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Antibacterial activity of four sulfonamide derivatives against multidrug-resistant *Staphylococcus aureus*

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ABSTRACT

*This study aims to evaluate, in vitro, antibacterial activity of four novel sulfonamide derivatives (**1a-d**) against Staphylococcus aureus: reference strain ATCC 25923 and 40 clinical isolates. Inhibition zones were performed with the disk diffusion method. The MIC values were determined by the dilution broth method. A 48 hours MIC-Kinetic curve was performed for the tested compounds. All compounds showed significant antibacterial activity. The mean values of the inhibition zones diameter for compounds **1a-d** were 22.15 ± 6.22, 16.39 ± 1.17, 15.42 ± 0.66 and 15.83 ± 1.28 mm, respectively (p value = 0.001). The MIC values were ranged between 64 and 512 µg/ml. The compound **1b** showed better activity. The 48 hours MIC-kinetic curve showed an inhibiting bacterial growth. The studied compounds **1a-d** showed a promising antibacterial effect to response to the urgent need for innovative drugs that could be more effective against resistant pathogens.*

Keywords: Antibacterial activity, Sulfonamides, *Staphylococcus aureus*, MIC.

INTRODUCTION

Sulfonamides are among the most widely used antibacterial agents in the world. They were the first effective chemotherapeutic agents used systematically for the prevention and cure of bacterial infections in humans and some animals, mainly because of their low cost, low toxicity and excellent activity against bacterial diseases [1]. The sulfonamide –SO2NH– group occurs in numerous biologically active compounds that constitute an important class of drugs used extensively as pharmaceutical and agricultural agents [2]. Many sulfonamide derivatives were synthesized, characterized and tested for antibacterial [3], anti-tumour [4,5] anti-carbonic anhydrase [6,7], diuretic [8,9], hypoglycemic properties [10], antithyroid [11], anti-inflammatory [5], and other biological activities [4,5].

Unfortunately, the abuse of antibiotics has led to the emergence of drug-resistant strains which have a significant impact on the patients' morbidity and mortality. In some cases, the formerly effective antimicrobial agents are no longer useful [12,13].

Staphylococcus aureus (S. aureus) is the most frequent resistant bacterium in the vast majority of the clinical isolates. It is a common human pathogen responsible for a significant number of infections worldwide such as skin and soft tissue infections, septicemia, pneumonia, endocarditis and deep abscesses; which have long been considered as hospital-acquired [14]. However, the epidemiology of *S. aureus* is changing because of its ability to adapt to varying environmental conditions [15]. New community-acquired strains, which differ from nosocomial strains in their susceptibility to various antibiotics, have appeared. *S. aureus* have rapidly become resistant above all

antibiotics, such as methicillin (MRSA: methicillin resistant *S. aureus*) [16,17], and recently to the vancomycin (VRSA: vancomycin resistant *S. aureus*), which previously represented the treatment of choice [18-20].

Even though the arsenal of antibacterial molecules available is considerable, it cannot solve all these problems [21]. Therefore, a clear need is required for the development of innovative antimicrobial agents with better pharmacological profiles. The aim of this study is to assess the *in vitro* activity of four innovative antimicrobial sulfonamide derivatives against *S. aureus*. We realize at the same time a MIC-kinetic curve for the inhibition activity of the new molecules.

EXPERIMENTAL SECTION

Bacterial strains

A total of 40 clinical *S. aureus* strains were used in this study. The isolates collected from public and private sanitary establishments were mainly isolated from different samples: 21 pus (52.50%), 9 urine (22.50%), 6 blood (15%), and 4 protected distal sampling (PDS) (10%).

