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Antimicrobial activity induced by a Sulfathiazole derivative on Staphylococcus aureus, and Vibrio cholerae.

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Abstract: In this work the antibacterial activity of asulfathiazole derivative was evaluated against both *Staphylococcus aureus* and *Vibrio cholerae* using cefotaxime, gentamicin, ciprofloxacin and sulfathiazole as controls. The evaluation of antimicrobial effect of the different compounds on the bacterial species was made by the method of microbial minimal inhibitory. The results indicate that bacterial growth of *Staphylococcus aureus*and *Vibrio cholerae*was inhibited with cefotaxime(MIC=5.23 × 10^{-4} mmol), gentamicin(MIC=2.68 × 10^{-5} mmol), ciprofloxacin (MIC=3.77 × 10^{-4} mmol) andsulphathiazole derivative(MIC= 4.10× 10^{-3} mmol).To delineate the structural chemical requirements of sulfathiazole derivative as antibacterial agent against*Staphylococcus aureus*and *Vibrio cholerae*, otherparameters such as the descriptors logP andπ were calculated.The results showed anincrease in the values of logP andπ for the sulfathiazole derivative in comparison with sulfathiazole. These data suggest a relationship between the physicochemical parameters evaluated and the degree of lipophilicityof the sulfathiazole derivative. Therefore, possibly the antibacterial activity of thesulfathiazole derivativecould depend of lipophilicity degree of sulfathiazole derivative in comparison with sulphathiazole.

Keywords: Sulfathiazole derivative, antibacterial activity, *Staphylococcus aureus*, and *Vibrio cholerae.*

Introduction

Infectious diseases are one of the main causes of morbidity-mortality in the world[1-3]. Several causal agents, such as *Staphylococcus Aureus*[4] and*Vibrio cholerae*[5] among others[6] have been shown to accelerate the progression of these pathologies. Although there are many therapeutic agents for treatment of these bacterial microorganisms[7,8] unfortunately, prolonged antibiotic therapy induces bacterialresistance[9,10], because somebacteria have developed ways to circumvent the effects of antibiotics[11,12]. For example, several studies indicate that *β-lactam* antibiotics (*methicilin/oxacillin*) predispose to patients for acquisition of resistance to *Staphylococcus Aureus*[13,14]. Other reports showed that antibiotic-resistant strains have emerged among Gram-negative bacilli such as *Vibrio cholerae*[15]. Therefore, antibiotic resistance can be considered a serious threat for the human health; this fact requires an international approach to its management. In this sense, new drugs have been developed for control of bacterial resistance[16,17]for example, the development of analidixic acid derivative which induces antibacterial activity against both Gram positive and Gram negative bacteria[18], Other reports show that a new cephalosporin induces antibacterial activity against *Staphylococcus aureus* in a rabbit endocartitis model[19]. Other data indicate that some sulfonamide derivatives exert antibacterial activity against *Staphylococcus Aureus*[20]. In addition, there reports which show that new aliphatic sulphonamide exert antibacterial activity against *Staphylococcus aureus*andother microorganisms[21]. Other studies indicate that a new Ni(II)-sulfonamide complex induce antibacterial activity against *Staphylococcus aureus*[22].Analyzing these data,the objective of this study was to evaluate the antimicrobial activity induced by a sulfathiazole derivative againstboth *Staphylococcus Aureus*and*Vibrio cholerae*using the method of microbial minimal inhibitory[23].In addition, in this study our aim was to have new drugs that can be used for treatment of infectious disease.

Materials And Methods

General methods:

Strains.

The microorganisms in this study belonged to the strain bank at the Departament of Pharmaco-Chemistry at the Faculty of Chemical Biological Sciences of the University Autonomous of Campeche. The strains are certified by Center for Disease Control in Atlanta and were as follows. *Staphylococcus aureus* (ATCC 25923) and *Vibrio cholerae* (ATCC 14547). The strains are kept under refrigeration at4^{o}C in special gel (BBL).

Antimicrobial agents.

4-{[(2-Hydroxy-naphtalen-1-yl)-phenyl-methyl]-amino}-*N*-thiazol-2-yl-benzenesulfonami-de (Figure 1) was synthesized by previouslymethod reported[24]. This compound was dissolved in methanol and diluted with distilled water. Cefotaxime, gentamicin, ciprofloxacin and sulfathiazole were used as the standard drugs.

