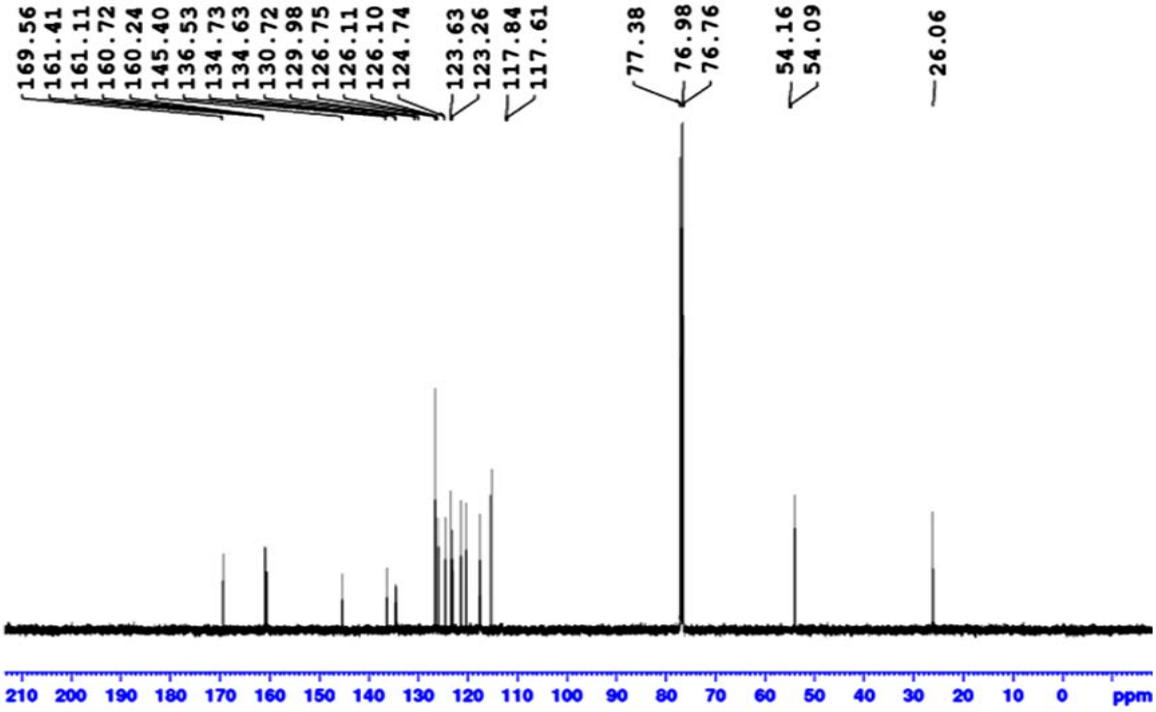

Figure S5. ^{13}C NMR spectrum of compound 3b

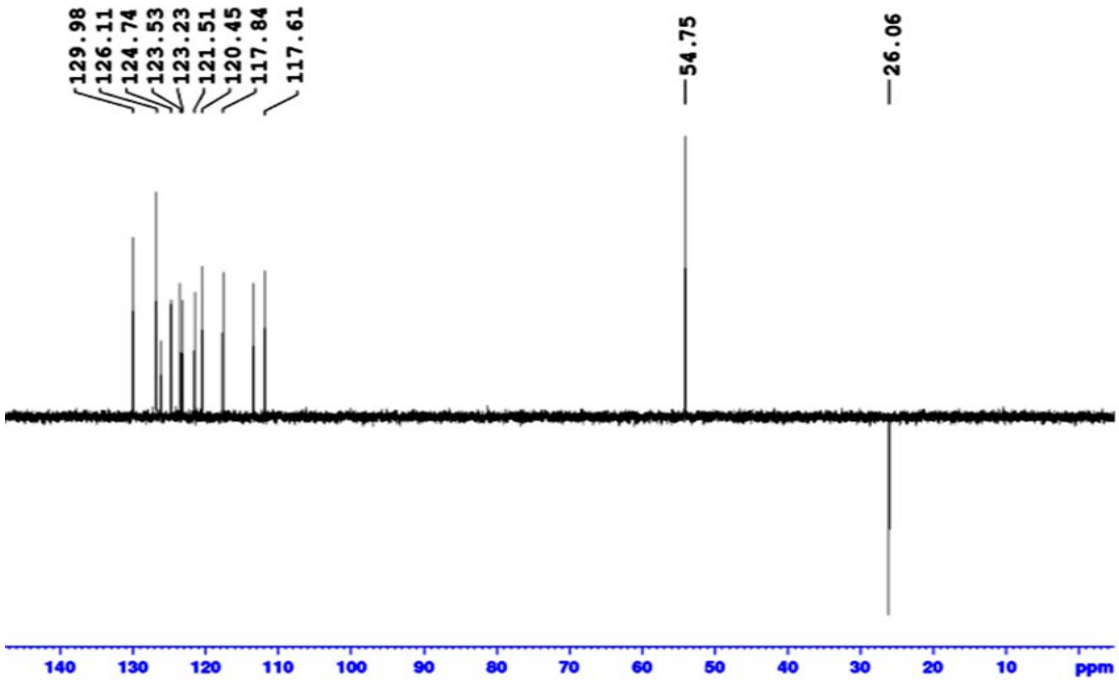

Figure S6. DEPT-135 NMR spectrum of compound 3b

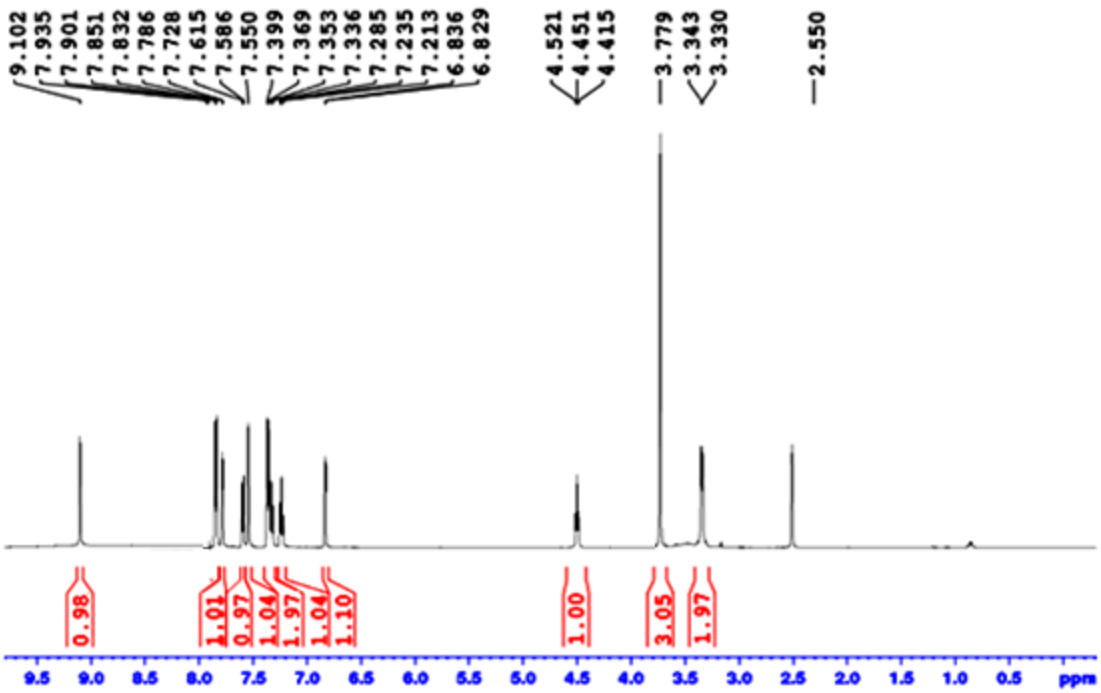

Figure S7. ^{1}H NMR spectrum of compound 3c

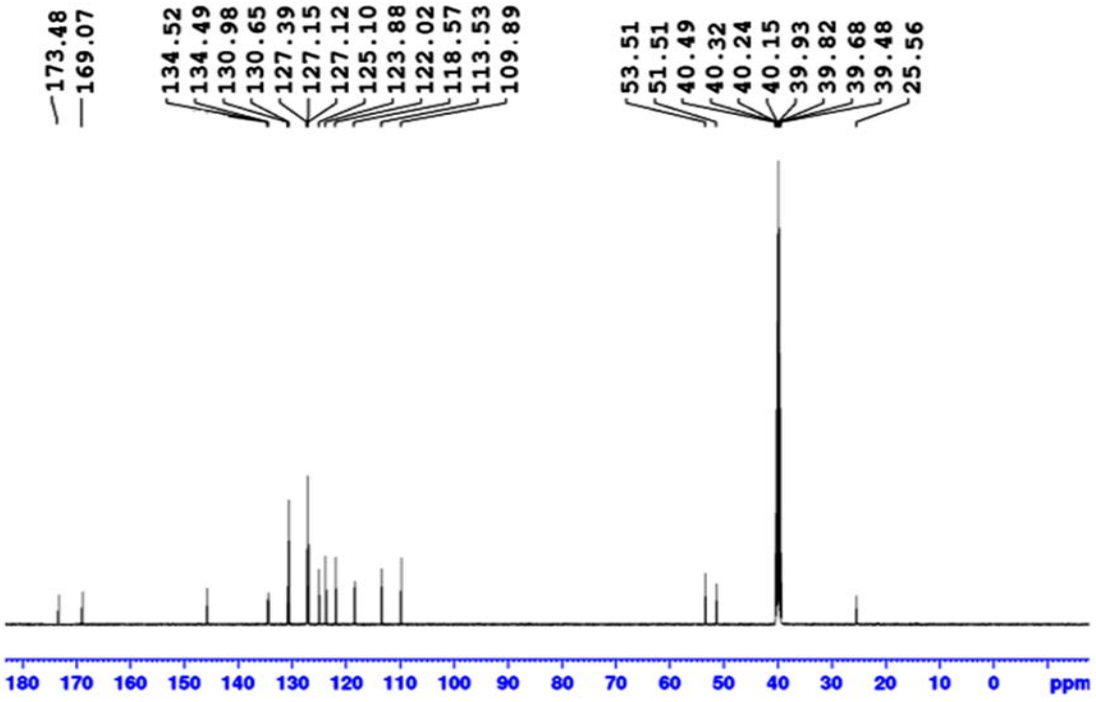

Figure S8. ^{13}C NMR spectrum of compound 3c

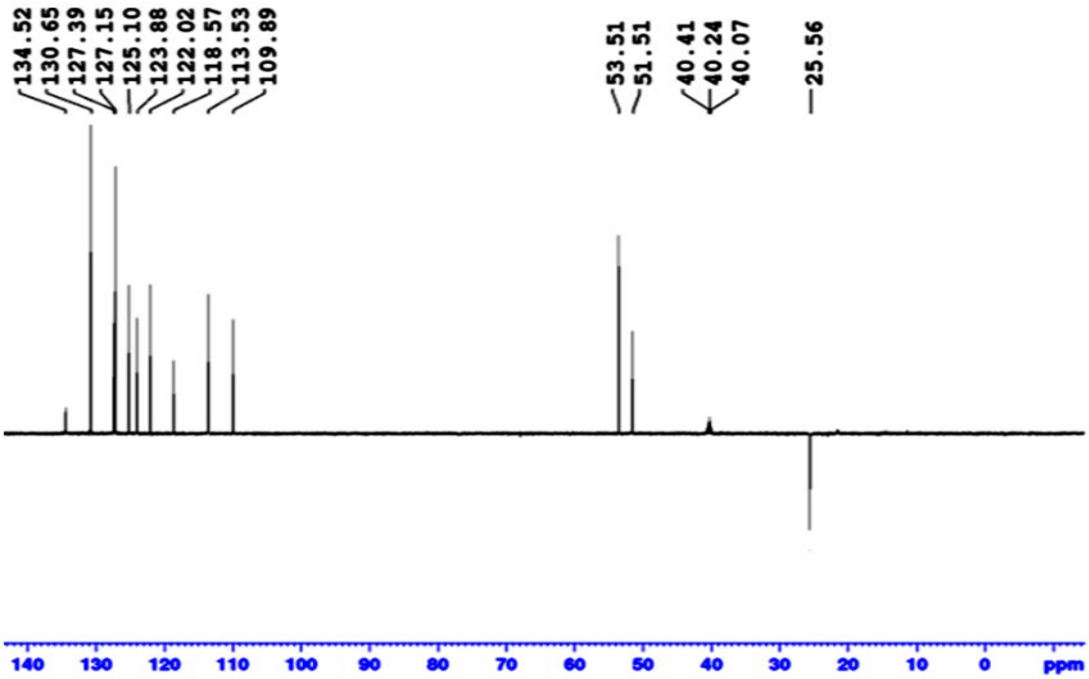

Figure S9. DEPT-135 NMR spectrum for 3c

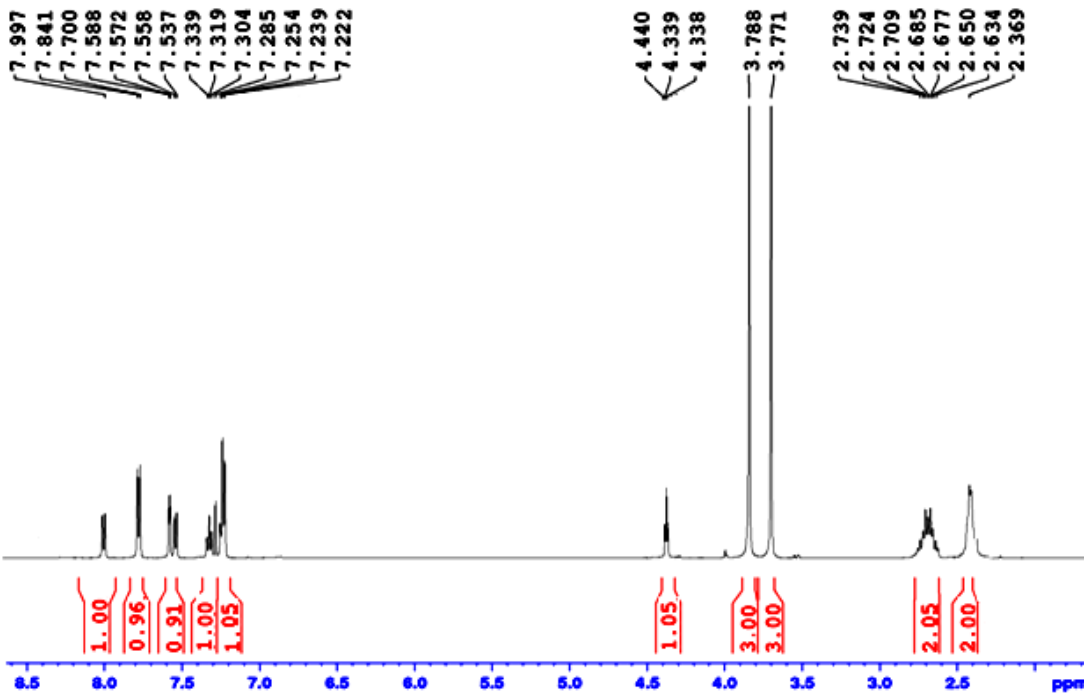

Figure S10. ^{1}H NMR spectrum of compound 3d

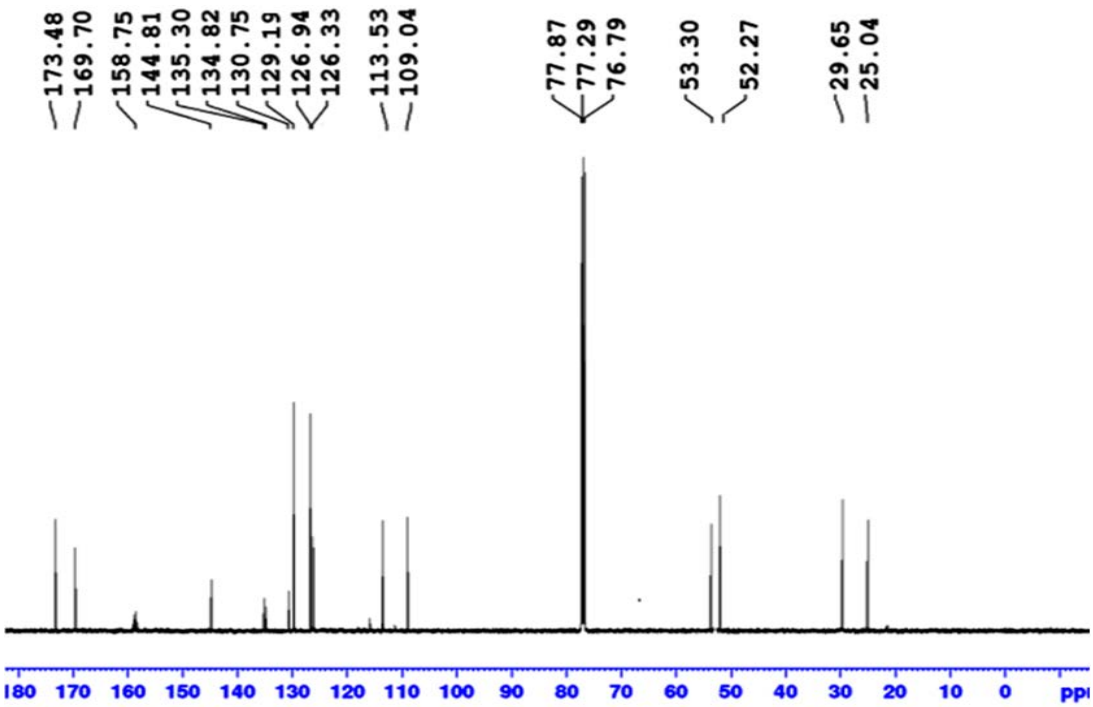

Figure S11. ^{13}C NMR spectrum of compound 3d

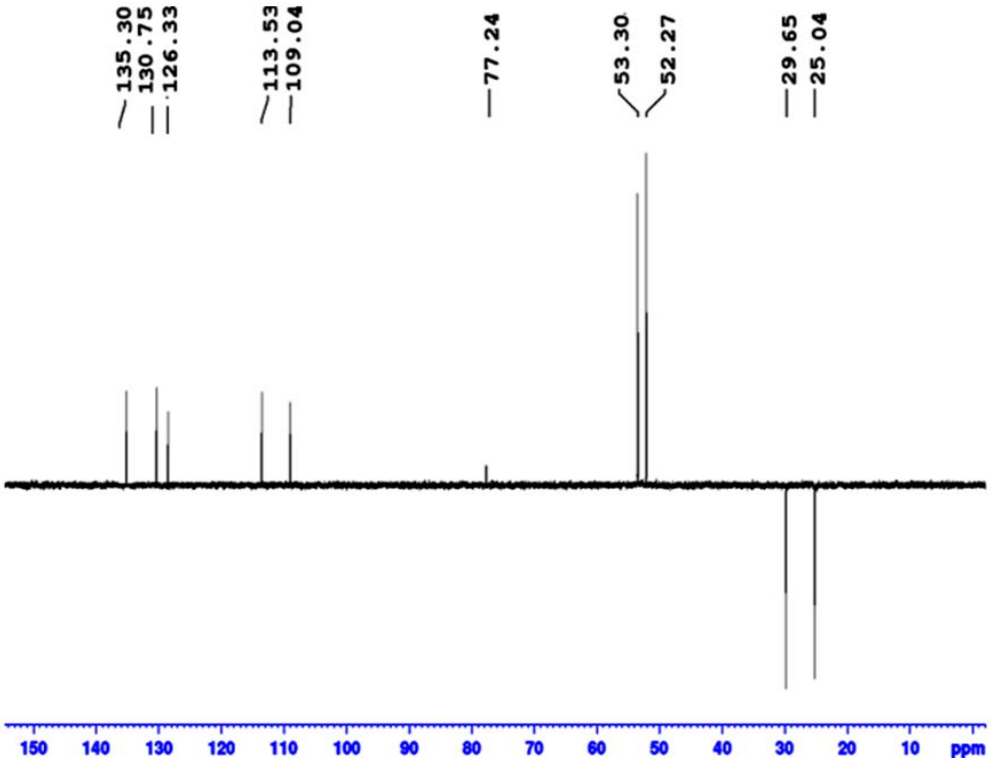

Figure S12. DEPT-135 NMR spectrum for 3d

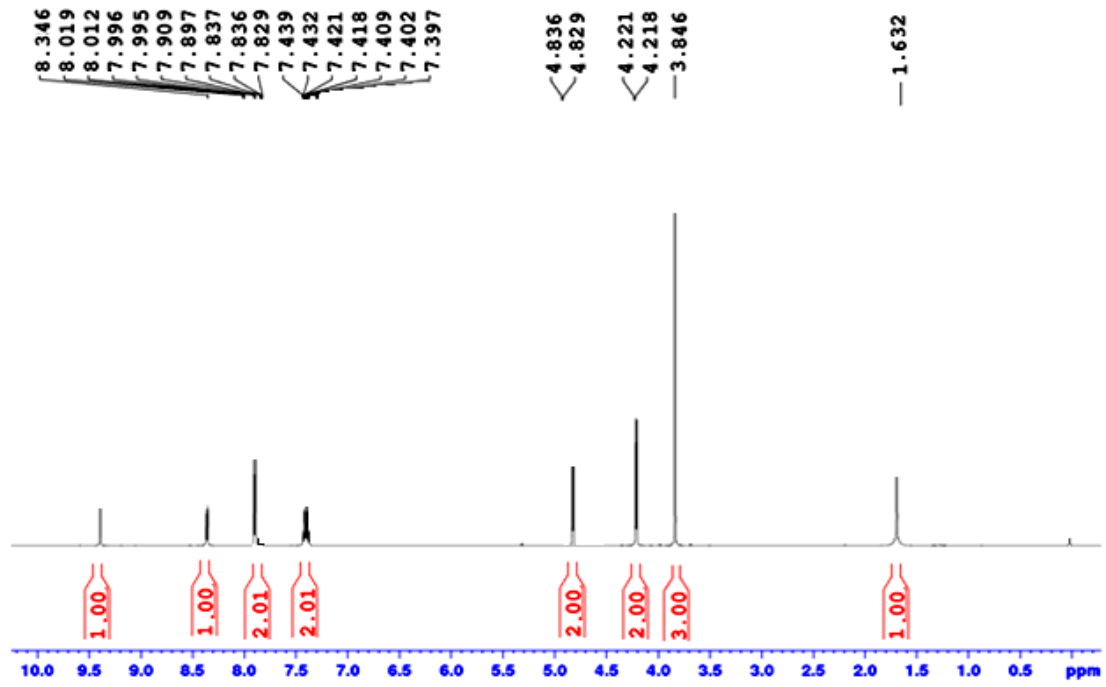

Figure S13. ^{1}H NMR spectrum of compound 4a

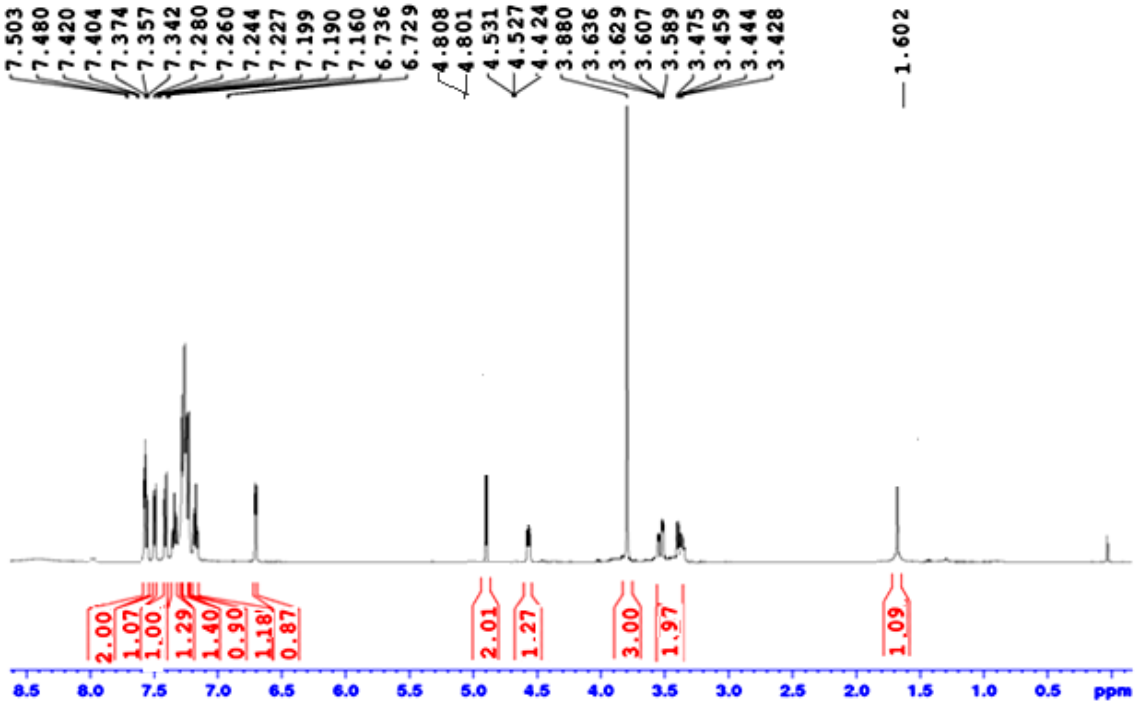

Figure S14. ^{1}H NMR spectrum of compound 4b

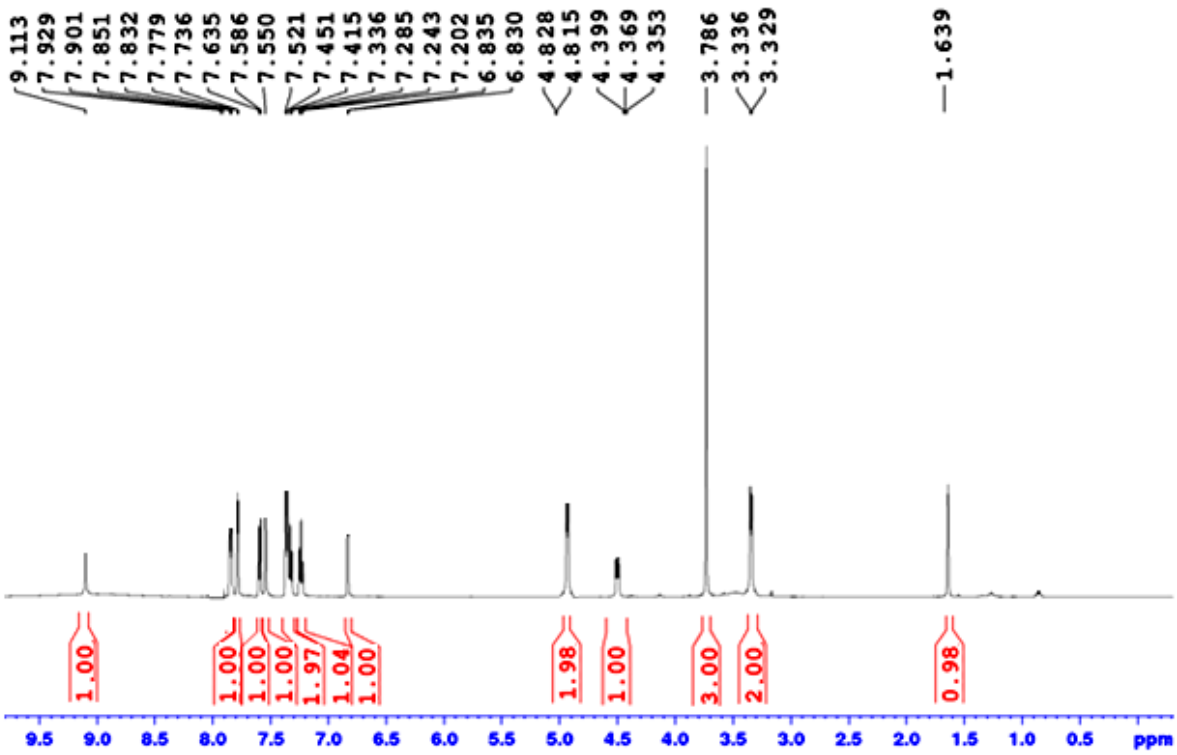

Figure S15. ^{1}H NMR spectrum of compound 4c

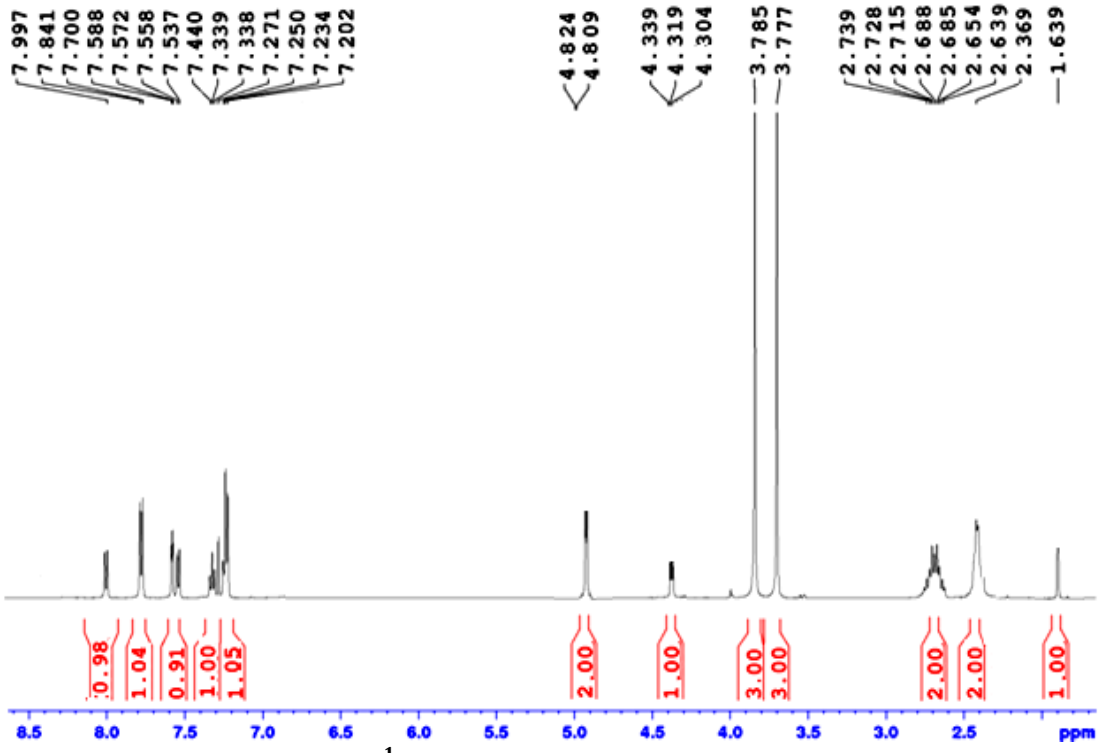

Figure S16. ^{1}H NMR spectrum of compound 4d

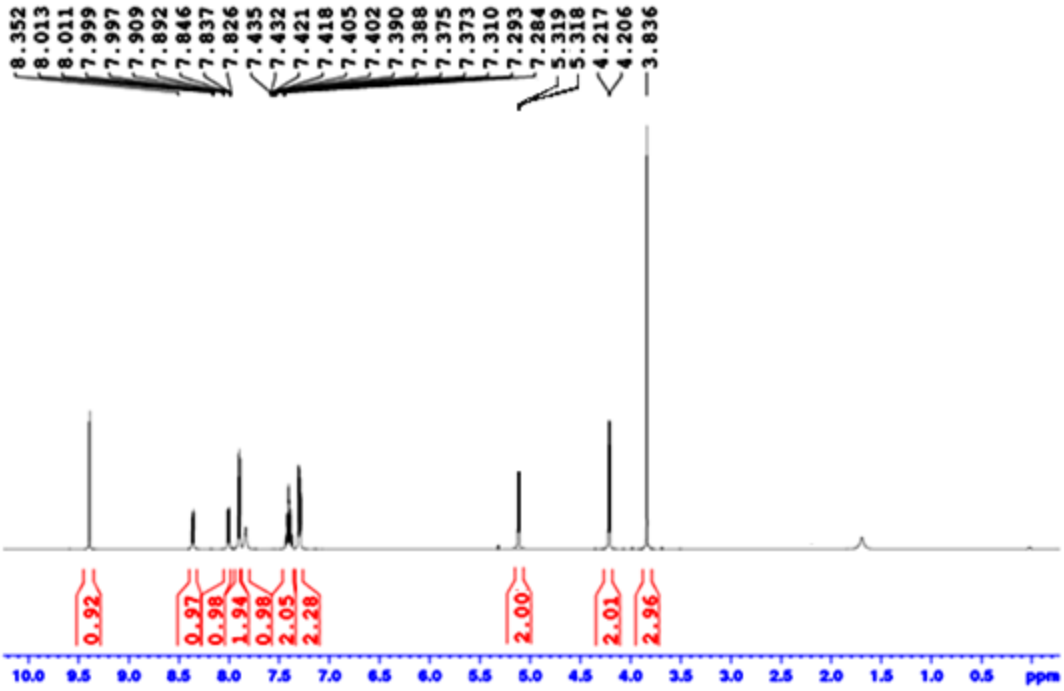

Figure S17. ^{1}H NMR spectrum of compound 5a

