Novel chalcone-thiazole Hybrids as Potent Inhibitors of Drug Resistant *Staphylococcus Aureus*

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1. General Information

Commercially available reagents, solvents and starting materials were used without further purification. Chromatography was executed with silica gel (60−120 or 230−400 mesh) using mixtures of ethyl acetate and hexane as eluants. All reactions were monitored by TLC; silica gel plates with fluorescence F254 were used. Melting points were taken in open capillaries on a Complab melting point apparatus and are presented uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined on 300, 400 MHz and 75, 100 MHz, respectively, using $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts were given in parts per million (ppm) downfield from internal standard tetramethylsilane (TMS). Electrospray ionization mass spectra were recorded on a Thermo Lcq Advantage Max-IT instrument. High resolution mass spectra were recorded on 6520 Agilent Q Tof LC− MS/MS (accurate mass). IR spectra were recorded on in the range of 500~4000 cm-1 and multiplicity (s = singlet, brs = broad singlet, d = doublet, brd = broad doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet). All final compounds (**22-34**) purity was checked by Shimadzu high performance liquid chromatography (HPLC) system and found to be > 95% pure. Purity was determined using analytical reverse-phase Phenomenex C18 column (250 mm x 4.6 mm, particle size (μ)-5) and a binary solvent system; solvent A, CH_3CN; solvent B, H_2O. Isocratic elution was carried out having 25min, 15% of H_2O and 85% of CH_3CN; at a flow rate of 0.8 mL/ min monitored at 302 nm using a SPD-M20A photodiode array detector.

1.1. General Synthesis of Compounds (4-6)

A solution of 2-alkyl substituted phenols (33.3 mmol) and hexamethylenetetramine (66.6 mmol) in 20 mL of TFA and reaction mixture was refluxed for 4 h at 120 °C. After cooling to room temperature and hydrolyzed by 10% aqueous H_2SO_4 (25 mL) and reaction mixture was heated further 2 h at 90-100 °C. The reaction mixture was basified with aqueous $NaHCO_3$ and extracted with chloroform. The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (4-5% EtOAc/hexane) to provide desired compounds as solids.

1.1.1. 5-*tert*-butyl-4-hydroxyisophthalaldehyde (**4**)

Oily; Yield: 55%; IR (KBr): 3381, 1692, 1653, 778 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.39 (s, 1H), 9.97 (s, 1H), 9.91 (s, 1H), 8.05 (d, J =1.4 Hz, 1H), 7.97(d, J = 1.4 Hz, 1H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 196.6, 189.9, 164.3, 138.9, 134.8, 133.2, 129.0, 119.9, 26.5, 22.1; ESI-MS (*m/z*): 207 $[M + H]^+$.

1.1.2. 4-hydroxy-5-isopropylisophthalaldehyde (**5**)

White solid; Yield: 55%, mp: 163-164 ºC; IR (KBr): 3023, 1693, 1655, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.94 (s, 1H), 9.97 (s, 1H), 9.91 (s, 1H), 7.99 (brs, 1H), 7.96 (s, 1H), 3.41-3.34 (m, 1H), 1.27 (d, J=6.92 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 191.0, 189.9, 164.4, 139.0, 131.8, 129.7, 129.4, 128.4, 27.0, 23.6 ; ESI-MS (*m/z*): 193 $[M+H]^+$.

1.2. General Synthesis of compounds (7-15)

To a solution of commercially available appropriate acetophenones (10.4 mmol) and aromatic dicarbaldehydes (10.4 mmol) in dioxane (15 mL) was added catalytic amount of hydrochloric acid (10 mL) and reaction mixture was refluxed at 120 °C for 4 h. After TLC showed the completion of reaction, the dioxane was removed under reduced pressure. The crude products obtained were purified by column chromatography (5-10% EtOAc/hexane) to give required compounds as solids.

1.2.1. (*E*)-3-(*tert*-butyl)-2-hydroxy-5-(3-oxo-3-(*p*-tolyl) prop-1-en-1-yl)benzaldehyde (**7**)

Yellow solid; Yield: 51%; mp 122-125 °C; IR (KBr): 3401, 3018, 1654, 1271,759 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 12.07 (s, 1H), 9.96 (s, 1H), 7.96 (d, J = 8.1 Hz, 2H), 7.83 (s, 1H),

7.78 (d, J = 15.6 Hz, 1H), 7.72 (d, J = 2.0 Hz, 1H), 7.46 (d, J = 15.6 Hz, 1H), 7.34 (d, J = 7.9 Hz, 2H), 2.47 (s, 3H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$,100 MHz): 197.0, 189.9, 163.0, 143.7, 143.4, 139.4, 135.7, 133.6, 132.5, 129.4, 128.7, 126.5, 120.7, 35.1, 29.2, 21.8; ESI-MS (*m/z)*: 323 $[M+H]^+$.

1.2.2. (*E*)-3-(*tert*-butyl)-2-hydroxy-5-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl) benzaldehyde (**8**)

Yellow solid; Yield: 42%; mp 142-143 °C; IR (KBr): 3430, 3034, 1678, 1254, 743 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 12.04 (s, 1H), 9.94 (s, 1H), 8.04 (d, J = 8.0 Hz, 2H), 7.80 (d, J = 2.1 Hz, 1H), 7.77 (d, J = 15.6 Hz, 1H), 7.69 (d, J = 2.1 Hz, 1H), 7.44 (d, J = 15.6 Hz, 1H), 6.99 (d, J = 8.8 Hz, 2H), 3.89 (s, 3H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$,100 MHz): 197.1, 188.5, 163.5, 163.0, 142.9, 139.4, 133.6, 132.4, 131.2, 130.9, 126.5, 120.8, 120.5, 114.0, 55.6, 35.1, 29.2; ESI-MS (*m/z)*: 339 $[M+H]^+$.

1.2.3. (E)-3-(tert-butyl)-5-(3-(furan-2-yl)-3-oxoprop-1-en-1-yl)-2-hydroxybenzaldehyde (**9**)

Yellow solid; Yield: 48%; mp 144-145 °C; IR (KBr): 3470, 3017, 1654, 1273, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 12.06 (s, 1H), 9.94 (s, 1H), 7.85 (d, J = 15.7 Hz, 1H), 7.81 (s, 1H), 7.71 (s, 1H), 7.66 (s, 1H), 7.38-7.34 (m, 2H), 6.60 (dd, $J_{1,2}$ = 1.6 Hz, $J_{1,3}$ = 3.5 Hz, 1H), 1.46 (s, 1H); ^{13}C NMR ($CDCl_3$,100 MHz): 197.0, 177.9, 163.2, 153.8, 146.5, 142.9, 139.4, 133.8, 132.7, 126.2, 120.8, 119.7, 117.5, 112.7, 35.1, 29.2; ESI-MS (*m/z)*: 299 $[M+H]^+$.

1.2.4. (*E*)-3-(*tert*-butyl)-2-hydroxy-5-(3-oxo-3-(thiophen-2-yl)prop-1-en-1-yl)benzaldehyde (**10**)

Yellow solid; Yield: 62%; mp 145-146 °C; IR (KBr): 3389, 3014, 1625, 1235, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 12.07 (s, 1H), 9.95 (s, 1H), 7.88-7.87 (m, 2H), 7.84-7.80 (m, 2H), 7.32 (d, J = 15.4 Hz, 1H), 7.19 (dd, $J_{1,2}$ = 3.8 Hz, $J_{1,3}$ = 4.8 Hz, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): 197.0, 181.9, 163.2, 145.6, 143.1, 139.5, 133.9, 133.7, 132.6, 131.8, 128.3, 126.2, 120.8, 120.2, 35.15, 29.2; ESI-MS (*m/z)*: 315 $[M+H]^+$.

1.2.5. *(E)-2-hydroxy-3-isopropyl-5-(3-oxo-3-(p-tolyl)prop-1-en-1-yl)benzaldehyde* (**11**)

Yellow solid; Yield: 59%; mp 134-135 °C; IR (KBr): 3401, 3019, 1655, 1216, 759 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 11.62 (s, 1H), 9.95 (s, 1H), 7.94 (d, J = 8.2 Hz, 2H), 7.77 (d, J = 15.6 Hz, 1H), 7.74 (s, 1H), 7.67 (s, 1H), 7.44 (d, J = 15.6 Hz, 1H), 7.32 (s, 1H), 7.30 (s, 1H), 3.44-3.34 (m, 1H), 2.45 (s, 1H), 1.30 (d,); ^{13}C NMR ($CDCl_3$,100 MHz): 196.8, 189.8, 161.2, 143.7, 143.2, 138.3, 135.7, 132.9, 132.1, 129.4, 128.7, 126.9, 120.8, 120.3, 26.6, 22.2, 21.7; ESI-MS (*m/z)*: 309 $[M+H]^+$.

1.2.6. *(E)-2-hydroxy-3-isopropyl-5-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)benzaldehyde* (**12**)

Yellow solid; Yield: 45%; mp 124-125 °C; IR (KBr): 3399, 3018, 1654, 1217, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 11.61 (s, 1H), 9.94 (s, 1H), 8.05 (d, J = 8.9 Hz, 2H), 7.77 (d, J = 15.6 Hz, 1H), 7.75-7.74 (m, 1H), 7.67 (s, 1H), 7.46 (d, J = 15.6 Hz, 1H), 6.99 (d, J = 8.9 Hz, 1H), 3.90 (s, 1H), 3.43-3.36 (m, 1H), 1.30 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$,100 MHz): 196.8, 188.5, 163.5, 161.1, 142.7, 138.2, 132.8, 132.0, 131.1, 130.9, 127.0, 120.6, 120.3, 113.9, 55.6, 26.6, 22.2; ESI-MS (*m/z)*: 325 $[M+H]^+$.

1.2.7. (*E*)-5-(3-(furan-2-yl)-3-oxoprop-1-en-1-yl)-2-hydroxy-3-*iso*propylbenzaldehyde (**13**)

Yellow solid; Yield: 47%; mp 119-120 °C; IR (KBr): 3421, 3147, 1623, 1216, 745 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz):δ 11.63 (s, 1H), 9.94 (s, 1H), 7.85 (d, J = 15.7 Hz, 1H), 7.75 (d, J = 1.8Hz, 1H), 7.69-7.66 (m, 2H), 7.38 (d, J = 15.7 Hz, 1H), 7.35-7.34 (m, 1H), 3.42-3.35 (m, 1H), 1.30 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 196.8, 177.9, 161.3, 153.8, 146.5, 142.7, 138.3, 133.0, 132.3, 126.7, 119.8, 117.5, 112.7, 26.6, 22.2; ESI-MS (*m/z)*: 285 $[M+H]^+$.

1.2.8. *(E)-2-hydroxy-3-isopropyl-5-(3-oxo-3-(thiophen-2-yl)prop-1-en-1-yl)benzaldehyde* (**14**)

Yellow solid; Yield: 49%; mp 133-134 °C; IR (KBr): 3405, 3020, 1651, 1216, 762 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 11.63 (s, 1H), 9.95 (s, 1H), 7.88 (d, J = 4.8 Hz, 1H), 7.82 (d, J = 15.5 Hz, 1H), 7.73 (s, 1H), 7.70-7.68 (m, 2H), 7.33 (d, J = 15.5 Hz, 1H), 7.19 (dd, $J_{1,2}$ = 3.8 Hz, $J_{1,3}$ = 4.9 Hz, 1H), 3.44-3.34 (m, 1H), 1.30 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 196.9, 181.7, 163.0, 145.5, 142.9, 139.3, 133.8, 133.6, 132.5, 131.7, 128.2, 126.0, 120.6, 120.1, 35.0, 29.1; ESI-MS (*m/z)*: 301 $[M+H]^+$.

1.2.9. *(E)-5-(3-(furan-2-yl)-3-oxoprop-1-en-1-yl)-2-hydroxy-3-propylbenzaldehyde* (**15**)

Yellow solid; Yield: 45%; mp 151-152 °C; IR (KBr): 3415, 3032, 1665, 1229, 747 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 11.53 (s, 1H), 9.93 (s, 1H), 7.83 (d, J = 15.7 Hz, 1H), 7.70-7.65 (m, 3H), 7.37 (d, J = 15.7 Hz, 1H), 7.35-7.34 (m, 1H), 6.60 (dd, $J_{1,2}$ = 1.6 Hz, $J_{1,3}$ = 3.5 Hz, 1H), 2.68 (t, J = 7.5 Hz, 2H), 1.71-1.67 (m, 2H), 0.99 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 196.6, 177.8, 161.9, 153.8, 146.5, 142.5, 136.1, 132.6, 132.5, 126.5, 120.3, 119.9, 117.5, 112.7, 31.3, 22.5, 14.0; ESI-MS (*m/z)*: 285 $[M+H]^+$.

1.3. General Synthesis of Compounds (19-21)

The appropriate 2-halo ketones (10.2 mmol) were dissolved in ethanol, which is found to be the best solvent, and heated to reflux for 24 h with an equimolar quantity of thiourea (10.2 mmol), led to the formation of the 2-aminothiazole derivatives in good yields. The removal of the ethanol in *vacuum* left solids, which were purified *via* silica gel chromatography (35-40% EtOAc/hexane) provided required compounds (**19-21**) as white solids.

1.3.1 *ethyl 2-aminothiazole-4-carboxylate* (**19**)

White solid; Yield: 69%; mp 162-163 °C; IR (KBr): 2931, 1695, 1220, 762 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 7.44 (s, 1H), 7.20 (s, 2H), 4.19 (q, J = 7.8 Hz, 2H), 1.24 (t, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 168.1, 161.0, 142.1, 116.9, 60.0, 14.1; ESI-MS (*m/z)*: 173 $[M+H]^+$.

1.3.2 *4-(4-methoxyphenyl)thiazol-2-amine* (**20**)

White solid; Yield: 67.6%; mp 192-193 °C; IR (KBr): 3400, 1626, 1219, 771 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 7.71 (d, J = 8.8 Hz, 2H), 6.98 (s, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.81 (s, 1H), 3.76 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 168.0, 158.5, 149.6, 127.8, 126.8, 113.7, 99.3, 55.0; ESI-MS (*m/z)*: 207 $[M+H]^+$.

1.3.3 *4-(3,4-dimethoxyphenyl)thiazol-2-amine* (**21**)

White solid; Yield: 68%; mp 195-196 °C; IR (KBr): 3405, 1650, 1215, 760 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 12.38 (s, 1H), 8.50 (s, 1H), 7.55 (s, 1H), 7.45-7.43 (m, 2H), 6.99 (d, J = 8.6 Hz, 1H), 3.81 (s, 3H), 3.78 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 159.6, 156.0, 148.9, 148.8, 148.7, 127.1, 118.2, 111.9, 109.4, 106.7, 55.5; ESI-MS (*m/z)*: 237 $[M+H]^+$.

1.4. General Synthesis of Compounds (22-34)

The appropriate chalcones (5.9 mmol) were dissolved in ethanol, which is found to be the best solvent, and heated to reflux for 24 h with an equimolar quantity of 2-aminothiazole derivative (5.9 mmol), led to the formation of the chalcone-thiazole derivatives in good yields. The removal of the ethanol in *vacuum* left solids, which were purified *via* silica gel chromatography (15-20% EtOAc/hexane) provided required compounds (**22-34**) as yellow solids.

1.4.1. Ethyl-2-((*E*)-(3-(*tert*-butyl)-2-hydroxy-5-((*E*)-3-oxo-3-(*p*-tolyl)prop-1-en-1-yl) benzylidene)aminothiazole-4-carboxylate (**22**)

Yellow solid; Yield: 60%; mp: 185-187^{o}C; IR (KBr): 3404, 3128, 2953, 2204, 1598, 1584, 1092 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz):δ 13.20 (s, 1H), 9.38 (s, 1H), 8.11 (s, 1H), 7.95 (d, J = 8.0 Hz, 2H), 7.80 (s, 1H), 7.75 (s, 1H), 7.67 (s, 1H), 7.44 (d, J = 15.6 Hz, 1H), 7.33-7.31 (m, 2H) 4.46 (q, J = 7.1 Hz, 2H), 2.44 (s, 3H), 1.49 (s, 9H), 1.43 (t, J=7.1 Hz, 3H); ^{13}C

NMR ($CDCl_3$, 100 MHz): δ 190.7, 159.3, 155.7, 150.0, 148.8, 139.0, 138.6, 132.1, 128.8, 128.7, 128.0, 126.6, 121.2, 120.4, 111.7, 111.1, 56.0, 55.9, 35.2, 29.6, 29.3 ; HRMS: calcd for $C_{27}H_{29}N_2O_4S$ $[M+H]^+$, 477.1848 ; found, 477.1840.

1.4.2. Ethyl-2-((*E*)-(3-(*tert*-butyl)-2-hydroxy-5-((*E*)-3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)amino)thiazole-4-carboxylate (**23**)

Light yellow solid; Yield: 61%; mp: 175-176 °C; IR (KBr): 3414, 3018, 2983, 2200, 1668, 1584, 1092 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz):δ 13.17 (s, 1H), 9.39 (s, 1H), 8.10 (s, 1H), 8.05 (d, J = 8.8 Hz, 2H), 7.79 (s, 1H), 7.75 (s, 1H), 7.79 (s, 1H), 7.75 (s, 1H), 7.67 (s, 1H), 7.44 (d, J = 15.5 Hz, 1H), 7.00 (d, J = 4.9 Hz, 2H), 4.46 (q, J = 7.1 Hz, 2H), 3.90 (s, 3H), 1.49 (s, 9H), 1.43 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 190.7, 159.2, 155.9, 153.3, 139.5, 139.2, 132.3, 129.5, 129.2, 128.9, 128.3, 120.4, 106.0, 61.0, 56.4, 35.3, 29.7; HRMS: calcd for $C_{27}H_{29}N_2O_5S$ $[M+H]^+$, 493.1797; found, 493.1791.

