Annals of the University of Craiova
The Chemistry Series
Volume XLIX, No. 2 (2023) 69-80
homepage: chimie.ucv.ro/annals/
10.52846/AUCCHEM.2023.2.16

Synthesizing, preliminary analyzing and investigating the potential veterinary use of two structurally related Zn(II) complexes with ligands of the benzenesulfonamides' class

Research article

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Received: 10.11.2023 / Accepted: 02.12.2023 / Published: 20.12.2023

Abstract
As a consequence of the fact that most bacteria types show increasing resistance to the conventional drugs, chemists and biochemists keep on trying to synthesize new potential medicines and to test their antibacterial activity. Under these circumstances, the present work's goal is to present the synthesis, the preliminary analysis and antibacterial tests' results for two structurally-related ligands belonging to the benzenesulfonamides' class, as well as for the two complex compounds formed by them through coordination to divalent zinc. Because of the usually high hepatotoxicity exhibited by benzenesulfonamides, they are unlikely suitable for human medical purposes, but they might present possibilities to be used as veterinary drugs. Consequently, we have focused on the possibility for our newly synthesized substances to be used in the treatment of urinary- and gastrointestinal tract bacterial infections, as well as galactophore channels' infections, on equine and cattle; actually, for that matter, all pathogenic agents were sampled from horses or cows suffering from different infectious diseases (from urinary- or gastrointestinal tract or from females' galactophore channels).

Keywords: complex compounds, benzenesulfonamides, divalent zinc, antibacterial tests, veterinary drugs

1. INTRODUCTION

It is well known [1, 2] that sulphonamides do not actually destroy bacteria, but they can efficiently interfere with the ability of these pathogenic agents to develop and to multiply (fact that is called „bacteriostatic effect"). Actually, it is Vitamin B9 the main component which is necessary for the bacteria to grow and multiply, sulfa drugs being effective in blocking the possibility of bacteria using Vitamin B9, therefore significantly inhibiting their growth and multiplication [1, 2].

Among other sulfa drugs, sulfonamides (such as furosemide, torsemide and so on) are frequently administered as drugs, especially in veterinary medicine [3-7], as they are generally effective against many bacterial strains, either Gram-positive or Gram-negative, but they exhibit a hepatotoxicity that is considered too high for human use.

Within the present work, we have focused on studying the action against several pathogenic agents performed by two benzenesulfonamide derivatives with similar structures – differing only by the nature of one heteroatom, *i.e.*, oxygen *vs*. sulfur – and also the action performed by their complex compounds formed with Zn(II) against the same pathogenic agents, knowing that sulfonamide derivatives – able to act as ligands – may show even better activity against pathogenic germs upon chelation with different transitional metals (zinc being known as an effective one).

More specifically, the study refers to sixteen bacterial strains, half of them being sampled from equine and the other half from cattle (also noting, in each case, which body region the bacterium was sampled from), which were subjected to the action of the four chemicals mentioned above, in order to analyze their sensitivity when exposed to these substances (all chemical compounds being synthesized in our laboratory).

These chemical compounds are under discussion to be used in the treatment of urinary- and gastrointestinal tract bacterial infections, as well as galactophore channels' infections on horses and cows [8-12]; they might be even more effective on ruminants smaller than cattle – namely sheep and goats, but they likely cannot be considered suitable for these last ones, because of their considerable hepatotoxicity [1, 2, 8].

2. MATERIALS AND METHODS

2.1. Materials used for the chemical syntheses and preliminary analyses

In order to synthesize the ligands and the complex compounds proposed for the antibacterial study, we have used the following chemicals:

- 4-aminobenzenesulfonamide
- 1-(furan-2-yl)ethan-1-one
- 1-(thiophen-2-yl)ethan-1-one
- zinc chloride
- sodium hydroxide
- tetrabutylammonium hydroxide
- ethyl alcohol
- acetic acid
- diethyl ether

All of them were Sigma-Aldrich pure reagents.

