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DRUG DISCOVERY AND RESISTANCE

Evaluation of antimycobacterial activity of a sulphonamide derivative

Vanessa Albertina Agertt [a,*], Lenice Lorenço Marques [b], Pauline Cordenonsi Bonez [a], Tanise Vendruscolo Dalmolin [a], Gelson Noe Manzoni de Oliveira [b], Marli Matiko Anraku de Campos [a]

[a] Programa de Pós-graduação em Ciências Farmacêuticas – Universidade Federal de Santa Maria, Avenida Roraima 1000, 97105900 Santa Maria, RS, Brazil
[b] Universidade Federal de Santa Maria, Departamento de química – 1000 Roraima Av., 9710597 Santa Maria, RS, Brazil

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SUMMARY

Mycobacterial infections including *Mycobacterium tuberculosis* have been increasing globally. The additional prevalence of multidrug-resistant (MDR-TB) strains and extensively drug-resistant tuberculosis (XDR-TB) stimulate an urgent need for the development of new drugs for the treatment of mycobacterial infections. It is very important to test the antimicrobial activity of novel compounds because they can be used in new with antimycobacterial drug formulation. Studies have shown that *Mycobacterium smegmatis* can be used in Minimum Inhibitory Concentration (MIC) assays with the advantage of rapidly and safely screen anti-tubercular compounds. This paper presents an evaluation of potential mycobacteriological compounds derived from inorganic synthesis and their microbiological performance along and in conjunction with Trimethoprim. Antimicrobial activity experiments were carried out by using the microdilution technique in broth to evaluate the sensibility against *M. smegmatis*. MIC values were between 0.153 and 4.88 μg/ml for the compounds tested. Tests of interaction between drugs were made by the method of Fractional Inhibitory Concentration Index (FICI). The compound [Au (sulfatiazolato)(PPh_3)] showed synergism FICI = 0.037 and was evaluated by isobols.

1. Introduction

Mycobacterial infections have been increasing globally and there has been an increased incidence of multidrug resistant *Mycobacterium tuberculosis* (MDR-TB). In addition, the increase in AIDS-associated infections and other diseases that cause immunodeficiency has contributed to the increase in the number of cases and severity of diseases caused by the bacillus.[1,2] Thus, it is necessary to develop new drugs and combinations of drugs to combat resistant strains. An alternative to this idea is to test new drugs with potential antimycobacterial and new combinations that increase the effectiveness of drugs even when their concentrations decrease.[3–5]

If two drugs are associated and synergism occurs between them, we note that lower concentrations of the drugs are associated with the same effect against microorganisms, when compared with the effect of both drugs separately. Thus, the side effects, which are often toxic to humans, can be reduced since lower concentrations generally diminish such effects.[6]

As an example of synergism related to anti-tuberculosis drugs we can cite two drugs: ethambutol, which possesses ocular toxicity, and the para-aminosalicylic acid, which may cause nausea, vomiting, severe diarrhea, hepatotoxicity, rash and fever; however, these two drugs have dose dependent side effects. Ethambutol and para-aminosalicylic acid co-administered with isocotinoilhidrazones allows the use of a reduced dose, which in turn reduces these side effects.[7]

Sulphonamides and Trimetoprim have been widely used to treat bacterial infections. These two drugs act synergically inhibiting serial steps in the synthesis of tetra-acid hydrofolic interacting with key enzymes in this pathway.[8] Forgacs et al.[9] reported that the combination of Trimethoprim/Sulfamethoxazole could be used in the treatment of tuberculosis cases, including the type caused by MDR-TB and extensively drug-resistant tuberculosis (XDR-TB) strains.

Several studies have reported significant results regarding the use of new sulphonamide derivative compounds against *M. tuberculosis*.[10,11] Suling et al.[12,13] proved the effectiveness of an analague Trimethoprim, a dihydrofolate reductase inhibitor, against the *Mycobacterium avium* complex, as well as synergistic activity with sulphamethoxazole. Given these facts, it is believed that new complexes of sulphonamide with metals may have a potential antimycobacterial as well as synergism with Trimethoprim.

* Corresponding author. Tel.: +55 5599322491; fax: +55 5532208753.
E-mail address: vaagertt@gmail.com (V.A. Agertt).

