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Fusion of Morpholine and Schiff Base on Novel Benzimidazole Scaffold as Anti-microbial Agents: A Computational Approach and *In-vitro* Evaluation

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1. S1: GENERAL INFORMATION

The starting reactants of chemical compounds, reagents and solvent were procured from Sigma Aldrich, Himedia Ltd and Merck Ltd, those were HPLC graded components. The evaluation of melting point for synthesised compounds were performed by digital melting point apparatus by open capillary approach. The chromatogram for synthesised compounds were performed by Silica gel G TLC plates (Merck Ltd) and it was detected by iodine chamber. Further various spectroscopic techniques were utilized for characterisation of synthesised compounds such as λ max (Schimadzu Ultraviolet spectrometer), IR spectrum (Schimadzu FT-IR spectrophotometer) on KBr pellets, elemental analysis (CHNO Analyser), mass spectrum (HRMS mass spectrometer) and ^{1}HNMR spectrum (FT-NMR 500 MHz spectrometer) were evaluated from Indian institute of technology, Chennai and VIT university, Vellore.

2. S2: SYNTHETIC PROCEDURES

2.1. First Step: Synthesis of 4-(1H-Benzimidazol-2-yl)benzamine (1)

The 0.05 M of *o*-phenylenediamine (5.4 gm) dissolved in water (20 ml) with continuous stirring on heating. The 0.05 M of *p*-amino benzoic acid (PABA) (6.9 gm) and concentrated hydrochloric acid (20 ml) were added to the above mixture and refluxed for 2 h in water bath. Cooled it and neutralized with ammonia solution results in precipitation of product, filtered by vacuum suction and recrystallized with ethanol.

2.2. Second Step: Synthesis of 4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl)benzamine (2)

The mixture of 0.01 M of 4-(1*H*-benzimidazol-2-yl) benzamine (2.09 gm), 0.015 M of formaldehyde (0.45 gm) and morpholine (0.87 g; 0.01 mol) in ethanol (25 ml) were stirred by magnetic stirrer for 2 h. The resultant solution was further refluxed for 4 h on water bath, after that poured into crushed ice for cooling and mixed well. The resultant solid was filtered and recrystallized with ethanol.

3. S3: CHARACTERISTIC DATA FOR SYNTHETIC COMPOUNDS

3.1. Characteristic Data for Compound 1

Yield (%): 74; m.p. (°C): 248-251; IR (cm^{-1}): 1632 (C=C), 1657 (C=N), 3021 (Ar-CH), 3374 (NH). ^{1}H-NMR (δ:

ppm): 4.76 (s, 1H, NH), 5.41 (s, 2H, NH_2), 7.13-8.07 (m, 8H, Ar-H). ^{13}C-NMR (δ: ppm): 158.52 (C-2), 150.94 (C'-1), 137.27 (C-8 & C-9), 135.76 (C'-3 & C'-5), 123.31 (C-5 & C-6), 121.05 (C'-4), 119.63 (C'-2 & C'-5), 110.41 (C-4 & C-7). MS (EI) *m/z*: 209.310 (M^+). *Anal*. Calcd for $C_{13}H_{11}N_3$: C, 74.62; H, 5.30; N, 20.08. Found: C, 74.87; H, 5.28; N, 20.01.

3.2. Characteristic Data for Compound 2

Yield (%): 77; m.p. (°C): 274-275; IR (cm^{-1}): 1059 (C-O-C), 1616 (C=C), 1671 (C=N), 2938 (CH_2-CH), 3005 (Ar-CH), 3309 (NH). ^{1}H-NMR (δ: ppm): 2.71 (t, 4H, CH_2 of morpholine), 3.58 (t, 4H, CH_2 of morpholine), 4.93 (s, 2H, CH_2 linkage), 5.69 (s, 2H, NH_2), 6.90-7.85 (m, 8H, Ar-H). ^{13}C-NMR (δ: ppm): 149.74 (C-2), 147.13 (C'-1), 139.06 (C-9), 137.41 (C-8), 131.28 (C'-3 & C'-5), 126.81 (C-5 & C-6), 117.02 (C'-4), 115.63 (C'-2 & C'-5), 112.90 (C-4 & C-7), 61.72 (CH_2 linkage), 62.16 (C-2 & C-6 of morpholine), 45.05 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 308.151 (M^+). *Anal*. Calcd for $C_{18}H_{20}N_4O$: C, 70.11; H, 6.54; N, 18.17. Found: C, 70.32; H, 6.52; N, 18.11.

3.3. Characteristic Data for Compound 3a-3o

3.3.1. N-(2,4-Dichlorobenzylidene)-4-(1-(morpholinomethyl)-1H-benzoimidazol-2-yl) benzenamine (3a)

Yield (%): 72; m.p. (°C): 183-185; IR (cm^{-1}): 746 (C-Cl), 1075 (C-O-C), 1607 (C=C), 1642 (C=N), 2874 (CH_2-CH), 3039 (Ar-CH), 3117 (=CH). ^{1}H NMR: δ 2.15 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.91 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 4.67 (s, 2H, CH_2 linkage), 7.03-7.41 [4H, ArH, (7.03 (ddd *J* = 6.4 Hz), 7.19 (dd, *J* = 8.3 Hz), 7.33 (ddd, *J* = 8.3 Hz), 7.41 (dd, *J* = 1.7 Hz)], 7.46 (2H, ddd, *J* = 8.7 Hz), 7.53-7.92 [4H, ArH, (7.53 (ddd, *J* = 8.7 Hz), 7.83 (ddd, *J* = 5.0, Hz), 7.92 (ddd, *J* = 8.3 Hz)], 8.29 (1H, dd, *J* = 8.3 Hz), 8.42 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 164.93 (N=CH), 155.06 (C-2), 151.72 (C'-1), 144.93 (C-9), 143.52 (C"-4), 138.15 (C"-2), 136.42 (C-8), 133.81 (C"-6), 131.05 (C"-1), 128.92 (C"-3), 128.32 (C'-4), 127.83 (C'-3 & C'-5), 126.06 (C"-5), 120.61 (C-5 & C-6), 118.29 (C'-2 & C'-5), 110.93 (C-4 & C-7), 63.77 (CH_2 linkage), 63.13 (C-2 & C-6 of morpholine), 48.54 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 464.2110 (M^+); 466.0162 (M^{+2}). *Anal*. Calcd for $C_{25}H_{22}Cl_2N_4O$: C, 64.52; H, 4.76; N, 12.04. Found: C, 64.30; H, 4.75; N, 12.09.

3.3.2. N-(2,4-Difluorobenzylidene)-4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl)benzenamine (3b)

Yield (%): 76; m.p. (°C): 159-161; IR (cm^{-1}): 1033.42 (C-O-C), 1141.78(C-F), 1628.51 (C=C), 1681.81 (C=N), 2909.81 (CH_2-CH), 3034.34 (Ar-CH), 3062.75 (=CH). ^{1}H NMR: δ 2.97 (4H, ddd, *J* = 15.2 Hz, CH_2 of morpholine), 3.86 (4H, ddd, *J* = 12.6 Hz, CH_2 of morpholine), 5.10 (s, 2H, CH_2 linkage), 7.12-7.31 [6H, ArH, (7.12 (ddd *J* = 6.3 Hz), 7.18 (dd, *J* = 7.8 Hz), 7.21 (ddd, *J* = 1.6 Hz), 7.24 (dd, *J* = 8.1 Hz), 7.31 (2H, ddd, *J* = 8.7 Hz)], 7.91-8.25 [4H, ArH, (7.91 (ddd, *J* = 8.8 Hz), 7.95 (ddd, *J* = 8.1, Hz), 8.25 (ddd, *J* = 7.8 Hz)], 8.26 (1H, dd, *J* = 5.1 Hz), 8.69 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 171.53 (C"-4), 163.26 (C"-2), 161.90 (N=CH), 150.04 (C-2), 149.07 (C'-1), 142.86 (C-9), 138.26 (C-8), 134.66 (C"-6), 129.71 (C'-4), 127.13 (C'-3 & C'-5), 126.90 (C-5 & C-6), 123.04 (C'-2 & C'-5), 112.86 (C-4 & C-7), 110.24 (C"-1), 107.58 (C"-5), 101.90 (C"-3), 64.34 (CH_2 linkage), 64.13 (C-2 & C-6 of morpholine), 50.64 (C-3 & C-5 of morpholine) MS (EI) *m/z*: 432.0271 (M^+). *Anal*. Calcd for $C_{25}H_{22}F_2N_4O$: C, 69.43; H, 5.13; N, 12.96. Found: C, 69.69; H, 5.11; N, 12.92.

3.3.3. 4-((4-(1-(Morpholinomethyl)-1H-benzimidazol-2-yl)phenylimino)methyl)benzene-1,3-diol (3c)

Yield (%): 81; m.p. (°C): 147-149; IR (cm^{-1}): 1058 (C-O-C), 1631 (C=C), 1666 (C=N), 2940 (CH_2-CH), 3005 (Ar-CH), 3082 (=CH), 3387 (OH). ^{1}H NMR: δ 2.48 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.62 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 5.10 (s, 2H, CH_2 linkage), 5.63 (s, 1H, OH), 5.87 (s, 1H, OH), 6.75 (1H, ArH, dd, *J* = 2.1 Hz), 6.89 (1H, ArH, dd, *J* = 8.1 Hz), 7.41-7.53 [4H, ArH, 7.41 (ddd, *J* = 5.9 Hz), 7.46 (ddd, *J* = 8.5 Hz), 7.53 (ddd, *J* = 8.0, 5.9, 1.4 Hz)], 7.67-7.83 [4H, ArH, (7.67 (ddd, *J* = 8.5 Hz), 7.78 (dd, *J* = 8.1Hz)), 7.83 (ddd, *J* = 8.0 Hz)], 7.91 (1H, ddd, *J* = 5.1Hz), 8.57 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 168.32 (C"-2), 167.64 (C"-4), 162.52 (N=CH), 157.93 (C-2), 155.42 (C'-1), 140.14 (C-9), 136.73 (C-8), 133.52 (C"-6), 130.83 (C'-4), 129.04 (C'-3 & C'-5), 121.63 (C-5 & C-6), 121.13 (C'-2 & C'-5), 119.45 (C-4 & C-7), 105.82 (C"-1), 103.25 (C"-5), 99.04 (C"-3), 61.43 (CH_2 linkage), 60.92 (C-2 & C-6 of morpholine), 47.63 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 428.1214 (M^+). *Anal*. Calcd for $C_{25}H_{24}N_4O_3$: C, 70.08; H, 5.65; N, 13.08. Found: C, 70.30; H, 5.67; N, 13.04.

3.3.4. N-(2,4-Dibromobenzylidene)-4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl)benzenamine (3d)

Yield (%): 75; m.p. (°C): 199-201; IR (cm^{-1}): 645 (C-Br), 1071 (C-O-C), 1609 (C=C), 1643 (C=N), 2920 (CH_2-CH), 3012 (Ar-CH), 3107 (=CH). ^{1}H NMR: δ 2.63 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.97 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 4.65 (s, 2H, CH_2 linkage), 6.98-7.02 [2H, 6.98 (ddd, *J* = 6.6 Hz), 7.02 (dd, *J* = 1.7 Hz)], 7.05-7.33 [4H, 7.05 (ddd, *J* = 8.6 Hz), 7.08 (dd, *J* = 8.3 Hz), 7.29 (ddd, *J* = 8.3 Hz)], 7.43-7.82 [4H, 7.43 (ddd, *J* = 8.6 Hz), 7.52 (ddd, *J* = 5.0 Hz), 7.65 (ddd, *J* = 8.3 Hz)], 7.97 (1H, dd, *J* = 8.3 Hz), 8.31 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 157.42 (N=CH), 154.27 (C-2), 152.53 (C'-1), 140.84 (C-9), 137.06 (C"-3), 136.64 (C"-1), 136.23 (C-8), 134.92 (C"-6), 132.14 (C"-5), 127.55 (C'-4), 126.94 (C'-3 & C'-5), 126.37 (C"-4), 122.04 (C"-2), 121.44 (C-5 & C-6), 120.83 (C'-2 & C'-5), 113.15 (C-4 & C-7), 60.92 (CH_2 linkage), 59.73 (C-2 & C-6 of morpholine), 48.25 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 552.1017 (M^+); 554.2173 (M^{+2}). *Anal*. Calcd for $C_{25}H_{22}Br_2N_4O$: C, 54.17; H, 4.00; N, 10.11. Found: C, 54.35; H, 3.99; N, 10.07.

3.3.5. N-(2,4-Dimethoxybenzylidene)-4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl) benzenamine (3e)

Yield (%): 73; m.p. (°C): 165-167; IR (cm^{-1}): 1141.90 (C-O-C), 1620.19 (C=C), 1681.81 (C=N), 2835.16 (CH_2-CH), 3012.75 (Ar-CH), 3105.10 (=CH). ^{1}H NMR: δ 2.07 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.76 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 3.95 (s, 3H, OCH_3), 4.12 (s, 3H, OCH_3), 4.95 (s, 2H, CH_2 linkage), 6.95-6.97 [2H, 6.95 (ddd, *J* = 2.1 Hz), 6.97 (dd, *J* = 8.1 Hz)], 7.41-7.78 [4H, 7.46 (ddd, *J* = 5.8 Hz), 7.53 (dd, *J* = 8.5 Hz), 7.67 (ddd, *J* = 7.8 Hz)], 7.81-7.90 [4H, 7.81 (ddd, *J* = 8.5 Hz), 7.83 (ddd, *J*

= 8.1 Hz), 7.90 (ddd, *J* = 7.8 Hz)], 7.93 (1H, dd, *J* = 5.8 Hz), 8.49 (s, 1H, N=CH).[13]C-NMR (δ: ppm): 168.10 (C”-4), 165.02 (C”-2), 162.12 (N=CH), 154.90 (C-2), 153.49 (C'-1), 143.02 (C-9), 138.26 (C-8), 133.61 (C”-6), 130.81 (C'-4), 129.52 (C'-3 & C'-5), 126.10 (C-5 & C-6), 125.75 (C'-2 & C'-5), 113.45 (C-4 & C-7), 108.90 (C”-1), 106.26 (C”-5), 93.52 (C”-3), 64.82 (CH_2 linkage), 64.32 (C-2 & C-6 of morpholine), 53.11 (OCH_3), 52.75 (OCH_3), 50.45 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 456.5338 (M^+). *Anal*. Calcd for $C_{27}H_{28}N_4O_3$: C, 71.03; H, 6.18; N, 12.27. Found: C, 70.79; H, 6.20; N, 12.32.

3.3.6. N-(2,4-Dinitrobenzylidene)-4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl)benzenamine (3f)

Yield (%): 80; m.p. (°C): 153-154; IR (cm^{-1}): 1048 (C-O-C), 1352 & 1589 (NO_2), 1605 (C=C), 1660 (C=N), 2917 (CH_2-CH), 3039 (Ar-CH), 3081 (=CH). ^{1}H NMR: δ 2.50 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.59 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 4.81 (s, 2H, CH_2 linkage), 6.72 (1H, ArH, ddd, *J* = 7.8 Hz), 7.41 (1H, ArH, ddd, *J* = 7.8 Hz), 7.46 (1H, ArH, ddd, *J* = 7.4 Hz), 7.46-7.81 [(6H, ArH, 7.53 (1H, dd, *J* = 7.3 Hz), 7.67 (2H, ddd, *J* = 6.9 Hz), 7.78 (2H, ddd, *J* = 6.9 Hz), 7.81 (1H, ddd, *J* = 5.0 Hz)], 7.83 (1H, ArH, dd, *J* = 7.3 Hz), 7.93 (1H, ArH, dd, *J* = 1.8 Hz), 8.35 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 160.53 (N=CH), 155.74 (C-2), 155.93 (C'-1), 154.22 (C”-4), 150.64 (C”-2), 139.43 (C-9), 133.02 (C-8), 130.87 (C”-1), 130.24 (C”-6), 127.54 (C'-4), 126.93 (C'-3 & C'-5), 126.77 (C”-5), 121.22 (C-5 & C-6), 120.46 (C'-2 & C'-5), 115.04 (C”-3), 114.91 (C-4 & C-7), 62.88 (CH_2 linkage), 62.36 (C-2 & C-6 of morpholine), 54.52 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 486.1065 (M^+). *Anal*. Calcd for $C_{25}H_{22}N_6O_5$: C, 61.72; H, 4.56; N, 17.28. Found: C, 61.91; H, 4.54; N, 17.22.

