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Synthesis, Characterization and Antimicrobial Activity Studies of New Amide Derivatives from Benzoyl derivatives of Amino Acid and Selected Sulfa Drugs by Using DCCI

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ABSTRACT

Several compounds which derived from condensation of N-benzoyl-DL-alanine, N-benzoyl glycine, N-benzoyl-D-valine and N-benzoyl-DL-isoleucine with selected sulfa drugs such as sulfamethoxazole, sulfanilamide, sulfamerazine, sulfamethazine and sulfapyridine by using N,N-dicyclohexyl carbodiimide (DCCI) as an enhancer for the condensation process were synthesis. These compounds that can be considered as amides have been characterized by elemental analysis (C,H,N,S), FT-IR and ^{1}HNMR. The synthesized compounds contain sulfa drug moiety shows excellent antimicrobial activity by using two kinds of bacteria, gram positive Staphylococcus aureus (S. aureus) and gram negative Aeromonas hydrophilic (A. hydrophilia) in different concentration and minimum inhibitory concentration MIC was founded.

Keywords: N-benzoyl amino acid, Sulfa drug, DCCI, Condensation, Antibacterial activity.

INTRODUCTION

The current study focuses on the importance of preparing some amino acid derivatives like N-benzoyl amino acid and the reaction of these derivatives with some sulfa drugs to obtain new organic compounds which can afford some of the properties of the amino acids and sulfa drugs. Amino acids are organic compounds that are naturally involved in the synthesis of enzymes and peptides as well as proteins because these compounds have an acidic group (-COOH), which are carboxylic acid and amino group (-NH_2). The amino acids show reactions of amine as well as carboxylic acid groups. In some cases, the amino group of these amino acids reacts with acid chlorides to form acyl derivatives such as N-benzoyl amino acid. Phthaloyl, phenyl methoxy carbonyl group (cbz), tert-butoxy carbonyl group (BOC) and N-benzoyl chloride which can be considered derivatives of amino acids.

Gramicidin and penicillin have been shown to be derivatives of D-amino acid [1]. Also, many complexes which prepared from amino acid have been reported in the literature for a long time [2-5]. Large number chemotherapeutically significant sulfa drugs such as sulfanilamide, sulfamerazine, sulfamethoxazole and sulfapyridine have been used as antibacterial agents which can be used to treat many diseases caused by some types of bacterial such as treatment urinary tract infections, throat and gum infections, eye infections and malaria [6,7]. Amide of sulfonic acids are called sulfonamides. The sulfa drugs are a class of sulfonamides. An amide is composite of a carboxylic acid and ammonia or its derivatives. Amides are least reactive of all acid derivatives because of delocalization which reduces the electrophilicity of carbon (of $CONH_2$) [8]. The formation of amides from amine and carboxylic acid can be accelerated by using N,N-Dicyclohexyl carbodiimide. DCCI, which activates the carboxylic carbon of the amino acid derivatives so that the nucleophilic attack of amino group of sulfa drugs occurs readily at carbon. DCCI acts as dehydrating agent [9]. Aim of this investigation is to synthesize new amide which derived from N-benzoyl amino acids and selected sulfa drugs at room temperature by using the reagent DCCI. This reagent is the anhydride of a disubstituted urea, and when treated with water, it is converted to N,N^1-dicyclohexyl urea (DCU) [10]. The reaction is efficient, and yields are generally very high.

EXPERIMENTAL

Materials and methods

The solvents, benzoyl chloride, glycine, DL-Alanine, DL-Valine and DL-Isoleucine were supplied by Merck.

Sulfamethoxazole, sulfanilamide, sulfamerazine, sulfamethazine and sulfapyridine were acquired from sigma-Aldrich product. Additionally, all solvent used in particularly this work were of analytical grade. All measurements of melting point were done on a Bauchi 510.

Instruments: The spectra of Infrared (IR) were recorded as solid pellets by using potassium bromide on Shimadzu FT-IR spectrophotometer model 8400s in range 4000-400 cm^{-1}. The spectra of 1HNMR were registered in a Bruker spectrophotometer (400 MHz) using DMSO-d_6 as solvent and TMS as internal standard. Elemental analysis (C,H,N,S) were evaluated by Euro vector model 3000 A (Italy).

Synthesis of the compounds: Genral procedure for synthesis of N-benzoyl amino acids

N-benzoyl amino acids are most favorable prepared by the influence of benzoyl chloride upon amino acids in aqueous solution in the existence of some alkali, such as sodium hydroxide, is used to neutralize the hydrochloric acid which released from this reaction. The method was inserted by Baum. The N-benzoyl amino acids by Baum's method, in the following way [11]. 20 mmol of amino acid dissolved in 60 ml of 2N sodium hydroxide and 30 ml of water. The solution that maintained below 30°C is treated with 20 mmol of benzoyl chloride with stirring for 15-30 minutes. Then the solution acidified with 10% of hydrochloric acid until reached acidic of the solution to pH (4-5). On starting for further 30 minutes, white precipitate was formed and filtered. The benzoyl derivative is purified with methanol and dried. White crystals were obtained in good yield. The following N-benzoyl amino acids are presented in (**Table 1**).