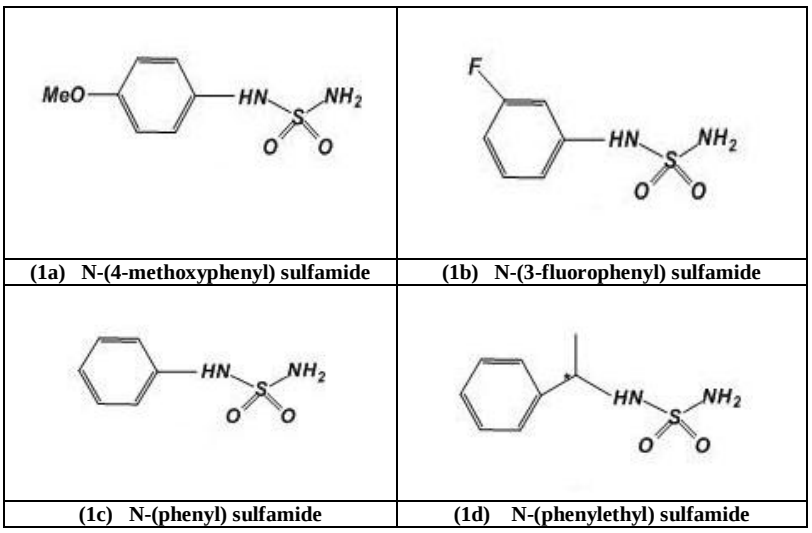
The identification of the bacterial strains was made on cultural and biochemical characters (API staph system, BioMérieux, France). The *S. aureus* ATCC 25923 was used as a control (Pasteur Institute, Algiers).

The commonly used method in routine laboratory practice for the detection of methicillin and vancomycine resistance is oxacilline (5mg, Bioanalyse®, Turkey) and vancomycine disc diffusion (30 mg, Bioanalyse®, Turkey).

Tested compounds

The tested sulfonamide derivatives **1a-d** (Figure 1) were prepared in acetone and then serial dilutions were made in a concentration range from 0.5 to 512 µg/ml.

Two commercial drugs were used as positive control and were diluted in the same manner: Control 1: Bactrim, sulfamethoxazole-trimethoprime (400/80mg) (Laboratoire Roche, France), and control 2: Enteropathyl, Sulfaguanidine (500 mg) (Merck, France).



(1a) N-(4-methoxyphenyl) sulfamide	(1b) N-(3-fluorophenyl) sulfamide
(1c) N-(phenyl) sulfamide	(1d) N-(phenylethyl) sulfamide

Figure 1: Chemical structure of the sulfonamide derivatives 1a-d

Determination of inhibition zones

The newly synthesized compounds **1a-d** were screened for antibacterial activity against *S. aureus* ATTC 25923 and clinical isolates. Inhibition zones of the compounds were performed with the disk diffusion method [22]. The antimicrobial screening was performed using Mueller–Hinton agar that was poured into each sterile Petri dish, allowed to solidify and finally seeded with a bacterial inoculums prepared in physiological sterile water with an OD_{625} about 0,08. Empty sterilized disks of 6 mm (Schleicher and Schule, Germany) were each impregnated with 20 µl of the different concentrations of the compounds. Disks were placed on agar plates and the cultures were incubated at 37°C for 24 hrs.

The standard drugs, control 1 and 2, were used as positive controls. Disks embedded with acetone were used as a negative one. Inhibition zones formed on the medium were evaluated in millimeter (mm). All tests were performed in duplicate, and experiment was repeated three times.

Minimal inhibitory concentration

The minimum inhibitory concentration (MIC) values of compounds **1a-d** were determined by the dilution broth method following the procedures recommended by the CLSI [22].

All tests were performed in Mueller–Hinton broth. Bacterial inoculum with an OD_{625} about 0.08 was added to each tube containing compound at geometric dilutions ranging from 0.5 to 512 µg/ml; a control tube without compound was used. The tubes were incubated at 37°C for 24 hrs. The results were recorded according to the presence or absence of bacteria growth comparatively to the controls. As previously, control 1 and 2 were used as positive controls. Two replicates were done for each compound, and experiment was repeated three times.

Minimal bactericidal concentration

The minimal bactericidal concentration (MBC) was carried out to assess the concentrations of the compounds that can kill or inhibit the growth of the tested organisms. Absence of growth was interpreted as bactericidal action while growth represented a bacteriostatic action [22]. The MBC was established on nutritive agar by sub-culturing, at 37°C for 24 hrs, 0.1 ml of tubes showing no turbidity at MIC concentration of the tested compounds.

MIC-kinetic curve

Bacterial suspensions were prepared in physiological sterile water (OD_{625} was approximately 0.08) and then inoculated in fresh Muller-Hinton broth. Compounds **1a-d** were added in MIC concentration as previously determined. A control was used to show bacterial growth without the presence of any compound.

The DO_{625} was taken before incubation and then each 2 hrs after incubation at 37°C: 0; 2; 4; 6; 8; 10; 12; 14; 16; 18; 24 and 48 hrs. Samples were removed from each tube at each time point indicated above.

