Article

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Synthesis,Antifungl and Antibacterial Evaluation for new Thiazolidinone and 1,3-Diazetidine Derivatives

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Abstract. This research,included prepare of some new derivatives of the thiazolidinone ,diazetidne and evaluation of their biological effectiveness.The first step includes formation of new azo compound,from diazotizing a primary aromatic amine from 2-amino-5-mercaptothiazole with sodium nitrite in HCl, followed by a reaction coupling reaction with 2-hydroxyl naphthaldehyde. The product is then reacted with (thiosemicarbazide) or (4-amino-5- fluoro-1H-pyrmidine-2-one)to form a Schiff base derivative B1 and B3, which B1 reacts with α-chloroethylacetate by[2+3] cycloadition to yield a thiazolidinone derivative and B3 reacts with (4-bromophenlisocyante) or (4-nitrophenylisothiocyante) by[2+2] cycloadition to yield diazetidine-2-on and diazetidine-2-thione . Their structures have been characterized by FT-IR, 13C-NMR, and 1H-NMR spectroscopic methods. The antibacterial activity of the prepared compounds were studied against Gram-positive bacteria (***Staphylococcus aureus***) and Gram-negative bacteria (***Acinetobacter baumannii***).Furthermore,some new derivatives were evaluated as antiofungals .against Yeast (***Candida Albicans***) And presenting it as a future study of the importance of heterocyclic compounds in many medical, biological, chemical and physical applications.

Keywords: SchiffBase;Diazetidne;Thiazolidinone;Spectroanalytical;Biological effectiveness

INTRODUCTION

In recent decades, the term "antibiotic resistance" has emerged, referring to the ability of bacteria to survive and grow despite being exposed to an antibiotic that was expected to kill or inhibit them. Bacterial resistance threatens the effectiveness of modern medicine, necessitating global collaboration to control it[1]. Consequently, increasing attention has been directed toward the chemistry of heterocyclic compounds, as they are among the most significant chemical components involved in the design of scientific and industrial innovations. This is due to their renewable properties, stemming from their structural diversity and reactive flexibility, which allow for precise manipulation of their composition and thus enable their use in various applications[2].

Schiff bases, characterized by the presence of an imine group (-CH=N-), are known for their dynamic nature and complex electronic properties, which play a central role in chemical activity, directing chemical reactions, and determining spectroscopic properties[3]. Additionally, they possess the ability to form metal complexes that contribute to the development of drugs capable of inhibiting vital enzymes in pathogenic organisms, rendering them effective against microbes resistant to conventional antibiotics[4].

Thiazolidinones, a class of heterocyclic compounds with the chemical formula C3H3SNO, have recently attracted significant attention due to their adaptability to various applications. They have become a cornerstone in fields ranging from drug design and pharmaceuticals to functional materials[5]. These compounds have contributed to the development of antioxidants, which act as a defense against oxidative stress and are crucial for protecting cells from damage and reducing the risk of chronic diseases[6].

The chemistry of quaternary rings, through understanding their deep structure and precise interaction mechanisms, has played a fundamental role and become a basic tool that has contributed to shaping the future of science and medicine as active vital structures in the drug discovery program and has contributed to enhancing physical and chemical properties.[7]. diazetidne have achieved unprecedented milestones in health and sustainability,their biological activity depends on the diversity of heteroatoms, particularly nitrogen, within the ring[8]. Although the chemistry and biology of four-membered rings remain relatively obscure, they have recently garnered attention from chemists for their strong stabilizing properties and versatile mechanisms of action, such as inhibiting bacterial cell wall synthesis and disrupting protein or DNA synthesis[9].. Diazetidin-2-one tetracyclic containing two nitrogen atoms in the 1,3 position and a carbonyl group (C=O) in the 2 position. It was first prepared in 1960 by Arbuzov and his colleagues from the addition reaction of acetyl isocyanate to a suitable imine (2+2 addition) via a zwitterionic iminium intermediate which is formed when the imine nitrogen attacks the carbonyl of the isocyanate [10]. Diazetidin-2-thione tetracyclic containing two nitrogen atoms in the 1,3 position and a thione group(C=S) in the 2 position. [11] The chemically strained diazetidne derivative is highly reactive and exhibits specific reactivity patterns, making it a lightweight molecular framework of considerable interest Because of the presence of two nitrogen atoms in these derivatives, they are considered intermediate compounds used to prepare more complex compounds. [12].

EXPERIMENTAL PARTS

Materials and Instruments

Reagents and reactants were obtained from commercial suppliers without further purification, while the solvents were previously purified. The melting points of the prepared organic compounds were determined using an electrothermal device (VOL 15 V, Watts 45 W), and the results were expressed in °C, uncorrected. Infrared spectra (FT-IR) were recorded with a Shimadzu FTIR-Prestige Fourier Transform Infrared Spectrophotometer at the University of Kufa, College of Pharmacy, using KBr disks within the range of 4000-400 cm^{-1}. NMR spectra of the derivatives were recorded at Shahid Beheshti University, Chemistry Department, Iran. Carbon spectra (^{13}C-NMR) were recorded on a Bruker spectrometer at 75 MHz with DMSO-d_6 as the solvent, and proton spectra (^{1}H-NMR) were recorded on a Bruker spectrometer at 300 MHz, also with DMSO-d_6 . The progress of the chemical reactions was monitored using thin-layer chromatography (TLC) on silica gel plates (stationary medium) with specific solvents (mobile medium), and iodine was used to visualize the spots.

Synthesis

Preparation for New Azo Compound [B]

Derivative (B) was prepared using 0.920 g (0.007 mole) of (2-amino-5-mercaptoThiazole) combined in a mixture of 5 ml of HCl and 20 ml of water, chilled to 0–5 °C, then sodium nitrite solution was added to it, which was formed by dissolving 0.485 g (0.007 mole) in 10 ml of cooled distilled water, was added drop by drop with vigorous stirring. The solution was left to settle for 15 minutes to complete the diazotization process and prepare new azo (B), according to the literature[13]. The diazonium salt solution that had been cooled down was added to a solution made by dissolving 1.204 g (0.007 moles) of (2-hydroxyl naphtaldehyde) in 20 ml of ethyl alcohol and 10 ml of sodium hydroxide NaOH (10%). The precipitate that forms is filtered, washed several times with distilled water, dried, and recrystallized with absolute ethanol, giving it an dark yellow color of wt=1.4799 gm. The physical properties of these compounds are listed in Table(1).

Preparation for New Schiff Base [B1,B3]

Derivative (1) was prepared by (0.63 g, 0.002 mol) of (azo B) combined in (30 ml) of absolute ethanol in a round-bottomed flask equipped with a magnetic stirrer with a few drops of (GAA) as catalyst with vigorous stirring, then adding (0.182 g, 0.002 mol) of (thiosemicarbazide) or (0.258 g, 0.002 mol)of(4-amino-5- fluoro-1H-pyrmidine-2-one(in batches and then refluxed and heated at 78 °C for 9-18 hrs[14]. Then, the product was cooled, filtered, dried, and recrystallized in absolute ethanol, followed by a TLC and monitoring reaction using benzene: ethanol at a volume ratio 2:3, yielding to(B1) an yellow color of wt=0.6636gm and (B3) giving it an light yellow color of wt=0.7442gm. The physical properties of these compounds are listed in Table(1).

