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The Effect of 1,3,4-Oxadiazol and 1,2,4-Triazole Compounds on Urease and Pepsin Enzymes

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Abstract

The helicobacter pylori affected urease and pepsin enzymes in the stomach, so the object of this study is to screen the effect of bis 1,3,4-oxadiazole and bis 1,2,4, triazole derivatives compounds on h-pylori urease enzyme in vitro. The results show that urease was inhibited by all produced compounds when the concentration increased. The most effective urease inhibitor is determined to be the compounds (A3e, A3c, A3d and B3c). The clinical study involved studying the pepsin enzyme activity level in H-pylori patients and control, the results found that the activity of pepsin elevated in h-pylori patients as compared with control. The prepared compounds were screened on pepsin enzyme in vitro and showed that all the prepared compounds decreased the pepsin activity as the concentration increased. The nitrogen heterocyclic compounds. This study concludes that nitrogen-containing heterocyclic compounds have an important future in the treatment of Helicobacter pylori disease.

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1. Introduction

Helicobacter pylori are a gram-negative spiral-shaped bacterium of the genus Epsilonproteobacteria that is found on the luminal surface of the gastric epithelium. It was isolated by warren and Marshall in 1983 [1]. If untreated, the illness, which is typically detected in the initial few years of life, can continue forever [2]. Its prevalence varies widely over the world and rises with age and declines with lower socioeconomic level during childhood [3]. H. pylori infection causes chronic gastritis can result in peptic ulcers, gastric adenocarcinomas, or gastric lymphomas of the mucosa-associated lymphoid tissue[4, 5]. As a result, H. pylori is one of the most significant human pathogens and blame for at least 500,000 fatalities annually [6, 7]. *In vivo*, the bacteria in the gastric mucus use a pH gradient to determine their spatial orientation [8]. Low gastric luminal pH elevated activity of pepsins in vivo severely restrict H. pylori motility [9,10]. The helical structure of this bacterium enables it to pass through the layers of mucus that coat the stomach. Helicobacter pylori has unusually strong urease activity helps it survive in the stomach's acidic environment; The urea in gastric juice is converted by urease to alkaline ammonia and carbon dioxide [11]. Ammonia forms a protective sheath around the bacteria, shielding it from stomach acid. As a result, large amounts of urease enzyme are required for H. pylori survival and pathogenicity [12].Urease is a (EC 3.5.1.5) acts as a nickel-containing enzyme which catalyzes urea hydrolysis to create ammonia and carbon dioxide, which is the final stage in the metabolism of nitrogen in living organisms [13,14]. Helicobacter pylori's urease activity is important in the pathogenesis of gastric and peptic ulcers [15]. In the other word, Ammonia

produced by urea hydrolysis may also have severe cytotoxic effects on gastric epithelial cells. The enzyme also shows a strong immune response during acute infection, implying that the immune system has easy access to this abundant antigen [15]. Because the bacterium the urease of H. pylori is one of the most critical enzymes linked with bacterial activity, as a result, urease inhibition could kill bacteria [16]. Oxadiazole is a five-membered aromatic heterocyclic molecule with the chemical formula $C_2H_2N_2O$. Oxadiazole has four distinct isomers [17]. 1,3,4-oxadiazole and 1,2,4-triazole compounds have grown to be an essential building motif for the development of novel medications due to their biological functions such as; HIV integrase inhibitory,[18] tyrosinase inhibitory [19], anti-inflammatory [20,21], anticancer [22], antimalarial [23], antibacterial [24], anticonvulsant [25], hypoglycaemic [26], antitubercular [27], cytotoxic [28], anti-urease [29], anti-allergic agent [30] and antifungal [31]. In this study, we synthesized bis-1,3,4-oxadiazol and bis-1,2,4-triazole and investigated its biological activity as a helicobacter urease enzyme inhibitor, also, the recent study showed that h-pylori effect on pepsin enzyme activity, so this compounds was tested in vitro on pepsin enzyme activity and compared the results with the clinical study that involved collecting blood samples from both genders of Iraqi H-pylori patients and comparing the efficacy of pepsin enzyme in them to those from healthy people.

2. Materials and Methods

Fourier Transform Infrared Spectrophotometer (FTIR), Bruker Spectrophotometer, Japan. Nuclear magnetic resonance was used to record ^{1}H-NMR spectra. Bruker Ultrasheild 400 MHz Spectrophotometer with tetramethylsilane internal standard and DMSO was used as solvent. The melting point was obtained by (FALC melting point equipment). Shimadzu UV-Vis. 1600A Ultraviolet-Visible Spectrophotometer was used to record spectra in the (200-1,100) nm range. The MTT assay was conducted at the Natural Product Research and Drug Discovery Center. Bio-red company's ELISA microplate reader model 680 with a wave length of 590 nm.

2.1. 1,3,4-oxadiazole derivatives Synthesis

2.1.1. Synthesis of (A1) compound:

Terephthalaldehyde (0.01 mol) has been dissolved in 25 ml of ethanol. Next, (0.02 mol) of semicarbazide-hydrochloride and 1 g of fused sodium acetate were added. The solution was refluxed while it is being constantly stirred. After the reaction had finished, allowed to cool at room temperature before it was added to the water. Glacial acetic acid was used to filter and recrystallize the separated solid. (yield: 72%, color: Pale yellow, M.w.: 248.2, m.p. 233, molecular formula: $C_{10}H_{12}N_6O_2$)

2.1.2. Synthesis of (A2) compound:

compound A1 (0.01 mol), anhydrous sodium acetate (3 g), and glacial acetic acid (18 ml) were mixed before Br_2 (0.55 ml) was *added*. Water was used to wash the mixture, and the precipitate was filtered and dried. (yield: 90%, color: orange, M.w: 244.2, m.p. 134, molecular formula: $C_{28}H_{22}N_8O_6$).

2.1.3. Synthesis of (A3) derivatives:

Thionyl chloride (5ml) and 2 drops DMF were added to just one gram of any of the subsequent acids: hippuric acid, 4-nitrobenzoic acid, 4-hydroxy benzoic acid, 4-chloro benzoic acid, or 4-amino hippuric acid. The addition of benzoyl chloride, dissolve of compound A2 (0.01 mol) in 10ml dry pyridine. The mixture was then boiled for an hour in a water bath before it was allowed to stand the next day. After being washed with cold water, it was treated using cold 1:1 hydrochloric acid solution, and the precipitate that formed was filtered, dried out, and recrystallized from ethanol. (A3a; yield: 86%, color: Brown-yellow,M.w: 566.5, m.p. 134, molecular formula: $C_{28}H_{22}N_8O_6$). (A3b; yield: 83%, color: Dark brown, M.w: 542.4, m.p. 133, molecular formula: $C_{24}H_{14}N_8O_8$). (A3c; yield: 92%, color: Pale brown, M.w: 454.4, m.p. 145, molecular formula: $C_{24}H_{16}N_6O_6$). (A3d; yield: 98%, color: brown, M.w: 521.3, m.p. 148, molecular formula: $C_{24}H_{14}Cl_2N_6O_4$). (A3e; yield: 88%, color: Pale yellow, M.w: 596.6, m.p. 143, molecular formula: $C_{28}H_{24}N_{10}O_6$).

2.1.4. Synthesis 1,2,4-triazole derivatives compounds

i. Synthesis of (B1) compound:

Dimethyl terephthalate (0.01mol) was dissolved in 20ml absolute ethanol, 5.8ml of the mixture has

been combined with hydrazine hydrate and refluxed for four hours. Let the mixture cooled, recrystallize with ethanol. (B1; yield: 90%, color: white, M.w: 194.2, m.p. 262dec., molecular formula: $C_8H_{10}N_4O_2$).

ii. Synthesis of (B2) compound:

The amount of KOH (0.03 mol) has been dissolved in 25 ml 100% ethanol, and chemical B1 (0.01 mol) was added. After cooling in an ice bath and stirring, 6 ml of carbon disulfide CS_2 (0.05 mol) was added. Salt was produced after 18 hours of stirring. After removing CS_2, 10ml of the hydrazine hydrate had been added to the mixture. Until the formation of hydrogen sulfide (H_2S). The solution was diluted with cold water and then acidified with strong HCl. The precipitate had been filtered and dried. (B2; yield: 79%, color: white, M.w: 306.4, m.p. 285dec c., molecular formula $C_{10}H_{10}N_8S_2$).

iii. Synthesis of (B3) derivatives:

To 5ml of thionyl chloride and two drops of DMF, 1g of each amino acid (Glycine, L-Valine, L-Arginine, and L-Cystine) was added. Refluxed for a period of time until undergoing cooling at a room temperature before it was used in the next stage. Each of the acids used in the previous stage was mixed with 0.01 mol of compound B2 diluted in ten milliliters of dry pyridine. The mixture heated in a water bath for one hour before being let to stand overnight. Then, cool 1:1 hydrochloric acid was applied. After being washed in cold water, the precipitate purified and recrystallized from ethanol. (B3a; yield: 91%, color: Pale yellow, M.w: 384.4, m.p. 180dec. c., molecular formula $C_{14}H_{12}N_{10}S_2$). (B3b; yield: 86%, color: Reddish-brown, M.w: 468.6, m.p. 178., molecular formula $C_{20}H_{24}N_{10}S_2$). (B3c; yield: 82%, color: Dark brown, M.w: 482.6, m.p. 190dec.., molecular formula $C_{18}H_{20}N_{13}S_2$). (B3d; yield: 86%, color: brown, M.w: 714.9, m.p. 185 dec.., molecular formula $C_{22}H_{26}N_{12}O_4S_6$).

2.2. Helicobacter Pylori Urease Activity Determination

Reagent A is an indophenol solution and Reagent B is a hypochlorite solution [32,33]. The solution for stock (Urea) was made by dissolving 0.3g in 10ml of distilled water and using it immediately. Phosphate solution [34] is buffer solution utilized. The synthesized compounds were dissolved in DMSO at various concentrations (50, 100, 150, and 200) M. The degree of inhibition rate was calculated as follows:

$$Inhibition\ rate\ at\ 625\ nm = \frac{Control\ Absorbance\ - Test\ Absorbance}{Control\ Absorbance\ at\ 625\ nm} \times 100\% \quad \dots(1)$$

Sample solution consisted from buffer solution 205 µL, inhibitor 10 µL, urea 25 µL, enzyme 10 µL. reagent A indophenol solution 5ml and reagent B hypochlorite solution 5ml. Enzyme solution prepare as the sample solution but without added inhibitor. In blank solution add the same additive in sample without added enzyme. All of them must shake and incubated at 37C° in water bath for (20 minute). The absorbance read at 625 nm.