We have used G4 fine porosity glass filters for obtaining all the four precipitates.

Finally, they were all dried into a vacuum oven.

The elemental analysis has been performed on a Perkin Elmer 2380 (USA) instrument.

To obtain the solutions destinated for the antibacterial tests, DMF (also Sigma-Aldrich pure) was used as a solvent.

2.2. Methods used for the chemical syntheses and preliminary analyses

The two benzenesulfonamide derivatives capable to act as ligands that we have synthesized for the purpose of this paper were obtained by direct condensation of 4-aminobenzenesulfonamide with two structurally similar heterocyclic ketones, the first being 1-(furan-2-yl)ethan-1-one and the second being 1-(thiophen-2-yl)ethan-1-one. The two products were obtained by ethyl alcohol refluxing during three hours and then they were concentrated to small volumes and diluted with the same volumes of distilled water. Afterwards, their solutions have been alkalized by adding sodium hydroxide and refluxed for another hour, then warmed in a water bath and acidified with acetic acid.

The precipitates were subsequently purified by dissolving them into a more concentrated sodium hydroxide solution and finally reprecipitating

them from a more concentrated acetic acid solution, with the purpose of using them both as ligands (yields: 73.02% and 77.05%, respectively). Both of them have been left to settle down for an hour, then filtered each on a fine porosity glass filter, washed with ethyl alcohol, as well as with diethyl ether and finally vacuum dried.

Then, the chemical compounds – *i.e.*, the ligands – thus synthesized were added to a tetrabutylammonium hydroxide solution, continuously stirring. Each of the two weakly alkaline liquids formed has been added to a solution that had previously been obtained by dissolving anhydrous powder of zinc chloride into the minimum necessary volume of ethyl alcohol. The stable products that have precipitated (yields: 70.02% and 76.03%, respectively) have been left to settle down for an hour, then filtered on a fine porosity glass filter, washed with ethyl alcohol, as well as with diethyl ether and finally vacuum dried.

2.3. Materials used for the antibacterial tests

In order to perform the antibacterial tests by Kirby-Bauer diffusion method – which will be further presented, we have used the following materials:

- substances to be tested (the two ligands and the two complex compounds that make the object of this paper)
- 7 cm Petri plates with agar medium
- bacterial material, sampled from equine or cattle (from urinary- or gastrointestinal tract or from females' galactophore channels).
- pipettes (for inoculating bacterial material on the agar medium)
- 6-mm blanc discs, high quality absorbent paper (for impregnation)
- sterile forceps (for placing the discs inside the Petri plates)
- ruler (for measuring the distance between discs and for lately estimating the results by performing the reading of the diameters of „growth inhibition zones", which will be defined in what follows)
- laboratory autoclave
- stainless steel laboratory tray (for holding the Petri plates into the autoclave)

Details about the bacterial material (referring to pathogenic agent's classification and provenience of samples) are found below, within Table 1.

Table 1. Studied pathogenic agent's classification and provenience of samples

No.	Pathogenic agent	Pathogenic agent's classification		Sampled from	
		Gram stain	Type/shape	Anatomical region	Animal species
1	*Salmonella enterica*	Gram-negative	bacillus/rod	GIT*	equine
2	*Actinobacillus equuli*	Gram-negative	bacillus/rod	GIT*	
3	*Clostridium difficile*	Gram-positive	bacillus/rod	GIT*	
4	*Enterococcus faecalis*	Gram-positive	coccus/sphere	GIT*	
5	*Lawsonia intracellularis*	Gram-negative	bacillus/rod	GIT*	
6	*Escherichia coli*	Gram-negative	bacillus/rod	UT**	
7	*Enterobacter cloacae*	Gram-negative	bacillus/rod	UT**	
8	*Rhodococcus equi*	Gram-positive	bacillus/rod	GCh***	
9	*Campylobacter jejuni*	Gram-negative	bacillus/rod	GIT*	cattle
10	*Yersinia enterocolitica*	Gram-negative	bacillus/rod	GIT*	
11	*Klebsiella pneumoniae*	Gram-negative	bacillus/rod	UT**	
12	*Corynebacterium renale*	Gram-positive	bacillus/rod	UT**	
13	*Pseudomonas aeruginosa*	Gram-negative	bacillus/rod	UT**	
14	*Trueperella pyogenes*	Gram-positive	coccobacillus/ intermediate	UT**	
15	*Staphylococcus aureus*	Gram-positive	coccus/sphere	UT**	
16	*Streptococcus agalactiae*	Gram-positive	coccus/sphere	GCh***	