1.4.3. Ethyl-2-((*E*)-(3-(tert-butyl)-5-((*E*)-3-(furan-2-yl)-3-oxoprop-1-en-1-yl)-2-hydroxybenzylidene)amino)thiazole-4-carboxylate (**24**)

Yellow solid; Yield: 59%; mp: 177-179 ^oC; IR (KBr): 3533, 3092, 2876, 2198, 1670, 1583, 1081 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 13.21 (s, 1H), 9.38 (s, 1H), 8.10 (s, 1H), 7.85 (d, J = 16.0 Hz, 1H), 7.75 (d, J = 4.0 Hz, 1H), 7.68 (s, 1H), 7.65 (d, J = 4.0 Hz, 1H),7.37 (s, 1H), 7.33 (d, J = 4.0 Hz, 1H), 6.61 (q, J = 4 Hz, 1H), 1.48 (s, 9H), 1.43 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 181.9, 168.3, 168.1, 163.2, 160.2, 146.7, 145.5, 142.1, 139.8, 137.8, 134.0, 132.9, 131.7, 127.9, 126.8, 126.0, 119.4, 111.4, 60.8, 37.2, 29.6, 29.3, 14.4; HRMS: calcd for $C_{24}H_{24}N_2O_5S$ $[M+H]^+$, 453.1484; found, 453.1482.

1.4.4. Ethyl-2-((*E*)-(3-(*tert*-butyl)-2-hydroxy-5-((*E*)-3-oxo-3-(thiophen-2-yl)prop-1-en-1-yl)benzylidene)amino)thiazole-4-carboxylate (**25**)

Light yellow solid, yield: 60%; mp: 185-186 °C; IR (KBr): 3314, 3008, 2883, 2224, 1678, 1544, 1102 cm-1; ^1H NMR ($CDCl_3$, 400 MHz) δ: 13.20 (s, 1H), 9.39 (s, 1H), 8.11 (s, 1H), 7.88 (s, 1H), 7.82 (d, J = 15.5 Hz, 1H), 7.74 (s, 1H), 7.68 (s, 1H), 7.32 (d, J = 15.5 Hz, 1H), 7.20 (t, J = 4.4 Hz, 1H), 4.45 (q, J = 7.0 Hz, 2H), 1.49 (s, 9H), 1.43 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 181.9, 169.4, 166.7, 163.4, 161.2, 145.7, 145.5, 143.4, 139.2, 133.8, 133.0, 132.4, 131.7, 128.3, 126.8, 126.1, 119.8, 118.4, 61.8, 35.2, 29.8, 29.3, 14.4; HRMS: calcd for $C_{24}H_{25}N_2O_4S_2$ $[M+H]^+$, 469.1256; found, 469.1252.

1.4.5. (E)-3-(3-(tert-butyl)-4-hydroxy-5-((E)-((4-(4-methoxyphenyl)thiazol-2-yl)imino)methyl)phenyl)-1-(p-tolyl)prop-2-en-1-one (**26**)

Light yellow solid, yield: 62%; mp: 185-187 °C; IR (KBr): 3316, 3018, 2983, 2223, 1678, 1541, 1103 cm-1; ^1H NMR ($CDCl_3$, 400 MHz) δ: 13.47 (s, 1H), 9.36 (s, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.87 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 15.6 Hz, 1H), 7.74 (s, 1H), 7.67 (s,1H) ,7.44 (d, J = 15.6, 1H), 7.32-7.31 (m, 3H), 6.98 (d, J = 8.8 Hz, 2H), 3.86 (s, 3H), 2.45 (s, 3H), 1.50 (s, 9H), ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 190.0, 168.6, 164.7, 163.2, 160.0, 153.9, 144.1, 143.6, 139.0, 135.9, 132.6, 131.7, 129.4, 128.7, 127.7, 127.2, 126.1, 120.0, 118.7, 114.3, 111.0, 55.5, 35.2, 29.3, 21.8; HRMS: calcd for $C_{31}H_{31}N_2O_3S$ $[M+H]^+$, 511.2055; found, 511.2049.

1.4.6. (*E*)-3-(3-(tert-butyl)-5-((*E*)-((4-(3, 4-dimethoxyphenyl)thiazol-2-yl)imino)methyl)-4-hydroxyphenyl)-1-(*p*-tolyl)prop-2-en-1-one (**27**)

Yellow solid; Yield: 60%; mp: 175-178 °C; IR (KBr): 3313, 3008, 2884, 2213, 1667, 1532, 1031 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 13.48 (s, 1H), 9.36 (s, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 4.0 Hz, 1H), 7.77 (s, 1H), 7.70 (s, 1H), 7.48 (m, 3H), 7.35 (s, 2H), 7.28 (s, 1H), 6.97 (d, J = 8.0 Hz, 1H), 4.03 (s, 3H), 3.96 (s, 3H), 2.47 (s, 3H), 1.53 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 189.9, 168.5, 164.7, 163.1, 153.9, 149.5, 149.2, 143.9, 138.9, 135.8, 132.5, 132.5, 131.6, 129.3, 128.6, 127.3, 126.0, 118.8, 118.6, 111.3, 111.1, 109.7, 56.0, 55.9, 35.1, 29.2, 21.6. HRMS: calcd for $C_{32}H_{32}N_2O_4S$ $[M+H]^+$, 541.2161; found, 541.2205.

1.4.7. (*E*)-3-(3-(*tert*-butyl)-5-((*E*)-((4-(3, 4-dimethoxyphenyl)thiazol-2-yl)imino)methyl)-4-hydroxy phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**28**)
Light yellow solid; Yield: 58%; mp: 155-156 °C; IR (KBr): 3214, 3118, 2973, 2189, 1700, 1604, 1062 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 13.45 (s, 1H), 9.34 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 15.5 Hz, 1H), 7.75 (s, 1H), 7.67 (s, 1H), 7.50-7.43 (m, 3H), 7.32 (s, 1H), 7.00 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.2 Hz, 1H), 4.00 (s, 3H), 3.94 (s, 3H), 3.90 (s, 3H), 1.51 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 188.7, 168.6, 164.8, 163.5, 163.1, 154.0, 149.6, 149.3, 143.6, 139.0, 132.6, 131.7, 131.4, 130.8, 127.5, 126.2, 119.9, 119.0, 118.7, 113.9, 111.4, 111.2, 109.8, 56.2, 56.1, 55.6, 35.2, 29.3; HRMS: calcd for $C_{32}H_{33}N_2O_5S$ [M+H]+, 557.2110; found, 557.2104.
1.4.8. (*E*)-3-(3-(*tert*-butyl)-5-((*E*)-((4-(3, 4-dimethoxyphenyl)thiazol-2-yl)imino)methyl)-4-hydroxy phenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**29**)
Light yellow solid; Yield: 59%; mp: 145-146 °C; IR (KBr): 3404, 3008, 2943, 2210, 1698, 1594, 1082 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 13.48 (s, 1H), 9.34 (s, 1H), 7.89-7.88 (m, 1H), 7.85 (d, J = 15.5 Hz, 1H), 7.74 (s, 1H), 7.69-7.67 (m, 2H), 7.50-7.46 (m, 2H), 7.33 (t, J = 8.8 Hz, 2H), 7.20 (dd, $J_{1,2}$ = 3.8 Hz, $J_{1,3}$ = 4.9 Hz, 1H), 6.94 (d, J = 8.2 Hz, 1H), 4.00 (s, 3H), 3.94 (s, 3H), 1.51 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.0, 168.6, 164.7, 163.3, 154.0, 149.3, 145.8, 143.7, 139.1, 133.7, 132.7, 131.8, 131.7, 128.3, 127.4, 125.8, 119.6, 119.0, 118.7, 111.4, 111.2, 109.8, 56.2, 56.1, 35.2, 29.3; HRMS: calcd for $C_{29}H_{29}N_2O_4S_2$ $[M+H]^+$, 533.1569; found, 533.1581.
1.4.9. (*E*)-3-(3-(*tert*-butyl)-4-hydroxy-5-((*E*)-((4-(4-methoxyphenyl)thiazol-2-yl)imino) methyl)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**30**)
Light yellow solid; Yield: 58%; mp: 171-172 °C; IR (KBr): 3398, 3098, 2990, 2190, 1700, 1590, 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 13.46 (s, 1H), 9.36 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H), 7.87 (d, J = 8.6 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 7.74 (s, 1H), 7.66 (s, 1H), 7.45 (d, J = 15.5 Hz, 1H), 7.28 (s, 1H), 6.99 (t, J = 8.6 Hz, 4H), 3.90 (s, 3H), 3.86 (s, 3H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 188.7, 168.6, 167.8, 164.7, 163.5, 163.1, 160.0, 153.9, 143.6, 139.0, 132.6, 131.7, 131.4, 131.0, 130.9, 128.9, 127.7, 127.2, 126.2, 119.8, 118.7, 114.3, 113.9, 110.9, 68.3, 55.6, 55.5, 38.8, 29.0; HRMS: calcd for $C_{31}H_{31}N_2O_4S$ $[M+H]^+$, 527.2005; found, 527.2011.
1.4.10. Ethyl-2-((*E*)-(2-hydroxy-3-isopropyl-5-((*E*)-3-oxo-3-(*p*-tolyl)prop-1-en-1-yl) benzylidene)amino)thiazole-4-carboxylate (**31**)
Light yellow solid; Yield: 60%; mp: 181-182 °C; IR (KBr): 3400, 3020, 2990, 2223, 1684, 1596, 1112 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 12.80 (s, 1H), 9.38 (s, 1H), 8.10 (s, 1H), 7.95 (d, J = 8.1 Hz, 2H), 7.77 (d, J = 15.6 Hz, 1H), 7.68 (s, 1H), 7.64 (s, 1H), 7.44 (d, J = 15.6 Hz, 1H), 7.32 (d, J = 7.9 Hz, 2H), 4.45 (q, J = 7.1 Hz, 2H), 3.46-3.39 (m, 1H), 2.44 (s, 3H), 1.43 (t, J = 7.1 Hz, 3H), 1.32 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 189.6, 169.4, 166.3, 161.3, 161.1, 145.4, 143.4, 143.3, 137.9, 135.7, 132.4, 131.3, 129.3, 128.5, 126.7, 120.2, 117.7, 61.6, 26.8, 22.1, 14.3; HRMS: calcd for $C_{26}H_{27}N_2O_4S$ $[M+H]^+$, 463.1692; found, 463.1684.
1.4.11. Ethyl-2-((*E*)-(2-hydroxy-3-isopropyl-5-((*E*)-3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)benzylidene)amino)thiazole-4-carboxylate (**32**)
Light yellow solid; Yield: 72%; mp: 142-143 °C; IR (KBr): 3431, 3032, 2943, 2234, 1620, 1524, 1068 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 12.79 (s, 1H), 9.38 (s, 1H), 8.10 (s, 1H), 8.05 (d, J = 8.8 Hz, 2H), 7.77 (d, J = 15.5 Hz, 1H), 7.68 (s, 1H), 7.64 (s, 1H), 7.45 (d, J = 15.5 Hz, 1H), 7.00 (d, J = 8.8 Hz, 2H), 4.45 (q, J = 7.1 Hz, 2H), 3.90 (s, 1H), 3.46-3.39 (m, 1H), 1.43 (t, J = 7.1Hz, 3H), 1.32 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 188.6, 169.6, 166.5, 163.5, 161.4, 161.2, 145.5, 143.1, 138.0, 132.5, 131.4, 131.3, 130.9, 126.9, 126.8, 120.2, 117.9, 113.9, 61.8, 55.6, 26.9, 22.3, 14.4; HRMS: calcd for $C_{26}H_{27}N_2O_5S$ $[M+H]^+$, 479.1641; found, 479.1631.

1.4.12. ethyl 2-((E)-(5-((E)-3-(furan-2-yl)-3-oxoprop-1-en-1-yl)-2-hydroxy-3-propylbenzylidene)amino)thiazole-4-carboxylate (**33**)
Light yellow solid; Yield: 59%; mp: 143-144 °C; IR (KBr): 3390, 3008, 2935, 2194, 1604, 1546, 1104 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 12.74 (s, 1H), 9.38 (s, 1H), 8.10 (s, 1H), 7.83 (d, J = 7.83 Hz, 1H), 7.67 (m, 3H), 7.38-7.34 (m, 2H), 6.61 (dd, $J_{1,2}$ = 1.6 Hz, $J_{1,3}$ = 3.5 Hz, 1H), 4.46 (q, J = 7.1 Hz, 2H), 2.72 (t, J = 7.4 Hz, 2H), 1.74-1.69 (m, 2H), 1.44 (t, J = 7.1 Hz, 3H), 1.01 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177.9, 169.6, 166.3, 162.2, 161.2, 153.9, 146.5, 145.5, 142.8, 134.6, 133.1, 132.2, 126.8, 126.5, 119.5, 117.9, 117.4, 112.6, 61.8, 31.7, 22.6, 14.4, 14.1; HRMS: calcd for $C_{23}H_{23}N_2O_5S$ $[M+H]^+$, 439.1328; found, 439.1321.
1.4.13. Ethyl-2-((*E*)-(2-hydroxy-3-isopropyl-5-((*E*)-3-oxo-3-(thiophen-2-yl)prop-1-en-1-yl)benzylidene)amino)thiazole-4-carboxylate (**34**)
Light yellow solid; Yield: 60%; mp: 177-178 °C; IR (KBr): 3410, 3010, 2980, 2206, 1660, 1580, 1090 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 12.84 (s, 1H), 9.39 (s, 1H), 8.14 (s, 1H), 7.94-7.92 (m, 1H), 7.84 (d, J = 15.5 Hz, 1H), 7.72-7.66 (m, 3H), 7.37 (d, J = 15.4 Hz, 1H), 7.23 (dd, J_{12} = 3.8 Hz, J_{13} = 4.8 Hz, 1H), 4.48 (q, J = 7.1 Hz, 2H), 3.50-3.41 (m, 1H), 1.46 (t, J = 7.1 Hz, 3H), 1.35 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 181.8, 169.4, 166.3, 161.5, 145.6, 145.4, 143.1, 137.9, 133.7, 132.5, 131.5, 128.2, 126.7, 126.5, 119.8, 117.8, 61.7, 29.7, 26.9, 22.1, 14.3; HRMS: calcd for $C_{23}H_{23}N_2O_4S_2$ [M+H]+, 455.1099; found, 455.1093.

2. Biological Materials and Methods

2.1. Ethics statement
The use of human blood (CDRI/IEC/2014/A1 dated 10th march 2014) and mice for infectious studies (IAEC/2013/91 dated 08th Nov 2013) were approved by both the human as well animal ethics committee at CDRI, Lucknow respectively.
2.2. Bacterial strains
Bacterial strains and growth conditions—All the *S. aureus* (ATCC 25923; 29213; 33592; 700699) were obtained from the ATCC, USA and were routinely grown in Todd-Hewitt (TH; Difco/Becton & Dickinson, Franklin Lakes, NJ) Agar medium. Just before the experiment, a loop full of bacteria was inoculated in liquid medium and incubated at 37 °C and 5% CO_2 for overnight to get the starter culture.
2.3. Minimum inhibitory concentration studies
MIC assay for each test compound was carried out by the microtiter broth dilution, CLSI (previously NCCLS) method.[1, 2] Test compounds concentrations used ranged from 50μg/mL to 0.19 μg/mL. For determination of MIC, test compounds were dissolved in DMSO 5 mg/mL and later diluted in water at a concentration 10 times higher than the required range by serial dilutions from a stock solution, and 10 μL of each concentration added to each well of a 96-well microtiter plate (Polypropylene, Corning Inc., Corning, USA). For CLSI, bacteria was grown overnight in 5% Mueller Hinton (MH) broth were rinsed twice with Tris, pH 7.4, and diluted in MH broth to a bacterial suspension with a concentration of $1\text{-}5\times10^5$ CFU/mL. Later, 90 μL of bacterial suspension in MH medium was added to each well containing the test compound and incubated at 37 °C for 16 h. MIC values were determined by visual inspection, as well as by measuring the absorbance at 492 nm using a microplate reader (SpectraMax, USA). MIC was taken as the concentration where the growth inhibition observed was greater than $\geq$90%. For each compound, MIC determinations were carried independently 2 times using triplicate samples each time.

