New Journal of Chemistry

Supplementary Information

PCl_3-Mediated Synthesis of Green/Cyan Fluorescent Proteins Chromophore using Amino Acids

Thokchom Prasanta Singh, and Raja Shunmugam*

Polymer Research Centre, Department of Chemical Sciences, Indian Institute of Science Education and Research Kolkata Mohanpur-741246, India.

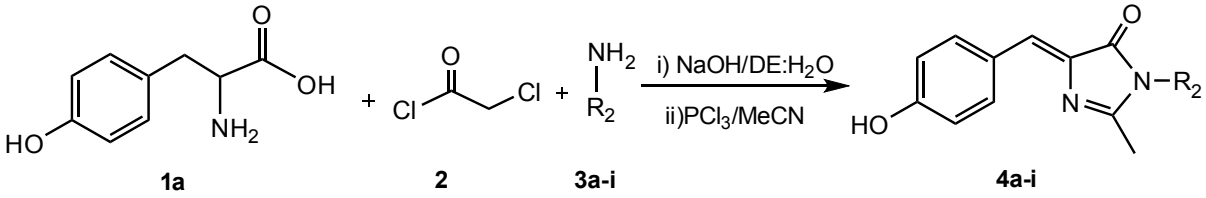
Email: sraja@iiserkol.ac.in

Table of Contents

General Information

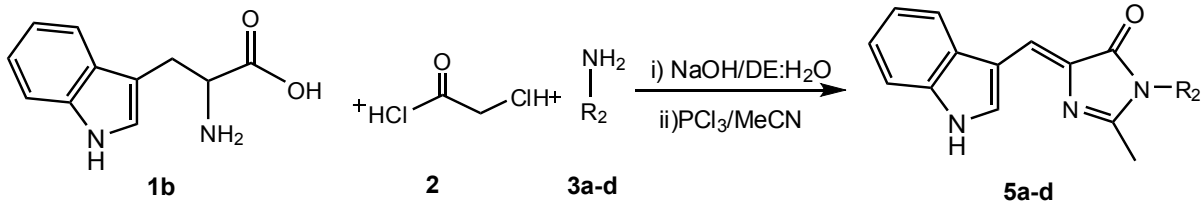
All reagents and solvents were obtained from commercial suppliers and used without further purification. All the ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on Bruker spectrometer using DMSO-d_6. Chemical shifts δ are in parts per million (ppm) and are relative to tetramethylsilane (TMS) as the internal reference. Data are reported as follows: chemical shift, multiplicity(s = singlet, d = doublet, dd= double doublet, t = triplet, m = multiplet) and coupling constants (*J*) in Hertz. The FT-IR spectra were recorded on a FT-IR Perkin-Elmer spectrometer (KBr). Melting points were determined using open capillary tube and are uncorrected. Column chromatography was performed on silica gel (60–120 mesh) using the reported eluents. Thin layer chromatography (TLC) was carried out on 5 x 20 cm plates with a layer thickness of 0.25 mm (Silica gel 60 F254). The crystal data was collected with SuperNova, X-ray diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) at 291 K.

Experiment 1. General procedure for the preparation of *Green Fluorescent Proteins Chromophore*



In a 25 mL round bottom flask, L-Tyrosine **1a** (2 mmol) was dissolved in diethyl ether/water (1:1 v/v) mixture (2 mL) in an ice bath (4 ^{o}C), and 2 mL of 4 M NaOH solution was added to mixture. Then, chloroacetyl chloride (2 mmol) dissolved in 1.0 mL of diethyl ether was added dropwise to the amino acid solution simultaneously with aqueous 4 M NaOH solution 2 mL over a 20-minute period under vigorous stirring. The aqueous layer was acidified to pH 1 with aqueous 4 M HCl and extracted with ethyl acetate. The combined organic layers were washed with saturated NaCl solution, dried with anhydrous Na_2SO_4, and concentrated under vacuum. To this solid product, anilines **3a-i** (2 mmol) in CH_3CN (2.5 mL) were taken. PCl_3 (1.0 mmol) was added and refluxed for 2-3 hour, where the reaction mixture turns yellow in colour as indicated by TLC. The reaction mixture was poured into ice-cold water and extracted with ethyl acetate (20 mL). The combined organic layer was washed with water for three times, dried over anhydrous Na_2SO_4 and organic layer was evaporated. The crude products were columned using hexane/ethyl acetate (80:20) and were recrystallized from EtOH to afford the pure products **4a-i** (65-85 % yield).

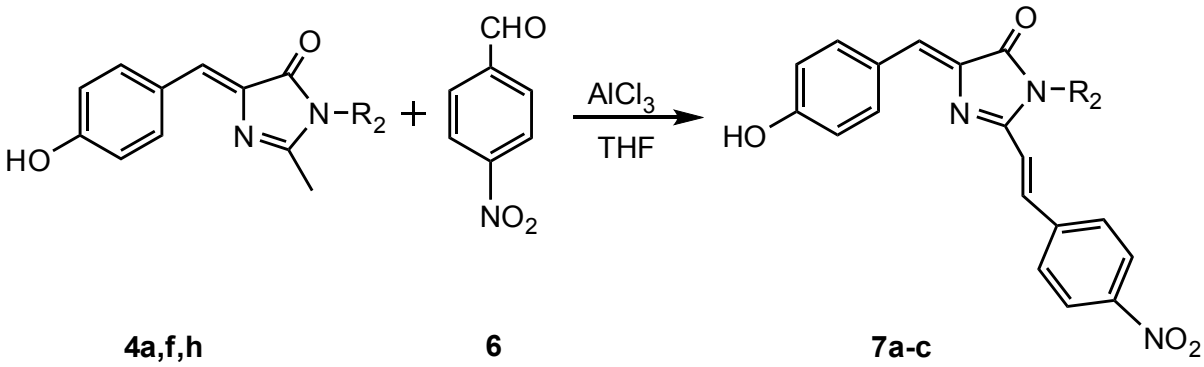
Experiment 2. General procedure for the preparation of *Cyan Fluorescent Proteins Chromophore*



1b **2** **3a-d** **5a-d**

Same as Experiment 1, L-Trytophan (**1b**) was used as aromatic amino acid. The crude products were columned using hexane/ethyl acetate (80:20) and were recrystallized from EtOH to afford the pure products **5a-d** (70-82 % yield).

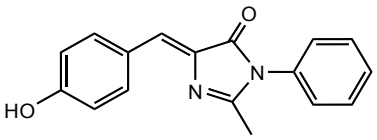
Experiment 3. General procedure for the preparation of synthetic analogues of *Red Fluorescent Proteins Chromophore*



4a,f,h **6** **7a-c**

The newly synthesized green FPs chromophore **4** (1 mmol) and *p*-nitrobenzaldehyde **6** (1 mmol) were taken in a 25 mL round bottom flask containing 2.5 mL of THF. Then, $AlCl_3$ (5 mol %) was added to mixture and allowed to reflux for 4 h. Then, the mixture was extracted with ethyl acetate. The combined organic layers were washed three times with saturated NaCl solution, dried with anhydrous Na_2SO_4, and concentrated under vacuum. The crude products were recrystallized from EtOH to afford the pure products **7** (85-91 % yield).

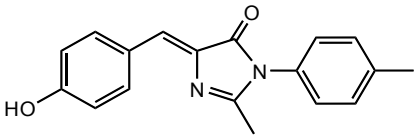
Characterization data



4a

(*Z*)-4-(4-hydroxybenzylidene)-2-methyl-1-phenyl-1*H*-imidazol-5(4*H*)-one (4a):

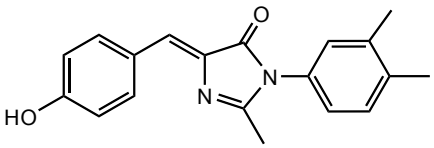
Yellow crystalline solid, M. P. 210-212 ^{o}C, (529 mg, 75 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.18(s, 1H); 8.14 (d, 2H, J = 8.7 Hz), 7.53 (t, 2H, J = 7.5 Hz), 7.46 (t, 1H, J = 7.4 Hz), 7.40 (t, 2H, J = 7.2 Hz), 7.01 (s, 1H), 6.87 (d, 2H, J = 8.7 Hz), 2.19(s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 169.1, 160.5, 159.8, 135.5, 134.2, 133.7, 129.3, 128.4, 127.6, 126.5, 125.3, 115.8, 16.1; IR (KBr) 1283, 1643, 1684, 2852, 2923, 3199 cm^{-1}; MS (ESI) m/z Calcd for $C_{17}H_{14}N_2O_2$: 278; Found: 279 $[M+H]^+$.



4b

(*Z*)-4-(4-hydroxybenzylidene)-2-methyl-1-p-tolyl-1*H*-imidazol-5(4*H*)-one(4b):

Yellow solid, M. P. 260-262 ^{o}C, (565 mg, 80 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.16(s, 1H); 8.13 (d, 2H, J = 8.7 Hz), 7.33 (d, 2H, J = 8.1 Hz), 7.26 (d, 2H, J = 8.3 Hz), 7.00 (s, 1H), 6.86 (d, 2H, J = 8.7 Hz), 2.37(s, 3H), 2.17(s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 169.1, 160.7, 159.8, 138.0, 135.6, 134.2, 131.0, 129.8, 127.4, 126.4, 125.3, 115.8, 20.7, 16.0; IR (KBr) 1272, 1638, 1694, 2920, 3054, 3292 cm^{-1}; MS (ESI) m/z Calcd for $C_{18}H_{16}N_2O_2$: 292; Found: 293 $[M+H]^+$.

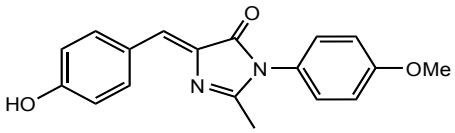


4c

(*Z*)-1-(3,4-dimethylphenyl)-4-(4-hydroxybenzylidene)-2-methyl-1*H*-imidazol-5(4*H*)-one(4c):

Yellow solid, M. P. 210-212 ^{o}C, (600 mg, 87 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.15(s, 1H); 8.13 (d, 2H, J = 8.2 Hz), 7.27 (d, 1H, J = 7.8 Hz), 7.14 (s, 1H), 7.09 (d, 1H, J = 7.8 Hz), 6.98 (s, 1H), 6.86 (d, 2H, J = 8.2 Hz), 2.27 (d, 6H, J = 3.9 Hz), 2.17(s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 169.2, 160.8, 159.8, 137.5, 136.8, 135.6, 134.2,

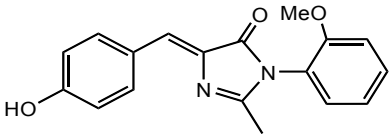
131.2, 130.2, 128.3, 126.3, 125.3, 124.8, 115.8, 19.3, 19.0, 16.0; IR (KBr) 1262, 1637, 1689, 2934, 3050, 3279 cm^{-1}; MS (ESI) m/z Calcd for $C_{19}H_{18}N_2O_2$: 306; Found: 307 $[M+H]^+$.



4d

(*Z*)-4-(4-hydroxybenzylidene)-1-(4-methoxyphenyl)-2-methyl-1*H*-imidazol-5(4*H*)-one (4d):

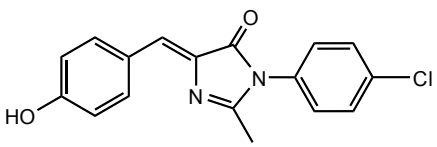
Yellow solid, M. P. 185-188 ^oC, (572 mg, 81 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.15(s, 1H); 8.13 (d, 2H, J = 8.7 Hz), 7.31 (d, 2H, J = 8.9 Hz), 7.06 (d, 2H, J = 8.9 Hz), 6.98 (s, 1H), 6.86 (d, 2H, J = 8.7 Hz), 3.86 (s, 3H, OCH_3), 2.16(s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 169.3, 161.0, 159.7, 159.1, 135.6, 134.2, 128.9, 126.2, 126.2, 125.3, 115.8, 114.5, 55.4, 16.0; IR (KBr) 1269, 1645, 1678, 2946, 3045, 3285 cm^{-1}; MS (ESI) m/z Calcd for $C_{18}H_{16}N_2O_3$: 308; Found: 309 $[M+H]^+$.



4e

(*Z*)-4-(4-hydroxybenzylidene)-1-(2-methoxyphenyl)-2-methyl-1*H*-imidazol-5(4*H*)-one (4e):

Yellow solid, M. P. 185-188 ^oC, (565 mg, 80 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.15(s, 1H); 8.12 (d, 2H, J = 8.7 Hz), 7.5-7.47 (m, 1H), 7.32 (dd, 1H, J = 1.7 Hz, J = 7.7 Hz), 7.22 (dd, 1H, J = 0.9 Hz, J = 8.4 Hz), 7.10-7.07 (m, 1H), 6.97 (s, 1H), 6.86 (d, 2H, J = 8.8 Hz), 3.79 (s, 3H, OCH_3), 2.06(s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 169.2, 161.2, 159.7, 154.8, 135.7, 134.2, 130.6, 129.9, 126.2, 125.2, 121.9, 120.8, 115.8, 112.5, 55.8, 15.4; IR (KBr) 1270, 1642, 1685, 2927, 3039, 3280 cm^{-1}; MS (ESI) m/z Calcd for $C_{18}H_{16}N_2O_3$: 308; Found: 309 $[M+H]^+$.

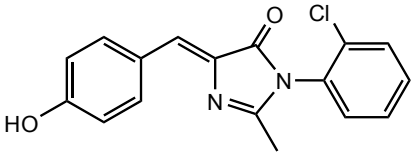


4f

(*Z*)-1-(4-chlorophenyl)-4-(4-hydroxybenzylidene)-2-methyl-1*H*-imidazol-5(4*H*)-one (4f):

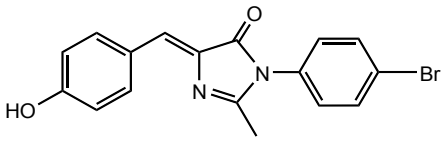
Yellow solid, M. P. 245-247 ^oC, (494 mg, 70 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.18(s, 1H); 8.14 (d, 2H, J = 8.6 Hz), 7.60 (d, 2H, J = 8.6 Hz), 7.45 (d, 2H, J = 8.6

Hz), 7.02 (s, 1H), 6.87 (d, 1H, *J* = 8.6 Hz), 2.20 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.9, 160.1, 159.9, 135.3, 134.3, 133.0, 132.6, 129.4, 129.4, 126.7, 125.2, 115.8, 16.0; IR (KBr) 1283, 1643, 1684, 2852, 2923, 3199 cm^{-1}; MS (ESI) m/z Calcd for $C_{17}H_{13}ClN_2O_2$: 312; Found: 313 $[M+H]^+$.



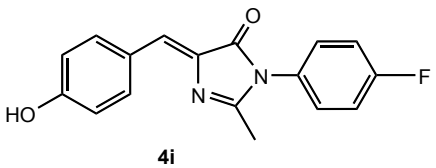
4g

(*Z*)-1-(2-chlorophenyl)-4-(4-hydroxybenzylidene)-2-methyl-1*H*-imidazol-5(4*H*)-one (4g): Yellow solid, M. P. 210-212 °C, (487 mg, 69 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.20(s, 1H); 8.14 (d, 2H, *J* = 8.7 Hz), 7.72 (dd, 1H, *J* = 1.6 Hz, *J* = 7.7 Hz), 7.60 (dd, 1H, *J* = 2.2 Hz, *J* = 7.3 Hz), 7.57-7.54 (m, 2H), 7.04 (s, 1H), 6.87 (d, 2H, *J* = 8.7 Hz), 2.10 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.6, 160.0, 159.7, 135.1, 134.4, 131.9, 131.2, 131.1, 130.1, 128.5, 127.1, 125.1, 115.9, 114.9, 15.5; IR (KBr) 1275, 1639, 1678, 2865, 2950, 3205 cm^{-1}; MS (ESI) m/z Calcd for $C_{17}H_{13}ClN_2O_2$: 312; Found: 313 $[M+H]^+$.



4h

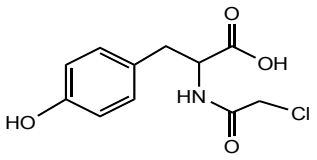
(*Z*)-1-(4-bromophenyl)-4-(4-hydroxybenzylidene)-2-methyl-1*H*-imidazol-5(4*H*)-one (4h): Yellow solid, M. P. 195-197 °C, (459 mg, 65 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.18(s, 1H); 8.14 (d, 2H, *J* = 8.8 Hz), 7.73 (d, 2H, *J* = 8.7 Hz), 7.39 (d, 2H, *J* = 8.7 Hz), 7.04 (s, 1H), 6.87 (d, 2H, *J* = 8.8 Hz), 2.20 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.8, 160.0, 159.9, 135.3, 134.3, 133.0, 132.3, 129.7, 126.7, 125.2, 121.5, 115.8, 16.0; IR (KBr) 1280, 1648, 1670, 2854, 2935, 3210 cm^{-1}; MS (ESI) m/z Calcd for $C_{17}H_{13}BrN_2O_2$: 356; Found: 357 $[M+H]^+$.