Synthesis of amides derivatives from sulfa drugs (S_1,S_2,S_5,S_8)

The new amide compounds (S_1,S_2,S_5) prepared from corresponding N-Benzoyl amino acids and sulfa drug by means of the general procedure introduced by Curni [12]. Quantity of 1.0 mmol (0.253 gm) of sulfamethoxazole was dissolved in 50 ml of ethyl acetate to this solution 1 mmol (0.221 gm, 0.193 gm and 0.235 gm) of N-benzoyl-D-Valine, N-benzoyl-DL-Alanine and N-benzoyl-DL-Isoleucine respectively, were added. A solution of DCCI 1.0 mmol (0.206 gm). In 5 ml of ethyl acetate was added dropwise to the mixture solution of sulfa drugs and N-benzoyl amino acids. With governorate 15-20 minute with constant stirring at room temperature. After the addition was completed, the mixture stirred overnight. The white precipitate formed (dicyclohexyl urea) was filtered. To the filtrate solution added 10 ml of 5% aqueous solution of citric acid, two layers formed. The organic layer was extracted vigorously, the solvent evaporated and the residue was dried and then re-crystallized from ethanol (**Table 2**).

Synthesis of amides derivatives from sulfa drugs (S_3,S_4,S_6,S_7)

Equimolar of sulfanilamide 1 mmol (0.172 gm) and 1 mmol (0.235 gm, 0.193 gm and 0.179 gm) of N-benzoyl-D-Valine, N-benzoyl-DL-Isoleucine, N-benzoyl-DL-Alanine and N-benzoyl glycine respectively were dissolved in 50 ml of ethyl acetate or acetone such as in compound S_7. A solution of DCCI 1 mmol (0.206 gm) in 5 ml of ethyl acetate was added gradually for 15-20 minutes with stirring to the previous solution while keeping the reaction at room temperature, the stirring continued overnight. A white precipitate formed (dicyclohexyl urea) was filtered 10 ml of 5% aqueous solution of citric acid was added to the filtrate solution, two layers formed. The organic layer was extracted power fully and the solvent evaporated. The residue which obtained was dried and re-crystallized from ethanol (**Table 2**).

Synthesis of amides derivatives from sulfa drugs (S_9,S_{10},S_{12})

Sulfamerazine 1 mmol (0.264 gm) and 1 mmol (0.179 gm, 0.221 gm and 0.193 gm) of N-benzoyl glycine, N-benzoyl-D-Valine and N-benzoyl-DL-Alanine were mixed and dissolved in 50 ml of acetone. A solution of DCCI 1 mmol (0.206 gm) in 5 ml of ethyl acetate was added dropwise over period 15-20 minutes to the previous solution with stirring until a white precipitate formed which it was dicyclohexyl urea. The mixture left stirred overnight at room

Table 1: Structural formula, IUPAC name and physical data of N-benzoyl amino acid.

Amino acid derivative	IUPAC name	Molecular formula	Molecular weight gm/mol	Melting point (°c)	Yield %
N-Benzoyl Glycine	2-benzamidoacetic acid	$C_9H_9NO_3$	179	187-188	55
N-Benzoyl-DL-Alanine	2-benzamidopropanoic acid	$C_{10}H_{11}NO_3$	193	164-165	57
N-Benzoyl-D-Valine	2-benzamido-3-methylbutanoic acid	$C_{12}H_{15}NO_3$	221	126-127	56
N-Benzoyl –DL-Isoleucine	2-benzamido-3-methylpentanoic acid	$C_{13}H17NO_3$	235	115-116	53

Table 2: The Symbol, Structural formula, IUPAC name, Analytical and physical data of the prepared compounds.

Symbol of com.	Colour	Molecular formula	Molecular weight gm/ mol	Melting point°c	Yield %	Elemental analysis CHNS Practical (Theoretical)			
						C%	H%	N%	S%
S_1	White	$C_{22}H_{24}N_4O_5S$	456.51	118-120	76.45	58.18 (57.88)	5.23 (5.3)	12.66 (12.27)	7.35 (7.02)
S_2	Light brown	$C_{20}H_{20}N_4O_5S$	428.46	188-190	75.5	56.29 (56.06)	4.76 (4.7)	13.14 (13.08)	7.74 (7.48)
S_3	White	$C_{18}H_{21}N_3O_4S$	375.44	196-198	43.6	57.32 (57.58)	5.52 (5.641)	11.07 (11.19)	8.31 (8.54)
S_4	Light brown	$C_{19}H_{23}N_3O_4S$	389.47	87-89	74	58.23 (58.59)	5.81 (5.95)	10.54 (10.79)	7.97 (8.23)
S_5	White	$C_{23}H_{26}N_4O_5S$	470.54	159-161	53.6	58.95 (58.71)	5.71 (5.57)	12.04 (11.91)	6.93 (6.81)
S_6	Light brown	$C_{16}H_{17}N_3O_4S$	347.39	148-150	41	55.63 (55.32)	4.81 (4.93)	12.08 (12.1)	9.12 (9.23)
S_7	Orange	$C_{15}H_{15}N_3O_4S$	333.36	97-99	88	54.65 (54.04)	4.50 (4.54)	12.49 (12.61)	9.58 (9.62)
S_8	Yellowish-brown	$C_{19}H_{18}N_4O5S$	414.43	187-189	61.1	55.7 (55.06)	4.3 (4.38)	13.48 (13.52)	7.78 (7.74)
S_9	Yellow	$C_{20}H_{19}N_5O_4S$	425.46	80-82	60	65.72 (65.46)	4.38 (4.50)	16.32 (16.46)	7.47 (7.58)
S_{10}	White	$C_{23}H_{25}N_5O_4S$	467.54	128-130	44.9	60.12 (59.09)	5.43 (5.39)	14.87 (14.98)	6.78 (6.86)
S_{11}	White	$C_{23}H_{24}N_4O_4S$	452.35	131-133	54.8	61.35 (61.04)	5.3 (5.35)	12.31 (12.38)	7.02 (7.09)
S_{12}	White	$C_{21}H_{21}N_5O_4S$	439.49	110-112	85.4	57.61 (57.39)	4.9 (4.82)	15.99 (15.94)	7.39 (7.30)
S_{13}	Yellow	$C_{24}H_{27}N_5O_4S$	481.57	52-54	83.6	59.63 (59.86)	5.59 (5.65)	14.48 (14.54)	6.21 (6.06)
S_{14}	White	$C_{25}H_{29}N_5O_4S$	495.59	130-132	89	60.72 (60.59)	5.87 (5.90)	14.3 (14.13)	6.61 (6.47)
S_{15}	Orange	$C_{21}H_{21}N_5O_4S$	439.49	46-48	60.9	57.68 (57.39)	4.73 (4.82)	15.86 (15.94)	7.4 (7.3)
S_{16}	Orange	$C_{24}H_{26}N_4O_4S$	466.55	105-107	77	61.9 (61.78)	5.56 (5.62)	12.11 (12.01)	6.91 (6.87)

