

Synthesis of Some New 4-[2-(2-methylbenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one Derivatives with Their Antioxidant Properties

ABSTRACT

Aims: To synthesize heterocyclic Schiff bases and evaluate their antioxidant activity.

Study design: Synthesizing some new 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives and determining their chemical structure via IR and NMR spectroscopy. The synthesized new compounds were analyzed for their *in vitro* potential antioxidant activities.

Place and Duration of Study: Department of Chemistry, Kafkas University, Kars, Turkey. Between February 2014 to February 2015.

Methodology: Nine new 3-alkyl(aryl)-4-[2-(2-methylbenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-ones (**3**) were synthesized by the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**1**) with 2-(2-methylbenzoxy)-benzaldehyde (**2**) which had also been synthesized by the reactions of 2-hydroxybenzaldehyde with 2-methylbenzoyl chloride by using triethylamine.

Results: Schiff bases were synthesized and their structures were determined with spectral methods. Antioxidant evaluation were carried out and discussed.

Conclusion: Synthesis and structural determination of the new 3-alkyl(aryl)-4-[2-(2-methylbenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-ones (**3**) was successfully done. The antioxidant evaluation was determined.

Keywords: 1,2,4-Triazol-5-one; synthesis; Schiff base; antioxidant activity

1. INTRODUCTION

In the last two decades there has been a growing attention in the role of reactive oxygen species (ROS) and nitrogen species (RNS) in food, drugs, and even living system. Therefore, scientists in diverse disciplines have become more curious about naturally-occurring antioxidant as well as in related synthetic derivatives that could supply active components which prohibit or decrease the effect of oxidative stress [1].

External chemicals and internal metabolic processes in the human body or in the food system may generate highly reactive free radicals. At high concentrations, they could be important mediators of damage among cell structures, including lipids and membranes, proteins, and nucleic acids [2]. In this regard, it is important to search for and synthesize new classes of compounds that have antioxidant properties.

1,2,4-Triazole derivatives are recorded to own a wide variety of pharmacological activities like antibacterial, antioxidant, anti-inflammatory, antiparasitic, analgesic, antiviral, antitumor, anti-HIV, antihypertensive and diuretic properties [3-11]. Besides, a few articles declaring the synthesis of several N-arylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives have been reported so far [3,4].

In this study, nine new 3-alkyl(aryl)-4-[2-(2-methylbenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-ones (**3a-i**) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**1a-i**) with 2-(2-methylbenzoxy)-benzaldehyde (**2**). In addition, due to a wide range of applications to find their possible antioxidant activity, the newly synthesized **3** type compounds were investigated by using different antioxidant assays like; reducing power, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity and iron binding effect.

2. METHODOLOGY

2.1 Chemical Reagents and Apparatus

Chemical reagents used in this study were purchased from Merck AG, Aldrich and Fluka. Melting points were determined in open glass capillaries using a Stuart SMP30 melting point apparatus and are uncorrected. The IR spectra were recorded on an Alpha-P Bruker FT-IR spectrometer. ^1H and ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as internal standard using a Bruker Ultrashield Plus Biospin spectrometer at 400 MHz and 100 MHz, respectively. UV absorption spectra were measured in 10 mm quartz cells between 200 and 400 nm using a PG Instruments Ltd T80 UV/Vis spectrometer. Extinction coefficients (ϵ) are expressed in $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$.

2.2 Synthesis

2.2.1 Procedure for the synthesis of 2-benzoxy-3-ethoxy-benzaldehyde (2)

2-hydroxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (100 mL) was treated with 2-methylbenzoyl chloride (0.01 mol), and to this solution was slowly added triethylamine (0.02 mol) with stirring at 0-5 °C. Stirring was continued for 2 h, and then the mixture was refluxed for 3 h and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from ethanol to afford compound **2**, yield 94.76%. Mp: 64°C. IR (cm^{-1}) 2863 and 2763 (ν_{CHO}), 1737, 1690 ($\nu_{\text{C=O}}$), 1237 (ν_{COO}); ^1H NMR (400MHz, $\text{DMSO}-d_6$) δ 2.62 (s, 3H, PhCH_3), 7.42-7.50 (m, 3H, ArH), 7.55-7.63 (m, 2H, ArH), 7.80-7.84 (m, 1H, ArH), 8.01 (d, 1H, ArH; $J=7.60$ Hz), 8.21 (d, 1H, ArH; $J=8.00$ Hz), 10.13 (s, 1H, CHO); ^{13}C NMR (100MHz, $\text{DMSO}-d_6$) δ 21.15 (PhCH_3), 123.99, 126.17, 126.72, 127.87, 128.18, 131.06, 131.76, 131.83, 133.08, 135.65, 140.46, 150.79 (Ar-C), 165.06 (C=O); UV ($\text{C}_2\text{H}_5\text{OH}$) λ_{max} (log ϵ) 286 (6597), 242 (15905) nm.

2.2.2 General procedure for the synthesis of compounds 3

The corresponding compound **1** (0.01 mol) was dissolved in acetic acid (15 mL) and treated with 2-(2-methylbenzoxy) benzaldehyde **2** (0.01 mol). The mixture was refluxed for 1.5 h and then evaporated at 50-55 °C *in vacuo*. Several recrystallizations of the residue from appropriate solvent gave pure compounds **3a-i** as colorless crystals.

2.2.2.1 3-Methyl-4-[2-(2-methylbenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (3a)

Yield 98.21%. Mp: 230°C. IR (cm^{-1}) 3169 (ν_{NH}), 1737, 1706 ($\nu_{\text{C=O}}$), 1601, 1578 ($\nu_{\text{C=N}}$), 1231 (ν_{COO}); ^1H NMR (400MHz, $\text{DMSO}-d_6$) δ 2.16 (s, 3H, CH_3), 2.60 (s, 3H, PhCH_3), 7.41-7.47 (m, 4H, ArH), 7.58-7.64 (m, 2H, ArH), 8.06 (d, 1H, ArH; $J=7.60$ Hz), 8.16-8.18 (m, 1H, ArH), 9.96 (s, 1H, N=CH), 11.79 (s, 1H, NH); ^{13}C NMR (100MHz, $\text{DMSO}-d_6$) δ 10.85 (CH_3), 21.13 (PhCH_3), 123.71, 126.19, 126.24, 126.67, 126.94, 127.79, 130.89, 131.88, 132.51, 133.19, 140.18, 149.89 (Ar-C), 144.19 (triazole C_3), 148.27 (N=CH), 151.22 (triazole C_5), 165.10 (C=O); UV ($\text{C}_2\text{H}_5\text{OH}$) λ_{max} (log ϵ) 292 (9636), 238(1600) nm.

2.2.2.2 3-Ethyl-4-[2-(2-methylbenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (3b)

Yield 99.41%. Mp: 202°C. IR (cm^{-1}) 3159 (ν_{NH}), 1727, 1702 ($\nu_{\text{C=O}}$), 1595, 1574 ($\nu_{\text{C=N}}$), 1232 (ν_{COO}); ^1H NMR (400MHz, $\text{DMSO}-d_6$) δ 1.22 (t, 3H, CH_2CH_3 ; $J=7.20$ Hz), 2.57 (q, 2H, CH_2CH_3 ; $J=7.20$ Hz), 2.61 (s, 3H, PhCH_3), 7.42-7.50 (m, 4H, ArH), 7.59-7.67 (m, 2H, ArH), 8.05 (d, 1H, ArH; $J=8.00$ Hz), 8.18 (d, 1H, ArH; $J=8.00$ Hz), 9.97 (s, 1H, N=CH), 11.83 (s, 1H, NH); ^{13}C NMR (100MHz, $\text{DMSO}-d_6$) δ 9.91 (CH_2CH_3), 18.47 (CH_2CH_3), 21.13 (PhCH_3), 123.74, 126.22, 126.35, 126.68, 127.01, 127.77, 130.91, 131.87, 132.77, 133.18, 140.42, 149.87 (Ar-C), 147.94 (triazole C_3), 148.38 (N=CH), 151.37 (triazole C_5), 165.08 (C=O); UV ($\text{C}_2\text{H}_5\text{OH}$) λ_{max} (log ϵ) 290 (13412), 240 (22315) nm.

2.2.2.3 3-Propyl-4-[2-(2-methylbenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (3c)

Yield 96.88%. Mp: 174°C. IR (cm⁻¹) 3163 (ν_{NH}), 1739, 1709 (ν_{C=O}), 1595 (ν_{C=N}), 1249 (ν_{COO}); ¹H NMR (400MHz, DMSO-*d*₆) δ 0.86 (t, 3H, CH₂CH₂CH₃; J=7.20 Hz), 1.60 (sext, 2H, CH₂CH₂CH₃; J=7.20 Hz), 2.50 (t, 2H, CH₂CH₂CH₃; J=7.20 Hz), 2.61 (s, 3H, PhCH₃), 7.42-7.51 (m, 4H, ArH), 7.59-7.67 (m, 2H, ArH), 8.04 (d, 1H, ArH; J=8.00 Hz), 8.17-8.19 (m, 1H, ArH), 9.96 (s, 1H, N=CH), 11.83 (s, 1H, NH); ¹³C NMR (100MHz, DMSO-*d*₆) δ 13.26 (CH₂CH₂CH₃), 18.90 (CH₂CH₂CH₃), 21.13 (PhCH₃), 26.48 (CH₂CH₂CH₃), 123.76, 126.23, 126.35, 126.71, 127.10, 127.79, 130.91, 131.88, 132.49, 133.18, 140.40, 149.85 (ArC), 146.80 (triazole C₃), 148.58 (N=CH), 151.05 (triazole C₅), 165.08 (C=O); UV (C₂H₅OH) λ_{max} (log ε) 290 (11491), 242 (14782), 224 (16205) nm.

2.2.2.4 3-Benzyl-4-[2-(2-methylbenzoxo)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (3d)

Yield 96.68%. Mp: 227°C. IR (cm⁻¹) 3161 (ν_{NH}), 1727, 1706 (ν_{C=O}), 1590 (ν_{C=N}), 1231 (ν_{COO}); ¹H NMR (400MHz, DMSO-*d*₆) δ 2.60 (s, 3H, PhCH₃), 3.99 (s, 2H, CH₂Ph), 7.23-7.35 (m, 5H, ArH), 7.41-7.50 (m, 4H, ArH), 7.58-7.67 (m, 2H, Ar-H), 8.01-8.03 (m, 1H, Ar-H), 8.11-8.18 (m, 1H, Ar-H), 9.96 (s, 1H, N=CH), 11.95 (s, 1H, NH); ¹³C NMR (100MHz, DMSO-*d*₆) δ 21.11 (PhCH₃), 30.88 (CH₂Ph), 123.67, 126.16, 126.24, 126.47, 126.71, 127.76, 128.43 (2C), 128.76, 128.91 (2C), 130.86, 131.88, 132.58, 133.20, 135.66, 140.37, 150.05 (Ar-C), 146.16 (triazole C₃), 147.87 (N=CH), 151.23 (triazole C₅), 165.14 (C=O); UV (C₂H₅OH) λ_{max} (log ε) 292 (7905), 238 (14198) nm.

2.2.2.5 3-*p*-Methylbenzyl-4-[2-(2-methylbenzoxo)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (3e)

Yield 98.73%. Mp: 226°C. IR (cm⁻¹) 3166 (ν_{NH}), 1732, 1701 (ν_{C=O}), 1594 (ν_{C=N}), 1231 (ν_{COO}); ¹H NMR (400MHz, DMSO-*d*₆) δ 2.25 (s, 3H, *p*-PhCH₃), 2.60 (s, 3H, *o*-PhCH₃), 3.93 (s, 2H, CH₂Ph), 7.12 (d, 2H, ArH; J=8.00 Hz), 7.19 (d, 2H, ArH; J=8.00 Hz), 7.40-7.50 (m, 4H, ArH), 7.58-7.66 (m, 2H, ArH), 8.03-8.05 (m, 1H, ArH), 8.16-8.18 (m, 1H, ArH), 9.67 (s, 1H, N=CH), 11.96 (s, 1H, NH); ¹³C NMR (100MHz, DMSO-*d*₆) δ 20.58 (*p*-PhCH₃), 21.11 (*o*-PhCH₃), 30.49 (CH₂Ph), 123.66, 126.18, 126.22, 126.46, 126.68, 127.76, 128.63 (2C), 128.98 (2C), 130.86, 131.87, 132.48, 132.54, 133.18, 135.77, 140.37, 150.05 (Ar-C), 146.30 (triazole C₃), 147.81 (N=CH), 151.24 (triazole C₅), 165.15 (C=O); UV (C₂H₅OH) λ_{max} (log ε) 290 (7834), 238 (14242), 222 (14844) nm.

2.2.2.6 3-*p*-Methoxybenzyl-4-[2-(2-methylbenzoxo)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (3f)

Yield 98.33%. Mp: 206°C. IR (cm⁻¹) 3163 (ν_{NH}), 1729, 1702 (ν_{C=O}), 1590 (ν_{C=N}), 1242 (ν_{COO}); ¹H NMR (400MHz, DMSO-*d*₆) δ 2.58 (s, 3H, PhCH₃), 3.71 (s, 3H, OCH₃), 3.89 (s, 2H, CH₂Ph), 6.87 (d, 2H, ArH; J=8.80 Hz), 7.20 (d, 2H, ArH; J=8.80 Hz), 7.39-7.50 (m, 4H, ArH), 7.57-7.66 (m, 2H, Ar-H), 8.02-8.05 (m, 1H, Ar-H), 8.14-8.16 (m, 1H, Ar-H), 9.94 (s, 1H, N=CH), 11.90 (s, 1H, NH); ¹³C NMR (100MHz, DMSO-*d*₆) δ 21.10 (PhCH₃), 30.25 (CH₂Ph), 54.99 (OCH₃), 113.85 (2C), 123.69, 126.17, 126.24, 126.53, 126.74, 127.41, 127.76, 129.83 (2C), 130.86, 131.86, 132.58, 133.20, 140.36, 150.03, 150.88 (Ar-C), 146.47 (triazole C₃), 147.88 (N=CH), 151.23 (triazole C₅), 165.14 (C=O); UV (C₂H₅OH) λ_{max} (log ε) 283 (11541), 230 (24715) nm.

2.2.2.7 3-*p*-Chlorobenzyl-4-[2-(2-methylbenzoxo)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (3g)

Yield 99.33%. Mp: 228°C. IR (cm⁻¹) 3163 (ν_{NH}), 1734, 1702 (ν_{C=O}), 1595 (ν_{C=N}), 1231 (ν_{COO}); ¹H NMR (400MHz, DMSO-*d*₆) δ 2.60 (s, 3H, PhCH₃), 3.99 (s, 2H, CH₂Ph), 7.32 (d, 2H, ArH; J=8.40 Hz), 7.38 (d, 2H, ArH; J=8.40 Hz), 7.39-7.49 (m, 4H, ArH), 7.58-7.67 (m, 2H, Ar-H), 8.00-8.03 (m, 1H, Ar-H), 8.16-8.18 (m, 1H, Ar-H), 9.97 (s, 1H, N=CH), 11.98 (s, 1H, NH); ¹³C NMR (100MHz, DMSO-*d*₆) δ 21.12 (PhCH₃), 30.20 (CH₂Ph), 123.68, 126.12, 126.23, 126.52, 126.69, 127.74, 128.35 (2C), 130.69 (2C), 130.86, 131.45, 131.87, 132.60, 133.19, 134.60, 140.38, 150.05 (Ar-C), 145.81 (triazole C₃), 148.01 (N=CH), 151.21 (triazole C₅), 165.11 (C=O); UV (C₂H₅OH) λ_{max} (log ε) 290 (10938), 226 (21988).

2.2.2.8 3-*m*-Chlorobenzyl-4-[2-(2-methylbenzoxo)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (3h)

Yield 96.67%. Mp: 196°C. IR (cm⁻¹) 3166 (ν_{NH}), 1728, 1708 (ν_{C=O}), 1587 (ν_{C=N}), 1229 (ν_{C=O}); ¹H NMR (400MHz, DMSO-*d*₆) δ 2.60 (s, 3H, PhCH₃), 4.02 (s, 2H, CH₂Ph), 7.25-7.29 (m, 4H, ArH), 7.25-7.49 (m, 4H, ArH), 7.57-7.67 (m, 2H, Ar-H), 8.01-8.03 (m, 1H, Ar-H), 8.16-8.19 (m, 1H, Ar-H), 9.97 (s, 1H, N=CH), 12.03 (s, 1H, NH); ¹³C NMR (100MHz, DMSO-*d*₆) δ 21.12 (PhCH₃), 30.50 (CH₂Ph), 123.67, 126.13, 126.22, 126.49, 126.64, 126.74, 127.54, 127.74, 128.93, 130.02, 130.87, 131.87, 132.59, 132.96, 133.17, 138.00, 140.39, 150.07 (Ar-C), 145.63 (triazole C₃), 147.93 (N=CH), 151.21 (triazole C₅), 165.11 (C=O); UV (C₂H₅OH) λ_{max} (log ε) 291 (12342), 239 (22237), 222 (24678).

2.2.2.9 3-Phenyl-4-[2-(2-methylbenzoxyl)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (3i)

Yield 98.53%. Mp: 238°C. IR (cm⁻¹) 3153 (ν_{NH}), 1737, 1705 (ν_{C=O}), 1602 (ν_{C=N}), 1234 (ν_{C=O}); ¹H NMR (400MHz, DMSO-*d*₆) δ 2.60 (s, 3H, PhCH₃), 7.38-7.67 (m, 5H, ArH), 7.38-7.67 (m, 4H, ArH), 7.87-7.90 (m, 2H, Ar-H), 7.99-8.01 (m, 1H, Ar-H), 8.13-8.15 (m, 1H, Ar-H), 9.94 (s, 1H, N=CH), 12.36 (s, 1H, NH); ¹³C NMR (100MHz, DMSO-*d*₆) δ 21.09 (PhCH₃), 123.79, 126.08, 126.16, 126.49, 126.58, 126.77, 126.79, 128.00 (2C), 128.45 (2C), 130.08, 130.84, 131.82, 132.74, 133.10, 140.33, 150.61 (Ar-C), 144.62 (triazole C₃), 150.20 (N=CH), 151.36 (triazole C₅), 165.12 (C=O); UV (C₂H₅OH) λ_{max} (log ε) 260 (13116), 234 (16566).

