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SYNTHESIS, CRYSTAL STRUCTURE AND ANTIBACTERIAL EVALUATION OF *N*-SUBSTITUTED PERHYDRO-1,3-OXAZIN-2-ONES CONTAINING *N*-PHENYLSULFONAMIDE

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Abstract - The synthesis of a new series of *N*-substituted perhydro-1,3-oxazin-2-ones containing *N*-phenylsulfonamide is described. The compounds **7a–7f** were obtained in a *one-pot* reaction from chlorosulfonyl isocyanate, selected 1,3-halogenoalcohols and various aromatic amines in alkaline conditions, to give the target *N*-heterocyclic 6-membered ring compounds with good yields. The X-ray crystal structure of *N*-[(*N*-4-fluorophenyl)sulfamoyl]perhydro-1,3-oxazin-2-one **7d** was solved. All the synthesized compounds have been screened for their *in-vitro* antibacterial activity against *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. Structures of **7d** and **6e** can be further optimized to give new potent antibacterial agents with structures significantly different from those of existing classes of antibiotics.

1. INTRODUCTION

The emergence of bacterial resistance to the antibiotics has posed a serious concern for medical professionals during the last decade.[1] In particular, multi-drug-resistant Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA),[2] *Staphylococcus epidermidis* (MRSE) and vancomycin-resistant *Enterococci* (VRE) are of major concern.[3] These bacteria are very dangerous and can be life-threatening especially for patients whose immune systems have been compromised due to HIV, surgery or any other diseases. Oxazolidinones and sulfonamides represent two classes of medicinally important compounds which are extensively used as antibacterial agents. Oxazolidinones are the only new class of synthetic antibacterial agents over the past 30 years that possess totally new structures compared to existing antibacterial agents.[4–7] It is well known that oxazolidinones bind to the 50S subunit of the bacterial ribosome and inhibit protein synthesis at a very early stage by preventing the initiation of mRNA translation. Because they target the bacterial protein synthesis at an early stage, drug resistance was expected to be rare. However, resistance to linezolid (Zyvox®) has already been reported.[8,9] For the sulfonamides -often called simply sulfa drugs-, it is well documented that this class of compounds interferes with PABA (*p*-aminobenzoic acid) in the biosynthesis of tetrahydrofolic acid[10] which is a basic growth factor that is essential for the metabolic process of bacteria. Many activities apart from carbonic anhydrase have been reviewed as anti-inflammatory, tubular transport inhibition and insulin release.[11]

In our days many new antibacterial agents are designed based on modification of the existing structural classes, since the antibiotic assay is easy to be carried out. The action modes of the agents are often discovered after finding them active. Identifying fragments features in existing drug classes and incorporating them into a de novo design of a new compound is an efficient recent method to discover new drugs with significantly different structure motifs.[12] For example, in some structures of recent biologically active small compounds shown in Figure 1 which include; linezolid 1,1,3-oxazinan-2-one **2**, Benzensulfonanilide, **3** indeglitazar (PAPAR) **4** and 4-azidomethyl-7-methylcoumarin-6-sulfonamide **5**. We can observe that the common general features of these agents are that they all contain phenyl sulfonamide and/or heterocyclic structures, and they have heteroatom substituent such as halo, amino and methoxy groups. Linezolid **1** has the oxazolidinone as the core structure, which is important for its activity. Bulk of literature reveals that several compounds containing *N*-heterocyclic 6-membered ring as 1,3-oxazinan-2-one **2** derivatives form compounds of pharmacological interest such as antibacterial activities,[13] anti-inflammatory[14] and anti-thrombotic.[15] Benzensulfonanilide **3** is a recent example of anti-MRSA/VRE type compounds containing phenylsulfonyl group.[16] Indeglitazar (PAPAR) **4** recently cited by Artis *et al*,[17] is expected to be useful in the treatment of Type 2 diabetes mellitus. Indeglitazar has now progressed to Phase II clinical evaluations. 4-azidomethyl-7-methylcoumarin-6-sulfonamide **5** is a novel sulfonamide containing 4-azidomethylcoumarin with a potent antibacterial activity.[18]

