Synthesis of New Amino Acid-Conjugated Derivatives of a Sulphonamide

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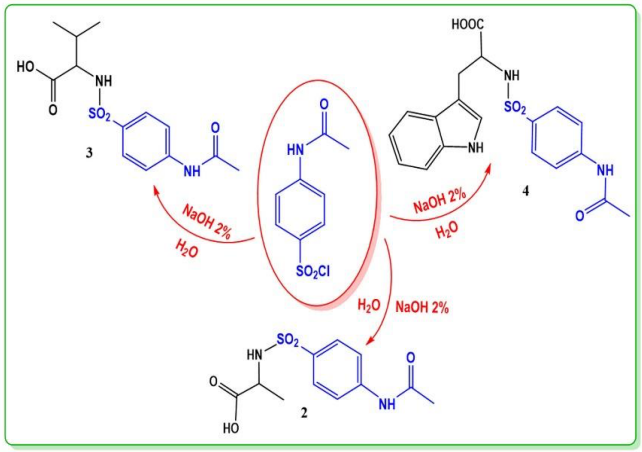
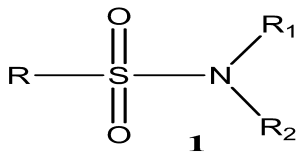
Abstract*— The subsequent synthesis involved the preparation of novel sulfonamide derivatives from 4-acetamidobenzenesulfanyl chloride. 4-acetamidobenzenesulfanyl chloride was synthesized as the initial compound, followed by the preparation of derivatives through the reaction of 4-acetamidobenzenesulfanyl with the amino acids valine, alanine, and tryptophan using a basic solution of sodium hydroxide. The structures of the derivatives determined by the using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and DEPT 135° analysis. In addition, the derivatives were also evaluated for their antimicrobial effectiveness against significant strains, specifically *S.aureus* and *E. coli.



Keywords: 4-Acetamidobenzenesulfonyl chloride, Sulfonamide, Amino acid, Derivatization, Spectroscopic techniques.

I. Introduction

Sulfonamides, a class of synthetic antimicrobial agents, have played a pivotal role in the field of medicine since their discovery in the mid-20th century. Also known as sulfa drugs, these compounds exhibit potent antibacterial properties and have been widely employed in the treatment of various bacterial infections. Its structure contains the function group R-SO_2-NR_2 **1.**



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The discovery of sulfonamides is credited to the groundbreaking work of German chemists Gerhard Domagk and his colleagues in the 1930s, who demonstrated the efficacy of the first clinically significant sulfonamide, Prontosil, in combating bacterial infections [1]. Sulfonamides function by interfering with bacterial folate synthesis, a crucial pathway for the production of nucleic acids.

By competitively inhibiting the enzyme dihydropteroate synthase, sulfonamides disrupt the synthesis of folic acid, an essential precursor for DNA and RNA synthesis in bacteria. This interference ultimately leads to the inhibition of bacterial growth and replication [2]. Sulphonamides have various therapeutic applications such as gastrointestinal [3], and urinary tract diseases [4-5]. Sulfonamide is the main focus of this study and is classified as sulfur compound [6] formerly known as sulfa drug.

The integration of amino acid derivatives into sulfonamide structures introduces a new dimension to their biological activity, offering the potential for improved efficacy and reduced side effects. This modification often targets specific pathways or mechanisms in bacteria, enhancing the selectivity and potency of the compounds. The rational design of sulfonamide-amino acid hybrids draws inspiration from the understanding of bacterial physiology and biochemistry, aiming to optimize antimicrobial activity. Researchers have explored the synthesis of sulfonamide-amino acid hybrids for their diverse applications, ranging from antimicrobial agents to anticancer drugs [7-8].

