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Synthesis and Characterization of New Azolidinone Derived from Creatinine and Study their Biological Activity

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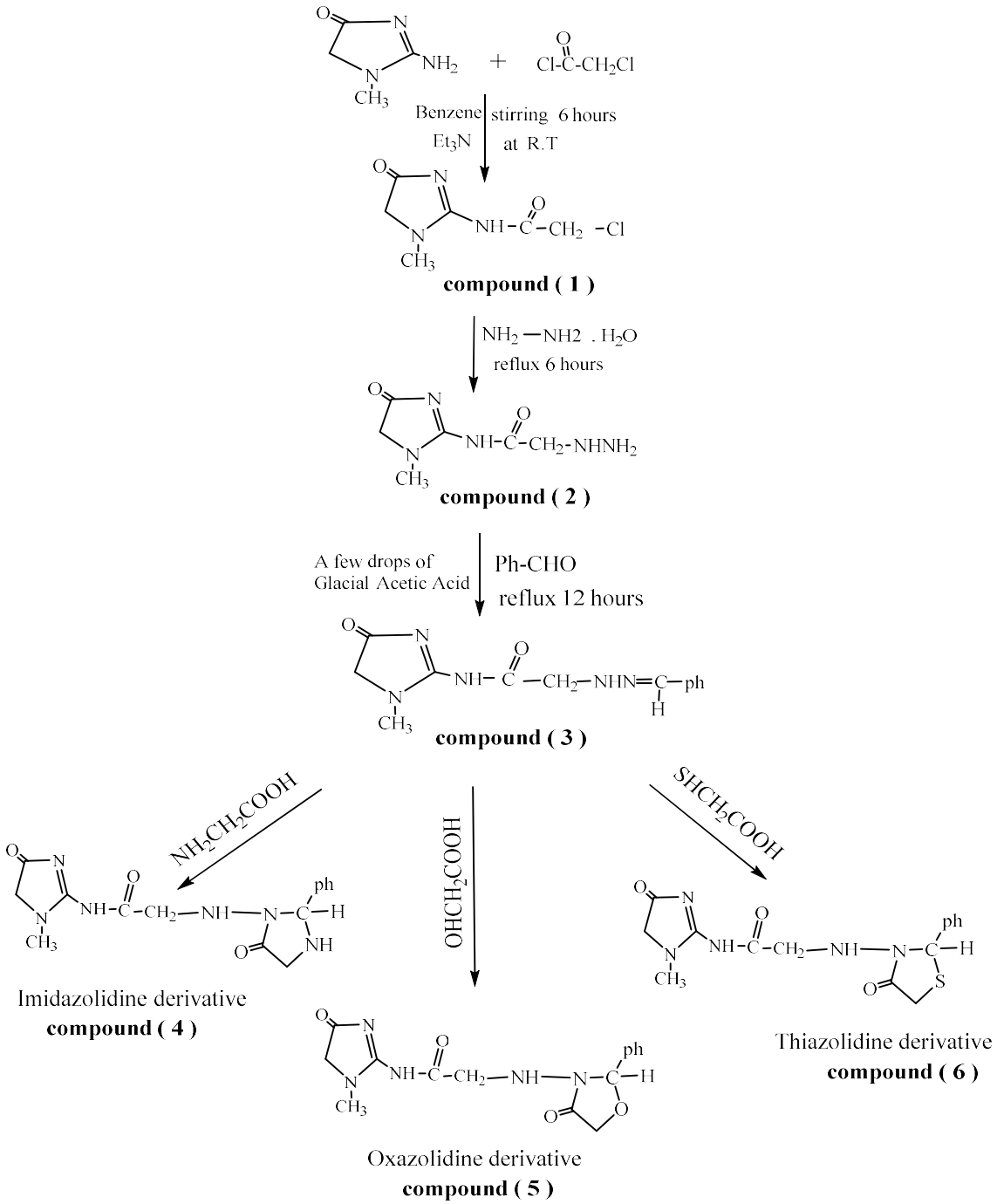
Abstract
This work consists of preparation new compounds using creatinine as a starting material carried out in four stages, where in the first stage, creatinine reacts with chloroacetyl chloride, leading to formation compound (**1**) which enters into the reaction of the second stage with hydrazine hydrate (99 percent), giving compound (**2**). In the third stage, aromatic aldehyde is used to prepare Schiff base compound(**3**).The resulting Schiff bases are used in ring-closing reactions with glycine, glycolic acid and thioglycolic acid to yield (**4, 5, 6**) respectively as final product. The organized compounds were uniqued by using IR spectroscopic method and ^{1}HNMR. The biological activity diagnosed for the prepared compounds and showed promising results.
The work aims to study the properties of creatinine derivatives and determine their effectiveness and biological attributes.

