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New imidazo[2,1-b]thiazole based aryl hydrazones: Unravelling their synthesis, antiproliferative and apoptosis inducing potential

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Experimental Section

Materials and methods

All the chemicals and reagents used in this study were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA), Finar (Finar chemicals, India), Avra (Avra Laboratories Private Limited, Hyderabad, India) and other commercial sources were used without further purification, unless otherwise specified. The reactions were monitored by TLC performed on silica gel glass plates containing 60 GF_{254}, and visualized by using a UV light or iodine indicator. Column chromatography was performed using Merck 60-120 mesh silica gel. ^{1}H NMR spectra were recorded on Bruker UXNMR/XWIN-NMR (300 MHz) or Innova Varian-VXR Unity (400, 500 MHz) instruments. ^{13}C NMR spectra were recorded on a Bruker UXNMR/XWIN-NMR (75, 100 and 125 MHz) instruments. Chemical shifts (δ) were reported in ppm downfield from an internal standard TMS and coupling constants are expressed in Hz. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), m (multiplet) and bs (broad singlet). ESI spectra were recorded on Micro mass Quattro LC using ESI+ software with a capillary voltage of 3.98 kV and an ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL hybrid MS-MS mass spectrometer. Melting points were determined with an electrothermal melting point apparatus and are uncorrected.

General procedure for the synthesis of compounds (4a–d)

The synthesis of intermediates (3a–d) to (8a–f) was carried out following the previously reported procedure and the spectroscopic data of these compounds were in agreement with the reported data. [19]

2-bromo-1-(substituted)ethanones (**1a**–**d**, 1.0 equiv) and thiazol-2-amine (**2**, 1.0 equiv) were dissolved in acetone and the reaction mixture was refluxed for 6–8 h. The resulting salts (**4a-d)** were collected by filtration, washed with acetone, dissolved in 3N HCl (200 mL) and refluxed again for 1–2 h. Before complete cooling, the solution was cautiously basified by drop wise addition of 15% aq. NH_4OH to pH 8. The resulting base was collected by filtration and crystallized from EtOH to afford compounds (**4a-d)** as solids.

6-Phenylimidazo[2,1-*b*]thiazole (4a)

The titled compound was prepared according to the general method described above, employing 2-bromo-1-phenylethan-1-one (**1a**, 1.0 equiv) and thiazol-2-amine (**2**, 1.0 equiv) to obtain the pure product **4a** as a white solid (80% yield); m.p. 148–150 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.85 (d, J = 4.34 Hz, 1H), 7.29 (d, J =7.36 Hz, 1H), 7.37–7.46 (m, 3H), 7.74 (s, 1H), 7.83 (d, J = 7.17 Hz, 2H); MS (ESI): 201 *m/z* [M+H]+.

6-(4-Methoxyphenyl)imidazo[2,1-*b*]thiazole (4b)

The titled compound was prepared according to the general method described above, employing 2-bromo-1-(4-methoxyphenyl)ethan-1-one (**1b**, 1.0 equiv) and thiazol-2-amine (**2**, 1.0 equiv) to obtain the pure product **4b** as a white solid (82% yield); m.p. 176–177 °C; ^{1}H NMR ($CDCl_3$,300 MHz): δ 3.83 (s, 3 H), 6.78 (d, J= 3.9 Hz, 1H), 6.93 (d, J= 8.5 Hz, 2H), 7.40 (d, J= 4.6 Hz, 2H), 7.63 (s, 1H), 7.74 (d, J= 8.5 Hz, 1H); MS (ESI): 231 *m/z* [M+H]+.

6-(4-Fluorophenyl)imidazo[2,1-*b*]thiazole (**4c**)

The titled compound was prepared according to the general method described above, employing 2-bromo-1-(4-fluorophenyl)ethan-1-one (**1c**, 1.0 equiv) and thiazol-2-amine (**2**, 1.0 equiv) to obtain the pure product **4c** as a white solid (84% yield); m.p. 114–116 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.73 (d, *J* = 6.7 Hz, 2H), 7.37 (s, 1H), 7.60 (s, 1H), 7.04 (t, *J* = 7.6 Hz, 2H), 6.77 (s, 1H); MS (ESI): 219 *m/z* [M+H]+.

6-(4-Chlorophenyl)imidazo[2,1-*b*]thiazole (**4d**)

The titled compound was prepared according to the method described above, employing 2-bromo-1-(4-chlorophenyl)ethan-1-one (**1d**, 1.0 equiv) and thiazol-2-amine (**2**, 1.0 equiv) to obtain the pure product **4d** as a white solid (86% yield); m.p. 163–164 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.84 (d, *J* = 4.5 Hz, 1H), 7.43 (d, *J* = 4.5 Hz), 7.52 (d, *J* = 8.4 Hz, 2H), 7.71 (t, *J* = 8.4 Hz, 3H); MS (ESI): 235 *m/z* [M+H]+

General procedure synthesis of compounds (**5a–d**)

Vilsmeier reagent was prepared by addition of $POCl_3$ (5.0 equiv) to a stirred solution of DMF (5.0 equiv) in $CHCl_3$ (10 mL) at 0–5 ^{o}C. To this reagent, compounds (**4a**–**d**) (1.0 equiv) in chloroform (20 mL) were added while maintaining cold conditions. After complete addition, the reaction mixture was stirred at room temperature for 3 h and at reflux conditions for 10–12 h. After completion of the reaction, as indicated on TLC, chloroform was removed under reduced pressure and the resulting oily liquid was poured onto ice. The obtained aldehydes were collected by filtration and crystallised from EtOH (5 mL) to obtain the desired products (**4a**–**d)** as solids.

6-Phenylimidazo[2,1-*b*]thiazole-5-carbaldehyde (5a)

The titled compound was prepared according to the general method described above employing 6-phenylimidazo[2,1-*b*]thiazole (**4a**, 1.0 equiv) to obtain the pure product **5a** as a white solid (87% yield); m.p: 139–141 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, *J* = 4.4 Hz, 1H), 7.46–7.57 (m, 3H), 7.79–7.82 (m, 2H), 8.40 (d, *J* = 4.4 Hz, 1H), 9.91 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 115.2, 121.2, 123.3, 124.3, 127.5, 130.9, 132.6, 133.3, 133.5, 155.1, 156.5, 178.1; MS (ESI): *m/z* 229 $[M + H]^+$.

6-(4-Methoxyphenyl)imidazo[2,1-*b*]thiazole-5-carbaldehyde (5b)

The titled compound was prepared according to the general method described above employing 6-(4-methoxyphenyl)imidazo[2,1-*b*]thiazole (**4b**, 1.0 equiv) to obtain the pure product **5b** as a brown solid (85% yield); m. p: 138-140 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.88 (s, 3H), 6.89–7.10 (m, 3H), 7.73–7.77 (m, 2H), 8.37–8.38 (m, 1H), 9.88 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.4, 114.3, 114.5, 121.6, 123.7, 125.4, 130.4, 155.7, 158.2, 160.9, 177.9; MS (ESI): *m/z* 259 $[M + H]^+$.

6-(4-Fluorophenyl)imidazo[2,1-*b*]thiazole-5-carbaldehyde (5c)

The titled compound was prepared according to the general method described above, employing 6-(4-fluorophenyl)imidazo[2,1-*b*]thiazole (**4c**, 1.0 equiv) to obtain the pure product **5c** as a yellow solid (84% yield); m.p. 169–171 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.05 (d, *J* = 4.5 Hz, 1H), 7.20 (t, *J* = 8.3 Hz, 2H), 7.78 (dd, *J* = 13.5, *J* = 3.0 Hz, 2H), 8.39 (d, *J* = 4.5 Hz, 1H), 9.87(s, 1H); MS (ESI): *m/z* 247 [M+H]+.

6-(4-Chlorophenyl)imidazo[2,1-*b*]thiazole-5-carbaldehyde (5d)

The titled compound was prepared according to the method described for compound **5a**, employing 6-(4-chlorophenyl)imidazo[2,1-*b*]thiazole (**4d**, 1.0 equiv) to obtain the pure product **5d** as a white solid (4.5g, 88% yield); m.p. 157–158 °C; ^{1}H NMR ($CDCl_3$,400 MHz): δ 7.09 (d, *J* = 4.4 Hz, 1H), 7.48–7.52 (m, 2H), 7.73–7.77 (m, 2H), 8.40 (d, *J* = 4.4 Hz, 1H), 9.90 (s, 1H); MS (ESI): *m/z* 263 [M+H]+.

5.4. General procedure for the synthesis of aryl hydrazides precursors (8a–f)

The aryl hydrazides precursors (**8a**–**f**) were prepared according to the previous reported procedure from our group.[20] Initially, substituted benzoic acids (**6a**–**f**) (1.0 eq), were dissolved in sufficient quantity of absolute ethanol and catalytic amount of sulphuric acid and the reaction was subjected to reflux conditions for 2–3 h. The progress of the reaction was monitored by TLC using ethyl acetate and hexane (1: 1) solvent system. Then the reaction was cooled to obtain the solid precipitate. The precipitate was filtered off, washed with ice cold water and recrystallized in small quantity of absolute ethanol to obtain the corresponding esters (**7a**–**f**) in excellent yields. The obtained compounds were used as such for the next step.

The obtained esters (**7a**–**f**) were dissolved in sufficient quantity of absolute ethanol and hydrazine hydrate (5.0 eq) and the reaction was subjected to reflux conditions for 6–8 h. [29] The progress of the reaction was monitored by TLC using ethyl acetate and hexane (1: 1) solvent system. Then the reaction was cooled to obtain the solid precipitate. The precipitate was filtered off, washed with ice cold water and recrystallized in small quantity of absolute ethanol to obtain the aryl hydrazides precursors (**8a**–**f**) in excellent yields. All the compounds were found were found to be in agreement with the reported spectroscopic data.

General procedure for the synthesis of titled imidazo[2,1-*b*]thiazole based aryl hydrazones (9a–w)

Initially, equimolar quantities of appropriate aldehydes (**5a**–**d**) (1.0 eq), substituted benzhydrazides (**8a**–**f**) (1.0 eq) were dissolved in sufficient quantity of absolute ethanol followed by addition of catalytic amount of glacial acetic acid and the reaction was subjected to reflux conditions for 3–6 h. The progress of the reaction was monitored by TLC using ethyl acetate and hexane (1: 1) solvent system. Then the reaction was cooled to obtain the solid precipitate. The precipitate was filtered off, washed with ice cold water and recrystallized in small quantity of absolute ethanol to furnish imidazo[2,1-*b*]thiazole linked N-acyl hydrazones (**9a**–**w**) in good to excellent yields.

3,4,5-trimethoxy-N'-((6-phenylimidazo[2,1-*b*]thiazol-5yl)methylene)benzohydrazide (9a)

Brown solid; 81%Yield; m.p: 234–236 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.72 (s, 3H), 3.86 (s, 6H), 7.24 (s, 2H), 7.37 (d, 1H, J = 7.8 Hz), 7.41–7.58 (m, 3H), 7.73 (d, 2H, J = 7.8 Hz), 8.52 (s, 1H), 8.77 (s, 1H), 11.82 (s, 1H);^{13}C NMR (125 MHz, CDCl$_3$ + DMSO-d_6); δ 34.4, 50.5, 103.5, 112.6, 117.7, 120.1, 126.4, 126.7, 127.0, 131.9, 135.4, 138.9, 147.5, 150.4, 151.0, 160.7; HRMS (ESI): m/z Calculated for $C_{24}H_{21}N_4O_4S$: 437.1278; found: 437.1284.

3,4-dimethoxy-N'-((6-phenylimidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9b)

Brown solid; 80% Yield; m.p: 236–237 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 6H), 7.08 (d, 1H, J = 8.2 Hz), 7.40–7.60 (m, 6H), 7.73 (d, 2H, J = 6.8 Hz), 8.54 (s, 1H), 8.75 (s, 1H), 11.81 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6); δ 55.75, 111.1, 114.8, 118.8, 120.9, 121.9, 125.3, 128.2, 128.9, 133.5, 137.0 148.5, 151.9, 162.5; HRMS (ESI): *m/z* Calculated for $C_{21}H_{19}BrN_4O_3S$:407.1172; found: 407.1178.

3-hydroxy-N'-((6-phenylimidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9c)

Yellow solid; 88% Yield; m.p: 268–270 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.99 (d, 1H, *J* = 7.7 Hz), 7.23 (d, 1H, *J* = 4.3 Hz), 7.29 (d, 1H, *J* = 7.1 Hz), 7.39 (t, 3H, *J* = 6.8, 8.6 Hz), 7.49 (t, 2H, *J* = 6.8, 7.2 Hz), 7.73 (d, 2H, *J* = 7.1 Hz), 8.62 (s, 1H), 8.85 (s, 1H), 9.54 (s, 1H), 11.75 (bs, 1H) ; ^{13}C NMR (75 MHz, DMSO-d_6); δ 51.0, 116.4, 118.8, 125.4, 140.4, 146.3, 150.4, 160.4, 160.9; HRMS (ESI): *m/z* Calculated for $C_{19}H_{15}N_4O_2S$: 363.0910; found: 363.0910.

4-methyl-N'-((6-phenylimidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9d)

Brown solid; 82% Yield; m.p: 258–260 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.43 (s, 3H), 7.24–7.33 (m, 3H), 7.41–7.53 (m, 2H), 7.73 (d, 2H, *J* = 7.1 Hz), 7.86 (d, 2H, *J* = 7.7 Hz) 7.94 (s, 1H), 8.65 (d, 1H, *J* = 4.4 Hz), 8.83 (s, 1H), 11.78 (s, 1H); ^{13}C NMR (100 Hz, $CDCl_3$+DMSO-d_6); δ 19.9, 112.3, 117.6, 120.8, 127.5, 127.6, 129.1, 132.3, 136.1, 140.5, 148.1, 150.9, 161.8; HRMS (ESI): *m/z* Calculated for $C_{20}H_{17}N_4OS$: 361.1118; found: 361.1131.

4-chloro-N'-((6-phenylimidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9e)

Yellow solid; 81% Yield; m.p: 245–247 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.45 (m, 1H), 7.51–7.58 (m, 3H), 7.63 (d, 2H, *J* = 8.3 Hz), 7.74 (d, 2H, *J* = 7.3 Hz), 7.96 (d, 2H, *J* = 8.32 Hz) 8.50 (d, 1H, *J* = 4.4 Hz), 8.81 (s, 1H), 11.78 (bs, 1H); ^{13}C NMR (100 Hz, DMSO-d_6); δ 114.8, 118.5, 121.4, 127.9, 128.1, 128.5, 128.6, 129.2, 131.8, 133.5, 136.5, 137.5, 149.0, 151.9, 161.4; HRMS (ESI): *m/z* Calculated for $C_{19}H_{14}ClN_4OS$: 381.0571; found: 381.0586.

4-nitro-N'-((6-phenylimidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9f)

Yellow solid; 84% Yield; m.p: 258–260 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47 (d, 1H, *J* = 7.1 Hz), 7.52 (d, 2H, *J* = 7.7 Hz), 7.57 (d, 1H, *J* = 4.4 Hz), 7.74 (d, 2H, *J* = 7.1 Hz), 8.17 (d, 2H, *J* = 8.0 Hz), 8.40 (d, 2H, *J* = 8.8 Hz), 8.49 (d, 1H, *J* = 4.4 Hz) 8.80 (s, 1H), 12.18 (bs, 1H); ^{13}C NMR (125 Hz, DMSO-d_6); δ 118.3, 121.4, 123.6, 128.0, 128.3, 128.7, 128.9, 133.2, 138.2, 138.4, 138.8, 149.7, 149.5, 152.2, 160.8; HRMS (ESI): *m/z* Calculated for $C_{19}H_{14}N_5O_3S$: 391.0180; found: 391.0162.

3,4,5-trimethoxy-N'-((6-(4-methoxyphenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9g)

Brown solid; 77% Yield; m.p: 237–239 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.73 (s, 3H), 3.82 (s, 3H), 3.86 (s, 6H), 7.08 (d, 2H, *J* = 8.4 Hz), 7.24 (s, 2H), 7.53 (d, 1H, *J* = 4.2 Hz), 7.65 (d, 2H, *J* = 8.5 Hz), 8.50 d, 1H, *J* = 4.2 Hz) 8.74 (s, 1H), 11.77 (bs, 1H); ^{13}C NMR (125 MHz, DMSO-d_6); δ 55.2, 56.1, 60.1, 105.1, 114.3, 118.0, 121.5, 125.8, 129.4, 128.4, 137.3, 140.4, 149.2, 151.4, 152.7, 159.4, 162.3; HRMS (ESI): *m/z* Calculated for $C_{23}H_{23}N_4O_5S$:467.1389; found: 467.1387.

3,4-dimethoxy-N'-((6-(4-methoxyphenyl)imidazo[2,1-*b*]thiazol-5 yl)methylene)benzohydrazide (9h)

Brown solid; 79% Yield; m.p: 232–233 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.83 (s, 3H), 3.84 (s, 6H), 7.08–7.13 (m, 3H), 7.51–7.56 (m, 2H), 7.59 (d, 1H, *J* = 8.5 Hz), 7.68 (d, 2H, *J* = 8.5 Hz), 8.53 (d, 1H, *J* = 4.1 Hz), 8.80 (s, 1H), 11.84 (s, 1H); ^{13}C NMR (125 MHz DMSO-d_6); δ 55.2, 56.6, 110.8, 110.9, 114.2, 114.6, 118.4, 120.7, 121.5, 125.2, 125.5, 129.3, 136.9, 148.3, 151.5, 151.7, 159.3, 162.1; HRMS (ESI): *m/z* Calculated for $C_{23}H_{21}N_4O_4S$: 437.1284; found: 437.1279.

3-hydroxy-N'-((6-(4-methoxyphenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9i)

Brown solid; 82% Yield; m.p: 259–260 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.86 (s, 3H), 6.95–7.05 (m, 3H), 7.21–7.31 (m, 2H), 7.38 (s, 2H), 7.66 (d, 2H, *J* = 7.9 Hz), 8.60 (d, 1H, *J* = 4.1 Hz), 8.82 (s, 1H), 9.65 (bs, 1H), 11.75 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6); δ 47.7, 53.9, 111.9, 112.7, 113.2, 116.8, 117.4, 120.8, 124.8, 128.1, 133.3, 136.5, 148.2, 150.8, 156.1, 158.2, 162.0; HRMS (ESI): *m/z* Calculated for $C_{20}H_{17}N_4O_3S$: 393.102; found: 393.1048.

N'-((6-(4-methoxyphenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-4-methylbenzohydrazide (9j)

Brown solid; 76% Yield; m.p: 249–251 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.39 (s, 3H), 3.84 (s, 3H), 7.08 (d, 2H, *J* = 8.5 Hz), 7.35 (d, 2H, *J* = 7.7 Hz), 7.52 (d, 1H, *J* = 4.2 Hz), 7.67 (d, 2H, *J* = 8.4 Hz), 7.85 (d, 2H, *J* = 7.7 Hz), 8.50 (d, 1H, *J* = 4.2 Hz), 8.79 (s, 1H), 11.71 (bs, 1H); ^{13}C NMR (100 MHz DMSO-d_6); δ 20.9, 55.1,114.1, 118.0, 121.4, 125.8, 127.3, 128.9, 130.3, 129.2, 137.1, 141.7, 148.9, 151.7, 159.2, 162.3; HRMS (ESI): *m/z* Calculated for $C_{21}H_{11}N_4O_2S$: 391.0180; found: 391.0162.