2.3. Determination of Pepsin Activity

The buffer solution used is (Glycine- HCl) [35] and the substrate is Casein 1%.Trichloroacetic acid (TCA) 5% is used as stop solution. Pepsin enzyme 1% prepared.

2.3.1. Measurement of Pepsin enzyme activity with inhibitors

The inhibitors were used in different concentrations (50, 100, 150, and 200) µM and dissolved in DMSO. The equation was utilized with constant substrate concentration to determine the impact of substances on pepsin enzyme activity

$$Enzyme\ Activity\ \left(\frac{U}{ml}\right) = \frac{\text{A}}{(0.1)(10)(0.01)} \quad \dots(1)$$

where

A: Absorbance at 280 nm.

0.1: Volume of enzyme solution in ml

10: Time of reaction.

0.01: From the definition of enzyme activity.

Table 1 lists the concentrations in the present work. Each solution must be shaken and filtered before taking the readings.

Table 1. Concentrations of the solutions.

	Blank µL	Enzyme µL	Sample µL
Enzyme	-	100	100
Buffer with casein	950	950	950
Compound	-	-	950
TCA	3ml	3ml	3ml

2.4. Specimen Collection and Preparation

Total of (100) Iraqi Helicobacter *Pylori* patients including both sexes were selected. Their age was between (18 and 60) years. The samples were obtained from Medical City, in Baghdad, Iraq. They were diagnosed by Helicobacter *Pylori* blood test, stool test and urea breath test. Patients who suffered from any chronic disease e.g., diabetes mellitus, hypertension, ischemic heart disease, thyroid diseases, thalassemia, inflammatory disease, and other malignant diseases were excluded from the study. Also, they had no history of alcohol.

3. Results and Discussion

All the reagents and starting materials utilized in this work were reagent grade and were employed without additional purification. Scheme 1 and scheme 2 show the synthesis steps of 1,3,4-oxadiazole and 1,2,4-triazole derivatives compounds respectively. The chemical structure proposed for the synthesized compound are show in Figure19 and Figure 20.

3.1. Infrared spectroscopy (FTIR)

3.1.1. Terephthaylsemicarbazone (A1):

Sharp absorption band is observed at 3457, 3282 and 3161 cm^{-1} for N-H and N-H of NH_3 stretching. The band at 1681 cm^{-1} assigned to carbonyl of amidic I, the bands at 3066, 1639, 1600, 1514 and 863 cm^{-1} for C-H aromatic stretching, H-C=N, C=C, N-H bending of amide II and *para*-substituted on benzene aromatic ring, respectively.

3.1.2. 5,5`-(1,4phenylene)bis(1,3,4-oxadiazol-2-amine) (A2):

Absorption bands are observed at: 3328 and 3227, 1197, 3079, 1597, 1390, 1503, 839 and 882 cm^{-1} . These bands indicate to presence of N-H stretching, C–O–C of oxadiazole ring, C – H aromatic stretching , C=C, C–N, N–H bending bond, C–H bending of benzene ring and *para*-substituted on the benzene ring, respectively.

3.1.3. N,N`-(1,4-phenylenebis(1,3,4-oxadiazole-5,2-diyl)diamide (A3a-Ae):

bands at (3388- 3366) cm^{-1}, (1673-1676) cm^{-1}, (3068- 3052), (1597-1586), (1588-1501) and (844-839) cm^{-1} . These bands may be for N – H stretching, carbonyl of amide I, C-H aromatic stretching, C=C aromatic, N–H bending and *para*-substituted on benzene ring, respectively. N-O bond is referenced by the band at (1519) cm^{-1} in compound A3b. The bond O-H in compound A3c may be indicated for the band at (3363) cm^{-1}. The C-Cl bond in compound A3d appears at (844) cm^{-1} . The FTIR spectrum of A3a-A3e compounds show in Figures (1,2,3,4 and 5).

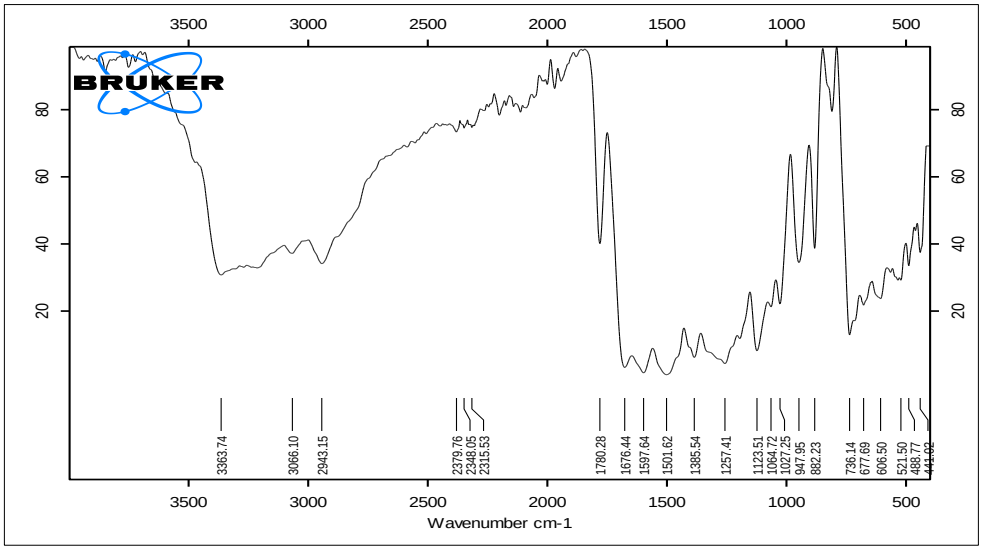


Figure1. FTIR spectrum of A3a compound

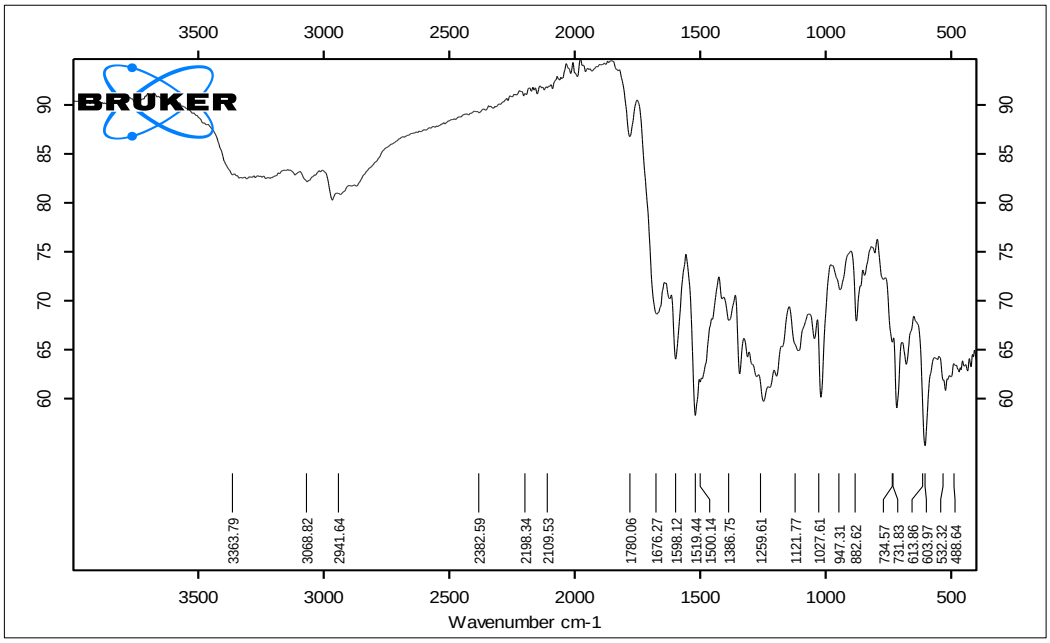


Figure 2. FTIR spectrum of A3b compound

3.1.4. Terephthlic dihydrazide (B1):
The FTIR spectrum of compound (B1) illustrate the bands at 3418, 3339, 3300, 3225, 3029, 1594, 1544 and 835 which could be assigned to N-H bond, ν C – H (aromatic), ν C=O (amide),ν C=C and *para*-disubstituted benzene, respectively.

3.1.5. 4,4`-(1,4-phenylenebis(5-mercapto-4H-1,2,4-triazole-3,4-diyl)diamine (B2):
The absorption bands at: (3363.9 and 3356) cm^{-1} , 3068.8, 1676, 1500, 1027 and 882.5 may be indicate to present of ν N- H 1^{o}, ν C-H.

aromatic bond, ν HC=N, ν C=C, ν C=S and bending *para*- disubstituted on benzene ring, respectively.

3.1.6. (1,4-phenylenebis([1,2,4]triazolo[3,4-b]1,3,4-thiadiazole-3,6-diyl)dimethanamine (B3a-B3d):

(3410 and 3358 cm^{-1}), 3042, 1689, 1581 and 844 cm^{-1} may be indicate to present for N – H bond stretching of NH_2, ν C-H of benzene ring, ν (C=N), ν (C = C) and *para*-disubstituted in benzene ring, respectively. Compound B3b, contains stretching bands at 3387 and 3329 cm^{-1} for N-H of NH_2 stretching. The bands at 3065.5, 1682, 1545.5 and 844 cm^{-1} , could be due to ν (C – H) aromatic, ν (C=N), ν (C = C) and bending of *para*-disubstituted on benzene ring respectively. Compound B3c bands at 3389 cm^{-1} sharp for N-H bond stretching and two bands at 3303.8 and 3273.9 cm^{-1} may be due to NH_2 stretching. bands at 3056, 2960.5, 2838, 1607.7, 1592, and 848 cm^{-1} for ν (C-H) aromatic, ν (C-H) aliphatic, (C=N), ν(C = C) and bending of *para*-disubstituted out of plain benzene ring substituted, respectively.