4i

(*Z*)-1-(4-fluorophenyl)-4-(4-hydroxybenzylidene)-2-methyl-1*H*-imidazol-5(4*H*)-one (4i): Yellow solid, M. P. 215-217 °C, (473 mg, 83 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.17(s, 1H); 8.14 (d, 2H, *J* = 8.6 Hz), 7.49-7.46 (m, 2H), 7.37 (t, 2H, *J* = 8.7 Hz), 7.01 (s, 1H), 6.87 (d, 2H, *J* = 8.6 Hz), 2.18 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 169.1, 162.6, 160.4, 159.8, 135.4, 134.2, 129.9, 129.8, 126.6, 125.2, 116.3, 116.1, 16.0; IR (KBr)

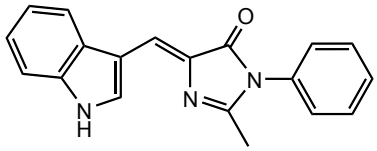
1283, 1639, 1720, 2854, 2924, 3375 cm^{-1}; MS (ESI) m/z Calcd for $C_{17}H_{13}FN_2O_2$: 296; Found: 297 $[M+H]^+$.



1a'

2-(2-chloroacetamido)-3-(4-hydroxyphenyl)propanoic acid(1a'):

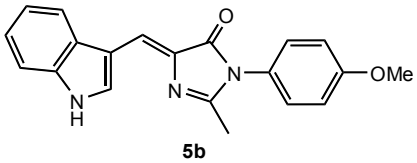
White solid, M. P. 98-101 ^oC, 80 % yield. ^1H-NMR (500 MHz, DMSO-d_6 δ ppm): 12.86(bs, 1H), 9.20(s, 1H), 8.40 (d, 1H, J = 7.9 Hz), 6.99 (d, 1H, J = 8.5 Hz), 6.65 (d, 1H, J = 8.5 Hz), 4.37-4.35 (m, 1H), 2.95 (dd, 1H, J = 5.0 Hz, J = 13.9 Hz), 2.80 (dd, 1H, J = 8.8 Hz, J = 13.9 Hz); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 172.5, 165.8, 156.0, 130.1, 127.2, 115.0, 54.0, 42.3, 35.8; IR (KBr) 1228, 1659, 1709, 2910, 3032, 3290 cm^{-1}; MS (ESI) m/z Calcd for $C_{11}H_{12}ClNO_4$: 257; Found: 258 $[M+H]^+$.



5a

(*Z*)-4-((1*H*-indol-3-yl)methylene)-2-methyl-1-phenyl-1*H*-imidazol-5(4*H*)-one (5a):

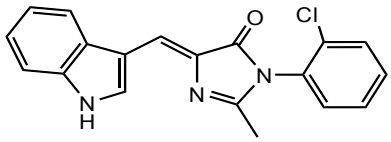
Yellow solid, M. P. 240-242 ^oC, (602 mg, 80 % yield). ^1H-NMR (500 MHz, DMSO-d_6 δ ppm): 12.00(s, 1H); 8.47 (d, 1H, J = 2.8 Hz), 8.26 (d, 1H, J = 7.7 Hz), 7.56-7.53 (m, 2H), 7.50-7.47 (m, 2H), 7.43-7.40(m, 3H), 7.24-7.16 (m, 2H), 2.22 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.4, 157.4, 136.4, 134.0, 133.1, 129.3, 128.2, 127.6, 127.5, 126.7, 122.6, 120.8, 120.6, 119.7, 112.2, 111.2, 16.0; IR (KBr) 1234, 1625, 1678, 2929, 3056, 3207 cm^{-1}; MS (ESI) m/z Calcd for $C_{19}H_{15}N_3O$: 301; Found: 302 $[M+H]^+$.



5b

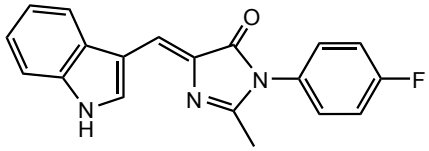
(*Z*)-4-((1*H*-indol-3-yl)methylene)-1-(4-methoxyphenyl)-2-methyl-1*H*-imidazol-5(4*H*)-one (5b): Yellow solid, M. P. 190-192 ^oC, (617 mg, 82 % yield). ^1H-NMR (500 MHz, DMSO-d_6 δ ppm): 11.98(s, 1H); 8.45 (d, 1H, J = 2.8 Hz), 8.24 (d, 1H, J = 7.7 Hz), 7.49 (d, 1H, J = 7.9 Hz), 7.40 (s, 1H), 7.32 (d, 2H, J = 8.9 Hz), 7.23-7.16 (m, 2H), 7.07 (d, 2H, J = 8.9 Hz), 3.62(s, 3H, OCH_3) 2.19 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.7, 159.0, 158.0, 136.4, 133.2, 133.0, 128.8, 126.7, 126.6, 122.5, 120.8, 120.3, 119.7, 114.5, 112.2, 111.2,

55.4, 16.0; IR (KBr) 1231, 1627, 1678, 2934, 3046, 3221 cm^{-1}; MS (ESI) m/z Calcd for $C_{20}H_{17}N_3O_2$: 331; Found: 332 $[M+H]^+$.



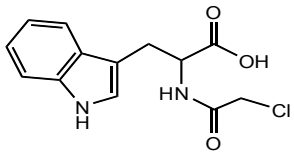
5c

(*Z*)-4-((1*H*-indol-3-yl)methylene)-1-(2-chlorophenyl)-2-methyl-1*H*-imidazol-5(4*H*)-one (5c): Yellow solid, M. P. 110-112 °C, (541 mg, 72 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 12.04(s, 1H); 8.47 (d, 1H, *J* = 2.8 Hz), 8.27 (d, 1H, *J* = 7.7 Hz), 7.74-7.72 (m, 1H), 7.61-7.54 (m, 3H), 7.50 (d, 1H, *J* = 7.9 Hz), 7.45 (s, 1H), 7.24-7.17 (m, 2H), 2.12 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.0, 156.7, 136.5, 133.4, 132.6, 132.0, 131.7, 131.1, 131.0, 130.1, 128.5, 126.7, 122.6, 121.2, 120.9, 119.8, 112.2, 111.1, 15.5; IR (KBr) 1277, 1691, 1720, 2924, 3052, 3318 cm^{-1}; MS (ESI) m/z Calcd for $C_{19}H_{14}ClN_3O$: 335; Found: 336 $[M+H]^+$.



5d

(*Z*)-4-((1*H*-indol-3-yl)methylene)-1-(4-fluorophenyl)-2-methyl-1*H*-imidazol-5(4*H*)-one (5d): Yellow solid, M. P. 120-122 °C, (526 mg, 70 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 12.00(s, 1H); 8.46 (d, 1H, *J* = 2.4 Hz), 8.26 (d, 1H, *J* = 7.5 Hz), 7.48 (dd, 3H, *J* = 5.6 Hz, *J* = 8.7 Hz), 7.43 (s, 1H), 7.38 (t, 2H, *J* = 8.7 Hz), 7.24-7.16 (m, 2H), 2.21 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.7, 160.8, 157.7, 136.7, 133.4, 130.1, 130.0, 127.0, 122.9, 121.1, 121.0, 120.0, 116.5, 116.4, 112.5, 111.5, 16.3; IR (KBr) 1275, 1695, 1709, 2941, 3048, 3298 cm^{-1}; MS (ESI) m/z Calcd for $C_{19}H_{14}FN_3O$: 319; Found: 320 $[M+H]^+$.

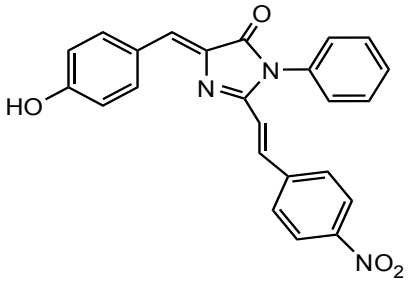


1b'

2-(2-chloroacetamido)-3-(1*H*-indol-3-yl)propanoic acid (1b'):

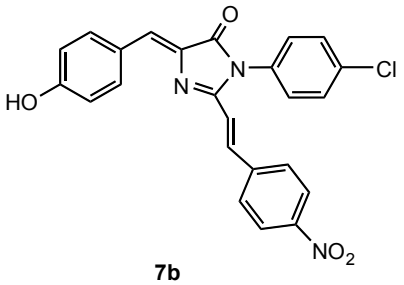
Light Brown solid, M. P. 135-138 °C, 80 % yield. ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.87(s, 1H); 8.44 (d, 1H, *J* = 7.8 Hz), 8.44 (d, 1H, *J* = 7.8 Hz), 7.33 (d, 1H, *J* = 8.1 Hz), 7.13

(d, 1H, *J* = 2.3 Hz), 7.08-7.05 (m, 1H), 7.00-6.97 (m, 1H), 4.52-4.48 (m, 1H), 4.08(s, 2H), 3.22-3.06(m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 172.8, 165.8, 136.1, 127.2, 123.6, 121.0, 118.4, 118.1, 111.4, 109.4, 53.3, 42.4, 26.9,; IR (KBr) 1254, 1637, 1708, 2910, 3032, 3319, 3424 cm^{-1}; MS (ESI) m/z Calcd for $C_{13}H_{13}ClN_2O_3$: 280; Found: 281$[M+H]^+$.



7a

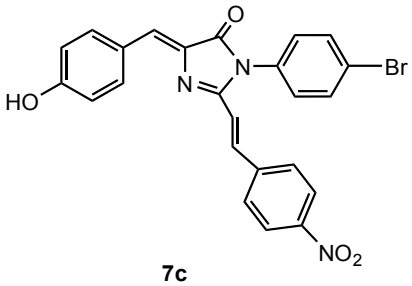
(*Z*)-4-(4-hydroxybenzylidene)-2-(4-nitrostyryl)-1-phenyl-1*H*-imidazol-5(4*H*)-one (7a): Red crystalline solid, M. P. 250-252 ^{o}C, (386 mg, 90 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.34(s, 1H); 8.28 (d, 2H, *J* = 8.6 Hz), 8.22 (d, 2H, *J* = 8.8 Hz), 8.01 (d, 1H, *J* = 16.0 Hz), 7.89 (d, 2H, *J* = 8.8 Hz), 7.59 (t, 2H, *J* = 7.5 Hz), 7.53 (d, 1H, *J* = 7.4 Hz), 7.43 (d, 2H, *J* = 7.3 Hz), 7.17(s, 1H), 6.92 (d, 2H, *J* = 8.7 Hz), 6.84 (d, 1H, *J* = 16.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.9, 160.4, 156.4, 147.6, 141.3, 136.9, 135.9, 135.0, 133.2, 129.6, 129.0, 128.7, 128.4, 127.7, 125.6, 124.1, 118.3, 116.1; IR (KBr) 1159, 1338, 1590, 1626, 1693, 2849, 2917, 3292 cm^{-1}; MS (ESI) m/z Calcd for $C_{24}H_{17}N_3O_4$: 411; Found: 412$[M+H]^+$.



7b

(*Z*)-1-(4-chlorophenyl)-4-(4-hydroxybenzylidene)-2-(4-nitrostyryl)-1*H*-imidazol-5(4*H*)-one (7b): Red crystalline solid, M. P. 285-287 ^{o}C, (421 mg, 91 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.35(s, 1H); 8.28 (d, 2H, *J* = 8.7 Hz), 8.23 (d, 2H, *J* = 8.9 Hz), 8.05 (d, 1H, *J* = 15.9 Hz), 7.96 (d, 2H, *J* = 8.8 Hz), 7.65 (d, 2H, *J* = 8.7 Hz), 7.48 (d, 2H, *J* = 8.7 Hz), 7.17 (s, 1H), 6.93-6.87 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.774, 160.461, 156.162, 147.625, 141.281, 137.049, 135.715, 135.045, 133.158, 132.080, 129.598, 129.558, 129.158, 128.540, 125.605, 124.009, 118.198, 116.085; IR (KBr) 1148, 1338, 1594,

1626, 1696, 2849, 2918, 3305 cm^{-1}; MS (ESI) m/z Calcd for $C_{24}H_{16}ClN_3O_4$: 445; Found: 446$[M+H]^+$.



7c

(*Z*)-1-(4-bromophenyl)-4-(4-hydroxybenzylidene)-2-(4-nitrostyryl)-1*H*-imidazol-5(4*H*)-one (7b): Red crystalline solid, M. P. 294-296 ^oC, (431 mg, 85 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6 δ ppm): 10.35(s, 1H), 8.28 (d, 2H, *J* = 8.7 Hz), 8.22 (d, 2H, *J* = 8.8 Hz), 8.05 (d, 1H, *J* = 15.9 Hz), 7.96 (d, 2H, *J* = 8.8 Hz), 7.78 (d, 2H, *J* = 8.6 Hz), 7.41 (d, 2H, *J* = 8.6 Hz), 7.17 (s, 1H), 6.90 (t, 3H, *J* = 12.8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6 δ ppm) 168.714, 160.459, 156.098, 147.621, 141.278, 137.056, 135.706, 135.049, 132.534, 132.492, 129.821, 129.164, 128.559, 125.603, 124.005, 121.678, 118.190, 116.083; IR (KBr) 1335, 1600, 1620, 1694, 2850, 2925, 3295 cm^{-1}; MS (ESI) m/z Calcd for $C_{24}H_{16}BrN_3O_4$: 489; Found: 490$[M+H]^+$.

Determination of Relative Configuration

The product **4a** was recrystallized from methanol. The crystal structure was solved using SuperNova, X-ray diffractometer with Mo−Kα radiation (λ = 0.71073 Å) and Using Olex2 [1], the structure was solved with the ShelXS structure solution program using Direct Methods and refined with the ShelXL [2] refinement package using Least Squares minimisation.

The crystallographic data were summarized in the following table.

System	**4a**
Formula	$C_{17}\,H_{14}\,N_2\,O_2$
*M*r	278.30
Crystal system	monoclinic
Space group	*P 1 21/c 1*
a (Å)	11.2845(8)
b (Å)	11.5944(8)
c (Å)	12.0744(9)
α (°)	90
β (°)	114.970(9)
γ (°)	90
V (Å^3)	1432.11(18)
Crystal size	0.3 × 0.2 × 0.1
T (K)	290.80(10)
Z	4
F(000)	584.0
μ (mm^{-1})	0.086
Ref. collected	4598
Parameters	180
Final R indices [I>2σ(I)]	0.0598
R indices (all data)	0.1637
Goodness of fit on F^2	1.050

The crystal structures have been deposited at the Cambridge Crystallographic Data Centre **4a** **(**CCDC 1415491**)**. The data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/data_request/cif. ORTEP view of the product **4a** with thermal ellipsoids drawn at the 50% probability level.

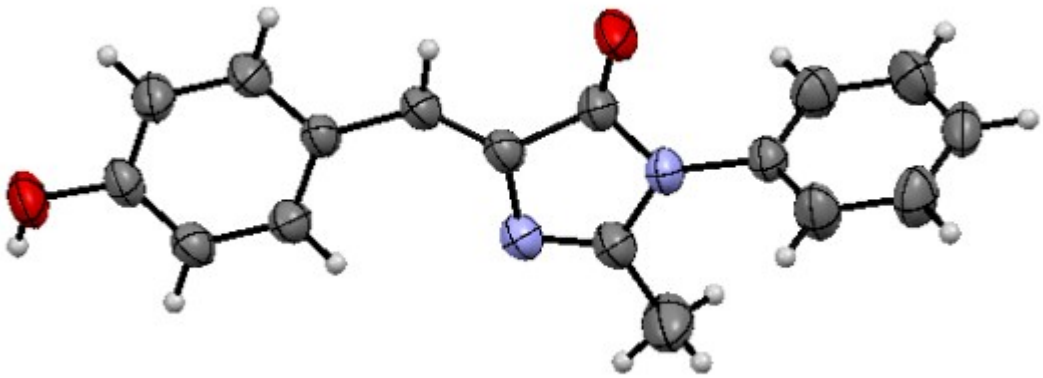


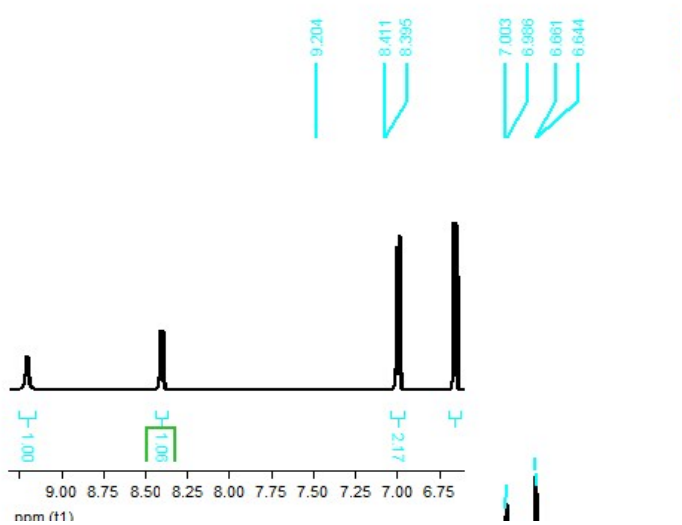
Figure 1. Thermal ellipsoid plots for **4a** at 50 % probability level.

References

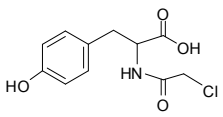
1. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.

2. Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.

