

Synthesis, Characterization and Biological Activity of New Amides Containing Azo Moieties

ABSTRACT

Three new amide compounds **6**, **8** and **9** which are derived from diazo dye **4** were synthesized, characterized, and tested against three types of bacteria. 4-Hydroxy-3-methoxy-5-(2,6-dinitrophenyl) benzaldehyde **4** was reacted separately with compounds **5** and **7** to obtain **6** and **8**, respectively. The amide compound **9** can be synthesized by two different methods. The reaction between **6** and a diacyl chloride compound **7** gave compound **9**. Also, it can be produced *via* the reaction of compound **8** and an *N*-terminus compound **5**. All compounds were characterized by their melting points, UV-Vis, and FTIR spectra. Moreover, the mass spectrum and elemental analysis of compound **9** were determined. Compound **6** displayed a high sensitivity for *P. mirabilis* at the most concentrations, while compound **8** exhibited positive tests for *E. coli* and *P. mirabilis* at many concentrations. Compound **9** showed an antibacterial activity against *S. aureus* at the concentration 0.25 mM, and against *E. coli* at 0.5 mM.

Keywords: Synthesis; Amide; Vanillin; Azo Functional Group; UV-Vis.; FTIR; MS.

1. INTRODUCTION

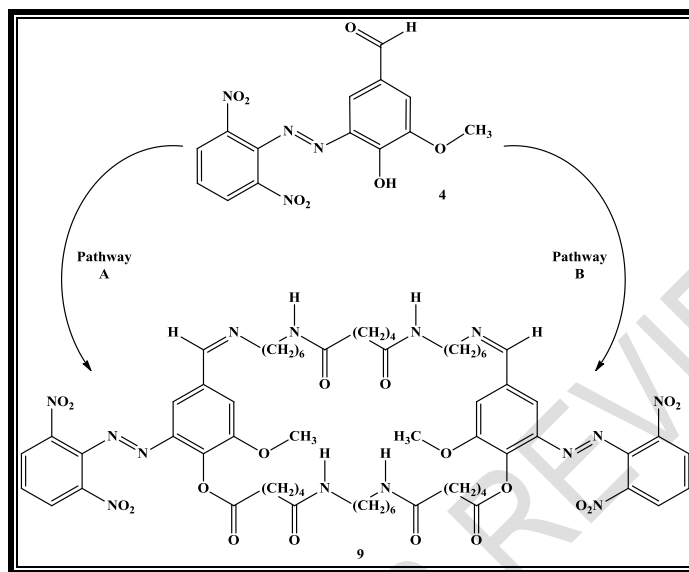
The amide group is one of the most important functional group in organic chemistry as well as in nature. This group is occurred in a wide variety of compounds. Proteins and enzymes have amide bonding structure. Medicinally, the amide group is one of the common groups in drug synthesis. About a quarter of available drugs contain at least one amide bond [1-3]. The amide compounds are thermally stable [4]. Amide bonds are also present in a huge molecules. The supramolecule compounds arises from the binding of a group of molecules of a well-defined structure and grouped together through non covalent interactions such as hydrogen bonds, dipole-dipole, van der Waals forces, π - π , and coordination bonds [5].

In recent years, supramolecular of aromatic systems have attracted attention of many researchers [6-9]. The diazo dye derivatives have undergone extensive studies in several fields, and exhibited an important applications such as dyes, biomedical studies, organic synthesis, biological activities including antineoplastics, antidiabetics, antiseptics, anti-inflammatory and other useful chemotherapeutics agents [10-15].

The supramolecular arrangement of azo dyes is based on non-covalent supramolecular interactions which keep packaged the whole systems. Also, it has raised particular interest in this chemical system [16]. Since the amide group, diazo dye, and supramolecular compounds are an important and have many applications. This is the motivation in our present study to synthesis three new amides derived from diazo dye and have a high molecular weight, as well as evaluate their biological activities against three types of bacteria.

Recently, we have been synthesized and characterized a new dinitro diazo dye **4**, Scheme 1 [11]. Additionally, the dipole interaction and the intramolecular hydrogen bonds of **4** are studied [12]. Based on previous information, we were interested to synthesize a new amide **9**, which produced from

the reaction between compound **4** with compounds **5** and **7** by two pathways, Scheme 1. All synthesized compounds were assigned using melting points, FTIR and UV–Vis spectroscopies. However, the microbial inhibitory of these derivatives against some pathogenic bacteria such as *Staphylococcus aureus* as gram +ve, *Escherichia coli* and *Proteus mirabilis* as gram –ve were tested.