3.3.7. N-Benzylidene-4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl)benzenamine (3g)

Yield (%): 78; m.p. (°C): 178-180; IR (cm^{-1}): 1036 (C-O-C), 1614 (C=C), 1635 (C=N), 2919 (CH_2-CH), 3017 (Ar-CH), 3128 (=CH). ^{1}H NMR: δ 2.19 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.85 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 4.63 (s, 2H, CH_2 linkage),7.20-7.65 [4H, 7.20 (ddd, *J* = 7.5 Hz), 7.43 (ddd, *J* = 8.1 Hz), 7.55 (2H), dddd, J = 7.8 Hz)], 7.72-8.44 [7H, ArH, 7.82 (tdd, *J* = 7.4 Hz), 7.98 (ddd, *J* = 5.0 Hz), 8.09 (2H, ddd, *J* = 8.3 Hz), 8.44 (2H, ArH, ddd, J = 8.3 Hz), 8.53 (ddd, J = 8.1 Hz)], 8.67 (2H, ArH, dtd, J = 7.8 Hz), 8.86 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 165.06 (N=CH), 156.72 (C-2), 155.43 (C'-1), 139.53 (C-9), 136.04 (C-8), 134.83 (C”-1), 130.42 (C”-4), 128.92 (C”-2 & C”-6), 128.33 (C'-4), 128.07 (C”-3 & C”-5), 126.63 (C'-3 & C'-5), 121.82 (C-5 & C-6), 120.54 (C'-2 & C'-5), 114.74 (C-4 & C-7), 63.13 (CH_2 linkage), 62.96 (C-2 & C-6 of morpholine), 44.33 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 396.1357 (M^+). *Anal*. Calcd for $C_{25}H_{24}N_4O$: C, 75.73; H, 6.10; N, 14.13. Found: C, 75.99; H, 6.08; N, 14.07.

3.3.8. 4-(1-(Morpholinomethyl)-1H-benzimidazol-2-yl)-N-(3-phenylallylidene)benzenamine (3h)

Yield (%): 72; m.p. (°C): 189-191; IR (cm^{-1}): 1052 (C-O-C), 1617 (C=C), 1639 (C=N), 2874 (CH_2-CH), 3013 (Ar-CH), 3135 (=CH). ^{1}H NMR: δ 2.82 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.70 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 5.27 (s, 2H, CH_2 linkage), 5.78 (1H, dd, *J* = 17.7 Hz, CH=CH), 6.50 (1H, d, *J* =17.7 Hz, CH=CH), 7.03-7.20 [3H, ArH, 7.03 (1H, ddd, *J* = 6.4 Hz), 7.20 (dddd, *J* = 8.0 Hz)], 7.21-8.36 [10H, ArH, 7.56 (2H, dtd, *J* = 8.0 Hz), 7.57 (1H, tt, *J* = 6.5 Hz), 7.60 (1H, ddd, *J* = 8.1 Hz), 7.79 (2H, ddd, *J* = 8.1 Hz), 7.83 (2H, ddd, *J* = 8.1 Hz), 8.12 (1H, ddd, *J* = 5.1 Hz), 8.22 (1H, ddd, *J* = 8.1 Hz)], 8.86 (d, 1H, *J* = 8.1 Hz, N=CH).[13]C-NMR (δ: ppm): 155.43 (N=CH), 150.04 (C-2), 148.72 (C'-1), 142.15 (C-9), 139.68 (N=CH-CH=CH-), 138.04 (C”-1), 137.93 (C-8), 133.52 (C'-4), 129.84 (C'-3 & C'-5), 129.42 (C”-3 & C”-5), 128.62 (C”-4), 122.13 (C”-2 & C”-6), 121.81 (C-5 & C-6), 122.12 (C'-2 & C'-5), 117.52 (N=CH-CH=CH-), 111.74 (C-4 & C-7), 66.90 (CH_2 linkage), 66.44 (C-2 & C-6 of morpholine), 50.22 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 422.3422 (M^+). *Anal*. Calcd for $C_{27}H_{26}N_4O$: C, 76.75; H, 6.20; N, 13.26. Found: C, 76.49; H, 6.22; N, 13.31.

3.3.9. 4-(1-(Morpholinomethyl)-1H-benzimidazol-2-yl)-N-(pyridin-4-ylmethylene)benzenamine (3i)

Yield (%): 79; m.p. (°C): 197-198; IR (cm^{-1}): 1040 (C-O-C), 1608 (C=C), 1653 (C=N), 2931 (CH_2-CH), 3006 (Ar-CH), 3059 (=CH). ^{1}H NMR: δ 2.30 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.63 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 4.95 (s, 2H, CH_2 linkage), 6.82-6.91 [4H, ArH, 6.82 (1H, ddd, *J* = 6.0 Hz), 6.90 (1H, ddd, *J* = 8.1 Hz), 6.91 (2H, ddd, *J* = 5.1 Hz)], 7.32-7.98 [6H, ArH, 7.32 (2H, ddd, *J* = 8.3 Hz), 7.65 (2H, ddd, *J* = 8.3 Hz), 7.75 (1H, ddd, *J* = 8.1 Hz), 7.94 (1H, ddd, *J* = 5.0 Hz)], 8.18 (2H, ddd, *J* = 5.1 Hz), 8.36 (s, 1H, N=CH).

^{13}C-NMR (δ: ppm): 169.66 (N=CH), 159.22 (C-2), 158.51 (C'-1), 152.02 (C”-2 & C”-6), 149.72 (C”-4), 144.31 (C-9), 139.61 (C-8), 130.43 (C'-4), 129.26(C'-3 & C'-5), 122.82 (C”-3 & C”-5), 122.53 (C-5 & C-6), 122.02 (C'-2 & C'-5), 112.96 (C-4 & C-7), 69.24 (CH_2 linkage), 68.71 (C-2 & C-6 of morpholine), 57.53 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 397.2314 (M^+). *Anal*. Calcd for $C_{24}H_{23}N_5O$: C, 72.52; H, 5.83; N, 17.62. Found: C, 72.29; H, 5.85; N, 17.68.

3.3.10. N-(4-Chlorobenzylidene)-4-(1-(morpholinomethyl)-1H-benzoimidazol-2-yl)benzenamine (3j)

Yield (%): 70; m.p. (°C): 162-163; IR (cm^{-1}): 708 (C-Cl), 1035 (C-O-C), 1620 (C=C), 1657 (C=N), 2943 (CH_2-CH), 3006 (Ar-CH), 3079 (=CH). ^{1}H NMR: δ 2.66 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.94 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 5.06 (s, 2H, CH_2 linkage), 7.29 (1H, ArH, ddd, *J* = 6.5 Hz), 7.33-7.51 [(5H, ArH, (7.33 (1H, ddd, *J* = 8.3 Hz), 7.44 (2H, ddd, *J* = 8.0 Hz), 7.51 (2H, ddd, *J* = 8.0 Hz)], 7.68 (2H, ArH, ddd, *J* = 8.3 Hz), 7.75-8.01 [(4H, ArH, (7.75 (2H, ddd, *J* = 8.3 Hz), 7.81 (1H, ddd, *J* = 8.3 Hz), 7.94 (1H, ddd, *J* = 5.0 Hz)], 8.68 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 165.34 (N=CH), 159.72 (C-2), 155.01 (C'-1), 142.54 (C-9), 139.13 (C”-4), 135.81 (C-8), 130.43 (C”-1), 129.20 (C”-2 & C”-6), 128.58 (C'-4), 128.14 (C”-3 & C”-5), 126.92 (C'-3 & C'-5), 122.31 (C-5 & C-6), 120.62 (C'-2 & C'-5), 115.94 (C-4 & C-7), 70.24 (CH_2 linkage), 69.07 (C-2 & C-6 of morpholine), 54.82 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 430.1464 (M^+), 432.1311 (M^{+2}). *Anal*. Calcd for $C_{25}H_{23}ClN_4O$: C, 69.68; H, 5.38; N, 13.00. Found: C, 69.93; H, 5.36; N, 12.95.

3.3.11. N-(4-Fluorobenzylidene)-4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl)benzenamine (3k)

Yield (%): 73; m.p. (°C): 141-142; IR (cm^{-1}): 1072 (C-O-C), 1184 (C-F), 1645 (C=C), 1679 (C=N), 2906 (CH_2-CH), 3024 (Ar-CH), 3114 (=CH). ^{1}H NMR: δ 2.54 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.47 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 4.81 (s, 2H, CH_2 linkage), 7.16-7.43 [(4H, ArH, (7.30 (1H, ddd, *J* = 6.5 Hz), 7.33 (2H, ddd, *J* = 8.3 Hz), 7.41 (1H, ddd, *J* = 8.3 Hz)], 7.53-8.14 [(8H, ArH, (7.63 (1H, ddd, *J* = 5.1 Hz), 7.67 (2H, ddd, *J* = 8.7 Hz), 7.78 (2H, ddd, *J* = 8.3 Hz), 7.83 (2H, ddd, *J* = 8.7 Hz), 7.93 (1H, ddd, *J* = 8.3 Hz)], 8.68 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 171.62 (C”-4), 165.91 (N=CH), 159.03 (C-2), 156.24 (C'-1), 140.56 (C-9), 137.14 (C-8), 136.72 (C”-2 & C”-6), 132.81 (C”-1), 129.53 (C'-4), 128.03 (C'-3 & C'-5), 126.64 (C-5 & C-6), 124.37 (C'-2 & C'-5), 110.31 (C”-3 & C”-5), 109.73 (C-4 & C-7), 73.22 (CH_2 linkage), 72.93 (C-2 & C-6 of morpholine), 45.53 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 414.1426 (M^+). *Anal*. Calcd for $C_{25}H_{23}FN_4O$: C, 72.45; H, 5.59; N, 13.52. Found: C, 72.20; H, 5.61; N, 13.56.

3.3.12. 4-((4-(1-(Morpholinomethyl)-1H-benzimidazol-2-yl)phenylimino)methyl)phenol (3l)

Yield (%): 78; m.p. (°C): 193-194; IR (cm^{-1}): 1067 (C-O-C), 1631 (C=C), 1674 (C=N), 2896 (CH_2-CH), 3028 (Ar-CH), 3123 (=CH), 3360 (OH). ^{1}H NMR: δ 2.78 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.41 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 4.76 (s, 2H, CH_2 linkage), 5.43 (s, 1H, OH), 6.86-6.98 [(4H, ArH, (6.88 (2H, ddd, *J* = 8.2 Hz), 6.94 (1H, ddd, *J* = 8.1 Hz), 6.98 (1H, ddd, *J* = 6.2 Hz)], 7.00-7.90 [(8H, ArH, (7.18 (2H, ddd, *J* = 8.6 Hz), 7.27 (2H, ddd, *J* = 8.2 Hz), 7.44 (2H, ddd, *J* = 8.6 Hz), 7.65 (1H, ddd, *J* = 5.1Hz), 7.88 (1H, ddd, *J* = 8.1Hz)], 8.57 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 164.51 (C”-4), 163.13 (N=CH), 157.86 (C-2), 156.49 (C'-1), 140.25 (C-9), 138.72 (C-8), 137.04 (C”-2 & C”-6), 133.93 (C'-4), 129.16 (C'-3 & C'-5), 126.83 (C”-1), 125.44 (C-5 & C-6), 121.04 (C'-2 & C'-5), 114.72 (C”-3 & C”-5), 111.24 (C-4 & C-7), 66.31 (CH_2 linkage), 65.96 (C-2 & C-6 of morpholine), 50.61 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 412.2411 (M^+). *Anal*. Calcd for $C_{25}H_{24}N_4O_2$: C, 72.80; H, 5.86; N, 13.58. Found: C, 73.06; H, 5.87; N, 13.53.

3.3.13. N-(4-Bromobenzylidene)-4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl)benzenamine (3m)

Yield (%): 75; m.p. (°C): 174-176; IR (cm^{-1}): 623 (C-Br), 1069 (C-O-C), 1623 (C=C), 1640 (C=N), 2948 (CH_2-CH), 3031 (Ar-CH), 3075 (=CH). ^{1}H NMR: δ 2.76 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.92 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 5.18 (s, 2H, CH_2 linkage), 6.97 (1H, ArH, ddd, *J* = 6.6 Hz), 7.41-7.53 [(5H, ArH, (7.41 (2H, ddd, *J* = 8.2 Hz), 7.46 (1H, ddd, *J* = 8.3 Hz), 7.53 (2H, ddd, *J* = 8.7 Hz)], 7.67-8.10 [(6H, ArH, (7.78 (2H, ddd, *J* = 8.2 Hz), 7.81 (2H, ddd, *J* = 8.7 Hz), 7.83 (1H, ddd, *J* = 8.3 Hz), 7.92 (1H, ddd, *J* = 5.0 Hz)], 8.34 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 158.94 (N=CH), 156.13 (C-2), 155.54 (C'-1), 142.02 (C-9), 139.84 (C-8), 134.26 (C”-1), 131.61 (C”-3 & C”-5), 131.04 (C”-2 & C”-6), 128.33 (C'-4), 127.94 (C'-3 & C'-5), 124.72 (C”-4), 120.83 (C-5 & C-6), 118.41 (C'-2 & C'-5), 112.94 (C-4 & C-7), 62.53 (CH_2 linkage), 62.13 (C-2 & C-6 of morpholine), 40.25 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 474.1325 (M^+), 476.0521 (M^{+2}). *Anal*. Calcd for $C_{25}H_{23}BrN_4O$: C, 63.16; H, 4.88; N, 11.79. Found: C, 63.39; H, 4.86; N, 11.75.

3.3.14. N-(4-Methoxybenzylidene)-4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl)benzenamine (3n)

Yield (%): 71; m.p. (°C): 156-158; IR (cm^{-1}): 1040.24 (C-O-C), 1604.66 (C=C), 1666.13 (C=N), 2921.43 (CH_2-CH), 3034.13 (Ar-CH), 3094.33 (=CH). ^{1}H NMR: δ 2.49 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.66 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 3.83 (s, 3H, OCH_3), 4.95 (s, 2H, CH_2 linkage), 7.12 (2H, ArH, ddd, *J* = 8.6 Hz), 7.38-7.40 [(2H, ArH, (7.38 (1H, ddd, *J* = 6.2 Hz), 7.40 (1H, ddd, *J* = 8.1 Hz)], 7.41-7.42 [(4H, ArH (7.41 (2H, ddd, *J* = 8.6 Hz), 7.42 (2H, ddd, *J* = 8.6 Hz)], 7.90 (2H, ArH, ddd, *J* = 8.6 Hz), 8.05 (1H, ArH, ddd, *J* = 8.1 Hz), 8.06 (1H, ArH, ddd, *J* = 5.1 Hz), 8.69 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 165.86 (C”-4), 164.66 (N=CH), 157.49 (C-2), 155.95 (C'-1), 144.07 (C-9), 138.26 (C-8), 136.13 (C”-2 & C”-6), 133.24 (C'-4), 129.30 (C'-3 & C'-5), 128.04 (C”-1), 126.53 (C-5 & C-6), 123.24 (C'-2 & C'-5), 115.32 (C-4 & C-7), 113.09 (C”-3 & C”-5), 68.34 (CH_2 linkage), 67.06 (C-2 & C-6 of morpholine), 58.87 (OCH_3), 53.81 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 426.5338 (M^+). *Anal*. Calcd for $C_{26}H_{26}N_4O_2$: C, 73.22; H, 6.14; N, 13.14. Found: C, 73.01; H, 6.16; N, 13.19.