temperature. The obtained products were separated by filtration. 10 ml of 5% aqueous solution of citric acid was added to the filtrate solution. Two layers formed, the organic layer extracted vigorously, then the solvent evaporated and the residue was dried and re-crystallized from ethanol (**Table 2**).

Synthesis of amides derivatives from sulfa drugs (S_{13},S_{14},S_{15})

Equimolar of sulfamerazine 1 mmol (0.278 gm) and 1 mmol (0.221 gm, 0.235 gm and 0.179 gm) of N-benzoyl-D-Valine, N-benzoyl-DL-Isoleucine and N-benzoyl glycine respectively were mixed and dissolved in ethyl acetate or in acetone S_{15}. A solution of DCCI 1 mmol (0.206 gm) in 5 ml of ethyl acetate was added dropwise over period 15-20 minutes to the previous solution with stirring until a white precipitate appears (dicyclohexyl urea). The mixture left stirred overnight at room temperature. The obtained products were separated by filtration, then 10 ml of 5% aqueous solution of citric acid was added to the filtrate solution. Two layers formed, the organic layer extracted and the residue was dried and re-crystallized from ethanol (**Table 2**).

Synthesis of amides derivatives from sulfa drugs (S_{11},S_{16})

Equimolar of sulfapyridine 1 mmol (0.249 gm) and 1 mmol (0.221 gm and 0.235 gm) of N-benzoyl-D-Valine and N-benzoyl isoleucine were mixed and dissolved in 50 ml of acetone. A solution of DCCI 1 mmol (0.206 gm) in 5 ml of ethyl acetate was added dropwise over period 15-20 minutes with stirring at room temperature until whit precipitate formed (dicyclohexyl urea). The mixture lifted overnight stirring. The obtained products were separated

by filtration. To the filtrate solution we added 10 ml 5% aqueous solution of citric acid was added. Two layers formed. The organic layer extracted, the residue dried and re-crystallized from ethanol (**Table 2**). The diversified synthetic amide derivatives compounds S_1-S_{16} are summarized in **Figure 1**.

***In vitro* antimicrobial activity:** The Antibacterial activity of the synthetic compounds S_1-S_{16} have been carried out with two kinds of bacteria, gram positive *Staphylococcus aureus (S. aureus)* and gram negative *Aeromonas hydrophilic (A. hydrophilia)* using disc diffusion method by using Dimethylsulphoxide (DMSO) as solvent. [13] The antibiotic tetracycline 25 mg/l was having been used to calibrate and to comparison with antibacterial tools. The method includes preparing Petri plates with 10 ml of sterile nutrient agar (NA) for testing the bacterial assay. Cultivation of bacteria have been done by providing the necessary material that encourages growth at 37 °C for full day. 5 ml of nutritious broth (NB) was inoculated and incubated at 37°C for six hours until the desired bacterial growth obtained. 0.1 ml of this pure culture of each type of studied bacteria was grown in Muller Hinton Broth. Agar well was cat using glass rod and allowed to dry for minutes. Different concentration of each synthetic compounds was prepared. 0.1 ml of each solution were poured in to labeled holes of 6 mm diameter. All the plates were incubated at 37 ± 0.5°C for 24 hours. Zone of inhibition of these compounds in (mm) were registering. In addition to that, values of minimum inhibitory concentration (MIC) of these compounds were noticed. The MIC end point is the lowest concentration at which growth was observed. The tests compounds are transmitted in concentration of (25,50,100,200,250,300) mg/l. The inhibition zones created by the synthetic compounds of various micro-organisms that we used were calculated.

