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Synthesis of Aryl Sulfonamides by Eco-Friendly Method and Evaluation of Their Anti-Bacterial Activity

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Abstract

Antibiotic resistance becomes a significant threat to serious infectious patients. In particular, the bacteria can develop multiple drug resistances to all known antibiotics. As a result, the development and synthesis of new sulfonamide derivatives is an urgent necessity for finding new antibiotics. Aryl sulfonamides 1a-1h were synthesized by reacting 4-acetamidobenzenesulfonyl chloride with amino derivatives via an easy method in an aqueous-alkaline medium with yields between 62-92%. The compounds 2a-2h were derived from acidic hydrolyses of 1a-1h with yields between 85-94%. The structure of synthesized compounds was confirmed by spectral analysis (IR, ^{1}HNMR, ^{13}CNMR and ESI-MS). Some of the physicochemical properties of the compounds have been calculated using software such as Marvin Sketch. The antibacterial activity of compounds was tested against clinical and reference strains of Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae, by two methods: MIC and agar-well diffusion. The antibacterial activity results were compared with sulfanilamide as a control. Compounds 2e and 2f were the most effective. The MIC values of compounds 2e and 2f ranged between 64 and 512 µg/ml and the zones of inhibition diameters of compounds 2e and 2f ranged between 11 and 24 mm. In this study, a series of sulfonamides were synthesized by an eco-friendly and cost-effective method. The relationship between antibacterial activity and some physicochemical properties was studied in order to contribute to the development of new antibiotics to overcome bacterial resistance.

Keywords: Aryl sulfonamides, antibacterial activity, Minimal Inhibitory Concentration (MIC), eco-friendly synthesis.

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1. Introduction

Bacterial infections have posed serious health threats to humans and animals since ancient times. It was one of the leading causes of death, especially before antibiotic discovery. The discovery of antibiotics marked a pivotal turning point in eliminating bacterial infections and reducing deaths. Unfortunately, in the past few decades, bacteria have developed several ways to overcome antibiotics, with randomly antibiotic use [1]. Recently, the problem of antibiotic resistance has increased, and it has spread among bacteria [2]. Therefore, it is necessary to think of ways to overcome this emerging problem, whether by trying to discover new antibiotics or developing the structure of existing ones [3]. Finding new antibiotics is a bit challenging and costing process. Thus, the development of current antibiotics by modifying their structure and studying their antimicrobial efficacy has attracted notable interest from medicinal chemistry workers [4]. Among the antibiotics that have attracted attention for development are sulfonamides due to their small structure, cheapness, and abundant availability in all countries, including developing ones. Antimicrobial sulfonamides act by inhibiting the enzyme Dihydropteroate synthetase, thereby inhibiting the synthesis of folic acid. Folic acid is essential for the synthesis of bacterial nucleic acids such as DNA and RNA, thus inhibiting the growth of the bacterial cell. These sulfonamides have a broad spectrum of action, affecting both gram-positive and gram-negative bacteria, chlamydia, nocardia, fungi, and some protozoa [5]. Sulfonamides have many biological activities, such as anti-inflammatory, anti-viral, anti-cancer, or anti-microbiological. Many experiments have been conducted on them since their first appearance in 1932 until now [6].

Due to the importance of sulfonamides, our research's focused on the synthesis of aryl sulfonamide derivatives [7] and assessed their anti-bacterial activity against several strains of clinical and reference obtained from Aleppo University Hospital.

2. Materials and methods

2.1. Chemicals, Equipment and materials

All chemical compounds and reagents were supplied from reliable companies such as Sigma-Aldrich, with purity of 98%. The culture media used were obtained from HiMedia company. The pka values were predicted by the Marvin

Sketch program. Log p and Topological Polar Surface Area (TPSA) were calculated by the Molecular Operating Environment 2015.10 (MOE) program. Melting points was measured by BÜCHI Melting Point B-540 apparatus. IR spectra (KBr disc) were scanned on ATR-FTIR Bruker spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were run on the JEOL-ECA NMR spectrophotometer at 400 MHz, using DMSO-d_6 as the solvent. Chemical shifts were presented as δ-values (ppm). The mass spectra were recorded on the triple quadrupole mass spectrometer with positive ionization and the stronger peaks' m/z values were noted.

2.2. Synthesis of sulfonamides

Step 1: The amino derivatives (10 mmol) were dissolved separately in 100 ml of sodium bicarbonate solution (10%) in a convenient round flask, 4-acetamidobenzenesulfonyl chloride (10 mmol) was slowly added and stirred at 0 C° temperature and the reaction was left to stir for overnight. The resultant solution was acidified to pH=3 with hydrochloric acid (HCl) (1M) using a pH meter. The precipitate formed was separated by filtration and recrystallized from an ethanol-water mixture (1:1).

Step 2: 1 gram of the compounds 1a-h was added to 50 ml HCl (4M) in a rounded flask equipped with a reflux distillation column. The mixture was stirred and heated at 85 C° for two hours. However, for compound 1h 10 ml of ethanol was also added. The reaction was cooled to room temperature and the pH was adjusted to pH=3 using sodium hydroxide. Similarly, to the previous step, the precipitate was filtered and recrystallized.