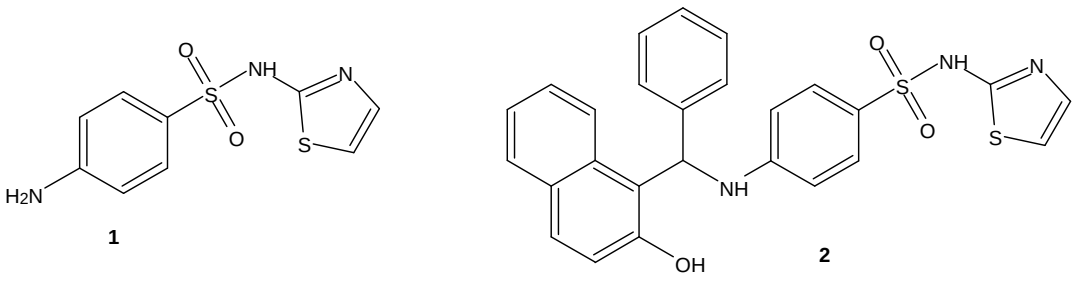

Figure 1. Chemical structure of sulfathiazole (**1**) and sulfathiazole derivative (**2**).

Antimicrobial activity.

The evaluation of antimicrobial effect of the different compounds on the bacterial species was made by method described by Figueroa[23]. The bacterial species were incubated on Brain-Heart Infusion (*Vibrio cholerae*) and *Staphylococcus* 110 (*Staphylococcus aureus*) agars for 24 hours at 37^{o}C. After such time, it was be determined whether growth had taken place or not. In addition, a series of tubes were prepared, the first of which contained 2 ml of culture medium (tripticasesoye) at double concentration and the remainder (11 tubes), contained the same quantity of medium at single concentrations. From the first tube (double concentration) an aliquot of 2 ml of the studied compound(1 mg/ml) was added and stirred, from this tube an aliquot of 2 ml was taken and added to the following tube (simple concentration) and the process was successively repeated until the last 2 ml of dissolution had been used up. After this process, each tube was inoculated with 0.1 ml of the bacterial suspension, whose concentration corresponded to Mc-Farland scale (9 x 10^{8} cells/ml) and all the tubes were incubated at 37^{o}C for 24 hours. Subsequently, a loop was taken from each of them and inoculated into the appropriate cultures for different bacterial organisms, and were incubated for 24 hours at 37 ^{o}C.All these process was done several times (n = 6). After such time, the minimum inhibitory concentration (MIC) was evaluated to consider the antimicrobial effect of the different compounds. In order to discard the effect of methanol (solvent) on the bacterial species studied, a series of the same number of tubes was prepared in parallel, to which 2 ml of methanol at 60% was added to the first and corresponding successive dilutions were added in the same way as before. In addition a control series was also performed using distilled water to pH 7.0.

Statistical analysis

The obtained values are expressed as average (n = 6). No adjustments were madefrom multiple comparisons.

Results

The bacterial activity of sulfathiazole derivativewas compared with the antibacterial effect of sulfathiazole, cefotaxime, gentamicin, and ciprofloxacin (controls) in such bacterial microorganism studied. The results obtained (Figure 2) indicate that bacterial growth of *Staphylococcus aureus* was inhibited by cefotaxime (MIC= 5.23×10^{-4} mmol), gentamicin (MIC= 2.68×10^{-5} mmol), and ciprofloxacin (MIC = 3.77×10^{-4} mmol). It is important tomention that bacterial growth of same microorganism was not inhibited by sulfathiazole. Nevertheless in presence of the sulfathiazole derivative (MIC= 4.10×10^{-3} mmol) the bacterial growth was blocked in a manner dosedependent.

On the other hand, alternative experimental were made in Gram-negative bacteria (*Vibrio cholerae*) using the same controls to evaluate the antibacterial effect of the sulfathiazole derivative. The results indicate that bacterial growth of *Vibrio cholerae* was inhibited (Figure 3) in presence of cefotaxime (MIC = 5.23×10^{-4} mmol), gentamicin (MIC = 1.34×10^{-5} mmol), ciprofloxacin (MIC = 3.01×10^{-3} mmol) and sulfathiazole derivative (MIC = 4.10×10^{-3} mmol).In addition, it's important to mention that *Vibrio cholerae* was not sensibility tosulfathiazole.

On the other hand, other results showed in the tables 1-3 indicate that physicochemical parameters logP and π were higher for the sulfathiazole derivative in comparison with sulfathiazole.