Preparation for Thiazole Derivatives [B2]

Derivative (B2) was prepared by 0.582 g (0.0015 mol) of Schiff base derivative (B1) combined in 40 ml of absolute ethanol in a round-bottomed flask equipped with a magnetic stirrer and adding 0.184 g (0.0015 mol) of (α-chloroethylacetate). With vigorous stirring on a magnetic stirrer at laboratory temperature, refluxed and heated at 78°C for 7 hrs[15].Then, the product was cooled, filtered, dried, and recrystall with absolute ethanol. Then, it was followed by TLC and a monitoring reaction using Benzene:Ethanol at a 2:3 volume ratio, yielding an brown color of wt=0.5530gm. The physical properties of these compounds are listed in Table(1).

Preparation for New Diazetidine Derivatives (B3A,B3B)

Derivative (B3A,B3B) were prepared by 0.2975 g (0.0007 mol) of Schiff base derivative (B3) combined in 25 ml of absolute ethanol in a round-bottomed flask equipped with a magnetic stirrer and adding 0.1386 g (0.0007 mol) of (1-bromo-4-isocyantobenzene) or 0.126 g (0.0007 mol) of(1-isothiocyante-4-nitrobezene).With vigorous stirring on a magnetic stirrer at laboratory temperature, refluxed and heated at 78°C for 12-14 hrs[16].Then, the product was cooled, filtered, dried, and recrystall with absolute ethanol. Then, it was followed by TLC and a monitoring reaction using Benzene:Ethanol at a 2:3 volume ratio, yielding to B3A an yellow color of wt=o.3640gm. and B3B giving it an light yellow color of wt=0.3963gm. The physical properties of these compounds are listed in Table(1).

***TABLE** 1*. Physical properties of prepared compound

No of Comp	M.F g/mol	M.wt	M.P C	R_f	Yield%	Color
B	$C_{14}H_9O_2S_2\ N_3$	315	84-86	/	67.11	**Dark yellow**
B1	$C_{15}H_{12}S_3N_6O$	388	227-229	0.72	85.52	**Yellow**
B2	$C_{17}H_{12}S_3N_6O_2$	428	205-207	0.70	86.14	**Brown**
B3	$C_{18}H_{10}FS_2N_6O_2$	425	239-241	0.68	87.55	**Light yellow**
B3A	$C_{25}H_{15}FBrS_2N_7O_3$	624	148-150	0.58	83.33	**yellow**
B3B	$C_{25}H_{15}FS_3\ {}_8O_4$	606	167-169	0.68	81.74	**light green**

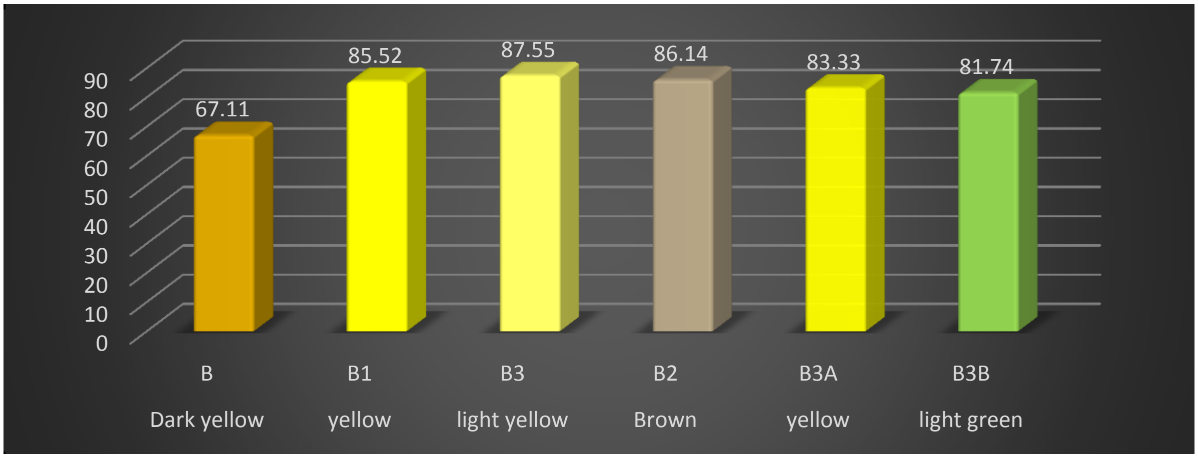


Figure. 1: Yield percentage (B-B3B)

Antifungal Assays

The well diffusion method was adopted by making four holes with a diameter of 6 mm using a sterile cork drill at equal distances from the center in the culture medium (PDA), followed by adding 10 microliters of compound solutions B3,B3A,B3B and testing their sensitivity at concentrations (1,0.5,0.25,0.125)µg/ml using a micropipette while leaving two dishes containing the sterile culture medium without adding solutions of the prepared compounds as a comparison factor (control), then inoculating each dish with the fungi Yeast (*Candida Albicans*). and the percentage of inhibition was calculated according to the following equation:

$$\%\ \text{Inhibition} = \frac{C-T}{C} \times 100 \qquad \text{Eq. (1)}$$

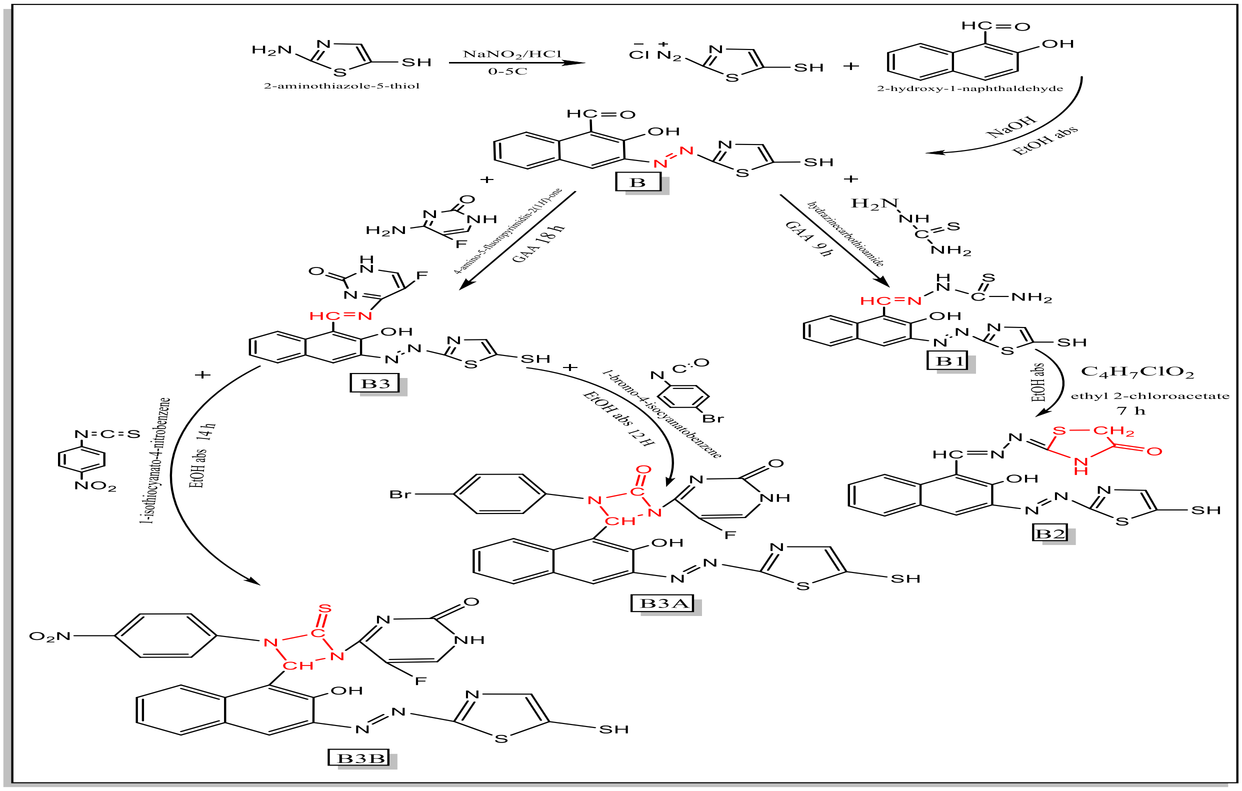
where:

C: Rate of diameter of growth of pathogenic fungi (comparison coefficient),

T: The diameter rate of growth of pathogenic fungi in dishes containing solutions of prepared test compounds (treatment) .

