Supporting Information

Magnetic sulfonated polysaccharides as efficient catalysts for synthesis of the isoxazole-5-one derivatives possessing substituted pyrrole ring, as anti-cancer agents

Zarrin Ghasemi*, Afsaneh Hamidian Amale, Sajjad Azizi, Sepideh Valizadeh and Jafar Soleymani.

1. Materials and instruments

All solvents, chemicals, and reagents were obtained from Merck, Fluka, and Aldrich chemical companies and used without further purification. The medium molecular weight of CS with an 85% degree of deacetylation was purchased from Sigma-Aldrich. The used benzyl azide was prepared from the reaction of benzyl chloride and sodium azid.[1] Melting points were measured on an Electrothermal MEL-TEMP apparatus (model 1202D) and are uncorrected. FT-IR spectra were obtained with a Bruker Tensor 27 spectrometer; υ in cm^{-1}. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Spectrospin Avance 400 spectrometer operating at 400 MHz and 100 MHz respectively. The absorption spectra were recorded on CYTATION 5 system (BIOTEK) with 2.8×10^{-5} M solutions in EtOH. Elemental analyses were measured by the Vario ELIII apparatus (Elementar Co.). Preparative thin layer chromatographies (PTLC) were done with prepared glass-backed plates (20×20 cm^2, 500 μ) using silica gel (Merck Kieselgel 60 $PF_{254+366}$). XRD measurements were performed on a Siemens D 500 diffractometer with Cu Kα radiation. The SEM images, mapping, and EDX were recorded with FEG-SEM MIRA3 TESCAN, Czech Republic at 30 kV. Vibrating sample magnetometry (VSM) was used to obtain magnetization measurements of samples at room temperature with a maximum magnetic field of 10 kOe (Maghnatis Daghigh Kavir Co., Iran). The Philips EM 208S microscope operating at an accelerating voltage of 100 KV was used to take TEM images. Phosphate buffer solution (PBS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. (Taufkirchen, Germany). Penicillin-streptomycin, trypsin-

EDTA (25%), Roswell Park Memorial Institute 1640 growth medium (RPMI), and Fetal bovine serum (FBS) were purchased from Gibco BRL Life Technologies (New York, USA). MCF-7 breast and HT-29 colon cancer and HEK 293 normal cell lines were provided from the standard cell banks of the National cell bank of Iran (NCBI) (Tehran, Iran). These cells were cultured in RPMI culture media supplemented by 10% FBS and 1% penicillin-streptomycin.

2. Experimental aspects and characterization of the synthesized products

Preparation of Fe_3O_4@Chitosan [2]

Chitosan powder (1 g) was added to a 1% (v/v) acetic acid solution (100 mL). The mixture was stirred vigorously until a homogeneous solution of chitosan was obtained. Then $FeCl_3 \cdot 6H_2O$ (2.5 g, 9.2 mmol) and $FeCl_2 \cdot 4H_2O$ (1 g, 5.0 mmol) were added. After stirring at 80 °C for 30 min, aqueous ammonia was added to the solution until the pH reached a value of 12. Then the solution was kept at 80 °C under vigorous stirring for a further 30 min. The dark brown precipitate was isolated by applying an external magnetic field, washed with deionized water (30 mL), and dried under vacuum at 60 °C for 6 h to afford Fe_3O_4@Chitosan nanoparticles (Fe_3O_4@CS NPs, 2 g).[2] Starch, cellulose, and pectin were also magnetized according to this method.[3-5]

Preparation of Fe_3O_4@Chitosan-SO_3H [6]

Fe_3O_4@CS NPs (1 g) is dispersed in dry CH_2Cl_2 (10 mL) in an ultrasonic bath for 30 min, then chlorosulfonic acid (1.6 mL) is added drop-wise over 10 min, at room temperature under N_2 atmosphere. Subsequently, the mixture is stirred for 30 min until HCl gas evolution is stopped. Finally, functionalized magnetic Fe_3O_4@CS-SO_3H NPs (Scheme 1) are separated by an external magnet, washed several times with dry CH_2Cl_2, until a neutral pH level is achieved, then dried under vacuum at room temperature. It is noteworthy that magnetic starch and cellulose were also sulfated in the same way,[7,8] but pectin was not sulfated due to its carboxylic acid groups.

Preparation of 1-alkyl-1H-pyrrol-2-carbaldehydes (1c, 1d)

A mixture of 1*H*-pyrrole-2-carbaldehyde **1a** (0.95 g, 10 mmol), alkyl halide [14 mmol, benzyl chloride (1.8 mL) or propargyl bromide (1.1 mL)] and K_2CO_3 (1.65 g, 12 mmol) in DMF (10 mL) was stirred at room temperature for 24 h. After completion of the reaction which was monitored by TLC (silica gel, EtOAc: *n*-hexane, 1:3), water (20 mL) was added and the mixture was extracted with EtOAc (3×10 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated under vacuum to give 1-benzyl-1*H*-pyrrol-2-carbaldehyde **1c** (1.5 g, 81% yield) as a yellow liquid [9] or 1-(2-propyn-1-yl)-1*H*-pyrrol-2-carbaldehyde **1d** (0.95 g, 71.5% yield) as a brown oily liquid.[10]

Synthesis of 1-(1-benzyl-1H-1,2,3-triazole-4-yl)methyl-1H-pyrrole-2-carbaldehyde (1e)

A mixture of 1-propargyl-pyrrole-2-carbaldehyde **1c** (1.33 g, 10 mmol), benzyl azide (1.33 g, 10 mmol) and CuI as catalyst (0.04 g, 2 % mol) in dried ethanol (10 mL) was stirred at 70 °C for 24 h. The hot mixture was filtered and slowly formed solid in the filtrate solution, was then separated. Recrystallization of the crude solid from ethanol gave the triazole derivative **1c** (1.7 g, 63.9 %) as a brown solid. m.p. 180-181 °C, **FT-IR (KBr, ν, cm^{-1}):** 3055, 2930, 2852, 2723, 1645, 1457, 1346, 1045. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm)**: 5.46 (s, 2H, CH_2), 5.59 (s, 2H, CH_2), 6.23 (dd, 1H, J = 4, 2.5 Hz, pyrrole-H), 6.92 (dd, 1H, J = 4, 1.6 Hz, pyrrole-H), 7.22-7.26 (m, 3H, Ar-H), 7.33-7.37 (m, 3H, Ar-H), 7.57 (s, 1H, triazole-H), 9.49 (d, J = 0.7 Hz, 1H, CHO). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm)**: 42.5, 53.2, 109.3, 121.8, 124.2, 127.0, 127.7, 128.1, 129.7, 130.9, 133.4, 143.3, 178.6. Anal. Calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04; Found: C, 67.39; H, 5.52; N, 20.88.

General procedure for the synthesis of isoxazole-5-(4H)-one derivatives (3a-k):

A mixture of aldehyde derivative **1a-e** (1 mmol), hydroxylamine hydrochloride (0.1 g, 1.5 mmol), *β*-keto esters (1.5 mmol), and Fe_3O_4@CS-SO_3H (0.04 g, 24 mol %) in solvent (10 mL) was stirred at room temperature (In the case of using methyl acetoacetate, the solvent was water, but for the reactions of ethyl benzoyl acetate, ethanol was used as solvent). After completion of the reaction, as monitored by TLC (silica gel, EtOAc : *n*-hexane, 1:2), the catalyst was separated from the mixture by an external magnet, and the solid product was

isolated by simple filtration and washed with water or ethanol (5 mL). The crude product was purified either by recrystallization from ethanol or by PTLC (EtOAc : *n*-hexane, 1:2) to give isoxazole-5-(4*H*)-one derivatives **3a-j**. The product **3k** was obtained by using 5-methylfuran-2-carboxaldehyde as an aldehyde component under the same conditions.

*4-[(1H-Pyrrole-2-yl) methylene]-3-methyl isoxzole-5-(4H)-one (**3a**):*

Reaction time: 2 h, recrystallization from ethanol gave dark brown solid (0.14 g, 80%); m.p. 185-187 ℃ (lit., 187-188 ℃).[11] **FT-IR (KBr, ν, cm^{-1})**: 3437, 3114, 2933, 1712, 1618, 1533, 1324, 1114, 1037. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 2.27 (s, 3H, CH_3), 6.51-6.53 (m, 1H, pyrrole-H), 7.02-7.04 (m, 1H, pyrrole-H), 7.25 (s, 1H, vinyl-H), 7.41-7.43 (m, 1H, pyrrole-H), 13.04 (b, 1H, NH). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 10.2, 107.4, 113.3, 126.0, 128.5, 130.5, 133.7, 159.9, 172.1. **UV-vis in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm:** 290, 413. Anal. Calcd for $C_9H_8N_2O_2$: C, 61.36; H, 4.58; N, 15.90; Found: C, 61.51; H, 4.80; N, 16.13.

*4-[(1H-Pyrrole-2-yl) methylene]-3-phenyl isoxzol-5-(4H)-one (**3b**):*

Reaction time: 4 h, purification by PTLC gave yellow solid (0.17 g, 72%); m.p. 144-146℃. **FT-IR (KBr, ν, cm^{-1})**: 3440, 2964, 1710, 1631, 1531, 1324, 1094, 1028, 803. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 6.52-6.54 (m, 1H, pyrrole-H), 7.00-7.01 (m, 1H, pyrrole-H), 7.40 (s, 1H, vinyl-H), 7.46 (b, 1H, pyrrole-H), 7.52-7.61 (m, 5H), 13.20 (b, 1H, NH). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 106.1, 113.6, 126.8, 126.9, 127.5, 128.2, 129.6, 129.8, 131.1, 135.7, 162.9, 172.4. **UV-vis in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm:** 279, 421. Anal. Calcd for $C_{14}H_{10}N_2O_2$: C, 70.58; H, 4.23; N, 11.76; Found: C, 70.29; H, 4.41; N, 11.59.

*4-[(1-Methyl-1H-Pyrrole-2-yl) methylene]-3-methyl isoxzole-5-(4H)-one (**3c**):*

Reaction time: 2 h, recrystallization from ethanol gave brownish orange solid (0.16 g, 83%); m.p. 211-212 ℃ (lit., 209-210 ℃).[11] **FT-IR (KBr, ν, cm^{-1})**: 3108, 2922, 1710, 1602, 1490, 1390, 1302, 1078, 1029, 874. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 2.25 (s, 3H, CH_3), 3.85 (s, 3H, N-CH_3), 6.41-6.43 (m, 1H, pyrrole-H), 7.13 (b, 1H, pyrrole-H), 7.16 (s, 1H, vinyl-H), 8.57 (dd, J = 4.2, 1.4 Hz, 1H, pyrrole-H). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 10.6, 33.6, 108.1, 111.7, 125.7, 128.5, 129.8, 133.5, 159.7, 168.4. **UV-vis in**

EtOH 2.8×10^{-5} mole/L, λ_{max}, nm: 276, 423. Anal. Calcd for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73; Found: C, 62.84; H, 5.46; N, 14.52.

*4-[(1-Methyl-1H-Pyrrole-2-yl) methylene]-3-phenyl isoxzol-5-(4H)-one (**3d**):*

Reaction time: 2 h, purification by PTLC gave brown solid (0.18 g, 70%); m.p.169-170 ℃. **FT-IR (KBr, ν, cm^{-1})**: 3115, 2924, 1726, 1625, 1463, 1389, 1286, 1116, 1053. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 3.71 (s, 3H, CH_3), 6.42-6.43 (m, 1H, pyrrole-H), 7.15 (b, 1H, pyrrole-H), 7.38 (s, 1H, vinyl-H), 7.52-7.60 (m, 5H, ph-H), 8.61 (dd, *J* = 4.4, 1.4 Hz, 1H, pyrrole-H). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 33.4, 106.7, 111.9, 126.1, 127.3, 127.5, 128.2, 128.8, 129.5, 132.1, 134.1, 162.9, 168.7. **UV-vis in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm:** 271, 425. Anal. Calcd for $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10; Found: C, 71.19; H, 4.51; N, 11.38.

*4-[(1-benzyl-1H-Pyrrole-2-yl) methylene]-3-methyl isoxzol-5-(4H)-one (**3e**):*

Reaction time: 2 h, recrystallization from ethanol gave orange solid (0.24 g, 89%); m.p. 170-171℃. **FT-IR (KBr, ν, cm^{-1})**: 3119, 2922, 2854, 1725, 1603, 1472, 1398, 1345, 1304, 1132, 1031, 867. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 1.99 (s, 3H, CH_3), 5.35 (s, 2H, CH_2), 6.48-6.49 (m, 1H, pyrrole-H), 7.07-7.10 (m, 3H), 7.26 (b, 1H, vinyl-H), 7.32-7.39 (m, 3H, ph-H), 8.58-8.59 (m, 1H, pyrrole-H). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 10.2, 50.7, 108.3, 111.8, 125.2, 126.1, 127.5, 127.7, 128.2, 130.6, 133.2, 135.2, 159.8, 168.3. **UV-vis in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm:** 276, 419. Anal. Calcd for $C_{16}H_14N_2O_2$: C, 72.17; H, 5.30; N, 10.52; Found: C, 72.37; H, 5.53; N, 10.23.

*4-[(1-benzyl-1H-Pyrrole-2-yl) methylene]-3-phenyl isoxzol-5-(4H)-one (**3f**)*

Reaction time: 2 h, recrystallization from ethanol gave yellow solid (0.26 g, 80%); m.p. 189-191℃. **FT-IR (KBr, ν, cm^{-1})**: 3124, 1735, 1593, 1472, 1351, 1307, 1150, 1078, 864. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 5.22 (s, 2H, CH_2), 6.51-6.53 (m, pyrrole-H), 6.88-6.91 (m, 2H), 7.15 (d, *J* = 7.2 Hz, 1H), 7.30-7.38 (m, 6H), 7.47-7.51 (m, 1H), 8.68-8.69 (m, 1H, pyrrole-H). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 50.9, 107.3, 107.1, 112.0, 124.9, 126.7, 127.1, 127.3, 127.5, 127.9, 128.0, 129.2, 132.9, 133.7, 135.1, 162.8, 168.6. **UV-vis**

in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm: 312, 423. Anal. Calcd for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53; Found: C, 76.68; H, 4.72; N, 8.31.

*4-{[1-(2-propyn-1-yl)-1H-pyrrol-2-yl] methylene}-3-methyl isoxzol-5-(4H)-one (**3g**):*

Reaction time: 2 h, recrystallization from ethanol gave yellow solid (0.18 g, 85%); m.p. 184-188 °C. **FT-IR (KBr, ν, cm^{-1})**: 3263, 3121, 2924, 2855, 1733, 1601, 1472, 1398, 1353, 1296, 1141, 1085, 872, 710. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 2.26 (s, 3H, CH_3), 2.57 (t, *J* = 2.5 Hz, 1H, acetylene-H), 4.90 (d, *J* = 2.4 Hz, 2H, CH_2), 6.44-6.46 (m, 1H, pyrrole-H), 7.25 (broad, 1H, pyrrole-H), 7.36 (s, 1H, vinyl-H), 8.57-8.59 (m, pyrrole-H). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 10.4, 36.4, 74.7, 75.5, 109.2, 111.9, 126.2, 127.6, 129.9, 131.9, 159.8, 168.2. **UV-vis in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm:** 303, 415. Anal. Calcd for $C_{12}H_{10}N_2O_2$: C, 63.15; C, 67.28; H, 4.71; N, 13.08; Found: C, 63.37; H, 4.96; N, 12.75.

*4-{[1-(2-propyn-1-yl)-1H-pyrrol-2-yl] methylene}-3-phenyl isoxzol-5-(4H)-one (**3h**):*

Reaction time: 3 h, recrystallization from ethanol gave brown solid (0.22 g, 80%), m.p. 151-152 °C. **FT-IR (KBr, ν, cm^{-1})**: 3267, 3109, 2924, 2853, 2124, 1721, 1588, 1470, 1344, 1297, 1132, 1078, 869, 687. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 2.53 (t, *J* = 2.3 Hz, 1H, acetylene-H), 4.76 (d, *J* = 2.3 Hz, 2H, CH_2), 6.46-6.48 (m, 1H, pyrrole-H), 7.53-7.63 (m, 7H), 8.65-8.67 (m, 1H, pyrrole-H). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 36.3, 74.4, 75.4, 107.8, 112.1, 126.8, 127.2, 127.6, 127.9, 128.1, 129.6, 132.2, 132.5, 162.9, 168.5. **UV-vis in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm:** 296, 421. Anal. Calcd for $C_{17}H_{12}N_2O_2$: C, 73.90; H, 4.38; N, 10.14; Found: C, 73.78; H, 4.41; N, 11.97.

*4-{[1-(1-benzyl-1H-1, 2, 3-triazol-4-yl) methyl-1H-pyrrol-2-yl] methylene}-3-methyl isoxazol-5-(4H)-one (**3i**):*

Reaction time: 2 h, purification by PTLC gave yellow solid (0.25 g, 71%); m.p. 202-205 °C. **FT-IR (KBr, ν, cm^{-1})**: 3106, 2924, 2853, 1715, 1605, 1466, 1344, 1289, 1125, 1079, 859. **^{1}H NMR (400 MHz, DMSO, δ, ppm):** 2.19 (s, 3H, CH_3), 5.57 (s, 2H, CH_2), 5.65 (s, 2H, CH_2), 6.47-6.49 (m, 1H, pyrrole-H), 7.25-7.36 (m, 5H, ph-H), 7.74 (b, 1H, pyrrole-H), 7.83 (s, 1H, vinyl-H), 8.13 (s, 1H, triazole-H), 8.34-8.35 (m, 1H, pyrrole-H). **^{13}C NMR (100 MHz, DMSO, δ, ppm):** 11.1, 41.9, 52.9, 112.6, 123.6, 125.7, 127.9, 128.2, 128.3,

128.7, 134.2, 135.4, 135.4, 135.8, 143.5, 161.7, 169.2. **UV-vis in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm:** 279, 416. Anal. Calcd for $C_{18}H_{15}N_5O_2$: C, 64.86; H, 4.54; N, 21.01; Found: C, 64.39; H, 4.66; N, 21.29.

*4-{[1-(1-benzyl-1H-1, 2, 3-triazol-4-yl) methyl-1H-pyrrol-2-yl] methylene}-3-phenyl isoxazol-5-(4H)-one (**3j**):*

Reaction time: 2 h, recrystallization from ethanol gave yellow solid (0.33 g, 81%); m.p. 175-177 ℃. **FT-IR (KBr, ν, cm^{-1})**: 3133, 2920, 2852, 1728, 1644, 1593, 1476, 1354, 1295, 1129, 1082, 866. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 5.26 (s, 2H, CH_2), 5.47 (s, 2H, CH_2), 6.44 (b, pyrrole-H), 7.16-7.55 (m, 13H), 8.62 (b, 1H, pyrrole-H). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 42.3, 53.4, 108.5, 112.2, 126.5, 126.5, 127.0, 127.6, 127.8, 128.1, 128.2, 128.2, 129.5, 132.6, 132.7, 132.8, 132.8, 133.0, 162.9. Anal. **UV-vis in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm:** 315, 423. Calcd for $C_{23}H_{17}N_5O_2$: C, 69.86; H, 4.33; N, 17.71; Found: C, 69.59; H, 4.52; N, 17.86.

*4-[(5-Methyl-furan-2-yl) methylene]-3-phenyl isoxzol-5-(4H)-one (**3k**):*

Reaction time: 1 h, recrystallization from ethanol gave orange solid (0.21 g, 85%); m.p. 133-136 ℃ (lit., 179-181 ℃).[11] **FT-IR (KBr, ν, cm^{-1})**: 3136, 2924, 1734, 1610, 1487, 1356, 1223, 1023, 854. **^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):** 2.45 (s, 3H, CH_3), 6.44 (d, J = 3.6 Hz, 1H, furan-H), 7.47 (s, 1H, vinyl-H), 7.52-7.61 (m, 5H, ph-H), 8.65 (d, J = 3.6 Hz, 1H, furan-H). **^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):** 13.4, 109.7, 112.2, 126.8, 127.1, 127.4, 128.2, 128.3, 129.7, 132.5, 148.7, 161.4, 162.3. **UV-vis in EtOH 2.8×10^{-5} mole/L, λ_{max}, nm:** 276, 412. Anal. Calcd for $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53; Found: C, 70.83; H, 4.09; N, 5.21.

Table 1. Absorption data of synthesized products

Product	λ_{max} (nm)	Absorption coefficient (L. cm^{-1}.Mol^{-1})
3a	290, 413	35850, 14771
3b	279, 421	12500, 44285
3c	276, 423	64678, 48767
3d	271, 425	3850, 15675
3e	276, 419	10714, 40714
3f	312, 423	1196, 9846
3g	303, 415	1807, 20767
3h	296, 421	5303, 18067
3i	279, 416	6150, 5717
3j	315, 423	2967, 20292
3k	276, 412	38953, 18135

FT-IR, ^{1}H NMR, ^{13}C NMR and UV-vis spectra of the target products

1-(1-Benzyl-1*H*-1,2,3-triazole-4-yl) methyl-1*H*-pyrrole-2-carbaldehyd (1e):

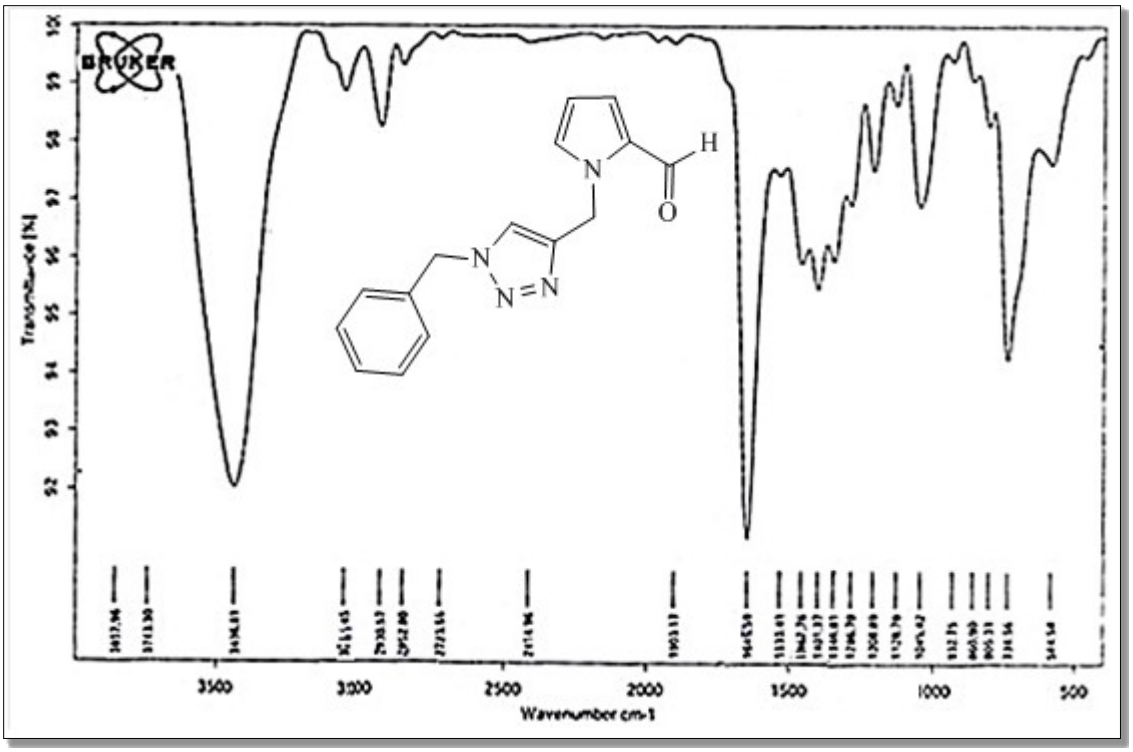


Figure 1: FTIR spectrum (KBr) of compound **1e**.