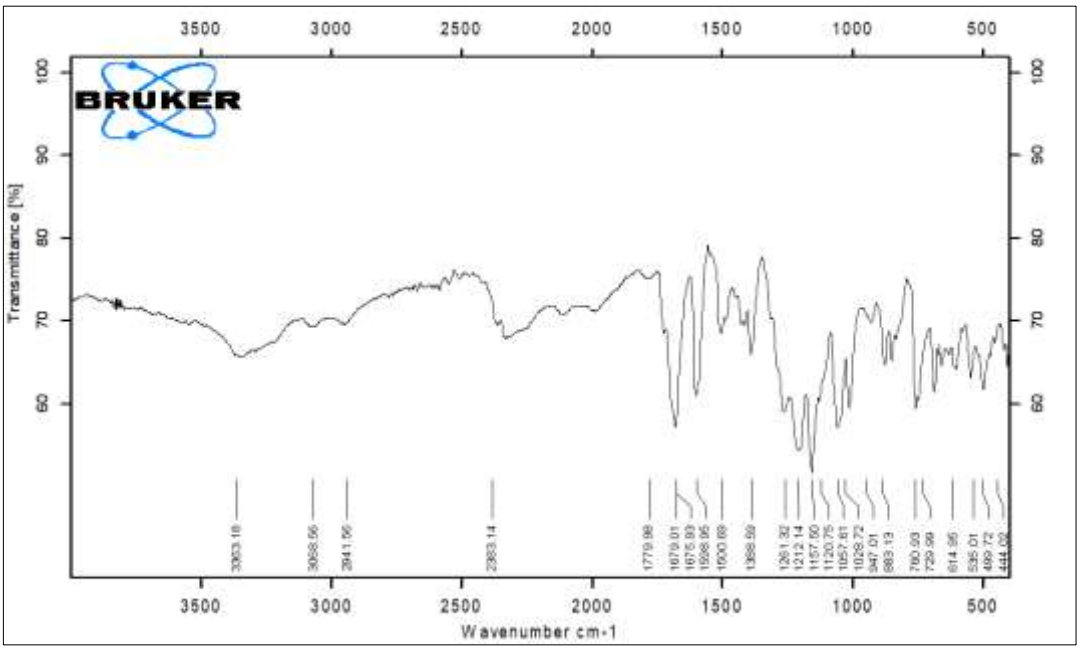


Figure 3. FTIR spectrum of A3c compound

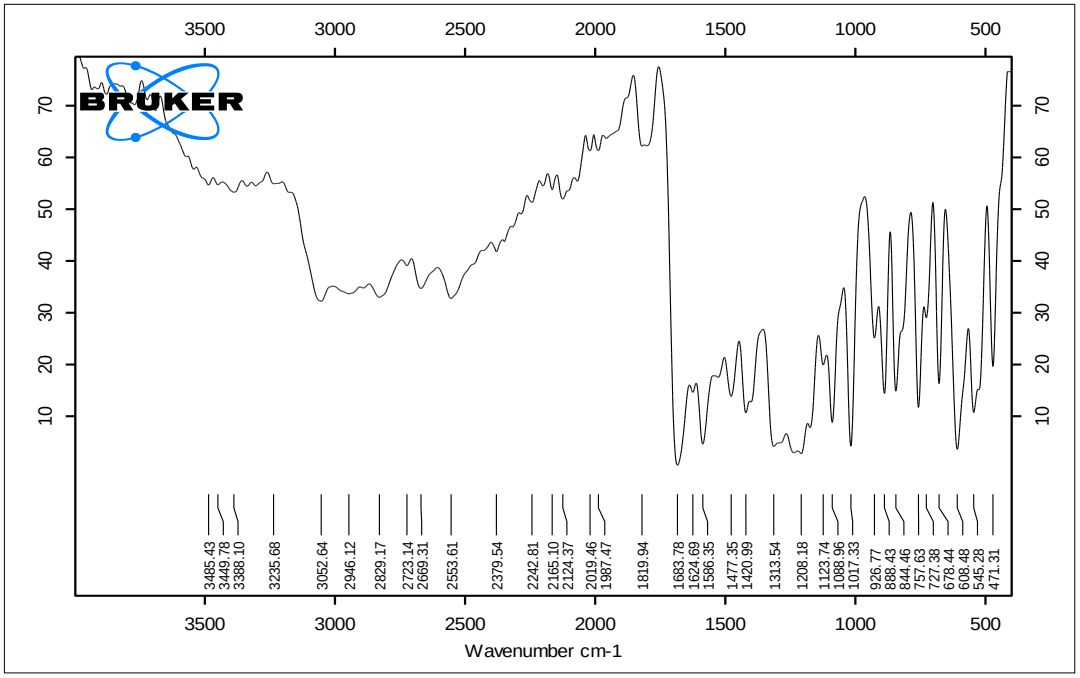


Figure 4. FTIR spectrum of A3d compound

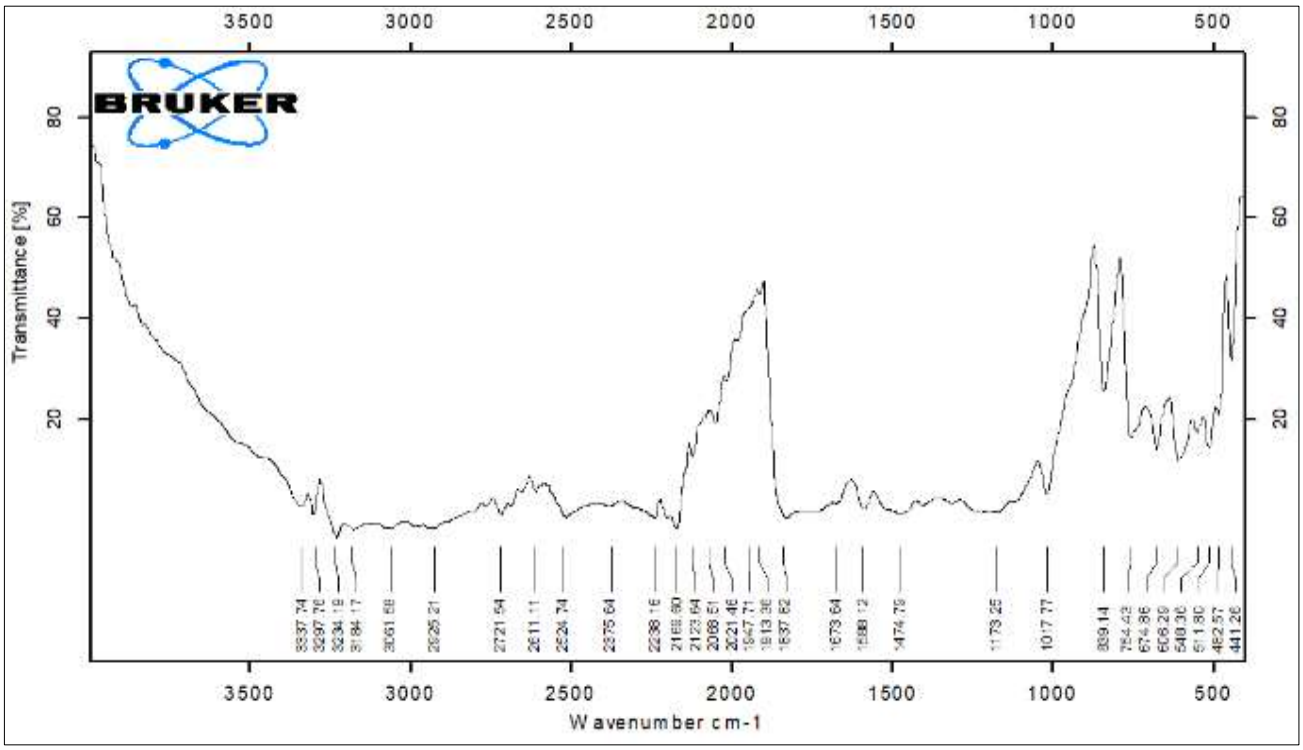


Figure 5. FTIR spectrum of A3e compound

Compound B3d shows sharp band at 3420.6 cm^{-1} this may be due to present of (O-H) bond stretching. The bands at 3223.7 and 3191.4 cm^{-1} for N – H bond could be attributed to stretching of NH_2. The spectrum also show bands at 3057, 1992, 1620 and 850 cm^{-1} due to ν (C-H) aromatic, ν (C=O) of carboxyl group, ν (C = C) aromatic and out of plane bending of *para*- disubstituted benzene ring, respectively. Figures (6,7,8 and 9) show the FTIR spectrum of B3a-B3b compounds.

3.2. Nuclear magnetic resonance spectroscopy (^{1}HNMR)

1HNMR (DMSO-d6)spectrum of Compound A3a showed (NH-CO connect with oxadiazole ring at 10.51 ppm, two protons H-N-CO singlet at (8.78) ppm, four CH_2 protons at 3.99 ppm singlet, twelve aromatic protons at 7.2-7.3 ppm. Compound A3b showed signals : NH-CO; singlet at δ 11.6 ppm, twelve protons of aromatic ring at δ 7.39-8.17 ppm. Compound A3c showed: two NH-CO protons singlet at δ 11.67 ppm, phenolic hydroxide at δ 8.77 ppm singlet of two protons, aromatic protons at δ 6.4-7.4 ppm.