RESULTS AND DISCUSSION

In general amides, the least reactive of the functional derivatives of carboxylic acid which can formed by nucleophilic acyl substitutions. The mechanism for connecting of an amine function (from sulfa drug derivatives) with carboxylic function (from N-benzoyl amino acid) by using DCCI can be epitomized in three steps, (**Figure 2**). The mechanism shows DCCI is a reagent commonly used to form amide bond which makes the free OH group of the carboxylic acid a better leaving group, therefore activating the carboxy group as a contribution to nucleophilic attack. In the first stage of the reaction, the carboxylic acid adds to DCCI (protonation). So, consists of o-acylisourea [1], the second step is the amine of sulfa drug derivatives added to the carbonyl group of o-acylisourea to give a tetrahedral intermediate [2], while the third step includes dissociated of a tetrahedral intermediate to form an amide and N,N-dicyclohexylurea. Mechanism synthesis of compounds S_1-S_{16} are summarized in **Figure 2**. The outcomes of elemental analysis as it shows in (**Table 2**), we will find congruence with the propose structure.

Spectroscopic analysis

FT-IR spectra: The infrared of the synthesized compounds (S_1-S_{16}) were recorded with the range (4000-400) cm^{-1} and they were summarized in **Table 3**. The entire spectroscopic test showed expected structures. The infrared spectra show the position and the intensities of the peaks which corresponds to various groups present in each compound. The FT-IR of compounds (S_1,S_{10}) shown in **Figures 3 and 4**. As seen from infrared spectra of these compounds disappearance, of bands which attributed to (O-H) moiety and instead of that, the compounds display a strong-medium band at (3394-3000) cm^{-1} which assigned to stretching ***υ***N-H. The formation of amide can be distinguished by appearance of strong

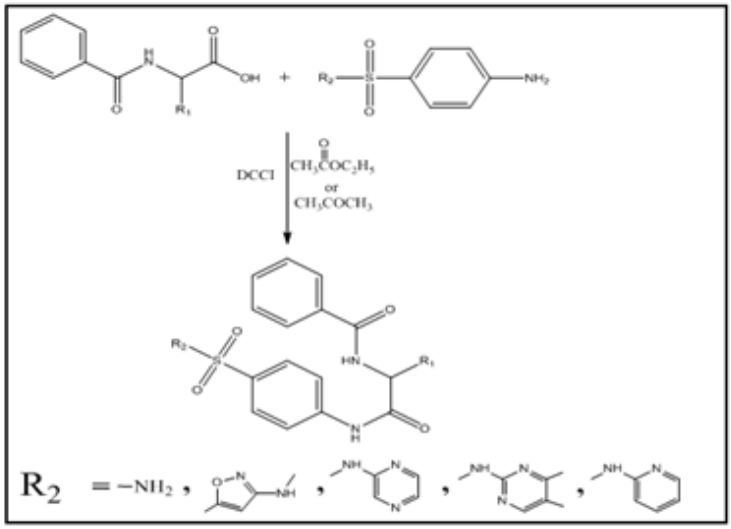


Figure 1: Preparation of amide derivatives from sulfa drug derivatives and N-benzoyl amino acid (S1-S16).

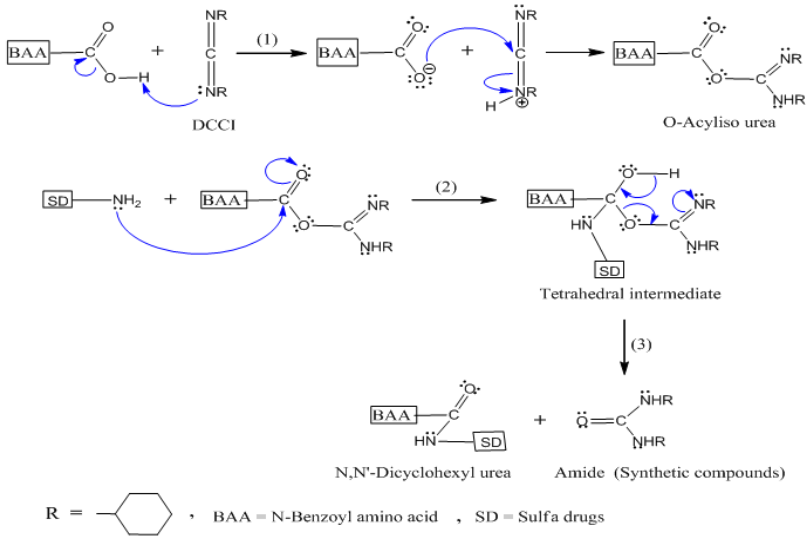


Figure 2: Mechanism preparation of amide derivatives from sulfa drug derivatives and N-benzoyl amino acid (compounds S1-S16).

Table 3: FT-IR data of synthetic compounds (cm^{-1}, KBr disc) (s: strong, vs: very strong, m: medium, w: weak, br: broad).

