ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-vitro Antibacterial Activity of two Novel Sulfonamide Derivatives against Urinary Strains of *Escherichia coli*.

Mabrouka Bouacha[1], Hajira Berredjem[1]*, Malika Berredjem[2] and Noureddine Bouzerna[1].

[1]Laboratory of Applied Biochemistry and Microbiology, Faculty of Sciences, Department of Biochemistry, University of Badji Mokhtar, Annaba, Algeria.
[2]Laboratory of Applied Organic Chemistry, Synthesis of Biomolecules and Molecular Modelling Group, Faculty of Sciences, Department of Chemistry, University of Badji Mokhtar, Annaba, Algeria.

ABSTRACT

Two novel sulfonamide derivatives, the 3,4-dihydroisoquinoline-2(1H)-sulfonamide (**1a**) and the 4-phenylpeperazine sulphonamide (**1b**), have been evaluated *in vitro* as antibacterial agents against urinary and standard strains of Gram-negative *Escherichia coli*, by both disk diffusion and dilution assay methods. These bacteria were screened against the novel compounds which were compared to a standard antibiotic, the sulfamethoxazol-trimethoprim (STX). The results revealed that the tested compounds showed a good antibacterial activity. The diameters of the growth inhibition area were in the range 10-40 mm. Antibacterial activity of compound **1a**, against all the bacterial strains, was superior to those of the compound **1b** and the commercial drug SXT. The diameters of the growth inhibition area of sulfonamide **1a** were in the range 15-40 mm and the MICs were ranged from 2 to 128 µg/ml. Compound **1b** was less active than compound **1a** but more active than antibiotic SXT. Diameters of inhibition of compound **1b** were in the range 4-26 mm and the MICs were ranged from 8 to 256 µg/ml. In conclusion, the newly synthesized sulfonamide derivatives showed a powerful interesting antibacterial activity against all strains of *Escherichia coli*. Better activity was obtained with compound **1a**.

Keywords: Sulfonamides, Antibacterial Activity, *Escherichia coli*, MIC.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Sulfonamides are among the most widely used antibacterial agents in the world [1], chiefly because of their low cost, low toxicity and excellent activity against common bacterial diseases [2]. They exhibit a wide spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria [3,4]. They have been used for many decades as efficient antibacterial agents for animals and man [5]. However, resistance has spread extensively and rapidly [1,2]. This is mainly due to the horizontal spread of resistance genes, expressing drug insensitive variants of the targets enzymes dihydropteroate synthase for sulfonamides [5,6].

The emergence of bacterial resistance to this antibiotic poses serious problem for medical professionals during the last decade [5] in particular, multi-drug resistant bacteria including *E.coli*, which could cause different intestinal and extra-intestinal infections in humans and animals: urinary tract infections, meningitis, peritonitis, and septicemia. Moreover, *E. coli* is also the most common cause of Gram-negative nosocomial and community-acquired infections [7]. It has been reported that the treatment of infections caused in particular by *E. coli*, is difficult and more expensive due to the multiple-drug resistance [7,8].

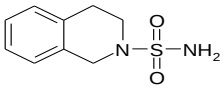
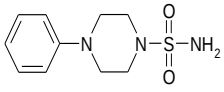
Due to these current problems of resistance associated with frequent use of antibiotics, the needs for new antimicrobials have increased; much attention is drawn to the search for new and effective antimicrobials.
In our study, we aimed to investigate the susceptibilities of *E. coli* strains isolated from urine cultures to the newly synthesized sulfonamide derivatives **1a-b** compared to the commercial antibiotics SXT.

MATERIALS AND METHODS

Tested compounds

The synthesized sulfonamide derivatives 3,4-dihydroisoquinoline-2(1H)-sulfonamide **(1a)** and 4-phenylpeperazine sulfonamide **(1b)** (Table 1), were investigated for their antibacterial activity. These compounds were synthesized by the Laboratory of Applied Organic Chemistry, Synthesis of Biomolecules and Molecular Modelling Group, University of Badji Mokhtar, Annaba (Algeria). The standard commercial antibiotic sulfamethoxazol-trimethoprim (SXT) (400/80 µg), was used as comparative drug.

