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Research Article

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SYNTHESIS AND MICROBIOLOGICAL STUDY OF NEW SULFONAMIDES

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ABSTRACT

In contrast to the classical antibacterial sulfa drugs that are unsubstituted or monosubstituted, our newly synthesized analogs were designed to obtain sulfonamide moiety containing disubstituted hetero nitrogen atom. These compounds were formed successfully by chlorosulfonation of acetanilide and the product was treated with different cyclic amines and finally amide hydrolysis was necessary to get agents that were analyzed for IR, UV, CHN, melting points and solubility. At last, we studied their antibacterial activity on certain types of bacteria and we noticed the inactivity due to possible steric factor. Principly, this means these products have no inhibiting action against the used microbes.

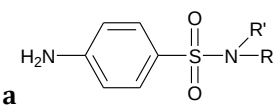
Keywords: sulfonamide; antimicrobial activity; dialkylated sulfonamide.

INTRODUCTION

The sulfonamide antimicrobial drugs were the first effective chemotherapeutic agents but the rapid development of widespread resistance diminished the usefulness of sulfonamides.[1a] An evaluation of azo dyes was done and prontosil was found to protect against, and cure, streptococcal infections in mice.[2-5] The structure-activity study on the sulfonamide azo dyes was performed and the reductive cleavage of azo linkage to release the active antibacterial product, sulfonamide, was concluded.[6,7] Today, sulfonamide - trimethoprim combinations are used extensively for opportunistic infections in patients with AIDS in addition to urinary tract infection and burn therapy.[8-12] Resistance is most likely a result of a compensatory increase in the biosynthesis of p-aminobenzoic acid (PABA) by bacteria although other mechanisms may play a role.[13,14] Resistance of *E Coli* strains to sulfonamide has been shown due to their containing sulfonamide- resistant dihydropteroate Synthase.[15]

The lipophilicity of the N_1 group has the largest effect on protein binding and, generally, the more lipid soluble a sulfonamide is the more of it will be protein bound.[16]

(N_4) (N_1)



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The aniline (N_4) amino group is very important for activity because any modification of it other than to make prodrugs results in a loss of activity.[4] More advanced studies revealed that condensation or alkylation of such side molecule with various chemicals yielded modified sulfonamides showing high to moderate antibacterial activity.[17] The active form of sulfonamide is the N_1- ionized salt.[18] Also an enough nonionized (i.e., more lipid soluble) drug must be present at physiological pH to be able to pass through bacterial cell walls.[18-22]

Aliphatic sulfonamides have highest powerful antibacterial activity for Gram negative bacteria than Gram- positive and antibacterial activity decreases as the length of the carbon chain increases.[23] Also, novel macrocyclic bis- sulfonamides were prepared and their antimicrobial activities were measured too. Bis-sulfonamide showed antibacterial activities against most strains tested.[24]

In this research, we are going to modify the classical monosubstituted sulfonamide side by making the N_1 dialkylated and within a ring (heterocycle) for the purpose of getting new analogs that may have possible improved physiochemical properties and, as a result, exert potent anti-infective effect. Microbiological study will also be investigated for their antimicrobial activity.

EXPERIMENTAL

The steps of synthesizing these compounds represented the classical method (scheme **1**)[25,26] which includes reaction of acetanilide (5gm, 37mmoles) with chlorosulfonic acid (12.5ml, 188.1mmoles) in 125ml conical flask on an ice-bath for 5-10 minutes to yield p-acetamidobenzene-sulfonyl chloride using gas trapping system. The intermediate was washed with distilled water on a Buchner funnel and the solid cake was transferred to the rinsed reaction flask. Piperidine (4gm, 47.1mmoles) and 15ml distilled water were added with occasional swirling for 5 minutes until appearance of the pastier product that is collected by filtration. The moist amide was transferred to a flask, 5ml of concentrated HCl and 10ml of water were added and the mixture was boiled