2.4. Hemolysis assay

Human blood was collected in EDTA (2mg/mL) containing vacutinaer as described previously.[3] Plasma and buffy coat was removed from the EDTA-blood by centrifugation at 800 X g for 10 mins. Later, the erythrocytes were washed three times by using normal saline (0.9%) and resuspended in saline to 5% erythrocytes suspension. The cells were incubated with end-over-end rotation for 1h at 37 °C in the presence of test compounds (100 µg/mL). After the incubation, the samples were centrifuged at 800 X g for 10 min and the supernatant was transferred to 96 well plates. 2% Triton X-100 (Sigma- Aldrich, St. Louis, USA) served as positive control. The absorbance of hemoglobin release was measured at 540 nm and is expressed as % of Triton X-100 induced hemolysis. The result was calculated by using the formula.

$$\% \text{ Hemolysis} = \frac{(\text{Absorbance of sample}) - (\text{Absorbance of blank})}{\text{Highest absorbance of positive control}} \text{ x } 100$$

2.5. MTT assay

Mammalian fibroblast cell line, L929 was used to evaluate the toxicity of synthetic compounds. Test compounds were prepared in DMSO as stock solutions (5 mg/mL). MTT (3-(4, 5-dimethylthiazolyl)- 2, 5-diphenyltetrazolium bromide; Sigma-Aldrich) solution (5 mg/mL) was prepared in PBS and stored at -20 °C after filter sterilisation by using 0.22 µm syringe filter. L929 Cells, 3000 cells/well, were seeded in 96-well plates and grown in RPMI 1640 medium supplemented with 10% FBS and 1 X antimycotic and antibacterial solution (sigma, USA) at 37 °C and 5% CO_2 to confluency. Test compounds (**22**, **27**) to be investigated were serially diluted (i.e., 50, 25, 12.5, 6.25, 3.12, 0.78, 0.39, 0.19 µg/mL) in serum free RPMI medium and added to the cells which were previously replaced with serum free RPMI along with appropriate controls. After incubation overnight, 200 µL of the MTT solution (0.5 mg in RPMI 1640 medium) was added to each well, and the plates were incubated for 1h in CO_2 at 37 °C. The supernatant from each well removed and 100 µL of the DMSO was added to each well to dissolve the blue formazan product generated. The plates were then gently swirled for 30 mins at room temperature to dissolve the precipitate. The absorbance was monitored at 570 nm and results given represent mean values from independent triplicate measurements.2% Triton X-100 (Sigma- Aldrich, St. Louis, USA) served as positive control.

2.6. Morphological analysis studies

The morphological analysis of the cells was observed using Giemsa stain under phase contrast microscope. After fixation of the cells in the wells of 96 well tissue culture plates, Giemsa stain was added to each well and incubated for 30 min at 37 °C. Culture plates were then air dried and observed under a phase contrast microscope.

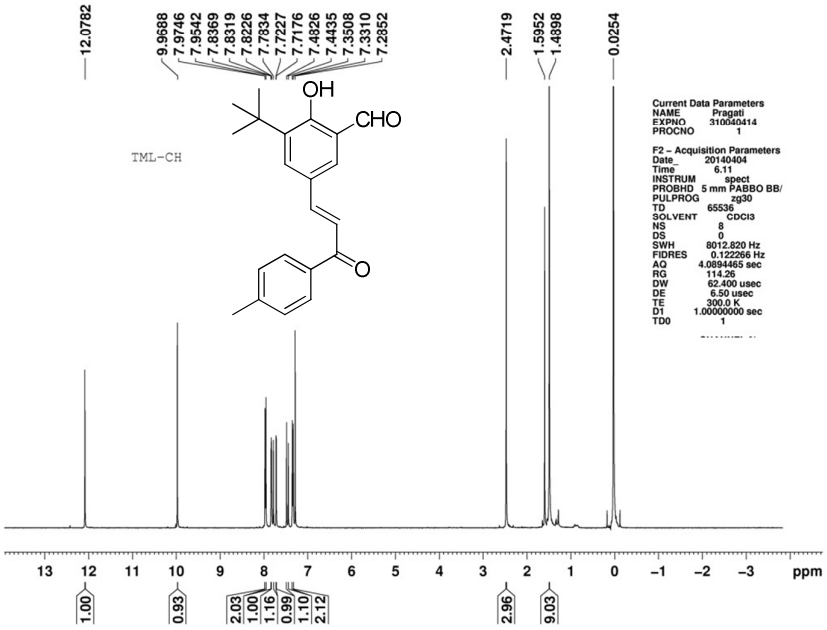
2.7. Animal models

For the *in vivo* studies, BALB/C mice were housed under standard conditions of light and temperature and had free access to standard laboratory chow and water. Animals were rendered neutropenic by injecting cyclophosphamide intraperitoneally before 24 h (150 mg/Kg of body weight) and 6h of the experiment. *S. aureus* ATCC 29213 was grown overnight at 37 °C in 3% *Todd Hewitt* (TH) medium and next day 200 µL of the overnight culture was inoculated in 10 mL of fresh medium 3% TH medium, followed by the incubation at 37 °C until the OD reached 0.4-0.5 at 620 nm. The bacteria were washed twice with PBS, pH 7.4 and resuspended in same buffer to 1-2 X 10^9 cfu/ml. one hundred µL of the bacterial suspension were injected intraperitoneally (i.p) into mice and after 2 hrs, test compound of 0.5 mg/mice (15 mg/Kg) and where as Vancomycin of 0.3 mg/mice (10mg/Kg) was injected I.P into the mice, far enough from the site of infection dose injection. This was repeated after 4 hrs.

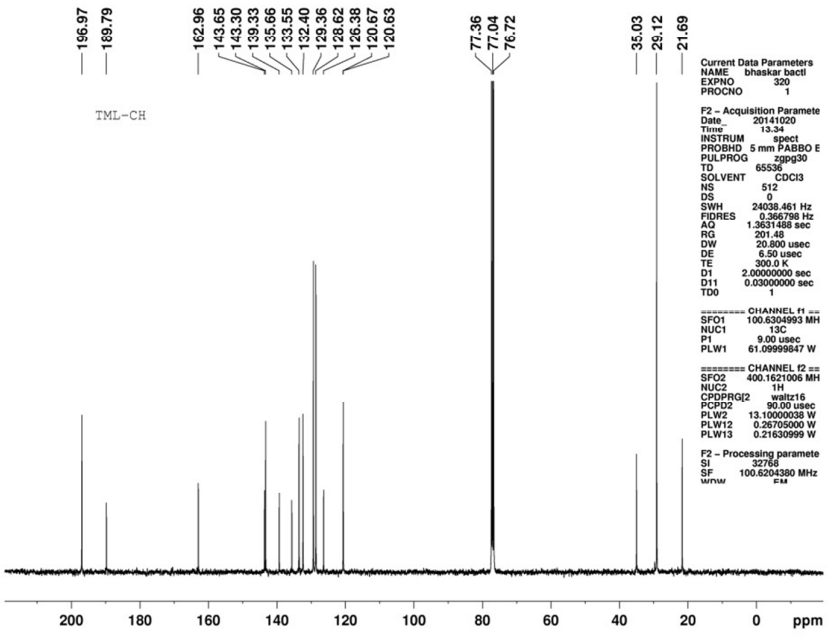
In order to investigate the bacterial dissemination to various organs, the mice were sacrificed after 12-16 h of post drug injection and spleen, liver and kidney were harvested. In order to determine the colony forming units, harvested organs were homogenized to get a bacterial suspension. The suspension was diluted in PBS and then plated on the TH agar medium and colony forming units were determined. The P-value was determined using the ANOVA on ranks. Data from three independent experiments were pooled.

3. NMR spectra of compounds

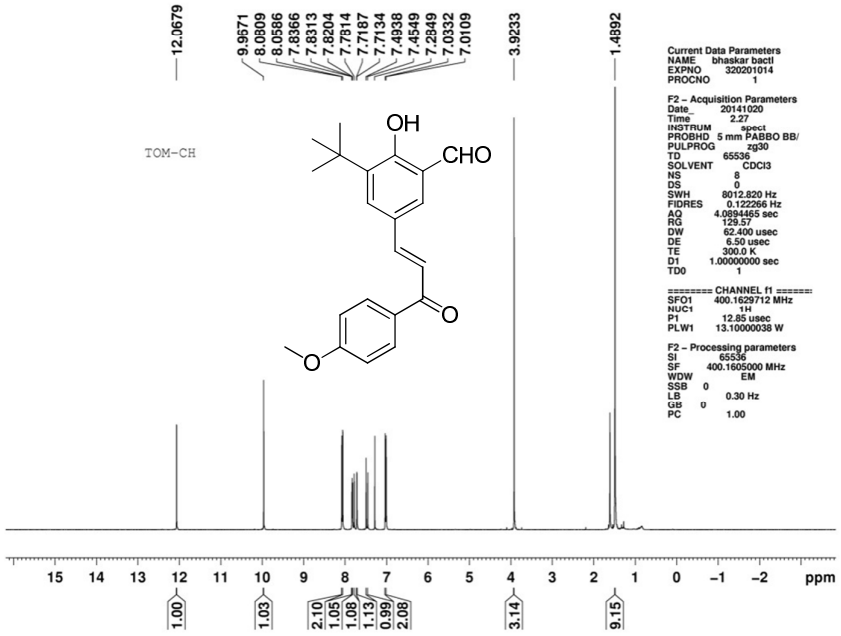
^{1}H NMR of compound **7** at 400 MHz ($CDCl_3$)



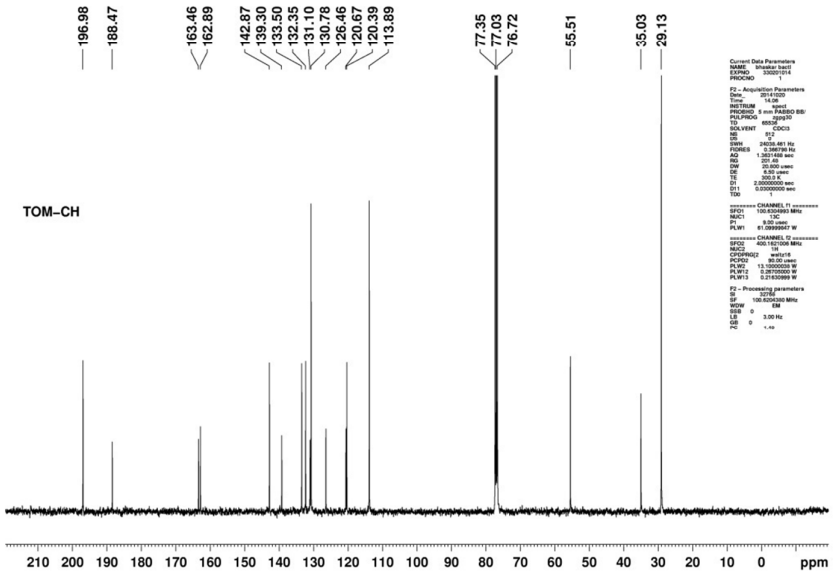
^{13}C NMR of compound **7** at 100 MHz ($CDCl_3$)



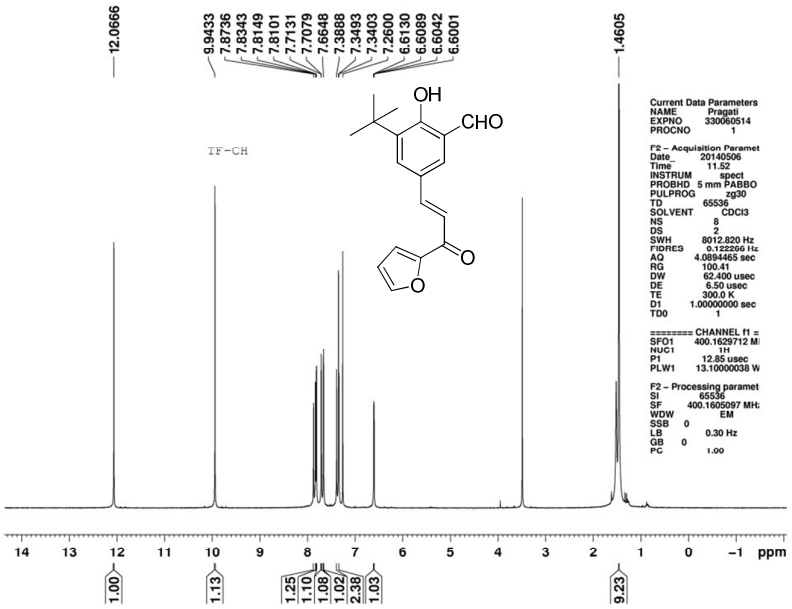
^{1}H NMR of compound **8** at 400 MHz ($CDCl_3$)



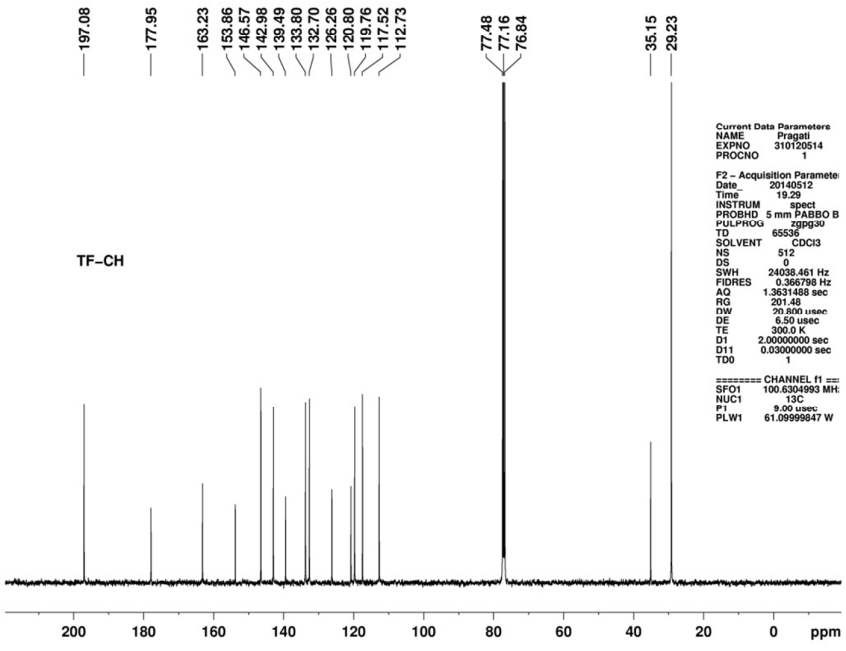
^{13}C NMR of compound **8** at 100 MHz ($CDCl_3$)



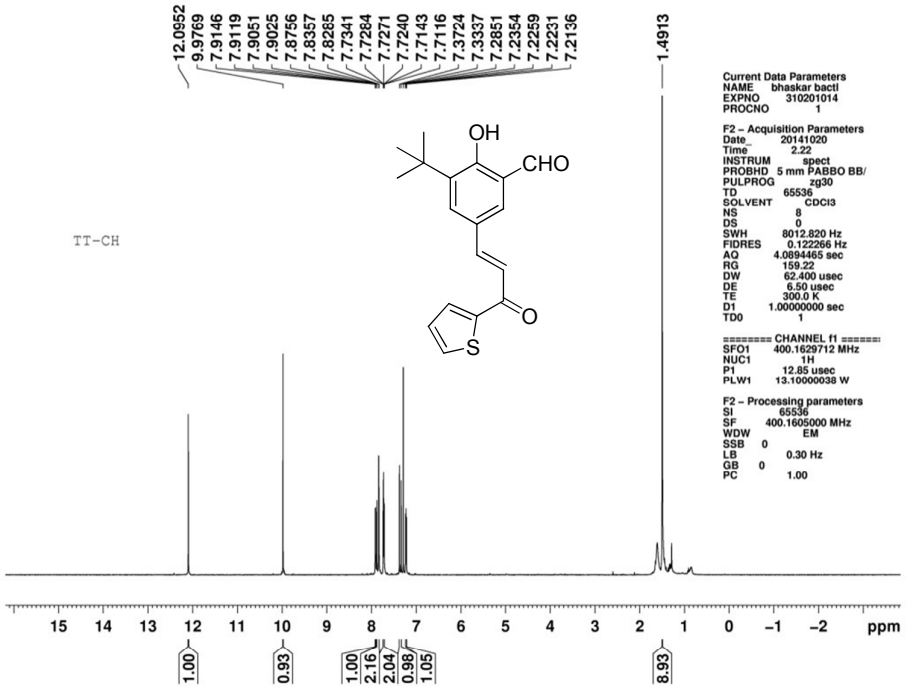
^{1}H NMR of compound **9** at 400 MHz ($CDCl_3$)



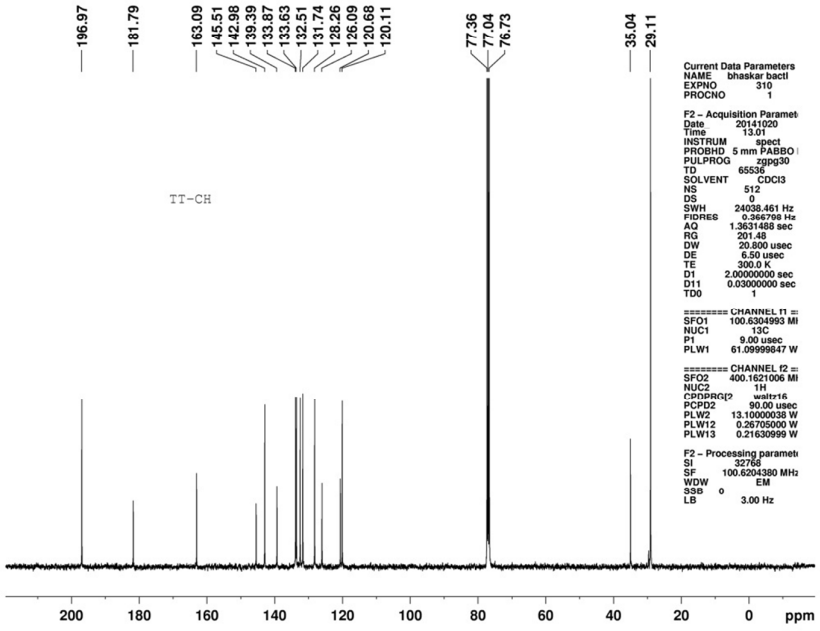
^{13}C NMR of compound **9** at 100 MHz ($CDCl_3$)