Antibacterial Agent

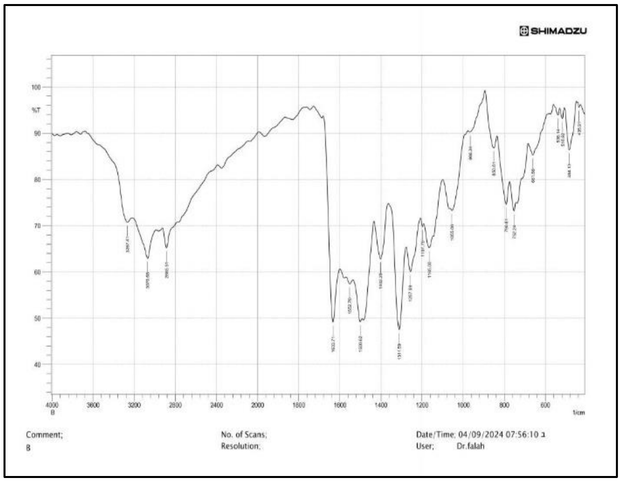
The biological effectiveness of some prepared compounds was studied at the Amin Center for Advanced Biotechnology and Research / Holy Shrine of Imam Ali. The study included the use of two types of isolated pathogenic bacteria, namely Gram-positive bacteria (***Staphylococcus aureus***) and Gram-negative bacteria (***Acinetobacter baumannii***). Solutions of the compounds were prepared and their biological activity against two types of bacteria was evaluated by taking concentrations of (0.125 µg/ml, 0.25 µg/ml, 0.5 µg/ml, and 1 µg/ml) of each compound and dissolving them in (5ml) of the solvent (DMSO). The bacteria were spread on the surface of the plates in the culture medium (Mueller-Hinton Agar) using (Loopful) and four holes were made in the plates with a diameter of 9 mm using a cork borer with a diameter of (9 mm) sterilized with alcohol, taking into consideration leaving a suitable distance between one hole and another to avoid overlapping of the inhibition zones between them. The prepared solutions were added to these holes in an amount of 0.1 ml using (Micropipette) and placed in the incubator for 24 hours at a temperature of 37 °C. The amount of inhibition zone of the compounds was then measured using a millimeter ruler.



