Synthesis, Antibacterial and Antifungal Activities for Novel Derivatives of 2,2'-(((1-benzylbenzoimidazol-2-yl) methyl) azanediyl) bis (ethan-1-ol)

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Abstract

The compound 2,2'-(((1H-benzo(d) imidazol-2-yl)methyl)azanediyl)bis(ethan-1-ol) was reacted with benzyl bromide to afford compound (1) which used as row material to prepare a series of compounds through condensation reaction, the starting compound were reacted with tosyl chloride to protect the OH group to afford compound 2, then reacted benzyl bromide to produce compound (2), then the compound (2) treated with three compounds (2-mercaptobenzthiazole, 2-mercaptobenimidazol and 2-chloromethyl benzimidazole) to form compounds 3a, b, 4a,b and 5a,b respectively. In the another step the click reaction of compound 2, 2'-(((1H-benzo(d)imidazol-2-yl) methyl) azanediyl)bis(ethan-1-ol) with Propargyl bromide produce compound 6 which reacted with sodium azide or benzyl azide to afford the compounds 7 and 8. The synthesized compounds were characterized and measured the physical properties via the FT-IR, HNMR, besides to the CHN analysis. These newly compounds were screened their antibacterial and antifungal activity. Compounds 1, 2a and 8 showed significant antibacterial activity as well these compounds exhibited either low or moderated antifungal activity.

Keywords: Benzimidazole Derivatives, diethanolamine, Benzothiazole, Triazole, Antibacterial, Antifungal.

1.Introduction

The heterocyclic compounds are considering very important compound in many fields like the agriculture, industry and medicine application, the benzimidazole compounds are derivatives from the heterocyclic compounds Benzimidazole can be described as fused bicycle between benzene and imidazole. The first synthesis of benzimidazole had been reported during 1872 by Hoebrecker whom synthesized 2,5-dimethylbenzimidazole and 2,6-dimethylbenzimidazole by the reduction of 2-nitro-4 methylacetanilide [1,2] the benzimidazoles and their derivatives exhibited remarkable biological activity and significant pharmacophore structure in therapeutic activities like antiulcers[3], antihypertensives, analgesic, anti-inflammatory [4,5], antiviral [6,7] , antifungals [8], anticancers [11-9] , and

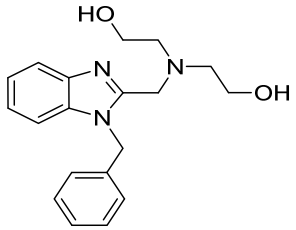
antihistaminics [12]. The most prominent benzimidazole compound in nature is N-ribosyl-dimethyl benzimidazole, which known as ligand for cobalt in vitamin B12 [13-15].

Due to the significant importance of benzimidazoles, a large numbers of methods have been reported for synthesizing benzimidazole derivatives and According to high biological activities of benzimidazole derivatives new benzimidazole containing some heterocyclic rings and test *in vitro* their preliminary antibacterial and antifungal activities.

2. Experimental

All chemicals and solvents utilized brand Sigma-Aldrich, Fisher and Merck to synthesize the target compounds. The synthesized compounds were characterized and measured the physical properties via the FT-IR, HNMR, spectroscopies and melting points.

2.1. Synthesis of 2,2'-(((1-benzylbenzoimidazol-2-yl) methyl)azanediyl)bis(ethan-1-ol) (1).



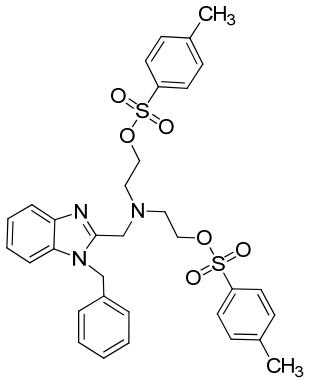
(1)

Benzyl bromide (4.1 g, 2.4 mmol) was added to a suspension of 2,2'-(((1H-benzo(d)imidazol-2-yl) methyl) azanediyl)bis(ethan-1-ol (2.35 g, 10 mmol) in 25 mL of acetone and anhydrous potassium carbonate (1.38 g ,10 mmol) drop wise within 30 mins. The mixture was refluxed for 48 hours and the excess of solvent was removed under reduce pressure. 50 mL of distilled water was added then transferred to a separating funnel. The mixture was extracted 25 × 3 by dichloromethane DCM. The combined organic layer washed with water and brine then dried under magnesium sulfate. After evaporating the solvent, the residue was purified by column chromatography ethyl acetate - hexane (5: 1) to afford 2.4 g (74%) as brown gummy precipitant.

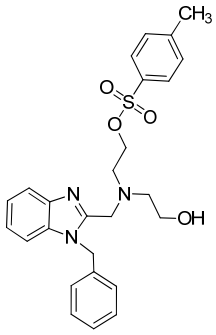
2.2. Synthesis of (((1-benzyl-1H-benzo[d]imidazol-2-yl) methyl) azanediyl)bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate)(2a) and 2-(((1-benzyl-1H-benzo[d]imidazol-2-yl)methyl)(2-hydroxyethyl)amino)ethyl 4-methylbenzenesulfonate (2b).

The 2.35 g (10 mmol) from compound **1** was taken in 40 mL dry triethylamine was stirred at 0 C for 15 mins. Tosyl Chloride (6.2 g, 32.52 mmol) was added in small portion in period of two hours with continues stirring and maintain temperature at 0 °C. after fulfillment the addition of the mixture is left to stirring at ambient temperature. The mixture poured into 50 mL crushed ice then acidified by 10% hydrochloric acid to pH = 5. Chloroform 100 mL is added, the mixture is transferred to separating funnel and extracted twice. The combined organic layer washed with distilled water several times then washed with brine. After daring the organic layer under anhydrous magnesium sulfate and evaporated under reduced pressure. The crude product was purified by column chromatograph using ethyl acetate - hexane (1: 8)

as eluent to obtain 4.1 g of compound 2a as brown precipitant and 1.83 g of compound 2b as brown precipitant.

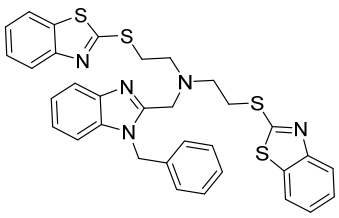


2 a

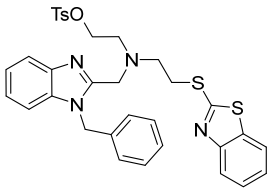


2 b

2.3. Synthesis of 2-(benzo[d]thiazol-2-ylthio)-N-(2-(benzo[d]thiazol-2-ylthio)ethyl)-N-((1-benzyl-1H-benzo[d]imidazol-2-yl)methyl)ethanamine (3a) and 2-((2-(benzo[d]thiazol-2-ylthio)ethyl)((1-benzyl-1H-benzo[d]imidazol-2-yl)methyl)amino)ethyl 4-methylbenzenesulfonate (3b).



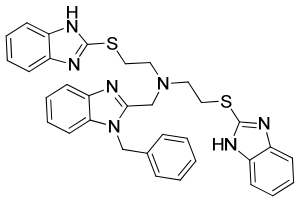
3a



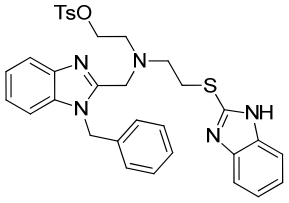
3 b

Benzimidazole-2-thiol (3.35 g, 4 mmol) was added with a few portions to a solution of 2a (1.4 g, 2 mmol) in dimethyl sulfoxide DMSO and sodium hydroxide (0.16ng, 4 mmol) in normal room temperature. The mixture was heated to 100 °C for 5 hrs. On cooling 100 mL distill water is added then the mixture acerbated at fridge overnight. The precipitated is collected, dried at 50 °C. The crude compound was purified by column chromatography ethyl acetate – hexane (1: 11) to obtain brown gummy priciptant.51% of compound 3a and 30 % of compound 3b as brown gummy precipitant.

2.4. Synthesis of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2-((1H-benzo[d]imidazol-2-yl)thio)ethyl)-N-((1-benzyl-1H-benzo[d]imidazol-2-yl)methyl)ethanamine (4a)and 2-((2-((1H-benzo[d]imidazol-2-yl)thio)ethyl)((1-benzyl-1H-benzo[d]imidazol-2-yl)methyl)amino)ethyl 4-methylbenzenesulfonate (4b).



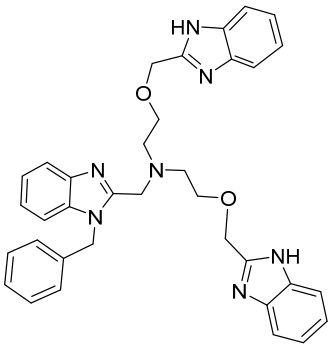
4a



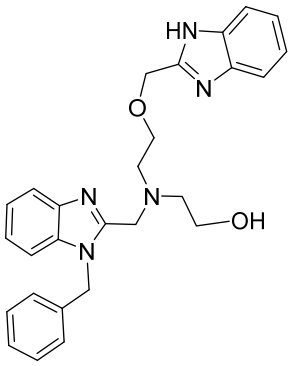
4 b

The target compounds were synthesized utilizing the same method mentioned in synthesized the compounds 3a, 3b and using 4 mmol of benzimidazole-2-thiol instead of Benzathiazole-2-thiol. The crude compound was purified by column chromatography ethyl acetate – hexane (1: 1) to afford 47% of compound 4a as brown gummy precipitant and 33 % of compound 4b as brown Gummy precipitant.