4-chloro-N'-((6-(4-methoxyphenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9k)

Yellow solid; 80% Yield; m.p: 239–240 °C; ^1H NMR (300 MHz, DMSO-*d*$_6$): δ 3.83 (s, 3H), 7.08 (d, 2H, *J* = 8.5 Hz), 7.53 (d, 1H, *J* = 4.4 Hz), 7.61–7.70 (m, 4H), 7.96 (d, 2H, *J* = 8.5 Hz), 8.48 (d 1H, *J* = 4.4 Hz), 8.78 (s, 1H), 11.96 (bs, 1H); ^{13}C NMR (100 MHz DMSO-*d*$_6$); δ 55.2 114.1, 117.8, 125.8, 128.5, 129.3, 131.9, 136.5, 137.7, 149.2, 151.9, 161.4, 159.3; HRMS (ESI): *m/z* Calculated for $C_{20}H_{16}N_4O_2S$: 411.0682; found: 411.0679.

N'-((6-(4-methoxyphenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-4-nitrobenzohydrazide (9l)

Yellow solid; 82% Yield; m.p: 256–258 °C; ^1H NMR (400 MHz, DMSO-*d*$_6$): δ 3.83 (s, 3H), 7.09 (d, 2H, *J* = 8.6 Hz), 7.55 (d, 1H, *J* = 4.2 Hz), 7.68 (d, 2H, *J* = 8.5 Hz), 8.18 (d, 2H, *J* = 8.6 Hz), 8.38 (d, 2H, *J* = 8.5 Hz), 8.48 (d, 1H, *J* = 4.4 Hz), 8.81 (s, 1H), 11.98 (bs, 1H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$); δ 55.1 114.1, 114.7, 117.3, 121.4, 123.5, 125.5, 128.9, 129.3, 138.4, 138.7, 149.1, 152.0, 160.9, 159.4; HRMS (ESI): *m/z* Calculated for $C_{20}H_{16}N_5O_4S$: 422.0923; found: 422.0917.

N'-((6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3,4,5-trimethoxybenzohydrazide (9m)

Yellow solid; 84% Yield; m.p: 253–255 °C; ^1H NMR (300 MHz, DMSO-*d*$_6$): δ 3.63 (s, 6H), 7.23 (s, 2H), 7.37 (t, 2H, *J* = 8.8 Hz), 7.56 (d, 1H, *J* = 4.4 Hz), 7.75–7.81 (m, 2H), 8.51 (d, 1H, *J* = 4.4 Hz), 8.72 (s, 1H), 11.82 (bs, 1H ; ^{13}C NMR (75 MHz DMSO-*d6*); δ 56.0, 60.0, 105.1, 114.8, 115.5, 115.8, 118.6, 121.4, 130.1, 128.3, 130.0, 136.7, 140.4, 147.8, 152.6, 151.8, 160.4; HRMS (ESI): *m/z* Calculated for $C_{20}H_{17}N_4O_2S$: 455.1205; found: 455.1204.

N'-((6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3,4-dimethoxybenzohydrazide (9n)

Yellow solid; 82% Yield; m.p: 251–252 °C; ^1H NMR (300 MHz, DMSO-*d*$_6$): δ 3.84 (s, 6H), 7.10 (d, 1H, *J* = 8.5Hz), 7.37 (t, 2H, *J* = 9.0, 8.8 Hz), 7.50 (s, 1H), 7.75–7.59 (m, 2H), 7.76–7.78 (m, 2H), 8.52 (d, 1H, *J* = 4.4 Hz), 8.76 (s, 1H), 11.80 (bs, 1H) ; ^{13}C NMR (125 MHz, DMSO-d_6); δ 55.6, 110.7, 114.8, 115.5, 115.7, 118.6, 120.7, 125.3, 130.4, 136.3, 147.6, 140.3, 151.7, 161.0, 162.1, 162.9; HRMS (ESI): *m/z* Calculated for $C_{21}H_{18}N_4FO_3S$: 425.0180; found: 425.0162.

N'-((6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3-hydroxybenzohydrazide (9o)

Yellow solid; 85% Yield; m.p: 257–259 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.97–7.03 (m, 1H), 7.33 (d, 2H, *J* = 6.0 Hz), 7.37 (d, 2H, *J* = 8.4 Hz), 7.40 (s, 1H), 7.56 (d, 1H, *J* = 4.4 Hz), 7.75-7.81 (m, 2H), 8.51 (d, 1H, *J* = 4.4 Hz), 8.74 (s, 1H), 9.81 (s,1H), 11.85 (s, 1H) ; ^{13}C NMR (75 MHz DMSO-d_6); δ 114.2, 114.8, 115.5, 115.7, 117.7, 117.8, 120.9, 121.4, 129.4, 130.0, 134.5, 136.9, 147.7, 151.8, 157.3, 160.3,162.6; HRMS (ESI): *m/z* Calculated for $C_{19}H_{14}N_4FO_2S$: 381.0821; found: 381.0818.

N'-((6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-4-methylbenzohydrazide (9p)

Yellow solid; 82% Yield; m.p: 248–249 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.40 (s, 3H), 7.33 (d, 4H, *J* = 7.7Hz), 7.48 (s, 1H), 7.77 (m,2H), 7.85 (d, 2H, *J* = 7.9 Hz), 8.55 (d, 1H, *J* = 4.4 Hz), 8.79 (s, 1H), 11.84 (bs, 1H) ; ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6); δ 112.9, 114.0, 116.9, 119.5, 125.7, 127.7, 128.3, 128.6, 135.2, 140.0, 146.1, 150.2, 159.4, 160.8, 161.4; HRMS (ESI): *m/z* Calculated for $C_{20}H_{16}N_4FOS$: 379.1029; found: 379.1022.

4-chloro-N'-((6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9q)

Yellow solid; 84% Yield; m.p: 252–253 °C; NMR (400 MHz, DMSO-d_6): δ 7.34 (d, 2H, *J* = 8.4 Hz), 7.55 (d, 1H, *J* = 4.4 Hz), 7.63 (d, 2H, *J* = 8.6 Hz), 7.75–7.79 (m, 2H), 7.95 (d, 2H, *J* = 8.5 Hz), 8.49 (d, 1H, *J* = 4.5 Hz), 8.76 (s, 1H), 11.97 (bs,1H) ; ^{13}C NMR (100 MHz, DMSO-d_6); δ 115.0, 115.6, 115.8, 117.2, 118.4, 121.4, 128.6, 129.3, 130.1, 130.0, 131.8, 136.5, 137.4, 148.1, 152.0, 160.8, 161.5, 163.3; HRMS (ESI): *m/z* Calculated for $C_{19}H_{13}ClN_4FOS$:399.048; found: 399.0482.

N'-((6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-4-nitrobenzohydrazide (9r)

Yellow solid; 84% Yield; m.p: 255–256 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.33–7.40 (m, 2H), 7.56 (d, 1H, *J* = 4.4Hz), 7.74–7.79 (m, 2H), 8.14 (d, 2H, *J* = 8.8 Hz), 8.34 (d, 2H, *J* = 8.5 Hz), 8.47 (d, 1H, *J* = 4.4 Hz), 8.76 (s, 1H), 12.16 (bs,1H) ; ^{13}C NMR (100 MHz DMSO-*d*$_6$); δ 115.1, 115.6, 115.8, 118.3, 121.4, 123.6, 128.9, 129.8, 130.0, 138.1, 138.7, 148.2, 149.2, 152.2, 160.9, 163.3; HRMS (ESI): *m/z* Calculated for $C_{19}H_{13}FN_3O_3S$: 410.0180; found: 410.0162.

N'-((6-(4-chlorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3,4,5 trimethoxybenzohydrazide (9s)

Brown solid; 74% Yield; m.p: 248–250 °C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 3.87 (s, 3H), 3.92 (s, 6H), 7.13 (d, 1H, *J* = 8.1 Hz), 7.27 (s, 2H), 7.46–7.48 (m, 2H), 7.71 (d, 2H, *J* = 7.8 Hz), 8.62 (s, 1H), 8.75 (s, 1H), 11.64 (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-*d*$_6$); δ 55.3, 59.6, 104.3, 112.5, 114.9, 118.2, 121.3, 127.8, 128.6, 129.3, 131.4, 132.8, 136.3, 139.9, 147.4, 151.9, 162.5; HRMS (ESI): *m/z* Calculated for $C_{22}H_{20}ClN_4O_4S$: 471.0180; found: 471.0162.

N'-((6-(4-chlorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3,4-dimethoxybenzohydrazide (9t)

Brown solid; 72% Yield; m.p: 246–247 °C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 3.84 (s, 6H), 7.10 (d, 1H, *J* = 8.2 Hz), 7.50 (s, 1H), 7.52–7.62 (m, 4H), 7.76 (d, 2H, *J* = 7.7 Hz), 8.52 (s, 1H), 8.77 (s, 1H), 11.79 (bs, 1H) ; ^{13}C NMR (100 MHz, DMSO-*d*$_6$); δ 55.1, 115.2, 118.7, 121.8, 127.4, 129.0, 130.1, 130.6, 132.2, 133.2, 136.7, 142.4, 149.9, 151.7, 162.5; HRMS (ESI): *m/z* Calculated for $C_{21}H_{18}ClN_4O_3S$: 441.0788; found: 441.0776.

N'-((6-(4-chlorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3-hydroxybenzohydrazide (9u)

Brown solid; 82% Yield; m.p: 258–260 °C; ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 6.97–7.00 (m, 1H), 7.30–7.34 (m, 3H), 7.51 (d, 1H, *J* = 4.4 Hz), 7.56 (d, 1H, *J* = 8.3 Hz), 7.74 (d, 1H, *J* = 8.3 Hz), 8.52 (d, 1H, *J* = 4.4 Hz), 8.77 (s, 1H), 9.90 (s, 1H), 11.84 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-*d*$_6$); δ 114.4, 115.3, 118.1, 118.9, 121.7, 128.9, 129.7, 132.2, 133.0, 134.6, 137.1, 147.5, 152.3, 157.4, 162.9; HRMS (ESI): *m/z* Calculated for $C_{19}H_{14}ClN_4O_3S$: 397.0521; found: 397.0516.

N'-((6-(4-chlorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-4-methylbenzohydrazide (9v)

Yellow solid; 81% Yield; m.p: 245–246 °C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 2.38 (s, 3H), 7.34 (d, 2H, *J* = 7.9 Hz), 7.74 (d, 2H, *J* = 8.3 Hz), 7.83 (d, 2H, *J* = 7.8 Hz), 8.52 (d, 1H, *J* = 4.4 Hz), 11.86 (bs, 1H), 8.71 (s, 1H), 7.53 (d, 1H, *J* = 4.4 Hz), 7.56 (d, 2H, *J* = 8.3 Hz) ; ^{13}C NMR (100 MHz, DMSO-*d*$_6$); δ 21.1, 115.1, 118.9, 121.6, 129.7, 127.5, 132.3, 133.2, 136.9, 142.0, 147.0, 152.1, 162.7; HRMS (ESI): *m/z* Calculated for $C_{20}H_{16}ClN_4OS$: 395.0733; found: 395.0728.

4-chloro-N'-((6-(4-chlorophenyl)imidazo[2,1-*b*]thiazol-5-yl)methylene)benzohydrazide (9w)

Yellow solid; 84% Yield; m.p: 256–258 °C ;^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 7.58 (d, 3H, J = 8.3 Hz), 7.64 (d, 2H, *J* = 7.4 Hz), 7.76 (d, 2H, *J* = 7.4 Hz), 7.95 (d, 2H, *J* = 7.6 Hz), 8.49 (d, 1H, *J* = 4.1 Hz), 8.79 (s, 1H), 11.98 (bs, 1H) ; ^{13}C NMR (100 MHz, DMSO-*d*$_6$); δ 109.6, 115.0, 118.6, 119.6, 121.4, 124.0, 127.5, 128.1, 128.4, 128.8, 132.0, 137.9, 149.2, 152.2, 161.1; HRMS (ESI): *m/z* Calculated for $C_{19}H_{13}Cl_2N_4OS$ $[M+H]^+$ 415.0180; found: 415.0162.

Biology

Cell line maintenance

The cell lines MDA-MB-231 (ATCC No. HTB-26), HEK-293 (ATCC No. CRL-1268), DU-145 (ATCC No. HTB-81), HeLa (ATCC No. CCL-2) and A549 (ATCC No. CCL-185) were purchased from ATCC. The cell lines were cultured in DMEM containing 10% FBS and antibiotics (penicillin and streptomycin). The cells were maintained at 37 °C, 5% CO_2 and 90% relative humidity.

Antiproliferative activity

The antiproliferative activity of the compounds was determined using MTT assay. 1×10^4 cells/well were seeded in 100 µL DMEM supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37 °C in a CO_2 incubator. After 24 h incubation, all the synthesized compounds were added to the respective wells and incubated for 48 h. After 48 h of drug treatment, 10 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (5 mg/mL) was added to each well and the plates were further incubated for 4 h. The supernatant from each well was carefully removed, formazan crystals were dissolved in 100 µL of DMSO and absorbance at wavelength of 570 nm was recorded.

Cell cycle analysis

Flow cytometric analysis (FACS) was performed to evaluate the distribution of the cells and growth arrest through the cell cycle phases as reported. In brief, MDA-MB-231 cells were plated at a density of 1 × 10^5 cells per well in 12 well format supplemented with 10% FBS containing media and incubated for 24 h at 37 ºC in 5% CO_2 atmosphere and replaced with serum free media for additional 6 h and treated with compounds and standard drugs. After 24 h incubation, the cells were trypsinized and washed twice with PBS and fixed in 70% ethanol and stained with propidium iodide (50 µg/ml, Sigma Aldrich) in the presence of RNAse A (20 µg/ml) containing 0.1% Triton-X 100 for 30 mins in dark at 37 ºC and 10,000 events were analyzed and quantified using Accuri C6 flow cytometer based software (BD Biosciences). All the values were expressed as mean ± SEM in three different experiments in which each treatment was performed in triplicates.

Quantification of apoptosis

In order to determine the apoptotic induced cell death, annexin V-FITC and propidium iodide staining was performed by using the Annexin V-FITC apoptosis detection kit according to manufacturer's protocol (Sigma Aldrich). In brief, cells were seeded at a density of 1 × 10^6 cells per well in a 6 well plate and allowed to adhere for overnight. The cells were treated with different concentrations of compounds along with the standard doxorubicin. After 24 h incubation, the cells were subjected to trypsinization and later trypsin was removed. It was followed by addition of 500 µL of staining buffer solution which was followed by incubation with Annexin V-FITC and propidium iodide at room temperature for 15 min. After 15 min of the incubation period, cells were analysed for a percentage of apoptosis using BD-C6 accuri flow-cytometer.as soon as possible. All the values were expressed as mean ± SEM in three different experiments in which each treatment was performed in triplicates.

DAPI staining

MDA-MB-231 cells were seeded on 24 well plates at the density of 25,000 cells/well and allowed to adhere overnight. Then, the cells were treated with compounds **9i** and **9m** (1 µM) for 48 h, washed with PBS and fixed with 4% neutral buffered formalin solution for 20 min. Doxorubicin (1 µM) was used as a standard. Again, the cells were washed twice with PBS and permeabilized with 0.2% Triton-X100 for 5 min and stained with DAPI (1 mg/mL) for 10 min at room temperature. Cells were examined for morphological changes under fluorescence microscope (Model: Nikon, Japan) using 350 nm excitation and 460 nm emission at 100x magnification.

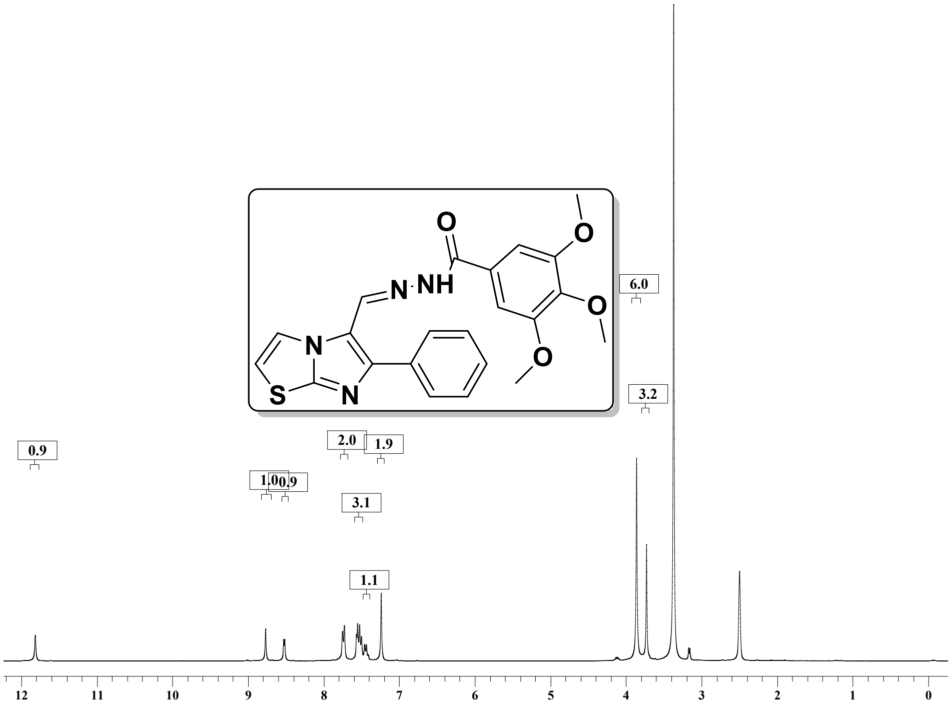
Quantitative determination of intracellular ROS level

The intracellular ROS level was determined quantitatively by using a cell permeant probe 2′7′-dichlorodihydrofluorescein diacetate (H2DCFDA) method**.** The cells were plated at a density of 3 × 10^5 cells per well in a 6 well plate and allowed to adhere for overnight. The medium was replaced with culture medium containing Carboxy-DCFDA (10 µM/well) and further incubated for 30 min at room temperature in dark and cells were treated with different concentrations of the compounds dissolved in the culture media and known standards for 6 h. The cells were collected and washed twice and resuspended in PBS. The fluorescence intensity from each sample was analyzed by flow cytometer at FL-1-H filter quantified using Accuri C6 flow cytometer based software (BD Biosciences). All the values were expressed as mean ± SEM in three different experiments in which each treatment was performed in triplicates.