2.3 Antioxidant activity

2.3.1 Chemicals

Butylated hydroxytoluene (BHT) was obtained from E. Merck. Ferrous chloride, α-tocopherol, DPPH[•], 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA), ethylenediaminetetraacetic acid (EDTA) and trichloroacetic acid (TCA) were obtained from Sigma.

2.3.2 Reducing Power

The reducing power of the synthesized **new** compounds was determined according to the method of Oyaizu [12]. Different concentrations of the samples (50-250 µg/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. after which a portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 1000 x g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%), and then **the absorbance was measured at 700 nm in a spectrophotometer**. Higher absorbance of the reaction mixture indicated greater reducing power.

2.3.3 Free Radical Scavenging Activity

Free radical scavenging activity of the **new** compounds was measured by DPPH[•], using the method of Blois [13]. Briefly, 0.1 mM solution of DPPH[•] in ethanol was prepared, and this solution (1 mL) was added to sample solutions in DMSO (3 mL) at different concentrations (50-250 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH[•] concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997):

$$\text{Absorbance} = 0.0003 \times \text{DPPH}^{\bullet} - 0.0174$$

The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH}^{\bullet} \text{ scavenging effect (\%)} = (A_0 - A_1/A_0) \times 100$$

where A₀ is the absorbance of the control reaction and A₁ is the absorbance in the presence of the samples or standards.

2.3.4 Metal Chelating Activity

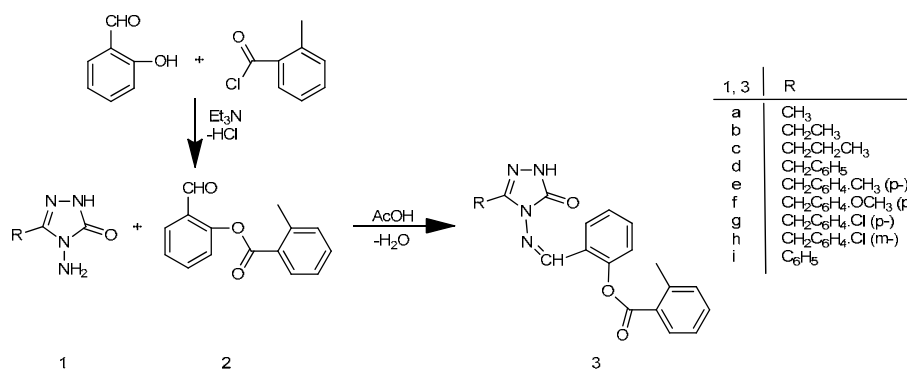
The chelation of ferrous ions by the synthesized **new** compounds and standards were estimated by the method of Dinis et al. [14]. Briefly, the synthesized compounds (30-90 µg/mL) were added to a 2 mM solution of FeCl₂·4H₂O (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and the mixture was shaken vigorously and left standing at the room temperature for 10 min.

After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm in a spectrophotometer. The percentage inhibition of ferrozine-Fe²⁺ complex formation was given by the formula: % Inhibition = (A₀ – A₁/A₀) x 100, where A₀ is the absorbance of the control, and A₁ is the absorbance in the presence of the samples or standards. The control did not contain compounds or standard.

3. RESULTS AND DISCUSSION

3.1 Chemistry

In this study the 3-alkyl(aryl)-4-[2-(2-methylbenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-ones **3a-i** were prepared. The starting compounds **1a-i** were prepared as described in the literature [15, 16]. Compounds **3a-i** were synthesized from the reactions of compounds **1a-i** with 2-(2-methylbenzoxy)-benzaldehyde (**2**) which had also been synthesized by the reactions of 2-hydroxybenzaldehyde with 2-methylbenzoyl chloride by using triethylamine (Scheme 1).



Scheme 1. Synthetic route of compounds **3a-i**

The structures of nine new 3-alkyl(aryl)-4-[2-(2-methylbenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-ones **3a-i** were characterized by using IR, ¹H-NMR, ¹³C-NMR and UV spectral data.

3.2 Antioxidant Activity

The antioxidant activity of nine new compounds **3a-i** was determined. Several methods have been used to determine antioxidant activities and the methods used in the study are given below:

3.2.1 Total reductive capability using the potassium ferricyanide reduction method

The reductive capabilities of compounds were assessed by the extent of conversion of the Fe³⁺ / ferricyanide complex to the Fe²⁺ / ferrous form. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHA, BHT and α-tocopherol. It has been observed that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [17]. The antioxidant activity of putative antioxidant has been attributed to various mechanisms, among which are prevention chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [18].

In this study, all the amount of the compounds showed lower absorbance than standard antioxidants. Hence, no activities were observed to reduce metal ions complexes to their lower oxidation state or to take part in any electron transfer reaction. In other words, synthesized compounds did not show the reductive activities.

3.2.2 DPPH[•] radical scavenging activity

The scavenging the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [19]. DPPH is a stable

free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [20]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidants because of reaction between antioxidant molecules and radical, progresses, which resulted in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants [21]. In the study, antiradical activities of compounds and standard antioxidants such as BHA and α -tocopherol were determined by using DPPH method. Scavenging effect values of compounds **3a-i**, BHA and α -tocopherol at different concentrations are given Figure 1. The newly synthesized compounds did not show a significant activity as a radical scavenger, but compound **3a** showed higher activities than the other compounds and its reductive ability was concentration-dependent as seen in Figure 1.

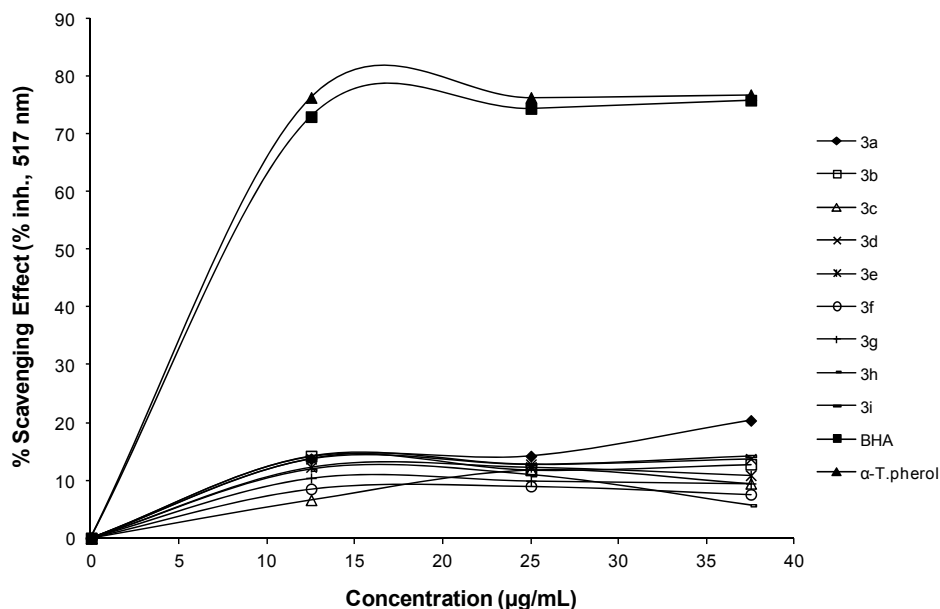
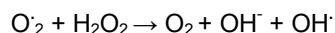


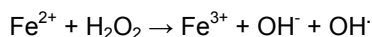
Fig. 1. Scavenging effect of compounds **3a-i, BHA and α -tocopherol at different concentrations (12.5-25-37.5 μ g/mL).**

3.2.3 Ferrous ion chelating activity

The chelating effect towards ferrous ions by the compounds and standards was determined. Ferrozine can quantitatively form complexes with Fe^{2+} . In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator [22]. Transition metals have significant role in the generation oxygen free radicals in living organism. The ferric iron (Fe^{3+}) is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+} , depending on condition, particularly pH [23] and oxidized back through Fenton type reactions with the production of hydroxyl radical or Haber-Weiss reactions with superoxide anions. The production of these radicals may lead to lipid peroxidation, protein modification and DNA damage. Chelating agents may not activate metal ions and potentially inhibit the metal-dependent processes [24]. Also, the production of highly active ROS such as $\text{O}_2^{\cdot-}$, H_2O_2 and $\text{OH}^{\cdot}$ is also catalyzed by free iron through Haber-Weiss reactions:



Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down the hydrogen and lipid peroxides to reactive free radicals via the Fenton reactions:



Fe³⁺ ion also produces radicals from peroxides, even though the rate is tenfold less than that of Fe²⁺ ion, which is the most powerful pro-oxidant among the various types of metal ions [25]. Ferrous ion chelating activities of compounds **3a-i** and standard antioxidants are shown in Figure 2. It was reported that chelating agents that form σ -bonds with a metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion [26].

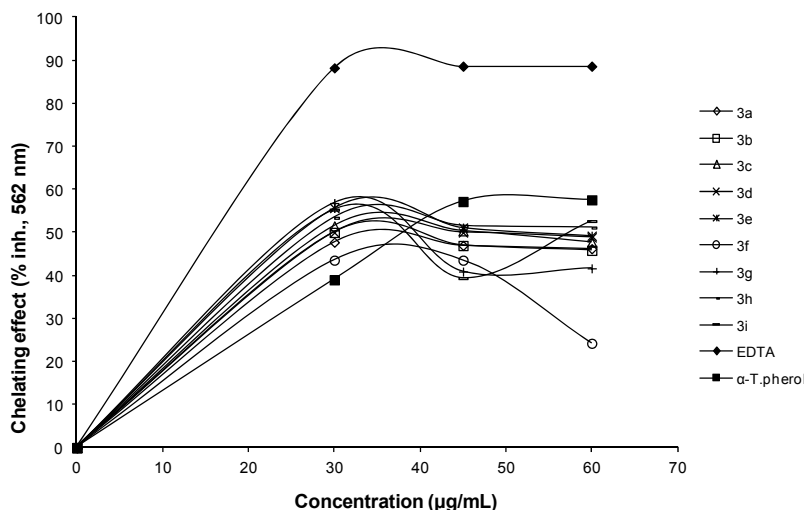


Fig. 2. Metal chelating effect of different amount of the compounds 3a-i, BHT, BHA and α-tocopherol on ferrous ions

Low absorbance at 562 nm indicates high metal chelating activity. The data obtained from Figure 2 reveal that the metal chelating effects of the compounds were not concentration-dependent. At the lowest concentration, all of the compounds showed better concentrations than α-tocopherol.

4. CONCLUSION

In this study, the structures of nine new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives synthesized from the reactions of **1** type compounds with a benzaldehyde derivative were identified using IR, ¹H NMR, ¹³C NMR, and UV spectral data. The newly synthesized compounds were screened for their antioxidant activities.

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Appendix:

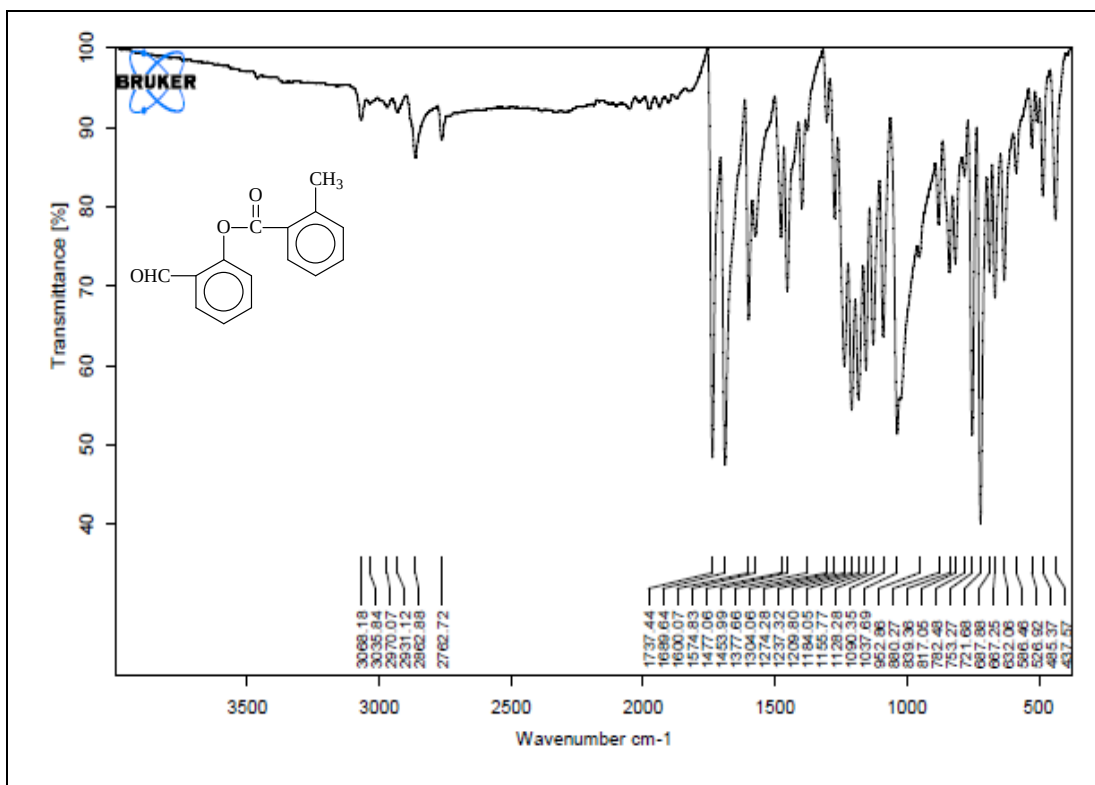


Figure 1. IR Spectrum of Compound 2

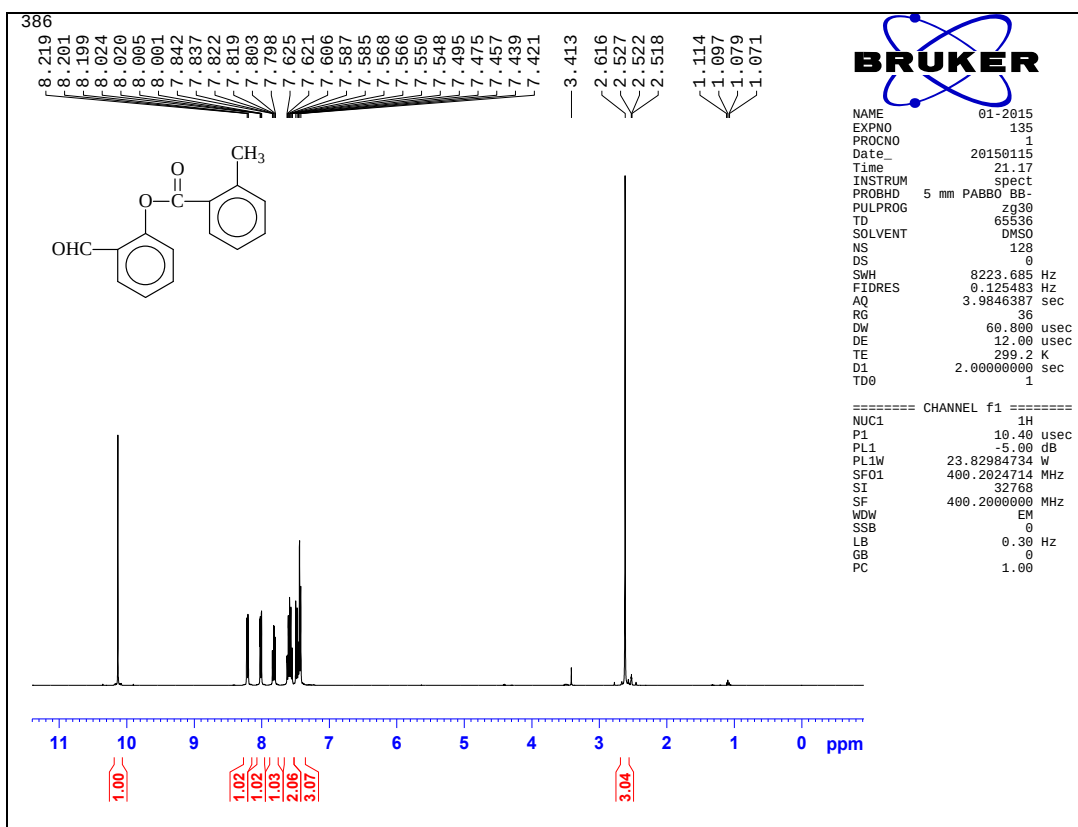


Figure 2. ¹H-NMR Spectrum of Compound 2

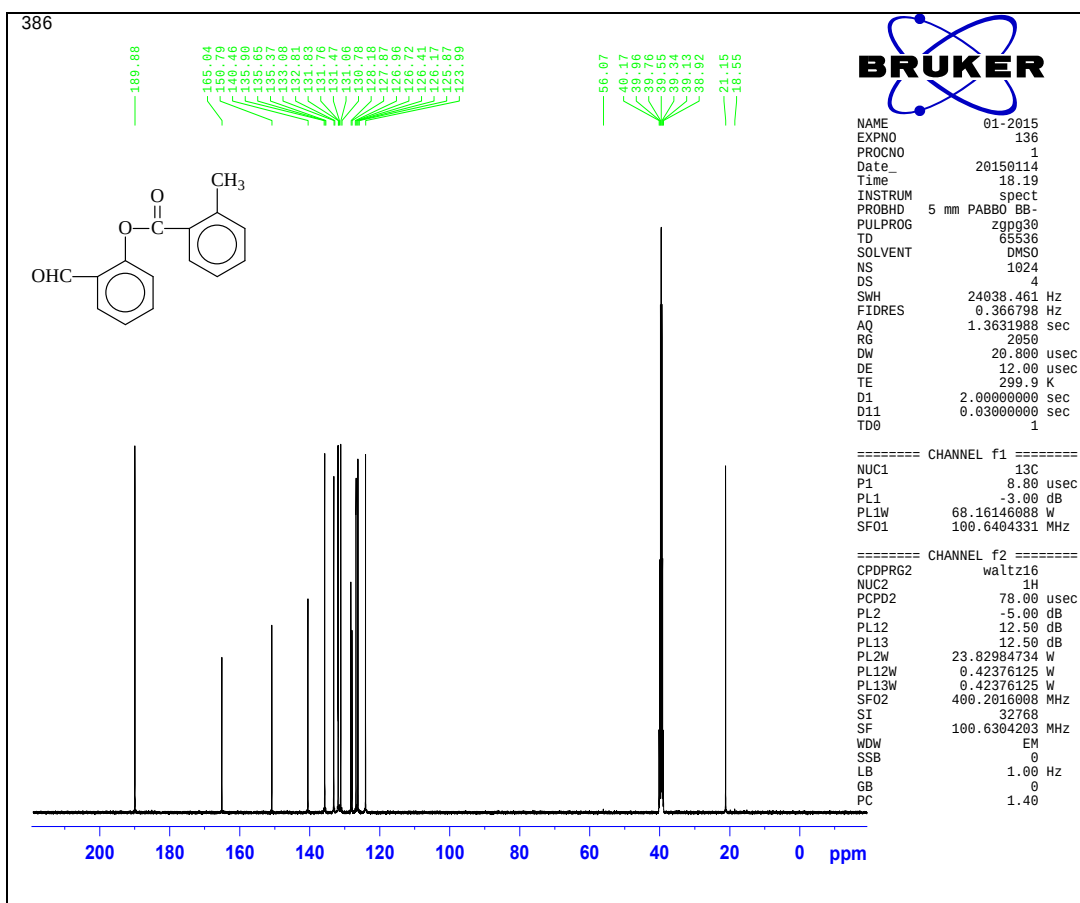


Figure 3. ¹³C-NMR Spectrum of Compound 2

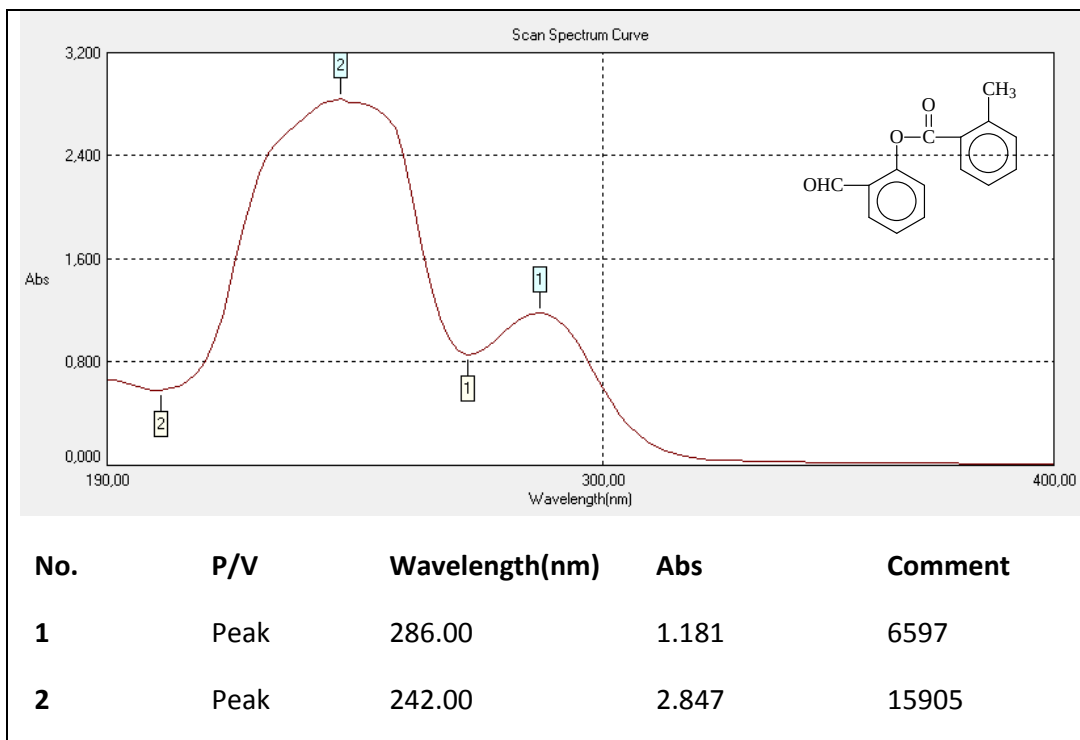


Figure 4. UV Spectrum of Compound 2

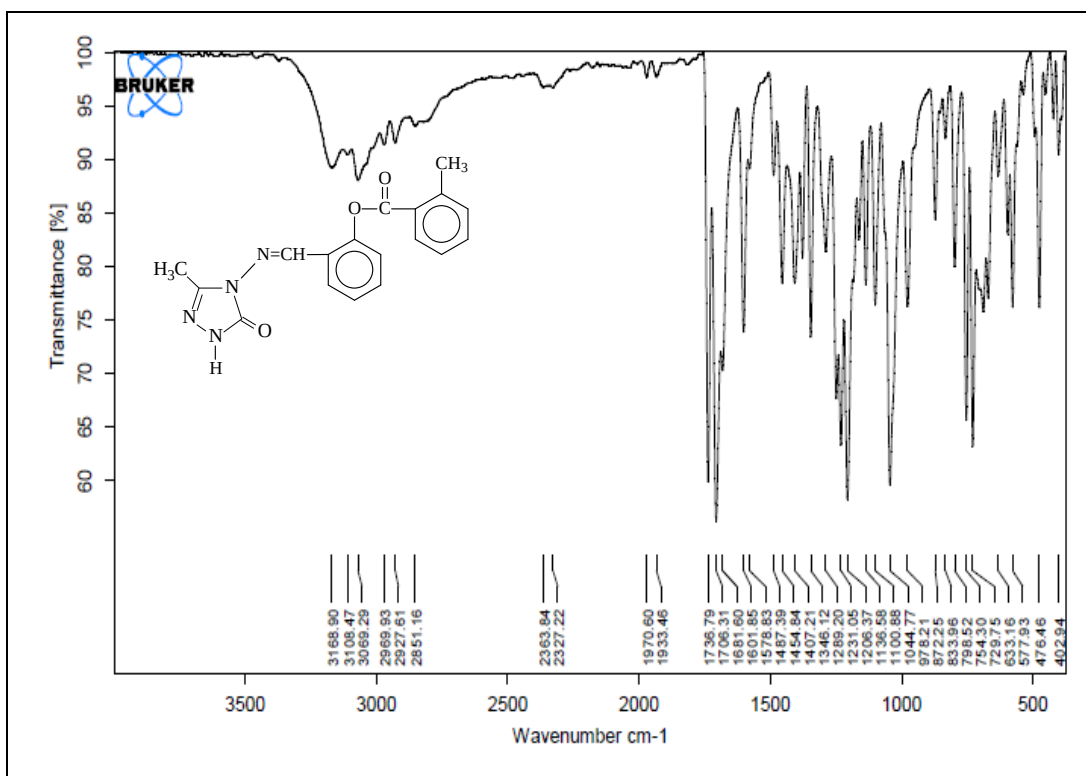
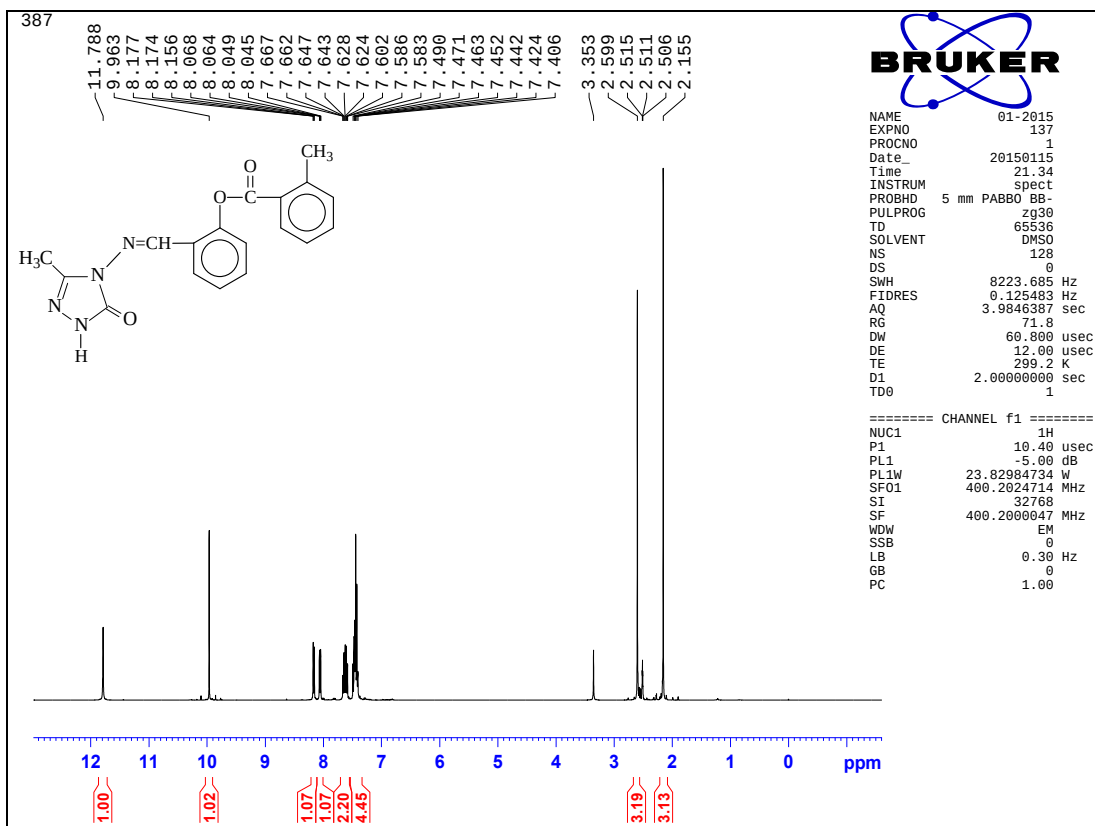
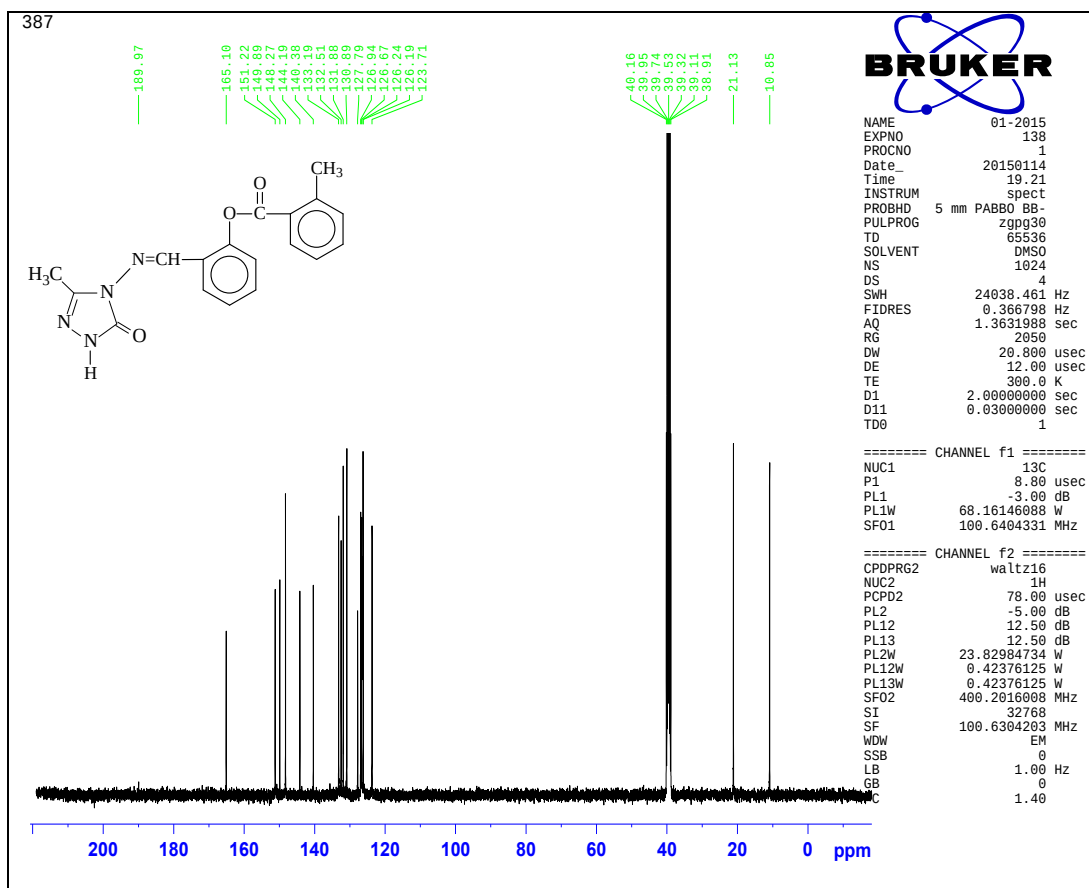