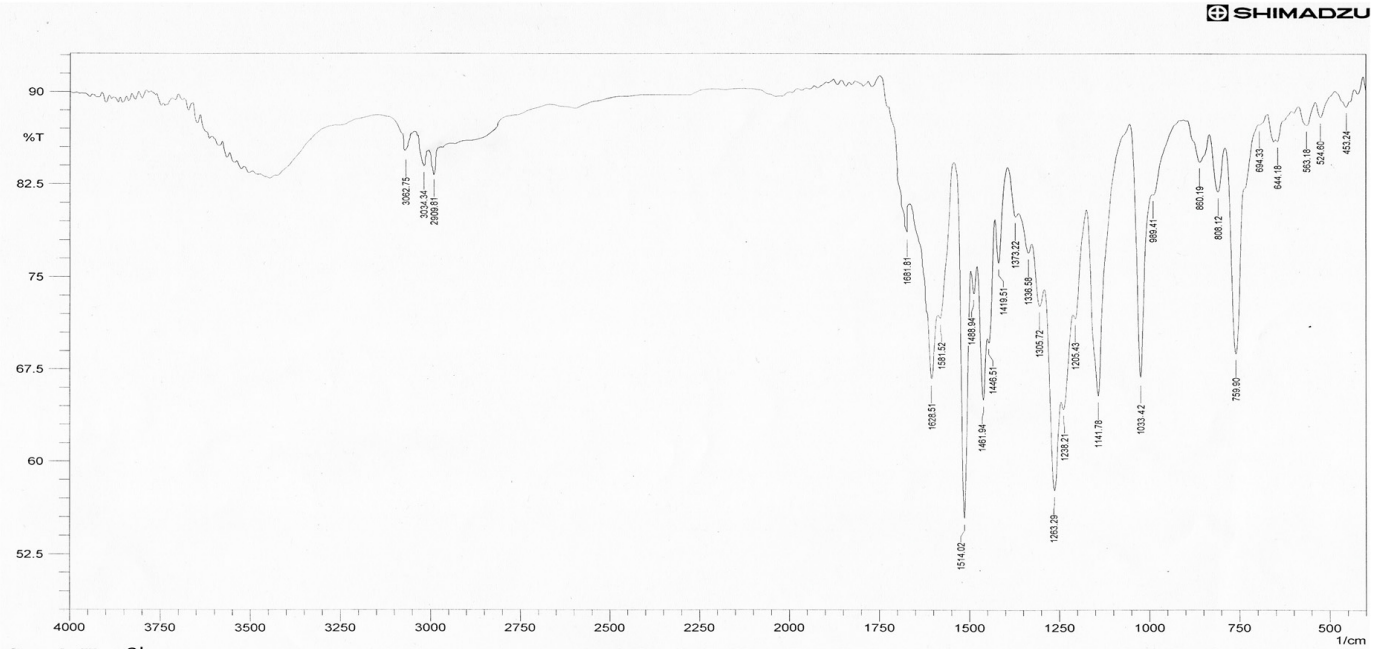
3.3.15. N-(2-nitrobenzylidene)-4-(1-(morpholinomethyl)-1H-benzimidazol-2-yl)benzenamine (3o)

Yield (%): 76; m.p. (°C): 186-187; IR (cm^{-1}): 1034 (C-O-C), 1375 & 1551 (NO_2), 1619 (C=C), 1651 (C=N), 2923 (CH_2-CH), 3015 (Ar-CH), 3096 (=CH). ^{1}H NMR: δ 2.53 (4H, ddd, *J* = 15.3 Hz, CH_2 of morpholine), 3.39 (4H, ddd, *J* = 12.5 Hz, CH_2 of morpholine), 5.04 (s, 2H, CH_2 linkage), 6.76 (2H, ArH, ddd, *J* = 5.9 Hz), 6.96 (2H, ArH, ddd, *J* = 8.3 Hz), 6.98-7.91 [(10H, ArH, (6.98 (2H, ddd, *J* = 8.0 Hz), 6.99 (2H, ddd, *J* = 8.0 Hz), 7.16 (1H, ddd, *J* = 8.5 Hz), 7.52 (1H, ddd, *J* = 8.3 Hz), 7.54 (1H, ddd, *J* = 7.8 Hz), 7.55 (1H, ddd, *J* = 5.0 Hz), 7.64 (1H, ddd, *J* = 8.5 Hz), 7.91 (1H, ddd, *J* = 7.8 Hz)], 8.50 (s, 1H, N=CH). ^{13}C-NMR (δ: ppm): 164.61 (N=CH), 155.43 (C-2), 154.85 (C'-1), 153.16 (C”-4), 141.25 (C”-1), 140.52 (C-9), 137.94 (C-8), 134.14 (C”-2 & C”-6), 132.62 (C'-4), 130.36 (C'-3 & C'-5), 128.59 (C-5 & C-6), 120.76 (C'-2 & C'-5), 119.42 (C”-3 & C”-5), 113.13 (C-4 & C-7), 60.87 (CH_2 linkage), 59.24 (C-2 & C-6 of morpholine), 54.76 (C-3 & C-5 of morpholine). MS (EI) *m/z*: 441.0431 (M^+). *Anal*. Calcd for $C_{25}H_{23}N_5O_3$: C, 68.01; H, 5.25; N, 15.86. Found: C, 68.19; H, 5.24; N, 15.80.