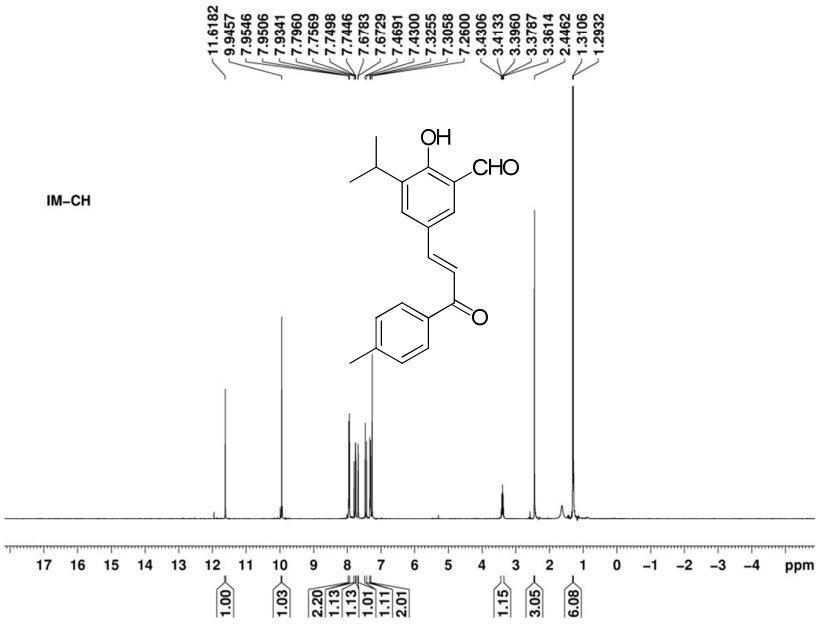
^{1}H NMR of compound **10** at 400 MHz ($CDCl_3$)



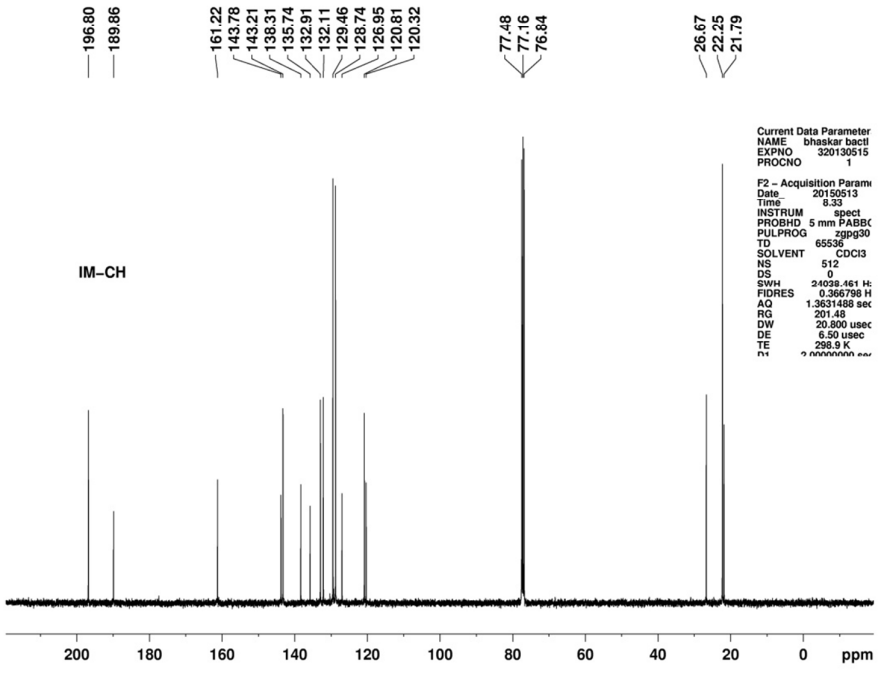
^{13}C NMR of compound **10** at 100 MHz ($CDCl_3$)



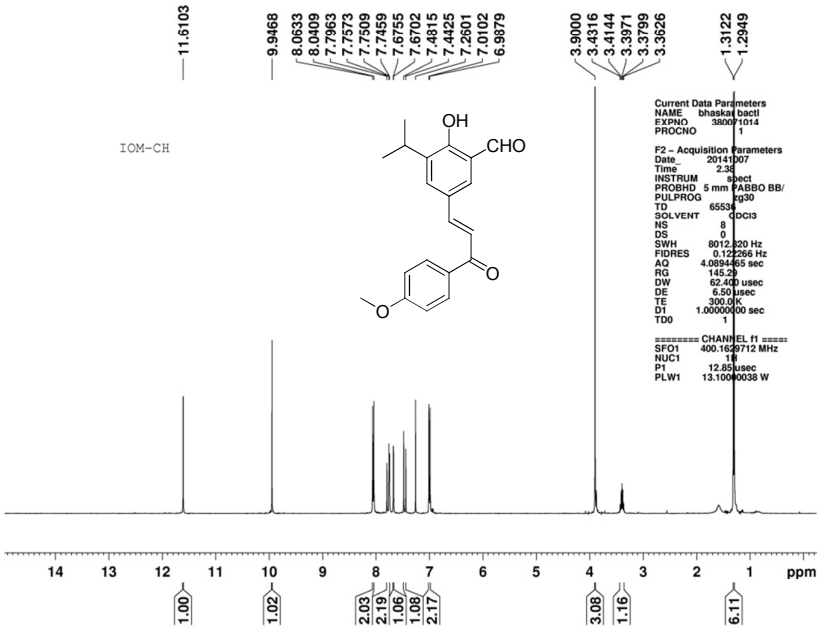
^{1}H NMR of compound **11** at 400 MHz ($CDCl_3$)



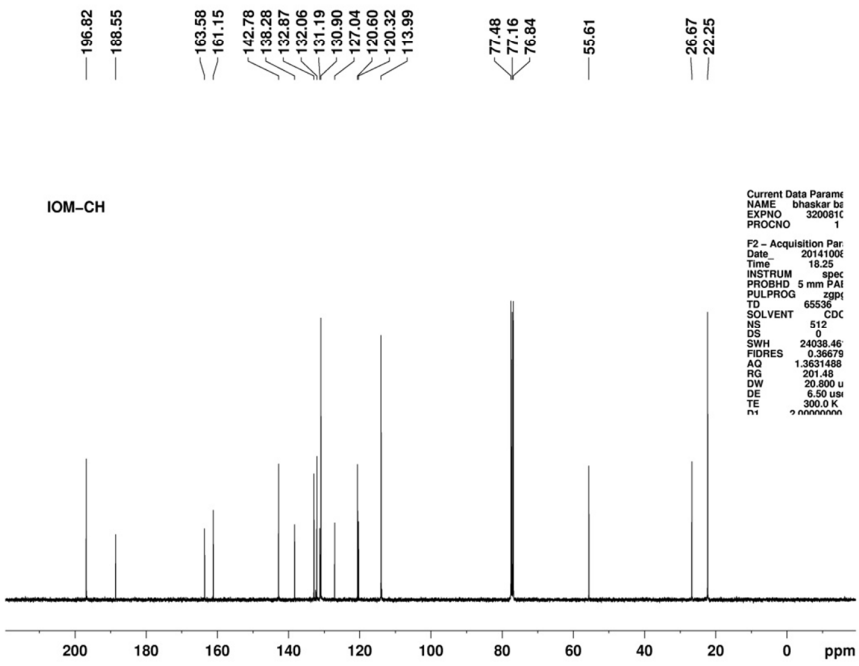
^{13}C NMR of compound **11** at 100 MHz ($CDCl_3$)



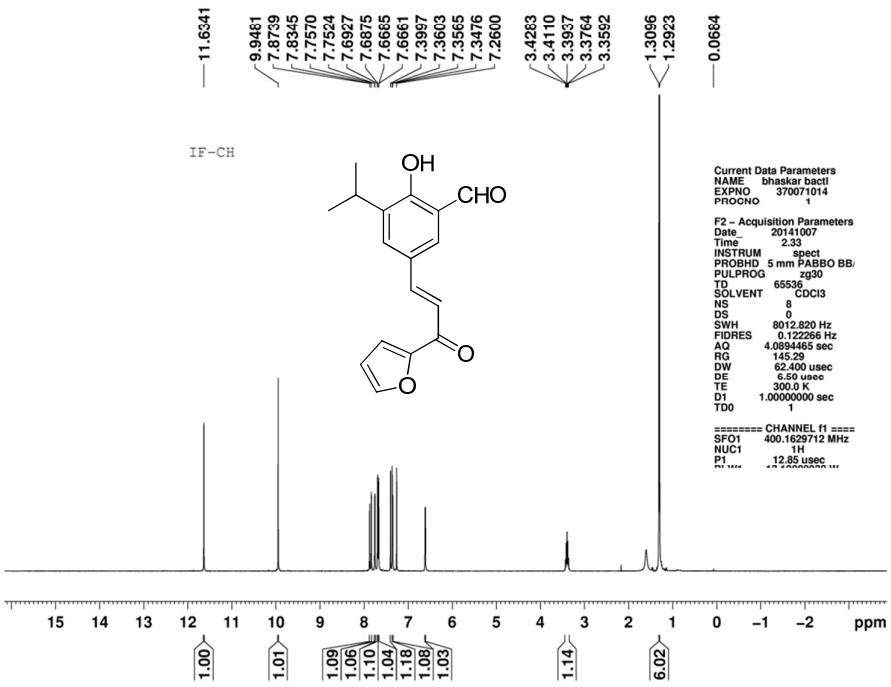
^{1}H NMR of compound **12** at 400 MHz ($CDCl_3$)



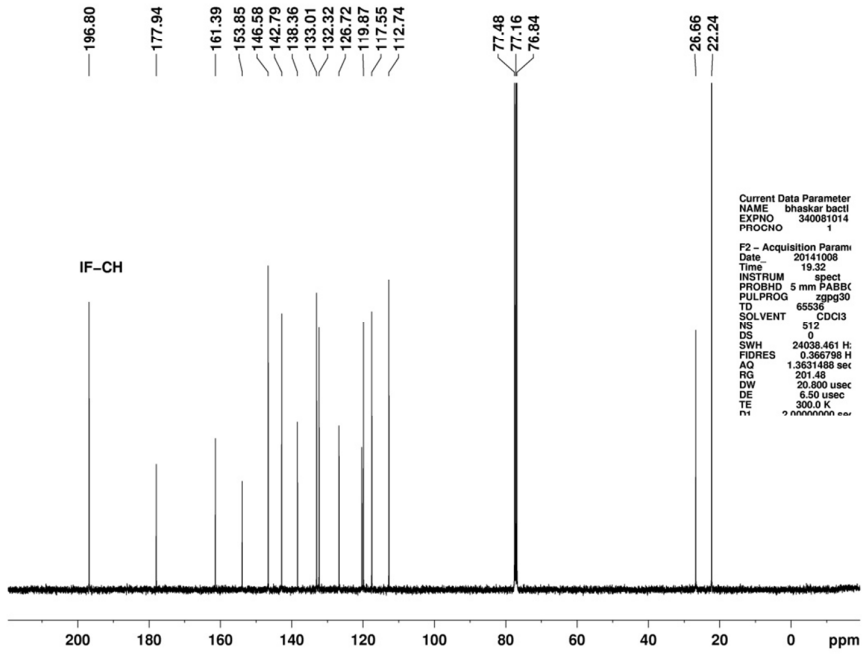
^{13}C NMR of compound **12** at 100 MHz ($CDCl_3$)



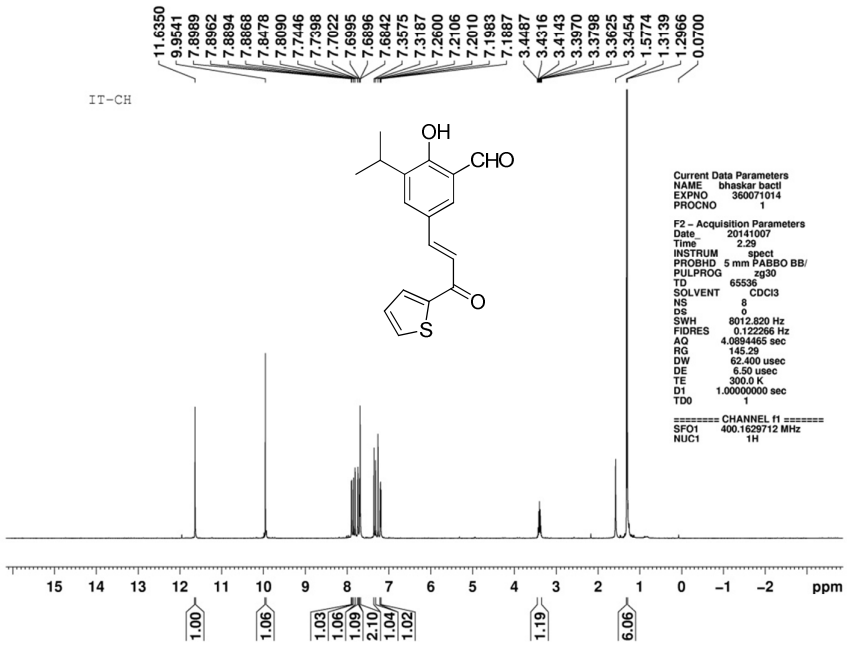
^{1}H NMR of compound **13** at 400 MHz ($CDCl_3$)



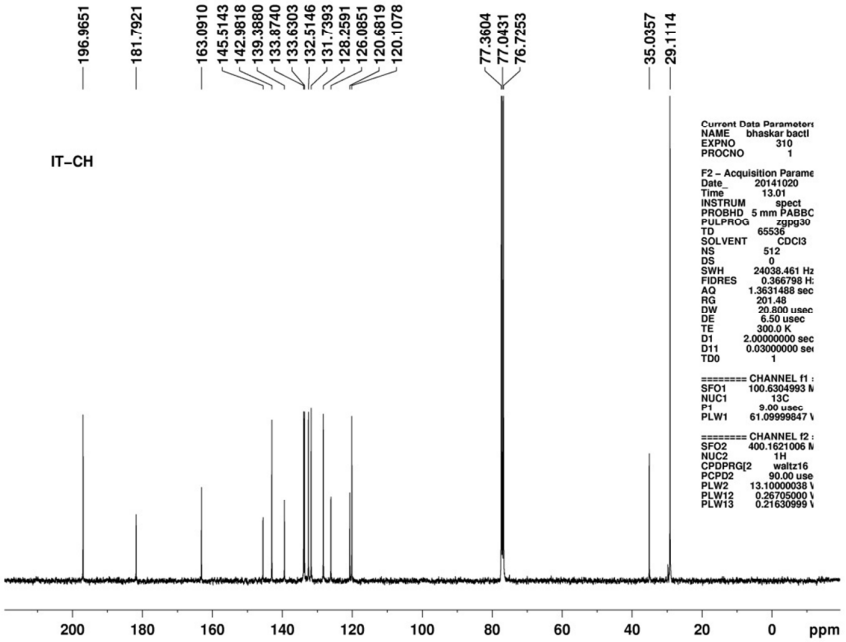
^{13}C NMR of compound **13** at 100 MHz ($CDCl_3$)



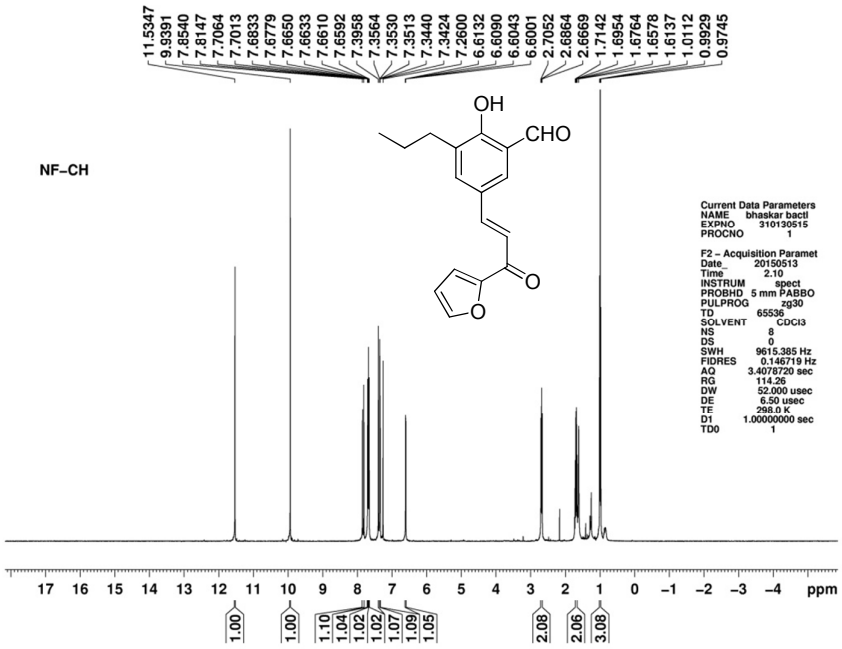
^{1}H NMR of compound **14** at 400 MHz ($CDCl_3$)