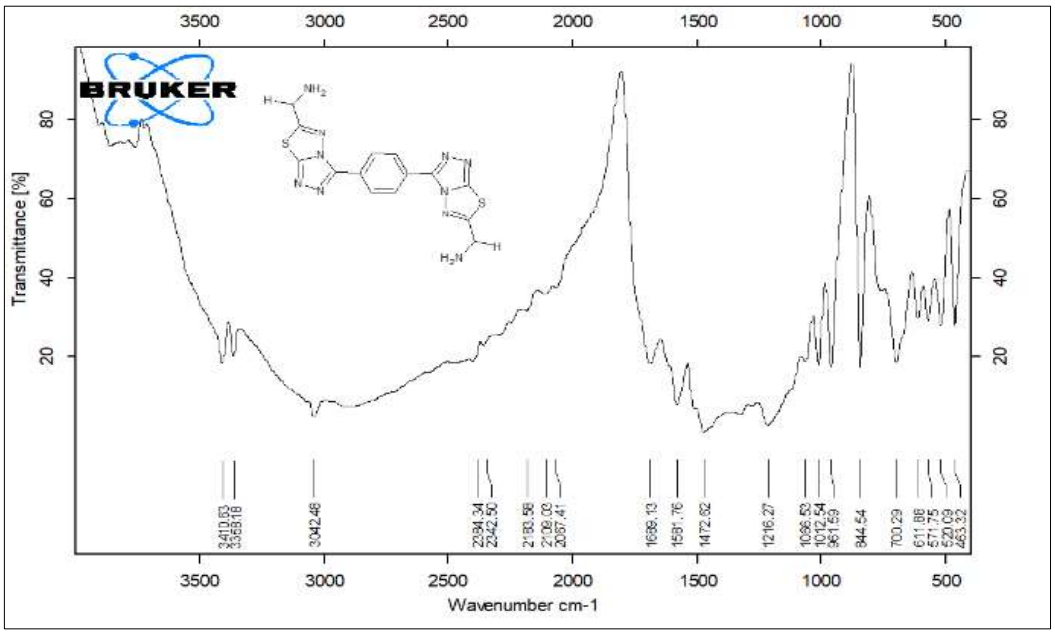


Figure 6. FTIR spectrum of B3a compound

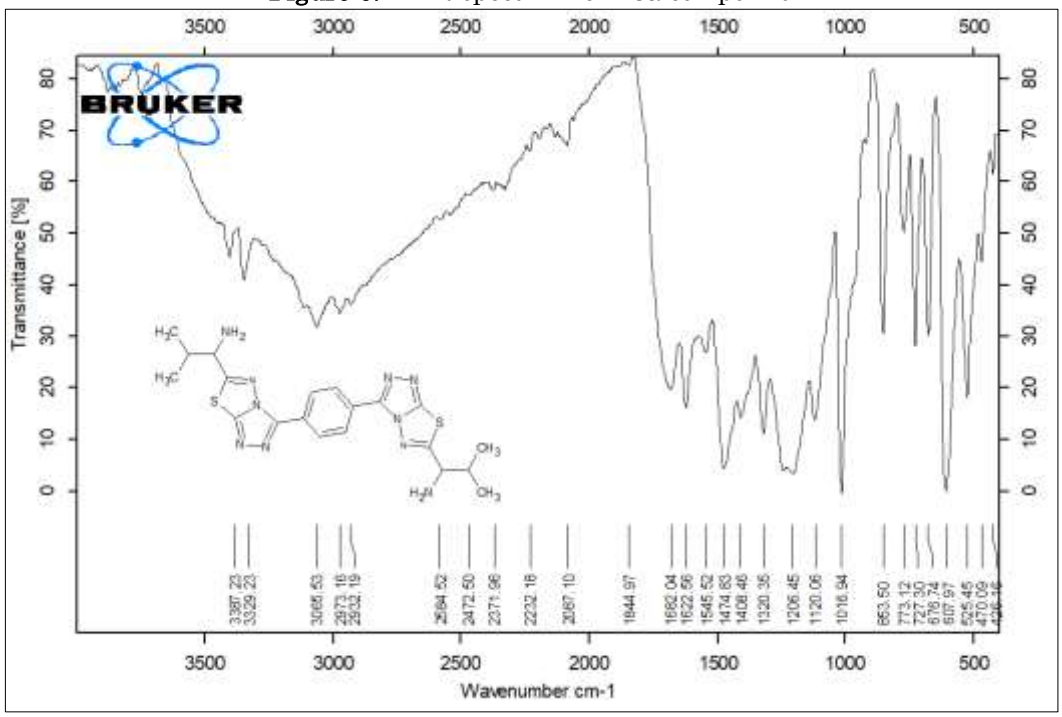


Figure 7. FTIR spectrum of B3b compound

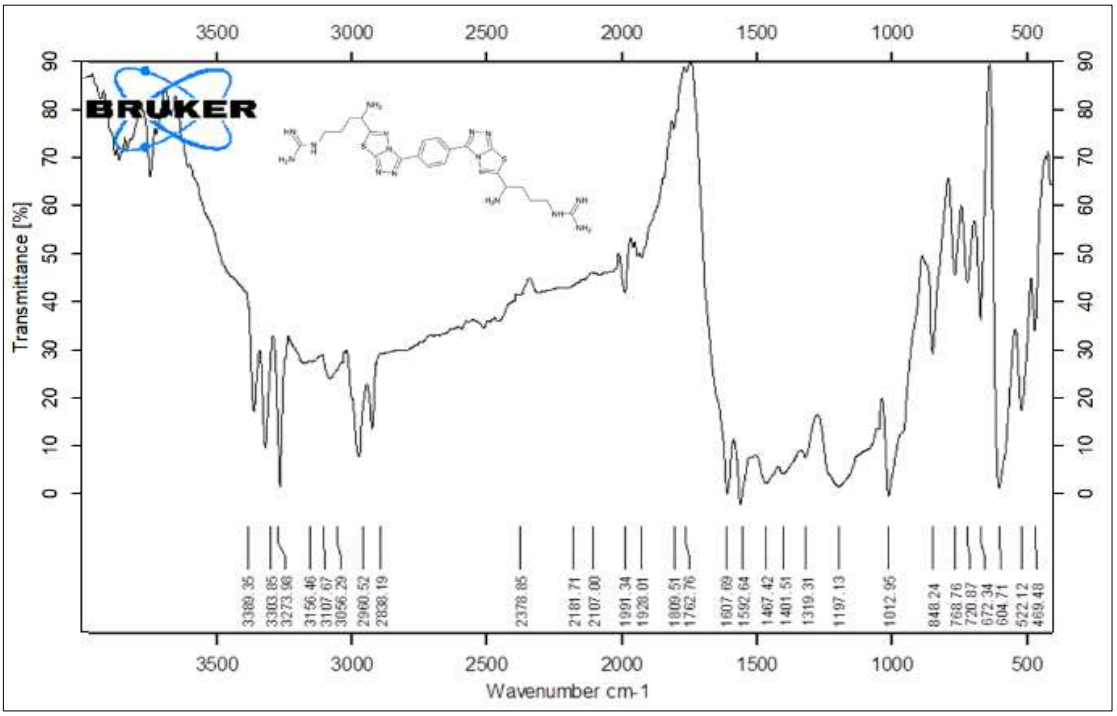


Figure 8. FTIR spectrum of B3c compound

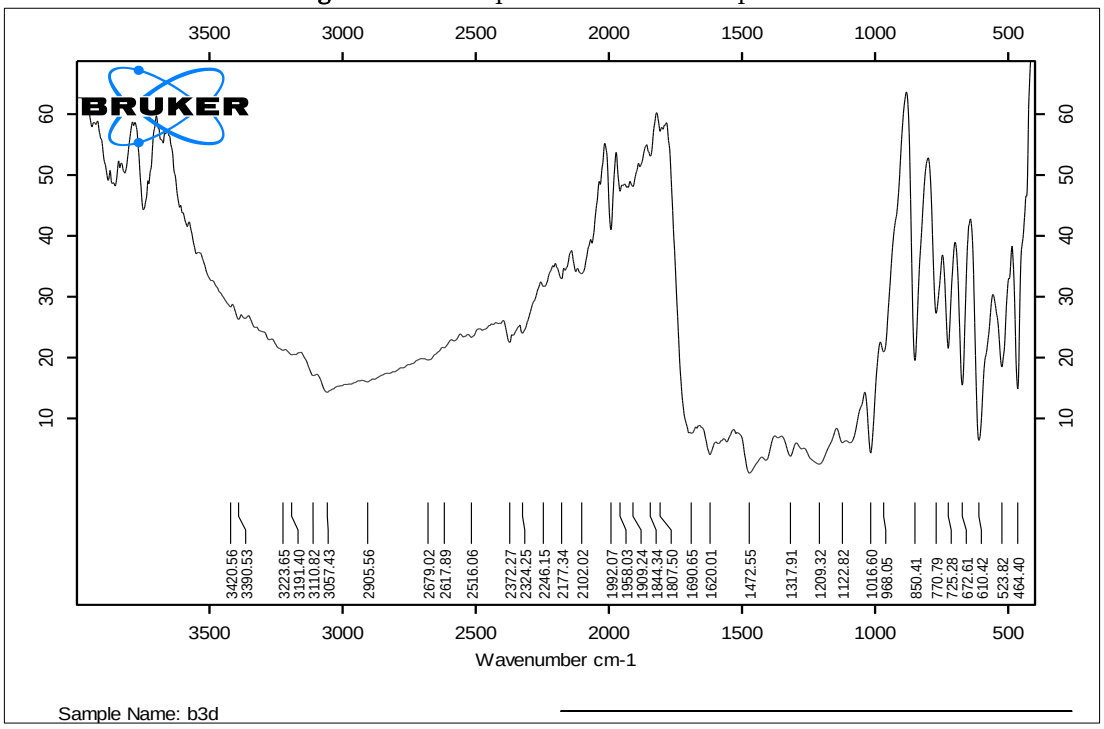


Figure 9. FTIR spectrum of B3d compound

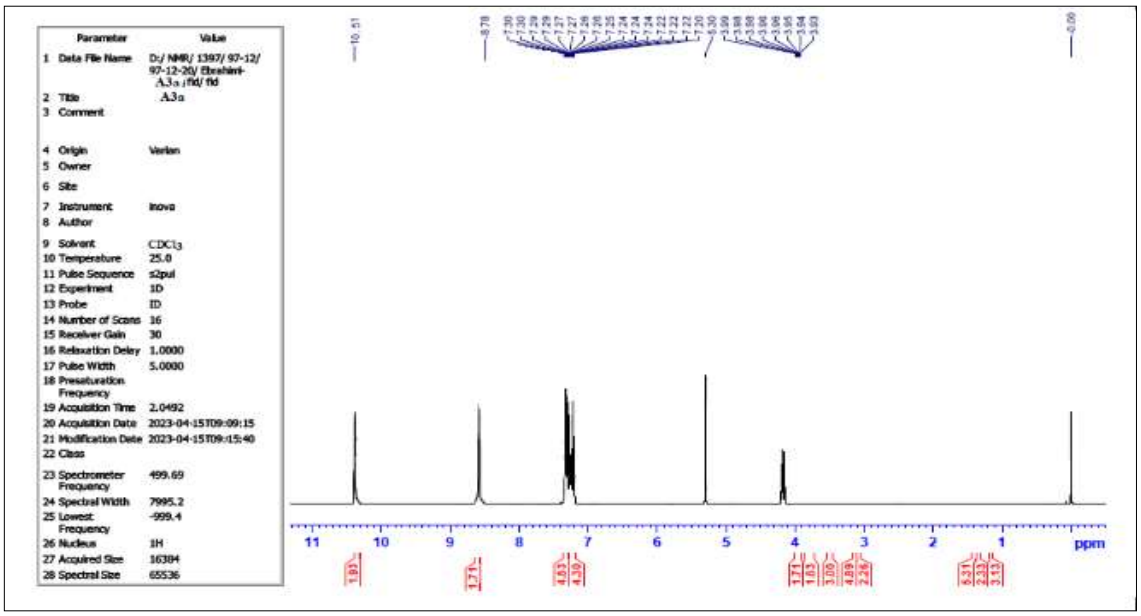