Scheme 1: The new synthesized of amide **9**

2. EXPERIMENTAL DETAILS

2.1 GENERAL

All experiments were made at PCB Research Laboratory, Faculty of Science, Hadhramout University, Mukalla, Hadhramout, Yemen. The melting points were determined with Electrothermal Melting Point Apparatus. FTIR spectra were performed using JASCO FTIR 4600, Single beam, Path Laser, by KBr disk technique in the frequency range of 4000–400 cm^{-1} . UV–Vis spectra of the compounds were recorded with JASCO V–750 Spectrophotometer in ethanol at the wavelength range of 900–200 nm. Mass spectra were carried out on Direct Inlet part to mass analyzer in Thermo Scientific GC/MS model ISQ, and Elemental analyses were done using FLASH 2000 CHNS/O analyzer, Thermo Scientific at the Regional Center for Mycology and Biotechnology RCMB, Al-Azhar University, Nasr City, Cairo, Egypt. All microbial experiments were carried out at Alsalam Medical Center, Mukalla, Hadhramout, Yemen.

2.2 SYNTHESIS

2.2.1 Synthesis of 4-Hydroxy-3-methoxy-5-(2,6-dinitrophenylazo) benzaldehyde **4**

It was synthesized according to our earlier published procedure [11]. The physical properties were tabulated in Table 1.

2.2.2 Preparation of Compounds **5** and **7**

Both compounds 1,6-*N,N*-di(6-aminohexyl)hexan diamide **5** [17] and diacyl chloride **7** [18] were prepared and characterized previously at PCB Research Laboratory, Faculty of Science, Hadhramout University, Mukalla, Hadhramout, Yemen.

2.2.3 Synthesis of Compounds 6

It was synthesized according to the method that mentioned in [19-21] with some modification. An equimolar amounts from **4** (3.50 g, 0.01 mol) and **5** (3.0 g, 0.01 mol) were dissolved in 15 mL absolute ethanol EtOH containing a drop of AcOH. The reaction mixture was refluxed on a water bath for 1 hr, after that allowed to cool at room temperature. The resulting solid was filtered off, washed with a little 2% HCl solution and distilled water. The physical properties were listed in Table 1.

2.2.4 Synthesis of Compounds 8

It was obtained as a method that described previously in the literature [22], with some modification. A (3.50 g, 0.01 mol) of **4**, (4.10 g, 0.01 mol) of **7**, and (7.45 g, 0.054 mol) of anhydrous K₂CO₃ in 70 mL ethanol 96% were refluxed for 6 hrs. The resulting solution was cooled at room temperature and poured into about 300 mL of cold water. The solid material immediately was formed, filtered off, washed several times with cold water, and dried. The physical properties were showed in Table 1.

2.2.5 Synthesis of Compounds 9

2.2.5.1 Pathway A

About (0.40 g, 0.001 mol) of **6**, (0.30 g, 0.001 mol) of **7**, and anhydrous K₂CO₃ (7.45 g, 0.054 mol) with 70 mL ethanol 96% were refluxed for 6 hrs., left at room temperature then poured into about 300 mL cold water. A deep green solid was formed, filtered, washed several times with cold water and dried. Yield: 21.6%, 0.14 g, and m.p. 150°C, Table 1. CHN elemental analysis: Found (calculated) for C₆₄H₈₂N₁₄O₁₈: C: 57.44(60.68), H: 6.45(6.54), N: 14.72(14.68).

2.2.5.2 Pathway B

About (0.50 g, 0.0005 mol) of **8** and (0.15 g, 0.0004 mol) of **5** were dissolved in 15 mL of absolute ethanol containing a drop of AcOH. The reaction mixture was refluxed on a water bath for 1 hr, then left to cool at room temperature. The resulting solid was filtered, washed with a little 2% HCl, distilled water, and left to dry. Yield: 21.6%, 0.14 g, m.p. 150°C, according to the Table 1. CHN elemental analysis: Found (calculated) for C₆₄H₈₂N₁₄O₁₈: C: 57.38(60.68), H: 6.41(6.54), N: 14.57(14.68).

