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Synthesis of Some Sulfa Drug Derivatives as Antibacterial Agents

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ABSTRACT

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Sulfa drugs are an important class of synthetic bacteriostatic antibiotics still used today for the treatment of bacterial infections and those caused by other microorganisms, sulfa drugs achieve this bacteriostatic action by inhibiting the synthesis of folic acid in bacteria. The current study has the aim of Synthesis of some of sulfa drug compounds and Study the biological activity of the synthesized compounds against two types of bacteria: *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative). These compounds were formed successfully by chlorosulfonation of acetanilide and the product was treated with different amines and finally amide hydrolysis was necessary to get agents that were analyzed for IR, UV and melting points. At last, we studied their antibacterial activity on certain types of bacteria. We carried out an antibacterial evaluation in vitro of a series of five synthetic sulfa drugs (IIIa, IIIb, IIIc, IIId and IIIe) and from the results, antibacterial activity studies indicate that halo substituted sulfonamide (compound IIIc) was more active than the other members.

Introduction

Sulfonamides are an important class of synthetic bacteriostatic antibiotics still used today for the treatment of bacterial infections and those caused by other microorganisms. They are also known as sulfa drugs and were the main source of therapy against bacterial infections before the introduction of penicillin in 1941. Although sulfonamides have for the most part been replaced by other agents, they still maintain considerable action in certain types of infection, for example in the urinary tract, eye and ear, and bronchitis (Mok, 2008). The activity of the sulfa drugs has been extensively studied and can be explained in

the following manner. Sulfonamides are typically administered in doses that are bacteriostatic, meaning they prevent or limit bacterial multiplication.

Sulfonamides achieve this bacteriostatic action by inhibiting the synthesis of folic acid in bacteria. Bacteria synthesize their own folic acid using endogenous compounds and enzymes, Endogenous compounds are those that occur naturally in the biological system, Specifically, sulfonamides inhibit the enzyme dihydropteroatesynthetase, an enzyme that catalyzes the conversion of p-

aminobenzoic acid (PABA) and dihydropteroate diphosphate to dihydropteroic acid, a precursor to folic acid and DNA. Sulfonamides compete with PABA for the “active site” in the dihydropteroatesynthase enzyme, and are considered to be “competitive inhibitors” of this enzyme. The structural similarity of the sulfonamides to PABA “tricks” the enzyme into binding with the drug (sulfonamide) instead of the endogenous compound (PABA). The displacement of the PABA by the sulfonamide leads to the formation of a “false” metabolite in the folic acid synthesis, which cannot continue through the synthetic sequence (Garcia *et al.*, 2009).

Sulfonamides can be classified into three major groups: oral absorbable agents, oral non absorbable agents and topical. Oral absorbable agents may be further classified as short, medium, or long acting sulfonamides. Sulfonamides are absorbed from the stomach and small intestine and widely distributed to tissues. Sulfonamides and inactivated metabolites are excreted by the kidney mainly through glomerular filtration (Abraham, 2003).

The toxic effects which may arise during sulfonamide therapy are: crystalluria, acute hemolytic anemia, agranulocytosis, a plastic anemia, hypersensitivity reactions, gastrointestinal upsets including anorexia, nausea and vomiting, headache, dizziness, mental depression, and other signs of central nervous system involvement. Hepatitis is a rare but potentially dangerous complication of sulfonamide therapy (Golzar, 2005).

Materials and Methods

Chemical

The steps of synthesizing these compounds represented the classical method (scheme 1)

which includes reaction of acetanilide (2.5 g, 0.018 moles) with chlorosulfonic acid (6.25ml, 0.093 moles) in 250 ml conical flask on an ice-bath. When the addition has been made, under stirring, heat the reaction mixture on a water bath (50-60C) for 1 hour in order to complete the reaction. Allow the flask to cool to room temperature and slowly pour the contents, with vigorous stirring, into a beaker containing 100g. Of crushed ice, a milky-white precipitate is formed. This suspension was filtered off, washed with a little cold water and air dried to give crude product (Vogel, 1956).

To crude product dissolved in diethyl ether (30 ml) was added dropwise o-methyl aniline (2.5 ml, 0.023moles). Under stirring it took one hour for the reaction to complete. After the completion of the reaction, the precipitate was collected by filtration and air dried. The same procedure was repeated with 0.023moles of each amine (2.56 ml o-methoxy aniline, 2.93g p-chloro aniline, 2.26 ml Piperidine, 2ml morpholine) (Hadizadeh *et al.*, 2007).