Table 1: Molecular structure of the sulfonamide derivatives 1a-b.

Tested compounds	1a	1b
Name	3,4-dihydroisoquinoline-2(1H)-sulfonamide	4-phenylpiperazine-1-sulfonamide
Structure		

The tested compounds were prepared by dissolving in acetone and were serially diluted in nutrient broth at 10 different concentrations in the range 1 to 512 µg/ml.

Chemistry

As part as the research for new derivatives of sulfonamide, the 4-phenylpeperazine sulfonamide and 3,4-dihydroisoquinoline-2(1H)-sulfonamide were prepared in tree steps (carbamoylation, sulfamoylation and deprotection) by the reaction of chlorosulfonyl isocyanate (1 equiv.) and tertiobutanol (1 equiv.) in anhydrous CH_2Cl_2 (20 ml) [9]. After 30 min, the *N*-Chlorosulfonylcarbamate was added to a solution of secondary amines; 4-phenylpeperazine, 3,4-dihydroisoquinoline-2(1H)-sulfonamide (1equiv) in the same solvent (20 ml) in the presence of triethylamine (1.1 equiv.) at 0°C. The resulting mixture was stirred for less than 2 hrs at the room temperature. The reaction mixture was washed with HCl 0.1 N and water; the organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuum. The residue was purified by chromatography on silica gel (eluted with CH_2Cl_2) to give 85% of *N*-Boc sulfonamide as white solid.

The protected sulfonamides, were refluxed in water for less than 15 min to afford deprotected sulfonamides with quantitative yield [9].

Tested bacteria

A total of 91 strains of *E. coli* were isolated from the urine of patients with urinary tract infection. We selected strains isolated at different times in different patients and with many different antibiotypes. The 91 urinary isolates of *E. coli*, and the standard strains *E.coli* ATCC 25922 (Institut Pasteur, Algiers), were used for the evaluation of the antibacterial activity of the synthesized compounds **(1a-b)**. An inoculum of approximately 10^6 CFU/ml was prepared in nutrient broth.

Evaluation of the antibacterial activity

Disk diffusion method

The diameters of growth inhibition area of compounds **1a**-**b** were determined by the disk diffusion method. Petri dishes were prepared with a base of Mueller Hinton agar medium (Bio Mérieux, France) inoculated with each bacteria suspension. 10 µl of each compound has been deposited on the inoculated medium. Disk containing SXT (23,75/1,25 µg) (Bio Mérieux, France), was used as positive control and disks embedded with acetone was used as a negative one. The inoculated plates were incubated for 24 hrs at 37°C. If the drugs were found to be active in the disc diffusion test (inhibition zone >10 mm), they were further evaluated for determining minimum inhibitory concentration (MIC) values [10]. All tests were performed in duplicate and experiments were repeated three times.

Minimal inhibitory concentration

The studied compounds were screened for their antibacterial activity against *E. coli*. The MIC of tested compounds (**1a**-**b** and SXT) were determined by the broth dilution method according to recommendation of the CLSI [11]; the standardized suspension of strains (0.1 ml) was added to each tube containing compounds at various concentrations from 2 to 512 µg/ml in nutrient broth (Bio Mérieux, France). The tubes were incubated at 37°C for 24 hrs. The lowest concentration of drug that completely inhibited visible bacteria growth was considered to be the MIC [12].

The standard antibiotic SXT was used as a positive control and the solvent (acetone) was used as a negative one. Two replicates were done for each compound and experiment was repeated three times.