2.3. Microbiological study

Escherichia coli ATCC 8739, *Staphylococcus aureus* ATCC 33591, *Klebsiella pneumoniae* ATCC 13885, and their clinical strains were obtained from Aleppo university hospital and were maintained in nutrient agar slant tube at 5 C° until use. Bacterial strains were cultured overnight at 35 C° in nutrient broth before use and the absorbance of bacterial suspension was adjusted to 0.09-0.11 at 620 nm by using a spectrophotometer to obtain fresh bacterial suspension equivalent to 0.5 McFarland 1.5×10^{8} CFU/ml. For the minimal inhibitory concentrations MIC study, the previous culture suspension was diluted a hundred times with Muller-Hinton Broth (MHB) to reach the final density 1.5×10^{6} CFU/ml. Stock solutions (which were used in the agar well diffusion method) were made by dissolving 100 mg of each compound separately in 10 ml of sterile dimethyl sulfoxide (DMSO) and their sterility was confirmed by filtering through a 0.22 µm membrane filter. While in the MIC method, 256 mg of the compound was dissolved in 12.5 ml of sterile DMSO and 1ml of that solution was diluted with 9 ml of Muller-Hinton Broth (MHB). Thus, the final concentration was 2048 µg/ml in 10% DMSO. The diameter of petri dishes was 9 mm and the depth of Muller-Hinton Agar (MHA) in dishes was 4-5 mm. MIC was applied to one strain of each of the reference and clinical bacteria. All procedures were done according to the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline [8-9].

2.3.1. Agar well diffusion method

At the first, 100 µl of the bacterial suspension (1×10^{8} CFU/ml) was spread on the surface of the agar plate (MHA) with a cotton swab, then wells with a diameter of 6 mm were dug into the agar by sterile pipette tips. those wells were filled with 40 µl of the studied substance solution (10 mg/ml in DMSO). Then the plates were incubated for 20 hours at a temperature of 37C°. Some wells were filled with DMSO as a blanc. The tests were carried out in triplicate and the zone of inhibition was measured with an accuracy of one mm, and the average of the three iterations for the measure was calculated.

2.3.2. Determination of Minimal Inhibitory Concentration method

The MIC of the compounds was determined using the serial microplate dilution method according to the following sequence: 100 µl of MHB medium was added to all wells of the microplate, then 100 µl of the compound solution was added to the first well then 100 µl from it was withdrawn, and added to the next one and mixed up and down 6-8 times, then 100 µl was transferred from the second to third well. The procedure was repeated down to obtain a two-fold serial dilution of tested compounds from 1024 to 8 µg/ml. The next step was dispensing 100 µl of fresh bacterial culture suspensions (1×10^{6} CFU/ml) into all wells except negative control wells. The plates were incubated for 20 hours at 37 C° and bacterial growth was detected by adding 20 µl of TTC (triphenyl tetrazolium chloride) (0.1%) to each well and the plates were scanned after incubation at 37 C° for 90 min [10].

3. Results and Discussions

3.1. Chemistry section:

Aryl sulfonamides compounds 1a-h were synthesized by reacting 4-acetamidobenzenesulfonyl chloride with primary amine derivatives in an aqueous alkaline medium. The reaction yield was ranged between 62-92%. The compounds 2a-h were synthesized by acidic hydrolysis of compounds 1a-h with yields between 85-94%.An ethyl ester derivative of compound 1h was obtained by adding ethanol to the hydrolysis medium. Figure 1 and Figure 2 summarize the synthesis reaction and the structure of compounds. The structure of compounds was identified using FT-IR, ES-MS, ^{1}HNMR and ^{13}CNMR spectra which were detailed in the identification section. The physicochemical properties of the molecules were predicted using the Molecular Operating Environment (MOE) and Marvin Sketch programs. The pKa_1 values of the amino group at the sulfonamide position ranged from 7.57 to 10.89 while the aryl amino group ranged from 2.03 to 2.27. The pka_3 values of compounds 2e, 2f, 2c and 2d were 12.79, 9.11, 3.31 and 4.49, respectively, where the pka_3 was related to hydroxyl and carboxyl groups. Topological Polar Surface Area (TPSA) and log P were calculated using MOE software. The TPSA ranged between 60.16 and 100.29 A°^{2} and the log P values ranged between 1.03 and 3.46.

3.2. Identification section:

***N*-(4-((phenylamino)sulfonyl)phenyl)acetamide (1a)**

White solid. Yield 85%. m.p 210 C°.

IR spectrum (vmax, cm^{-1}): 3235.47 (NH_{str}), 1669.97 ($C{=}O_{str}$), 1589.83 (NH_{bend}), 1538.32 ($C{=}C_{Ar\ str}$), 1315.08 ($S{=}O_{asym\ str}$), 1151.95 ($S{=}O_{sym\ str}$).

^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.22 (s, 1H, NH), 10.08 (s, 1H, NH), 7.64 (d, *J* = 0.9 Hz, 4H, Ar-H), 7.17 (dd, J = 8.5,

7.3 Hz, 2H, Ar-H), 7.03 (dd, *J* = 8.5, 1.0 Hz, 2H, Ar-H), 6.96 (tt, *J* = 7.0, 1.2 Hz, 1H, Ar-H), 2.01 (s, 3H, $COCH_3$).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 169.52, 143.61, 138.37, 133.60, 129.63 (d, *J* = 22.5 Hz), 128.45, 124.35, 120.55 (d, *J* = 20.6 Hz), 119.09, 24.59.
ESI-MS: *m/z* 291.07$[M+H]^+$, 313.09$[M+Na]^+$.

N-(4-(((4-fluorophenyl)amino)sulfonyl)phenyl)acetamide (1b)

white solid. Yield 90%. m.p 191 C°.
IR spectrum (νmax, cm^{-1}): 3339.78 (NH_{str}), 3181.04 (NH_{str}), 1651.79 ($C=O_{str}$), 1589.42 (NH_{bend}), 1533.98 ($C=C_{Ar\ str}$), 1318.32 ($S=O_{asym\ str}$), 1152.30 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.23 (s, 1H, NH), 10.03 (s, 1H, NH), 7.70 – 7.55 (m, 4H, Ar-H), 7.22 – 6.87 (m, 4H, Ar-H), 2.02 (s, 3H, $COCH_3$).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 169.54, 159.56 (d, J = 240.5 Hz), 143.67, 134.56 (d, J = 3.0 Hz), 133.29, 128.44, 123.34 (d, J = 8.4 Hz), 119.07, 116.32 (d, J = 22.8 Hz), 24.62.
ESI-MS: *m/z* 309.14$[M+H]^+$, 331.15$[M+Na]^+$.