2.5. Synthesis of 2-((1H-benzo[d]imidazol-2-yl)methoxy)-N-(2-((1H-benzo[d]imidazol-2-yl)methoxy)ethyl)-N-((1H-benzo[d]imidazol-2-yl)methyl)ethanamine (5a)and 2-((2-((1H-benzo[d]imidazol-2-yl)methoxy)ethyl)((1-((1H-benzo[d]imidazol-2-yl)methyl)-1H-benzo[d]imidazol-2-yl)methyl)amino)ethanol (5b).



5 a

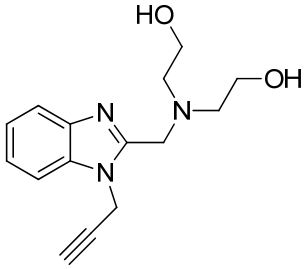


5 b

2-Chloromethyl benzimidazole (1 g, 6 mmol) was added with small portions to a solution of compound 1 (0.71g, 3 mmol) in dry pyridine at ambient temperature. The mixture was heated under reflux overnight. Upon cooling the mixture poured into 50 mL crashed ice then acidified to pH 6-5 by 5% cooled solution of hydrochloric acid. The resulting product was extracted with dichloromethane DCM (30 x 3) and washed with water then brine. The combine organic layer dried under magnesium sulfate and evaporated, the crude product

purified by column chromatograph to afford brown gummy precipitant yield 44% of compound 5a and 34 % of compound 5b as brown gummy precipitant.

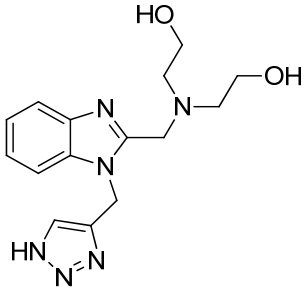
2.6. Synthesis of 2,2'-(((1-(prop-2-yn-1-yl)-1H-benzo[d]imidazol-2-yl)methyl)azanediyl)diethanol (compound 6).



(6)

Propargyl bromide (2.6 g, 22 mmol) is added dropwise to a solution of 2,2'-(((1H-benzo[d]imidazol-2-yl)methyl)azanediyl)diethanol (4.7 g, 20 mmol) in 10 mL of dimethyl sulfoxide with sodium hydroxide (0.8 g, 20 mmol) at ambient temperature. The mixture is heated to 70 °C under reflux for 6 hrs. After cooling the mixture, it quenched with 40 mL distilled water then transferred to separate funnel and extract three times with 100 mL of dichloromethane DCM. The organic layer is washed with distilled water several time, dried under anhydrous magnesium sulfate. The organic solvent is removed under reduced pressure. The crude product was purified by column chromatography using ethyl acetate – hexane (1: 1) As eluent to afford yellow precipitant with 87% yield.

2.7. Synthesis of 2,2'-(((1-((1H-1,2,3-triazol-4-yl)methyl)-1H-benzo[d]imidazol-2-yl)methyl)azanediyl)diethanol (compound 7).

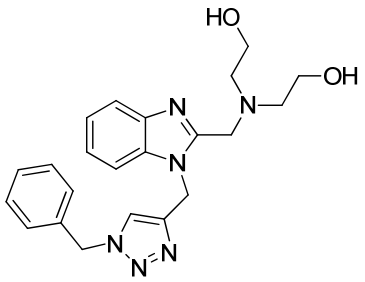


(7)

Sodium azide (0.2 g, 3.1 mmol) is added to a stirring solution of compound **6** (0.41g, .1.5mmol) in 25 mL Methanol - H_2O (5:1). Copper sulfate five hydrates (0.08 g, 0.5 mmol) is added followed by (0.59 g, 3 mmol) of sodium ascorbate. The mixture has left under vigorously stirring at ambient temperature for 24 hours. The resulting solution is filtered, evaporated under reduced pressure.

The crude product was purified by column chromatograph using ethyl acetate – hexane (5: 1) to give white precipitant of yield 66%.

2.8. Synthesis of 2,2'-(((1-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-1H-benzo[d]imidazol-2-yl)methyl)azanediyl)diethanol (8).



8

Freshly prepared Benzyl azide (0.2 g, 1.5 mmol) is added to a stirring solution of compound **6** (0.41g, 1.5 mmol) in 25 mL Methanol Copper sulfate five hydrate (0.08 g, 0.5 mmol) then followed by adding (0.59 g, 3 mmol) of sodium ascorbate. The mixture left under vigorously stirring at ambient temperature for 24 hours. The resulting is filtered, evaporated under reduced pressure. The crude product was purified by column chromatography using ethyl acetate – hexane (5 : 1) as eluent to give white precipitant.

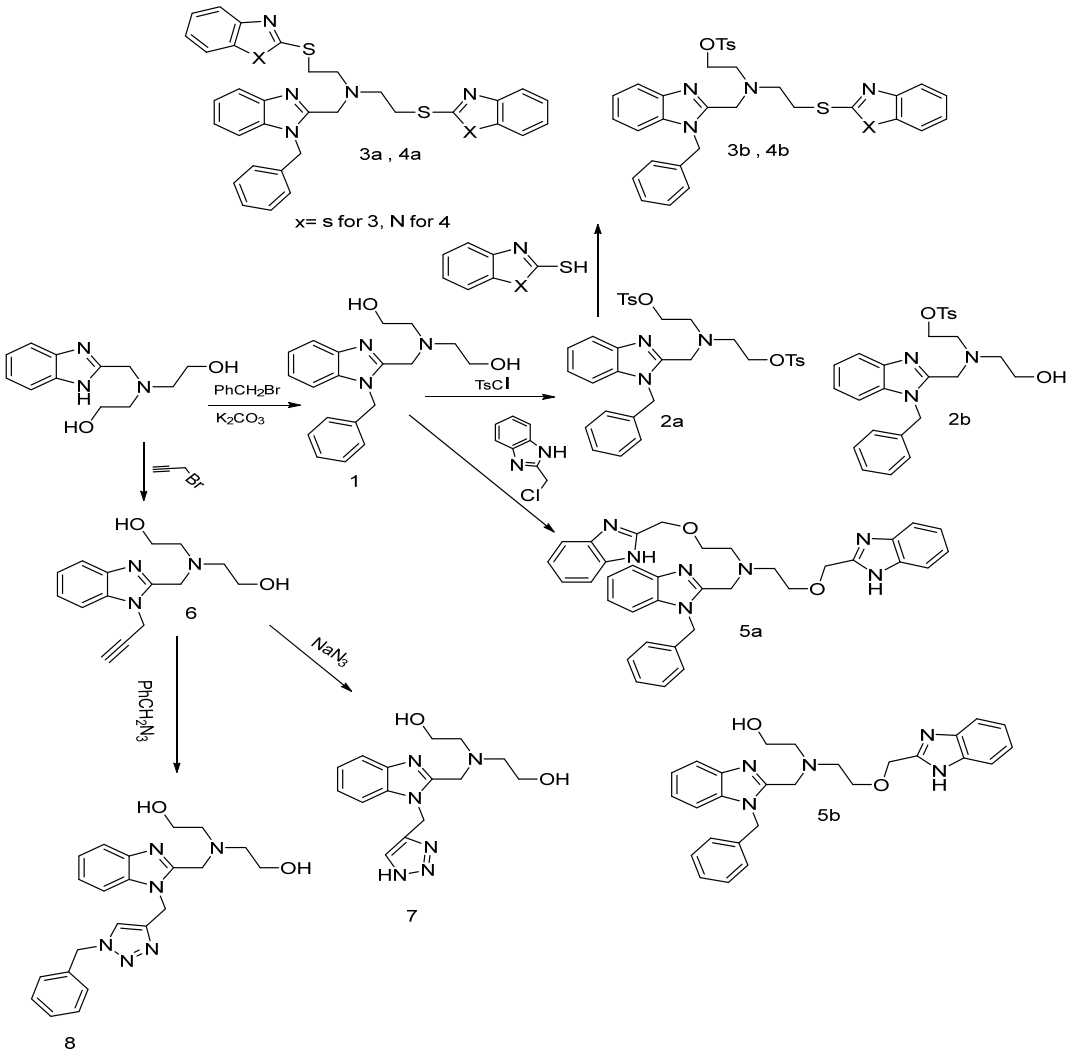
3. Results and Discussion

3.1 Chemistry

The compound **1** is synthesized by benzylation reaction in acetone and in the presence of potassium carbonate as demonstrated in **Scheme 1.**

This compound is characterized by FTIR and ^{1}H NMR besides to the CHN analysis. The FTIR spectrum of this compound showed the broad band for OH at 3404 cm^{-1}, while the NH of benzimidazole was disappeared from 3384 cm^{-1}. The band of C=N located at 1651 cm^{-1} and the C=C at 1485 cm^{-1} as displayed in **Figure 1**.

The ^{1}H NMR spectrum of this compound showed multiplet peak at 4.16 ppm for nine protons, eighth of them for CH_2 and one broad peak for OH. The second OH did not appeared and that could be due to engage with intramolecular hydrogen bonding or to deuterium exchange[16-18]. The CH_2 of benzyl appeared as singlet at 4.75 ppm with integral of two protons. Moreover, the singlet of CH_2 attached with benzimidazole appeared at 5.49 ppm. The aromatic protons located as multiplet peak at 6.84-7.66 ppm as displayed in **Figure 2**.