Statistical analysis

Data analysis was performed using one-way analysis of variance (ANOVA) followed by *t* student test.
All the results were expressed as mean ± S.E.M. (standard error of the mean). Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Determination of inhibition zones

As shown in Table 1, the diameter of inhibition zones values of tested compounds **1a-d** against the reference strain *S. aureus* ATCC 25923 were ranged between 15 and 34 mm. Values for control 1 and control 2 were 24 and 16 mm respectively.

Table 1: The MIC and the diameter of growth inhibition zones values of tested compounds 1a-d against *S. aureus* ATCC 25923

Tested compounds	Diameter of inhibition zones (mm)	MIC (µg/ml)
1a	34	256
1b	15	64
1c	15	128
1d	16	128
Control 1	24	32
Control 2	16	512

Table 2: Percentage (%) of *S.aureus* resistance/sensibility against tested compounds 1a-d

Tested compounds	resistant Strains		sensible strains	
	(%)	(N)	(%)	(N)
1a	21,96	9	**78,04**	32
1b	21,96	9	**78,04**	32
1c	21,96	9	**78,04**	32
1d	19,52	8	**80,48**	33
Control 1	34,17	14	**65,83**	27
Control 2	**85,37**	35	14,63	6

Among the 40 clinical strains, 9 (21.96%) were resistant towards the new compounds whereas resistance towards control 1 and 2 were 34.17% and 85.37% respectively (Table 2). Therefore, all the synthesized compounds **1a-d**

exhibited a good antibacterial activity with a varying degree of inhibitory effect on the growth of the tested microbial strains. These results were comparative to the reference strain ones.

The diameters of inhibition zones values were expressed as an interval of measures (Table 3). The highest diameters of inhibition zones for the test compounds **1a-d** were obtained in the interval [30-34] (31.8 ± 1.83 mm) for **1a**, [15-19] (16.54 ± 1.00 mm) for **1b**, [15-19] (15.51 ± 0.55 mm) for **1c** and [15-19] (16.5 ± 1.08 mm) for **1d**.

The results vary between 14-34 mm for the compound **1a**, 14-18 mm for the compounds **1b** and **1d,** and 14-17 mm for the compound **1c**; the highest percentage of sensitive strains is obtained in the interval [15-19].

Compound **1a** inhibited the growth of pathogens, particularly MRSA (28 strains) and VRSA (7 strains), better than compounds **1b-d** and the control 2.

The solvent control (acetone) did not show any antimicrobial activity.

Table 3: Percentage (%) of *S. aureus* strains relative to the intervals of the inhibition zones of the tested compounds 1a-d

Tested compounds	Percentage (%) of *S. aureus* strains relative to the inhibition zones intervals (mm)						
	[0-4]	[5-9]	[10-14]	[15-19]	[20-24]	[25-29]	[30-34]
1a	21,97	0	7,31	**24,39**	14,63	19,51	12,19
1b	19,51	0	4,89	**75,60**	0	0	0
1c	19,51	0	4,89	**75,60**	0	0	0
1d	19,52	0	7,31	**73,17**	0	0	0
Control 1	**39,04**	0	0	14,63	21,95	14,63	9,75
Control 2	**53,67**	0	34,14	12,19	0	0	0

Determination of the MIC

As shown in Table 2, the MIC values of tested compounds **1a-d** against the reference strain *S. aureus* ATCC 25923 were ranged between 64 and 256 µg/ml. Values for control 1 and 2 were respectively 32 and 512 µg/ml.

For the clinical strains, the antibacterial results (Table 4) evidently shown that the new series of sulfonamide derivatives possess a good concentration-dependent antibacterial activity against the tested bacteria at MIC values between 64 and 512 µg/ml. Among the screened compounds, **1b-d** showed good activity against all the bacterial strains, compared to the control 2.

Antibacterial screening revealed that the tested compound **1b** showed promising activity with the lowest MIC 64 µg/ml for 51.61% of tested strains whereas the compounds **1c-d** showed activity for the same concentration respectively for 12.12% and 24.24% of tested strains. The compound **1a** was found to be active on 53.12% of the tested strains at higher concentration 512 µg/ml.