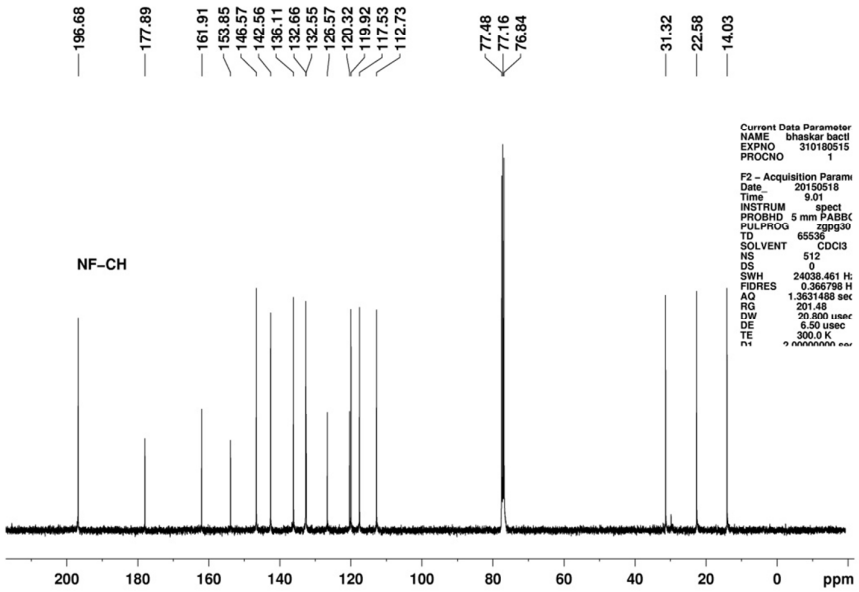
^{13}C NMR of compound **14** at 100 MHz ($CDCl_3$)



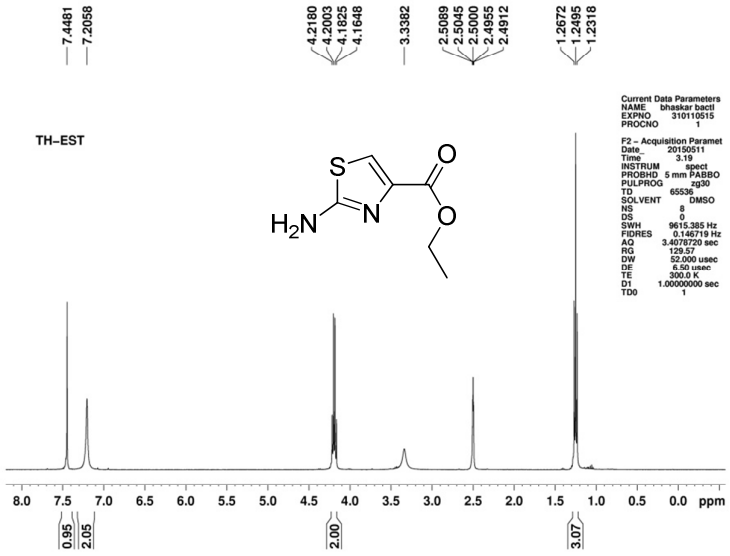
^{1}H NMR of compound **15** at 400 MHz ($CDCl_3$)



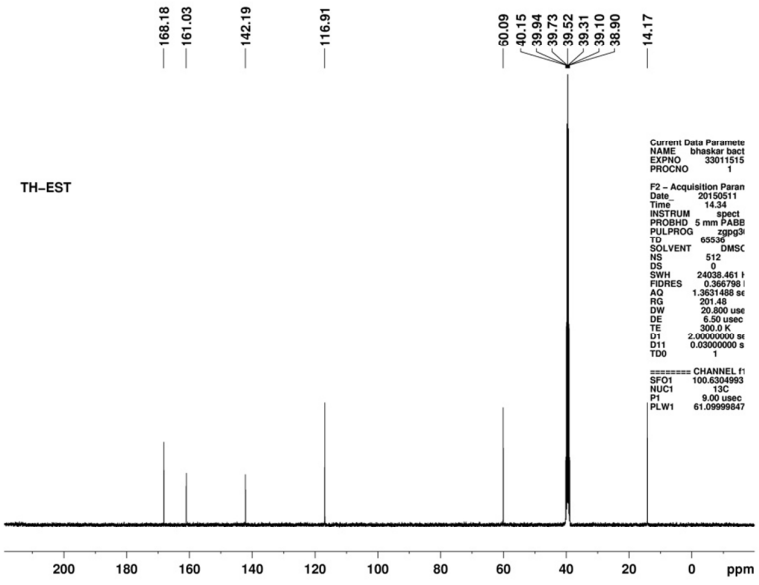
^{13}C NMR of compound **15** at 100 MHz ($CDCl_3$)



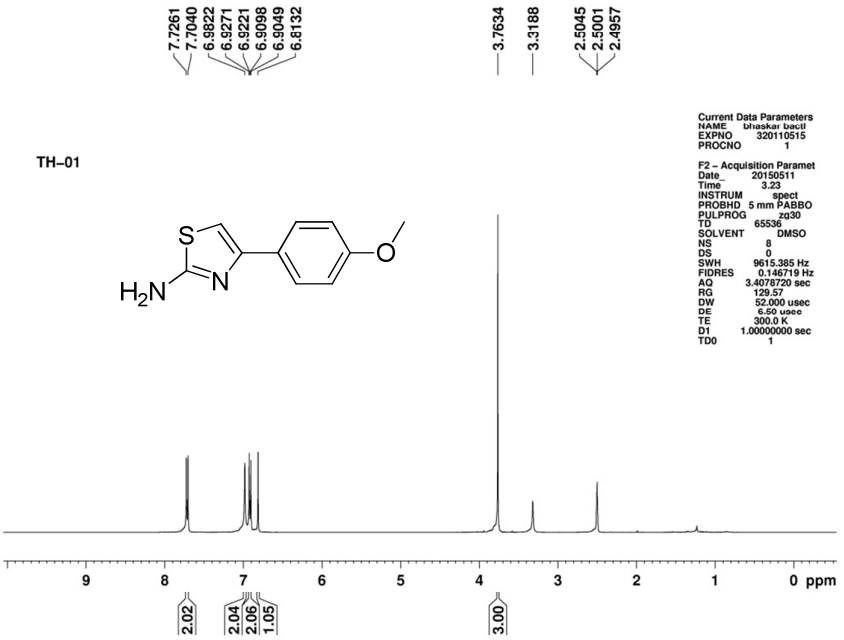
^{1}H NMR of compound **19** at 400 MHz (DMSO-d_6)



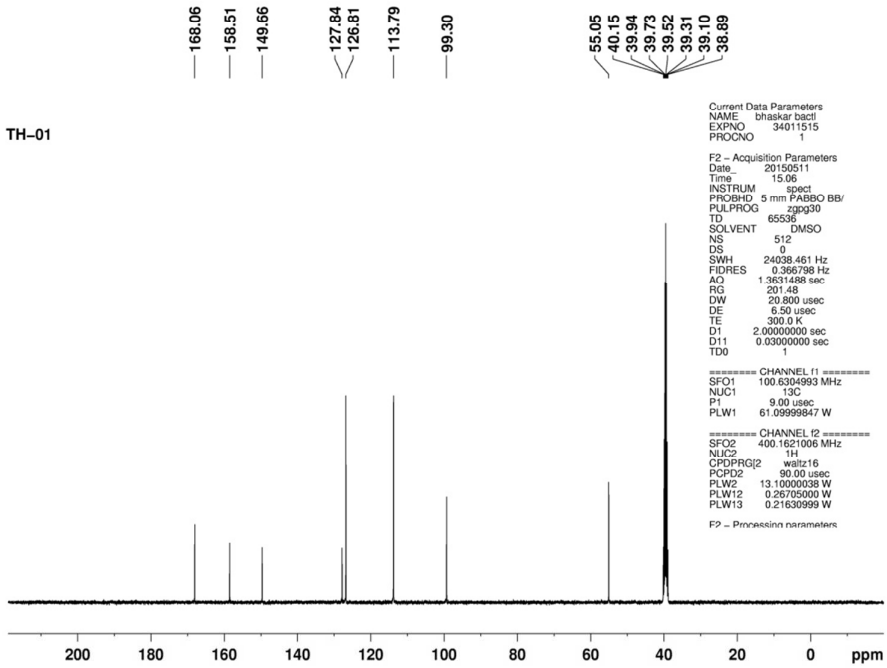
^{13}C NMR of compound **19** at 100 MHz (DMSO-d_6)



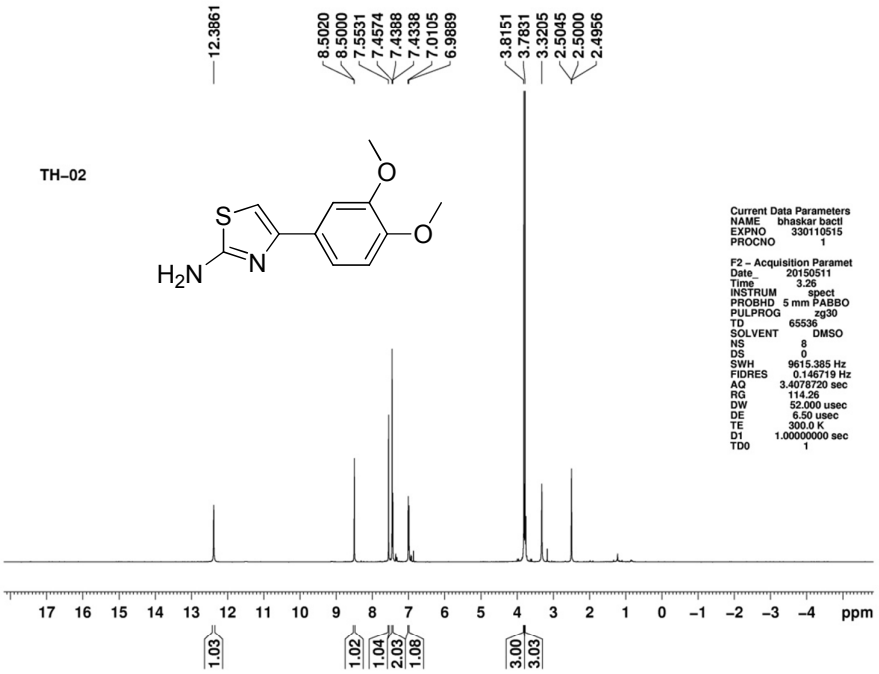
^{1}H NMR of compound **20** at 400 MHz (DMSO-d_6)



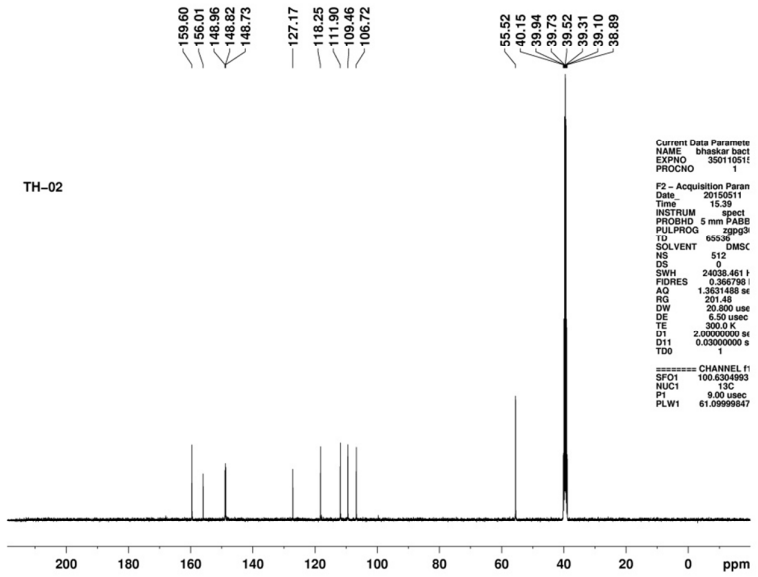
^{13}C NMR of compound **20** at 100 MHz (DMSO-d_6)



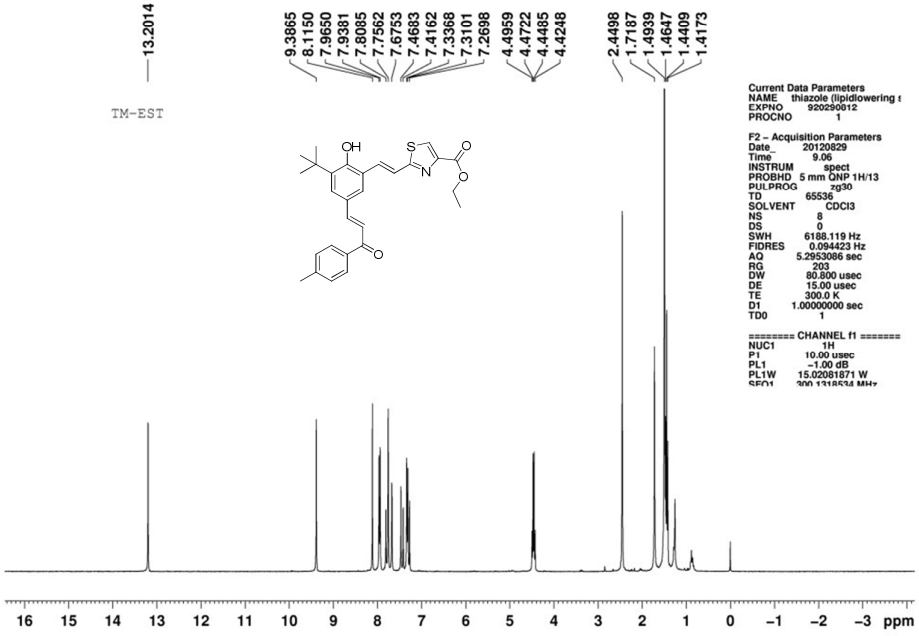
^{1}H NMR of compound **21** at 400 MHz (DMSO-d_6)



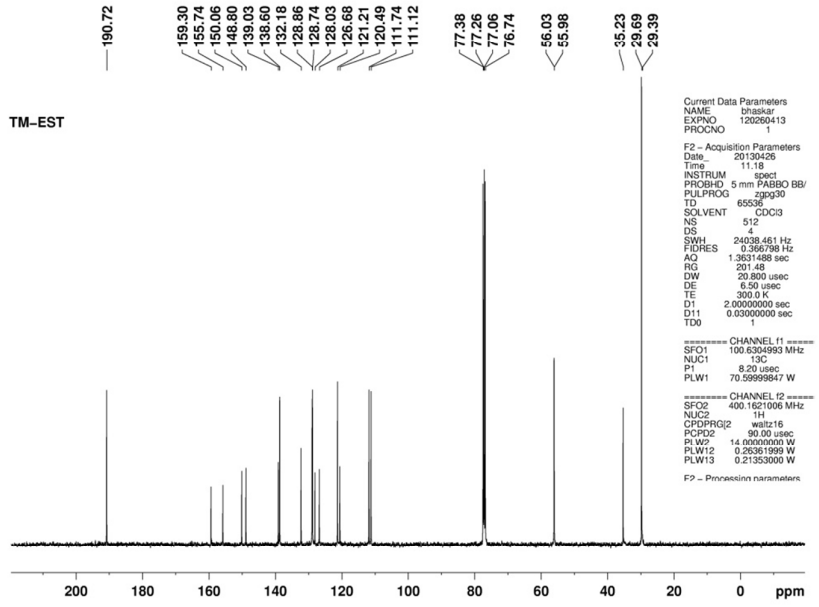
^{13}C NMR of compound **21** at 100 MHz (DMSO-d_6)



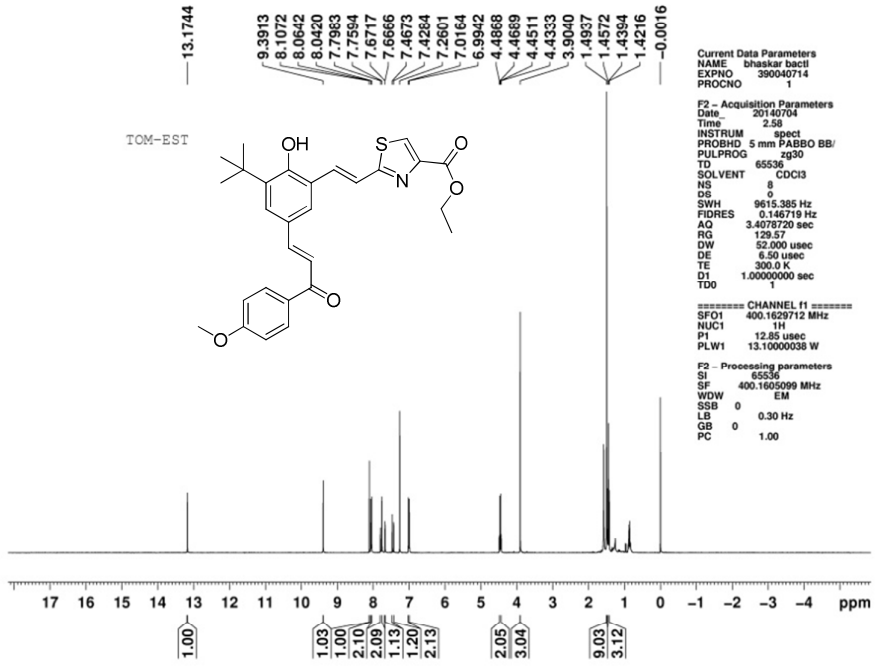
^{1}H NMR of compound **22** at 300 MHz ($CDCl_3$)



