Original Research Article

Synthesis, docking study and antifungal activity evaluation of some 1,3-benzo[*d*]thiazole analogs: a promotion in synthetic method with nano-γ-Al_2O_3/BF_{3-n} under solvent free conditions

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ABSTRACT

Aim: Nano-γ-Al_2O_3/BF_{3-n} was applied for the synthesis of 1,3-benzo[*d*]thiazole derivatives as a new solid acid catalyst. Benzothiazole is used as a pharmacological agent with a wide variety of biological activities such as anticancer, antimicrobial, antitumor and antiviral properties. In the view we have synthesized a series of 1,3-benzo[*d*]thiazole derivatives (***T1-T10***) and screened for their antibacterial and antifungal abilities.

Methods: In this work, nano-γ-Al_2O_3/BF_{3-n} was applied for the synthesis of 1,3-benzo[*d*]thiazole derivatives. Ten compounds (***T1-T10***) were screened for antimicrobial activity by broth micro dilution methods as recommended by CLSI.

Results: We have demonstrated a simple method for the synthesis of 1,3-benzo[*d*]thiazoles with using nano-γ-Al_2O_3/BF_{3-n} as a new solid acid catalyst under solvent free condition at 110 ℃ .Also, of the tested compounds *N1,N1*-dimethyl-4-(1,3-benzothiazol-2-yl)aniline (***T8***) and 2(2-(4-nitrophenyl)-1,3-benzothiazole (***T10***) inhibited the growth of all examined fungi. Determination the probable binding model of compounds ***T8*** and ***T10*** in to Mycobacterium tuberculosis enzyme CYP51 active site was performed with docking simulation.

Conclusion: We have demonstrated a simple method for the synthesis of 1,3-benzo[*d*]thiazoles with using nano-γ-Al_2O_3/BF_3-n as a new heterogeneous solid acid. Biological studies showed that some of the synthetic compounds including ***T8*** and ***T10*** exhibited a great activity against tested candida and dermatophytes.

Keywords: 1,3-Benzo[d]thiazole; 2-Aminothiophenol; Nano-γ-Al_2O_3/BF_{3-n}; Solvent free; Antifungal; Docking Study.

1. INTRODUCTION

Benzothiazole, is a heterocyclic aromatic molecule with electron rich sulfur and nitrogen atoms. Its derivatives have many biological and pharmaceutical applications such as antiviral [1], antitumor [2], antimicrobial [3,4], antibacterial [5], Antifungal [4], and anticancer activity [6]. Systemic fungal infections have increased dramatically in the past few decades, especially in immune-compromised individuals suffering from tuberculosis, cancer, AIDS, and in organ transplant recipients. The widespread use of antifungal drugs and their resistance against fungal infections have led to serious health concerns. Although varieties drugs such as novel azole compounds are available for the treatment of superficial and systemic mycoses but they are not completely effective in all cases. In addition, they all possess a certain degree of toxicity and quickly develop resistance due to the large-scale usage. Therefore, an urgent need for new antifungal chemical structures as alternative agents to the existing ones is required. In this sense, the azole ring system is presented in biologically active compounds which possess high antifungal properties. Benzothiazoles also have industrial applications both as antioxidants [7] and accelerators in vulcanization. Some of them have liquid crystalline [8] and ionic liquid [9] properties. Due to their unique structure, some benzothiazole derivatives in radioactive are used both as amyloid radiographic and anticancer agents, with the former serving as a major diagnostic technique for Alzheimer's disease [10].They are extensively employed in developing new pharmaceutical products to counter inflammation [11,12], pain and fever [13], stress and depression [14], convulsion [15,16], Parkinson's disease [17], malaria [18], tuberculosis [19, 20], diabetes [21], and ALS [14], while acting as antipsychotic [22], antileishmania [23], and anthelmintic [24]. Other applications include low-carbonsteel erosion inhibitors in acidic environments [25], textile color synthesis [26], and reinforcement agent in manufacturing tires [27]. Also, they are used in the structure of organic light-emitting diodes [28], nonlinear light chromophore, and heat resistant [29]. Benzothiazoles have been synthesized via a two-component coupling of 2-aminothiophenol with aldehydes [30,31], carboxylic acids [32,33], esters [34], Benzanilids [35], Nitrils [36] and alkyl amines [37]. Previously, numerous catalysts were applied in the protocol such as ceric ammonium nitrate (CAN) [30], montmorillonite K10 [38], zinc triflate [39], acetic acid [40], p-oxalic acid [41], silica sulfuric acid [41,42], $Co(NO_3)_3/H_2O_2$[43], Trichloroisocyanuric acid [44] and $AlCl_3.6H_2O$ [41]. Nanostructure materials are chemically very active, because the number of molecules or atoms on their surface is very large compared to that in the sample mass. These materials, in fact, exhibit the largest increased section as compared to the common materials; this feature causes the catalyst to act more efficiently and quickly. Besides increasing the section, placing the catalyst on the bed turns it into a solvent free acidic one. Nano-γ-Al_2O_3/BF_{3-n} is prepared *via* reaction of nano-alumina with boron tri-fluoride-diethyl ether in chloroform at room temperature.

In the view of having a wide scope to find new potentially active agents, we have synthesized a series of 1,3-benzo[*d*]thiazole derivatives (***T1-T10***) and screened for their antibacterial and antifungal abilities.

2. MATERIAL AND METHODS

2.1 General

The chemicals were used without any additional purification. The products were characterized by FT-IR, ^{1}H-NMR, and a comparison of their physical properties with those reported in the literature. FT-IR spectra were run on a Bruker, Eqinox 55 spectrometer. A Bruker (DRX-400 Avanes) NMR was used to record the ^{1}HNMR spectra. Afterwards, melting points were determined by a Buchi melting point B-540 B.V.CHI apparatus. BANDELIN Sonopuls HD 3200 ultrasonic apparatus (20 kHz, 150 W) was used for sonication. The microwave oven Kenwood, 1300W was used for running the described reactions. In order to

specify the reaction progress, TLC plates manufactured by MERC, containing fluorescence active in 254nm wavelength were employed. Furthermore, MIRA TESCAN device was utilized to examine boron terifluoride that is placed on Nano-γ-alumina *via* SEM. EDS(EDX) analysis was conducted by means of Phoneme Pro X device. In addition, the thermogram was recorded by TGA by Iris F1 TG 209 NETZSCH, and in diffraction of x-ray by XRD device with the model of Philips Xpert MPD diffract meter was used. HyperChem software (Version 7, Hypercube Inc), then geometry optimization were done with semi-empirical AM1 method and saved in pdb file format. Molecular vina docking studies were done using PYRX software [Wolf LK, ChemEng News. 2009 87: 31], The X-ray structure of Mycobacterium tuberculosis-CYP51 enzyme in complex with Fluconazole (PDB ID: 1EA1) was gained from Protein Data Bank (http://www.rcsb.org), Water molecules and cognate ligand were removed from the receptor. Binding mode views were created with PYMOL [44].

2.2 General procedure for the preparation of nano- γ-Al_2O_3/BF_{3-n}

To a mixture of nano-Al_2O_3 (5 g) and $CHCl_3$ (10 ml), $BF_3.Et_2O$ (5 ml) was added drop wise. The resulting suspension was stirred for 1 h at room temperature, filtered, washed with chloroform, and dried at room temperature.

2.3 General procedure for the synthesis of 1,3-benzo[*d*]thiazole derivatives under solvent free conditions

[Nano- γ-Al_2O_3/BF_{3-n}] (0.04 g) is added to the balloon containing (1mmol) aldehyde and (1mmol) 2-aminothiophenol. The reaction mixture was then kept in 110˚C under solvent free condition. The reaction progress was proceeded by TLC (ethylacetate: *n*-hexane 20:80).As the reaction completed, the mixture was dissolved in acetone so that the insoluble catalyst be extracted in it by filtering. Subsequently, water was added to the solution under filter; the resultant product in the form of sediment was collected through filtration as good efficient benzothiazole derivatives were synthesized.

2.4 Selected spectroscopic data

2-(4-Isopropyl)-1,3-benzo[d]thiazole (T1)

Yield: 88%, Green solid, m.p. 65-67 ℃; FT-IR (ATR) ῡ =1589 (C=N stretch), 1484 (C=C stretch), 1434, 1312, 967 (C-H bend), 838 (C-H bend), 755 (C-H bend), 726 (C-H bend) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 1.35 (d, *J*=7 Hz, 6H), 3.03 (sept, *J*=7.1 Hz, 1H), 7.4 (d, *J*=8.3 Hz, 2H), 7.42 (t, *J*=7.5 Hz, 1H), 7.52 (t, *J*=8 Hz, 1H), 7.94 (d, *J*=7.5 Hz, 1H), 8.07 (d, *J*=8.2 Hz, 2H), 8.12 (d, *J*=8.5Hz , 1H) ppm.

2-(3-Nitrophenyl)-1,3-benzo[d]thiazole (T2)

Yield: 82%, Yellow solid, m.p. 181-183 (182-184)[43]

FT-IR (ATR) ῡ =1617 (C=N stretch), 1527, 1344 (N=O stretch), 988 (C-H bend), 888 (C-H bend), 842 (C-H bend), 760 (C-H bend), 729 (C-H bend), 670 (C-H bend) cm^{-1}; ^{1}HNMR (500 MHz,$CDCl_3$): 7.5 (t, *J*=8 Hz, 1H), 7.59 (t, *J*=10Hz, 1H), 7.74 (t, *J*=8Hz, 1H), 8 (d, *J*=8Hz, 1H), 8.17 (d, *J*=8.1Hz, 1H), 8.39 (dd, *J*=7.9 and 1.5Hz, 1H), 8.48 (d, *J*=7.8Hz, 1H), 8.98 (dd, *J*=1.9 and 1.5 Hz, 1H) ppm.

2-(2-Furyl)-1,3-benzo[d]thiazole (T3)

Yield: 80%, Brown solid, m.p. 101-103 ℃; FT-IR (ATR) ῡ =1582 (C=N stretch), 1503 (C=C stretch), 1434, 1312, 1245 (C-O stretch), 1011, 896 (C-H bend), 744 (C-H bend) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 6.8 (m, 1H), 7.37 (d, *J*= 3.4Hz, 1H), 7.46 (td, *J*= 7.5 and 0.85 Hz, 1H), 7.56 (td, *J*= 7.6 and 0.9Hz, 1H), 8.01 (s, 1H), 8.03 (d, *J*= 7.9 Hz, 1H), 8.15 (d, *J*= 7.9 Hz, 1H) ppm.

2-(2,4-Dichlorophenyl)-1,3-benzo[d]thiazole (T4)

Yield: 86%, Cream solid, m.p. 133-134℃; FT-IR (ATR) ῡ =3067 (C-H stretch), 1583 (C=N stretch), 1470 (C=C stretch), 1376, 1316, 1258, 1105, 1060, 963 (C-H bend), 825 (C-H bend), 795 (C-H bend), 752 (C-H bend), 725 (C-H bend), 692 (C-Cl stretch) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): 7.41 (brs, *J*=6.4 Hz , 1H) 7.45 (d, *J*=8 Hz, 1H),7.53 (d, *J*=7.2, 1H),7.57 (d, *J*=1.6 Hz, 1H), 7.96 (d, *J*=8Hz, 1H), 8.13 (d, *J*=8 Hz, 1H), 8.24 (d, *J*=8.4 Hz, 1H) ppm.

2-pheny-1,3-2-(benzo[d]thiazol-2-yl) (T5)

Yield: 92%, brown solid, m.p.179-181℃; FT-IR (ATR) ῡ = 1450 (C=N stretch), 1363, 1251 (C-O stretch), 743 (C-H bend) cm^{-1};.^1H NMR (400 MHz, Acetone,d_6): 6.98 (brs, 1H), 7.13 (brs, 1H), 7.27 (brs, 2H), 7.43 (brs, 1H), 7.53 (brs, 1H), 7.73 (brs, 1H), 7.93 (brs, 1H), 8.02 (brs, 1H), 12.54 (s, 1H) ppm.

2-(4-Bromophenyl)-1,3-benzo[d]thiazole (T6)

Yield: 85%, Green solid, m.p. 132-134 (133-134)[42]; FT-IR (ATR) ῡ =3059 (C-H stretch), 1583 (C=N stretch), 1475 (C=C stretch), 1395, 966 (C-H bend), 826 (C-H bend), 842 (C-H bend), 751 (C-H bend), 720 (C-H bend), 683 (C-Br stretch) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.49 (d, *J*=8 Hz 1H), 7.57 (t, *J*=8 Hz, 1H), 7.76 (d, *J*=1 Hz, 1H), 7.78 (d, *J*=1 Hz, 1H), 8.09 (m, 4H) ppm.