There are two general procedures for this synthesis, one is using a two-phase solvent system, consisting of water and an organic solvent using modified Schotten-Baumann conditions, the other procedure is using organic solvents and organic bases (amine) to remove the HCl that is formed, and this procedure often require high temperature, especially for the less reactive substrates. Recently, one of the most important ways for the good efficiency and simplicity of the method has been the traditional synthesis method of sulfonamides, which involves the synthesis at room temperature under pH control with NaOH or Na_2CO_3, in addition, this method limits the use of expensive. organic solvents. The main obstacle to this method is the use of excess bases to remove the HCl produced during the reaction. In recent years, green chemistry supports the synthesis of products and methods that reduce the use

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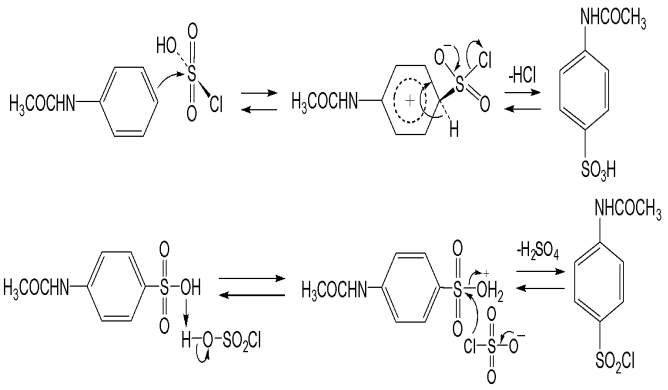
and generation of dangerous substances to protect nature [9-10].

II. Results and Discussion

The compounds **2**, **3** and **4** sourced from 4-acetamidobenzenesulfanyl chloride. The 4-acetamidobenzenesulfanyl chloride was obtained from acetanilide with chlorosulfonic acid according to the aromatic electrophilic substitution reaction, the melting point of the product was identical to the reference product (148 °C). The reaction mechanism is shown in scheme (**1**), the aromatic π electrons can bind to the partially positively charged sulfur atom and the Cl will leave to generate the HCl.

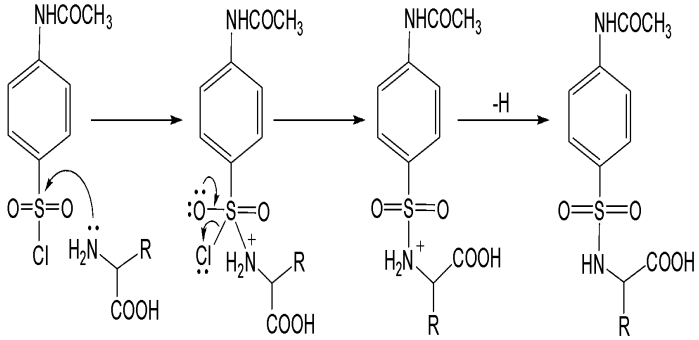


Scheme (1)

Another molecule of chlorosulfonic acid binds its chloride to an aromatic sulfonic acid to produce a more stable sulfuric acid and transforms the aromatic acid to the corresponding chloride, which was immediately used in the next step without further refinement. The IR spectrum afford peaks identical to the reference spectrum which showed two medium peak at 3307 cm^{-1} and 3263 cm^{-1} due to the stretching vibration of hydrogen–nitrogen in amide group. Stretching vibration of SO_2 was identified by the peak at 1370 and 1167 cm^{-1}.

The crude 4-acetamidobenzenesulfonyl chloride that used in this step without further purification reacts with amino acids alanine, valine, and tryptophan to produce the compounds **2**, **3** and **4** in the same manner by a nucleophilic substitution reaction using an alkaline aqueous solution of NaOH, which was used to enhance the nucleophilicty of the amine on the on hand, and modify the released HCl on the other hand. Diluted HCl was added slowly to set the pH to 2. The precipitate was collected by filtration. Products formation was confirmed through TLC, and purified by column chromatography.

The reaction mechanism was proposed through nucleophilic substitution according to the S_N2 mechanism is illustrated in the scheme (**2**)



Scheme (2)

We use ^{1}H-NMR, ^{13}C-NMR and DEPT-135° spectroscopy beside TLC to confirm the identity and purity of all reported products. The IR and NMR spectroscopic data details are presented below.