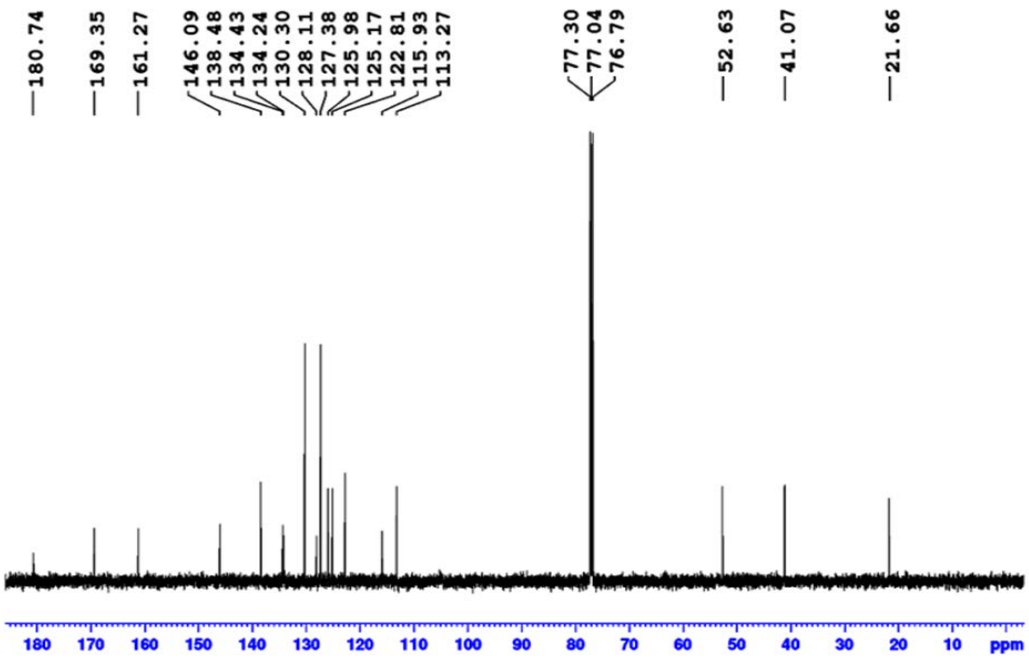

Figure S18. ^{13}C NMR spectrum of compound 5a

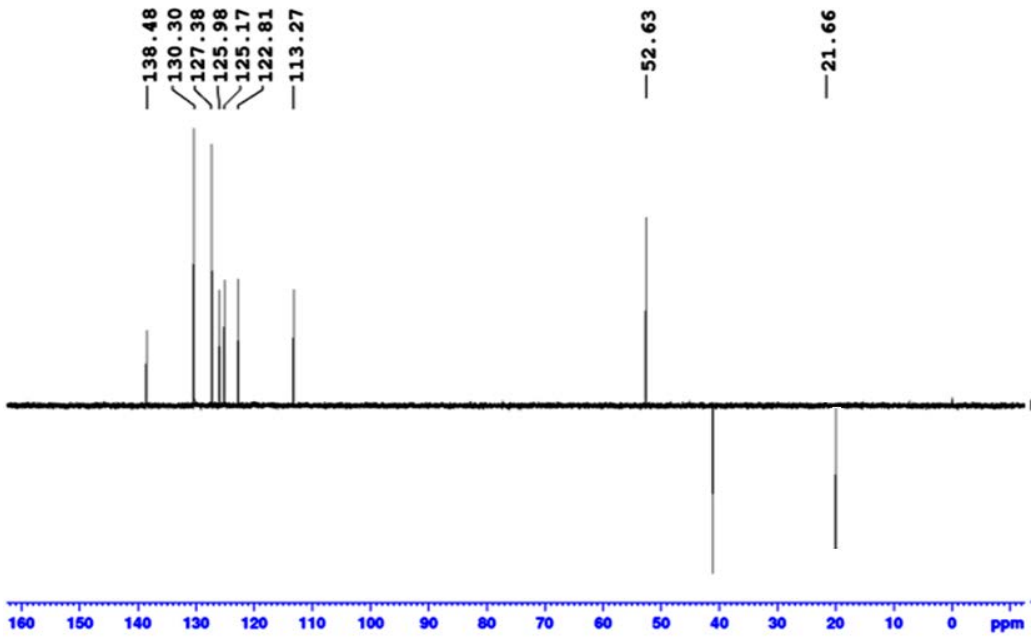

Figure S19. DEPT-135 NMR spectrum of 5a

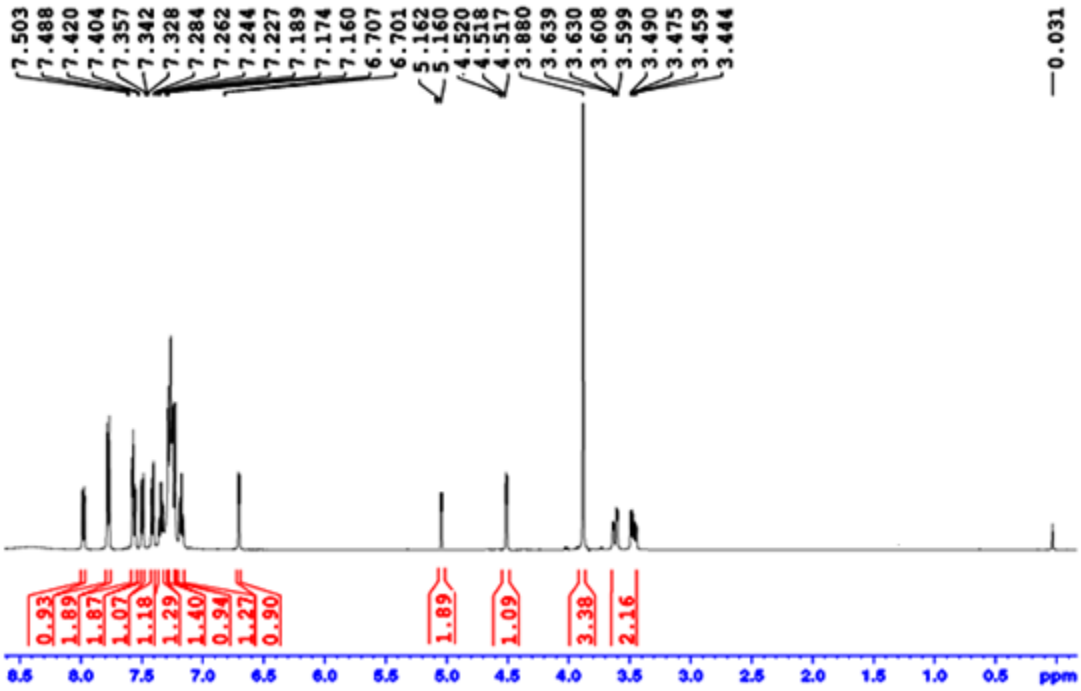

Figure S20. ^{1}H NMR spectrum of compound 5b

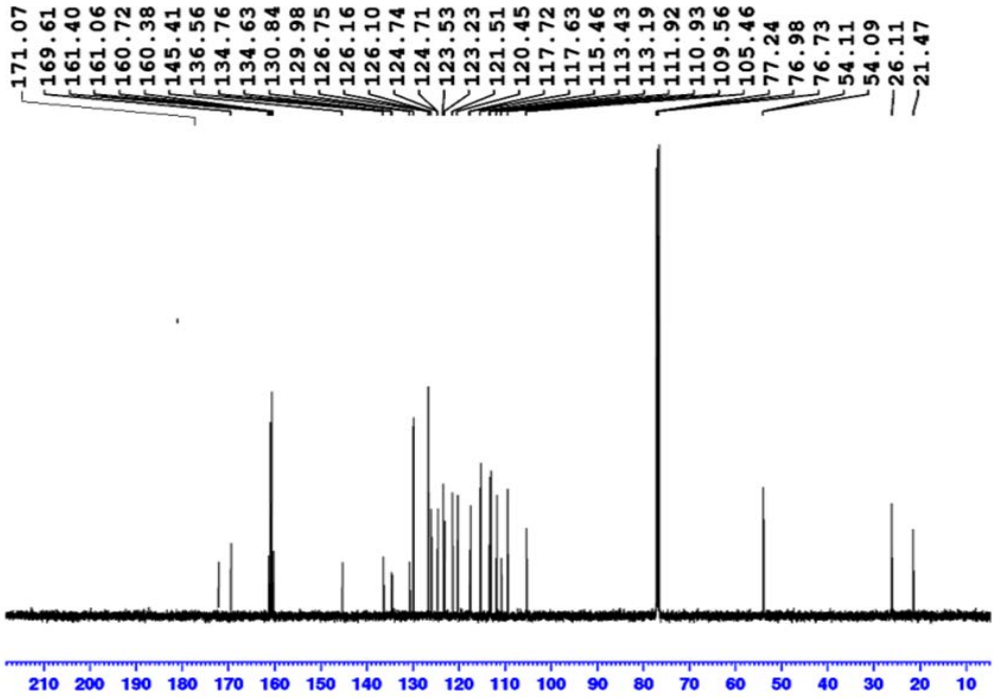

Figure S21. ^{13}C NMR spectrum for 5b

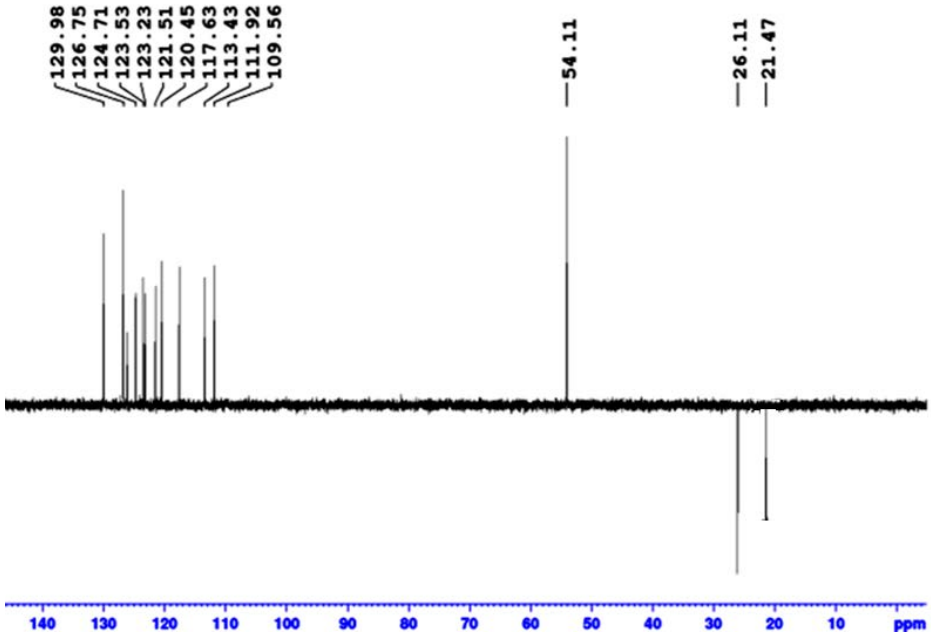

Figure S22. DEPT-135 NMR spectrum for 5b

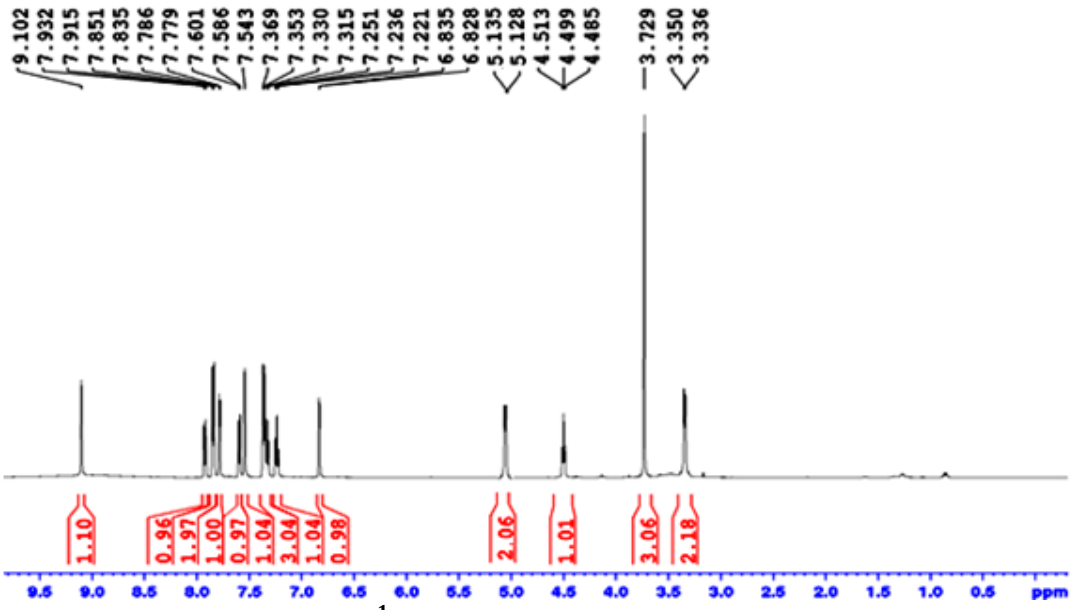

Figure S23. ^{1}H NMR spectrum of compound 5c

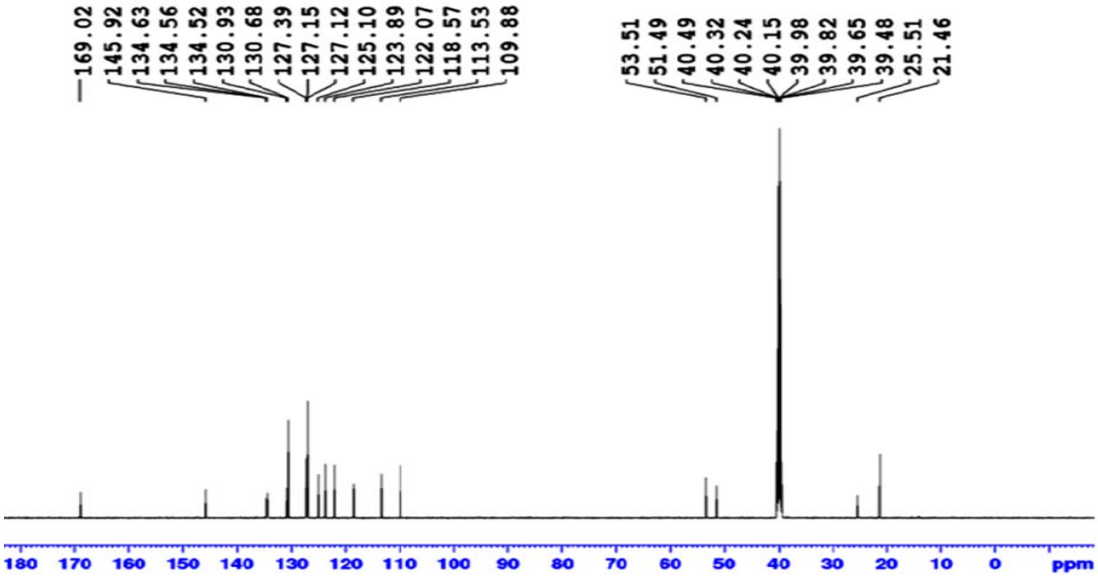

Figure S24. ^{13}C NMR spectrum of compound 5c

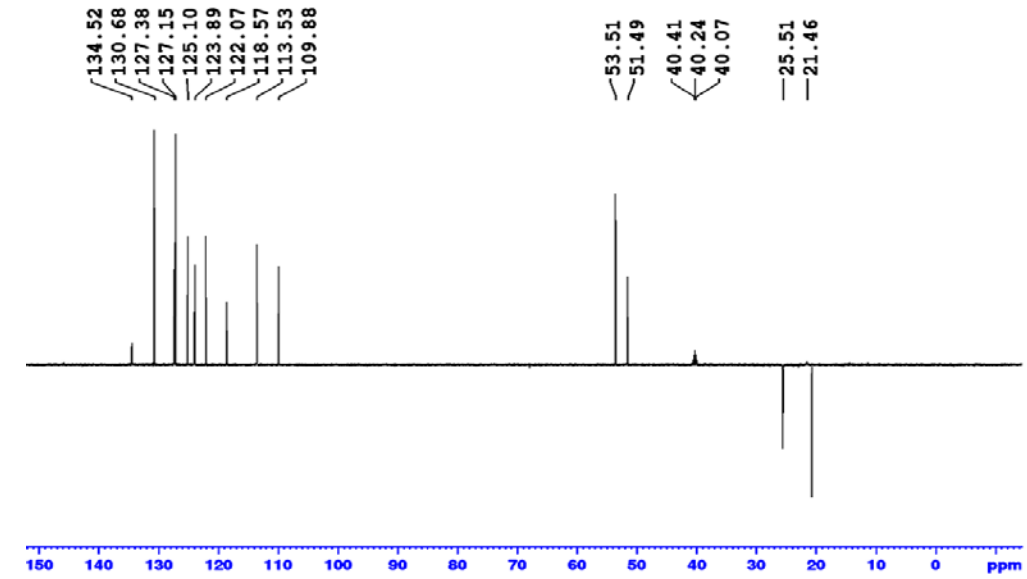

Figure S25. DEPT-135 NMR spectrum for 5c

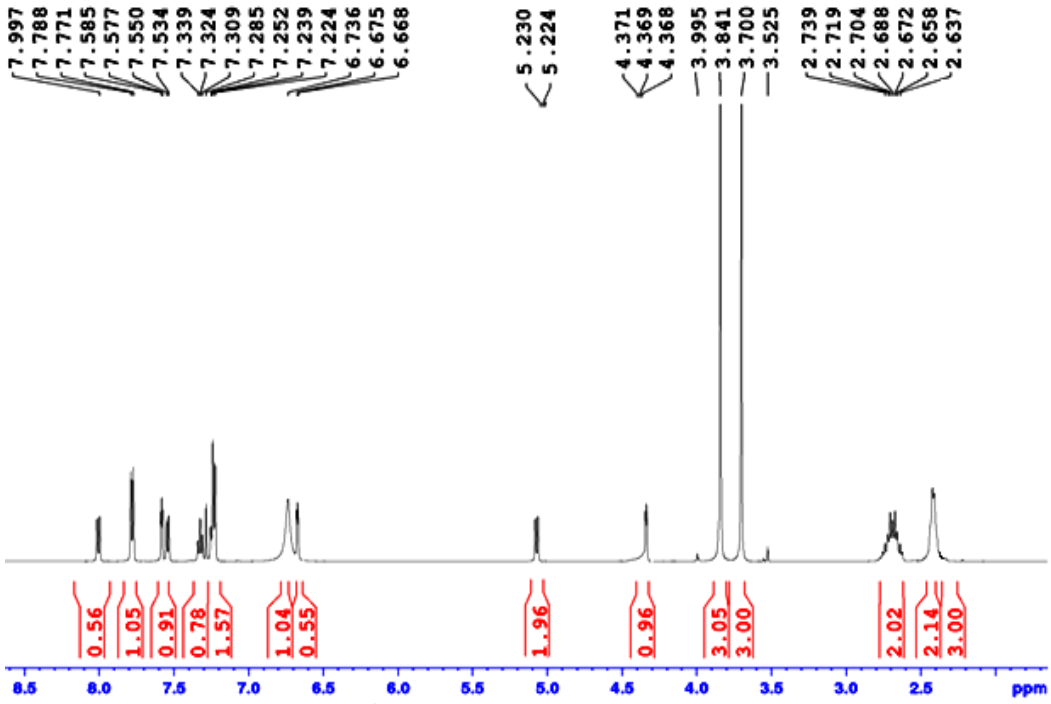

Figure S26. ^{1}H NMR spectrum of compound 5d

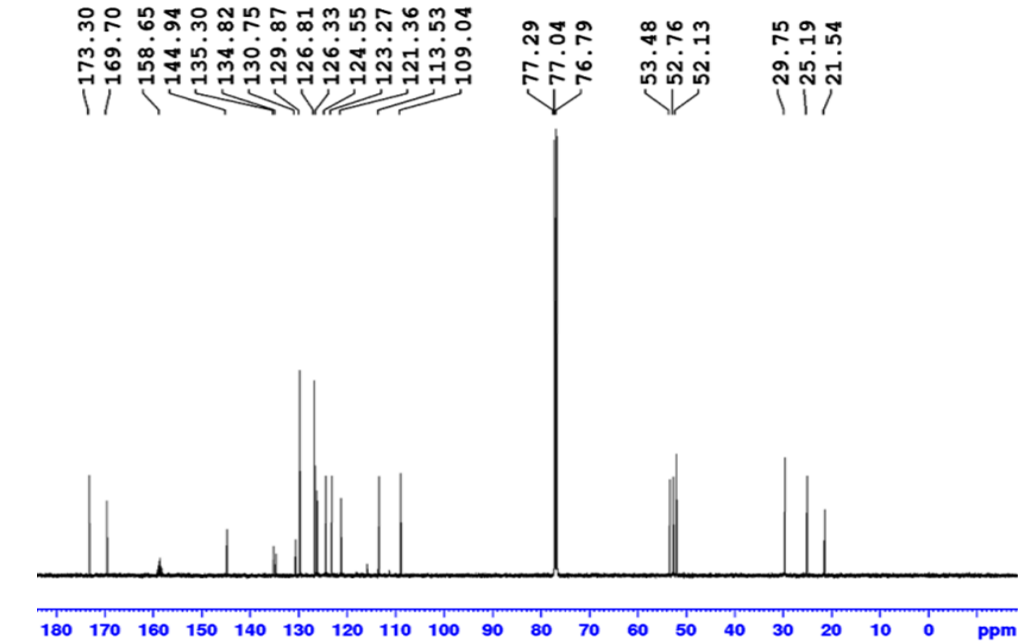

Figure S27. ^{13}C NMR spectrum of 5d

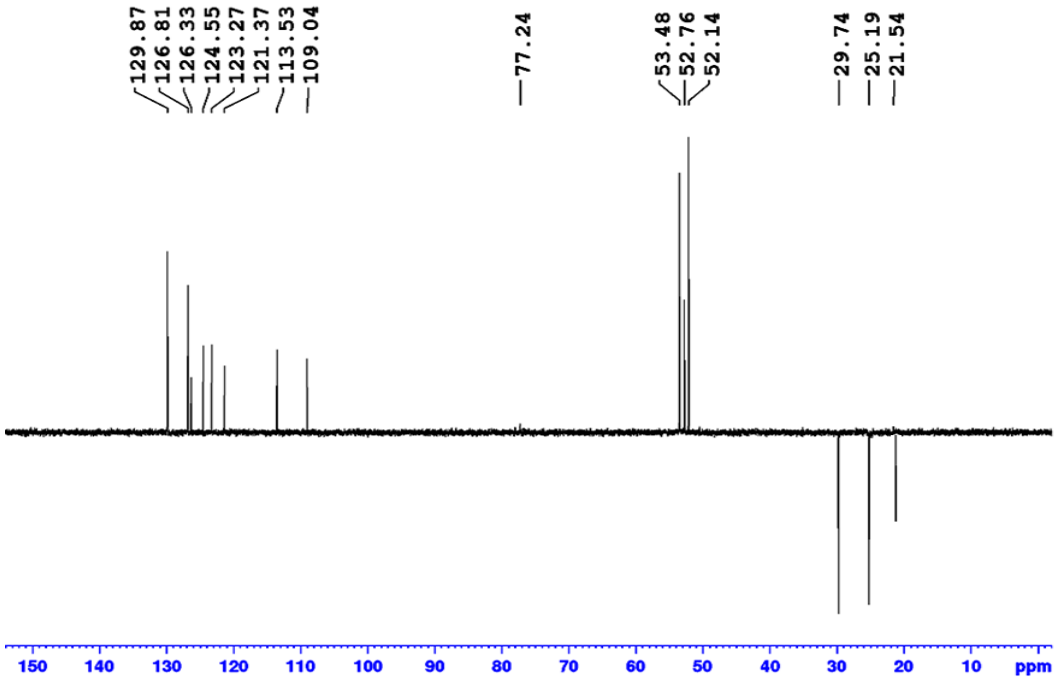

Figure S28. DEPT-135 NMR spectrum of 5d

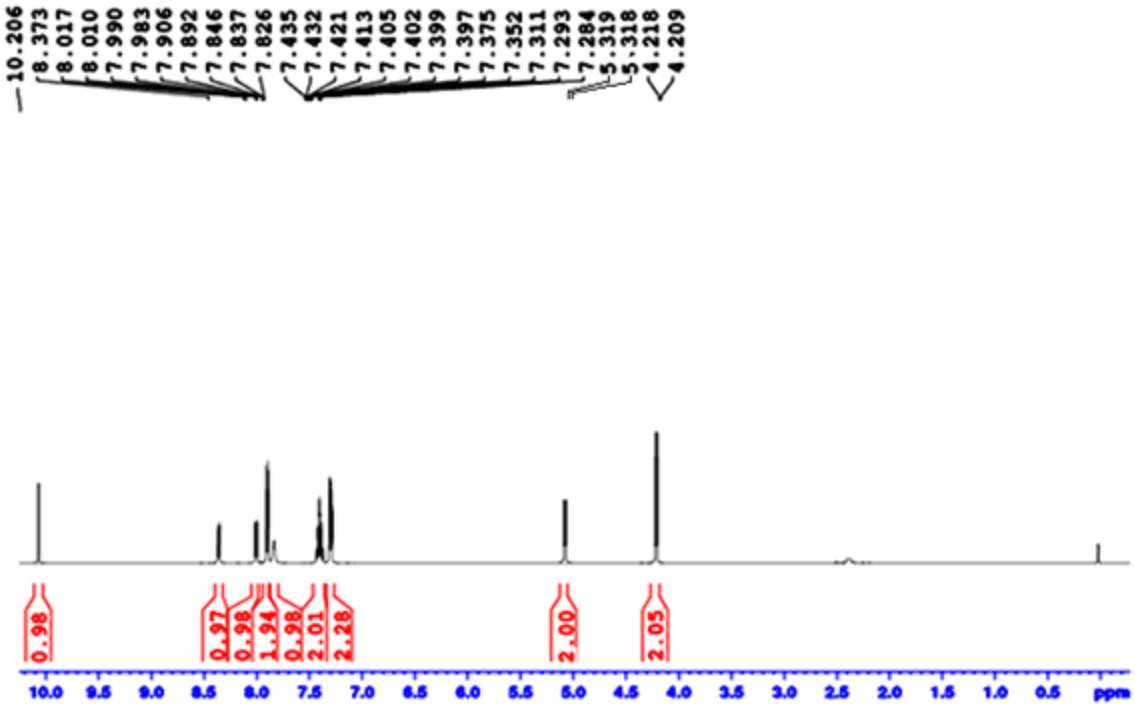

Figure S29. ^{1}H NMR spectrum of compound 6a

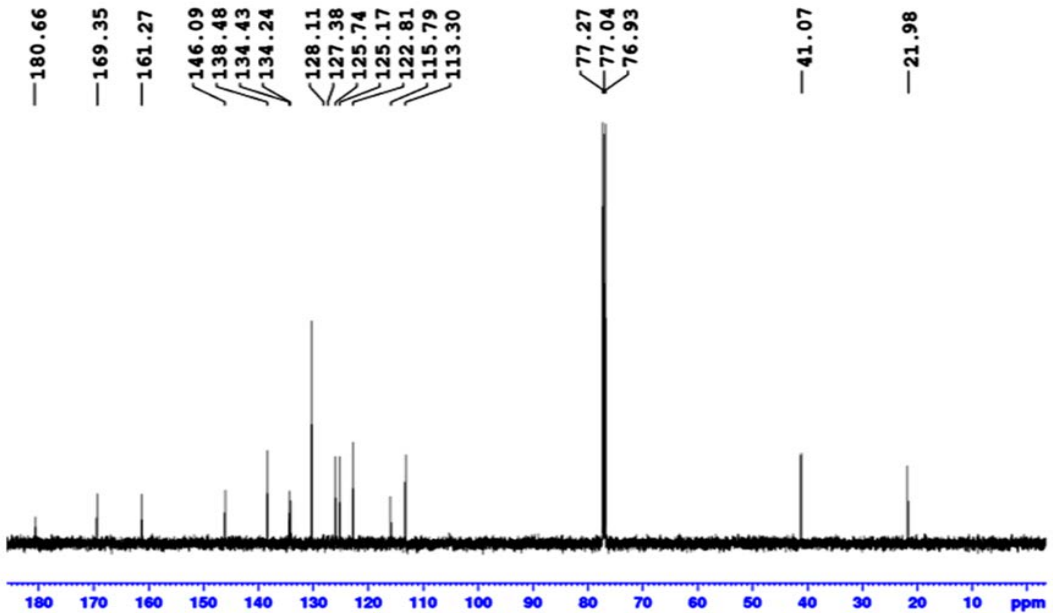

Figure S30. ^{13}C NMR spectrum of 6a

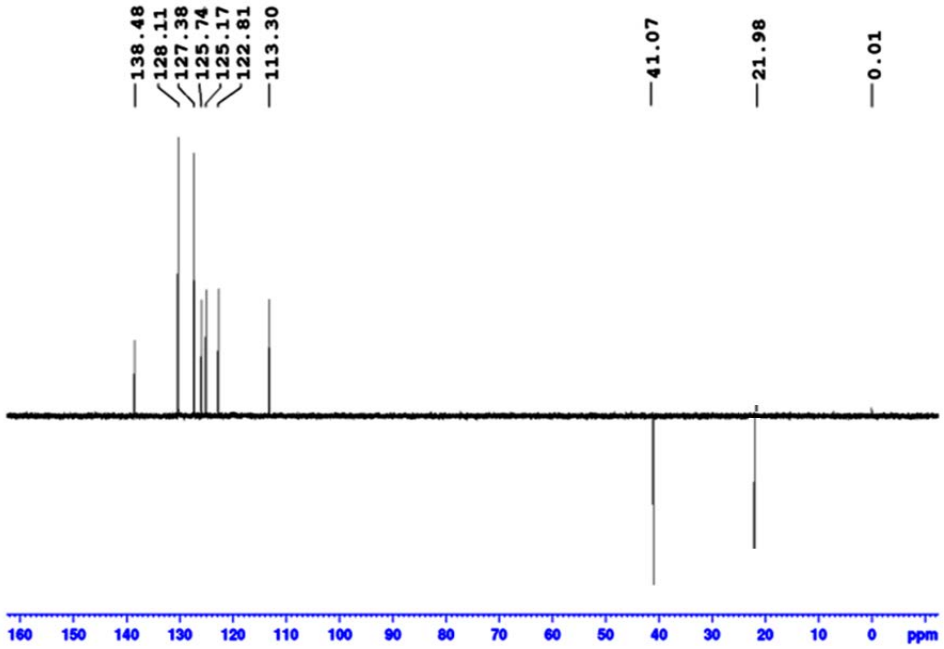