Scheme 1. Preparationof the new Thiazolidin-4-one and diazetidne derivatives

RESULTS AND DISCUSSION

The compounds B-B3B were synthesized according to the chemical procedures outlined in Scheme 1.The current study included the use of a compound (2-hydroxyl naphtaldehyde) as a starting material to prepare derivatives of the thiazole and diazetidine ring, where the prepared compounds were identified by a number of spectra: FTIR (cm-1), 1HNMR (δ ppm), and 13CNMR (δ ppm). The biological activity of all the prepared compounds was also evaluated. This study included the use of two types of pathogenic bacteria isolated and diagnosed in the laboratory using biochemical and microscopic tests. It includes two different types of Gram-positive (Staphylococcus aureus) and Gram-negative bacteria (Acinetobacter baumannil). These isolates are considered pathogenic for many diseases. Some of the prepared derivatives were also studied as antifungals against (***Candida Albicans***), and then a new azo compound B (E)-2-hydroxy-3-((5-mercaptothiazol-2-yl)diazenyl)-1-naphthaldehyde, FT-IR analysis showed the disappearance of the amine group that appears at 3400-3200 cm-1 and the appearance of the azo group at 1402cm-1, the stretching as well as the 3375 cm-1, 3070 cm-1,2885 cm-1,1633m-1 that many attributed to OH naphthol,CH aromatic,CH aldehyde, and C=O aldehyde [17], respectively, 1552cm-1to C=N endocyclic and 1500cm-1 (C=C aromatic),as shown in fig.2.The 1H-NMR spectrum for compound B showed singlet for OH proton at δ11.41 ppm, singlet for SH proton at δ13.22 ppm, singlet for CH proton of thiazol ring at δ6.93 ppm and singlet for CH proton of aldehyde at δ 12.50 ppm. Mutilate signals from 7.4-7.7 are belong to the aromatic protons,as shown in fig.3 .13C-NMR showed the aldehyde's carbonyl at δ 192.30 ppm, the peak of -C=N five ring at δ 157.48 ppm, the peak of N-C=N at δ 157.48 ppm, the peak of HS-C=C at δ 153.51 ppm, the aromatic carbon of rings at δ 112-130 ppm,as shown in fig.4.Then a new Schiff base derivative B1(Z)-2-((2-hydroxy-3-((E)-(5-mercaptothiazol-2-yl)diazenyl)naphthalen-1-yl)methylene)hydrazine-1-carbothioamide. FT-IR, 1H-NMR, and 13C-NMR confirmed the structure of the newly synthesized derivative. The FTIR spectra showed the disappearance of the carbonyl group of the aldehyde at 1633 cm-1 and the appearance of the azomethine group an absorption band at 1608 cm-1, the (C=N) endo group at 1516 cm-1 and showed the band at 3477 cm-1 OH naphthol, 3446- 3259 cm-1 due to NH2 stretching of thioamide, 3165 cm-1,3072 cm-1 and 2885 cm-1 that many attributed to NH stretching of thioamide, CH aromatic, CH aliphatic,1496 cm-1 due to the C=C aromatic, 1394 cm-1 due to the (N=N), and 1278 cm-1 due to the C=S thionyl,as shown in fig.5 .The 1H-NMR spectrum for compound B showed disappearance of the singlet for the C-H proton of aldehyde at δ 12.5 ppm and appearance of the singlet of the CH=N proton of azomethine at δ8.8 ppm, singlet for OH proton at δ11.69 ppm, singlet for SH proton at δ13.29 ppm, singlet for NH_2 proton of thioamide at δ 6.55 ppm,singlet for NH proton of thioamide at δ 10.98 ppm. Mutilate signals from 7.0-7.6 are belong to the aromatic protons,as shown in fig.6. 13C-NMR showed the peak of -C=N imine at δ 159.20 ppm, the peak of -C=S at δ 177.46 ppm, the peak of N-C=N at δ 157.22 ppm, the peak of HS-C=C at δ 152.20 ppm. the aromatic carbon of rings at δ 105-142 ppm,as shown in fig.7.A new Schiff base derivative B3 (2-(2-((E)-(2-hydroxy-3-((E)-(5-mercaptothiazol-2-yl)diazenyl)naphthalen-1-yl)methylene)hydrazineyl)thiazolidin-4-one). The FTIR spectra showed the disappearance of the carbonyl group of the aldehyde at 1633 cm-1 and the appearance of the azomethine group an absorption band at 1639 cm-1 ,the C=N endo group at 1587cm-1at five ring and 1558cm-1 at six ring showed the band at 3379 cm-1 OH naphthol, 3124 cm-1, 3078 cm-1 , 2885-2808cm-1 and 2600 cm-1 that many attributed to (=CH) stretching of six ring ,CH aromatic, CH aliphatic, SH thiole, 1678cm-1 due to the C=O pyrimidin-2-on ,1517 cm-1 due to the C=C aromatic and 1465 cm-1 due to the N=N,as shown in fig.8.The 1H-NMR spectrum for compound B showed disappearance of the singlet for the (C-H) proton of aldehyde at δ 12.5 ppm and appearance of the singlet of the (CH=N) proton of azomethine at δ 8.3 ppm. singlet for the (C-H) proton of thiazole at δ 6.1 ppm, singlet for the (C-H) proton of pyrimidin-2-on at δ 6.3 ppm, singlet for the (SH) proton of thiol at δ 12.1 ppm , singlet for the (OH) proton of naphthol at δ 11 ppm, singlet for the (NH) proton of six ring at δ 10.2 ppm and Mutilate signals from 7.0-7.3 are belong to the aromatic protons,as shown in fig.9 . 13C-NMR showed the peak of -C=N imine at δ 163 ppm, the peak of -C=O at δ 169.36 ppm, the peak of N-C=N six ring at δ 157.61 ppm, the peak of N-C=N five ring at δ 161.61 ppm the peak of F-C=C six ring at δ 152.59 ppm,. the aromatic carbon of rings at δ 115-130 ppm,as shown in fig.10.Derivative B2 (5-fluoro-4-(((Z)-(2-hydroxy-3-((E)-(5-mercaptothiazol-2-yl)diazenyl)naphthalen-1-yl)methylene)amino)pyrimidin-2(1H)-one,was prepared of Schiff base derivative (B1) combined in 40 ml of absolute ethanol and adding of ethyl chloroacetate. The compound B2 FT-IR spectrum data showed that the band was C=O thiazolidine-4-on in the frequency range of 1714cm-1, the azomethine band at 1608 cm-1, and the appearances were 3446 cm-1 OH naphthol, 3259 cm-1 NH for thiazole, 3057 cm-1 CH aromatic, 2885 cm-1 CH aliphatic,1517 cm-1 C=C aromatic, 1396 cm-1 N=N azo, and 1571cm-1 at C=N endo group,as shown in fig.11. A singlet peak at 3.79 ppm of the CH2 proton with five rings, the singlet of the (CH=N) proton of azomethine at δ 8.46 ppm. singlet for the (C-H) proton of thiazole at δ 6.5 ppm, singlet for the (NH) proton of thiazolidinon at δ 10.9 ppm, singlet for the (SH) proton of thiol at δ 12.9 ppm , singlet for the (OH) proton of naphthol at δ 11.5 ppm, and Mutilate signals from 7.6-7.7 are belong to the aromatic protons can be seen in the 1H-NMR spectrum results,as shown in fig.12 . The 13C-NMR spectrum revealed the peak of CH2 in the five rings at approximately 53.48, while the carbonyl group for thiazolidine-4-one appeared to peak at approximately 178.71. the peak of -C=N imine at δ 161.16 ppm, the peak of -C=O at δ 178.41 ppm, the peak of N-C=N at δ 159.17 ppm, the peak of HS-C=C at δ 152.16 ppm, . the peak of N=C-S endo at δ 157.16 ppm, the aromatic carbon of rings at δ 116-142 ppm,as shown in fig.13. Derivative new diazetidine-2-on B3A (E)-5-fluoro-4-(3-(4-fluorophenyl)-2-(2-hydroxy-3-((5-mercaptothiazol-2-yl)diazenyl)naphthalen-1-yl)-4-oxo-1,3-diazetidin-1-yl)pyrimidin-2(1H)-one.IR analysis (KBr,cm-1) showed the disappearance of the imine group at 1639 cm-1 and the appearance of C=O diazetidine-2-on at 1697 cm-1, the C=O six ring at 1635cm-1,the C=N endocyclic at 1533cm-1 , showed the band at 3414 cm-1 (OH), 3315 cm-1, 3068 cm-1, 2983-2819 cm-1that many attributed to NH stretching of six ring ,CH aromatic,CH aliphatic,1471 cm-1 due to the C=C aromatic, 1402 cm-1 due to the N=N azo, and 756 cm-1 due to the C-Br,as shown in fig.14 .The 1H-NMR spectrum for compound B3A showed disappearance of the singlet for the (C-H) proton of azomethine and appearance of the singlet of the (C-H) proton of diazetidine-2-on at δ 5.3 ppm , singlet for the (OH) proton of naphthol at δ 11.4 ppm, singlet for the (NH) proton of six ring at δ 10.1 ppm, singlet for the (C-H) proton of thiazole at δ 6.4 ppm, singlet for the (C-H) proton of pyrimidin-2-on at δ 6.6 ppm, singlet for the (SH) proton of thiol at δ 12.2 ppm , singlet for the (OH) proton of naphthol at δ 11 ppm and Mutilate signals from 7.0-7.7 are belong to the aromatic protons,as shown in fig.15 . 13C-NMR showed the peak of -C=O diazetidine-2-on at δ 170 ppm, the peak of -C=O six ring at δ 169 ppm, the peak of -C=N five ring at δ 161 ppm, the peak of CH diazetidine-2-on at δ 79 ppm, the aromatic carbon of rings at δ 110-130 ppm,as shown in fig.16. Derivative new diazetidine-2-thion B3B (E)-5-fluoro-4-(2-(2-hydroxy-3-((5-mercaptothiazol-2-yl)diazenyl)naphthalen-1-yl)-3-(4-nitrophenyl)-4-thioxo-1,3-diazetidin-1-yl)pyrimidin-2(1H)-one FT-IR, 1H-NMR, and 13C-NMR confirmed the structure of the newly synthesized derivative.. IR analysis showed the disappearance of the imine group at 1639 cm-1 and the appearance of C=S diazetidine-2-thion at 1209 cm-1, the C=O six ring at 1639 cm-1,the C=N endocyclic at 1608cm-1 , showed the band at 3388 cm-1 (OH), 3294 cm-1, 3076 cm-1, 2983-2823 cm-1that many attributed to NH stretching of six ring ,CH aromatic,CH aliphatic,1552 cm-1 due to the C=C aromatic, 1396 cm-1 due to the N=N azo, and 1506,1321 cm-1 due to the NO_2 symmetric and asymmetric streching,as shown in fig.17 .The 1H-NMR spectrum for compound B3B showed disappearance of the singlet for the (C-H) proton of azomethine and appearance of the singlet of the (C-H) proton of diazetidine-2-thion at δ 5.3 ppm , singlet for the (OH) proton of naphthol at δ 11.4 ppm, singlet for the (NH) proton of six ring at δ 10.1 ppm, singlet for the (C-H) proton of thiazole at δ 6.2 ppm, singlet for the (C-H) proton of pyrimidin-2-on at δ 6.4 ppm, singlet for the (SH) proton of thiol at δ 12.3 ppm , singlet for the (OH) proton of naphthol at δ 11.4 ppm and Mutilate signals from 7.4-7.9 are belong to the aromatic protons ,as shown in fig.18. 13C-NMR showed the peak of -C=S diazetidine-2-thion at δ 168 ppm, the peak of -C=O six ring at δ 183 ppm, the peak of -C=N five ring at δ 162 ppm, the peak of CH diazetidine-2-on at δ 78 ppm, the peak of HS-C=C at δ 144 ppm, the peak of C=C-NH at δ 142 ppm, the peak of F-C=C at δ 149 ppm, the aromatic carbon of rings at δ 117-130 ppm,as shown in fig.19.