2-[(4-acetamidophenyl) sulfonamide] propanoic acid **2** was prepared via reaction of alanine with 4-acetamidobenzenesulfanyl chloride. The important functional groups of compound **2** were confirmed from FT-IR spectroscopy. The functional group area of the IR spectrum shows a high vibrational frequency (3451 cm^{-1}) of O-H *str.* relative to N-H stretching's (sulfonamide N-H *str.*: 3338, amide N-H *str.*: 3260 cm^{-1}). Peaks at 1543 cm^{-1} and 1593 back to the C=C stretching, stretching vibration of SO2 was identified by the peak at 1326 and 1151 cm^{-1}. The most significant signal in the spectrum is related to C=O stretching. The C=O stretching carboxylic signal at 1726 cm^{-1} confirm the formation of the compound **2** which the appearance of carboxylic carbonyl stretching at higher vibrational absorption frequencies, while the resonant effect is stronger in starting compound alanine due to it exists on the form of a double charge so appears at a lower wavenumber C=O stretching carboxylic at 1625 cm^{-1}. Moreover, the hydrogen configuration of this compound was confirmed by ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectrum analysis shows quartet peak to (H-2) at δ = 3.56 ppm and a doublet signal to proton (H-3) at δ = 1.13 ppm besides a singlet signal of proton (H-2") at δ = 2.07 ppm. The aromatic protons in the range of (δ = 7-8 ppm). The exchangeable proton O-H are clearly seen at δ = 10.42 ppm and protons of N-H are very broad at δ = 5.48 ppm due to the electric quadruple moment of the nitrogen nuclei, which enhances spin relaxation, leading to a very broad peak. The carbons configuration of the compound **2** was determined by ^{13}C-NMR spectroscopy. The carbons of C-3 at (δ = 18.84 ppm), C-2" at (δ = 23.87 ppm) and C-2 at δ = 51.19 ppm. The aromatic carbons were in the range of (115-130 ppm) that possess four signal represent six carbons. The carbons of carbonyl C-1 and C-1" at δ = 173.02 and 168.55 ppm. Furthermore, spectroscopic confirmation about the formation of compound **2** has been obtained from the DEPT-135 spectrum, all quaternary carbons (δ = 173.02, 168.66, 142.60,

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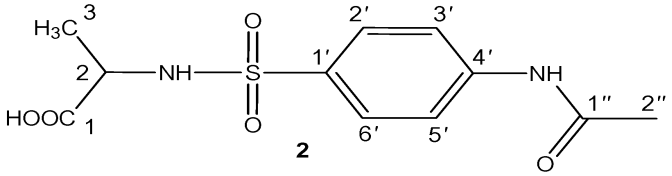

and 134.66 ppm) vanish from the spectrum, the carbons CH and CH_3 at (δ = 127.43, 118.35, 51.19, 23.87 and 18.84 ppm) at the top of spectrum. All the chemical shift and coupling constants determined for the compound **2** in the Table **1**.

To confirm the completion of the reaction and obtain compound **2**, we can compare the spectroscopic data of ^{13}C-NMR spectroscopy for reactant compound alanine and product compound **2**. The differences in chemical shift were ($\Delta\delta$ = -0.9) for C-1, ($\Delta\delta$ = +1.09) for C-2, and ($\Delta\delta$ = +2.34) for C-3. All the above data pointed toward a structure were complete agreement with the compound **2**.

TABLE **1**, ^{1}H, ^{13}C-NMR AND DEPT-135^{O} DATA FOR COMPOUND (**2**)

No	^{13}C	DEPT	^{1}H
1	173.02	C	10.42, *s*
2	51.19	CH	3.56 (*q*, *J* =6.96 Hz, 1H)
3	18.84	CH_3	1.13 (*d, J* =7.08 Hz, 3H)
1'	134.66	C	
2', 6'	127.43	CH	7.74 (*d*, *J* = 8.84 Hz, 2H)
3', 5'	118.35	CH	7.69 (*d*, *J* = 8.84 Hz, 2H)
4'	142.60	C	
1"	168.66	C	
2"	23.87	CH_3	2.07 (*s*, 3H)
NH			5.48 *br. S*
CO-NH			5.48 *br. S*

2-[(4-acetamidophenyl) sulfonamide]-3-methyl butanoic acid **3** was prepared from amino acid valine with 4-acetamidobenzenesulfonyl chloride. The functional group area of the IR spectrum shows a high vibrational frequency (3441 cm^{-1}) of O-H stretching relative to N-H stretching's (sulfonamide N-H *str*.: 3363 cm^{-1}, amide N-H *str*.: 3254 cm^{-1}).

