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Synthesis, crystal structure and potential antimicrobial activities of di (4-sulfamoyl-phenyl-ammonium) sulphate

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ABSTRACT

A new organic–inorganic hybrid $SO_4[C_6H_9N_2O_2S]_2$, has been synthesized and characterized by X-ray diffraction. This compound crystallizes in the orthorhombic system, spaces group P*bcn*. In the title compound, the packing is stabilized by intermolecular N—H···O hydrogen bonds and π–π stacking interactions between the phenyl rings, linking the molecules into three-dimensional network. The *in vitro* antimicrobial activity of di (4-sulfamoyl-phenyl-ammonium) sulphate was determined by the broth dilution method against several strains selected to define their spectrum and potency. Here we show that the synthetic sulfanilamide exhibits promising antibacterial potency. High inhibition was also detected against *Candida albicans*. In this paper we firstly showed the antifungal activity of the sulfanilamide against two serious phytopathogenic fungi. Interestingly, the new compound was able to suppress mycelial growth as well as the spores germination of tested fungi, values of spore germination vary from 97.6% to 37.5%, respectively for *Botrytis cinerea* and *Fusarium* species. The minimal inhibitory concentrations (MIC) ranging from 8 to 100 $\mu g\,ml^{-1}$ and IC50 values varying from 5.81 to less than 100 $\mu g\,ml^{-1}$), showed that the sulfanilamide sulphate had high activity against bacteria, yeast and fungi, compared to others published antifungal compounds.

1. Introduction

The alarming rates of arising microbial and fungal infections coupled with their increasing resistance have been motivating scientific communities worldwide to find novel antimicrobial agents that must be more effective, or to optimize the existing agents by improving both their binding affinity and their spectrum of activity. To achieve this goal, a new class of antimicrobial agents named Sulfa drugs has seen light. They are synthetic antimicrobial agents that derived from Sulfanilamide which are synthetic bacteriostatic antibiotics with a wide spectrum against gram-positive and gram-negative bacteria. They were widely used as effective chemotherapeutic agents to prevent and cure bacterial infections in humans (Ajibade et al. 2006). They also were employed as anhydrase inhibitors (Song et al. 2007), antifungal (Isik et al., 2009), antiviral (Gawin et al. 2008), antitumor (Bouissane et al. 2006), and anti-inflammatory agents (Weber et al. 2004). Besides, previous studies investigated the fungicidal activity of a sulfanilamide compound against *Botrytis cinerea*; the pathogen responsible for gray mold disease of a variety of fruits, vegetables, and field crops (Li et al. 2010), *Fusarium oxysporum* the causal agent of fusariosis in cereals (Everitt and Sullivan 1940), *Puccinia graminis* responsible for the wheat stem rust (Tapke, 1950).

In fact, the enzyme dihydropteroate synthase which is the key of folate biosynthetic pathway in plant and bacteria is known to be the target of sulfanilamides (*e.g.* Loizeau et al. 2007). Sulfanilamides inhibit this enzyme by competing with para-aminobenzoic acid (PABA) for enzyme binding sites (Storozhenko et al. 2007; Kornfeld and Nichols 2005). Therefore, sulfanilamide activity does not appear to be only antimicrobial or antifungal but it might be a general inducer of plant defense. But the mechanism of sulfanilamide-induced resistance remains to be determined. In this work, we aimed at the synthesis of new compounds of sulfanilamide sulphate and the *in vitro* estimation of its potential antimicrobial activity against microorganisms such as Gram-positive and Gram-negative bacteria, yeasts and fungi. In this context, sulfanilamide and its derivatives have long been considered due to their antibacterial activity. This reactivity is due to the presence of two chelating sites in the structure, a particularity which procures them important properties in the extraction and complication of various metals. In this frame, the crystal structure of a shape of sulfanilamide has been widely studied in this work (Scheme 1).

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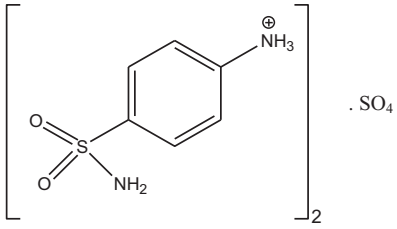

Scheme 1. The title compound.

2. Materials and methods

2.1. 2.1.Chemistry

2.1.1. Analytical and physical measurements

The infrared (IR) spectrum was recorded within the 4000–400 cm^{-1} region on a FT-IR Paragon 1000 PC spectrometer using KBr pellets. NMR spectra were recorded on NMR Varian Inova 300 MHz. The presence of the elements was confirmed by qualitative energy dispersive spectroscopy (EDX) analysis, performed on JEOL-JSM 5400 scanning electron microscope.

2.1.2. Synthesis

The title compound was prepared as good quality colorless single crystals from a mixture of sodium sulfate (RIEDEL-DE HAEN), sulfanilamide (PROLABO) and phosphoric acid (85% PROLABO), respectively (1:2:1) molar ratio in water. The resulting solution is left to evaporate at room temperature. After many days good single crystals were obtained. ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.04 (d, 2H, NH_2); 7.25 (s, 1H, —CH); 7.79 (s, 1H, —CH); 8.02 (t, 3H, NH_3^+).