2.3 METHODOLOGY OF ANTIMICROBIAL ACTIVITY

2.3.1 Bacterial Strains

The bacteria strains that used in this study are most commonly involved in causing gastroenteritis, wound and burn exudates, urinary tract infection and ear secretions. Three bacteria strains *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis* were isolated from different patients attending Alsalam Medical laboratory in Mukalla, Hadhramout – Yemen, and used throughout this study. The isolated bacteria were subcultured on Nutrient agar (Difco) and incubated aerobically at 37°C for 24 hrs. Organisms were maintained in the laboratory on nutrient agar slopes at 4°C [23].

2.3.2 Determination the Minimal Inhibitory Concentration of 4, 6, 8 and 9

The minimum inhibitory concentration MIC of **4**, **6**, **8** and **9** were determined by using a different dilution for those compounds, which diluted with sterile ethanol 75%. It was weighed 0.07 g, 0.21 g, 0.20 g and 0.27 g at the respective of **4**, **6**, **8** and **9** in 100 mL of ethanol 75%. The following concentration was prepared (1:2, 1:4, 1:8, 1:16 and 1:32) from the stock of each solution [23-24].

2.3.3 Antimicrobial activity

The antibiotic susceptibility tests were carried out by the Kirby–Bauer disk diffusion technique according to Clinical Laboratory Standard Institute guidelines [25-26]. About 20 mL of the sterilized medium was poured into each sterile 9 cm diameter of Petri dish and allowed to solidify. Bacterial suspension for each strain tested was adjusted at 3×10^8 CFU/mL by McFarland scale which prepared by mixing 0.1 mL of 1.0% dehydrate barium chloride with 9.9 mL of 1.0% Sulfuric acid H₂SO₄ as described by McFarland [27].

About 0.1 mL of the prepared bacterial suspension was spread evenly onto the agar surface using a cotton swab and kept in a refrigerator for 2 hrs. The compound diffuses into the agar resulting in a concentration gradient that is inversely proportional to the distance from the disc. The test medium was used Mueller–Hinton agar [28]. Thereafter, all prepared plates were incubated at 37°C for 24 hrs. [29]. After that, the size of the inhibition zone around the disc is a measure in mm of the degree of inhibition [25-26].

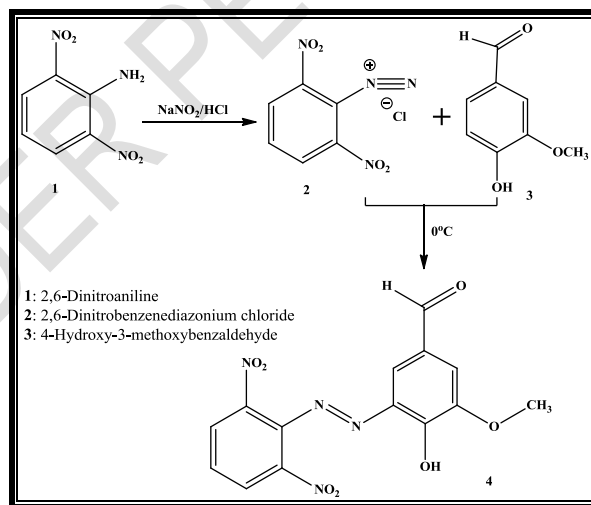
The Discs of Ciprofloxacin Cip, Amikaci Ami, Clarithromycin Cit, Cefaclor Cef, Cefepime Cep, Azithromycin Azt and Co-trimoxazole Ctm were used as a reference for evaluation of antibacterial [30]. By this manner, some of the synthesized compounds were screened *in vitro* for their antibacterial activity against *S. aureus*, *E. coli* and *P. mirabilis*.

3. RESULTS AND DISCUSSION

Amide **9** was synthesized by two different pathways **A** and **B**, in which the compound **4** was first synthesized by coupling reaction between 2,6-dinitroaniline **1** and 4-hydroxy-3-methoxybenzaldehyde **3** as shown in Scheme 2. The diazo dye **4** was obtained as solid compound, and its structure was fitted with the published data [11].