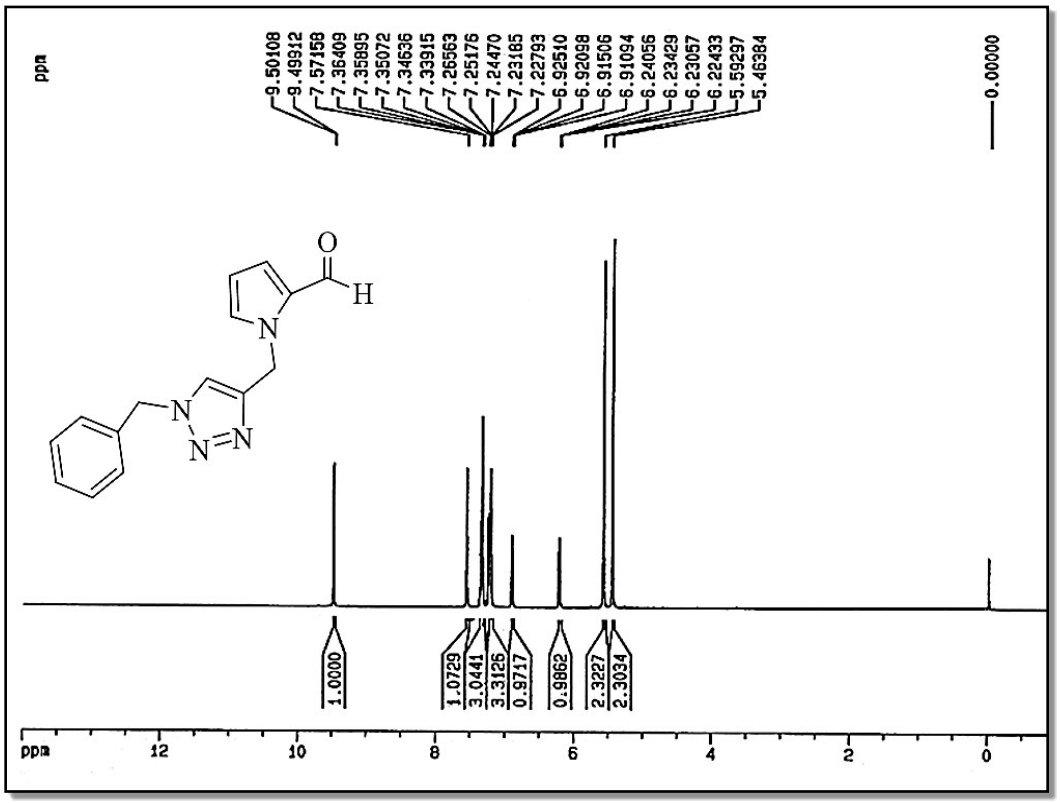


Figure 2: ^{1}H NMR spectrum (400 MHz) of compound **1e** in $CDCl_3$.

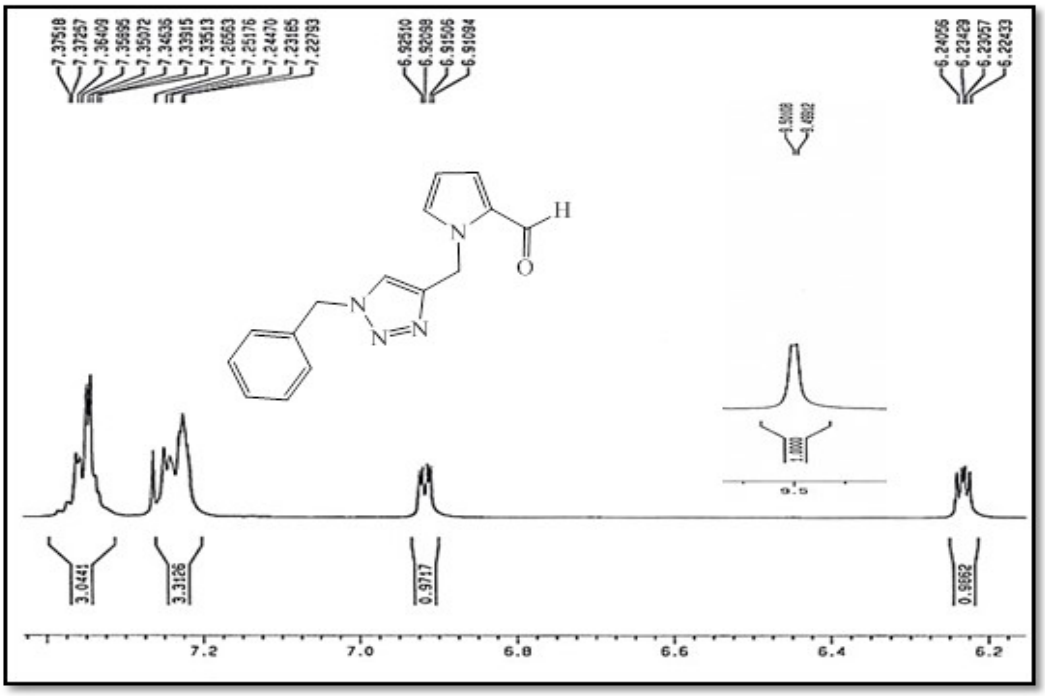


Figure 3: Expanded ^{1}H NMR spectrum (400 MHz) of compound **1e** in $CDCl_3$.

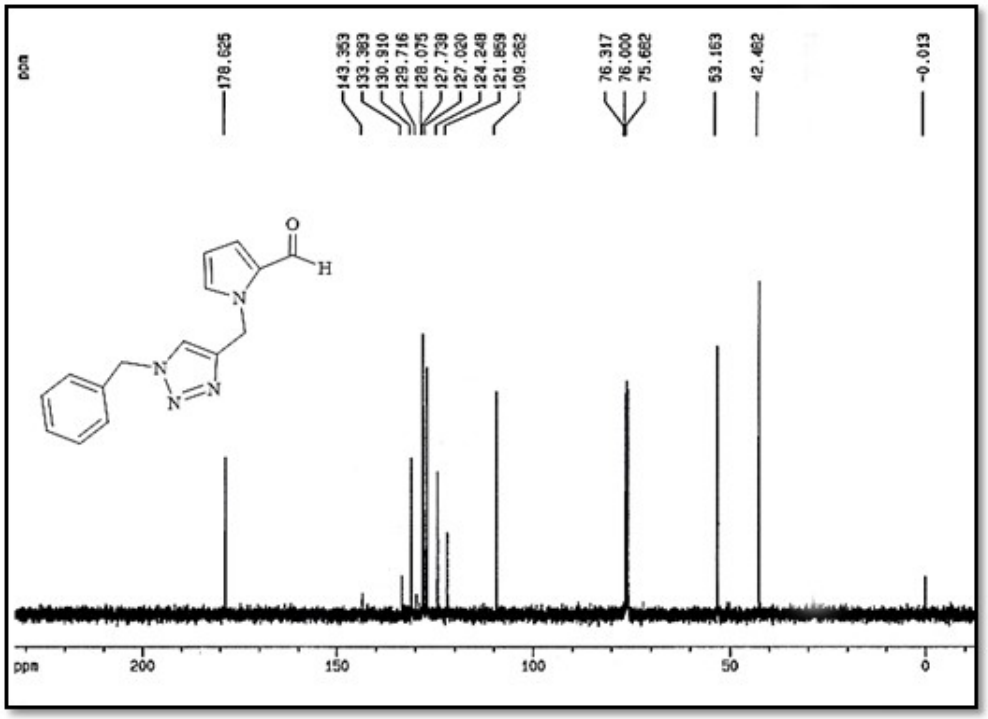


Figure 4: ^{13}C NMR spectrum (100 MHz) of compound **1e** in $CDCl_3$.

4-[(1*H*-Pyrrole-2-yl) methylene]-3-methyl isoxzol-5-(4*H*)-one (3a):

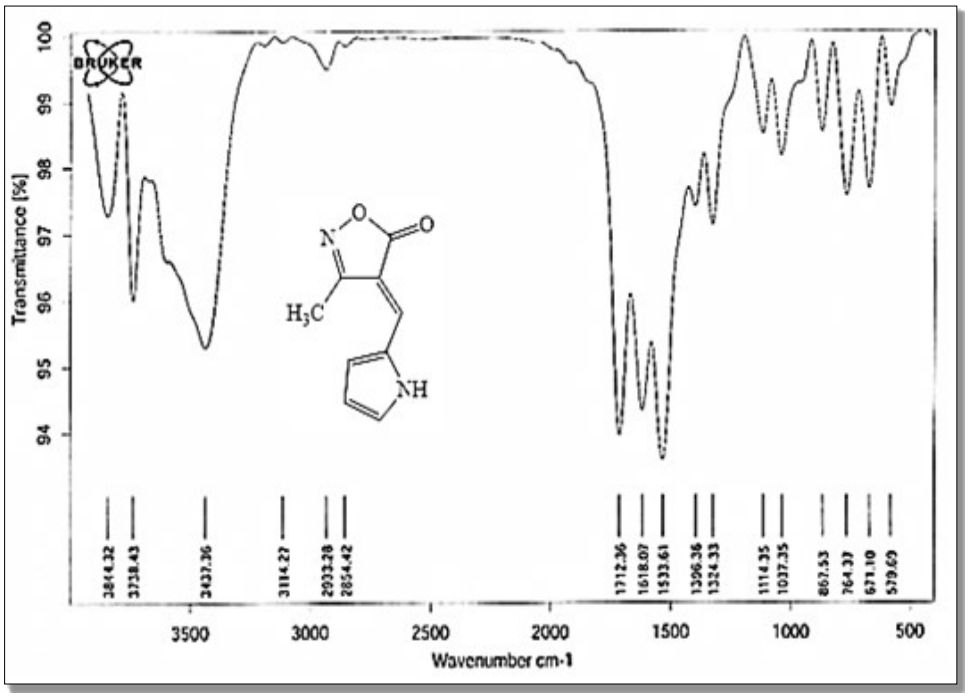


Figure 5: FTIR spectrum (KBr) of compound **3a**.

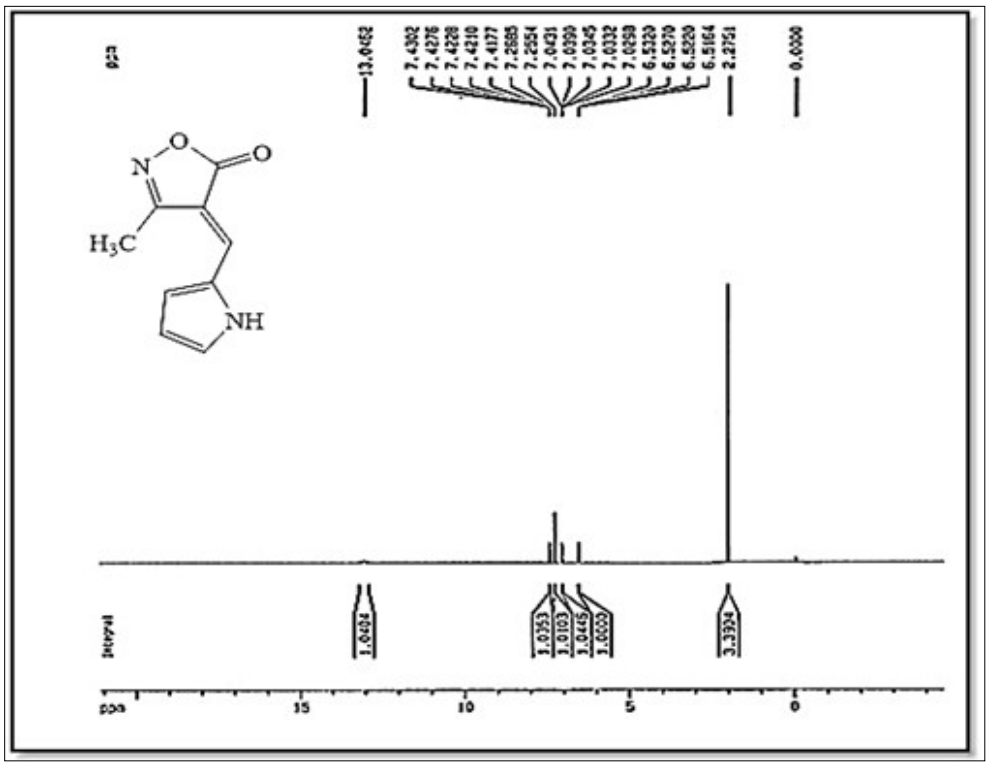


Figure 6: ^{1}H NMR spectrum (400 MHz) of compound **3a** in $CDCl_3$.

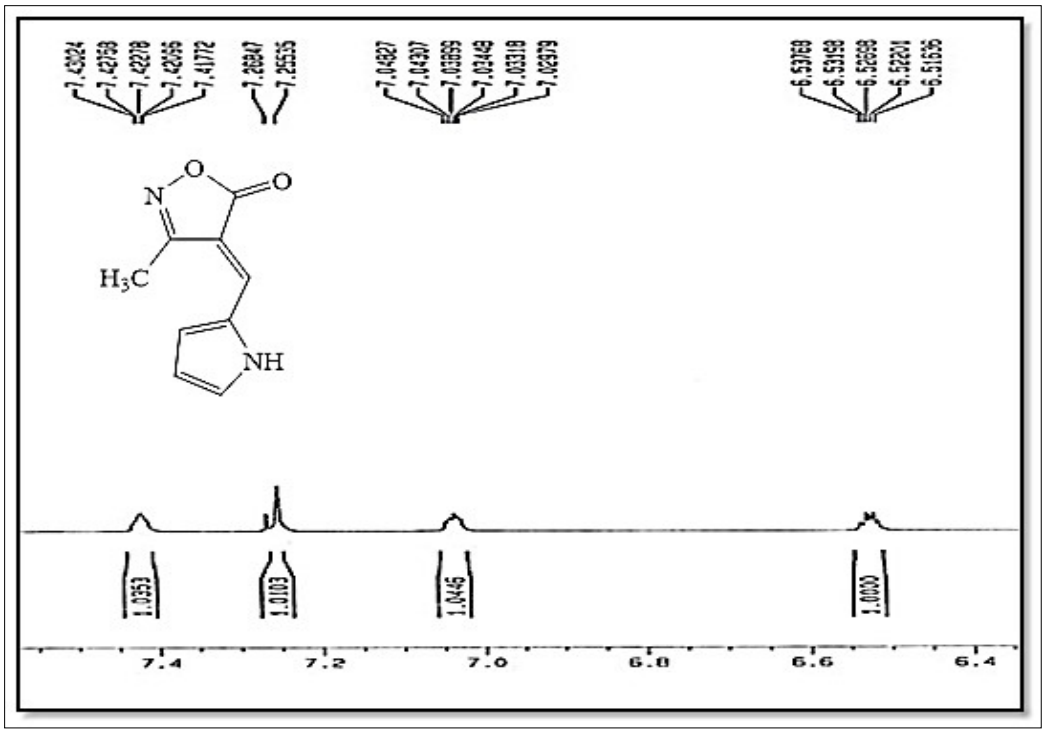


Figure 7: Expanded ^{1}H NMR spectrum (400 MHz) of compound **3a** in $CDCl_3$.

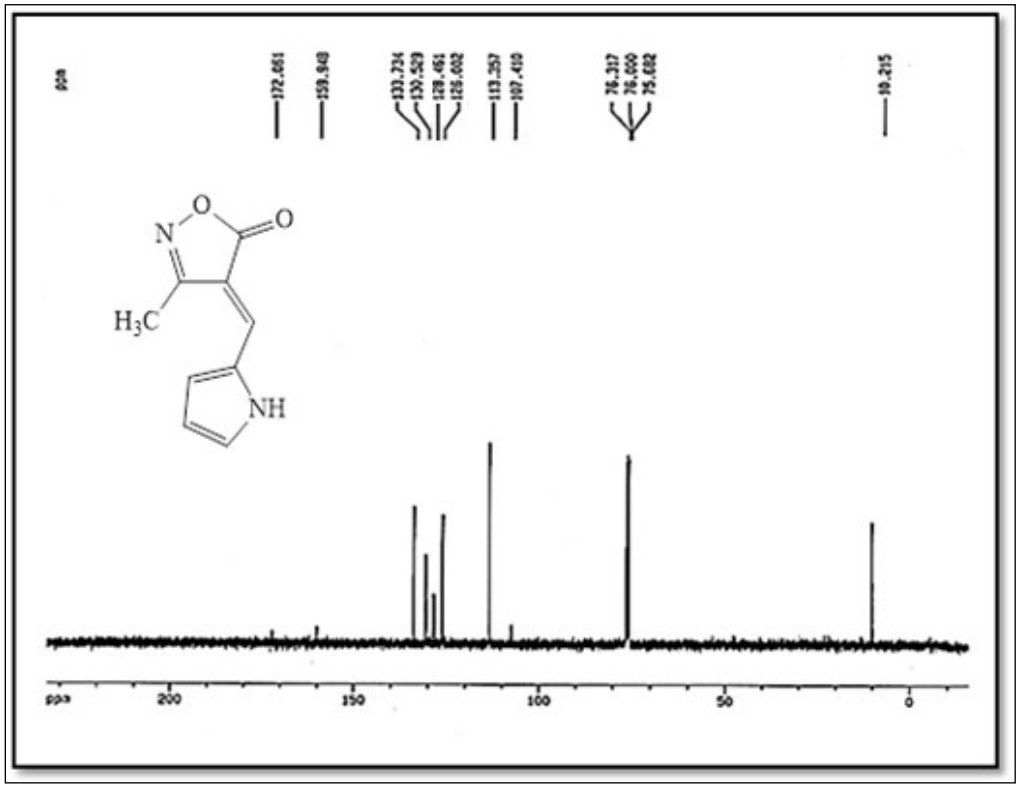


Figure 8: ^{13}C NMR spectrum (100 MHz) of compound **3a** in $CDCl_3$.

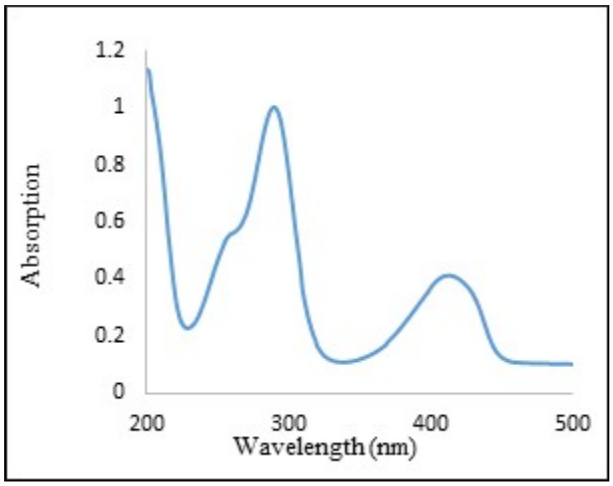


Figure 9: UV-Vis Spectrum of compound **3a** ($2.8\mathcal{S}10^{-5}$ M) in EtOH.

4-[(1*H*-Pyrrole-2-yl) methylene]-3-phenyl isoxzol-5-(4*H*)-one (3b):

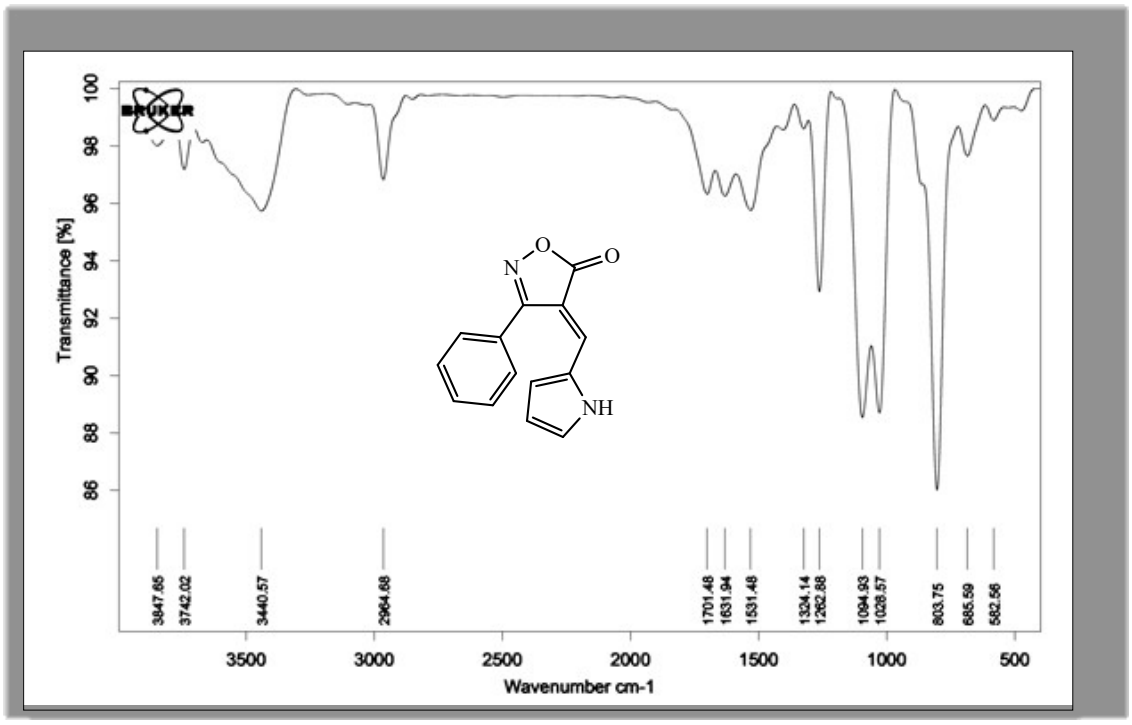


Figure 10: FTIR spectrum (KBr) of compound **3b**.