Com.	υ (N-H)	υ (C-H) Arom. Stretching	υ (C-H) aliph.	υ (C=O)	υ (C=N) sulfa	υ (S=O) Asym.	υ (S=O) Sym.	υ (S-N)	Sub.Arom
S_1	3184 m	3064 w	2933 m	1627 vs	1533 s	1330 m	1161 s	925 m	840 m
S_2	3327 m	3072 w	2850 m	1653 vs	1593 s	1313 w	1163 m	927 m	831 w
S_3	3251 m	3072 w	2931 m	1624 vs	…..	1313 w	1159 m	902 m	827 w
S_4	3000 m	3000 m	2900 m	1662 vs	…..	1285 w	1045 m	879 m	859 w
S_5	3265 s	3000 m	2929 m	1620 vs	1537 s	1319 s	1033 m	933 m	829 w
S_6	3323 s	3000 m	2933 m	1639 vs	…..	1307 m	1160 w	925 w	825 w
S_7	3400 m	3000 w	2899 m	1633 vs	…..	1311 m	1045 m	920 w	879 w
S_8	3288 s	3000 w	2852 m	1647 vs	1521 s	1319 m	1168 m	925 w	820 w
S_9	3342 br	3000 w	2937 m	1732 vs	1598 s	1303 m	1161 m	879 m	839 w
S_{10}	3292 s	3000 w	2929 m	1714 vs	1597 s	1300 m	1151 m	891 m	783 w
S_{11}	3390 m	3000 w	2899 m	1710 s	1595 m	1370 m	1047 m	879 m	780 w
S_{12}	3327 s	3000 w	2850 m	1627 vs	1597 s	1311 m	1089 m	893 m	783 w
S_{13}	3250 m	3000 w	2927 m	1635 vs	1520 m	1375 m	1087 m	920 m	879 m
S_{14}	3335 m	3061 w	2933 m	1683 vs	1593 s	1311 m	1159 m	975 w	837 m
S_{15}	3250 m	3000 w	2897 m	1653 s	1590 m	1313 m	1047 m	879 m	780 w
S_{16}	3385 m	3000 w	2897 m	1653 vs	1590 m	1382 m	1047 w	879 m	785 w

υN-H (amide II) at the range (1616-1600) cm^{-1}, while the (amide) I **υ**C=O appear as very strong bands at the range (1718-1624) cm^{-1}. In addition, the bands present at the range (1382-1285) cm^{-1} and (1161-1033) cm^{-1} are assigned to asymmetrical and symmetric **υ**SO_2 respectively [9]. Also, from IR spectra the bands appear at the range (1598-1520) cm^{-1} are assigned to **υ**C=N moiety of aromatic ring in sulfa drugs [14,15]. The spectral data have been shows in **Table 3**.

^{1}HNMR: The ^{1}HNMR spectral data of all the synthesized compounds (S_1-S_{16}) were summarized in **Table 4**. Generally, all protons signals were found as to be in their predictable regions. The interpretation of these data gave further information and maintenance to the manner of bonding discussed in their IR spectra. All the compounds display a singlet signal at δ(12.24-12.29) ppm attributed to the proton of $NHCO_{(amide)}$ [16]. The multiple signals at δ(6.54-8.89) ppm can be assigned to the protons of phenyl group of these synthesized compounds. Due to ^{1}HNMR spectrum of compounds the CH_3 protons appear in different situation, as we seen in compounds S_1,S_2,S_3,S_6,S_{10},S_{12} and S_{13}, the signal of these protons which existent in the amino acids part appeared at as a doublet signal at the range δ(0.98-1.42)

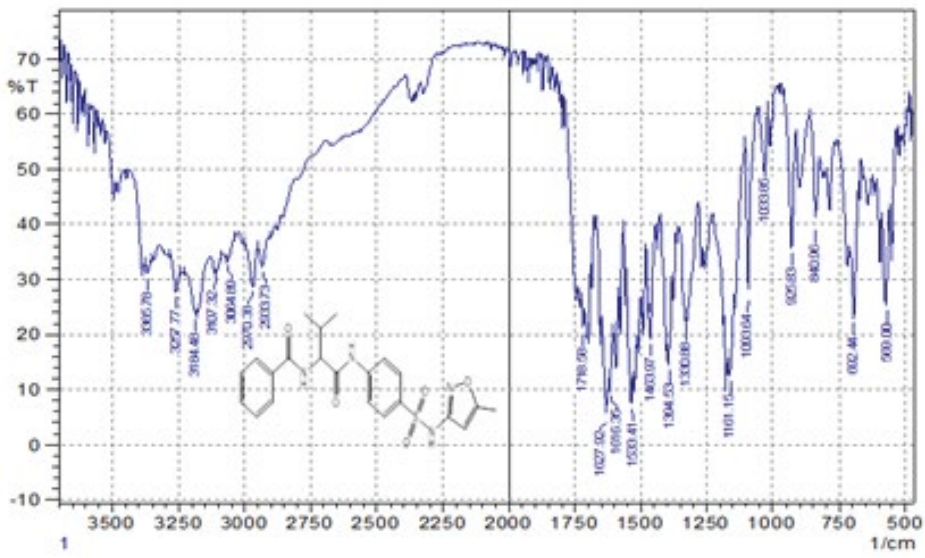


Figure 3: IR Spectrum of the compound S_1.