2-(3-Pyridyl)-1,3-benzo[d]thiazole (T7)

Yield: 85%, White solid, m.p. 113-115 ℃; FT-IR (ATR) ῡ =1573 (C=N stretch), 1503, 1428 (C=C stretch), 1310, 963(C-H bend), 763(C-H bend), 700 (C-H bend) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 7.45 (t, *J*= 7.4 Hz, 2H), 7.5 (t, *J*=7.2Hz, 1H), 7.95 (d, *J*= 7.9 Hz, 1H), 8.1 (d, *J*=8.1 Hz, 1H), 8.38 (d, *J*= 8.1 Hz, 1H), 8.7 (d, *J*= 8.1 Hz, 1H), 9.35 (s, 1H) ppm.

2-(4-Dimethylamino phenyl)-1,3-benzo[d]thiazole (T8)

Yield: 85%, White solid, m.p. 153-155 (157-159)[41]; FT-IR (ATR) ῡ =1606 (C=N stretch), 1476 (C=C stretch), 1430, 1368, 1227 (C-N stretch), 1186, 943 (C-H bend), 816 (C-H bend), 750 (C-H bend), 720 (C-H bend) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 3.07 (s, 6H), 6.76 (d, *J*=8 Hz, 2H), 7.32 (t, *J*=7.5 Hz, 1H), 7.44 (t, *J*=7.4 Hz, 1H), 7.85 (d, *J*=8 Hz, 1H), 7.97 (d, *J*=8.4 Hz, 1H), 8 (d, *J*=8 Hz, 2H) ppm.

2-(3-Bromophenyl)-1,3-benzo[d]thiazole (T9)

Yield: 83%, Green solid, m.p. 81-83 (83-84)[41]; FT-IR (ATR) ῡ =3061 (C-H stretch), 1561 (C=N stretch), 1503 (C=C stretch), 1466, 1422, 1312, 1219, 1068, 973 (C-H bend), 860 (C-H bend), 749 (C-H bend), 722(C-H bend), 674(C-H bend) cm^{-1}; ^1HNMR (400 MHz, $CDCl_3$): 7.4 (m,2H), 7.53 (t, *J*=7.6 Hz, 1H), 7.63 (d, *J*=8 Hz 1H), 7.93 (d, *J*=8.4 Hz, 1H), 8.005 (d, *J*=4 Hz, 1H), 8.09 (d, *J*=8 Hz, 1H), 8.3 (s, 1H) ppm.

2-(4-Nitrophenyl)benzo[d]thiazole (T10)

Yield: 87%, Yellow solid, m.p. 229-230 (226-228)[42]; FT-IR (ATR) ῡ =1605(C=N stretch), 1518, 1341(N=O stretch), 1341, 1250, 1107, 968(C-H bend), 851 (C-H bend)1, 765(C-H bend), 751 (C-H bend), 729 (C-H bend), 685 (C-H bend) cm^{-1}; ^1H NMR (400 MHz, Acetone-d_6): 7.58 (t, *J*=7.3Hz, 1H), 7.64 (t, *J*=7.1Hz, 1H), 8.17 (d, *J*=7.2 Hz, 1H), 8.19 (d, *J*=7.2Hz, 1H), 8.44 (brs, 4H) ppm.

2.5 Determination of antifungal activities

2.5.1 *Microorganisms*

The antifungal activities of the synthetic compounds against some standard strains of fungi, including *Candida. ablicans* (ATCC 10261, 1905, 2730, 1912), *C. tropicalis* (ATCC 750), *C.*

krusei (ATCC 6258), *C. glabrata* (ATCC 90030, CBS 863, CBS 2192), *C. dubliniensis* (CBS 8500, 8500, 8501, 7988, 7987), *C. neoformance* (ATCC 9011), *Aspergillus. flavus* (ATCC 64025), *A. clavatus* (CBS 514.65), *A. fumigatus* (ATCC 14110) and *Exophiala* dermatitidis (ATCC 109136) were determined. In addition, the antifungal activities of the compounds were tested against six clinical isolates of yeasts identified by polymerase chain reaction-restriction fragment length polymorphism(PCR–RFLP) and three clinical isolates of dermatophytes (*Epidermophyton floccosum, Microsporumcanis and Trichophytonrubrum*) identified by both morphological and molecular methods [45]. The antifungal susceptibility of the tested yeasts and *Aspergillus* species against fluconazole (Sigma, St. Louis, MO, USA) and dermatophytes against griseofulvin (sigma) were examined by microdilution methods [46,47].

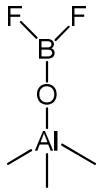
2.5.2 *Determination of minimum inhibitory concentration*

MICs were determined by using the broth micro dilution method recommended by the CLSI with some modifications. In order to determine the antimicrobial activities against fungi, serial dilutions of the synthetic compounds (1–1024 µg/mL) were prepared in 96-well micro titer plates using RPMI-1640 media (Sigma, St. Louis, MO, USA) buffered with MOPS (Sigma). Stock inoculums were prepared by suspending three colonies of the examined yeast in 5 mL sterile 0.85% NaCl, and adjusting the turbidity of the inoculums to 0.5 McFarland standards at 530 nm wavelengths (this yields stock suspension of 1-5 × 10^6 cells/mL). For moulds (*Aspergillus* spp. and dermatophytes), conidia were recovered from the 7-day old cultures grown on potato dextrose agar by a wetting loop with tween-20. The collected conidia were transferred in sterile saline and their turbidity was adjusted to OD=0.09-0.11 that yields 0.4-5 × 10^6 conidia/mL. Working suspension was prepared by making a 1/50 and 1/1000 dilution with RPMI of the stock suspension for moulds and yeasts, respectively. Working inoculums (0.1 mL) were added to the micro titer plates, which were incubated in a humid environment at 30ºC for 24-48 h. Uninoculated medium (200 µL) was included as a sterility control. In addition, growth controls (medium with inoculums but without antibiotics or the synthetic compounds) were also included. The growth in each well was compared with that of the growth in the control well. MICs were visually determined and defined as the lowest concentration of the compounds produced ≥95% growth reduction compared with the growth in the control well. Each experiment was performed in triplicate.

In addition, media from the wells with fungi showing no visible growth were further cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany) to determine the minimum fungicidal concentration (MFC). MFCs were determined as the lowest concentration yielding no more than 4 colonies, which resulted in mortality of 98% of the microbes in the initial inoculums.

3. RESULTS AND DISCUSSION

In continuation of our research on the applications of solid acids in organic synthesis, we have investigated Nano-γ-Al_2O_3/BF_{3-n} efficiency in the reaction of Benzothiazole condensation at 110˚C under solvent free condition. For identification of the structure of Nano-γ-Al_2O_3/BF_{3-n}, we have studied FT-IR (ATR) spectra of nano-γ-Al_2O_3 and Nano-γ-Al_2O_3/BF_{3-n} (Figure 1). In nano-γ-Al_2O_3 FT-IR spectrum, strong bands at 1742, 1370 and 1216 cm^{-1} was observed. In Nano-γ-Al_2O_3/BF_{3-n}, in addition to the above mentioned bands, three bands also appeared at 1627, 1410 and 1071 cm^{-1}. The peaks at 1410 and 1071 cm^{-1} verify the B-O and Al-O-B bonds on Nano-γ-Al_2O_3/BF_{3-n} respectively. Based on these results, we have also suggested the following structure for nano-γ-Al_2O_3/BF_{3-n} (Scheme 1) [49]. The Field Emission Scanning Electron Microscopy (FESEM) image of Nano-γ-Al_2O_3/BF_{3-n} is shown in Figure 2.



Scheme 1. The proposed structure for nano-γ-Al_2O_3/BF_{3-n}

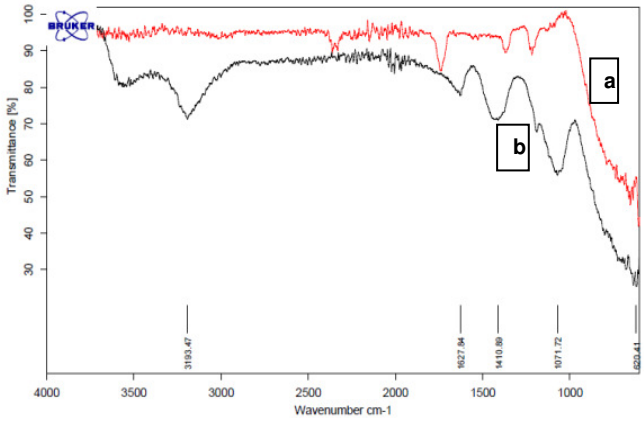


Figure 1. FT-IR (ATR) spectrum of: (a) nano-Al_2O_3, (b) nano-γ-Al_2O_3/BF_{3-n}

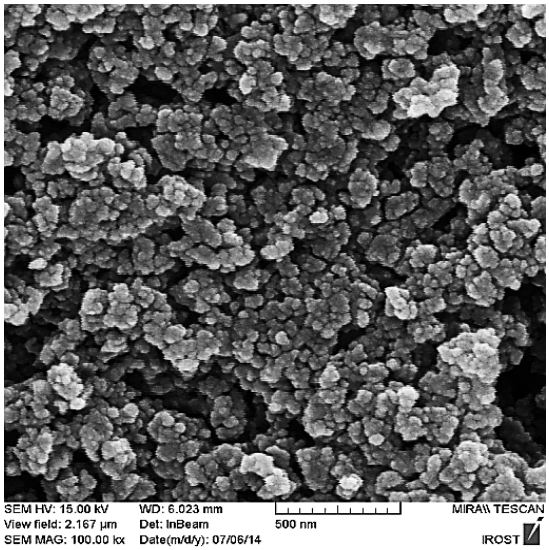


Figure 2. The FESEM image of Nano-γ-Al_2O_3/BF_{3-n}

Energy-Dispersive X-ray Spectroscopy (EDS) of Nano-γ-Al_2O_3/BF_{3-n} was measured by EDS instrument (Figure 3). According to this data, the weight percentage of O, Al and F are 42.8, 34.9 and 22.3, respectively.

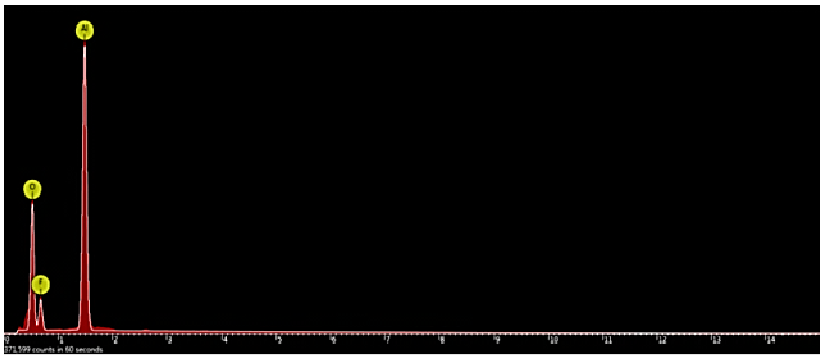


Figure 3. EDS analysis diagram of Nano-γ-Al_2O_3/BF_{3-n}

The amount of boron in Nano-γ-Al_2O_3/BF_{3-n} was determined. For this purpose, a mixture of Nano-γ-Al_2O_3/BF_{3-n} (0.1 g) and water (50 ml) was stirred and boiled for 20 minutes. Then, the mixture was cooled and titrated with 23 ml of standard NaOH (0.009 N) in the presence of phenolphetalein. The boron amount in catalyst was found to be 2.1 meq.g^{-1}. In this process, the attached boron in nano-γ-Al_2O_3/BF_{3-n} was reacted with water, captured OH^- from water to produce $B(OH)_4^-$ and H^+. The amount of H^+ that evolved during titration is equivalent to Boron (Scheme 2).

$$\text{nano-}\gamma\text{-}Al_2O_3/BF_{3-n} \xrightarrow[\Delta]{H_2O} B(OH)_3 + HF \xrightarrow[\Delta]{H_2O} B(OH)_4^{\ominus} + H^{\oplus}$$

Scheme 2.

The X-ray diffraction (XRD) pattern of Nano-γ-$Al_2O_3/BF_{3-n}$is shown in Figure 4. According to XRD pattern of catalyst, the values of 2θ and Full width at half maximum (FWHM) are shown in table 1. According to XRD pattern, the two signals at 2θ equal to 14.57 and 27.96 with FWHM equal to 0.2952 and 0.1771 respectively, is similar to HBO_3 with B-O bonds. The signals at 2θ equal to 25.09, 45.91 and 66.99 are shown γ-Al_2O_3 structure.