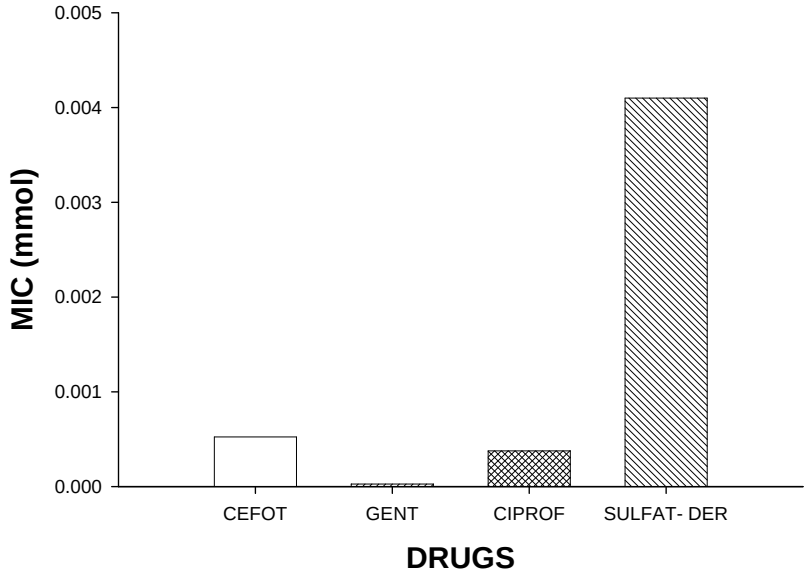

Figure 2. Antibacterial effect exerted by the sulfatiazole derivative (SULFAT-DER) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) on *Staphylococcus aureus*. Experimental data showed that *Staphylococcus aureus* was susceptibly to CEFOT (MIC = 5.23×10^{-4} mmol), GENT (MIC = 2.68×10^{-5} mmol), CIPROF (MIC = 3.77×10^{-4} mmol). Nevertheless, in presence of SULFAT-DER the MIC was of 4.10×10^{-3} mmol. Each bar are expressed as average (n = 6).

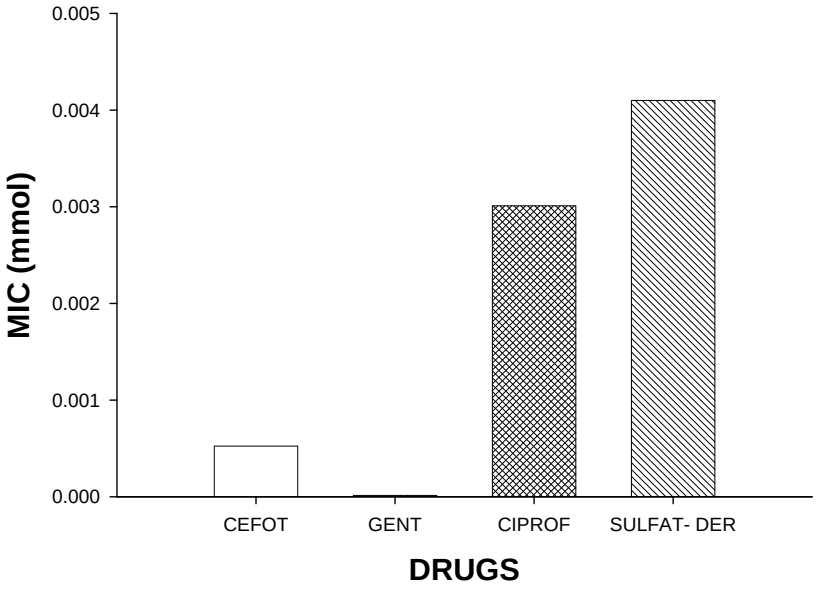

Figure 3. Antibacterial activity induced by the sulfatiazole derivative (SULFAT-DER) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin and CIPROF) on *Vibriocholerae*. Experimental data showed that *Vibrio cholerae*was susceptibly to CEFOT (MIC = 5.23×10^{-4} mmol), GENT (MIC = 1.34×10^{-5} mmol), CIPROF (MIC = 3.01×10^{-3} mmol). Nevertheless, in presence of SULFAT-DER the MIC was of 4.10×10^{-3} mmol and for SULFAT the MIC was of 7.83×10^{-3} mmol.Each bar are expressed as average (n = 6).

Table 1. Theoretical calculating of physicochemical parameters LogPforsulphatiazole (**1**) and its derivative (**2**) using several programs.

Program	Compounds	
12		
ALOGPs	0.88	5.37
AC logP	1.13	5.27
ALOGP	1.04	5.43
MLOGP	0.63	3.68
KOWWIN	0.72	5.00
XLOGP2	-0.03	5.08
XLOGP3	0.05	5.88
Average LogP	0.63 ± 0.46	5.10 ± 0.69

Table 2. Theoretical calculating of physicochemical parameters LogPand π of sulphatiazole.