2-(((4-(acetylamino)phenyl)sulfonyl)amino)benzoic acid (1c)

white solid. Yield 91%. m.p 243 C°.
IR spectrum (νmax, cm^{-1}): 3327.20 (NH_{str}), 1681.28 ($C=O_{str}$), 1651.05 ($C=O_{str}$), 1589.21 (NH_{bend}), 1537.89 ($C=C_{Ar\ str}$), 1315.70 ($S=O_{asym\ str}$), 1156.09 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 13.82 (s, 1H, OH), 10.98 (s, 1H, NH), 10.27 (s, 1H, NH), 7.85 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-H), 7.76 – 7.62 (m, 4H, Ar-H), 7.54 – 7.42 (m, 2H, Ar-H), 7.07 (ddd, *J* = 8.2, 6.9, 1.6 Hz, 1H, Ar-H), 2.01 (s, 3H, $COCH_3$).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 170.27, 169.62, 144.22, 140.47, 135.00, 132.40, 132.02, 128.76, 123.71, 119.19, 118.91, 117.09, 24.63.
ESI-MS: *m/z* 335.16$[M+H]^+$, 357.14$[M+Na]^+$.

4-(((4-(acetylamino)phenyl)sulfonyl)amino)benzoic acid (1d)

white solid. Yield 92%. m.p 248 C°.
IR spectrum (νmax, cm^{-1}): 3340.16 (NH_{str}), 1694.03 ($C=O_{str}$), 1668.03 ($C=O_{str}$), 1590.97 (NH_{bend}), 1532.43 ($C=C_{Ar\ str}$), 1325.57 ($S=O_{asym\ str}$), 1153.27 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 12.65 (s, 1H, OH), 10.63 (s, 1H, NH), 10.25 (s, 1H, NH), 7.79 – 7.66 (m, 6H, Ar-H), 7.15 (d, *J* = 8.9 Hz, 2H, Ar-H), 2.02 (s, 3H, $COCH_3$).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 169.56, 167.26, 143.92, 142.64, 133.27, 131.21, 128.53, 126.01, 119.20, 118.59, 24.62.
ESI-MS: *m/z* 335.17$[M+H]^+$, 357.12$[M+Na]^+$.

***N*-(4-(((2-hydroxyphenyl)amino)sulfonyl)phenyl)acetamide (1e)**

reddish solid. Yield 62%. m.p 223 C°.
IR spectrum (νmax, cm^{-1}): 3401.64 (OH_{str}), 3356.85 (NH_{str}), 3211.74 (NH_{str}), 1694.83 ($C=O_{str}$), 1590.13 (NH_{bend}), 1529.83 ($C=C_{Ar\ str}$), 1304.03 ($S=O_{asym\ str}$), 1145.74 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.20 (s, 1H, NH), 9.45 (s, 1H, NH), 8.95 (s, 1H, OH), 7.68 – 7.55 (m, 4H, Ar-H), 7.08 (dd, *J* = 8.0, 1.9 Hz, 1H, Ar-H), 6.88 (ddd, *J* = 8.2, 7.4, 1.8 Hz, 1H, Ar-H), 6.73 – 6.59 (m, 2H, Ar-H), 2.02 (s, 3H, $COCH_3$).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 169.48, 150.75, 143.39, 134.63, 128.44, 126.65, 125.13, 124.70, 119.45, 118.74, 116.04, 24.63.
ESI-MS: *m/z* 307.14$[M+H]^+$, 329.13$[M+Na]^+$.

***N*-(4-(((4-hydroxyphenyl)amino)sulfonyl)phenyl)acetamide (1f)**

reddish solid. Yield 65%. m.p 267 C°.
IR spectrum (νmax, cm−1): 3550.42 (OH_{str}), 3377.45 (NH_{str}), 1680.75 ($C=O_{str}$), 3317.71 (NH_{str}), 1592.11 (NH_{bend}), 1536.24 ($C=C_{Ar\ str}$), 1324.80 ($S=O_{asym\ str}$), 1147.42 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.22 (s, 1H, NH), 9.52 (s, 1H, NH), 9.25 (s, 1H, OH), 7.63 (d, *J* = 8.9 Hz, 2H), 7.53 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.79 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.56 (d, *J* = 8.8 Hz, 2H, Ar-H), 2.02 (s, 3H, $COCH_3$).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 169.57, 155.34, 143.38, 133.73, 129.15, 128.44, 124.68, 118.95, 116.03, 24.61.
ESI-MS: *m/z* 307.15$[M+H]^+$, 329.13$[M+Na]^+$.

***N*-(4-(((3-chloro-4-fluorophenyl)amino)sulfonyl)phenyl)acetamide (1g)**

white solid. Yield 88%. m.p 231 C°.
IR spectrum (νmax, cm−1): 3357.11 (NH_{str}), 1679.99 ($C=O_{str}$), 1592.69 (NH_{bend}), 1531.16 ($C=C_{Ar\ str}$), 1309.36 ($S=O_{asym\ str}$), 1182.00 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.30 (s, 1H, NH), 10.26 (s, 1H, NH), 7.73 – 7.59 (m, 4H, Ar-H), 7.26 (d, *J* = 9.1 Hz, 1H, Ar-H), 7.16 (dd, *J* = 6.6, 2.6 Hz, 1H, Ar-H), 7.01 (ddd, *J* = 8.9, 4.2, 2.7 Hz, 1H, Ar-H), 2.02 (s, 3H, $COCH_3$).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 169.60, 154.59 (d, J = 243.8 Hz), 143.91, 135.64 (d, J = 3.0 Hz), 132.92, 128.51, 122.44, 121.29 (d, J = 7.3 Hz), 120.17 (d, J = 19.2 Hz), 119.20, 117.97 (d, J = 22.0 Hz), 24.64.
ESI-MS: *m/z* 343.09$[M+H]^+$, 345.06$[M+2]^+$, 365.02, 366.97$[M+Na]^+$.