Figure 10. FTIR spectrum of A3a compound

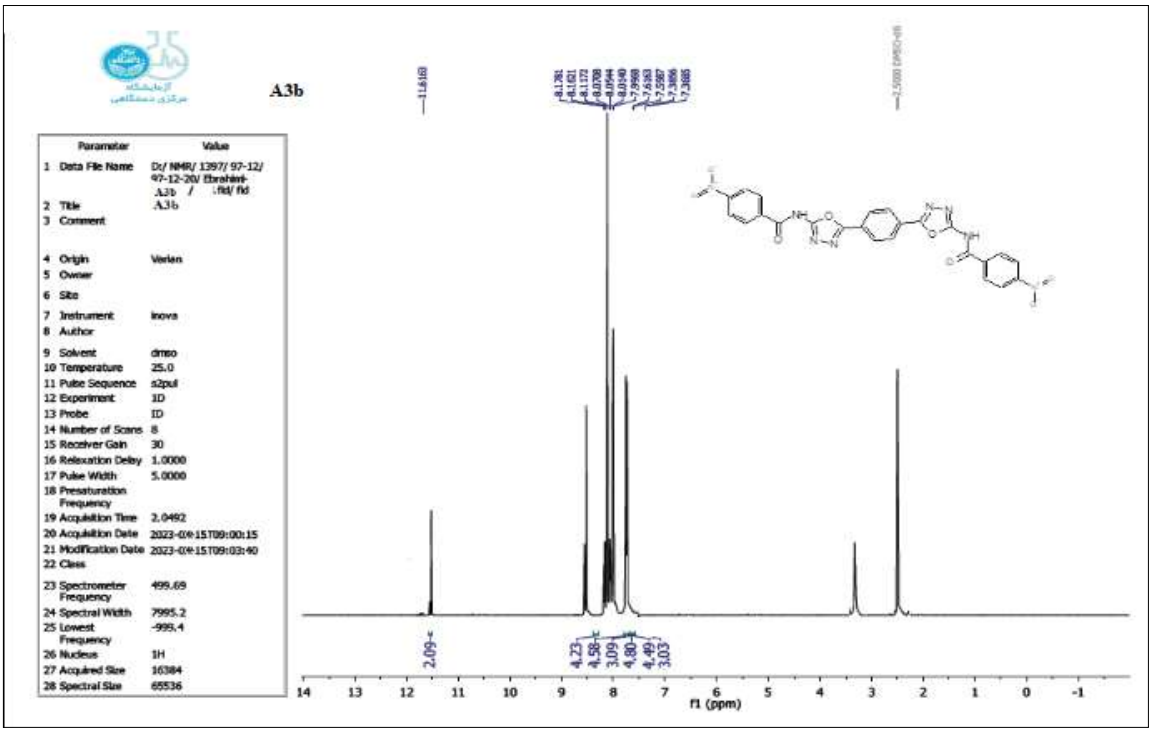


Figure 11. FTIR spectrum of A3b compound

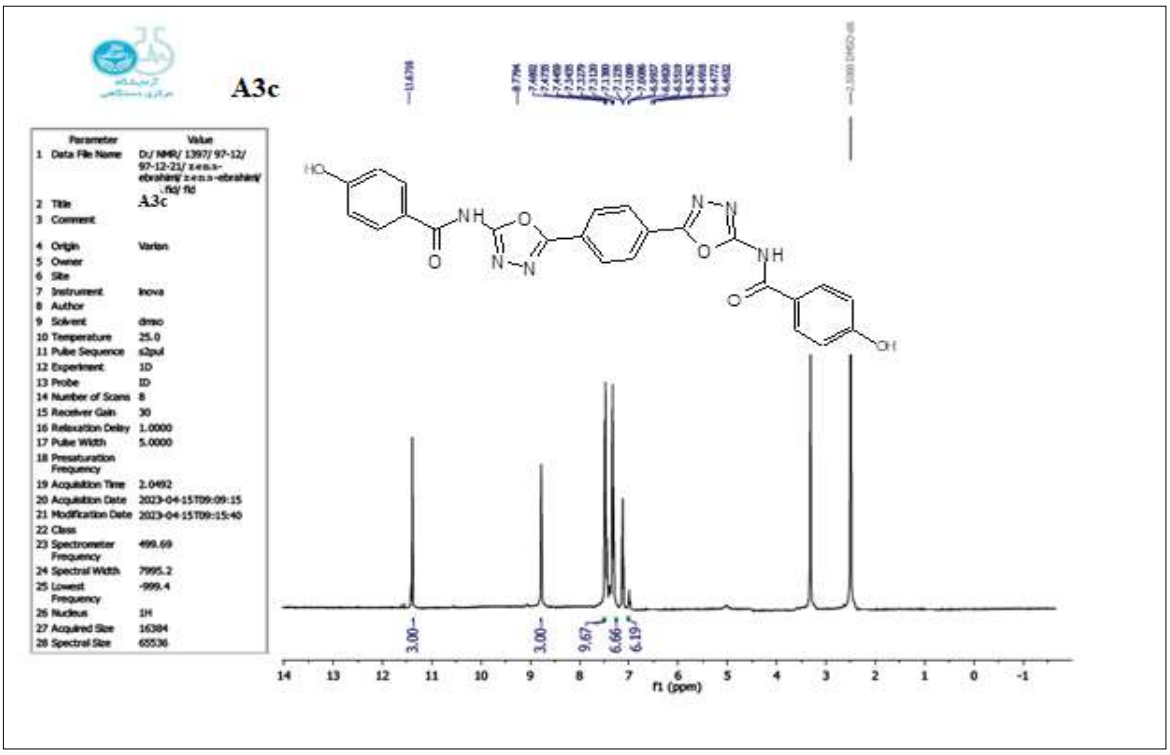


Figure 12. FTIR spectrum of A3c compound

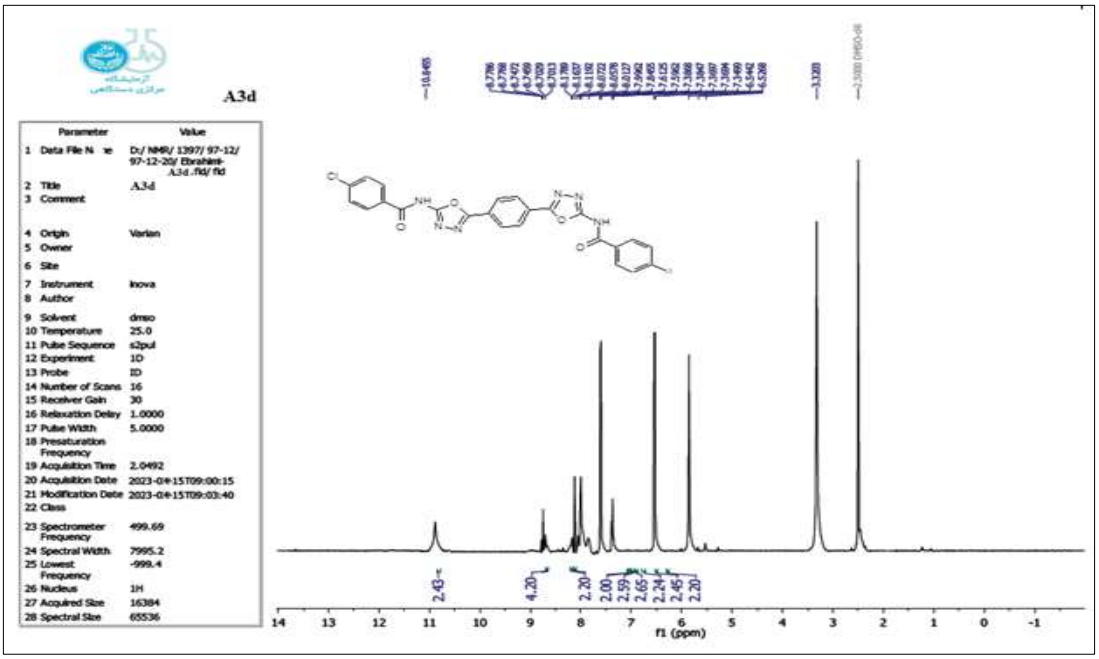


Figure 13. FTIR spectrum of A3d compound

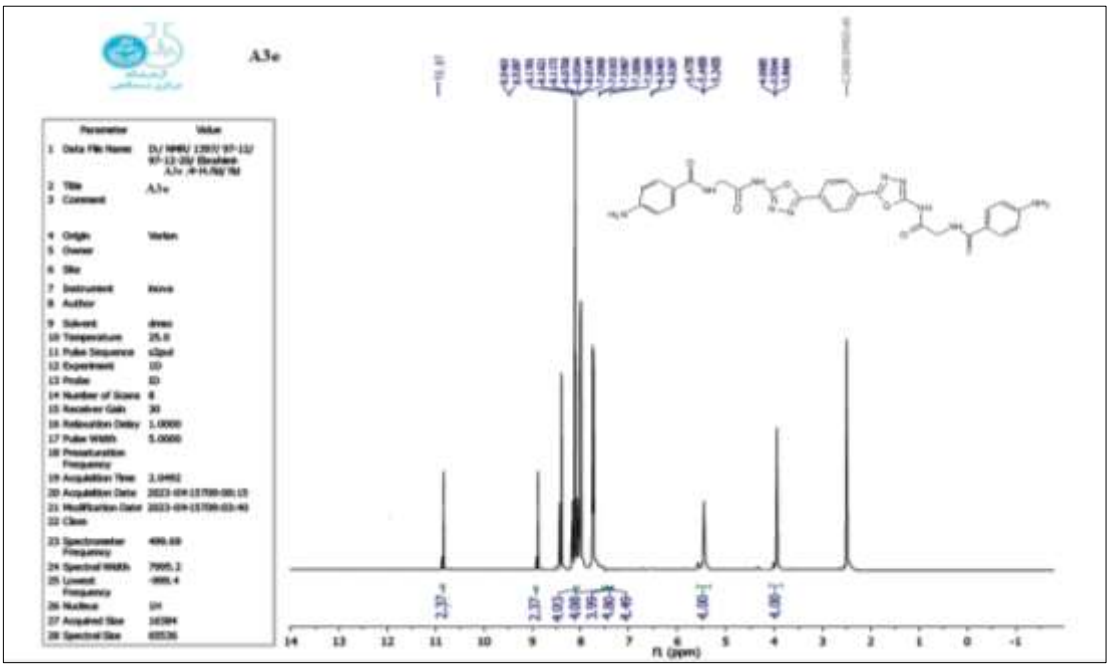