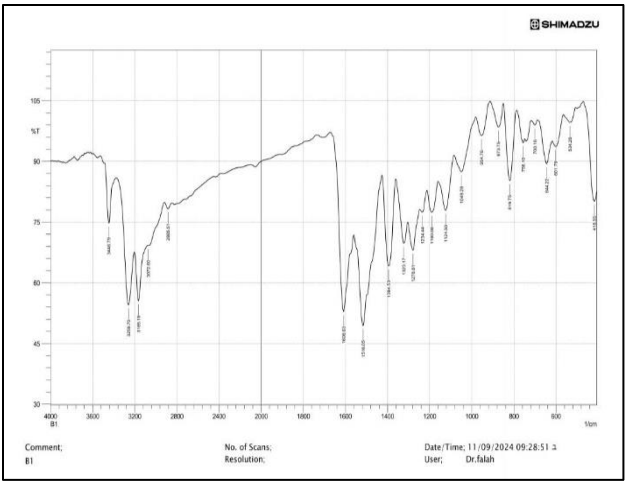
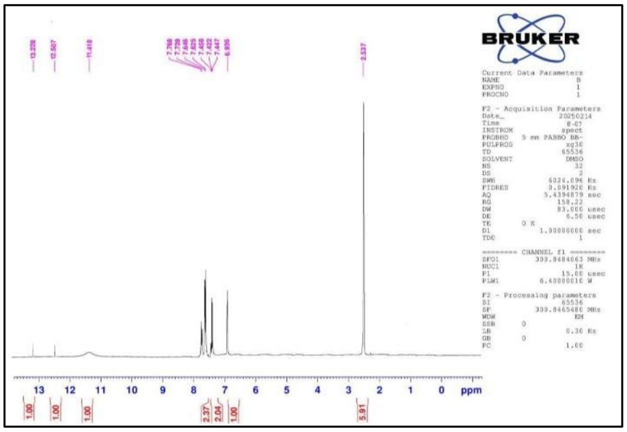


Figure 2: FTIR of compound B

Figure 5: FTIR of compound B1



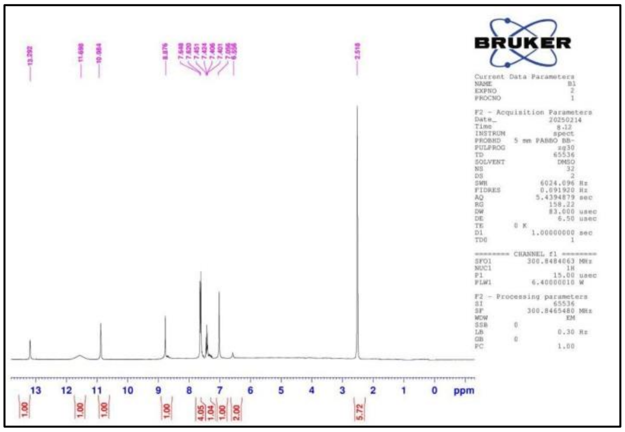
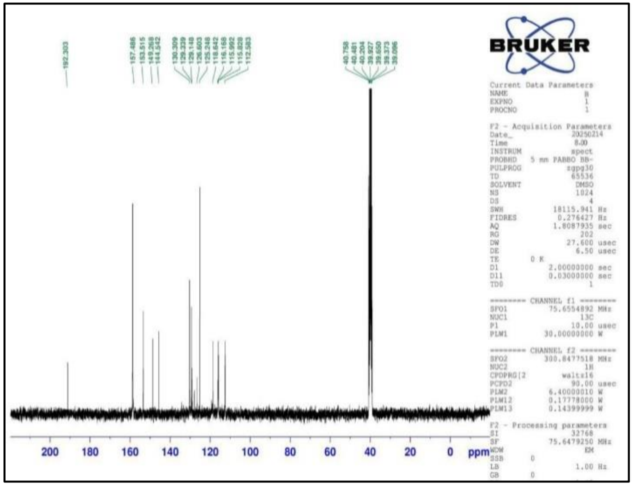