Scheme 1: Route of synthesized compounds 1, 2a-b, 3a-b, 4a-b, 5a-b, 6 ,7 and 8.

Tosylation of compound 1 in pyridine afforded two products. The major one was with two tosyl group, while the minor one was with on tosyl group. Both compounds 2a and 2b were characterized from their FTIR and ^{1}H NMR spectra.

The FTIR for 2a exhibited disappearance of the hydroxyl group and that was good indicator for the success of tosylation reaction. As well as the rest band was located at their expected region. For instance, the CH aliphatic and C=C were located at 2900 and 1662 cm^{-1} respectively as depicted in **Figure 3**.

The ^{1}H NMR of compound 2a showed singlet for six protons at 2.3 ppm attributed to two CH_3 of tosyl as well multiplet peak at 3.42-3.5 ppm for eight protons for four CH_2 group. The CH_2 of benzyl is located at 4.27 ppm as singlet peak and the CH_2 attached benzimidazole located at 4.29 ppm as singlet peak. The aromatic protons of the benzimidazole and benzyl appeared as multiplet at 6.4-6.69 ppm while the aromatic protons of two tosyl group appeared as two doublet peak at 7.22 ppm and 7.52 ppm respectively as shown in **Figure 4**. Existence of hydroxyl group at FTIR of compound 2b besides the integration of methyl group of tosyl at 2.27 ppm and the appearance of OH in ^{1}H NMR spectrum for this compound confirms that one tosyl group was substituted. Furthermore, the results of CHN analysis harmonized with the proposed structure, as shown in **Figure 5**.

The compounds 3a and 3b were characterized by FTIR and ^{1}H NMR spectra. The FTIR spectrum of compound 3a showed the aromatic CH at 3097 cm^{-1} and the aliphatic CH at 2924-2852 cm^{-1}.the band of C=N is located at 1610 cm^{-1} and the C=C at 1516, 1425 cm^{-1} as demonstrated in **Figure 6**.

The ^{1}H NMR of this compound showed disappearing to the CH_3 of tosyl group as well the four group of CH_2 appeared as multiplet: with integration equal to eight protons. The two CH_2 at high field 4.16-4.26 ppm with integral of four protons at 4.97 and 5 ppm attributed to CH_2 of benzyl and to CH_2 attached the benzimidazole ring respectively. The integration of aromatic protons was identical to judge that the two benzothiazole substituted as shown in **Figure 7**.

Expectance singlet at 2.3 ppm for three protons of CH_3 belong to tosyl quit enough evidence that the minor product (3b) is substituted with one group of benzothiazole. The FTIR and ^{1}H NMR was depicted in **Figures 8. and 9.** respectively.

2- Thiobenzimidazole also was substituted as depicted in **scheme 1**. As well as this reaction proceed as earlier mentioned and afforded two products 4a as major product while 4b was isolated by column chromatography as minor product.

Both compounds (a, b) were characterized by FTIR and ^{1}H NMR spectra. The FTIR of compound 4a exhibited the NH of benzimidazole at 3105 cm^{-1} and the CH aliphatic at 2979. 2873 cm^{-1}. The band of C=N was located at 1633 cm^{-1} and the C=C at 1606, 1531 cm^{-1} as demonstrated in **Figure 10**.

The ^{1}H NMR of compound 4a showed three multiplet peak at high magnetic field two of them for four CH_2 of ethanol amine while the third one belongs CH_2 of benzyl and CH_2 attached Benzimidazole at position two. The aromatic protons were assigned as multiplet with integral equal seventeen protons. The interested NH of benzimidazole appeared as two protons at low magnetic field with integration equal to two. These two peaks refer that the two NH were unsymmetrical due to the geometrical orientation[19-20] as demonstrated in **Figure 11**.

The FTIR of compound 4b (**Figure 12.**) showed the band of NH benzimidazole at 3323 cm^{-1} . The band of the CH aromatic was located at 3032cm^{-1} and the CH aliphatic at 2927, 2852 cm^{-1}. The band of C=N and C=C was located at 1620, 1568 and 1460 cm^{-1} respectively. Furthermore, the ^{1}H NMR of compound 4b illustrated the structure and confirmed existence one tosyl group. Whereas the peak at 2.21 ppm with integral three refer for one tosyl group.

All rest protons appeared at their expected region with their expected integration (**Figure 13.**).

The route of synthesis of compounds 5a and 5b is demonstrated in scheme 1. These compounds were identified from their FTIR and ^{1}H NMR spectrum besides to the CHN analysis. The FTIR spectrum of compound 5b displayed the hydroxyl group and rising new band at 3141 cm^{-1} for NH of benzimidazole. As well the CH aliphatic were assigned at 2981, 2931 cm^{-1}. The strong band located at 1670 cm^{-1} attributed to C=N cm^{-1} and the C=C located at 1614 cm^{-1} as shown in **Figure 14**.

The ^{1}H NMR spectrum of this compound illustrated the structure by exhibiting all expected peak and their correct integrations. For instance, the ^{1}H NMR spectrum showed multiplet peak at 4.19-4.93 ppm for fourteen protons attributed to seven set of CH_2 as well as the integration of aromatic proton for the multiplet peak at 6.91-7.71 ppm equal seventeen protons.

These two multiplet peaks harmonized with the structure. Furthermore, the two NH of benzimidazole located at 10.39 ppm for wit integration equal to two protons as depicted in **Figure 15**. The results of CHN analysis also were in agreement with IR and ^{1}H NMR results.

The FTIR **Figure 16.** of the second compound 5b clarified the existence of the hydroxyl group through the band at 3244 cm^{-1} as well existence of the NH of benzimidazole. These bands refer to reaction one hydroxyl group with 2-chloromethyl benzimidazole. The band of aliphatic CH located at 2958 cm^{-1} and the C=N at 1647 cm^{-1} moreover the band of C=C were located at1603, 1520 cm^{-1}. The ^{1}H NMR spectrum of this compound exhibited one broad peak at 3.32 ppm integral equals one for alcoholic hydroxyl as well as multiplet peak at 5.22-5.64 ppm for five set of CH_2. The two singlet peaks appeared as multiplet at 5.83-5.95 ppm due to overlap for two set of CH_2, CH_2OH and CH_2 of benzyl group as demonstrated in **Figure 17**.

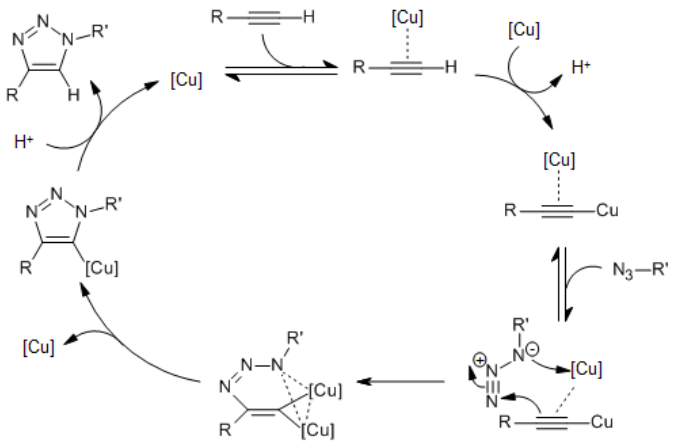
Finally, the multiplet peak at 6.50-7.30 ppm showed integration equals thirteen aromatic protons and the integration of NH benzimidazole ring was one proton assigned at 10.00 ppm. All these peaks illustrate the proposed structure and it in agreement with the results of CHN analysis. Reaction of Propargyl chloride with compound 2,2'-(((1H-benzo[d]imidazol-2-yl)methyl)azanediyl) diethanol in dimethyl sulfoxide DMSO and sodium hydroxide afford compound 6 as depicted in **Scheme 1**.

This compound was characterized by FTIR and ^{1}H NMR. The FTIR showed broad band at 3417 cm^{-1}which consists of the two groups of alcohol and the band of acetylenic hydrogen (≡CH), as well as the aromatic CH band and the aliphatic CH band were located at 2999,2817 cm^{-1} the band of C≡C was located at 2224 cm^{-1}. The band of C=N assigned at 1658 cm^{-1} and the C=C assigned at 1599, 1533 cm^{-1} as demonstrated in **Figure 18**.

The ^{1}HNMR spectrum confirm success of the reaction where it displays the propynyl group. The protons of this group were located as triplet at 3.35 ppm with J= 6.5 Hz for ≡CH and doublet at 4.285 ppm with J=7 Hz due to long range coupling[21]. The ^{1}H NMR spectrum showed two triplets at 3.34-3.38 ppm and 5.40-5.42 ppm attributed to $N\underline{CH_2}CH_2$ and CH_2OH respectively. The CH_2 attached position two of benzimidazole located as singlet peak at 5.83 ppm and the aromatic protons appeared as multiplet peak at 7.45-7.52 ppm. The integrations of all protons were compatible with number of protons in chemical structure of compound 6 as depicted in **Figure 19**.

The compound 7 was synthesized by click chemistry[22,23] utilizing sodium ascorbate and $CuSO_4.5H_2O$ as depicted in **Scheme 1**.