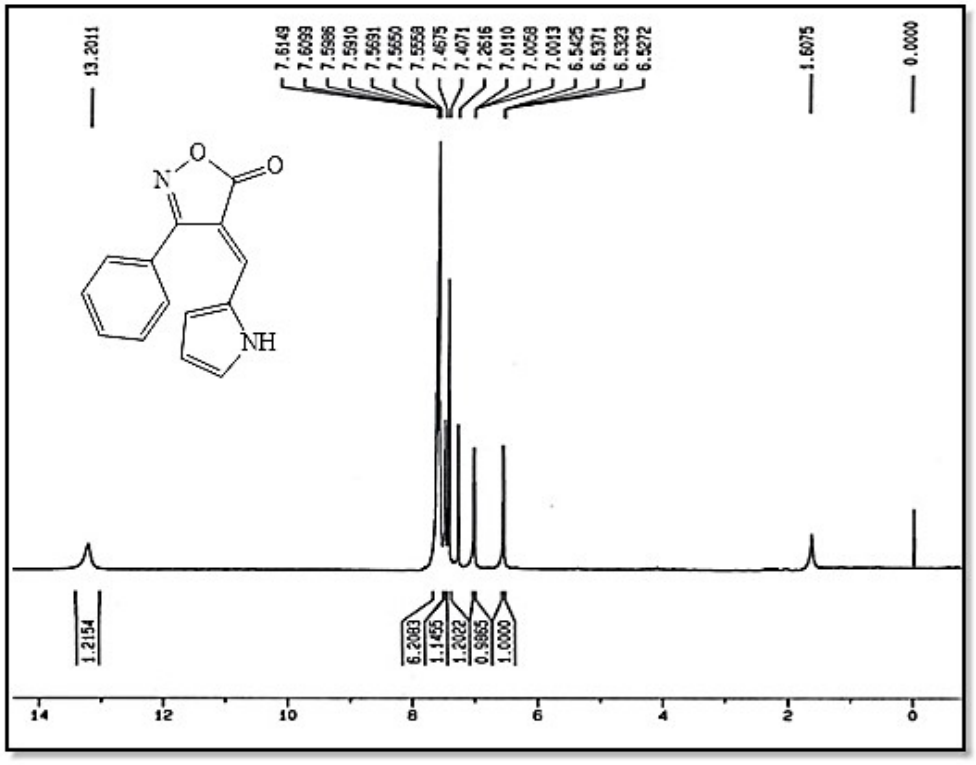


Figure 11: ^{1}H NMR spectrum (400 MHz) of compound **3b** in $CDCl_3$.

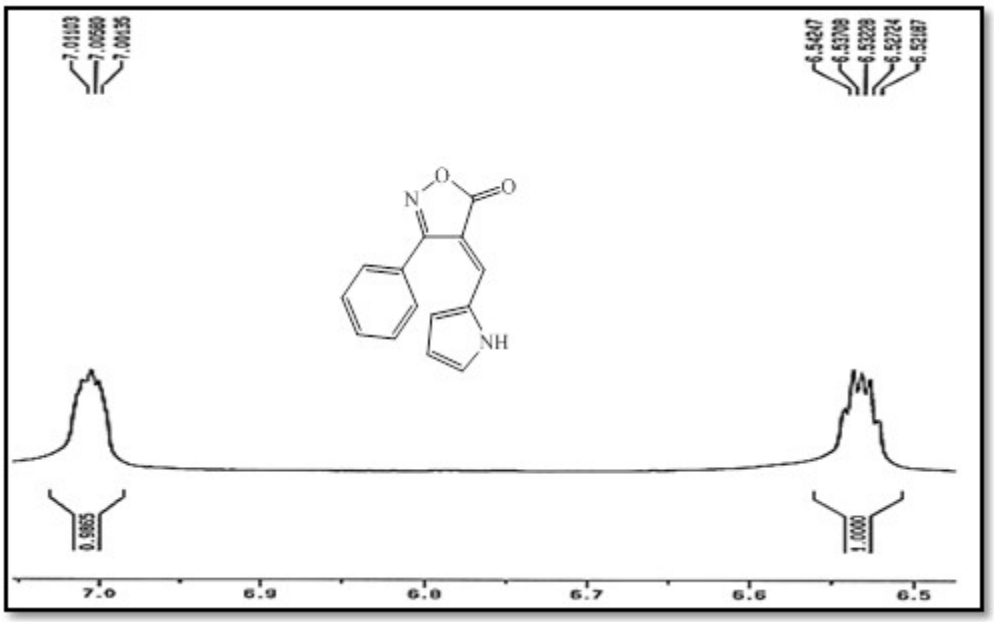


Figure 12: Expanded (1) ^{1}H NMR spectrum (400 MHz) of compound **3b** in $CDCl_3$.

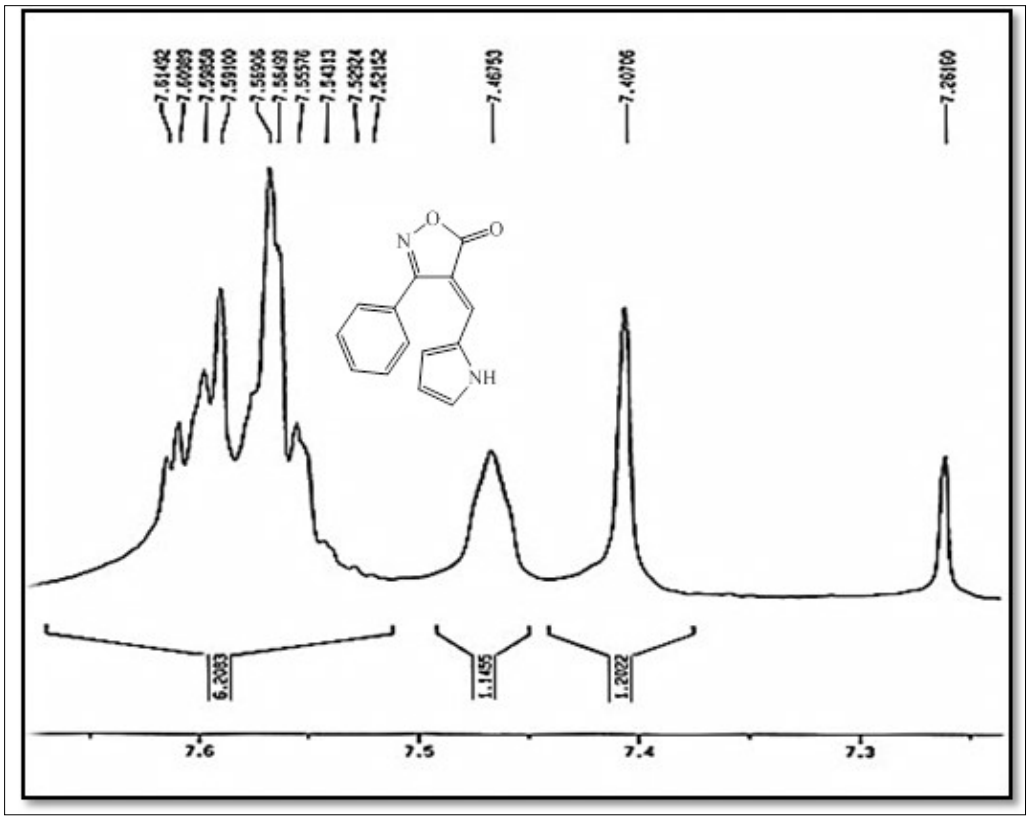


Figure 13: Expanded (2) ^{1}H NMR spectrum (400 MHz) of compound **3b** in $CDCl_3$.

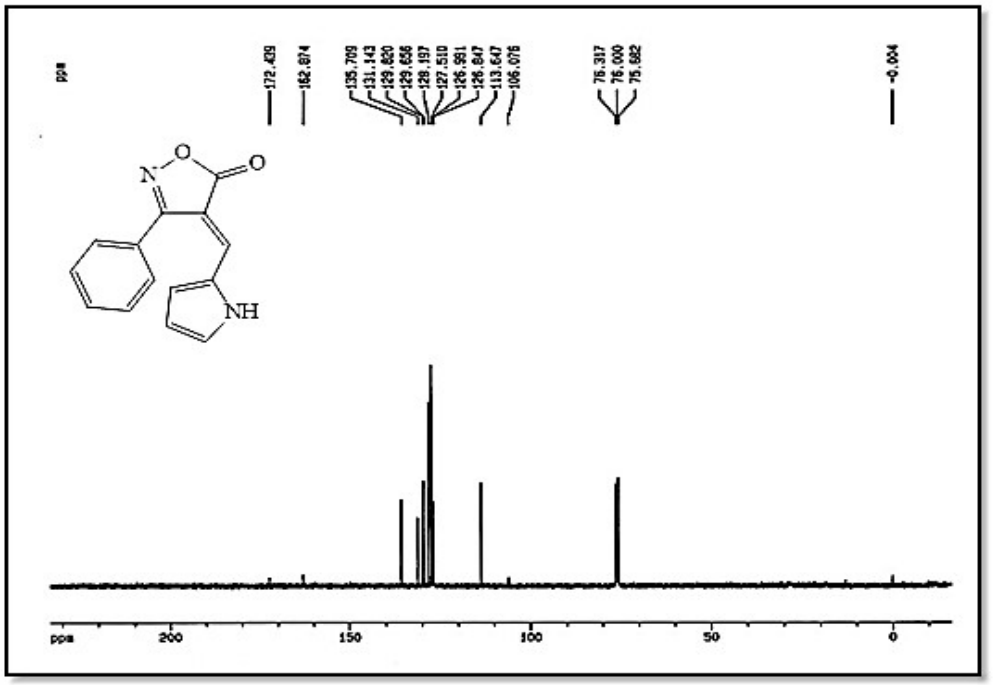


Figure 14: ^{13}C NMR spectrum (100 MHz) of compound **3b** in $CDCl_3$.

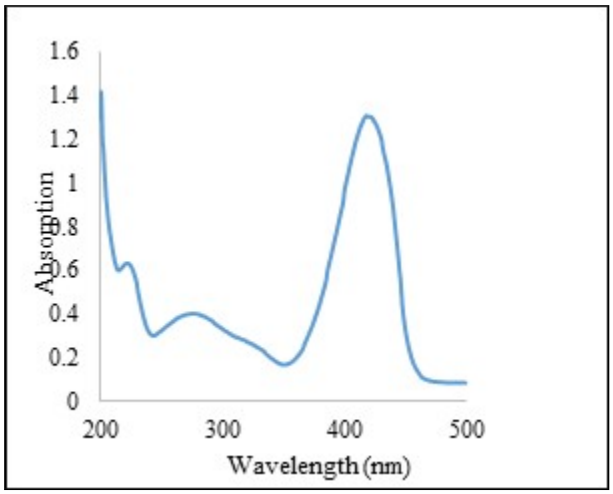


Figure 15: UV-Vis Spectrum of compound **3b** (2.8×10^{-5} M) in EtOH.

4-[(1-Methyl-1*H*-pyrrole-2-yl) methylene]-3-methyl isoxzol-5-(4*H*)-one (3c):

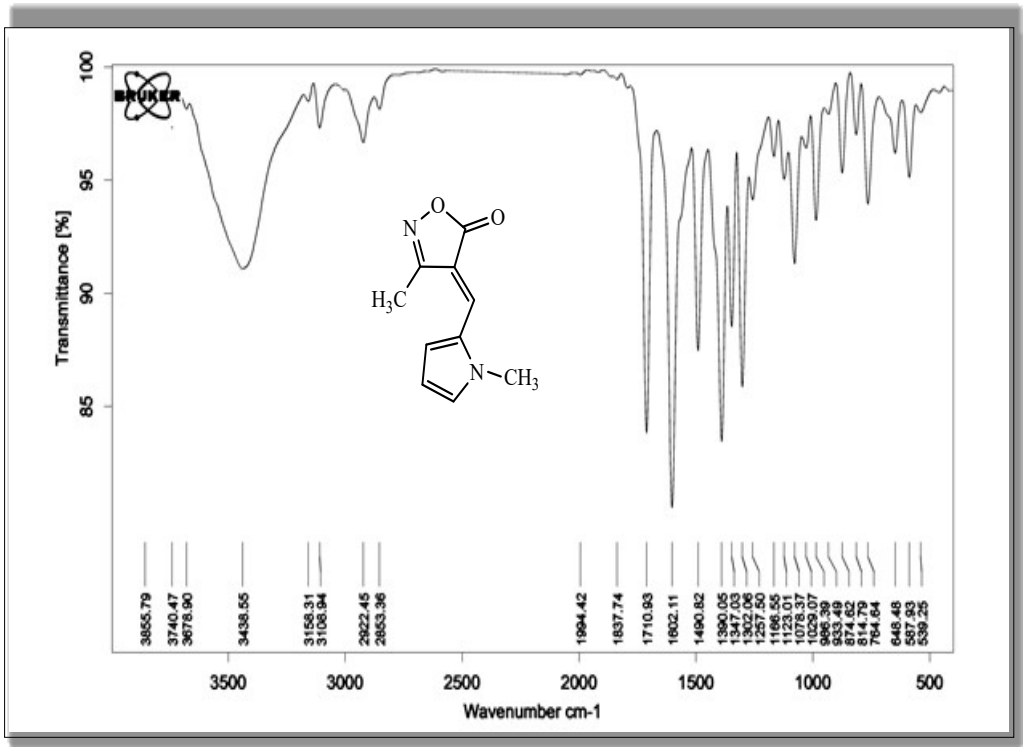


Figure 16: FTIR spectrum (KBr) of compound **3c**.

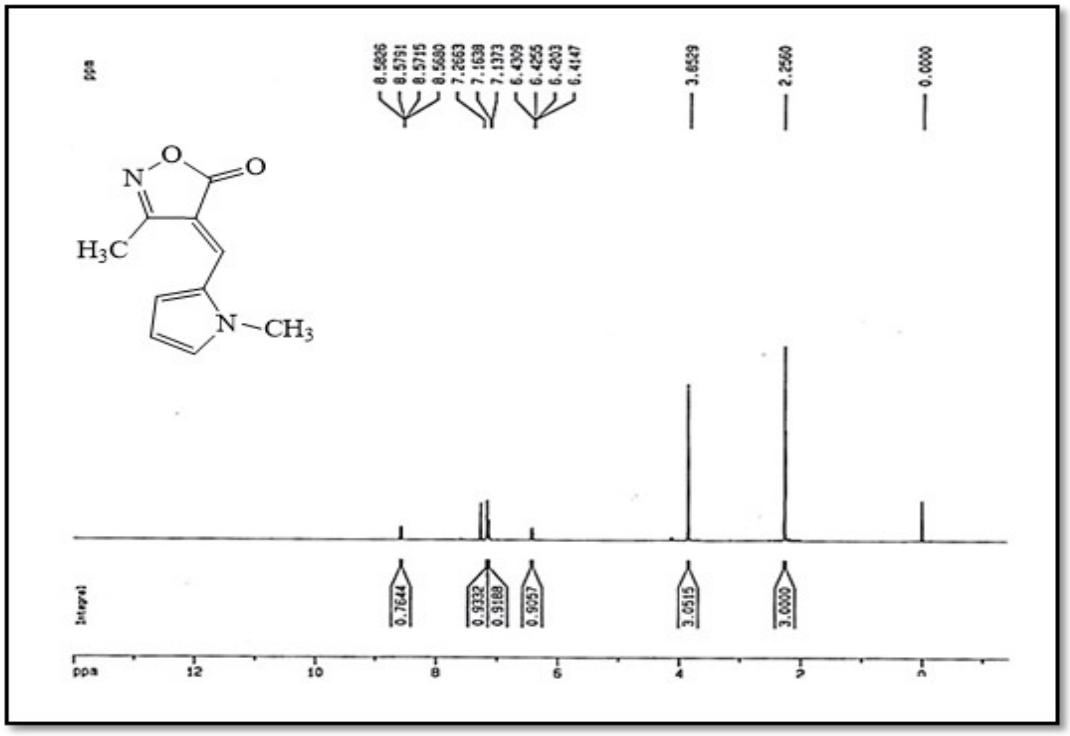


Figure 17: ^{1}H NMR spectrum (400 MHz) of compound **3c** in $CDCl_3$.

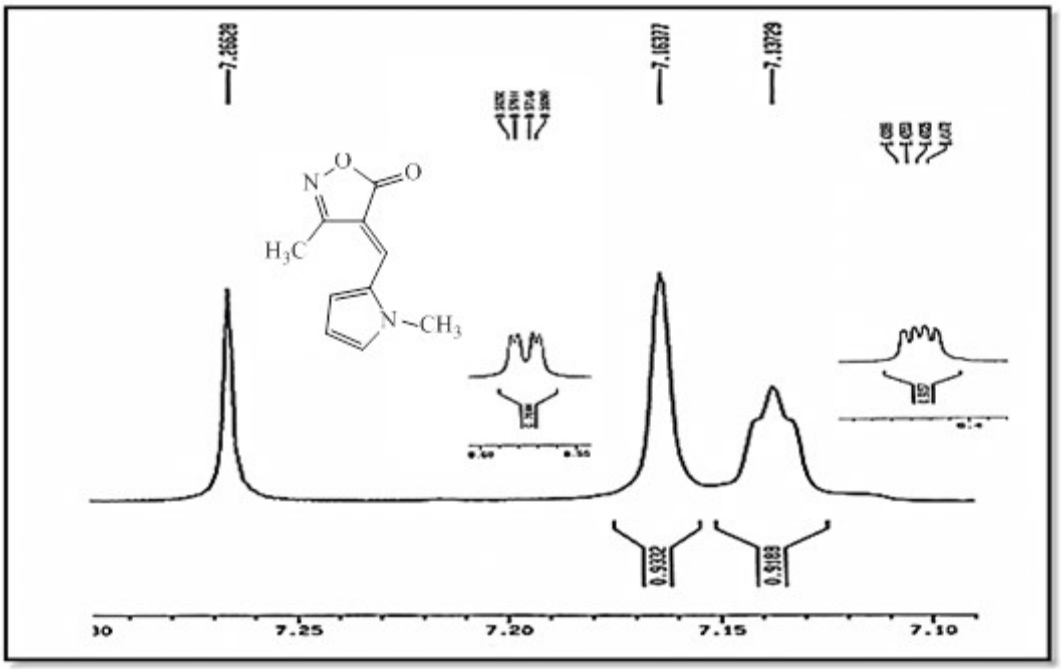


Figure 18: Expanded ^{1}H NMR spectrum (400 MHz) of compound **3c** in $CDCl_3$.

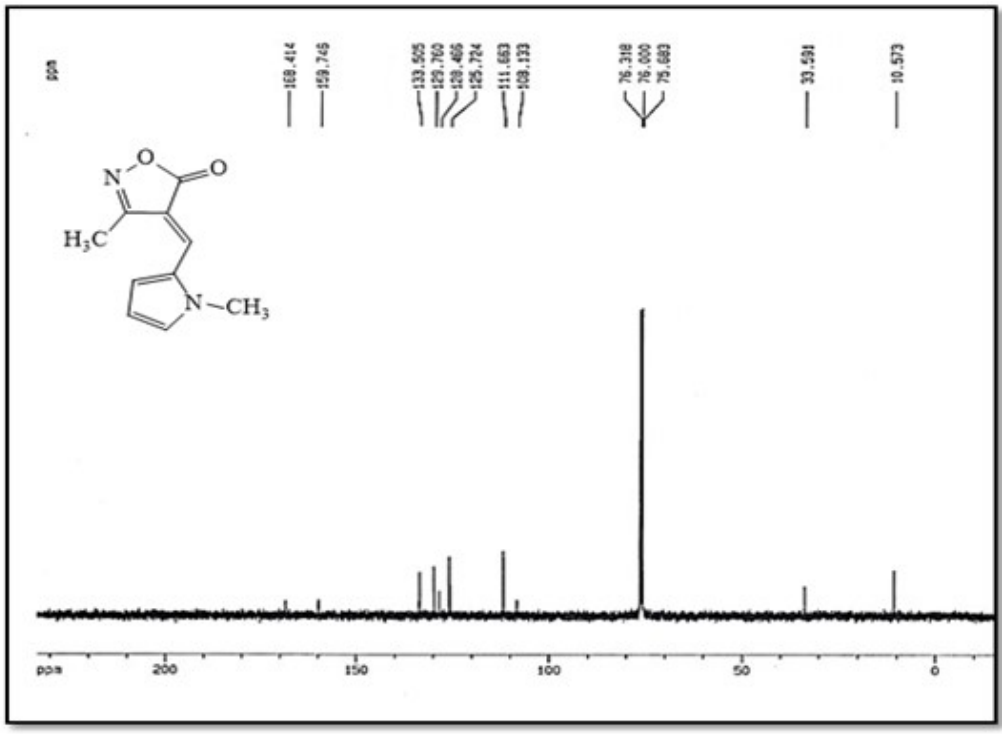


Figure 19: ^{13}C NMR spectrum (100 MHz) of compound **3c** in $CDCl_3$.

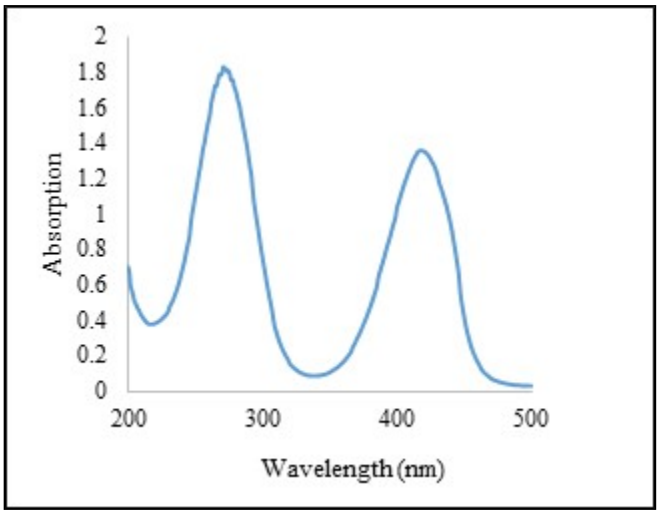


Figure 20: UV-Vis Spectrum of compound **3c** (2.8×10^{-5} M) in EtOH.