Peaks at 1546 cm^{-1} and 1593 cm^{-1} could be attributed to the stretching C=C, stretching vibration of SO_2 was identified by the peak at 1332 cm^{-1} and 1165 cm^{-1}. The appearance of carboxylic carbonyl stretching at higher vibrational absorption frequencies at 1715 cm^{-1} relative to the carbonyl amino acid valine at 1596 cm^{-1} for the same reason mentioned in the previous compound. Moreover, the ^{1}H-NMR spectrum analysis shows two doublet peaks to methyl groups (H-4) and (H-5) at δ = 0.76 ppm and 0.85 ppm and multiple signal to proton (H-3) at δ = 2.51 ppm that overlap with solvent signal and singlet signal to methyl protons (H-2") at δ = 2.07 ppm. Besides, a doublet signal to proton (H-2) at δ = 2.98 ppm and the aromatic protons in the range of (δ = 7-8 ppm). The exchangeable proton O-H are clearly seen at δ = 10.72 ppm and protons of N-H are very broad at δ = 6.62 ppm. The ^{13}C-NMR spectrum analysis shows eleven signal to thirteen of carbons. The carbons of carbonyl C-1 and C-1" at δ = 172.29 ppm and 169.48 ppm. The aromatic carbons in the range of (115-145 ppm) that possess four signal to six of carbons. In addition to signal to C-2 at δ = 62.99 ppm and signal to C-3 at δ = 31.38 ppm. The carbons of methyl (C-2") (C-5) (C-4) at δ = 24.51, 20.01, 18.28 ppm. All the chemical shifts and coupling constants determined for the compound **3** in the Table **2**. Furthermore, spectroscopic confirmation about the formation of compound **3** has been obtained from the DEPT-135 spectrum, all quaternary carbons (δ = 172.29, 169.48, 143.15, and 134.43 ppm) vanish from the spectrum, the carbons CH and CH_3 at (δ = 128.21, 118.83, 62.99, 31.38, 24.51, 20.01 and 18.28 ppm) at the top of spectrum (the data shown in Table **2**). Moreover, comparison between the ^{13}C-NMR data of compound **3** and valine showed these compounds were different in chemical shift of C-1 ($\Delta\delta$ = -0.41), C-2 ($\Delta\delta$ = +3.39), C-3 ($\Delta\delta$ = +1.39), C-4 ($\Delta\delta$ = +0.38), C-5 ($\Delta\delta$ = +1.51). All the above data pointed toward a structure were complete agreement with the compound **3**.

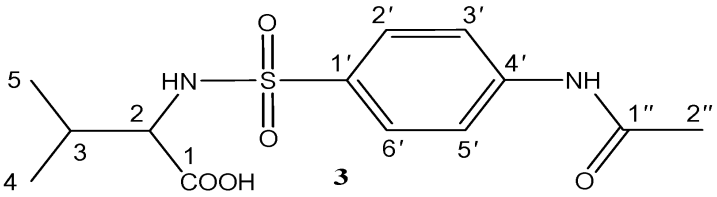

TABLE **2**, ^{1}H, ^{13}C-NMR AND DEPT-135^{O} DATA FOR COMPOUND (**3**)

No	^{13}C	DEPT	^{1}H
1	172.29	C	10.72, *s*
2	62.99	CH	2.980 (*d, J* = 3.4 Hz, 1H)
3	31.38	CH	2.510, *m*
4	18.28	CH_3	0.760 (*d, J* = 6.8 Hz, 3H)
5	20.01	CH_3	0.850 (*d, J* = 6.8 Hz, 3H)
1'	143.15	C	
2', 6'	128.21	CH	7.719 (*d, J* = 8.76 Hz, 2H)
3', 5'	118.83	CH	7.640 (*d, J* = 8.36 Hz, 2H)
4'	134.43	C	
1"	169.48		
2"	24.51	CH_3	2.07(*s*,3H)
NH			6.620 (*br. s*)
CO-NH			6.620 (*br. s*)

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2-[(4-acetamidophenyl) sulfonamide]-3-(1H-indol-3-yl) propanoic acid **4** was prepared from tryptophan with 4-acetamidobenzenesulfonyl chloride.