Keywords: Creatinine, Azolidinone, Schiff base , Biological activity

1. Introduction

Creatinine a product derived either from creatine phosphate in the muscles or directly from creatine by non-enzymatic catalysis, and is typically created at a persistent ratio by the body[1]. Traditional reference levels for humans are 0.5to1.0 mg/dL (nearly 45-90 µmol/L) for females and 0.7 to1.2mg/dL(60-110 µmol/L) for male[2], Creatinine level higher than indicate kidney dysfunction that blood creatinine helps assess the efficiency of kidney function. Recently, many studies have emerged that confirm the importance of creatinine, as some studies have proven that it has effective biological advantages[3]. while other studies have shown that it has antioxidant and anti cancer properties[4]. Creatinine molecule contains a good active group, the amine group, which reacts with chloroacetyl chloride, the resulting compound reacts with hydrazine hydrate, forming the primary amine which reacts easily with the aromatic aldehyde to obtain the Schiff bases. Schiff bases are an important compounds owing to their extremely broad range of biological apps, straightforwardness of synthesis and chelating properties [5]. Schiff bases are renowned dating back to 1864 when HugoSchiff stated the condensation of primary amines with carbonyl

compounds [6]. Currently, this field copes with Schiff base coordination chemistry has extended immensely. The prominence of Schiffbase have been proven in bioorganicchemistry, biomedical applications, supramolecular chemistry, catalysis and material science[7]. In this work, cyclo-addition of Schiff base or hydrazone with glycine, glycolic acid and thioglycolic acid to synthesis new azolidinone derivatives[8]. Azolidinone is a 5-membered heterocyclic ring having a nitrogen atom and a carbonyl group in addition to another atom which may be oxygen, nitrogen or sulfur[9]. It shows potential medicinal properties with favourable antibacterial activity[10,11]. Many studies have studied the properties of this class of compounds, which have proven their therapeutic potential.These derivatives include a broader range of biological actions such as, antibacterial, antifungal, hypnoticmusclerelaxant, antagonistic, antiepileptic inflammatory and antimicrobial properties[12,13,14].



Scheme 1: Synthetic rote of synthesized compound

2. Experimental part

2.1. Materials and Methods

With no prior purification, all chemicals were taken from Sigma-Aldrich. The SHIMADZU FTIR-8400 Foureir transform IR spectrophotometer was meticulously adopted with KBr disc in

the Department of Chemistry, University of Baghdad. 1HNMR spectra were documented on Ultra Sheild 400 MH, with tetramethyl silane as the interior standard and DMSO as solvent.

2.2. Methods of the synthesis of compounds

*Compound Synthsis (**1**)* [15]

A mixture of (1g , 0.008mol) creatinine , (0.03 mL) chloroacetylchloride , and few drops of Et_3N in 20mL dry benzne was stirring for 6 hrs at room temperature and after then the mixture evaporated the solvent and recrystallized from Dioxan.

*Synthesis of compound (**2**)* [16]

To a solution of compounds [1] (0.01 mole) in 20 mL absolute ethanol, hydrazine hydrate (0.03 mole, 99 percent, 1.5 mL) was added. Later, refluxation was made to the reaction mixture for 6 hrs, then evaporating solvent, and the created precipitate was recrystallized from Dioxan.

*Synthesis of compound (**3**)* [17]

To a solution of aromatic aldehydes (0.01 mole) (benzaldehyde) in manifestation of a scarce drops of glacial acetic acid, (0.01 mole) of compound(2) in 15 mL of dry 1,4-dioxane were added. Refluxation was made to this mixture for 12 hours, evaporating the excess solvent and the created precipitate was recrystallized from chloroform.

*Synthesis of compound (**4**)* [18]

2-aminoaceticacid (0.001 mole) mixed in a round-bottomed flask with Schiff bases (compound3) (0.001mole)in(10 mL) dry Benzene. The reaction mixture got heated at $80C^0$ for (14-16) hrs. Once cooled, the precipitate was collected and recrystallized via ethanol.