(2*S*)-2-(((4-(acetylamino)phenyl)sulfonyl)amino)-3-phenylpropanoic acid (1h)

white solid. Yield 78%. m.p 207 C°.
IR spectrum (νmax, cm^{-1}): 3357.11 (NH_{str}), 3297.01 (NH_{str}), 1725.78 ($C=O_{str}$), 1659.95 ($C=O_{str}$), 1589.83 (NH_{bend}), 1535.46 ($C=C_{Ar\ str}$), 1315.08 ($S=O_{asym\ str}$), 1151.95($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 12.62 (s, 1H, OH), 10.18 (s, 1H, NH), 8.05 (d, *J* = 9.0 Hz, 1H, NH), 7.62 – 7.53 (m, 2H, Ar-H), 7.50 – 7.42 (m, 2H, Ar-H), 7.20 – 7.06 (m, 5H, Ar-H), 3.81 (td, *J* = 8.9, 5.9 Hz, 1H), 2.88 (dd, *J* = 13.7, 5.9 Hz, 1H), 2.67 (dd, *J* = 13.7, 8.8 Hz, 1H), 2.05 (s, 3H, $COCH_3$).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 172.80, 169.45, 143.09, 137.32, 135.21, 129.70, 128.68, 127.96, 127.02, 118.82, 57.85, 38.40, 24.66.
ESI-MS: *m/z* 363.12$[M+H]^+$, 385.06$[M+Na]^+$.

4-amino-*N*-phenylbenzene-1-sulfonamide (2a)

White solid. Yield 94%. m.p 196 C°.
IR spectrum (νmax, cm^{-1}): 3421.76 (NH_{str}), 3350.60 (NH_{str}), 1595.85 (NH_{bend}), 1492.94 ($C=C_{Ar\ str}$), 1318.73 ($S=O_{asym\ str}$), 1151.61 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 9.78 (s, 1H, $NHSO_2$), 7.35 (d, J = 8.8 Hz, 2H, Ar-H), 7.16 (dd, J = 8.6, 7.4 Hz, 2H, Ar-H), 7.02 (dd, J = 8.7, 1.2 Hz, 2H, Ar-H), 6.93 (tt, J = 7.4, 1.1 Hz, 1H, Ar-H), 6.49 (d, J = 8.9 Hz, 2H, Ar-H), 5.87 (s, 1H).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 153.32, 139.02, 129.22, 125.08, 123.87, 120.04, 113.13.
ESI-MS: *m/z* 249.07$[M+H]^+$, 271.05$[M+Na]^+$.

4-amino-*N*-(4-fluorophenyl)benzene-1-sulfonamide (2b)

White solid. Yield 92%. m.p 165 C°.
IR spectrum (νmax, cm^{-1}): 3393.54 (NH_{str}), 3343.70(NH_{str}), 1595.04 (NH_{bend}), 1509.01 ($C=C_{Ar\ str}$), 1310.02 ($S=O_{asym\ str}$), 1151.48 ($S=O_{sym\ str}$).

^{1}H NMR (DMSO-d_6, δ, ppm): δ 9.73 (s, 1H, $NHSO_2$), 7.31 (d, J = 8.7 Hz, 2H, Ar-H), 7.07 – 6.94 (m, 4H, Ar-H), 6.50 (d, J = 8.8 Hz, 2H, Ar-H), 5.91 (s, 2H, NH_2).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 159.24 (d, J = 239.9 Hz), 153.40, 135.28 (d, J = 2.4 Hz), 129.22, 124.72, 122.73 (d, J = 8.4 Hz), 116.15 (d, J = 22.8 Hz), 113.13.
ESI-MS: *m/z* 267.09$[M+H]^+$, 289.07$[M+Na]^+$.

2-(((4-aminophenyl)sulfonyl)amino)benzoic acid (2c)

White solid. Yield 93%. m.p 222 C°.
IR spectrum (νmax, cm^{-1}): 3483.24 (OH_{str}), 3382.33 (NH_{str}), 1678.40 ($C=O_{str}$), 1624.54 (NH_{bend}), 1488.98 ($C=C_{Ar\ str}$), 1317.73 ($S=O_{asym\ str}$), 1146.22 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.80 (s, 1H, $NHSO_2$), 7.86 (ddd, *J* = 8.0, 1.6, 0.5 Hz, 1H, Ar-H), 7.49 – 7.46 (m, 2H, Ar-H), 7.40 (d, J = 8.9 Hz, 2H, Ar-H), 7.04 (ddd, *J* = 8.0, 6.9, 1.6 Hz, 1H, Ar-H), 6.51 (d, *J* = 8.8 Hz, 2H, Ar-H).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 169.81, 153.40, 140.69, 134.39, 131.44, 129.01, 123.01, 122.62, 118.04, 116.01, 112.73.
ESI-MS: *m/z* 293.03$[M+H]^+$, 315.08$[M+Na]^+$.

4-(((4-aminophenyl)sulfonyl)amino)benzoic acid (2d)

White solid. Yield 92%. m.p 204 C°.
IR spectrum (νmax, cm^{-1}): 3417.88(NH_{str}), 3321.56(NH_{str}), 1590.92($NH_{bending}$), 1672.82 ($C=O_{str}$) 1509.83($C=C_{Ar\ str}$), 1314.94($S=O_{asym\ str}$), 1150.18($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.34 (s, 1H, $NHSO_2$), 7.74 (d, J = 8.7 Hz, 2H, Ar-H), 7.42 (d, J = 8.8 Hz, 2H, Ar-H), 7.12 (d, J = 8.8 Hz, 2H, Ar-H), 6.53 (d, J = 8.7 Hz, 2H, Ar-H), 5.38 (s, 2H, NH_2).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 167.36, 153.48, 143.29, 131.13, 129.34, 125.39, 124.72, 118.07, 113.33.
ESI-MS: *m/z* 293.03$[M+H]^+$, 315.05$[M+Na]^+$.