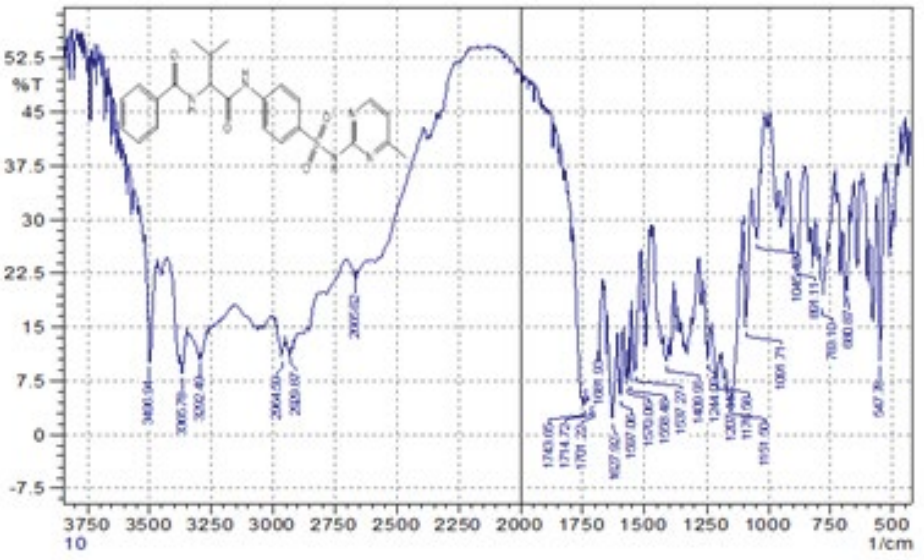
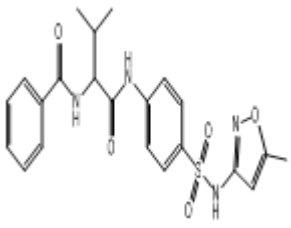
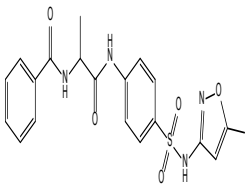
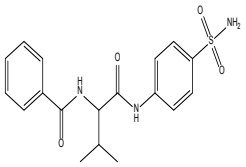
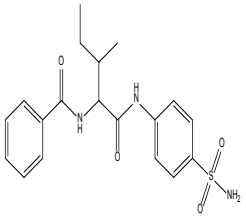
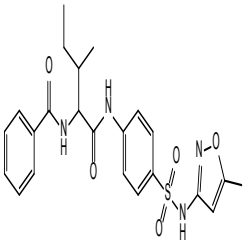
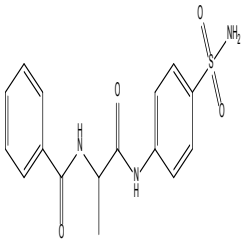
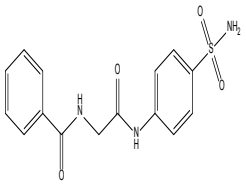
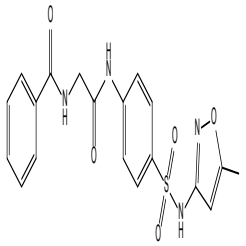


Figure 4: IR Spectrum of the compound S10.

Chemical shift δ (ppm)	Structural formula	Symbol of com.
12.27 (br, 2H, 2NHCO) 6.54-8.4 (m, 10H, Ar) 4.28 (s, 1H, $NH)_{sulfa}$ 2.78 (d, 1H, CH-CO) 2.61 (m, 1H, CH) 2.31 (s, 3H, $CH_3)_{sulfa\ ring}$ 1.0 (d, 6H, $2CH_3$)		**S_1**

12.27 (br, 2H, 2NHCO) 6.7-8.9 (m, 10H, Ar) 4.28 (s, 1H, $NH)_{sulfa}$ 2.76 (q, 1H, CH-CO) 2.32 (s, 3H, $CH_3)_{sulfa\ ring}$ 1.09 (d, 3H, CH_3)		**S_2**
12.29 (br, 2H, 2NHCO) 8.20 (br, 2H, $NH_2)_{sulfa}$ 6.75-8.89 (m, 9H, Ar) 2.78 (d, 1H, CH-CO) 2.61 (m, 1H, CH) 1.05 (d, 6H, $2CH_3$)		**S_3**
12.28 (br, 2H, 2NHCO) 8.15 (br, 2H, $NH_2)_{sulfa}$ 6.72-8.50 (m, 9H, Ar) 2.77 (d, 1H, CH-CO) 2.601 (m, 1H, CH) 2.13 (m, 2H, CH_2) 1.84 (d, 3H, CH_3) 0.95 (t, 3H, CH_3)		**S_4**
12.28 (br, 2H, 2NHCO) 6.88-8.65 (m, 10H, Ar) 4.27 (s, 1H, $NH)_{sulfa}$ 2.73 (d, 1H, CH-CO) 2.29 (s, 3H, $CH_3)_{sulfa\ ring}$ 2.22 (m, 2H, CH_2) 2.075 (m, 1H, CH) 1.82 (d, 3H, CH_3) 0.99 (t, 3H, CH_3)		**S_5**
12.29 (br, 2H, 2NHCO) 8.25 (br, 2H, $NH_2)_{sulfa}$ 6.77-8.69 (m, 9H, Ar) 2.73 (q, 1H, CH-CO) 1.42 (d, 3H, CH_3)		**S_6**
12.27 (br, 2H, 2NHCO) 8.05 (br, 2H, $NH_2)_{sulfa}$ 6.59-8.43 (m, 9H, Ar) 2.69 (s, 2H, CH_2)		**S_7**
12.24 (br, 2H, 2NHCO) 6.77-8.69 (m, 10H, Ar) 4.25 (s, 1H, $NH)_{sulfa}$ 2.75 (s, 2H, CH_2) 2.36 (s, 3H, $CH_3)_{sulfa\ ring}$		**S_8**
Chemical shift δ (ppm)	**Structural formula**	**Symbol of com.**

