Preparation of Some Oxazepine Derivatives Derived from Benzimidazole Substitutes and Study of their Biological Activity

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ABSTRACT
Despite significant advancements in drug discovery, many diseases still lack effective treatments. Oxazepine derivatives, a class of heterocyclic compounds, have shown promising potential in many therapeutic areas, however, their discovery and development are still relatively limited. Accordingly, the research aims to prepare, characterize, and biologically evaluate new oxazepine derivatives derived from benzimidazole substitutes. The aim was to identify compounds with biologically active potential. Several oxazepine derivatives were prepared starting from the preparation of substituted benzimidazole (SA1) as a starting material with high productivity and then reacting it with ethyl chloroacetate in the presence of a base to obtain the corresponding ester (SA2). The ester (SA2) reacts with hydrazine to obtain hydrazide (SA3). Hydrazones (SA4-5) were obtained from the reaction of hydrazide (SA3) with different Benzaldehydes in the presence of glacial acetic acid as a catalyst. Finally, oxazepine derivatives (SA6-11) were obtained from the reaction of hydrazones with maleic anhydride, succinic anhydride, or phthalic anhydride. The prepared compounds were confirmed by physical methods like melting point and color and by spectroscopic methods using infrared spectroscopy (FT-IR) and proton magnetic resonance spectroscopy (1H-NMR). Some of the prepared compounds were biologically evaluated. Against three strains of bacteria: Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, and a type of yeast: Candida albicans, and compared with different antibiotics and antifungals. Some compounds showed biological activity against Gram-positive and Gram-negative bacteria and yeast, these results highlight the potential of this class of compounds for further exploration as potential drug candidates. Future studies could

1. Introduction

Benzimidazole was discovered during research on vitamin B12, where the benzimidazole nucleus was found to be a suitable structure on which drugs can be developed [1]. Benzimidazole derivatives are more effective and useful compounds from a medical point of view in addition to being broad-spectrum biochemicals. Benzimidazole derivatives have shown practical applications in various fields, where benzimidazole derivatives have been found to have many pharmacological activities, for example, antimicrobial [2] , antiviral [3], and powerful antidote for various types of cancer [4], analgesic[5] , antihypertensive [6] . On the other hand, Schiff bases or hydrazones are considered a source of oxazepines and other heterocyclic rings. Oxazepines refer to seven-ring heterocyclic compounds containing oxygen at the first position and nitrogen at the third position in addition to five carbon atoms [7] . The various pharmacological and biological functions that heterocyclic systems may perform have attracted the attention of researchers due to their broad pharmacological effects. Oxazepine derivatives (benzodiazepines) were originally used in the treatment of psychiatric disorders characterized by anxiety and tension. Oxazepines and their derivatives have been linked to a variety of biological and pharmacological effects, including antiepileptic, antifungal, anti-inflammatory, muscle relaxant, analgesic, and antibacterial activity [8].

Based on that, the objective of the research is to develop a series of novel oxazepine derivatives derived from various substituted benzimidazoles through a series of reactions and under specific conditions and the structural diversity and potential modifications that can enhance biological activity to characterize the synthesized oxazepine derivatives using spectroscopic techniques (e.g., 1H-NMR- FT-IR) and assess their purity and monitor the reaction through chromatographic methods.

2. Experimental Section

Material and methods

All chemicals used were produced by Fluka, BDH, and Aldrich Companies. Melting points were recorded in open capillaries using an electrothermal digital Stuart SMP30 melting point apparatus and were uncorrected. The infrared spectrum of the prepared compounds was measured in the central laboratory of the College of

Science/University of Mosul using an FT-IR Spectrophotometer, Bruker. 1H-NMR spectra were measured on a Bruker Ascend 400 MHZ spectrometer at the University of Basra/College of Education for Pure Sciences, in deuterated dimethyl sulfoxide (DMSO-d6) as a solvent and tetramethylsilane (TMS) as an internal standard. Thin layer chromatography (TLC) was used to monitor the reaction's progress and product purity. On pre-coated silica gel (60 F254) aluminum plates visualization was achieved in a UV chamber using a solvent mixture of Ethyl acetate and n-hexane (3:7). The biological activity of the compounds prepared was studied at the Department of Biology/College of Science/ University of Mosul.