RESULTS

Diameters of inhibition

The results of the comparative *in vitro* activities of the new compounds and the commercial antibiotic against standard strain (*Escherichia coli* ATCC 25922) using disk diffusion method and dilution method are reported in Table 2. We considered a sensitive strain if the diameter of the inhibition area is superior to 17 mm and resistant strains if the diameter of the inhibition is less than 12 mm [13]. The two sulfonamide derivatives have showed a good antibacterial activity against the standard strain. The diameter of growth inhibition area for the compound **1a** (34 mm) was superior than compound **1b** (23 mm) and SXT (27 mm).

Table 2: The MIC and the diameter of growth inhibition area values of tested compounds 1a-b against *Escherichia coli* ATCC 25922.

Tested compounds	MIC (mg/ml)	Diameter of inhibition (mm)
1a	4	34
1b	16	23
SXT	8	27

1a: 3,4-dihydroisoquinoline-2(1H)-sulfonamide**.**
1b: 4-phenylpeperazine sulfonamide.
SXT: Sulfamethoxazol-Trimethoprim

ISSN: 0975-8585

These results could be confirmed on clinical urinary strains of *E. coli*; the sensitivity of the compounds **1a**-**b**, against urinary strains of *E. coli* was compared with the control antibiotic SXT (Table 3). Compound **1a** displays more activity against all urinary strains of *E. coli* than compound **1b** and SXT. The diameters of growth inhibition area for compound **1a** were in the range 15-40 mm for the majority of the tested strains (94,50%) including strains resistant to SXT, while the diameters of growth inhibition area for compound **1b** were in the range 4-26 mm. These results are better than those obtained with the standard antibiotic SXT.

Table 3: Percentage (%) of the clinical urinary *E. coli* strains for the different intervals of inhibition area diameters values of tested compounds 1a-b.

Tested compounds	Diameters of inhibition area (mm)							
	[0-4]	[5-9]	[10-14]	[15-19]	[20-24]	[25-29]	[30-34]	[35-40]
1a	0	0	0	12,08	25,27	**46,15**	8,79	6,59
1b	2,19	1,09	2,19	26,37	**52,74**	15,38	0	0
SXT	**36,26**	25,27	7,69	12,08	5,49	9,89	1,09	2,19

1a: 3,4-dihydroisoquinoline-2(1H)-sulfonamide.
1b: 4-phenylpeperazine sulfonamide.
SXT: sulfamethoxazol-trimethoprim

The Minimal inhibitors concentrations (MIC)

The results of the comparative *in vitro* activities of the new compounds and the commercial antibiotic against urinary strain of *E. coli* using dilution method are reported in Table 4.

Table 4: Percentage (%) of the clinical urinary *E. coli* strains for the MIC concentrations values of tested compounds 1a-b.

Tested compounds	MIC (µg/ml)								
	1	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**
1a	0	1,09	6,59	20,87	**48,35**	18,68	1,09	3,29	0
1b	0	0	0	4,39	15,38	**71,42**	3,29	2,19	3,29
SXT	0	0	8,79	9,89	3,29	8,79	3,29	**25,27**	40,7

1a: 3,4-dihydroisoquinoline-2(1H)-sulfonamide**.**
1b: 4-phenylpeperazine sulfonamide.
SXT: sulfamethoxazol-trimethoprim