Aromatic Carbon	2.6460
N [aliphatic N, one aromatic attach]	-1.8340
Aromatic Sulfur	0.4082
-SO2-N [aromatic attach]	-0.2079
Aromatic Nitrogen [5-member ring]	-0.5262
Equation Constant	0.2290
π	-0.9170
Log Kow	0.7151

Table 3. Theoretical calculating of physicochemical parameters Log P for sulphathiazole derivative.

-CH [aliphatic carbon]	0.3614
Aromatic Carbon	7.3500
-OH [hydroxy, aromatic attach]	-0.4802
-N [aliphatic N, one aromatic attach]	-1.8340
Aromatic Sulfur	0.4082
-SO2-N [aromatic attach]	-0.2079
Aromatic Nitrogen [5-member ring]	-0.5262
aromatic-C-N-aromatic correction	-0.3000
Equation Constant	0.2290
π	4.2852
Log Kow	5.0003

Discussion

In this study, the antibacterial activity of sulfathiazole and its derivative against *Staphylococcus aureus* and *Vibrio cholerae* was evaluated using cefotaxime, gentamicin, and ciprofloxacin as controls.The experimental data obtained indicate that bacterial growth of *Staphylococcus aureus* was inhibited by the controls; nevertheless, this microorganism was not sensibility to sulfathiazole. Bacterial resistance to sulfathiazole could be mediated by mutational or recombinational changes in the target enzyme (dihydropteroate synthase) involved in the folic acid pathway, such as happening to other *Staphylococcus aureus* strains resistant to sulphonamides[25].

To assess whether this phenomenon is also present in Gram negative bacteria; in this study, the antibacterial activity of sulphathiazole against *Vibrio cholerae* was evaluated using as biological tools the same controls.The results obtained indicate that *Vibrio cholerae* only was sensibility to controls in comparison with sulfathiazole. Possibly, the antibacterial resistant exerted by*Vibrio cholerae*to sulfathiazole could be to changes in the structure of genes encoding to dihydropteroate synthase; this hypothesis is availed by other reports which show that *Vibrio cholerae* strains resistant to sulphonamides by horizontal gene transfer via self-transmissible mobile genetic elements, including SXT elements-mobile DNA elements belonging to the class of integrative conjugating elements[26]. Here, it is important to mention that in the search for alternative therapeutic to decrease bacterial resistance to sulfonamides since several years ago, have developed new sulfonamide derivatives[27,28] with high lipophilic properties[29]. In this sense in this study was evaluated a sulfathiazole derivativeagainst *Staphylococcus aureus* and *Vibrio cholerae*.The results obtained indicate that differences exist of antibacterial activity against *Staphylococcus aureus* and *Vibrio cholerae* between sulfathiazolederivative and the controls. These data indicate that antibacterial effect induced by sulphathiazole derivative is through of a molecular mechanism different in comparison with the controls and sulfathiazole.

Analyzing all this results in this study wasconsidering that antibacterial activity induced by the sulfathiazole derivative against *Staphylococcus aureus*and *Vibrio cholerae* could depend of the hydrophobic region involved in their chemical structure in comparison with sulfathiazole, in order to interact with some components of bacterial cell to induce decrease of bacterial growth and exertcell death. This premise is availed by some studies which suggest that antibacterial activity of some compounds can depend of their physicochemical characteristics which bring consequently induce cell death[30,31].

Therefore, to delineate the structural chemical requirements of the sulfathiazole derivative as antibacterial agents against *Staphylococcus aureus* and *Vibrio cholerae*, some physicochemical parameters such as the descriptors logP and π were calculated. LogP describes the logarithmic octanol-water partition coefficient; therefore, it represents the lipophilic effects of a molecule that includes the sum of the lipophilic contributions of the parent molecule and its substituents[32]. The difference between the substituted and unsubstituted logP values is conditioned by the π value for a particular substituent. Hammett showed that π values measure the free energy change caused by a particular substituent to relate to biological activity[33]. Therefore, in this study, the logP and π parameters were calculated by the method reported by Mannholdand Waterbeemd[34]. The results (Table 1-3) showed an increase in logP and π values in sulfathiazole derivative with respect to sulfathiazole. This phenomenon is conditioned mainly by the contribution of all substituent atoms involved in the chemical structure of the sulfathiazole derivative. These results showed that both aliphatic and aromatic carbons involved inthe sulfathiazole derivative contribute to the highlipophilicity in comparison with sulfathiazole. All dataindicate that an increase in the degree oflipophilicity is related to the antibacterial activity induced by the sulfathiazole derivative on the microorganisms studied such happening with other antibacterial reagents[35].

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