Figure 5. IR Spectrum of Compound 3a



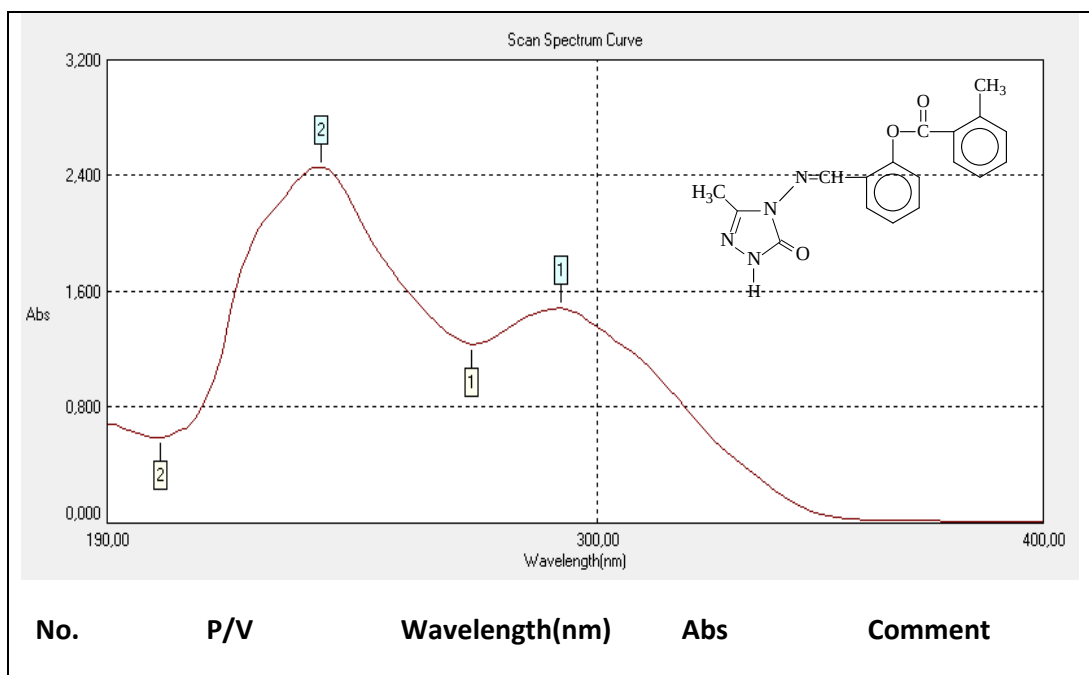
398

Figure 6. ¹H-NMR Spectrum of Compound 3a

399

Figure 7. ¹³C-NMR Spectrum of Compound 3a

400



1	Peak	292.00	1.484	9636
2	Peak	238.00	2.464	16000

Figure 8. UV Spectrum of Compound 3a

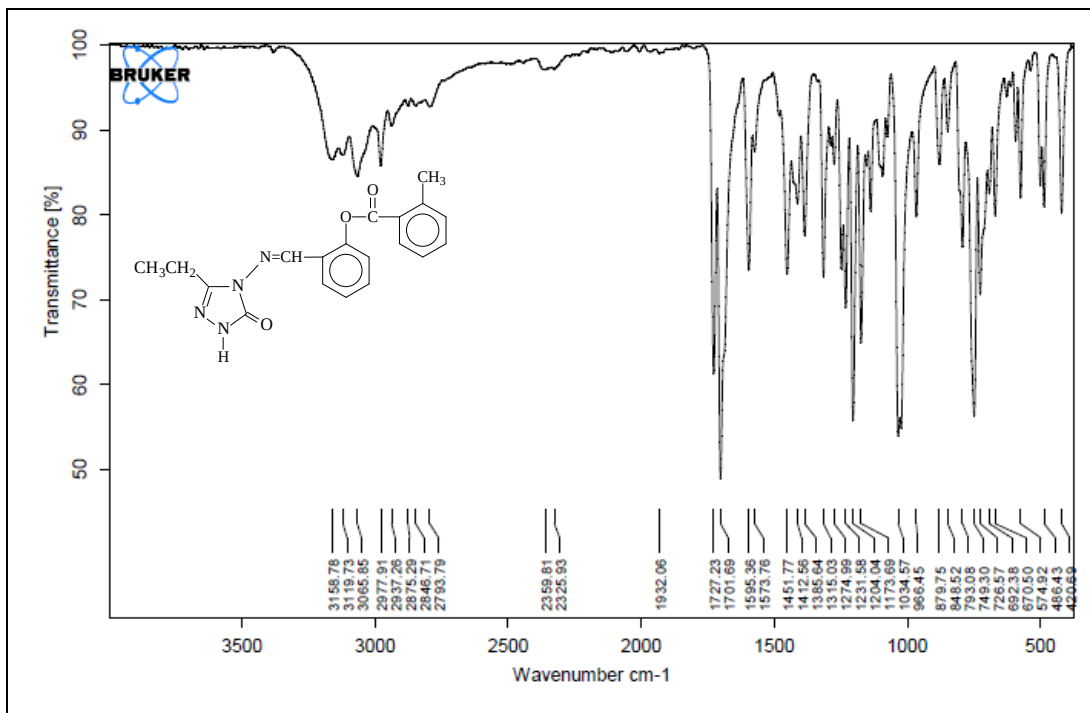


Figure 9. IR Spectrum of Compound 3b

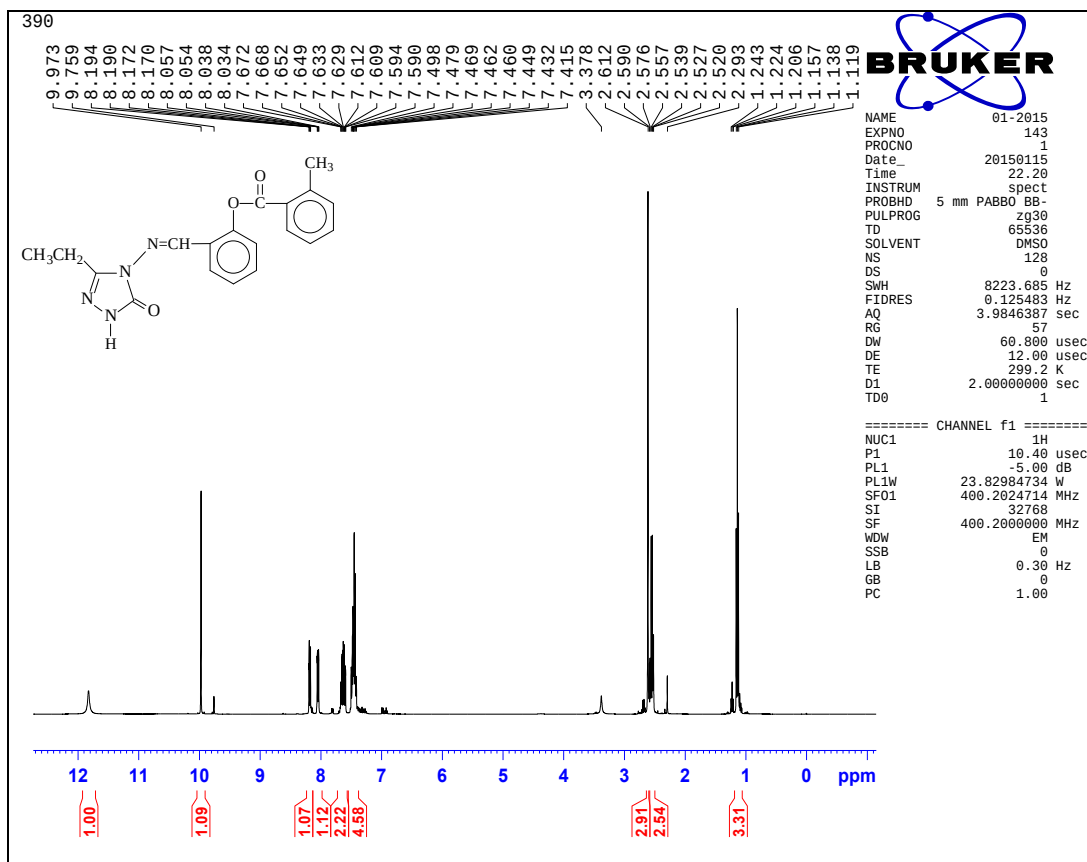


Figure 10. ¹H-NMR Spectrum of Compound 3b

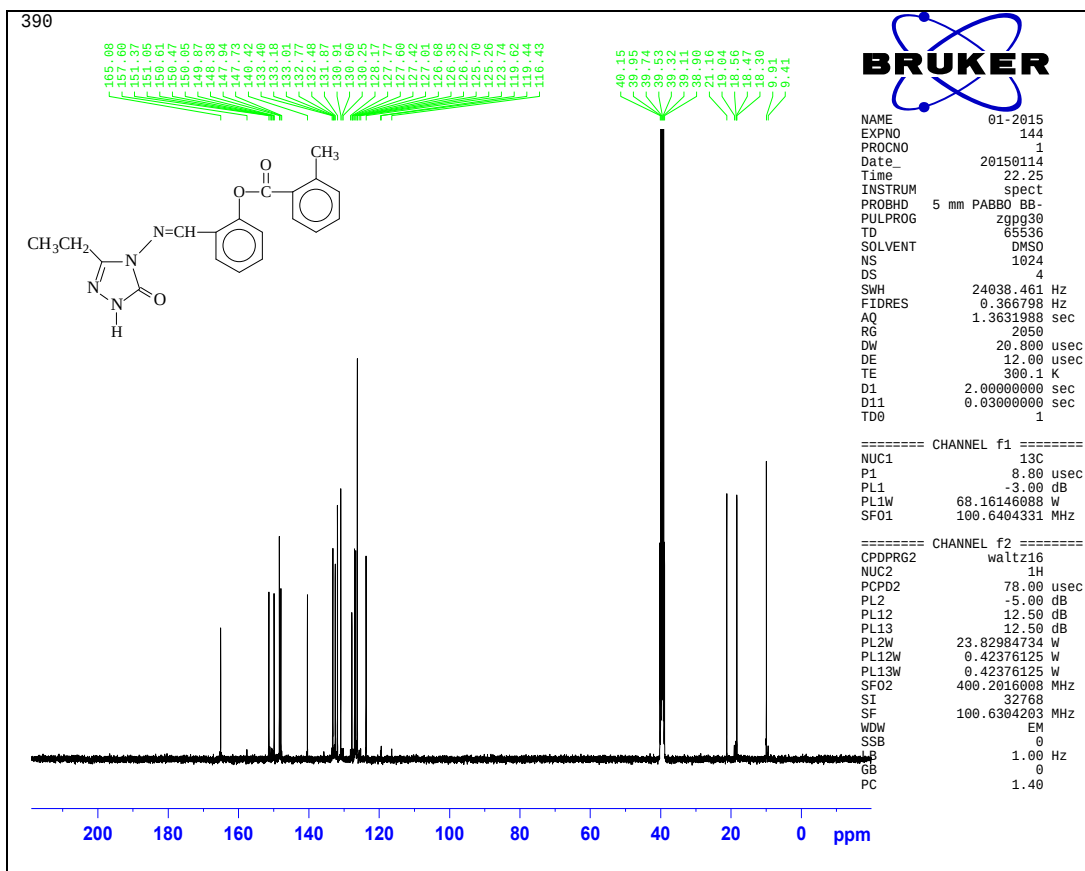


Figure 11. ¹³C-NMR Spectrum of Compound 3b

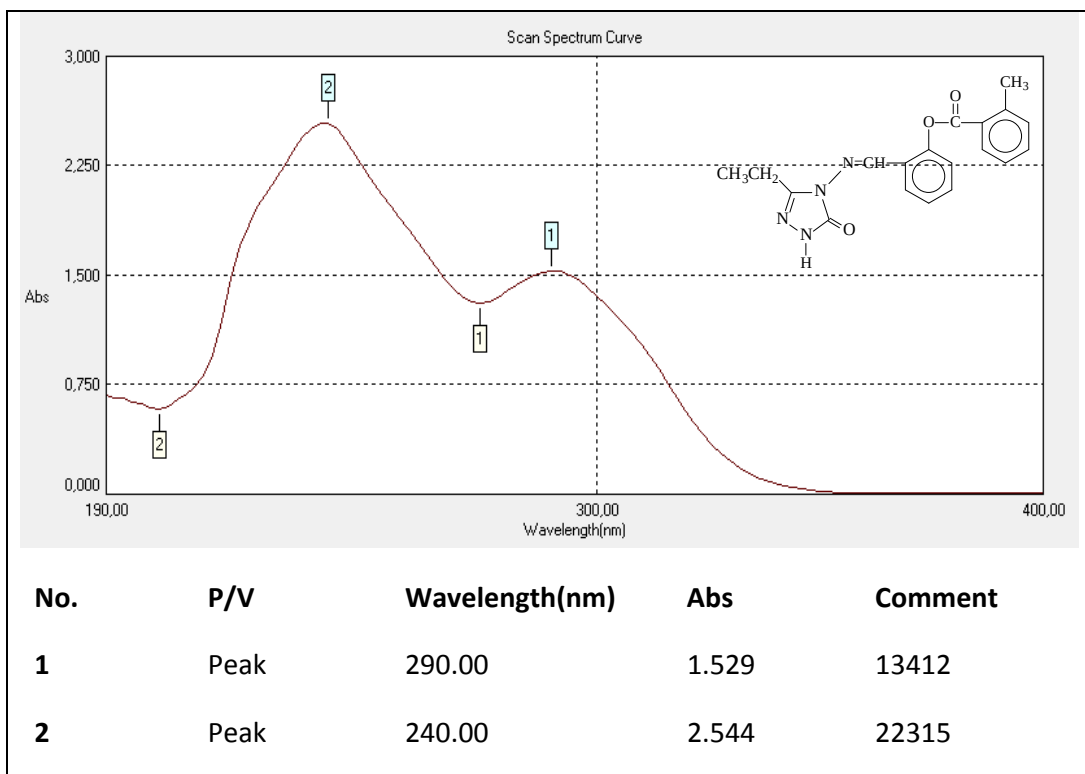


Figure 12. UV Spectrum of Compound 3b

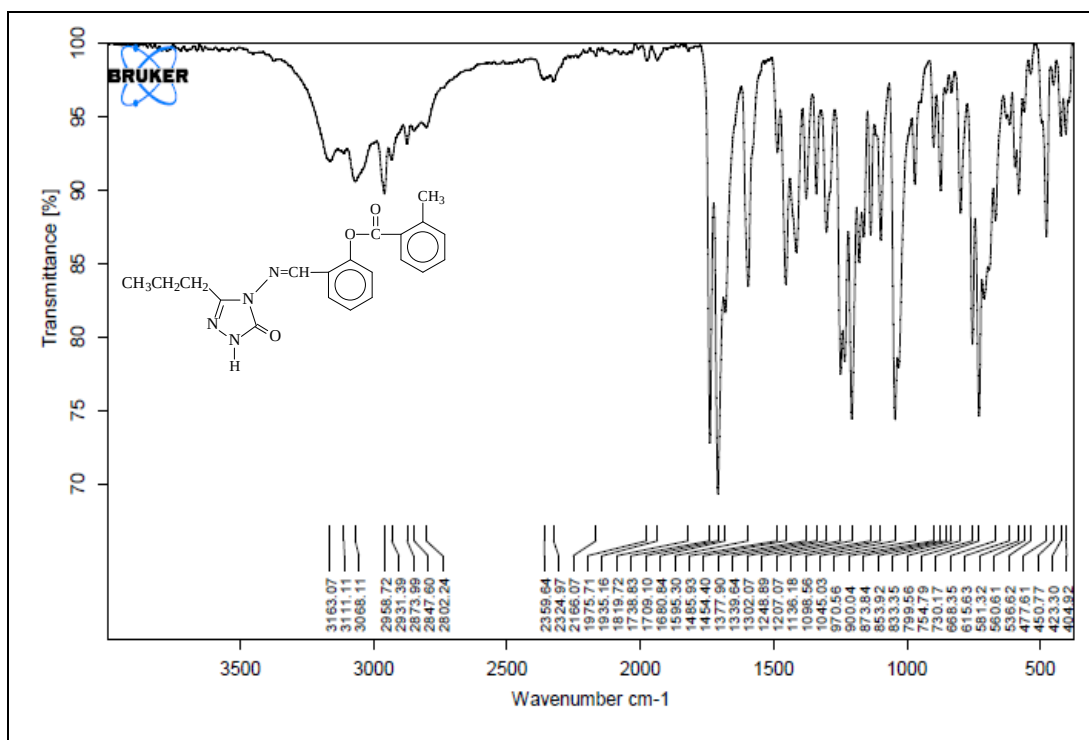
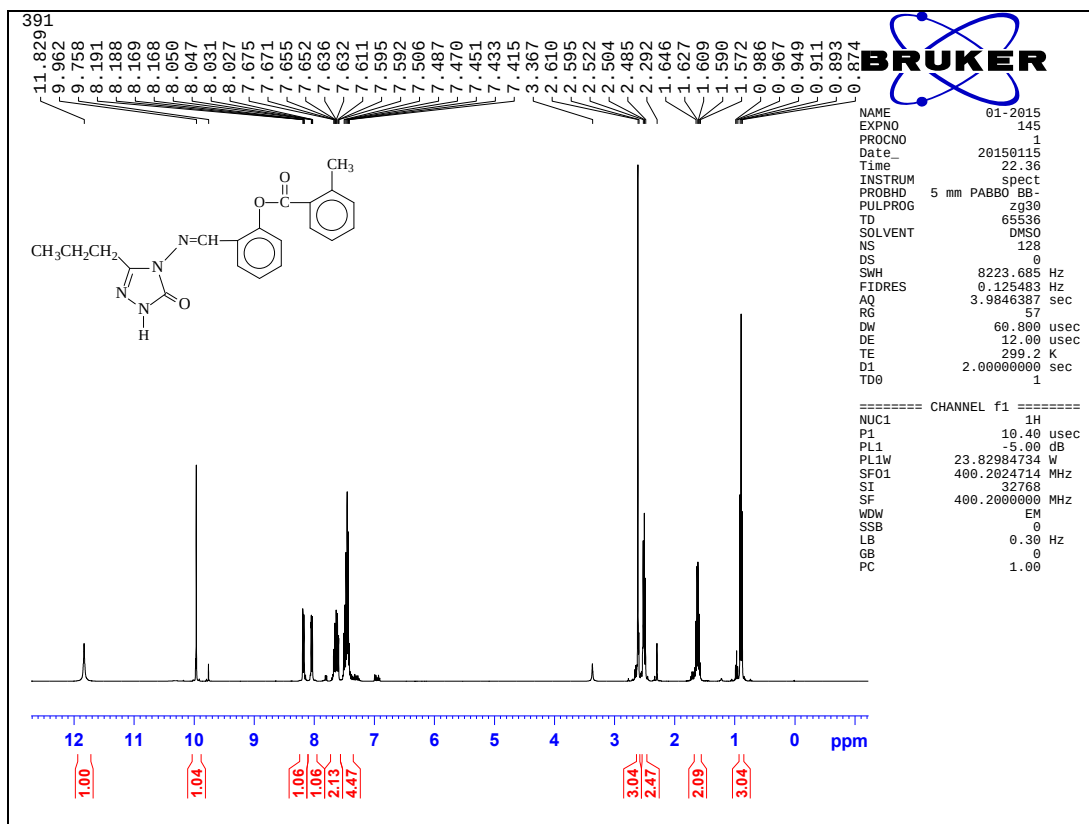
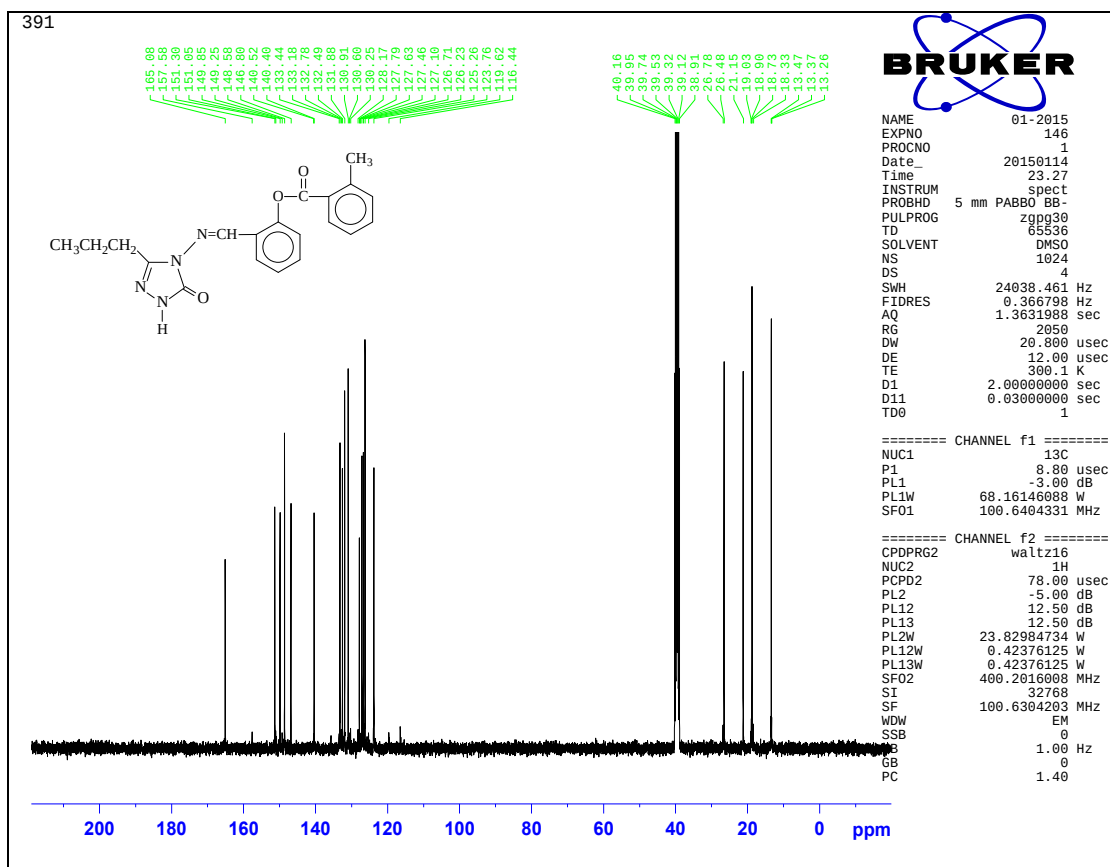