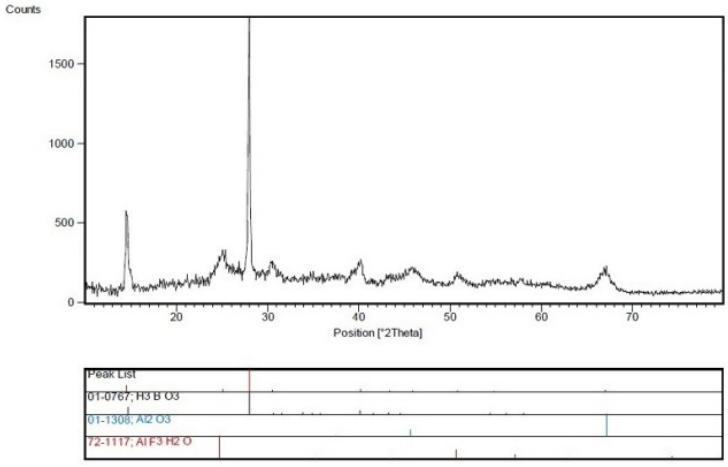


Figure 4. X-ray diffraction (XRD) pattern of Nano-γ-Al_2O_3/BF_{3-n}

Thermal gravimetric analysis (TGA) pattern of Nano-γ-Al_2O_3/BF_{3-n} was detected from 50 to 800 ℃ (Figure 5). The catalyst is stable until 100℃ and only 10% of its weight was reduced in 115 ℃. This initial reducing mass (10%) of catalyst is related to removal of catalyst

moisture. By heating of catalyst between 600 ℃ to 660 ℃, the reducing amount of its weight is 6% *via* cleavage of B-F bonds. According to TGA diagram of BF_3/nano-γ-Al_2O_3, this catalyst is stable until 115 ℃.

Table 1. Nano-γ-Al_2O_3/BF_{3-n} reflexes in XRD diffractogram

No.	Pos. [2θ]	FWHM [2θ]
1	14.5780	0.2952
2	25.0940	0.8266
3	27.9663	0.1771
4	30.4779	0.5904
5	40.2502	0.3542
6	43.4113	0.7085
7	45.9193	1.4170
8	50.7719	0.4723
9	54.8168	1.4170
10	66.9918	0.8640

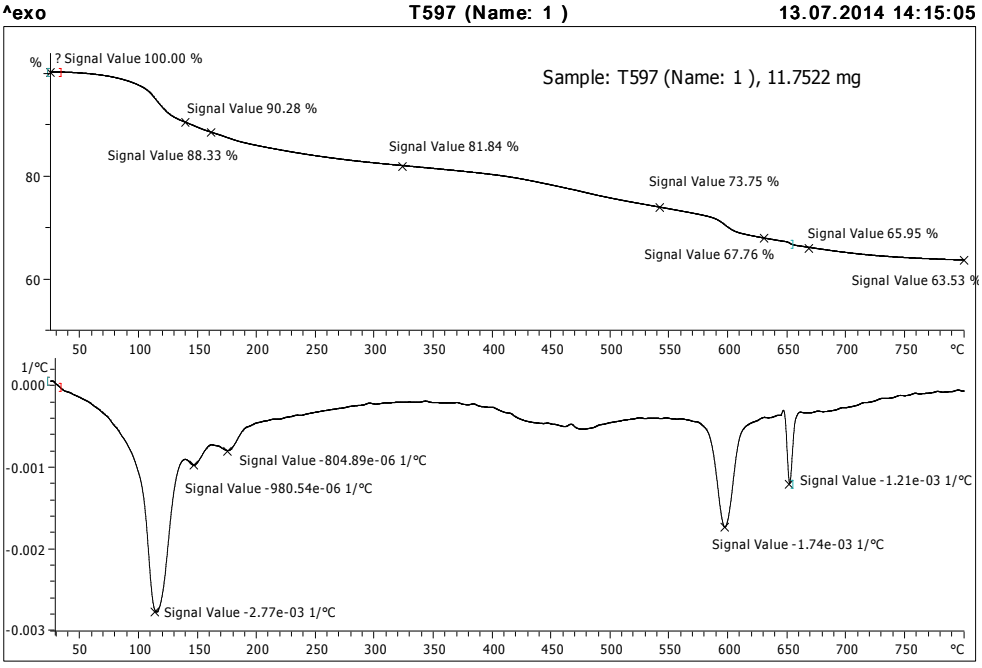


Figure 5. Thermal gravimetric analysis (TGA-DTG) pattern of Nano-γ-Al_2O_3/BF_{3-n}

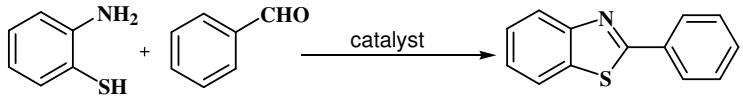
In this research project we used nanocatalyst as the solid acidic catalyst for synthesis of benzothiazoles *via* condensation of different aldehydes and 2-aminothyophenol in the presence of Nano-γ-Al_2O_3/BF_{3-n} as catalyst. As a consequence, the reaction of benzaldehyde (1 mmol) with 2-aminothiophenol (1.2 mmol) was investigated for optimization of the reaction conditions (Table 2). We found that the best condition was solvent free at 110

℃ and a molar ratio of benzaldehyde: 2-aminothiophenol: Nano-γ-Al_2O_3/BF_{3-n} equal to 1:1.2:0.04.

As shown in table 2, the most yield of reaction was acquired at 110˚C under solvent free condition in the presence of 0.04 g Nano-γ-Al_2O_3/BF_{3-n} after 60 minutes (Table 2, Entry 1). 2-aminothiophenol and benzaldehydes were used as substrates for the synthesis of benzothiazoles under sonication conditions in ethanol (Table 2, Entry 10).The Benzothiazole condensation with different aldehhydes and 2-aminothiophenol to give the desired products in good yields. The results are summarized in Table 3.

For synthesis of benzo[*d*]thiazole, we have used trioxane as formaldehyde source (Table 3, Entry 9). The aromatic aldehydes containing electron releasing or electron withdrawing groups have reacted in this protocol. The reaction of 2-amino thiophenol with various aromatic aldehydes of both electron-releasing and with drawing groups was investigated. It is observed that the groups replaced in the ring have no explicit impact on reaction time or yield under optimal condition. However, aldehydes containing strong electron withdrawing group such as nitro group in para position give good yield.

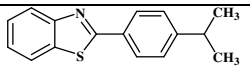
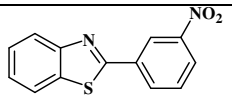
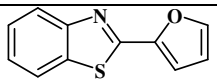
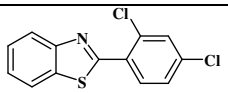
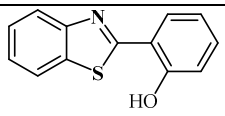
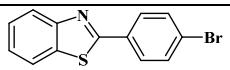
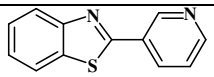
Table 2. Condensation of benzothiazole under different conditions[a]

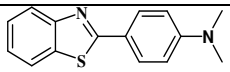
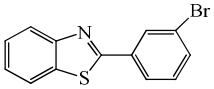
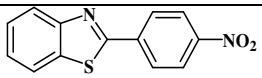


Entry	Catalyst (g)	Solvent	Condition	Time (min)	Yield (%)	Ref.
1	Nano-γ-Al_2O_3/BF_{3-n}	-	110℃	60	92	-
2	Nano-γ-Al_2O_3/BF_{3-n}	EtOH	Reflux	130	45	-
3	Nano-γ-Al_2O_3/BF_{3-n}	EtOAc	Reflux	120	84	-
4	Nano-γ-Al_2O_3/BF_{3-n}	CH3Cl	Reflux	160	50	-
5	Nano-γ-Al_2O_3/BF_{3-n}	MeOH	Reflux	140	60	
6	Nano-γ-Al_2O_3/BF_{3-n}	n-hexane	Reflux	150	40	-
7	Nano-γ-Al_2O_3/BF_{3-n}	H_2O	Reflux	360	12	-
8	Nano-γ-Al_2O_3/BF_{3-n}	EtOH / H_2O	Reflux	300	32	
9	Nano-γ-Al_2O_3/BF_{3-n}	-	M.W.	3	45	-
10	Nano-γ-Al_2O_3/BF_{3-n}	EtOH	Sonication	15	83	-
11	montmorillonite K10	$PhNO_2$	M.W.	5	92	[38]
12	$Co(NO_3)_2.6H_2O$	DMF	80 ℃	35	88	[43]
13	Acetic acid	Acetic acid	Reflux	300	76	[40]
14	Silica Sulfuric Acid	-	M.W.	12	90	[42]
15	CAN	MeOH	r.t.	1440	75	[30]
16	Oxalic acid	$EtOH/H_2O$	80℃	30	80	[41]
17	$AlCl_3.6H_2O$	$MeOH:H_2O$ (20:1)	r.t.	30	90	[41]
18	Silica Sulfuric Acid (SSA)	CH_3CN	80 ℃	25	82	[41]
19	Zinc triflate	EtOH	Reflux	300	92	[39]
20	Trichloroisocyanuric acid	THF	r.t.	120	80	[48]

[a]*The molar ratio of 2-aminothiophenol : benzaldehyde is 1.2:1*
[b]*Isolated yield*

316 **Table 3. Synthesis of 2-substituted benzothiazoles in the presence of Nano-γ-**
317 **Al_2O_3/BF_{3-n} under solvent free conditions at 110 ℃[a]**

Entry	Product	Yield %	M.P. °C (Lit)[Ref]
T1	 2-(4-isopropyl)-1,3-benzo[*d*]thiazole	88	65-67
T2	 2-(3-Nitrophenyl)-1,3-benzo[*d*]thiazole	82	181-183 (182-184)[43]
T3	 2-(Furan-2-yl)-1,3-benzo[*d*]thiazole	80	101-103
T4	 2-(2,4-dichlorophenyl)-1,3-benzo[*d*]thiazole	86	133-134
T5	 2-pheny-1,3-2-(benzo[d]thiazol-2-yl)	92	179-181
T6	 2-(4-Bromophenyl)-1,3-benzo[*d*]thiazole	85	132-134 (133-134)[42]
T7	 2-(3-Pyridin-3-yl)-1,3-benzo[*d*]thiazole	85	113-115

T8	 2-(4-Dimethylamino phenyl)-1,3-benzo[*d*]thiazole	85	153-155 (157-159)[41]
T9	 2-(3-Bromophenyl)-1,3-benzo[*d*]thiazole	83	81-83 (83-84)[41]
T10		87	229-230 (226-228)[42]

[a]A mixture of 2-aminothiophenol (1.2 mmol), aldehyde (1 mmol), Nano-γ-Al_2O_3/BF_{3-n} (0.04 g) was heated at 110 °C under solvent free condition.
[b]Isolated yield

Moreover, the reaction of 2-aminothiophenol with such heterocyclic aldehydes as furfural, 3-pyridine, and so on was scrutinized where good-yielding products were observed. Conducting the reaction with aliphatic aldehydes like butyraldehyde, an admixture of reactors and materials was discerned at TLC which was oily at the time of sedimentation. (In this protocol many aliphatic aldehydes were examined but oily liquids with difficult purification method were obtained). A mechanism for the catalytic activity of Nano-γ-Al_2O_3/BF_{3-n} in the reaction of Benzothiazole condensation may be postulated as shown in scheme 3.

3.1 Antifungal activities of the synthetic compounds

Table 4 summarizes the inhibitory activities of the synthetic compounds and control drugs against the tested fungi. In comparing MIC values of the synthetic compounds, ***T8*** and ***T10*** exhibited strong inhibitory activities against all of the tested fungi including *yeasts* fungi at concentration ranging from 8 to 128 µg/mL and 16-128 µg/mL, respectively.