4-[(1-Methyl-1*H*-pyrrole-2-yl) methylene]-3-phenyl isoxzol-5-(4*H*)-one (3d):

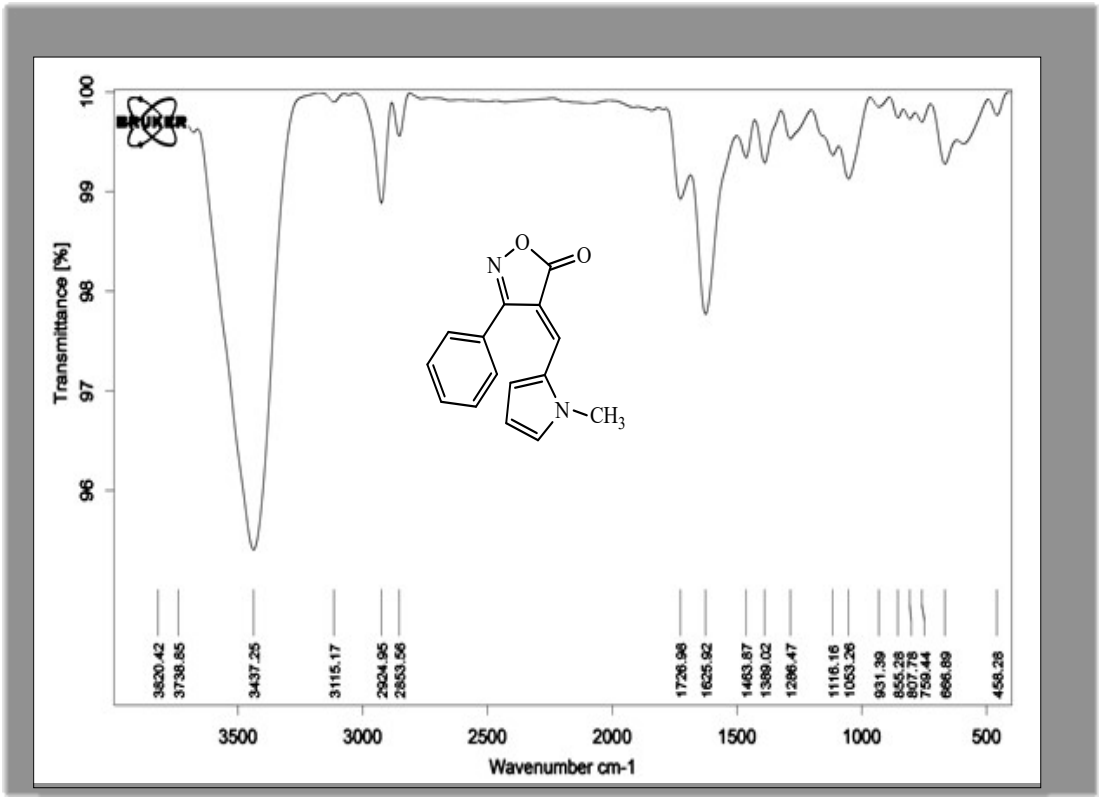


Figure 21: FTIR spectrum (KBr) of compound **3d**.

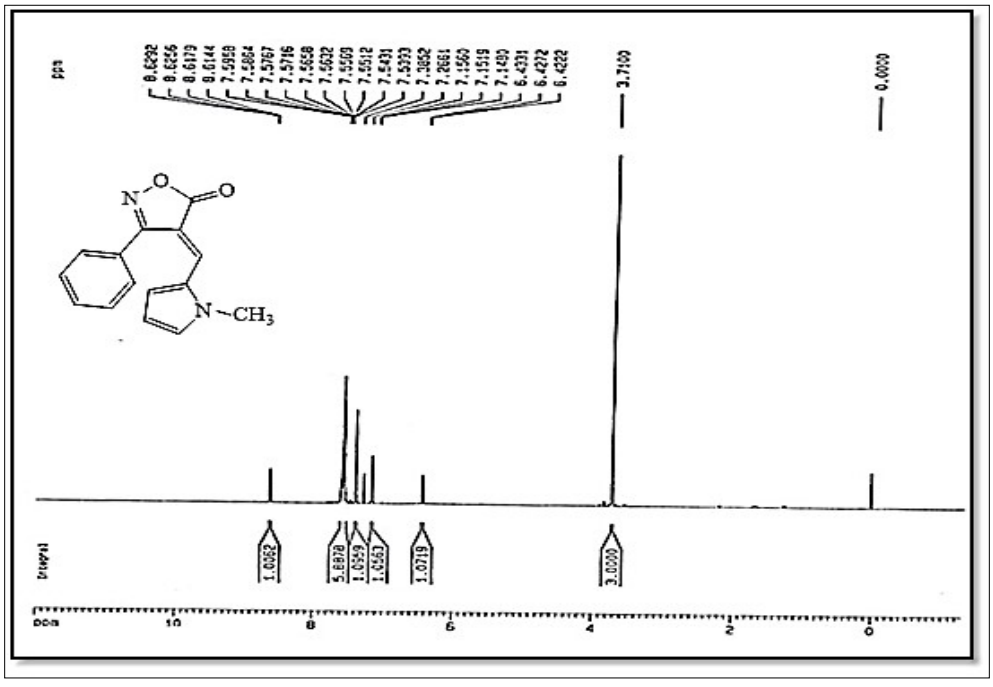


Figure 22: ^{1}H NMR spectrum (400 MHz) of compound **3d** in $CDCl_3$.

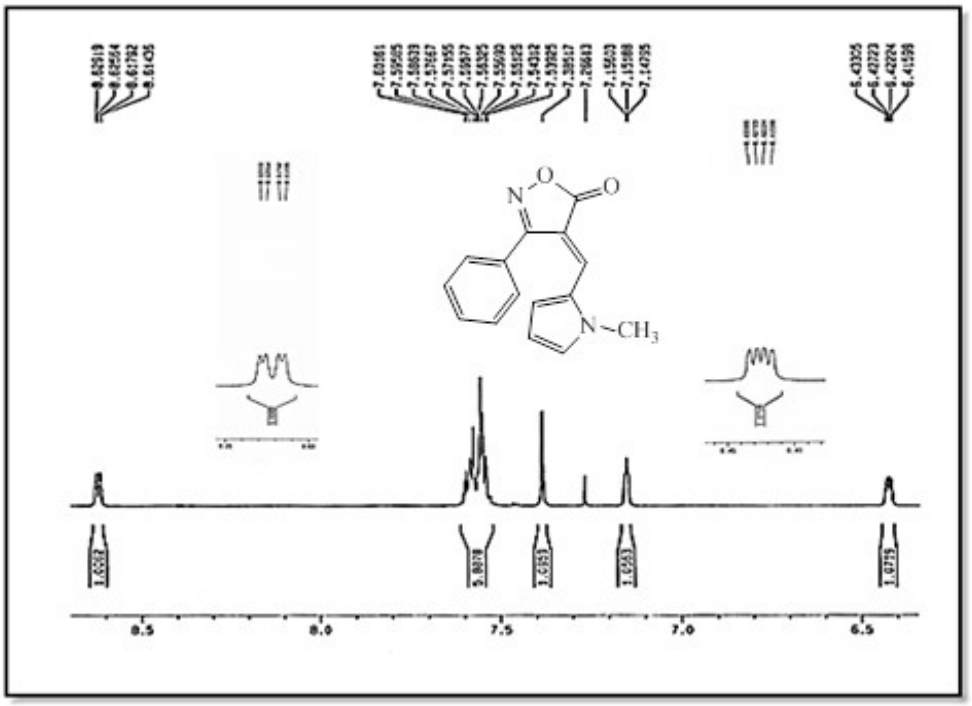


Figure 23: Expanded (1) ^{1}H NMR spectrum (400 MHz) of compound **3d** in $CDCl_3$.

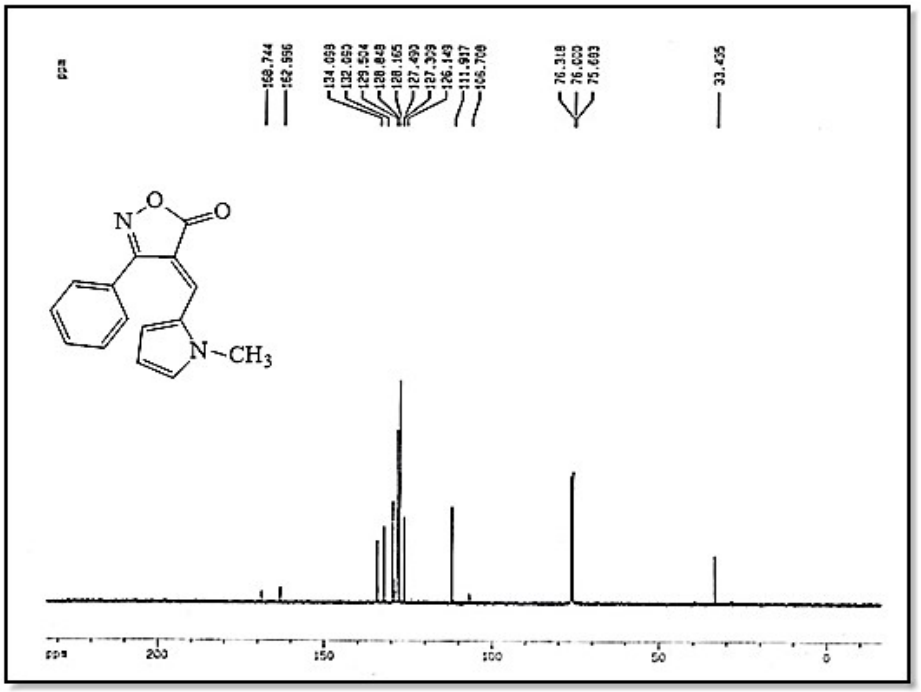


Figure 24: ^{13}C NMR spectrum (100 MHz) of compound **3d** in $CDCl_3$.

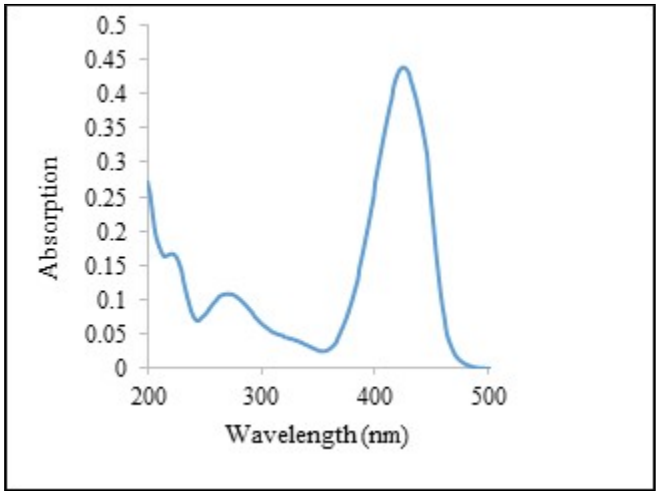


Figure 25: UV-Vis Spectrum of compound **3d** (2.8ß10^{-5} M) in EtOH.

4-[(1-Benzyl-1*H*-pyrrole-2-yl) methylene]-3-methyl isoxzol-5-(4*H*)-one (3e):

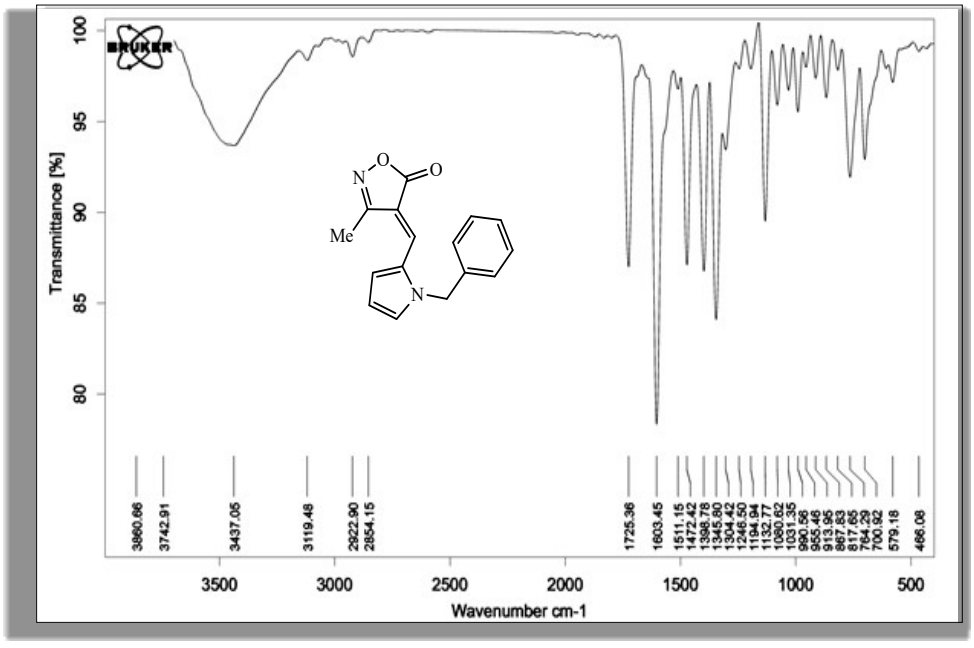


Figure 26: FTIR spectrum (KBr) of compound **3e.**

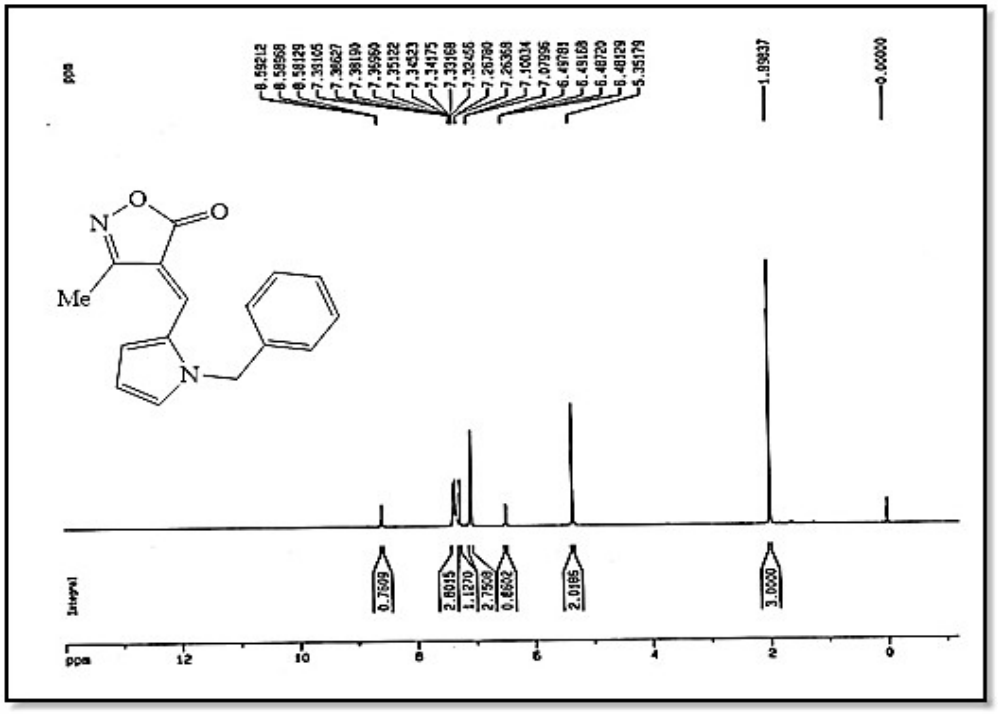


Figure 27: ^{1}H NMR spectrum (400 MHz) of compound **3e** in $CDCl_3$.

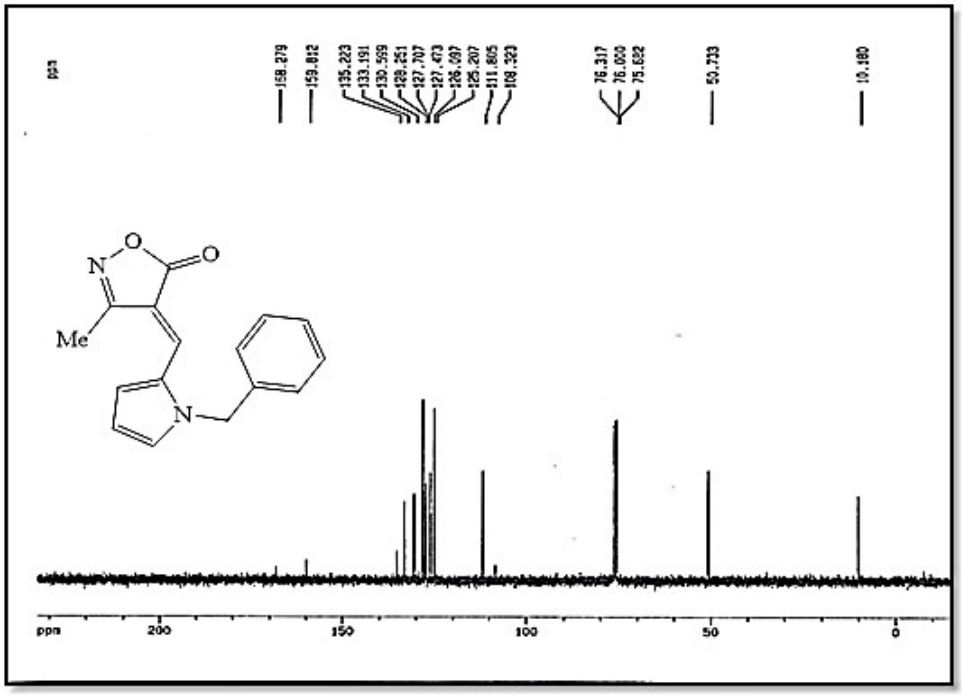


Figure 28: ^{13}C NMR spectrum (100 MHz) of compound **3e** in $CDCl_3$.

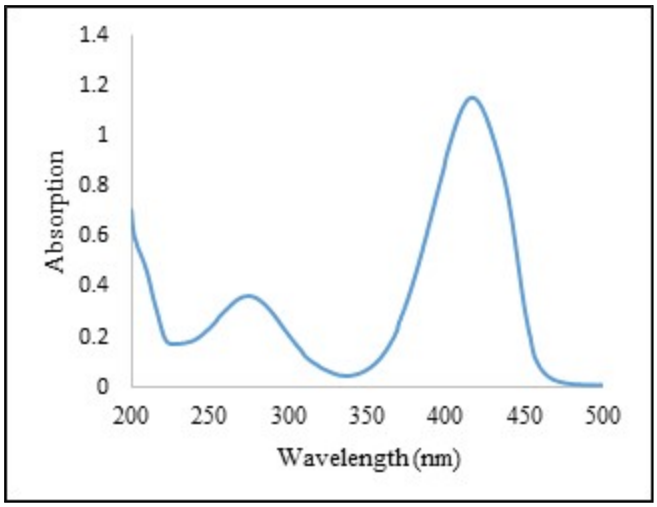


Figure 29: UV-Vis Spectrum of compound **3e** (2.8×10^{-5} M) in EtOH.

4-[(1-Benzyl-1*H*-pyrrole-2-yl) methylene]-3-phenyl isoxzol-5-(4*H*)-one (3f):

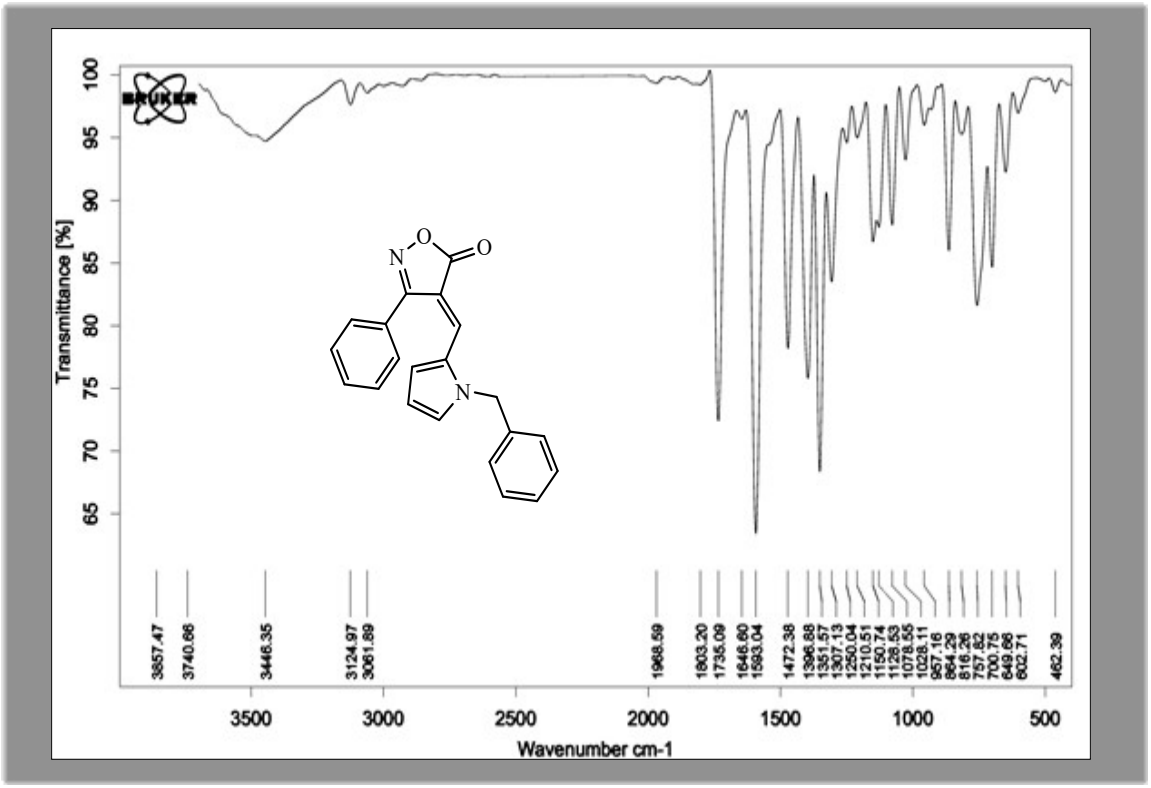


Figure 30: FTIR spectrum (KBr) of compound **3f**.

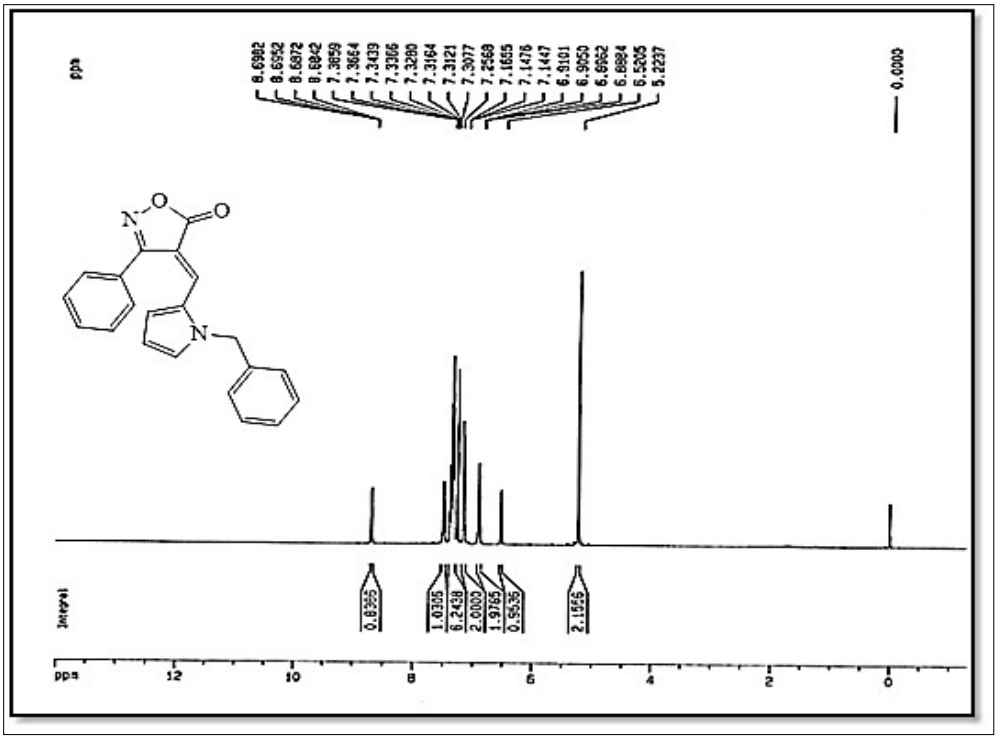


Figure 31: ^{1}H NMR spectrum (400 MHz) of compound **3f** in $CDCl_3$.