Figure 13. IR Spectrum of Compound 3c



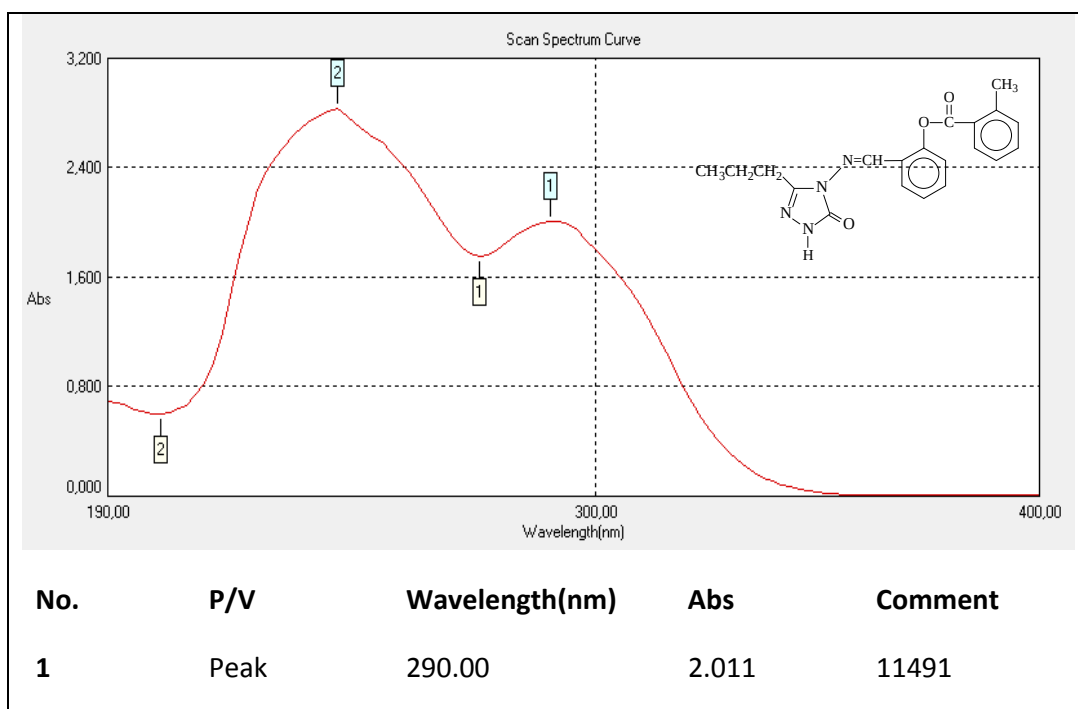
410

Figure 14. ^1H -NMR Spectrum of Compound 3c

411

Figure 15. ^{13}C -NMR Spectrum of Compound 3c

412



2	Peak	252.00	2.587	14782
3	Peak	242.00	2.836	16205

Figure 16. UV Spectrum of Compound 3c

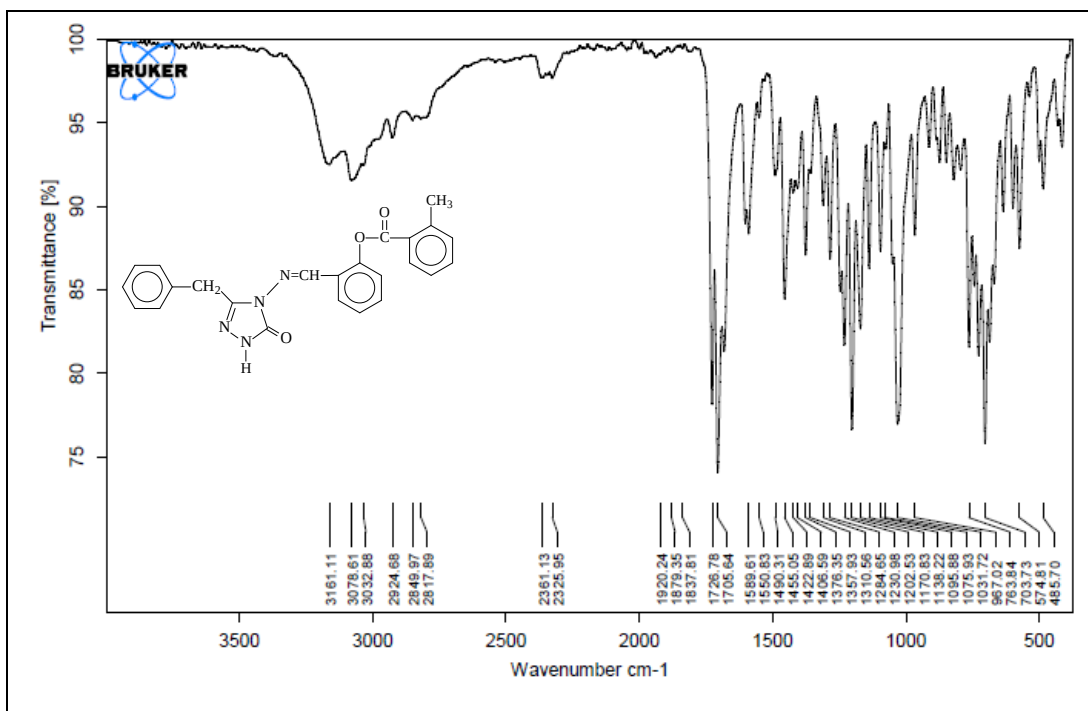
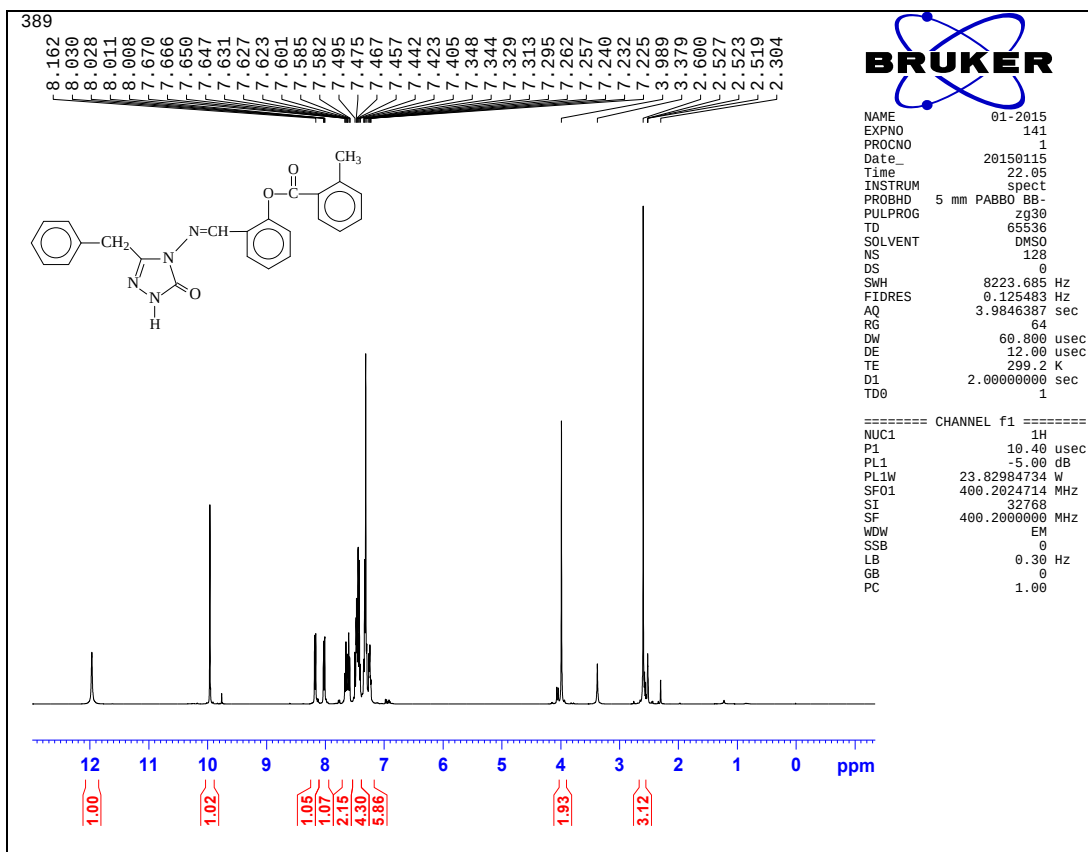


Figure 17. IR Spectrum of Compound 3d



416

Figure 18. ¹H-NMR Spectrum of Compound 3d

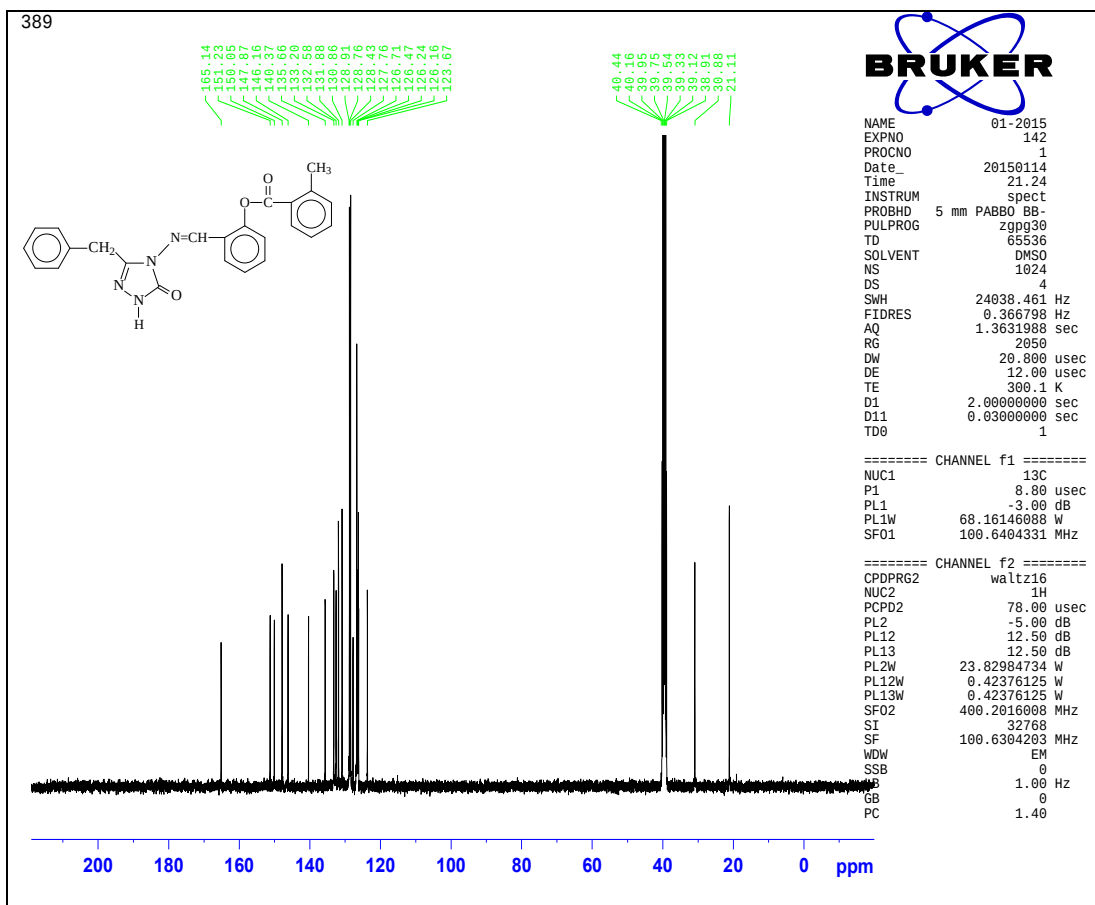
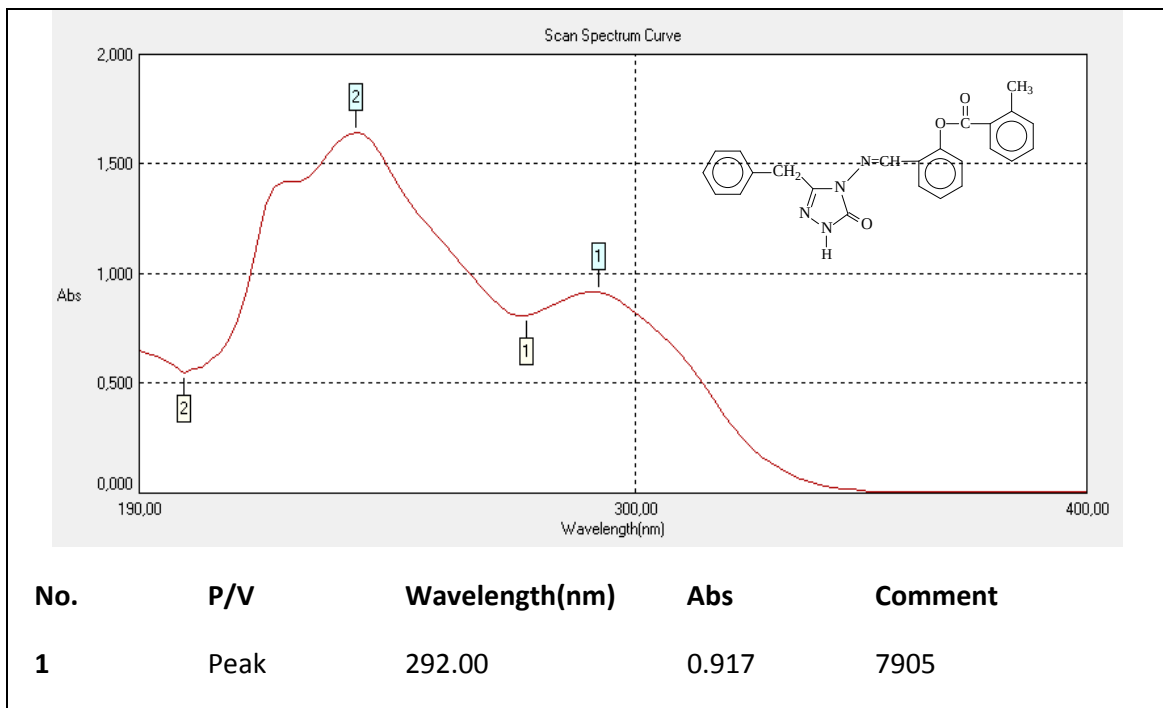