Figure S31. DEPT-135 NMR spectrum of 6a

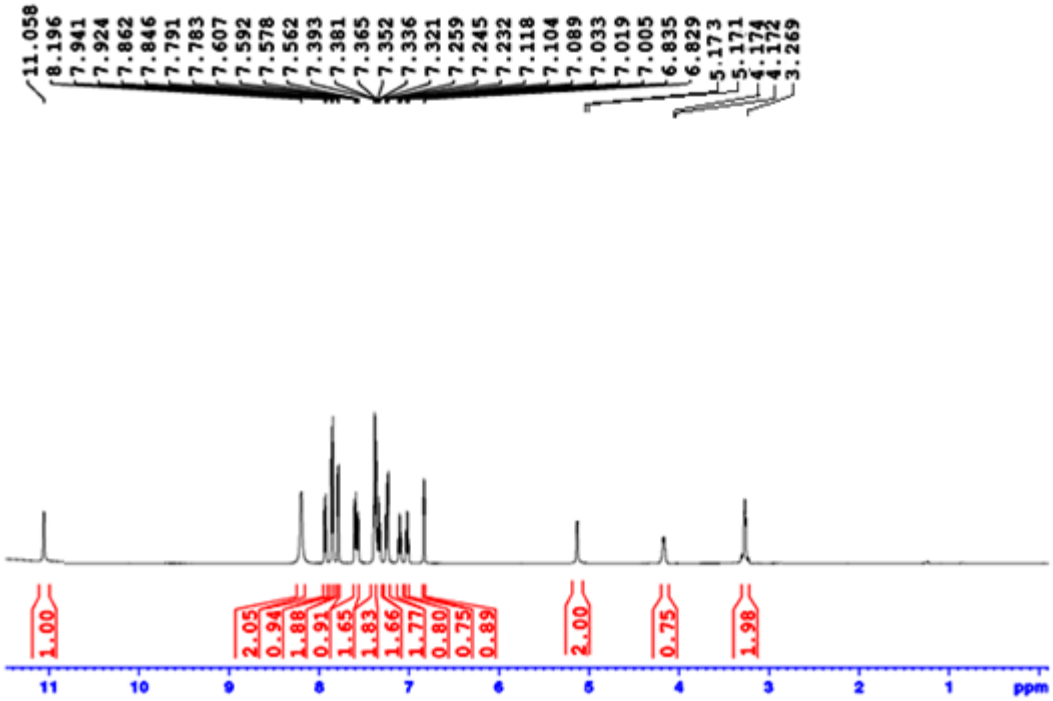

Figure S32. ^{1}H NMR spectrum of compound 6b

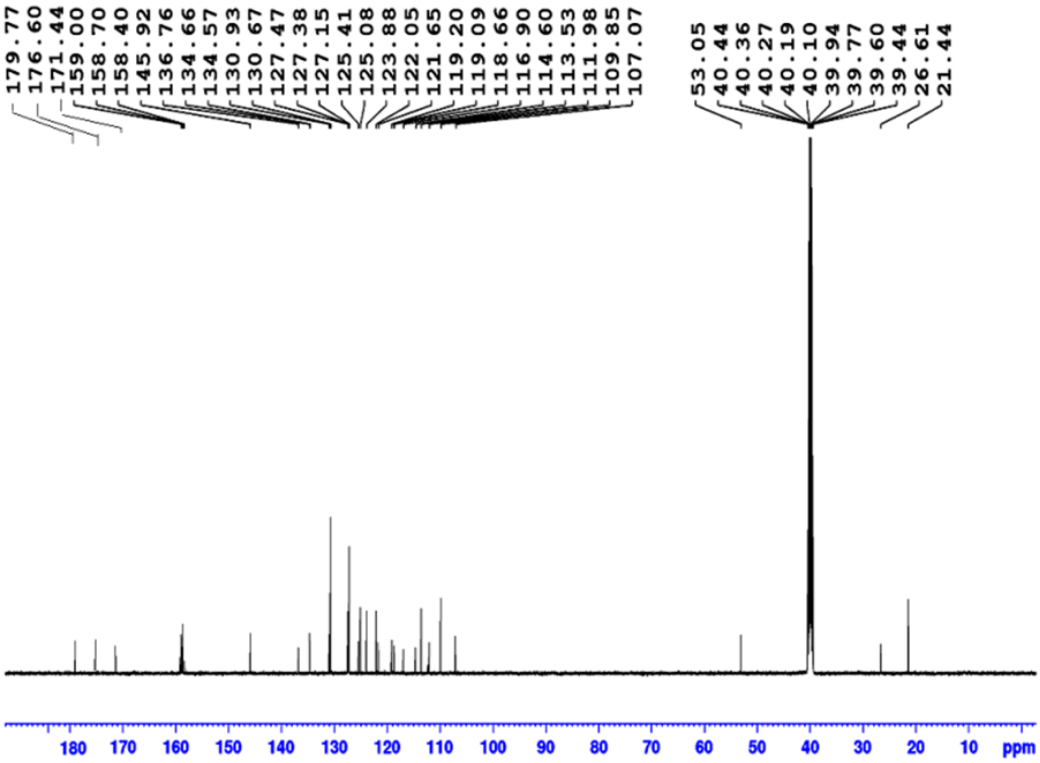

Figure S33. ^{13}C NMR spectrum of 6b

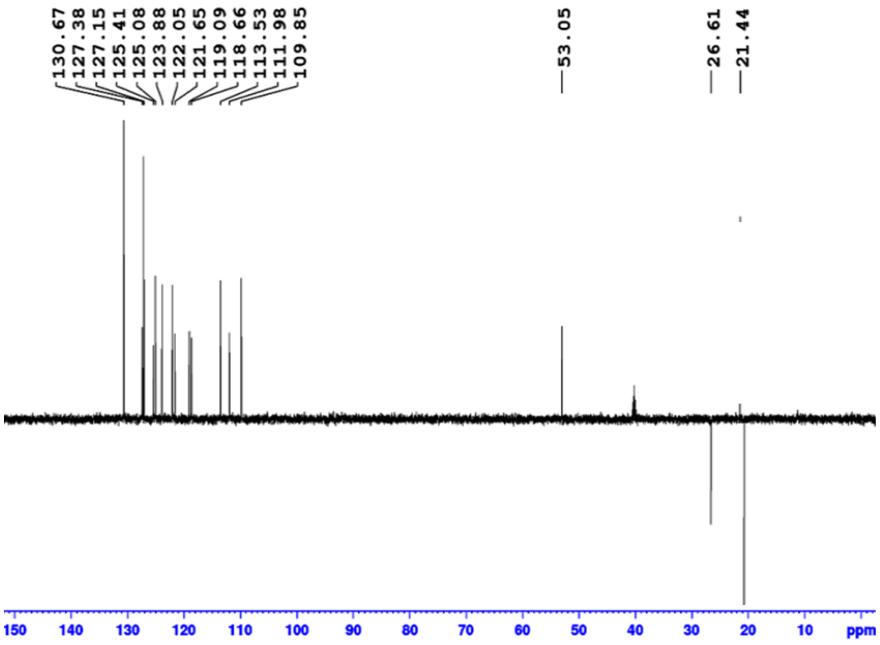

Figure S34. DEPT-135 NMR spectrum of 6b

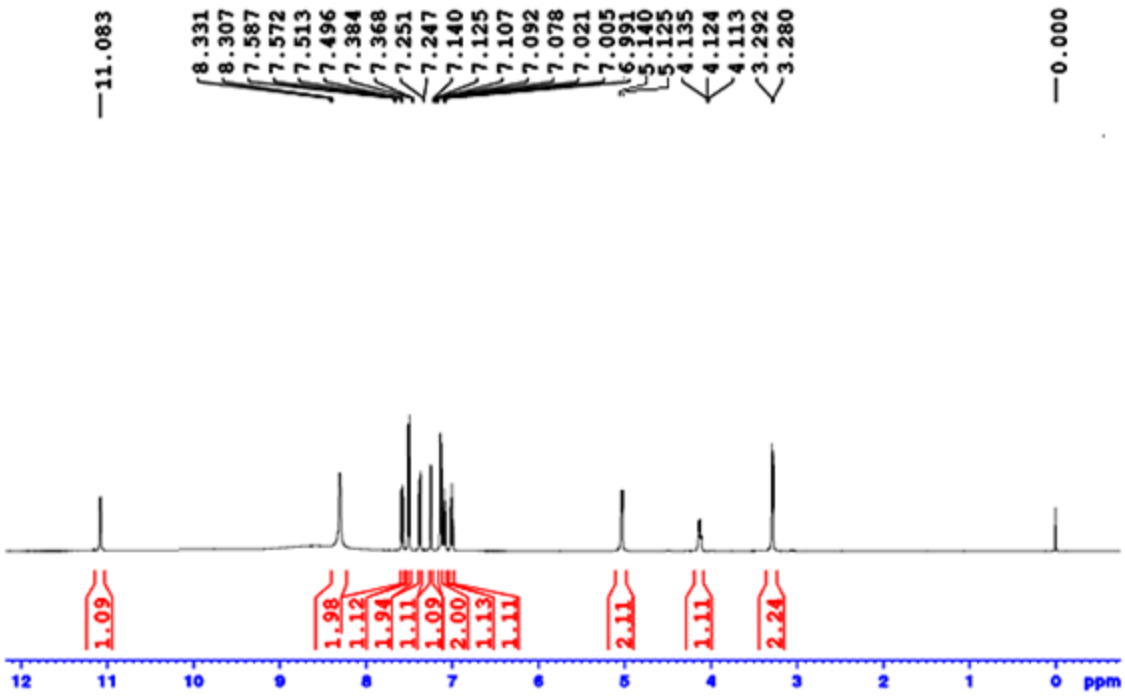

Figure S35. ^{1}H NMR spectrum of compound 6c

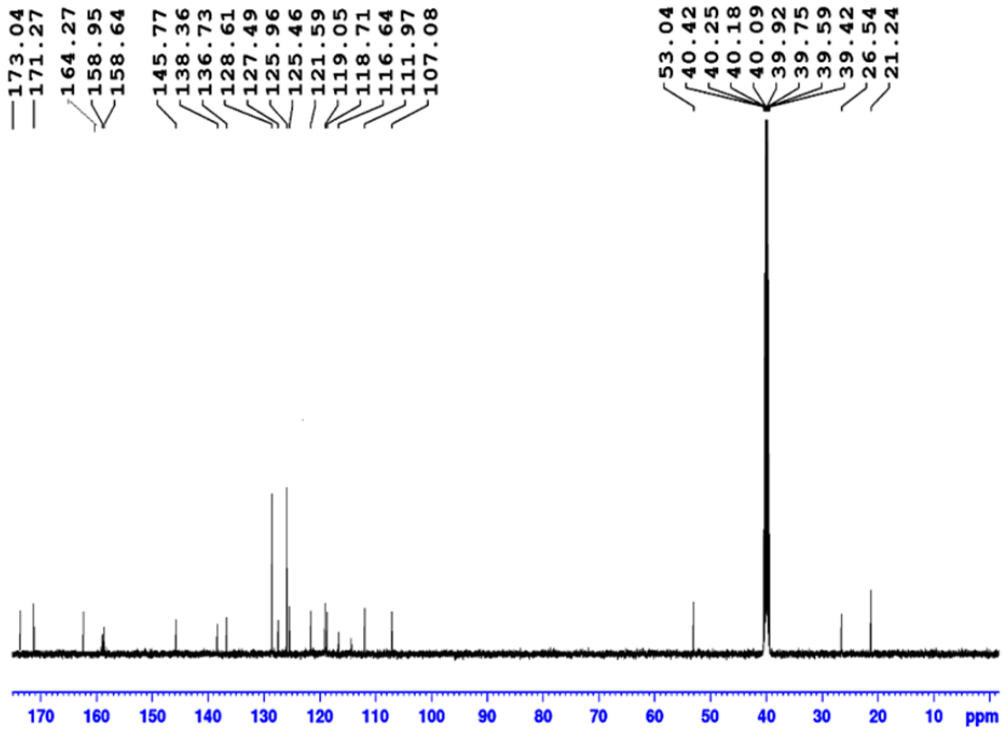

Figure S36. ^{13}C NMR spectrum of 6c

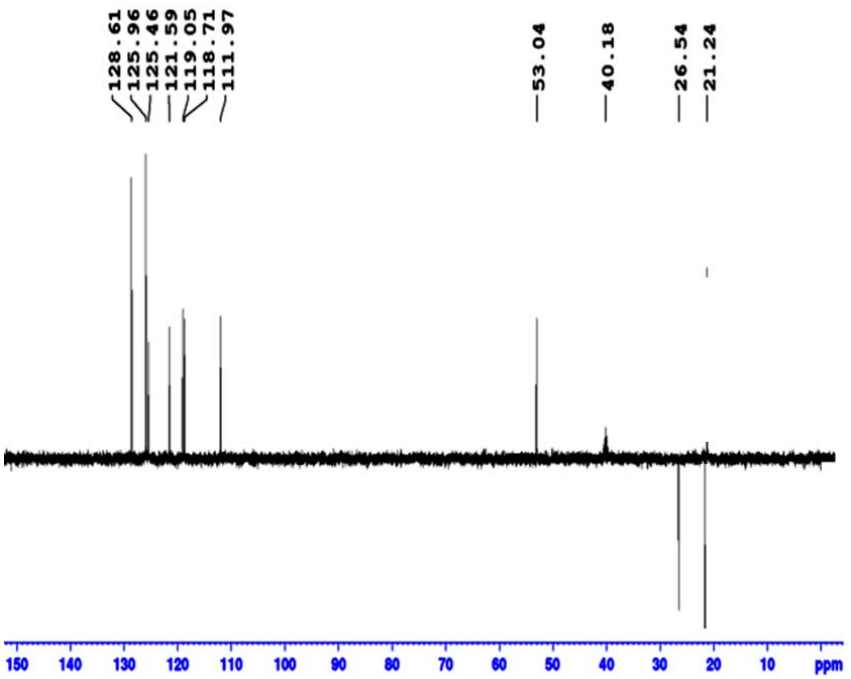

Figure S37. DEPT-135 NMR spectrum of 6c

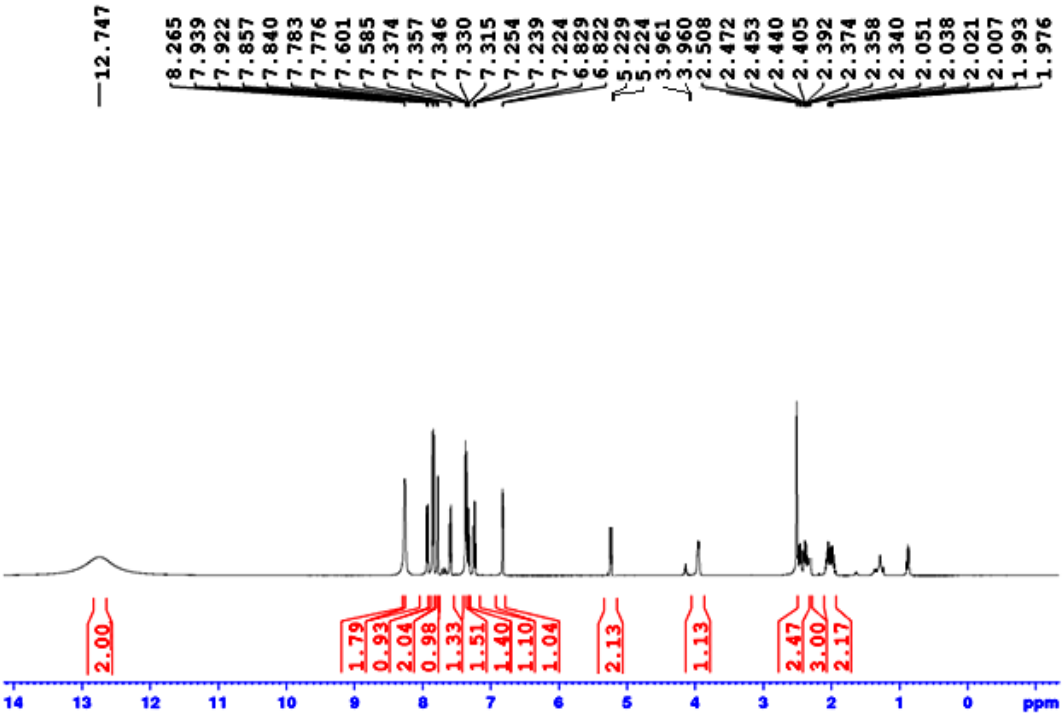

Figure S38. ^{1}H NMR spectrum of compound 6d

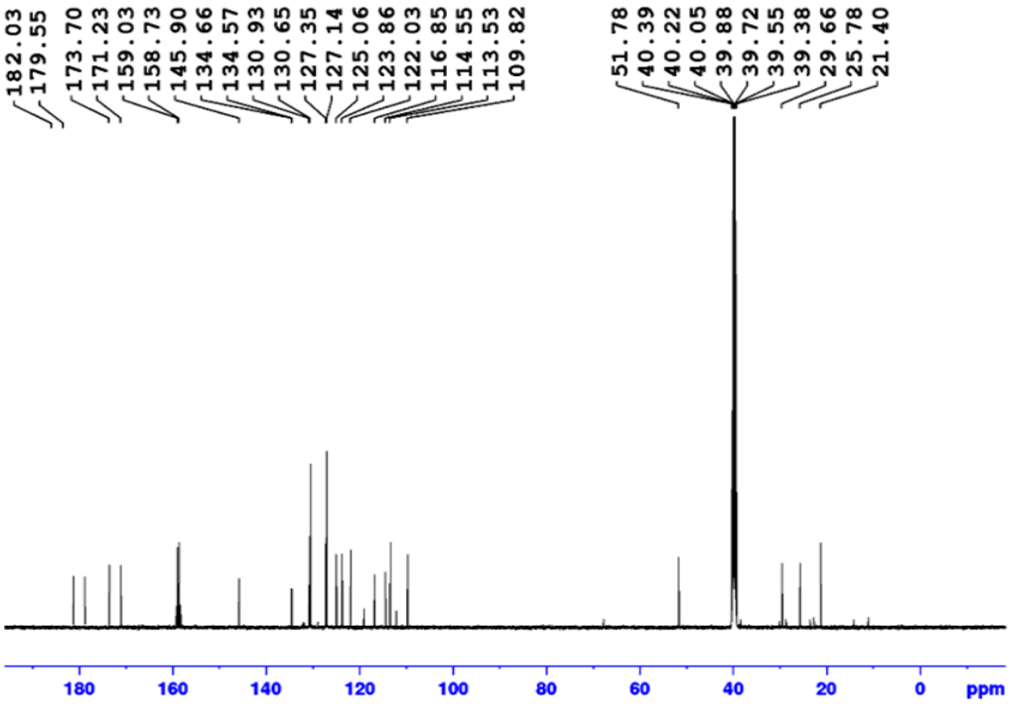

Figure S39. ^{13}C NMR spectrum of 6d

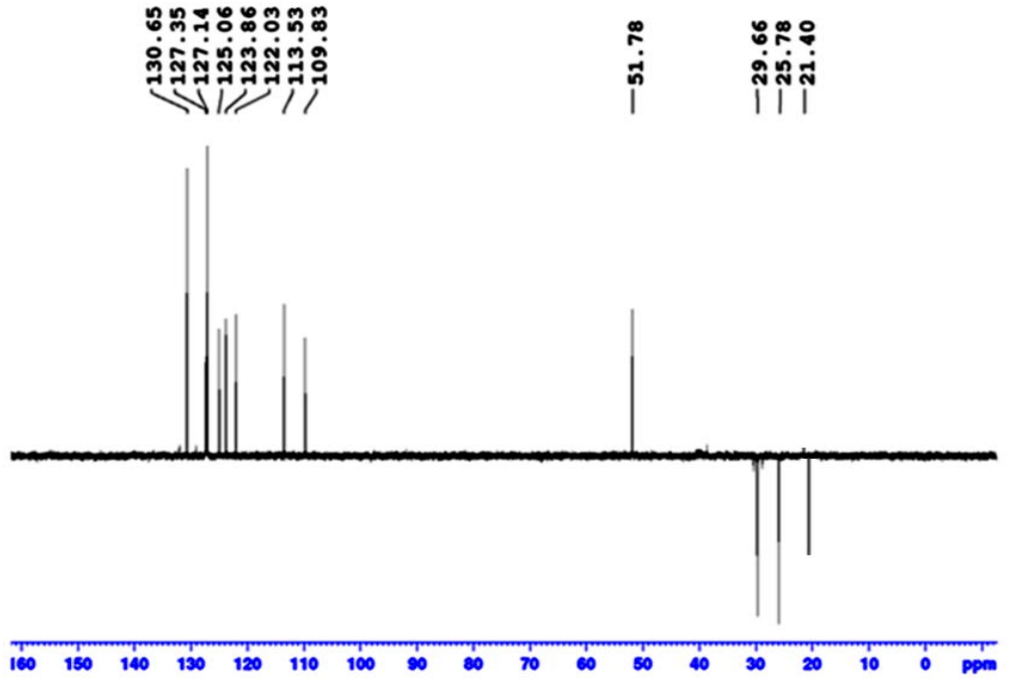

Figure S40. DEPT-135 NMR spectrum of 6d

HRMS analysis of compounds

a) **HRMS of the compound**

b) **Simulated HRMS for the compound**

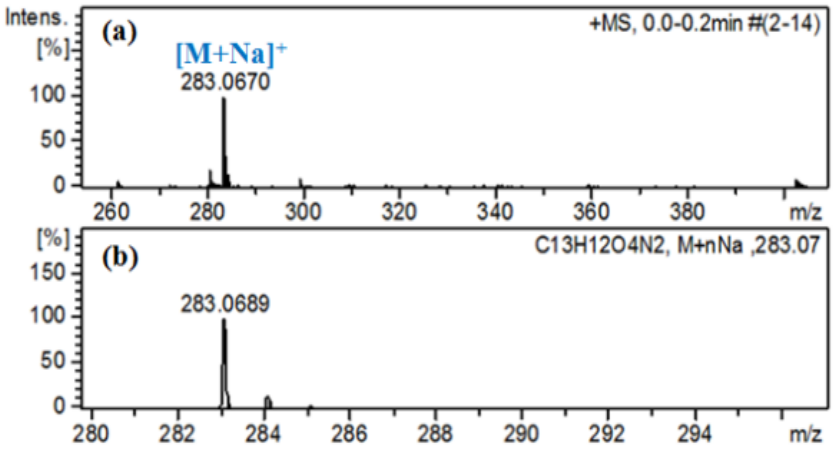

Figure S41. Compound **3a**

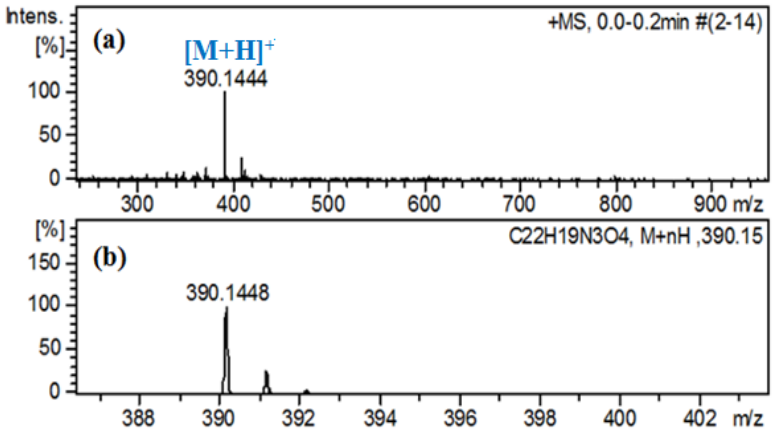

Figure S42. Compound **3b**

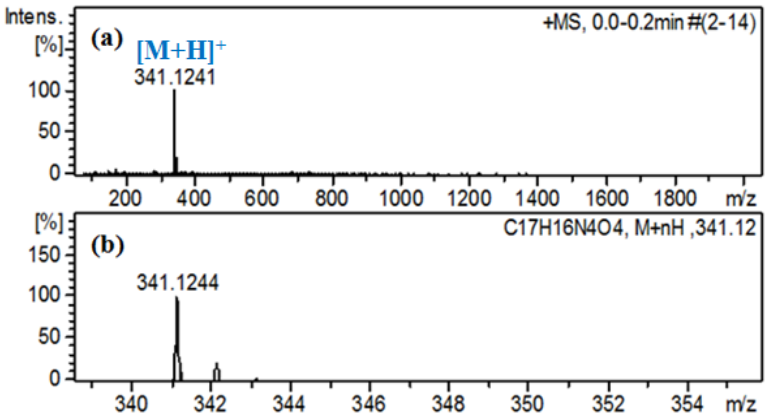

Figure S43. Compound **3c**

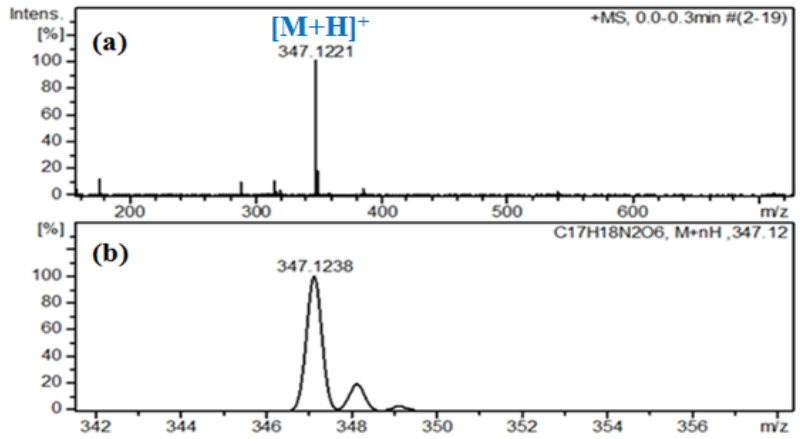

Figure S44. Compound **3d**