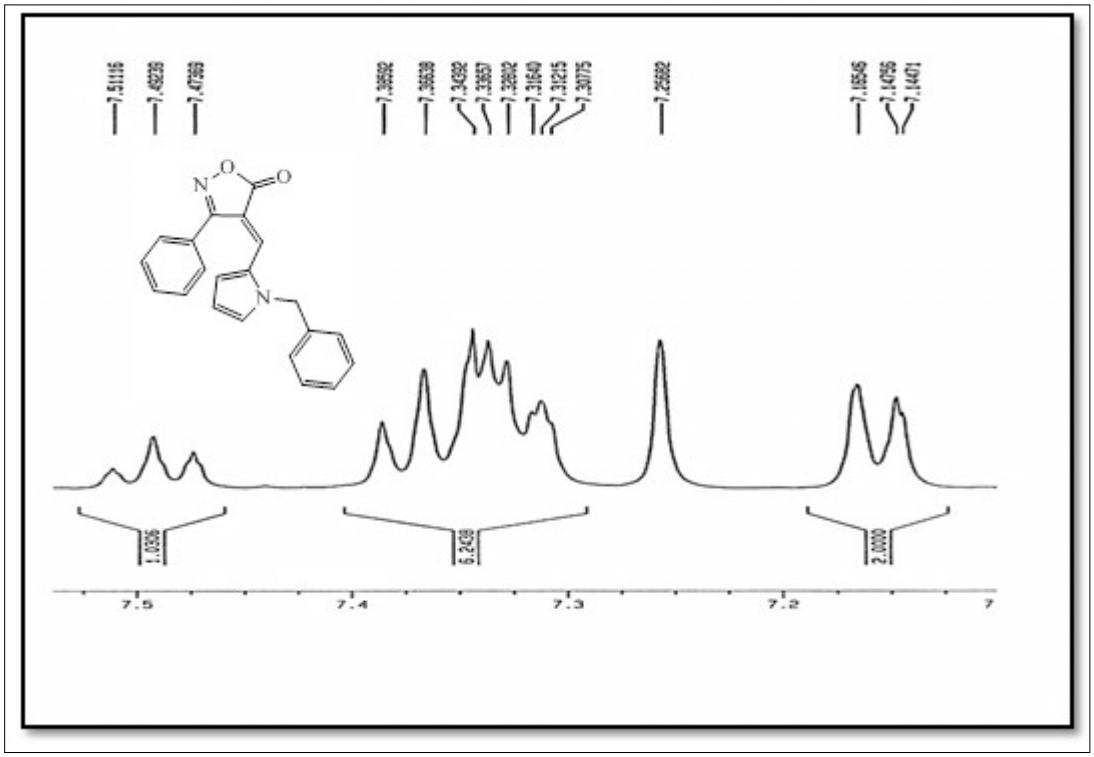


Figure 32: Expanded (1) ^{1}H NMR spectrum (400 MHz) of compound **3f** in $CDCl_3$.

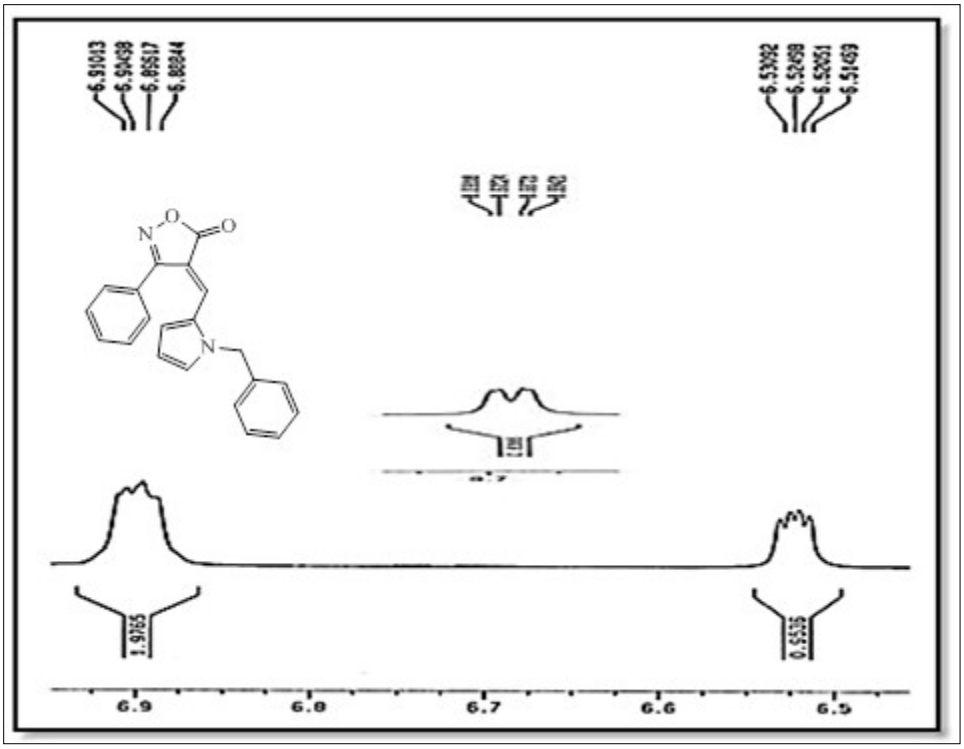


Figure 33: Expanded (2) ^{1}H NMR spectrum (400 MHz) of compound **3f** in $CDCl_3$.

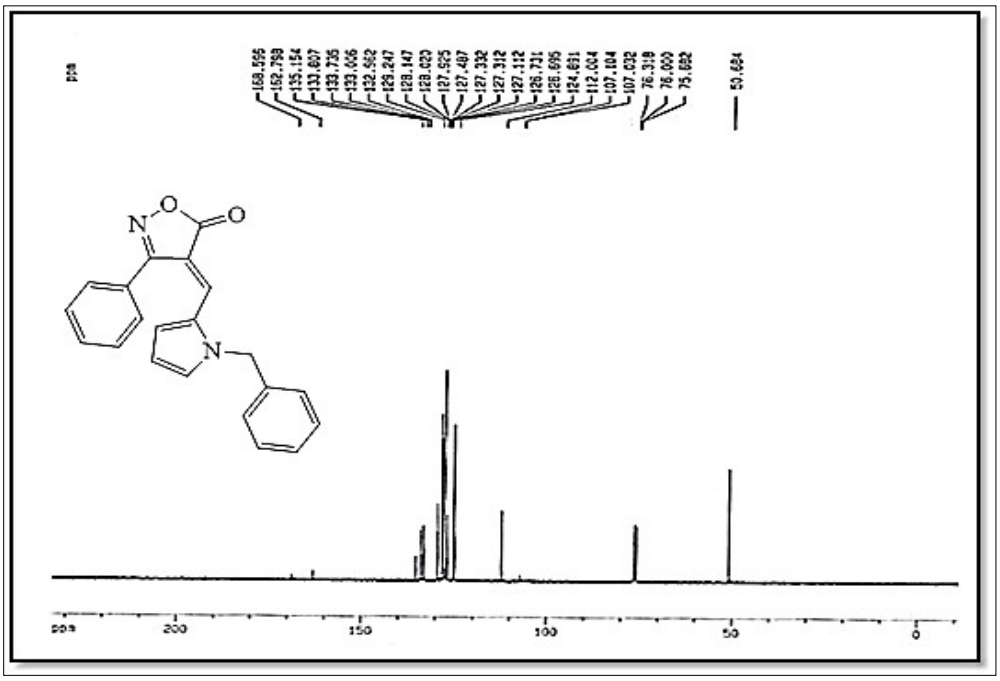


Figure 34: ^{13}C NMR spectrum (100 MHz) of compound **3f** in $CDCl_3$.

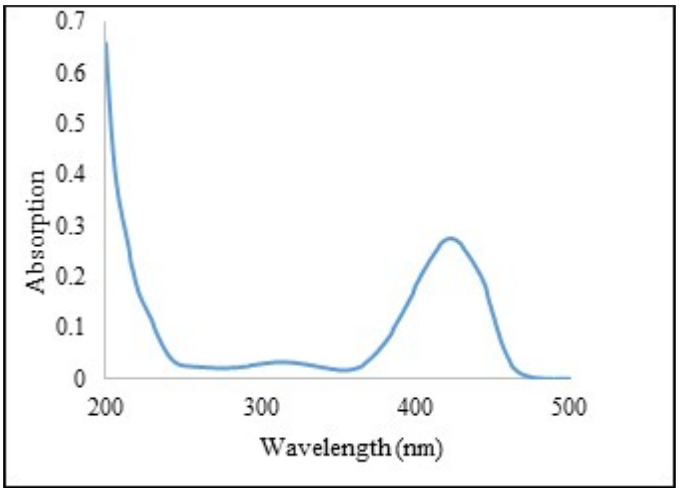


Figure 35: UV-Vis Spectrum of compound **3f** (2.8×10^{-5} M) in EtOH.

4-{[1-(2-Propyn-1-yl)-1*H*-pyrrol-2-yl] methylene}-3-methyl isoxzol-5-(4*H*)-one (3g):

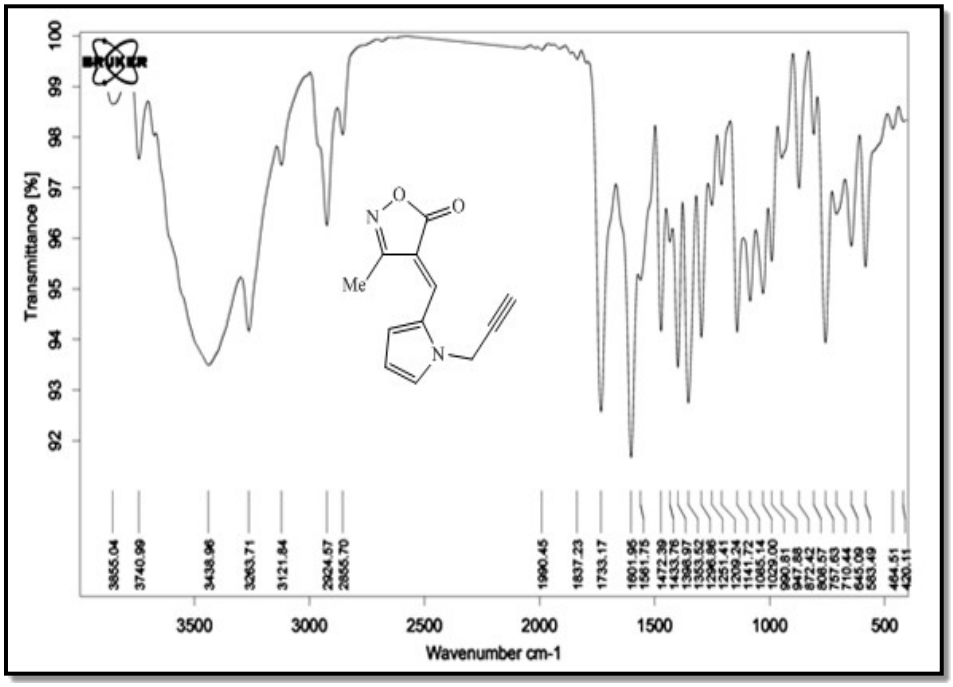


Figure 36: FTIR spectrum (KBr) of compound **3g**.

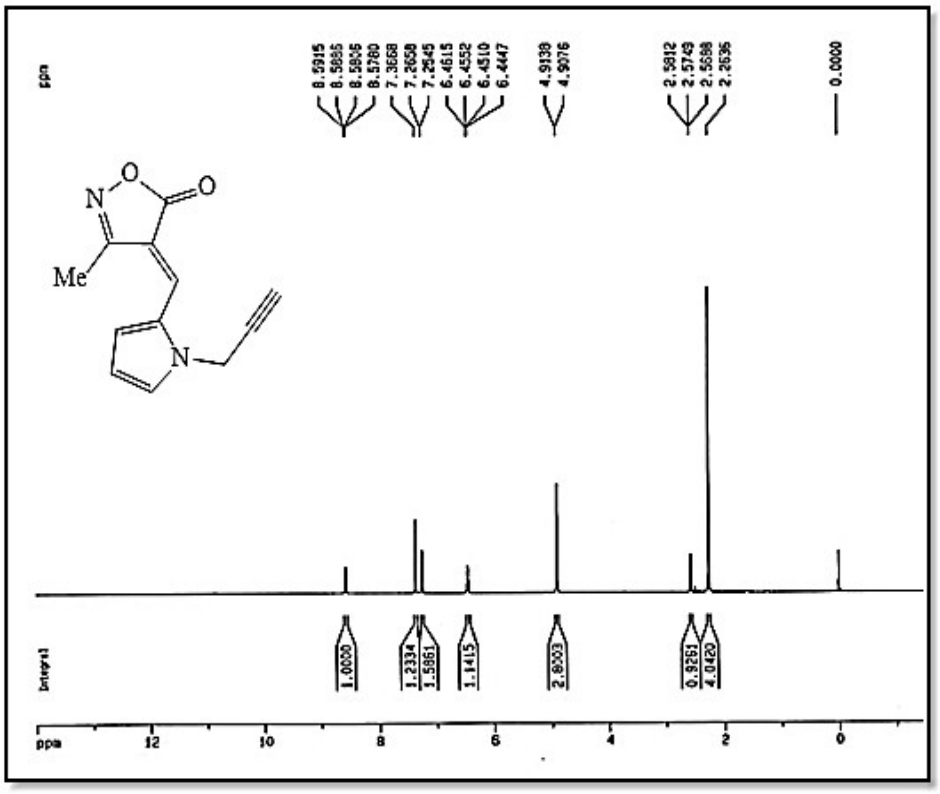


Figure 37: ^{1}H NMR spectrum (400 MHz) of compound **3g** in $CDCl_3$.

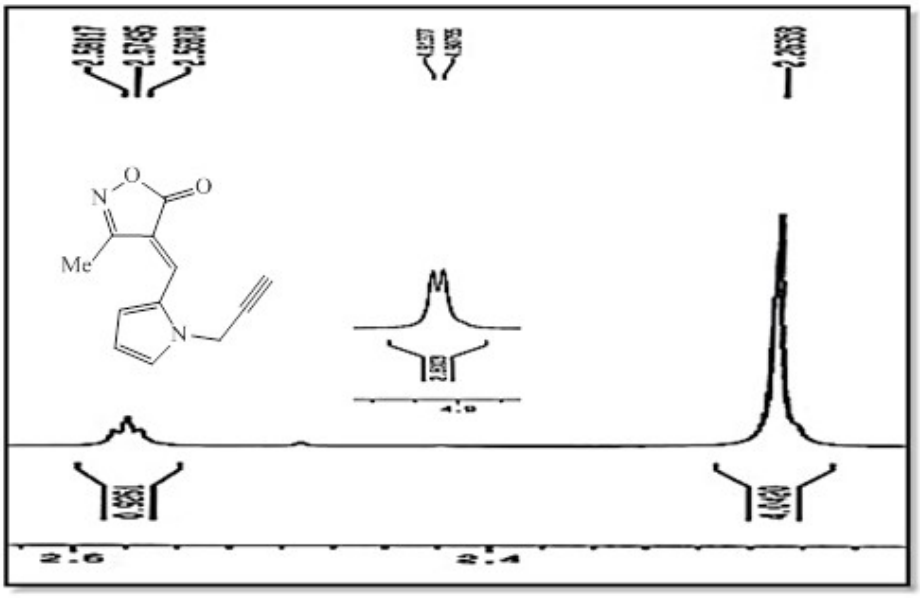


Figure 38: Expanded (1) ^{1}H NMR spectrum (400 MHz) of compound **3g** in $CDCl_3$.

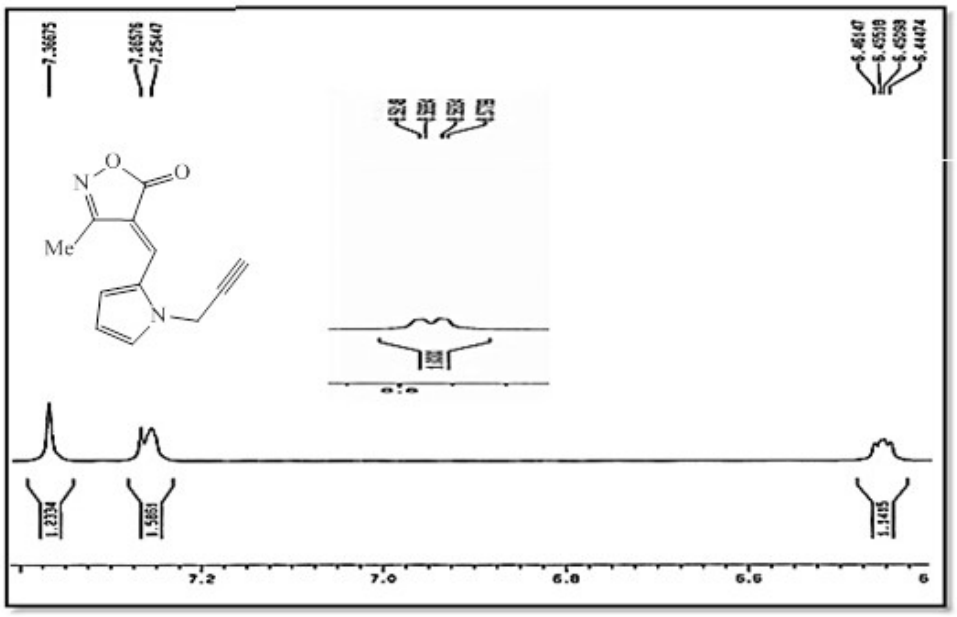


Figure 39: Expanded (2) ^{1}H NMR spectrum (400 MHz) of compound **3g** in $CDCl_3$.

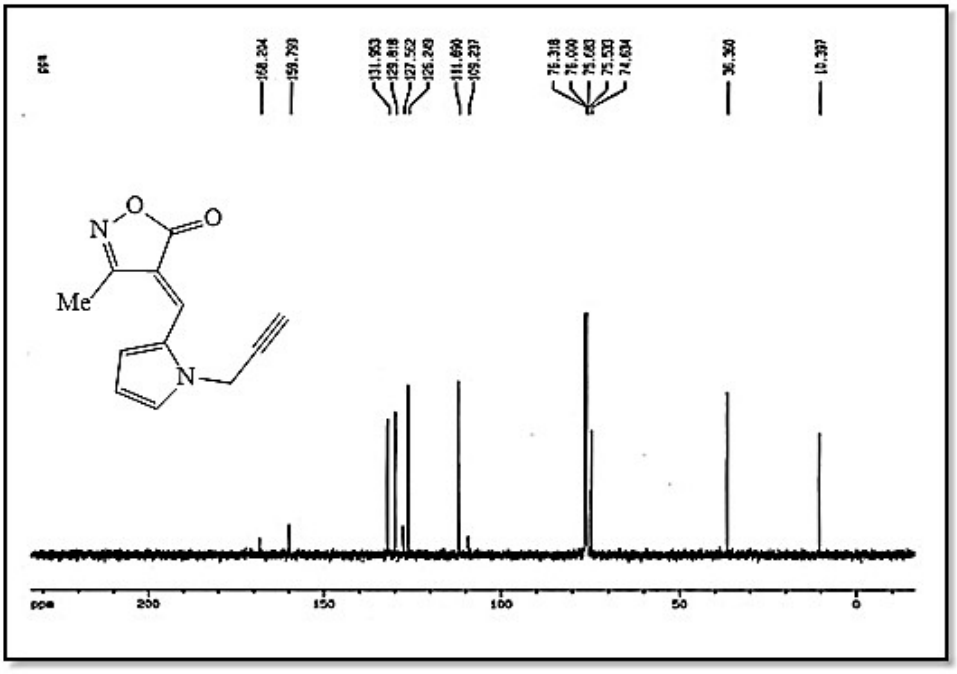


Figure 40: ^{13}C NMR spectrum (100 MHz) of compound **3g** in $CDCl_3$.

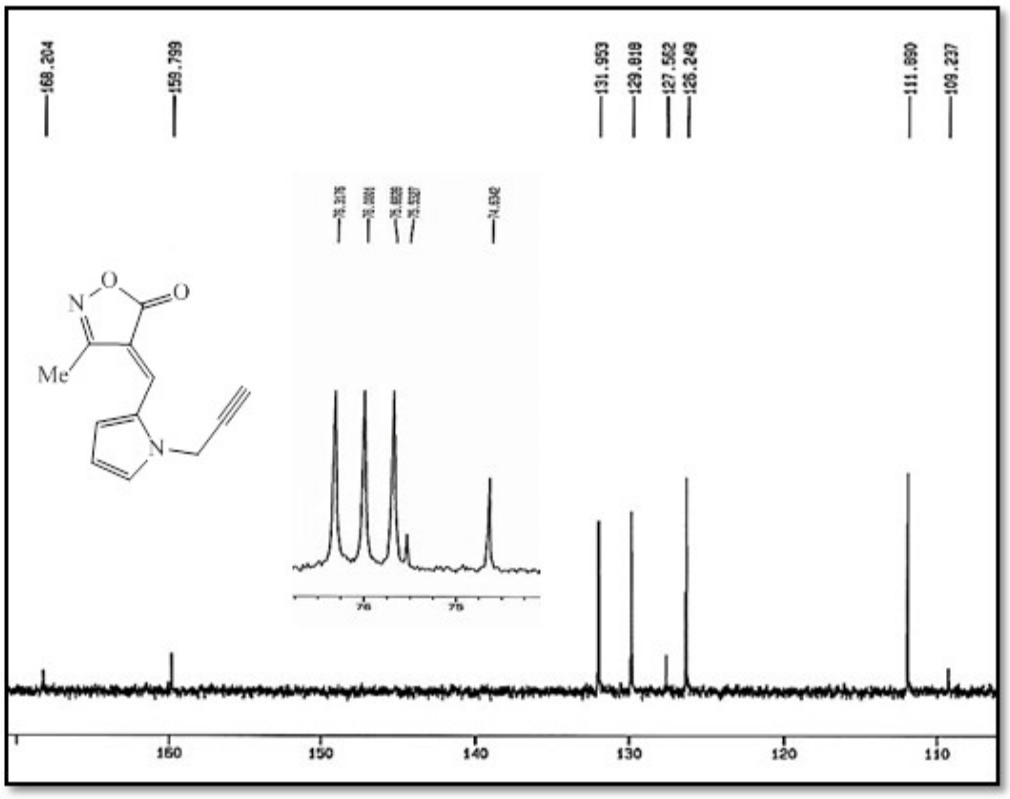


Figure 41: Expanded (1) ^{13}CNMR spectrum (100 MHz) of compound **3g** in $CDCl_3$.