Figure 3: 1HNMR of compound B

Figure 6: 1HNMR of compound B1



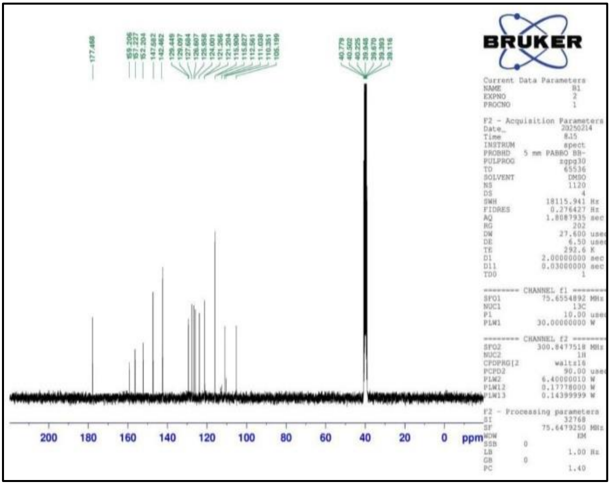


Figure 4: 13CNMR of compound B

Figure 7: 13CNMR of compound B1

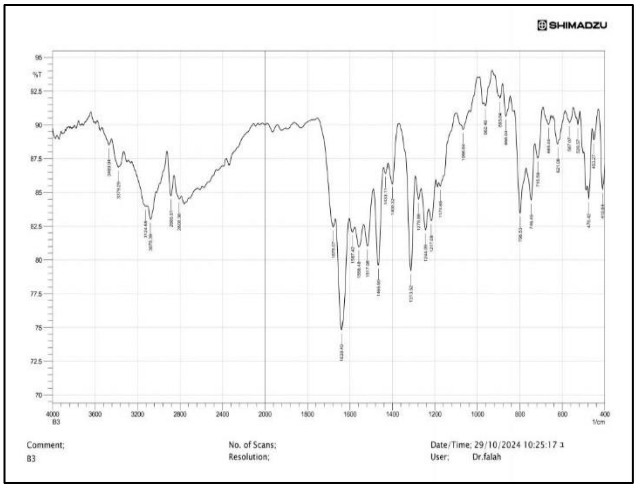


Figure 8: FTIR of compound B3

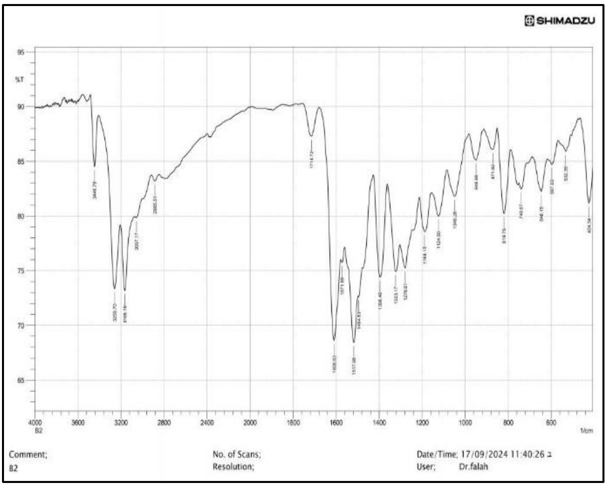


Figure 11: FTIR of compound B2

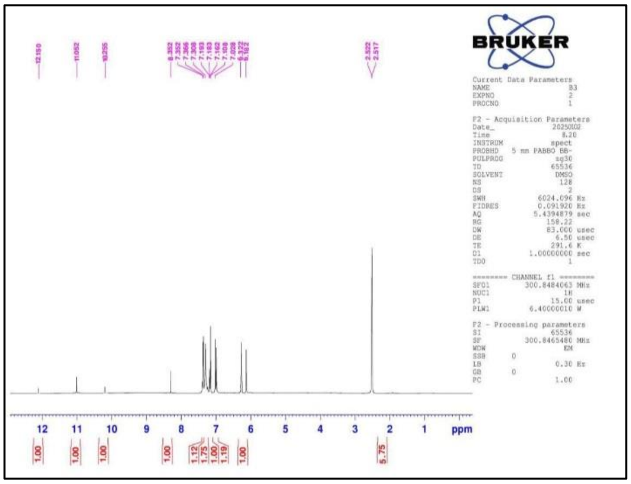


Figure 9: 1HNMR of compound B3

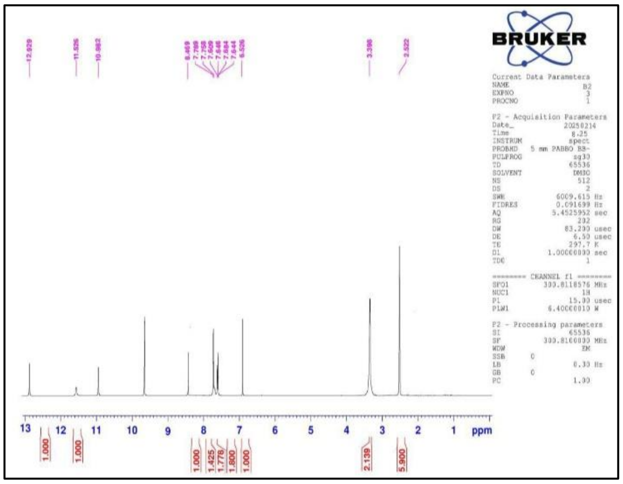


Figure 12: 1HNMR of compound B2

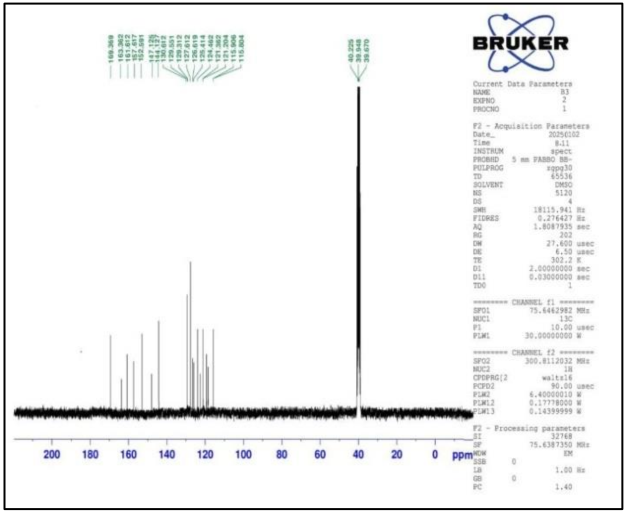


Figure 10: 13CNMR of compound B3

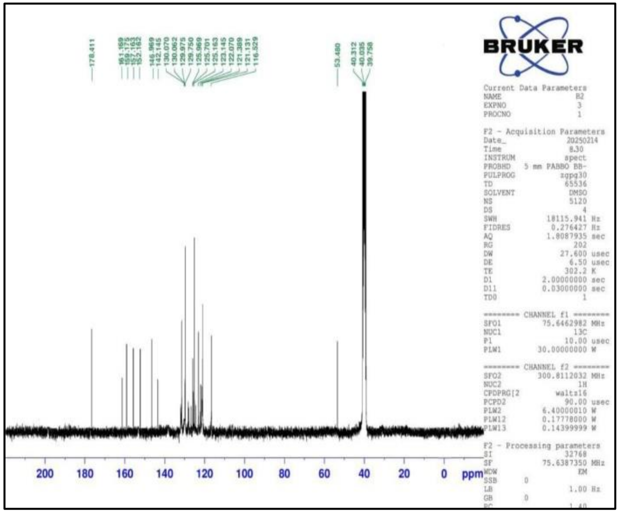


Figure 13: 13CNMR of compound B2

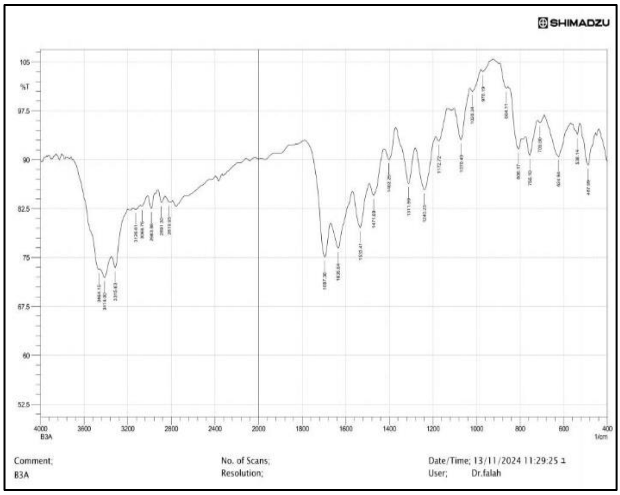


Figure 14: FTIR of compound B3A

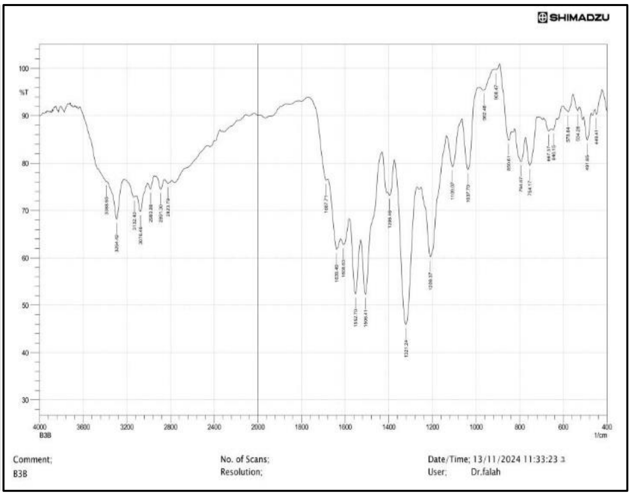


Figure 17 : FTIR of compound B3B

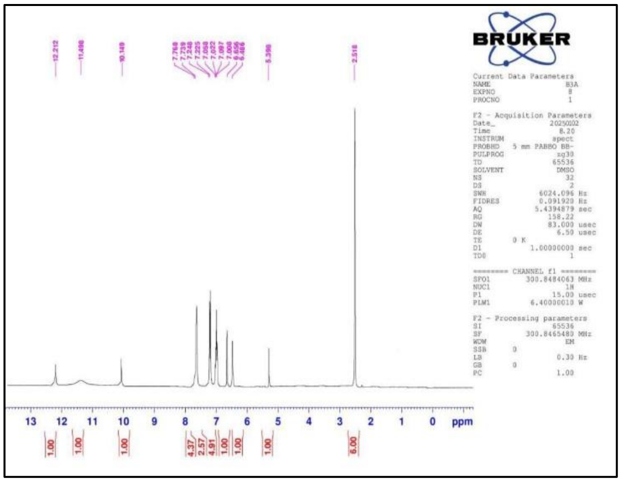


Figure 15 : 1HNMR of compound B3A

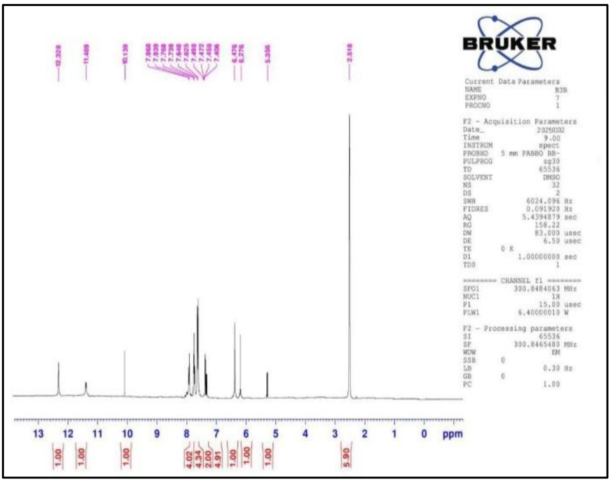


Figure 18:1HNMR of compound B3B

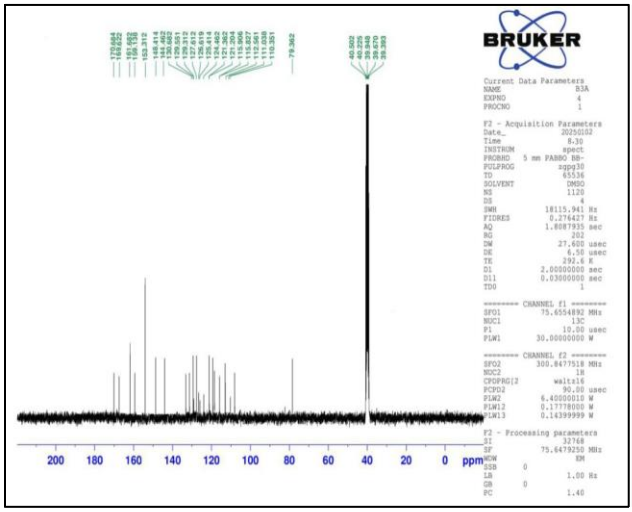


Figure 16: 13CNMR of compound B3A

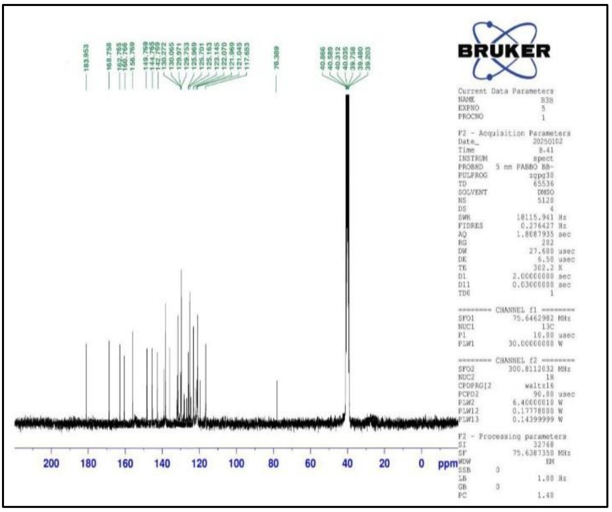


Figure 19 : 13CNMR of compound B3B

Antifungal Activity Study