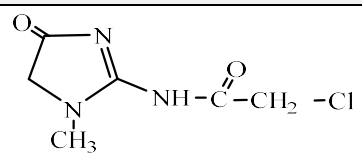
*Synthesis of compound (**5**)* [19]

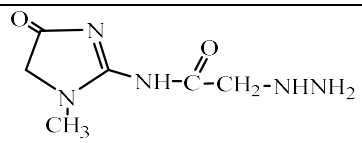
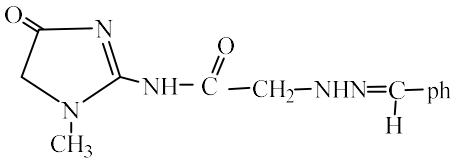
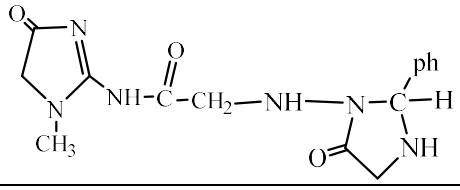
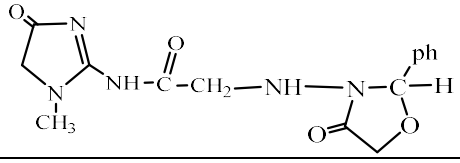
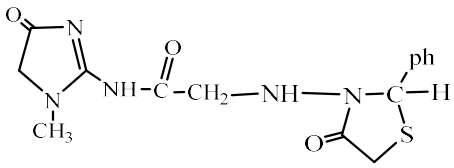
Compound3 (0.001 mol), (10 mL) dry Benzene, glycolic acid (0.001 mole) and anhydrous zinc chloride (0.0016 mole). The mixture got heated at $80C^0$ for (14-16) hrs. Then the mixtureof reaction pourly discharged onto crushed ice, and the outcome precipitate filtered and recrystallized from ethanol.

*Synthesis of compound (**6**)* [20]

In a round-bottomed flask, Schiff bases (compound3) (0.001 mol) , 2- mercapto acetic acid (0.001mole) was dissolved in(10)mL of dry Benzen, and anhydrous zinc chloride (0.0016 mole) were put. The mixture got heated at 80 C^0 for (14-16) hours. We carefully poured the reaction mixture over crushed ice, which caused a solid to form. We then filtered out this solid, let it dry, and finally purified it by recrystallizing it from ethanol.

Table 1: Physical properties for the synthesized compounds (1-6)

Com pNo.	Structure	Molecular formula	M.wt g/mol	Yiel d (%)	mp (°C)	Color
1		$C_6H_8N_3O_2Cl$	189. 52	82	210-212	White

2		$C_6H_{11}N_5O_2$	185.09	68	185-187	dark Brown
3		$C_{13}H_{15}N_5O_2$	273.61	78	166-168	Off White
4		$C_{15}H_{18}N_6O_3$	330.63	72	178-180	Yellow
5		$C_{15}H_{17}N_5O_4$	331.61	63	135-137	Pale Yellow
6		$C_{15}H_{17}N_5O_3S$	347.23	67	123-125	White

biological activity

To test how effective certain materials (1-6) were against various bacteria and fungi, we used a culture medium called Müller-Hinton[21]. First, we dissolved the Müller-Hinton agar and sterilized it in an autoclave at 121°C for 15 minutes. Once sterilized, we poured it into Petri dishes and let it solidify. After the agar was ready, we introduced specific microorganisms onto the plates . Next, we added 0.1 mm of each material to the center of the plates, which had already been contaminated with bacteria or fungi, to see how well they could inhibit microbial growth. Then placing plates in an incubator at 37°C—24 hrs for bacteria and 48 hrs for fungi. After incubation, we observed clear zones around the materials where the microorganisms couldn't grow. The size of these zones varied depending on the material and the type of microorganism being tested . All the results from these experiments are neatly summarized in Table (4).

3. Results and discussion :

In the initial reaction, creatinine reacts with chloraocetyl chlorid to give a creatinine derivative compound(**1**). The derivative of creatinine was reacted with hydrazine hydrate to form acid hydrazide derivative compound (**2**)which then reacted with aromatic aldeheydes to form Schiffe bases derivatives compound (**3**). Then, the resulting Schiff bases were given a cyclization with glycine, glycolic acid, and thioglycolic acid to produce imidazolidine-4-one compound (**4**), oxazolidine-4- one compound (**5**) and thiazolidine-4-one compound (**6**) respectively . The FITR of the compounds revealed the appearance of the N-H band, NH2 band, and (C=O) band of

hydrazide. While, in Schiff bases the existence of C=N bands were revealed, as shown in Table (2) which includes these bands as well as additional bands.