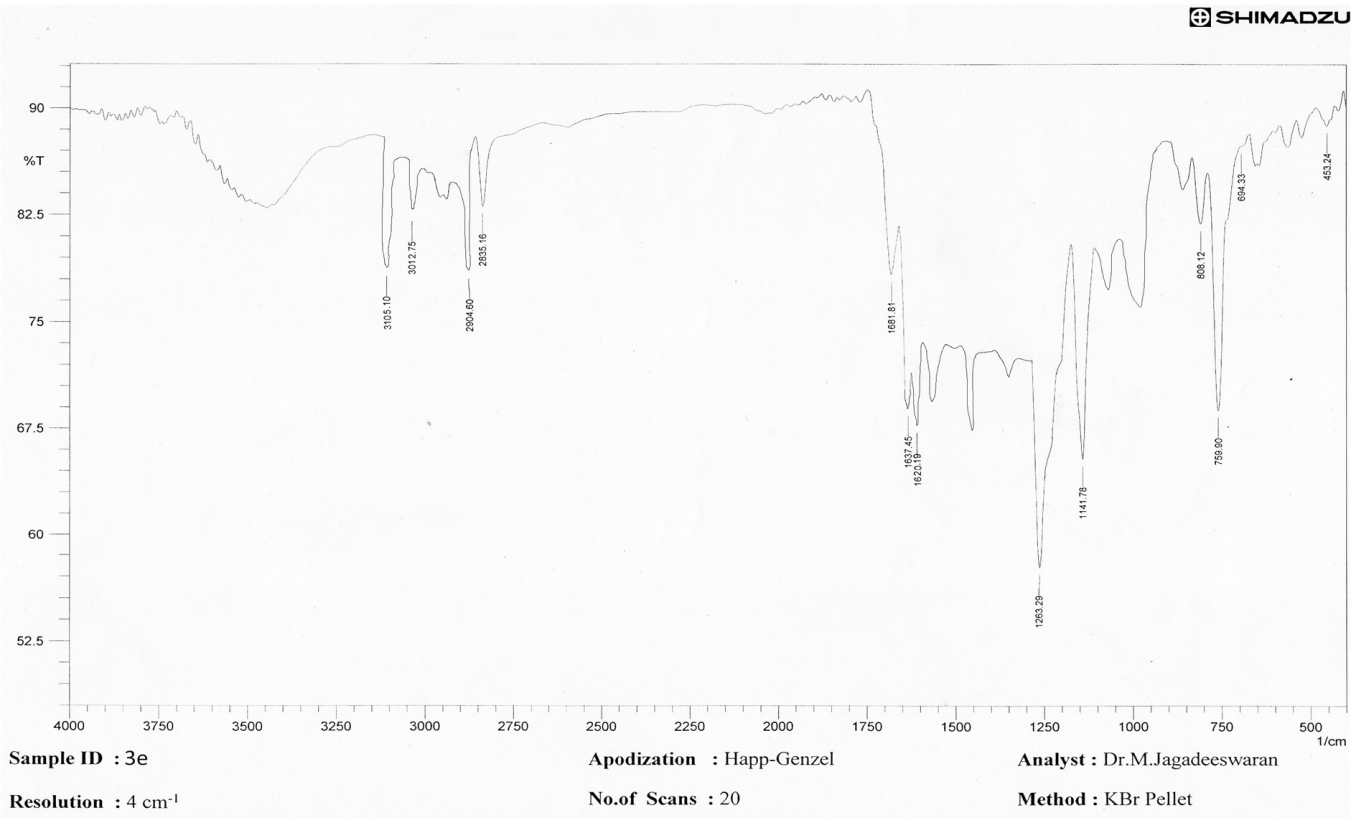
4. S4: SPECTRUM DETAILS FOR SYNTHESISED ACTIVE COMPOUNDS

4.1. IR SPECTRUM DETAILS

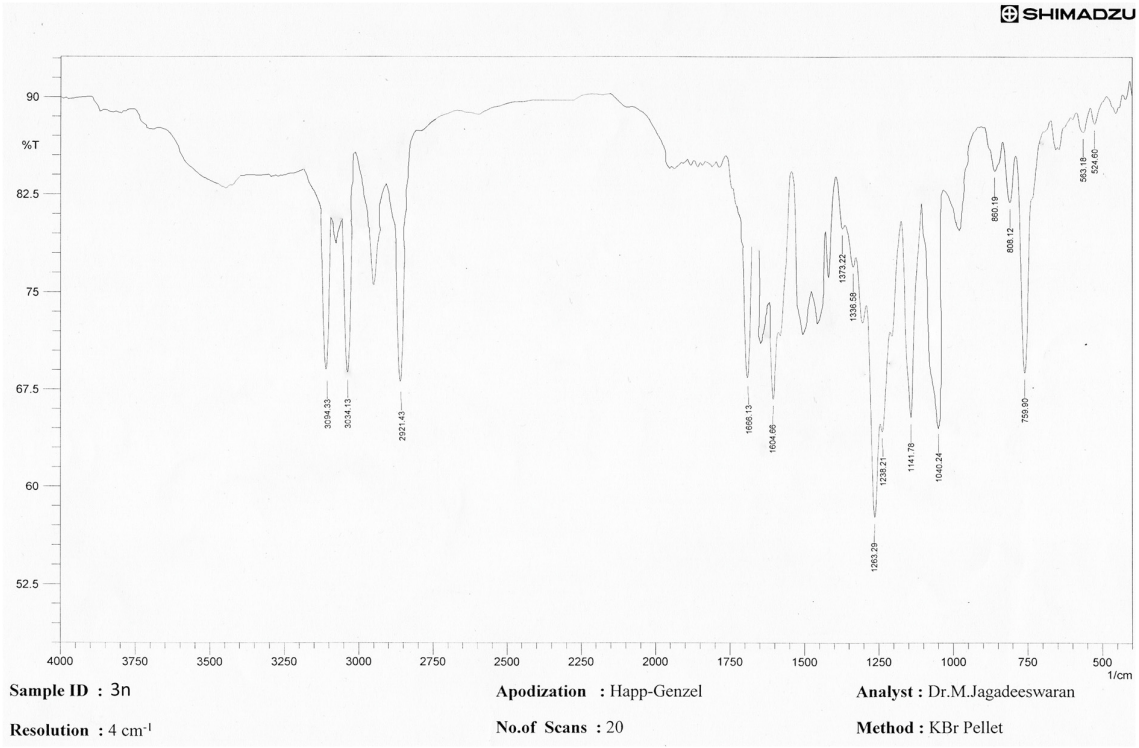
4.1.1. Compound 3b



4.1.2. Compound 3e

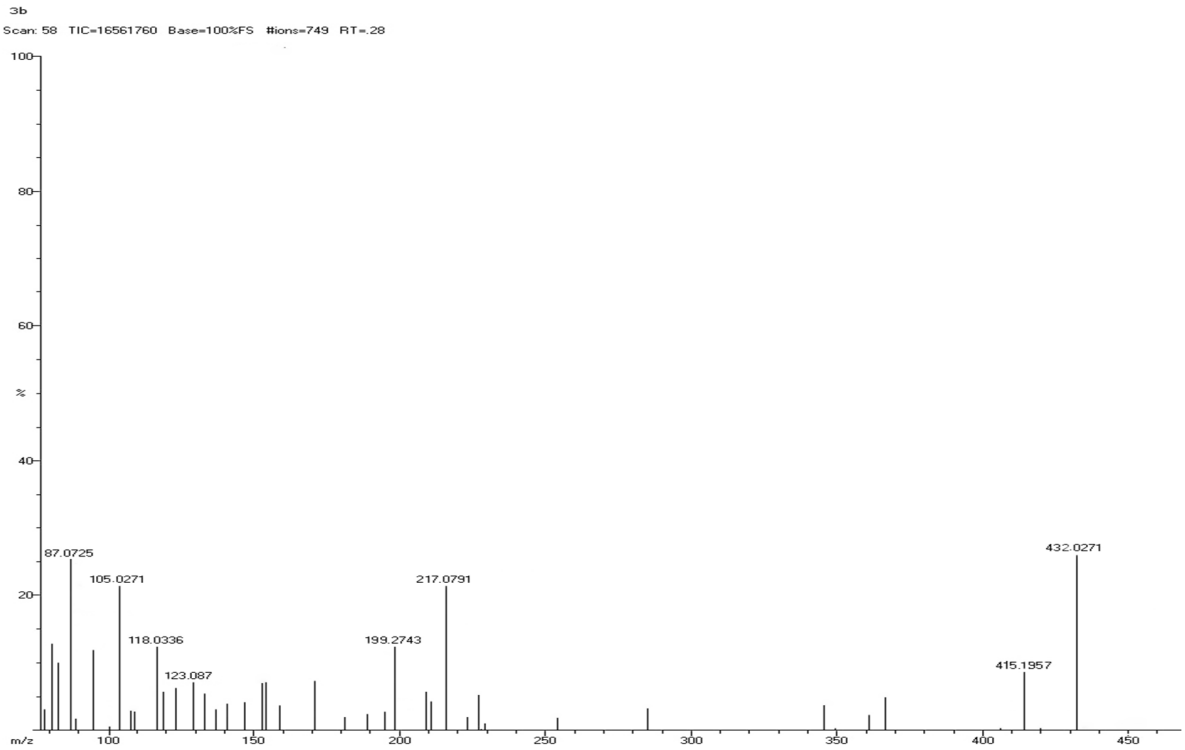


4.1.3. Compound 3n

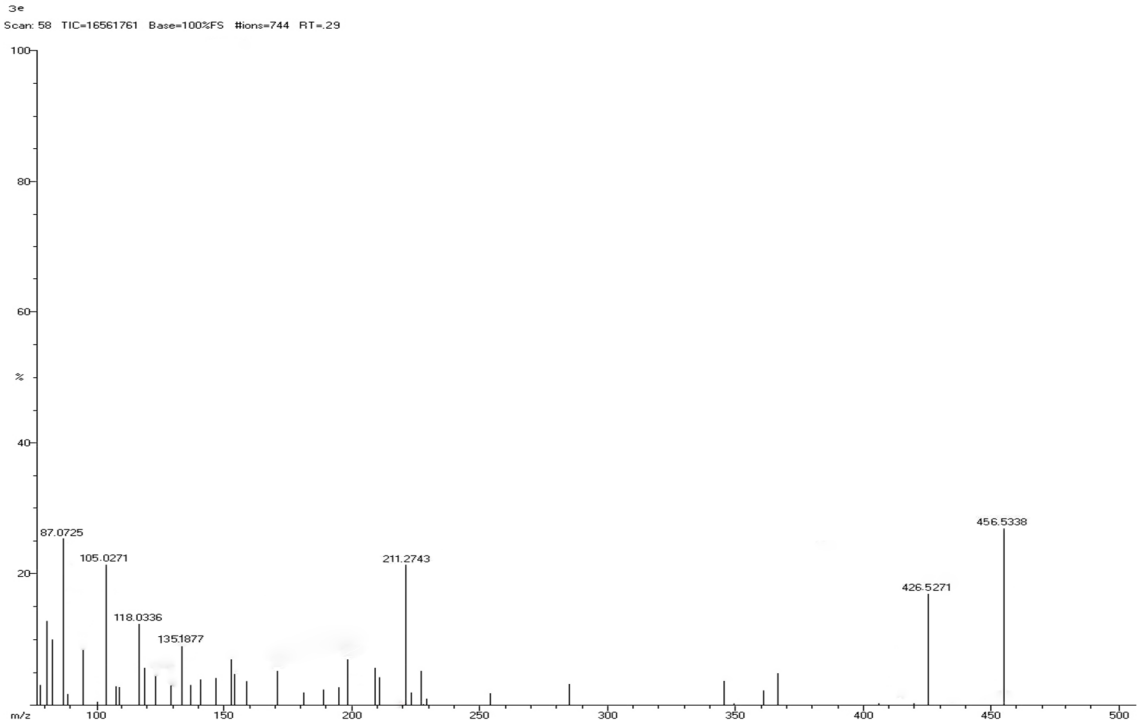


4.2. MASS SPECTRUM DETAILS

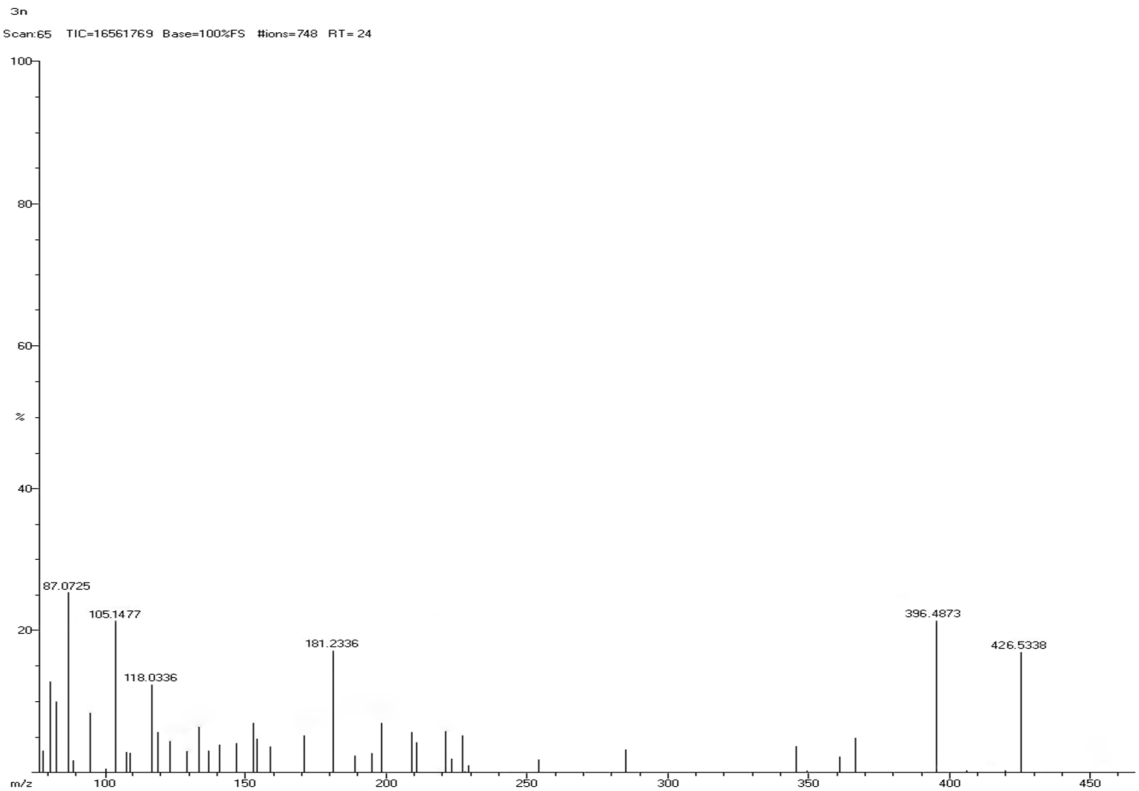
4.2.1. Compound 3b



4.2.2. Compound 3e

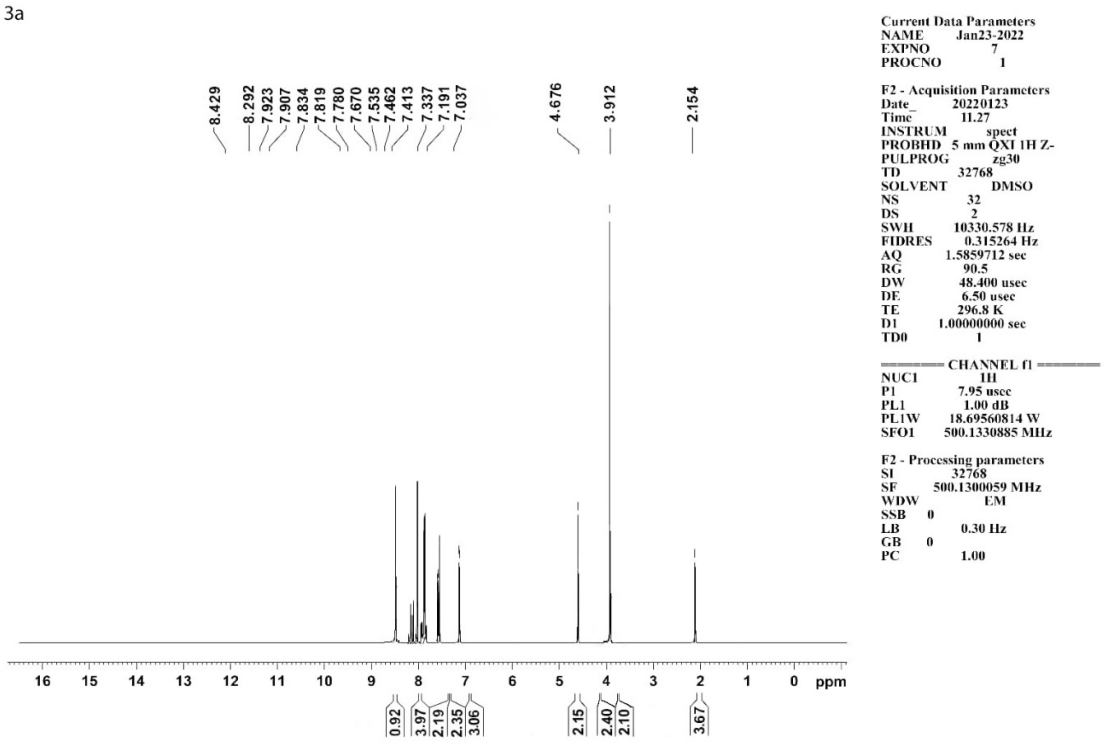


4.2.3. Compound 3n

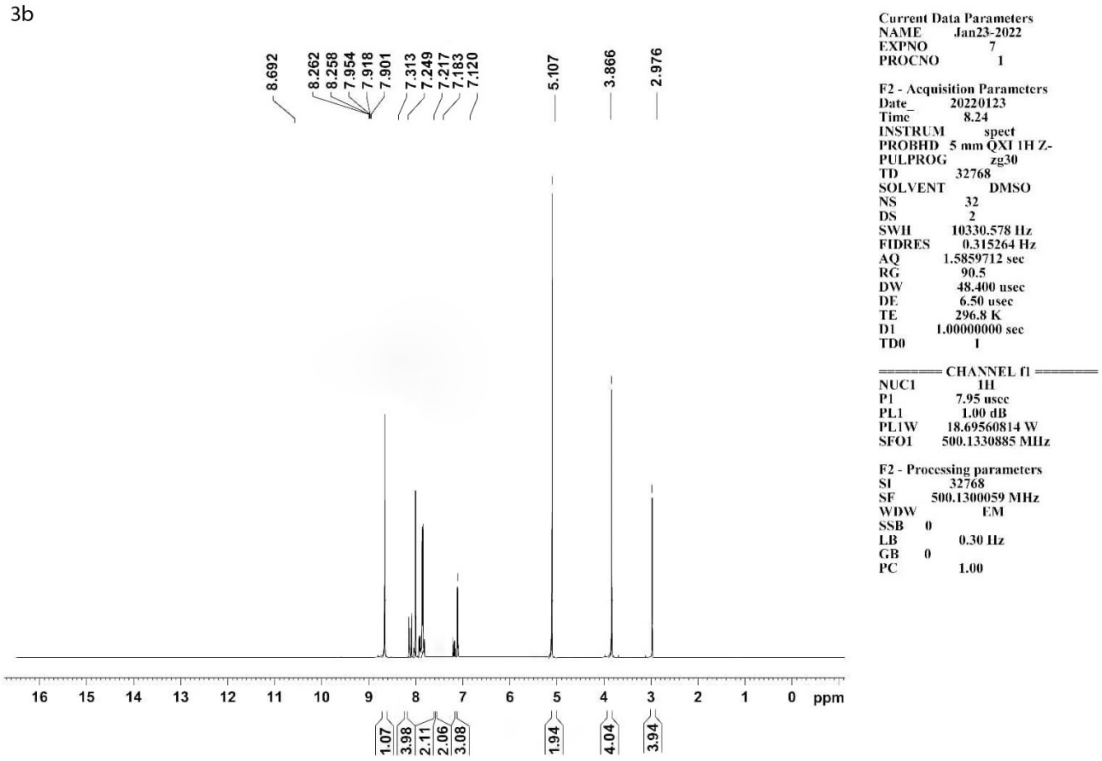


4.3. [1]HNMR SPECTRUM DETAILS

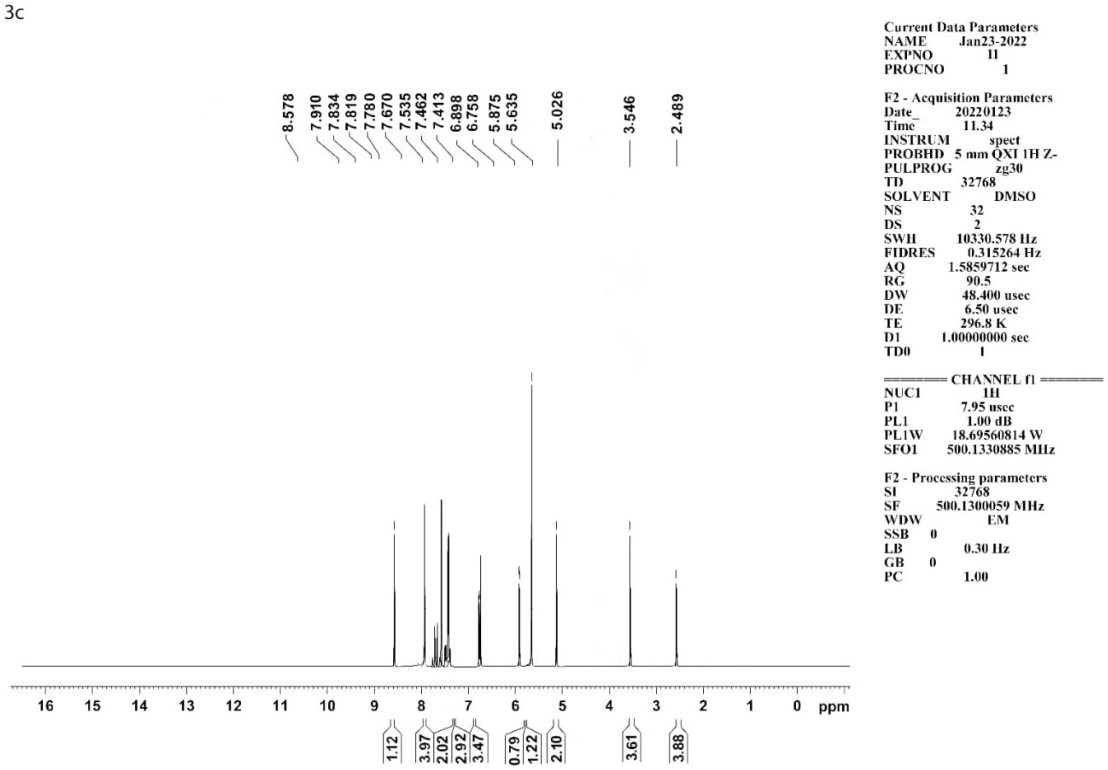
4.3.1. Compound 3a



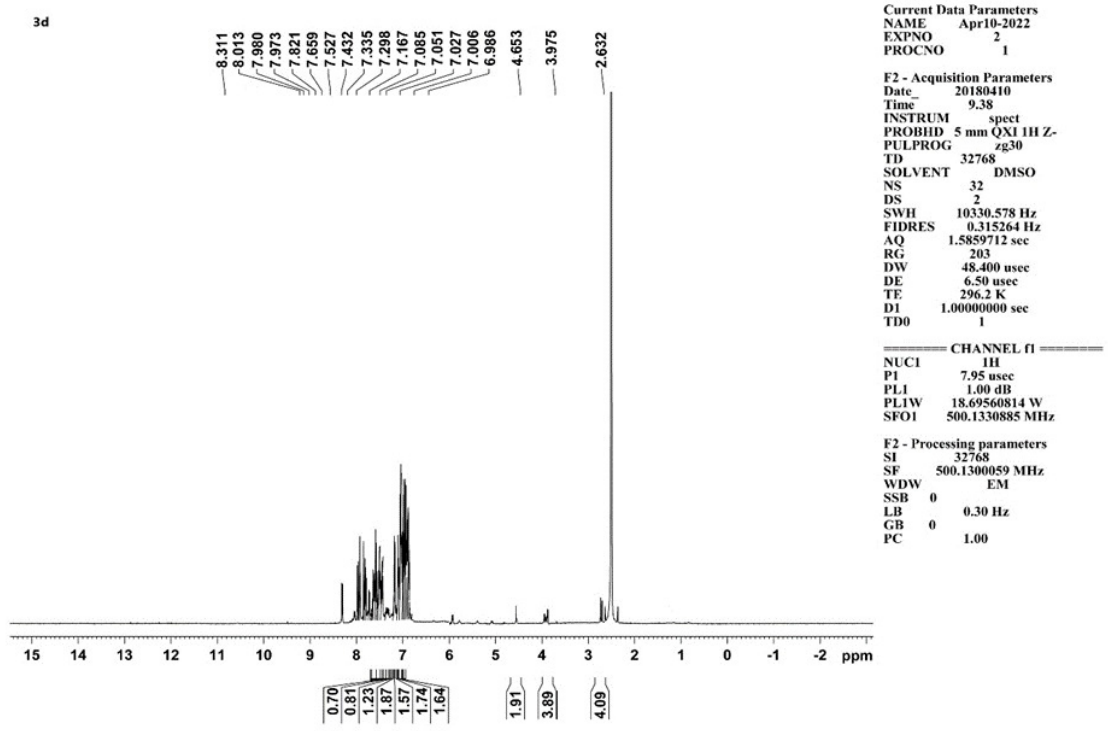
4.3.2. Compound 3b



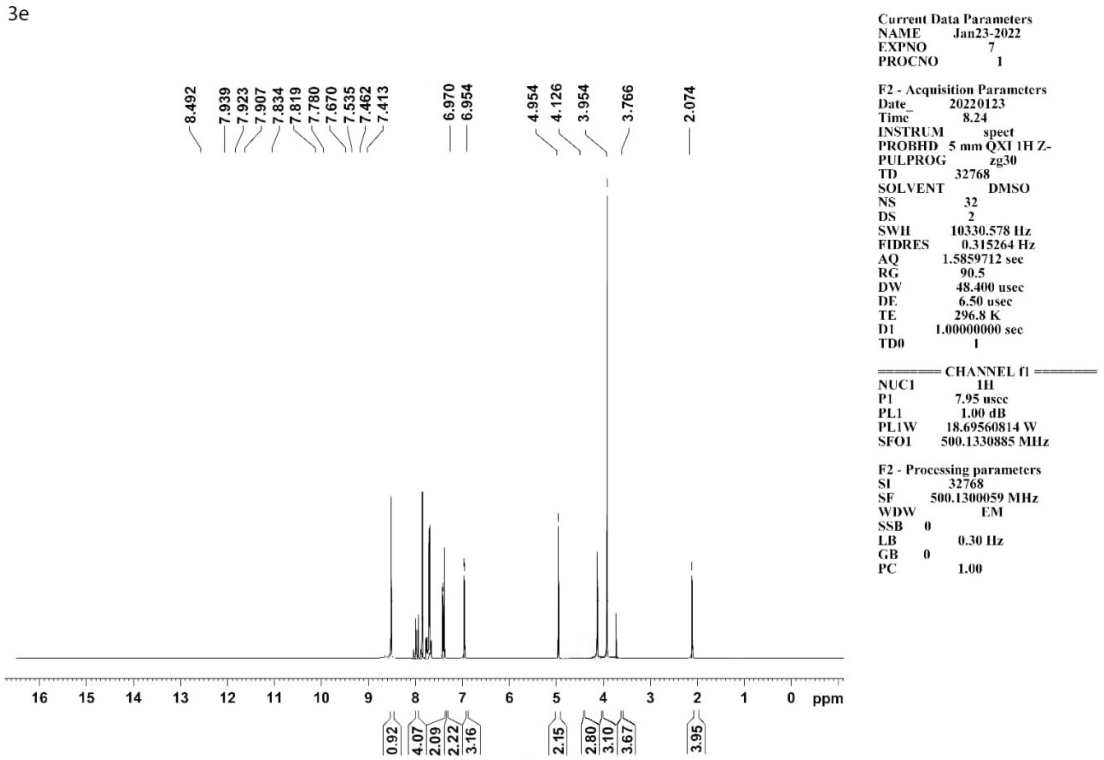
4.3.3. Compound 3c



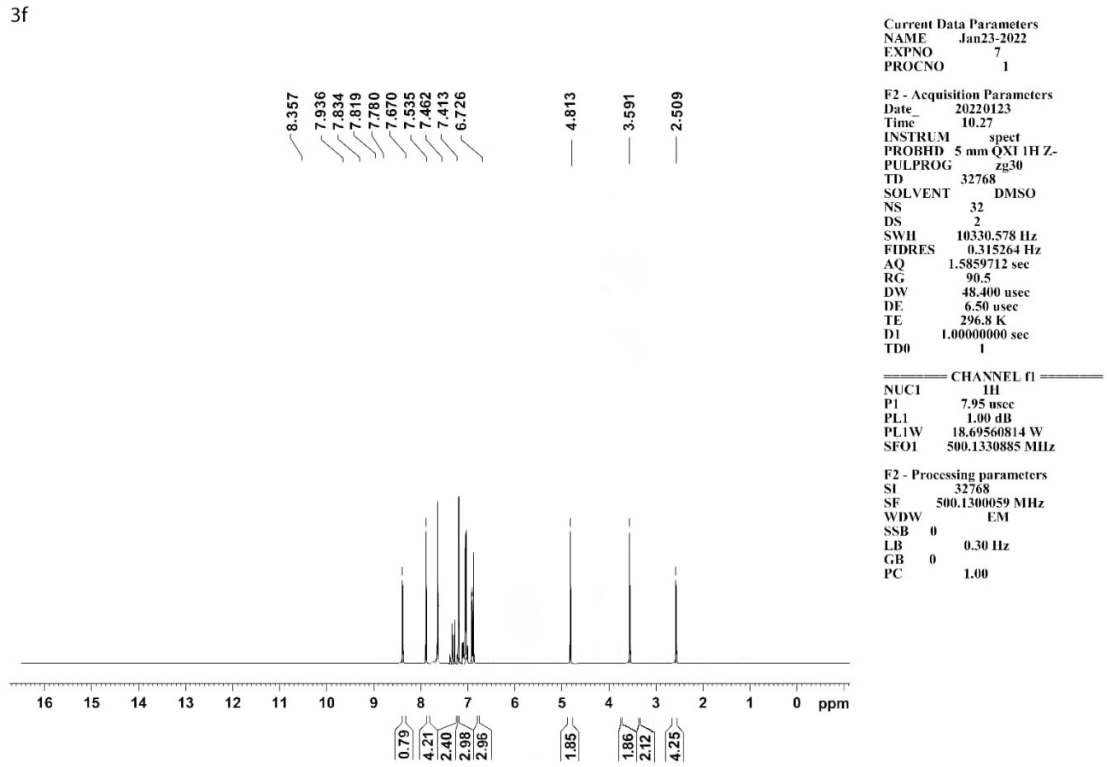
4.3.4. Compound 3d



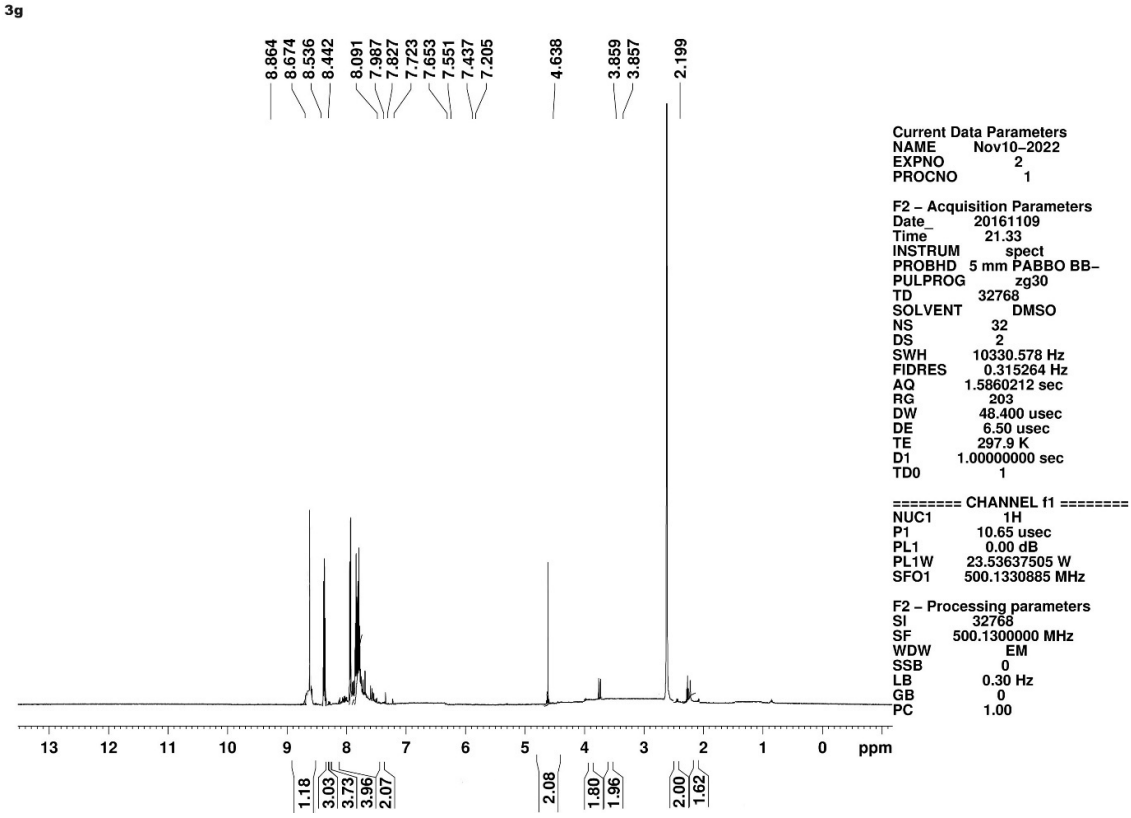
4.3.5. Compound 3e



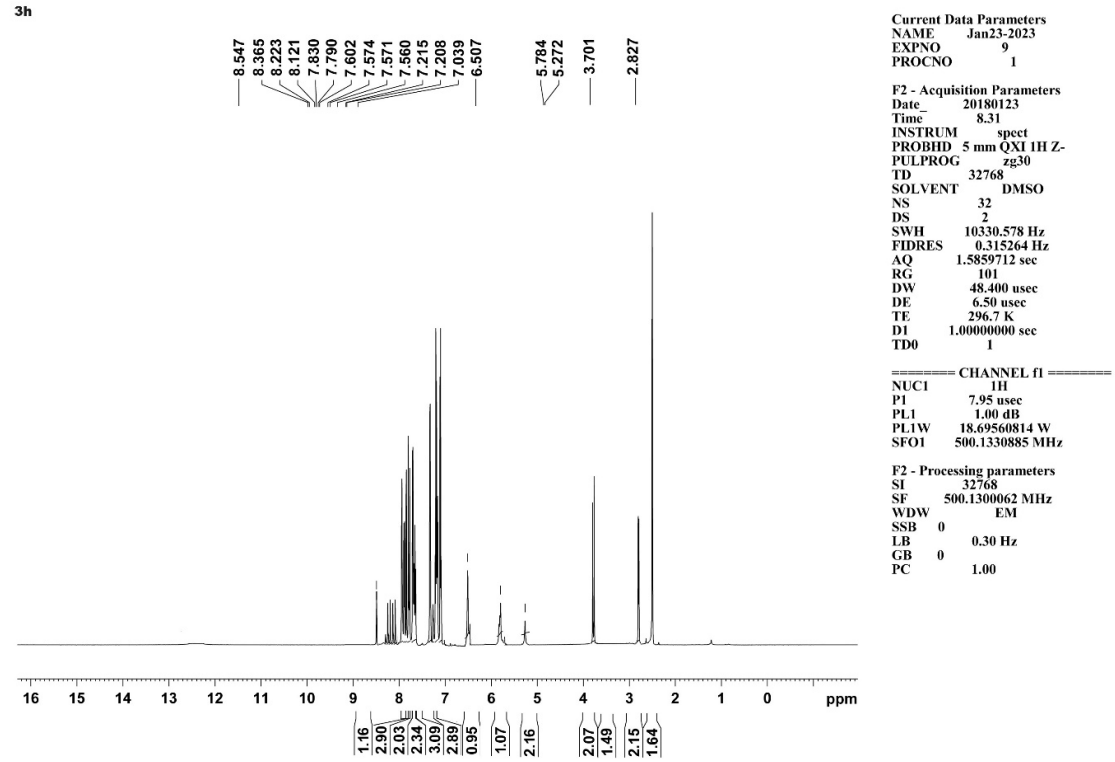
4.3.6. Compound 3f



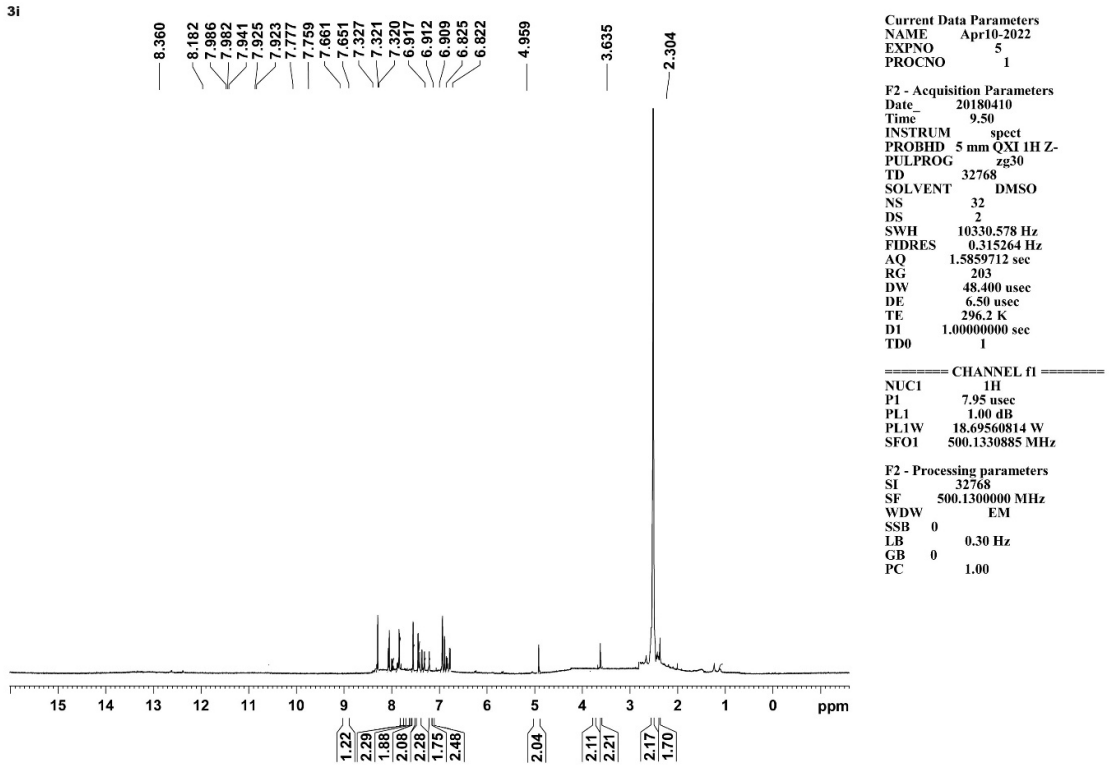
4.3.7. Compound 3g



4.3.8. Compound 3h