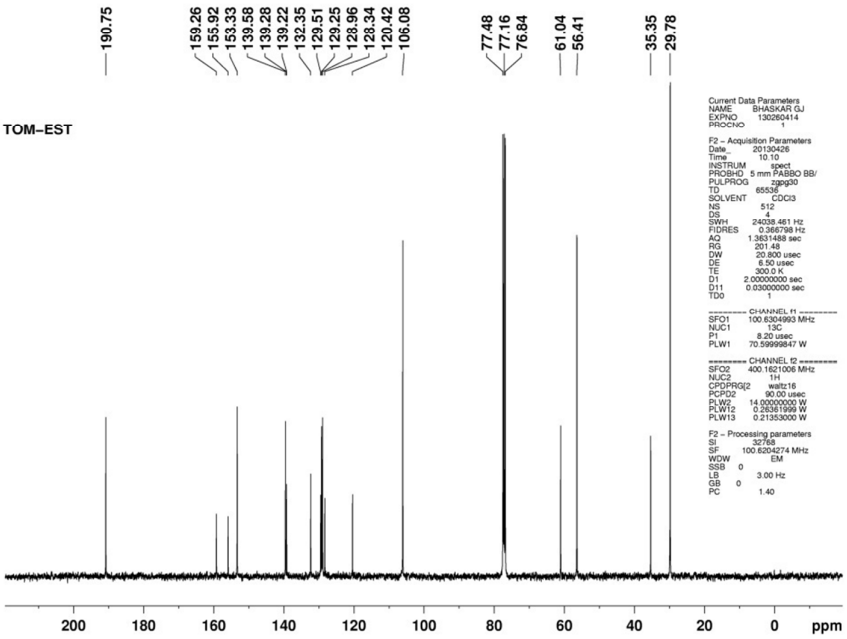
^{13}C NMR of compound **22** at 100 MHz ($CDCl_3$)



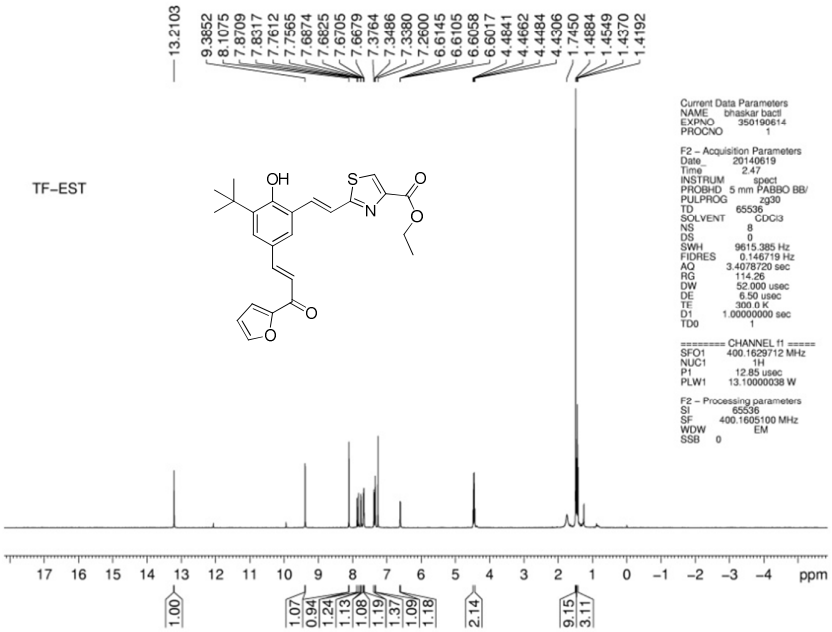
^{1}H NMR of compound **23** at 400 MHz ($CDCl_3$)



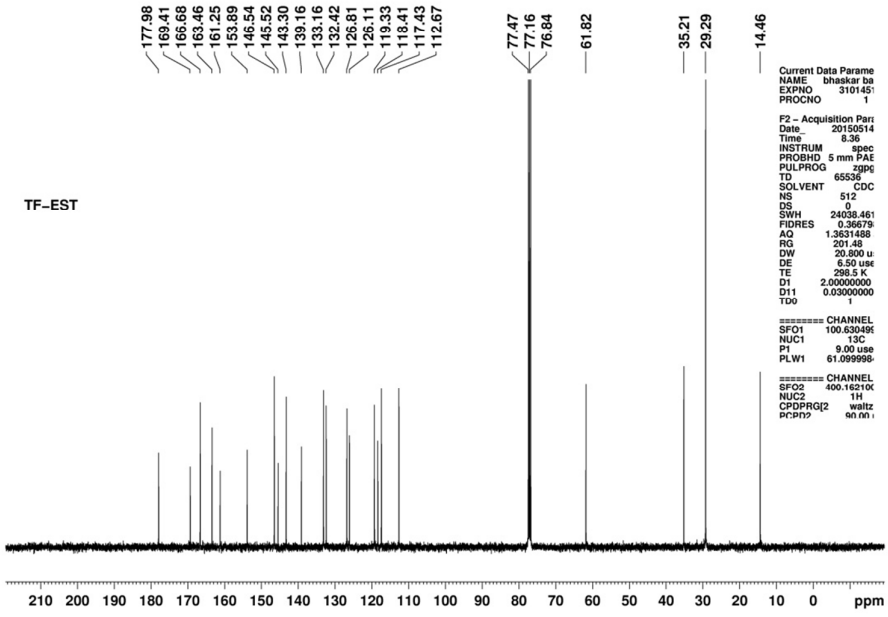
^{13}C NMR of compound **23** at 100 MHz ($CDCl_3$)



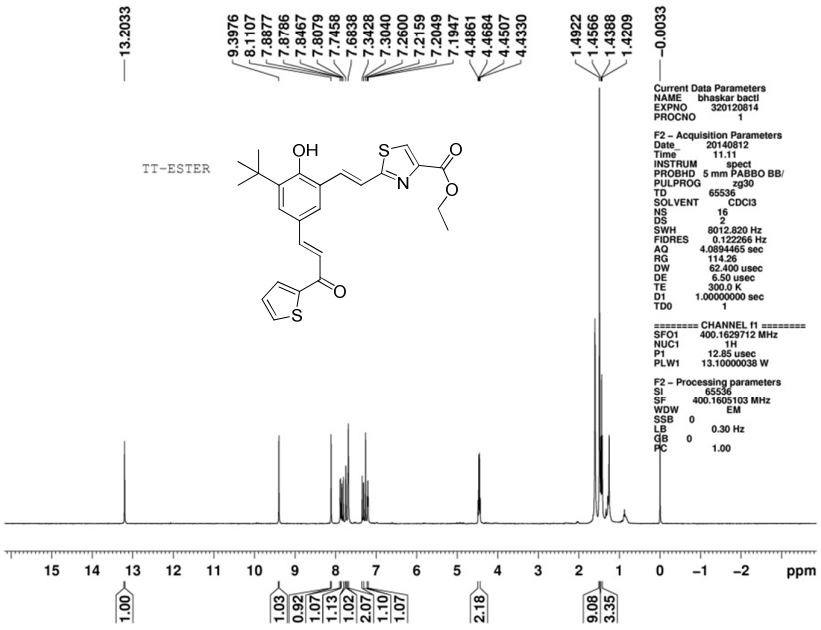
^{1}H NMR of compound **24** at 400 MHz ($CDCl_3$)



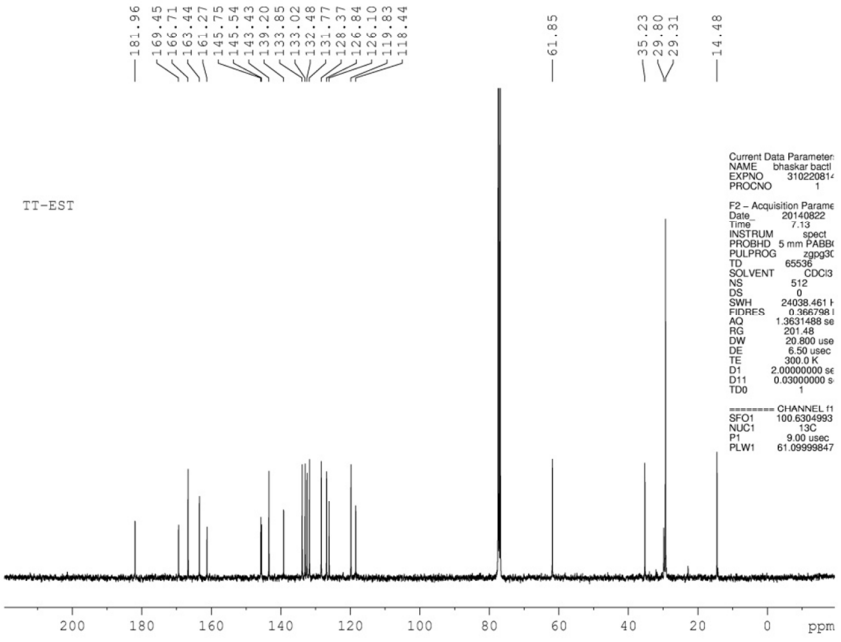
^{13}C NMR of compound **24** at 100 MHz ($CDCl_3$)



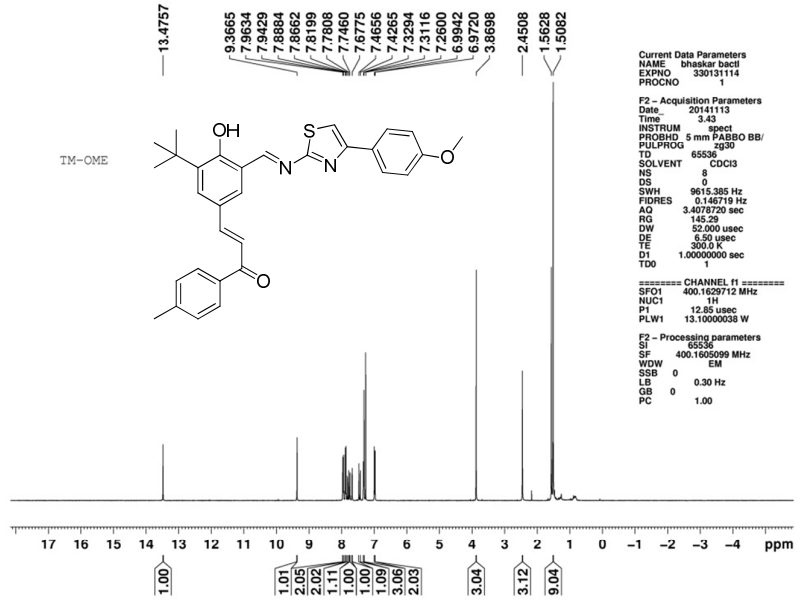
^{1}H NMR of compound **25** at 400 MHz ($CDCl_3$)



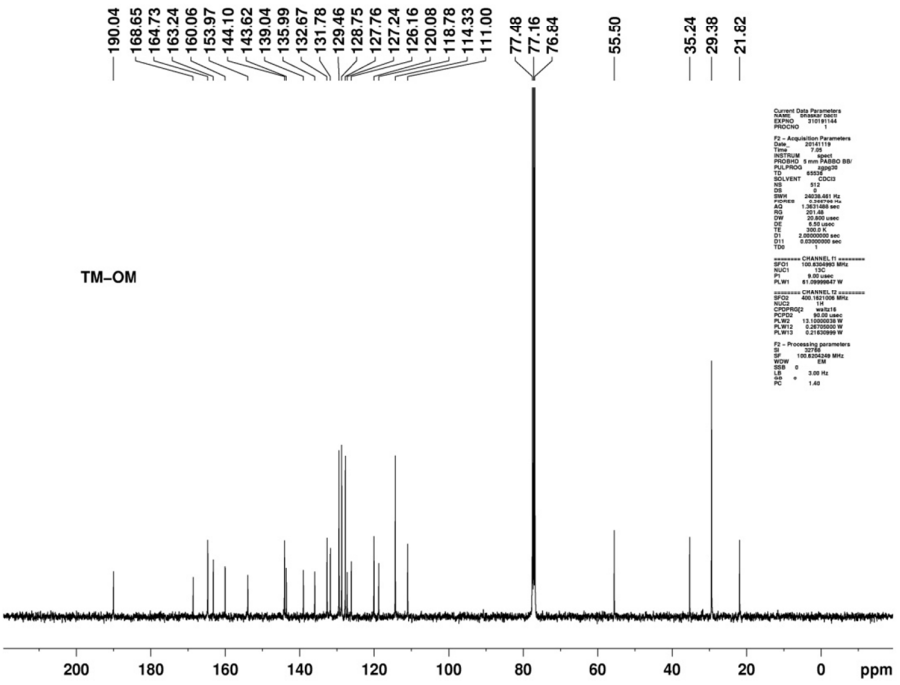
^{13}C NMR of compound **25** at 100 MHz ($CDCl_3$)



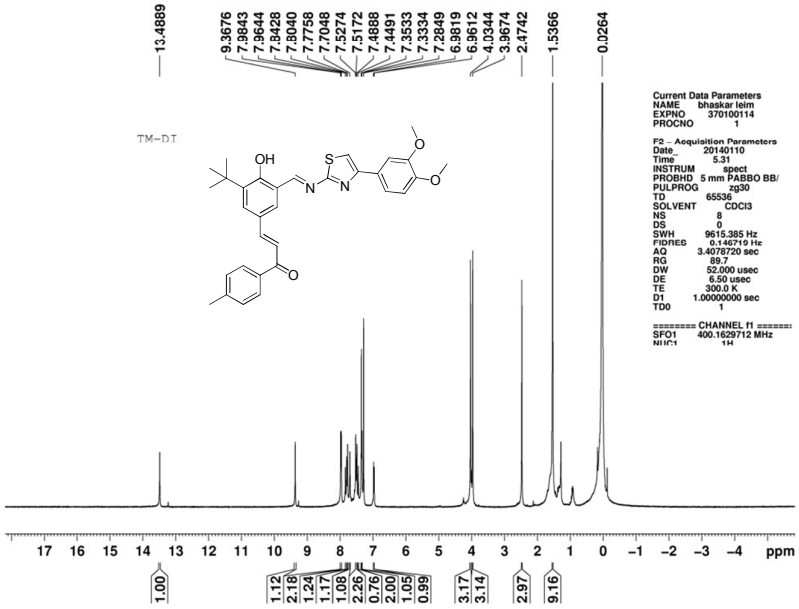
^{1}H NMR of compound **26** at 400 MHz ($CDCl_3$)



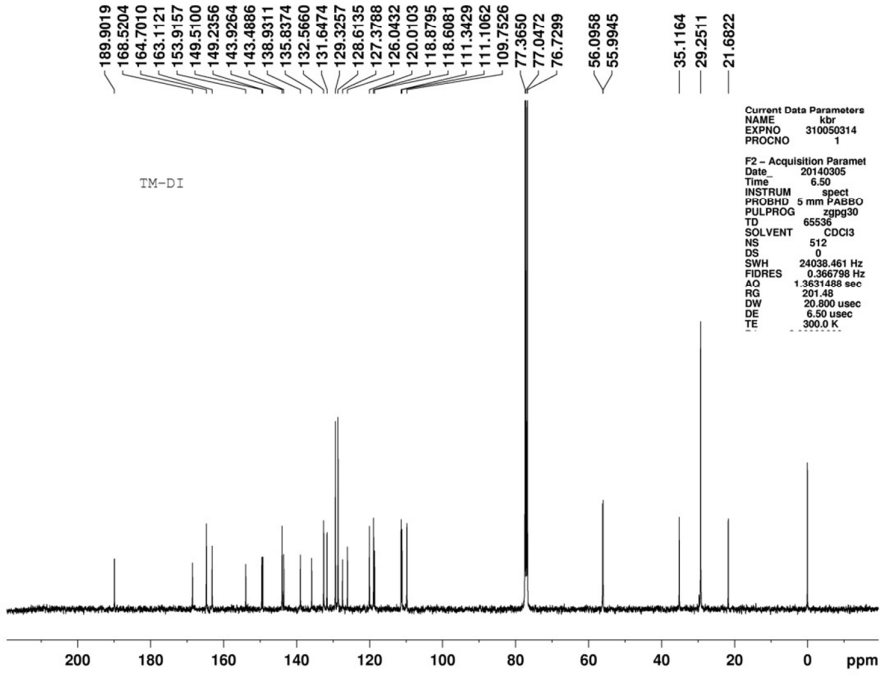
^{13}C NMR of compound **26** at 100 MHz ($CDCl_3$)



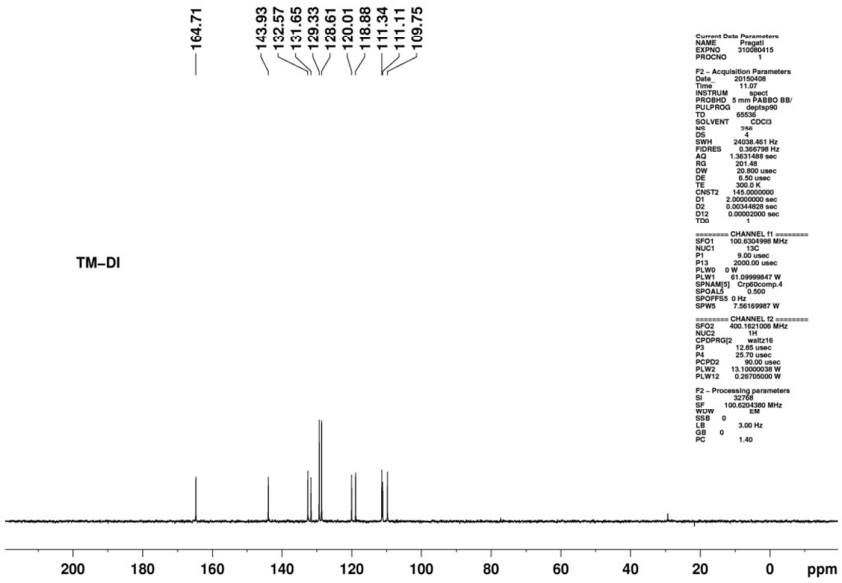
^{1}H NMR of compound **27** at 400 MHz ($CDCl_3$)