337 **Table 4. Minimum inhibitory and fungicidal concentrations of the synthetic compounds (µg/mL) against the examined fungi.**

Microorganism		*(T1)*			*(T2)*			*(T3)*			*(T4)*			*(T5)*			*(T6)*		
		MIC 50	MIC 99	MFC	MIC 50	MIC 99	MFC	MIC 50	MIC 99	MFC	MIC 50	MIC 99	MFC	MIC 50	MIC 99	MFC	MIC 50	MIC 99	MFC
Yeasts	*C.albicans*(ATCC 10261)	128	>512	>512	>512	>512	>512	128	256	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
	C.albicans(CBS 1905)	256	>512	>512	128	>512	>512	256	>512	>512	256	>512	>512	>512	>512	>512	256	>512	>512
	C.albicans(CBS 2730)	128	>512	>512	64	256	>512	64	128	256	32	128	>512	256	>512	>512	256	>512	>512
	C.albicans(CBS1912)	128	128	>512	>512	>512	>512	64	64	256	64	64	>512	128	>512	>512	256	>512	>512
	C.dubliniensis(CBS8500)	64	128	256	>512	>512	>512	128	256	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
	C.dubliniensis(CBS8501)	256	>512	>512	>512	>512	>512	128	256	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
	C.dubliniensis(CBS7988)	128	128	>512	64	128	>512	64	128	256	64	128	>512	128	>512	>512	256	>512	>512
	C.dubliniensis(CBS7987)	128	128	256	128	128	256	128	256	>512	128	>512	>512	256	>512	>512	256	>512	>512
	C.glabrata(ATCC 90030)	32	64	>512	32	64	>512	32	64	256	64	128	>512	64	128	>512	64	128	>512
	C.glabrata(CBS 863)	>512	>512	>512	>512	>512	>512	128	128	256	>512	>512	>512	>512	>512	>512	>512	>512	>512
	C.glabrata(CBS 2192)	64	256	>512	32	128	256	32	128	256	64	256	>512	128	>512	>512	128	128	>512
	C.krusei(ATCC 6258)	128	>512	>512	128	>512	>512	64	256	>512	256	>512	>512	>512	>512	>512	>512	>512	>512
	C.parapilopsis(ATCC 4344)	>512	>512	>512	>512	>512	>512	128	256	>512	64	>512	>512	256	>512	>512	>512	>512	>512
	C.tropicalis(ATCC 750)	64	64	256	32	64	256	64	64	256	64	64	>512	32	64	>512	32	64	>512
Filamentous fungi	*A.fumigatus*(ATCC 14110)	>512	>512	>512	>512	>512	>512	>512	>512	>512	64	128	>512	64	128	>512	>512	>512	>512
	A.flavus(ATCC 64025)	>512	>512	>512	>512	>512	>512	>512	>512	>512	128	256	>512	128	>512	>512	>512	>512	>512
	A.clavatus(CBS 514.65)	128	256	>512	32	64	256	32	64	256	>512	>512	>512	128	256	>512	>512	>512	>512

	Exophiala dermatitidis(ATCC 109136)	64	128	>512	64	128	256	32	64	>512	128	128	64	128	>512	64	256	256	256
	M.canis(clinical isolate)	>512	>512	>512	8	16	>512	8	16	>512	256	>512	>512	256	>512	>512	>512	>512	>512
	T.rubrum(clinical isolate)	8	16	>512	>512	>512	>512	>512	>512	>512	64	64	>512	8	16	>512	>512	>512	>512
	E.flucusom(clinical isolate)	16	32	64	32	128	256	16	32	64	16	64	128	32	128	256	32	128	256

338

Microorganism		***(T7)***			***(T8)***			***(T9)***			***(T10)***			***Control****
		MIC 50	MIC 99	MFC	MIC 50	MIC 99	MFC	MIC 50	MIC 99	MFC	MIC 50	MIC 99	MFC	MIC
Yeasts	*C.albicans*(ATCC 10261)	128	256	>512	16	32	64	128	>512	>512	16	32	64	0.5
	C.albicans(CBS 1905)	128	>512	>512	32	64	128	256	>512	>512	16	32	64	0.25
	C.albicans(CBS 2730)	64	128	256	16	32	128	64	128	>512	64	32	128	1
	C.albicans(CBS1912)	64	64	>512	32	32	256	128	128	>512	64	64	256	1
	C.dubliniensis(CBS8500)	128	128	256	32	32	64	256	>512	>512	32	32	64	0.25
	C.dubliniensis(CBS8501)	64	128	>512	64	64	256	128	>512	>512	128	256	>512	0.5
	C.dubliniensis(CBS7988)	64	64	>512	32	32	256	128	128	>512	32	32	128	1
	C.dubliniensis(CBS7987)	128	256	>512	32	64	128	128	>512	>512	16	32	64	1
	C.glabrata(ATCC 90030)	16	32	256	16	32	128	64	64	>512	32	64	128	0.5
	C.glabrata(CBS 863)	32	64	>512	64	64	128	128	>512	>512	128	256	>512	0.5
	C.glabrata(CBS 2192)	32	64	256	32	64	128	32	64	>512	16	32	64	0.25

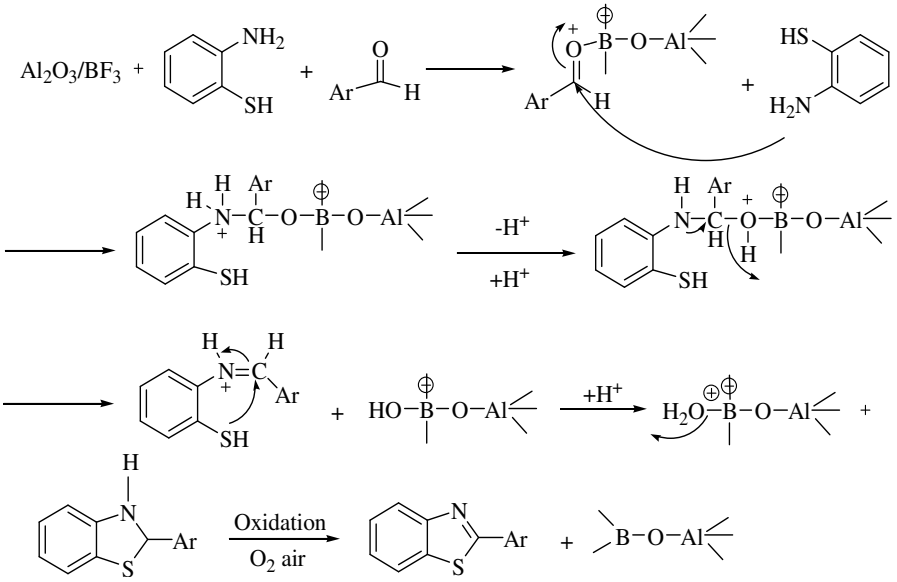
	C.krusei(ATCC 6258)	128	256	>512	128	256	>512	256	>512	>512	64	128	256	64
	C.parapilopsis(ATCC 4344)	128	>512	>512	8	32	256	128	>512	>512	128	>512	>512	0.25
	C.tropicalis(ATCC 750)	64	128	>512	16	32	64	32	64	256	16	32	64	2
Filamentous fungi	*A.fumigatus*(ATCC 14110)	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	32
	A.flavus(ATCC 64025)	>512	>512	>512	>512	>512	>512	256	>512	>512	>512	>512	>512	32
	A.clavatus(CBS 514.65)	32	64	256	16	64	128	32	64	256	32	64	128	16
	Exophiala dermatitidis(ATCC 109136)	64	128	>512	16	32	128	128	128	256	32	64	128	1
	M.canis(clinical isolate)	2	4	>512	8	16	>512	64	128	>512	1	2	>512	8
	T.rubrum(clinical isolate)	16	32	128	8	16	32	64	64	256	16	32	32	64
	E.flucusom(clinical isolate)	16	32	64	8	32	64	16	64	128	16	64	256	8

**Fluconazole was used as positive control for Candida and Aspergillus spp. and Griseofulvin for dermatophytes.*

MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration

Among the tested *filamentous* fungi, Compound ***T8*** completely inhibited the growth of *M. canis, T. rubrum, E. flucusom, E. dermatitidis* at concentration ranging from 8 to 32 µg/mL. Of the synthetic compound, ***T10*** exhibited the best inhibitory and fungicidal activities against *M. canis* followed by ***T7*** at concentrations ranging from 1 µg/mL to 4 µg/mL. In comparison of the antifungal activities of the synthetic compounds ***T1***, ***T2*** and ***T3*** were all effective against azole-resistant strains *of C. glabrata* at concentrations ranging from 32-64 µg/mL, suggesting that the modes of action of this compound are different from the examined antibiotics. Also compounds, ***T1***, ***T2***, ***T3***, and ***T5*** exhibited the best inhibitory and fungicidal activities against *M. canis*and *T. rubrum*. In comparison of the antifungal activities of the synthetic compounds based on variation of substitutions on 2,3 and 4-position of phenyl ring, we found that the base compound ***T8*** exhibited a better antifungal activity against the tested fungi than the other compounds except ***T10***. Replacement of hydrogen with nitrogen residue in 4-position of phenyl ring (***T8*** and ***T10***) increase its antifungal and azole-resistant strains activity compared other compounds.

In order to understand the antifungal activity, the potent compounds ***T8*** and ***T10*** were docked into the *Mycobacterium tuberculosis* enzyme CYP51 (PDB ID: 1EA1) structure. Earlier researches show the perpendicular binding model of fluconazole to the heme iron of CYP51 and it was an important key for the antifungal activity [50]. Figure 6 indicates the overlaid of two potent compounds (***T8*** and ***T10***) in active site of target. Docking strongly suggested that the orientation of phenyl rings between adjacent the heme iron and pharmacophore residuesareon to the contrary (turned upside down) sides; So the phenyl rings with poor electron density of nitro group in ***T8*** and fused phenyl ring in ***T10*** are inhydrophobic interaction with Leu 321, Ile 323, Tyr 76, Met 433 and Phe78. It Proposed position of nitroin ***T8*** and dimethyl amine substituted in ***T10*** with difference in electron density could active as antifungal agent by suitable direction in active site.



Scheme 3. The mechanism for the catalytic activity of nano-γ-Al_2O_3/BF_{3-n}in the reaction of benzothiazole condensation.

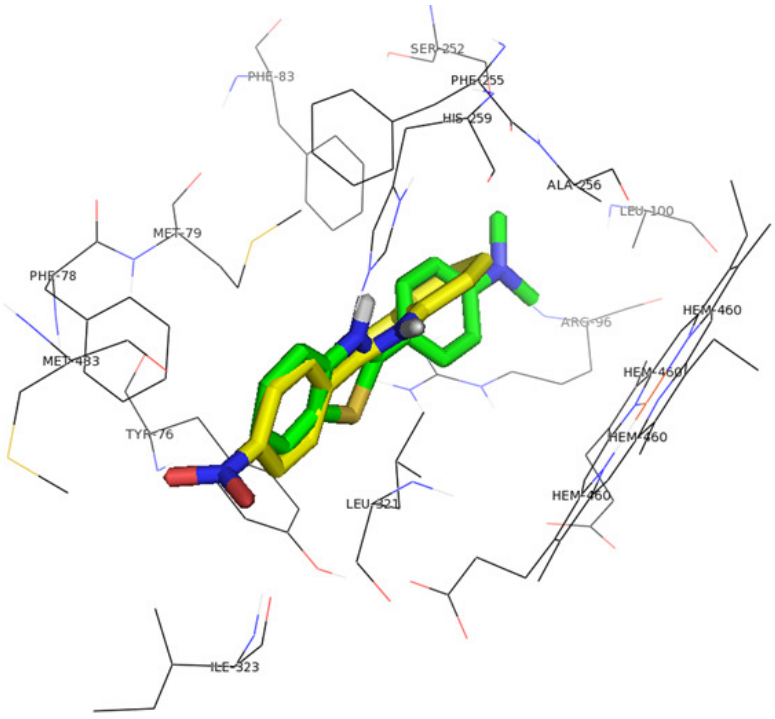


Figure 6. Molecular modeling of overlaid compound ***T8*** and ***T10*** to the heme iron of CYP51. Note: For clarity, only interacting residues in 8 Å were displayed.