Figure 19. ^{13}C -NMR Spectrum of Compound 3d



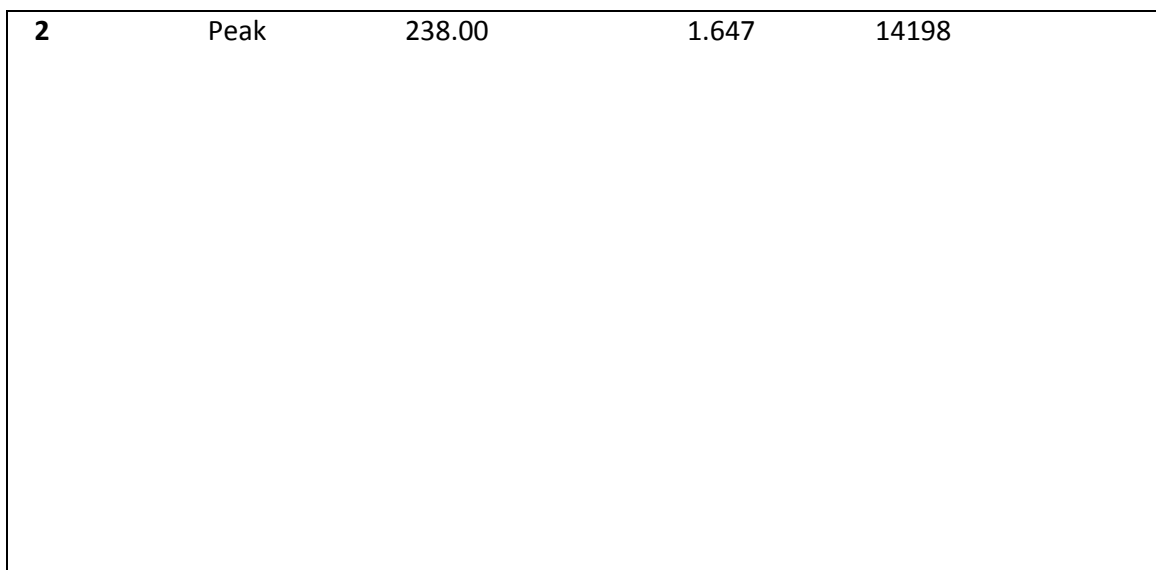


Figure 20. UV Spectrum of Compound 3d

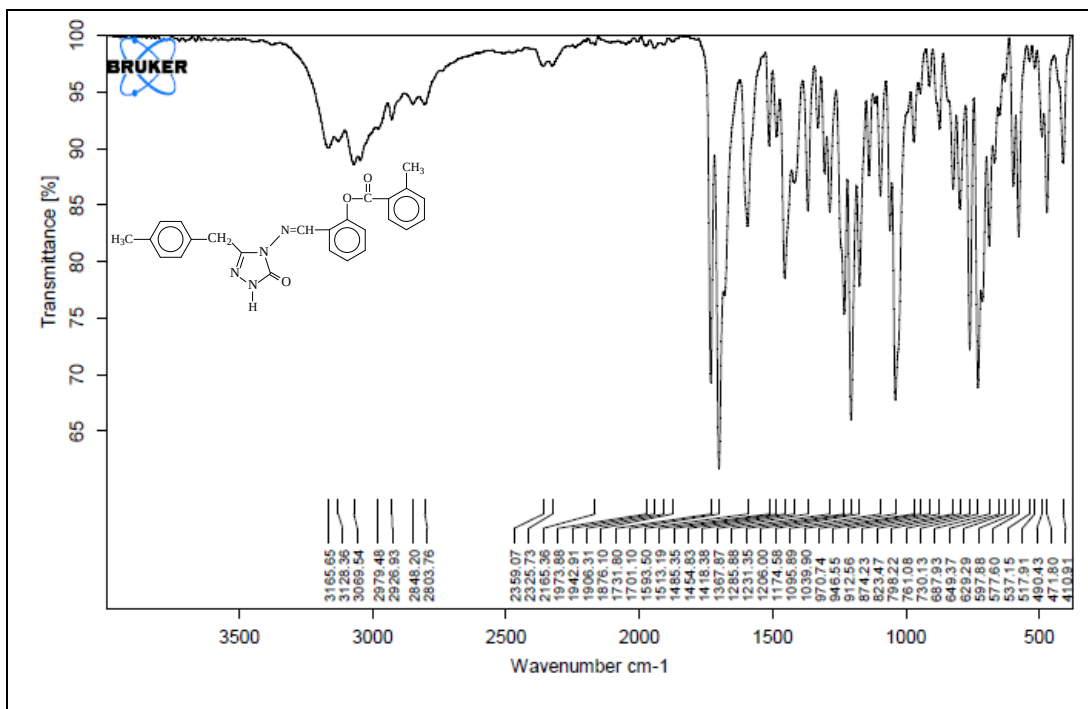


Figure 21. IR Spectrum of Compound 3e

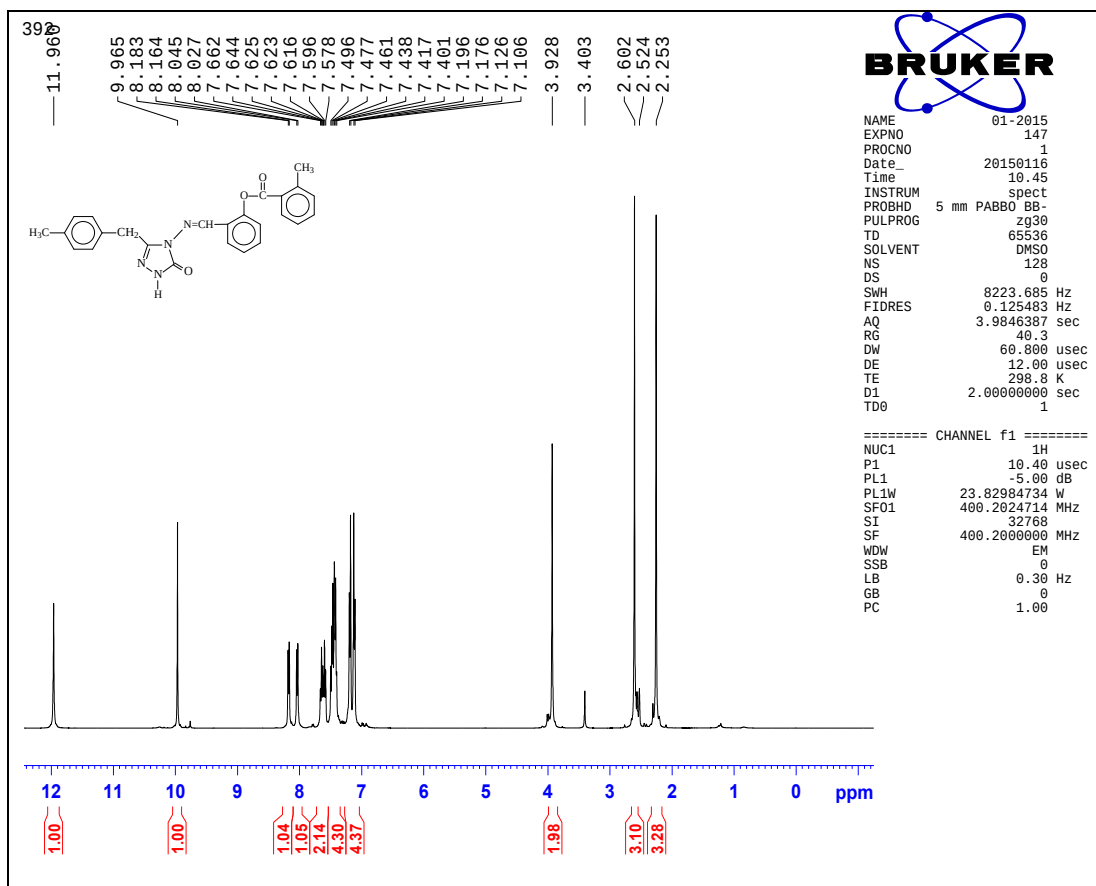


Figure 22. ¹H-NMR Spectrum of Compound 3e

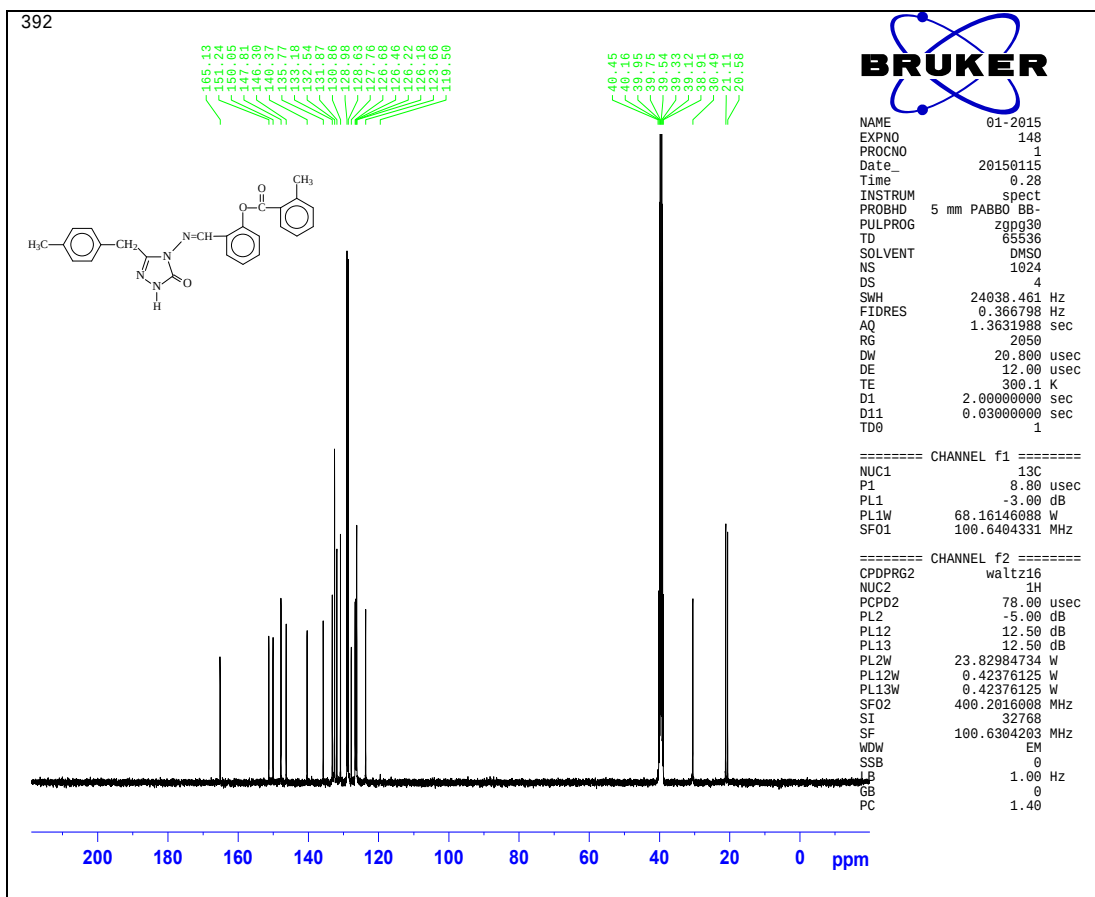
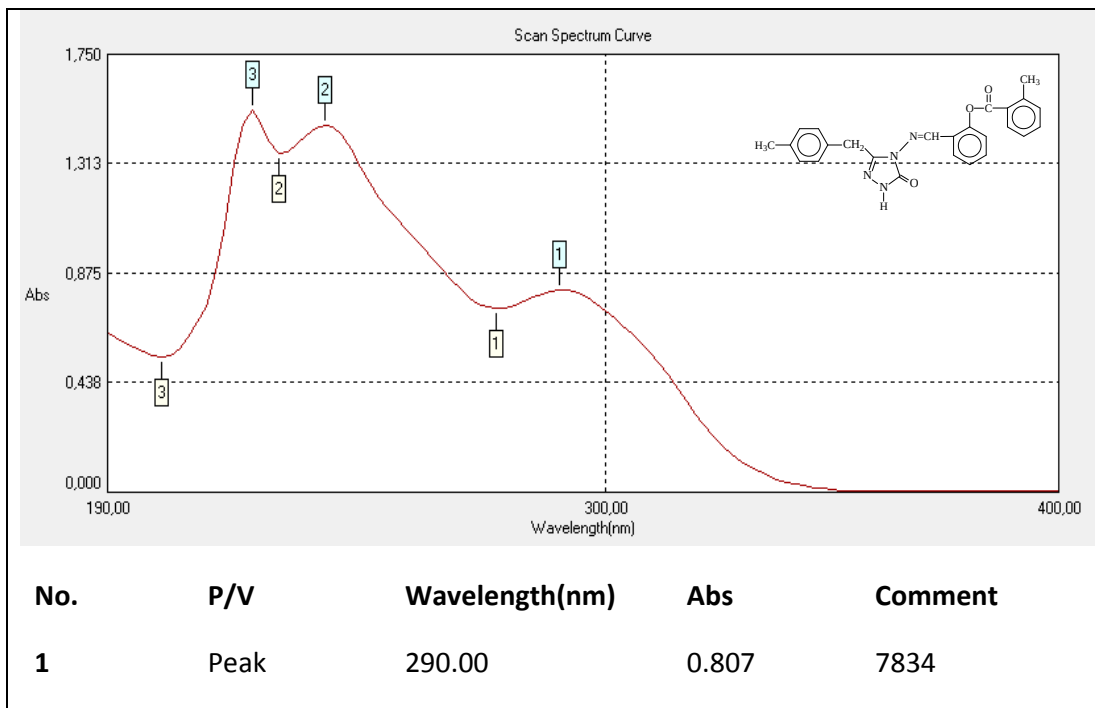


Figure 23. ¹³C-NMR Spectrum of Compound 3e



2	Peak	238.00	1.467	14242
3	Peak	222.00	1.529	148448

Figure 24. UV Spectrum of Compound 3e

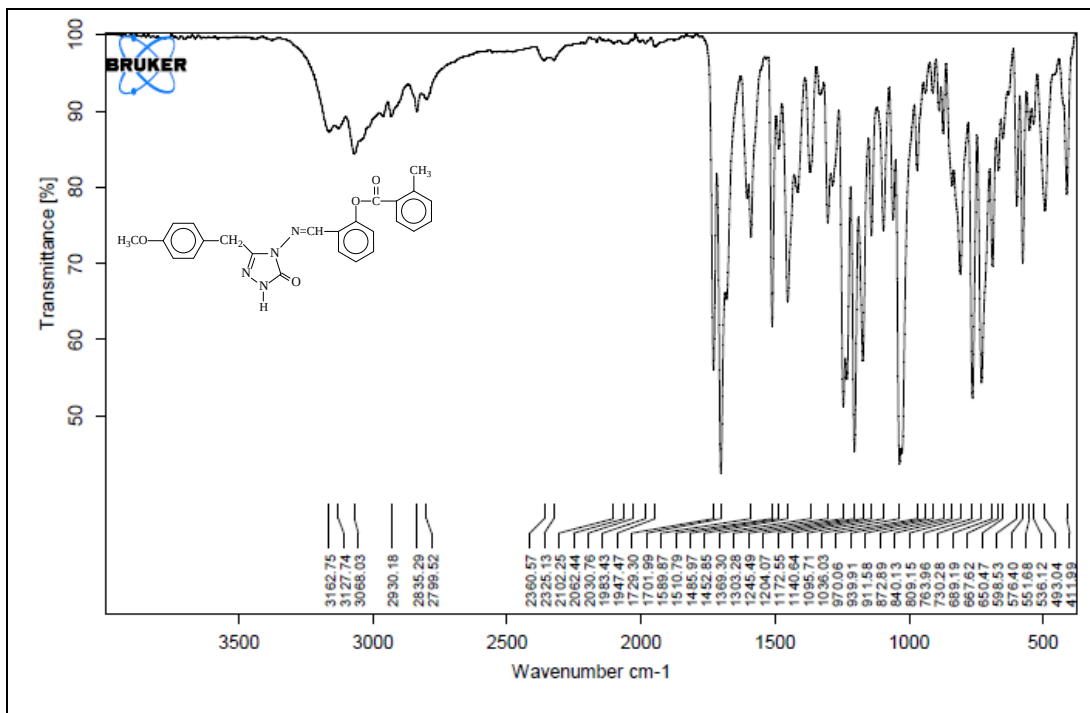
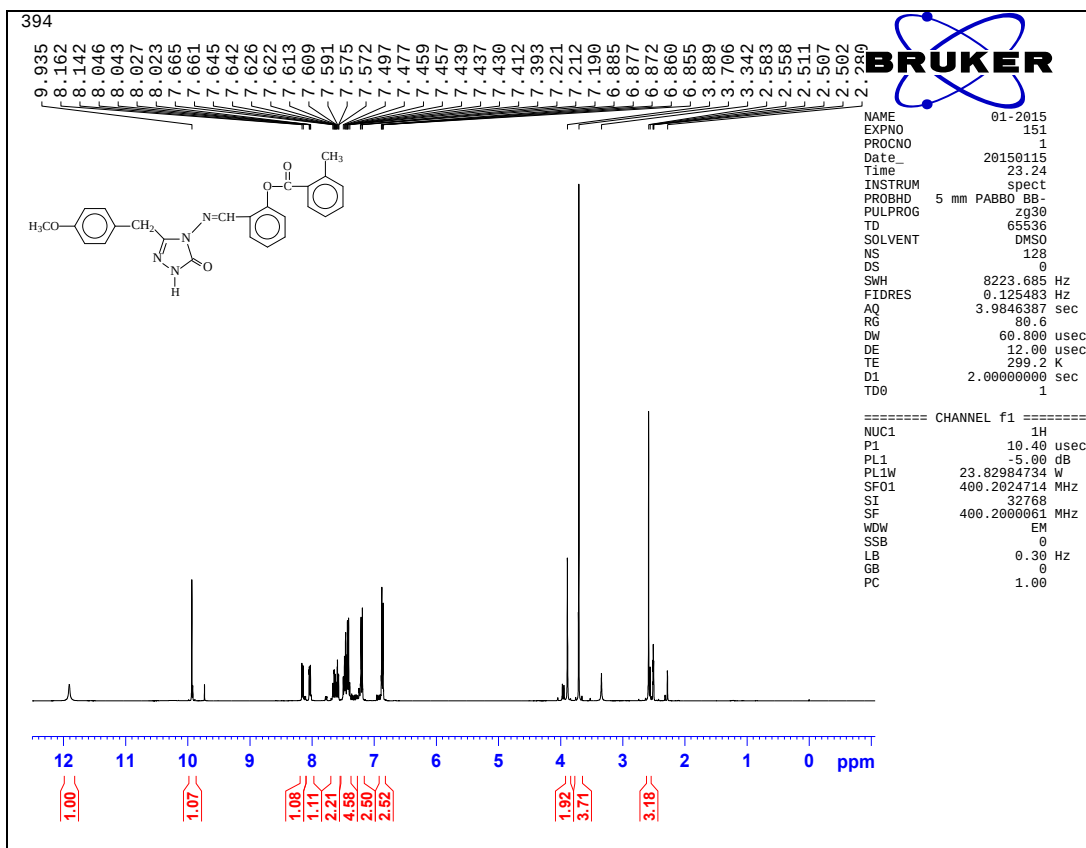


Figure 25. IR Spectrum of Compound 3f



428

Figure 26. ¹H-NMR Spectrum of Compound 3f

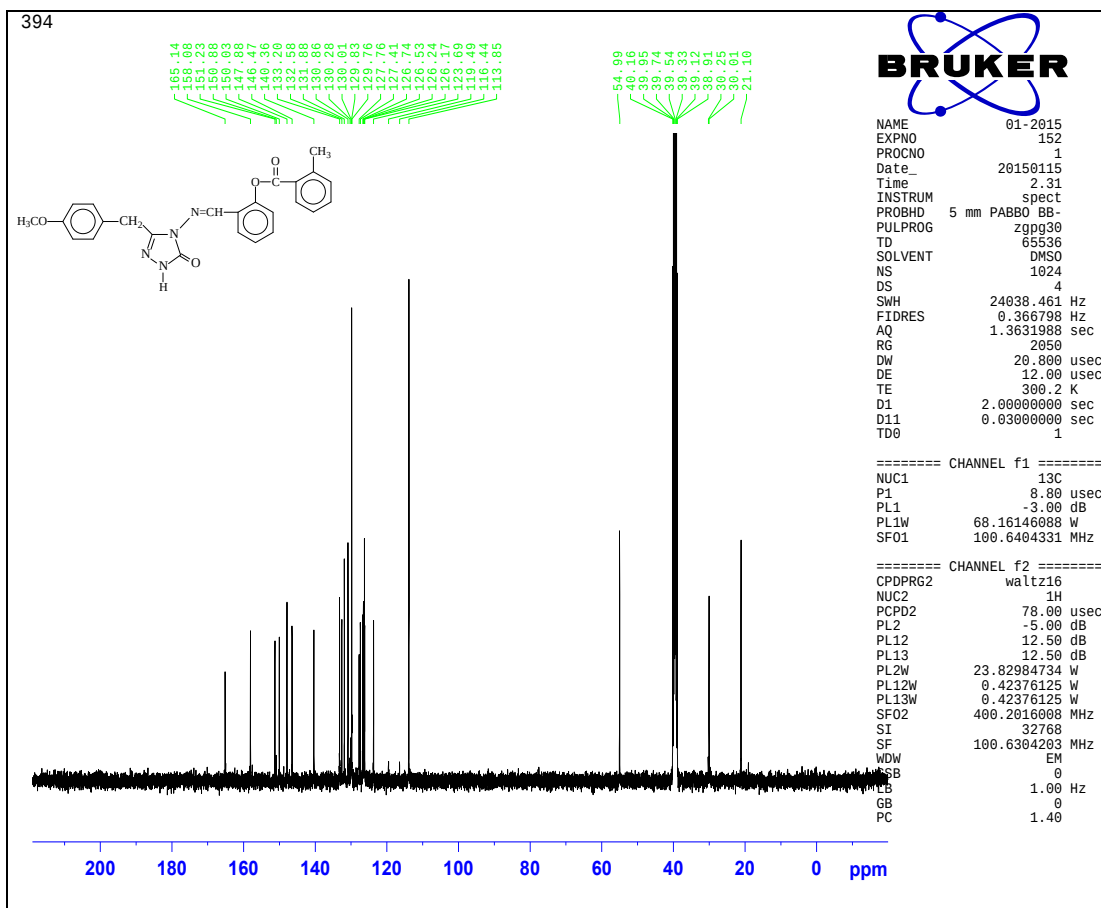
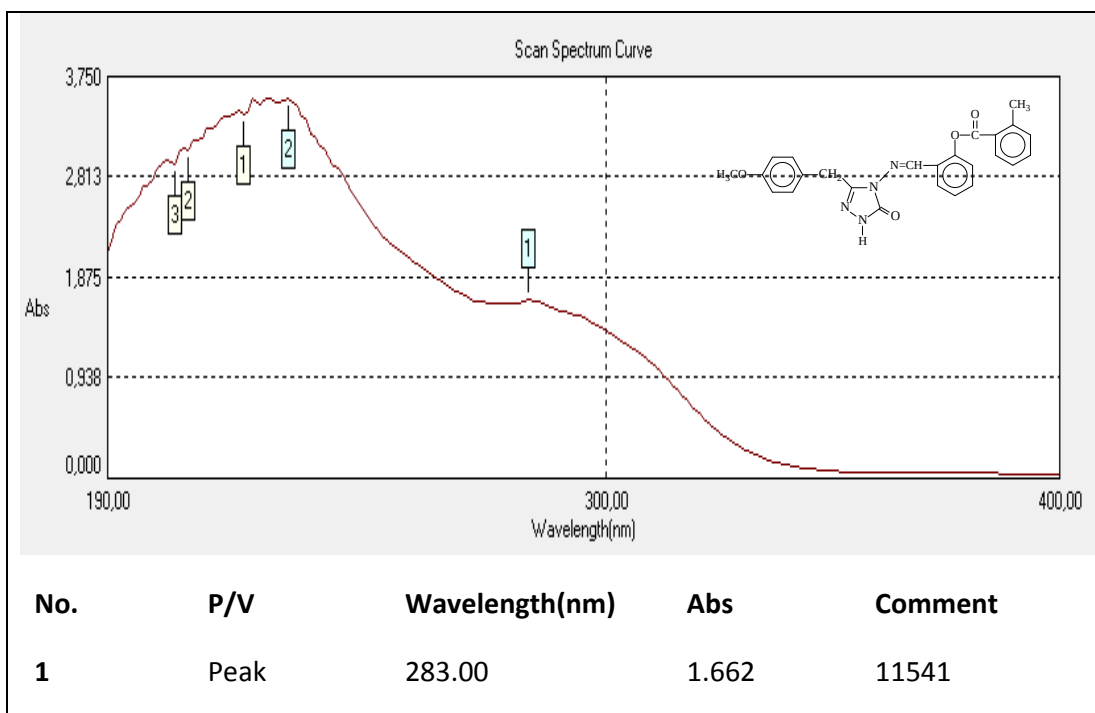