4-amino-*N*-(2-hydroxyphenyl)benzene-1-sulfonamide (2e)

Brown solid. Yield 88%. m.p 184-188 C°.
IR spectrum (νmax, cm^{-1}): 3474.77 (OH_{str}), 3415.81 (NH_{str}), 1595.48 (NH_{bend}), 1497.47 ($C=C_{Ar\ str}$), 1308.01 ($S=O_{asym\ str}$), 1153.37 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.33 (s, 1H, OH), 8.95 (s, 1H, $NHSO_2$), 7.32 (d, J = 8.7 Hz, 2H, Ar-H), 7.09 (dd, *J* = 7.9, 1.6 Hz, 1H, Ar-H), 6.84 (ddd, *J* = 8.1, 7.4, 1.6 Hz, 1H, Ar-H), 6.70 (dd, *J* = 8.0, 1.4 Hz, 1H, Ar-H), 6.63 (ddd, *J* = 7.9, 7.3, 1.5 Hz, 1H, Ar-H), 6.48 (d, J = 8.8 Hz, 2H, Ar-H), 5.86 (s, 2H, NH_2).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 153.23, 149.84, 129.26, 125.80, 125.57, 123.50, 119.47, 116.01, 112.98.
ESI-MS: *m/z* 265.09$[M+H]^+$, 287.05$[M+Na]^+$.

4-amino-*N*-(4-hydroxyphenyl)benzene-1-sulfonamide (2f)

Brown solid. Yield 89%. m.p 195 C°.
IR spectrum (νmax, cm^{-1}): 3473.78 (OH_{str}), 3377.21 (NH_{str}), 3203.86 (NH_{str}), 1597.74 (NH_{bend}), 1508.42 ($C=C_{Ar\ str}$), 1307.95 ($S=O_{asym\ str}$), 1151.55 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 9.20 (s, 1H, $NHSO_2$), 7.24 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.78 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.55 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.48 (d, *J* = 8.7 Hz, 2H, Ar-H), 5.83 (s, 2H, NH_2).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 154.94, 153.06, 129.92, 129.17, 125.30, 124.21, 115.89, 113.03.
ESI-MS: *m/z* 265.11$[M+H]^+$, 287.09$[M+Na]^+$.

4-amino-*N*-(3-chloro-4-fluorophenyl)benzene-1-sulfonamide (2g)

white solid. Yield 90%. m.p 171 C°.
IR spectrum (νmax, cm^{-1}): 3412.92 (NH_{str}), 3339.94 (NH_{str}), 1591.26 (NH_{bend}), 1496.82 ($C=C_{Ar\ str}$), 1307.93 ($S=O_{asym\ str}$), 1149.09 ($S=O_{sym\ str}$).
^{1}H NMR (400 MHz, DMSO-d_6): δ 9.98 (s, 1H, $NHSO_2$), 7.33 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.24 (t, *J* = 9.1 Hz, 1H, Ar-H), 7.13 (dd, *J* = 6.6, 2.7 Hz, 1H, Ar-H), 7.00 (ddd, *J* = 8.9, 4.2, 2.7 Hz, 1H, Ar-H), 6.51 (d, *J* = 8.8 Hz, 2H), 5.97 (s, 2H, NH_2).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 155.41, 153.64, 152.99, 136.33 (d, *J* = 2.9 Hz), 129.28, 124.16, 121.75, 120.67 (d, *J* = 7.2 Hz), 119.98 (d, *J* = 18.7 Hz), 117.82 (d, *J* = 22.0 Hz), 113.21.
ESI-MS: *m/z* 301.03$[M+H]^+$, 303.08$[M+2]^+$, 323.03$[M+Na]^+$

ethyl (2*S*)-2-(((4-aminophenyl)sulfonyl)amino)-3-phenylpropanoate (2h)

White solid. Yield 75%. m.p 117 C°.
IR spectrum (νmax, cm^{-1}): 3408.21 (NH_{str}), 3313.72 (NH_{str}), 1736.42 ($C=O_{str}$), 1592.09 (NH_{bend}), 1501.06 ($C=C_{Ar\ str}$), 1318.58 ($S=O_{asym\ str}$), 1152.38 ($S=O_{sym\ str}$).
^{1}H NMR (DMSO-d_6, δ, ppm): δ 7.85 (d, *J* = 9.0 Hz, 1H, $NHSO_2$), 7.28 – 7.10 (m, 5H, Ar-H), 7.10 – 7.02 (m, 2H, Ar-H), 6.49 (d, J = 8.7 Hz, 2H, Ar-H), 5.84 (s, 2H, NH_2), 3.83 – 3.67 (m, 3H), 2.79 (dd, *J* = 13.5, 7.5 Hz, 1H, CH_2-Ar), 2.71 (dd, *J* = 13.5, 7.7 Hz, 1H, CH_2-Ar), 0.91 (t, *J* = 7 .1 Hz, 3H, CH_3 for ethyl ester).
^{13}C NMR (DMSO-d_6, δ, ppm): δ 171.45, 153.08, 136.92, 129.63, 128.99, 128.76, 127.16, 126.15, 112.99, 60.90, 57.78, 38.54, 14.19.
ESI-MS: *m/z* 349.16$[M+H]^+$, 371.13$[M+Na]^+$.

3.3. Microbiological section:

The agar well diffusion method and the Minimal Inhibitory Concentration (MIC) method were used to assess the antibacterial activity and the results were as follows:

3.3.1. Antibacterial activity results against *Staphylococcus aureus*:

The antibacterial activity findings against clinical and reference *S. aureu*s are shown in tables 2 and 3.