4. CONCLUSION

We have demonstrated a simple method for the synthesis of 1,3-benzo[*d*]thiazoles with using Nano-γ-Al_2O_3/BF_{3-n} as a new solid acid catalyst under solvent free condition at 110 °C. The short reaction times, good yields, a clean process, simple methodology, easy work-up are some advantages of this protocol. In the present study, some of the synthetic compounds including ***T8*** and ***T10*** exhibited a great activity against tested *Candida* and *dermatophytes*. Comparing the structure and activity of these two compounds with the others revealed, we found the containing $-N(CH_3)_2$ group as electron releasing and NO_2 group as electron withdrawing in *para* position of phenyl ring ***T8*** and ***T10*** enhance the antifungal activity, respectively. Altogether, regarding a broad spectrum antifungal activities of some of the tested compounds (even against azole resistant strains), they might be a good candidate for further *in vivo* studies to elucidate their effects and toxicity as a novel antifungal drug. Docking study simulates the interaction of compound ***T8*** and ***T10*** with the *Mycobacterium tuberculosis* enzyme CYP51 binding pocket nicely. It was proposed the different chemical structures in reverse binding mode in active site of target.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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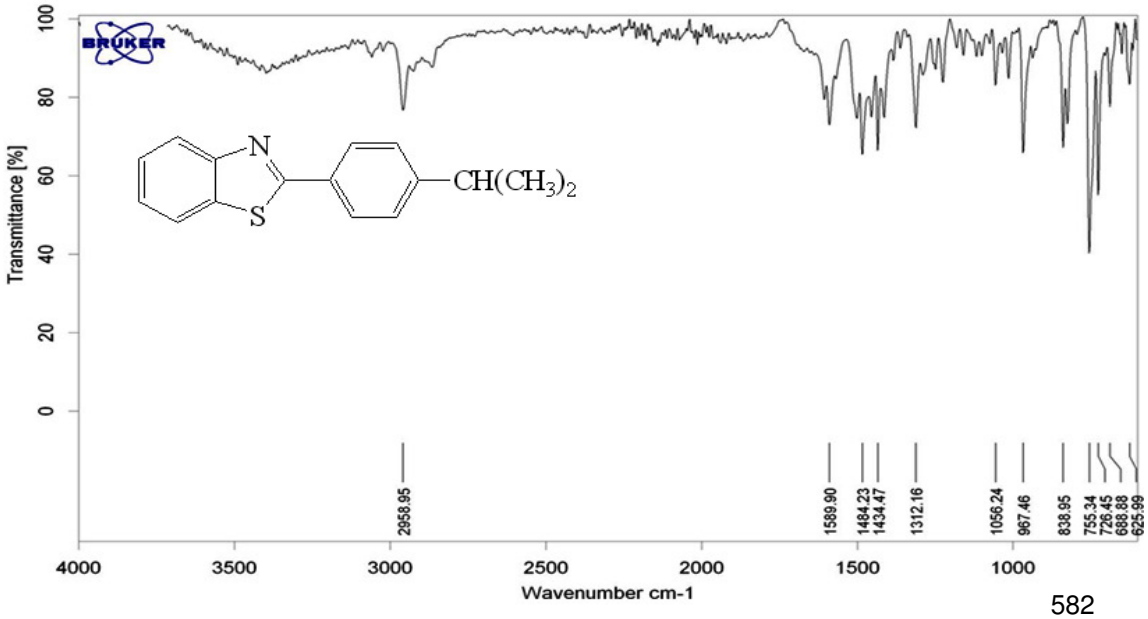

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583 (FT-IR） ATR of ***T1***

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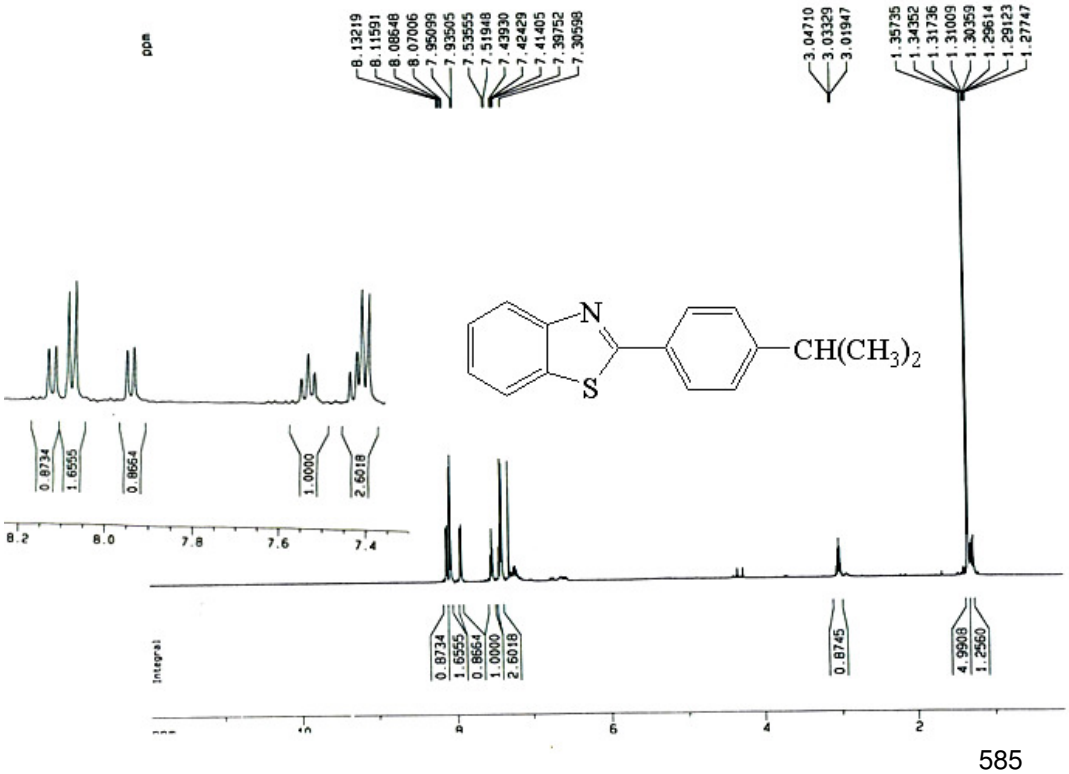

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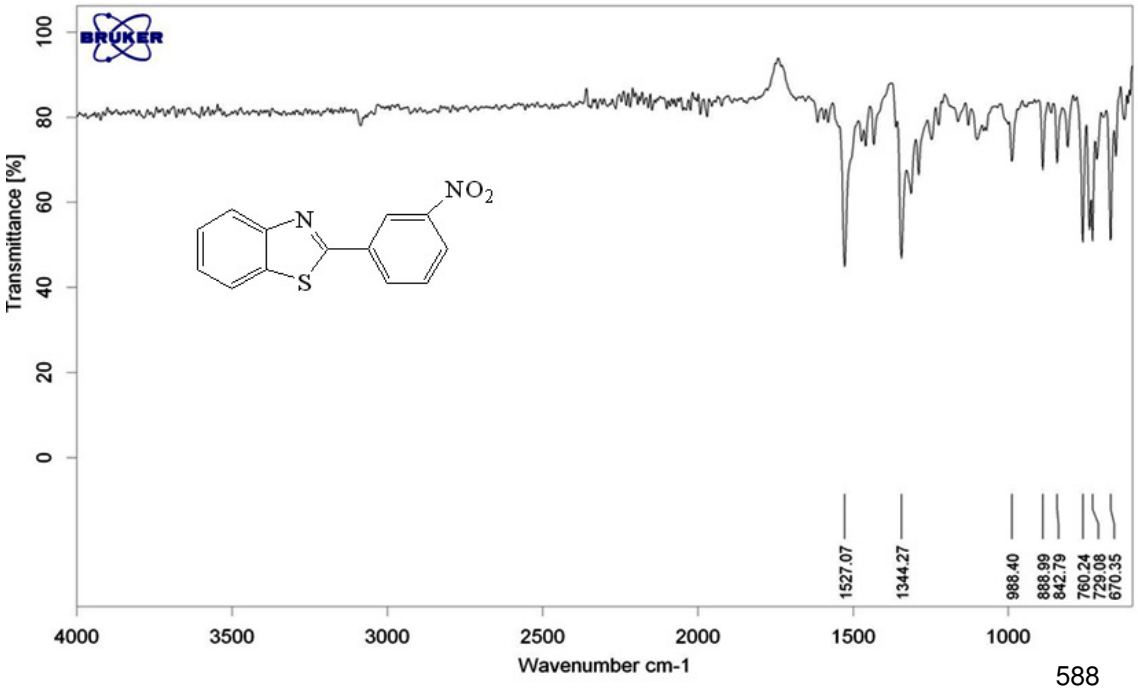
586 ^{1}H-NMR (400MHz, $CDCl_3$) of ***T1***

587



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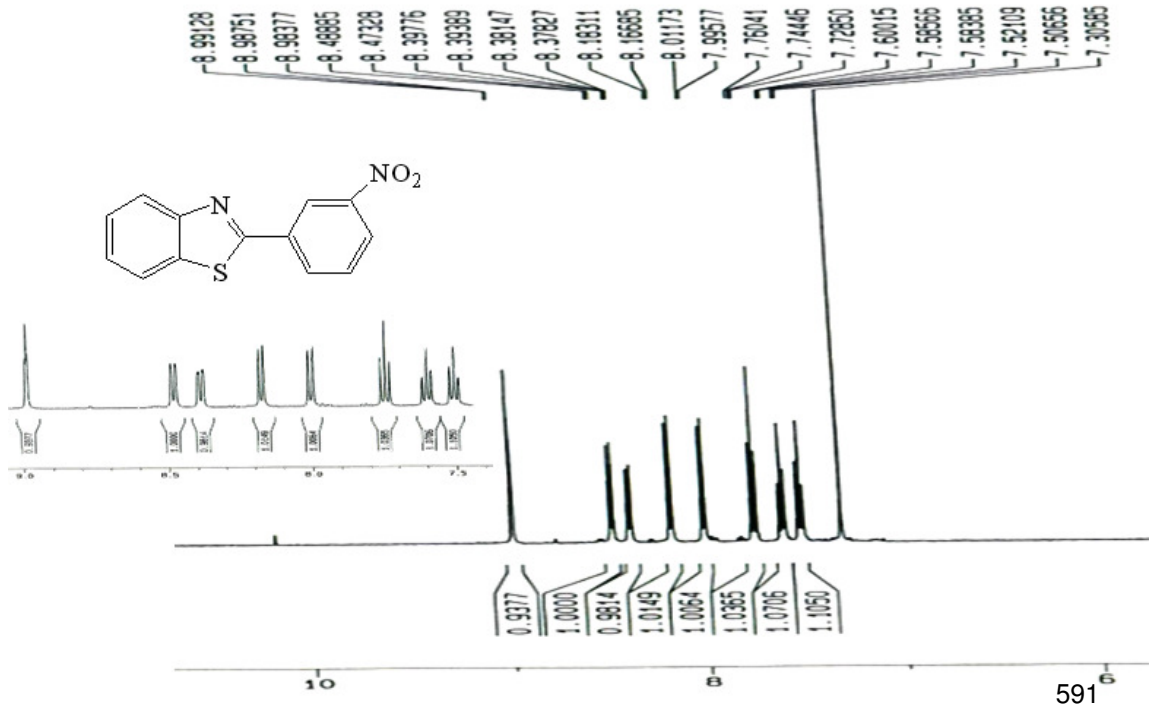

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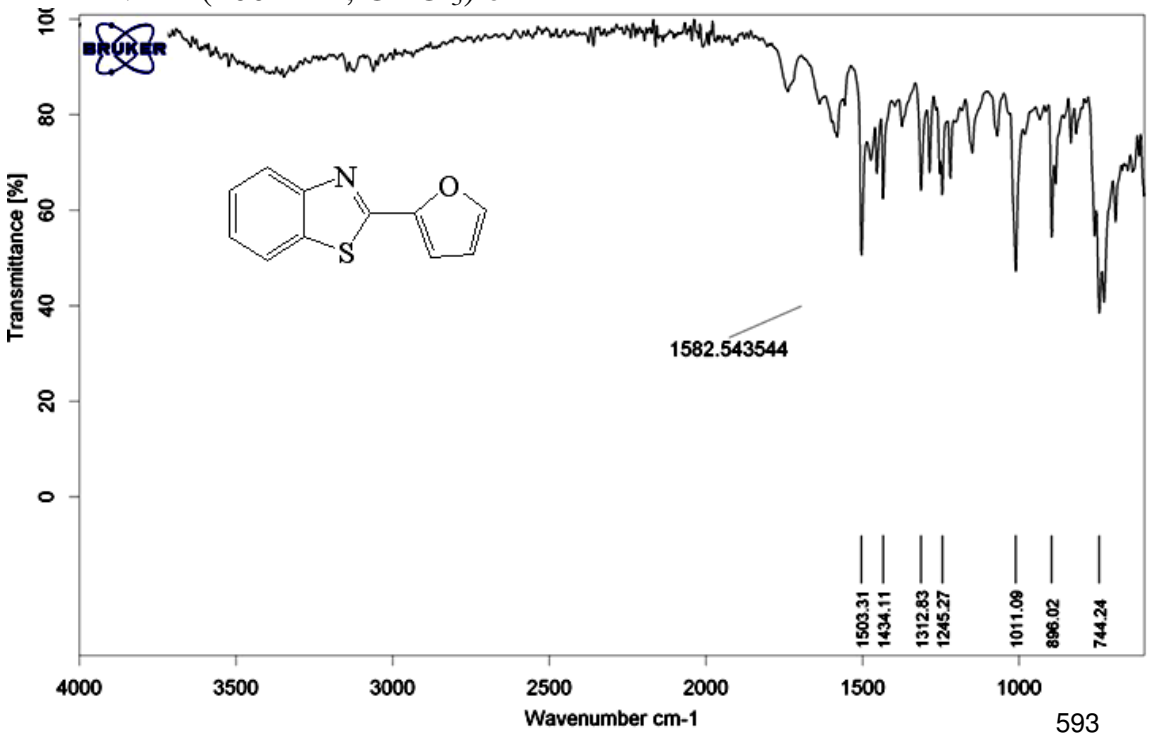