* GIT = gastrointestinal tract

** UT = urinary tract

*** GCh = galactophore channels

2.4. Methods used for the antibacterial tests

Kirby-Bauer diffusion method has numerous variants, from which we have chosen for the present study the standardized technique of impregnated discs disposed on Petri plates [13].

The main principle of it is the following: seeding the surface of the agar medium inside each Petri plate – usually by means of a pipette – with a standardized inoculum which was obtained from the bacteria submitted to the test and then placing in each Petri plate blanc discs impregnated with the solutions of the studied substances (quite symmetrically, by keeping at least a distance of 3 cm between each two discs' centers and at least a distance of 1.5 cm between any disc and the edge of the plate, as the maximum diameter of growth inhibition zone seems to be around 3 cm).

Sixteen 7 cm Petri plates were used for each determination, on each of which four 6 mm discs were placed, being impregnated with the four substances subjected to the investigation, *i.e.*, the two ligands and the two complex compounds formed by them with divalent zinc., the discs have to be placed inside the Petri plate approximately in the vertices of a square whose side does not exceed 3 cm, so as its diagonal not to exceed 4.2 cm, for a proper reading in estimation of the results (otherwise, the distance between a disc and the edge of the Petri plate might be less than 1.5 cm).

Then, the Petri plates were left to rest at room temperature for half an hour, so as to get a uniform diffusion of substances in the medium.

Afterwards, they were placed on a stainless steel laboratory tray and introduced into the autoclave, to be incubated for a day at 38 °C (as we wanted to propose these substances as potential veterinary drugs for horses and cows, and this is the normal body temperature for both equine and cattle).

Finally, the reading of the „growth inhibition zone" diameters should be done, by using a ruler. The results were labeled and will be further presented.

Figure 1 presents, for exemplification, the Petri plate used to test the activity of the four studied substances (denoted A, B, C and D – as explained within its caption) against *Staphylococcus aureus*, seeded on agar medium, which was chosen because it was the one with the best results.

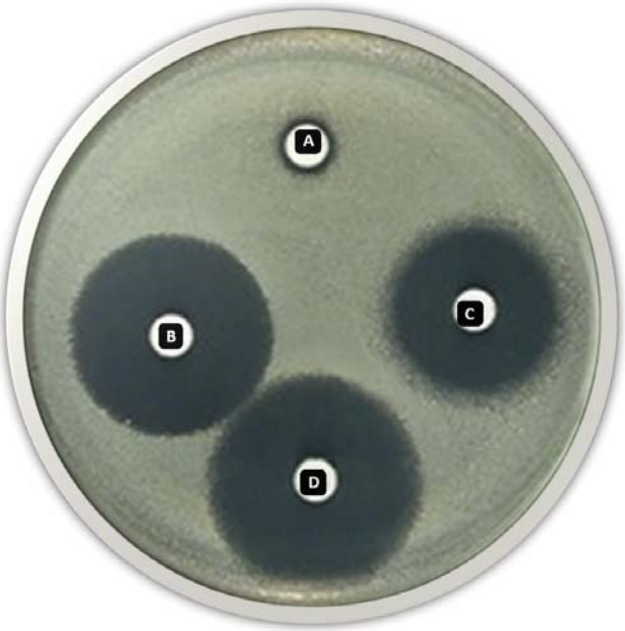


Figure 1. Petri plate with *Staphylococcus aureus* inoculated on agar medium, the discs being impregnated with the two ligands and the two complex compounds that are tested against it (A = L; B = L′; C = $[ZnL_2(H_2O)_2]Cl_2$; D = $[ZnL'_2(H_2O)_2]Cl_2$)