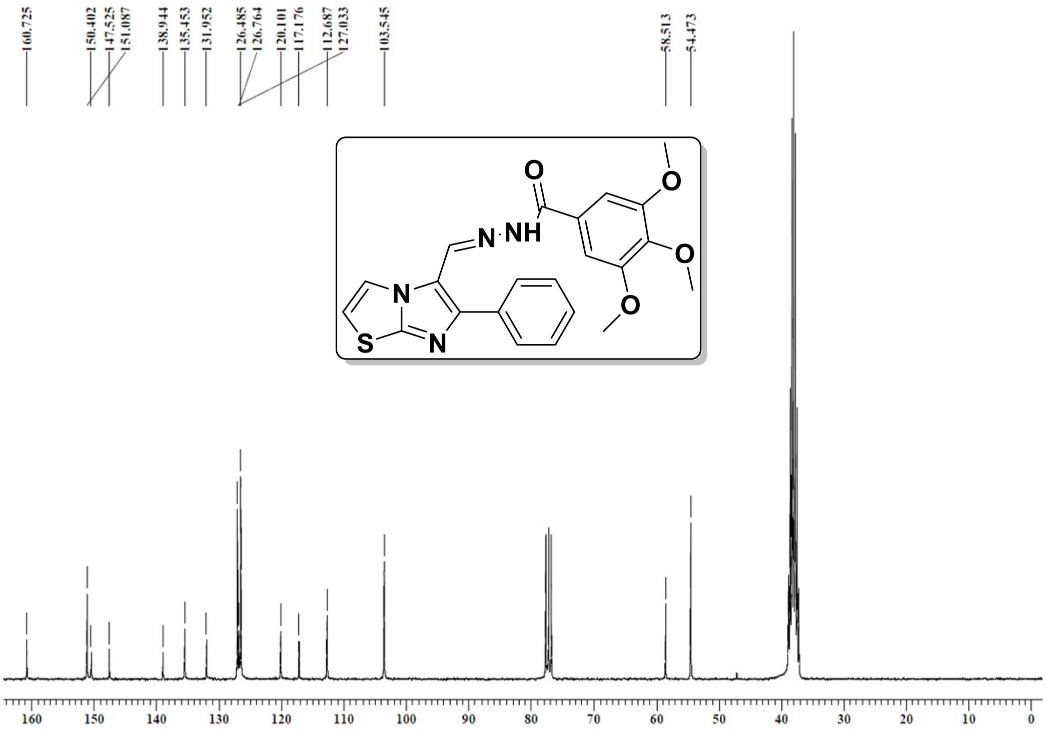
Quantitative determination of mitochondrial membrane potential by JC-1 dye

The cells were plated at a density of 3 × 10^5 cells per well in a 6 well plate and allowed to adhere for overnight. The cells were treated with different concentration of the compounds (**9i** and **9m**) dissolved in the culture media and known standards for 24 h. The medium was replaced with culture medium containing JC-1 dye (5 µM) and further incubated for 20 min at room temperature in dark. The cells were washed twice with PBS and were subjected to flow cytometric analysis through flow cytometer using FL2 channel to detect loss of mitochondrial membrane potential in treated samples. All the values were expressed as mean ± SEM in three different experiments in which each treatment was performed in triplicates.

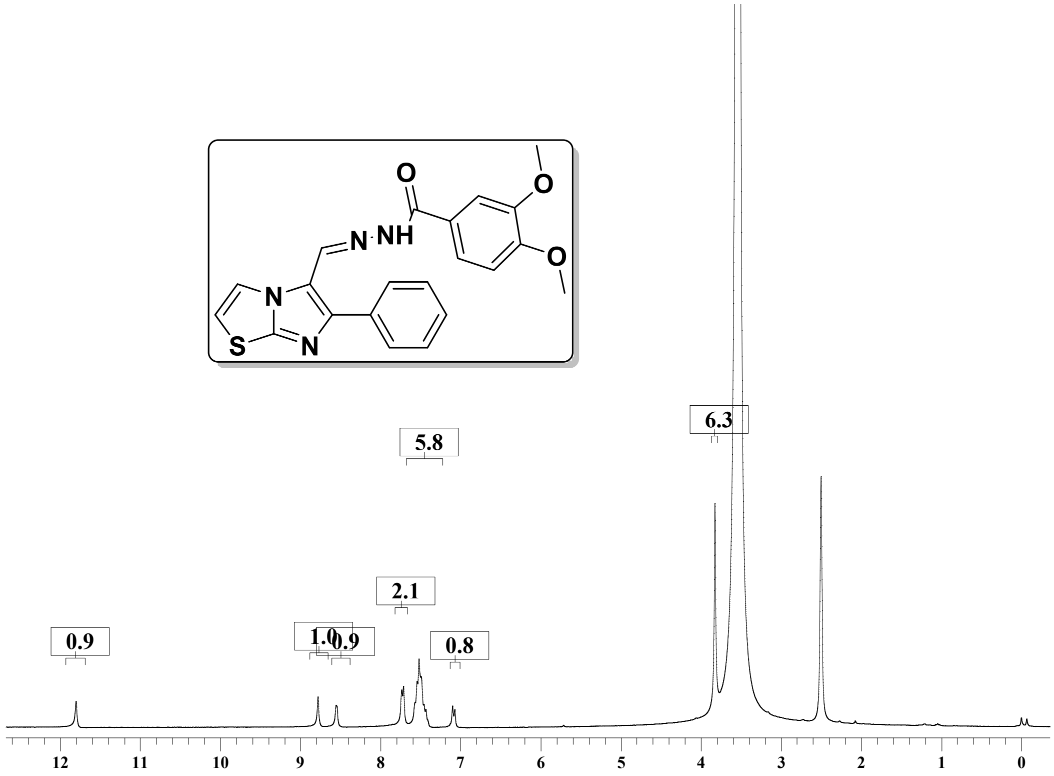
<u>**Representative Spectra of final Compounds**</u>



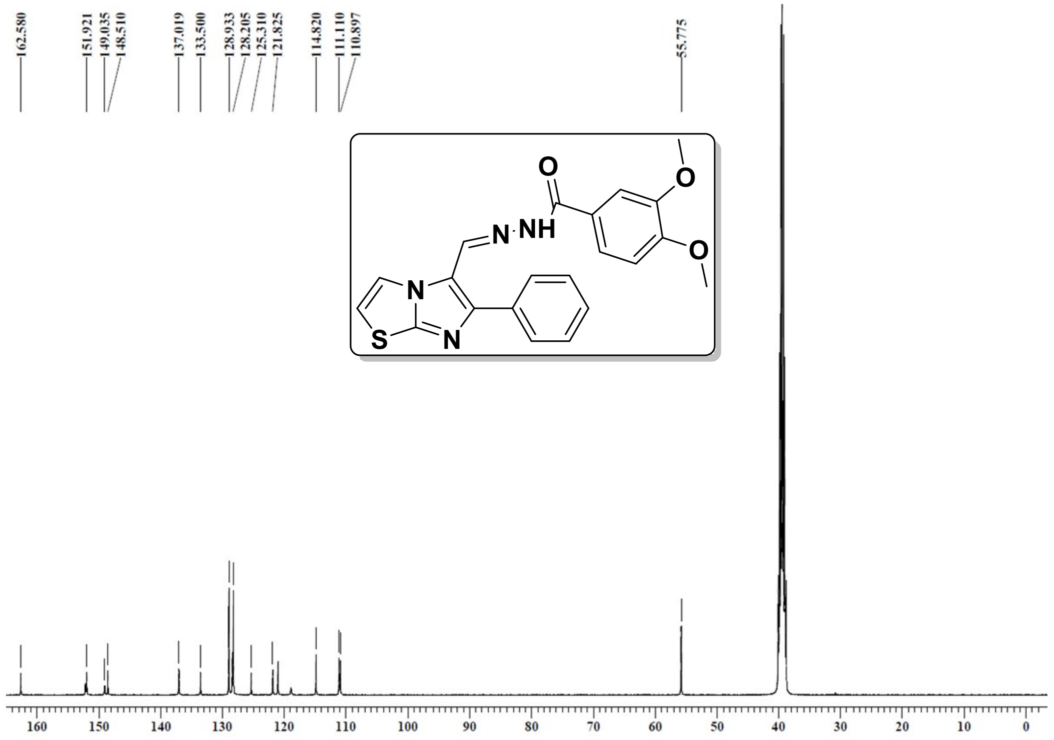
^{1}H NMR of compound 9a



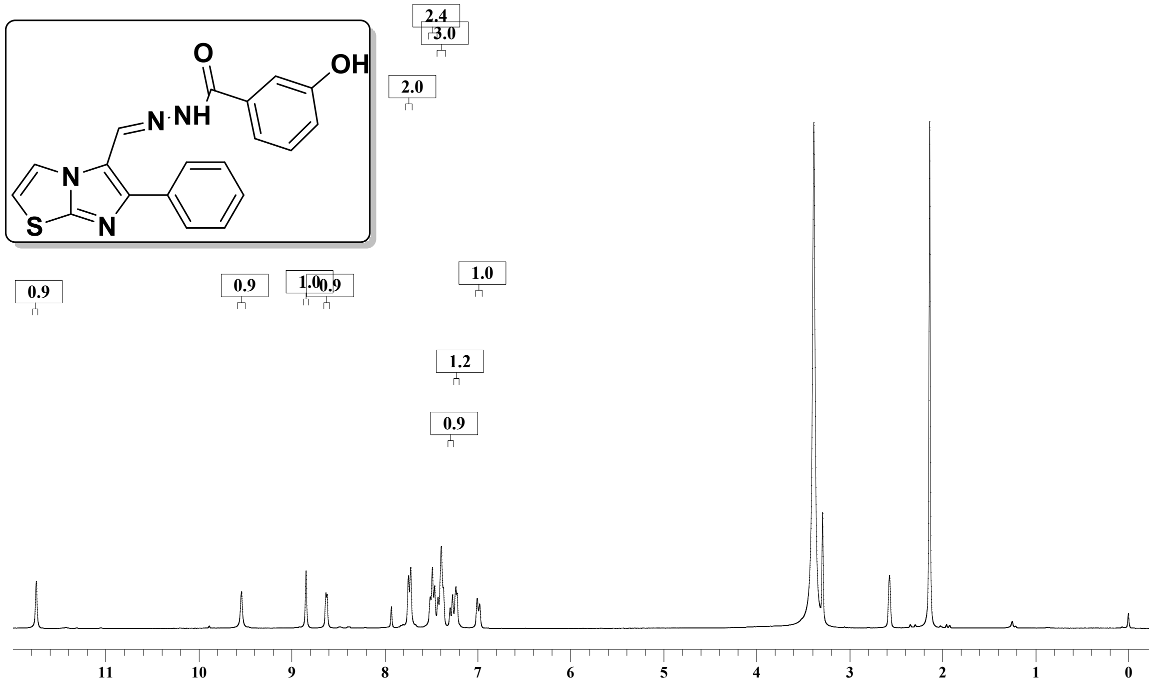
^{13}C NMR of compound 9a



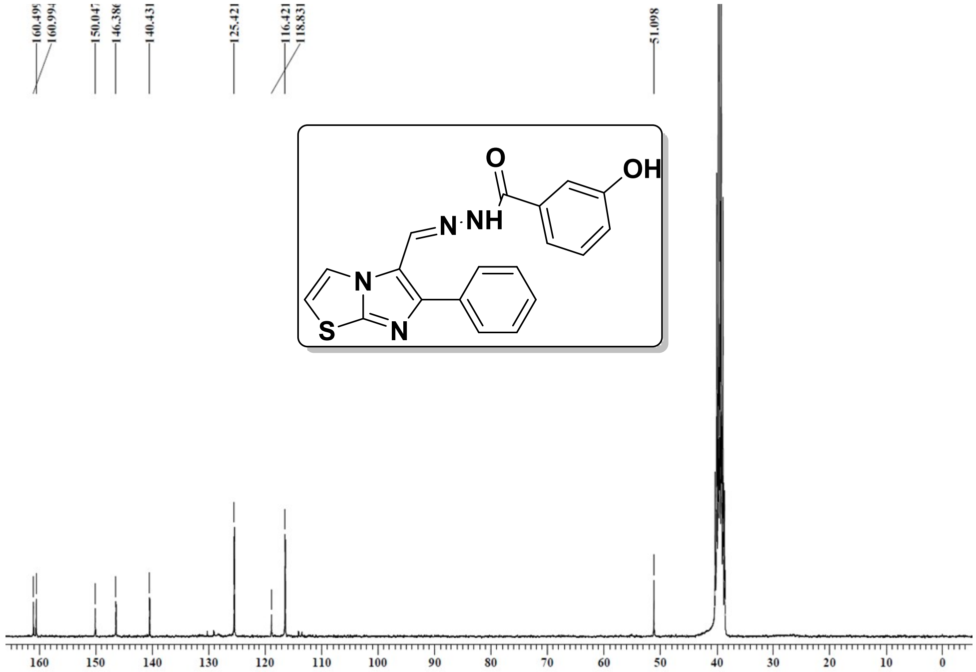
^{1}H NMR of compound 9b



^{13}C NMR of compound 9b



^{1}H NMR of compound 9c



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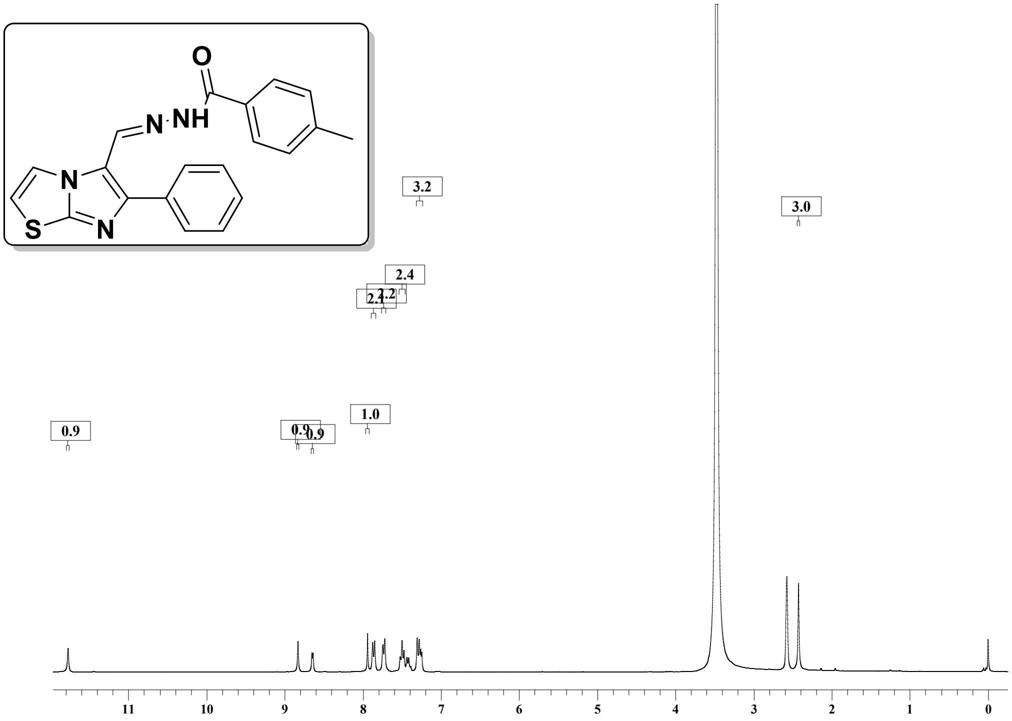

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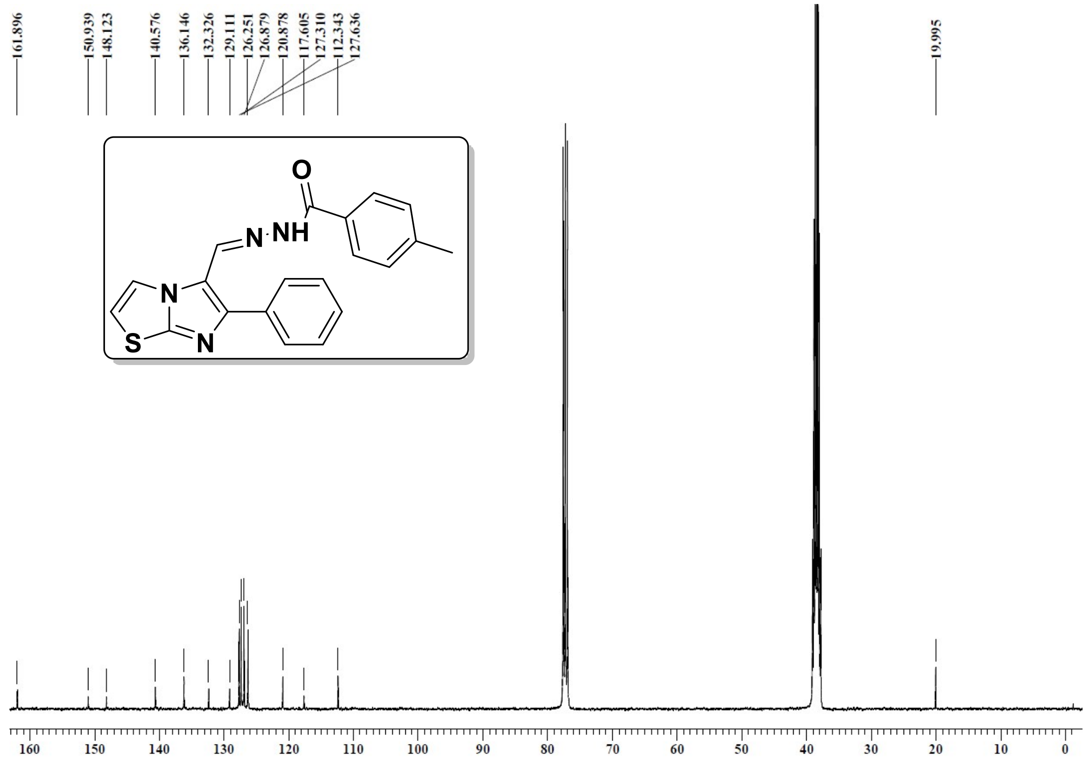