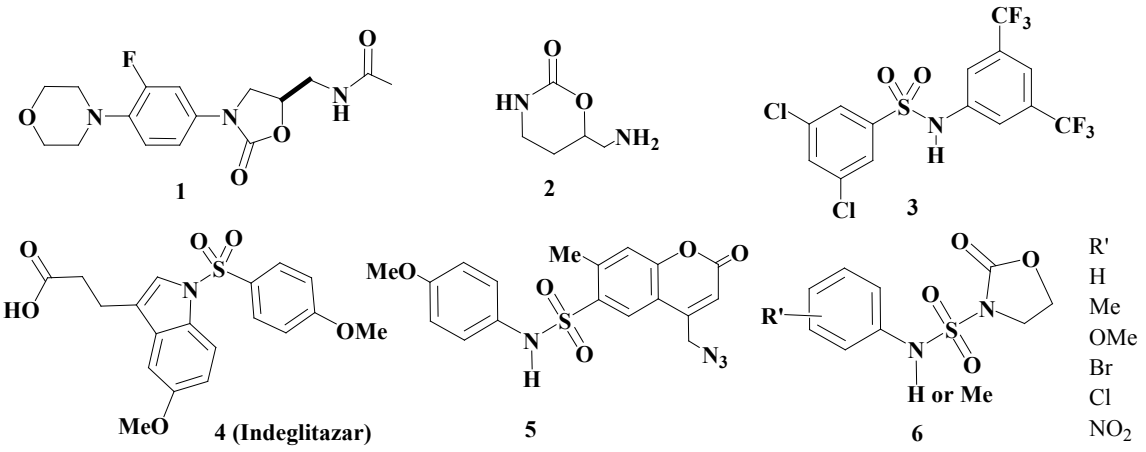


Figure 1. Some Structures of Recent Biologically Active Small Compounds

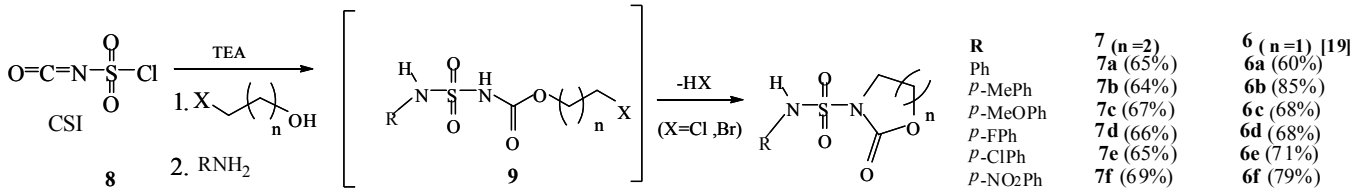
The general features of these compounds can be used to design new structures that can be potentially useful as antibacterial agents. For this purpose, at present, many efforts are focused on substituted phenyloxazolidinones and/or phenylsulfonamide. Previously,[19] we described the synthesis and antibacterial activity of some *N*-[(*N*-aryl)sulfamoyl]oxazolidin-2-ones **6** (Figure1) derivatives against only *Staphylococcus aureus* strain; Based on the positive effect of sulfonamide moieties on the activity of oxazolidinones, especially against both Gram-positive and Gram-negative bacteria.[20-22] In addition, Wang *et al*[13] described that synthesized compounds having 6-membered ring 1,3-oxazinan-2-one instead of 5-membered ring oxazolidinones have slightly more potent antibacterial activity. Considering this background, the objective of this study is to obtain more potent antibacterial compounds, by combining the chemistry of sulfonamide with perhydro-1,3-oxazin-2-one. A new class of *N*-[(*N*-aryl)sulfamoyl]-perhydro-1,3-oxazin-2-ones 7 (n=2) (Scheme 1) is reported in this paper. To our knowledge, antibacterial activity of these compounds and their analogs has not been investigated. Their use is limited to the preparation of various 2-chloroethylsulfamides,[23,24] transfamoylation reaction,[25] or as ligands in inclusion complex by β-cyclodextrin.[26]

In this work, we present the synthesis, characterization, crystal structure and antibacterial activity evaluation of some *N*-[(*N*-aryl)sulfamoyl]perhydro-1,3-oxazin-2-ones isomers **7a-7f** (Scheme 1) obtained in one-pot reaction starting from chlorosulfonylisocyanate (CSI) 8 (Scheme 1), selected 1,3-halogenopropanol (n=2) and various aromatic amines in alkaline conditions, to give the synthesis of the target *N*-heterocyclic 6-membered ring in good yields. Several of these molecules exhibit potent antibacterial activities against tested *Staphylococcus aureus*, *E.coli and Pseudomonas aeruginosa* clinical strains.

2. RESULTS AND DISCUSSION

2.1 CHEMISTRY

The Preparation of *N*-[(*N*-aryl)sulfamoyl]perhydro-1,3-oxazin-2-ones **7a-7f** was carried out starting from chlorosulfonylisocyanate (CSI) **8**, 1-3 halogenoalcohols **(n=2)**, aromatic amines and triethylamine (TEA) through a previously described one-pot procedure.[23] The isolation of the linear sulfamoylcarbamates **9** is not the aim of this study and it reduces the total yield. The used halogenoalcohols was 3-bromo-1-propanol or 3-chloro-1-propanol (Scheme 1).



R	7 (n =2)	6 (n =1) [19]
Ph	**7a** (65%)	**6a** (60%)
p-MePh	**7b** (64%)	**6b** (85%)
p-MeOPh	**7c** (67%)	**6c** (68%)
p-FPh	**7d** (66%)	**6d** (68%)
p-ClPh	**7e** (65%)	**6e** (71%)
p-NO_2Ph	**7f** (69%)	**6f** (79%)

Scheme 1. Preparation of perhydro-1,3-oxazin-2-ones derivatives **7a-7f**

Insitu cyclization is observed for the linear *N*-sulfamoylcarbamate **9** (n=2) which is achieved by triethylamine (TEA) addition, to give perhydrooxazinones **7**. This intramolecular cyclization was reported by Dewynter *et al*,[23] These compounds were formed by spontaneous cyclization during the first step related to the excellent leaving group character of Cl and Br. No cyclization is observed related to the sulfamide moiety. This is probably in relation with the rotational barrier level around the sulfamido group.[27,28] Some physical and spectral data of the synthesized compounds **7a-7f** were summarized in section 4. All of the perhydrooxazinones isomers **7** are crystalline and thermally stable compounds. The structural study was completed by crystallographic analysis.