3.3.1.1. Results against *Staphylococcus aureus* ATCC 33591:

Zones of inhibition diameters for compounds 2e and 2f were 20 and 19 mm respectively and MIC value was 128 µg/ml for both; these approximate the outputs of sulfadiazine (SDZ) and were better than those of sulfanilamide (SAA). As for compounds 2a, 2b and 2g they had less activity, zones of inhibition diameters were 7, 8 and 13 mm respectively and MIC values were higher than 256 µg/ml. Finally, the compounds 2c, 2d and 2h were ineffective.

3.3.1.2. Results against clinical *Staphylococcus aureus*:

The compounds 2e and 2f showed good activity against clinical S. aureus, as it was better than sulfanilamide and close to the activity of sulfadiazine, with zones of inhibition ranged between 15 and 24 mm and MIC values ranged between 64 to 128 µg/ml. The compounds 2a, 2b and 2g were moderately effective with zones of inhibition diameters between 9 to 13 mm and MIC value was 256 µg/ml for all. The compounds 2c, 2d and 2h were also ineffective.

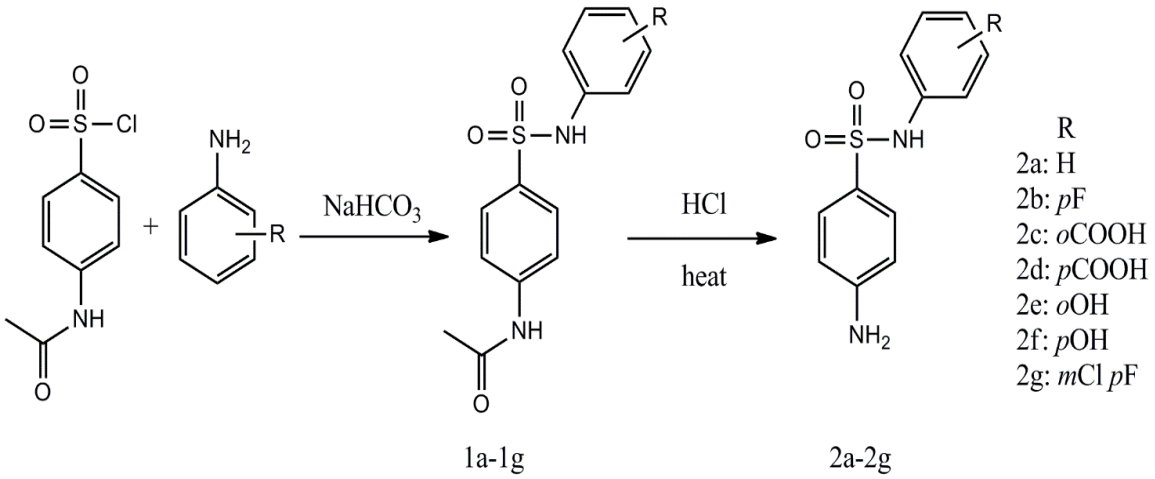

Figure 1: Synthesis of compounds (1a-1g) and (2a-2g).

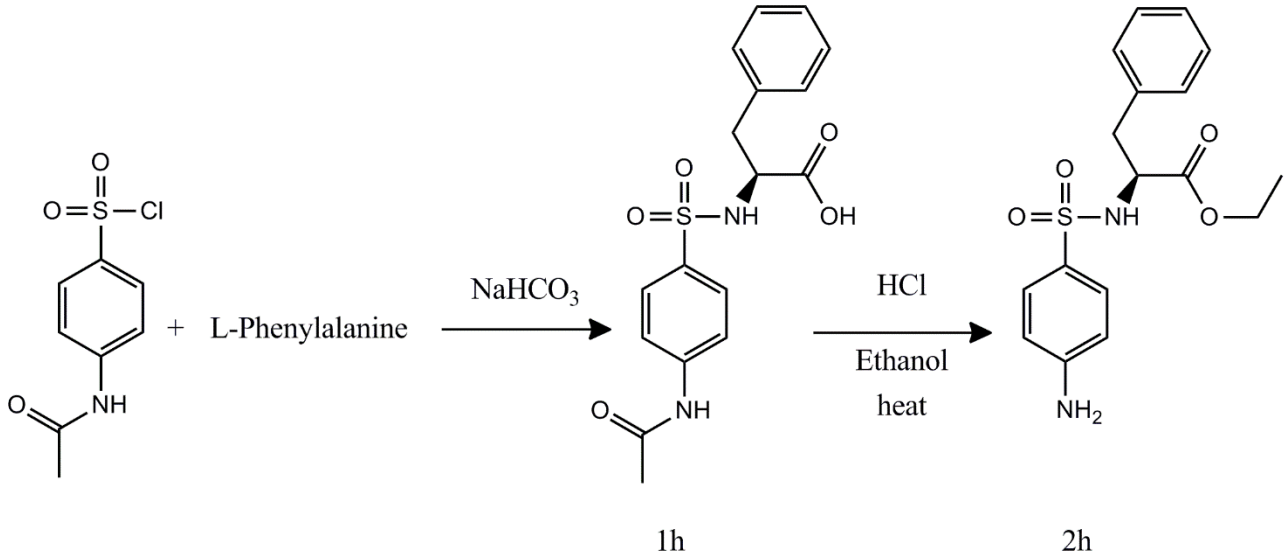

Figure 2: Synthesis of compounds 1h and 2h.

Table 1. Some practical and computational physiochemical properties of synthesized compounds.