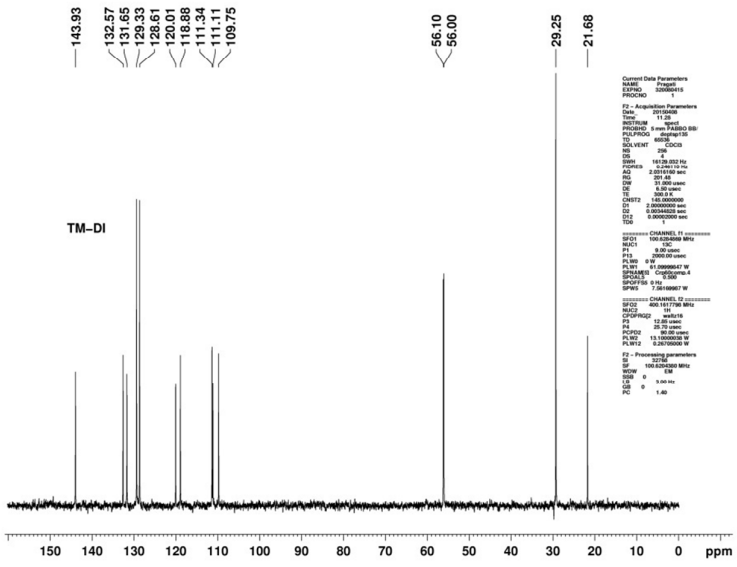
^{13}C NMR of compound **27** at 100 MHz ($CDCl_3$)



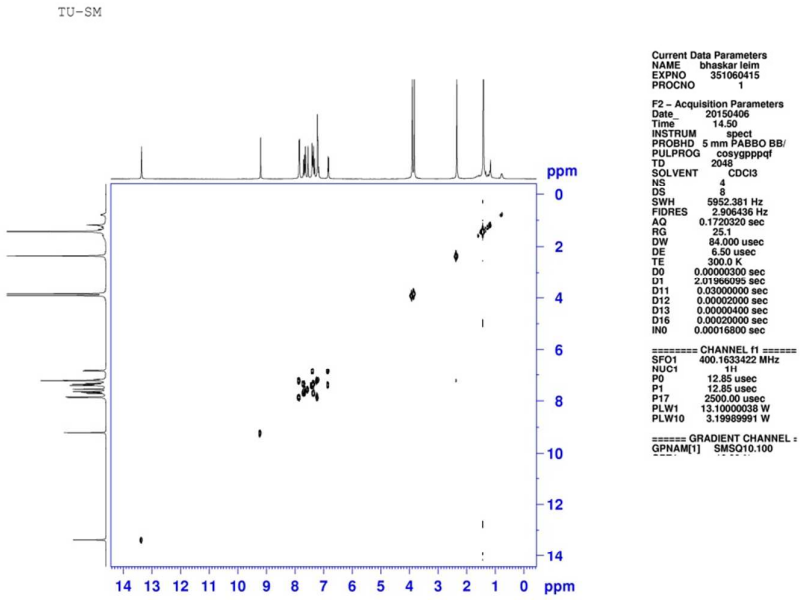
DEPT 90 of compound **27** at 100 MHz ($CDCl_3$)



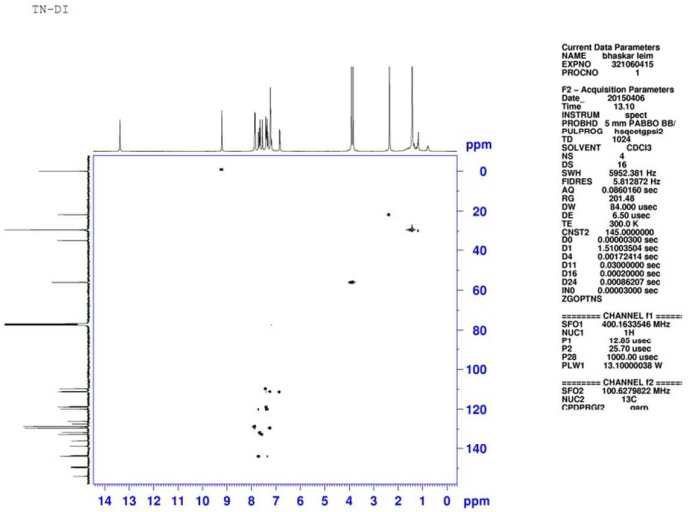
DEPT 135 of compound **27** at 100 MHz ($CDCl_3$)



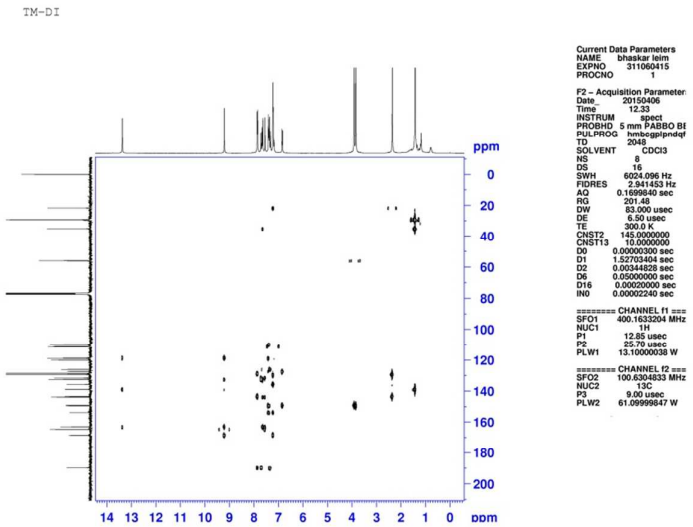
COSY of compound **27** at 400 MHz ($CDCl_3$)



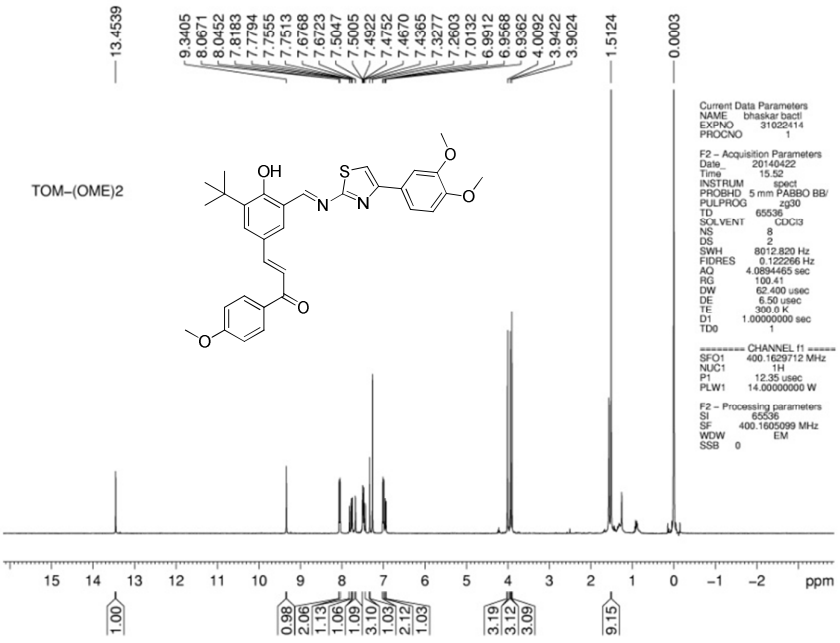
HSQC of compound **27** at 300 MHz ($CDCl_3$)



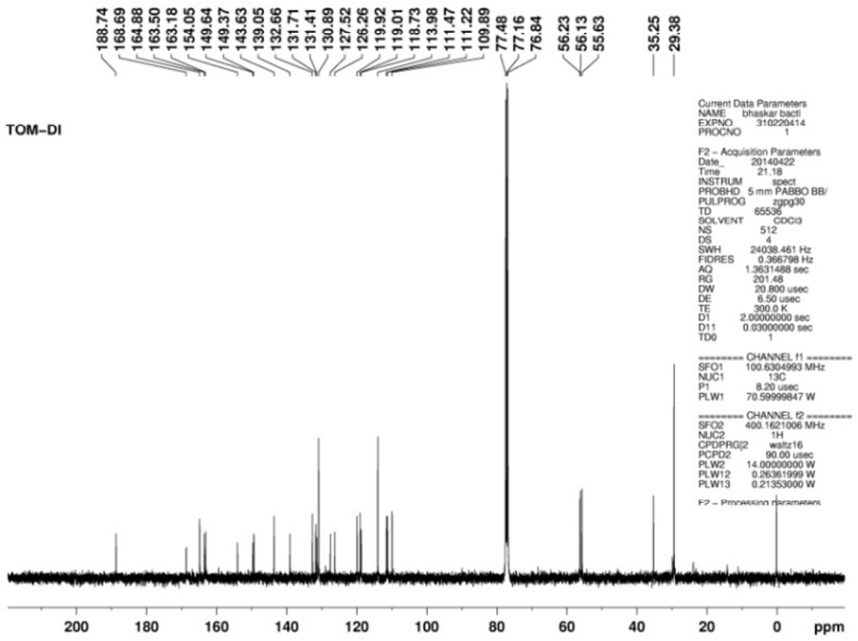
HMBC of compound **27** at 400 MHz ($CDCl_3$)



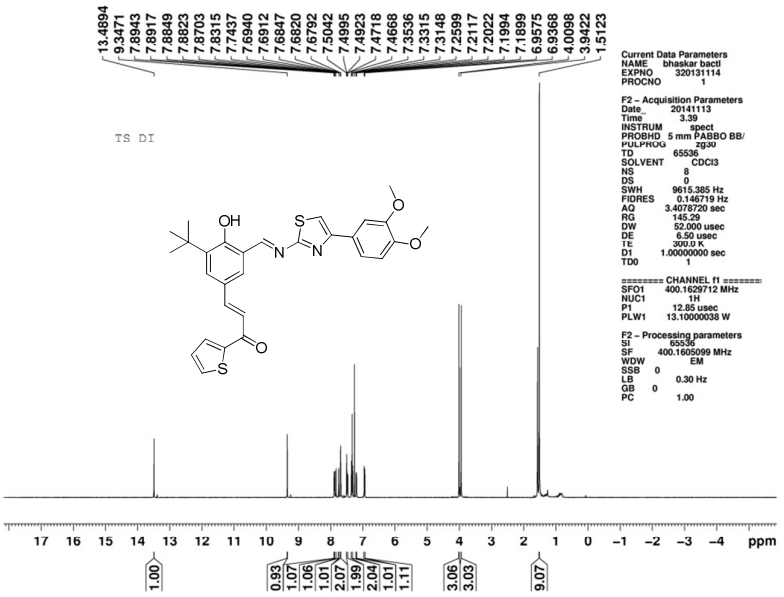
^{1}H NMR of compound **28** at 400 MHz ($CDCl_3$)



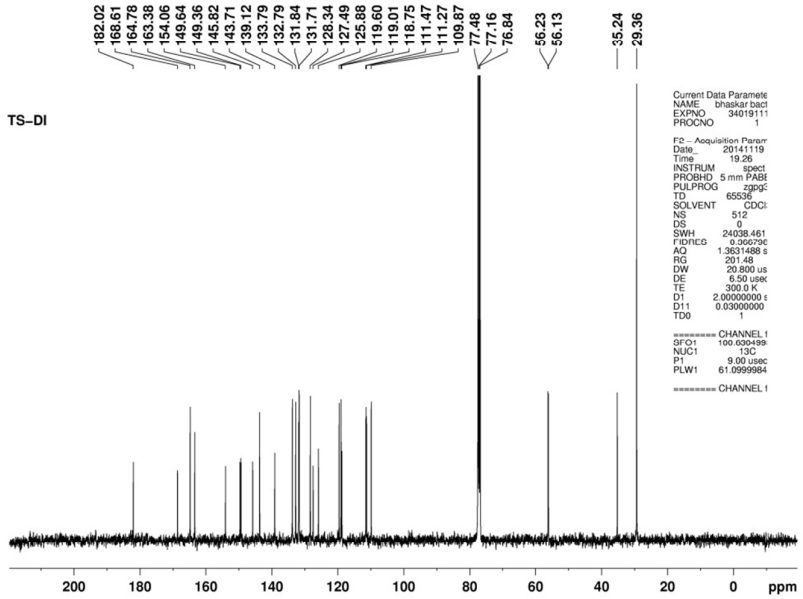
^{13}C NMR of compound **28** at 100 MHz ($CDCl_3$)



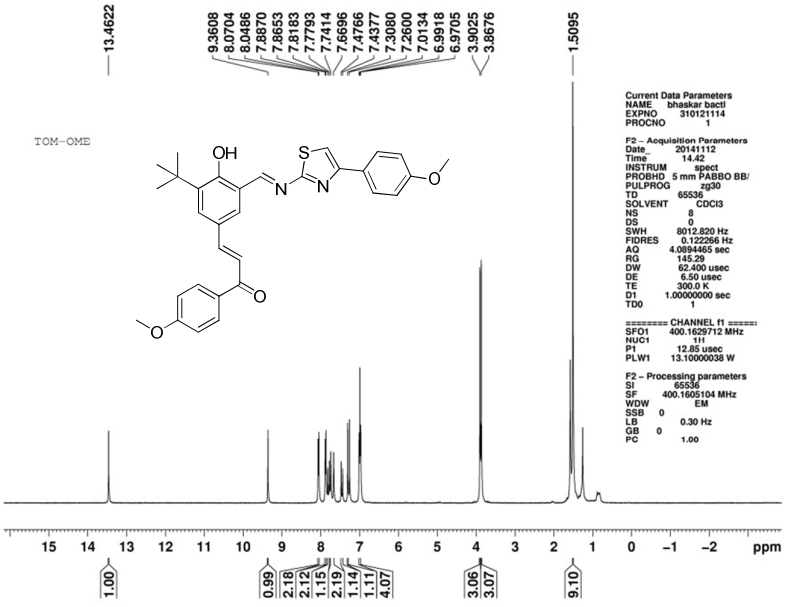
^{1}H NMR of compound **29** at 400 MHz ($CDCl_3$)



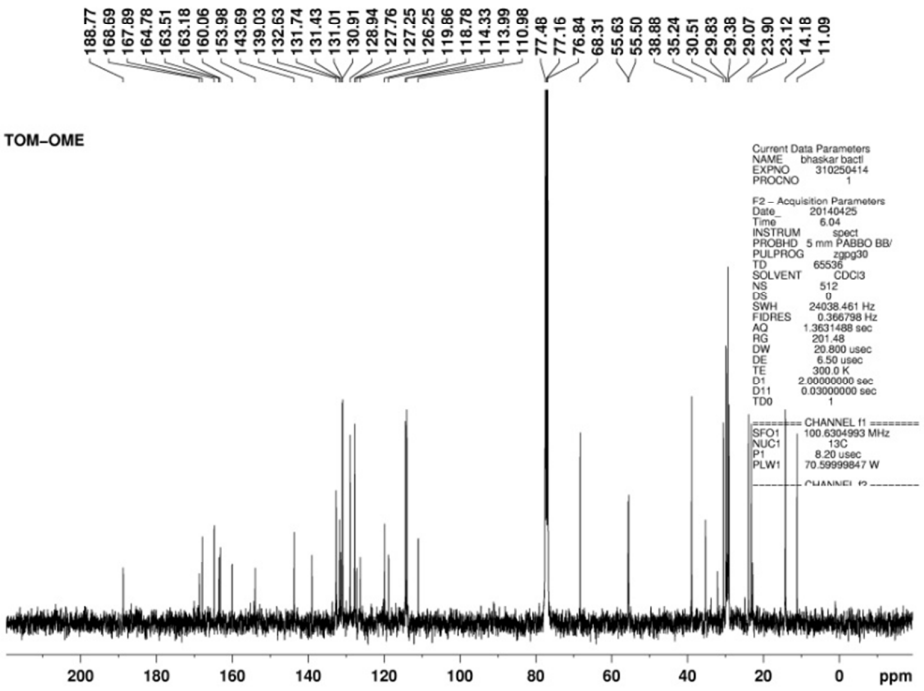
^{13}C NMR of compound **29** at 100 MHz ($CDCl_3$)