Table 4: Percentage (%) of *S. aureus* strains relative to the different MIC (µg/ml) of the tested compounds 1a-d

tested compounds	Percentage (%) of *S. aureus* strains relative to the different MIC (µg/ml)										
	0,5	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**
1a	-	-	-	-	-	-	-	-	-	46,87	**53,12**
1b	-	-	-	3,22	-	-	-	**51,61**	22,58	19,35	3,22
1c	-	-	-	-	-	-	-	12,12	**42,42**	27,27	18,18
1d	-	-	-	-	-	-	3,03	24,24	**39,39**	21,21	12,12
Control 1	-	-	-	**33,33**	11,11	7,40	18,51	29,62	-	-	-
Control 2	-	-	-	-	-	-	-	-	-	-	**14,63**

(-): negative.

Determination of the MBC

The bacterial count determined a number greater than 10^2 UFC/ml in all Petri dishes, which corresponds to 0.01% of the initially number of bacteria. According to these results of MBC study, the synthesized compounds **1a-d** are bacteriostatic.

Kinetic Curve

The kinetic curve of the MIC for 48 hrs showed that the antibacterial activity of the new series of sulfonamide derivatives occurs during the first hours of incubation by inhibiting bacterial growth. The optical density (OD) was low and equal to the initial seeding rate of bacterial strains, as compared to the control one, which showed an exponential growth of the bacteria (Figure 2).

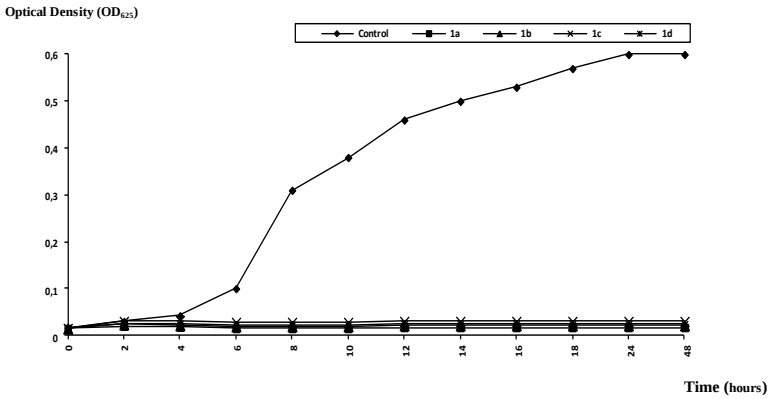


Figure 2: MIC-kinetic curves of clinical *S. aureus* strains for the tested compounds 1a-d

In the 1960s, antibiotics have emerged as a revolution; they healed in a few days deadly infections, wound infections, and food intoxications. Diseases such as syphilis or tuberculosis appeared to be eradicated, and ancient scourges, such as plague and cholera, were mastered [23]. The treatment of bacterial infections is made more complex because of the ability of bacteria to develop a variety of resistance mechanisms to numerous therapeutic agents. Many authors have described this phenomenon as the end of the era of antibiotics [24]. In reality, emerging and re-emerging infectious diseases have left us facing drug resistant organisms, which remain an important problem in clinical practice that is difficult to solve [16]. Drug-resistance bacteria, especially the *Staphylococcus aureus*, *Staphylococcus pneumoniae* … kill more than two million people each year and endanger human health seriously [25]. However, the number of new antibiotics has precipitously declined over the last 25 years. A decrease of almost 75% was observed for systemic antibiotics, approved by the FDA (American Food and Drug Administration) between 1983 and 2007, and this decline is particularly important for five years (2003-2007) [26]. This rapid evolution of bacterial resistance to the most marketed antibiotics encourages the discovery of new molecules with a good pharmacokinetic profile. Therefore, developing new antimicrobial agents continues to attract attention and is an area of rigorous research. Although a large number of antibiotics and chemotherapeutics are available for medical use, the antimicrobial resistance created an increasing need of new antimicrobial agents [27,28].

Sulfonamides were the first effective chemotherapeutic agents employed systematically for the prevention and cure of bacterial infections in humans and other animal systems [29]. The importance of the sulfonamide has been achieved when the sulfonylamide, sulfonamide analogue key, has been reported to be the first antibacterial drug. Later, many sulfonylamide derivatives were synthesized, characterized and tested for their biological activities [30,31].

In this study, new series of four synthetic sulfonamide derivatives **1a-d** were screened for the *in vitro* antimicrobial activity against Gram positive bacteria, a reference strain *Staphylococcus aureus* ATCC 25923 and 40 clinical strains of *S. aureus*. There has been a predominance of strains isolated from pus (51.28% of samples), comparatively to the other biological samples. These findings corroborate the previous study done by Elhamzaoui et al. [32] which showed a predominance of 62.70% for *S. aureus* strains isolated from pus.