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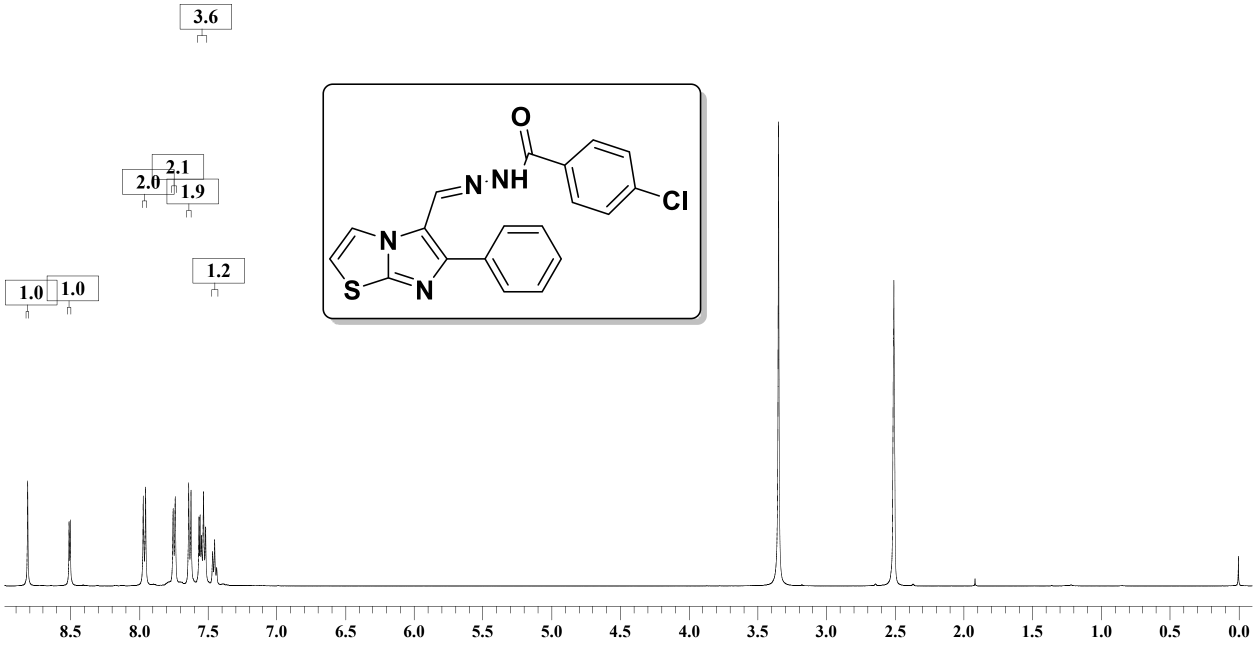

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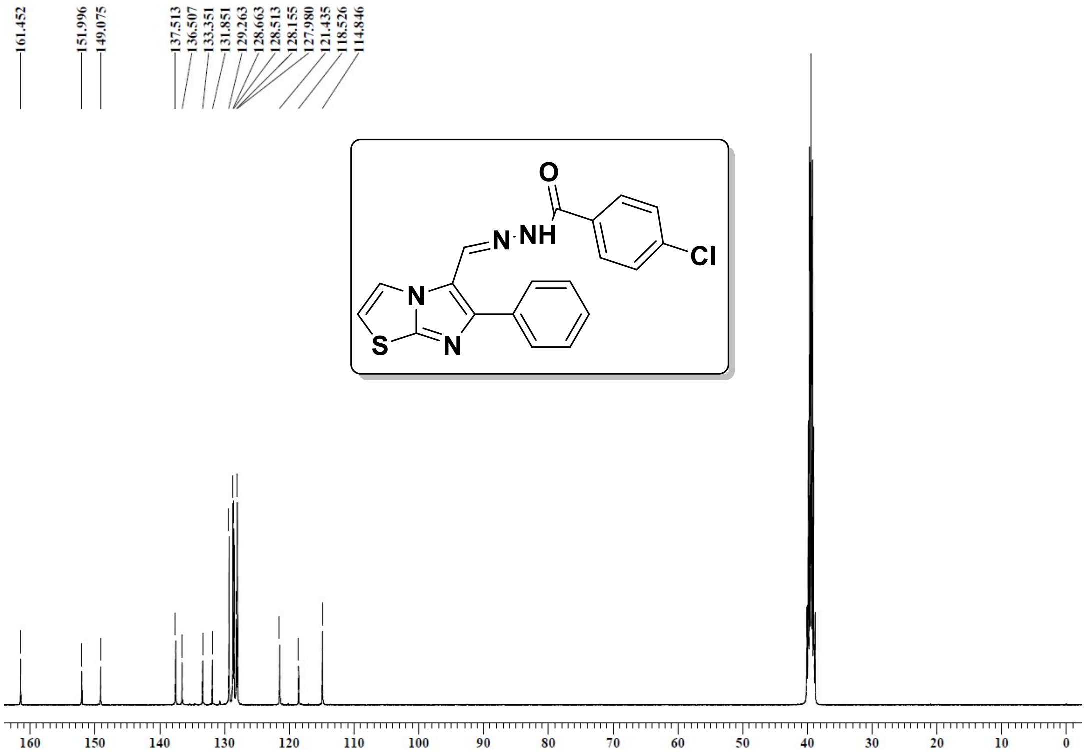

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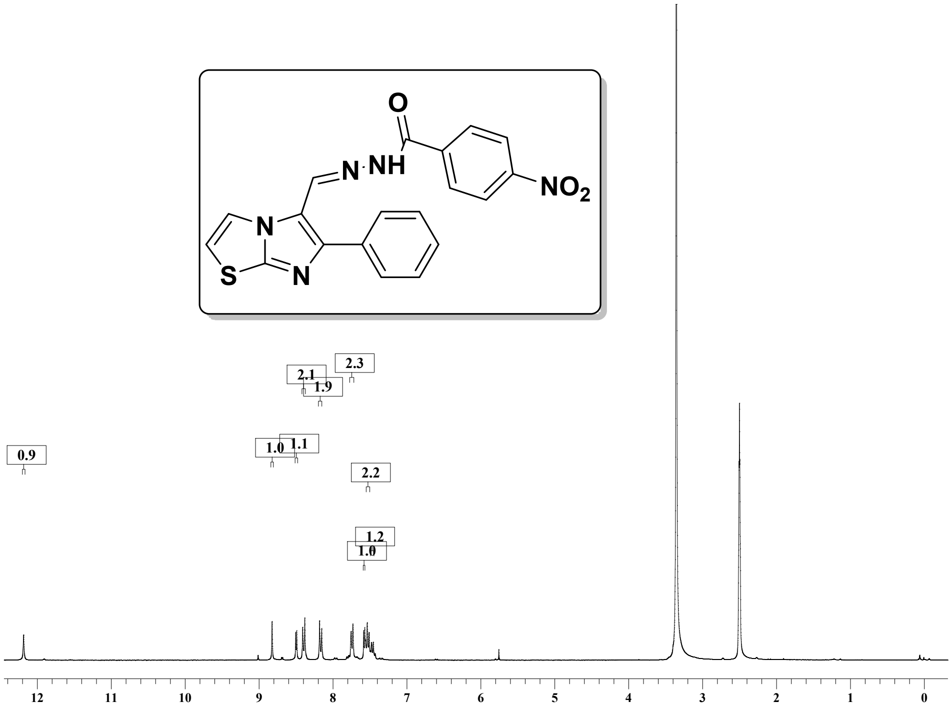
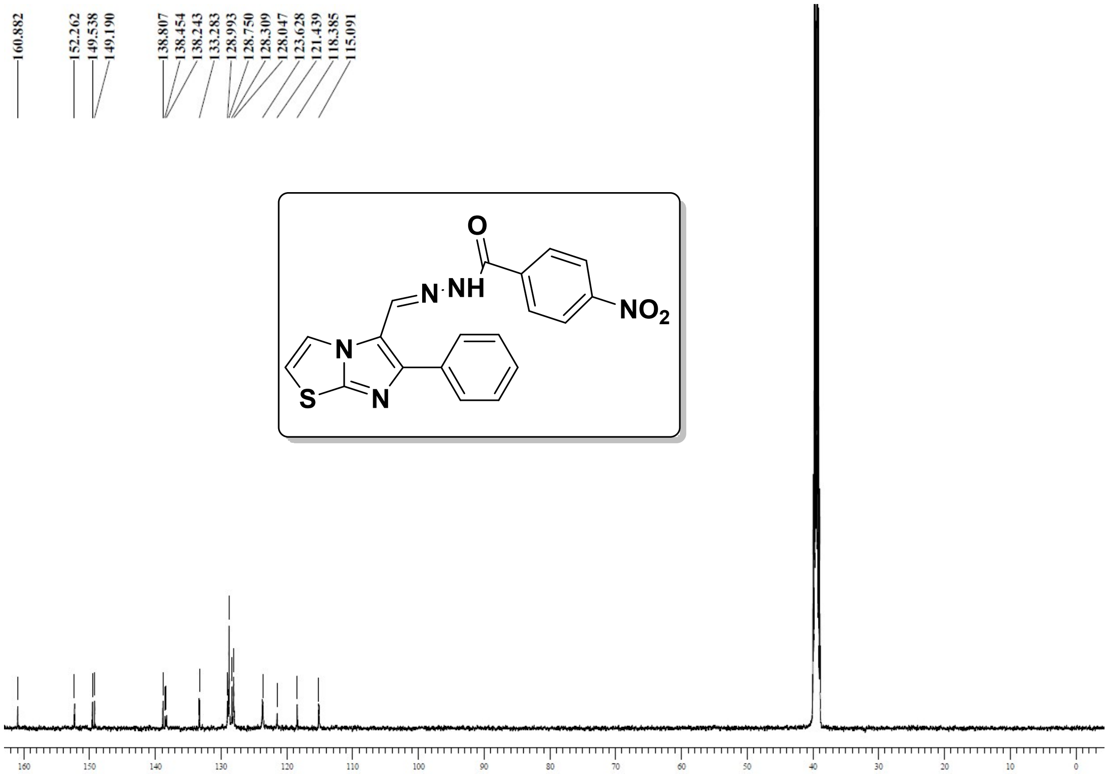
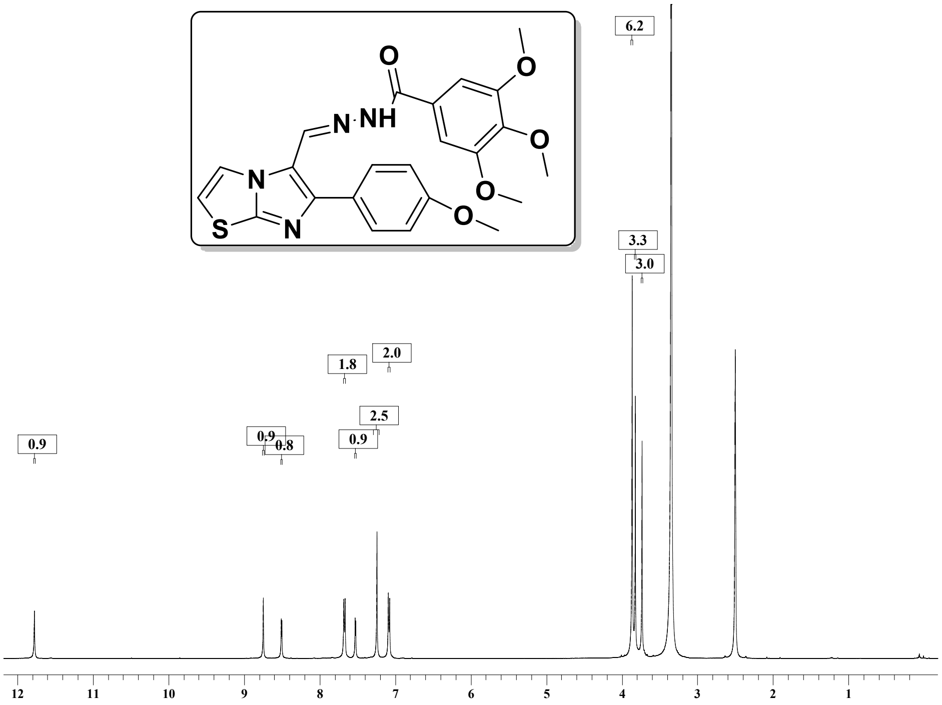