2.2 X-RAY ANALYSIS OF COMPOUND 7d

Apart from the fluoro derivative of *N*-[(*N*-aryl)sulfamoyl]perhydro-1,3-oxazin-2-one, the other isomers were not well crystallized to be suitable for X-ray diffraction in spite of several attempts of recrystallization. In view of that, a colorless prism-shaped crystal of **7d**, 0.2 × 0.1 × 0.1, were carefully selected under a polarizing microscope and glued at the tip of a thin glass fiber. Data collection was carried out using Oxford Diffraction Xcalibur CCD diffractometer equipped with sealed tube X-ray source (Mo-K_α radiation, λ = 0.71073 Å). All calculations, i.e., data collection, cell refinement and data reduction were performed using CrysAlis CCD and CrysAlis RED.[29] Significant details of the data collection and crystal data for **7d** are presented in Table 1.

Table 1. Experimental crystallographic parameters for **7d**

Crystal data	
Empirical formula	$C_{10}\,H_{11}\,F\,N_2\,O_4\,S$
Formula weight	274.27
System	monoclinic
Space group	$P2_1/c$ (14)
a (Å)	7.815(5)
b (Å)	13.072(5)
c (Å)	12.094(5)
β (°)	106.077(5)
V ($Å^3$)	1187.2(10)
Z, Calculated density ($g.cm^{-3}$)	4, 1.535
Crystal habit, colour	Prism, colourless
Crystal size (mm)	0.2 × 0.1 × 0.1
Absorption coefficient (mm^{-1})	0.295
F(000)	568
Data collection	
Temperature (K)	293
Diffractometer	Xcalibur Oxford diffraction
Wavelength Mo(Kα) (Å)	0.71073
θ range (°)	3.12 - 30.02
Limiting indices	$-11 \le h \le 10$, $-15 \le k \le 18$, $-16 \le l \le 17$
Reflections collected/unique	10938 / 3462 [R(int) = 0.0206]
Structure refinement	
Refinement method	Full-matrix least-squares on $\|F^2\|$
Data / restraints / parameters	3462/1/166
Goodness-of-fit on F^2	1.079
Final R indices [I>2sigma(I)]	R1 = 0.0455, wR2 = 0.1096
R indices (all data)	R1 = 0.0598, wR2 = 0.1187
Largest difference peak and Hole (e $Å^{-3}$)	0.379 and -0.368

The structure was solved by direct methods and refined by full-matrix least-squares techniques using the SIR-97[30] and SHELXL-97[31] programs respectively. Sulfur, nitrogen, oxygen and carbon atoms were located on the basis of 2787 independent reflections and anisotropically refined. All H atoms attached to

C atoms were fixed geometrically and treated as riding with C---H = 0.97 Å (methylene) and 0.89 Å (phenyl) with U_{iso}(H) = 1.2U_{eq}(C) times U_{eq}(C). The coordinates of the H atom attached to N2 were refined using N-H restraint [0.86(1) Å] and with U_{iso}(H) = 1.2 times U_{eq}(N). Pertinent details of the data collection and crystal data for **7d** are presented in Table 1. Selected bond lengths and angles are given in Table 2.

Table 2. Selected bond lengths (A°) and angles (°) with their standard deviations relevant to **7d**

Bond lengths (A°)		**Bond angles (°)**	
S1 – O11	1.414(2)	O11 – S1 – O12	120.46(10)
S1 – O12	1.417(2)	N1 – S1 – N2	106.25(8)
S1 – N1	1.681(2)	O11 – S1 – N1	103.04(9)
S1 – N2	1.613(2)	O11 – S1 – N2	109.33(9)
N1 – C11	1.371(2)	O12 – S1 – N1	109.58(9)
N1–C14	1.485(2)	O12 – S1 – N2	107.35(9)
N2–C21	1.435(2)		
C24–F1	1.354(2)		

2.3 CRYSTAL STRUCTURE STUDY

The structure of **7d** is shown in Figure 2. From Table 1, it is clear that the molecular geometry of **7d** is in good agreement with related structure.[33,34] Accordingly, the asymmetry of S-N bond lengths is shown within the sulfamid moiety N1-S1-N2, with values of 1.681(2) A° for the first bond and 1.613(2) Å for the second.