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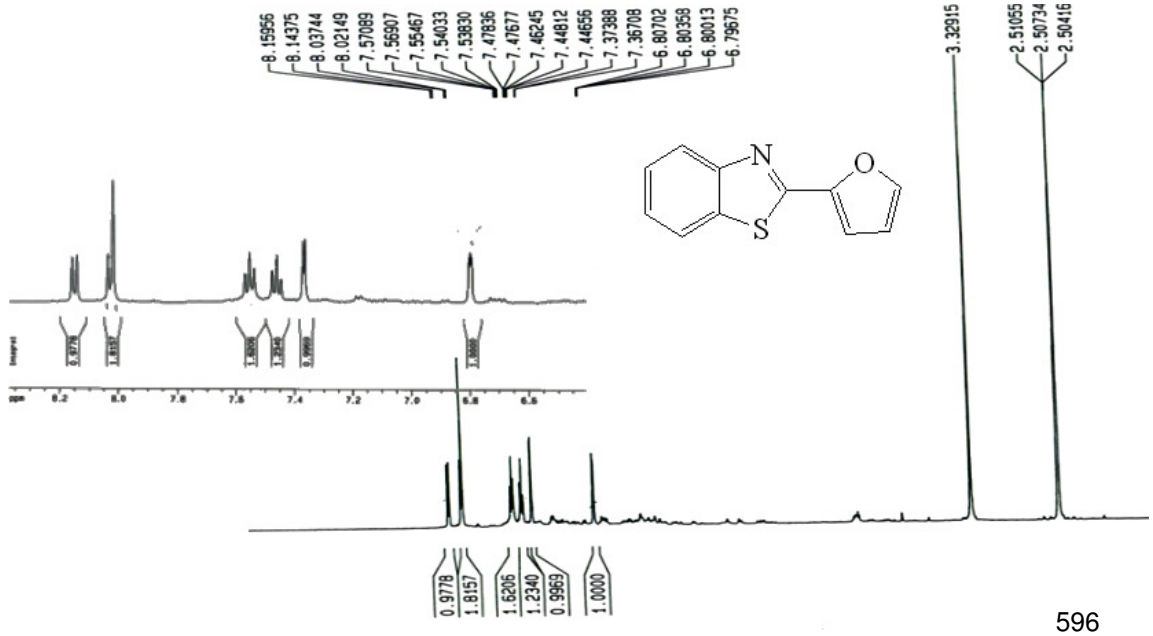

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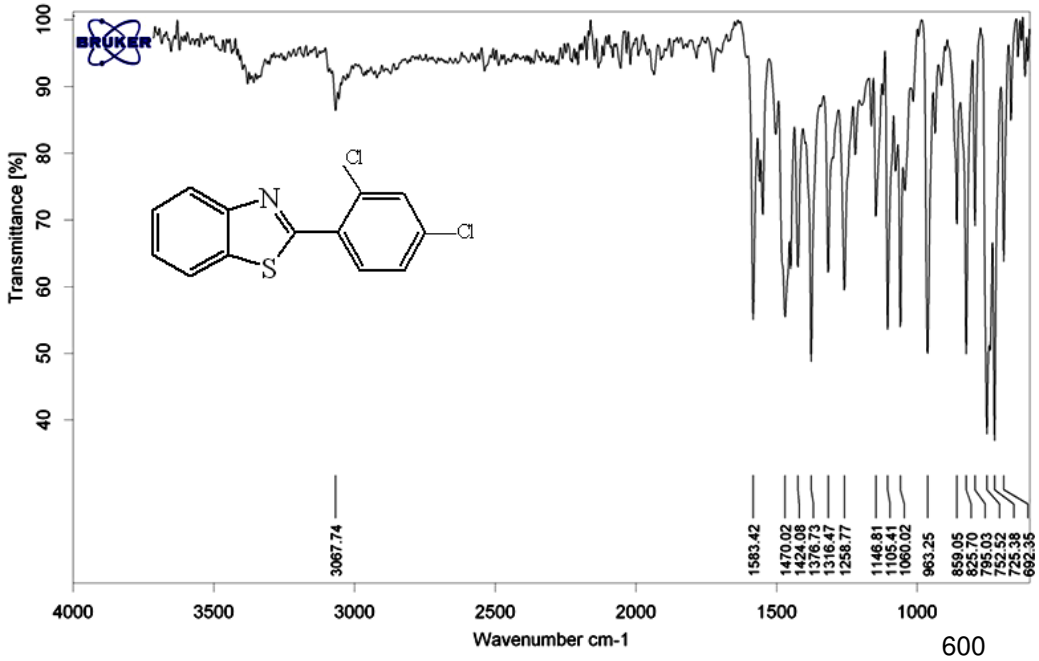
597 ^{1}H-NMR (400MHz, $CDCl_3$) of ***T3***

598



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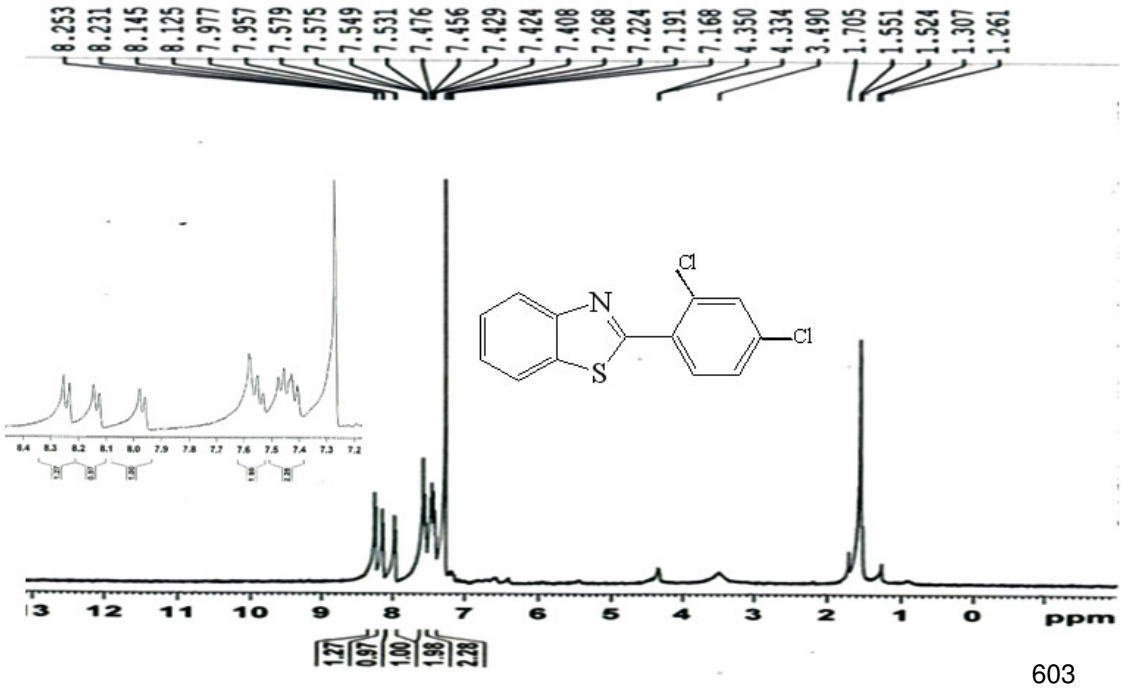

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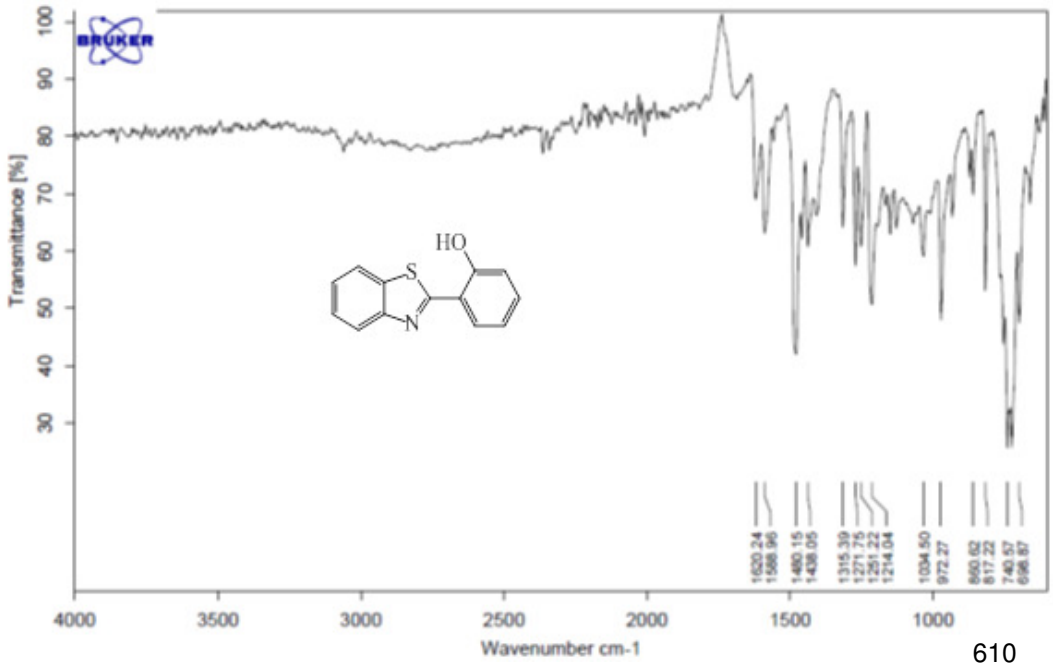