3. RESULTS AND DISCUSSION

3.1. Physical properties and results of the preliminary analyses

For now, we have resumed ourselves to report only the physical properties and results of the preliminary analyses for the studied compounds. Spectral characterization (able to lead to all the statements regarding the structures) is still ongoing and will be further reported.

Whereas the ligands were white to cream microcrystalline powders, their Zn(II) complexes were yellowish-white microcrystalline powders.

The complex compounds obtained, having the melting points at 238°C and 242°C respectively, were proved as being easily soluble in DMF.

The two benzenesulfonamide derivatives capable to act as ligands that we have synthesized within this work will be denoted as L and L′. Thus, L = (*E*)-4-amino-N-(1-(furan-2-yl)ethylidene)benzenesulfonamide,
L′ = (*E*)-4-amino-N-(1-(thiophen-2-yl)ethylidene)benzenesulfonamide.

The results of the elemental analysis of the complex compound formed by the two ligands with divalent zinc are presented in Table 2.

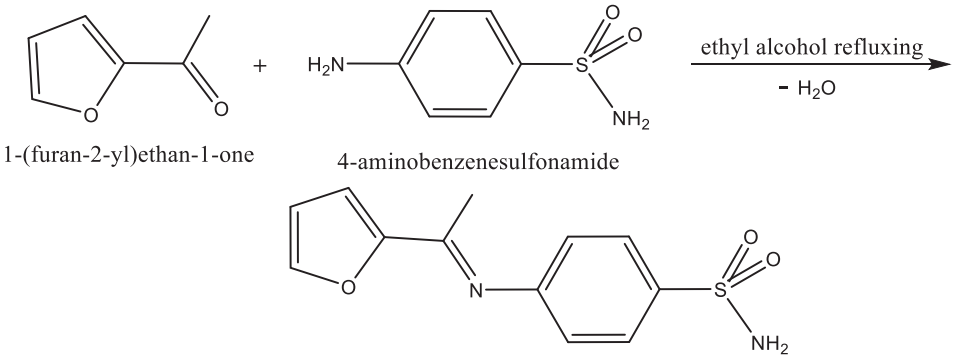
Table 2. Main results of the elemental analysis of the complex compound

Complex compound	Yield, %	Molar mass (g/mol)	Molecular formula	Calculated/found, %			
				C	H	N	Zn
$[ZnL_2(H_2O)_2]Cl_2$	70.02	700.93	$C_{24}H_{28}N_4O_8S_2Cl_2Zn$	41.18/ 41.30	4.03/ 4.05	7.99/ 8.02	9.33/ 9.23
$[ZnL'_2(H_2O)_2]Cl_2$	76.03	733.06	$C_{24}H_{28}N_4O_6S_4Cl_2Zn$	39.32/ 39.48	3.85/ 3.90	7.64/ 7.68	8.92/ 8.89