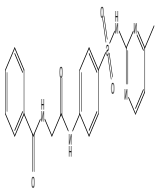
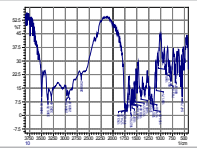
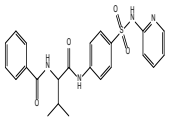
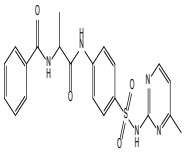
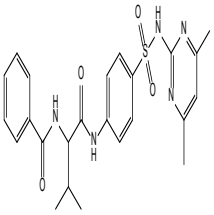
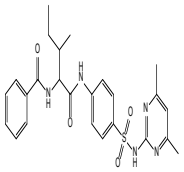
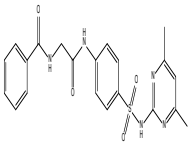
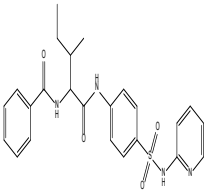
12.27 (br, 2H, 2NHCO) 6.65-8.71 (m, 11H, Ar) 4.25 (s, 1H, $NH)_{sulfa}$ 2.77 (s, 2H, CH_2) 2.34 (s, 3H, $CH_3)_{sulfa\ ring}$		**S_9**
12.28 (br, 2H, 2NHCO) 6.54-8.30 (m, 11H, Ar) 4.28 (s, 1H, $NH)_{sulfa}$ 2.77 (d, 1H, CH-CO) 2.63 (m, 1H, CH) 2.30 (s, 3H, $CH_3)_{sulfa\ ring}$ 0.98 (d, 6H, $2CH_3$)		**S_{10}**
12.28 (br, 2H, 2NHCO) 6.67-8.82 (m, 13H, Ar) 4.27 (s, 1H, $NH)_{sulfa}$ 2.78 (d, 1H, CH-CO) 2.59 (m, 1H, CH) 1.01 (d, 6H, $2CH_3$)		**S_{11}**
12.27 (br, 2H, 2NHCO) 6.65-8.79 (m, 11H, Ar) 4.28 (s, 1H, $NH)_{sulfa}$ 2.79 (q, 1H, CH-CO) 2.29 (s, 3H, $CH_3)_{sulfa\ ring}$ 0.99 (d, 3H, CH_3)		**S_{12}**
12.25 (br, 2H, 2NHCO) 6.58-8.78 (m, 10H, Ar) 4.28 (s, 1H, $NH)_{sulfa}$ 2.78 (d, 1H, CH-CO) 2.56 (m, 1H, CH) 2.27 (s, 6H, $2CH_3)_{sulfa\ ring}$ 0.99 (d, 6H, $2CH_3$)		**S_{13}**
12.27 (br, 2H, 2NHCO) 6.79-8.70 (m, 10H, Ar) 4.29 (s, 1H, $NH)_{sulfa}$ 2.77 (d, 1H, CH-CO) 2.29 (m, 1H, CH) 2.29 (s, 6H, $2CH_3)_{sulfa\ ring}$ 2.07 (m, 2H, CH_2) 1.81 (d, 3H, CH_3) 0.98 (t, 3H, CH_3)		**S_{14}**
12.29 (br, 2H, 2NHCO) 6.68-8.79 (m, 10H, Ar) 4.28 (s, 1H, $NH)_{sulfa}$ 2.68 (s, 2H, CH_2) 2.30 (s, 6H, $2CH_3)_{sulfa\ ring}$		**S_{15}**
12.27 (br, 2H, NHCO) 6.74-8.68 (m, 13H, Ar) 4.28 (s, 1H, $NH)_{sulfa}$ 2.78 (d, 1H, CH-CO) 2.34 (m, 1H, CH) 2.09 (m, 2H, CH_2) 1.07 (d, 3H, CH_3) 0.99 (t, 3H, CH_3)		**S_{16}**

Table 4: ^{1}HNMR spectral data of synthetic compounds (S_1-S_{16})

ppm while the protons of CH_3 of the compounds S_4,S_5,S_{14} and S_{16} appeared as triplet signal at the range δ(0.95-0.99) ppm and the signals of CH_3 protons which is part of sulfa drugs appeared as singlet signals at the range δ(2.29-2.36)ppm as in compounds (S_1,S_2,S_5,S_8,S_9,S_{10} and S_{12}). Furthermore, all the signals of the groups, -CH_2, CH were pointed out and indicated in the **Table 4 and Figure 5**. Also due to the ^{1}HNMR spectra of the compounds S_3,S_4,S_6 and S_7 we can observed broad signal at the range δ(8.05-8.12)ppm which confirm the two protons of terminal amine group NH_2 which affiliate to sulfanilamide [10,17].

Antibacterial activity: *In vitro* antibacterial activity of the compounds (S_1-S_{16}) was done in comparison with tetracycline as standard to disclose the effectiveness of the synthesized compounds against two selected strain Gram-positive *(S. aureus)* and Gram-negative *(A. hydrophilia)*. The inhibition zones were measured in mm and results are shown in **Table 5**. The results of antimicrobial screening indicate that these compounds show more activity against Gram-negative