CHEMISTRY

Synthesis 6-methyl-2-(3-nitrophenyl)-1H-1,3-benzimidazole (SA1)

Dissolve (0.0327 mol, 4 g) of 4-methyl-ortho-phenylenediamine in (30 ml) of absolute ethanol and add (0.0372 mol, 4.952 g) of 3-nitrobenzaldehyde and (0.0327 mol, 1.75 g) of ammonium chloride as a catalyst. Then the reaction mixture refluxed with stirring for (12) hours, this reaction was monitored by TLC, and the contents were poured into ice-cold water. Filtered, dried, and recrystallized by absolute ethanol, to give a dark brown precipitate M.P.135-137 °C, yielding 94% [9].

Synthesis ethyl [6-methyl-2-(3-nitrophenyl)-1H-1,3-benzimidazol-1-yl] acetate (SA2)

A mixture of (SA1) (0.0118 mol, 3 g) with (0.0118 mol, 1.64 g) of anhydrous potassium carbonate is heated in (40 ml) of dry acetone with continuous stirring for (15) minutes, then (0.028 mol, 3.43 g) is added of ethyl chloroacetate gradually, the mixture is refluxed for (9) hours this reaction was monitored by TLC, the contents were poured into ice-cold water. Filtered (20 ml x 3) is washed with distilled water and dried then washed several times using petroleum ether, afforded solid as brown M.P. 98-100 °C, yielding 83% [10].

Synthesis 2-[6-methyl-2-(3-nitrophenyl)-1H-1,3-benzimidazol-1yl] acetohydrazide (SA3)

A mixture of (0.0029 mol, 1 g) of ester (SA2) with (0.0118 mol, 0.59 g) of hydrazine hydrate (80%) was refluxed in (20 ml) of absolute ethanol for (6) hours, completion of the reaction was monitored by TLC, the reaction mixture was cooled, poured on to crushed ice the precipitate was filtered, dried and recrystallized from ethanol, to give a white precipitate M.P. 122-124 °C, yield 94% [11]

General procedure for Synthesis of hydrazone derivatives (SA4-5)

With stirring (0.003 mol, 1 g) of hydrazide (SA3) in (20 ml) of absolute ethanol, was added to the substituted benzaldehyde mixture (0.003mol), and add to it few drops of glacial acetic acid the mixture was refluxed for (9-11) hours. Monitored by TLC, Then the reaction mixture is poured into a beaker containing ice water. The resulting precipitate is separated by filtration and washed (10 ml x 3) of distilled water and dried, then washed several times using ether. It is recrystallized using a mixture of benzene/1,4-dioxane The properties of the compounds are shown in (Table 1) [12]

Table 1. Physical constants of hydrazine derivatives (SA4-5).

Comp.No.	R	m.p.(ºC)	Yield%	Color
SA_4	3-CH_3	129-131	86	Light brown
SA_5	4-$N(CH_3)_2$	202-204	72	Yellowish brown