2.1.3. Spectral studies

The region of IR spectrum of title compound, exhibiting the CH stretching modes is around 3050–2300 cm^{-1}. The assignments of NH_2 and NH_3 stretching modes are in agreement with our previous study. The bands corresponding to the NH_3 and NH_2 scissoring vibrations belong to the ammonium ring and sulfamoyl group are expected at 1625 and 1703 cm^{-1}, respectively. In addition, the asymmetric and symmetric stretching modes of SO_2 group appear in the region 1398–1310 cm^{-1} and 1204–1135 cm^{-1}, respectively (Evans, 1960). The band at 1222 cm^{-1} is assigned to the C—S stretching and rocking of sulfamoyl group. The ring planar deformation modes are observed 716, 558 and 526 cm^{-1}, the band observed at 848 cm^{-1} is not pure υ(SN) vibration and contains a significant contribution of π(CH) mode, in the same way from the pick at 801 cm^{-1} where there is a combination of π(CS) vibration and π(CN) mode.

2.1.4. Crystal structure determination and refinement

A suitable crystal with dimensional (0.2 × 0.3 × 0.3) mm^3 was chosen for X-ray diffraction studies. The measurements were made on an Enraf-Nonius CAD-4 diffractomter with graphite monochromated radiation (Mo Kα) at 293 K. The numbers of collecting and independent reflections were 2117 and 1898, respectively. Unit-cell dimensions were obtained by least-square refinement of the angular settings in the $3 < \theta < 27$. Lorentz and polarization correction were applied.

The structure was solved by direct methods, expanded using Fourier techniques and refined with anisotropic temperature factors for non-hydrogen atoms, by a full matrix least-squares based on F^2 using SHELXL (Sheldrick 1997). Further details of X-ray structural analysis are given in Table 1. Drawing figures are made with Diamond 2.1 supplied by Crystal Impact (Brandenburg and Diamon, 2001). Any oxygen atoms for sulphate anions occupy two positions each. The relevant crystallographic data for the title compound are listed in Table 1.

Table 1
Crystal data and structure refinement for the title compound.

Empirical formula	$SO_4[C_6H_9N_2O_2S]_2$
Formula weight (g mol^{-1})	442.48
Crystal size (mm)	0.2 × 0.3 × 0.3
Crystal system	Orthorhombic
Space group	P *b c n*
a (Å)	9.77(4)
b (Å)	9.64(4)
c (Å)	18.47(4)
V (Å^3)	1740(11)
Z	4
D_C (g cm^{-3})	1.746
μ (mm^{-1})	0.479
F (0 0 0)	920
hkl ranges	$0 \le h \le 12$; $-1 \le k \le 12$; $-23 \le l \le 0$
T (K)	293 (3)
Refl. measured	2117
Refl. unique	1889
R_{int}	0.0112
Parameters	169
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0309$, $wR_2 = 0.0887$
R indices (all data)	$R_1 = 0.0315$, $wR_2 = 0.0899$
Goodness of fit on F^2	1.075
Residual electron density (eÅ^{-3})	−0.362; 0.317

2.2. Microbiology

2.2.1. Microorganisms and growth conditions

To determine the antimicrobial activity of the new sulfanilamide sulphate described in this paper; gram-negative bacteria (*Pseudomonas aeruginosa*; *Escherichia coli DH5α*), gram-positive bacteria *(Listeria innocua*, *Staphylococcus aureus*), yeast (*Candida albicans*) and the dermatophyte (*Microsporum canis*); were taken from the culture collection of laboratory of Microorganisms and Biomolecules Actives, Faculty of Sciences in Tunisia. Some *Phytopathogenic fungi*: *Fusarium oxysporum* sp.; *Botrytis cinerea*, *Fusarium oxysporum culmorum* and *Fusarium oxysporum graminearum*, were also employed. There have been obtained from the laboratory of Mycology of the Institut National de la Recherche Agronomique de Tunisie (INRAT), Tunisia. Broth cultures were prepared from the above mentioned bacteria in Tryptone Soy Broth (TSB; 50 ml), inoculated with overnight stationary-phase cultures and held in 100 ml Erlenmeyer flask inside an orbital incubator (110 rpm, 37 °C and 30 °C for *Listeria innocua*). TSB (25 ml) held within 100 ml Erlenmeyer flasks were used to prepare bacterial cultures for the suspension tests.

2.2.1.1. Chemicals. TSB and TSA were employed throughout the experiments with bacteria. In the experiments with *Candida albicans*, yeast malt extract broth (YMB) and agar (YMA) were used. Potato Dextrose Agar (PDA) was used for experiments with fungi.

2.2.2. Agar diffusion method

The *in vitro* antimicrobial test utilized in the investigation is based on the diffusion method on agar plates (Collins et al. 1989). Therefore, media agar was autoclaved, cooled to 45 °C, seeded at 1% (v/v) with a culture of the overnight indicator strain in the appropriate medium. After homogenizing, the agar was poured in Petri dishes (90 mm diameter, 25 ml each). Plates were then placed at 25 °C for solidification. Before use, the sulfanilamide sulphate was diluted in distilled water, adjusted to 100 μg ml^{-1}, and sterilized by filtration through a 0.2 μm pore size filter. A 50 μl aliquot of filtered sulfanilamide sulphate was placed into paper discs. After overnight pre-diffusion at 4 °C, the plates were incubated at appropriate