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2. Material and methods

Mycobacterium smegmatis mc2155 (ATCC 700084) was used in a preliminary study. After this we used the standard strain of *M. tuberculosis* H37Rv (ATCC 25618) and nine *M. tuberculosis* isolates susceptible to rifampicin and isoniazid. Both standard strains were maintained on Löwenstein-Jensen (HiMedia Laboratories Pvt. Ltd, India) agar until needed.

The inoculum was prepared in base medium Middlebrook 7H9 supplemented with 10% OADC (oleic acid-albumin-dextrose-catalase) (Difco Laboratories, Detriod, Mich.) and 0.2% glycerol (MD7H9) and homogenized in ultrasound for one minute. The concentration of bacteria in this medium was determined by optical density on spectrophotometer (0.08–0.1 of absorbance at 625 nm) of 0.5 McFarland scale and then diluted with MD7H9 up to 10^5 CFU/mL for *M. smegmatis,* to reach the inoculum. The *M. tuberculosis* was used in a concentration of 0.5 McFarland (not diluted) because they are slow growing.

The compounds used in this study [Au (sulfatiazolato)(PPh_3)], [Au (sulfametoxazolato)(PPh_3)], [AuCl(PPh_3)], [Cu_2(μ-$CH_3COO)_4$(sulfametoxazolato)$_2$], Cu(ac)$_2$.H_2O, [Cd (sulfametoxazolato)$_2$($CH_3OH)_2$] n.x(CH_3OH), Cd(ac)$_2H_2O$, [Hg (sulfametoxazolato)$_2$].2DMSO, Hg(ac)$_2$, [Ag (sulfametoxazolato)], and AgCl were synthesized at the Laboratory of Inorganic Materials (LMI), Department of Chemistry – Universidade Federal de Santa Maria as reported previously.[15] These compounds, isoniazid, rifampicin and Trimethoprim (Sigma®, USA) were dissolved in dimethilsulfoxide (DMSO) (Sigma®, USA), from which further dilutions were made in MD7H9. To avoid interference by the solvent, the highest DMSO concentration was 0.5%.

The antimycobacterial activity was evaluated with a colorimetric broth microdilution assay as reported previously,[16–18]with some modifications, as a result we obtained a minimum inhibitory concentrations (MICs) of each compound alone and in combinations was carried out as Checkboard dilution.[14] Triplicate wells were used for each experimental condition. One hundred microliters of the standardized bacterial suspension was placed in each test well of a microtiter plate of 96 wells and an equal volume of compound to be tested in different concentrations. Each compound in the proper dilution was tested in triplicate. We performed a broth control, a growth control and a compound control for each compound concentration, which was used later to compare the results. The plates were incubated for 48 h for *M. smegmatis* and 7 days for *M. tuberculosis* at 37 °C. To check the growth or no colonies of microorganisms Tetrazolium bromide [3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (MTT) (Sigma®, USA) dye was used to read the plates.[19]

The *in vitro* synergic activity was evaluated by using the fractional inhibitory concentration index (FICI). FICI was calculated using the following formula: $FICI = FIC_A + FIC_B$, where $FIC_A = (MIC_{Drug\ A}$ in combination/$MIC_{Drug\ A}$ alone) and $FIC_B = (MIC_{Drug\ B}$ in combination/$MIC_{Drug\ B}$ alone). $FICI < 0.5$, $0.5 \leq FICI \geq 1$, $1 < FICI \leq 4$, and $FICI > 4$ were defined as synergy, additive effect, indifference, and antagonism, respectively.[12,20–23]

3. Results

The MIC of the complexes and salts were determined for a colorimetric broth microdilution assay (Table 1). The MIC of the complexes and salts associated with trimethoprim at a ratio of 5:1 was determined (Table 2).

From the results cited above, the MIC, the fractional inhibitory concentrations (FIC) and the FICI of the compound that showed synergism at the concentration of 5:1, in varying concentrations (Table 3) were determined. From these values, we developed an isobologram (Figure 1) to better visualize the results. To better assess the profile of synergism between [Au (sulfatiazolato)(PPh_3)] and Trimethoprim, we determined the MIC and FICI for the salt, [AuCl (PPh_3)] (Table 4).