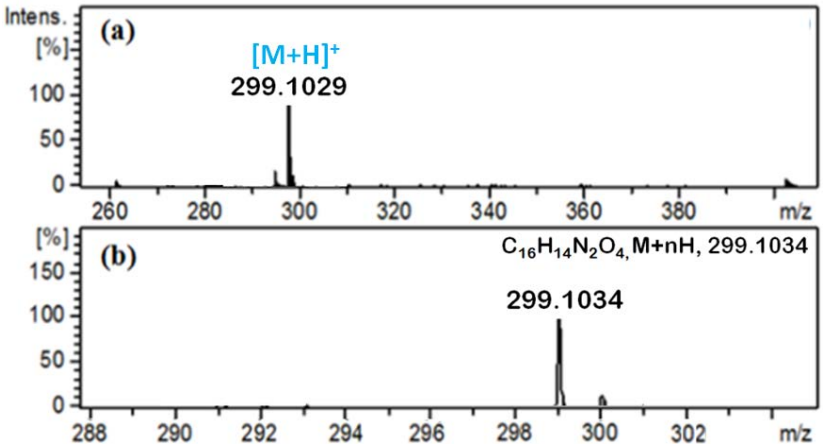

Figure S45. Compound **4a**

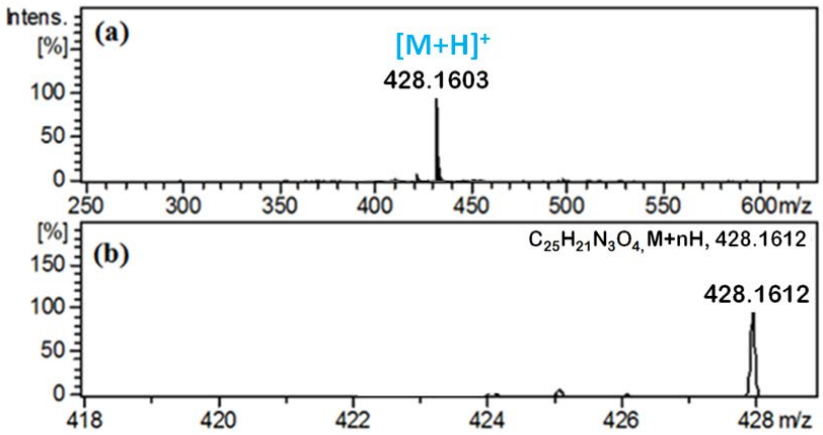

Figure S46. Compound **4b**

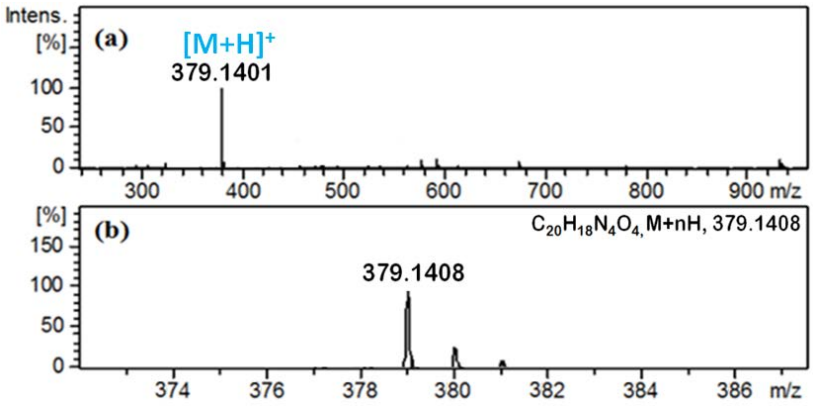

Figure S47. Compound **4c**

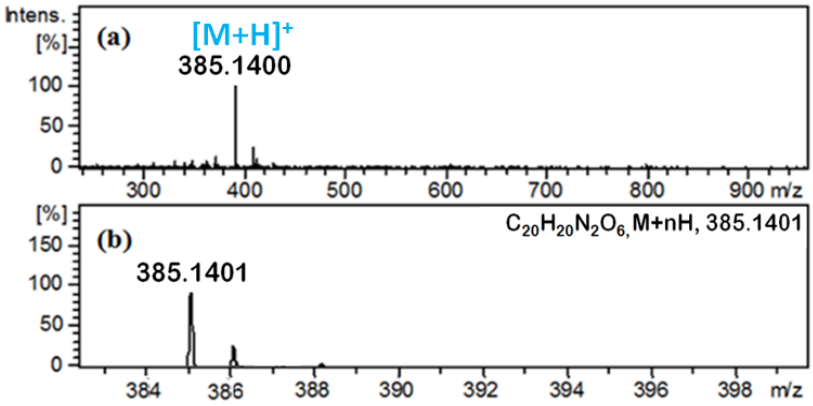

Figure S48. Compound **4d**

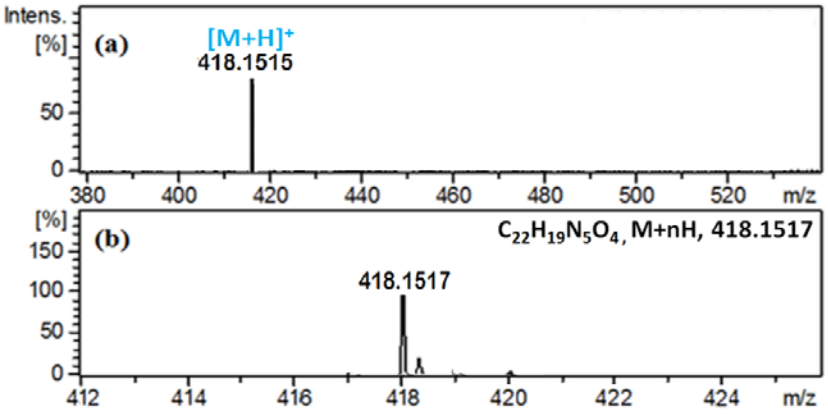

Figure S49. Compound **5a**

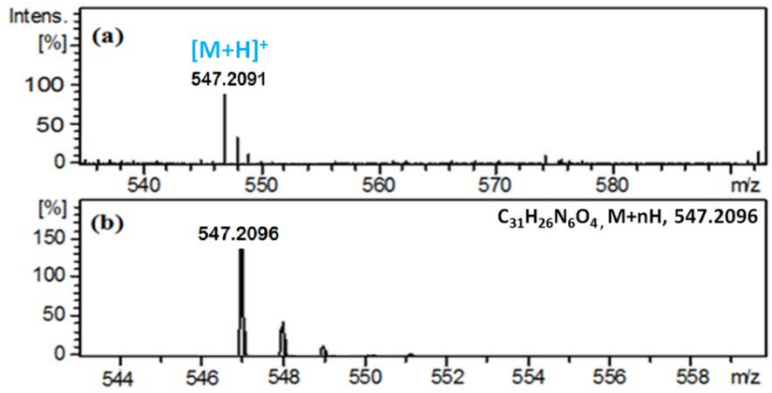

Figure S50. Compound **5b**

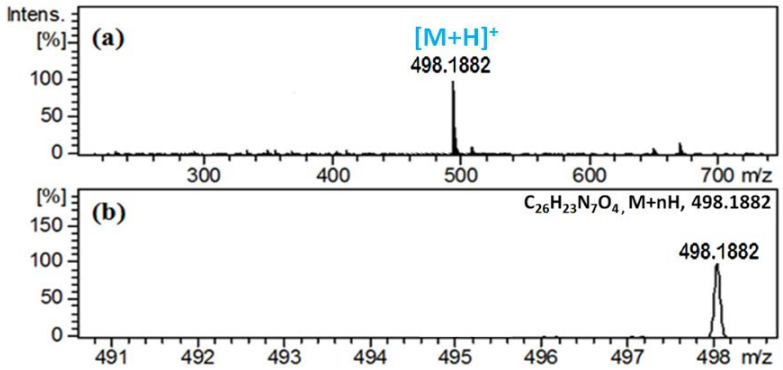

Figure S51. Compound **5c**

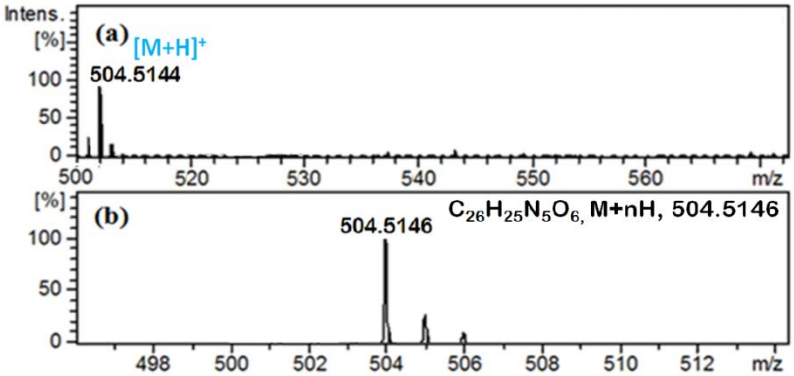

Figure S52. Compound **5d**

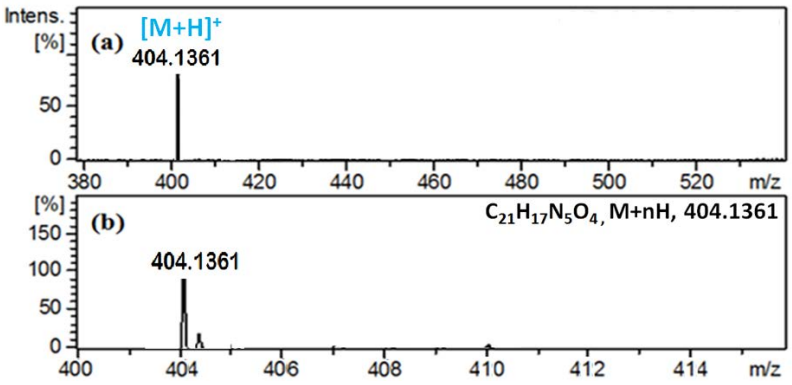

Figure S53. Compound **6a**

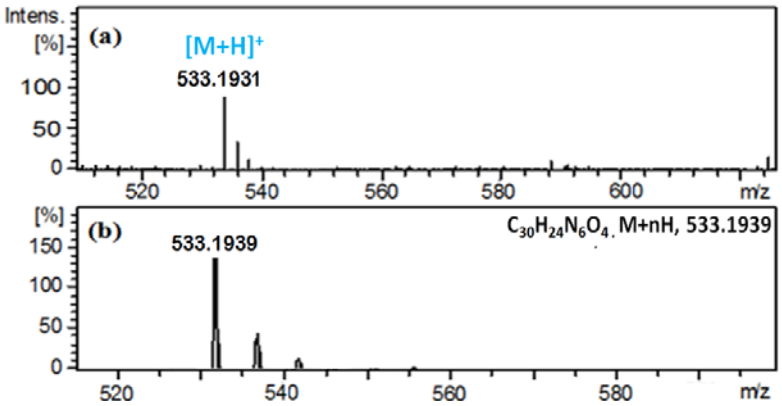

Figure S54. Compound **6b**

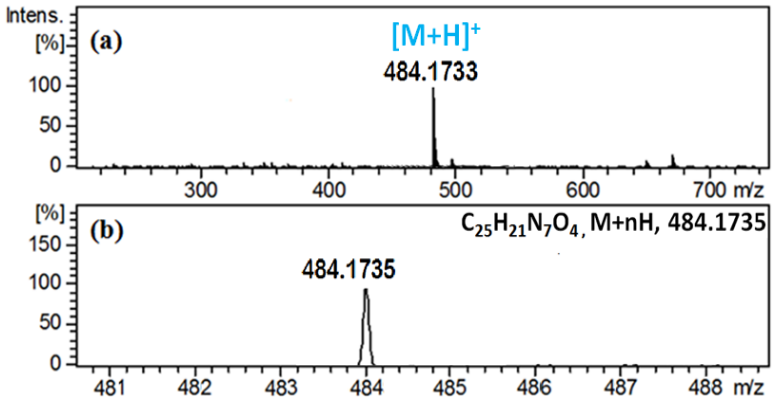

Figure S55. Compound **6c**

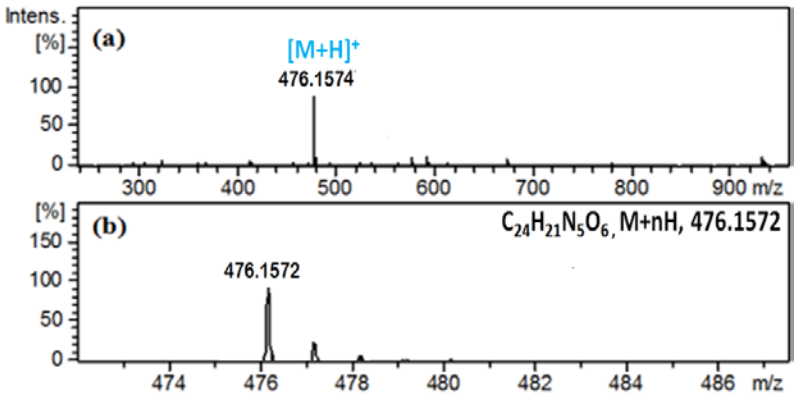

Figure S56. Compound **6d**

1.2 Biology

1.2.1 Disc Diffusion Assay

A 2% inoculum from overnight grown BWP17 cells grown in SD-Ura minimal medium was added to 10 ml of fresh media to set up secondary culture. After 5 h of growth 0.1 $O.D._{600nm}$ (10^7) cells were taken and spread on 90 mm SD-Ura plate. Plate was allowed to dry