Figure 14. FTIR spectrum of A3e compound

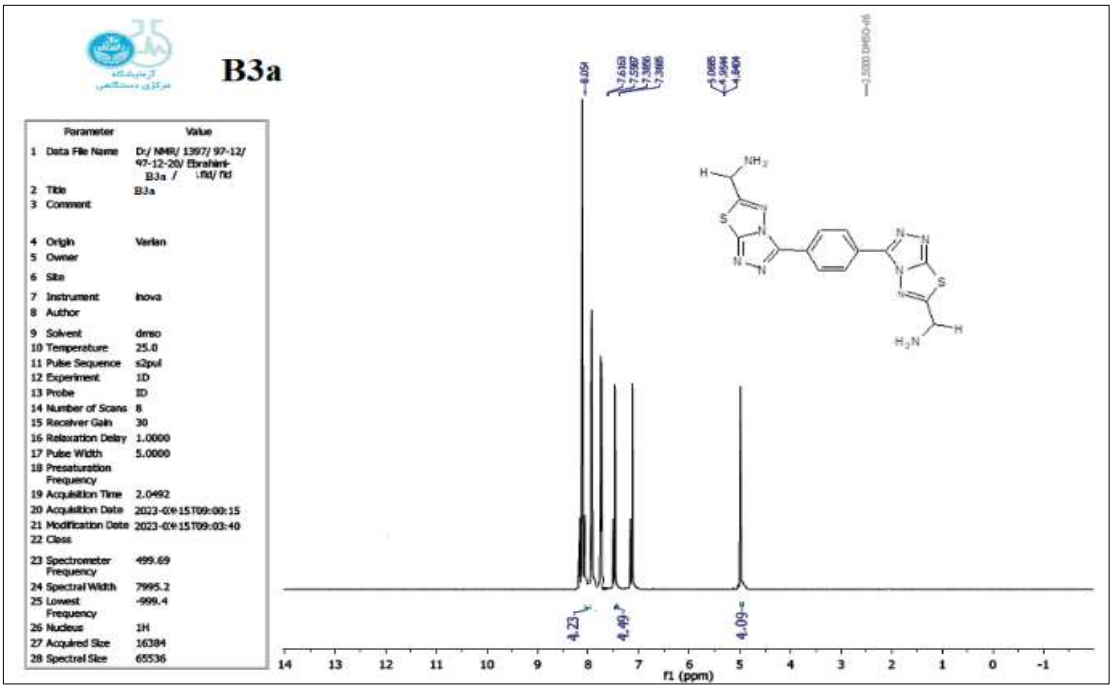


Figure 15. FTIR spectrum of B3a compound

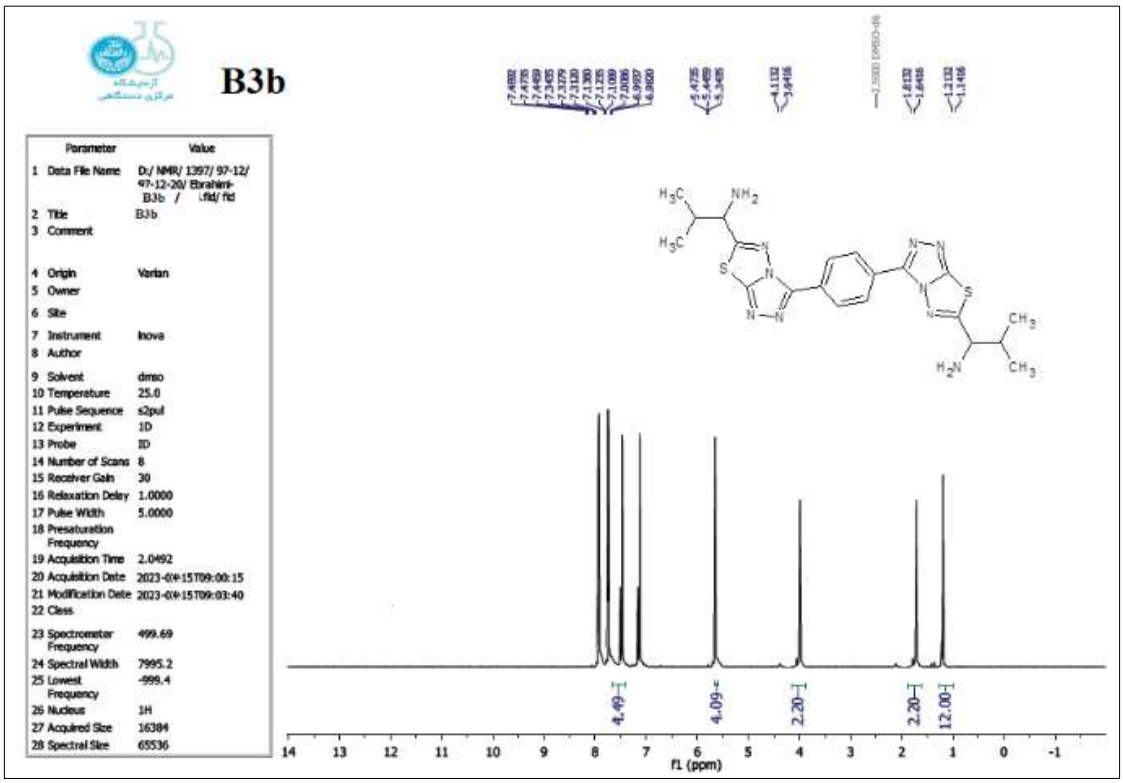


Figure 16. FTIR spectrum of B3b compound

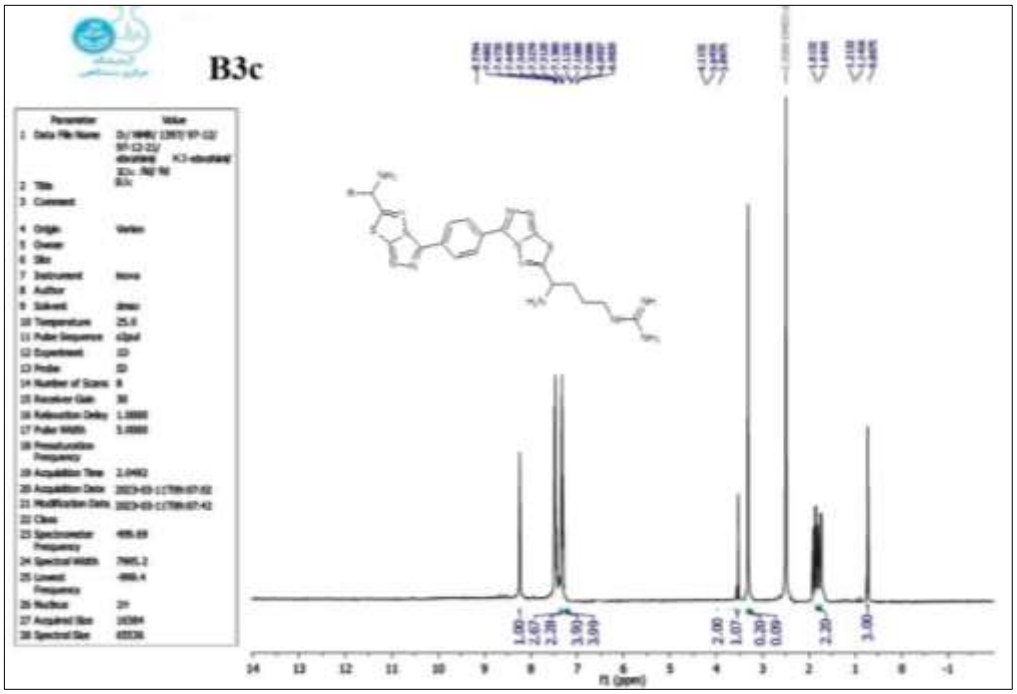


Figure 17. FTIR spectrum of B3c compound

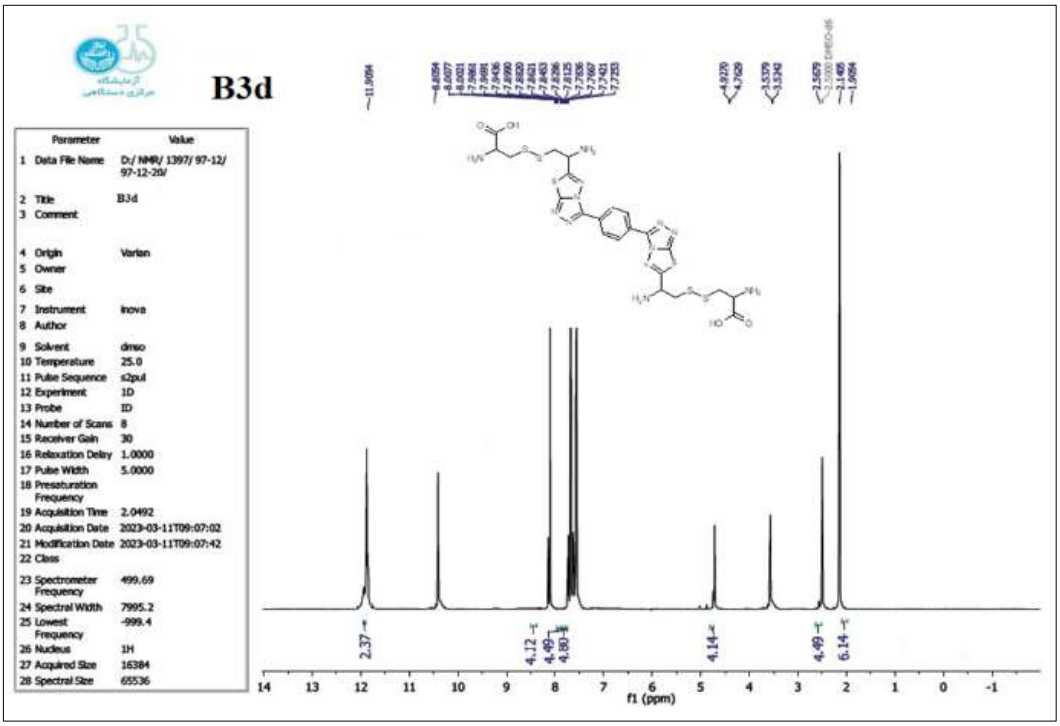
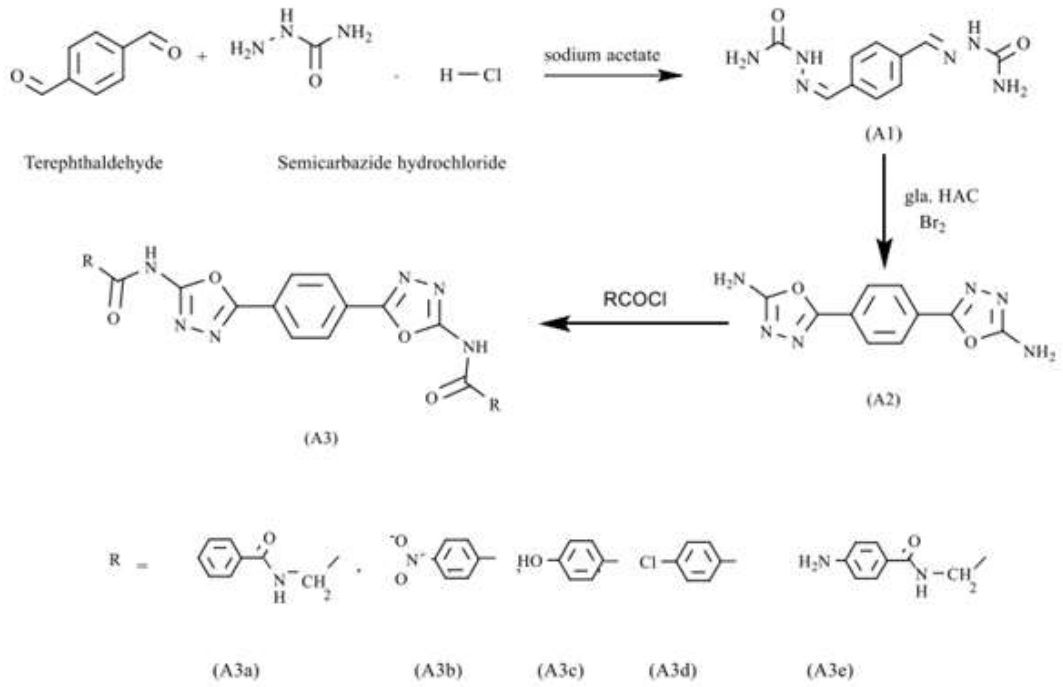