The proposed mechanism of this reaction was in agreement with the description of by Himo *et al* [24] and as shown in **scheme 2** below :



Scheme 2: Proposed mechanism of Synthesis of compound 7

This compound was characterized by FTIR and ^{1}H NMR spectra besides to the CHN analysis. All data confirm the success of the reaction. The FTIR showed disappearance of both band of propynyl group (≡CH and C≡C) as well rising new band for NH of 1,2,3-triazole ring at 3323 cm^{-1} while the OH appeared at 3496 cm^{-1}. The CH aliphatic located at 2970, 2935 cm^{-1} and the C=N located at 1633 and C=C at 1562,1527cm^{-1} as depicted in **Figure 20**.

The ^{1}H NMR spectrum of compound **7 Figure 21.** exhibited convert the doublet of CH_2 of propynyl to singlet at 5.12 ppm besides to the change in pattern of aromatic protons and exhibited integration equal five protons quit good evidence for the success of the cyclization reaction. As well as all rest peaks located at their expected area. Although the one hydroxyl group wasn't recognized in this spectrum, It could be either masked with the peak of water for DMSO-d_6, or undergoes deuterium exchange [25 , 26]

The percentage of nitrogen in CHN analysis (**Table 1.**) besides to the percentage of H and C were too persuasive with convert the propynyl group to corresponding 1,2,3-triazole ring. The compound **8** was synthesized by using the same method mentioned earlier. The difference in this reaction was by utilizing freshly synthesized of benzyl azide instead of sodium azide. The route of synthesizing this compound illustrated by **scheme 1**.

This compound successfully characterized by FTIR and ^{1}H NMR besides to the elemental analysis. The FTIR spectrum of is exhibited disappearing of the bands of propynyl, as well as the band of OH at 3191 cm^{-1}. The aliphatic CH bands were located at 2956, 2871 cm^{-1} and the C=N at 1610 cm^{-1} besides to C=C bands were located at 1543, 1477 cm^{-1} as demonstrated in **Figure 22**.

The 1H NMR spectrum displayed multiplet at 7.07 –7.25 ppm for six aromatic protons which was attributed to the benzimidazole and two protons of Meta benzyl. Furthermore, one triplet at 7.45 ppm with *J*=8.22 Hz for one proton of para benzyl besides two protons of *ortho* benzyl located at 7.81 ppm as doublet with *J*=7.97 Hz. Two singlet peaks for two CH_2 groups attached with benzimidazole ring were assigned at 5.65 and 5.74 ppm. Five protons for two CH_2 attached with hydroxyl and on hydroxyl located as multiplet peak at 4.12-4.23 ppm. The second hydroxyl did not recognize for the same reasons mention earlier. The triplet of $N(\underline{CH_2}CH_2OH)_2$ assigned at 3.35 with *J* =6.86 Hz as demonstrated in **Figure 23**.

Table 1. Elemental Analysis (Theoretical and Practical Values) for synthesized Compounds

Compounds No	CHN Analysis	C %	H %	N %	O %	S %	Formula
1	Theoretical Value	70.13	7.12	12.91	9.83	-	$C_{19}H_{23}N_3O_2$
	Practical Value	70.42	7.03	13.12	9.64	-	
2a	Theoretical Value	62.54	5.57	6.63	15.15	10.12	$C_{33}H_{35}N_3O_6S_2$
	Practical Value	62.33	5.90	6.82	15.24	10.27	
2b	Theoretical Value	65.11	6.10	8.76	13.34	5.68	$C_{26}H_{29}N_3O_4S$
	Practical Value	65.56	6.24	8.98	13.11	5.87	
3a	Theoretical Value	63.53	4.69	11.23	-	20.56	$C_{33}H_{29}N_5S_4$
	Practical Value	63.21	4. 98	11.54	-	21.03	
3b	Theoretical Value	63.03	5.13	8.91	7.63	15.30	$C_{33}H_{32}N_4O_3S_3$
	Practical Value	63.52	5.48	8.34	7.51	15.39	
4a	Theoretical Value	67.21	5.30	16.62	-	10.87	$C_{33}H_{31}N_7S_2$
	Practical Value	67.84	5.55	16.41	-	10.57	
4b	Theoretical Value	64.79	5.44	11.45	7.85	10.48	$C_{33}H_{33}N_5O_3S_2$
	Practical Value	65.11	5.82	11.76	7.29	11.13	
5a	Theoretical Value	71.77	6.02	16.74	5.46	-	$C_{35}H_{35}N_7O_2$
	Practical Value	71.95	6.47	16.85	5.97	-	
5b	Theoretical Value	71.19	6.42	15.37	7.02	-	$C_{27}H_{29}N_5O_2$
	Practical Value	71.04	6.36	15.76	7.39	-	
6	Theoretical Value	65.91	7.01	15.37	11.71	-	$C_{15}H_{19}N_3O_2$
	Practical Value	66.17	7.25	15.41	11.98	-	
7	Theoretical Value	55.95	6.13	26.56	10.11	-	$C_{15}H_{20}N_6O_2$
	Practical Value	65.12	6.74	26.89	10.26	-	
8	Theoretical Value	65.01	4.45	20.68	7.87	-	$C_{22}H_{26}N_6O_2$

3.2 Antimicrobial Susceptibility and Antifungal Activity

Antimicrobial susceptibility testing is one of the factors could be utilized in drug discovery, epidemiology and expectation of therapeutic value. The Antimicrobial Susceptibility was carried out according to procedure of McFarland et al. [27]. Two groups of Bacterial isolates were selected for biological activity. The first group is Gram Positive Bacteria (Vancomycin – Resistant *Staphylococcus aureus* (VRSA), while the second group is gram negative bacteria (*Enterotoxigenic Eschrichia coli (*ETEC), *Psedumonas, Shigella and Vibrio*). The antibacterial activity of the synthesized compounds was determined by minimum inhibitory concentration (MIC) method according to Flórez *et al*. (2006) [28]. The fungal conidia were harvested by scraping the sporulating colonies and suspended in sterile distilled water containing 1.0% Tween80 (v/v aqueous solution, as wetting agent) [29]. The conidia were suspended in sterile ddH_2O. The resulted conidial suspensions were filtered for hyphal debris, a sterilized piece of clothes is used, and centrifugation the suspension for 5 min at 3000 rpm then washed two times with 0.05% Tween80 or ddH_2O with intervening centrifugation. The Resulting conidia are resuspended in 1.0% Tween80 or ddH_2O and the numbers of conidia are determined by using a haemacytometer before they were diluted with sterile water containing 1.0% Tween80 or ddH_2O to reach the appropriate concentrations (1×10^6, 1×10^7 and 1×10^8 conidia/ml^{-1}). Conidial viability is examined before to the experiments by placing three drops of a 1 × 106 conidia spores/ml^{-1} suspension onto agar medium followed by incubation in 30°C for 24h, after which, their germination is examined under a microscope (10 X) by observing > 90% growth for all isolates. Currently, microbial diseases are causing problems world-wide, because of their resistance to antimicrobial agents. A variety of clinically important species of microorganisms has become an important health problem globally[30] .One way to fight this challenge is the suitable usage of the marketed antibiotics the other is the evolution of novel antibiotics (8). At this point, there will be forever a vital need to discover new antimicrobial agents to control the resistance and shorten the duration of therapy. Because of the benzimidazoles similarity to purine, antibacterial ability of benzimidazoles are explained by their contest with purines resulting in growth inhibition by inhibit synthesis of bacterial nucleic acids and proteins [31].

Worldwide biochemical and pharmacological studies have proved that its derivatives are active against various strains of microorganisms [32]. For example, change the amide group to the anilide on the 2-phenyl benzimidazole produce antimicrobial activity[33].Hydrazone has antimicrobial activity and its considerable pharmacophore group. Furacilin, furazolidone and ftivazide are antibacterial drugs known to contain this group [31].

Our study **Tables 2.** and **3.** showed that the derivatives (**1**) have a great activity against *Vibrio cholera*, the derivatives (**1** and **8**) have a great activity against *Shigella* while (**1**) derivative have a strongest activity against *Pseudomonas aeruginosa* and most of derivative (**2a**) have a strong activity against Vancomycin Resistant *Staphylococcus aureus.*

Table 2. Antibacterial activity of tested compounds against some Enterobacteriaceae and Vancomycin Resistant Staphylococcus aureus (VRSA)

Test organisms	Gram stain specify	Compound No.	Conc. (µL/ml)	Zone of inhibition(mm)	MIC (µL/ml)
Vibrio cholerae	**G-ve**	1	10^{-1}-10^{-4}	18	($1x10^{-3}$)
		2a	10^{-1}-10^{-4}	15	($1x10^{-2}$)
Shigella spp.	**G-ve**	1	10^{-1}-10^{-4}	17	($1x10^{-3}$)
		8	10^{-1}-10^{-4}	19	($1x10^{-2}$)
Pseudomonas aeruginosa	**G-ve**	1	10^{-1}-10^{-4}	20	($1x10^{-2}$)
S. aureus (VRSA)	**G+ve**	1	10^{-1}-10^{-4}	19	10 ($1x10^{-2}$)
		2a	10^{-1}-10^{-4}	20	($1x10^{-3}$)
E.coli (UTEC)	**G-ve**	1	10^{-1}-10^{-4}	No inhibition zone	------

It was found that the antibacterial of benzimidazole product expresses the excellent activity in gram positive bacteria with the inhibizone area around 18 mm and gives the moderate result in gram negative bacterial with the inhibizone area around 17 mm. As the result, when comparing with Gentamicin which is the antibiotic amino glycoside drug, the benzimidazole gives more excellent antibacterial than the drug.