for 10 min and after drying sterile filter paper discs were placed. Compounds with different concentrations was spotted on these discs. Plates were dried, incubated at 30 ˚C for 48 h and photographs were taken. The formation of clear zone around the disc indicates the inhibitory effect of the compound.

1.2.2 Determination of Minimum Inhibitory Concentration for 80% Reduction of Growth (MIC_{80})

MIC_{80} was calculated by micro broth dilution method in 96 well flat bottomed ELISA plate of capacity 200 µl. 100 µl of SD-Ura minimal media was added in each well of row except 11th well where 200 µl of media was added and it acts as a positive control. 5.0 mg/ml of compound was added in the first well and two fold serial dilution was done from 1st well to 10th well. 100 µl of BWP17 0.01 OD_{600nm} (10^6) cells were added in each well of the row except 11th well. The concentration of compound now reduces to half in each well and becomes 2.5 mg/ml from 1st

well to 0.0048 mg/ml in 10^{th} well of the row.12^{th} well of the row where no compound was added acts as a positive control. Screening of the compound along with DMSO (solvent control) was done in duplicates. Plate was incubated at 30 ˚C for 48 h and growth was monitored by measuring the Optical density at 600nm using microplate reader. The percentage of fungal growth inhibition for each concentration of compound was calculated by using the formula (8).

Percentage inhibition= [{1-(OD_{600}C/OD_{600}P)}-{(1-(OD_{600}S/OD_{600}P)}] ×100

OD_{600}C, OD_{600}S andOD_{600}P corresponds to OD_{600nm} of compound well, solvent well and positive control well. The well having concentration of compound corresponding to 80% inhibition was taken as MIC_{80}.

1.2.3 Spot Assays

Spot Assays were done on different SD-Ura agar culture plates containing different concentrations of PPG2, azoles, CFW and their different combinations. 2% inoculum from overnight grown primary culture of BWP17 was added in fresh media to set up secondary culture. After 5 h of growth $0.1OD_{600nm}$ cells were taken and five times serial dilutions was done in 0.9% saline. 5 µl of cell suspension from each dilution corresponding to cell numbers 1×10^7, 2×10^6, 4×10^5, 8×10^4 and 1.6×10^4 was spotted on agar culture plates. Plates were allowed to incubate at 30 ˚C for 4-5 days and images were taken.

