**RESEARCH ARTICLE**

# Synthesis and Biological Activity of New Sulfonamide Derivatives

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**ABSTRACT**

The widespread of resistance to antibacterial agents that became threatening made it essential to search for new antibacterial agents with new mechanisms of action. Different amino acids, dipeptides, and polypeptides derivatives were synthesized and tried as antibacterial agents, some of which were found to possess antibacterial activity. Five derivatives were prepared, the Ethyl-Para amino benzoate, ethyl 4-(phenylsulfonamido) benzoate, N-(4-(hydrazinecarbonyl) phenyl) benzene and N-(4-(2-(4-(dimethylamino) benbenzylidene) hydrazine carbonyl) phephenyl) benzene. The methods used for the identification of synthesized derivatives include elemental analysis, thin layer chromatography, infrared spectroscopy and melting point determination. The Preliminary study of antimicrobial activity for the target compounds showed that the compounds (4 and 5) had no activity against *Staphylococcus aureus*, while showed moderate activity against *Escherichia coli*, when compare to Ciprofloxacin. These compounds are thus promising targets for further researches in the antibacterial wide field.

**Keywords:** Biological, Sulfonamide, Synthesis.

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**Conflict of interest:** None

## INTRODUCTION

The sulfonamides (sulfa drugs) were the first medicines commonly used for these uses, including in the urinary tract, skin, and ears, and for curing bronchitis.[1] despite being a synthetic bacteriostatic antibiotic no longer in usage today, sulfonamides are also used as synthetic bacteriostatic antibiotics.[2]

Approximately 30 medications possess this capability, among them an antihypertensive agent named bosentan,[3] antiprotozoal,[4] antibacterial,[5] anti-inflammatory,[6] antifungal,[7] non-peptidic vasopressin receptor antagonists[8] as well as translation initiation inhibitors.[9] The sulfonamide derivatives serve as important carbonic anhydrase inhibitors, such as octadecyl trimethyl ammonium.[10] Although it's most widely used to treat urinary, intestinal, and ophthalmic diseases, it's also useful for treating skin wounds, ulcers, and colitis,[11] rheumatoid arthritis,[12] one form of male erectile dysfunction is the inhibition of phosphodiesterase-5 (PDE-5). The drug sildenafil, or its commercial name, Viagra, is a PDE-5 inhibitor[13] and obesity.[14] As a cancer-fighting tool, sulfur-containing medications such as sulfonamides are used nowadays,[15] and also used as antiviral against HIV by inhibition of protease.

In 1908,[16] was studying azo dyes when he synthesized the first sulfonamides. A short time after the conclusion of this study,[17] discovered dyes that included the sulfanyl group had a preference for silk and wool proteins. Liggett, in 1913, noticed that one of the azo dyes they had tested, chrysolidine, was very successful against bacteria in vitro.[18] Sulfonamides were not shown to have medicinal effects until 1932. a strong in vivo antibacterial action was discovered for Prontisil, (p-[2,4-diaminophenylazo] sulfanilamide), in animals.[19]

Although Prontisil possessed the Sulfanilamide as the key ingredient, which later contributed to substantial research into sulfonamide drug testing, Sulfanilamide was discovered to be the active portion of the Prontisil molecule.[20]

Sulfonamides mainly function as antimetabolites, directly inhibiting the biosynthesis of tetrahydrofolic acid.[21] Since sulfonamides inhibit para-aminobenzoic (PABA)-processing enzymes, PABA is unable to be synthesized. Inhibition of dihydrofolic acid synthetase will reduce thymidine and uridine for bacteria because they cannot consume tetrahydrofolic acid from their surroundings. For deoxyribonucleic acid (DNA) replication and transcription to occur, the cell requires these two nucleosides. This creates disturbance of cell development and differentiation, which gives the body's immune system ample time to remove the bacterial threat.[22,23] This study aimed to the synthesis of new sulfonamide derivative.

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## MATERIALS AND METHODS

### Synthesis of Ethyl-Para Amino Benzoate (EPAB) Compound[1]

Transfer the para-amino benzoic acid (12 g, 88 mmol) and 80 mL of absolute ethanol to a jar, and then add the condensed $H_2SO_4$ steadily and heat the mixture to 60ºC on a water tank. Keep the mixture at this temperature for 2 hours, then cool the flask for many minutes. In the end, 150 mL of 10% NaOH solution was applied, and with that, significant gas evolved. Removed impurities from the solution and obtained the precipitate. The residual spirit that has been rectified and dried in a desiccator under a vacuum to obtain crystals with a particular yield percentage produces white crystals.