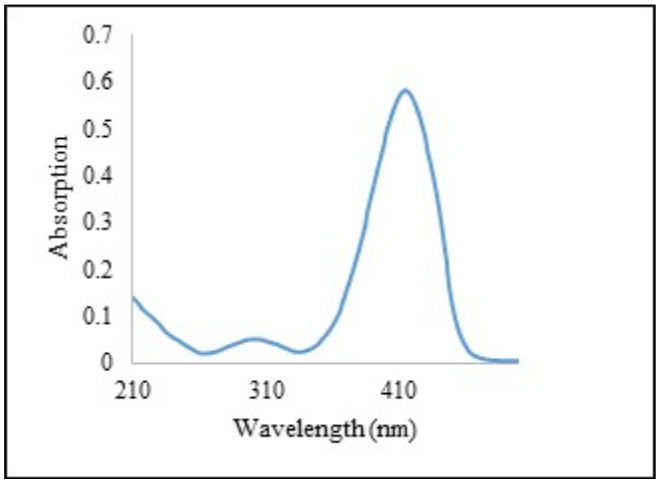


Figure 42: UV-Vis Spectrum of compound **3g** ($2.8\text{ß}10^{-5}$ M) in EtOH.

4-{[1-(2-Propyn-1-yl)-1*H*-pyrrol-2-yl] methylene}-3-phenyl isoxzol-5-(4*H*)-one (3h):

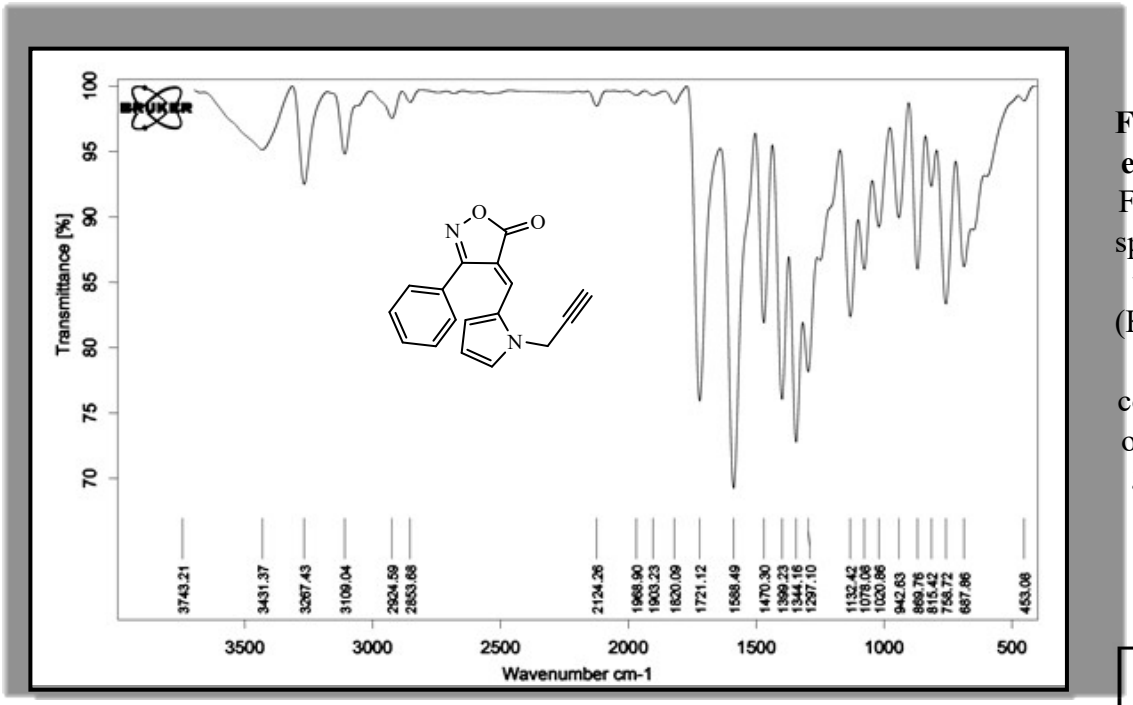


Figure 43: FTIR spectrum (KBr) of compound **3h**.

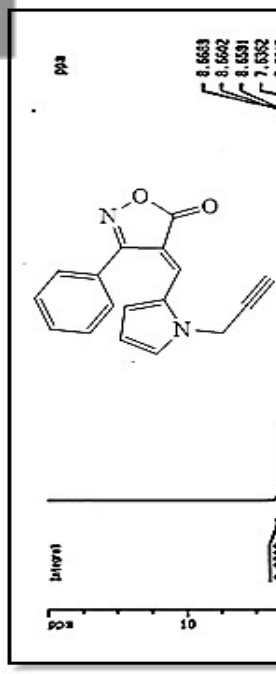


Figure 44: ^{1}H NMR spectrum (400 MHz) of compound **3h** in $CDCl_3$.

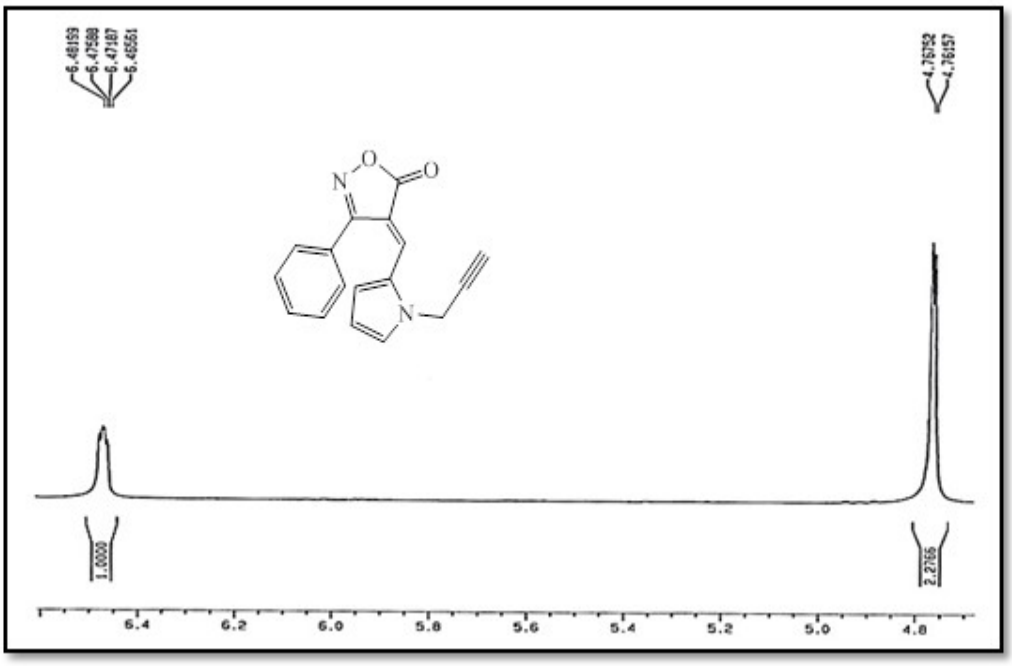


Figure 45: Expanded (1) ^{1}H NMR spectrum (400 MHz) of compound **3h** in $CDCl_3$.

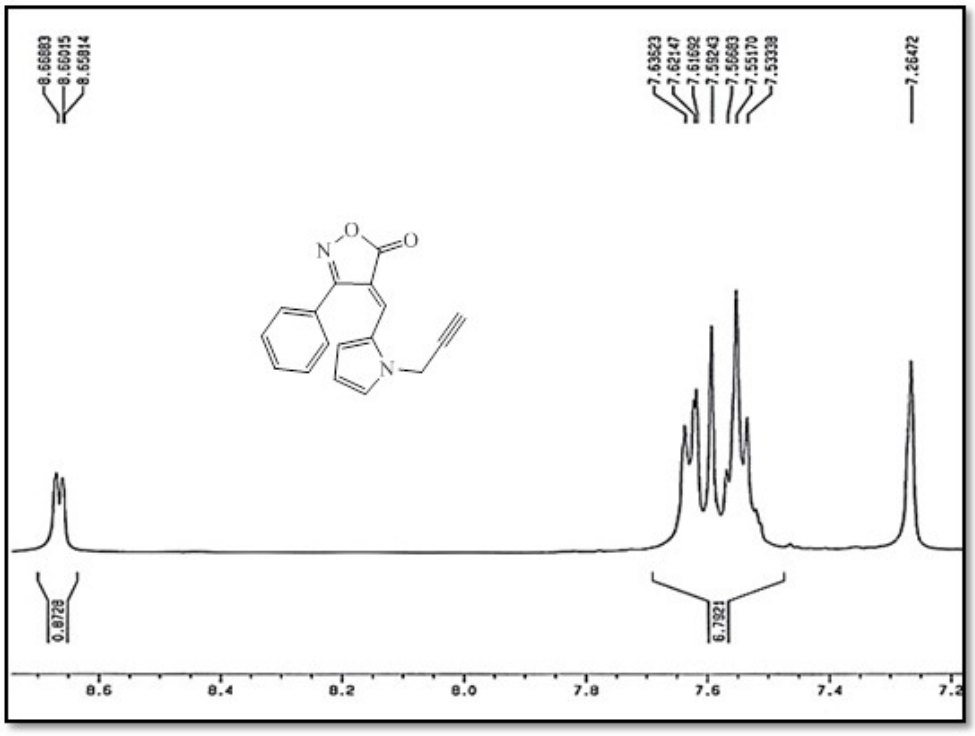


Figure 46: Expanded (2) ^{1}H NMR spectrum (400 MHz) of compound **3h** in $CDCl_3$.

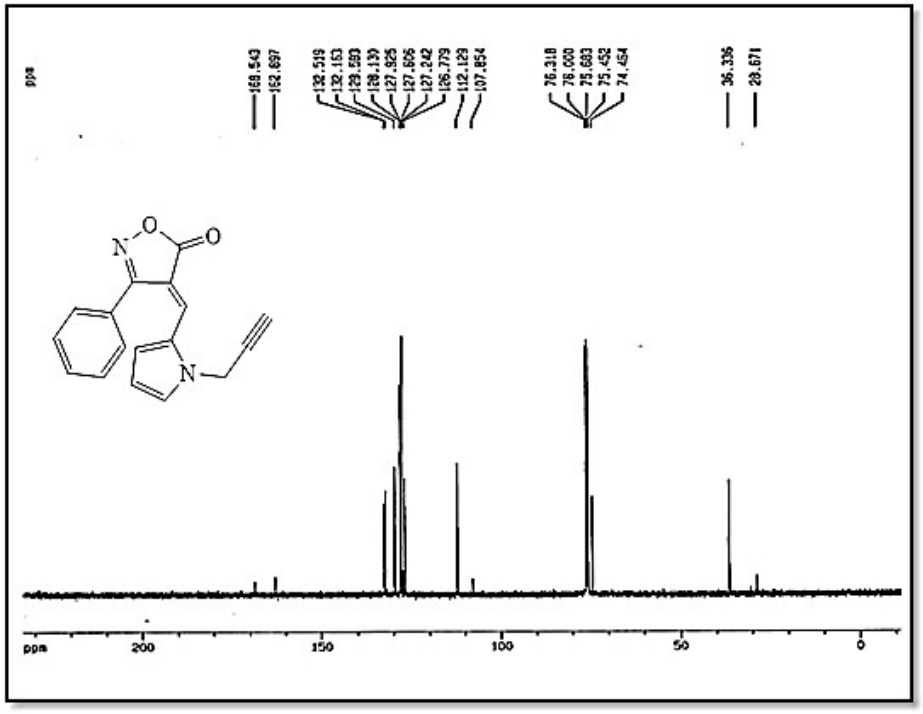


Figure 47: ^{13}C NMR spectrum (100 MHz) of compound **3h** in $CDCl_3$.

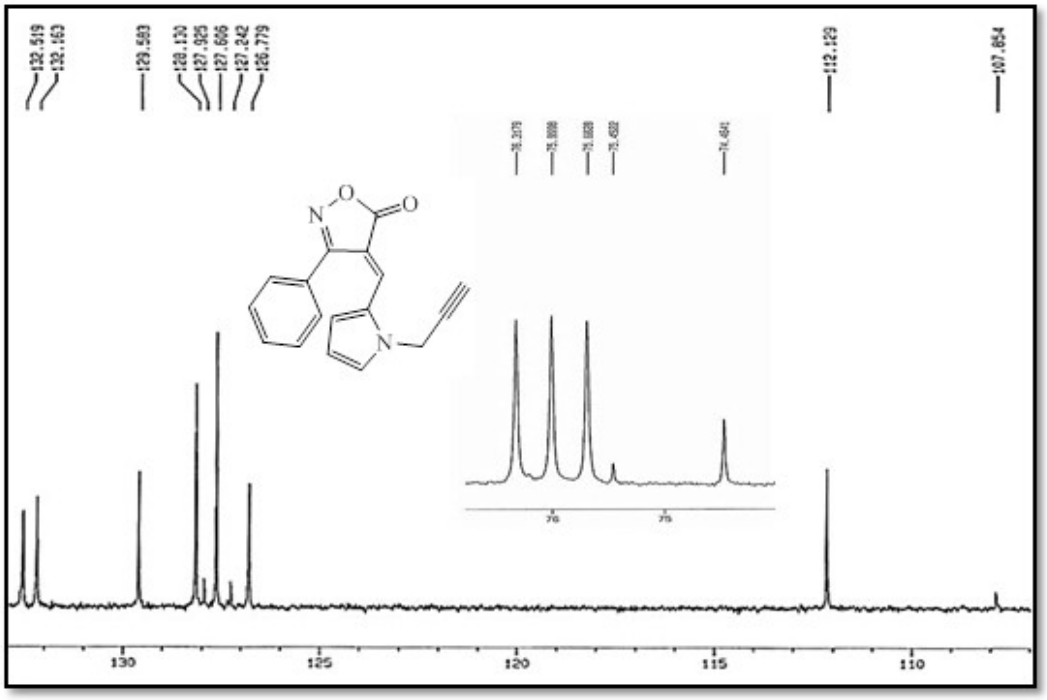


Figure 48: Expanded (1) $^{13}CNMR$ spectrum (100 MHz) of compound **3h** in $CDCl_3$.

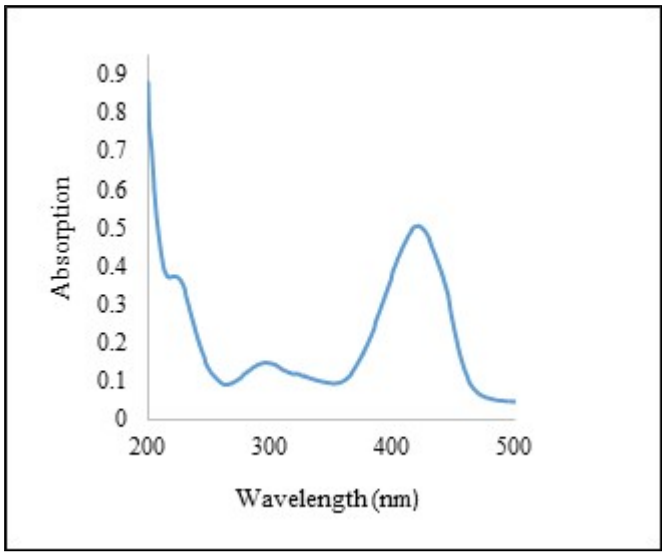


Figure 49: UV-Vis Spectrum of compound **3h** (2.8×10^{-5} M) in EtOH.

4-{[1-(1-Benzyl-1*H*-1,2,3-triazol-4-yl) methyl-1*H*-pyrrol-2-yl] methylene}-3-methyl isoxazol-5-(4*H*)-one (3i):

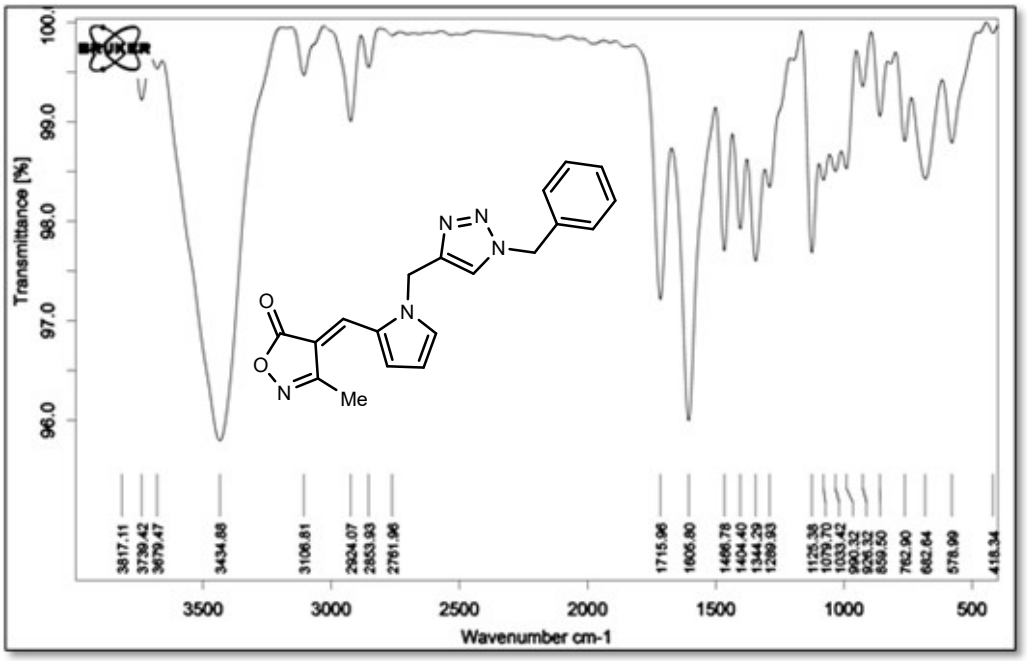


Figure 50: FTIR spectrum (KBr) of compound **3i**.

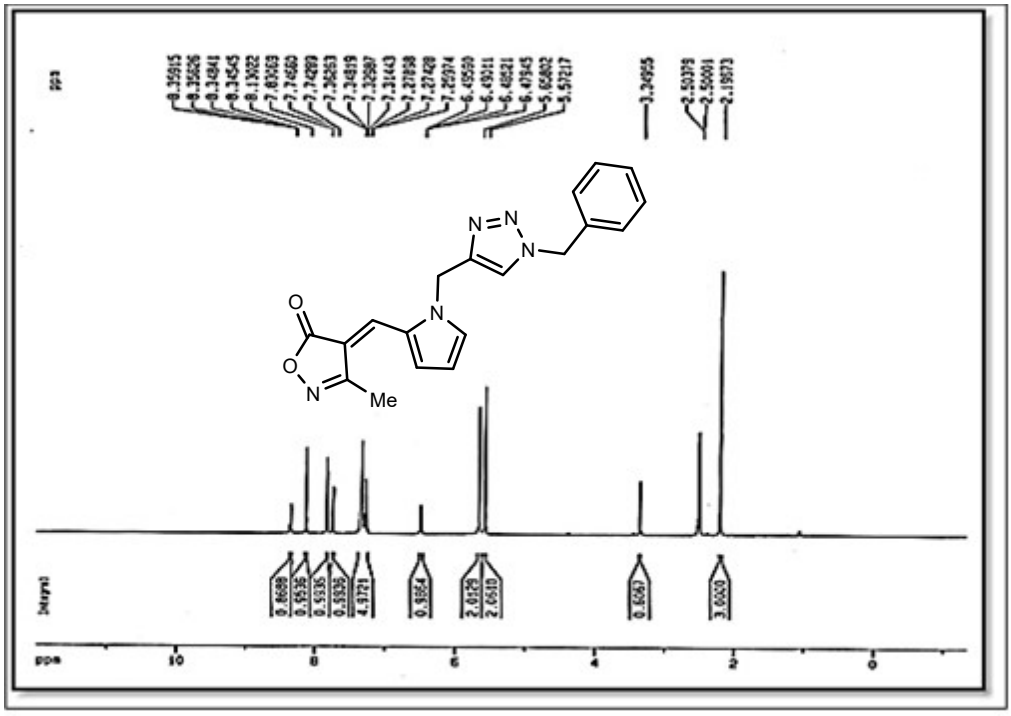


Figure 51: ^{1}H NMR spectrum (400 MHz) of compound **3i** in $CDCl_3$.

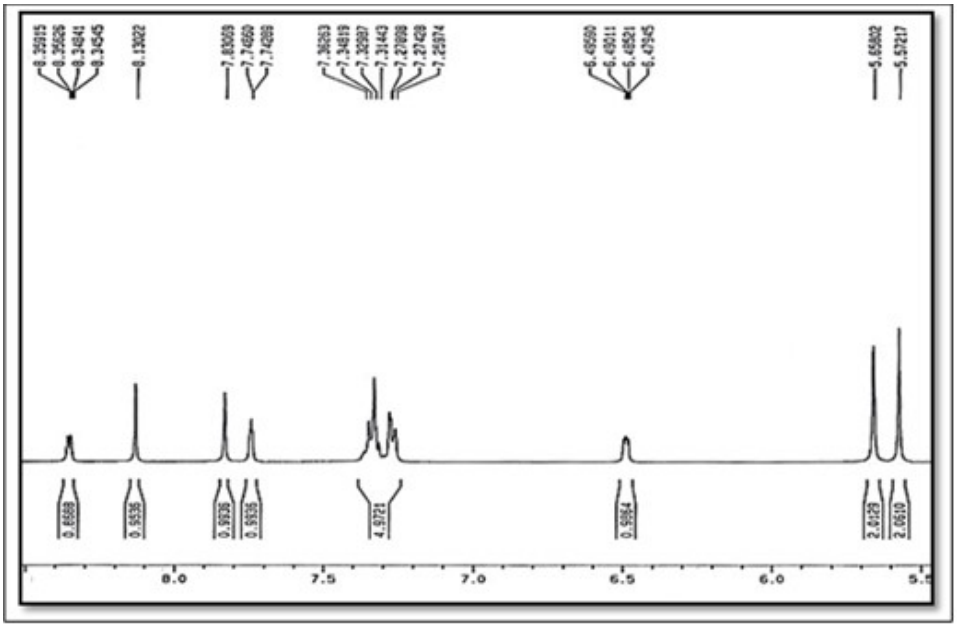


Figure 52: Expanded (1) ^{1}H NMR spectrum (400 MHz) of compound **3i** in $CDCl_3$.