The functional group area of the IR spectrum shows a high vibrational frequency (3451cm^{-1}) of O-H *str.* relative to N-H stretching (sulfonamide N-H *str.*: 3401 cm^{-1}, amide N-H *str.*: 3313 cm^{-1}). Peaks at 1586 cm^{-1} and 1528 cm^{-1} could be attributed to the stretching C=C, stretching vibration of SO_2 was identified by the peak at 1319 cm^{-1} and 1150 cm^{-1}. The appearance of carboxylic carbonyl stretching at higher vibrational absorption frequencies at 1706 cm^{-1} relative to the carbonyl amino acid tryptophan for the same reason mentioned in the compound **2**. Moreover, The ^{1}H-NMR spectrum analysis shows a singlet signal to methyl group (H-2") at δ = 2.07 ppm, two signals for diastereotopic protons (H-3), (H-3') at δ = 2.81 ppm, 3.03 ppm and signal to (H-2) at δ = 3.87 ppm.

The aromatic protons and indole protons in the range at (6-8 ppm).

The exchangeable protons (O-H) at δ = 10.81 ppm and N-H protons at δ = 7.29, 10.24, 12.57 ppm. The ^{13}C-NMR spectrum analysis shows eighteen signal to twenty of carbons. The carbon of carbonyl C-1 and C-1" at δ = 172.99 ppm and 169.33 ppm. The aromatic carbons in the range of (105-145 ppm) that possess four signal to six of carbons, signal to C-2 at δ = 57.04 ppm and signal to C-3 at δ = 28.81 ppm. The carbons of methyl (C-2") at δ = 24.60 ppm. Furthermore, spectroscopic confirmation about the formation of compound (4) has been obtained from the DEPT-135 spectrum, all quaternary carbons (δ = 172.99, 169.33, 143.04, 136.50, 134.99, 127.41, and 109.37 ppm) vanish from the spectrum, the carbons CH and CH_3 at (δ = 127.92, 124.31, 121.31, 118.80, 118.65, 118.33, 111.84, 57.04, and 24.60 ppm) at the top of spectrum, and the carbon CH_2 at δ = 28.81 ppm at the bottom of spectrum. All the chemical shift and coupling constants determined for the compound **4** in the Table **3**. The comparison between the ^{13}C-NMR spectra of compound **4** and tryptophan confirms the formation of compound **4**. The differences in chemical shifts were at C-1 ($\Delta\delta$ = -1.71), C-2 ($\Delta\delta$ = +1.04), C-3 ($\Delta\delta$ = +0.61), C-3" ($\Delta\delta$ = -3.29), C-4" ($\Delta\delta$ = -4.83), C-5" ($\Delta\delta$ = -5.85), C-6" ($\Delta\delta$ = -3), C-7" ($\Delta\delta$ = +0.81), C-8" ($\Delta\delta$ = -2.36), C-9" ($\Delta\delta$ = -3.61), C-10" ($\Delta\delta$ = -1.49). All the above data pointed toward a structure were complete agreement with the compound **4**.

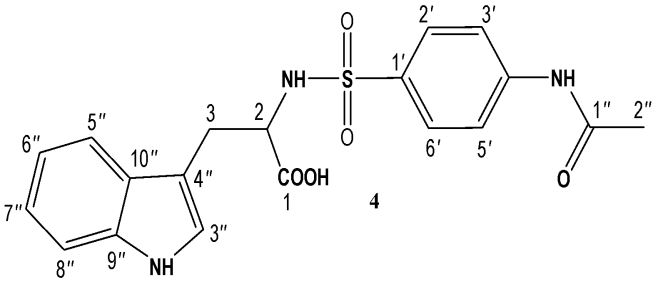

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TABLE **3**, ^{1}H, ^{13}C-NMR AND DEPT-135^O DATA FOR COMPOUND (**4**)

No	^{13}C	DEPT	^{1}H
1	172.99	C	10.81, *s*
2	57.04	CH	3.89 (*dd*, *J* =7.2 Hz, 1H)
3	28.81	CH_2	2.81 (*dd*, *J* =7.3 Hz, 1H)
3*	28.81	CH_2	3.03 (*dd*, *J* =6.8 Hz, 1H)
1'	136.50	C	
2', 6'	127.92	CH	7.62 (*d*, *J* =8.8 Hz, 2H)
3', 5'	118.33	CH	7.55 (*d*, *J* =8.8 Hz, 2H)
4'	143.04	C	
1"	169.33	C	
2"	24.60	CH_3	2.07(*s*,3H)
3"	124.31	CH	7.07 (*d*, *J* =2.4 Hz, 1H)
4"	109.37	C	
5"	118.65	CH	7.29 (*d*, *J* =8,0 Hz, 1H)
6"	118.80	CH	7.03 (*t*, J_1 = 8,0 Hz, J_2 =7.2 Hz 1H)
7"	121.31	CH	6.93 (*t*, J_1 =8,0 Hz, J_2 = 7.2 Hz 1H)
8"	111.84	CH	8.09 (*d*, *J* = 8.0 Hz, 1H)
9"	134.99	C	
10"	127.41	C	
NH			12.57 (*br. s*)
NH			10.24 *s*
NH			7.29 (*br. s*)