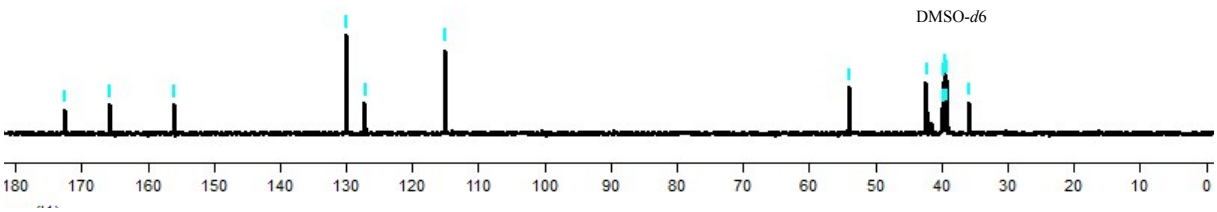
^{1}H NMR of 1a'



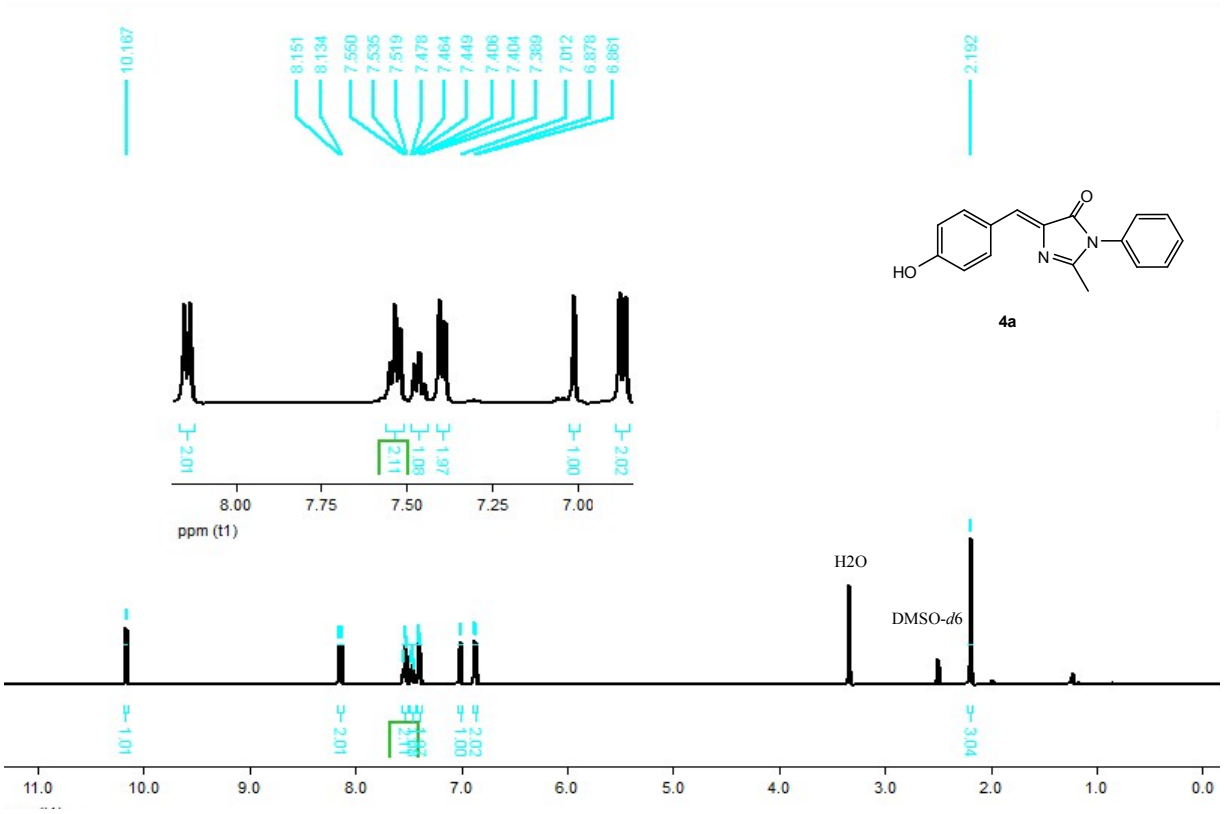
^{13}C NMR of 1a'



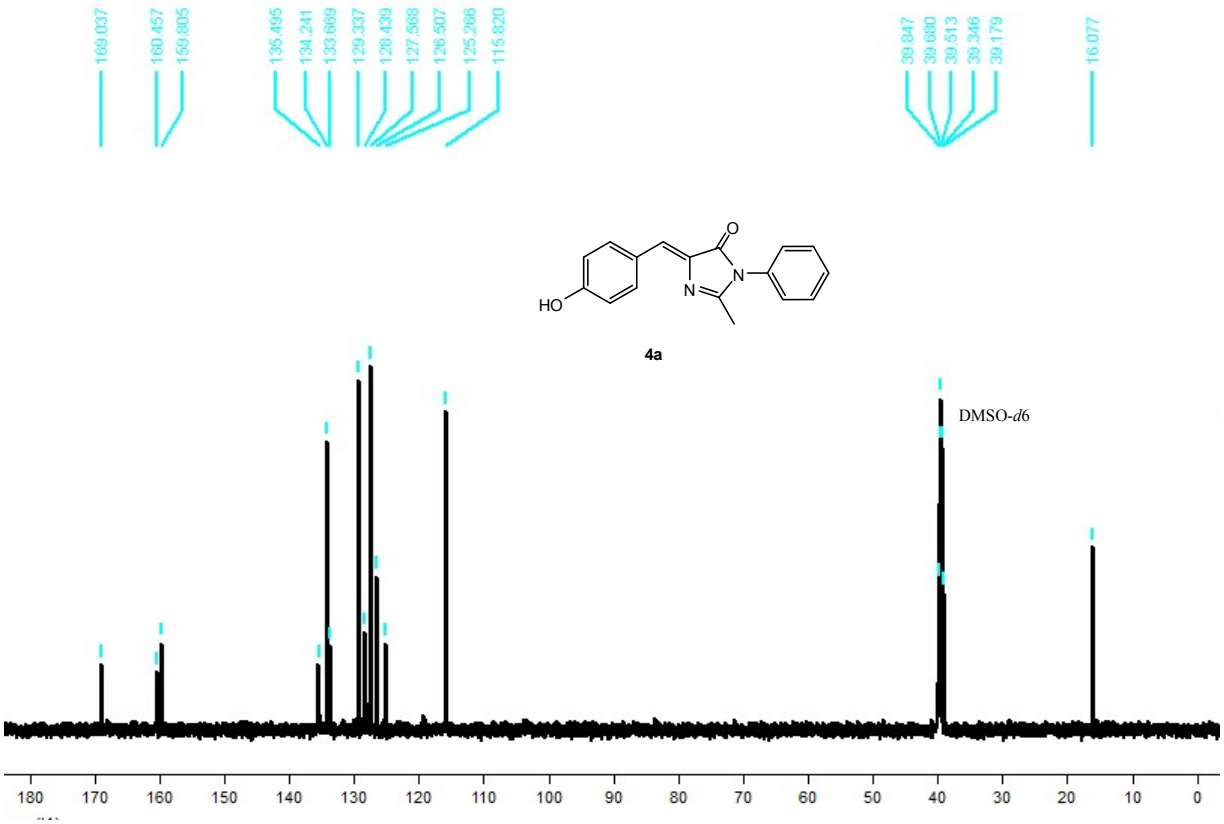
1a'