4.3.9. Compound 3i



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PROCNO 1

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TD 32768
SOLVENT DMSO
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DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5859712 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 296.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
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P1 7.95 usec
PL1 1.00 dB
PL1W 18.69560814 W
SFO1 500.1330885 MHz

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WDW EM
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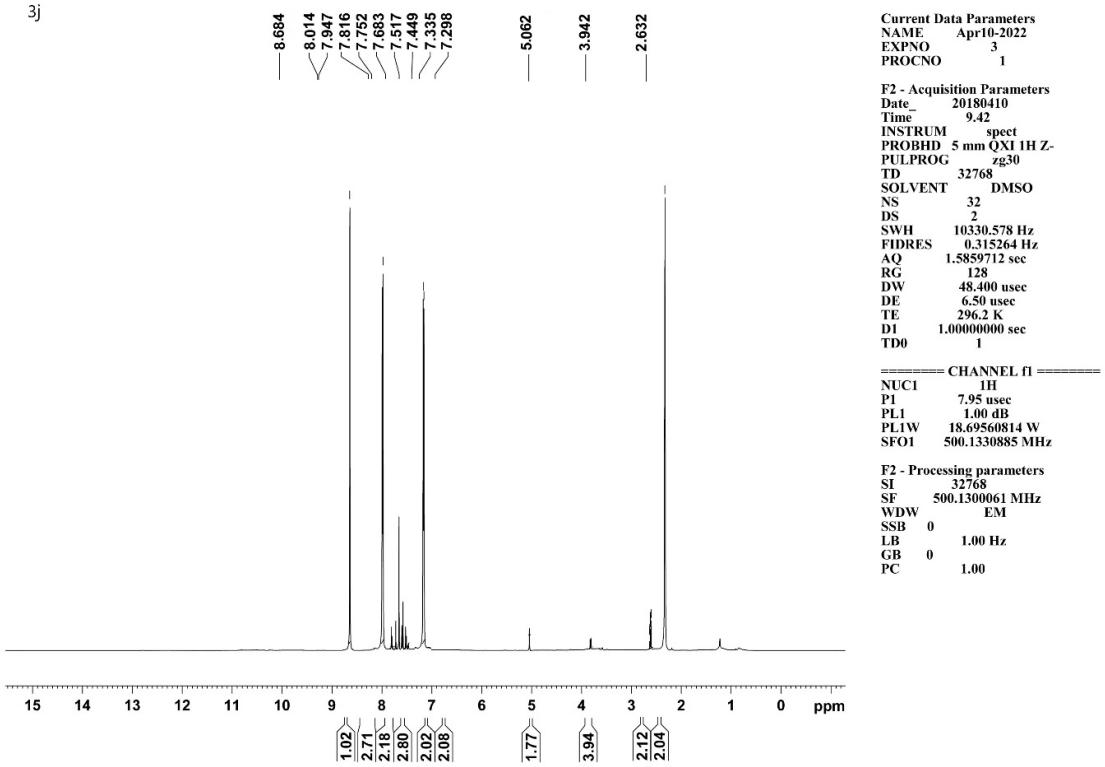
PC 1.00

4.3.10. Compound 3j



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PROCNO 1

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AQ 1.5859712 sec
RG 128
DW 48.400 usec
DE 6.50 usec
TE 296.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
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PL1 1.00 dB
PL1W 18.69560814 W
SFO1 500.1330885 MHz