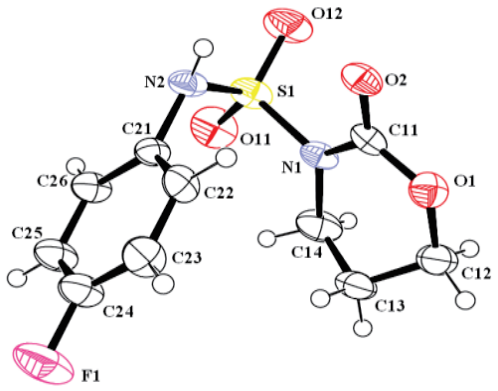


Figure 2. ORTEP[32] plot of the molecule **7d** in the crystal with atomic numbering scheme. Ellipsoids correspond to 50% probability levels and H atoms are shown as small spheres of arbitrary radii.

The interesting feature in the crystal packing is that the arrangement of the molecules gives rise to a pseudo dimer through N-H…O hydrogen bonds building a $R_2^2(12)$ ring according to the graph set theory[35,36] as shown in Figure 3. Several weak intermolecular interactions participate in the molecular interconnection.

These interactions are set up via C–H···O sulfonamide (O11 and O12) and carbonyl (O2) functions in addition to C–H···F intermolecular interaction via halogen function as shown in Table 3. All these interactions lead to an infinite three-dimensional network.

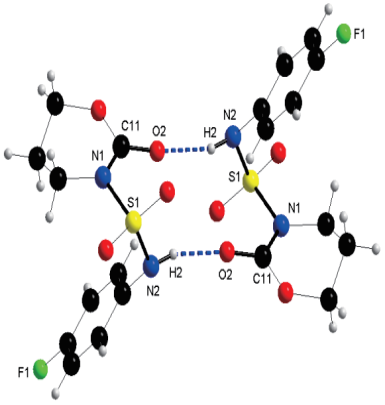


Figure 3. DIAMOND[37] plot of a 10-member hydrogen-bonded head-to-head cyclic dimer [$R_2^2(12)$] involving N-H…O intermolecular interaction. Hydrogen bonds are shown as blue dashed lines.

Table 3. Strongest hydrogen bonds and interactions for **7d**

D–H···A[a]	d(DH) (Å)	d(H···A) (Å)	d(D···A)	d DH···A (°)(Å)
N2 – H2···O2^{I}	0.854(18)	2.04(2)	2.863(3)	162(2)
C12 – H12A···F1^{II}	0.97	2.42	3.234(3)	141
C12 – H12B···O2^{III}	0.97	2.46	3.283(3)	143
C14 – H14B···O12^{IV}	0.97	2.44	3.234(3)	138
C23 – H23···O11^{V}	0.93	2.42	3.282(3)	154

[a]Symmetry code : (I) -x+2, -y, -z; (II) -x+2, -y, -z+1; (III) -x+1, -y, -z; (IV) x, -y-1/2, z-1/2; (V) -x+2, -y+1/2, -z+1/2

2.4. *IN-VITRO* ANTIMICROBIAL TESTS (FIRST SCREENING).

All compounds **7a-7f** were tested against one Gram-positive (*S. aureus*) and two Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) clinical strains, isolated from patients with various (cutaneous, urinary, and pulmonary) infections at Annaba University Hospital. Using the disc diffusion method as described in Section 4.4, all the synthesized compounds exhibited varying degree of inhibitory effect on the growth

of different tested strains (Table 4). A significant activity was observed for the compound **7d** against *S. aureus*. Compounds **7a** and **7b** showed moderate activity. Whereas, compounds **7a**, **7c**, **7d** showed moderate activity against *E. Coli*. For *P. aeruginosa*, Compounds **7c** and **7d** showed moderate activity, whereas compounds **7e**, **7f** showed weak activity against all Gram-negative and positive-gram bacterial strains.

Table 4. Antibacterial Bioassay (used 300 μ mol/L in DMSO[a]) of **7a-7f**

		Inhibitory zone[b] (mm) at 300 μ mol/L		
Compounds	R^1	*S. aureus*	*E. coli*	*P. aeruginosa*
7a	Ph	13,1	10,2	8,1
7b	*p*-Me Ph	10,2	6,4	6
7c	*p*-MeO Ph	8,1	12,1	10
7d	*p*-FPh	21,1	11,1	14,1
7e	*p*-ClPh	6	8,1	8,3
7f	*p*-NO_2Ph	7,4	7,9	6,5
SD[C]	-	26	20	24

[a]Antibacterial Activity was determined by disc diffusion method as described in **Section 4.4**
[b][zone of inhibition (mm) by disc diffusion method]:<10: weak; >10: moderate; >16: Significant.
[C]Standard drug (Sulfisoxazole)

Although, for structure comparison purposes, we have also resynthesized and tested **6a-6f** analogs[19] (n=1, shown in Scheme 1) for their antibacterial activity against the same test strains. These compounds contain an oxazolidin-2-one ring instead of a perhydro-1,3-oxazinan-2-one ring. The biological assay data in Table 5 showed that compounds **6e**, **6a** and **6b** have a significant activity against *P. aeruginosa* strain of bacteria. Whereas, compound **6d** showed moderate activity against all Gram-negative and Gram-positive bacterial strains. All other compounds showed weak activity against all other tested strains.