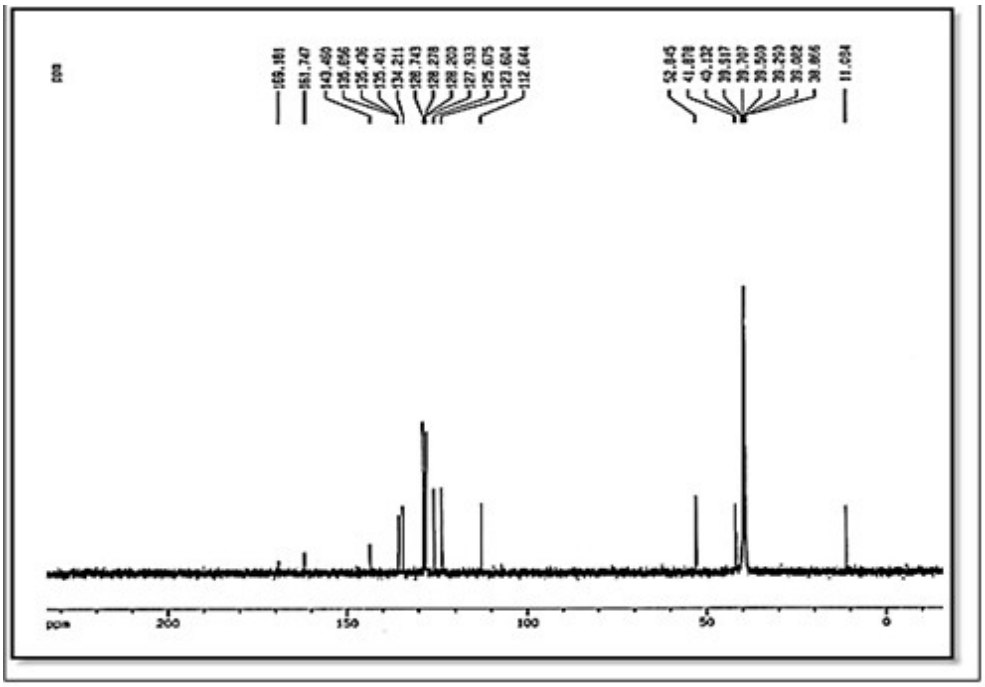


Figure 53: ^{13}C NMR spectrum (100 MHz) of compound **3i** in $CDCl_3$.

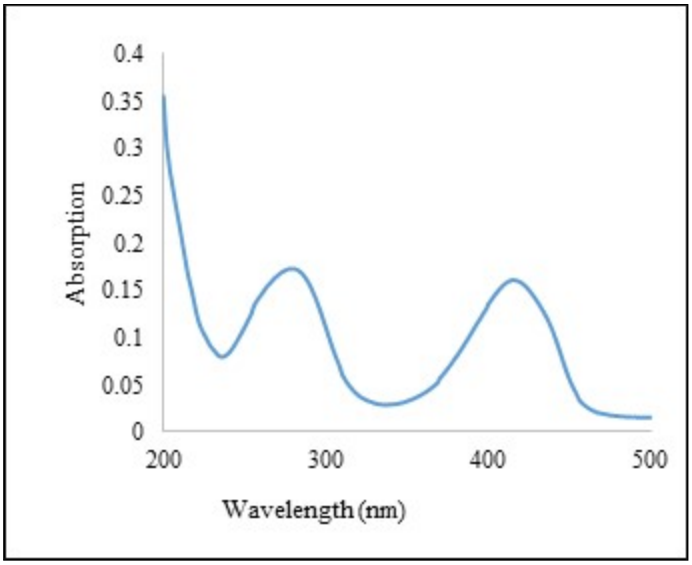


Figure 54: UV-Vis Spectrum of compound **3i** ($2.8\text{ß}10^{-5}$ M) in EtOH.

4-{[1-(1-Benzyl-1*H*-1,2,3-triazol-4-yl) methyl-1*H*-pyrrol-2-yl] methylene}-3-phenyl isoxazol-5-(4*H*)-one (3j):

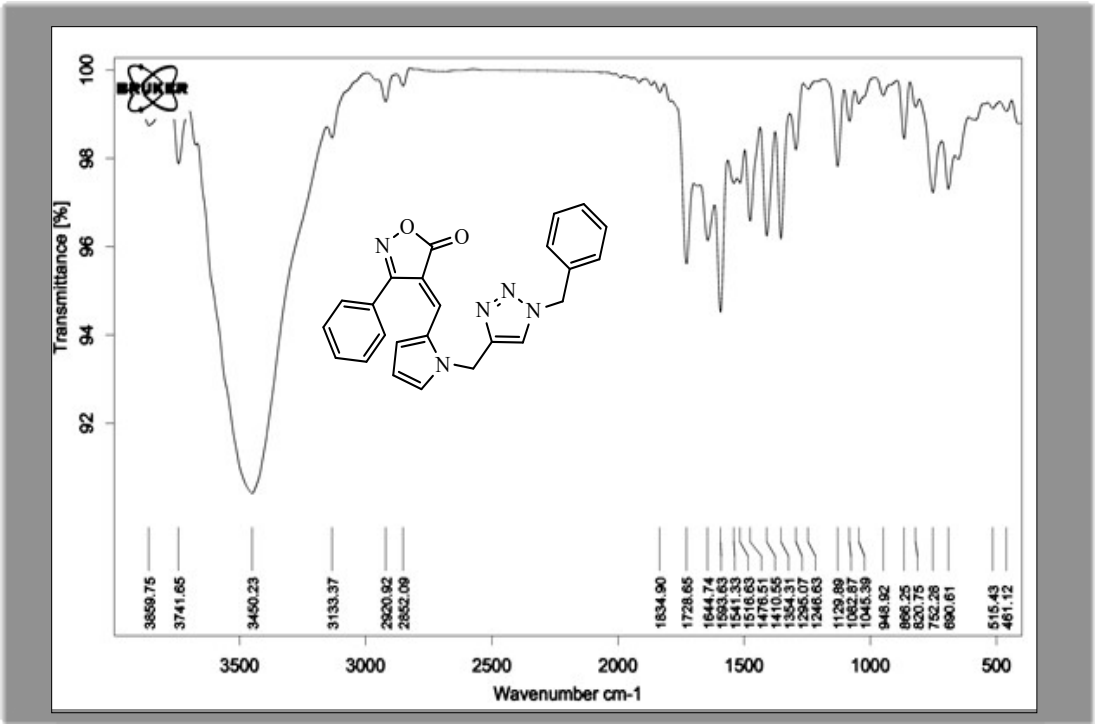


Figure 55: FTIR spectrum (KBr) of compound **3j** (before of elimination H_2O).

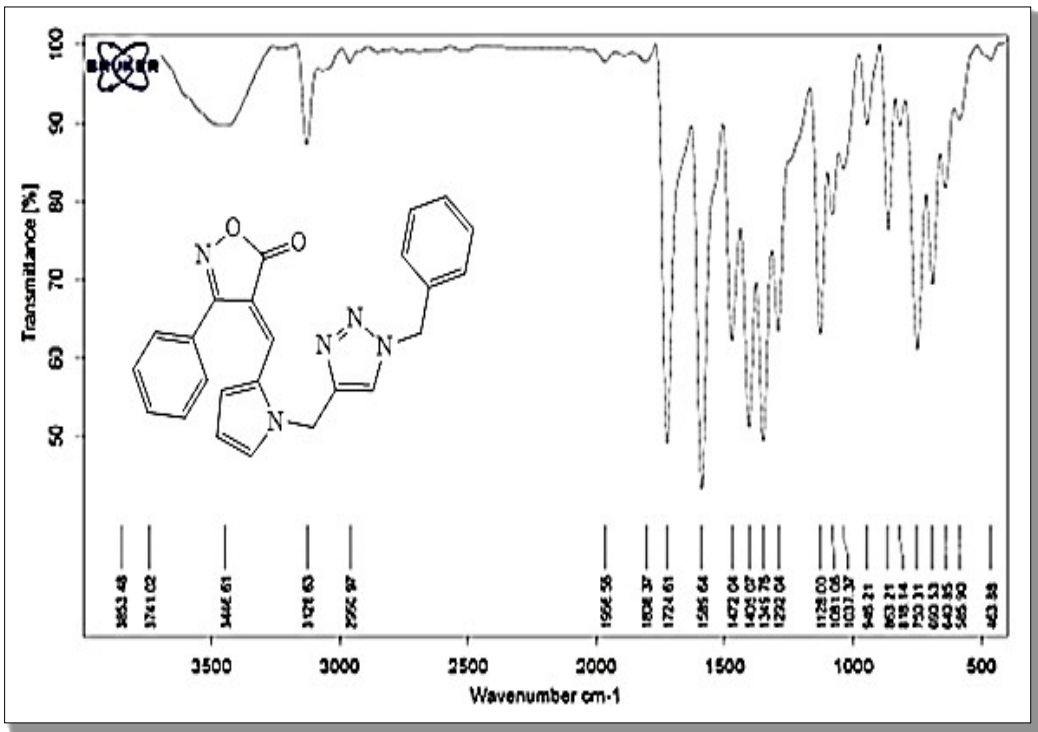


Figure 56: FTIR spectrum (KBr) of compound **3j** (after of elimination H_2O).

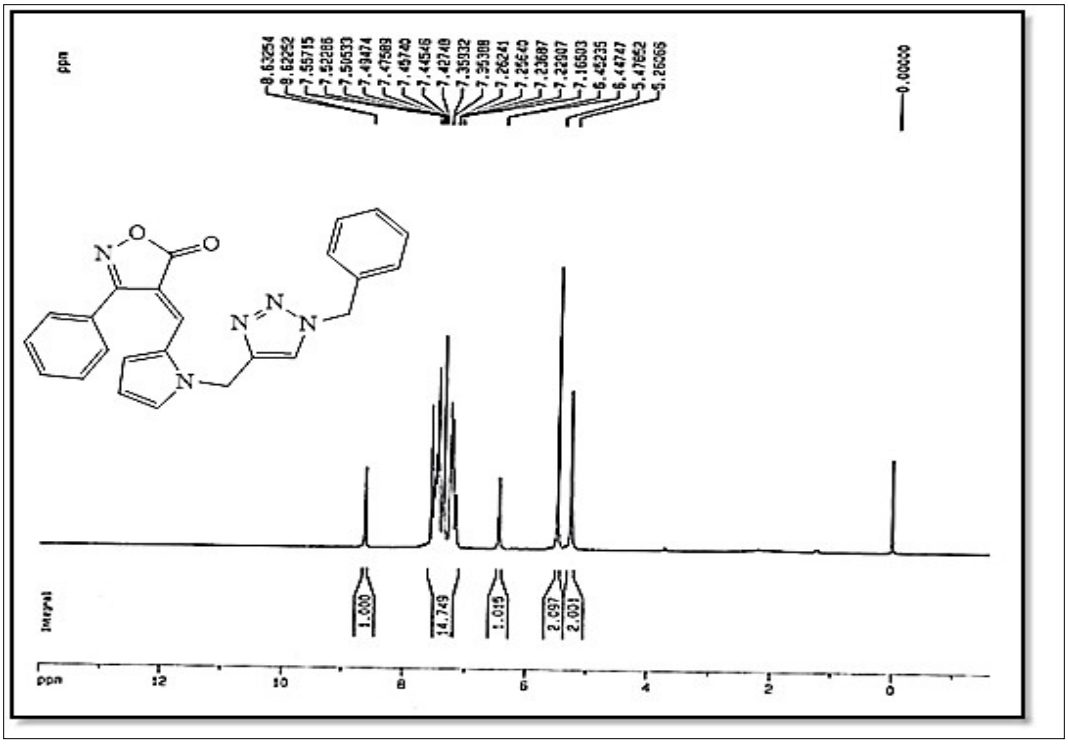


Figure 57: ^{1}H NMR spectrum (400 MHz) of compound **3j** in $CDCl_3$.

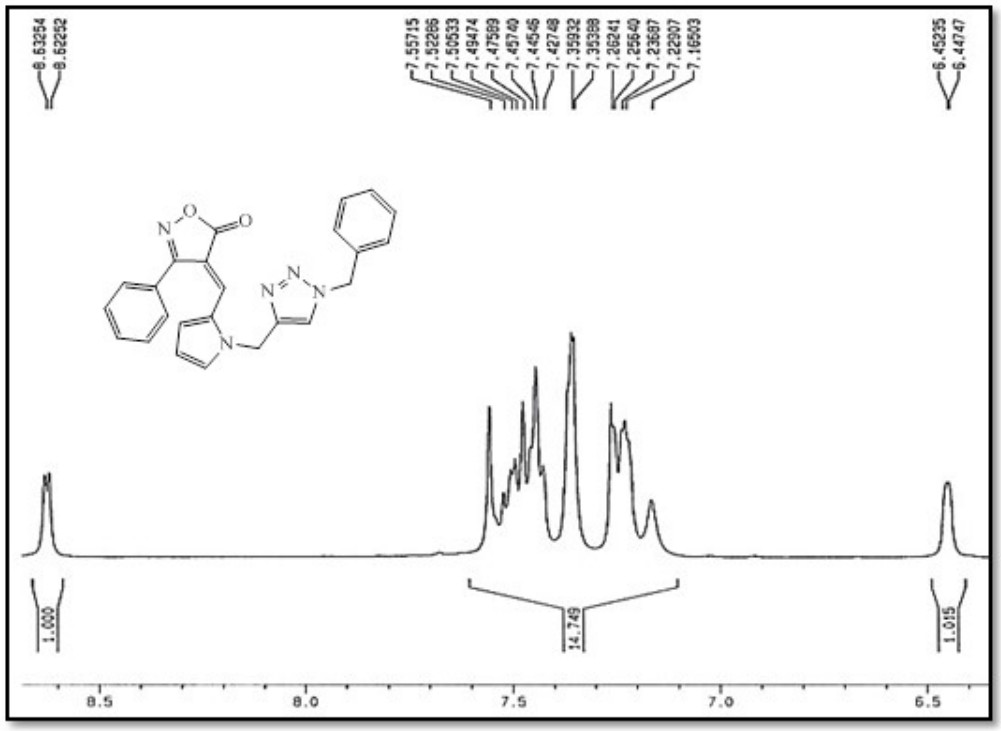


Figure 58: Expanded (1) ^{1}H NMR spectrum (400 MHz) of compound **3j** in $CDCl_3$.

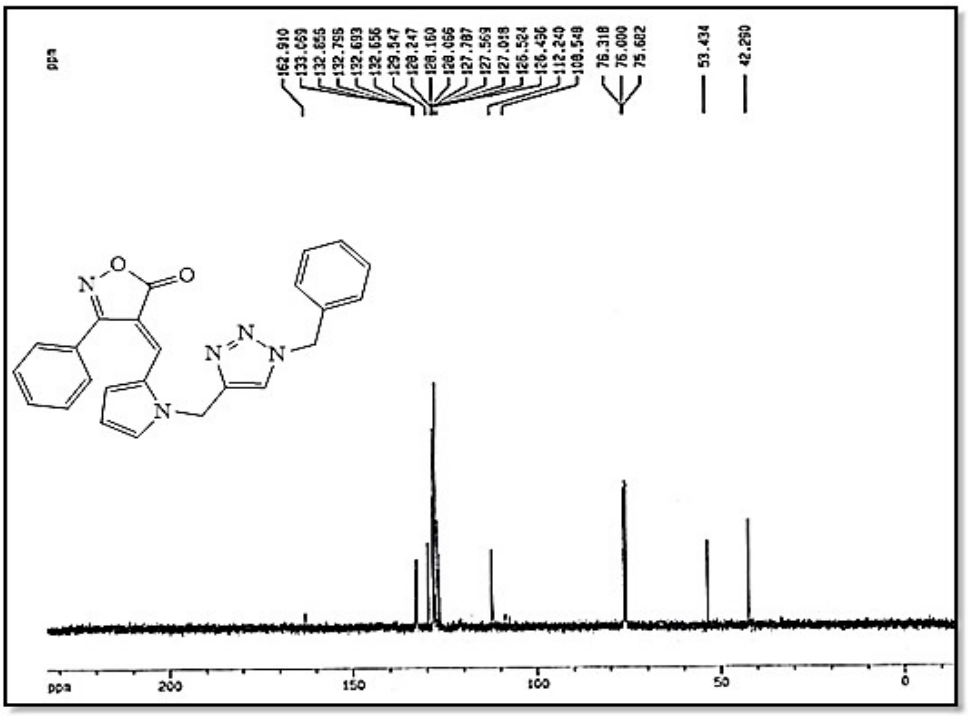


Figure 59: ^{13}C NMR spectrum (100 MHz) of compound **3j** in $CDCl_3$.

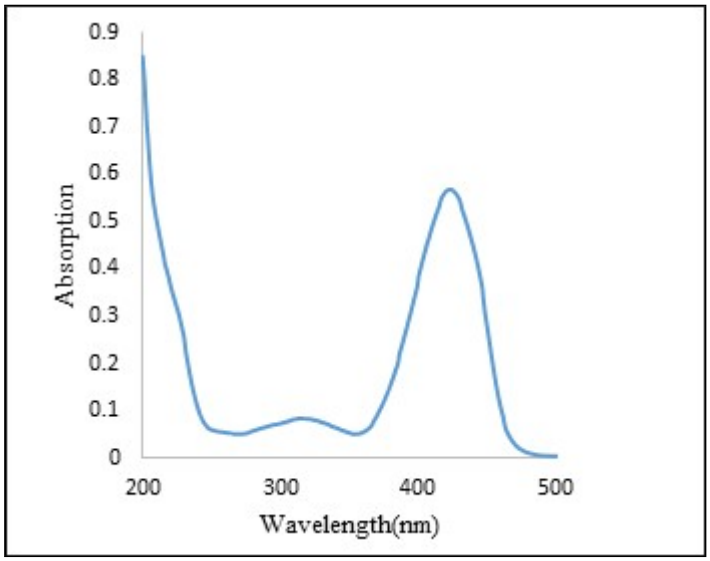


Figure 60: UV-Vis Spectrum of compound **3j** (2.8×10^{-5} M) in EtOH.

4-[(5-Methyl-furan-2-yl) methylene]-3-phenyl isoxzol-5-(4*H*)-one (3k):

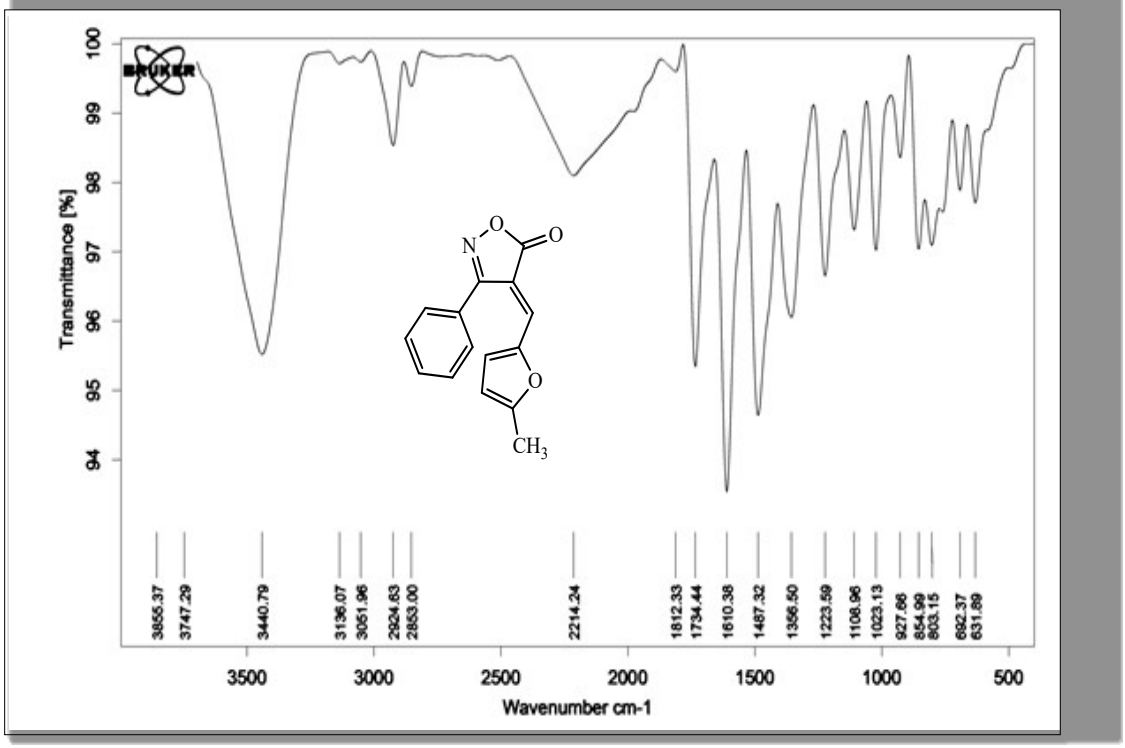


Figure 61: FTIR spectrum (KBr) of compound **3k**.

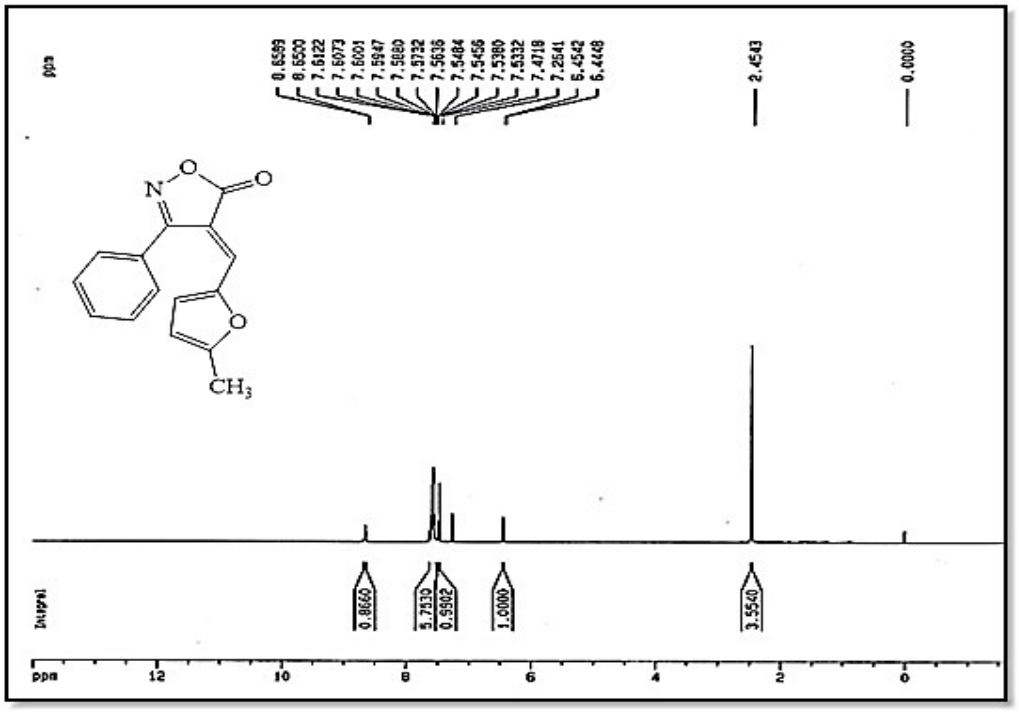


Figure 62: ^{1}H NMR spectrum (400 MHz) of compound **3k** in $CDCl_3$.