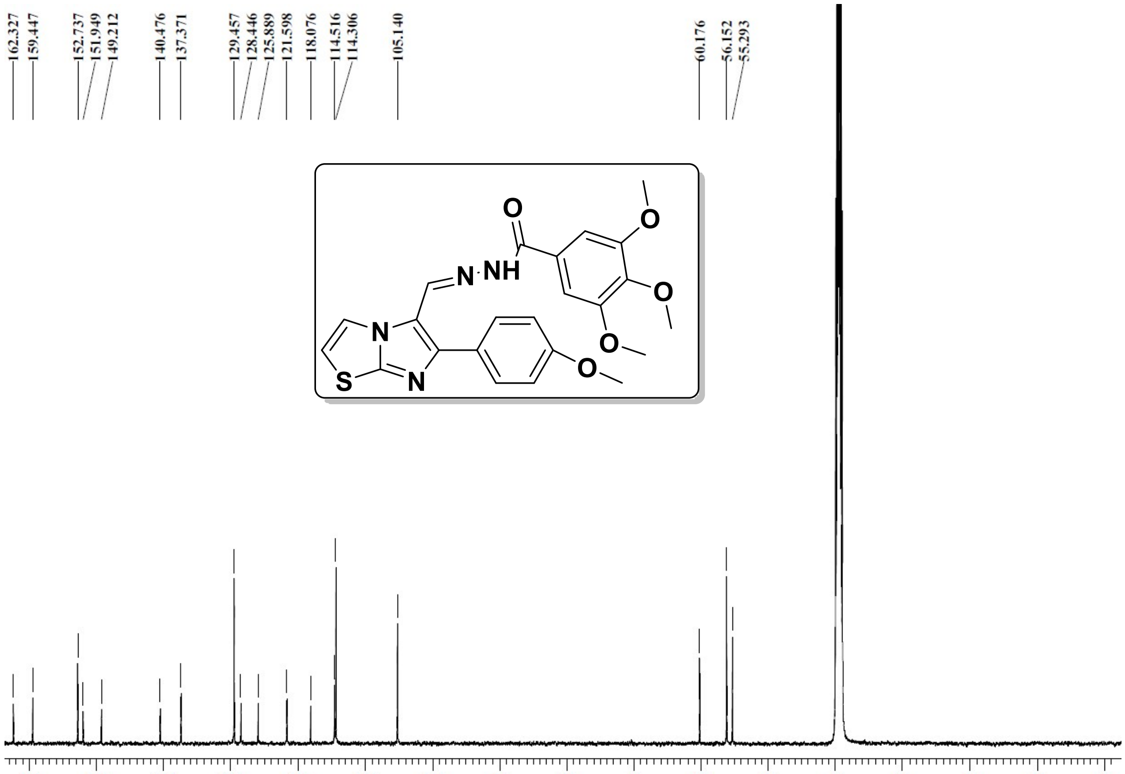
^{1}H NMR of Compound 9f



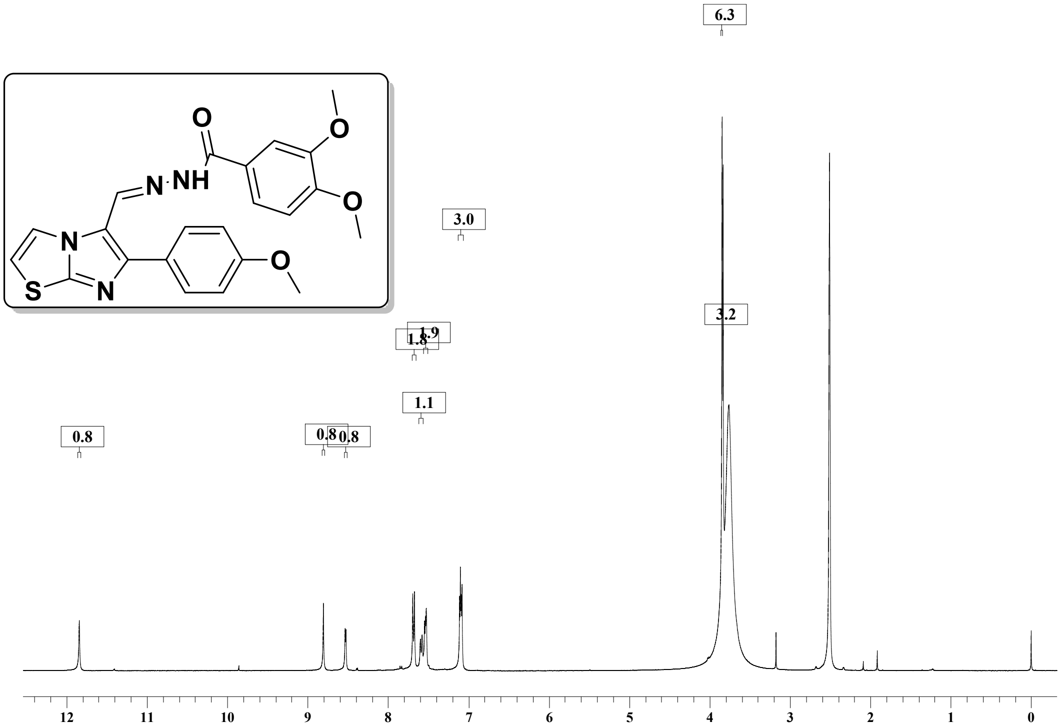
^{13}C NMR of Compound 9f



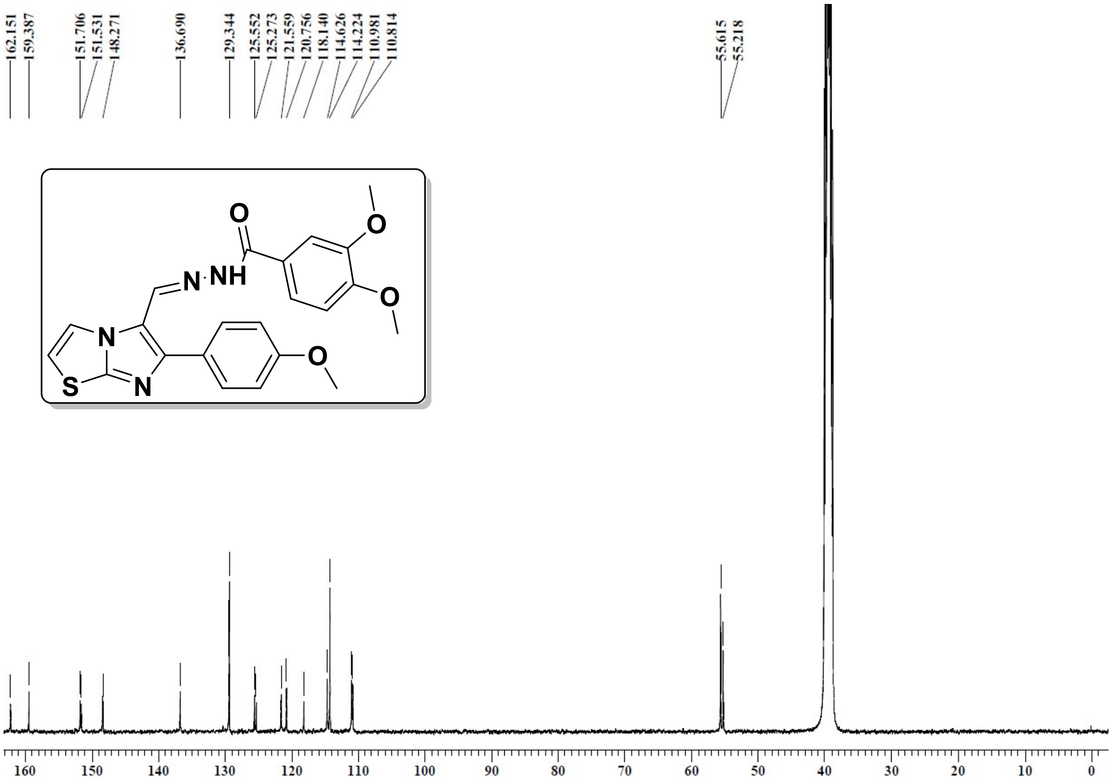
^{1}H NMR of Compound 9g



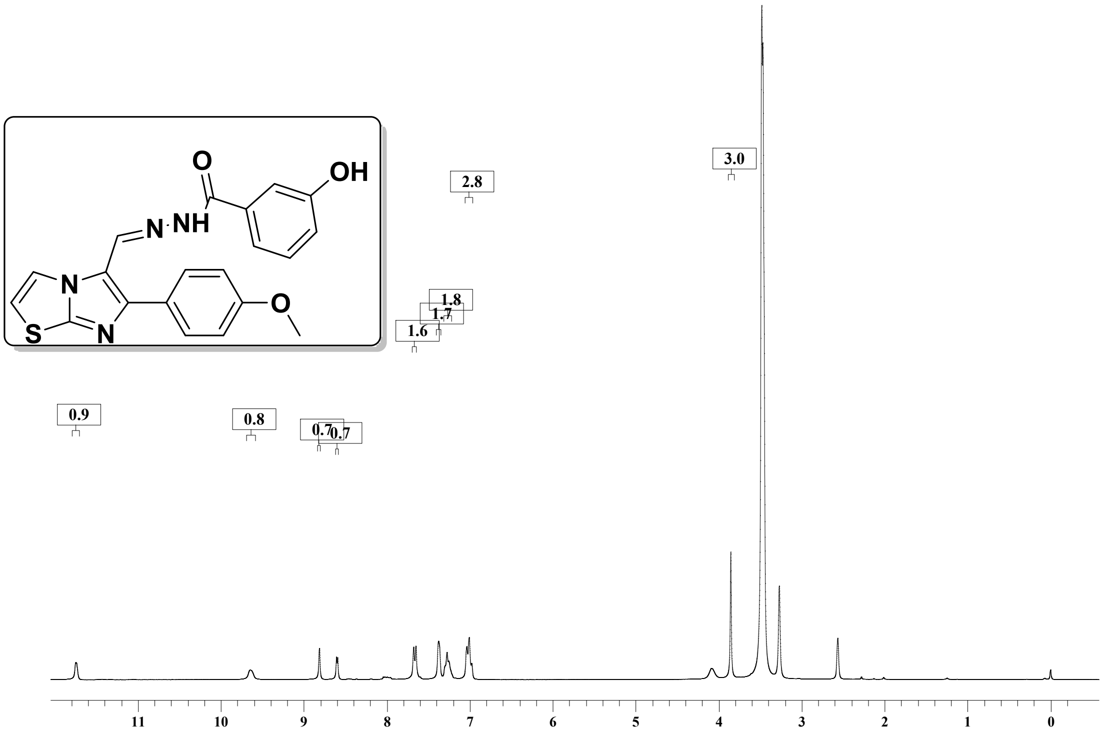
^{13}C NMR of Compound 9g



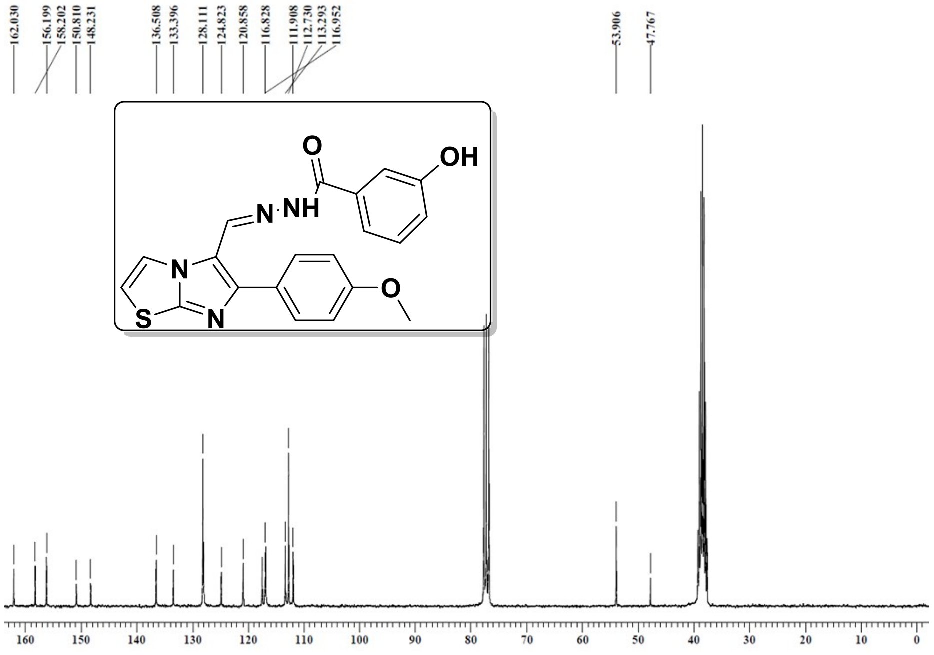
^{1}H NMR of compound 9h



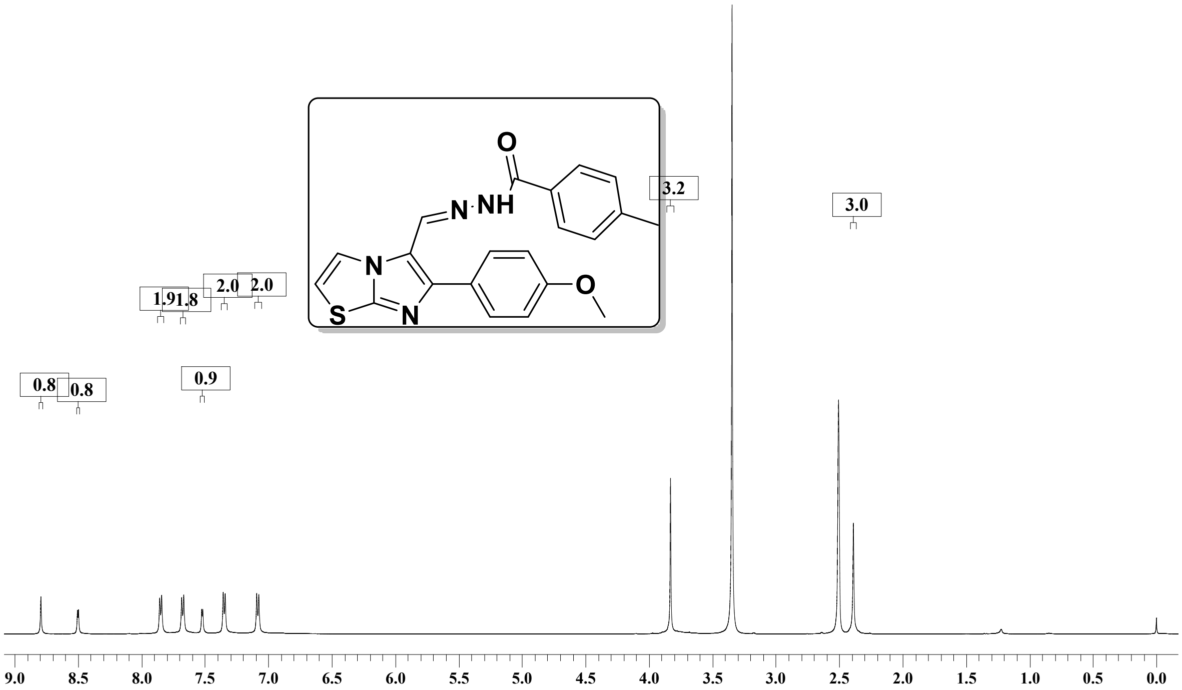
^{13}C NMR of compound 9h



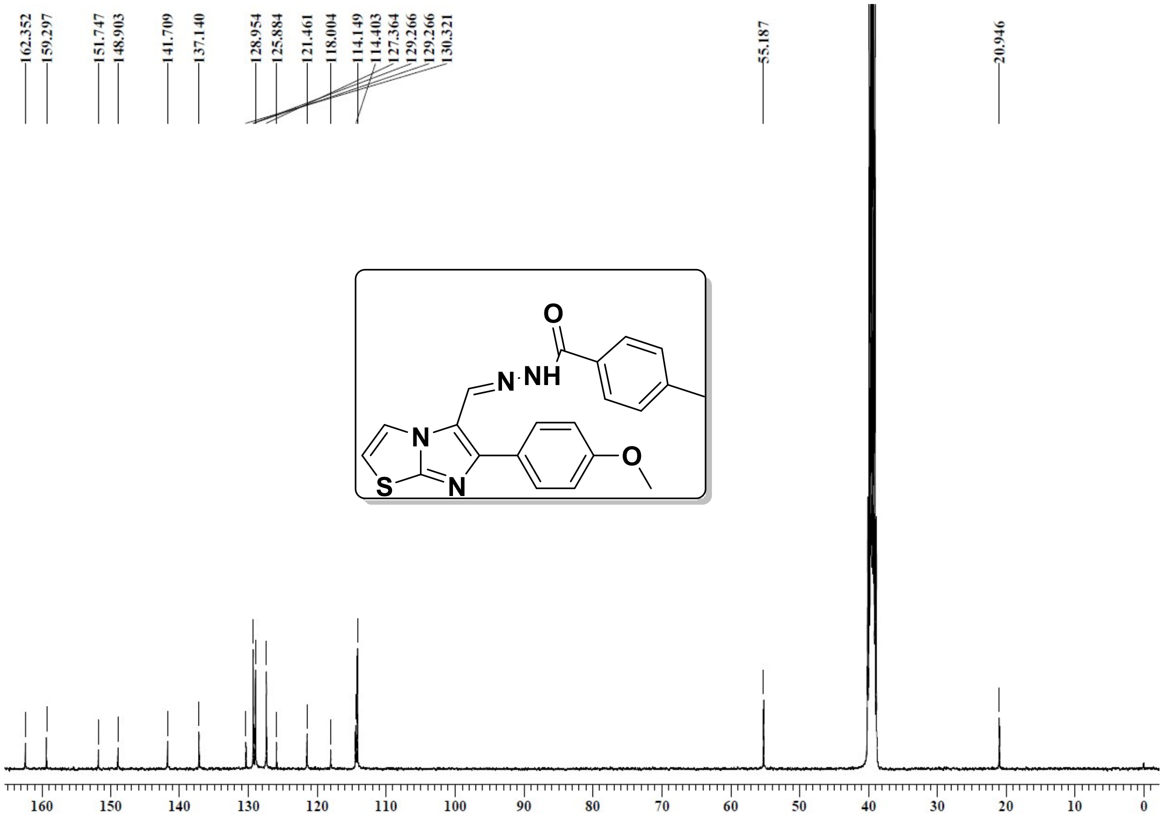
^{1}H NMR of compound 9i



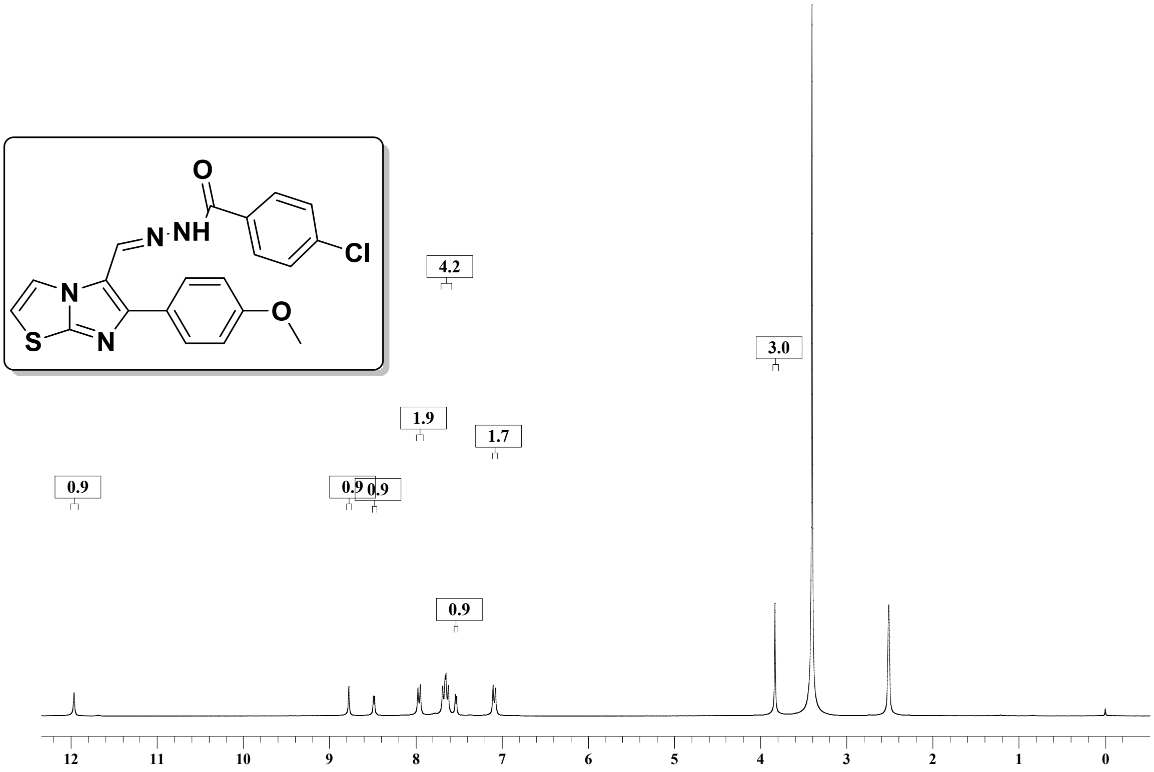
^{13}C NMR of compound 9i



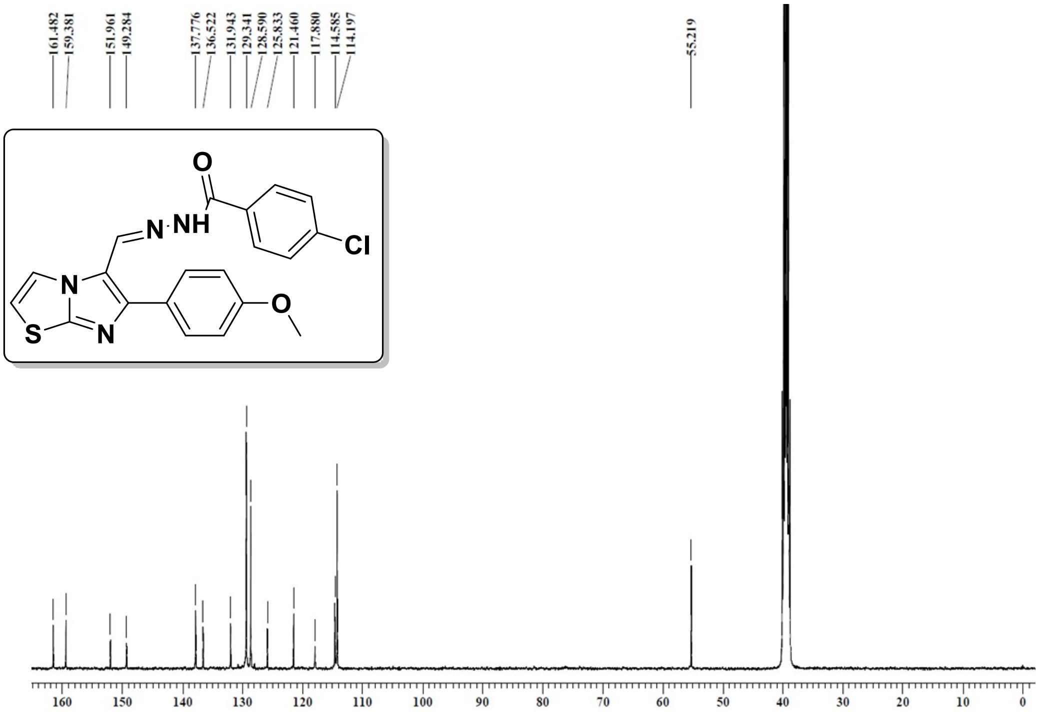