Figure 27. ^{13}C -NMR Spectrum of Compound 3f



No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	283.00	1.662	11541

2	Peak	230.00	3.559	24715
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Figure 28. UV Spectrum of Compound 3f

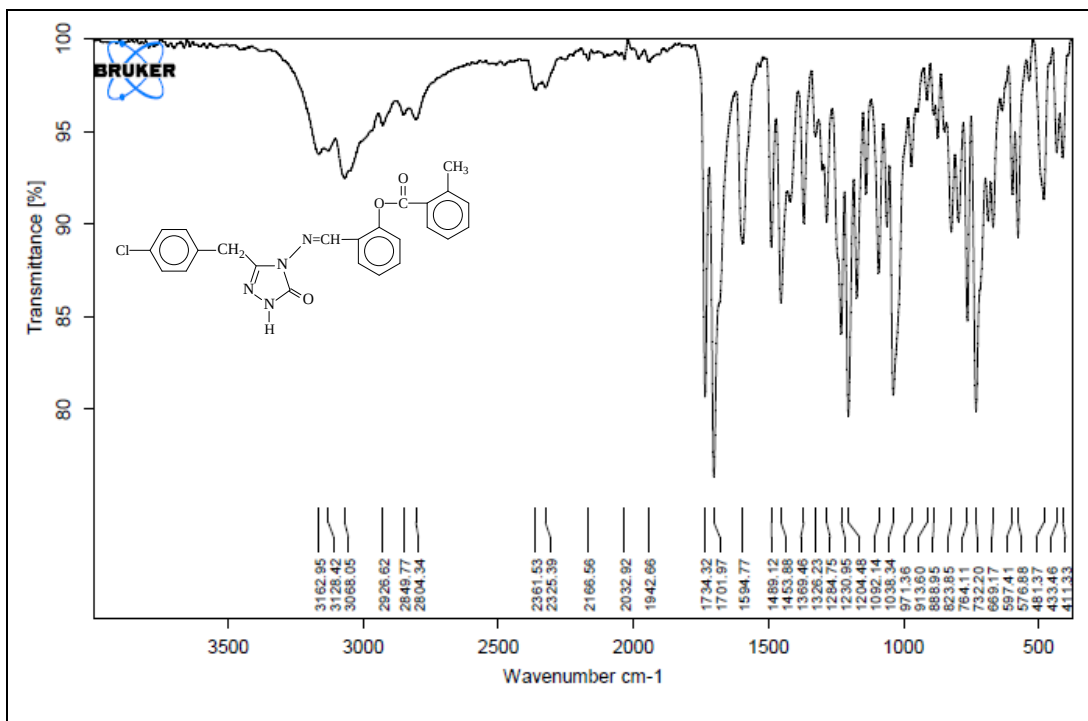


Figure 29. IR Spectrum of Compound 3g

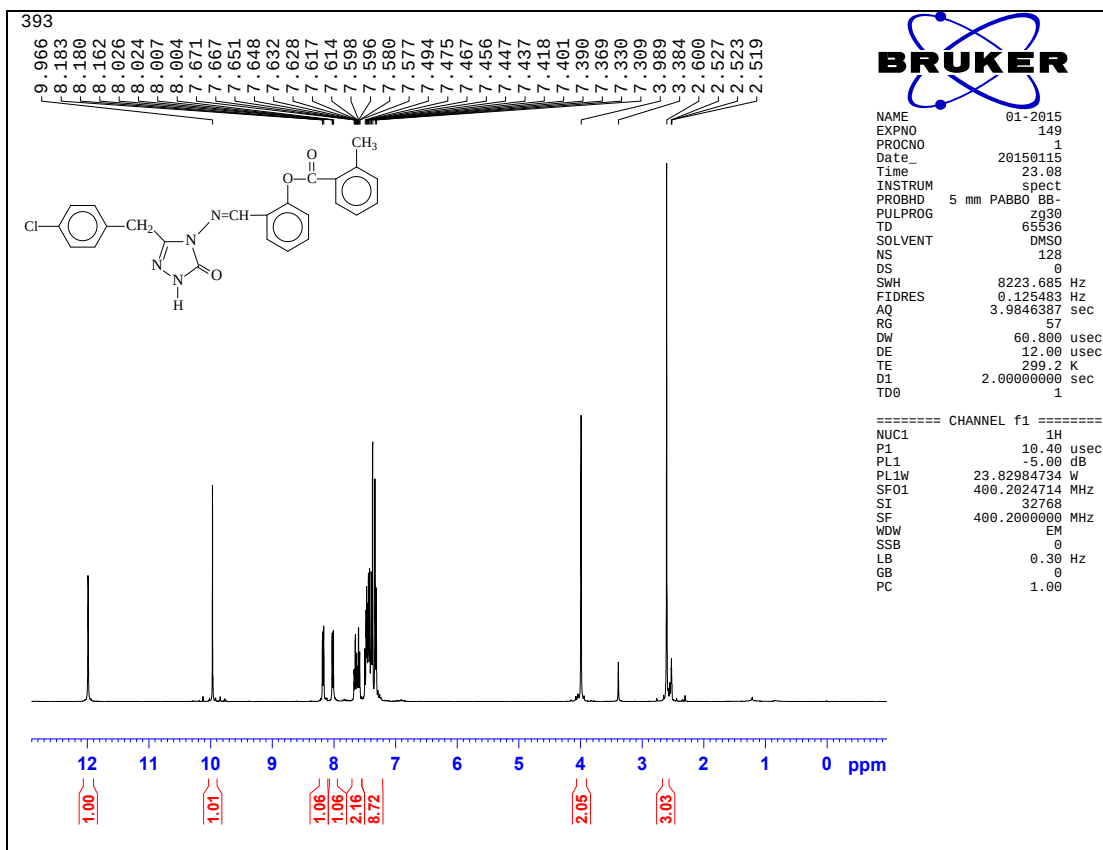


Figure 30. ¹H-NMR Spectrum of Compound 3g

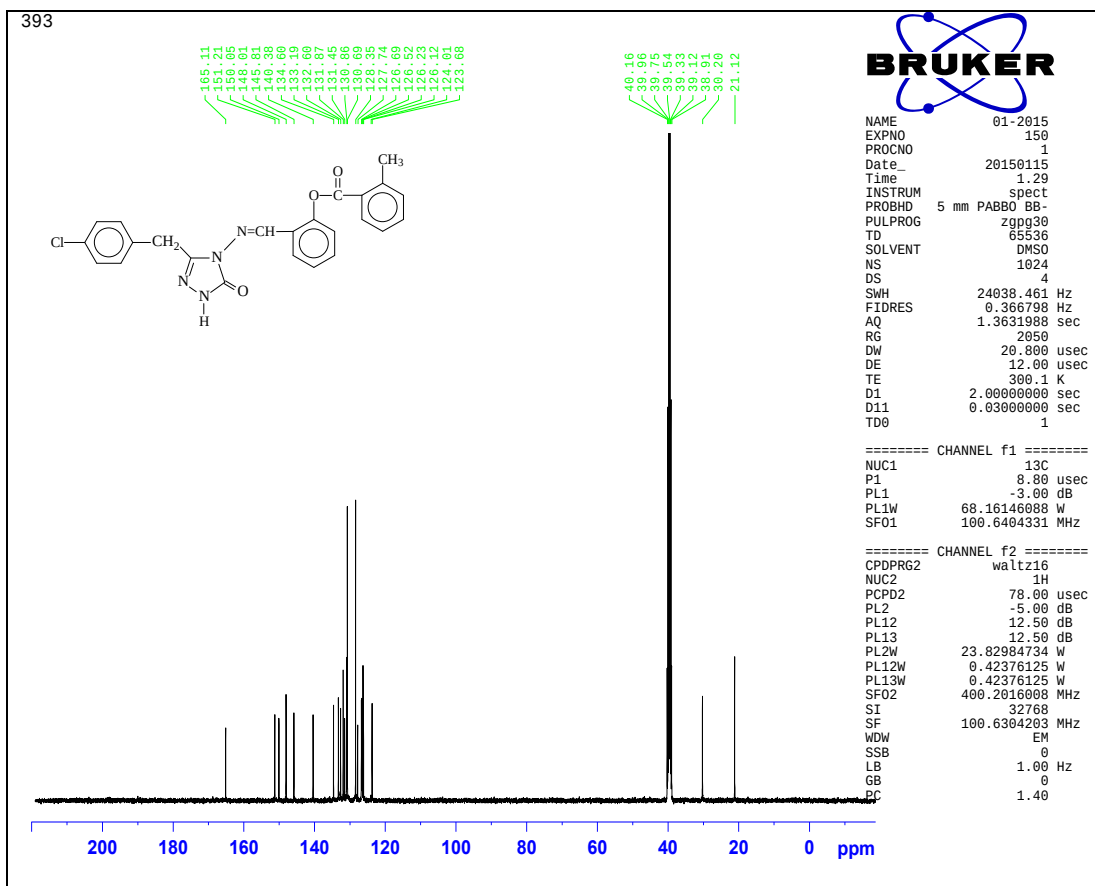
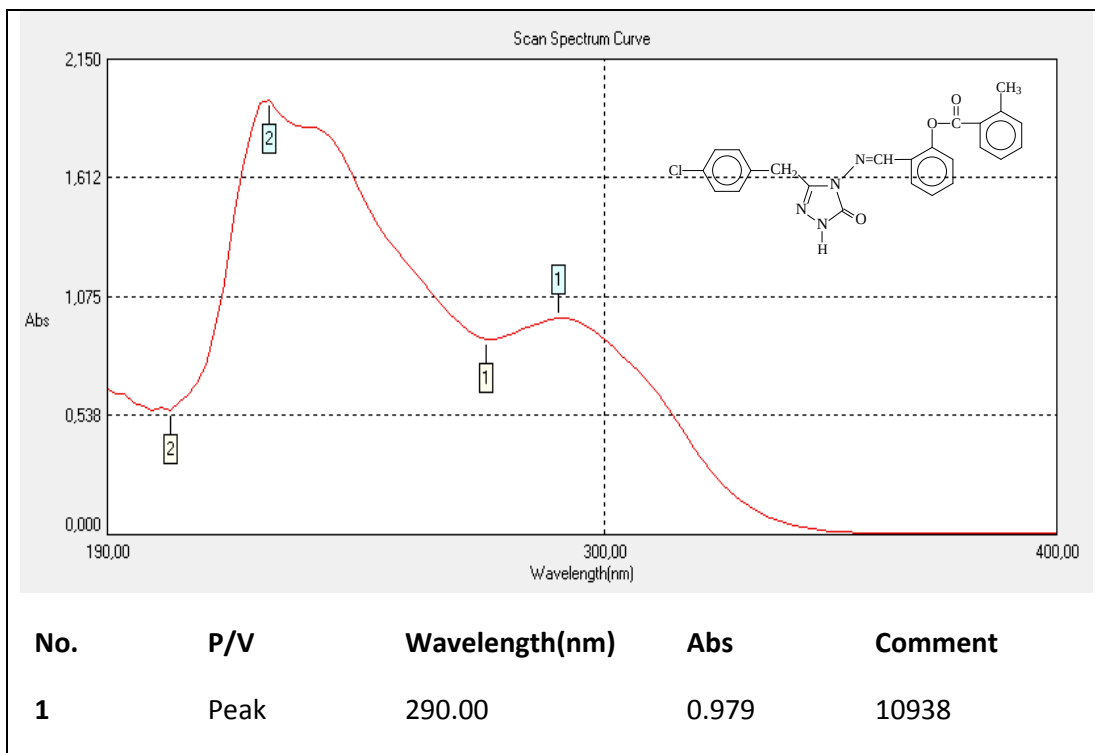