	Melting point	pka_1	pka_2	pka_3	TPSA	Log p
2a	196	8.46	2.27		60.16	2.66
2b	165	8.56	2.18		60.16	2.80
2c	222	8.37	2.11	3.31	100.29	1.03
2d	204	8.59	2.18	4.49	97.46	2.36
2e	184-188	7.57	2.20	12.79	80.39	2.37
2f	195	8.64	2.22	9.11	80.39	2.37
2g	171	8.43	2.03		60.16	3.46
2h	117	10.89	2.25		98.49	1.72

Table 2. Zones of inhibition for sulfonamides against *S. aureus.*

	2a	2b	2c	2d	2e	2f	2g	2h	SAA	SDZ	SXT
S. aureus ATCC (33591)	7	8	-	-	20	19	13	-	12	20	25
S. aureus Isolate 1	10	11	-	-	24	23	13	-	18	25	29
S. aureus Isolate 2	9	9	-	-	23	23	8	-	15	24	28
S. aureus Isolate 2	-	-	-	-	15	15	-	-	6	20	22

(–) No zone of inhibition was observed.

Table 3. MIC for sulfonamides against *S. aureus*

	2a	2b	2c	2d	2e	2f	2g	2h	SAA	SDZ	SXT
S. aureus ATCC(33591)	512	512	*	*	128	128	256	*	256	128	64
S. aureus Isolated 1	256	256	*	*	64	64	256	*	128	64	32

(*) >512µg/ml.

Table 4. Zones of inhibition for sulfonamides against *E. coli*

	2a	2b	2c	2d	2e	2f	2g	2h	SAA	SDZ	SXT
E. coli ATCC (8739)	-	-	-	-	-	-	-	-	-	-	-
E. coli Isolate 1	8	-	-	-	20	14	7	11	8	25	26
E. coli Isolate 2	4	3	-	-	18	12	-	-	-	24	24
E. coli Isolate 3	-	-	-	-	14	11	-	-	6	18	23

(–) No zone of inhibition was observed.

Table 5. MIC for sulfonamides against *E. coli*

	2a	2b	2c	2d	2e	2f	2g	2h	SAA	SDZ	SXT
E. coli Isolated 1	256	*	*	*	64	128	256	128	256	16	16

(*) >512µg/ml.

Table 6. Zones of inhibition for sulfonamides against *K. pneumoniae*

	2a	2b	2c	2d	2e	2f	2g	2h	SAA	SDZ	SXT
K. pneumoniae ATCC 13885	-	-	-	-	10	10	-	-	-	24	28
K. pneumoniae Isolate 1	-	-	-	-	15	14	-	-	9	21	25
K. pneumoniae Isolate 2	-	-	-	-	8	7	-	-	-	12	15

(–) No zone of inhibition was observed.

Table 7. MIC for sulfonamides against *K. pneumoniae*

	2a	2b	2c	2d	2e	2f	2g	2h	SAA	SDZ	SXT
K. pneumoniae ATCC 13885	*	*	*	*	512	512	*	*	*	64	32
K. pneumoniae Isolate 1	*	*	*	*	256	256	*	*	512	128	64

(*) >512µg/ml.

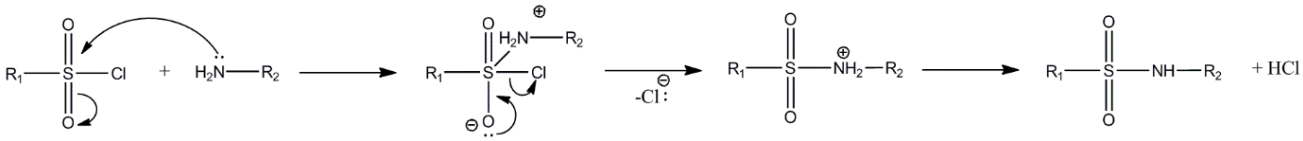

Figure 3: addition-elimination reaction mechanism in the synthesis of sulfonamides.

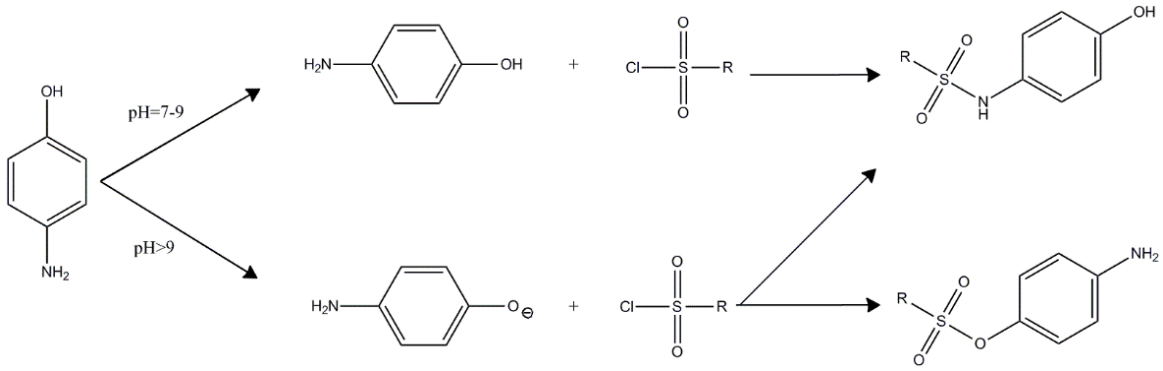

Figure 4: Reaction of phenols with sulfonyl chloride under different pH conditions.

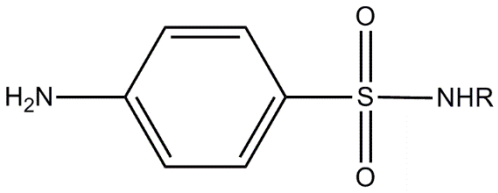

Figure 5: the general structure of synthesized compounds

3.3.2. Antibacterial activity results against *Escherichia coli:*

Tables 4 and 5 show the antibacterial efficacy results against the clinical and reference *E. coli*.