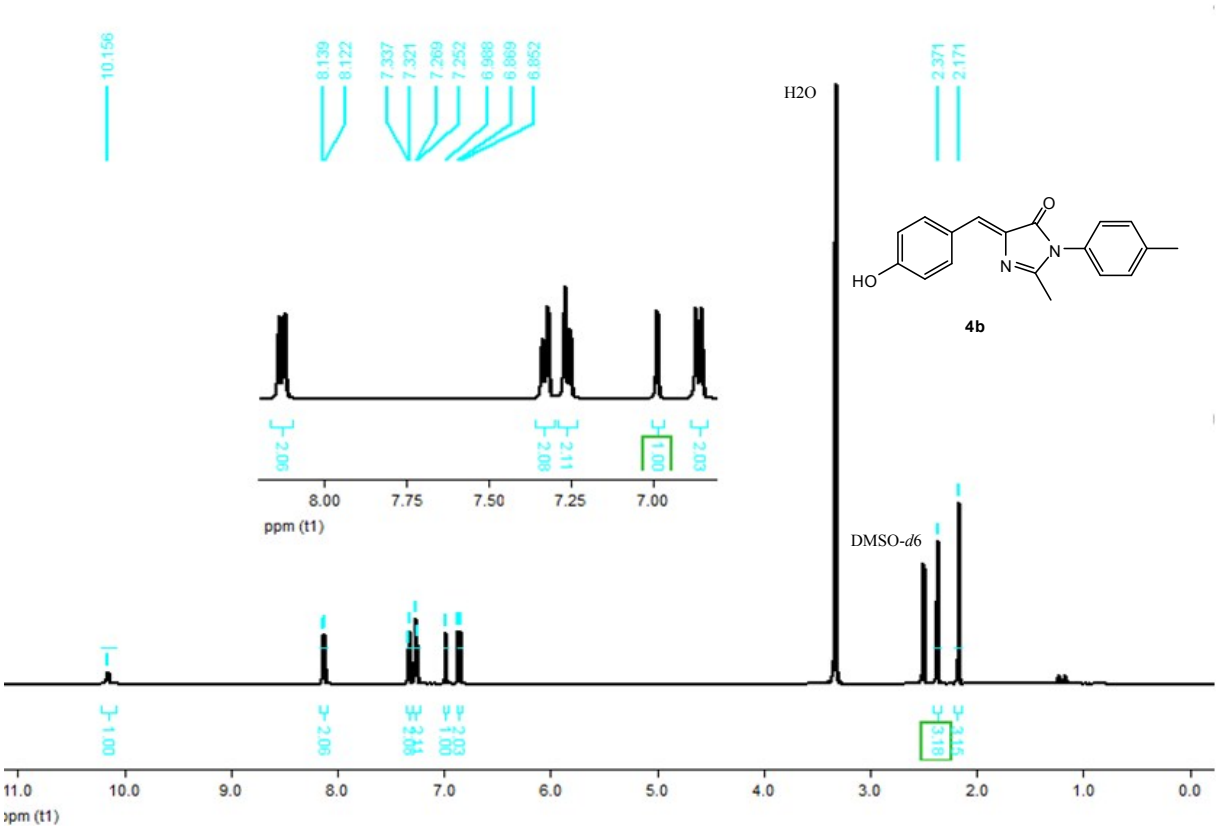
^{1}H NMR of 4a



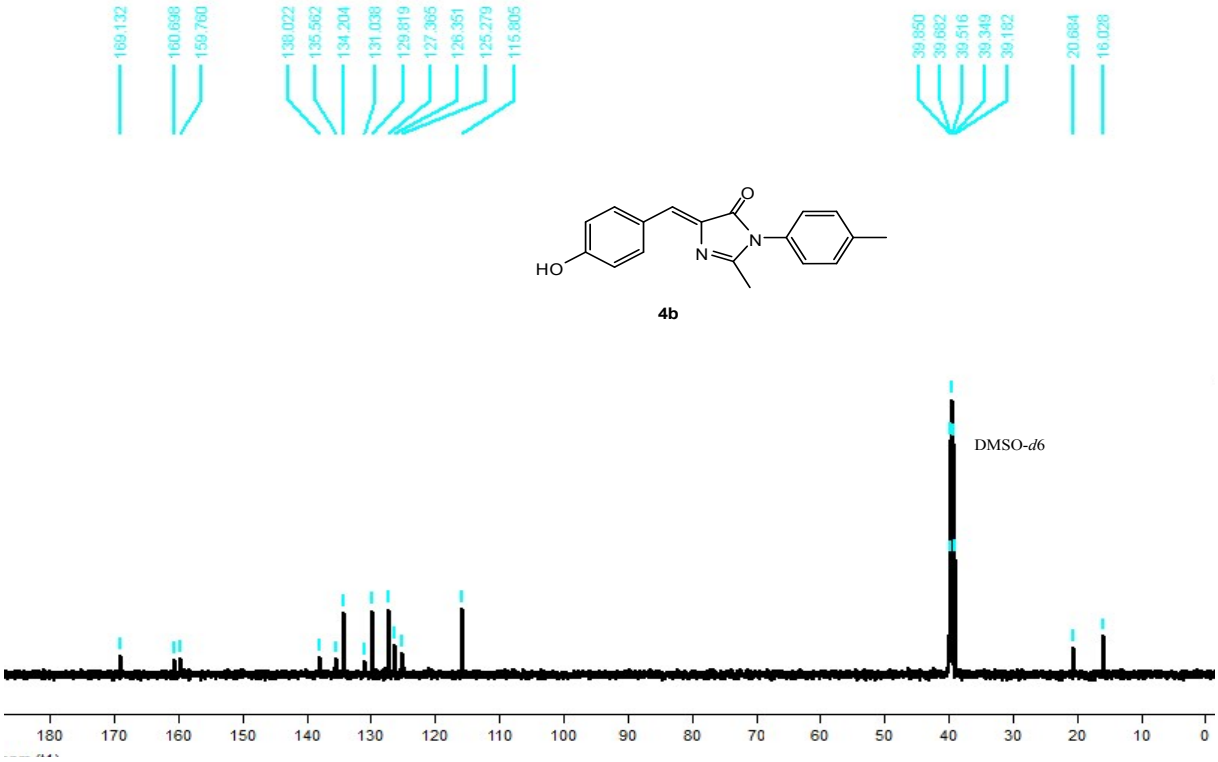
^{13}C NMR of 4a



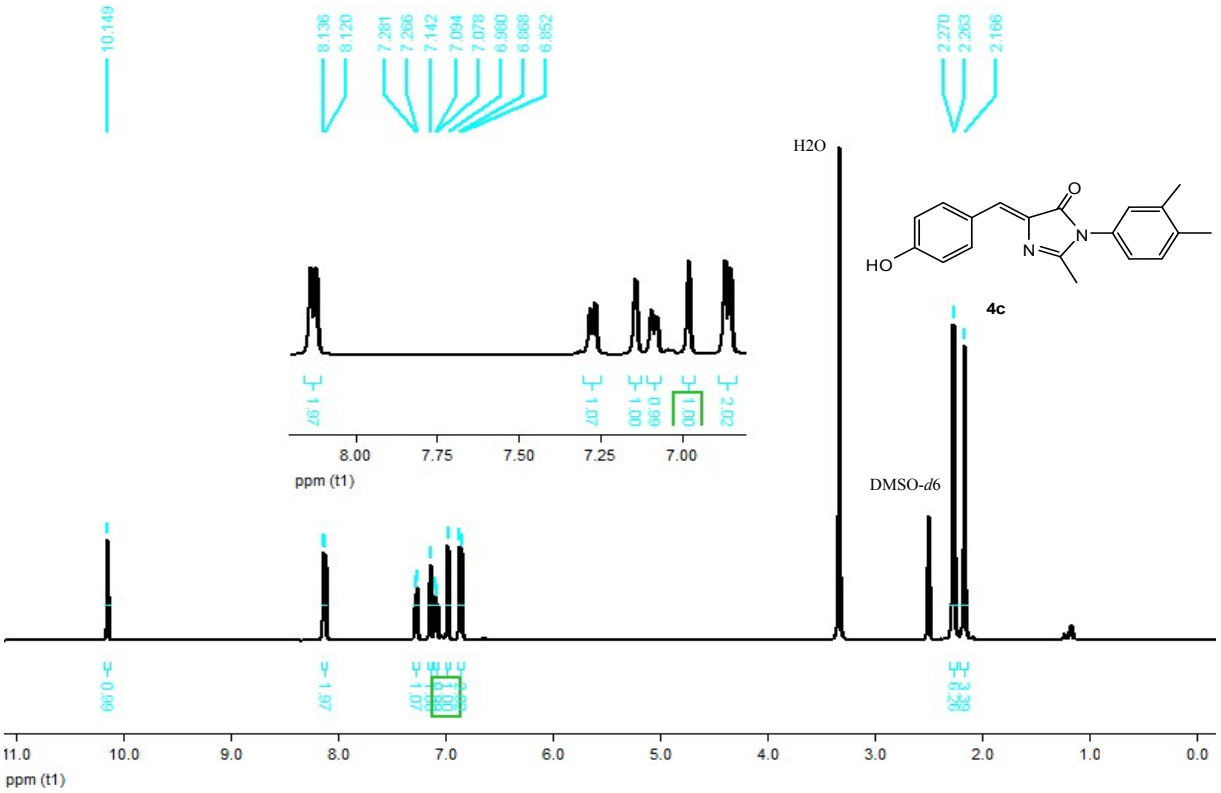
^{1}H NMR of 4b



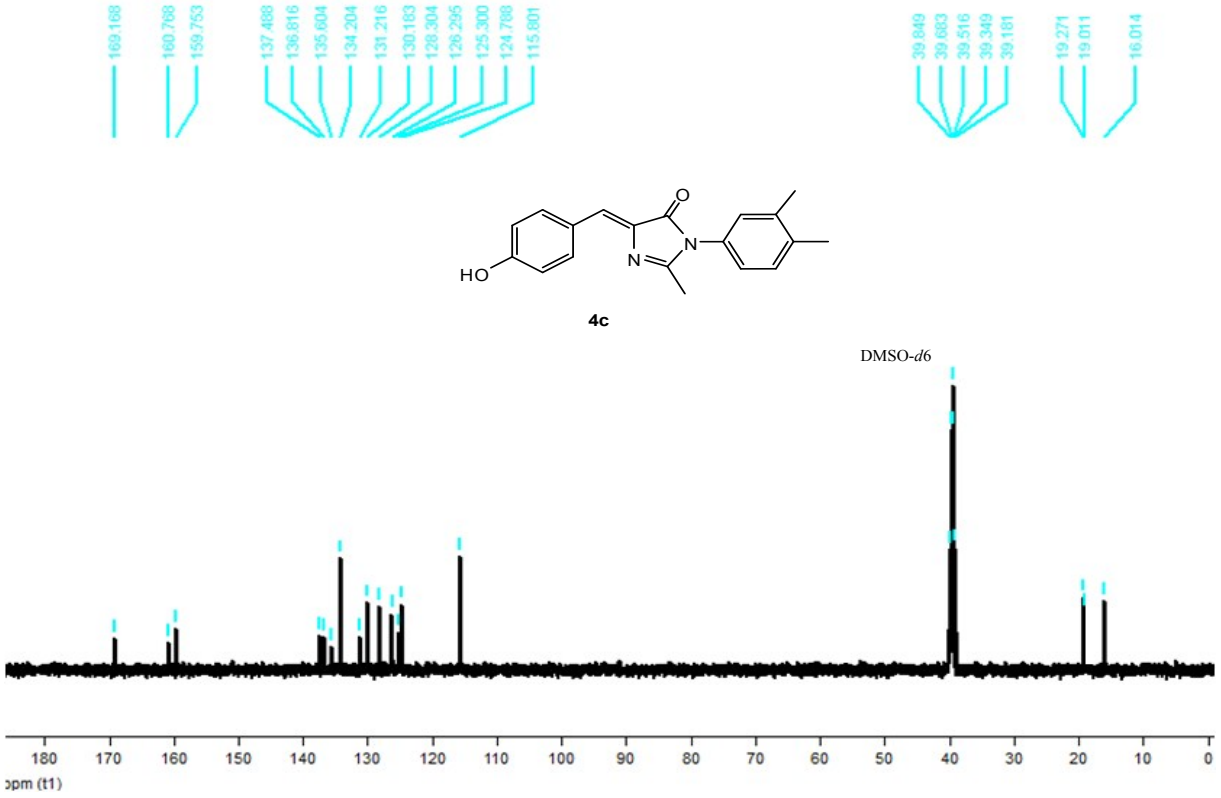
^{13}C NMR of 4b



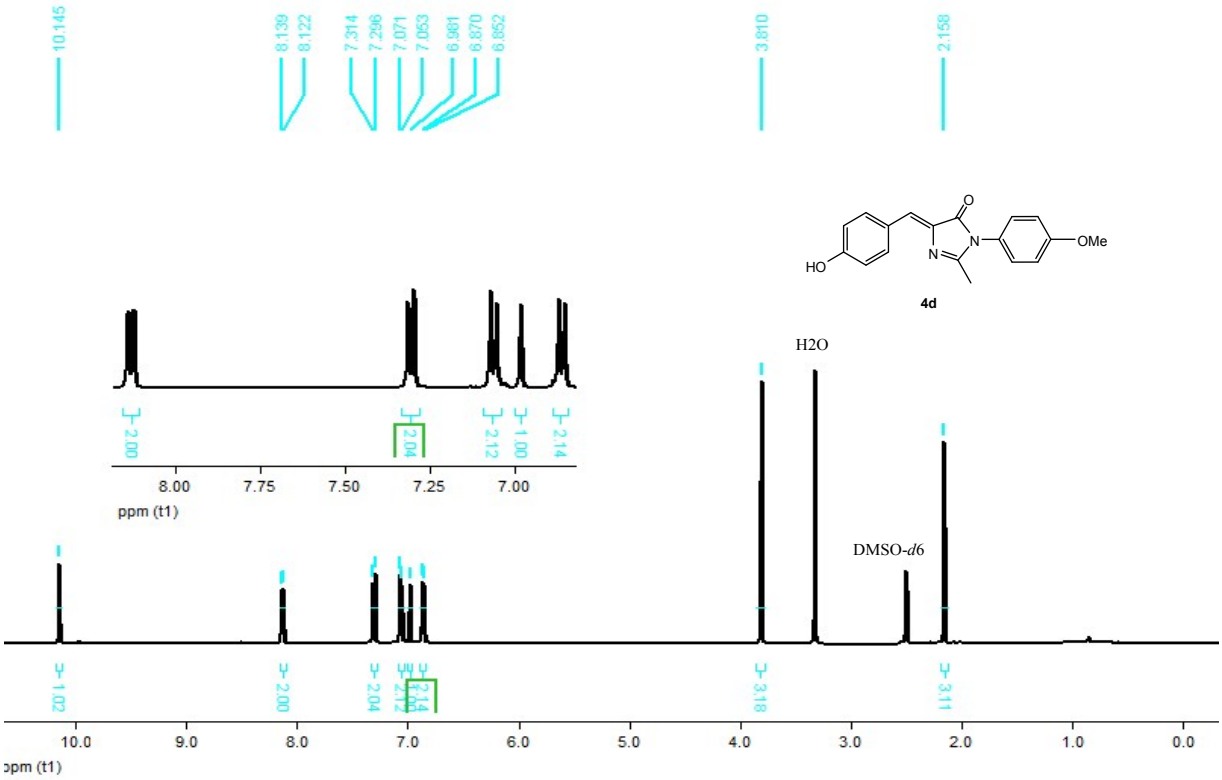
^{1}H NMR of 4c



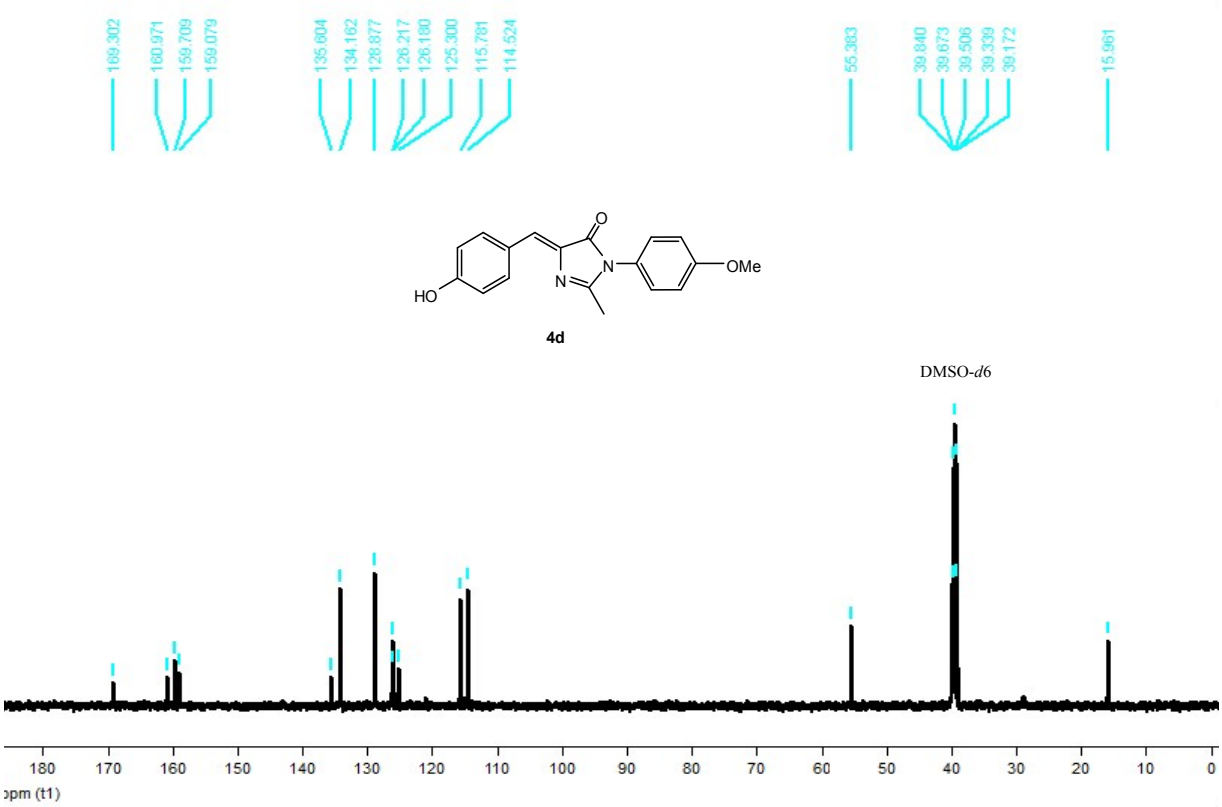
^{13}C NMR of 4c



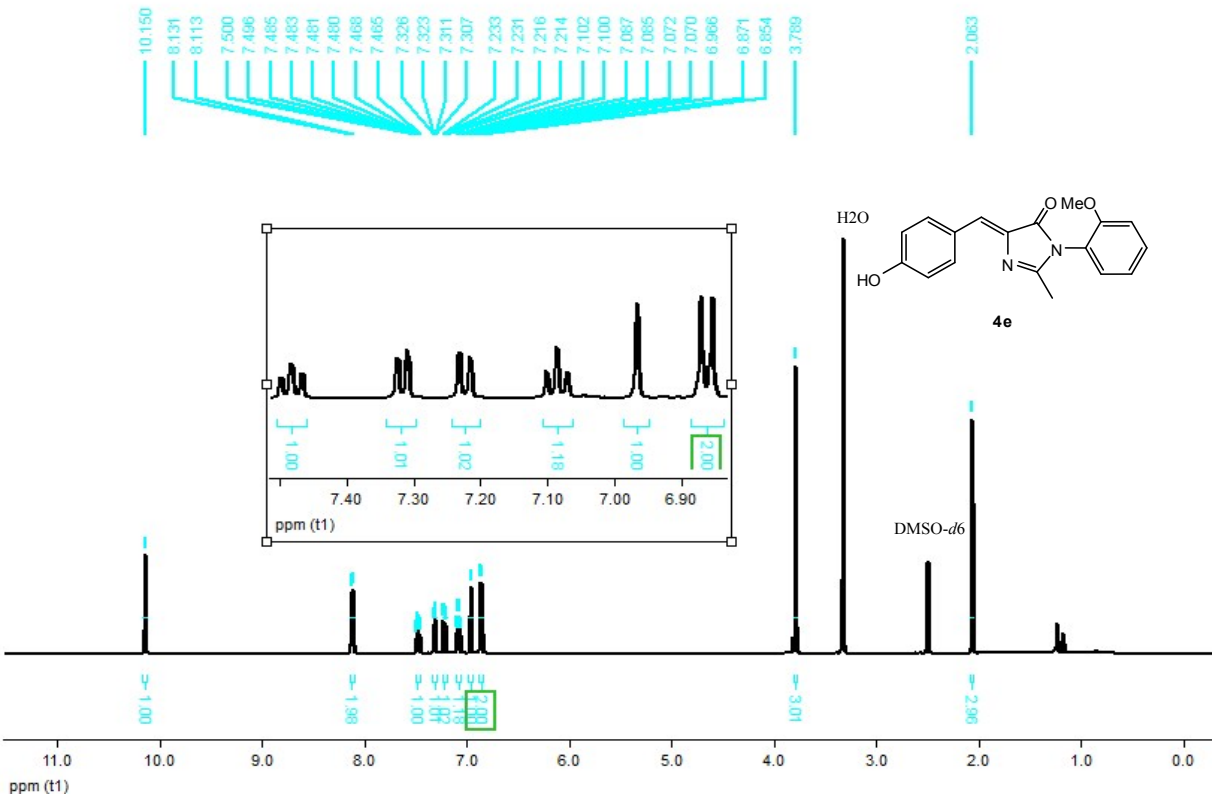