The effectiveness of the prepared compounds (B3, B3A, B3B) as antifungals against Yeast Candida albicans was evaluated using biochemical, microscopic and fungal tests, as this fungus causes many common diseases that affect living organisms. Using DMSO as a solvent and using the disk diffusion method in the laboratory at concentrations (1, 0.5, 0.25, 0.125 mg/ml), the effect of the solvent on the isolation was determined and deducted from the final result, as the result of the biological test, as shown in Figure (20-21), indicated a high level of inhibition, as all compounds are characterized by high activity against (C. albicans) resistant to multiple drugs. Compound B3B showed the highest inhibition at 32 mm. By studying the relationship between the compounds, we conclude that the high activity is attributed to the presence of the NO2 functional group in the para position of the phenyl group, which withdraws the electron on the ring to increase the antifungal activity compared to the other substitution.

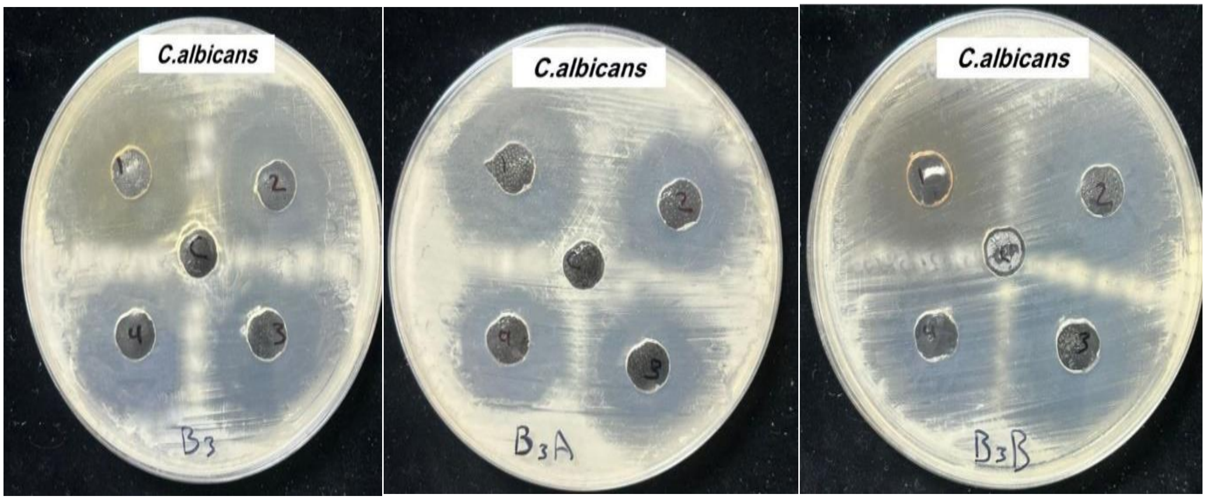
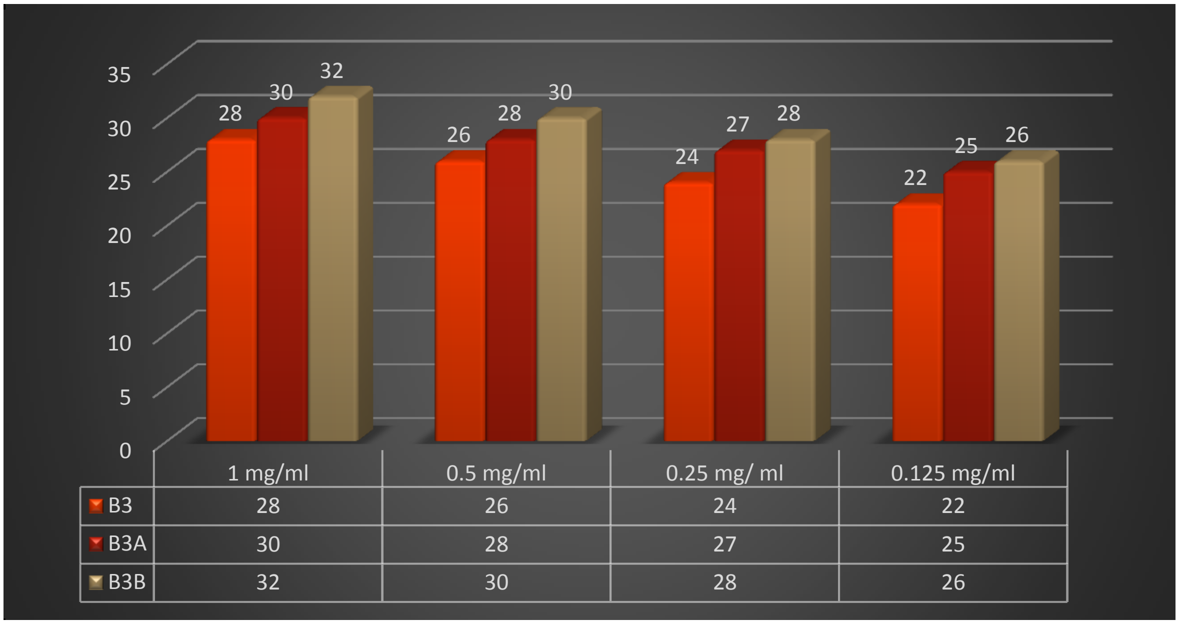