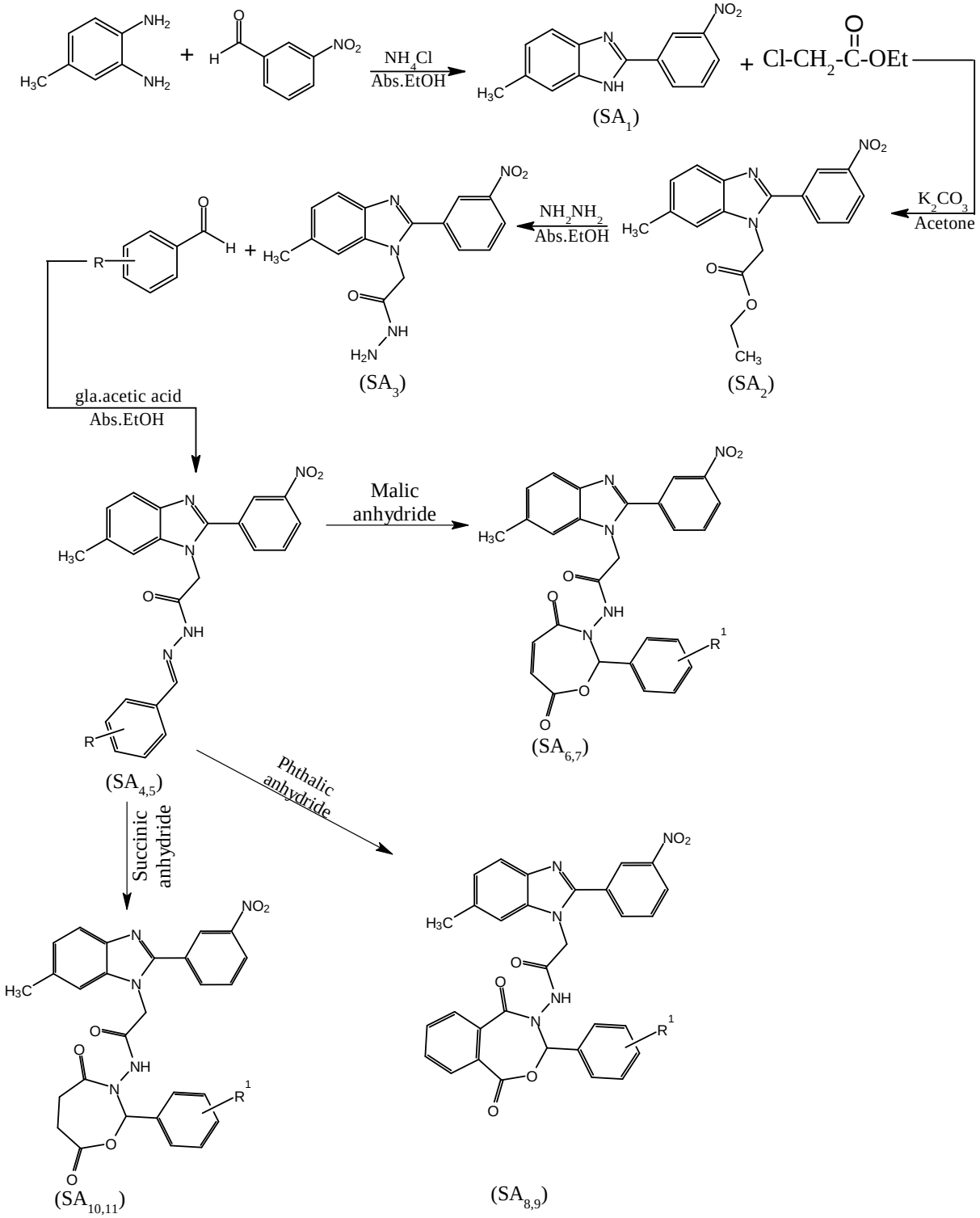
General Procedure for Preparing Substitutes Oxazepane. (SA6-11)

Dissolve (0.001 mol) of one of the hydrazone substitutes (SA6-11) in (15 ml) of dimethyl formamide, then gradually added to it (0.005 mol) of anhydrous maleic anhydride, succinic anhydride, or phthalic anhydride dissolved in (3 ml). of dimethylformamide, The mixture is refluxed with stirring for (18) hour, completion of the reaction was monitored by TLC, poured on to crushed ice. The resulting precipitate is separated by filtration and washed with (15 ml x 3) of distilled water. Then wash several times using benzene. The properties of compounds are shown in the (Table 2) and in scheme (1) [13]

Table 2. Physical constants of compounds (SA6-11).

Comp.No.	R	m.p.(ºC)	Yield%	Color
SA_6	4-$N(CH_3)_2$	209-211	63	Dark Brown
SA_7	3-CH_3	144-146	42	Dark Brown
SA_8	4-$N(CH_3)_2$	160-162	58	Light Brown
SA_9	3-CH_3	137-140	49	Light Brown
SA_{10}	4-$N(CH_3)_2$	94-96	88	Brown

SA_{11}	3-CH_3	254-256	75	Brown



R= 3-CH3 (SA4,7,9,11), 4-N(CH3)2 (SA5,6,8,10).

Scheme 1. The route of synthesis of benzimidazole substitute, hydrazone derivatives, and oxazepine derivatives.

Biological activity

In this study, we assessed the inhibitory activity of several compounds (SA3, SA4, SA5, SA6, SA11), against various Gram-positive and Gram-negative bacteria, including: Staphylococcus aureus 6538, Escherichia coli 11229, Pseudomonas aeruginosa 95110 as well as yeast, including: Candida albicans . The inhibitory

effectiveness was compared using the antibiotics listed in (Table 3).

Table 3. Shows the antibiotics and antifungals used.