Table 5. Antibacterial Bioassay (used 300 μ mol/L in DMSO[a]) of **6a-6f**

		Inhibitory zone[b] (mm) at 300 μ mol/L		
Compounds	R^1	*S. aureus*	*E. coli*	*P. aeruginosa*
6a	Ph	8,7	9,1	19,2
6b	*p*-Me Ph	8,3	7,6	17,4
6c	*p*-MeO Ph	8,8	7,1	6
6d	*p*-FPh	10,3	11,2	11,1
6e	*p*-ClPh	8,2	8,5	26,2
6f	*p*-NO_2Ph	9,1	7,9	6
SD[C]	-	26	20	24

[a]Antibacterial Activity was determined by disc diffusion Method as described in section 4.4
[b][zone of inhibition (mm) by disc diffusion method]<10: weak; >10: moderate; >16: Significant.
[C]Standard drug (Sulfisoxazole)

Relying on these results, we propose that active compounds exhibit certain potent strain-specificity. From the literature, it is common for some bacteria strains to be resistant and others is susceptible to a particular antibiotic.[38,39] The comparison of Inhibition results of synthesized compounds **6a-6f** and **7a-7f** (Figure 4) showed that the compound **7d** was found to be the most active against Gram-positive (*S. aureus*), and the compound **6e** was the most active against Gram-negative (*P. aeruginosa*). But for *E. coli*, all compounds showed weak and/or moderate activity. The preliminary structure–activity relationship (SAR) of compounds containing perhydro 1,3-oxazin-2-one ring showed that the nature of substituent in *para* positions of the phenyl ring affected the activity significantly. A general trend is that non-polar groups are more favorable than polar groups on the benzyl ring. Possessing H, Methoxy (-OMe) and halogen groups (F and Cl) substituent, frequently appear in many antibacterial drug structures, exhibited potent antibacterial activity against all tested strains. Introducing the polar nitro group on *para* position reduces this activity.[40] Basing on the crystal structure study, this observation may be attributed to the electron-withdrawing (EW) character of the *para*-substituents.[25]

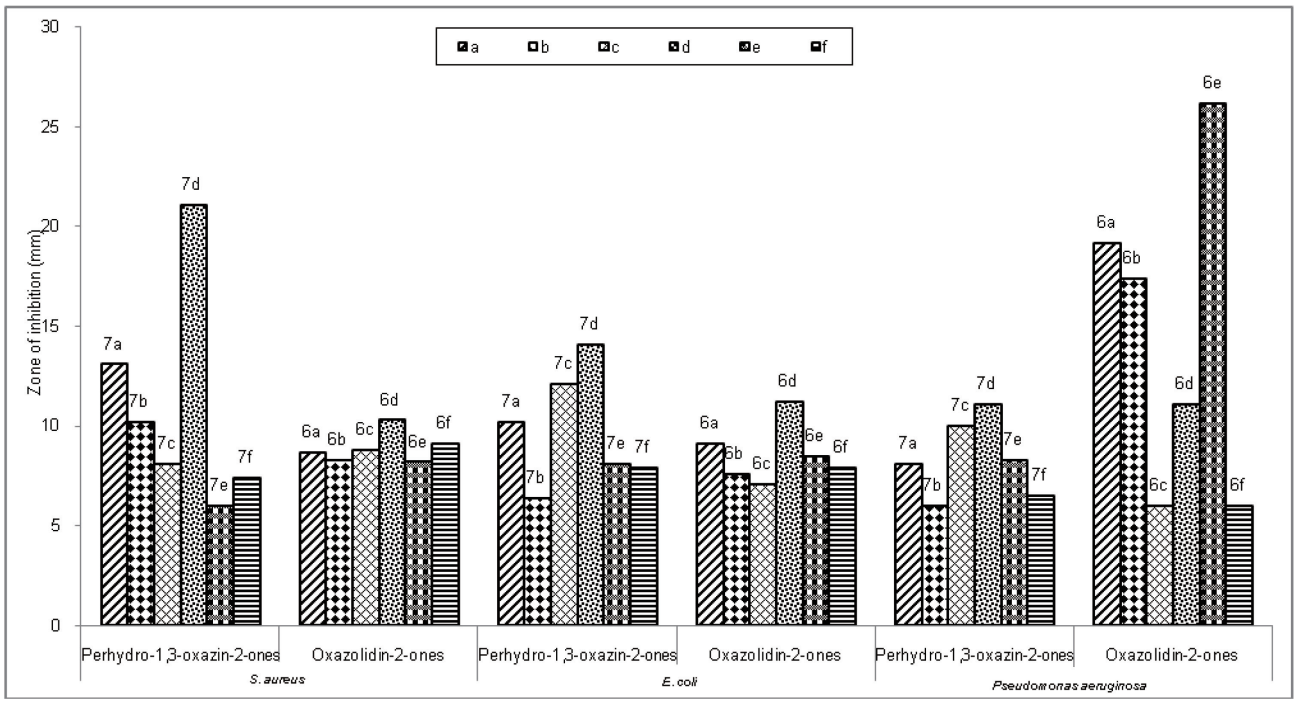


Figure 4. Antibacterial activity comparison of synthesized compounds **6a-6f** and **7a-7f**

Although, In Figure 4, we can observe that the 6-membered ring perhydro-1,3-oxazinan-2-one is slightly more potent than the 5-membered ring oxazolidin-2-ones against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*).[13] But for **6a**, **6b** and **6e** with H, *p*-Me, *p*-Cl respectively substituents on the phenyl ring, oxazolidin-2-ones compounds still showed a good activity against Gram-negative (*P. aeruginosa*).