### Synthesis of Ethyl 4-(phenylsulfonamido) Benzoate Compound[2]

To the mixture of compound 1 (0.66 g, 0.004 mol) in dichloromethane (20 mL) benzenesulfonyl chloride (0.7 g, 0.004 mol) was added dropwise at 0◦C, in the presence of triethylamine (0.01 mol, 1.4 mL). After 5 hours at room temperature, the resultant solution was stirred. When the reaction was finished, the solution was placed into an ice bath. After filtration, drying, and recrystallization, the precipitate was isolated: ethanol to water.

### Synthesis of N-(4-(hydrazinecarbonyl) phenyl) benzene sulfonamide Compound[3]

The reaction mixture was refluxed for 4 hours in ethanol and was made up of a solution of compound 2 (0.7g, 0.002 mol) and hydrazine hydrate 99% (1g, 0.02 mol). Cooling, refining, and drying the resulting firm, accompanied by recrystallization from a mixture of $CHCl_3$ and MeOH gave white crystals.

### Synthesis of Compounds[4,5]

A mixture of compound 3 (0.291 g, 0.001 mol) and appropriate aromatic aldehyde (0.001 mol) in 25 mL of absolute ethanol was refluxed for eight hours. The reaction mixture was cooled, and the product obtained was separated and recrystallized from ethanol.

### Biological Activity

According to the Agar well Diffusion Process, tentative antibacterial and antifungal activity has been done.

A variety of them was previously tested for their antimicrobial effects, which were successful against two gram-positive bacteria (*S. aureus*) and one gram-negative bacteria (*E. Coli*). Dissolved dimethyl fumarate (DMF) was used to remove the substances.

Ciprofloxacin was used as a standard antibiotic for antibacterial activity.[61,62] $1.510^8$ × CFU/mL of bacteria prepared from McFarland turbidity norm is used to perform the Agar well diffusion assay (number 0.5).

This was used to inoculate Mueller Hinton agar (MHA) plates by swabbing the soil. The extra air-dried and placed under a sterile hood. Each agar plate had five wells for the bacteria to expand, with 30 µL of each concentration placed in each well.

Antimicrobial activity was calculated by assessing the diameter of the inhibition zone (IZ) across the disk (plates incubated at 30°C for 72 hours or 37°C for 24 hours).

## RESULTS AND DISCUSSION

### Chemical Synthesis

The designed goal compounds' reaction sequences are diagrammed in the flowchart 1.

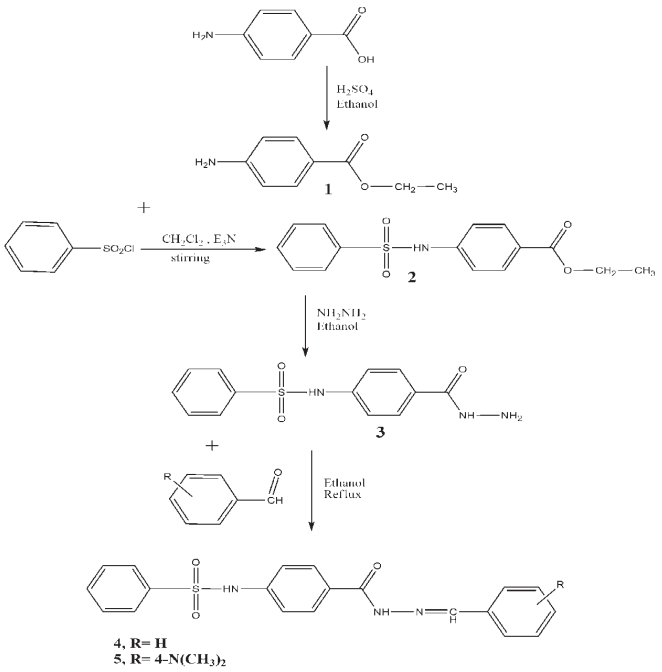
The overall synthesized compounds are designed to be as follows:

1. *Compounds 1:* was prepared by the reaction of p-amino-benzoic acid with ethanol (Scheme1).
2. *Compound (2):* Sulfonamide was prepared by the reaction of the ethyl 4-aminobenzoate (1) with benzenesulfonyl chloride, (Scheme 1).
3. *Compound (3):* was prepared by the reaction of the ethyl 4-(phenylsulfonamido) benzoate (2) with hydrazine hydrate, (Scheme 1).
4. Compounds (4,5): Schiff bases compounds were prepared by the reaction of N-(4-(hydrazinecarbonyl) phenyl) benzenesulfonamide3 with different aromatic aldehydes, (Scheme 1).

The synthesized compounds have been identified by their melting points (Table 1 to 3), IR data Figures 1 to 4. The purity of the compounds has been checked by TLC as illustrated in Table 1.