^{1}H NMR of 4d



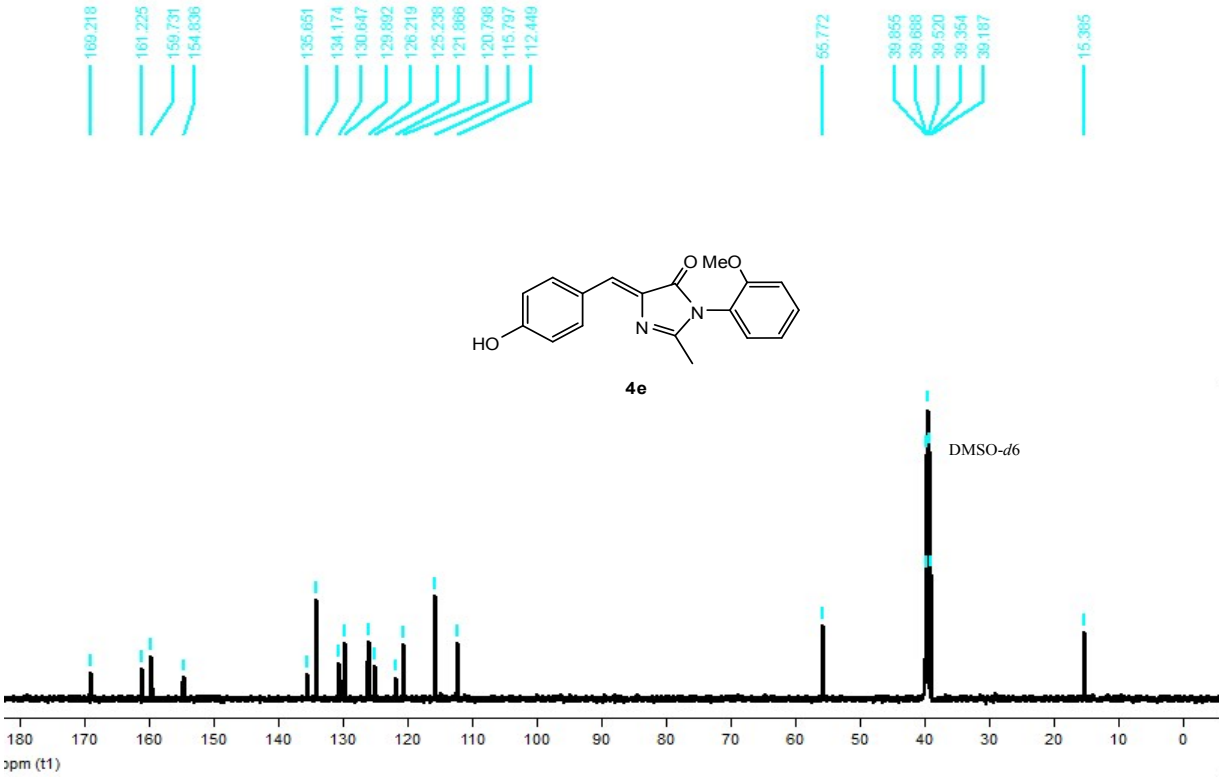
^{13}C NMR of 4d



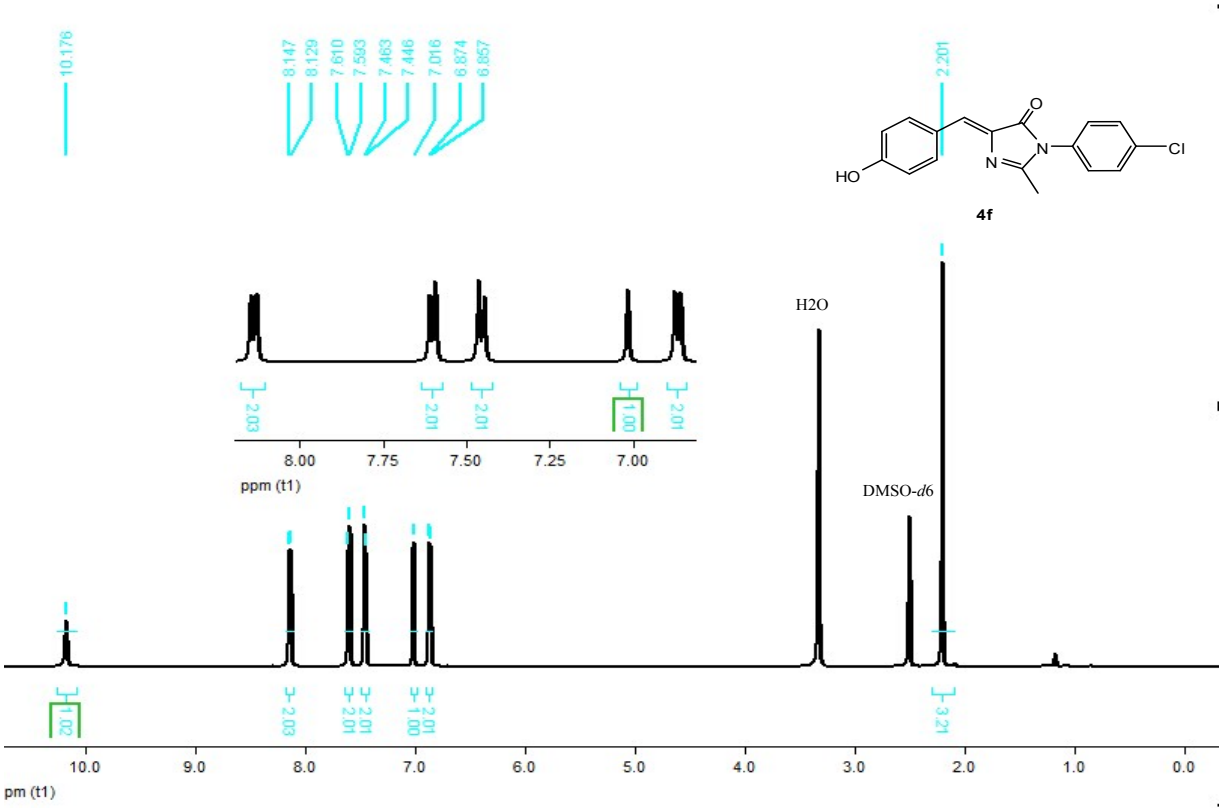
^{1}H NMR of 4e



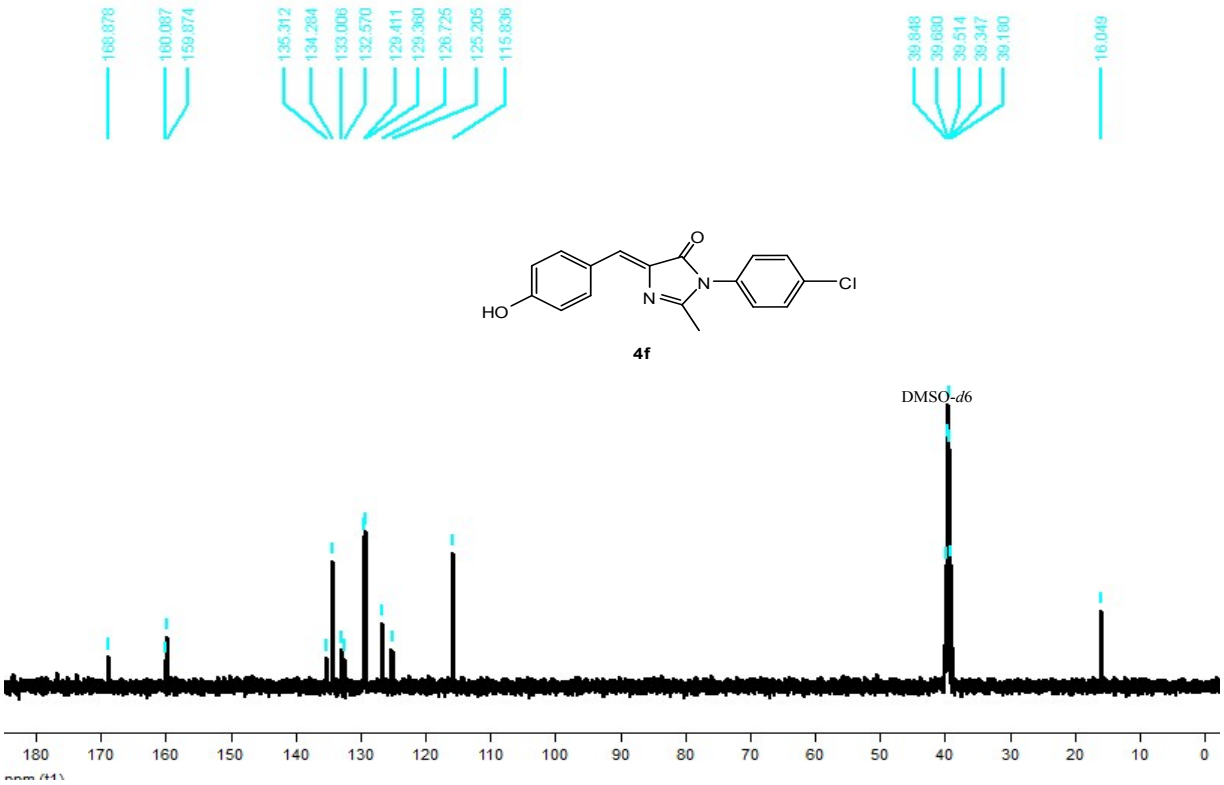
^{13}C NMR of 4e



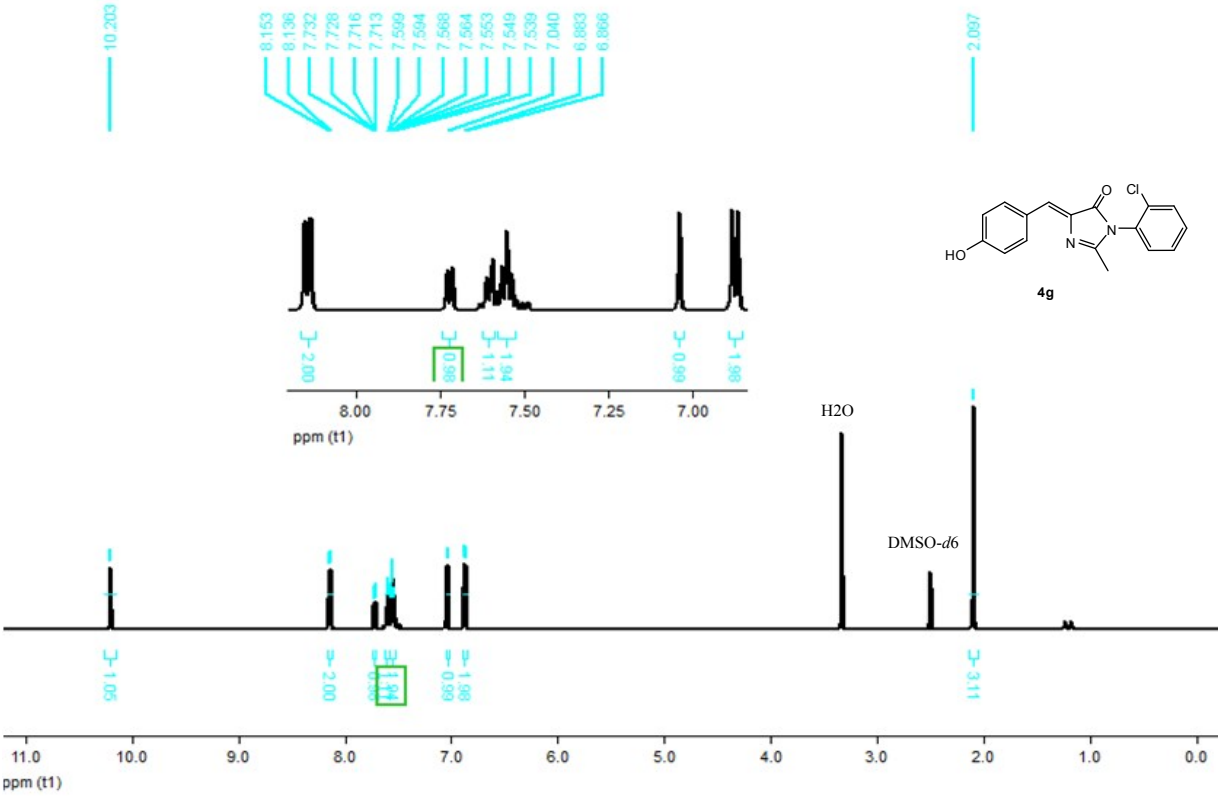
^{1}H NMR of 4f



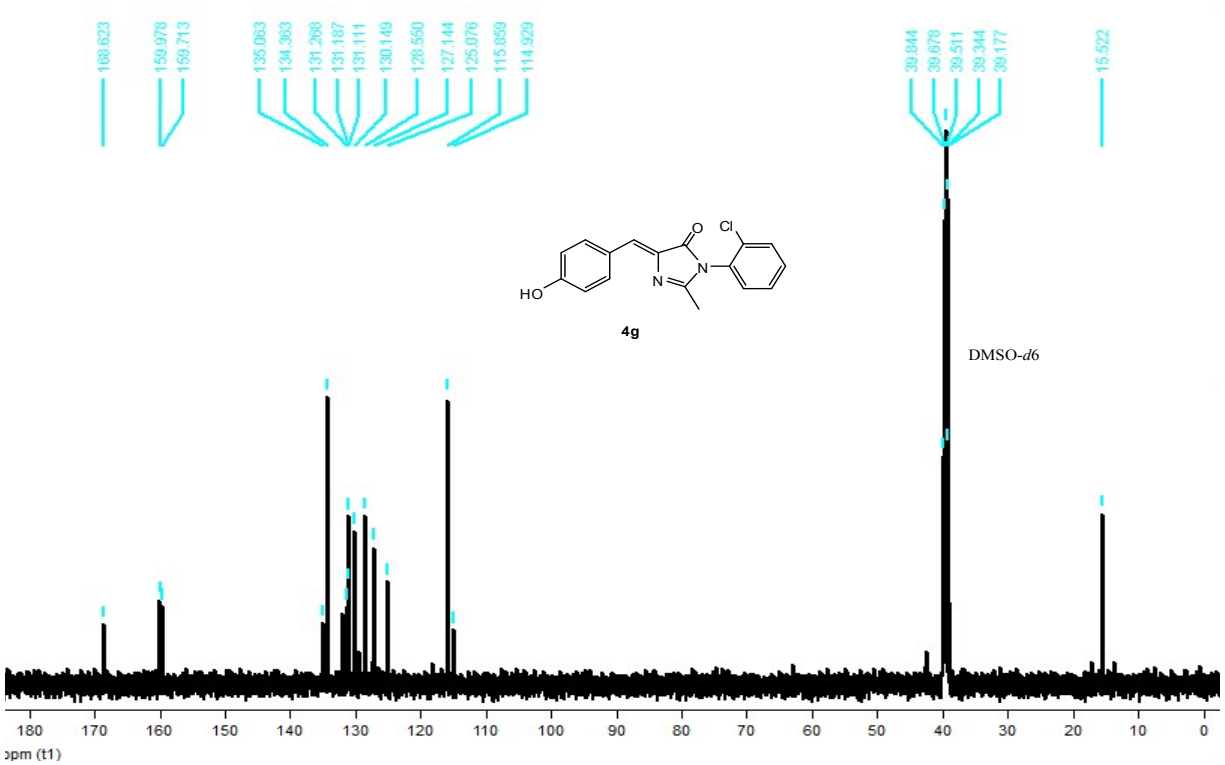
^{13}C NMR of 4f



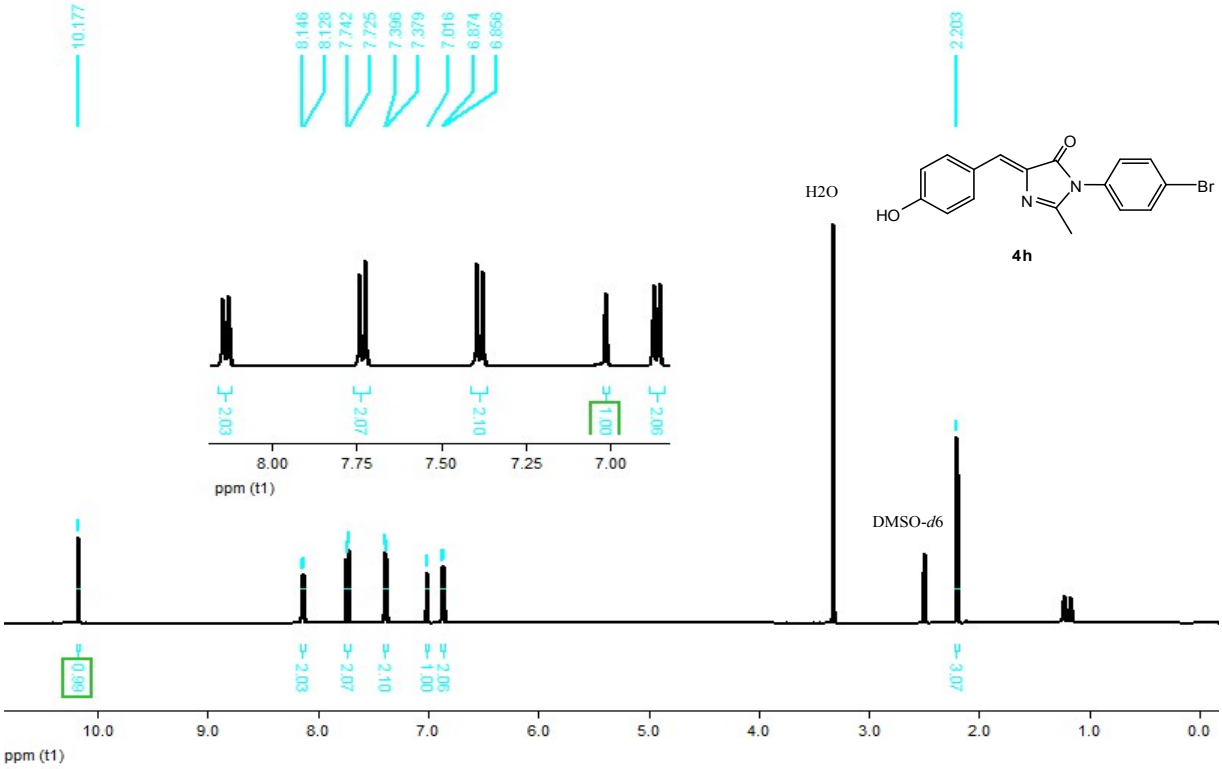
^{1}H NMR of 4g



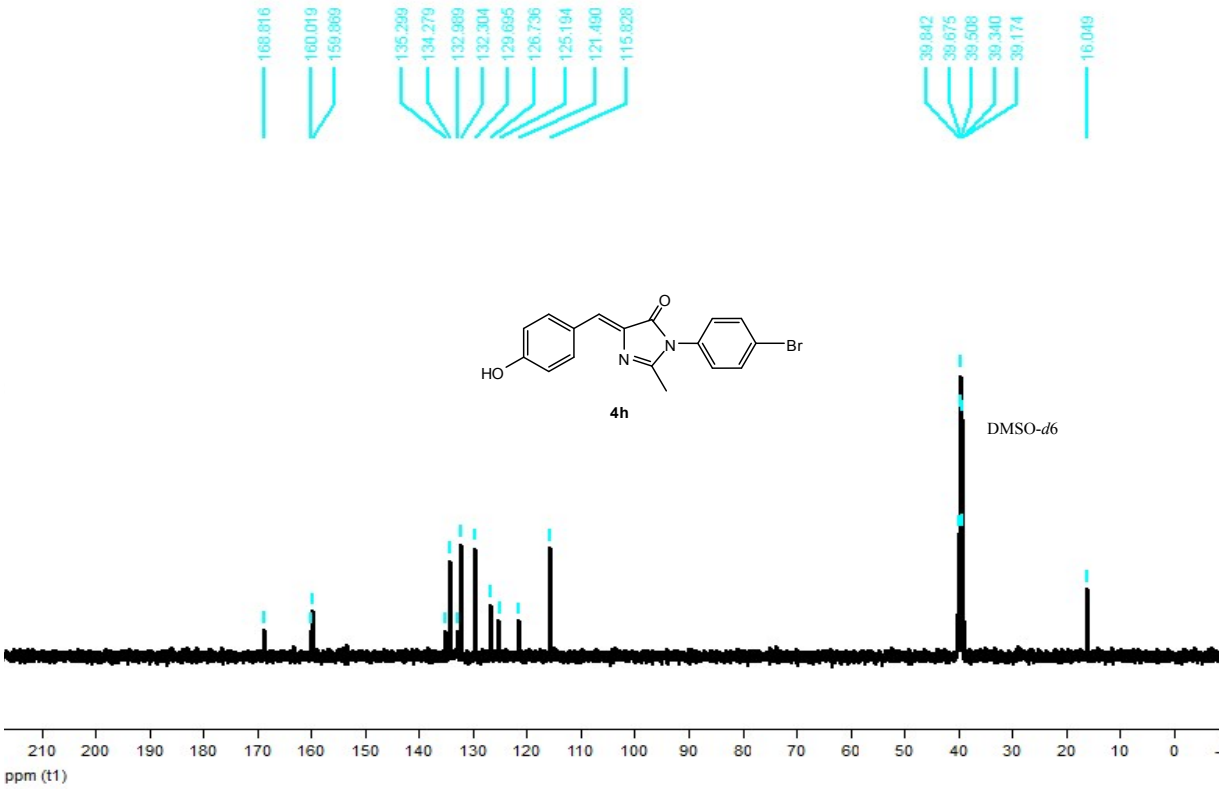