The tested compounds demonstrate a significant antibacterial activity against the *S. aureus* strains which showed a high sensibility of 78.04% and 80.48% comparatively to the standard antibiotics. In fact, when conventional antibiotics were used for an antibiogram (data not shown), the clinical *S. aureus* strains presented an important multidrug-resistance; the rates of resistance were as follows: B-Lactam antibiotics (89.02%), aminoglycosides (Aminosides) (75.12%) and quinolones (60.97%). High resistance was obtained with different antibiotics: Rifampicin (95.12%), fusidic acid (80.48%), sulphonamides (90.24%), pristinamycin (56.09%) and tetracyclin (60.97%).

The *in vitro* evaluation of the antibacterial activity of the sulfonamide derivatives **1a-d** has highlighted an important dose-dependent antibacterial activity, which results in the appearance of the inhibition zones. The MIC kinetic curve during 48 h showed that the antibacterial activity of the new molecules appears since the first hours of incubation and inhibits the bacterial growth. Among the 40 clinical isolated strains, 32 (80%) showed inhibition zones $\geq$ 14

mm, reflecting their sensitivity to the new effective compounds. Regarding control 2, 86.37% of the tested strains were resistant.

Compounds **1b, 1c** and **1d** inhibited the growth of pathogen particularly MRSA and VRSA. Even though the synthesized compounds showed a good antibacterial activity, control 1 exhibited a better activity with a MIC equal to 4 µg/ml.

Among the four molecules, the compounds **1b-d** showed good antibacterial activity as indicated by MIC values equal to 64 µg/ml while the compound **1a** showed moderate antibacterial activity with a MIC value equal to 256-512 µg/ml. When compounds are compared with each other, **1b** was found to be more active at the lower concentration 64 µg/ml against 51.61% of studied strains. The presence of electron donating and withdrawing groups, size and shape of molecule, might be influencing the selective antibacterial activity. Aromatic fluorine substituent improves bioavailability and increases potency. It was therefore concluded that the presence of fluor moiety, in addition to phenyl group, was found to be essential for its high antibacterial activity**.** Kumar M. et al. [33] indicate in their study that the presence of phenyl ring attached to the sulfonamide moiety increased the antimicrobial potential of the synthesized compounds against the tested microbial strains; these results are coherent with our results. Ozdemir et al. [30] carried out a study on a *S. aureus* ATCC 25923 strain; six sulfonamide derivatives and their complexes gave MIC values between 220 and 413 µg/ml. These values are higher than ours (128- 64 µg/ml). Ever more, Chohan et al. [34] determinate the antibacterial activity of some new biologically active metal-based sulfonamides on a *S. aureus* strain; the diameters of the inhibition zones vary between 12 and 26 mm. and corroborate our results (15 and 34mm). Another study was carried out by Messah et al. [35] on a reference strain *Staphylococcus aureus* ATCC 25923 and a clinical strain for a new series of five N-acylsulfonamides. When compared to our findings, the reference strain showed the same results. The MICs obtained for the clinical strain vary between 256-512 µg/ml for three compounds as the results of compound **1a**. Among the five N-acylsulfonamides, two had MIC between 128-64 µg/ml, which were comparable with our tested compounds **1b-c**.

Compared to the antibacterial potential of our studied sulfonamide compounds **1a-d**, N-acylsolfonamide synthesized by Berredjem et al. [36] didn't show any activity on the Gram positive bacteria (*Staphylococcus aureus* ATCC 25923 and *S. aureus* isolates).

However, traditional methods of measuring antibiotic efficacy such as the MIC are insufficient for understanding the complex dynamics that lead to the rapid development and spread of antibiotic resistance within bacterial populations. The ability to investigate the relationship between individual molecular components of the system and the overall treatment outcome can lead to a better understanding of how to optimize antibiotic performance and to predict treatment outcome [37].

CONCLUSION

The antibacterial activity results of the studied compounds revealed that all the synthesized sulfonamides showed very good inhibitory characteristics. Among the screened molecules, compound **1b** with stronger conjugation effect of fluor in the benzene ring, was noticeable as the most active antibacterial agent against MRSA, VRSA. The studied products are still under investigation. Their antibiotic properties have promising applications in the control of infections.

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