The product from the previous step is placed in a 50 mL round-bottom flask equipped with a magnetic stir bar. Dilute hydrochloric acid (5 ml of conc. HCl in 10ml of water) is added to the flask. The flask is fitted with a condenser and heated at reflux with constant stirring for (30-45) minutes, after which it is allowed to cool to room temperature. After cooling, the reaction mixture is neutralized by slow addition of a 10% NaHCO₃ solution with stirring until it tests slightly alkaline to pH paper. A precipitate may have begun to form during neutralization. Cool the beaker in an ice bath to complete the precipitation of product. It may be necessary to gently scratch the inside bottom of the beaker to induce crystallization. The product is collected by filtration, washed with a

small amount of ice cold water and air dried. The same procedure was repeated with each compounds (Vogel, 1956; Pavia and Lampman, 2011).

All the starting materials and reagents were obtained from commercial sources and were used without further purification. Acetanilide was supplied by Merck, Chlorosulfonic acid was from Fluka and the other chemicals were procured by BDH.

Antimicrobial Activity

In this study, the synthesized compounds were evaluated for their in vitro antimicrobial activity against the pathogenic bacteria, two bacterial species were used: one of them gram positive bacteria which was *Staphylococcus aureus*, the other gram negative bacteria which was *Pseudomonas aeruginosa*. Well diffusion method was used to determine the inhibiting power of the prepared compounds against the pathogenic bacteria (Moumita and Thankamani, 2013). Sulfamethoxazole was used to compare the power of inhibition.

This method depends upon the variation in concentration of the prepared compounds. A series of concentrations were made from the already prepared compounds with the range from (50, 25, 12, 6, and 3 mg/ml). Sulfamethoxazole was used as a reference antibiotic drugs and DMSO was used as a solvent for these compounds.

Antibacterial Evaluation

Medium Inoculated bacteria suspension were diluted by 1/100 by using normal saline solution with concentration of (0.85%) to prevent crowded growth. (0.1 mL) of bacteria diluted suspension was transported to each plate and spread by using sterilized cotton spreader on test

medium surface. The Plates were left for (15-20) min. at 37°C to make absorption. The plates of Mueller Hinton agar were inoculated by *S.aureus* and *P.aeruginosa*. In each medium six pores were made by the use of a sterile dry rod with a diameter of 5 mm, these pores were made with equal distance between each other, then the solutions of different concentrations of the prepared compounds were added using fixed amount of 0.1 mL from each concentration in one pore and DMSO as control, to see the effect of solvent for each type of bacteria. These plates were incubated at 37°C⁰ for 24 hrs. After the incubation, the inhibition zone was measured for each pore using a ruler in millimeter (mm). The zone of inhibition is defined as the translucent area which surrounds the disc including the diameter of the disc that lacks bacterial growth. The bacteria was considered sensitive, mean-sensitive or resistant depending on inhibition zone.

Results and Discussion

Chemistry

Acetanilide was chosen as a starting material based on its amino group which is already protected by acetyl and later on can be deprotected to get the free amino group again for further synthesis when the desired sulfa compounds could be obtained. Acetanilide was converted to its corresponding sulfonyl chloride by chlorosulfonation by the direct reaction of chlorosulfonic acid with it⁽⁵⁶⁾. The suggested amino compounds for preparation of sulfa derivatives are verified from aromatic with either activated and deactivated group or aliphatic residue like piperidine and morpholine. These products were prepared by the condensation of precursor (I) with the amines, i.e., (2-methylaniline, 2-methoxyaniline, 4-chloroaniline, morpholine

and piperidine) to furnish the 4-acetamido-N substituted benzenesulfonamides (IIa, IIb, IIc, IId and IId) respectively. The acetyl group was removed to afford the 4-amino-N-substituted benzenesulfonamides (IIIa, IIIb, IIIc, IIId and IIIe). The synthesis routes of the compounds (IIIa, IIIb, IIIc, IIId and IIIe) are shown in scheme.1.

The percent yield, physical description and the corresponding melting point of any compound are shown in table1. Infrared identification was done by KBr disc aiming the proof of their formation, functional groups and structures and the bands are listed on table2. Ultraviolet spectroscopy was applied on DMSO solutions and the related λ_{max} s were appeared as per table.3.

Antibacterial Activity

Pathogenic microorganisms cause different kinds of diseases to human and animals. Discovery of chemotherapeutic agents played a very important role in controlling and preventing such diseases. Chemotherapeutic agents are isolated either from living organisms known as antibiotics like penicillin and tetracycline etc., or they are chemical compounds prepared by chemists such as sulfa drugs. Microorganisms have the ability to develop resistance to these chemotherapeutic agents and such strains which are resistant causing major problem in treatment of microbial infections. For this reason searching for new antimicrobial agents is continuous process

and great efforts have been employed to find new antibiotics or new compounds with good antimicrobial activity which might be suitable to be used as chemotherapeutic agents (Endimiani *et al.*, 2005).