^{13}C NMR of 4g



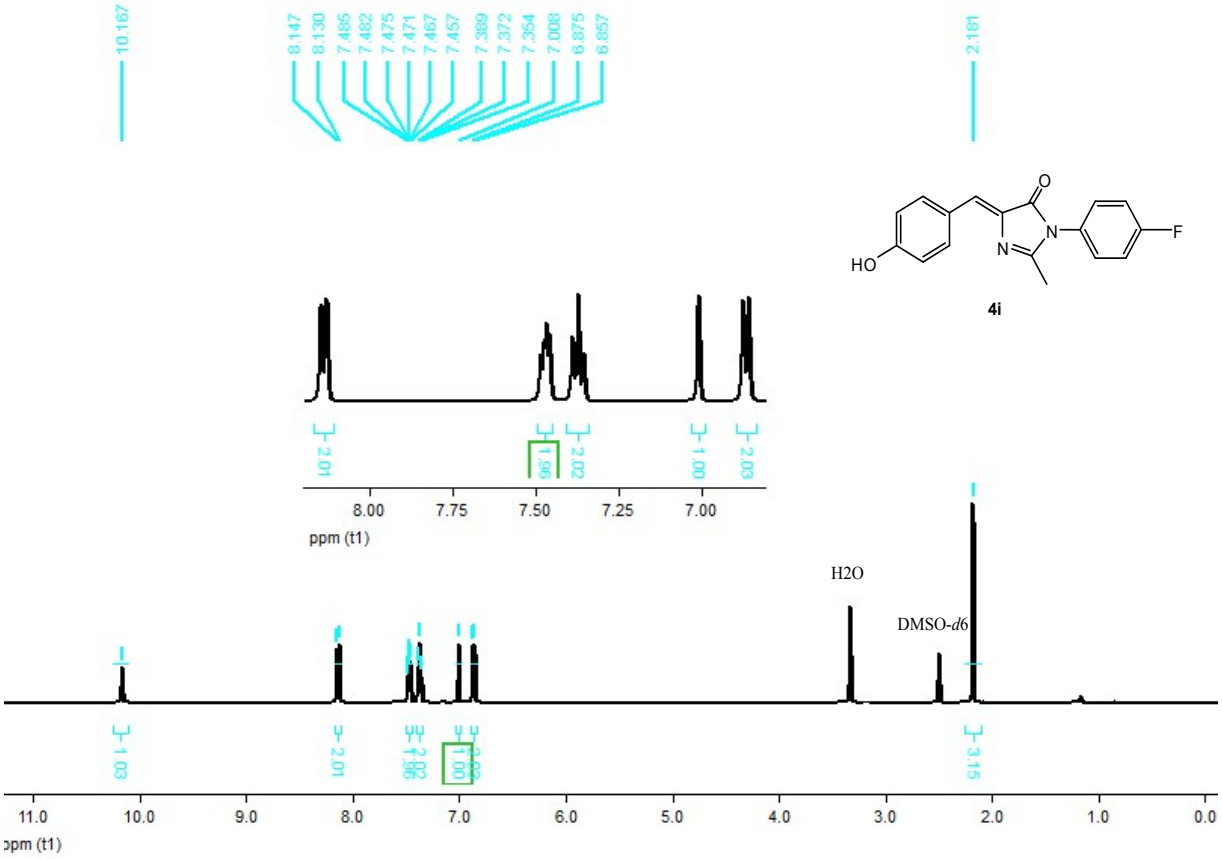
^{1}H NMR of 4h



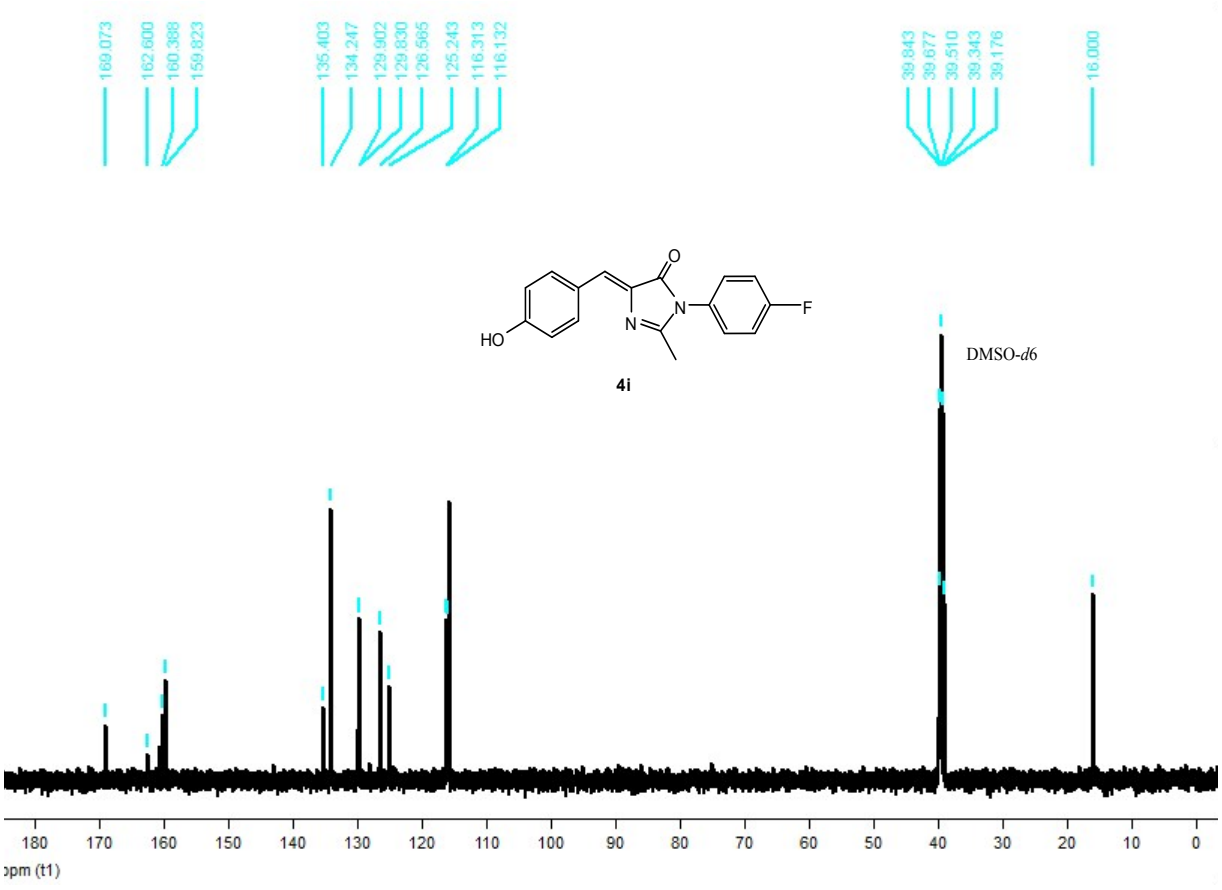
^{13}C NMR of 4h



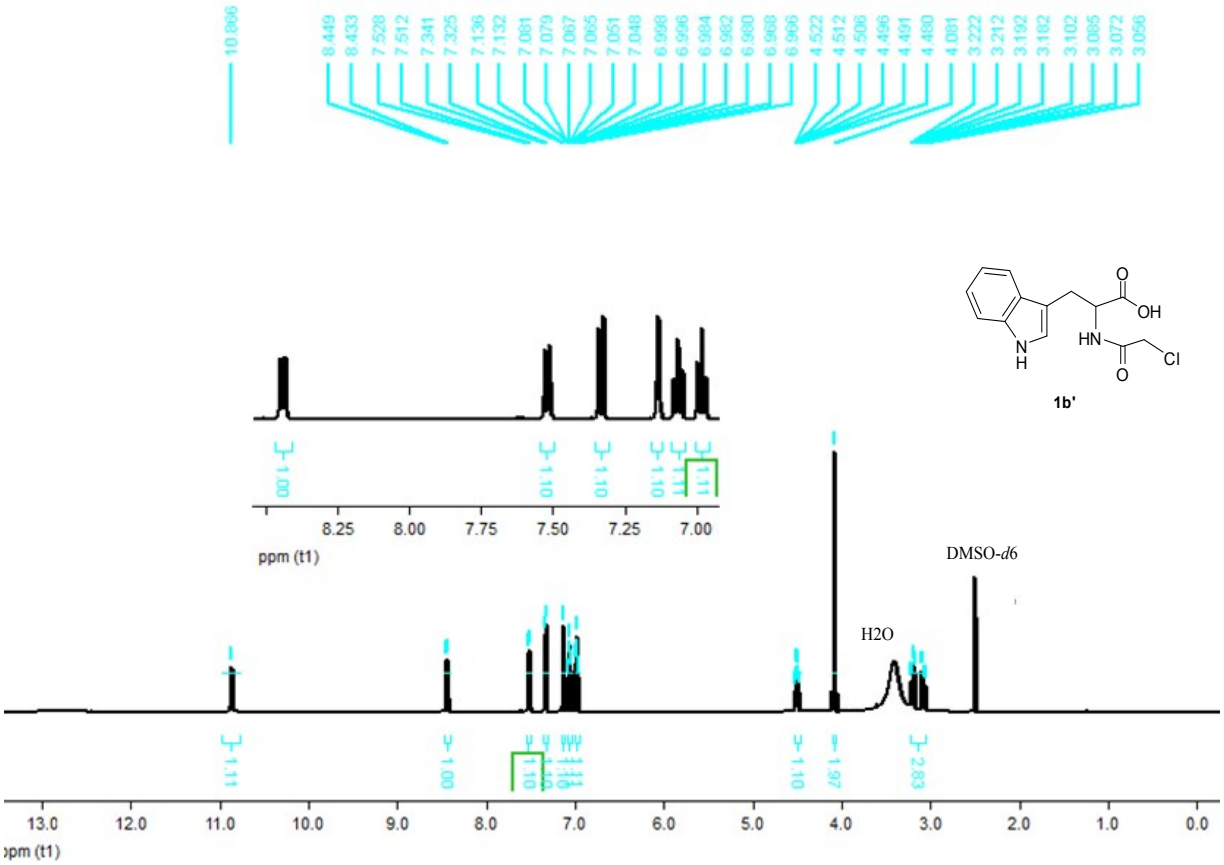
^{1}H NMR of 4i



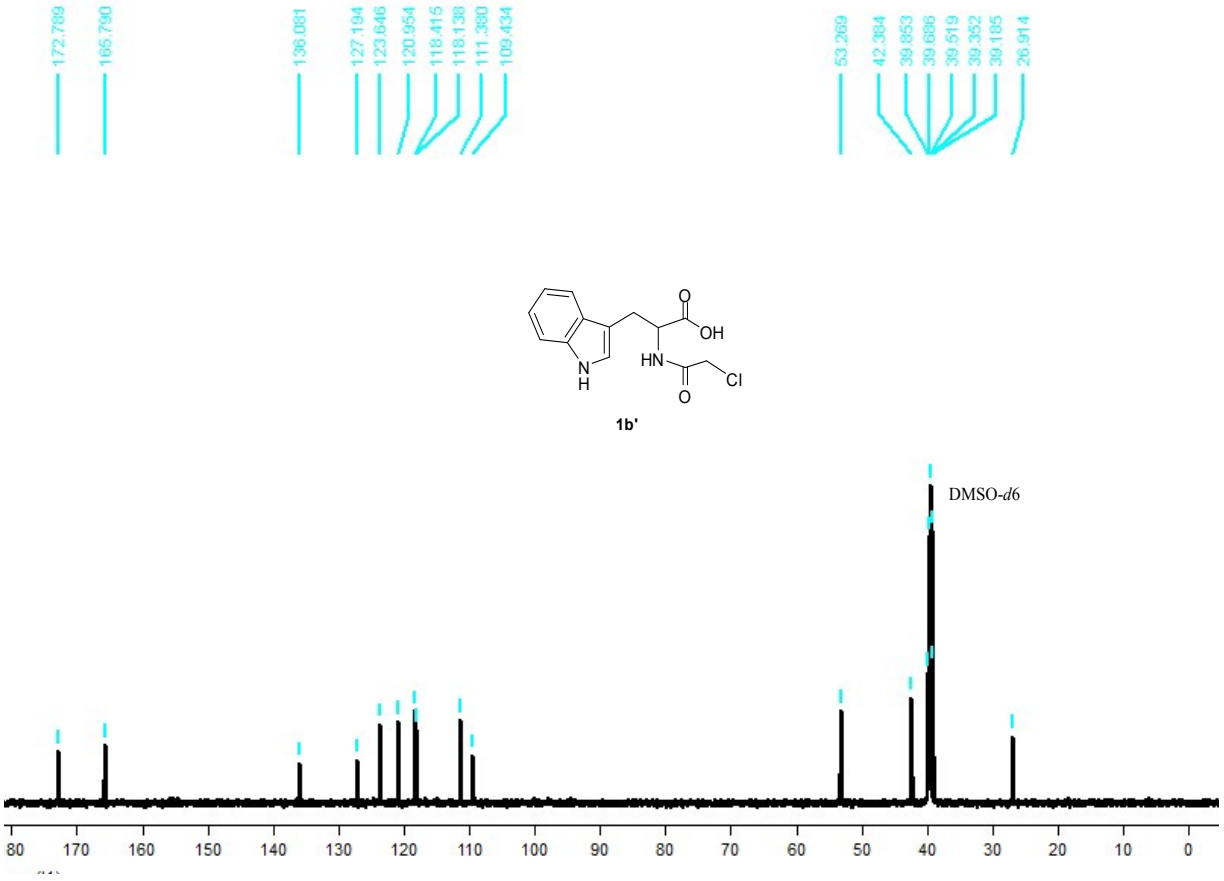
^{13}C NMR of 4i



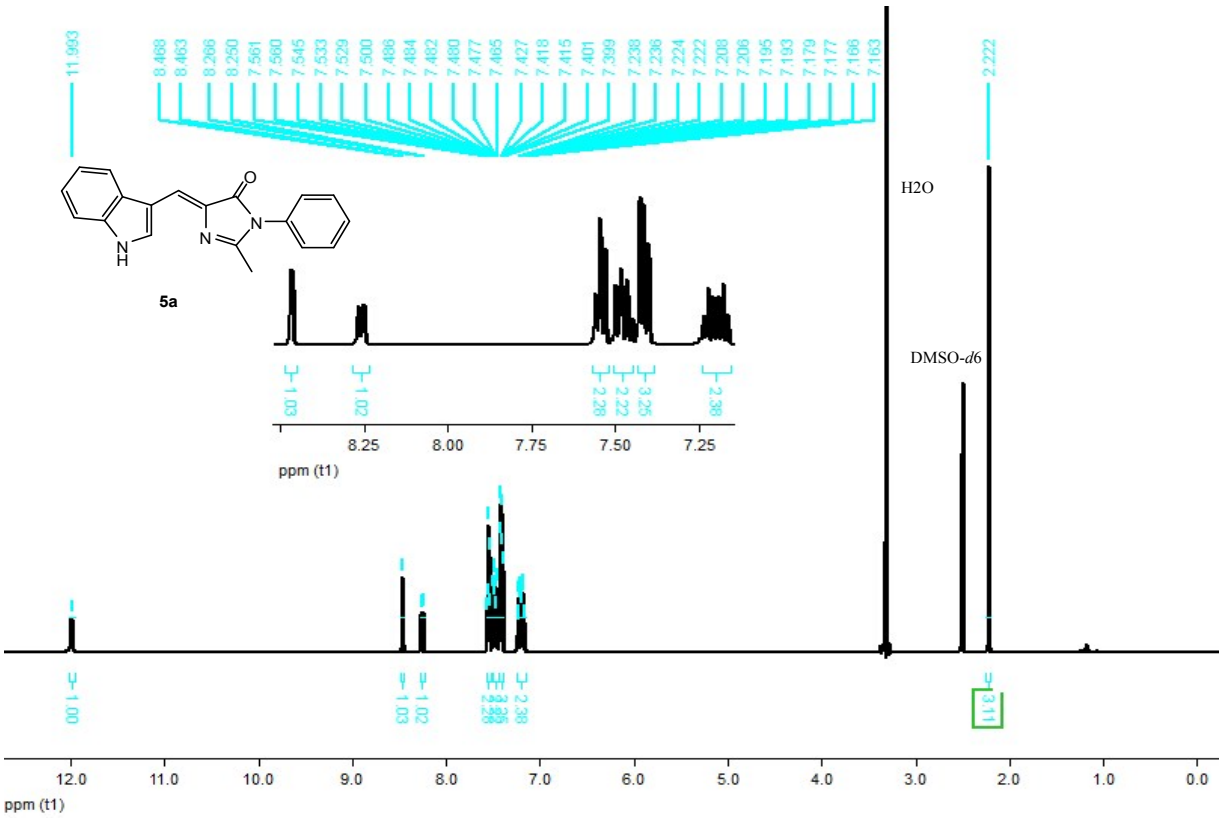
^{1}H NMR of 1b'



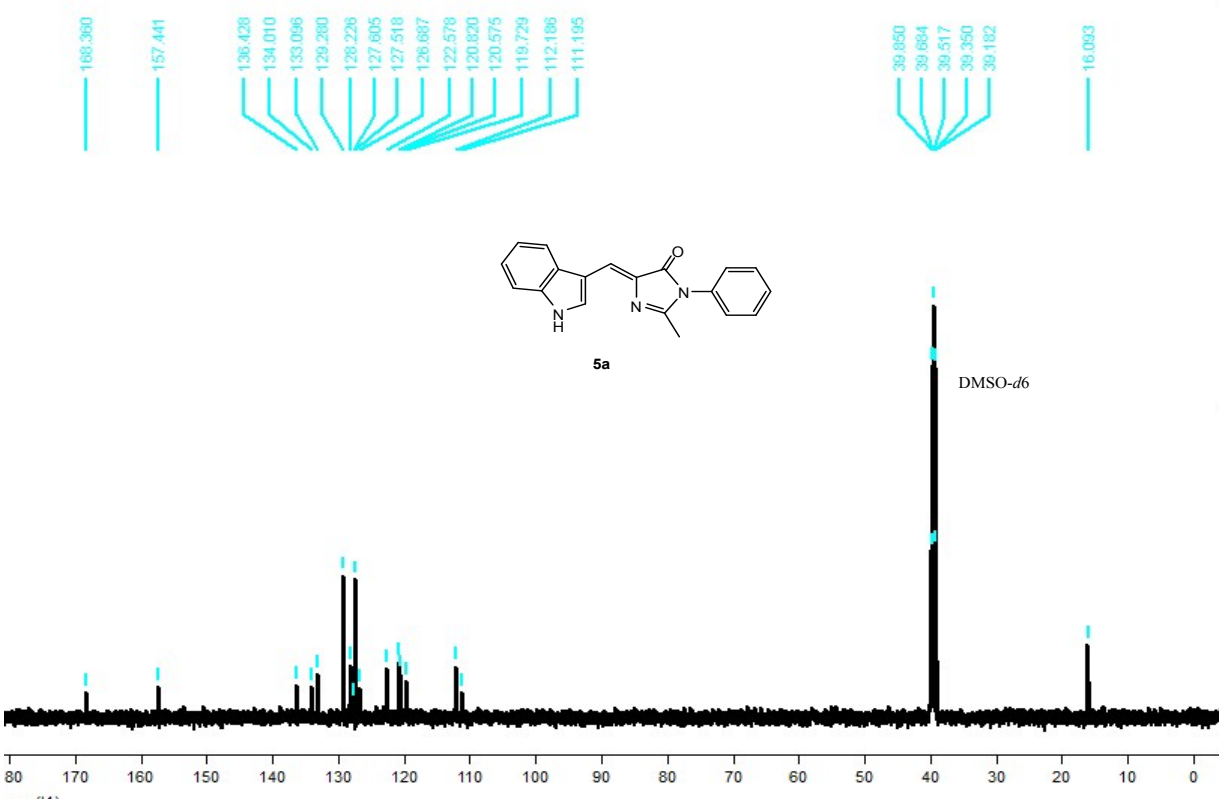
^{13}C NMR of 1b'