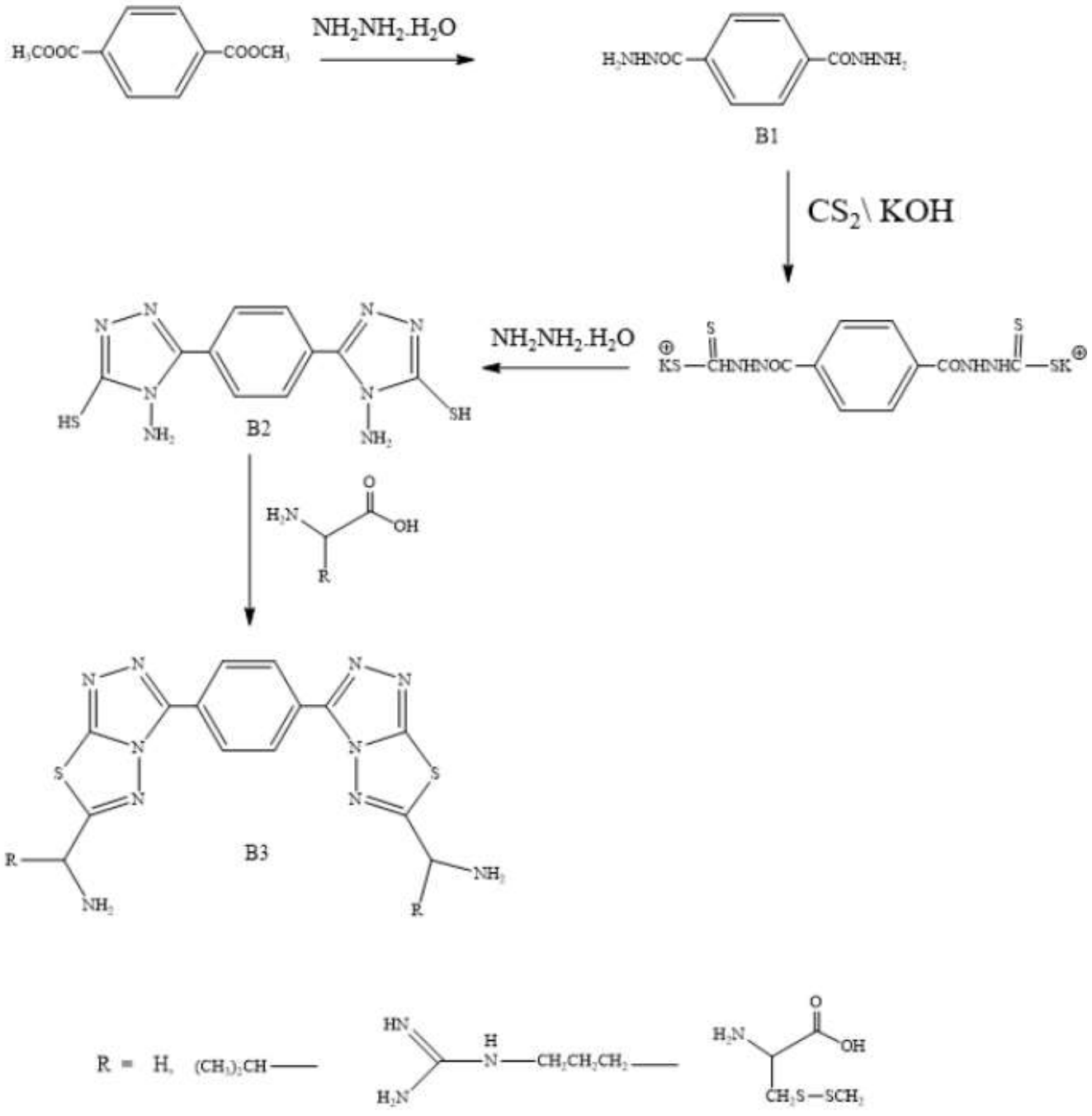


Figure 18. FTIR spectrum of B3d compound



Scheme1. Proposed the synthesis steps for 1,3,4-oxadiazole derivatives compounds



Scheme2. Proposed the synthesis steps for 1,2,4-triazole derivatives compounds

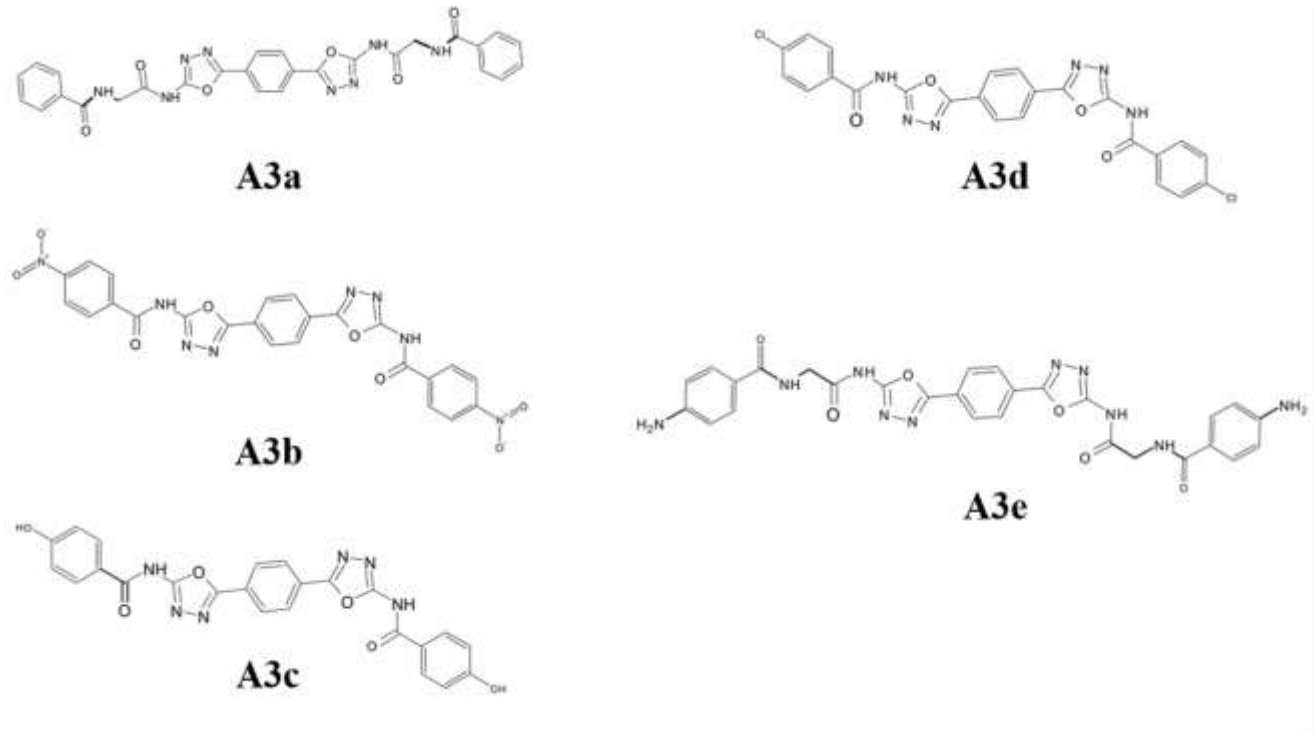


Figure 19. Proposed chemical structure for 1,3,4-oxadiazole derivatives compounds