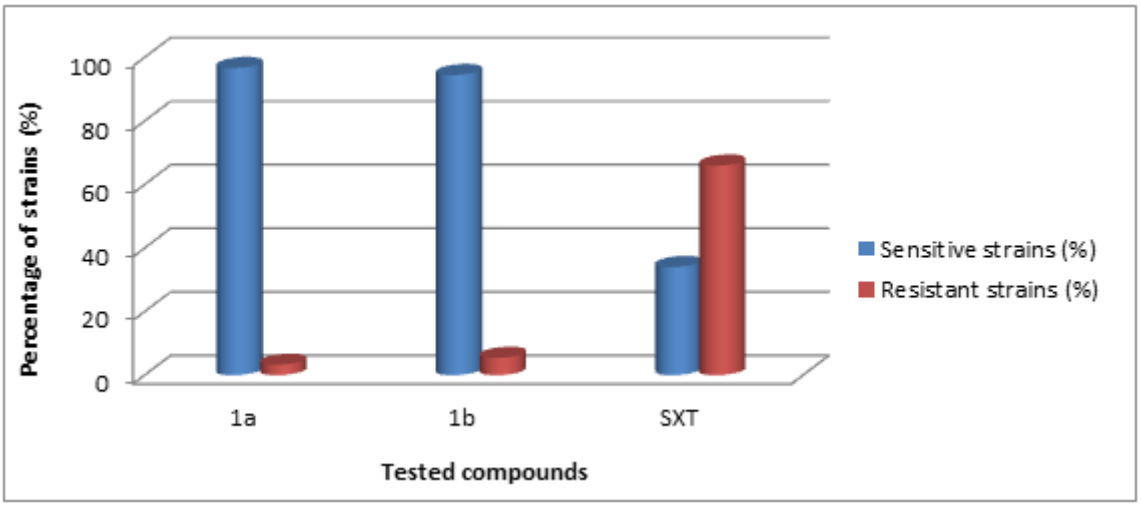
Compound **1a** showed highly potent activity against all urinary strains of *E. coli*; the majority of strains was inhibited with MIC ranged from 2 to 128 µg/ml. These results are better than those of compounds **1b** which have MIC ranged from 8 to 256 µg/ml and better than those of SXT which have MIC ranged from 4 to 256 µg/ml.

According to the MIC of sulfonamides recommended by the CLSI, we considered a sensitive strain if MIC was ≤ 64µg/ml and resistant strains when MIC ≥ 256 µg/ml.

According to the MIC's results shown in Figure 1, the sulfonamide derivatives tested possess a broad spectrum of activity against all urinary strains of *E. coli*, whereas sulfamethoxazol-trimethoprim, the drug used as standard, has been found less active against *E. coli* strains. Also, the antimicrobial activity is highly influenced by the nature of the reactive group.

ISSN: 0975-8585

Figure 1: Percentage (%) of the sensitive clinical *E. coli* strains for the tested compounds 1a-b.



1a: 3,4-dihydroisoquinoline-2(1H)-sulfonamide.
1b: 4-phenylpeperazine sulfonamide.
SXT: sulfamethoxazol-trimethoprim

DISCUSSION

The sulfonamides act as competitive enzyme inhibitors and block the biosynthesis of the vitamin folic acid in bacterial cells [1]. They do this by inhibiting the enzyme responsible for linking together the component parts of folic acid. The consequences of this are disastrous for the cell [1,14]. The success of sulfonamides is due to two metabolic differences between mammalian and bacterial cells. In the first place, bacteria have a susceptible enzyme which is not present in mammalian cells. In the second place, bacteria lack the transport protein which allows them to acquire folic acid from outside the cell [1].

In this study, two synthesized sulfonamide derivatives **1a**-**b** were evaluated for their antibacterial activity against clinical and standard strains of *E.coli* using disk diffusion method, and dilution assay method. These compounds exhibited much higher antibacterial activity than commercial antibiotic SXT which is an agent that has been used in the treatment of urinary tract infections for a very long period. However, because of widespread resistance rates, fluoroquinolones are now more frequently preferred in these infections [7].

The antibacterial activities of the sulfonamides seem to be depending to the degree of ionization of the sulfonamides in the agar medium [14]. They were also related to the substitution of different reactive groups [15-17].

Indeed, synthesized compounds **1a-b** have a structure activity relationship; compound **1a** with substitution of 3,4-dihydroisoquinoline-2(1H) showed enhanced activity against *E. coli.* This is shows how biological activities are influenced by structure modification.

A study reported by Yinjie C. and *al*. (2003) indicated that the introduction of a sulfonyl group in the structure of the novel antibacterial compounds such as oxazolidinone has increased their antibacterial activity [18]. The antibacterial activity should be related to the modification on the structure of the sulfonamides derivatives [17]. In our study the two compounds carry a benzene group, which could be necessary for the biological activities of any sulfonamides derivatives. These findings agree with the previous reports of Johnson and *al*. They have showed that the presence of benzene group can give ionized forms which are necessary for the antibacterial activity. Replacement or substitution of the benzene ring affords compounds biologically inactive [19].