Antibiotics and antifungals	Abbreviation	Concentration µg/Disc	Producing company
Ciprofloxacin	CIP	10	General Company for the Manufacture of Medicines and Medical Supplies / Samarra-Iraq
Amoxicillin	AX	10	
Azithromycin	AZM	15	
Itraconazole	ITR	50	Bioanalyse(turkey)
Amphotericin B	AMB	20	
Nystatin	NY	100	

inhibitory activity test

The inhibitory activity of the prepared compounds under study was tested using the sensitivity test method (disc diffusion method) and based on the method of Bauer and his group [14] according to CLSI [15], where the bacterial suspension was prepared in the nutrient broth medium at a concentration of (108) cells/cm3 compared to McFarland tube No. (0.5) cells/cm3 of the suspension and inoculated using a sterile cotton swab on (Mueller Hinton agar-Oxoid) medium, then the plates were incubated at room temperature for (10-15) minutes, for impregnation to occur. To study the antibacterial efficacy of the prepared materials on the growth of bacteria, filter paper discs (Whatman No. 1) with a diameter of (6 mm) were prepared, saturated with a concentration of (200 mg/ml) of the selected prepared compounds, and dimethyl sulfoxide DMSO was used as a solvent. Then, the discs were fixed with sterile forceps on the surface of the inoculated plates with the use of antibiotics and fungicides shown in Table (6) for comparison. The plates were incubated at a temperature of (37 0C) for (18-24) hours. After the incubation period ended, the diameters of inhibition around the discs were measured [16]

3. Results and Discussion

Chemistry

The synthesis of oxazepine derivatives from benzimidazole substituents typically involves a multi-step approach as in scheme (1). The starting material (SA1) was prepared by reacting 4-methyl-ortho-phenylenediamine with 3-nitrobenzaldehyde in the presence of ammonium chloride as a catalyst which is an environmentally friendly and inexpensive catalyst, as it works to shorten the reaction time and increase productivity up to 94%. Following its diagnosis by (1H-NMR), the compound was characterized (FT-IR) by the appearance of a characteristic nitro band at (1345 cm-1) belonging to (C-NO2 sym.) and at (1519 cm-1) belonging to (C-NO2 asy.), in addition to other bands. Then, the ester (SA2) was prepared by reacting the starting material with ethyl chloroacetate in dry acetone in the presence of K2CO3 as a base. The reaction includes the nucleophilic displacement of chlorine in the ethyl chloroacetate compound by the amine group in the starting material. The ester is characterized by the appearance of an ester carbonyl stretching band at (1739 Cm-1)[17]. Then, the ester was reacted with aqueous hydrazine to obtain hydrazide (SA3), which is adsorbed by the appearance of an amide carbonyl stretching band at (1665 Cm-1). It was observed that the absorption of the carbonyl group of the hydrazide was shifted to a lower frequency compared to the absorption of the ester group. This is attributed to the presence of the resonance phenomenon in the hydrazide, which leads to the reduction of the double bond character (C=O), and thus the force constant of this bond decreases and its frequency decreases [18]. The Hydrazide compound (SA3) was converted to a Hydrazone (SA4-5) derivative via the reaction with different aromatic aldehydes in ethanol absolute as a solvent and glacial acetic acid as a catalyst. FTIR spectral data of compounds, the appearance of clear absorption bands at (1618,1602Cm-1). for (C=N) amine and carbonyl band in rang at (1665,1650 Cm-1)[19]. All details of FTIR spectral data of compound (3-7) were listed in Table 4. In addition to diagnosis by1H-NMR in table 6. Oxazepine substitutes (SA6-11) were prepared by ring-closure, which includes the reaction of the prepared hydrazones (SA4-5) with anhydrous maleic anhydride or succinic anhydride or phthalic anhydride in the presence of dimethylformamide as the solvent to form the product, which is the oxazepine compound with a heterogeneous seven-membered ring. The prepared compounds were identified spectroscopically by 1H-NMR- FT-IR, as shown in the following (Table 4-6) and figure (1,2).

Table 4. FT-IR absorption spectra data (Cm-1) of the prepared compounds.

Comp. No.	vN-H	vC-H arom.	vC-H alph.	vC=O	vC=N	others
SA_1	3153	3097	2981,2923,	-	1651	as.1519(C-NO_2)

SA_2	-	3088	2858 2978,2919 2863	1739	1621	sy.1345(C-NO_2) as.1202(C-O-C) sy.1165(C-O-C)
SA_3	3087	3034	2920,2861	1665	1621	as.3304(NH_2) sy.3195(NH_2)
SA_4	3198	3040	2920,2862	1665	1602	-
SA_5	3187	3035	2918,2893	1650	1618	-

Table 5. FT-IR absorption spectra data (Cm-1) of the prepared compounds.