Antimicrobial Assay

The antimicrobial activity of the synthesized compounds were also evaluated against two types of microorganisms gram positive *S. aureus* and the other gram negative bacterial *E. coli* with the use gentamicin as reference antimicrobial. The compounds **2**, **3** and **4** have good antimicrobial activities against both *S. aureus* and *E. coli*. The concentrations values and results of measuring the diameter of the inhibition zones of are presented in Table **4** and **5**.

III. Conclusions

4-acetamidobenzenesulfonyl chloride was prepared as a starting compound by the electrophilic aromatic substitution reaction using excess of chlorosulfonic acid and its structure

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was proven by FT-IR and it matches the reference spectrum, In the next step, the new derivatives have been synthesized by reaction of 4-acetamidobenzenesulfonyl chloride with the amino acids alanine, valine, tryptophan by the nucleophilic substitution reaction using sodium basic solution. The compounds were purified using column chromatography. The structures of the derivatives have been described by different spectroscopy manners: ^{1}H-NMR, ^{13}C-NMR, DEPT- 135º, FT-IR.

TABLE **4**, ANTIMICROBIAL ACTIVITY OF PREPARED COMPOUNDS AGAINST *S. AUREUS.*

	Zone of inhabitation (mm)[a]			
Concentration	Gentamicin	2	3	4
350 µg\ml	24	14	13	22
300 µg\ml	23	12	10	18
250 µg\ml	21	9	7	17

[a]: Values are means of triplicate determination (n =3).

TABLE **5**, ANTIMICROBIAL ACTIVITY OF PREPARED COMPOUNDS AGAINST *E .COLI.*

	Zone of inhabitation (mm)[a]			
Concentration	Gentamicin	2	3	4
350 µg\ml	26	15	16	23
300 µg\ml	24	14	15	21
250 µg\ml	22	12	13	18

[a]: Values are means of triplicate determination (n =3).

Antimicrobial study of the derivatives was also evaluated against important strains, namely, *S. aureus* and *E. coli.* it was found that compound 4 is the most effective compounds toward both *E. coli* and *S. aureus* and the compounds **2** and **3** are moderately efficient toward both *E. coli* and *S. aureus.*

IV. Experimental Section

Chemicals and Instrumentation

Chemicals and solvents used in current work were of a high degree of purity, and the water used was also of high purity. The compounds alanine, valine, tryptophan, and chlorosulfunic acid were obtained from Sigma Aldrich. The progress of reaction was detected by TLC on 60 F_{254} aluminum plates (Merck) with detection by UV light. Purification performed using a chromatographic column 20 cm long and have a diameter of 2 cm using silica gel 230 mesh as stationary phase obtained from Merck. The IR spectra were recorded by Jasco IR (FT-IR), and we used NMR spectrometer from Bruker with ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz). The ^{1}H-NMR and ^{13}C-NMR spectra of all the synthesized compounds were recorded using DMSO.

Synthesis

General Procedure for Synthesis of 4-acetamidobenzenesulfonyl chloride:

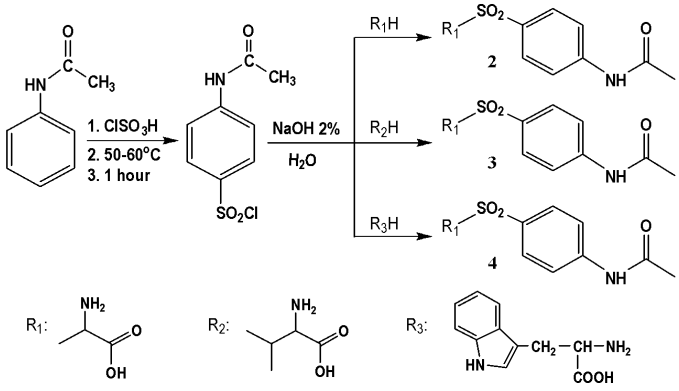
0.18 mol of chlorosulfonic acid (11.96 ml) was added to 0.02 mol of dry acetanilide (3 gr). The reaction components were placed in a flask with a magnetic stirrer on an ice bath, then the reaction was heated gently on a water bath to 60° C for 1 hour in order to finish the reaction. A flask cooled down to room temperature and the contents slowly poured into a beaker containing 100 gr of crushed ice, the pale white precipitate was removed by filtration, washed with a cold water, dried to give a solid product used in the next stage [11]. Yield 85% (3.97 gr), m.p. = 147-148 ºC. IR (KBr, cm^{-1}): 3307 and 3263 (NH $_{\text{stretching}}$), 3186 (C_{sp2}-H), 1683 (C=O amide), 1585 and 1533 (C=C aromatic), 1370 and 1167 (O=S=O).