2.5. MINIMUM INHIBITORY CONCENTRATION (MIC) FOR ANTIBACTERIAL ACTIVITY

The data obtained after preliminary antibacterial screening showed that compounds **7d**, **6a**, **6b** and **6e** were the most active, their inhibitory zone values were 21.1, 19.2, 17.4 and 26.2 (>16 mm) respectively. These compounds were therefore, selected for antibacterial minimum inhibitory concentration (MIC) studies (Table 6).

Table 6. MIC **(μg/mL**) of the selected compounds (**7d**), (**6a**), (**6b**) and (**6e**) against selected bacteria

Compounds	Gram-positive	Gram-negative	
	S. aureus	*E. coli*	*P. aeruginosa*
7d	2	8	16
6a	(-)	(-)	≥ 250
6b	(-)	(-)	16
6e	(-)	(-)	8
SD*	8	>125	62,5

***** Standard drug (Sulfisoxazole)
(-) No tested

The biological assay data in Table 6 showed that the MIC of these most active compounds was in the range of 7,292 x10^{-6} M to 1,031 x10^{-3} M. The Compound **7d** proved to be the most active one against Gram-positive and Gram-negative isolated clinical strains; It inhibited the growth of *S. aureus* at 2 μg/mL (7,292 x10^{-6} M) and *E. coli* at 8 μg/mL (2,916 x10^{-5} M) while compound **6e** proved to be the most active one against Gram-negative strain; It inhibited the growth of *P. aeruginosa* at 8 μg/mL (2,891 x10^{-5} M). These are good activities, considering the simplicity of the structure and the differences with Sulfisoxazole or other existing antibacterial agents.

It is well known that sulfa drugs act as dihydropteroate synthase (DHPS) inhibitiors in the synthetic pathway of folate, thereby causing DNA and RNA synthesis inhibition.[41,42] But all of these compounds do not possess an amino group on the benzene moiety. Though, an amino group is essential for Dihydropteroate synthase (DHPS) inhibition. With these Preliminary results we suggest that the mechanism of the antibacterial action of **7d** and **6e** is different from that of sulfa drugs.

3. CONCLUSIONS

The results of the present investigation support the suggested structures of *N*-substituted perhydro-1, 3-oxazin-2-ones containing *N*-phenylsulfonamide and their analogs oxazolidin-2-ones. It has been

suggested that some functional groups such as methoxy (-OMe) or halo-atoms (F, Cl) substituents on the benzene ring present in these compounds, play a chief role in biological activity.[13,16-18] The potent antibacterial properties of synthesized compounds encouraging us for purposes of new drug design suggesting that very likely most of these compounds could be used as potential antibacterial activity after minor modifications. From these results, several pertinent observations are revealed: (i) inhibition effect of tested compounds depends on substituent nature in *para* position on the benzene ring, (ii) H, fluoro, chloro, and methoxy are good substituents, (iii) The 6-membered ring 1,3-oxazin-2-one seems to have a slightly better activity than the oxazolidin-2-ones. Moreover, experimental data show that the compound **7d** and **6e** may also be used to further optimization of structures to obtain potent novel antibacterial drugs against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *P. aeruginosa*) strains.

4. EXPERIMENTAL

4.1. General

Starting materials were obtained from commercial suppliers and were used without further purification. Before use, aniline was purified by distillation over NaOH under reduced pressure. The substituted anilines were used without further purification. Melting points were determined with a Electrothermal melting point apparatus (IA9000). IR spectra were recorded on Perkin Elmer FT-IR 1725 (KBr). ^{1}H NMR spectra were recorded on a Brüker Avance (300 MHz) spectrometer using DMSO-d_6 or $CDCl_3$ as solvent. Proton chemical shifts were referenced to the TMS internal standard. Electrospray Ionization mass spectra (ESI-MS) (30eV and 20eV) were recorded in positive mode on a Water MicroMass ZQ. Thin layer chromatography (TLC) analysis was performed on silica gel/TLC-cards (Fluka). Spots were visualized under 254 nm UV illuminations by ninhydrin solution spraying. Chromatography columns were carried out using Silica Gel Merck 60. X-Ray crystal diffraction data was cited in Section 2.2.