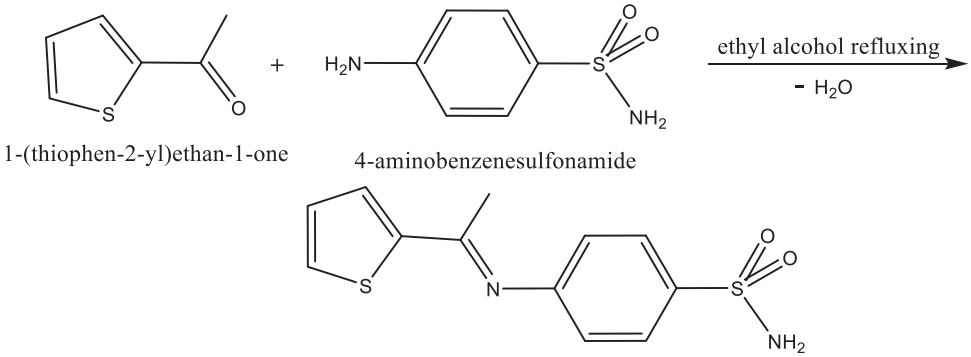
3.2. Reaction schemes

As earlier mentioned, the spectral characterization being not yet finished, statements regarding the structures of the complex compounds are in progress and cannot be yet reported, so we only present the two similar reaction schemes corresponding to the synteses of the structurally related ligands.

The direct condensation reactions that have taken place between 4-aminobenzenesulfonamide and the two structurally related heterocyclic ketones, *i.e.*, 1-(furan-2-yl)ethan-1-one and 1-(thiophen-2-yl)ethan-1-one, leaded to (*E*)-4-amino-N-(1-(furan-2-yl)ethylidene)benzenesulfonamide and to (*E*)-4-amino-N-(1-(thiophen-2-yl)ethylidene)benzenesulfonamide, respectively.



Scheme 1. Direct condensation reaction leading to the ligand L



Scheme 2. Direct condensation reaction leading to the ligand L′

3.3. Results of the antibacterial tests

Inhibition zone diameters obtained by treating bacteria with the two benzenesulfonamide derivatives and their complexes are shown in Table 3.

Table 3. Main results of the antibacterial tests for the four studied substances

No.	Chemical compound / Pathogenic agent	Growth inhibition zone diameter (mm)			
		Ligands		Complex compounds	
		L	L′	$[ZnL_2(H_2O)_2]Cl_2$	$[ZnL'_2(H_2O)_2]Cl_2$
1	*Salmonella enterica*	10	16	14	18
2	*Actinobacillus equuli*	8	9	10	12
3	*Clostridium difficile*	9	12	11	13
4	*Enterococcus faecalis*	8	10	9	11
5	*Lawsonia intracellularis*	8	9	9	12
6	*Escherichia coli*	11	16	22	24
7	*Enterobacter cloacae*	10	22	22	25
8	*Rhodococcus equi*	9	11	12	13
9	*Campylobacter jejuni*	9	11	10	12
10	*Yersinia enterocolitica*	12	19	18	20
11	*Klebsiella pneumoniae*	11	23	23	26
12	*Corynebacterium renale*	13	18	19	22
13	*Pseudomonas aeruginosa*	7	9	8	10
14	*Trueperella pyogenes*	11	14	19	23
15	*Staphylococcus aureus*	10	28	22	29
16	*Streptococcus agalactiae*	11	16	18	23

3.4. Discussion on the antibacterial tests

If the inoculed pathogenic agent is „sensitive" or even „moderately sensitive" to a certain substance, bacterial growth will be inhibited on a big round surface centred on the respective disc, this surface being usually called „growth inhibition zone"; otherwise, *i.e.*, if the inoculed pathogenic agent is resistant to that substance, bacterial growth will not be inhibited, so there will be no „growth inhibition zone".

In other words, a strain is called „sensitive" to the action of a substance if it exhibits a big „growth inhibition zone" diameter (*i.e.,* the substance, if regularly administrated as a drug, has a high chance of eradicating the infection); it is called „moderately sensitive" if it exhibits a medium „growth inhibition zone" diameter (*i.e.,* the drug might be effective, but only in high concentrations and locally applied) and it is called „resistant" if it exhibits a negligible „growth inhibition zone" diameter (*i.e.*, it is more likely for the drug not to affect bacterial growth).