3.3.2.1. Results against *Escherichia coli* ATCC 8739:

The synthesized and reference compounds could not show any activity against this strain.

3.3.2.2. Results against clinical *Escherichia coli:*

The compounds 2e and 2f were effective against all clinical isolates with superiority for compound 2e, where zones of inhibition diameters for compound 2e were between 14 and 20 mm and MIC value was 64 µg/ml, while zones of inhibition for compound 2f ranged between 11 and 14 mm and MIC value was 128 µg/ml. The compounds 2a, 2b, 2g and 2h were effective with zones of inhibition diameters between 11 and 14 mm and MIC values between 64 and 128 µg/ml, whereas the compounds 2c and 2d were ineffective.

3.3.3. Antibacterial activity results against *Klebsiella pneumoniae:*

The antibacterial activity data against the clinical and reference *K. pneumoniae* are presented in tables 6 and 7.

3.3.3.1. Results against *Klebsiella pneumoniae* ATCC 13885:

All synthesized compounds were ineffective except compounds 2e and 2f with zone of inhibition diameter of 10 mm and MIC value of 512 µg/ml, which is poor compared to sulfamethoxazole (SXT) and sulfadiazine.

3.3.3.2. Results against clinical *Klebsiella pneumoniae*:

The compounds 2e and 2f were with zones of inhibition diameter ranging from 7 to 15 mm and MIC value of 256 µg/ml, while the rest of the compounds were ineffective.

Discussions: The compounds 1a-h have been synthesized by reacting to 4-acetamidobenzene sulfonyl chloride with primary amine derivatives via an addition-elimination reaction (Figure 3). The amines have nucleophilic properties due to their electron pair and this pair disappears in acidic media so the reaction was carried out in an alkaline condition [11]. Hydrochloric acid is produced during the reaction, and this may reduce the pH so the pH of the reaction must be constantly monitored. In previous studies, some of our compounds were synthesized using toxic organic solvents, such as chloroform, pyridine, and acetone, which are harmful to the environment. Unlike the above, this study used water as a solvent instead of organic solvents and doesn't require heating. As a result, this method is both environmentally friendly and cost-effective. Synthesis of compounds 1e and 1f must be done under strict pH control (pH=7-9) which gives

us only sulfonamide derivatives. But at high pH (pH >9) a phenoxy ion is formed which may react with a sulfonyl chloride according to the Schotten-Baumann reaction to give sulfonate ester [12] as shown in (Figure 4).

The esterified derivative was obtained in the synthesis of compound 2h by catalyzing hydrochloric acid via the Fisher reaction [13]. All synthesized compounds are acidic and can be separated from the reaction mixture by precipitation at pH = 3. In the spectra of compounds 2a-h, the disappearance of acetyl group peaks confirmed the success of the hydrolysis process. In addition, the appearance of ethyl ester group peaks also confirmed the success of the esterification process. The NH and OH peaks did not appear in some ^{1}H NMR spectra due to moisture in the solvent. Carbon-Fluorine coupling was noted in the ^{13}C NMR spectrum of compounds 1b, 1g, 2b and 2g. In the mass spectrum of compounds 1g and 2g, a peak of $[M+2]^{+}$ appeared due to the presence of the isotope chlorine Cl^{37}. The results of the microbiological study showed a variation in the effectiveness of the studied compounds, some of which were better than sulfanilamide and some less. The studied compounds have a general structure which is shown in (Figure 5). Substituent R is a variable and it plays an important role in efficacy and pharmacokinetics as it alters the physicochemical properties of compounds such as hydrophilicity and pKa values [14]. Previous studies showed that the ionized form of sulfonamides is the active form that binds to the DHPS enzyme [15]. Hence, the pKa value had a major role in determining effectiveness. It was found that there is a relationship between sulfonamide pKa values and MIC values. This relationship will give a parabolic curve and the best antibacterial activity is at pKa values between 6-7 [16]. Compounds 2a-2g had convergent pKa values for the sulfonamide group but differed in effectiveness. Therefore, other factors, such as polarity and hydrophilicity, could also affect antibacterial activity [17].

Compounds 2e and 2f showed good activity against gram-negative and gram-positive bacteria. These two compounds have a hydroxyl group, which is an electron-donating and polar group that gives the compound hydrophilic properties. It is likely that the hydroxyl group improved the passage of the compound through bacterial membranes [18]. Hydroxyl likely had no role in binding to DHPS enzyme because there was no difference when it was added to the ortho or para position. Compounds 2a, 2b and 2g have hydrophobic properties. The three compounds were less effective than sulfanilamide. The addition of halogens did not improve the effectiveness. As for compounds 2c and 2d, they have a carboxyl group, which is an electron-withdrawing and polar group. But, they were ineffective against all bacteria tested. The carboxylic group is highly ionized, with an acid dissociation constant of 3.1-4.49, which may have hindered the passage of the compounds through bacteria membranes.

So, esterification of the carboxylic group may be a rational idea to improve effectiveness. It was applied to compound 2h, but the results were disappointing. 2h was effective against just one *Escherichia coli* strain. However, compound 2h has a large substitute and a high pka value (pka=10.89), which may be a reason for making the enzyme less affinity for it. Resistance to sulfonamides arises in several ways, such as enzyme modification. This explains the different results of the bacterial activity of the same compound according to different bacterial strains [19].

4. Conclusions

In summary, sulfonamide derivatives can be synthesized from aryl or alkyl aryl amines easily using an economical and environmentally friendly method. Esterification and hydrolysis processes were simultaneously carried out in compound 2h synthesis. The antibacterial activity of the synthesized compounds has been tested against several strains of *E. coli*, *S. aureus* and *K. pneumoniae.* Their effectiveness was compared with sulfanilamide. Some hypotheses were developed linking the effectiveness to the physicochemical properties of the compounds. But the interpretation of the results remains complicated due to the overlapping of several factors in determining effectiveness.

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