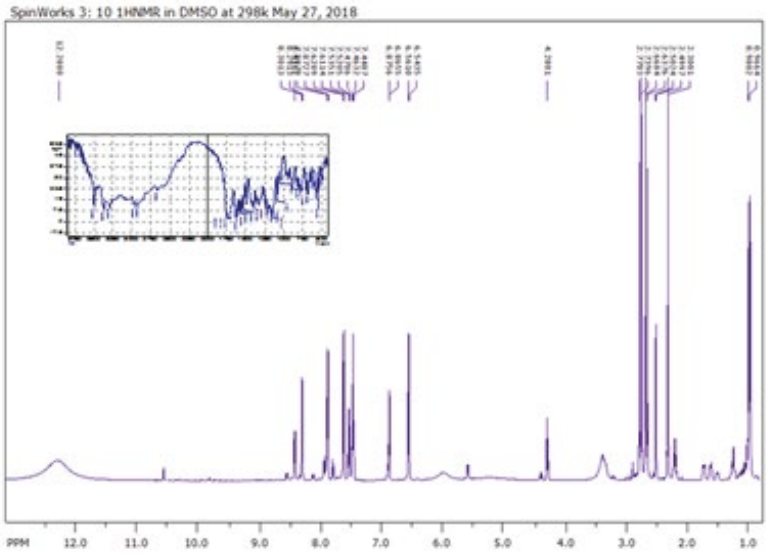


Figure 5: [1]HNMR Spectrum of the compound $S_{10.}$

Table 5: Inhibition diameters of compounds S_1-S_{16} against *Staphylococcus aureus* and *Aeromonas hydrophilic.*

	Diameter of inhibition zone (mm) *Staphylococcus aureus*								Diameter of inhibition zone (mm) *Aeromonas hydrophilic*						
	Concentration (mg/l)								Concentration (mg/l)						
Compounds	25	50	100	200	250	300	MIC	**Compounds**	25	50	100	200	250	300	MIC
S_1	0	0	0	5	9	17	200	S_1	0	5	7	11	16	22	50
S_2	0	0	0	5	7	15	200	S_2	0	5	8	13	18	22	50
S_3	0	0	0	0	8	13	250	S_3	0	0	5	11	15	20	100
S_4	0	0	0	5	9	16	200	S_4	0	0	4	7	11	18	100
S_5	0	0	5	8	11	17	100	S_5	0	5	8	11	18	23	50
S_6	O	0	0	0	8	17	250	S_6	0	0	5	9	14	21	100
S_7	0	0	0	0	6	15	250	S_7	5	8	12	15	21	26	25
S_8	0	0	0	7	13	18	200	S_8	4	7	10	14	19	22	25
S_9	0	0	5	13	19	22	100	S_9	5	9	13	17	20	24	25
S_{10}	0	0	5	8	11	15	100	S_{10}	0	5	8	12	18	22	50
S_{11}	0	0	4	6	10	14	100	S_{11}	0	0	0	5	9	15	200
S_{12}	0	0	0	5	9	15	200	S_{12}	0	6	10	17	19	23	50
S_{13}	0	0	0	0	6	10	250	S_{13}	0	5	8	13	19	22	50
S_{14}	0	0	0	0	5	11	250	S_{14}	0	5	9	12	18	20	50
S_{15}	0	0	0	0	5	13	250	S_{15}	0	5	8	11	16	21	50
S_{16}	0	0	0	5	9	14	200	S_{16}	0	0	0	7	16	21	200
tetracycline	4	7	11	17	21	24	25	**tetracycline**	8	12	17	21	26	29	25

bacteria. A minimum inhibitory concentration (MIC) also done which can define it as the lowest concentration of the compound in a particular medium which we can located the growth of the tested strain in concentration straighten from 25-300 mg/l. Invest with data, these compounds have mutable antibacterial activity against the two species of these organism. As mentioned earlier and as a comparison to these kinds of study microorganism we can observed that these compounds have effectiveness with Gram-negative bacteria, than another kind Gram-positive especially compounds S_7,S_8 and S_9, so the perception of antimicrobial screening denote that the synthesized compounds may be have a great effect on the cell membrane of the bacteria. According to intelligible of cell walls of bacteria, although even though

that the bacterial cell wall differs from that of all other organism by the presence of peptidoglycan which is located outside of the cytoplasmic membrane and lipid bilayer membrane. In Gram-positive bacteria cell walls, the matrix substance in this wall is poly saccharides, peptidoglycan (also known as murein layer) and the major component of that wall is lipoteichoic acid, the characteristic grade for this wall thick, while the Gram-negative bacteria cell walls are thin and unlike the Gram-positive cell walls, contain a thin peptidoglycans layer . An ordinary lipid layer is very weak and may be these compounds have the ability to penetrate the cell wall and thus lead to a difference in osmotic pressure [18]. On other hand the wall membrane of the cell composed of peptidoglycans long chain of carbohydrate cross-linked by short peptides (7-12 amino acids), for this there is a possibility of some of synthesized compounds (unnatural) are incorporated into these peptides and prevents bacteria from multiplying and blocking the formation of the cross-links results in rupture of the cell [19].

CONCLUSION

Consequently, in this work we have synthesized new amide which can be considered as new sulfa drugs derivatives which in turn derived from available and well-known drugs. These synthesized compounds were characterized and confirmed by the spectroscopy methods and elemental analysis and it enhances that the reaction of sulfa drugs with benzoyl amino acid is a kind of acyl substitution reaction to form new amides compounds. This reaction updated by using DCCI as promoting reagent which accelerating the condensation reaction successfully. Also, antimicrobial activity of synthesized compounds and MIC was done in comparison with tetracycline as standard to detect the capability of the synthesized compounds. Two selected strain *Staphylococcus aureus* and *Aeromonas hydrophilic* showed good effectiveness at higher concentrations (300 mg/l), also the negative-Gram bacteria showed good sensitivity even in lowest concentration (25 mg/l).

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