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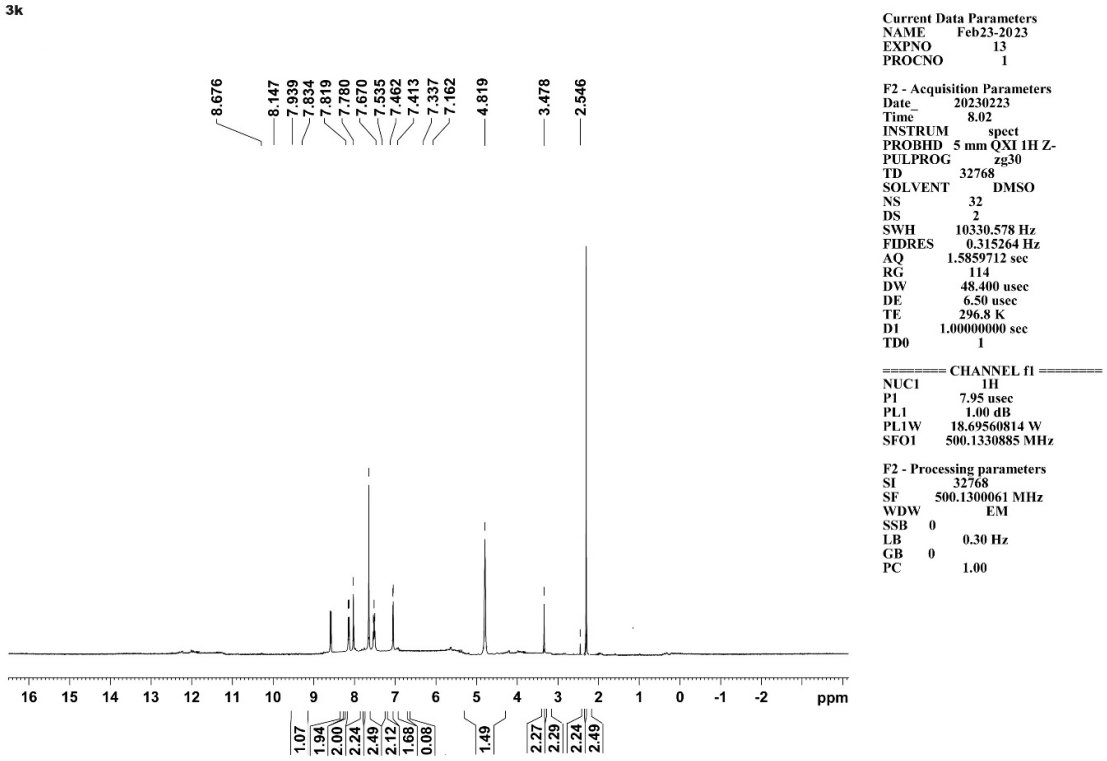
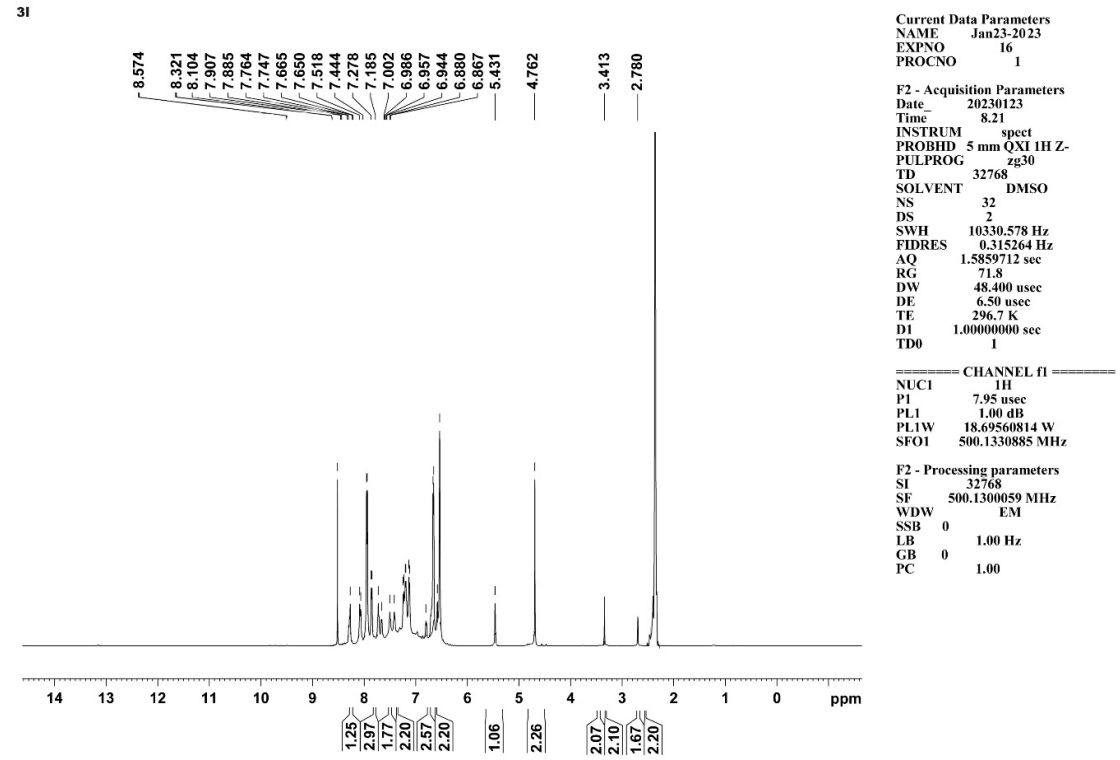
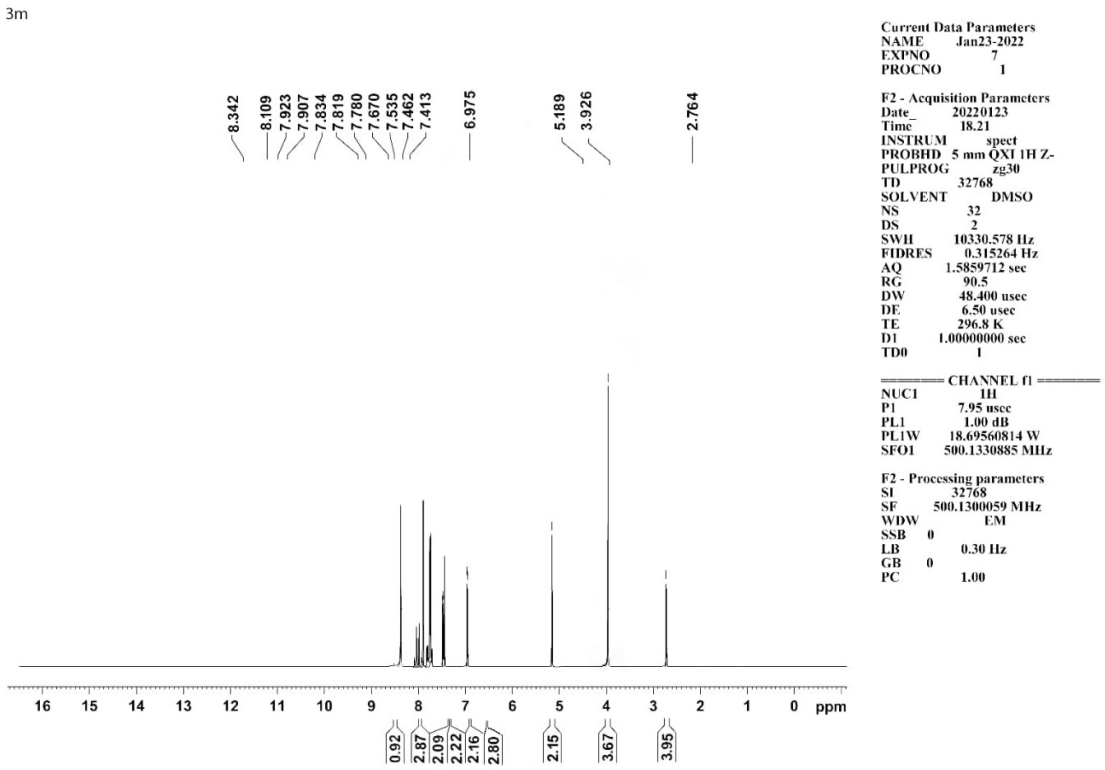
4.3.11. Compound 3k