4.2 Typical Operating Procedure for Synthesis of *N*-[(*N*-Aryl)sulfamoyl]perhydro-1,3-oxazin-2-ones 7a-7f : To a solution of chlorosulfonyl isocyanate (CSI) (4.5g, 31.8 mmol) in CH_2Cl_2 (40 mL) at room temperature and under a nitrogen atmosphere. The reaction mixture was cooled to about 1 °C, and a solution of halogeno-alcohol (4.00 g, 32 mmol, 1,1 equiv) in dried CH_2Cl_2 (10 mL) was slowly added over 5 min. at 0 °C. Stirring of the reaction mixture was continued at the same temperature for a minimum of 20 min. This reagent of carbamoylation reaction and 2.2 equiv of triethylamine (TEA) was slowly added (dropwise) at 0 °C into a solution containing the primary amine (aniline or *para*-substituted anilines) (0.05 mol 1.1 equiv in 100 mL of CH_2Cl_2). Progress of this reaction was monitored by TLC. The reaction was achieved in 45 min. The medium was diluted with CH_2Cl_2 (100 mL), washed with two fractions of HCI 0.1 N. Then the decanted and separated organic layer was washed with water, dried and concentrated. The crude residue of *N*-[(*N*-aryl)sulfamoyl]perhydro-1,3-oxazin-2-ones was then purified

by column chromatography (yields 64-69%).

4.2.1. ***N*-[(*N*-Phenyl)sulfamoyl]perhydro-1,3-oxazin-2-one (7a).** According to the typical operating procedure (**4.2**) using the 1,3 chloropropanol as halogeno-alcohol, **7a** was obtained in 65.02% yield as white solid after column chromatography (eluent: CH_2Cl_2/MeOH = 95:5) and recrystallization from CH_2Cl_2/*n*-hexane. Mp 163-165 °C; R_f: 0.65 (CH_2Cl_2/MeOH = 95:5); IR (KBr, cm^{-1}): 3219 (NH), 1697 (C=O), 1271, 1190 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$) 1.89 (q, 2**H**), 3.59 (t, *J* = 6.26 Hz, 2**H**), 4.22 (t, *J* = 5.17 Hz, 2**H**), 7.26-7.31 (m, **3H**), 7.35-7.38 (m, **2H**), 7.6 (br s, 1**H**), [M+Na^+] = 279.05.

4.2.2. *N*-[(*N*-*P*-Toloyl)sulfamoyl]perhydro-1,3-oxazin-2-one 7b. According to the typical operating procedure **(4.2)** using the 1,3-chloropropanol as halogeno-alcohol, **7b** was obtained in 64.69% yield as colorless solid after column chromatography (eluent: CH_2Cl_2/MeOH = 95:5) and recrystallization from CH_2Cl_2/*n*-Hexane. Mp 177-179 °C; R_f: 0.63 (CH_2Cl_2/MeOH = 95:5); IR (KBr, cm^{-1}): 3212 (NH), 1702 (C=O), 1273, 1163 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$) 1.90 (q, 2**H**), 2.36 (s, 3**H**), 3.57 (t, *J* = 6.18 Hz, 2**H**), 4.23 (t, *J* = 5.36 Hz, 2**H**), 7.17-7.28 (m, **4H**), 7.47 (br s, 1**H**), [M+Na^+] = 293.06.

4.2.3. *N*-[(*N*-4-Methoxyphenyl)sulfamoyl]perhydro-1,3-oxazin-2-one 7c. According to the typical operating procedure **(4.2)** using the 1,3-chloropropanol as halogeno-alcohol, **7c** was obtained in 67.99 % yield as gray solid after column chromatography (eluent: CH_2Cl_2/MeOH = 95:5) and recrystallization from CH_2Cl_2/*n*-hexane. Mp 183-185 °C; R_f: 0.55 (CH_2Cl_2/MeOH = 95:5); IR (KBr, cm^{-1}): 3201 (NH), 1669 (C=O) 1269, 1155 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$) 1.91 (q, 2**H**), 3.54 (t, *J* = 6.21 Hz, 2**H**), 3.82 (s, 3**H**), 4.24 (t, *J* = 5.32 Hz, 2**H**), 6.88-6.91 (dm, **2H**), 7.21-7.28 (dm, **2H**), 7.41 (br s, 1**H**), [M+Na^+] = 309.06.

4.2.4. ***N*-[(*N*-4-Fluorophenyl)sulfamoyl]perhydro-1,3-oxazin-2-one 7d.** According to the typical Operating Procedure **(4.2)** using the 1,3-chloropropanol as halogeno-alcohol, **7d** was obtained in 66.63% yield as colorless solid after column chromatography (eluent: CH_2Cl_2/MeOH = 95:5) and recrystallization from CH_2Cl_2/*n*-hexane. Mp 190-192 °C; R_f: 0.62 (CH_2Cl_2/MeOH = 95:5); IR (KBr, cm^{-1}): 3292 (NH), 1703 (C=O) 1273, 1163 (SO2); ^{1}H NMR (300 MHz, $CDCl_3$) 1.93 (q, 2**H**), 3.58 (t, *J* = 6.20 Hz, 2**H**), 4.25 (t, *J* = 5.36 Hz, 2**H**), 7.03-7.11 (m, **2H**), 7.26-7.29 (m, **2H**), 7.65 (br s, 1**H**), [M+Na^+] = 296.67.