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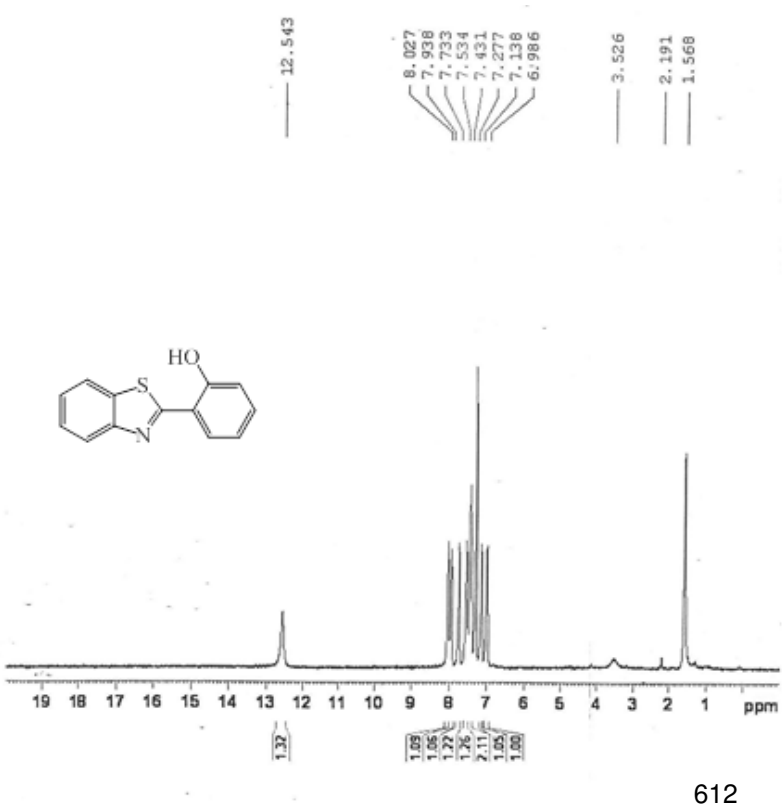
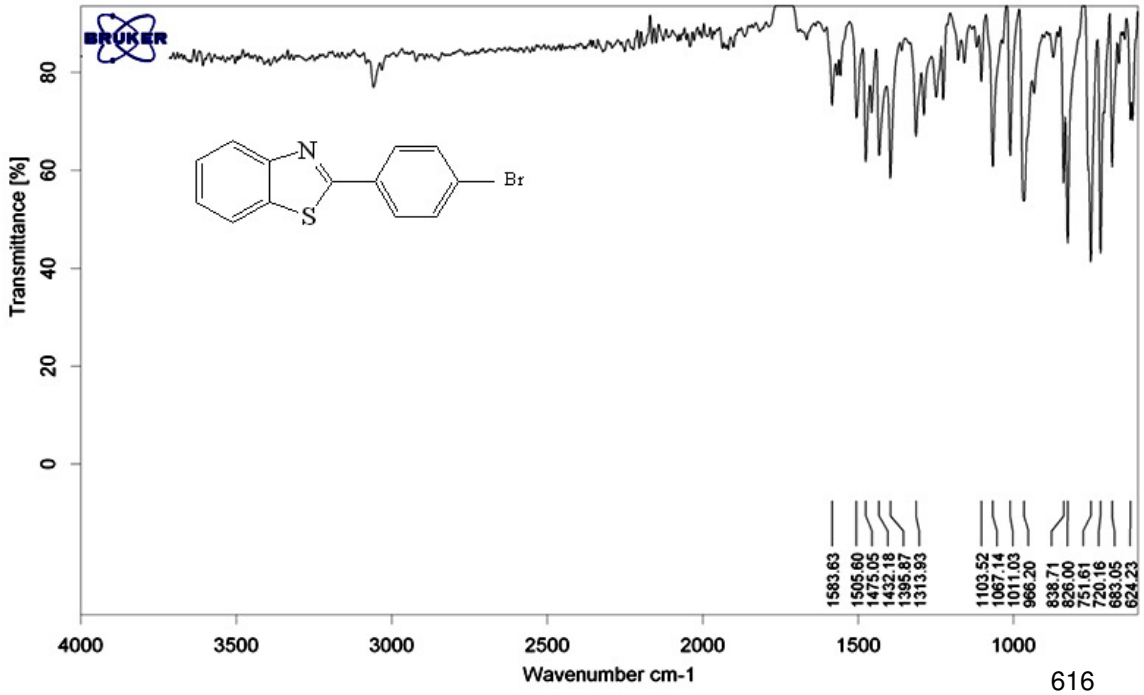


612

613 1H-NMR (400MHz, Acetone-*d6*) Of ***T5***

614

615



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617 (FT-IR） ATR of ***T6***

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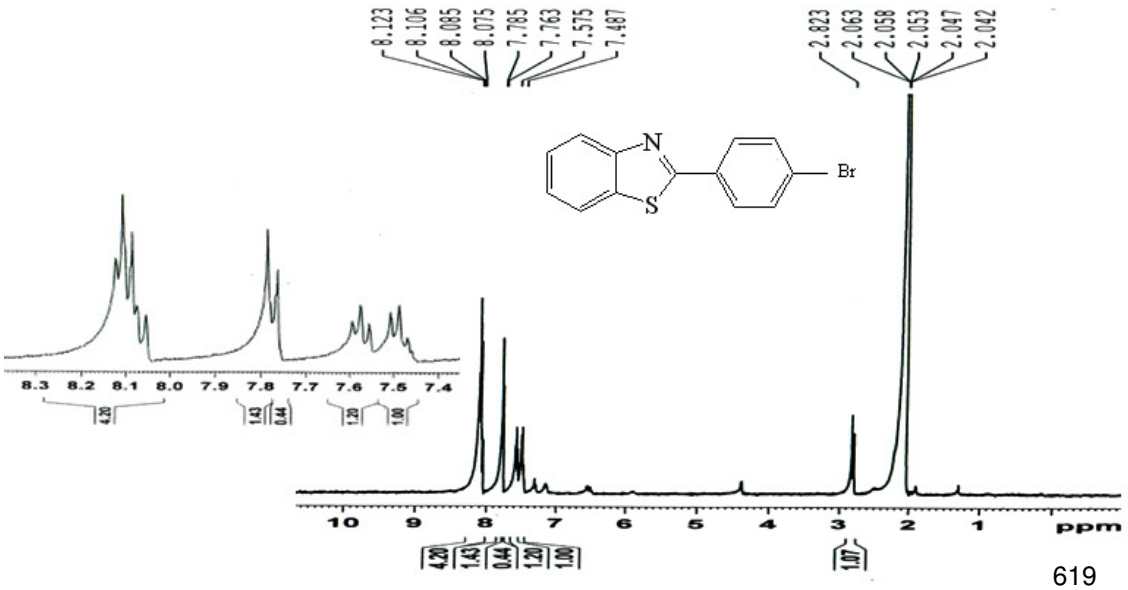

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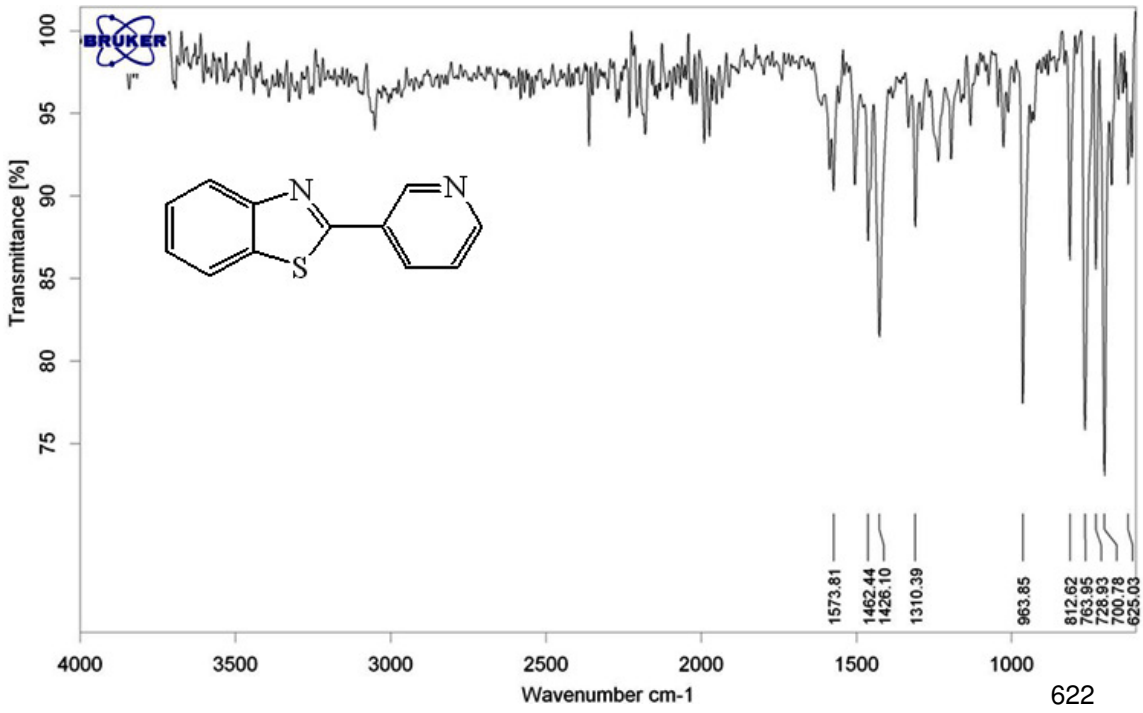
620 ^{1}H-NMR (400MHz, Acetone-d_6) of ***T6***

621



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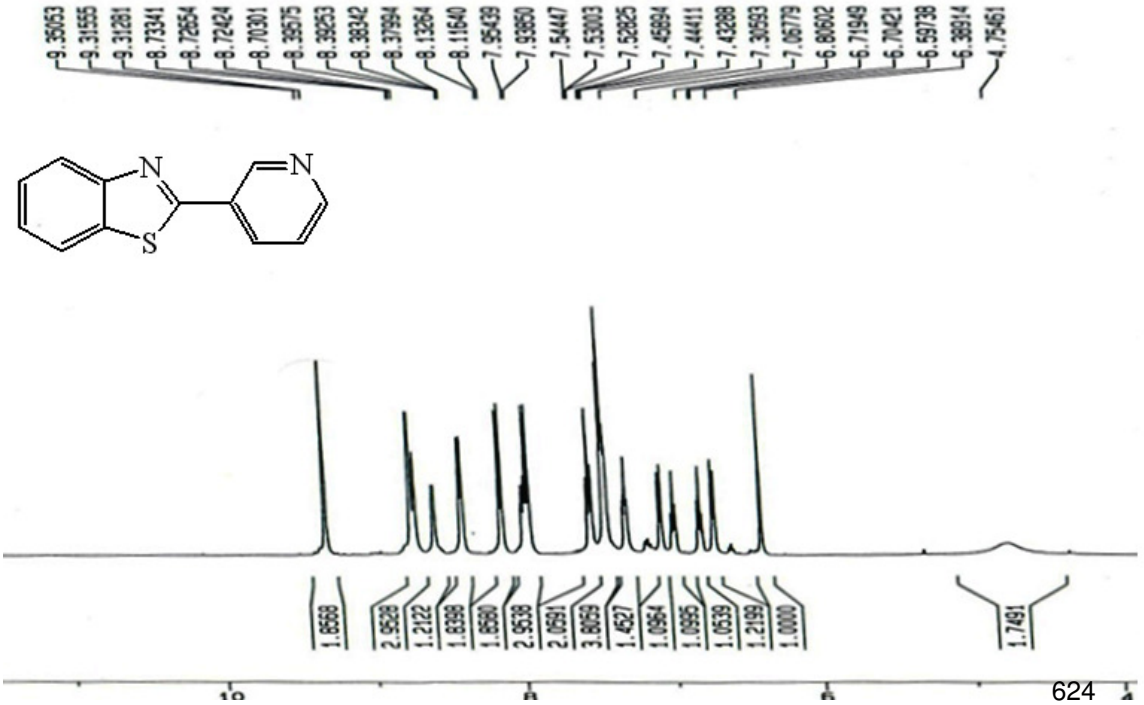
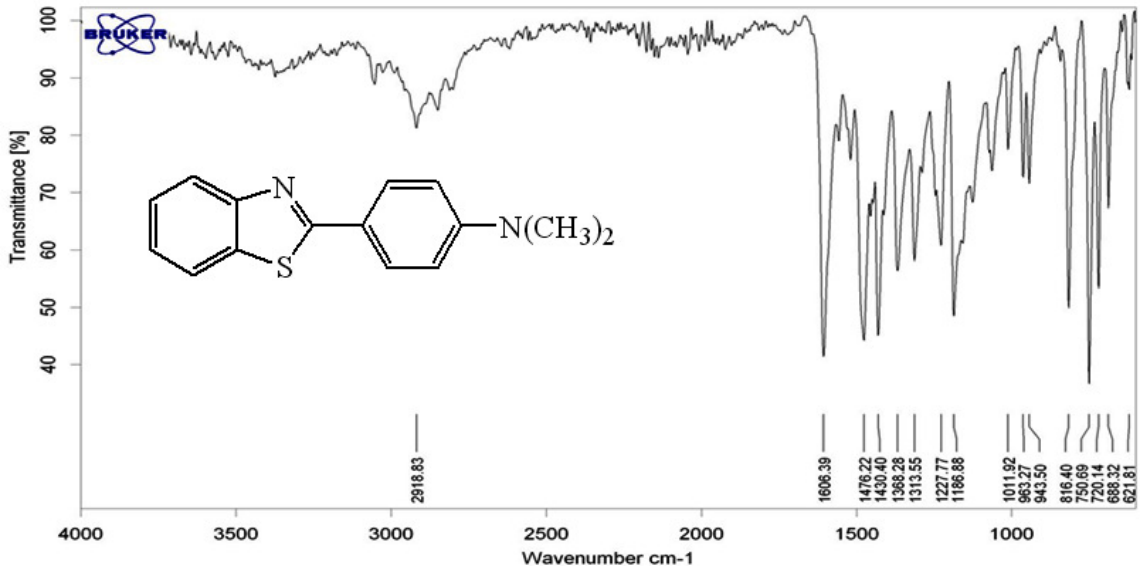