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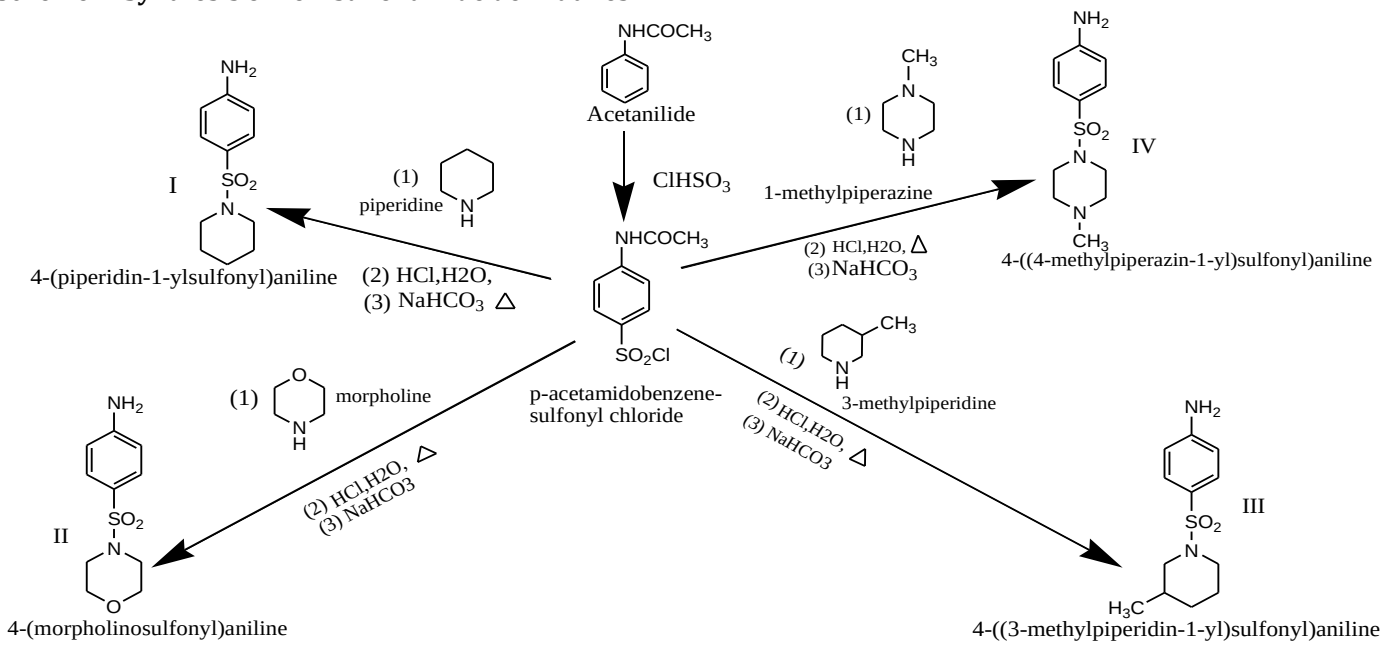
gently until the solid has all dissolved. Heating was continued at the boiling point for 10 minutes. The solution of sulfonamide hydrochloride was cooled, filtered; the filtrate was placed in a beaker and a solution of 5gm sodium bicarbonate was cautiously added with stirring. The solution was tested for basicity of with litmus, cooled thoroughly in ice; the granular white precipitate of the sulfonamide was collected by filtration. The product was washed with some distilled water and the product was recrystallized from ethanol. The same procedure was repeated with 47.1mmoles of each cyclic amine (morpholine 4.1 gm, n-methyl piperazine 4.52gm and 3-methylpiperidine 4.43gm) hoping the preparation of other sulfa drugs.

Scheme 1. Synthesis of new sulfonamide derivatives



All the starting materials and reagents were obtained from commercial sources and were used without further purification. The melting points of the synthesized compounds were determined on open capillary tubes and are uncorrected.

Acetanilide was supplied by E Merck AG, Germany; Chlorosulfonic acid and sulfanilamide were from Fluka, Switzerland; Morpholine, Piperidine, N- methyl piperazine, 3-methyl piperidine and ethanol were procured by BDH, England; sodium bicarbonate was supplied by SDI, Iraq; Hydrochloric acid (36% w/w) was supplied by Riedel-Deltaen, Germany and Muller Hinton agar was procured by Himedia Laboratories Pvt Ltd, India. The percent yield, physical description and the corresponding melting point of any compound are shown in table **1**, while the solubilities (tested using various solvents) are given with table **2**. Infrared identification was done by KBr disc aiming the proof of their formation, functional groups and structures and the bands are listed on table **3**. Ultraviolet spectroscopy was applied on equimolar ethanolic solutions and the related λ_{maxs} were appeared as per table **4**. CHN analysis was also necessary for the purpose of identifying the elements of the highly purified products and the percentage is shown in table **5**.

Table 1. Melting points, physical description and yield percent of the new four compounds

Compound	Melting point °C	Physical description	Yield percent
Acetanilide	114	Colourless glistening crystals	-
I	161-162	White powder	83%
II	202-203	White powder	81%
III	169-170	Yellow powder	76%
IV	216-218	Yellow powder	79%

Table 2. Solubilities of the new four compounds and sulfanilamide

Compound	Solvents					
	water	methanol	Ethanol	acetone	5%NaOH	5%HCl
I	insoluble	soluble	Soluble	soluble	Insoluble	soluble
II	insoluble	soluble	Soluble	soluble	Insoluble	soluble
III	insoluble	soluble	Soluble	soluble	Insoluble	soluble
IV	insoluble	soluble	Soluble	soluble	Insoluble	soluble
sulfanilamide	insoluble	soluble	Soluble	soluble	Soluble	soluble