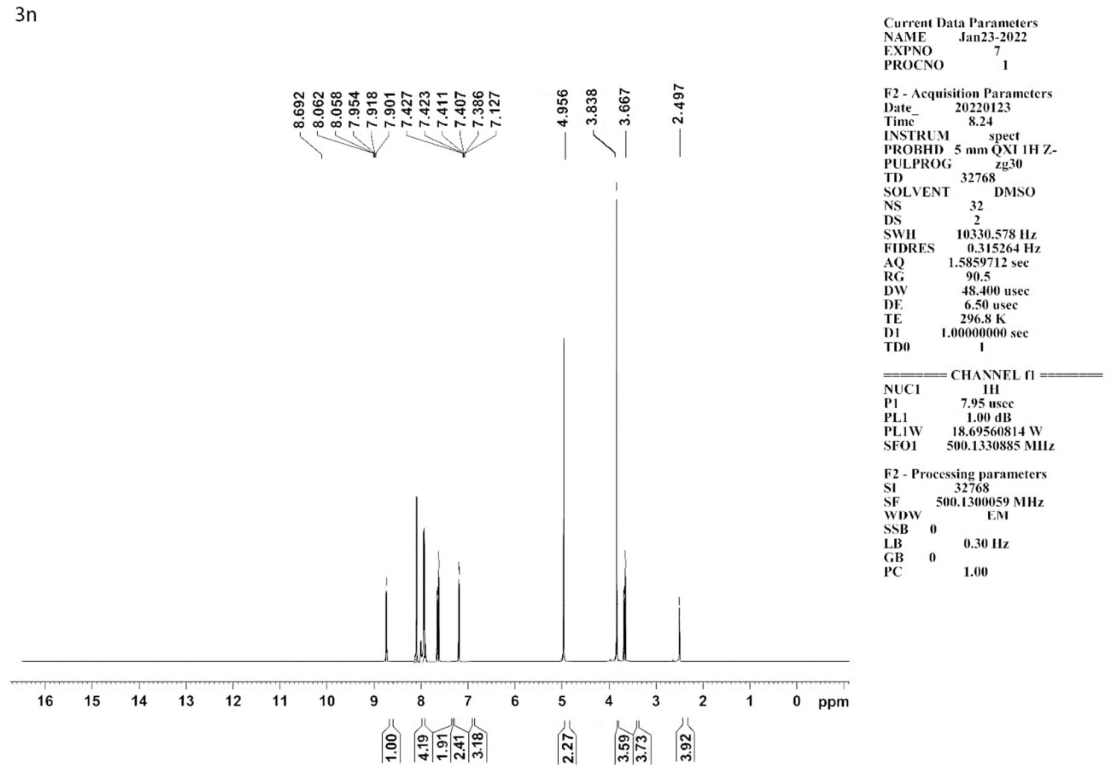
4.3.12. Compound 3l



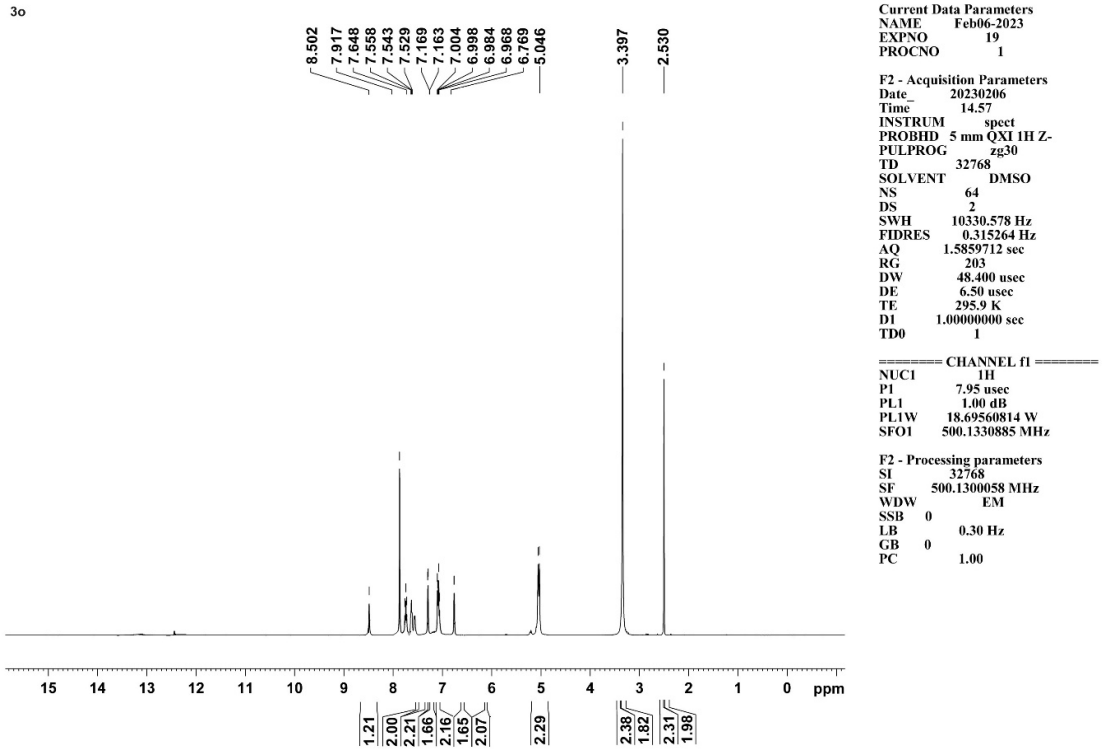
4.3.13. Compound 3m



4.3.14. Compound 3n

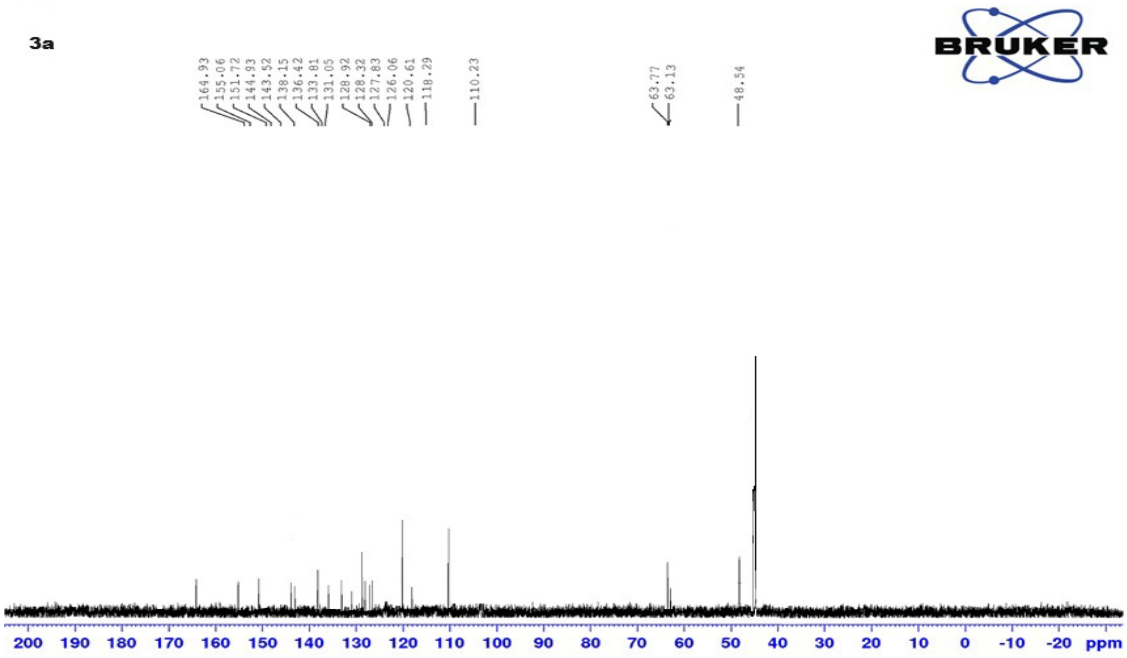


4.3.15. Compound 3o

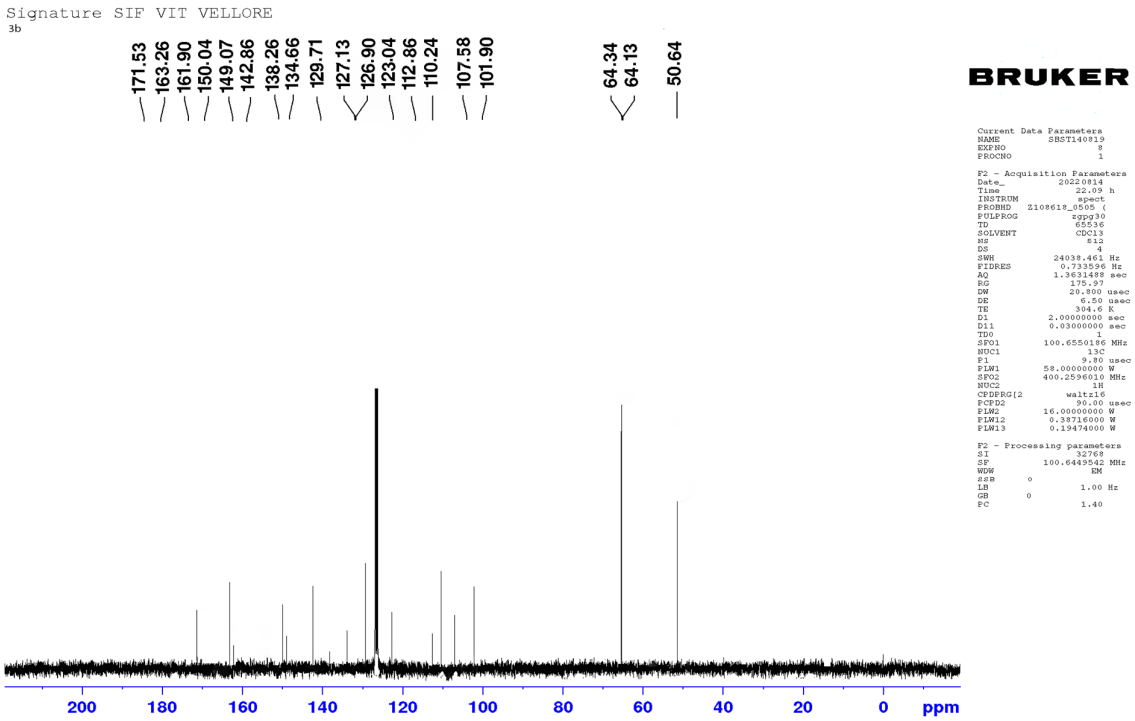


4.4. ^{13}CNMR SPECTRUM DETAILS

4.4.1. Compound 3a



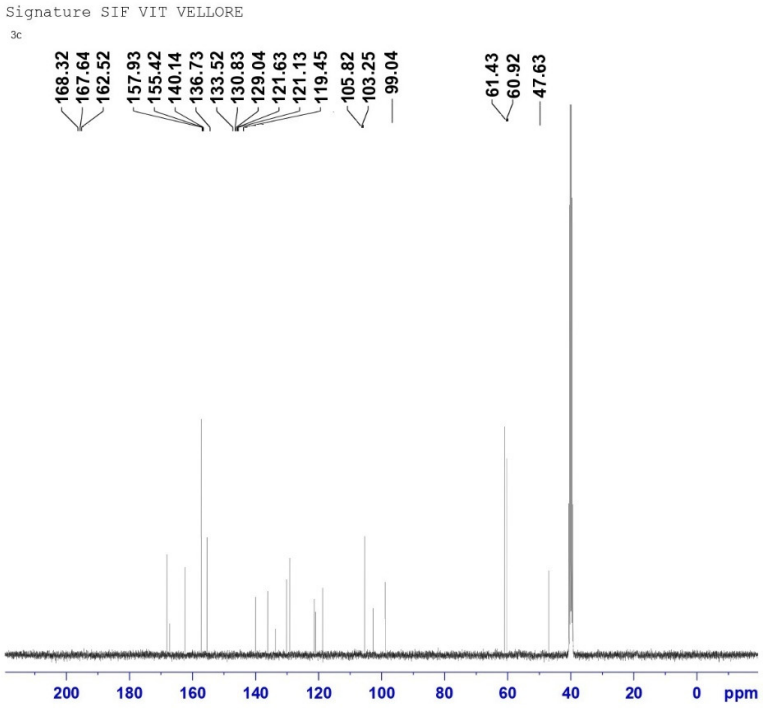
4.4.2. Compound 3b



BRUKER

```
Current Data Parameters
NAME          SBST140819
EXPNO                  8
PROCNO                 1

F2 - Acquisition Parameters
Date_           20220814
Time              22.09 h
INSTRUM             spect
PROBHD   Z108618_0505 (
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    4
SWH           24038.461 Hz
FIDRES         0.733596 Hz
AQ             1.3631488 sec
RG                175.97
DW               20.800 usec
DE                6.50 usec
TE                304.6 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1
SFO1        100.6550186 MHz
NUC1                13C
P1                9.80 usec
PLW1         58.00000000 W
SFO2        400.2596010 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         16.00000000 W
PLW12         0.38716000 W
PLW13         0.19474000 W

F2 - Processing parameters
SI                 32768
SF          100.6449542 MHz
WDW                   EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

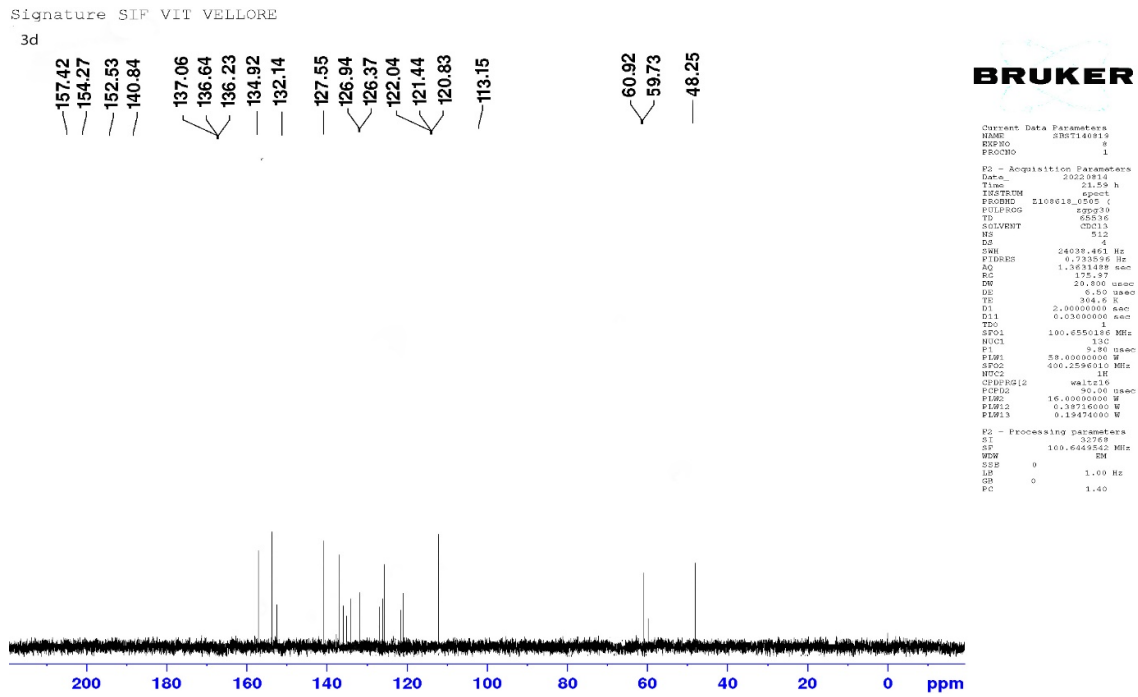
4.4.3. Compound 3c



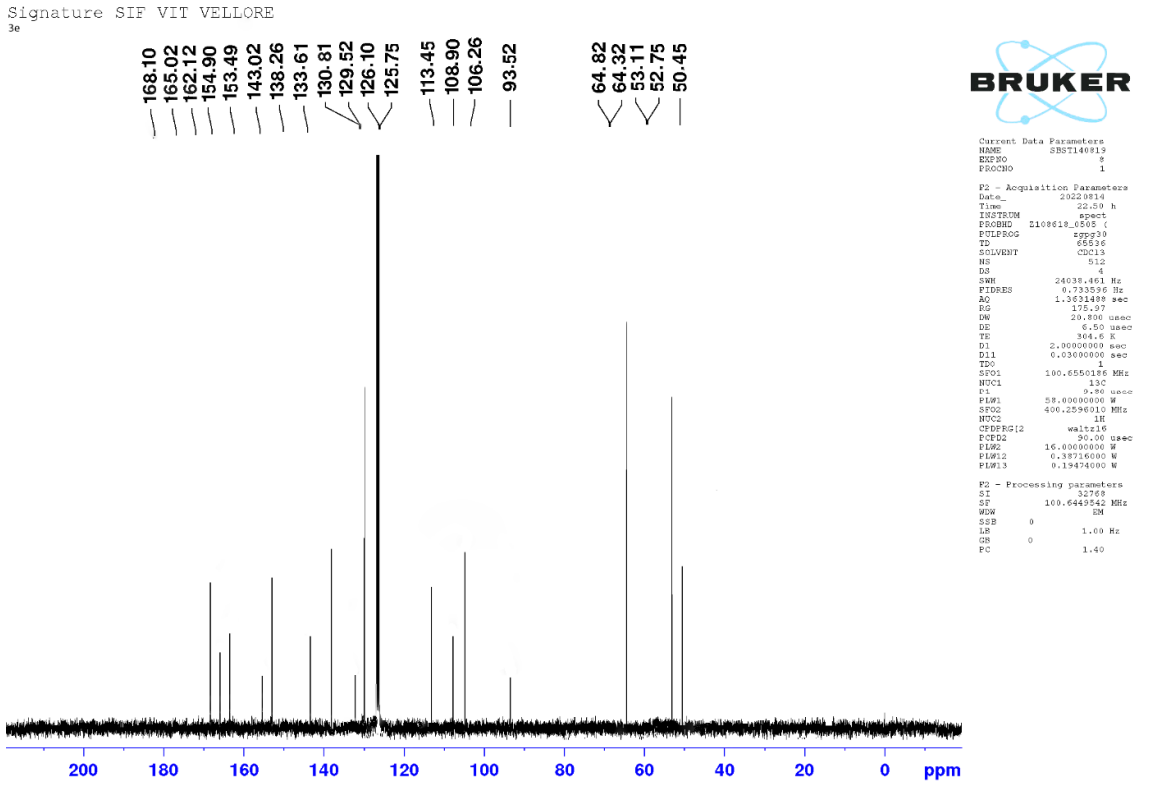
BRUKER

```
Current Data Parameters
NAME          SBST010719
EXPNO                 16
PROCNO                 1

F2 - Acquisition Parameters
Date_           20220701
Time              13.33 h
INSTRUM             spect
PROBHD   Z108618_0505 (
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                  512
DS                    4
SWH           24038.461 Hz
FIDRES         0.733596 Hz
AQ             1.3631488 sec
RG                156.91
DW               20.800 usec
DE                6.50 usec
TE                300.8 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1
SFO1        100.6550186 MHz
NUC1                13C
P1                9.80 usec
PLW1         58.00000000 W
SFO2        400.2596010 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         16.00000000 W
PLW12         0.38716000 W
PLW13         0.19474000 W

F2 - Processing parameters
SI                 32768
SF          100.6449542 MHz
WDW                   EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