The prepared compounds (IIIa and IIIb) had no inhibiting action on growth of the used germs and, therefore, these compounds possessed no antibacterial activity against the previously mentioned types of gram positive and gram negative bacteria with all concentrations (resistance). The most interesting results were with compound (IIIc) which showed the best antibacterial activity on staphylococcus aureus with inhibition zones 17mm, 14mm and 8mm at concentrations (50, 25 and 12 mg/ml) respectively. On pseudomonas aeruginosa, the compound (IIIc) showed one inhibition zone 13mm at (50 mg/ml). The compound (IIId) showed two inhibition zones 16mm and 10mm at (50, 25 mg/ml) respectively on staphylococcus aureus, while compound (IIId) showed one inhibition zone 16mm at (50) mg/ml on pseudomonas aeruginosa. The compound (IIIe) have inhibition ability 9mm at (50) mg/ml on staphylococcus aureus, while on pseudomonas aeruginosa the inhibition zone was not observed (resistance) (Badampudi *et al.*, 2014).

The inhibition zones caused by the various compounds on the staphylococcus aureus and pseudomonas aeruginosa were examined and the results were listed in table 4 and table 5 respectively.

Table.1 Physical Properties of Synthesized Compounds

Compounds	Colour	M.P.(°C)	Yield (%)	M. Wt.	Formula
IIIa	White	185-187	86	262.33	C ₁₃ H ₁₄ N ₂ SO ₂
IIIb	Pale White	204-206	80	278.33	C ₁₃ H ₁₄ N ₂ SO ₃
IIIc	Yellowish white	196-198	52	282.75	C ₁₂ H ₁₁ N ₂ SO ₂ Cl
IIId	White	174-176	76	242.29	C ₁₀ H ₁₄ N ₂ SO ₃
IIIe	White	160-162	78	240.32	C ₁₁ H ₁₆ N ₂ SO ₂

Table.2 Infrared Spectra of the Prepared Compounds

Compounds	Vibration bands Cm^{-1}
IIIa	SO ₂ =1315, 1149 NH ₂ =3444, 3423 CH ₃ =2902, 2864 Stretching CH ₃ = 1465, 1398 bending
IIIb	SO ₂ =1323, 1157 NH ₂ =3367, 3313 O – CH ₃ =1111
IIIc	SO ₂ =1323, 1157 NH ₂ =3383, 3228 C – Cl= 640
III d	SO ₂ = 1315, 1165 NH ₂ =3309,3263 C – O= 1053
IIIe	SO ₂ = 1323, 1161 NH ₂ = 3294, 3259 CH ₂ = 2927,2854 Stretching

Table.3 Electronic Spectra of the Prepared Compounds

Comps.	Absorption Bands(nm)	Assignment
IIIa	208	π - π^*
	233	π - π^*
	263	n- π^*
IIIb	208	π - π^*
	231	π - π^*
	266	n- π^*
IIIc	210	π - π^*
	237	π - π^*
	265	n- π^*
III d	208	π - π^*
	260	π - π^*
	283	n- π^*
IIIe	209	π - π^*
	263	n- π^*