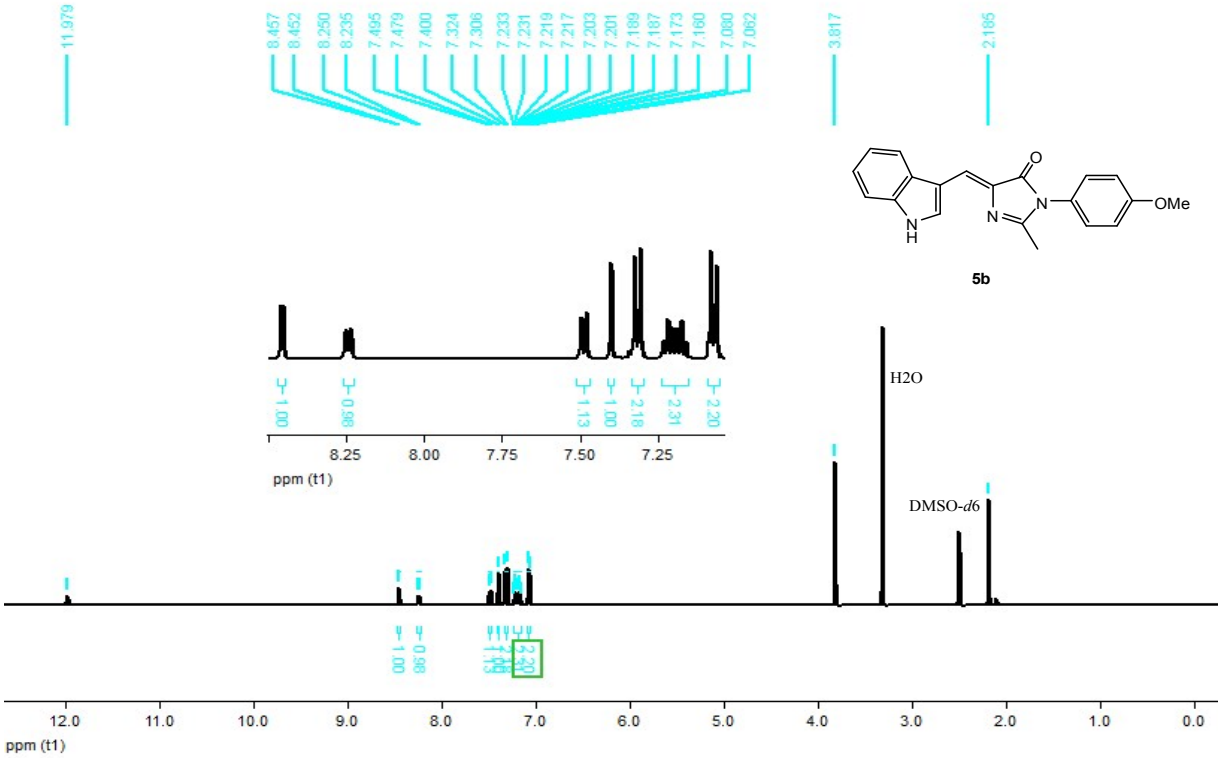
^{1}H NMR of 5a



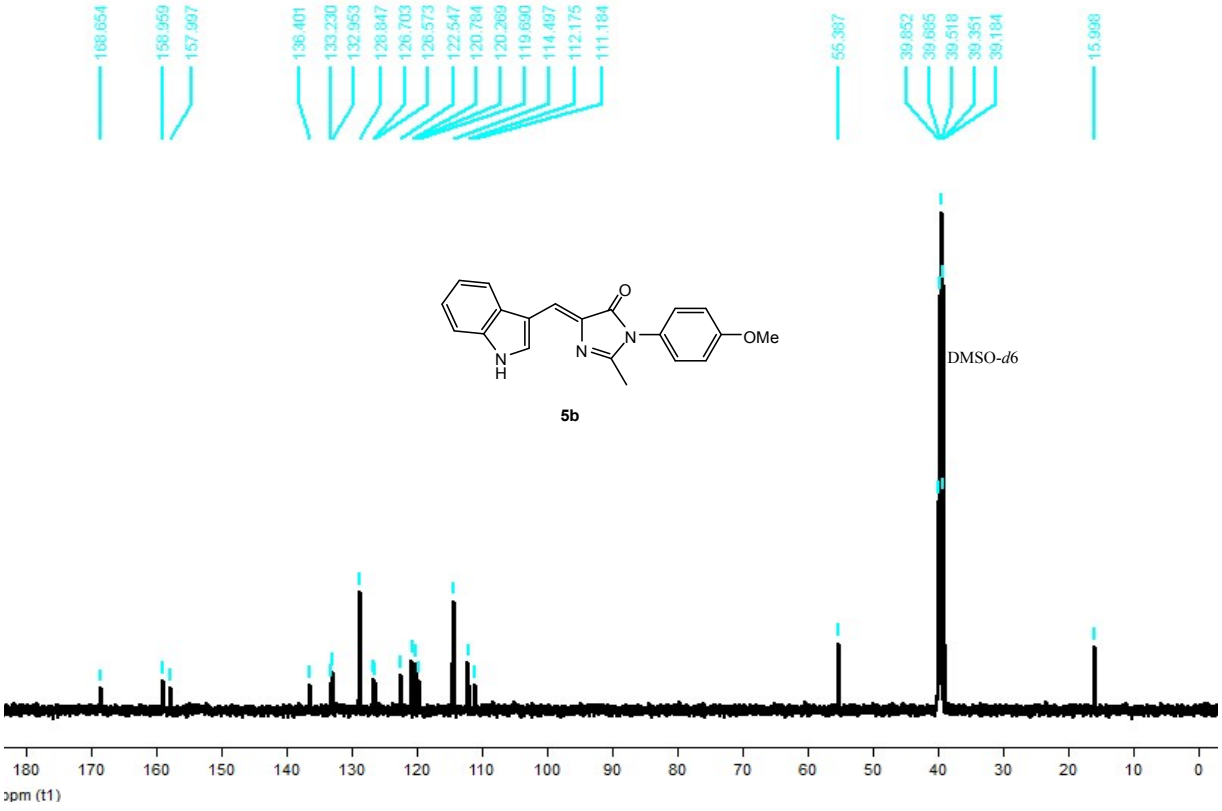
^{13}C NMR of 5a



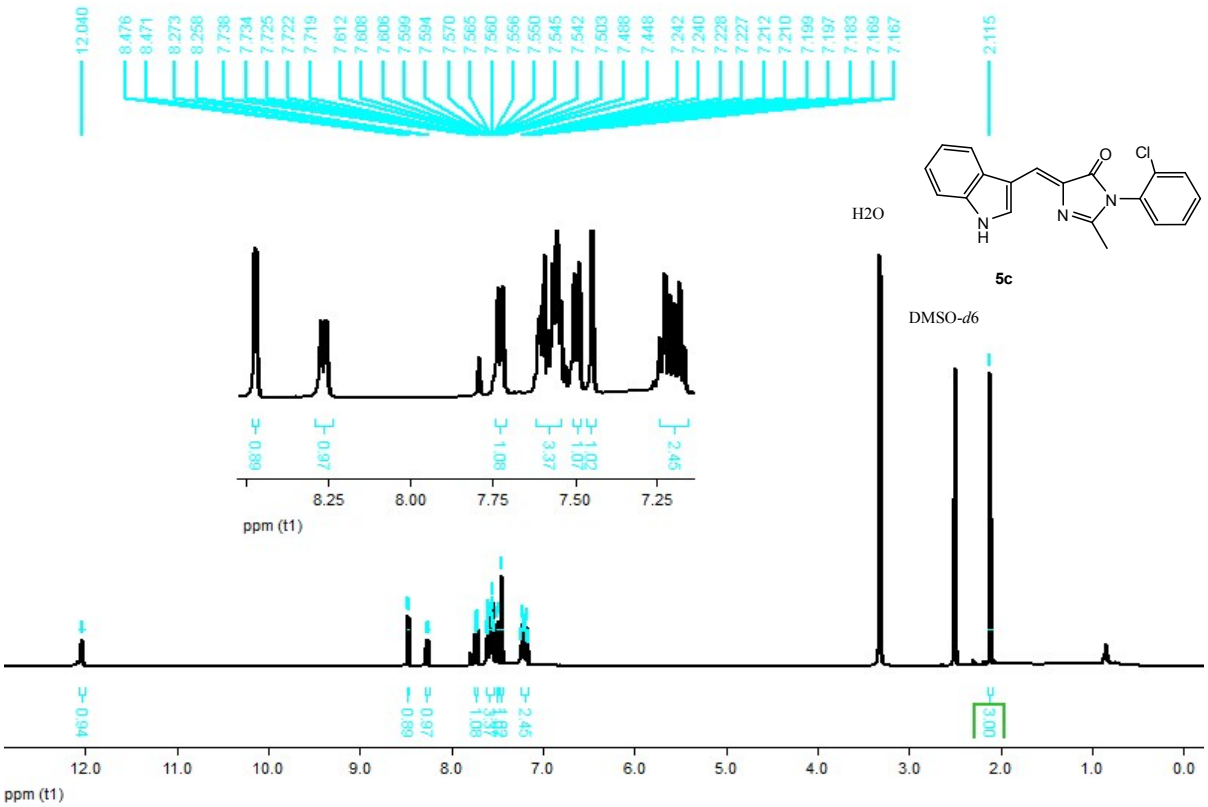
^{1}H NMR of 5b



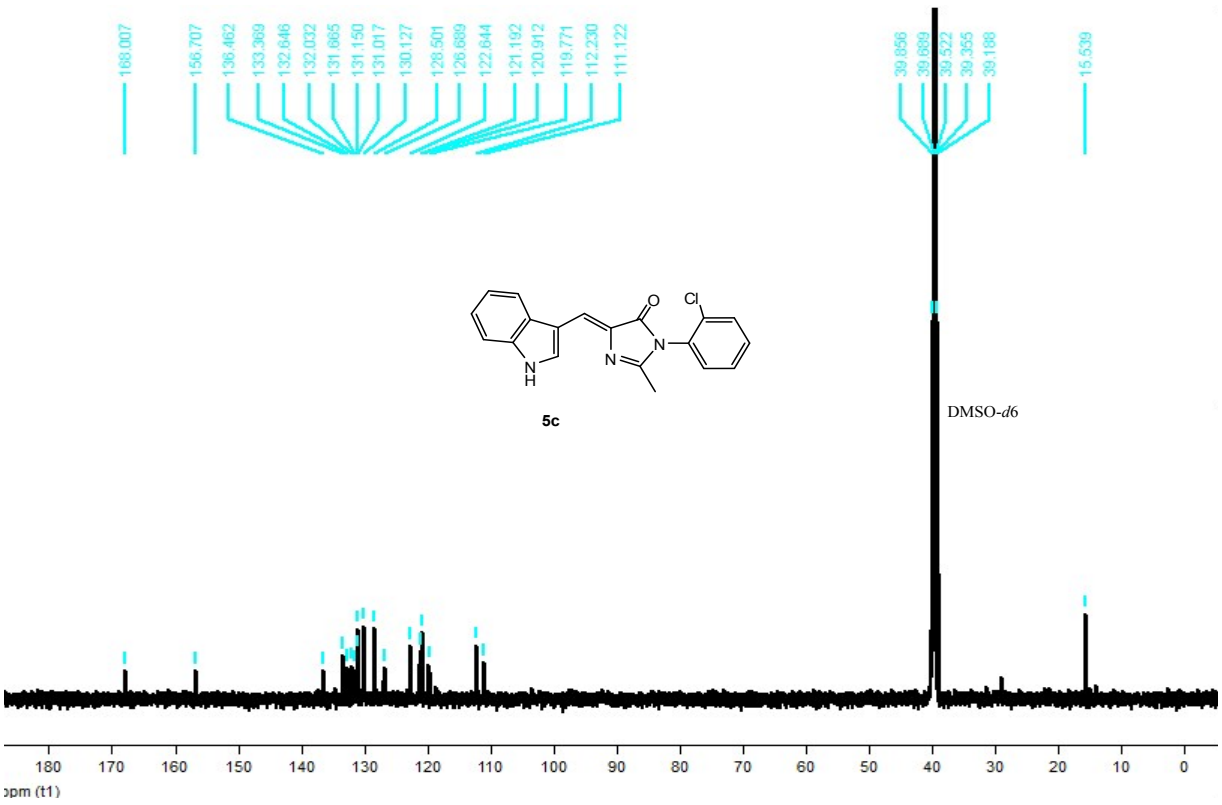
^{13}C NMR of 5b



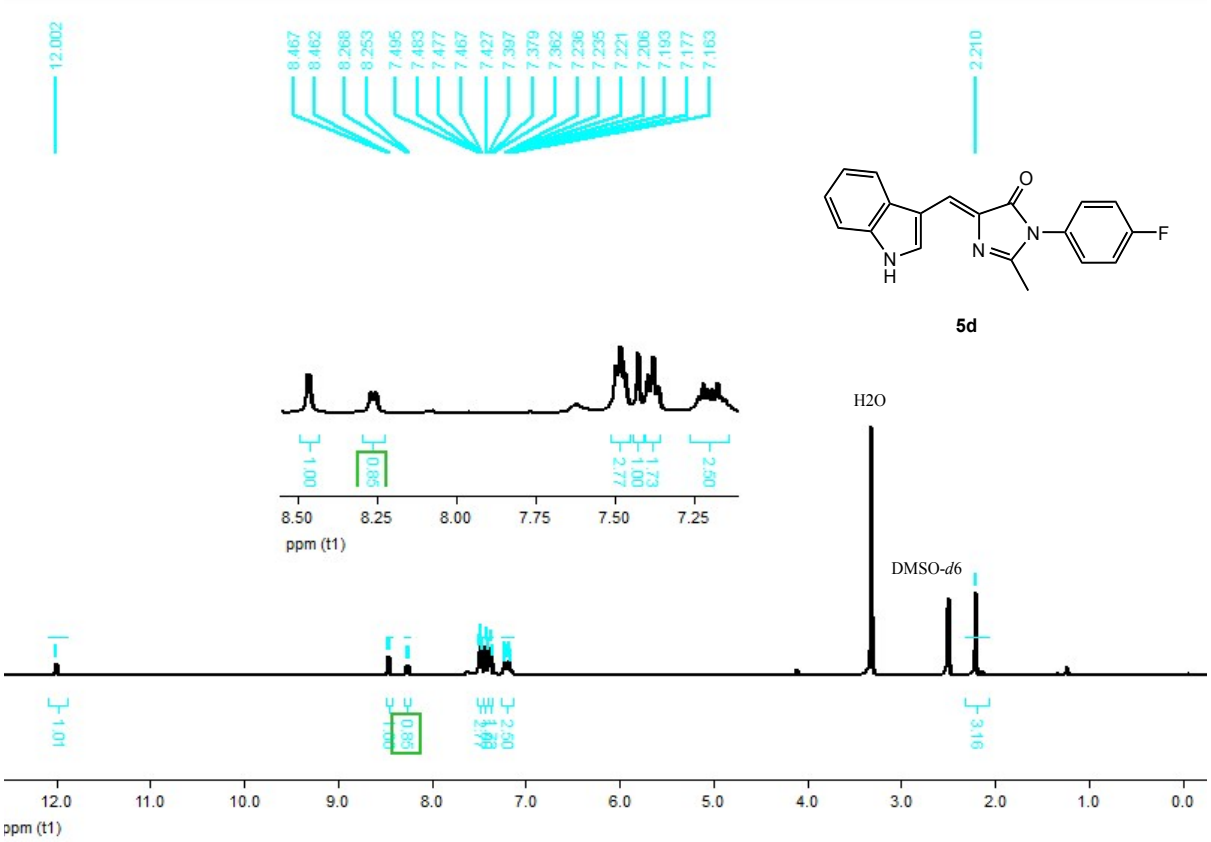
^{1}H NMR of 5c



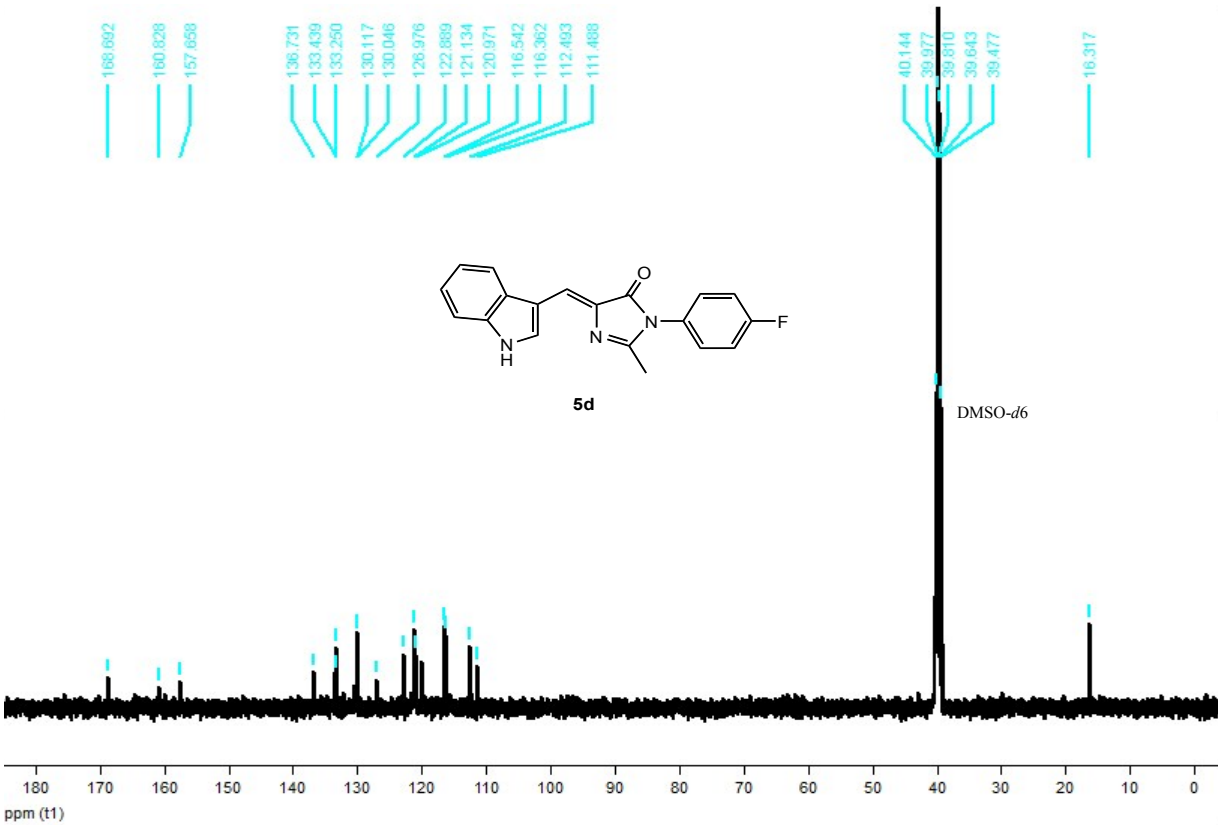
^{13}C NMR of 5c