As the compound [Au (sulfatiazolato) (PPh_3)] had its best activity associated with Trimethoprim in the ratio 1:5 were tested against this standard strain of *M. tuberculosis* and nine clinical isolates (Table 5).

Table 1
MIC values for compounds tested against *Mycobacterium smegmatis*.

Compounds	MIC (μg/ml)
[Au (sulfatiazolato)(PPh_3)]	4.88
[Au (sulfametoxazolato)(PPh_3)]	2.44
[AuCl (PPh_3)]	9.76
[Cu_2 (μ-$CH_3COO)_4$(sulfametoxazolato)$_2$]	0.153
Cu(ac)$_2$.H_2O	2.44
[Cd (sulfametoxazolato)$_2$($CH_3OH)_2]_n$.x(CH_3OH)	0.153
Cd (ac)$_2H_2O$	2.44
[Hg (sulfametoxazolato)$_2$].2DMSO	0.305
Hg (ac)$_2$	19.53
[Ag (sulfametoxazolato)]	0.305
AgCl	9.76
Trimethoprim	4.88

4. Discussion

We observed that the MIC values of the compounds [Cu(μ-$CH_3COO)_4$(sulfametoxazolato)$_2$], [Cd (sulfametoxazolato)$_2$ ($CH_3OH)_2]_n$.x(CH_3OH), [Hg (sulfametoxazolato)$_2$].2DMSO, [Ag (sulfametoxazolato)] and [Au (sulfametoxazolato)(PPh_3)] were similar to those found for the drugs mentioned in the literature against *M. smegmatis*: 0.153 μg/ml, 0.153 μg/ml, 0.305 μg/ml, 0.305 μg/ml, 2.44 μg/ml, respectively for the compounds named above (Table 1), as well as 0.78 μg/ml for isoniazid.

The MICs obtained by the salt that originated each complexed compound had higher values than their complex. [AuCl(PPh_3)] (9.76 μg/ml) had an MIC greater than its complexes, [Au (sulfatiazolato)(PPh_3)] (4.88 μg/ml) and [Au (sulfametoxazolato)(PPh_3)] (2.44 μg/ml); Cu(ac)2.H_2O (2.44 μg/ml) greater than [Cu(μ-$CH_3COO)_4$(sulfametoxazolato)$_2$] (0.153 μg/ml), Cd (ac)$2H_2O$ (2.44 μg/ml) greater than [Cd (sulfametoxazolato)$_2$(CH3OH)$_2$] *n.x*(CH3OH) (0.153 μg/ml); Hg(ac)$_2$ (19.53 μg/ml)greater than [Hg (sulfametoxazolato)$_2$].2DMSO (0.305 μg/ml) and AgCl (9.76 μg/ml) greater than [Ag (sulfametoxazolato)] (0.305 μg/ml). This leads us to believe that the mechanism of inhibition of compounds is related to the same mechanism of sulphonamides.

Available preparations of Trimethoprim/Sulphamethoxazole are manufactured in a fixed ratio of 1:5 that results in a desired peak serum ratio of approximately 1:20. Our preliminary tests for the screening were based on a concentration of 1:5 as the commercial

Table 2
Values of FICI for the compounds tested against *Mycobacterium smegmatis*.

Compounds in combination with trimethoprim	FICI
[Au (sulfatiazolato)(PPh_3)]	0.037 synergism
[Au (sulfametoxazolato)(PPh_3)]	0.550 additive effect
[AuCl (PPh_3)]	0.700 additive effect
[Cu_2 (μ-$CH_3COO)_4$(sulfametoxazolato)$_2$]	1.024 indifference
Cu (ac)$_2$.H_2O	8.312 antagonism
[Cd (sulfametoxazolato)$_2$($CH_3OH)_2]_n$.x(CH_3OH)	0.512 additive effect
Cd (ac)$_2H_2O$	2.976 indifference
[Hg (sulfametoxazolato)$_2$].2DMSO	1.061 indifference
Hg (ac)$_2$	9.812 antagonism
[Ag (sulfametoxazolato)]	1.061 indifference
AgCl	5.907 antagonism