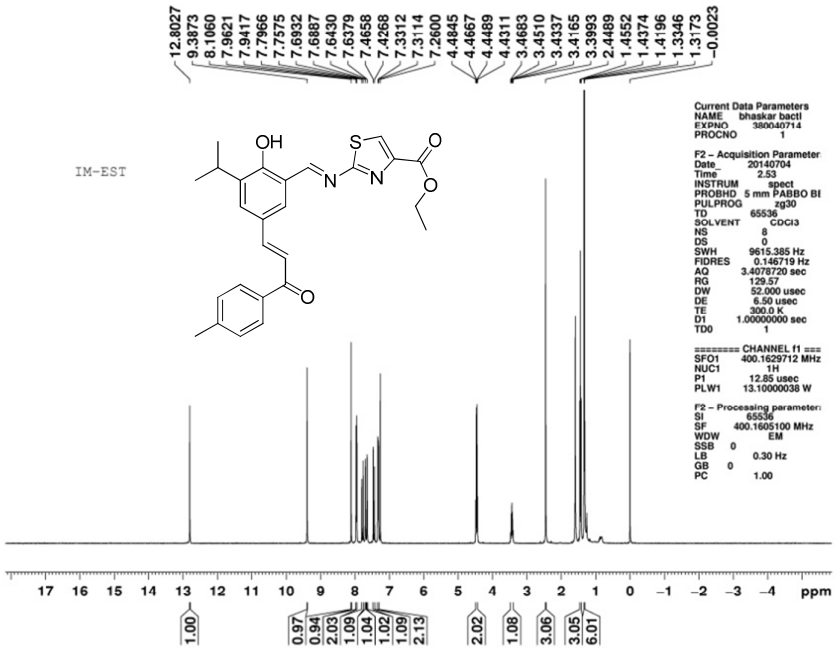
^{1}H NMR of compound **30** at 400 MHz ($CDCl_3$)



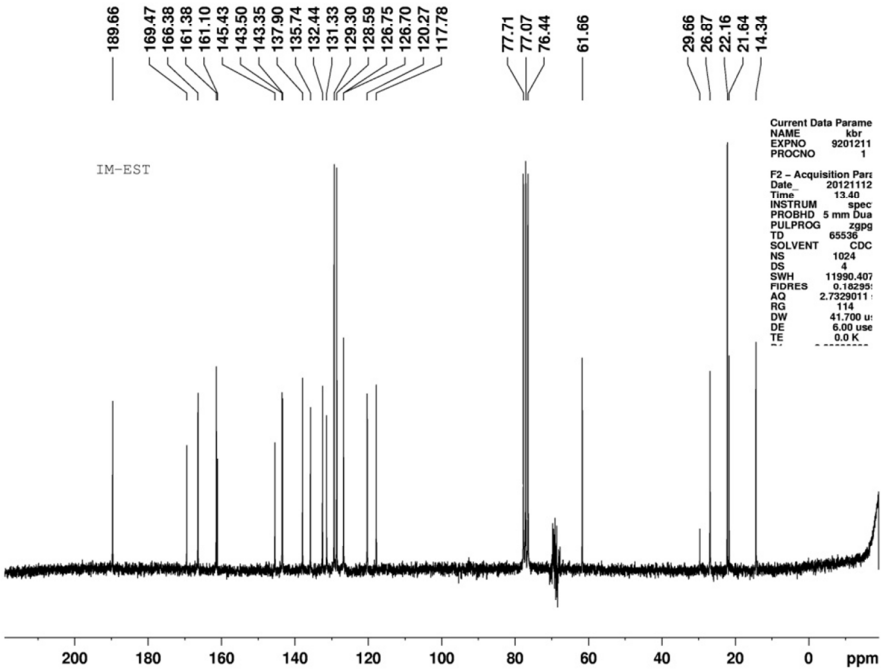
^{13}C NMR of compound **30** at 100 MHz ($CDCl_3$)



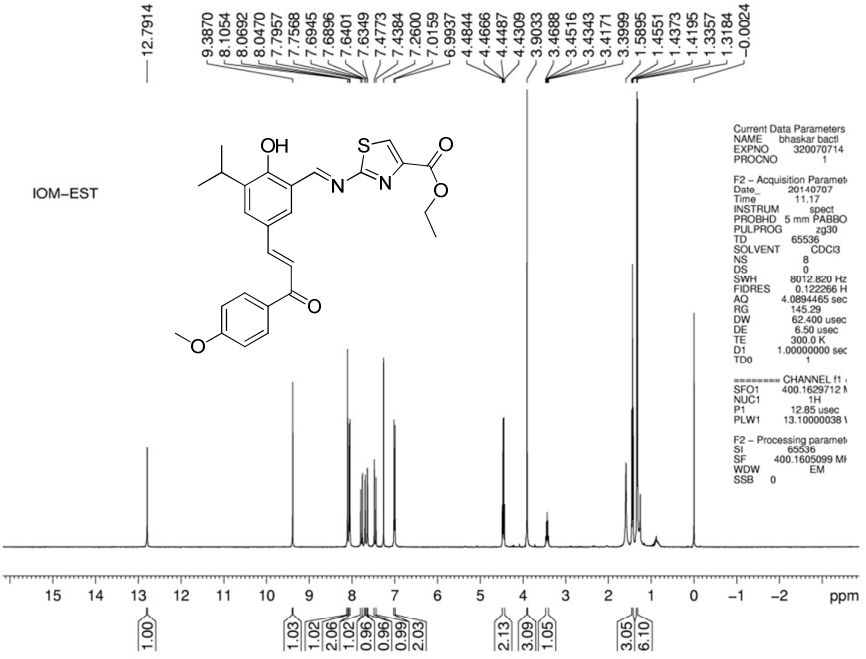
^{1}H NMR of compound **31** at 400 MHz ($CDCl_3$)



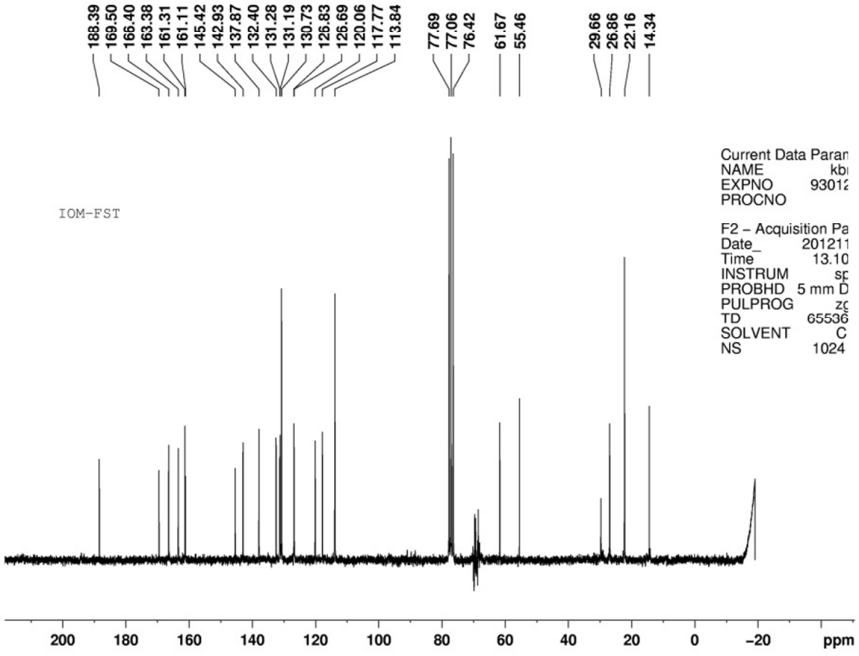
^{13}C NMR of compound **31** at 100 MHz ($CDCl_3$)



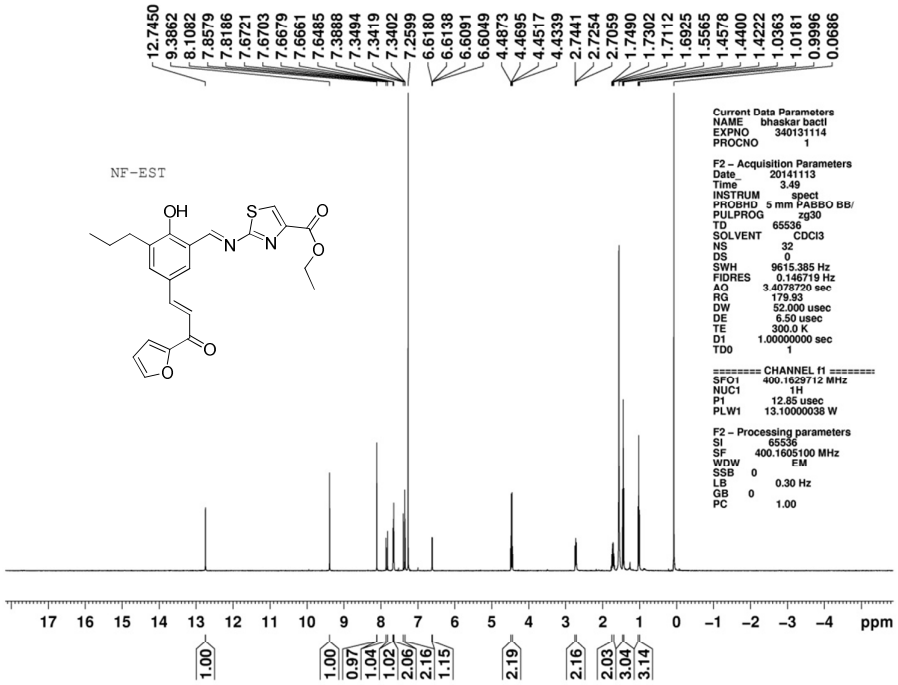
^{1}H NMR of compound **32** at 400 MHz ($CDCl_3$)



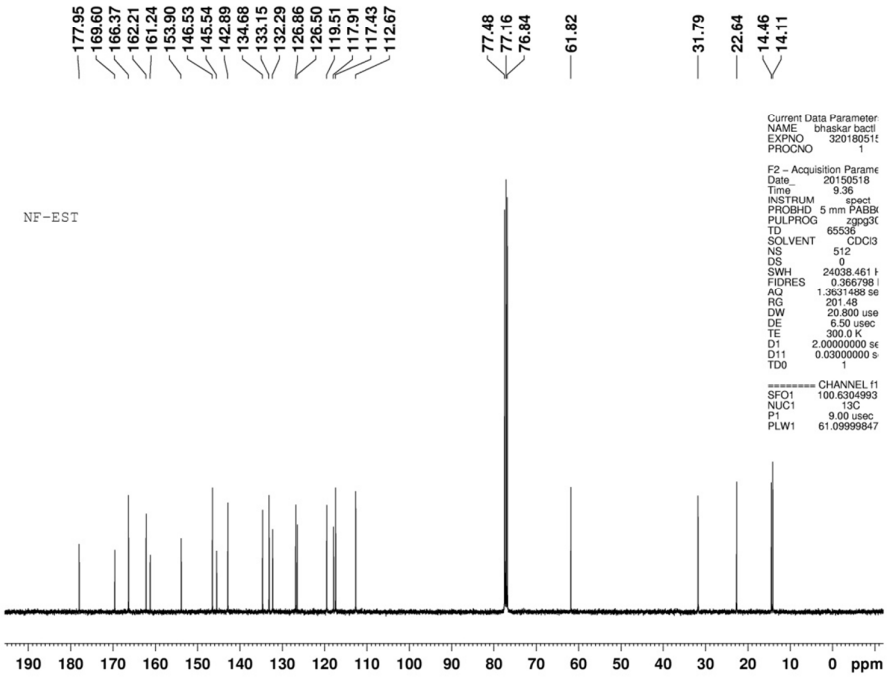
^{13}C NMR of compound **32** at 100 MHz ($CDCl_3$)



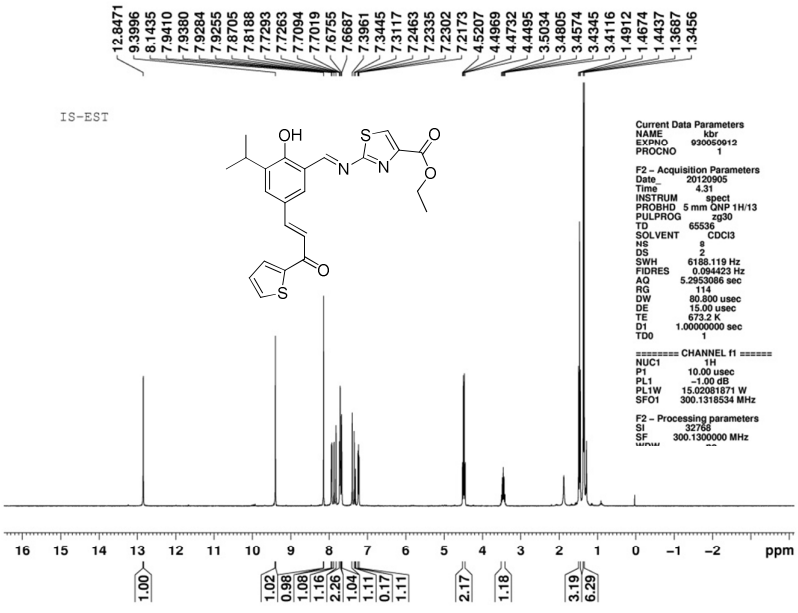
^{1}H NMR of compound **33** at 400 MHz ($CDCl_3$)



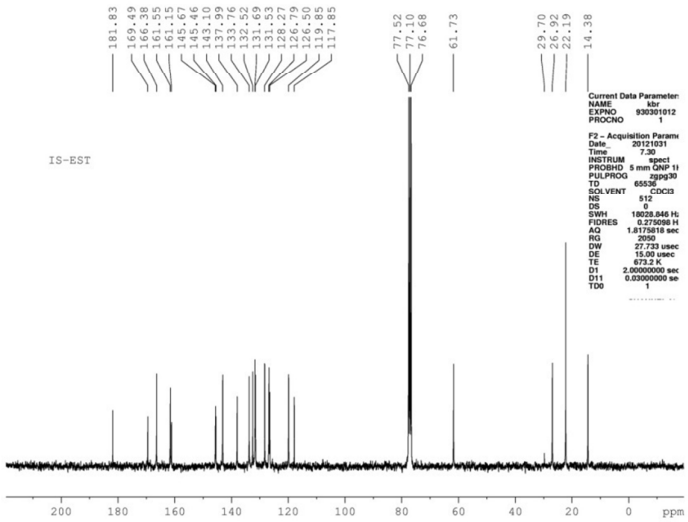
^{13}C NMR of compound **33** at 100 MHz ($CDCl_3$)



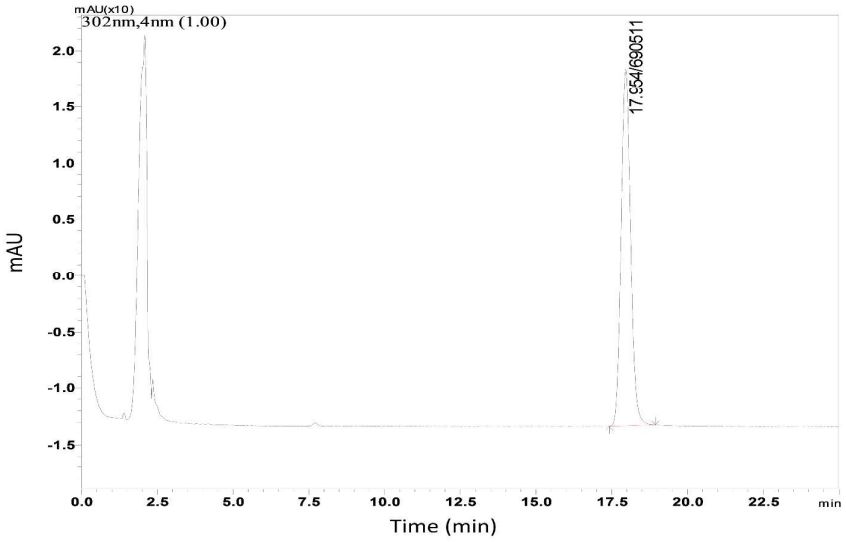
^{1}H NMR of compound **34** at 300 MHz ($CDCl_3$)



^{13}C NMR of compound **34** at 75 MHz ($CDCl_3$)



4. HPLC Report of most active compounds **27**



Peak results

S.No	Name	RT	Area	Height	% area
1	Peak1	17.95	690511	31690	100

5.Table 1 : IC 50 values for the compounds

Compound		Bacteria μM			
		S.a. ATCC 25923	*S.a.* ATCC 29213	*S.a.* ATCC 33592	*S.a.* ATCC 700699
	Molecular weight	IC50	IC50	IC50	IC50
22	476.59	2.06	1.47	1.68	0.82
23	492.59	3.65	3.65	6.50	1.58
24	542.52	5.52	4.86	6.85	3.40
25	468.59	1.92	1.49	1.71	0.83
26	510.65	6.07	4.31	6.07	2.94
27	540.67	3.51	6.10	2.77	0.92
28	556.67	5.57	5.57	6.11	2.16
29	532.67	6.01	3.75	5.44	2.25
30	526.65	3.04	3.04	3.23	3.04
31	462.56	6.70	6.70	6.70	3.24
32	478.56	6.48	6.48	7.10	2.51
33	438.50	13.00	14.60	14.82	17.56
34	454.56	9.68	10.78	11.44	7.04

6. References

(1) National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard, NCCLS Document M27-A. National Committee for Clinical Laboratory Standards, Wayne, PA, USA, 1997.

(2) National Committee for Clinical Laboratory Standards, Methods for Dilution antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard, fifth ed. NCCLS, Villanova, PA.; 2000.

(3) Pasupuleti, M.; Schmidtchen, A.; Chalupka, A.; Ringstad, L.; Malmsten, M. End-tagging of ultra-short antimicrobial peptides by W/F stretches to facilitate bacterial killing. *PLoS One* **2009,** *4*, e5285.