In the first pathway, the amide **9** was synthesized by reaction of diazo dye **4** with *N*-terminus diamine **5** by refluxing in absolute ethanol using a drop of acetic acid to yield **6**. After that, compound **6** was treated with diacyl chloride **7** to obtain amide **9**, Scheme 3. The physical data of compounds **4**, **6**, and **9** were tabulated in Table 1.

The amide **9** was obtained also by the second different pathway, in which the diazo dye **4** treated with a diacyl chloride compound **7** and refluxing in 96% ethanol to give compound **8**, then the resulted **8** was reacted with *N*-terminus diamine **5** to obtain **9**, Scheme 4 and Table 1.



Scheme 2: Synthesis of 4-hydroxy-3-methoxy-5-(2,6-dinitrophenylazo) benzaldehyde **4**

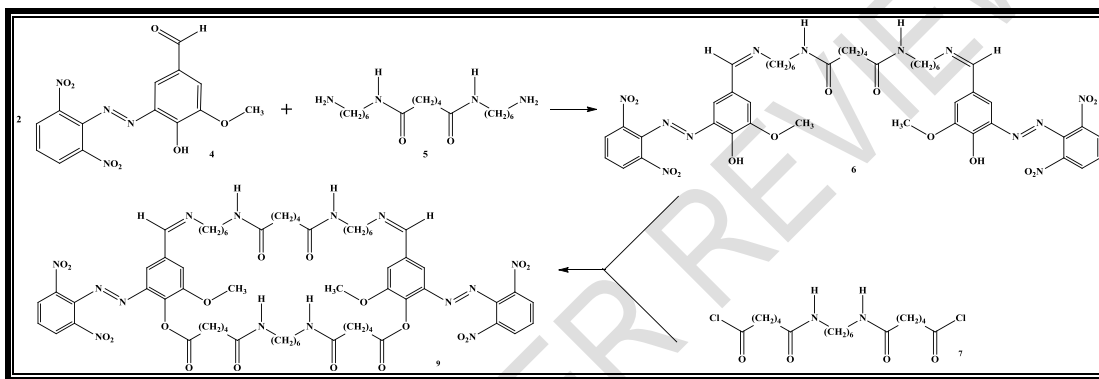
Table 1. Physical properties of compounds **4**, **6**, **8** and **9**

Physical Properties	Compounds			
	4	6	8	9
Molecular formula	C ₁₄ H ₁₀ O ₇ N ₄	C ₄₆ H ₅₄ O ₁₄ N ₁₂	C ₄₆ H ₄₈ O ₁₈ N ₁₀	C ₆₄ H ₈₂ N ₁₄ O ₁₈

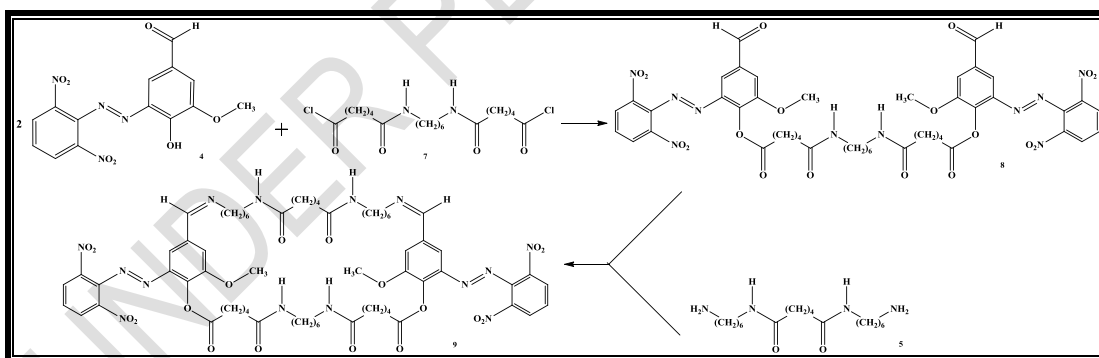
Molecular weight (g/mol)	346.25	998.99	1028.93	1335.42
Melting point (°C)	88	191	153.4	150
Color	Light orange	Yellowish brown	Brown	Deep green
Yield %	46.2	27	6.5	21.6

3.1 FTIR SPECTROSCOPY

The compound **6** was characterized by infrared spectroscopy, Table 2. The important infrared bands of **6** compared to the FTIR of **4** were the appearance of strong band at 1658 cm^{-1} which was attributed to the imine functional group $\nu\text{ C=N}$, accompanied by disappearance of aldehydic hydrogen stretching frequencies, as well as the two spikes of primary amine. The FTIR spectrum of **8** displayed a new band at 1765 cm^{-1} corresponding to an ester functional group stretching frequency as well as disappearing of the $\nu\text{ OH}$ band as comparing with the FTIR spectrum of diazo dye **4**, Table 2.