Figure 31. ¹³C-NMR Spectrum of Compound 3g



2	Peak	226.00	1.968	21988
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Figure 32. UV Spectrum of Compound 3g

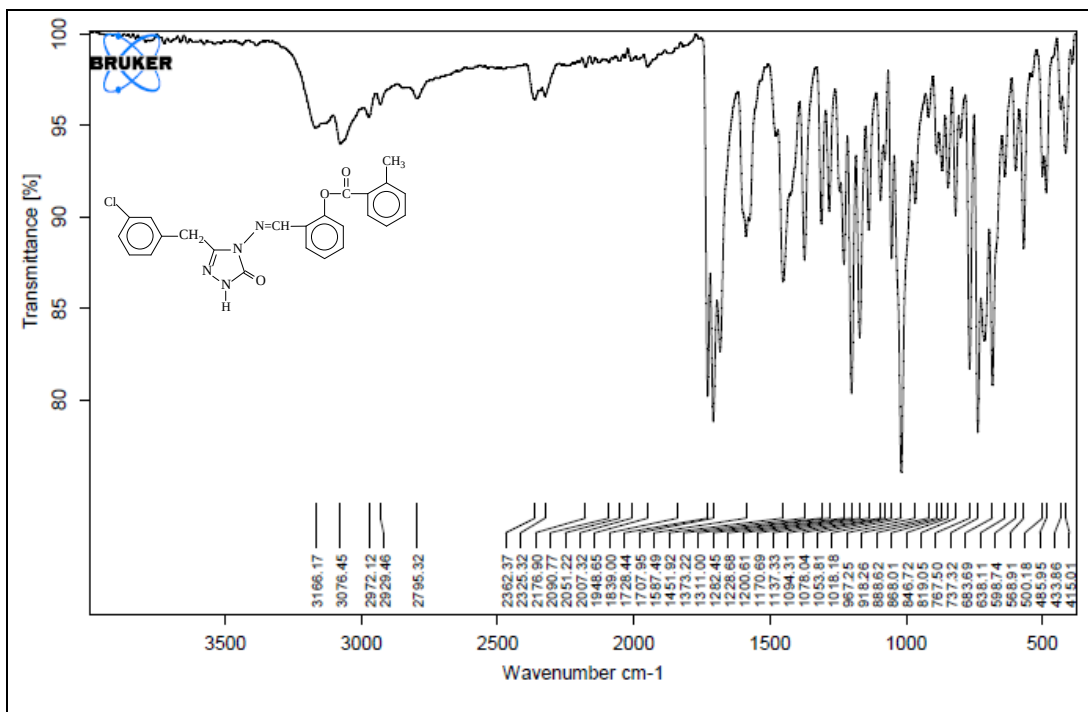


Figure 33. IR Spectrum of Compound 3h

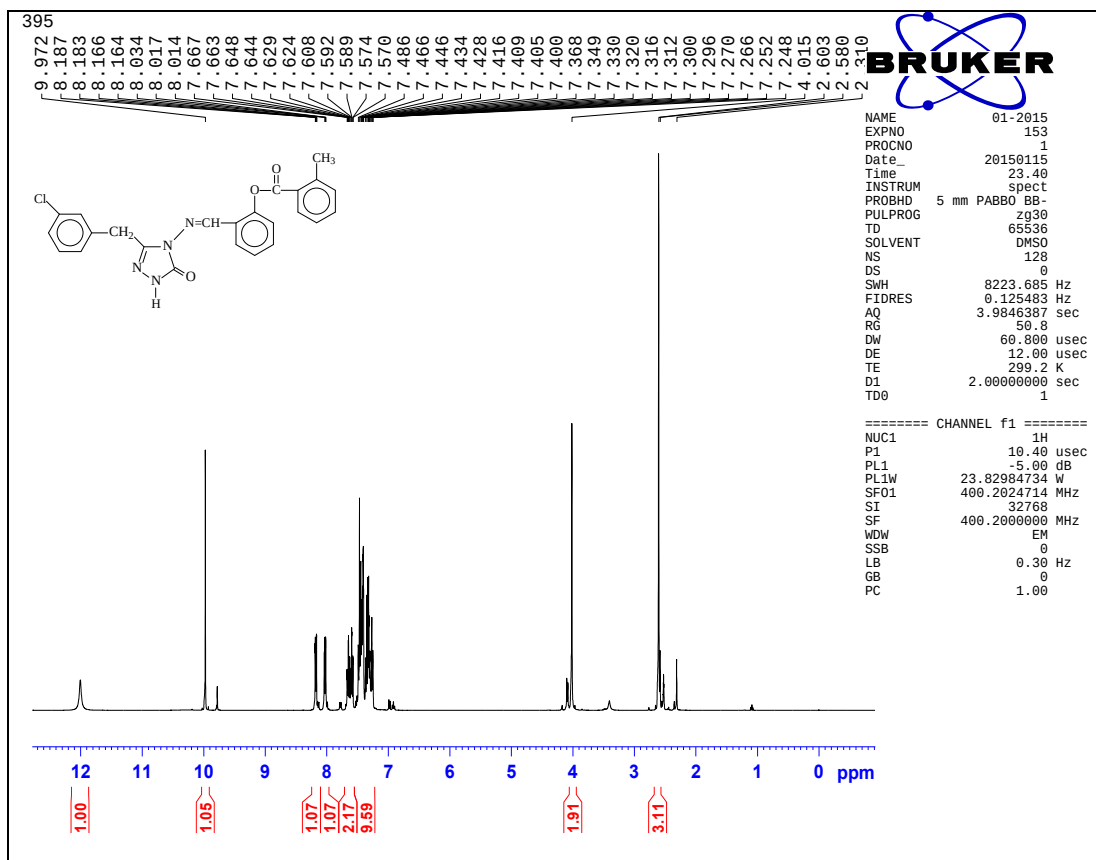


Figure 34. ^1H -NMR Spectrum of Compound 3h

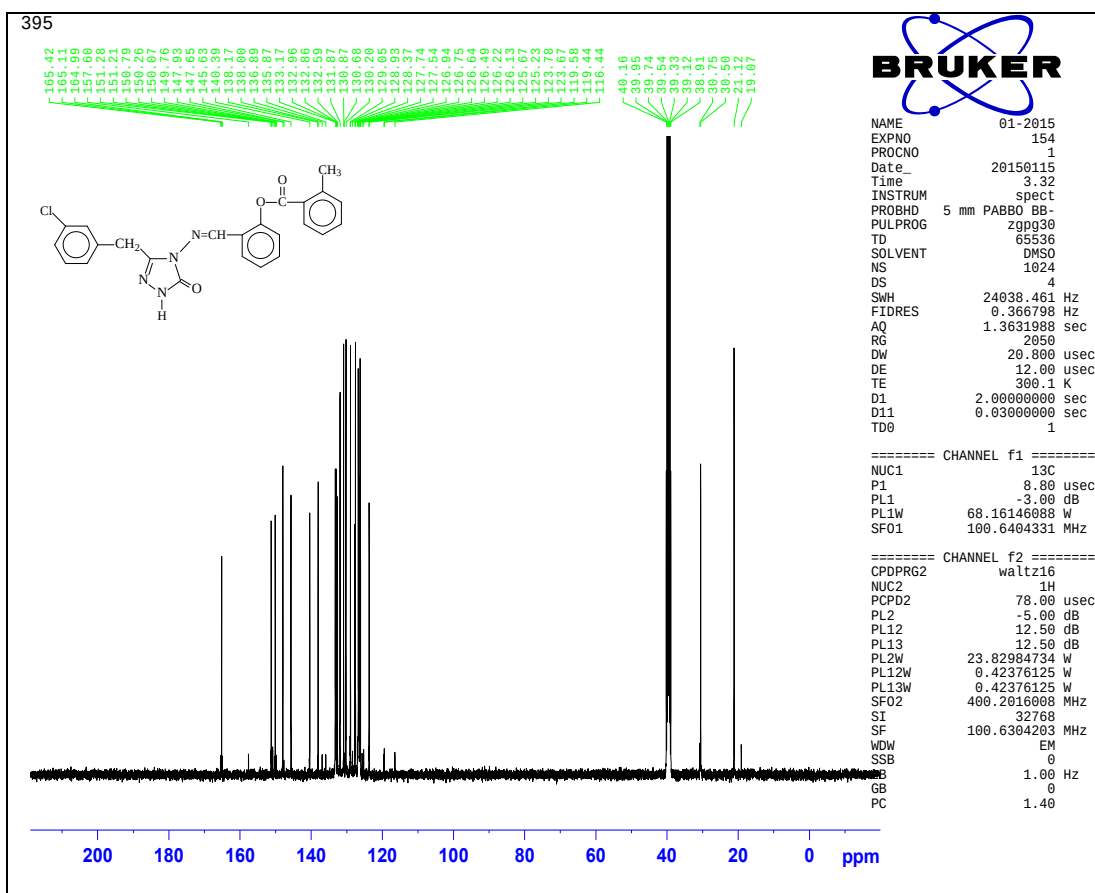
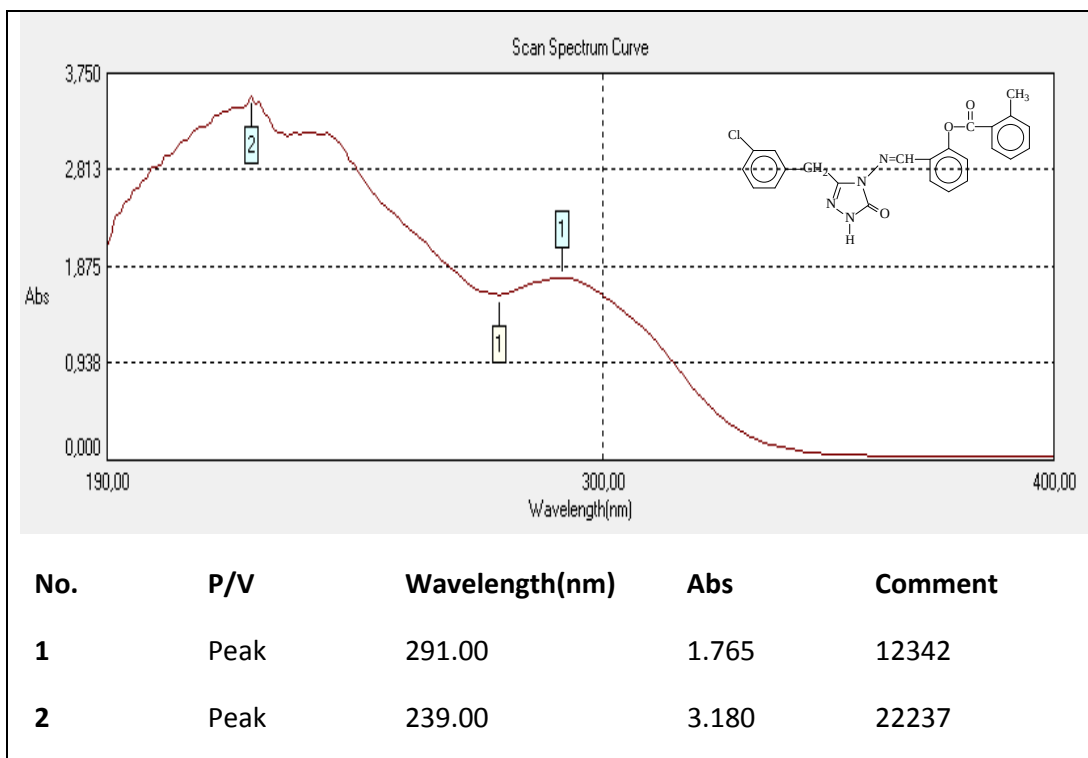


Figure 35. ¹³C-NMR Spectrum of Compound 3h



3	Peak	222.00	3.529	24678
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Figure 36. UV Spectrum of Compound 3h

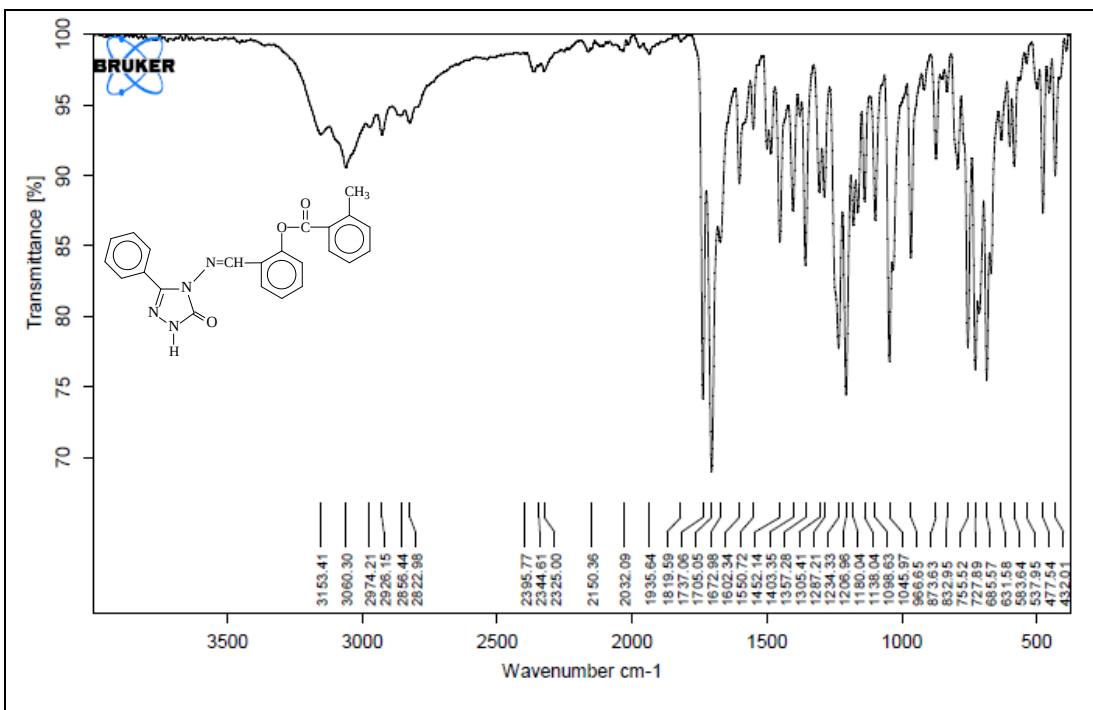


Figure 37. IR Spectrum of Compound 3i

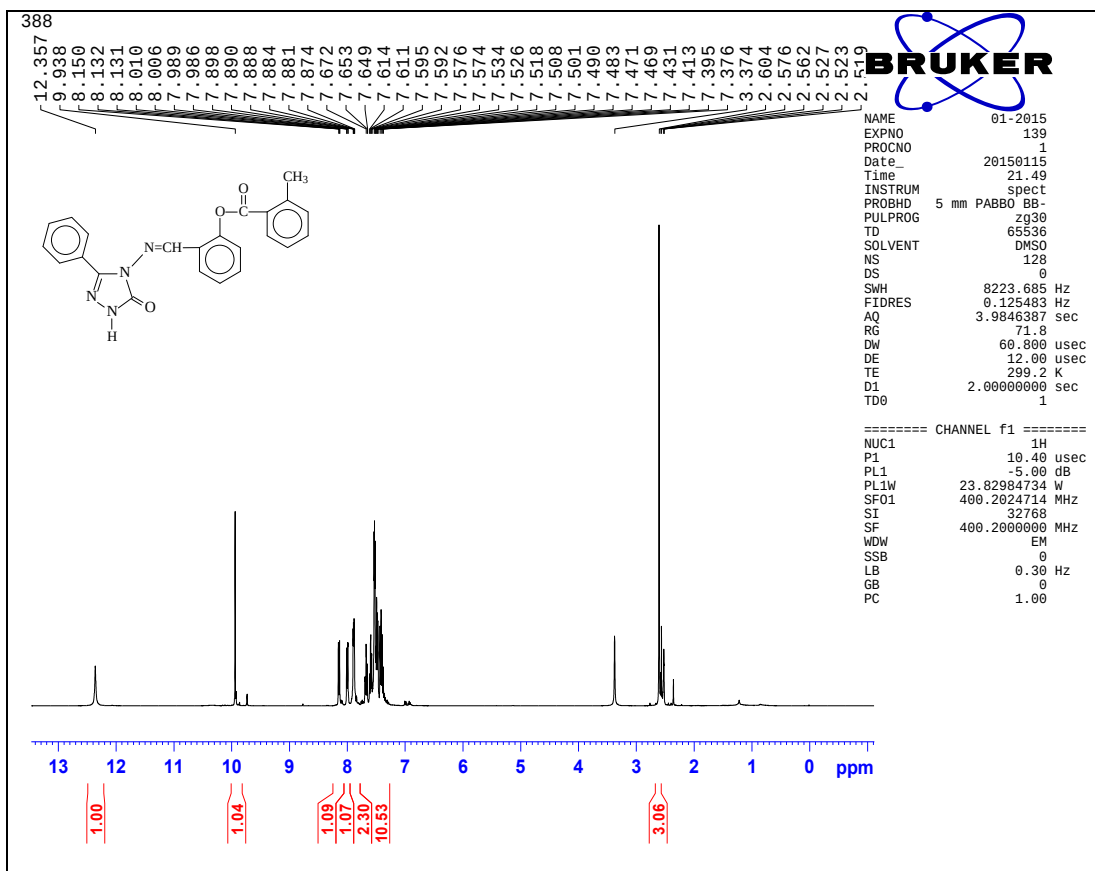


Figure 38. ¹H-NMR Spectrum of Compound 3i

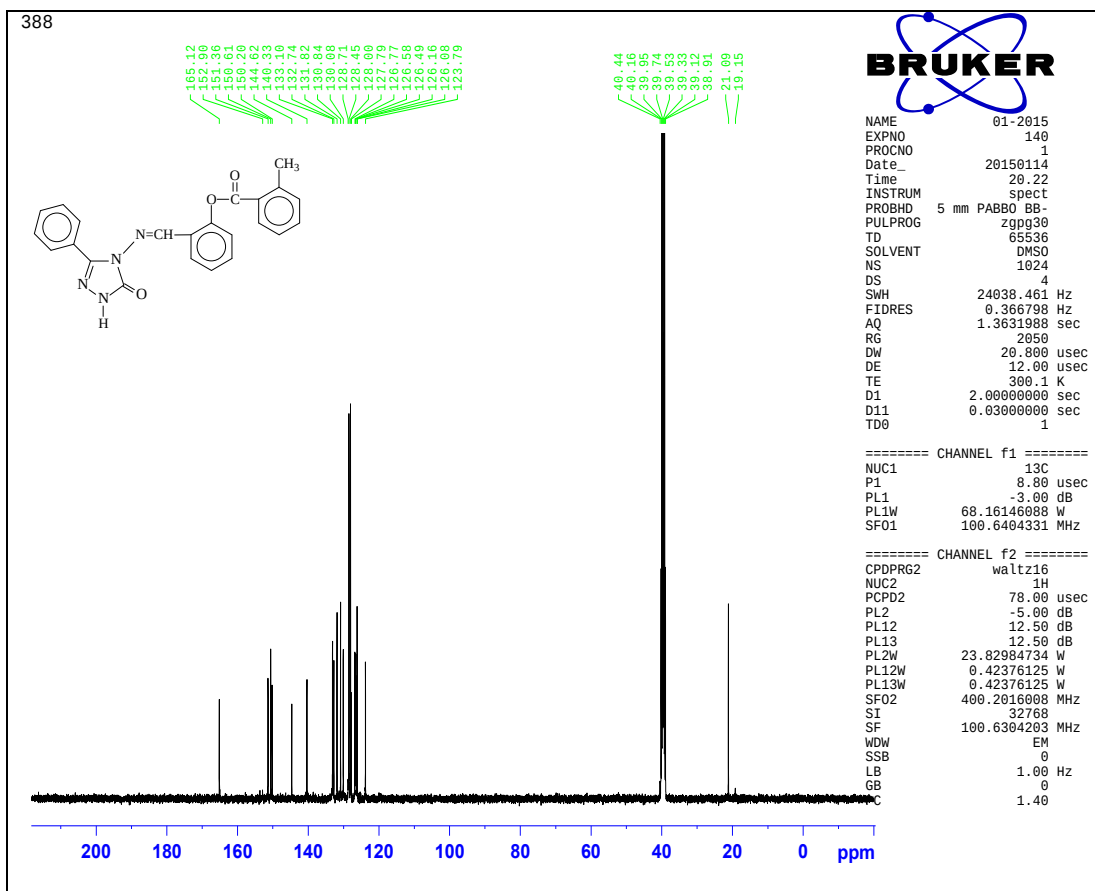
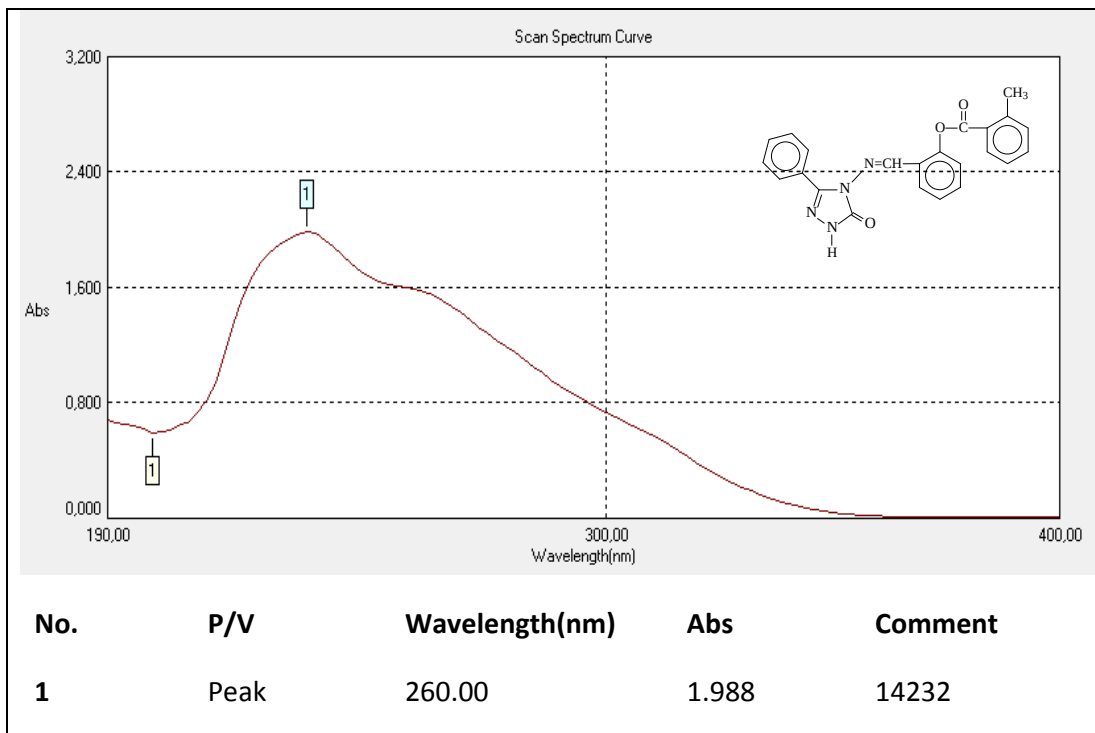


Figure 39. ¹³C-NMR Spectrum of Compound 3i



2	Peak	234.00	1.574	32223
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Figure 40. UV Spectrum of Compound 3i