Table 3. Activty Against *Enterobacteriaceae Vancomycin Resistant Staphylococcus aureus* (VRSA).

Test organisms	Substance	Zone of inhibition(mm)	MIC (µl/ml)
Vibrio cholerae	1	18	($1x10^{-3}$)
Shigella spp.	8	19	($1x10^{-2}$)
	1	20	($1x10^{-1}$)
Pseudomonas aeruginosa	1	19	($1x10^{-2}$)
S. aureus (VRSA)	2a	20	($1x10^{-3}$)

Infectious diseases caused by fungi are called mycoses, and they are often chronic in nature. Mycotic infections may be superficial and involve only the skin (cutaneous mycoses extending into the epidermis), while others may penetrate the skin, causing subcutaneous or systemic infections. The characteristics of fungi are so unique and diverse that they are classified in their own kingdom. Unlike bacteria, fungi are eukaryotic, with rigid cell walls composed largely of chitin rather than peptidoglycan (a characteristic component of most bacterial cell walls). In addition, the fungal cell membrane contains Ergosterol rather than the cholesterol found in mammalian membranes. These structural characteristics are useful in targeting chemotherapeutic agents against fungal infections.

In the present work, the results in **Table 4.** showed that a great activity against Aspergillus and Penicillium where the antibiotic Nystatin© have activity less than benzimidazole derivatives (3a, 5a and 8).

Studies has showed that benzimidazole derivatives are specific inhibitor for microtubule assembly that act by binding to their heterodimeric subunit, the tubulin molecule.

Table 4. Challenge study between Nystatin and some chemical substances against *Aspergillus* and *Penicillium* fungi

Test organism	Tested compound	Conc. (µl/ml)	Zone of inhibition(mm)	MIC (µl/ml)
Aspergillus	Nystatin®	10^{-1} - 10^{-5}	12	100 IU
	3a	10^{-1} - 10^{-5}	14	$1x10^{-3}$
Pencillium	Nystatin®	10^{-1} - 10^{-5}	11	100 IU
	3a	10^{-1} - 10^{-5}	15	$1x10^{-3}$
	5a	10^{-1} - 10^{-5}	13	$1x10^{-3}$
	8	10^{-1} - 10^{-5}	3	$1x10^{-3}$

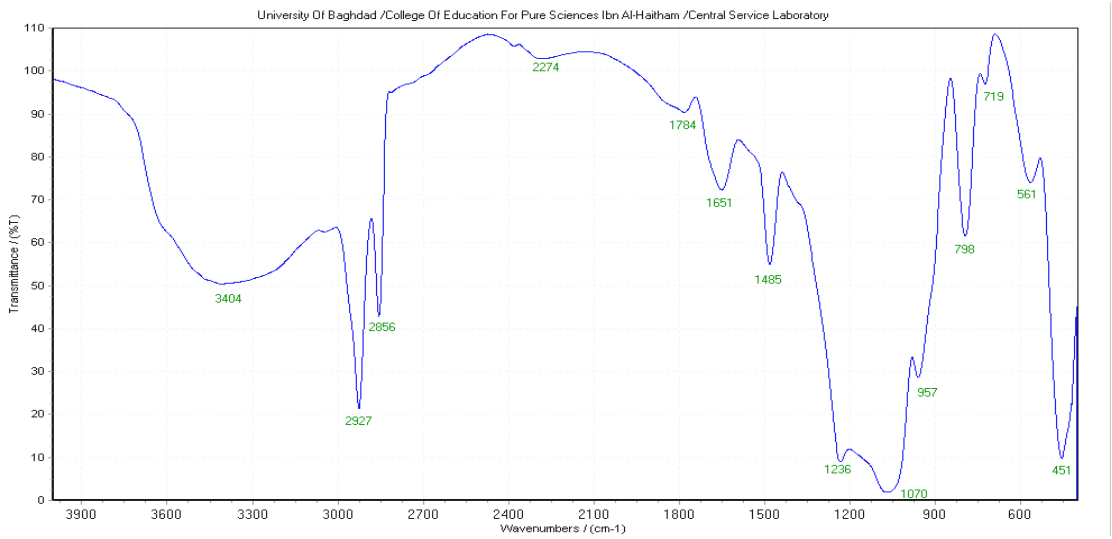


Figure 1. FTIR spectrum of compound 1

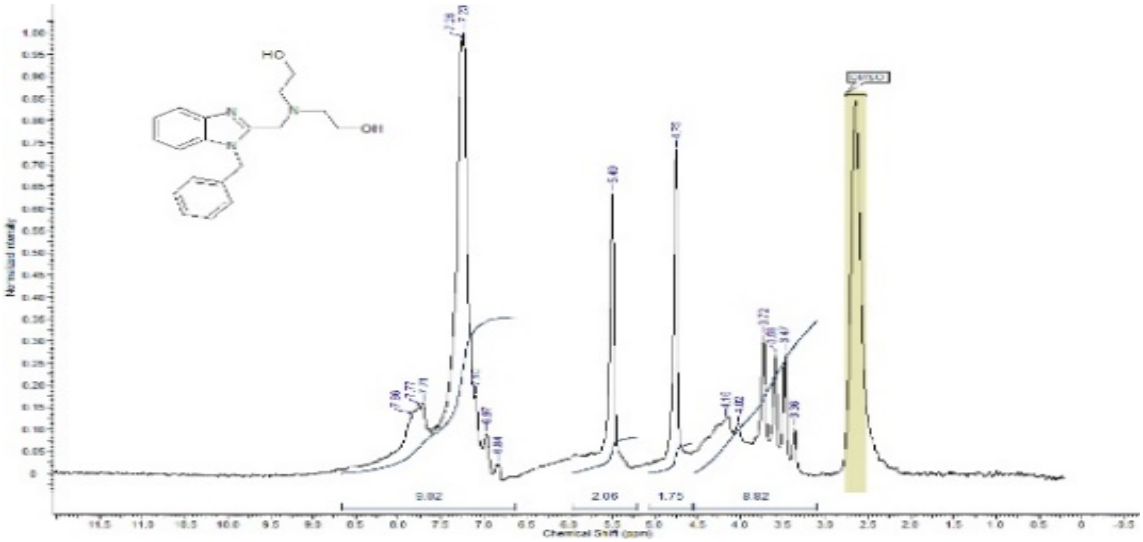


Figure 2. ^{1}H NMR spectrum of Compound 1.

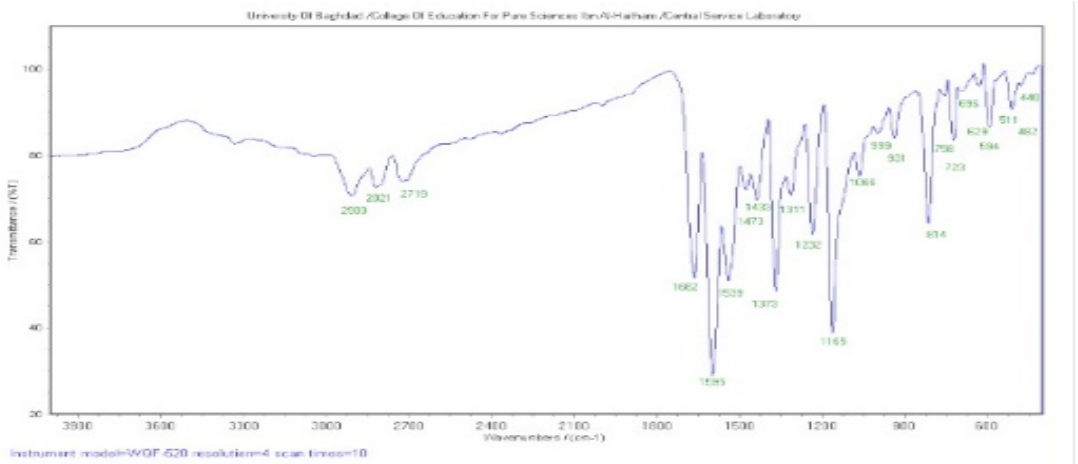


Figure Error! No text of specified style in document.**.** FTIR spectrum of compound 2a.