Scheme 3: The first pathway for synthesis of **9**.



Scheme 4: The second pathway for synthesis of **9**.

Table 2. Physical properties of compounds **4**, **6**, **8** and **9**

Functional Group	Compounds			
	4	6	8	9
$\nu\text{-OH}$ arom.	3277	3111	-	-
C=O aldehyde	1672	-	1720	-
$\nu\text{ C=C}$	1589	1494	1494	1549

νCH_3 aliph.	<i>as</i> 2976	<i>as</i> 1694	<i>as</i> 2948	<i>as</i> 2925
	<i>sy</i> 2858	<i>sy</i> 1590	<i>sy</i> 2366	<i>sy</i> 2360
$\nu\text{-N=N-}$	<i>as</i> 1589	<i>as</i> 1511	<i>as</i> 1598	<i>as</i> 1604
	<i>sy</i> 1430	<i>sy</i> 1465	<i>sy</i> 1403	<i>sy</i> 1503
δCH_3	<i>as</i> 1454	<i>as</i> 1428	<i>as</i> 1403	<i>as</i> 1504
	<i>sy</i> 1372	<i>sy</i> 1345	<i>sy</i> 1362	<i>sy</i> 1403
$\nu\text{-NO}_2$	<i>as</i> 1508	<i>as</i> 1428	<i>as</i> 1530	<i>as</i> 1530
	<i>sy</i> 1301	<i>sy</i> 1345	<i>sy</i> 1463	<i>sy</i> 1400
$\nu\text{C-O-C}$ aliph.	<i>as</i> 1266	<i>as</i> 1267	<i>as</i> 1268	<i>as</i> 1255
	<i>sy</i> 1153	<i>sy</i> 1141	<i>sy</i> 1113	<i>sy</i> 1114
$\nu\text{C-N-C}$	-	1027	-	1004
$\nu\text{C=O}$ amide	-	1672	1615	1681
$\nu\text{C=N}$	-	1590	-	1619
$\nu\text{C=O}$ arom. ester	-	-	1765	1787

The comparison of the FTIR spectra of the compounds **6** and **8** as well as the amide spectrum. Table 2 gives important information about the suggested structure of **9**. The FTIR spectrum of compound **9** synthesized from the first pathway, Table 2 characterized by the observance of the band at 1787 cm^{-1} indicated an ester functional group and disappearance of $\nu\text{ OH}$ stretching frequency as compared to the FTIR spectrum of **6**. In contrast, the FTIR spectrum of **9** produced from the second pathway characterized by the absorption band at 1619 cm^{-1} assigned for imine functional group as well as the absence of two spikes of primary amine that comparing with the FTIR spectrum of **8**.

3.2 UV-VIS. SPECTROSCOPY

The UV-Vis of **6** exhibited a high intense absorption peak at 390-477 nm assigned to $\pi \rightarrow \pi^*$ electronic transition of the azo group -N=N- , the spectrum also contained a shoulder significant peak at the range of 360-340 nm which attributed to $n \rightarrow \pi^*$ transition of the C=N- imine group [31], Table 3.

The UV-Vis spectrum of **8**, Table 3, showed bands at 391.2 nm and 230 nm indicated of $\pi \rightarrow \pi^*$ electronic transitions, and 442.8 nm assigned for $n \rightarrow \pi^*$ transitions. While the UV-Vis spectrum of **9** was recorded in ethanol and exhibited bands at 390 nm and 230 nm that assigned for $\pi \rightarrow \pi^*$ electronic transitions, and 445 nm, 340.4 nm assigned for $n \rightarrow \pi^*$ electronic transitions, Table 3.