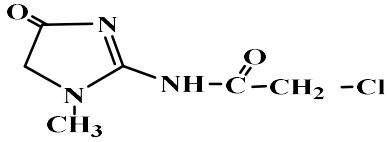
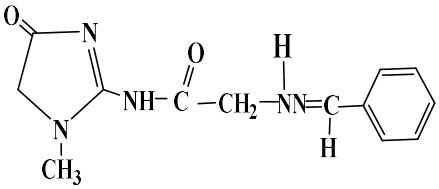
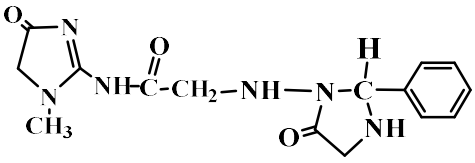
Table 2 : FT-IR spectral data of synthesized compuonds (1-6) in cm-1

Comp No.	υ NH	υ C-H aromatic	υ **C-H** aliphatic	**υ C=O** cyclic amide	**υ C=O** amide	**υC=N** Creatinine ring	other
1	3281	----	Asym 2945 Sym 2833	1720	1689	1643	C-Cl 648
2	3263	----	Asym 2971 Sym 2825	1703	1693	1656	NH_2 ASym 3438 Sym 3368
3	3277	3089	Asym 2911 Sym 2793	1716	1686	1661	C=N imine 1638
4	3225	3073	Asym 2949 Sym 2789	1696	1688	1668	N-H 3236
5	3234	3097	Asym 2928 Sym 2842	1722	1695	1654	C-O 1252
6	3289	3105	Asym 2932 Sym 2795	1708	1683	1663	C-S 648

The FTIR spectrum of compound [**1**] showed the appearance of significant characteristic stretching bands at (3281 cm -1), which corresponded to υ(NH), (1689 cm-1) due to υ(C=O) amide, (648 cm -1) due to υ(C-Cl), (1720 cm-1) due to υ(C=O) cyclic amide and (2945, 2833 cm-1) due to υ(C-H) aliphatic. It has been observed that the υ (NH_2) band for starting material creatinine has disappeared. Compound [**2**] have been found to have FTIR spectra where the (C-Cl) band has disappeared, the appearance of absorption in the (3438,3368 cm^{-1}) due to υ(NH_2) band. Compound [**3**] significant characteristic bands at (3089 cm^{-1}) ownig to aromatic group and (1638 cm^{-1}) which corresponded to υ(C=N) imine group. Compounds [**4,5,6**]showed disappeared of significant characteristic for υ(C=N) imine group. Compound [**5**] showed the appearance of absorption in the (1252 cm^{-1})due to the υ(C- O) band While Compound [**6**] appearance of significant at (648 cm^{-1}) due to the υ(C- S) band.

The 1HNMR spectra of compounds [1,2,3, and 4] revealed signals at δ (2.04 – 2.76 ppm) due to (s,3H, N-CH3), other signals at δ (2.80 – 3.31ppm) due to (s,2H, CH3-N-CH_2) of imidazoline ring, signals at δ (2.03-2.4) due to (m,1H,C=O-CH_2) of amide and signal at δ (6.3ppm) due to(s,1H, N=CH) of Schiff bases[22] , also signal at δ (4.21– 4.36 ppm) to (s,1H, NH-C=O), at δ (2.47ppm) due to (d,2H, -NH_2) . These signals and others are revealed in table (3), and showed in Figures (1,2).