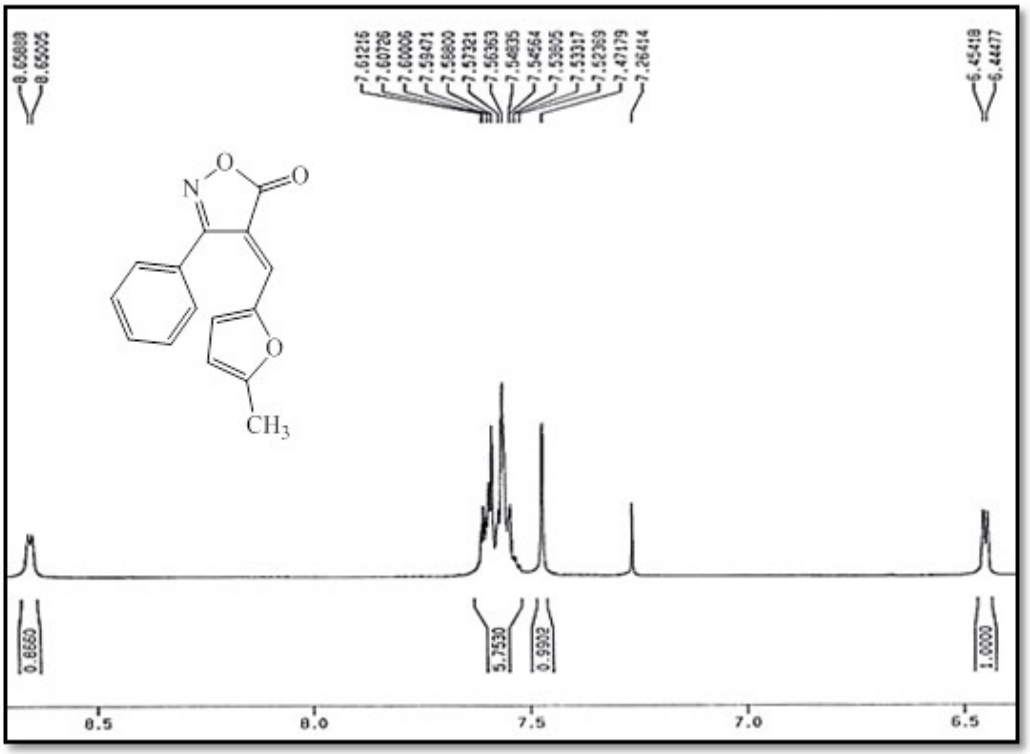


Figure 63: Expanded (1) ^{1}H NMR spectrum (400 MHz) of compound **3k** in $CDCl_3$.

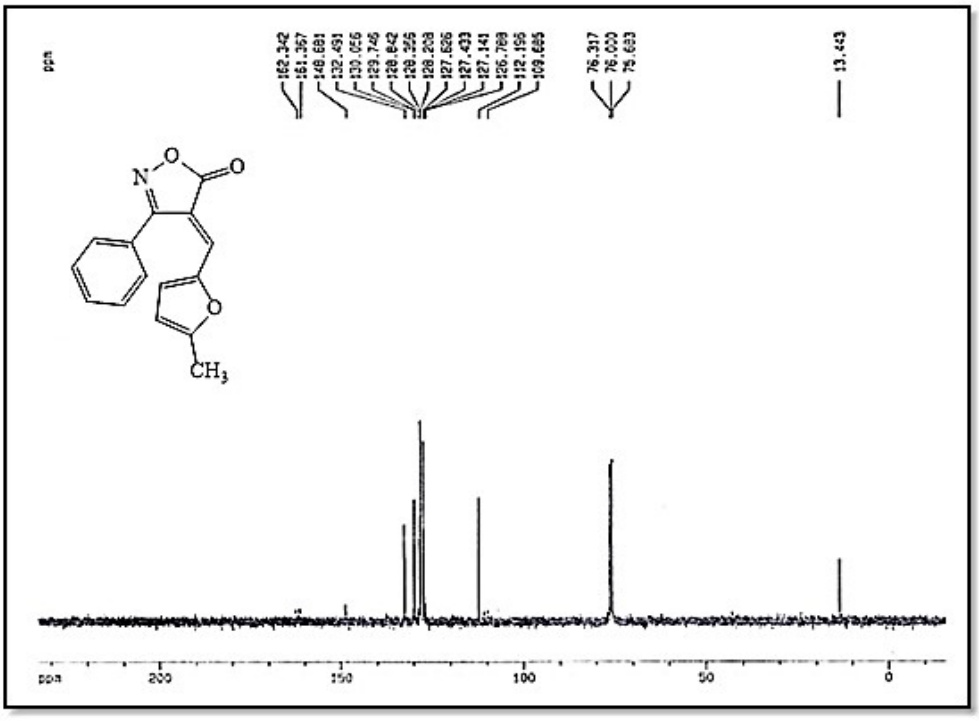


Figure 64: ^{13}C NMR spectrum (100 MHz) of compound **3k** in $CDCl_3$.

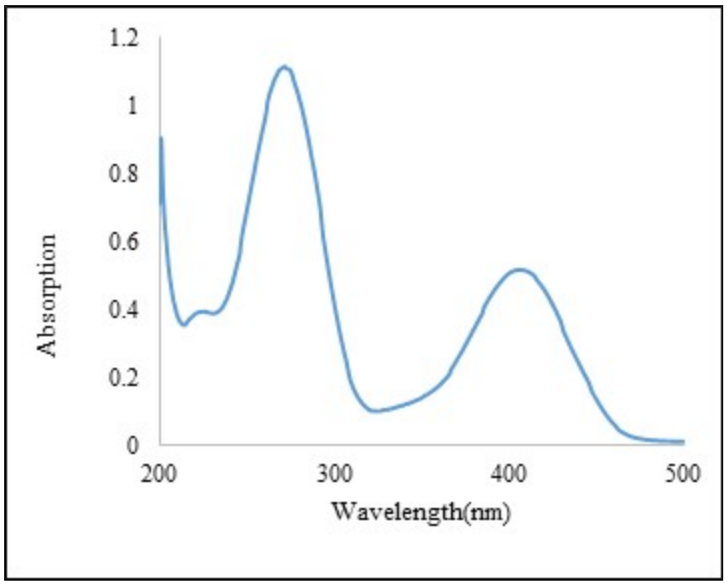


Figure 65: UV-Vis Spectrum of compound **3k** (2.8×10^{-5} M) in EtOH.

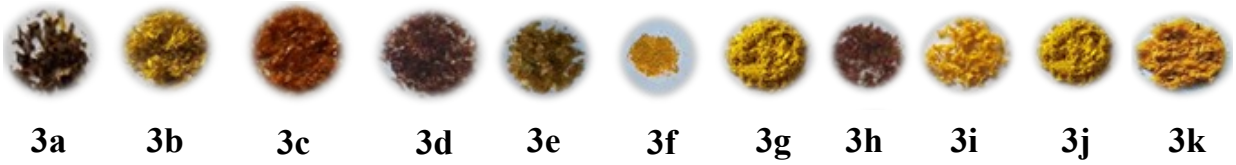


Figure 66: The appearance of synthesized isoxazole-5-one derivatives.

3. Some characterization data of the magnetic sulfonated polysaccharides

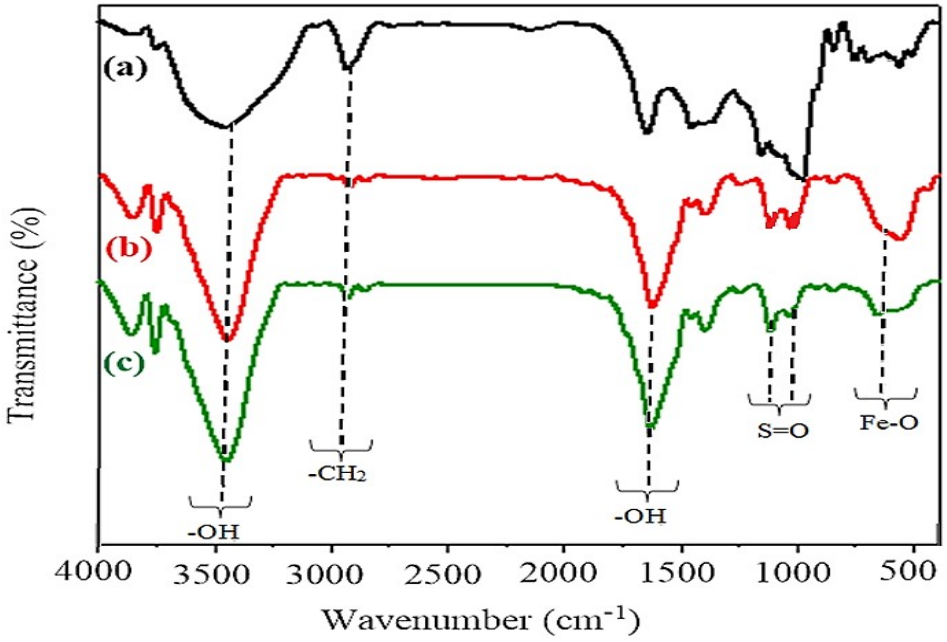


Figure 67: FT-IR spectra for starch (a), Fe_3O_4@starch (b), and Fe_3O_4@starch-SO_3H (c)

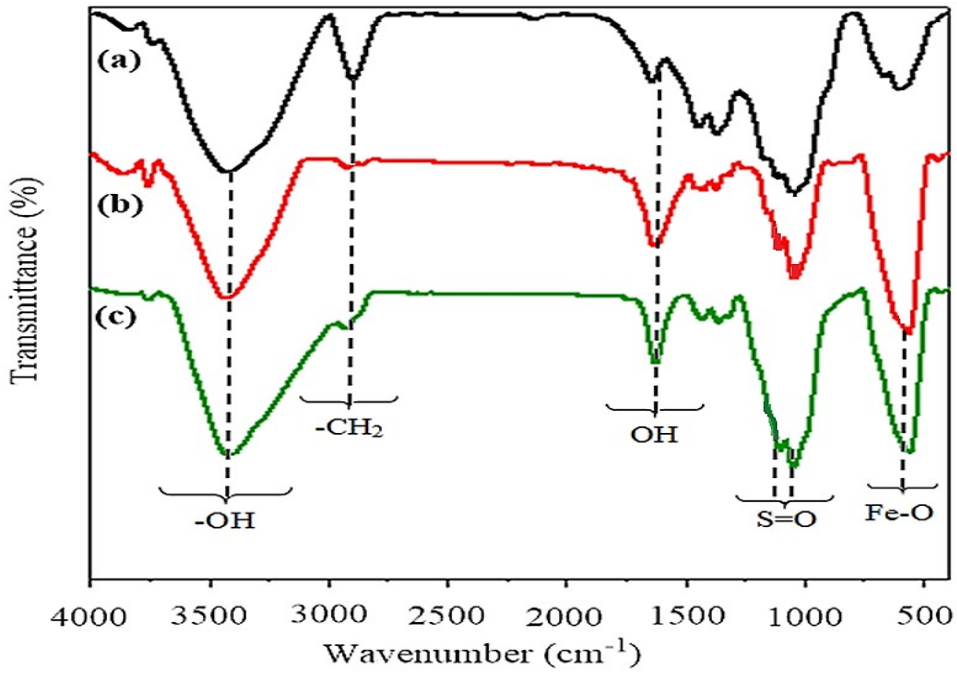


Figure 68: FT-IR spectra for cellulose (a), Fe_3O_4@cellulose (b), and Fe_3O_4@ cellulose-SO_3H (c)

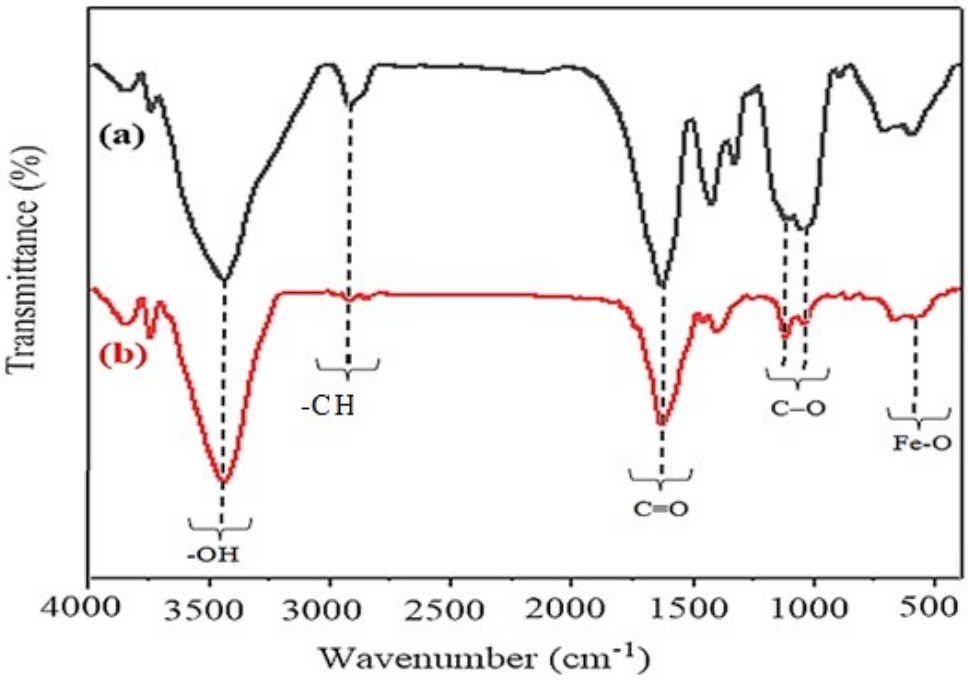


Figure 69: FT-IR spectra for pectin (a), Fe_3O_4@pectin (b)

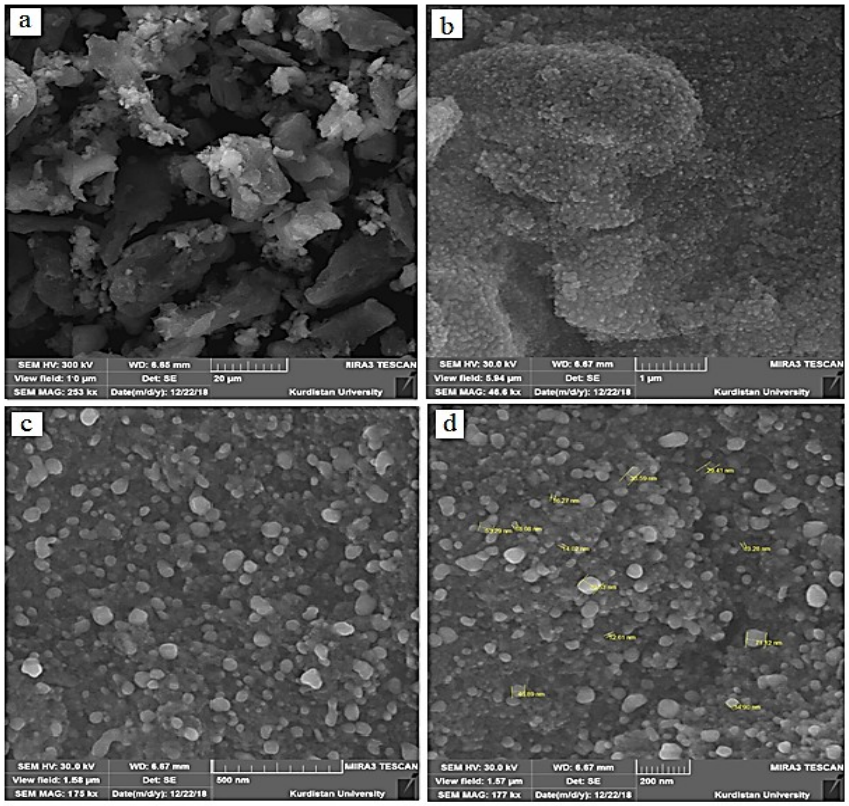
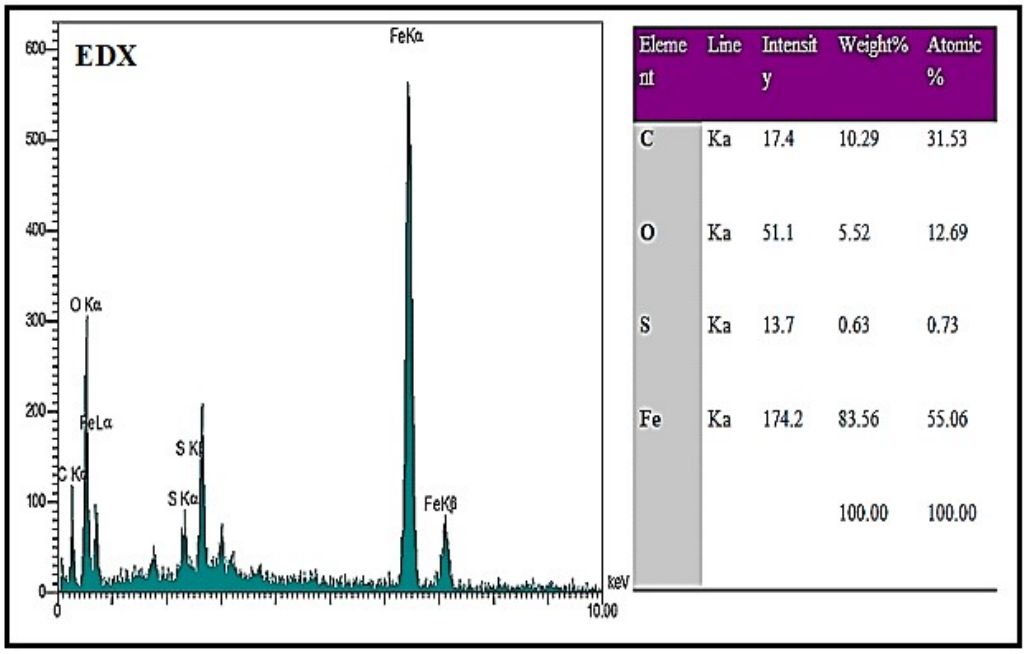


Figure 70: SEM images of the Fe_3O_4@Cellulose-SO_3H: 20 µm (a), 1 µm (b), 500 nm (c) and 200 nm (d)**,**
Average particle size is **37 nm**



Element	Line	Intensity	Weight%	Atomic %
C	Ka	17.4	10.29	31.53
O	Ka	51.1	5.52	12.69
S	Ka	13.7	0.63	0.73
Fe	Ka	174.2	83.56	55.06
			100.00	100.00

Figure 71: EDX pattern of the Fe_3O_4@Cellulose-SO_3H

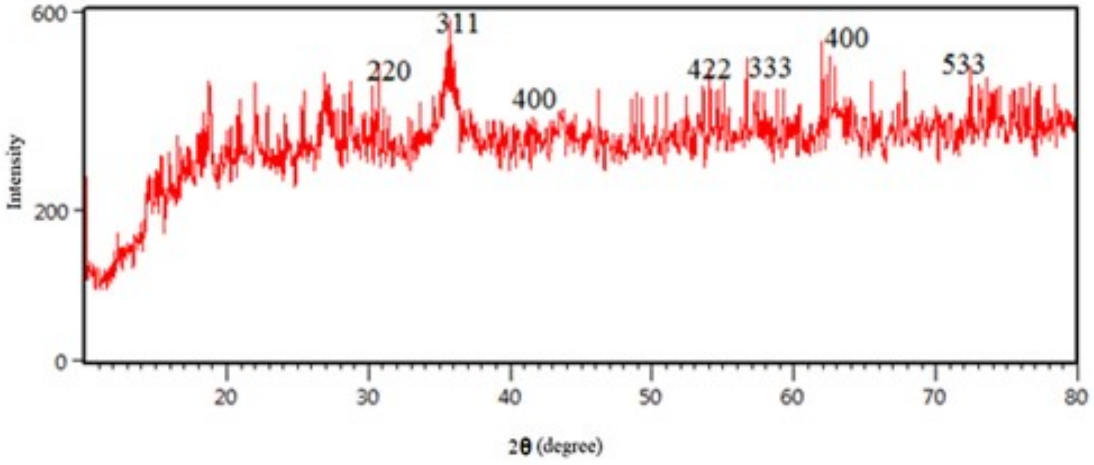


Figure 72: XRD patterns of Fe_3O_4@Cellulose-SO_3H

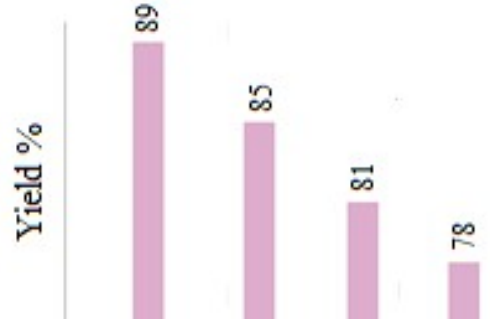


Figure 73: Reusability of recovered Fe_3O_4@Chitosan-SO_3H catalyst.

4. Cell cytotoxicity assay by MTT

To investigate in vitro cytotoxicity, we used HEK-293 normal and MCF-7 and HT-29 cancer cell lines which cultured in RPMI media supplemented with 10% of FBS and streptomycin and 1% penicillin in 5% CO_2 at 37 °C. After about 3 days, the unused media were removed and phosphate buffer solution (PBS) was added to the flask to delete the extra RPMI. Trypsin-EDTA solution (about 350 µL) was then added and the flask was incubated for 5 min at 37 °C in 5% of CO_2 to detach the cells from the flask bottom. After that, suspended cancer and normal cells were moved to a 20 mL tube and then centrifuged to remove extra trypsin-EDTA. Eventually, precipitated cancer and normal cells were suspended to the fresh medium in PBS buffer.

Cell cytotoxicity of HEK 293 normal and MCF-7 and HT-29 cancer cells towards synthesized compounds were investigated by MTT assay. For this aim, cells were seeded at a concentration of 1.0×104 cells per well in each well of a 96-well texture plate. Culture plate was then incubated for about 24 h and treated with various concentrations of the 4-arylmethylene-isoxazole-5(4*H*)-ones and incubated for another 48 h. Afterward, the solution of the plate was detached and washed with PBS solution. Next, 180 µL of media and 20 µL of MTT reagent (3.0 mg/mL) were added to each well of the plate and incubated at 37 °C in 5% of CO_2 for 4 h. The solution was then detached and DMSO (200 µL) was added to each well and incubated for about 30 min at room temperature. Finally, the absorbance of the wells was read at 570 nm by the MTT-assay plate reader.

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