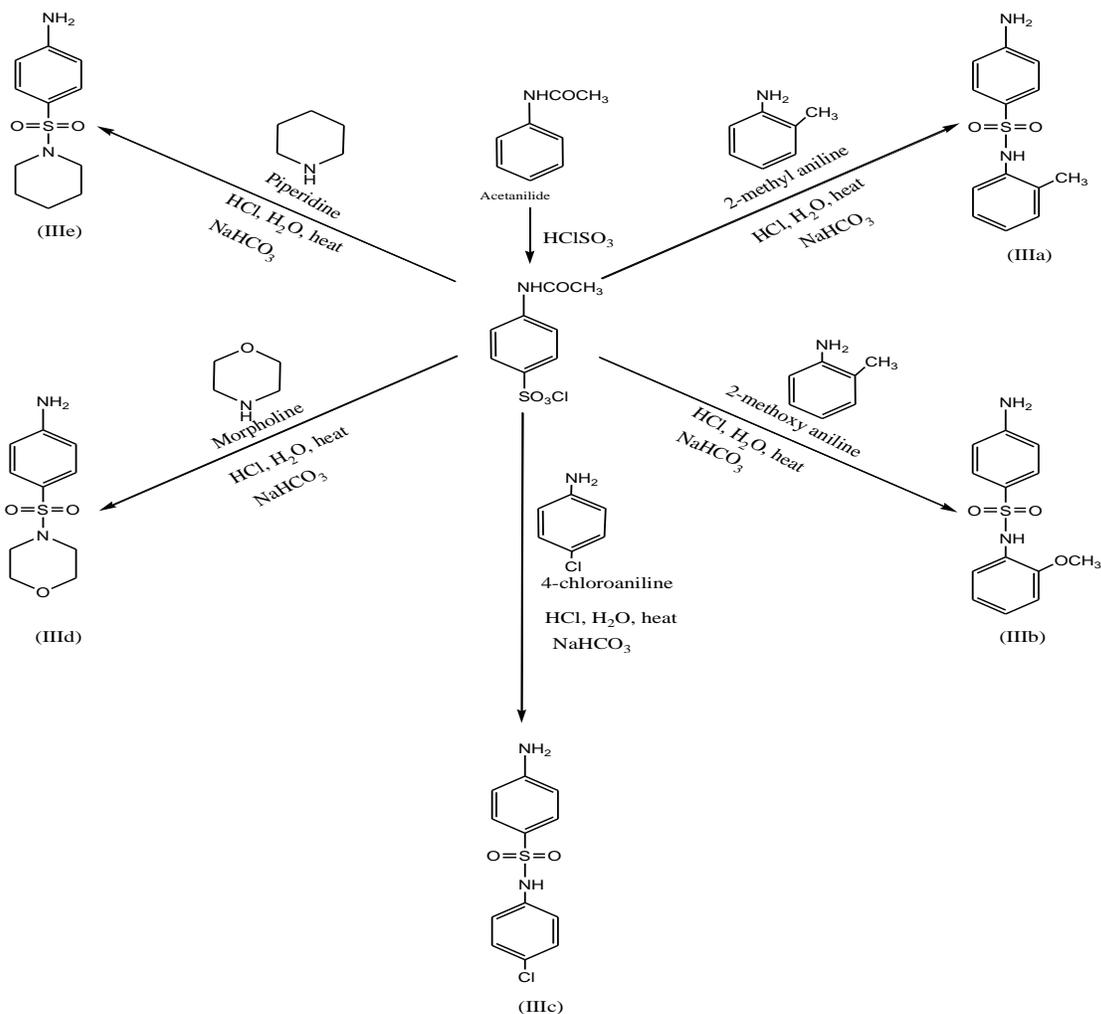
Table.4 The Inhibition Zones in MM of Synthesized Compounds against *Staphylococcus aureus*

Compounds	Concentrations mg/ml	Inhibition Zones in mm
IIIa	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
IIIb	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
IIIc	50	17
	25	14
	12	8
	6	No Inhibition
	3	No Inhibition
III d	50	16
	25	10
	12	No Inhibition
	6	No Inhibition
	3	No Inhibition
IIIe	50	10
	25	No Inhibition
	12	No Inhibition
	6	No Inhibition
	3	No Inhibition
Sulfamethoxazole as Antibiotic Control	50	Synergistic
	25	Same
	12	Same
	6	Same
	3	Same
DMSO as Solvent	—	No Inhibition

Table.5 The Inhibition Zones in MM of Synthesized Compounds against *Pseudomonas aeruginosa*

Compounds	Concentrations mg/ml	Inhibition Zones in mm
IIIa	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
IIIb	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
IIIc	50	13
	25	No Inhibition
	12	No Inhibition
	6	No Inhibition
	3	No Inhibition
III d	50	16
	25	No Inhibition
	12	No Inhibition
	6	No Inhibition
	3	No Inhibition
IIIe	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
Sulfamethoxazole as Antibiotic Control	50	Synergistic
	25	Same
	12	Same
	6	Same
	3	Same
DMSO as Solvent	-----	No Inhibition

Scheme.1 The Chemical Steps of the Synthesized Compounds



In conclusion, for this study, we carried out an antibacterial evaluation in vitro of a series of five synthetic sulfonamides (IIIa, IIIb, IIIc, IIId and IIIe), against gram positive and gram negative clinical strains isolated from patients presenting burns infections: *Staphylococcus aureus* and *Pseudomonas aeruginosa*. From the results, antibacterial activity studies indicate that halo substituted sulfonamide (compound IIIc) was more active than the other members (IIIa, IIIb, IIId and IIIe). The prepared compounds (IIIc, IIId and IIIe) showed inhibition activity as antibacterial agents less than sulfamethoxazole (the reference drug showed better biological

activity than our compounds in all tested strains "synergistic effect" against *Staphylococcus aureus* and *Pseudomonas aeruginosa*).

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