Table 3: 1H-NMR of some new prepared compounds

Compo No.	Compound structure	1H-NMR spectral data (δ ppm)
1		1H-NMR (400 MHz, DMSO): δ 2.25 (m, 2H, CH2C=O) amide δ 2.75 (s,3H,N-CH3)of imidazolin ring δ 3.29 (s,2H,CH2-C=O)imidazolin ring δ 4.36 (s ,1H,NH)
2		1H-NMR (400 MHz, DMSO): δ 2.3 (m, 2H, CH2C=O) amide δ 2.47 (d,2H, -NH2) δ 2.57 (s,3H,N-CH3)imidazoline ring δ 2.80(s,2H,CH2-C=Oimidazoline ring δ 4.21 (s ,1H,NH) δ 4.15-4.18 (m,1H,NH)
3		1H-NMR (400 MHz, DMSO): δ 2.1 (m, 2H, CH2C=O) amide δ 2.76(s,3H,N-CH3)imidazoline ring δ 3.31(s,2H,CH2-C=O)imidazoline ring δ 4.12- 4.14(m,1H, NHN=CH-Ph) δ 4.32 (s ,1H, NH) amide δ 6.3 (s,1H,N=CH-ph) of Schiff bases δ 8.15-8.39 (m,5H,Ar-H.)
4		δ 2.03 (m, 2H, CH2C=O) amide δ 2.04 (s,3H,N-CH3)imidazolin ring δ 2.50 (s,1H,N-CH-Ar) δ 2.92 (s,2H,CH2-C=O) imidazolin ring δ2.99(s,2H,CH2-C=O) imidazolidin ring δ 4.26 (s ,1H, NH) amide δ 4.31- 4.18(m,1H, NH-N) δ4.33(q,1H,C-NH) imidazolidin ring δ 7.21-7.63 (dd,5H,Ar-H.)

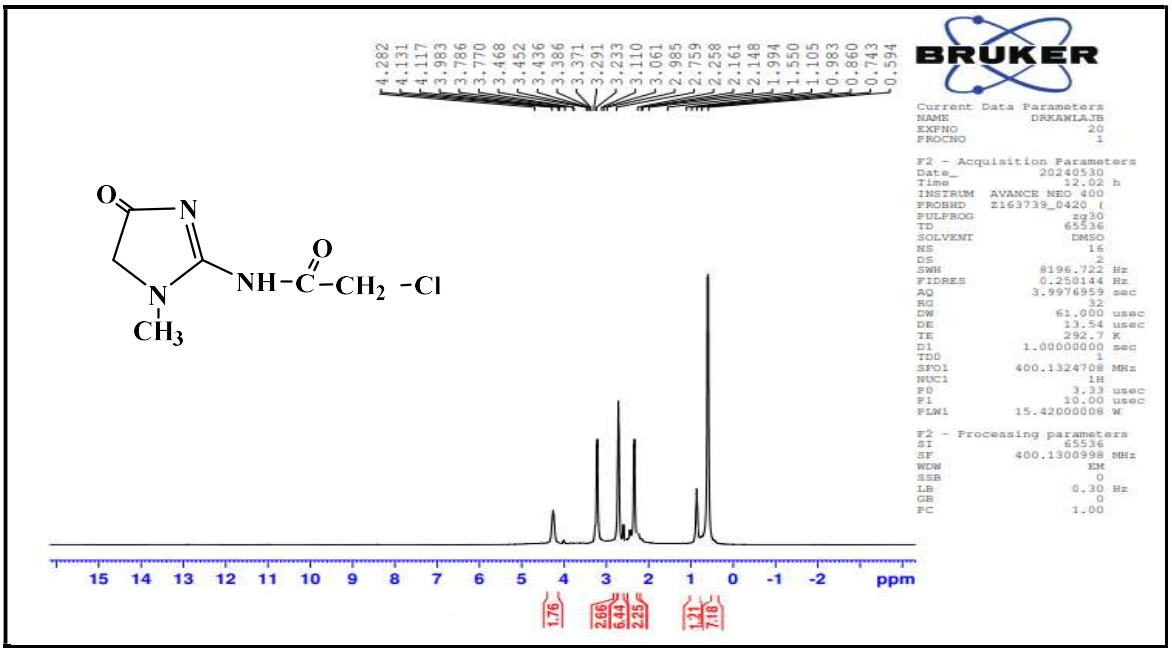


Figure (1): ^{1}H-NMR spectrum for compuond [1]