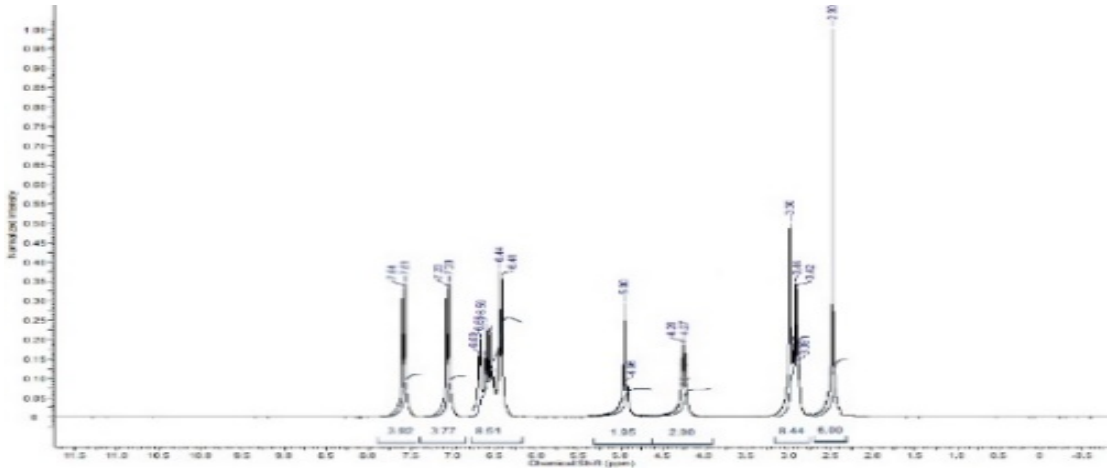


Figure 4. ^{1}H NMR spectrum of Compound 2a

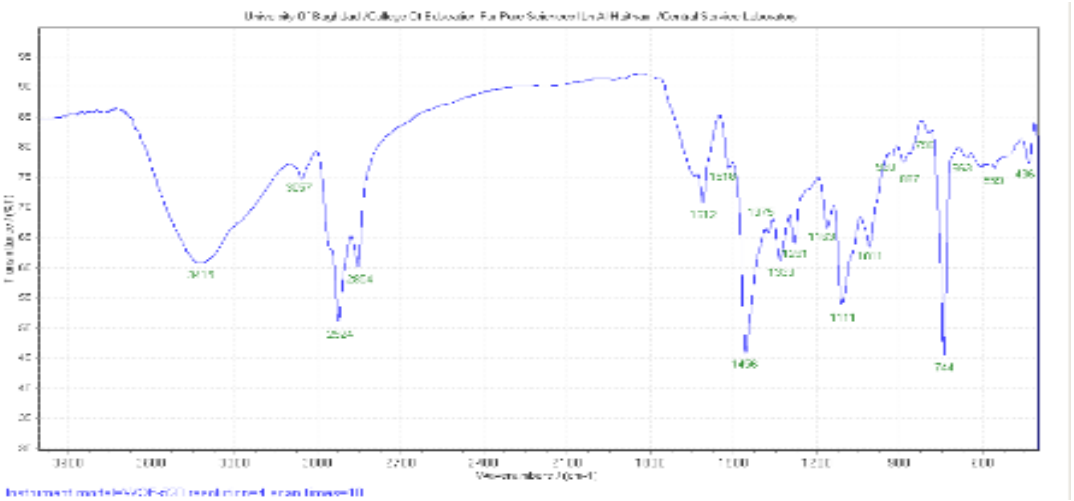


Figure 5. FTIR Spectrum of compound 2 b.

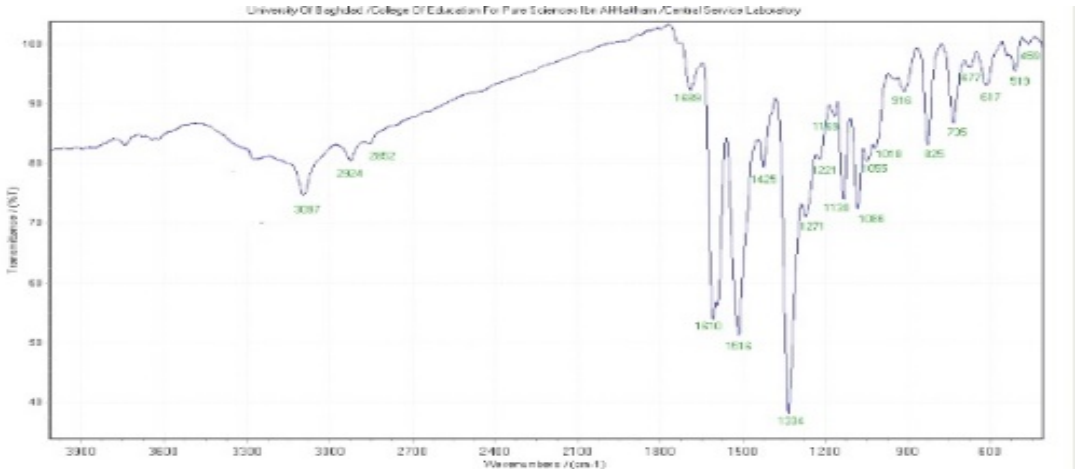


Figure 6. FTIR spectrum of compound 3a.

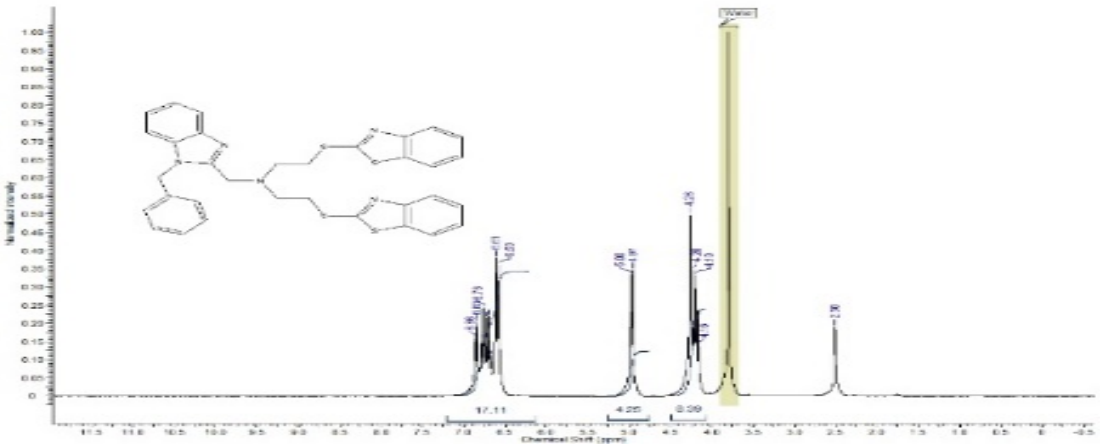


Figure 7. [1]H NMR spectrum of Compound 3a.

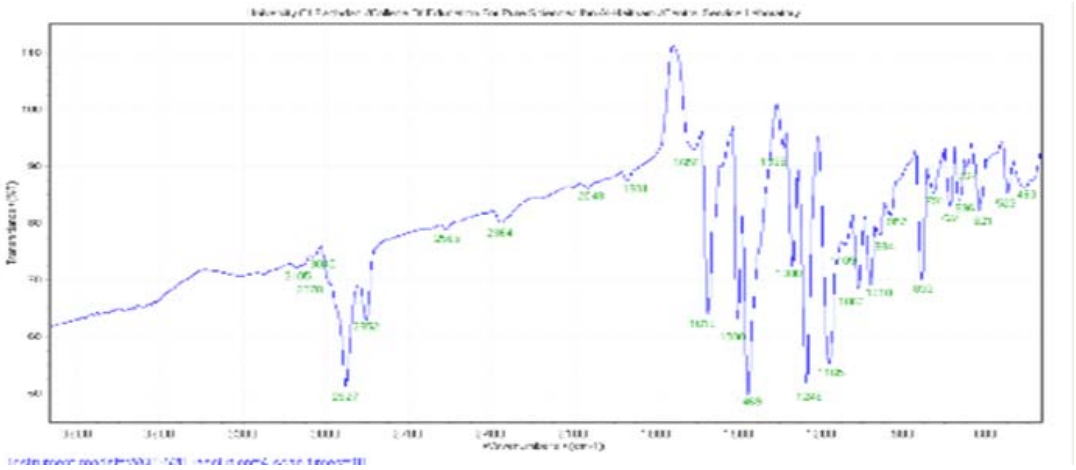


Figure 8. FTIR Spectrum of compound 3b.

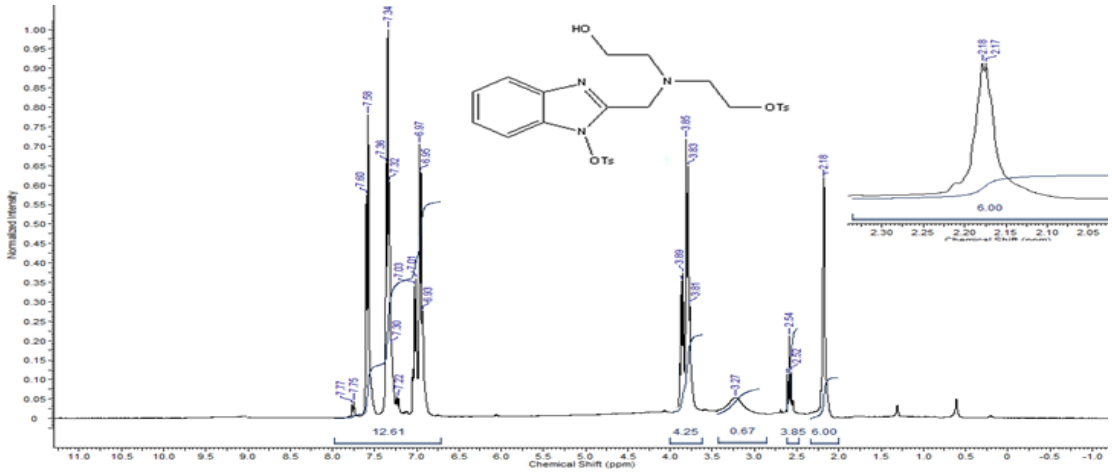


Figure 9. 1H NMR spectrum of compound 3b.