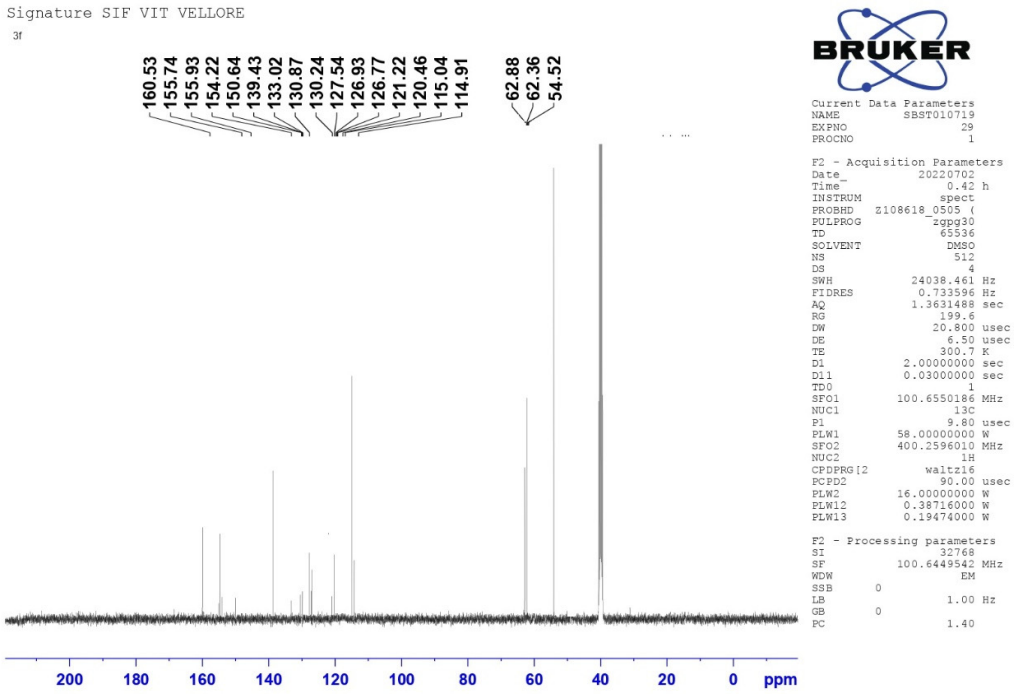
4.4.4. Compound 3d



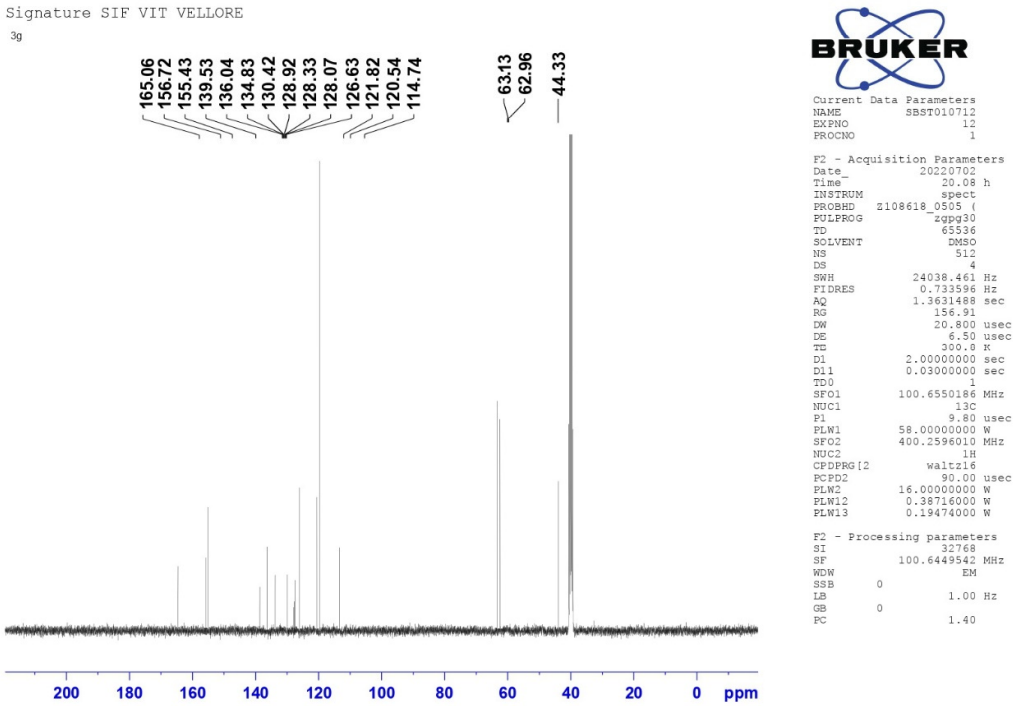
4.4.5. Compound 3e



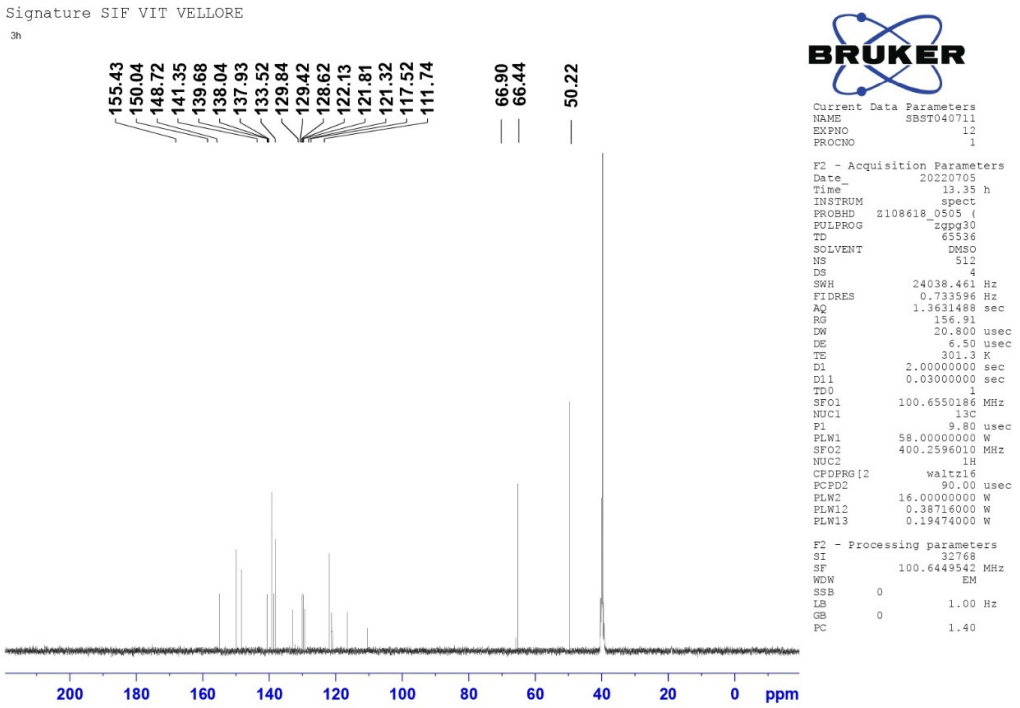
4.4.6. Compound 3f



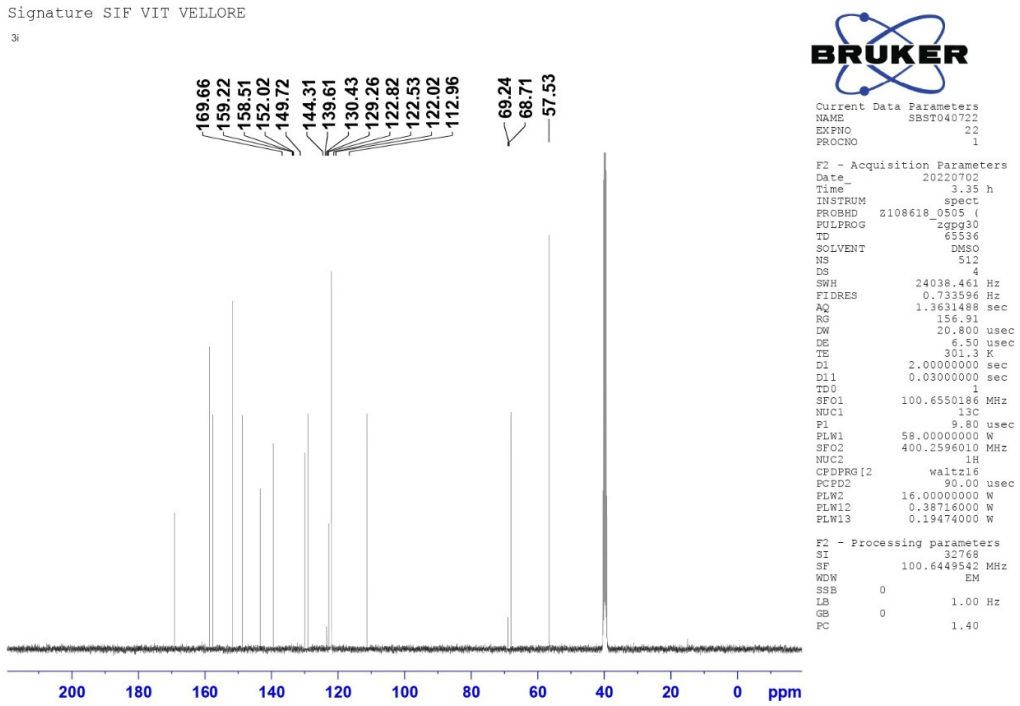
4.4.7. Compound 3g



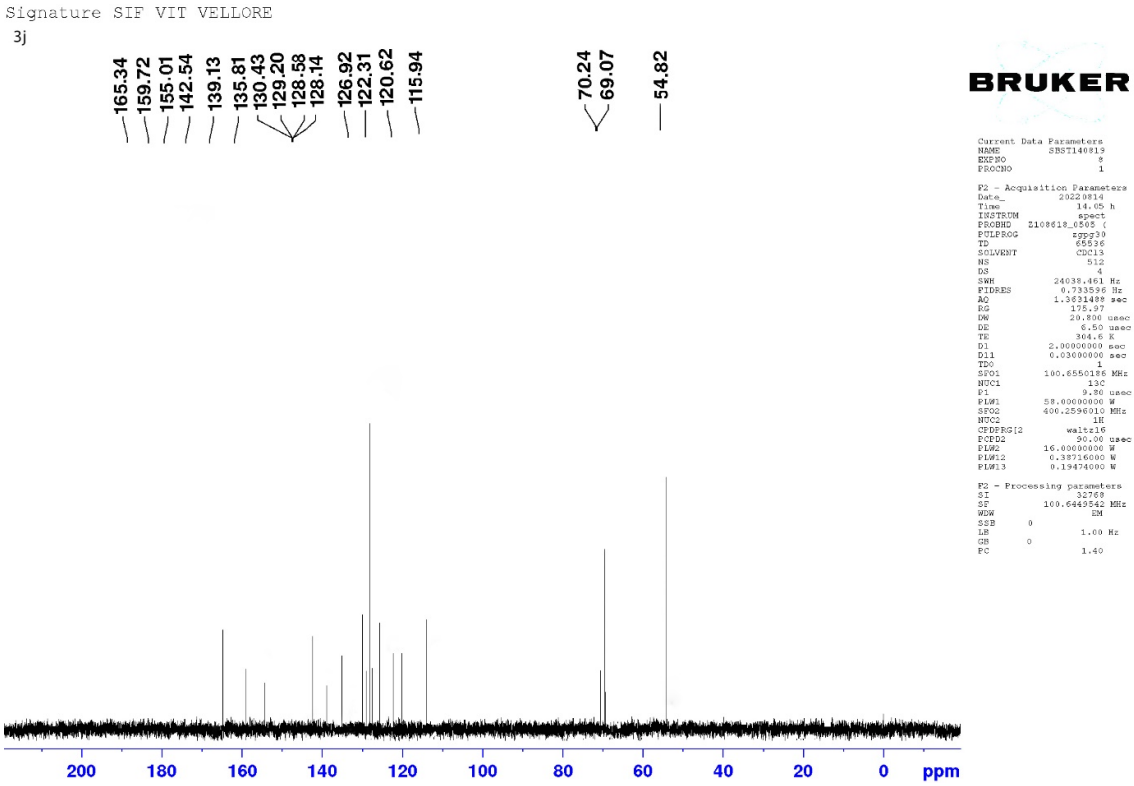
4.4.8. Compound 3h



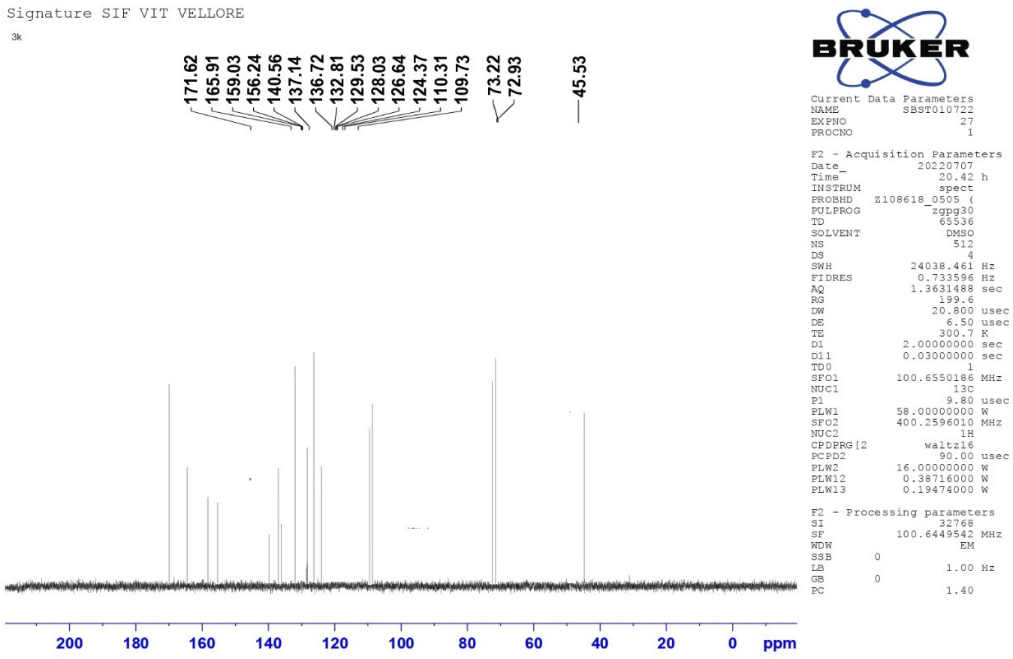
4.4.9. Compound 3i



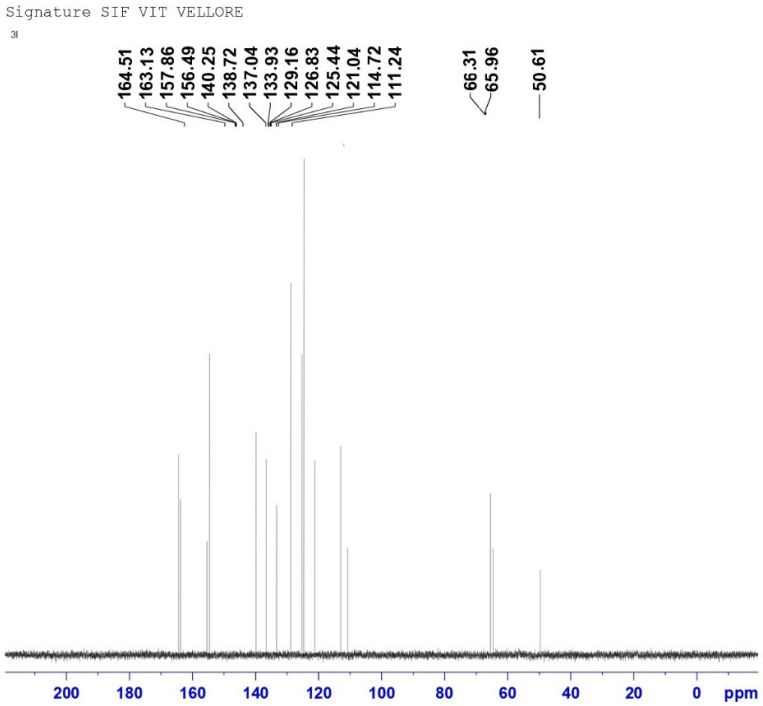
4.4.10. Compound 3j

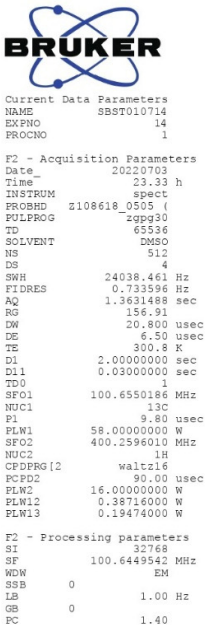


4.4.11. Compound 3k

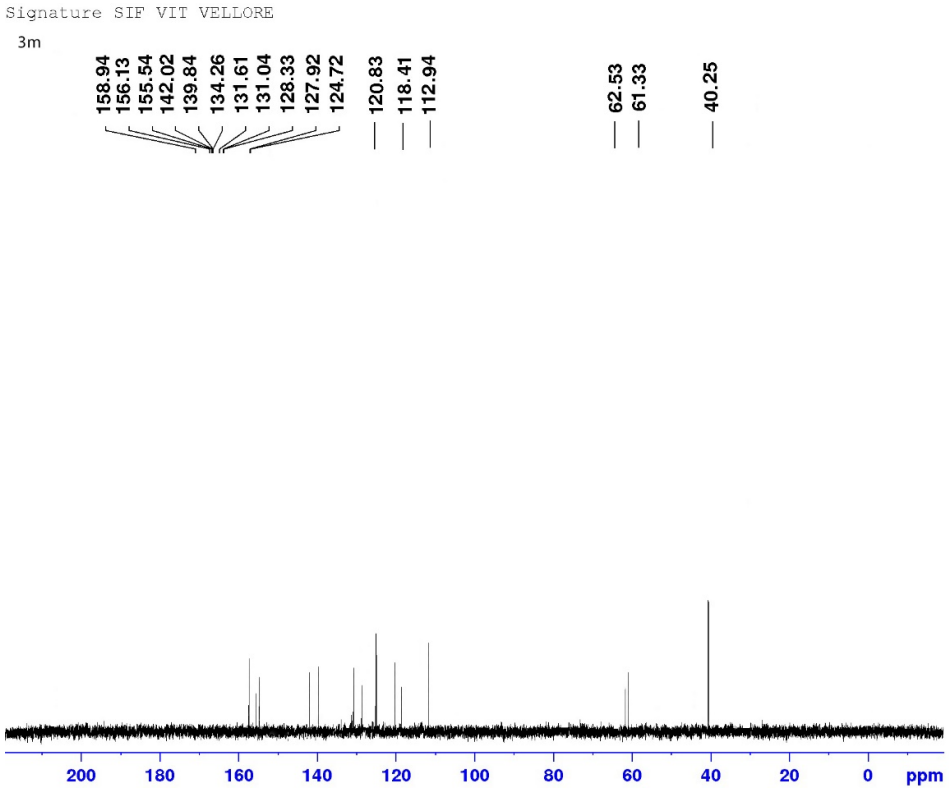


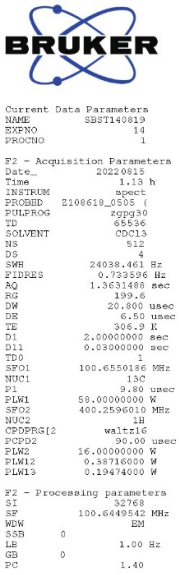
4.4.12. Compound 3l



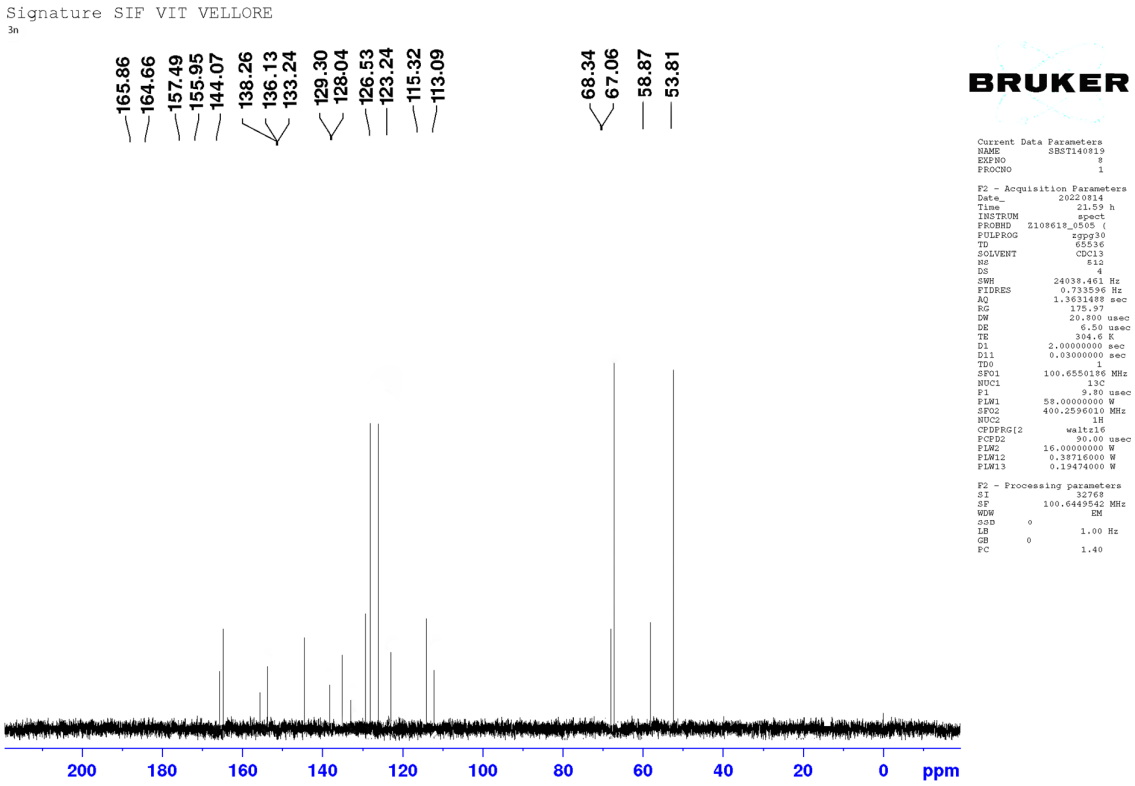


4.4.13. Compound 3m

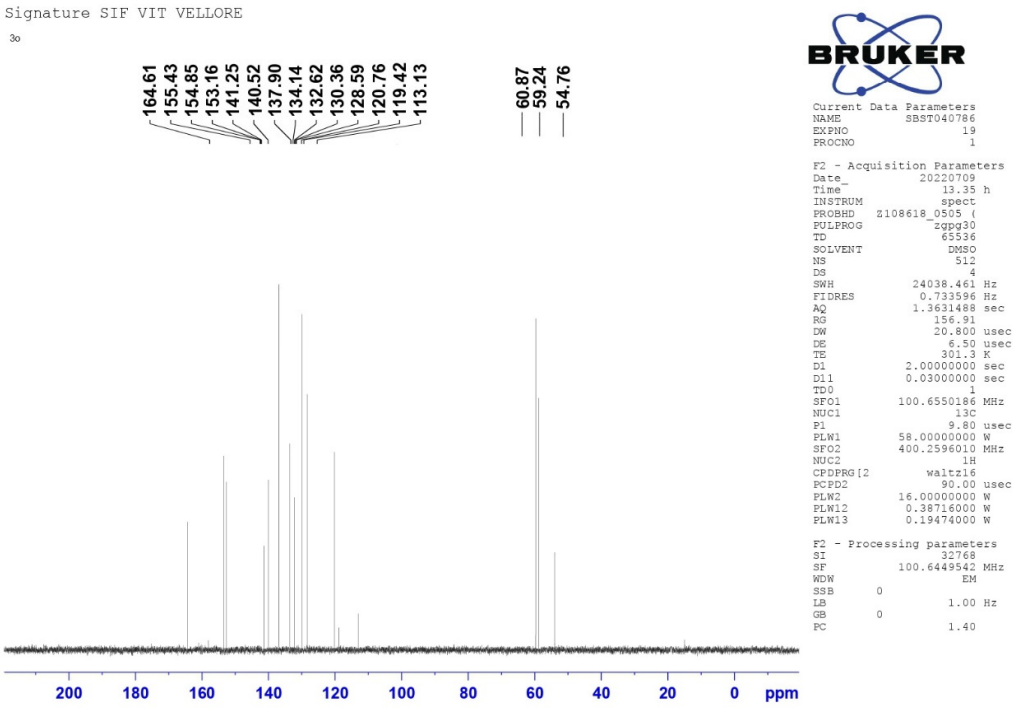




4.4.14. Compound 3n



4.4.15. Compound 3o



4.5. Selected Images for Anti-microbial Activity