1.2.4 CFW Staining

BWP17 cells were allowed to grow at 30 ˚C in presence of PPG2, azole, CFW and their different combination. From each culture $0.2OD_{600nm}$ cells were taken for two different sets stained and unstained. Cells were pelleted, washed with PBS and 100 µg/ml of CFW was added in stained set. Both sets were incubated at 30 ˚C by keeping on rocker for 30 minutes. After 30 minutes

cells were again pelleted, washed and resuspended in 50% glycerol. 5 µl of cells were spotted on glass slides and observed under confocal microscope.

1.2.5 Propidium Iodide (PI) Staining

BWP17 cells were allowed to grow at 30 ˚C in the presence of PPG2, azole and their different combinations. From each culture $0.2OD_{600nm}$ cells were taken for two different sets stained and unstained. Cells were pelleted and washed with PBS. 5 µg/ml of PI was added in stained set. Both sets were incubated at 30 ˚C for 15 min. Cells were pelleted again, washed and resuspended in 50% glycerol. 5µl of cells were spotted on glass slides and observed under confocal microscope.

1.2.6 DAPI staining

BWP17 cells were allowed to grow in the presence of PPG2, azole and their combination at 30 ˚C. $0.2OD_{600nm}$ cells from each culture were taken for stained and unstained sets. Cells were pelleted and washed with PBS. 1 µg/ml of DAPI was added in stained set only and both sets were incubated at 30 ˚C for 30 min. Cells were again pelleted, washed and resuspended in 50% glycerol. 5µl of cells were spotted on glass slides and observed under confocal microscope.

1.2.7 Scanning Electron Microscopy

Scanning Electron Microscopy was done to monitor the morphological changes taking place in the cell.BWP17 cells were allowed to grow in presence of PPG2, ketoconazole and in combinations. Cells were pelleted, washed and fixed in 2.5% glutaraldehyde and 2% Paraformaldehyde in 0.1M phosphate buffer at 4 ˚C. Secondary fixing was done in Osmium tetraoxide (OsO_4) to enhance staining of intracellular membranes. Sample was dehydrated by successively treating the cells with more concentrated acetone (50-70-90-95%). Sample was then

dried with critical point drier and mounted on the stub SCM. Gold coating of cells was done under Spatter coater and viewed under Carl Zeiss Microscope EVO40 at 30 KV.