Comp. No.	R	vC=O Lactone	vC=O Lactam	vC=O amide	C-N	C-O-C	Ar C-C
SA_6	4-N(CH_3)$_2$	1778	1693	1682	1277	as.1229 sy.1127	1455
SA_7	3-CH_3	1755	1680	1657	1286	as.1253 sy.1125	1487
SA_8	4-N(CH_3)	1730	1692	1655	1239	as.1254 sy.1161	1454
SA_9	3-CH_3	1774	1678	1657	1286	as.1254 sy.1125	1488
SA_{10}	4-N(CH_3)$_2$	1795	1739	1661	1278	as.1217 sy.1125	1455
SA_{11}	3-CH_3	1798	1747	1684	1287	as.1254 sy.1104	1456

Table 6. 1H-NMR spectra data (ppm) of the prepared compounds.

Comp. No.	^{1}H-NMR δ (ppm) DMSO-d_6
SA_1	9.04 (s, 1H), 8.64 (d,1H), 8.34 (d, 1H), 7.87 (t, 1H), 7.53 (s, 1H), 7.43 (d, [13.22 (s, 1H) NH, Ar-H, 2.43(s,3H) CH_3.] 1H), 7.10 (d,1H)
SA_2	8.53 (s, 1H) , 8.43 (d, 1H), 8.24 (d, 1H), 7.90 (t, 1H), 7.50 (s, 1H), 7.21 (d, 1H), 7.12 (d, 1H[Ar-H, 5.34 (s, 2H) CH_2, 4.25 – 4.14 (q, 2H) CH_2-O, 2.50 (s,3H) CH_3, 1.23 (t, 3H) CH_3ester.]
SA_3	8.79 (s, 1H), 8.64 (d, 1H), 8.36 (d, 1H), 7.89 (t, 1H), 7.59 (s, 1H), 7.47 (d, [9.04 (t, 2H) NH, Ar-H , 4.94 (s, 1H) CH_2, 4.51 (d, 1H) NH_2, 2.48 (s, 3H) CH_3.]1H), 7.22 (d, 1H)
SA_4	7.55 ، 7.62 (s, 1H)، 7.83 (t, 1H)، 8.21 (d, 1H)، 8.34 (d, 1H)،8.53 (s, 1H)[11.88 (s, 1H) NH, Ar-H, 8.02 (s, 1H) CH=N, 5.58,] 7.43(d, 1H) , 7. 31(s, 1H), 7.24(d, 1H)، 7.49 (t, 1H)،(s, 1H) .(s, 2H) CH_2CO, 2.45 (s, 3H) CH_3Ph, 2.32 (s, 3H) CH_3
SA_5	8.60 (s, 1H), 8.39 (d, 1H), 8.26 (d, 1H), 7.92 (t, 1H), 7.55 (d, 2H), 7.45 (d, [11.64 (s, 1H) NH, Ar-H, 8.15 (S, 1H) CH=N, 5.56 (s, 2H) CH_2CO,]1H), 7.21 (s, 1H), 7.18 (d,1H), 6.76 (d, 2H) 3.00 (s, 6H)2CH_3Ph, 2.53 (s, 3H) CH_3Ph.
SA_6	8.60 (s, 1H), 8.40 (d, 1H), 8.26 (d, 1H), 7.83 (t, 1H), 7.74 (s, 1H), 7.63 (d, [11.64 (s, 1H) NH, Ar-H, 8.10 (s,1H)CH-]2H), 7.52 (d, 2H), 7.41 (d, 1H), 7.14 (s, 1H), 6.76 (s, 1H), 6.72 (d, 1H) N, 5.52 (s, 2H)CH_2CO, 3.00 (s, 6H)2CH_3, 2.54 (s, 3H)CH_3Ph.
SA_7	8.54 (s, 1H), 8.37 (d, 1H), 8.21 (d, 1H), 7.85 (t, 1H), 7.64 (s, 1H), 7.52(d, [11.88 (s, 1H)NH, 1H), 7.49 (d, 1H), 7.43 (s, 1H), 7.31 (t, 1H), 7.24 (d, 1H), 7.13 (d, 1H), 5.59 (s, 1H), 5.58 (s, Ar-H, 8.03 (s, 1H)CH-N, 5.10 (s, 2H)CH_2CO, 2.43 (s, 3H)CH_3Ph, 2.32 (s, 3H)CH_3.]1H)
SA_8	8.99 (s, 1H), 8.61 (d, 1H), 8.41 (d, 1H), 8.21 (t, 1H), 7.94 (d, 2H), 7.82 [11.66 (s, 1H)NH, Ar-H, 8.28 (s,](d,2H), 7.62 (d, 1H), 7.54 (s, 1H), 7.41 (d, 2H), 7.21 (d, 1H), 7.15 (d, 2H) 1H)CH-N, 5.32 (s, 2H)CH_2CO, 2.84 (s, 6H)2CH_3, 2.45 (s, 3H)CH_3Ph.
SA_9	8.66 (s, 1H), 8.35 (d, 1H), 8.30 (d, 1H), 8.21 (t, 1H), 7.81 (d, 2H), 7.76 [11.27 (s, 1H)NH, Ar-H, 8.05 (s,]H)1(d,2H), 7.62 (d, 1H), 7.50 (s, 1H), 7.41 (d, 2H), 7.19 (d, 1H), 7.15 (d, 1H) CH-N, 5.20 (s, 2H) CH_2CO, 2.45 (s, 3H)CH_3,2.31 (s, 3H)CH_3 Ph.