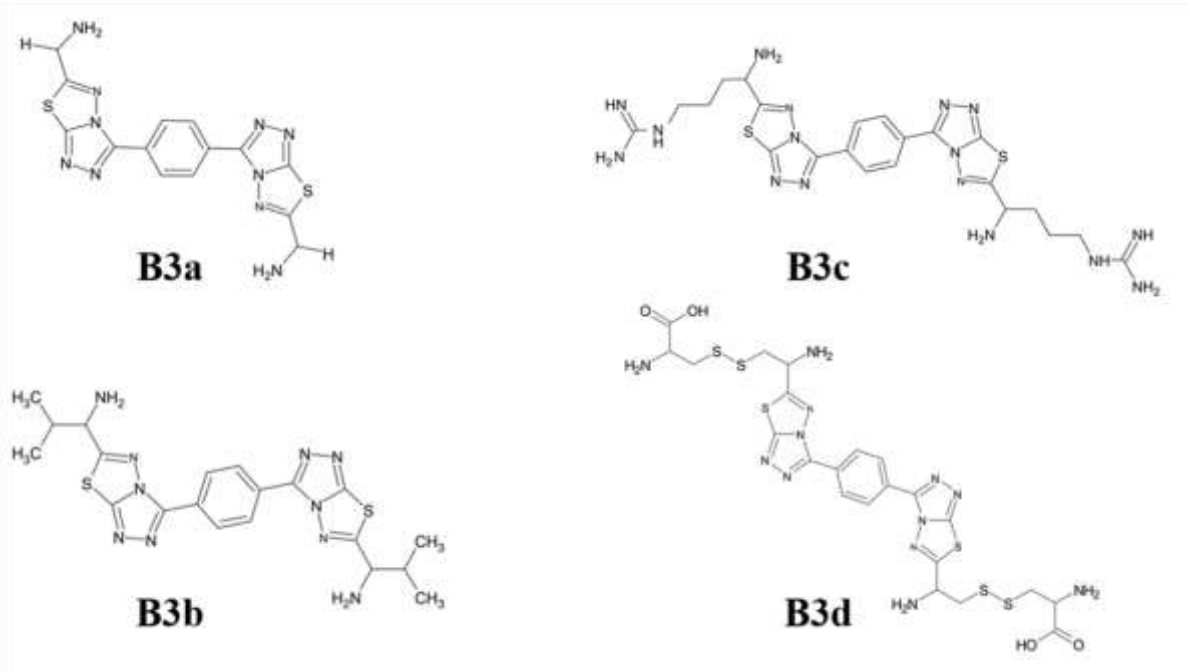


Figure 20. Proposed chemical structure for 1,2,4-triazole derivatives compounds

Table 2. FTIR spectral data of synthesized compounds

Comp.	FT-IR Spectral data, cm^{-1}						
	vN-H	vC-H aromatic	vC=O	vC=C aromatic	N-H	*Para* sub.	Other
A1	3457 3282 3161	3066	1681	1600	1514	836	v (CH=N) 1639
A2	3328 3227	3079	------	1597	1503	882	v (C-O-N)1197 v (C-N) 1390 δ (C-H) 839
A3 (a-e)	3388- 3366	3068- 3052	1673- 1676	1597- 1586	1588- 1501	844- 839	v (N-O) 1519 v (O-H) 3363 v (C-Cl) 844
B1	3300 3225	3029	1594	1544	----	835	------
B2	3364 3356	3068.8	-----	1500	----	883	vC=N (1676) (C=S) 1027
B3a	3411 3358	3043	-----	1581	----	844	C=N (1689)
B3b	3387 3329	3066	-----	1546	----	854	C=N (1682)
B3c	3389 3304 3274	3056	-----	1592		848	C=N (1608) $(C-H)_{aliphatic}$ 2961 & 2838 (C=S) 1197
B3d	3224 3191	3057	1992	1620	----	850	(O-H) 3421

Table 3. Inhibitory activity of synthesize compounds (A3a-B3d) against urease enzyme.

Compound	Concentration (µg/ml)	Absorbance (nm)	inhibition %
A3a	200	0.055	35.29
	150	0.062	27.05
	100	0.067	21.17
	50	0.07	17.64
A3b	200	0.061	28.23
	150	0.068	20
	100	0.073	14.12
	50	0.081	4.7
A3c	200	0.034	60
	150	0.038	55.29
	100	0.042	50.58
	50	0.049	42.35
A3d	200	0.036	57.64
	150	0.04	52.94
	100	0.044	48.23

	50	0.048	43.52
A3e	200	0.022	74.11
	150	0.027	68.23
	100	0.031	63.52
	50	0.036	57.64
B3a	200	0.072	15.29
	150	0.075	11.76
	100	0.079	7.06
	50	0.082	3.53
B3b	200	0.076	10.59
	150	0.079	7.06
	100	0.081	4.71
	50	0.084	1.18
B3c	200	0.061	28.24
	150	0.067	21.18
	100	0.069	18.82
	50	0.072	15.29
B3d	200	0.065	23.53
	150	0.068	20
	100	0.07	17.64
	50	0.074	12.94

Table 4. Activity of pepsin enzyme of synthesize compounds (A3a-B3d) at different concentration

Compound	Concentration (µg/ml)	Absorbance (nm)	Activity (U/ml)
A3a	50	0.513	51.3
	100	0.321	32.1
	150	0.118	11.8
	200	0.004	0.4
A3b	50	0.003	0.3
	100	0.001	0.1
	150	0.000	0
	200	0.000	0
A3c	50	0.544	54.4
	100	0.372	37.2
	150	0.143	14.3
	200	0.031	0.31
A3d	50	0.004	0.4
	100	0.002	0.2
	150	0.000	0
	200	0.000	0
A3e	50	0.342	34.2
	100	0.301	30.1
	150	0.175	17.5
	200	0.091	9.1
B3a	50	0.390	39.0

	100	0.306	30.6
	150	0.271	27.1
	200	0.151	15.1
B3b	50	0.153	15.3
	100	0.056	5.6
	150	0.020	2
	200	0.000	0
B3c	50	0.002	0.2
	100	0.000	0
	150	0.000	0
	200	0.000	0
B3d	50	0.013	1.3
	100	0.006	0.6
	150	0.002	0.1
	200	0.000	0

Table 5. Serum pepsin enzyme levels for H Pylori bacteria patients and healthy control

Group	Mean ± SD pepsin	P value
Patient	0.2236 ± 0.04174	0.02
Control	0.1262 ± 0.005983	

Compound A3d showed HN-CO a singlet at δ 10.84 ppm. Aromatic protons appear at δ 6.5-8.7 ppm. Compound A3e signals consist of HN-CO attached to oxadiazole ring at δ 10.81 ppm singlet, four protons of CH_2 at δ 3.8-4.0 ppm, NH_2 at δ 5.3-5.6 ppm , aromatic protons at δ 6.5-8.7 ppm. As shown in figures (10,11,12,13 and 15). B3a compound appear to has δ 8.05 ppm singlet for NH_2 four protons that attached to thiazole ring, a singlet signal at δ 5.0-4.8 ppm for four protons of CH_2. Doublet of doublets signals at δ 7.3-7.6 ppm for four aromatic protons. B3b compound: singlet signal at δ 5.3- 5.4 ppm (NH_2 groups) attached to thiadiazol ring. δ 3.9-4.1 ppm a doublet signal of (C-H) connected to amino group. 1.6-1.8 Multiplet signal for isopropyl proton. 1.1-1.2 Doublet for 20 protons of (CH_3 groups) terminal. δ 6.9-7.4 ppm doublets for aromatic protons. B3c compound: δ 8.3 ppm a singlet for NH_2 groups four protons connected to thiadiazol ring. δ 3.8-4.1 ppm a triplet for CH_2 four protons connect to amino group. The multiplet signals at 1.6-1.8 for $CH_2 - CH_2 - CH_2$-proton. The triplet signals at 1.1-1.2 for terminal CH_2 groups four protons. Doublets signals at δ 6.9-7.4 ppm for four aromatic protons. B3d compound has δ 8.8 ppm a singlet for 4 protons NH_2 groups connect to thiadiazol ring, δ 4.7-4.9 ppm a triplet seems to be for 4 protons of NH_2. δ3.5 Singlet for CH_2 proton. δ1.9-2.1 Singlet for CH_2 groups 4 protons. δ 7.7-8.0 ppm doublets are indicated to present of aromatic protons. δ 11.9 singlet for CO-OH group. As shown in figures (16,17,18 and 19)

3.3. Urease Inhibitors

The urease inhibition by produced compounds was investigated in vitro. Thiourea was used as reference compound. The tested compounds were prepared in concentrations (50, 100, 150, 200) µg/ml and their inhibition data were listed in Table 3. General pattern, inhibitory activity of 1,3,4-oxadiazol derivatives was increased with increase concentration. In Based on obtained results, the compound (A3e, A3c and A3d) is the most potent inhibitor with maximum inhibition (74.1, 60 and 57) %, respectively, the high inhibition percentage of A3e compound compared to other compounds is due to the percent NH_2-group in compound [36]. The compound (B3c) is the most effective inhibitor with a maximum inhibition of 28.24%, as shown in table 6 . This is because the compound (inhibitor) contains of more than NH_2-groups, which compete with substrate to bind in the active site of the

enzyme: as a result , the enzyme's inhibition increase[36].

3.4. Pepsin Enzyme Activity

The study of *in vitro* pepsin enzyme activity on synthesized derivatives compounds of 1,3,4-oxadiazol and 1,2,4-triazol has been determined separately. Pepsin's enzyme activity was calculated to be 53 U/ml at acidic conditions corresponding to the normal pH of the stomach (pH 2.5). The result in Table 4 shows the activity pepsin enzyme activity with inhibitors , as compared with the activity of enzyme before added inhibitor. It will be decrease as the concentration increase. The compounds (A3b, A3d,B3b, B3c and B3d) are reduce the activity of pepsin enzyme to (0) U/ml at high concentration 200 µg/ml, this compound are good inhibitors at low concentration. The compounds (A3a, A3c, A3e and B3a) show slightly reduce for pepsin enzyme and this is more acceptable in the concentrations chosen in this study because the presence of the enzyme pepsin in the stomach is necessary to complete the process of digesting food, so these compounds reduce its effectiveness by a small percentage, but do not stop its work in high concentrations.

3.5. Determination of Pepsin Activity in *H-Pylori* Patients

Blood samples were collected and separated from people with Helicobacter pylori infection and compared with healthy subjects after making urea breath test and confirmation of infection with h. pylori bacteria. The study included calculating their levels of pepsin enzyme activity. The result in Figure 21 and Table 4 shows the pepsin enzyme activity in h.pylori patients and healthy control. The elevated pepsin enzyme levels in h.pylori patients (0.2236 ± 0.04174) were (p value 0.02) compared to healthy control (0.1262 ± 0.005983) [37]. The reason is due to in normal cases, the mucous layer that lines the stomach wall and contains bicarbonate molecules that balance the pH in the stomach, neutralize the effect of pepsin, and protect the stomach from hydrochloric acid. However in cases of infection with H. pylori in the stomach, it settles in the mucous layer above the gastric epithelial cells in humans and prevents the secretion of this mucous layer that lines the stomach wall, This causes A rise in pH caused pepsinogen to become pepsin, So the activity of the pepsin enzyme increases, allowing it to create voids in the stomach wall that lead to ulcers in the stomach, inflammation of the stomach lining, or stomach cancer [38,39].

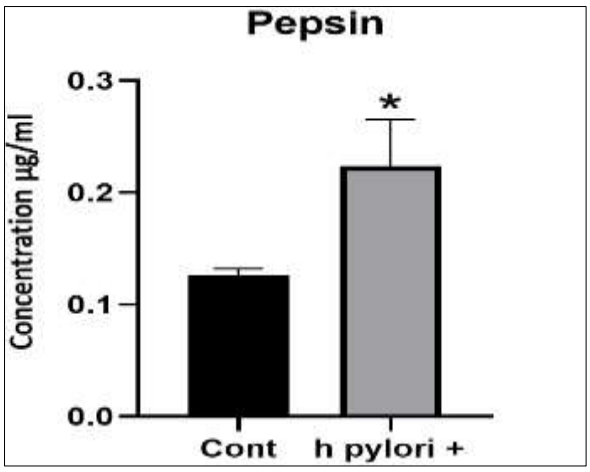


Figure 21. Concentration levels of serum Pepsin enzyme in patients and healthy.

4. Conclusions

This study concludes that the triazole and oxadiazole derivative compounds prepared could be used to treat Helicobacter pylori disease due to their effectiveness as urease enzyme inhibitors and also because they reduce the activity of the pepsin enzyme, that increases in the presence of h.pylori bacteria.

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