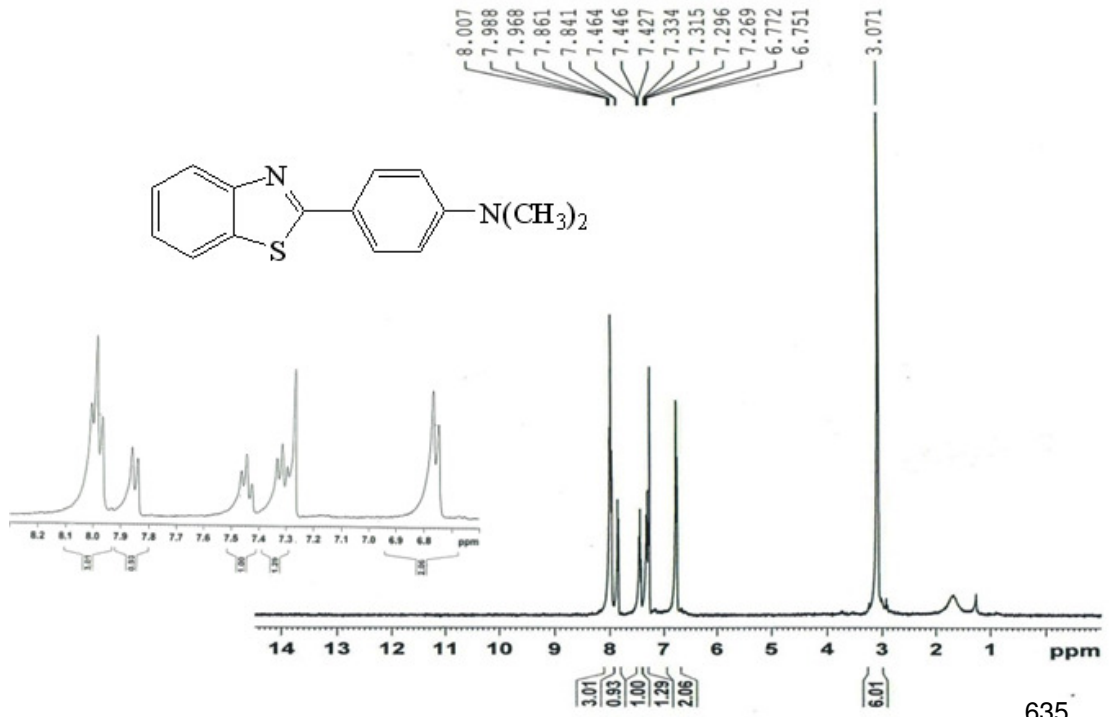
624

625 ^{1}H-NMR (400MHz, $CDCl_3$) of ***T7***

626



(FT-IR） ATR of ***T8***



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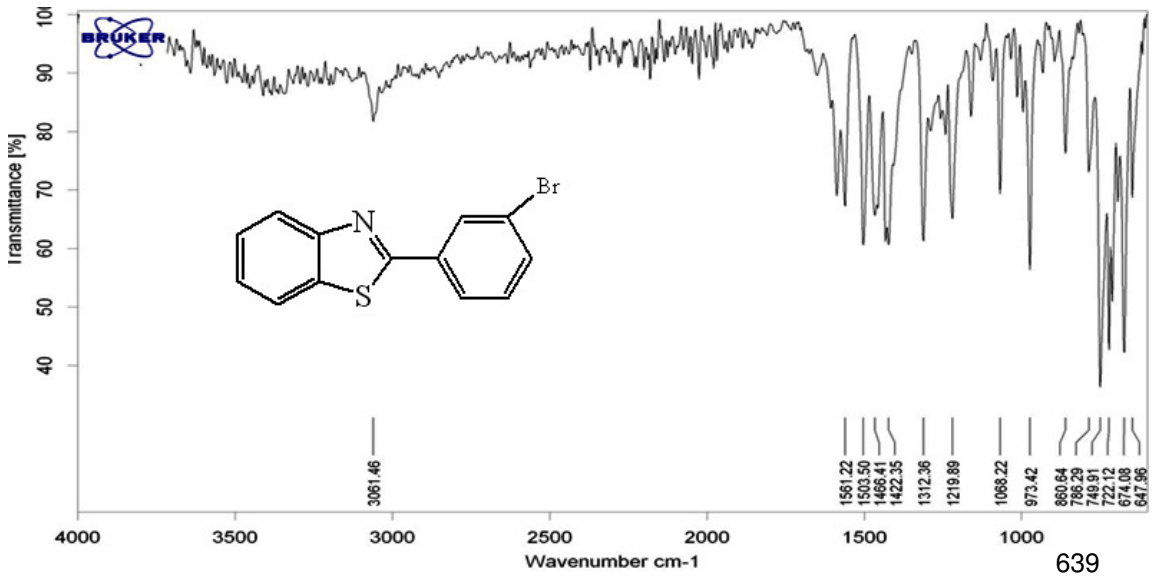
636 ^{1}H-NMR (400MHz, $CDCl_3$) of ***T8***

637

638



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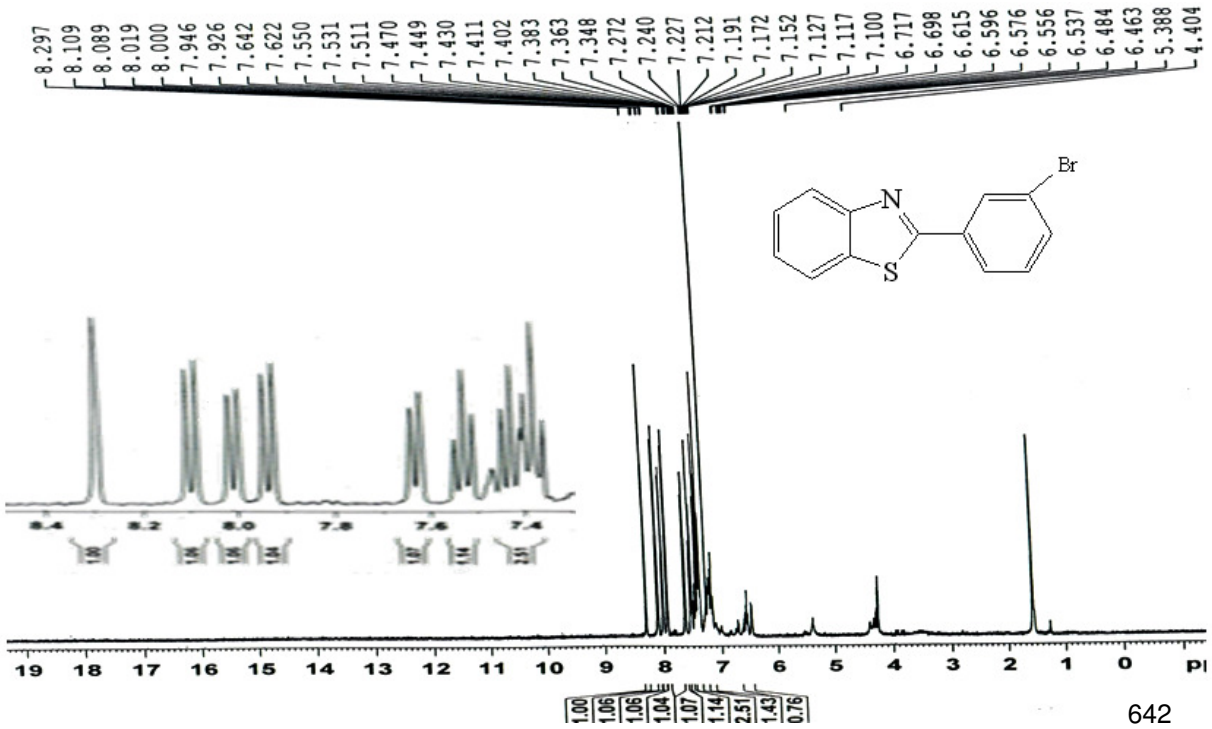

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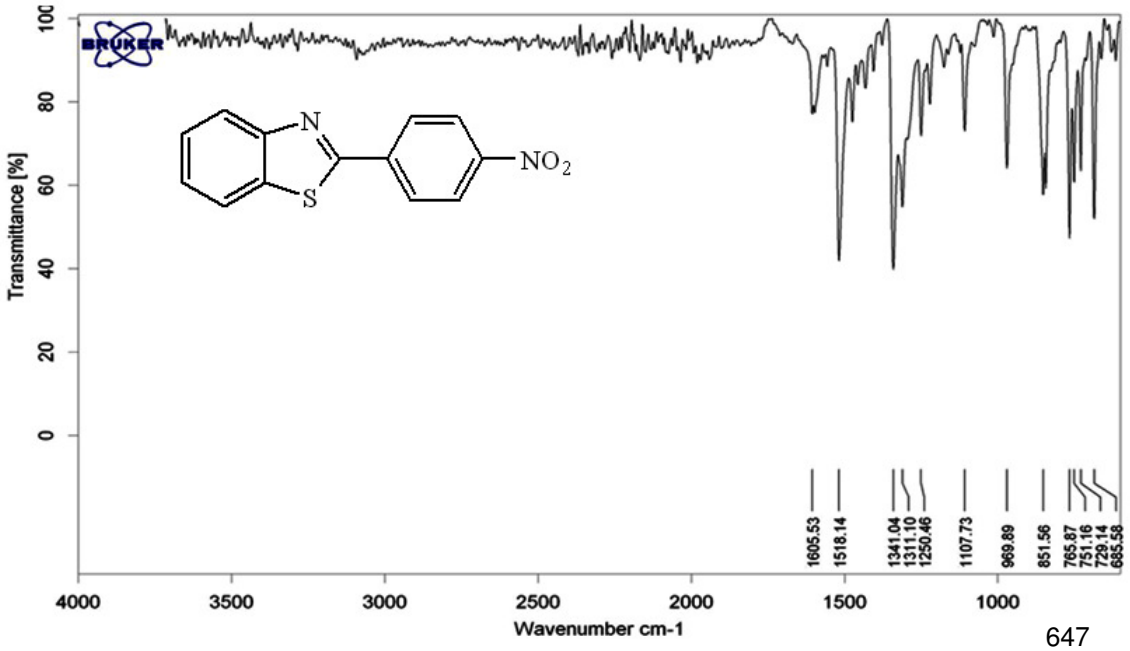
644

645 ^{1}H-NMR (400MHz, $CDCl_3$) of ***T9***

646



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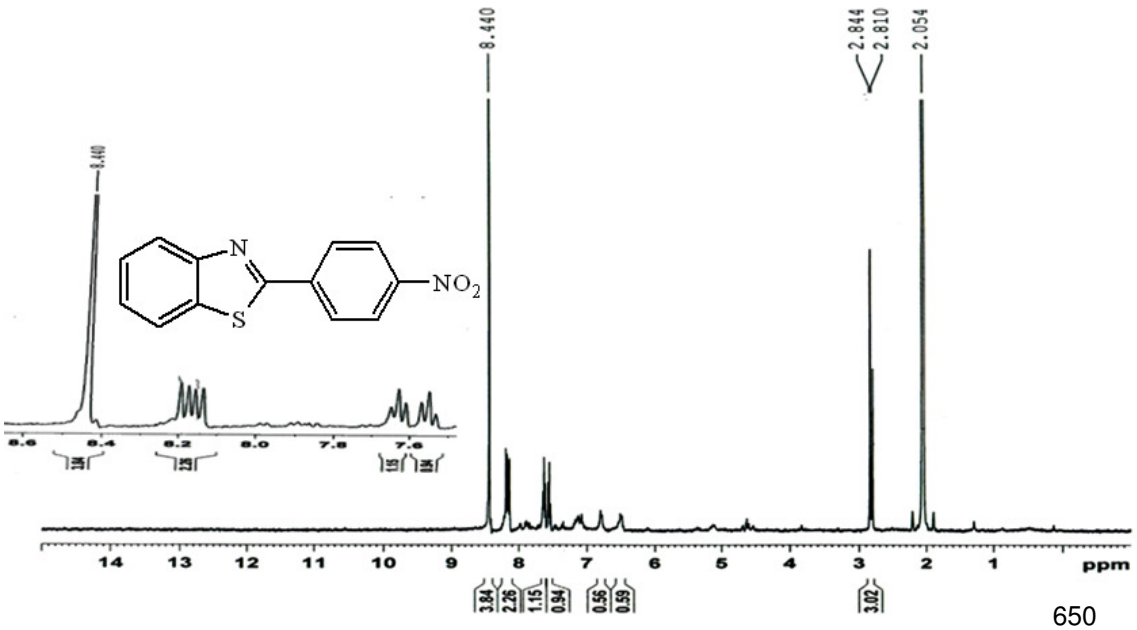

^{1}H-NMR (400MHz, Acetone-d_6) of ***T10***