Nevertheless, we must draw the attention to the fact that the limits to classify strains from the sensitivity point of view depend on their types and, moreover, on the substance concentration, these being labeled in literature [16].

Within our experiments, *Staphylococcus aureus* has proved itself to be the pathogenic agent with best inhibition results (as earlier mentioned, this is the reason for us to present the Petri plate seeded with this inoculum as an example).

Very good results were also obtained for *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Escherichia coli*.

Satisfactory behaviors were recorded for *Streptococcus agalactiae*, *Trueperella pyogenes*, *Corynebacterium renale* and *Yersinia enterocolitica*.

Salmonella enterica gave a worse result that we had expected [8].

Actinobacillus equuli, *Clostridium difficile*, *Enterococcus faecalis*, *Lawsonia intracellularis*, *Rhodococcus equi* and *Campylobacter jejuni* also gave unsatisfactory results.

As far as *Pseudomonas aeruginosa* is concerned, this was the worst among all the results of the current study.

These observations become more evident by regarding the plots in Figure 2, within which all results are comparatively viewed.

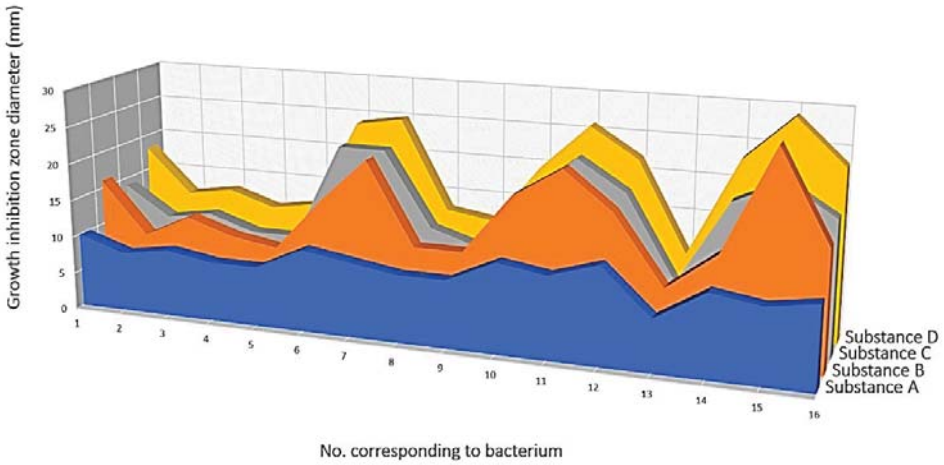


Figure 2. Comparative plots showing the results of antibacterial tests performed on the two investigated ligands and the two complex compounds (A = L; B = L′; C = $[ZnL_2(H_2O)_2]Cl_2$; D = $[ZnL'_2(H_2O)_2]Cl_2$), when tested against the chosen bacteria (the number corresponding to each bacterium being according to tables above)

4. CONCLUSION

The potential veterinary use of both complex compounds which constituted the object of this study being put into discussion, it has been proved that they seemed suitable for this purpose, as it was ascertained that half of the pathogenic agents are sensitive to their action.

As an additional statement, we might note that the set of the bacteria that provided good or even very good results was surprisingly regardless of Gram stain or bacterial shape.

The action of each complex compound was proved to be stronger than the action of the corresponding free ligand.

It is important to stress out the fact that, within all tests, the results obtained for the complex compound formed – with divalent zinc – by L = (*E*)-4-amino-N-(1-(furan-2-yl)ethylidene)benzenesulfonamide) were better than the ones gained for the complex compound – similarly formed – by L′ = (*E*)-4-amino-N-(1-(thiophen-2-yl)ethylidene)benzenesulfonamide, just as the results obtained for the first ligand were always better than the ones gained for the second ligand, clearly suggesting that the presence of a supplementary sulfur atom instead of an oxygen one enhances the action exercised against bacteria by the potential veterinary drug.

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