FIGURE 20: Antifungal evaluation of some synthetic compounds against ***Candida Albicans***



	1 mg/ml	0.5 mg/ml	0.25 mg/ ml	0.125 mg/ml
B3	28	26	24	22
B3A	30	28	27	25
B3B	32	30	28	26

FIGURE 21**.** Show the percentage of effectiveness of some prepared compounds

Study of Biological Activity Against Bacteria

The microbiological activity of synthesized compounds(B,B1,B2) was evaluated against two types of bacteria: Gram (-ve) bacteria (Acinetobacter baumannii) and (+ve) bacteria (Staphylococcus aureus), which showed greater effectiveness in killing bacteria. The potency was concentration-dependent, with the highest inhibition observed at 26 mm. The B1 compound exhibited the most significant activity against Staphylococcus aurous.Meanwhile, compounds (B1,B2)showed antibacterial activity at 24mm against Acinetobacter baumannii.The selected bacteria are thought to be the most dangerous and lethal variety. Thus, a thorough screening is conducted for these microorganisms in the hospital's operating rooms. Additionally, the synthesized compounds demonstrated superior action against bacterial strains, as shown in Table 2and (Figure 22,23).

TABLE 2. Biological activity against Bacteria of prepared compounds

Mate	Con µg /mL	Acinetobacter baumannii (-ve)	Staphylococcus aureus (+ve)
con	control	0	0
B	1	20	22
	0.5	16	17
	0.25	12	13
	0.125	0	11
B1	1	24	26
	0.5	17	20
	0.25	11	16
	0.125	0	11
B2	1	24	20
	0.5	20	18
	0.25	18	13
	0.125	0	0

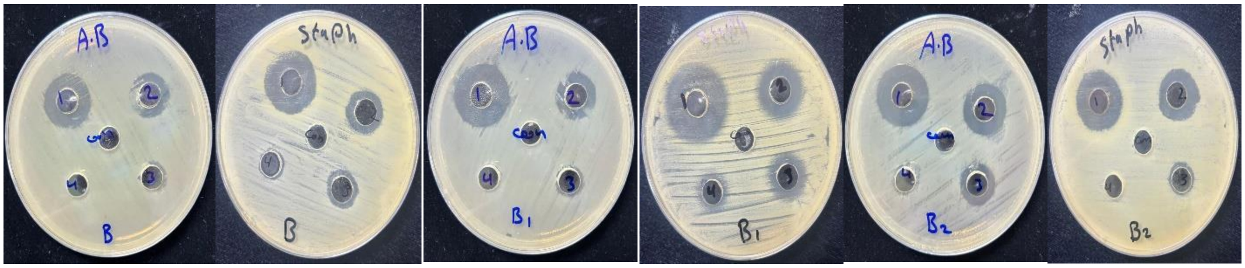


FIGURE 22**.** Inhibition zone of compounds (B,B1,B2) on *Acinetobacter baumannii* and *Staphylococcus aurous*

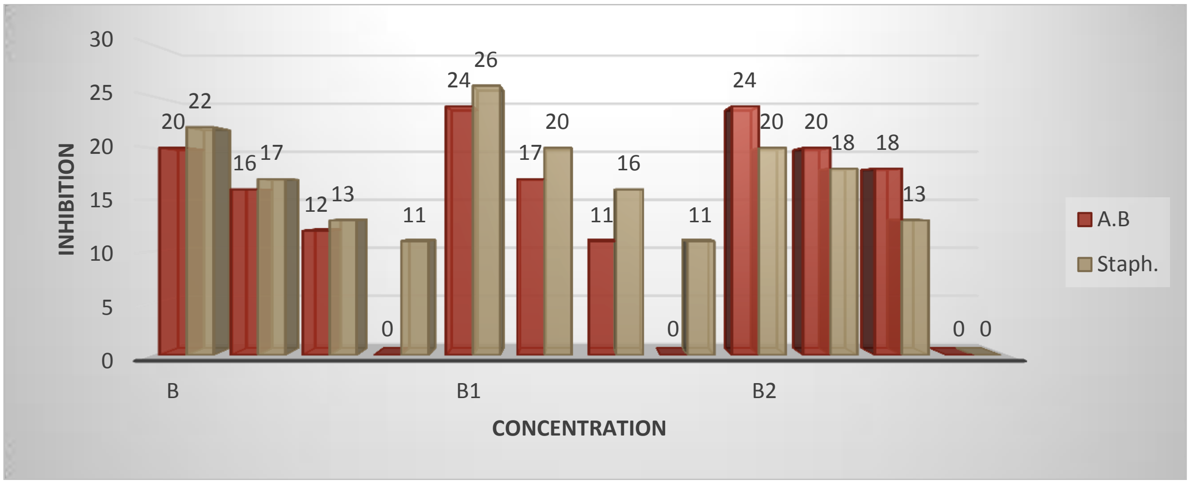


FIGURE 23**.** Antibacterial evaluation of prepared compounds against *Acinetobacter baumannii* and *Staphylococcus aurous*

CONCLUSIONS

- In this research, new heterocyclic derivatives were prepared from the reaction of two types Schiff base derivative with chloroethylacetate or (4-amino-5- fluoro-1H-pyrmidine-2-one).
- It was found that these derivatives are stable at room temperature. The other hand a high melting point. All these derivatives were confirmed by analysis of spectroscopic data
- Some of them were evaluated for their antifungal Yeast Candida albicans activity and others as antibacterial against two types of bacteria: Gram *(-ve*) bacteria (*Acinetobacter baumannii*) and (+*ve*) bacteria (*Staphylococcus aureus*) in the laboratory. The results of the bioassay indicated that some of the prepared compounds are very effective in killing fungi, as the derivative B3B showed the highest inhibition at 32 mm at the concentration (1 mg/mL).As for the other compounds that were tested as antibacterials, it was noted that they have inhibitory activity, as they proved effective in killing bacteria.
- The biological evaluation of the antibacterial and antfungi properties of the prepared compounds is attributed to the presence of a large number of heterogeneous atoms carrying electron pairs.

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