^{1}H NMR of compound 9j



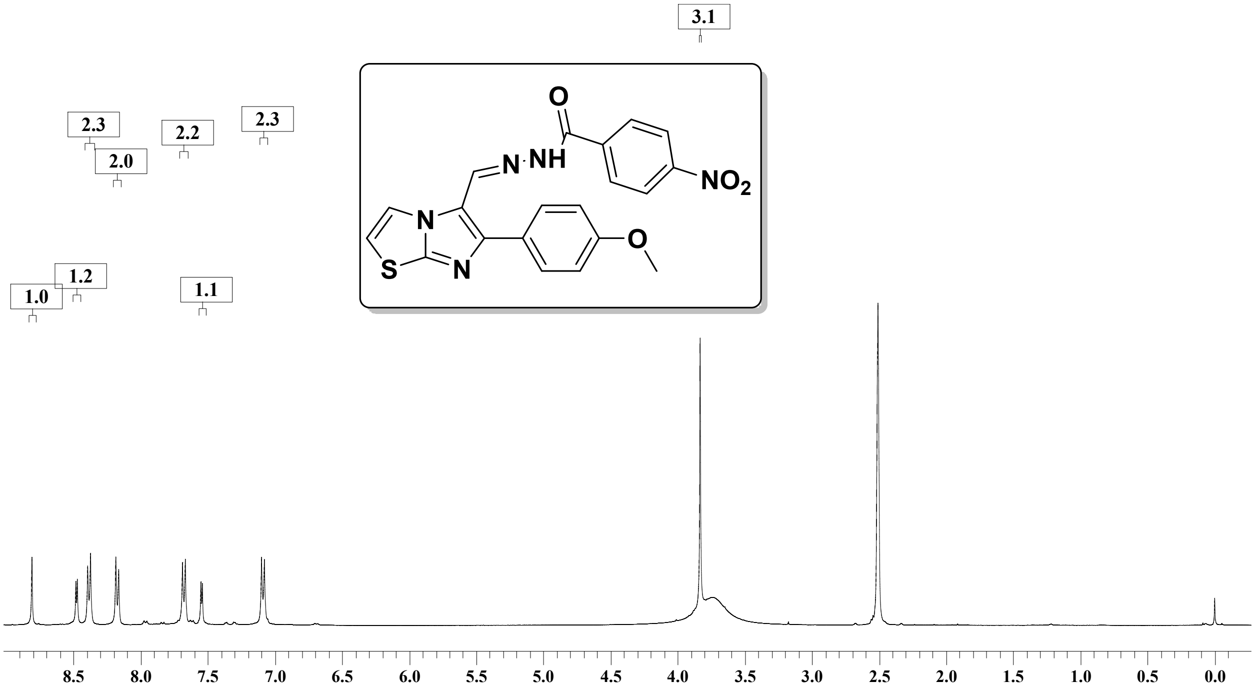
^{13}C NMR of compound 9j



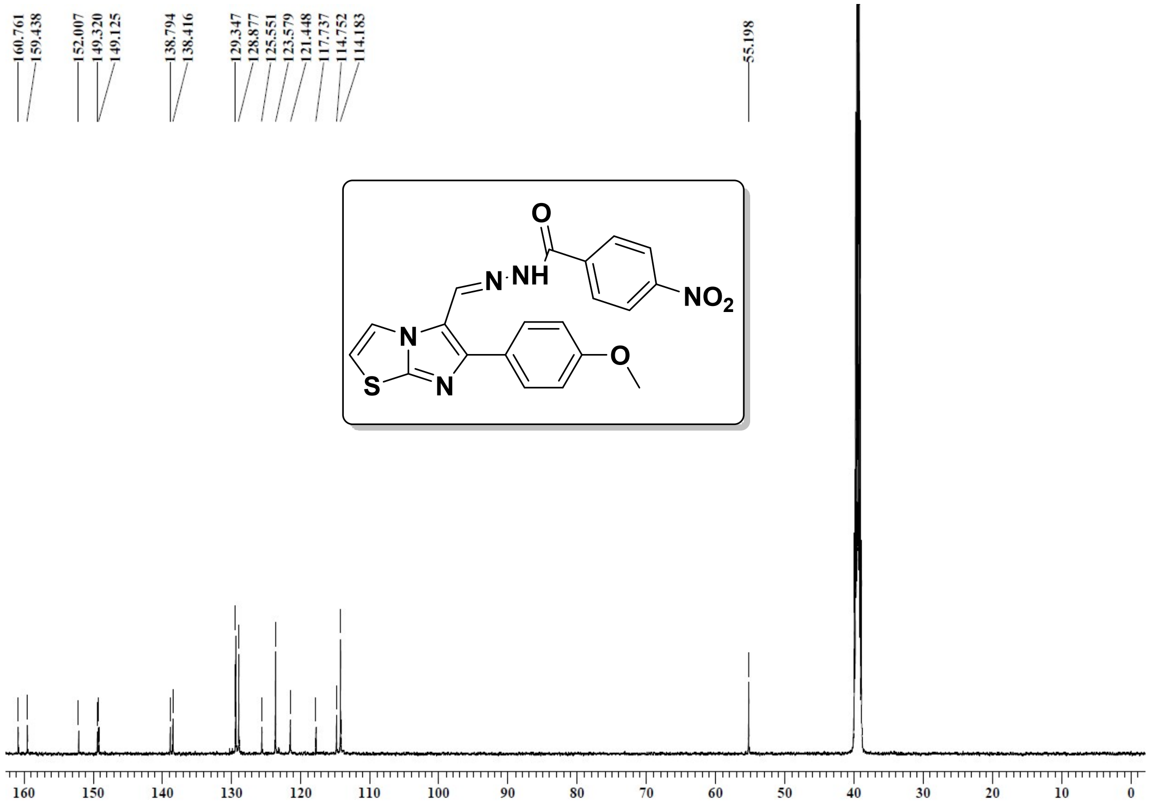
^{1}H NMR of compound 9k



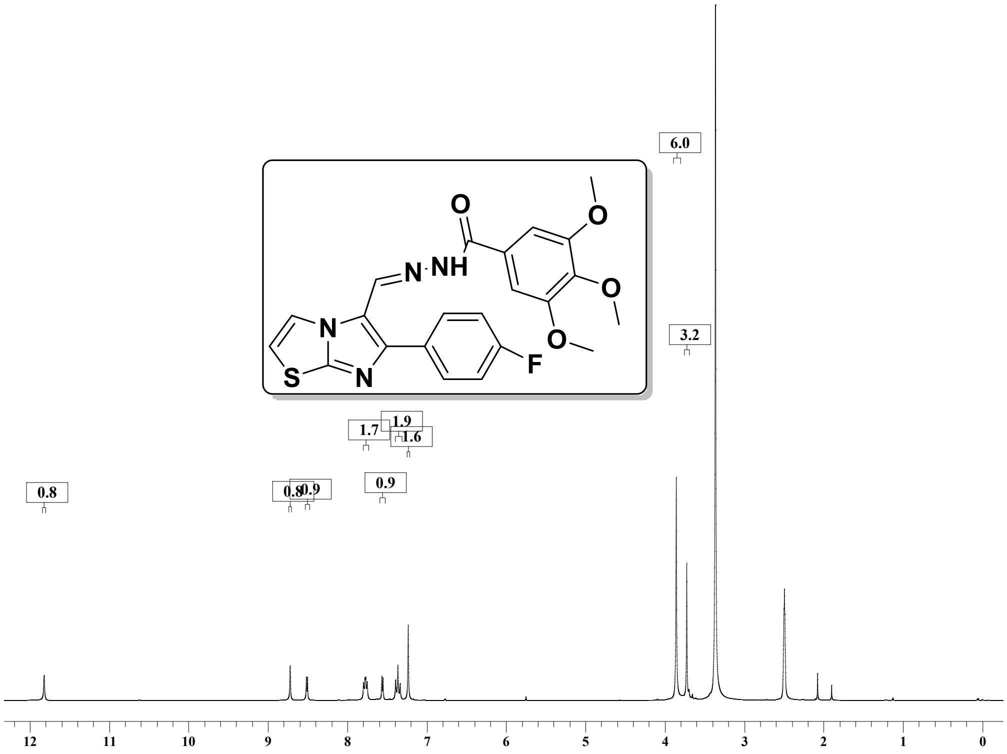
^{13}C NMR of compound 9k



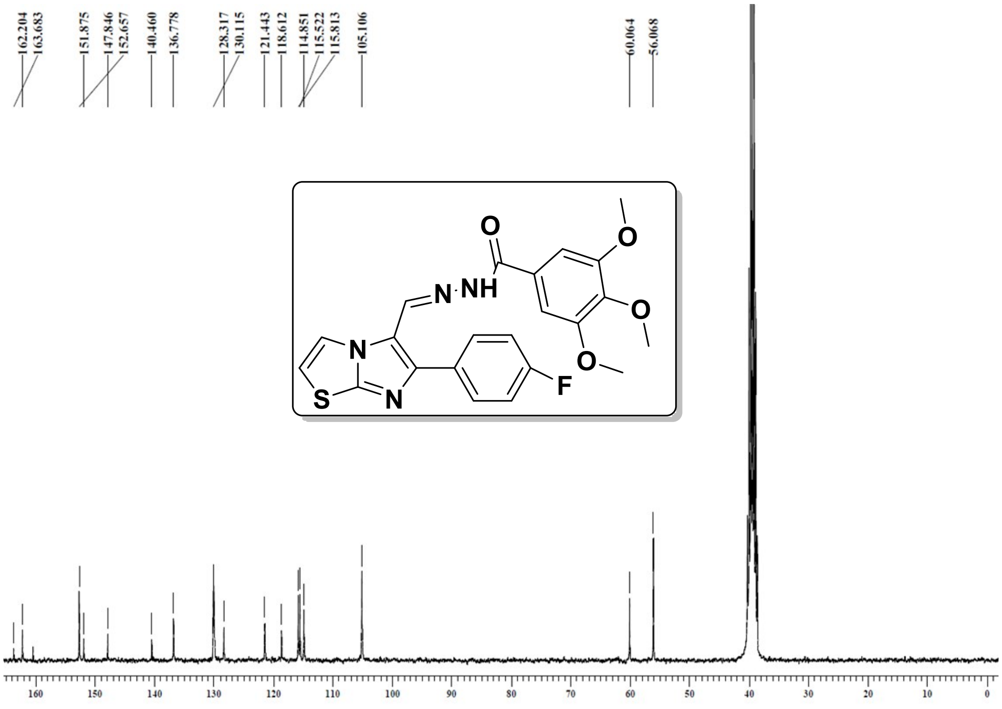
^{1}H NMR of compound 9l



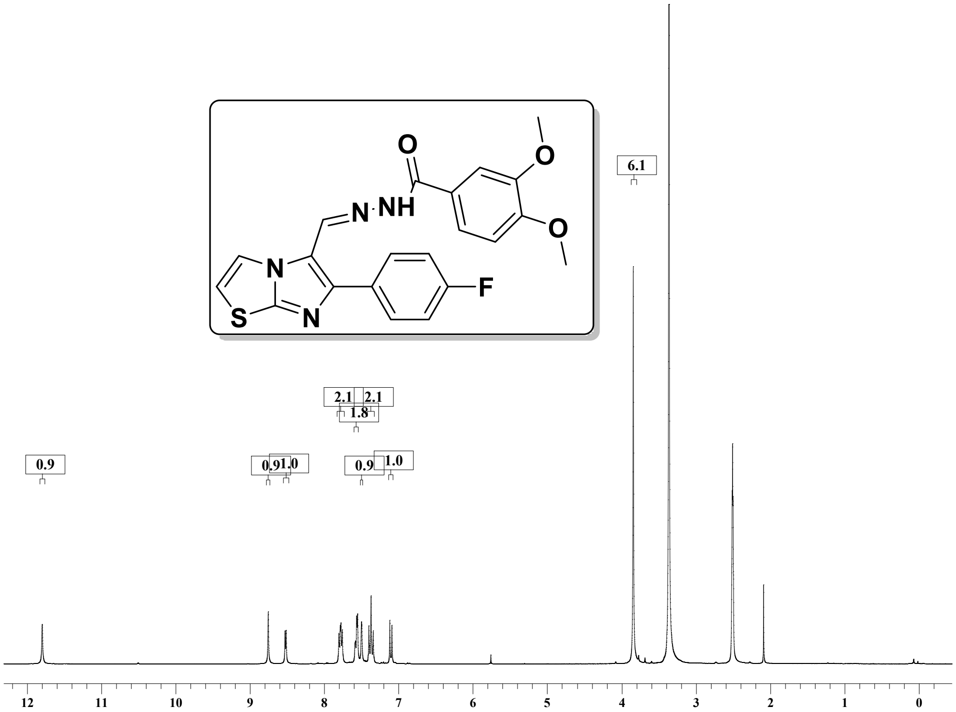
^{13}C NMR of compound 9l



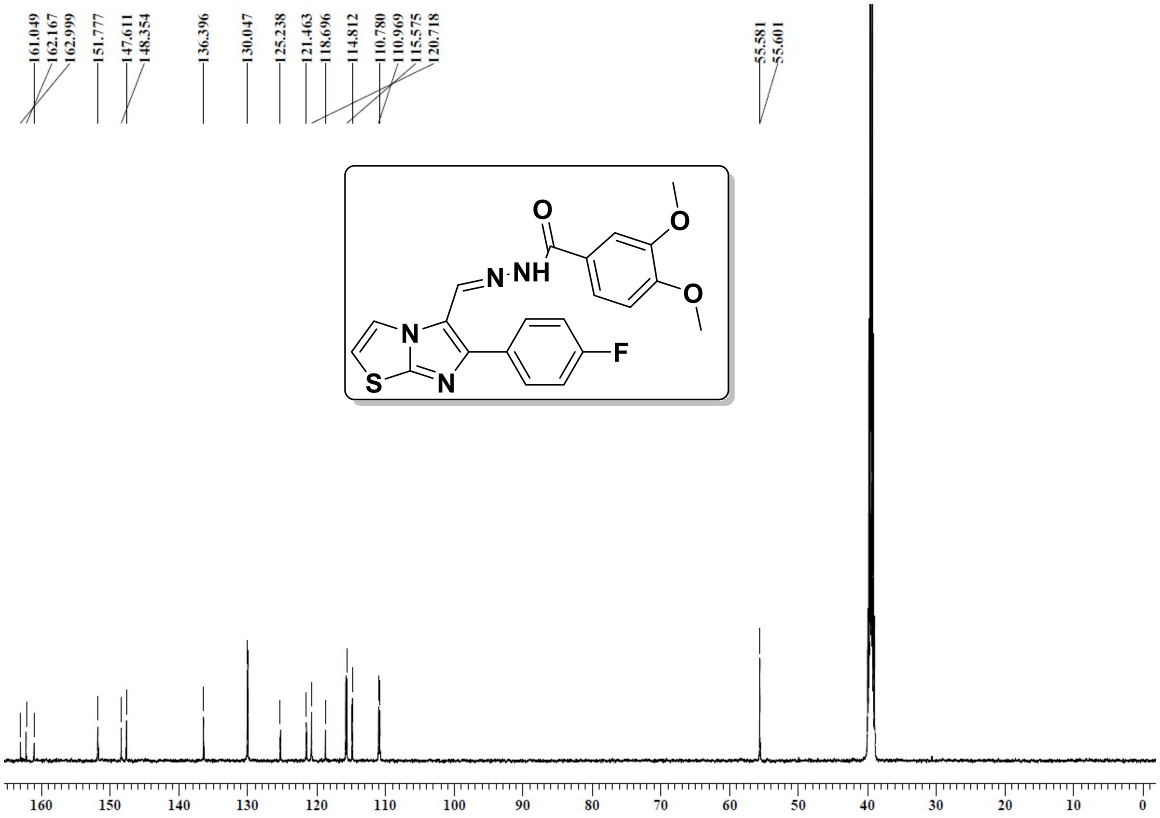
^{1}H NMR of compound 9m



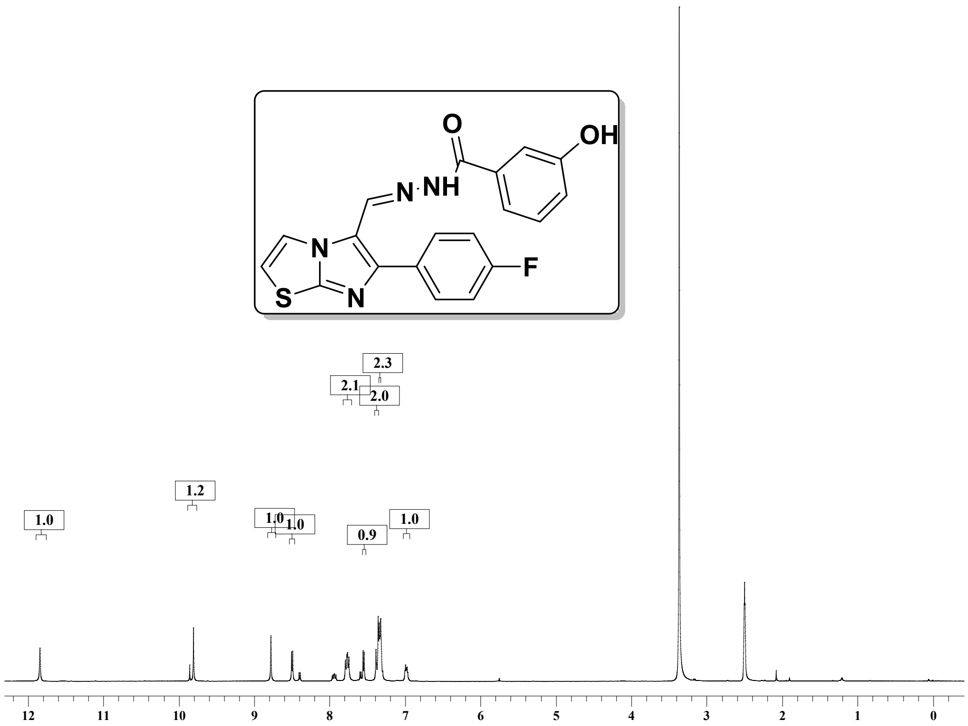
^{13}C NMR of compound 9m



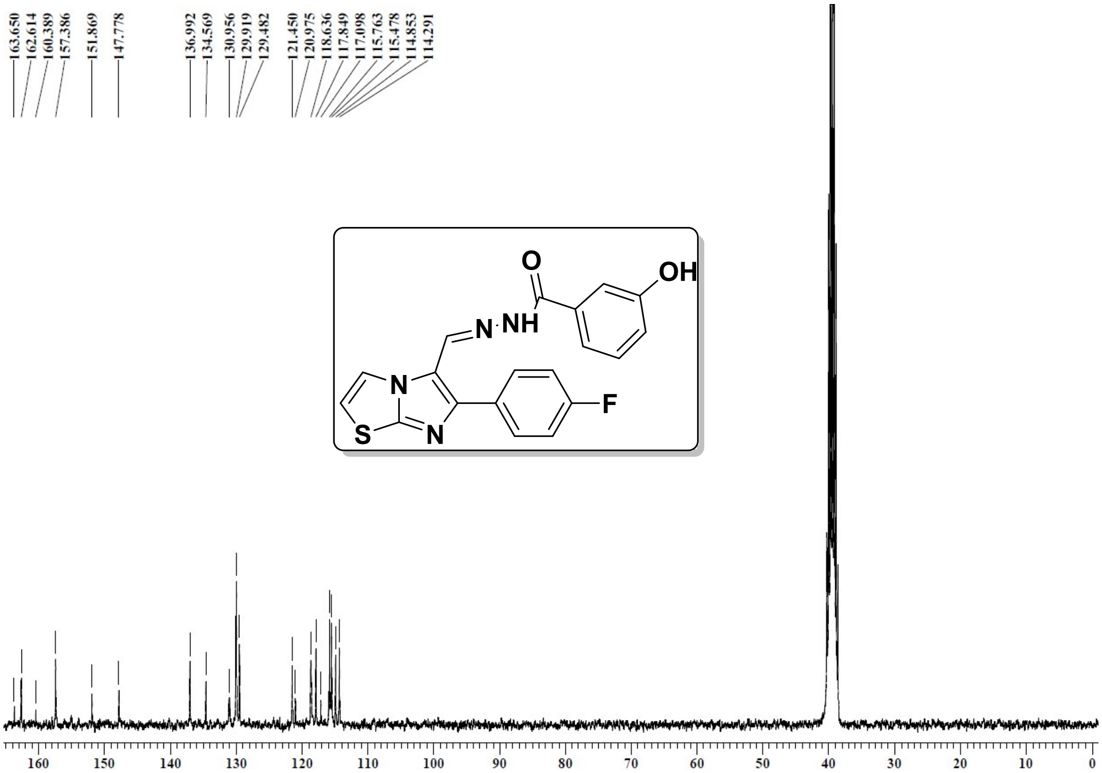
^{1}H NMR of compound 9n