Table 3. Physical properties of compounds 4, 6, 8 and 9

Electronic Transition λ_{max} (nm)	Compounds			
	4	6	8	9
$\pi \rightarrow \pi^*$ Benzene	200.2	230	230	230
$\pi \rightarrow \pi^*$ azo	390	390.4	391.2	390
$n \rightarrow \pi^*$ aromatic ester	-	-	442.8	442.4-448
$n \rightarrow \pi^*$ imine	-	340.4	-	340.4
$\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ peptide	-	200	205.2	205.2

3.3 MASS SPECTRUM

The MS spectrum of amide **9**, Fig. 1, showed the ion base peak **10** at m/z 343.94 which was corresponded to M^+ the mass of **11**, Fig. 2. The molecular weight of the suggested structure amide

m/z 1335.42 was outside the range of the used MS spectrometer. However, the CHN elemental analyses of **9** showed a good agreement with suggested molecular formula $C_{64}H_{82}N_{14}O_{18}$ for **9**.

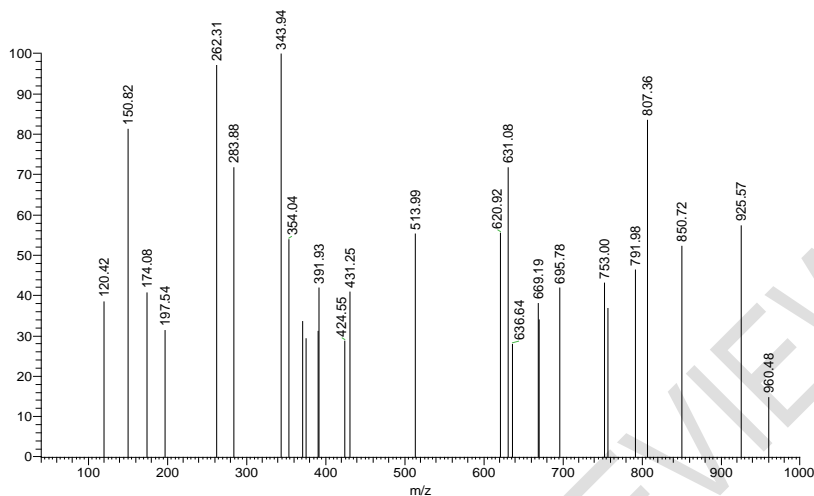


Figure 1: The Mass Spectrum of new synthesized amide **9**.

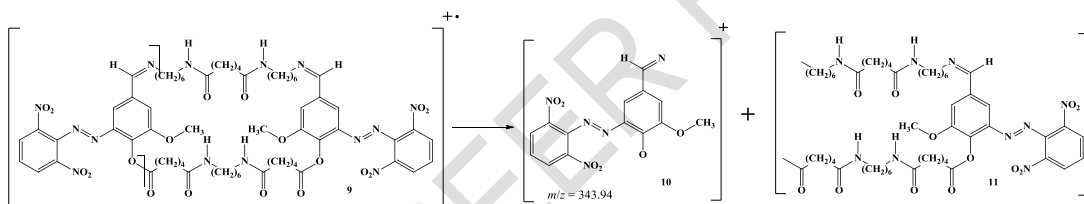


Figure 2: The fragment $M^+ - 991.48$ of amide **9**.

3.4 MICROBIOLOGY ACTIVITY

The synthesized compounds **6**, **8**, and **9** are showed most sensitive to a many kind of bacteria due to the type of the functional groups of these compounds, which had an increasing effect on the levels of toxicity of bacterial species [11]. In fact, the imine compounds are very important in industrial application and in biological activities. They have found a good effect to the pharmacological activities such as antimicrobial [32].

The results of antibacterial activity of these compounds against *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis* are showed in Table 4. Compound **6** showed active of *P. mirabilis* at the concentrations 2.0 mM [$+$ = IZ 7–13 mm], 1.0 mM [$+$ = IZ 7–13 mm], 0.25 mM [$+$ = IZ 7–13 mm], 0.125 mM [$+$ = IZ 7–13 mm] and 0.0625 mM [$+$ = IZ 7–13 mm]. On other hand, compound **8** was effective against two types of pathogenic bacteria, *E. coli* at concentration 0.25 mM [$+$ = IZ 7–13 mm] and 0.125 mM [$+$ = IZ 7–13 mm]. Also, *P. mirabilis* at concentrations 2.0 mM [$+$ = IZ 7–13 mm], 1.0 mM [$+$ = IZ 7–13 mm], 0.25 mM [$+$ = IZ 7–13 mm], 0.125 mM [$+$ = IZ 7–13 mm] and 0.0625 mM [$+$ = IZ 7–13 mm].