### Synthesis of ethyl 4-(phenylsulfonamido) benzoate (2)

Sulfonamides have been synthesized by reaction of compound (1) with benzenesulfonyl chloride in dichloromethane and triethylamine. The molecule's reaction follows a nucleophilic assault on the amine group and the subsequent release of HCl (Scheme 2).



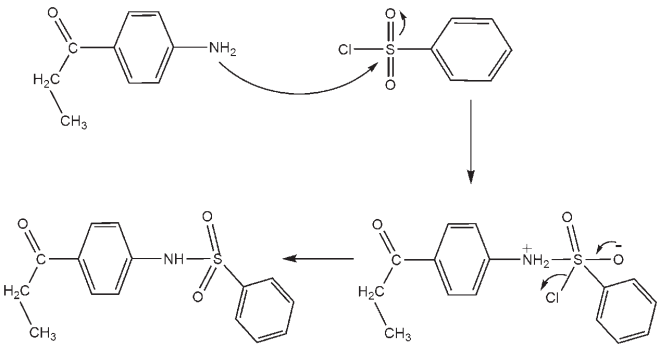

**Scheme 1:** Chemical synthesis of compounds (1–5)

The structures of this compound have been characterized by the disappearance of symmetric and asymmetric absorption bands for ($NH_2$) of compound (2) and the appearance of a new absorption band in the synthesized compound (3242) $cm^{-1}$ belong to (NH) group. Other IR characteristics absorption bands were listed in Table 2, Melting points and Rf value were listed in Table 1, and IR spectrum was shown in Figure 1.

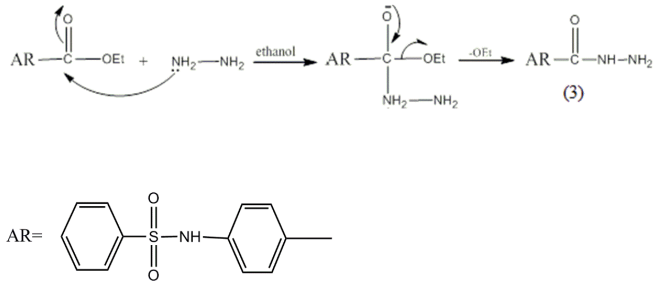
**Synthesis of Compound (3)**

N-(4-(hydrazinecarbonyl) phenyl) benzene sulfonamide was prepared by the treatment of the ethyl 4-(phenylsulfonamido) benzoate with Hydrazine hydrate in ethanol. The reaction proceeds via nucleophilic attack of the amine on the carbon atom of the carbonyl of compound 2 to give the combination 3.

The structures of this compound have been characterized by the disappearance of absorption bands for (C=O) of an ester of compound 2 and the appearance of a new absorption band in the synthesized compound (1674) $cm^{-1}$ and belong to (C=O) and ($NH_2$) of acid hydrazide. Other IR characteristics absorption bands were listed in Table 2, Melting points and Rf value were listed in Table 1, and IR spectrum was shown in Figures 2A and B).



**Scheme 2:** Mechanism of sulfonamide formation



**Scheme 2:** Mechanism of formation of Compound 3

**Synthesis of Compounds (4 and 5)**

Schiff bases have been synthesized by condensing compound 1 with the appropriate aromatic aldehydes in absolute ethanol. The reaction proceeds via nucleophilic attack of the amine on carbonyl carbon of the aldehyde with loss of a water molecule, as shown in Scheme 3.

The structures of these compounds have been confirmed by the disappearance of absorption bands for ($NH_2$) of compound 3 and the appearance of a new absorption band in synthesized compounds between (16-16) $cm^{-1}$ belong to (C=N) group. Other IR characteristics absorption bands were listed in Table (3-2), Melting points and Rf values were listed in Table 1, and IR spectrum was shown in Figures 3 and 4.

**Characterization and Identification of the Synthesized Compounds**

*Determination of Melting point*

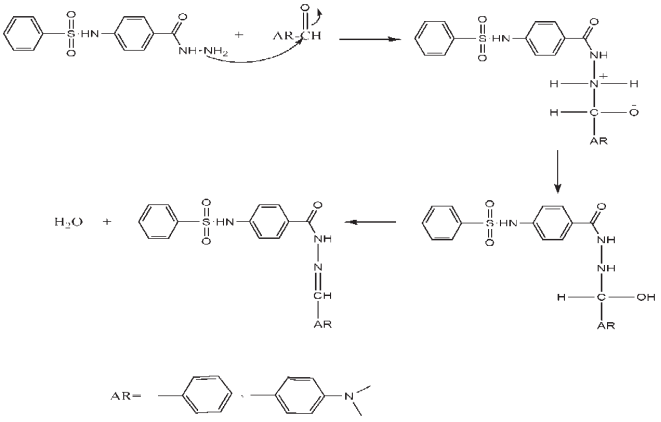
The synthesized substances had different melting points from the starting materials, suggesting that an erroneous adjustment has been implemented, as shown in Table 1.