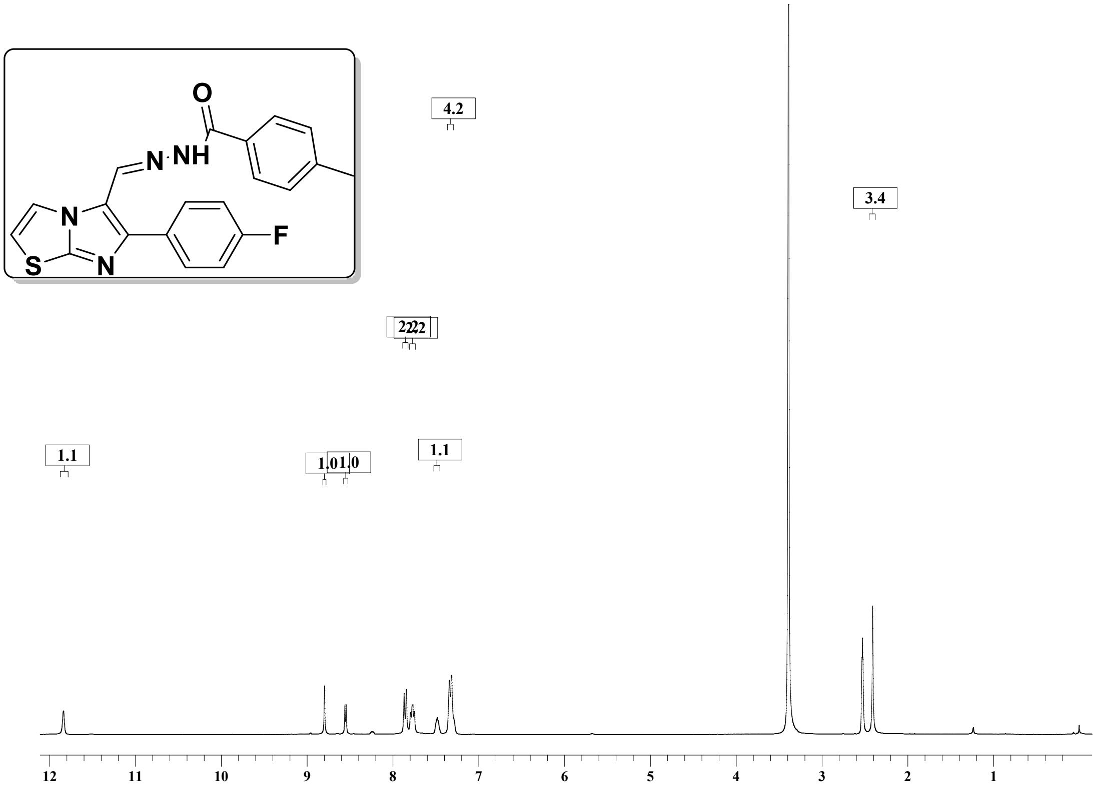
^{13}C NMR of compound 9n



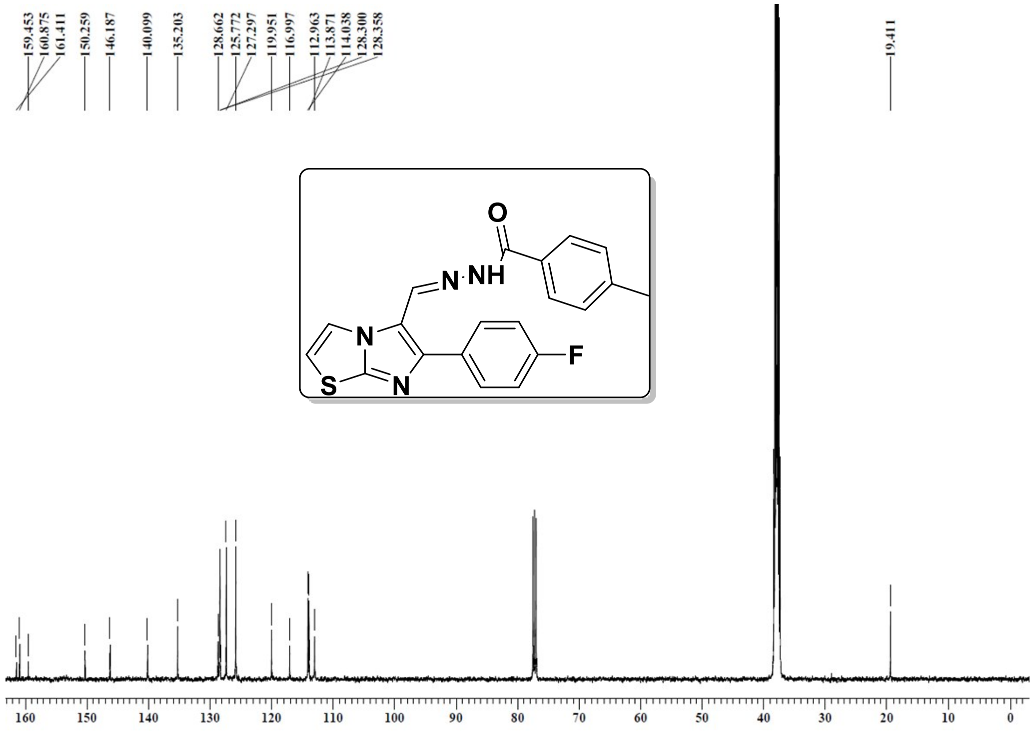
^{1}H NMR of Compound 9o



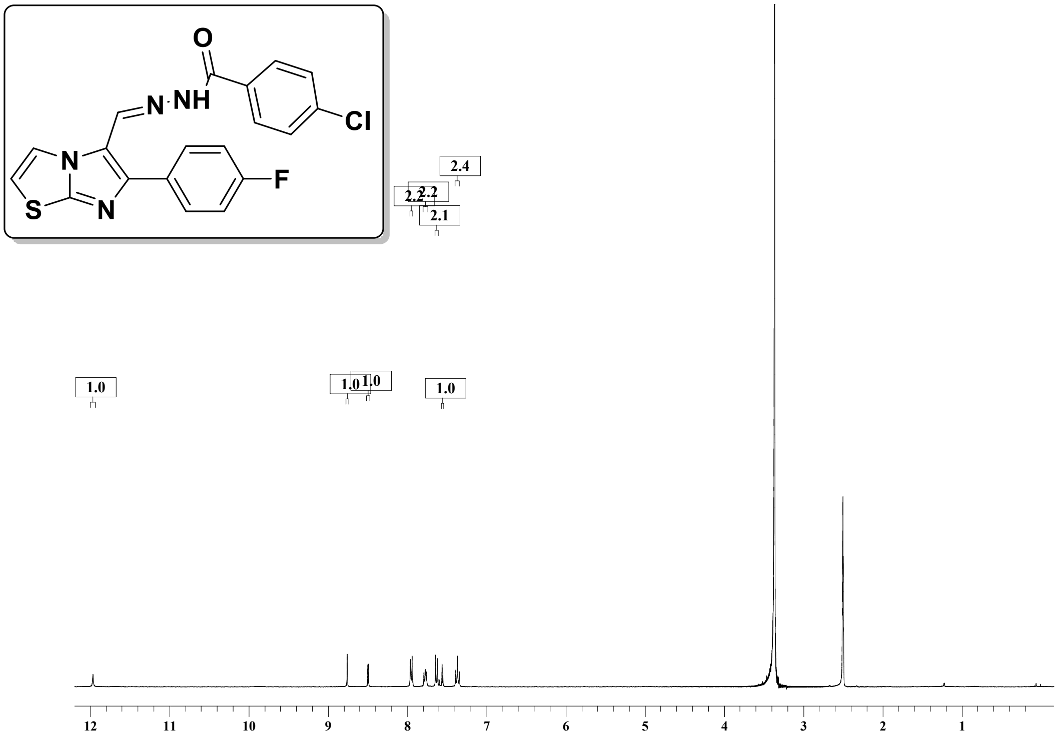
^{13}C NMR of Compound 9o



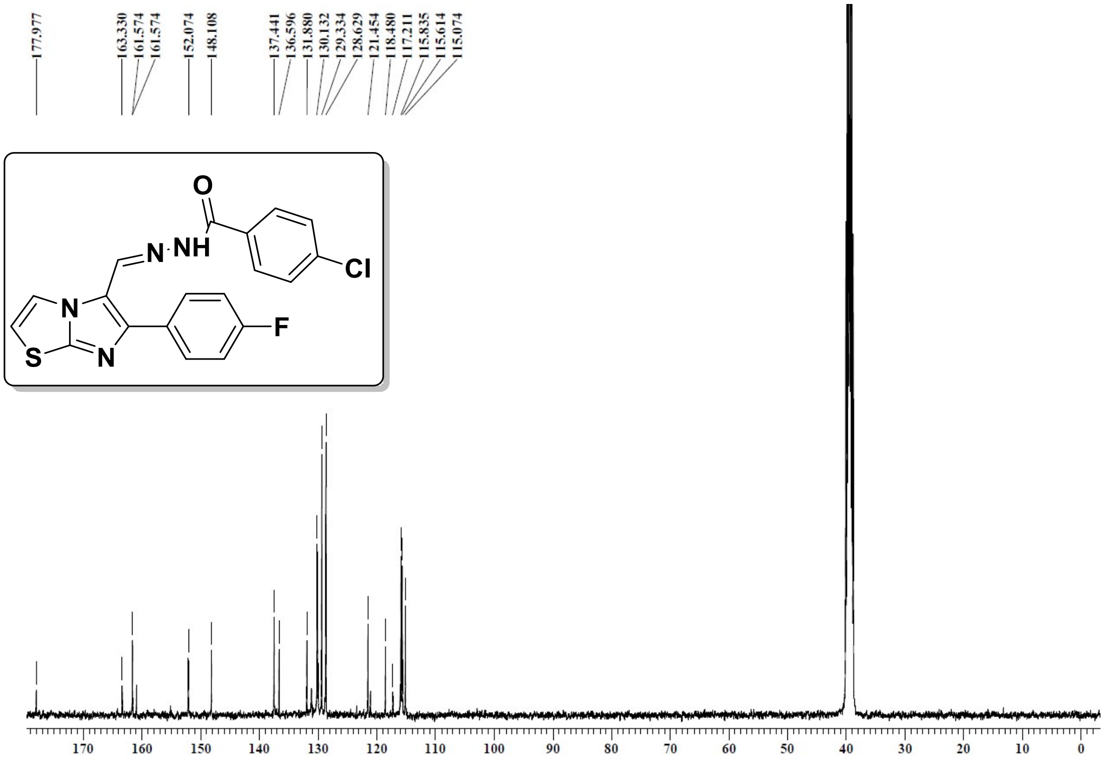
^{1}H NMR of compound 9p



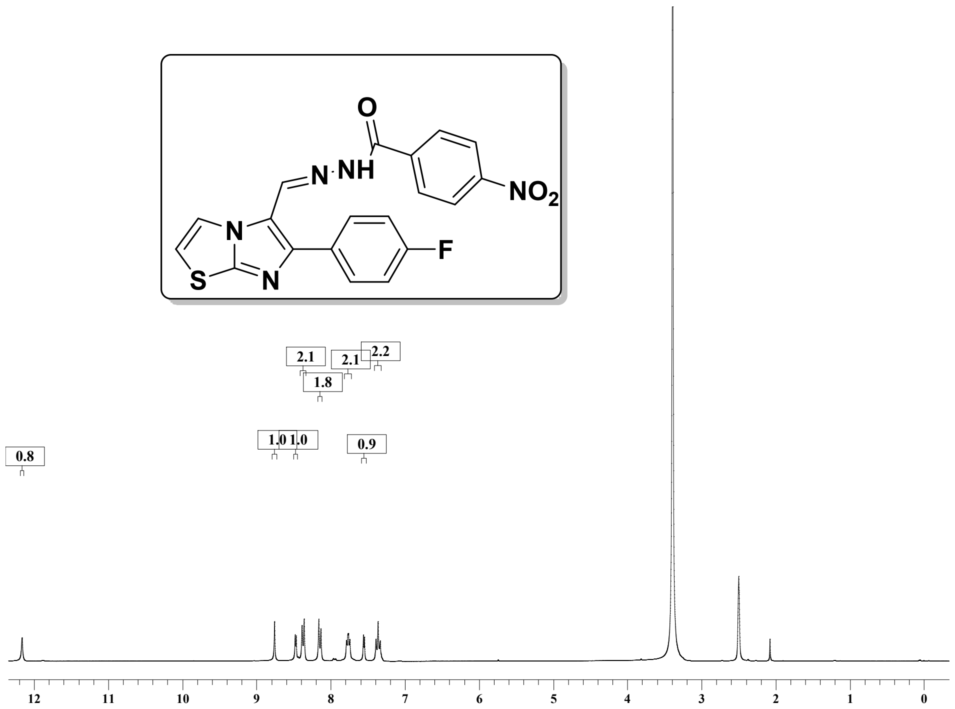
^{13}C NMR of compound 9p



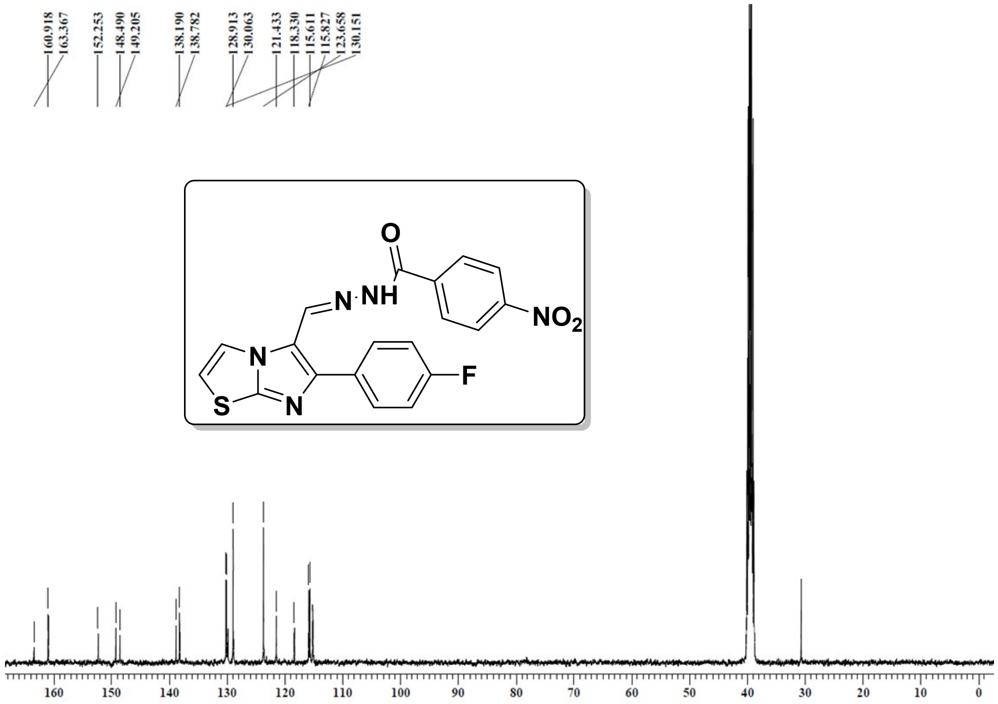
^{1}H NMR of compound 9q



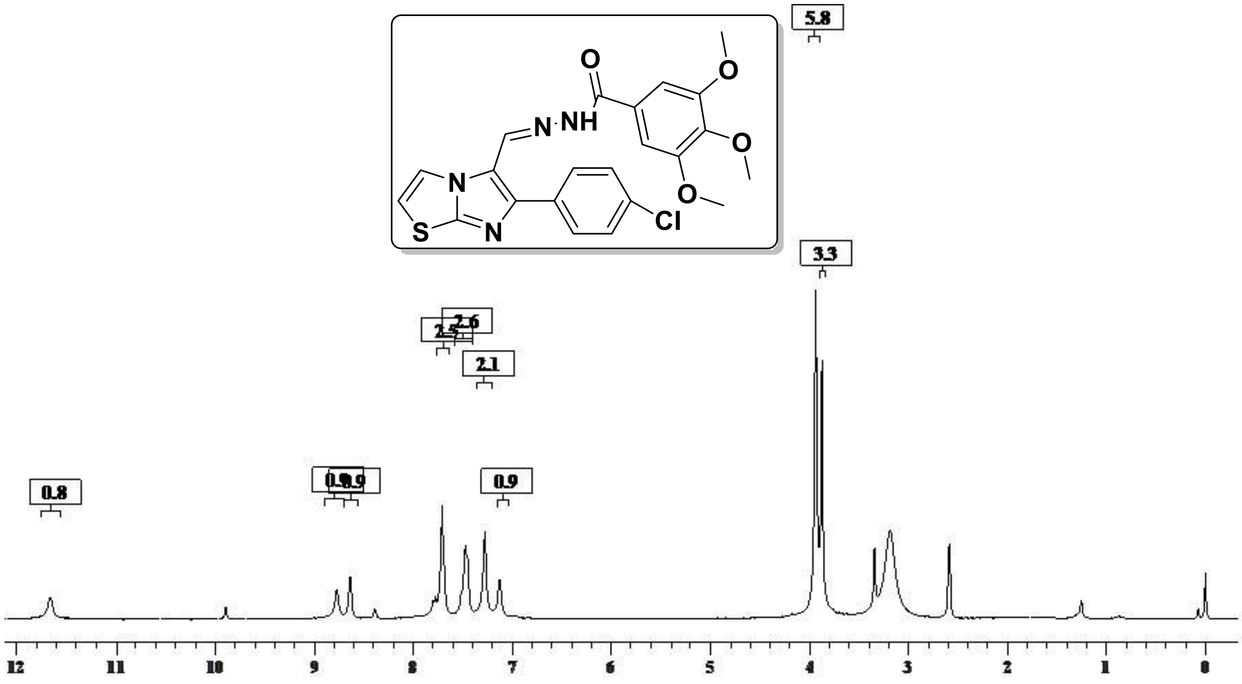
^{13}C NMR of compound 9q



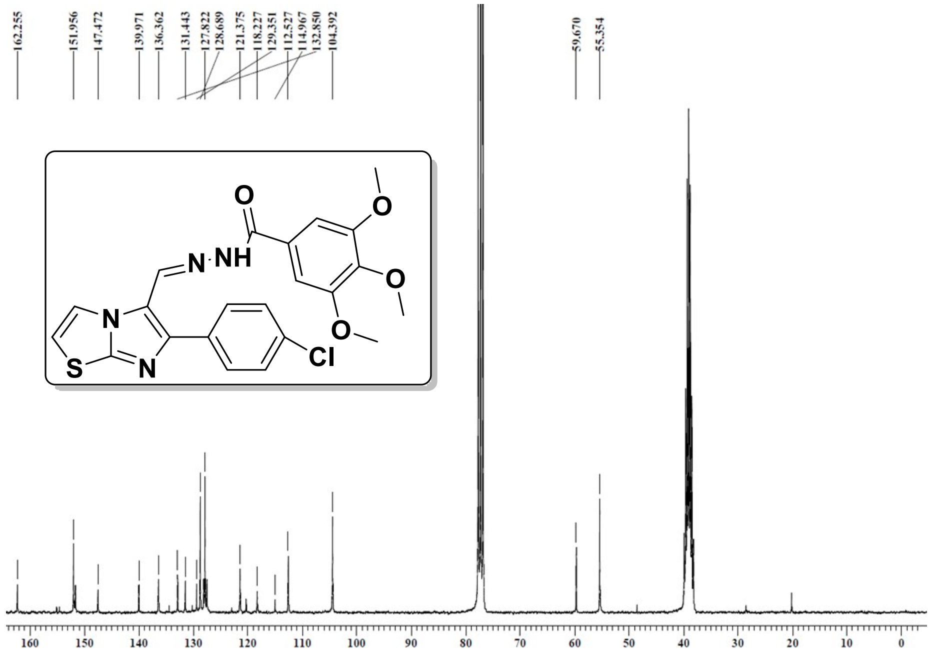
^{1}H NMR of compound 9r



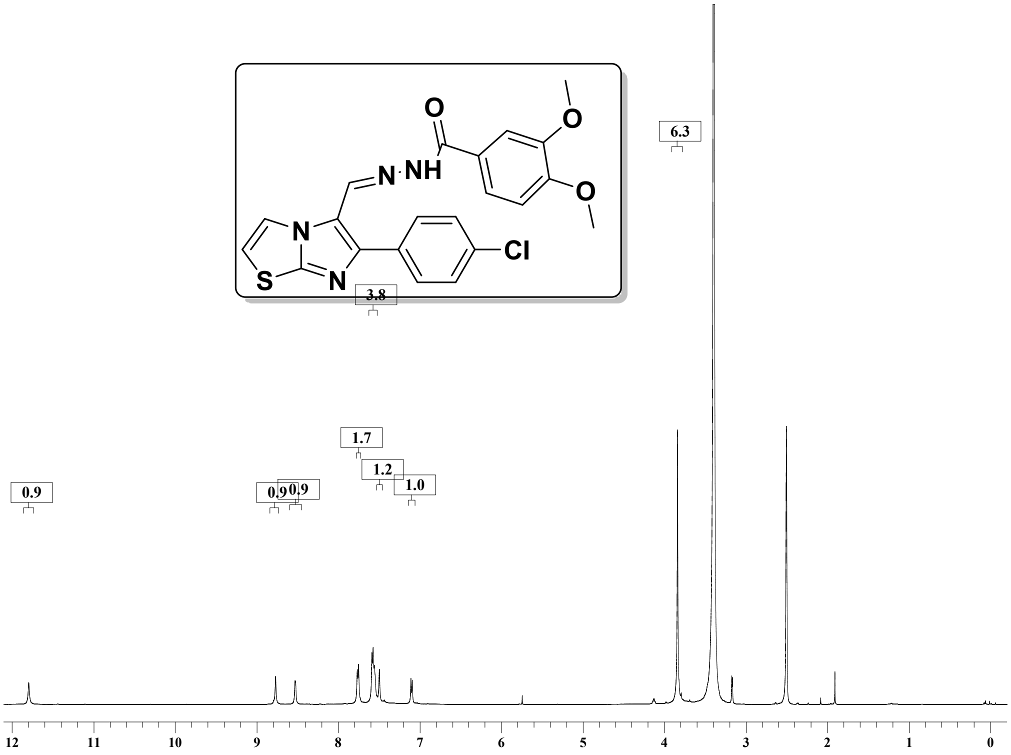