4.2.5. ***N*-[(*N*-4-Chlorophenyl)sulfamoyl]perhydro-1,3-oxazin-2-one 7e.** According to the typical operating procedure **(4.2)** using the 1,3-chloropropanol as halogeno-alcohol, **7e** was obtained in 65.13% yield as white solid after column chromatography (eluent: CH_2Cl_2/MeOH = 95:5) and recrystallization from CH_2Cl_2/*n*-hexane. Mp 173-175 °C; R_f: 0.60 (CH_2Cl_2/MeOH = 95:5); IR (KBr, cm^{-1}): 3291 (NH), 1702 (C=O), 1272, 1161 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$) 1.92 (q, 2**H**), 3.62 (t, *J* = 6.20 Hz, 2**H**), 4.26 (t, *J* = 5.36 Hz, 2**H**), 7.23-7.27 (m, **2H**), 7.40-7.43 (m, **2H**), 7.82 (br s, 1**H**), [M+Na^+] = 313.52.

4.2.6. ***N*-[(*N*-4-Nitrophenyl)sulfamoyl]perhydro-1,3-oxazin-2-one 7f.** According to the typical operating procedure **(4.2)** using the 1,3-chloropropanol as halogeno-alcohol, **7f** was obtained in 69.22%

yield as light yellow solid after column chromatography (eluent: CH_3Cl/MeOH = 95:5) and recrystallization from CH_3Cl/*n*-hexane. Mp 186-188 °C; R_f: 0.48 (CH_2Cl_2/MeOH = 95:5); IR (KBr, cm^{-1}): 3165 (NH), 1693 (C=O), 1387, 1227 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) 1.99 (q, **2H**), 3.78 (t, J = 6.41 Hz, **2H**), 4,30 (t, J = 5.35 Hz, **2H**), 7.83-7.88 (m, **2H**), 8.17-8.20 (m, **2H**); 7.49 (br s, **1H**), [M+Na^+] = 323.56.

4.3. Preparation of bacterial culture

Test clinical strains were *S. aureus*, *E. coli* and *P. aeruginosa.* three to five similar colony of each strain was transferred into 3 ml of nutrient broth in a 22 ml test tube, then a suspensions in physiologic water was prepared. Test strains were spread on solid nutrient agar surface by using sterile ekùvyon rod. The growth obtained was diluted 1:10 so as to give an approximate concentration of 10^6-10^8 CFU/mL (0.5 McFarland standard) in conformity with the recommendations of the Clinical and Laboratory Standards institute (CLSI, 2008). *S. aureus* ATCC 25923, *E. coli* 25922 and *P.aeruginosa* 27853 references strains were used as control strains in order to monitor the antibacterial test.

4.4. Disc diffusion method (first screening)

The compounds **7a-7f** were dissolved in dimethyl sulfoxyde (DMSO) (Merck) and then their antibacterial effect were tested using selected concentration. Petri dishes (measuring 90 mm each side) containing 20ml Muller-Hinton agar (DIFCO Laboratories, IPP, France) were prepared as described above and then dried at 35 °C for about 30 min in an incubator. At the same time, absorbent paper discs (6 mm) were placed on the agar surface and then appropriate concentration of the compounds in DMSO were applied onto the disks, and 300 μmol/L final concentrations were obtained for each disc. Sulfisoxazole ST (300 μmol/L) antibiotic disks were also used for all test microorganisms as positive control for evaluating the data. The plates were incubated at 35 °C for 24 h. After that, the diameter of inhibition zone was measured in millimeters by compass (mm). The size of inhibition zone was categorized into three classes: <10: weak activity; >10: moderate activity; >16: Significant activity. In order to clarify any participating role of DMSO in the bacterial screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against all bacterial strains. All tests were repeated three times to minimize test error.

4.5. Minimum inhibitory concentration (MIC)

Compounds containing significant antibacterial activity (>16 mm) were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the broth microdilution method.[43] The MIC determination of the tested compounds was investigated in comparison with Sulfisoxazole. Double dilutions of the test compounds and reference drugs were prepared in Muller-Hinton agar. 10 mg of each test compound were dissolved in 1 mL of dimethyl sulfoxide (DMSO)

separately to prepare stock solution. Further, progressive dilutions with melted Mueller–Hinton agar were performed to obtain the required concentrations of 500, 250, 125, 62.5, 31.25,16, 8, 4, 2,1 μg/mL. The Petri dishes were inoculated with 1–5 x 10^4 colonies forming units (cfu/mL) and incubated at 37 °C for 18 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the tested compounds that yield no visible growth on the plate was recorded in Table 6. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments.

SUPPLEMENTARY MATERIAL

Crystallographic data for the structural analysis of **7d** have been deposited with the Cambridge Crystallographic Data Centre (CCDC-N° 790885). Copies may be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; by quoting the publication citation and the deposit numbers. [Fax: (+44) 1223-336-033; E-mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk]

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