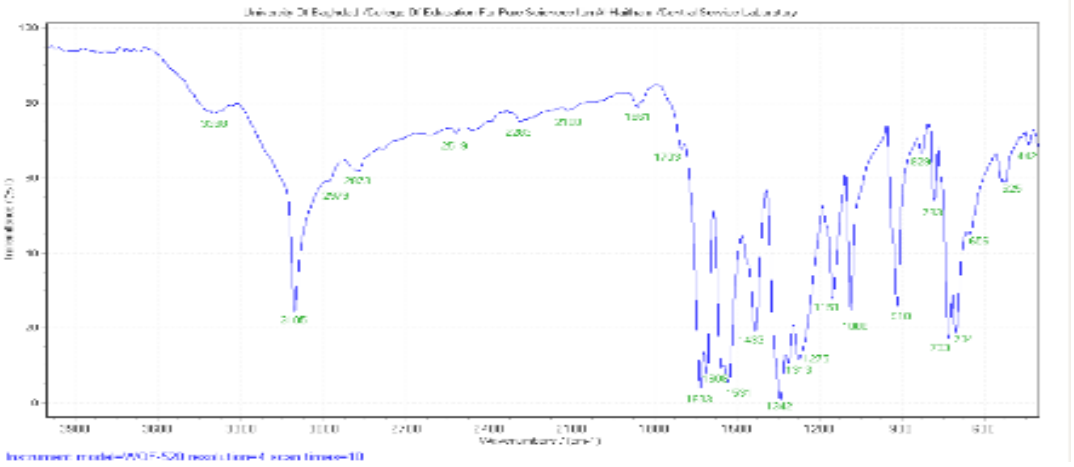


Figure 10. FTIR spectrum of compound 4a.

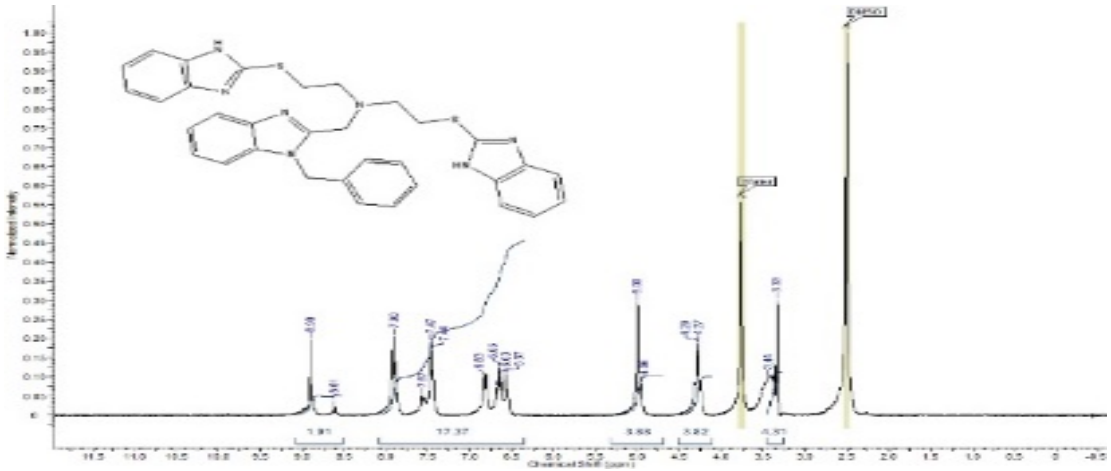


Figure 11. [1]H NMR spectrum of Compound 4a.

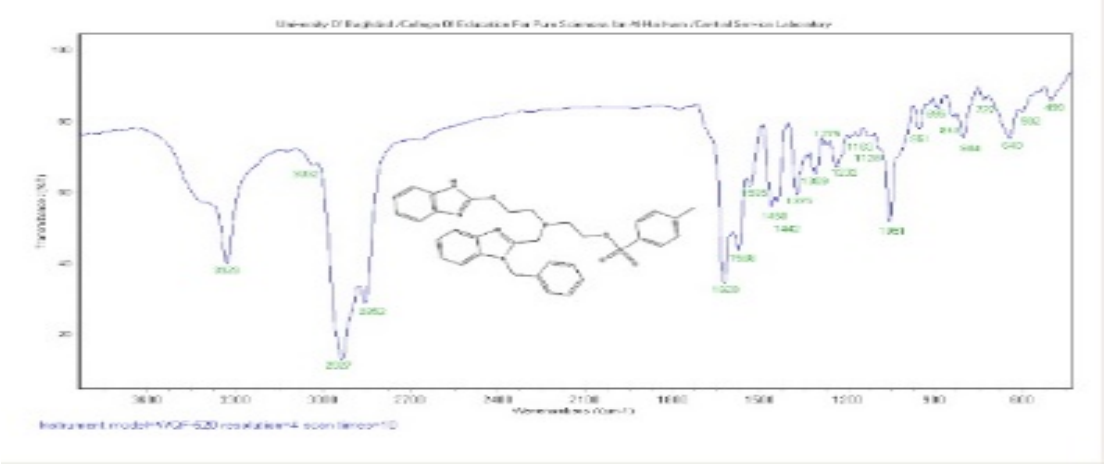


Figure 12. FTIR Spectrum of compound 4 b

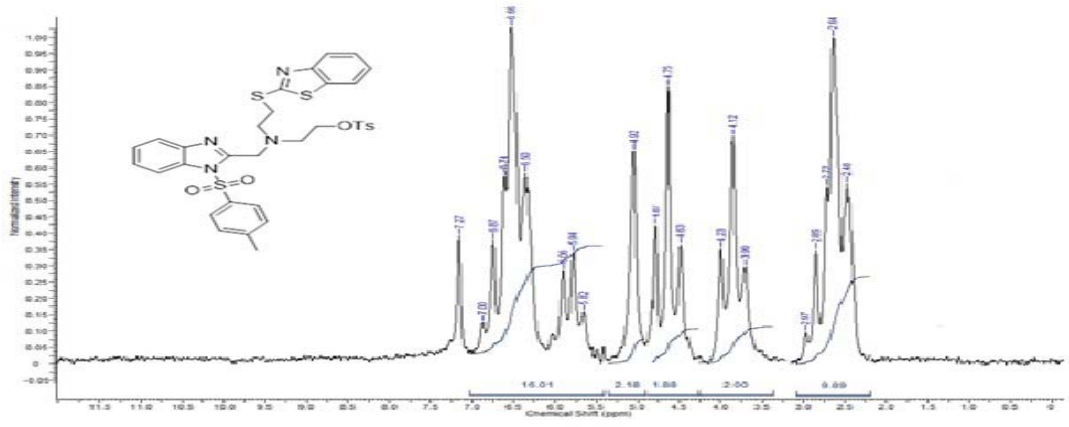


Figure 13. [1]H NMR spectrum of compound 4b

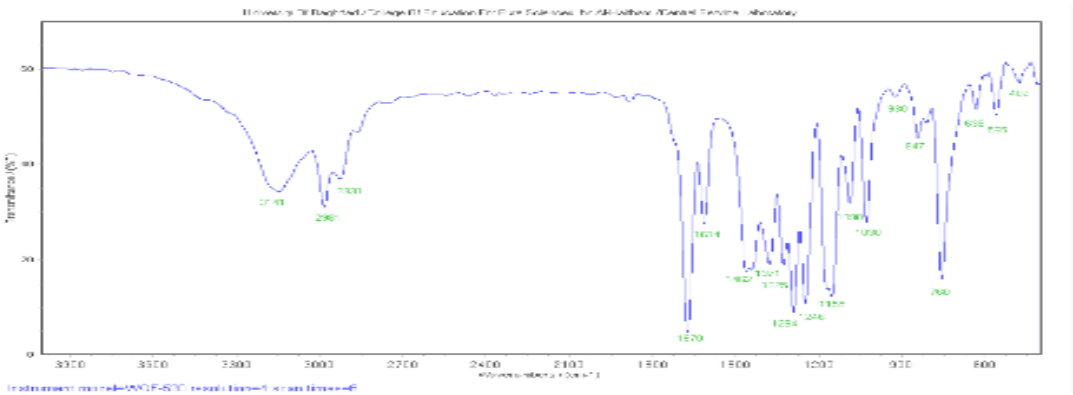


Figure 14. FTIR spectrum of compound 5a.