*Thin Layer Chromatography (TLC)*

Table 1 showed the $R_f$ values of the intermediates and final products, which presented single round spots looked afterwards revealing the chromatograms to iodine vapour indicating the purity and the completion of the reactions.

**Infrared Spectra**

The fourier-transform infrared spectroscopy (FTIR) spectra of the synthesized compounds and their intermediates, Figures 1 to 4. As can be seen in the chemical structures that were



**Scheme 3:** Mechanism of schiff bases formation

**Table 1:** Physical appearance, percent yield, melting points and $R_f$ values of intermediates and compounds.

| *No* | *Compound Name* | *Yield %* | *Physical appearance* | *m.p. Co* | *Rf value* |
|---|---|---|---|---|---|
| 1 | Ethyl-Para amino benzoate | 70 | White crystals | 86–88 | 0.70 A |
| 2 | Ethyl 4-(phenylsulfonamido) benzoate | 60 | Faint-yellow crystals | 145–147 | 0.81A |
| 3 | N-(4-(hydrazinecarbonyl) phephenyl) benzene sulfonamide | 67 | | | |
| 4 | N-(4-(2-benzylidenehydrazine carcarbonyl) phenyl) benzene sulsulfonamide | 82 | | | |
| 5 | N-(4-(2-(4-(dimethylamino) benbenzylidene) hydrazine carbonyl) phephenyl) benzene sulfonamide | 80 | | | |

**Table 2:** The characteristic IR bands of synthesis compound

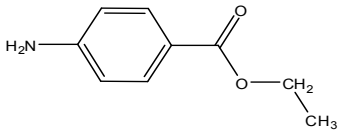
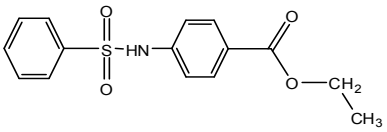
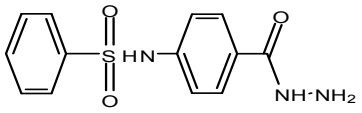
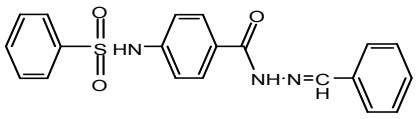
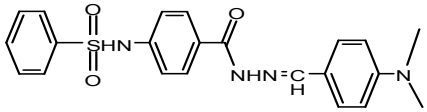
| *CPD No.* | *Compound* | *Characteristic IR bands Cm$^{-1}$* |
|---|---|---|
| 1 |  | (3344-3223 assym. & sym. NH2 str); (3060 C-H ar. Str.); (2980-2897 C-H alf. str.) ; (1694 C=O str. of ester); (1635-1597 C=C ar. str.); 1282 C-O str. |
| 2 |  | (3254 N-H Str.), (1716 C=O Str. ester), (3024 CH aro Str.), (2935assym, 2865sym C-Halif. Str.) (1597 - 1456 C=Caro Str.), (1340assym, 1165sym S=O Str.), (813,758 CHar out of plane). |
| 3 |  | (3392, 3355 NHNH2 Str.), (1674 C=O –CONHNH2) (3055 CHar Str.), (1610 - 1446 C=Car Str.), (1340assy, 1157sy S=O Str.),, (740-686 CHar out of plane). |
| 4 |  | |
| 5 |  | |

**Table 3:** The antibacterial activity of the tested compounds

| *Compound No.* | | *Zone of Inhibition in mm* | |
|---|---|---|---|
| *Staphy. aureus* | | *E. Coli* | |
| 4 | 50 µg/mL | No activity | 15 |
| | 100 µg/mL | No activity | 15 |
| 5 | 50 µg/mL | No activity | 15 |
| | 100 µg/mL | No activity | 16 |
| Ciprofloxacin | 100 µg/mL | 53 | 34 |

proposed in this paper, the bands of absorption observed in this experiment were inconsistent with the compounds presented. The Table displaying IR data with their interpretation was shown in Table 2.

### The Antimicrobial Activity

Synthesized compounds were screened for their antimicrobial activity. From the result in Table 3, Compounds 4 and 5 showed no activity against *Staphylococcus aureus,* while showed moderate activity against *Escherichia coli***.** When compare to Ciprofloxacin.

## CONCLUSIONS

On the bases of the results arrived at, the study concluded the following:

- The synthesized compounds have been put together successfully.
- Titration curves, refractive index, and FTIR analysis confirmed the purity and character of the synthesized compounds.
- The Preliminary study of antimicrobial activity for the target compounds showed that the compounds (4, and 5) had no activity against *S. aureus,* while showed moderate activity against *Escherichia coli***,** when compare to Ciprofloxacin.

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