Table 3. Infrared spectroscopy vibration values for the new compounds

Compound	Vibration bands cm^{-1}
I	Two medium bands at 3340 and 3367 due to N-H stretching
	Medium band at 2949 due to aromatic C-H stretching
	Medium band at 2846 due to C-H stretching of $-CH_2$--
	Two medium bands at 1601 and 1645 due to N-H bending
	Medium band at 1503 due to C-H bending of $-CH_2$-
	Two strong bands at 1304 and 1153 due to S=O stretching
	Medium band at 822 due to two adjacent aromatic hydrogen atoms
II	Approximately, it has the same bands above but it has strong band at 1140 due to C-O stretching
III	Again, it has the same bands but in addition it has variable bands at 1367 and 1386 due to C-H bending of $-CH_3$ and at 1407 and 1469 due to C-H bending of tertiary carbon
IV	It has, in addition to the closely related bands values of compound I, variable bands at 1361 and 1387 due to C-H bending of $-CH_3$ group

Table 4. λ_{max} of the new compounds using Ultraviolet- visible Scanning

Compound	Corresponding λ_{max} nm
Acetanilide	242
I	267
II	268
III	263
IV	268

Table 5. CHN elemental analysis of the four new products

Compound	Elemental Analysis				
	C	H	N	S	
I	55.223	5.991	12.121	12.542	observed
	54.98	6.71	11.6	13.34	calculated
II	47.351	6.094	12.342	14.311	observed
	49.87	5.82	11.56	13.23	calculated
III	58.802	7.095	10.295	13.714	observed
	56.67	7.13	11.01	12.6	calculated
IV	53.346	7.244	15.951	10.892	observed
	51.74	6.71	16.46	12.56	Calculated

RESULTS AND DISCUSSION

Chemistry

Acetanilide reacted with the moisture sensitive chlorosulfonic acid at dry condition. The acetamido group modulates the reactivity of the ring toward chlorosulfonation as an electrophilic aromatic substitution reaction (electrophilic effect). This is together with the steric factor which potentiates formation of p-nucleophiles (saturated heterocyclic amines) that are added in excess moles to serve as base picking the released HCl and yielding water soluble salt and enhancing the yield of products that were now ready for the next step i.e. acid hydrolysis of amide linkage rather than sulfonamide. This was followed by alkalinization to get our final compounds that were purified with washing and recrystallization. The resulted dialkylated hetero N^1 moiety may have more nucleophilic and basic tendency to bind with and inhibit the dihydrofolate enzyme than the ordinary monoalkylated ones.

This is, surely, less than or, at least, equal to the ionized sulfonamides (anions) that have, on N^1 atom, intensive electronic density rendering them the most active drugs. In addition, the dialkyl groups surrounding N^1 may add steric effect that hinders the binding with degradating enzymes of pathogens and this will prohibit the resistance exerted by the microbes to the usual sulfa drugs.

The differences observed between the melting points values of the newly synthesized compounds as well as acetanilide proved that these molecules differ from each other in their chemical constitutions. This also had been supported by significant IR identification to confirm their structures and the resulted bands were compatible with the functional groups of each molecule. UV spectroscopy revealed that changing acetanilide to these compounds had given additional value on λ_{max} resulting in bathochromic shift to longer wave length. The approximating results of compounds **I-IV** λ_{max} proved their similarity in structural formula. CHN analysis was run to prove their own empirical formula and the observed percents of elements were acceptable with those calculated. In respect to solubilities, these four compounds were water insoluble but soluble in organic solvents. In contrast to sulfanilamide, they were insoluble in NaOH solution and we conclude the N^1, N^1 dialkylation, the absence of free hydrogen atom and the difficulty of sulfonamide anion formation. Therefore, these analogs are expected to have partition coefficients higher than that of classical N^1 monosubstituted sulfa drugs and, as a result, they may be more potent as antibacterials by their too high penetrating cross bacterial cell membranes.

Bacteriological study

Testing the antibacterial effect of these molecules was to be qualified using culture-sensitivity procedure as diffusion method which was applied on *S aureus, E coli* and *P mirablis* that were grown on Muller Hinton agar. The discs were prepared from Whatmann filter paper #1 and then dipped in, separately for 24hrs, 500µg /100µl and 1000µg /100µl solutions of each compound in ethanol. Sulfanilamide discs of same concentrations were also used as control for comparing their inhibition zone with.

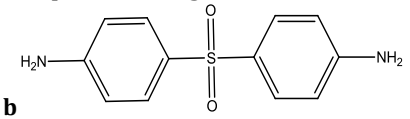
All the synthesized products had no inhibiting action on growth of the used germs and, therefore, these compounds possessed no antibacterial activity against the previously mentioned types of gram positive and gram negative bacteria. Also mixing sulfanilamide with each compound in single disc gave the same inhibition due to the former one. This cancels the probability of potentiation effect. The results are presented with table **6**.

Table 6. Comparative bacteriological study results

Compound	*S aureus*	*E coli*	*P mirablis*
Sulfanilamide	inhibition	inhibition	inhibition
Sulfanilamide-each compound combination	same	same	same
I	No inhibition	No inhibition	No inhibition
II	same	same	same
III	same	same	same
IV	same	same	same

CONCLUSION

With this research, we conclude that making the disubstituded hetero N_1 atom of the sulfonamide side will give compounds having no anti-infective property on those selected bacteria but we can do a future study to test their action against other microorganisms(for example leprosy causative agents) due to their closely related structures as dapsone analogs.[1b]



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