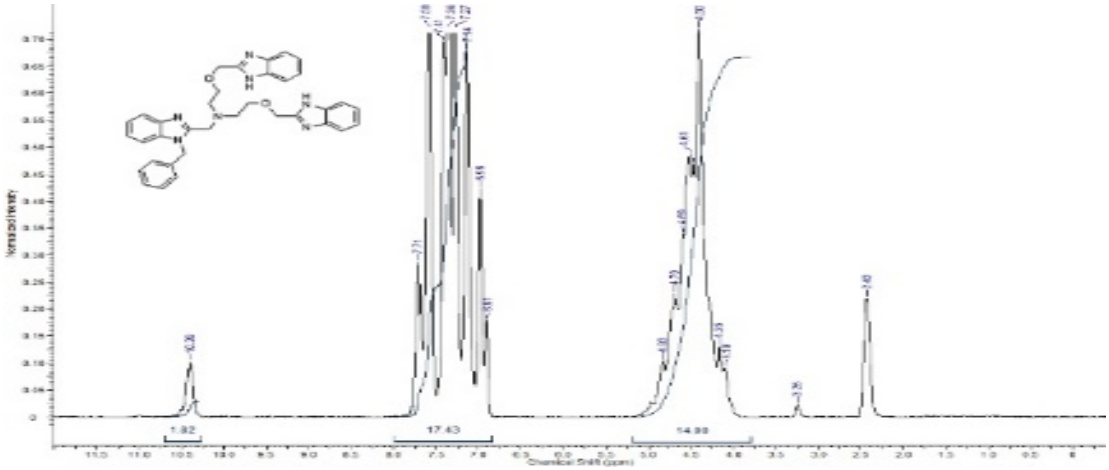


Figure 15. ^{1}H NMR spectrum of Compound 5a

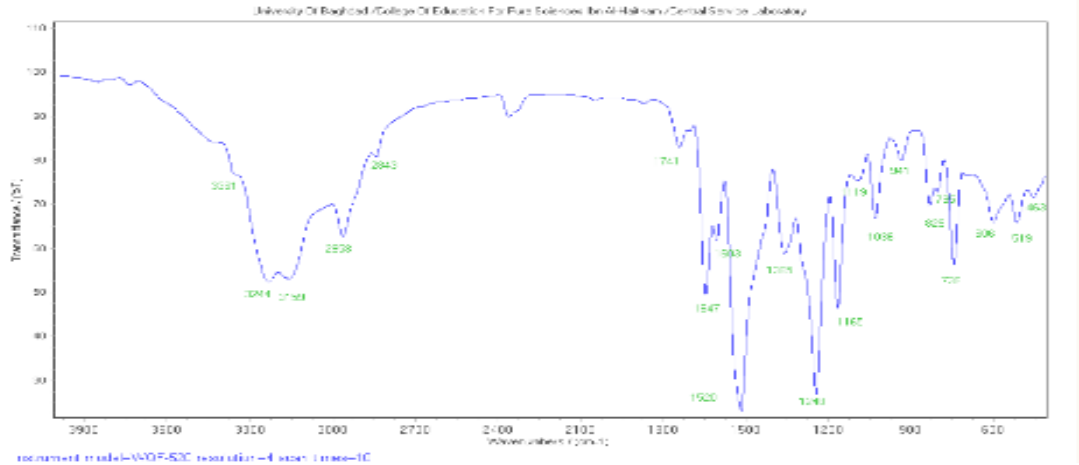


Figure 16. FTIR spectrum of compound 5b.

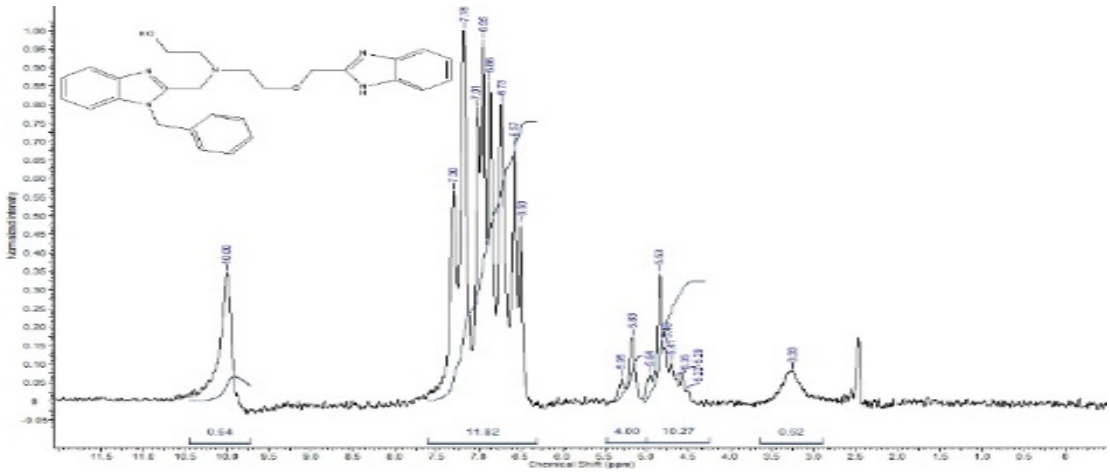


Figure Error! No text of specified style in document.**17.** ^{1}H NMR spectrum of Compound 5b.

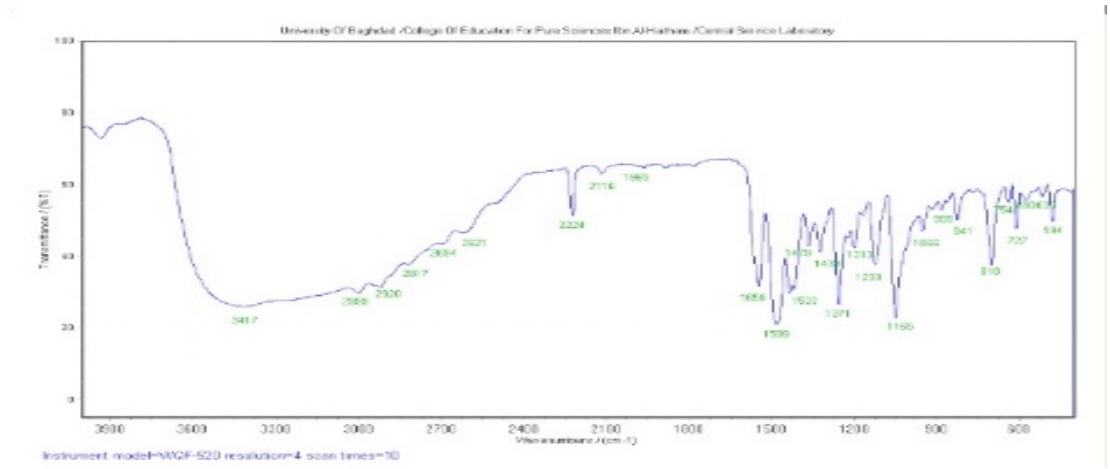


Figure 18. FTIR spectrum of compound 6.

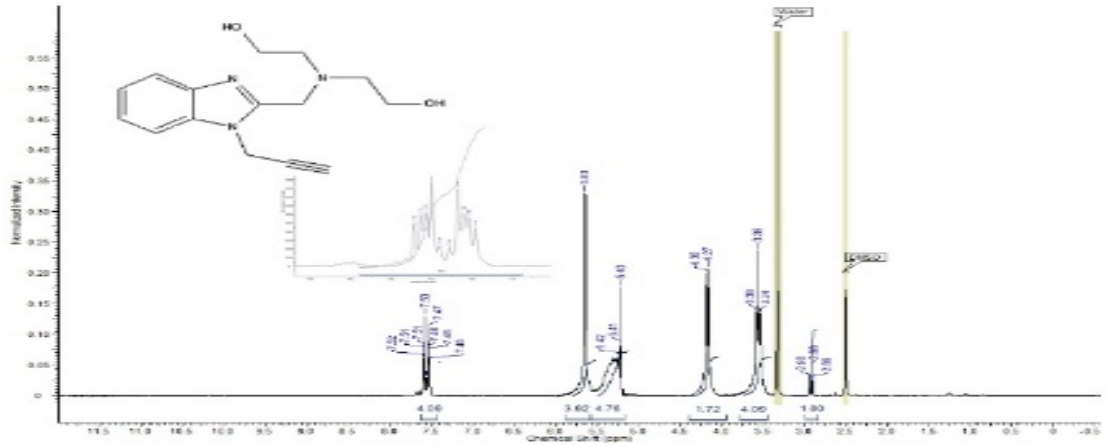


Figure 19. ^{1}H NMR spectrum of Compound 6.

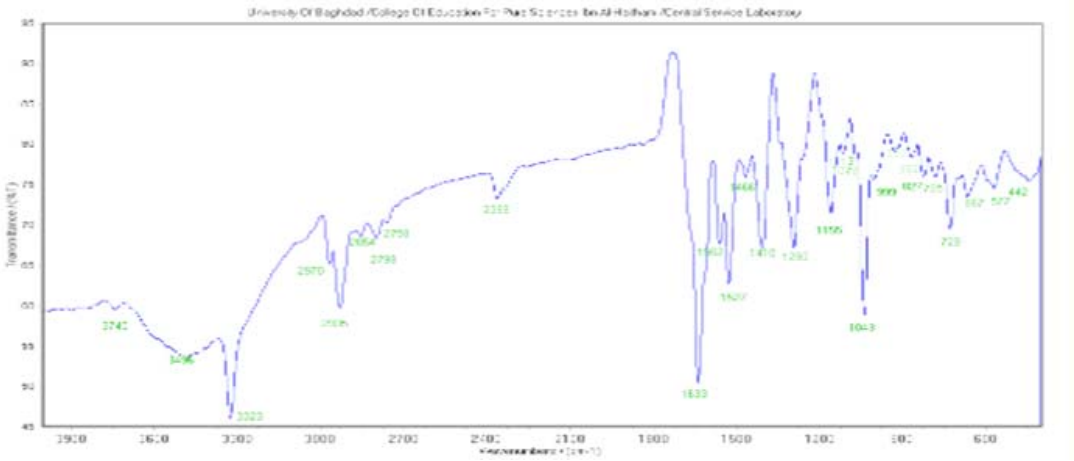


Figure 20. FTIR spectrum of compound 7.

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