It is known that the susceptibility of a microorganism to some agents depends first of all, on the properties of the agents and the microorganism itself. The present study demonstrated that the best antibacterial activity among the synthetic analogues was shown with compound **1a**; it has demonstrated

enhanced activity against *E. coli* strains, including strains resistant to other classes of commercial antibiotics (data not shown).

CONCLUSION

In this study, we have reported the antibacterial activity of two novel sulfonamides derivatives which demonstrated potent inhibition against all strains of *E. coli*.

Compound **1a** showed a better antibacterial activity than compound **1b** and commercial antibiotic and both can be considered as potential candidates for the treatment of some bacterial infectious diseases. MIC determination proved that the tested compounds presented different profiles against bacterial strains, due to their substituent, being the 3,4-dihydroisoquinoline-2(1H) the best one. Further investigations in this area are in progress in our laboratory.

REFERENCES

[1] Graham L, Patrick J. Chimie pharmaceutique. Paris: Deboek, 2003, pp. 380-386.
[2] Beronica I, Malin G, Mattias L, Charlotte K, Pallecchi L, Rossolini G. M, Goran K. Inter J Antimicrob Agents 2005; 25: 308-312.
[3] Ahmad S, Ebrahim S and Ali Hossein R. Tetrahedron Letters 2007; 48: 2185-2188.
[4] Skold O. Drug Resist Updat 2000; 3: 155-160.
[5] Skold O. INRA veterinaire research 2001; 32(3-4): 261-273.
[6] George A. Jacoby. History of Drug-Resistant Microbes, Antimicrobial Drug Resistance, Vol 1: Mechanisms of Drug Resistance Edited by Douglas L. Mayers 2008, pp. 124-131.
[7] Pullukcu H, Aydem F, Ifiikgoz M, Tafibakan F, Alper Tunger, Ulusoy S. Turk J Med Sci 2008; 38(2): 175-180.
[8] Perreten V, Boerlin P. Antimicrob Agents Chemother. 2003; 47: 1169-1172.
[9] Bouasla R, Berredjem M, Hessainia S, Chereait Z, Berredjem H and Aouf N. J Chem Chem Eng 2011; 5: 1153-1159.
[10] Andrews JM. JAC 2001; 48: 420-429.
[11] Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Wayne, PA; 2012 (CLSI publication M7-A9).
[12] Andrews JM. J Antimicrob Chemoth 2001; 48:15-16.
[13] Cavallo J.D, Chardon H, Chidiac C, Choutet P, Courvalin P, Debernat H et al. Comité de l'Antibiogramme de la Société Française de Microbiologie 2013; pp :13.
[14] Kratky Martin, Jarmila Vinsova, Marie Volkova, Vladimir Buchta, Frantisek Trejtnar. Jirina Stolarkova. Europ J Medic Chem 2012; 2:1-8
[15] Mengelers MJ, Hougee PE, Janssen LH, Van Miert AS. J Vet Pharmacol Ther. 1997; 20 (4): 276-283.
[16] Nieto MJ, Alovero FL, Manzo RH, Mazzieri Maria R. Eur J Med Chem 2005; 40: 361-369.
[17] Seoung Jong Kim, Myung-Ho Jung, Kyung Ho Yoo, Jung-Hyuck Cho, Chang-Hyun Oh. Bioorg Med Chem Lett 2008; 18: 5815-5818.
[18] Yinjie C, Yushe Y, Kaixian C, Ruyum J, Shuhua Z. Bioorg Med Chem Lett 2003; 13: 2311-2313.
[19] Johnson T, Khani A, Avery M.A, Grant S, Meshnick SR. AAC 1998; 42(6): 1454-1458.