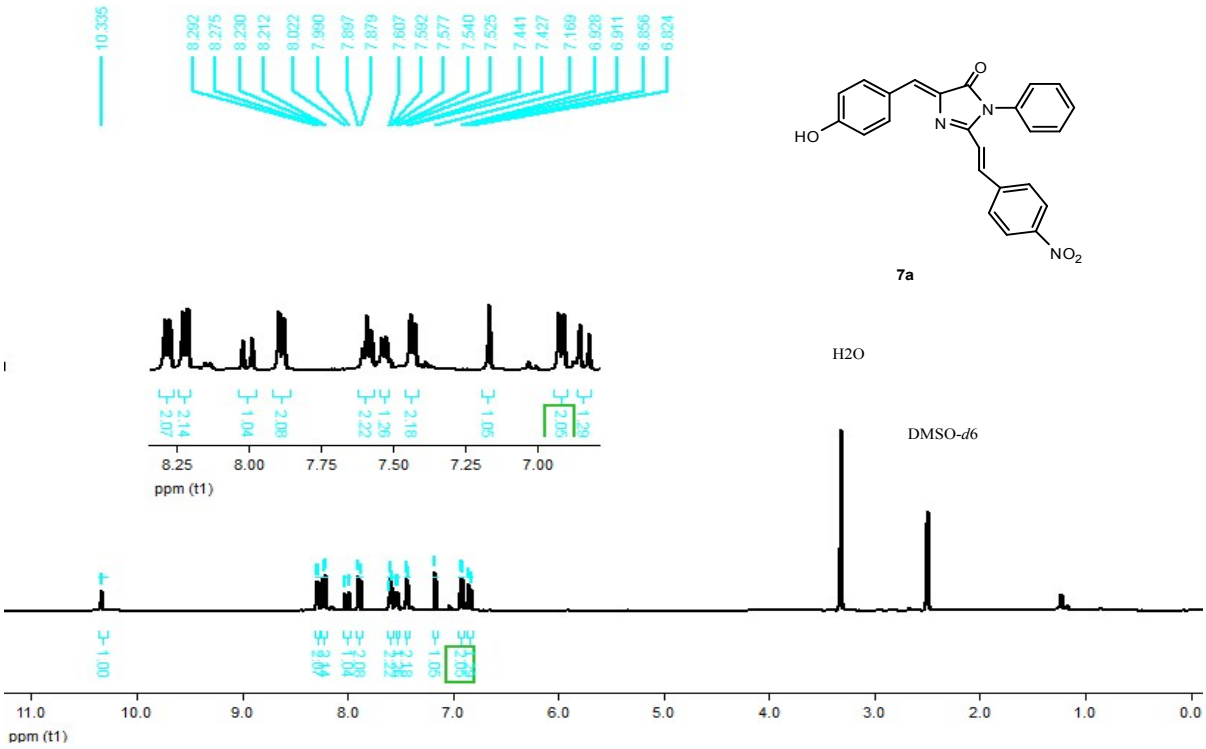
^{1}H NMR of 5d



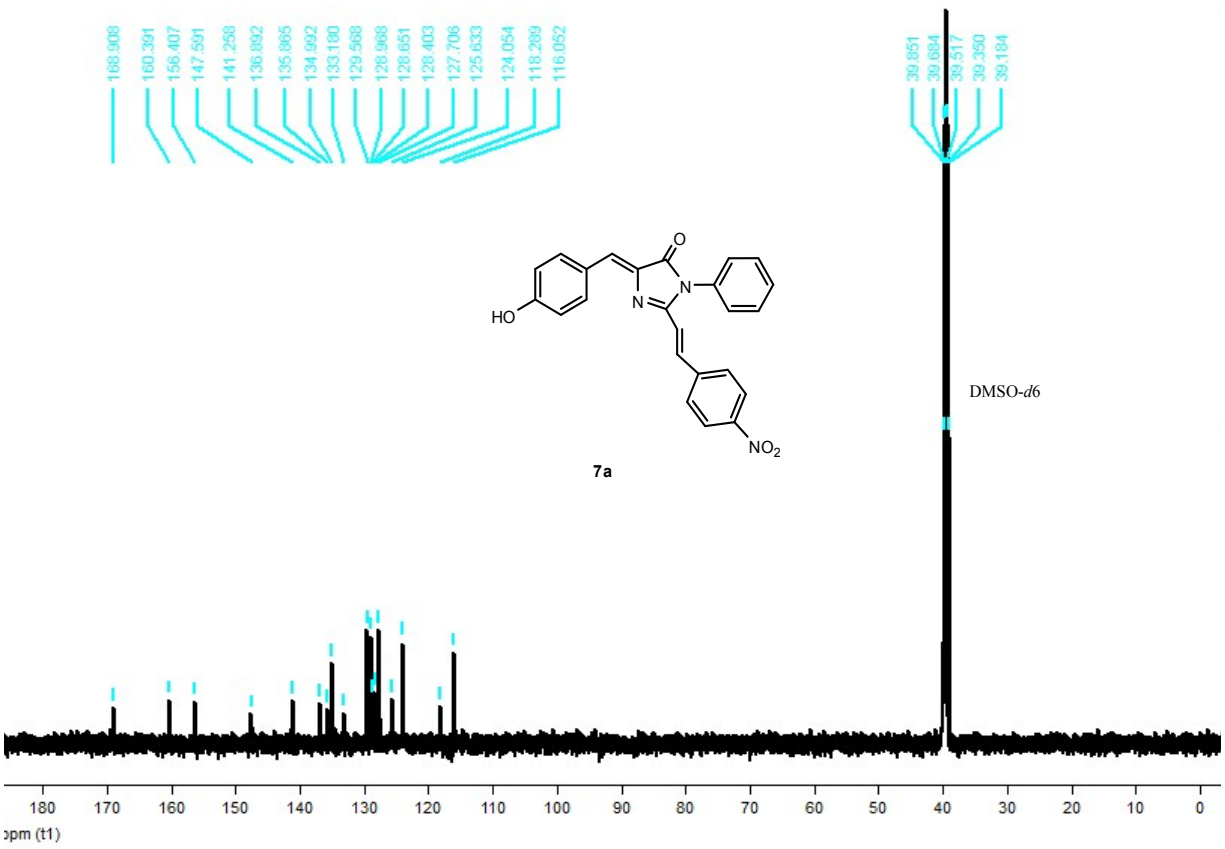
^{13}C NMR of 5d



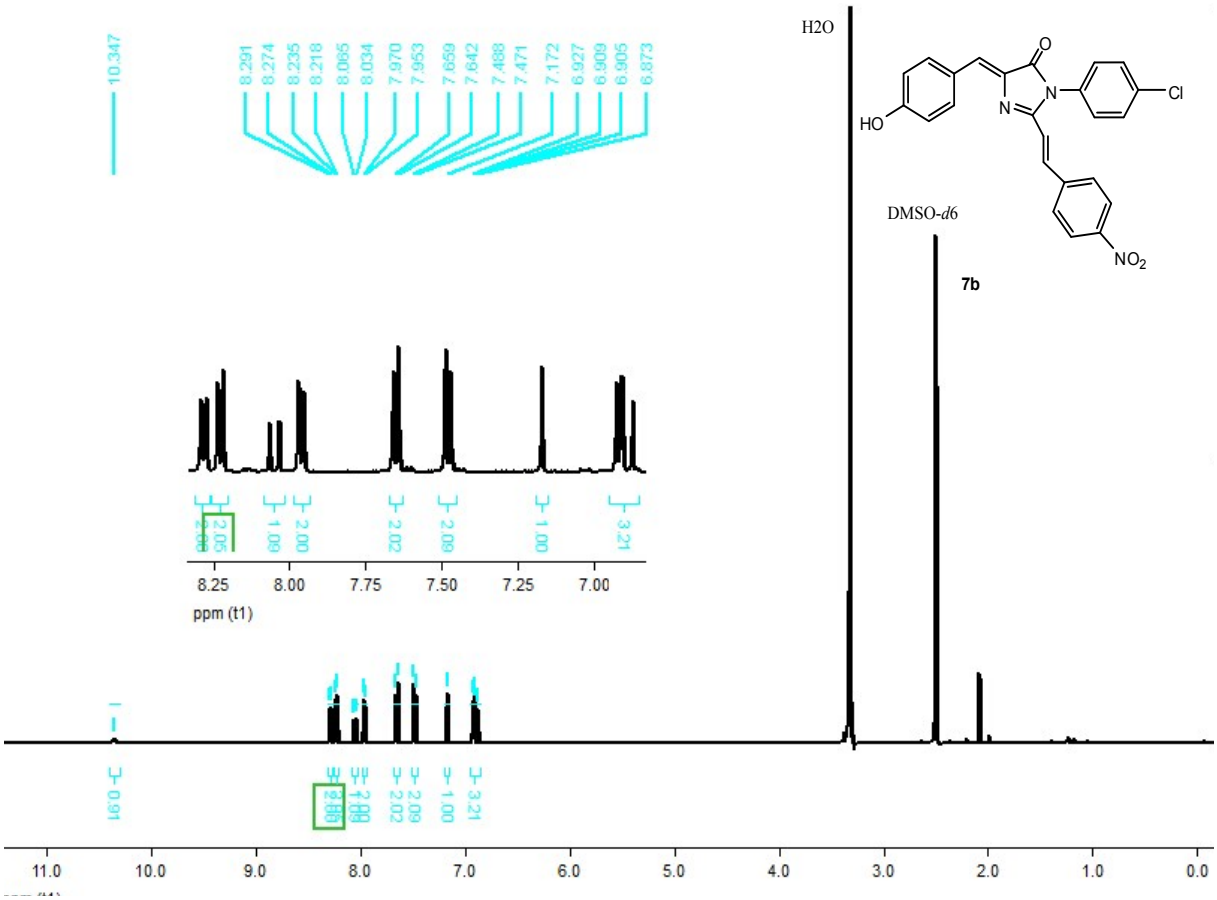
^{1}H NMR of 7a



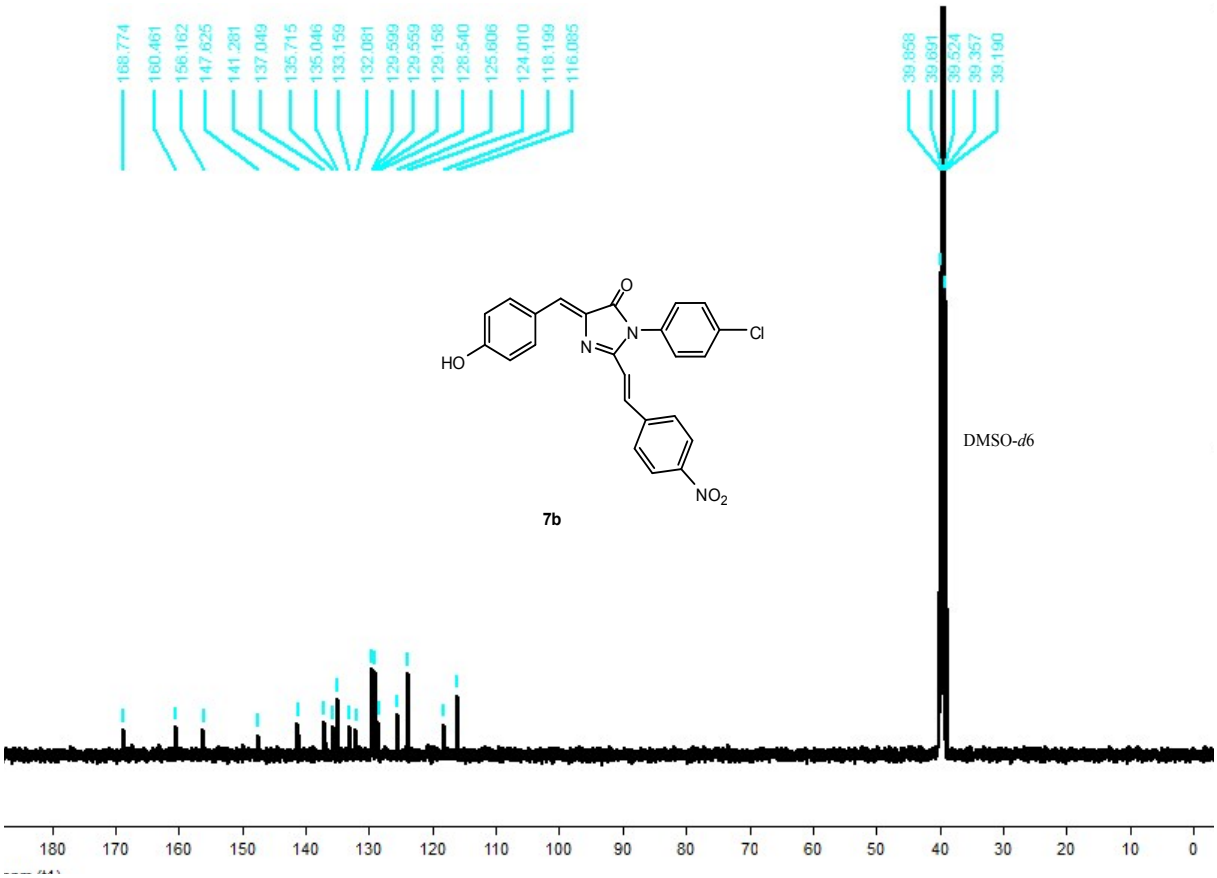
^{13}C NMR of 7a



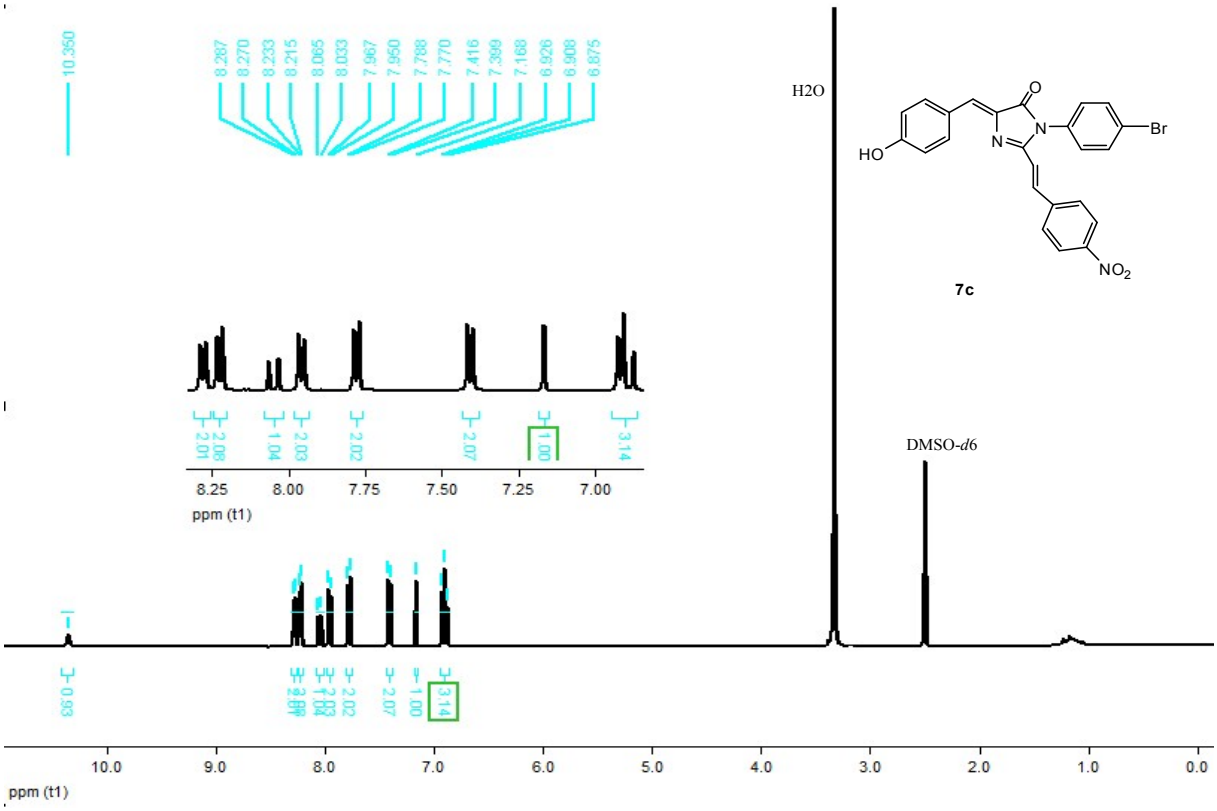
^{1}H NMR of 7b



^{13}C NMR of 7b



^{1}H NMR of 7c



^{13}C NMR of 7c