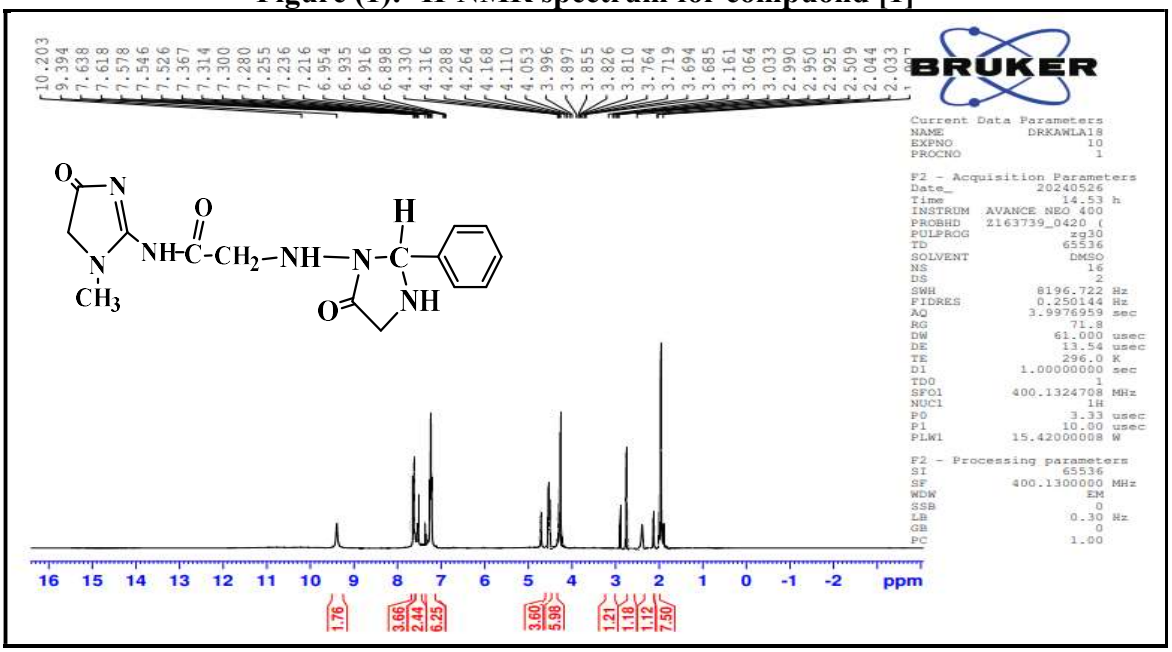


Figure (2): 1H-NMR spectrum for compound [4]

Biological activity

The synthetic compounds(**1–6**) demonstrated different biological effects against the gram-positive *Streptococus aureus*, *Bacillus* and gram-negative bacteria *Escherchia coli*, *Klebsiella* with (Amoxicillin) as standard as well as type of fungi *Candida albicons* in contrast to the standard(Fluconazole drug)[23]. As seen in the results, compound (**5**) has the highest activity against *Streptococcus* aureus. While,compounds (**2,4**) showed the highest activity against *Bacillus*. compounds (**4,5**) showed highest effect against *Escherichia coli*, and *Klebsiella*.As for *Candida albicans*, compounds (**1,5,6**) have shown their strong effectiveness against this type of fungus. While, compound (**3**) showed moderate activity against all type of bacteria and fungi, as shown

in table 4 and Figures (3).

Table 4: Biological activities of prepared compounds

Comp. no	Streptococcus *(+ve)*	*Bacillus (+ve)*	*Escherichia coli (- ve)*	*Klebsiella (- ve)*	*Candida (fungus)*
1	12	6	----	5	25
2	16	27	12	12	9
3	11	8	8	6	14
4	8	27	25	24	----
5	25	13	22	21	27
6	10	9	----	-----	25
Amoxicillin	18	25	20	27	-----
Fluconazole	------	-----	-----	-----	30

Conc. (0.01 g/ml)
Inhibition zone was measured in (mm)
(DMSO) used as solvent

Figure (3): Biological activities of prepared compounds

4. CONCLUSIONS

In the present research, new Creatinine derivative (**1,2**) and Schiff bases (**3**) were used to synthesize imidazolidine-4-one(**4**), oxazolidine-4-one(**5**), thiazolidine-4-one(**6**) compounds. The identification of these novel compounds was centered on spectrum datum (FT-IR and 1H-NMR). Additionally, The biological activity test was conducted, which included antibacterial and antifungal properties, and it was found that the compound (**4,5**) have activity against gram-positive bacteria *Streptocccus* aureus, *Bacillus* and gram-negative bacteria *Escherchia coli*, *Klebsiella*. While, compound (**1,5,6**) showed antifungal activity. So the biological activity of creatinine derivatives was confirmed by this study.

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