Synthesis of Amino Acids Sulfonamides 2, 3, And 4.

We used the general method describe above to prepare the compounds **2**, **3**, and **4**. 0.0029 mol of 4-acetamidobenzenesulfanyl chloride (0.669 gr) was added to 10 ml of amino acid solution prepared in 2% NaOH (0.003 mol) at 25°C with constant stirring. The pH of the reaction contents was strictly monitored and maintained at 8-10 at regular intervals during reaction using NaOH 2%. The reaction takes 7 h for alanine, 9 h for valine, and 4 h for tryptophan. Then the aqueous solution was acidified to pH = 2-3 with hydrochloric acid (6 M), Scheme (3). Products formation was confirmed through TLC, purified by column chromatography using as eluent a mixture of (ethanol: chloroform 60:40) for alanine, (ethanol: chloroform 40:60) for valine, (ethanol: chloroform 50:50) for tryptophan.

2-[(4-acetamidophenyl) sulfonamide] propanoic acid **2**

White solid in yield 60% (0.5 gr), m.p. = 210-211 ºC. IR (KBr, cm^{-1}): 3451 (OH), 3338 (NH sulfonamide), 3260 (NH amide), 1726 (C=O carboxylic), 1644 (C=O amide), 1593 and 1543 (C=C aromatic), 1326 and 1151 (O=S=O). ^{1}H-NMR, ^{13}C-NMR and DEPT-135º spectra were recorded in DMSO-d_6 (Table **1**).



Scheme (**3**)

2-[(4-acetamidophenyl) sulfonamide]-3-methyl butanoic acid **3**

White solid in yield 70% (0.64 gr), m.p. = 222-224 ºC. IR (KBr, cm^{-1}): 3441 (OH), 3363 (NH sulfonamide), 3254 (NH amide), 1715 (C=O carboxylic), 1644 (C=O amide), 1593 and 1546 (C=C aromatic), 1332 and 1165 (O=S=O). ^{1}H-NMR, ^{13}C-

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NMR and DEPT-135° spectra were recorded in DMSO-d_6 (Table **2**).

2-[(4-acetamidophenyl) sulfonamide]-3-(1H-indol-3-yl) propanoic acid **4**

White solid in yield 64% (0.512 gr), m.p. = 250-252 °C. IR (KBr, cm^{-1}): 3451 (OH), 3401 (NH sulfonamide), 3313 (NH amide), 1706 (C=O carboxylic), 1662 (C=O amide), 1586 and 1528 (C=C aromatic), 1319 and 1150 (O=S=O). ^{1}H-NMR, ^{13}C-NMR and DEPT-135° spectra were recorded in DMSO-d_6 (Table **3**).

In Vitro Antibacterial Activity

Synthesized compounds were checked for antimicrobial activity by agar disk diffusion method. The standard strains of pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli* were collected from Microbiology Laboratory of the Department of Biological Sciences, Albath University. The checked compounds were dissolved in DMSO (6%). Bacterial suspension was added to each Mueller-Hinton agar plates. Blank paper discs impregnated with Different concentrations (250, 300, 350 $\mu g.ml^{-1}$) of stock solutions of synthesized compounds to be tested were placed on the agar plate and the growth of the bacteria was evaluated after 24 h incubation at 37 °C. The compounds were tested and Antibacterial activities were appreciated by measuring the growth inhibition zone against test organisms and compare to gentamicin used as reference and the average values are illustrated in Table **4** for *Staphylococcus aureus* and the Table **5** for *Escherichia coli*.

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