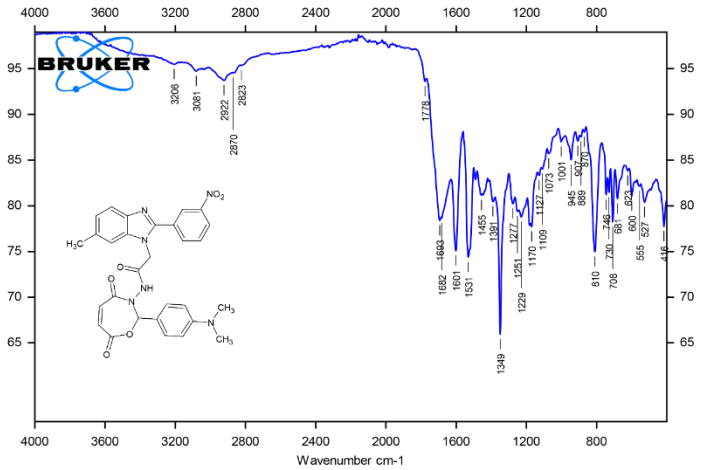


Figure 1. FT-IR. Spectrum of Compound (SA6).

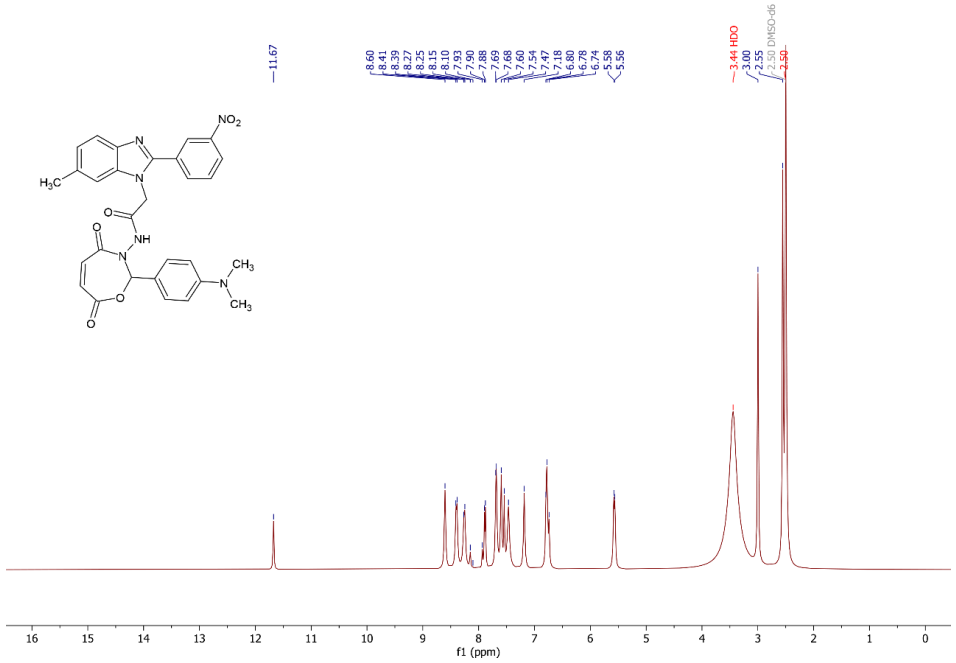


Figure 2. 1HNMR. Spectrum of Compound (SA6).