The amide **9** showed no activated on *P. mirabilis* with all concentrations. It exhibited the most sensitive for *S. aureus* at concentration 0.25 mM [$+$ = IZ 7–13 mm] and *E. coli* at concentration 0.5 mM [$+$ = IZ 7–13 mm]. However, amide **9** is inactive of other concentrations that indicated all bacteria tested is resistance of this compound, as shown in Table 4. A similar result is previously

reported by Al-Shara'ey *et al.* They have been reported that most bacteria tested are resistance to some synthesized polycyclic compounds [33-34].

Seven antibiotics were used in this study that the highest percentage of antibiotics active was to Ciprofloxacin, Amikaci and Clarithromycin to *S. aureus*, while inactive to both *E. coli* and *P. mirabilis*. Each of Cefaclor, Cefepime, Azithromycin and Co-trimoxazole were shown more active to *E. coli* and *P. mirabilis* except *S. aureus*. The studies indicated to better understand virulence and multidrug resistance, revealed that most of the antibiotic resistance genes and many of the virulence genes reside on mobile genetic elements, such as plasmids, transposons, and prophages, indicating substantial horizontal gene transfer from other bacteria [35]. We believe that the functional groups of these compounds are an increasing effect on the levels of toxicity of bacterial species.

UNDER PEER REVIEW

Table 4. Physical properties of compounds 4, 6, 8 and 9

Compound	Conc. mM	microorganisms		
		gram +ve	gram -ve	
		<i>S. aureus</i>	<i>E. coli</i>	<i>P. mirabilis</i>
6	2.0	—	—	+
	1.0	—	—	+
	0.5	—	—	+
	0.25	—	—	—
	0.125	—	—	+
	0.0625	—	—	+
8	2.0	—	—	+
	1.0	—	—	+
	0.5	—	—	—
	0.25	—	+	+
	0.125	—	+	+
	0.0625	—	—	+
9	2.0	—	—	—
	1.0	—	—	—
	0.5	—	+	—
	0.25	+	—	—
	0.125	—	—	—
	0.0625	—	—	—
Ethanol 75%	—	—	—	—
Cip	5*	+++	—	—
Ami	30*	+++	—	—
Cit	15*	+++	—	—
Cef	30*	—	++	+++
Cep	30*	—	++	++
Azt	15*	—	+	—
Ctm	25*	—	+++	+++

Key to symbols: *ug, Cip: Ciprofloxacin, Ami: Amikaci, Cit: Clarithromycin, Cef: Cefaclor, Cep: Cefepime, Azt: Azithromycin and Ctm: Co-trimoxazole as antibacterial, Disc diameter = 6 mm, Highly active: +++ [IZ > 19 mm], Moderately active: ++ [IZ 13-19 mm], Slightly active: + [IZ 7-13 mm], Inactive: — [IZ < 7 mm].

4. CONCLUSION

In this paper, we have synthesized three new amide compounds **6**, **8**, and **9**. Compound **6** has been synthesized *via* the condensation reaction between **4** and **5**. Compound **8** has been obtained through the nucleophilic substitution reaction between **4** and **7**. Both compounds **6** and **8** can be used to synthesize amide **9** through two different pathways. Amide **9** can be obtained either by the reaction of **6** and **7**, or by the reaction of **8** with **5**. All synthesized compounds have been characterized by spectroscopic UV-Vis, and FTIR technique, as well as elemental analysis CHN and mass spectrometry of the compound **9**. The antibacterial activity has been evaluated by using minimum inhibitory concentration MIC method. The results showed that **6** exhibited a high sensitivity for *P. mirabilis* at the most concentrations, while **8** had positive results for *E. coli* and *P. mirabilis* at many concentrations. Amide **9** displayed antibacterial activity against *S. aureus* at the concentration 0.25 mM, and against *E. coli* at 0.5 mM.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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