^{13}C NMR of compound 9r



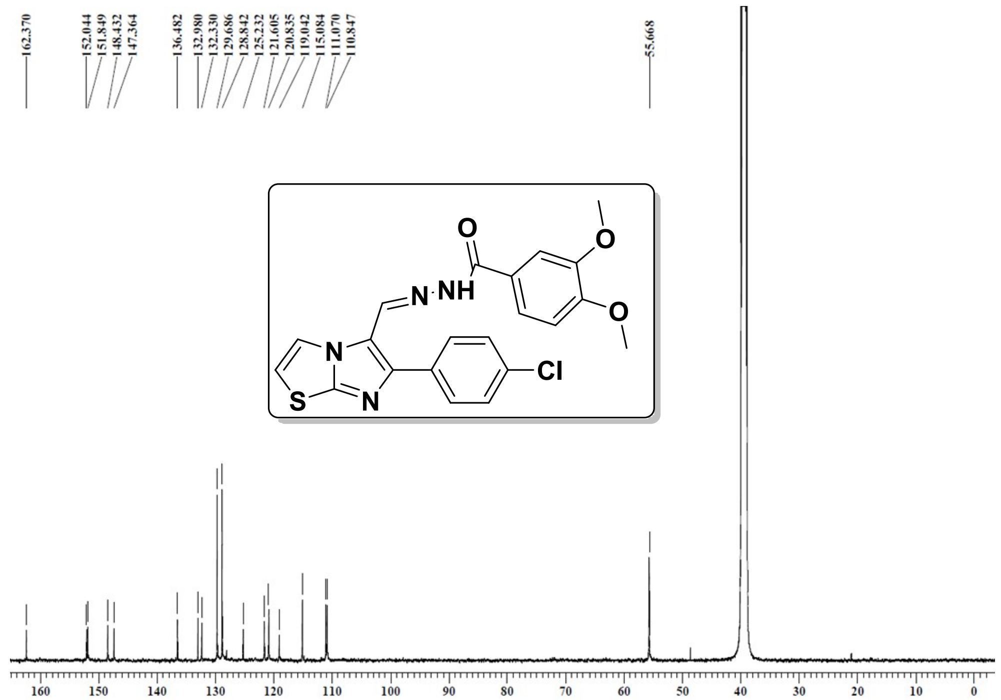
^{1}H NMR of compound 9s



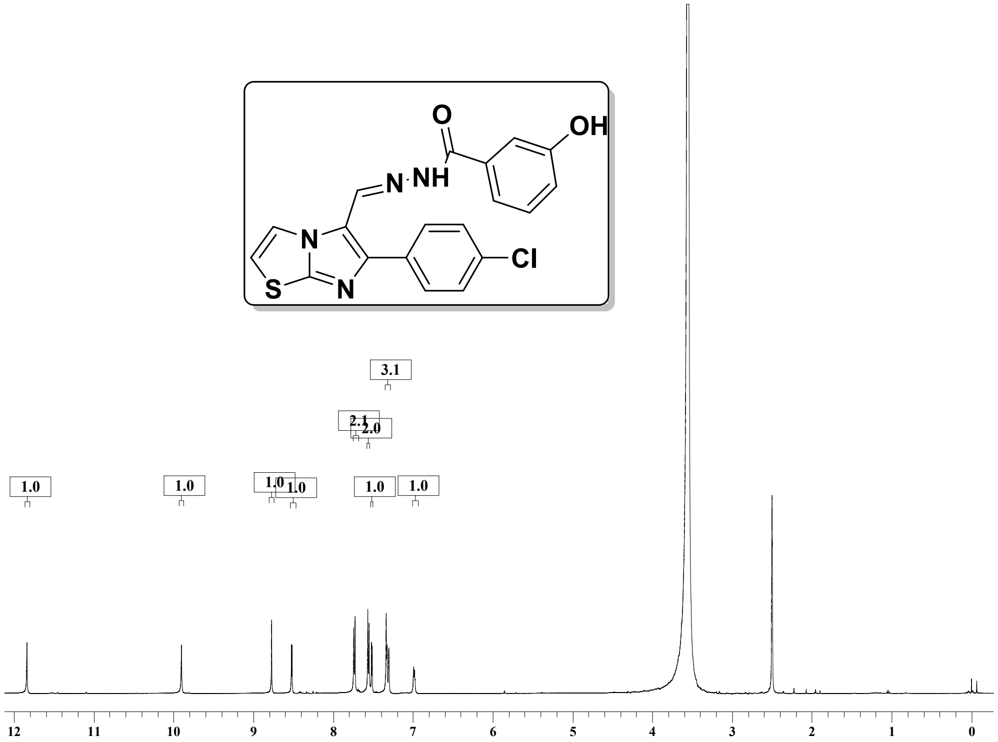
^{13}C NMR of compound 9s



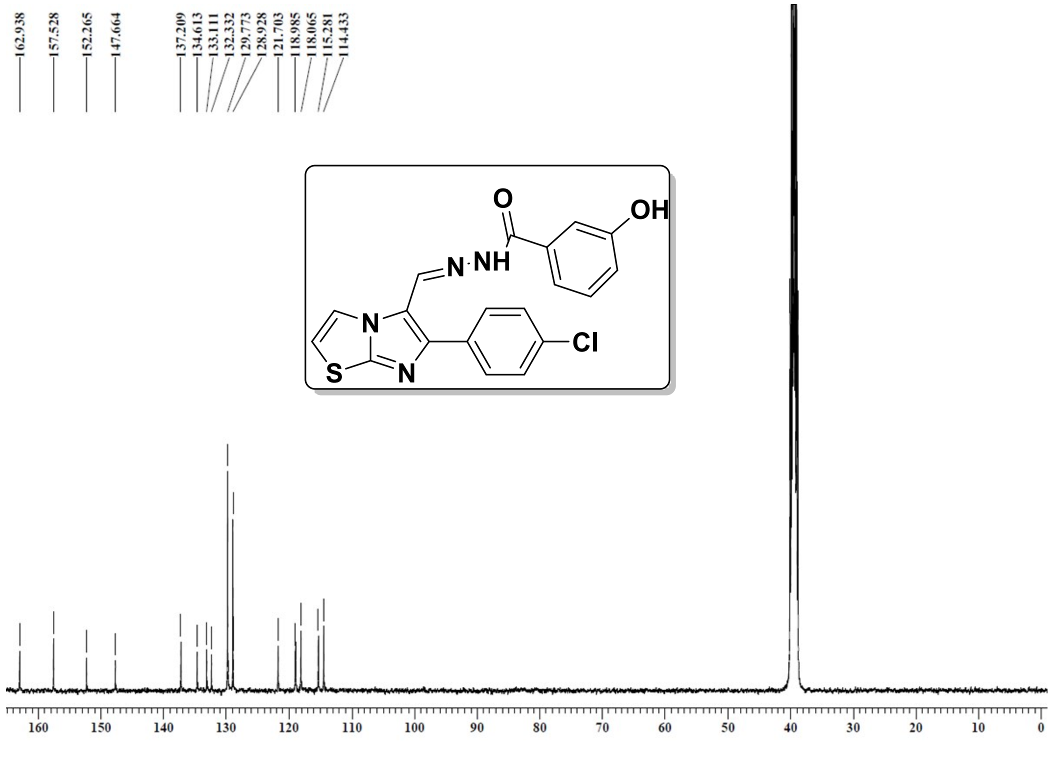
^{1}H NMR of compound 9t



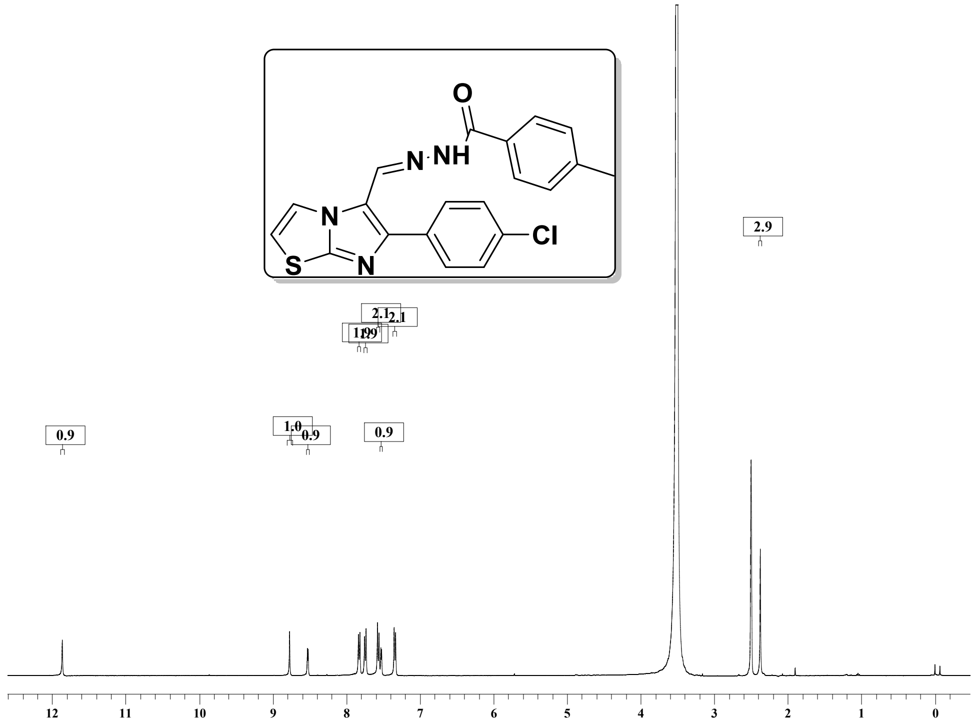
^{13}C NMR of compound 9t



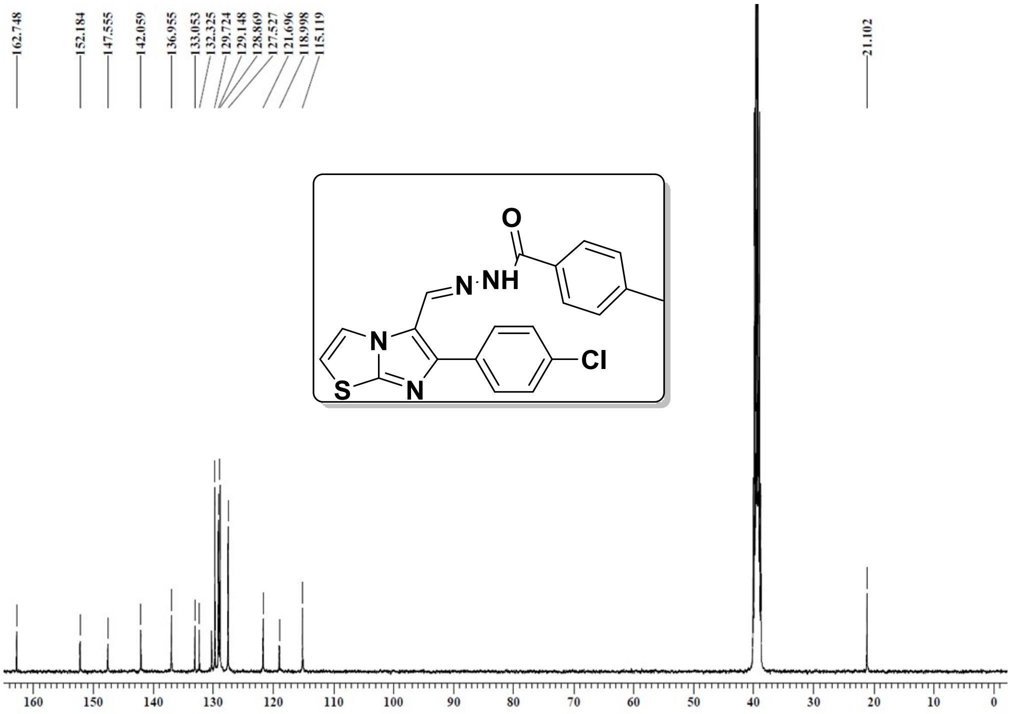
^{1}H NMR of compound 9u



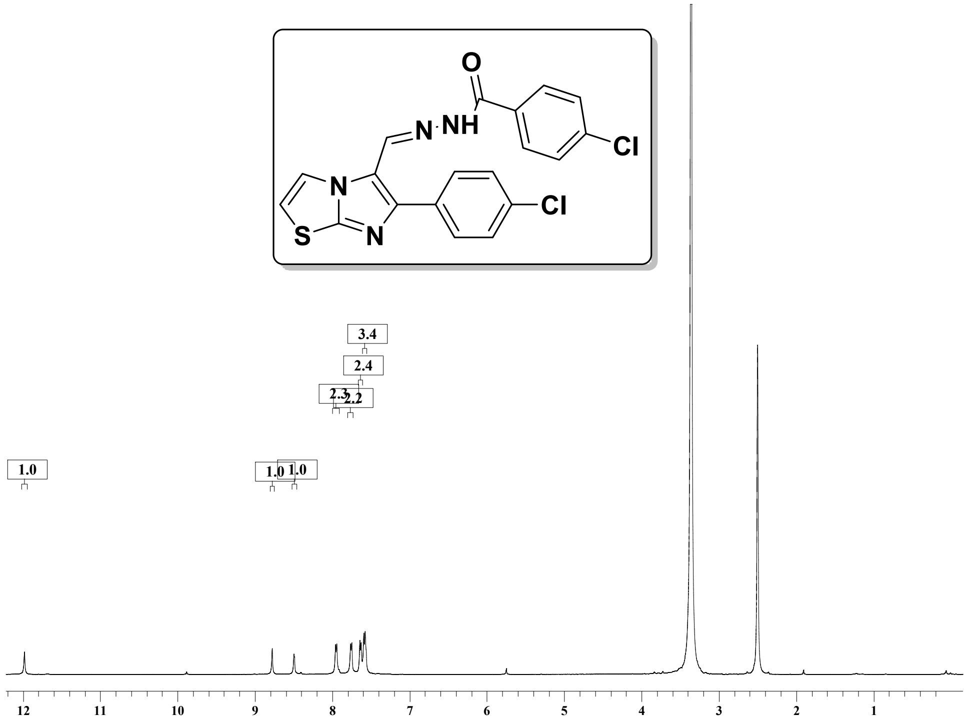
^{13}C NMR of compound 9u



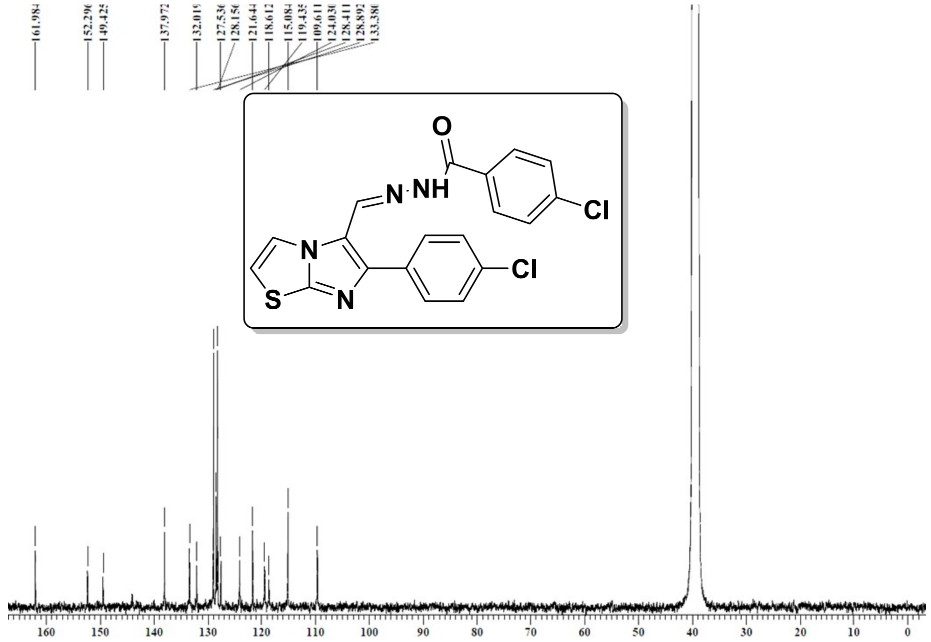
¹H NMR of compound 9v



¹³C NMR of compound 9v



^{1}H NMR of compound 9w



^{13}C NMR of compound 9w