BIOLOGICAL

The results of the inhibitory activity test of the prepared compounds (SA3, SA4, SA5, SA6, SA11), under study and using the disk diffusion method, showed a varying effect on the bacteria and yeast under study, which were chosen due to their importance in the medical field, as they cause several diseases. In addition, they differ in the nature of their resistance to antibiotics and therapeutic chemicals, and this effect differs according to the type of prepared compounds as well as the type of microorganisms used. The results shown in (Table 7) indicate that some of the tested compounds can inhibit the bacteria and yeast used.

Table 7. Inhibitory activity of the prepared compounds on the growth of some Gram-negative and Gram-positive bacteria and yeast compared to antibiotics (inhibition circle diameter measured in mm.

Comp.NO. / Microbiology	SA_3	SA_4	SA_5	SA_6	SA_{11}	CIP	AX	AZM
Staphylococcus aureus	R	R	15	10	R	45	30	30
Escherichia coli	33	R	R	R	R	40	R	20
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R
Candida albicans	R	R	R	9	R	ITR R	AMB R	NY R

R: Resistance, CIP: Ciprofloxacin, AX: Amoxicillin, AZM: Azithromycin.

ITR: Itraconazole, AMB: Amphotericin B, NY: Nystatin.

The compounds (SA5, SA6) showed inhibitory values against Staphylococcus aureus bacteria ranging from (15, 10) mm, respectively, and these values were less than the values of the control factor (antibiotics) under study. As for the compound (SA3), it showed high inhibitory activity against Gram-negative Escherichia coli bacteria, which was (33) mm, which was close to the effect of Ciprofloxacin, which was (40) mm, and better than the effect of Azithromycin, which reached (20) mm, and Amoxicillin, which showed resistance to it. As for Candida albicans yeast, the compound (SA6) showed an inhibition diameter of (9) mm, and the yeast was resistant to all the antifungals used as in Figure (3), while all the compounds and antibiotics were resistant to Pseudomonas aeruginosa bacteria, this effectiveness is due to the presence of heterocyclic compounds and the functional groups within them.

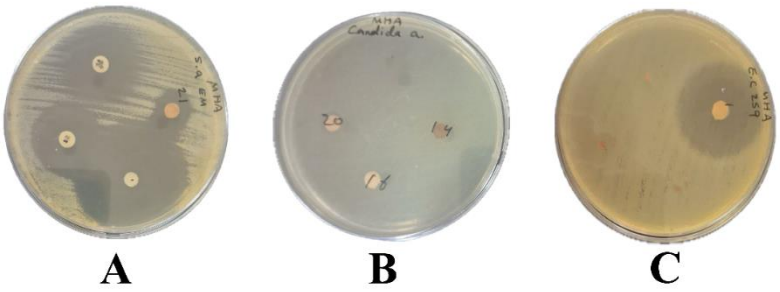


Figure 3. Biological activity of: A. (SA6, Ciprofloxacin, Amoxicillin, Azithromycin) against Staphylococcus aureus, B. (SA4,6,11) against Candida albicans, C. (SA3) against Escherichia coli.

4. Conclusion

Based on our findings, we have prepared the compound 6-methyl-2-(3-nitrophenyl)-1H-1,3-benzimidazole using the ammonium chloride catalyst, which is environmentally friendly, low cost, and gives an excellent yield of (94%). We have proven that different hydrazones of benzimidazole compound can be prepared after converting it to the ester and the corresponding hydrazide and then reacting it with several aldehydes. Hydrazones prepared several oxazepine derivatives by reacting with some available carboxylic acid anhydrides. The results obtained proved the accuracy and purity of the prepared compounds. On the other hand, the biological activities of the prepared compounds were evaluated against three strains of bacteria: Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, and against a type of yeast: Candida albicans, and compared with different antibiotics and antifungals. Finally, the validity of the preparation of all the prepared compounds was studied using the physical properties as well as conducting spectral measurements including the FT-IR spectrum and the 1H-NMR spectrum of the protons present in the prepared compounds.

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