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ORIGINAL ARTICLE

Modulation of the antibiotic activity against multidrug resistant strains of 4-(phenylsulfonyl) morpholine

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Abstract The compound 4-(Phenylsulfonyl) morpholine belongs to the class of sulfonamides, which are widely used in the treatment of a large number of diseases caused by microorganisms. This compound has a morpholine group, which is also known for its antimicrobial properties. The aim of the present study was to investigate the antimicrobial and modulating activity of 4-(Phenylsulfonyl) morpholine against standard and multi-resistant strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and strains of the fungi *Candida albicans*, *C. tropicalis* and *C. krusei*. Antimicrobial activity was assessed based on the minimum inhibitory concentration (MIC) using the microdilution method. MIC was $\geqslant$1024 μg/mL for all microorganisms. Regarding modulating activity, the most representative effect occurred with the combination of 4-(Phenylsulfonyl) morpholine at a concentration of 128 μg/mL (MIC 1/8) and amikacin against *P. aeruginosa* 03, with a reduction in MIC from 312.5 to 39.06 μg/mL.

1. Introduction

Sulfonamides are substances with structures correlative to that of *p*-aminobenzoic acid. As competitive antagonists, sulfonamides impede their use by bacteria in the synthesis of folic acid, thereby affecting microorganisms that need to synthesize their own folic acid. As mammals do not synthesize folic acid, sulfonamides do not affect their metabolism (Alaburda et al., 2007). The importance of the sulfonamide nucleus is well

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established in pharmaceutical chemistry. New synthesized 5-substituted amino pyrazole sulfates in an attempt to find a therapeutic alternative for combating infection (Borges et al., 2004). A number of sulfonamides, especially those derived from *p*-aminobenzenesulfonamide, have structural variations that enhance their efficacy to obtain a greater action spectrum and increase their solubility in biologic systems (Coutinho et al., 2008a).

Morpholine derivatives constitute a new antifungal chemical group not correlated with other currently available medications with antifungal activity. These derivatives inhibit the biosynthesis of sterol by blocking two successive enzymatic processes: (1) inhibiting the biotransformation of lanosterol into zymosterol by blocking the enzyme C-14 sterol reductase and; (2) inhibiting the synthesis of ergosterol from the biotransformation of fecosterol into episterol by blocking the enzyme C-8 sterol isomerase; these enzymes are different from those inhibited by allylamines or azoles (Kerkenaar, 1987; Polak, 1988). The advantage in preparing morpholine derivatives resides in the fact that these compounds provide chlorhydrates that are soluble in water for pharmacological assays (Pinto et al., 2013). Different sulfonyl-hydrazones obtained from sulfonyl chloride exhibit anti-neoplasm, antibacterial, antinociceptive and other pharmacological activities against several and different targets (Oliveira, 2012). Synthetic substances have demonstrated efficacious antimicrobial action against resistant microorganisms. The determination of synergism or antagonism between antimicrobial agents is important to understanding the action mechanisms of these substances as well as resistance mechanisms. Moreover, *in vivo* and *in vitro* analyses are performed for the detection of therapeutic potential with the aim of finding alternative pathogen control methods (Catão et al., 2010).

The aim of the present study was to investigate the antimicrobial and modulating activity of 4-(Phenylsulfonyl) morpholine against standard and multi-resistant strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and strains of the fungi *Candida albicans*, *Candida. tropicalis* and *Candida. krusei*.

2. Materials and methods

2.1. Synthesis

The precursor's benzenesulfonyl chloride and morpholine were purchased commercially from Sigma–Aldrich (St. Louis, USA). The precursors were used without further purification. The title compound 4-(Phenylsulfonyl) morpholine was prepared by reaction of 1 equivalent of benzenesulfonyl chloride (1 mmol) and morpholine (2 mmol) in methanolic mixture of pyridine (5 mL) at low temperature (~0 °C) under stirring by 2 h as a previously described procedure (Buchmann and Schalinatus, 1962). The material thus formed was filtered and washed with methanol solution then dried. The resulting material was recrystallized by using heating methanol solution. The crystals were formed by slow solvent evaporation at room temperature. The complete structure elucidation was confirmed by NMR ^{1}H and ^{13}C spectroscopy analysis by comparison with literature data (Modarresi-Alam et al., 2009). The NMR spectra in $CDCl_3$, were recorded in Varian-Mercury 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometer, using tetramethylsilane (TMS) as internal standard. Analytical Data: ^{1}H RMN ($CDCl_3$, 300 MHz): t (4H; 3.01 ppm); t (4H; 3.74 ppm); t (2H, J = 7.56 Hz); t (1H; 7.64 ppm); d (2H; 7.77 ppm) ^{13}C RMN ($CDCl_3$, 75 MHz): δ = 46; 66.10; 127.85; 129.15; 133.09; 135.13. The synthesis is described by the scheme present in Fig. 1.

2.2. Microorganisms

The following strains were used: *E. coli* ATCC10536 and *E. coli* EC 27; *S. aureus* ATCC25923 and *S. aureus* SA358; *P. aeruginosa* ATCC15442 and *P. aeruginosa* PA; *C. albicans* ATCC40006; *C. krusei* ATCC6258 and *C. tropicalis* ATCC13803. Table 1 displays the resistance profile of the microorganisms. All strains were maintained on heart infusion agar (HIA, Difco Laboratories Ltd., San Diego, USA). Prior to the assays, the strains were cultivated in Brain-Heart Infusion broth (BHI, Difco Laboratories Ltd., San Diego, USA) for 18 h at 37 °C.

2.3. Antimicrobial activity and antibiotic modulating activity

The minimum inhibitory concentration (MIC) of all microorganisms was determined in broth microdilution assays (CLSI, 2005) using an inoculum of 100 μL of each strain suspended in BHI broth at a concentration of 10^5 colony forming units/mL in 96-well microtitration plates, with dilutions in ½ series. An aliquot of 100 μL of 4-(Phenylsulfonyl) morpholine was added to each well. The final concentrations of the substance ranged from 512 to 8 μg/mL. The standard antibiotics (amikacin, gentamicin and neomycin) and antifungals (amphotericin B, benzoilmetronidazol, mebendazole and nystatin) were assayed at concentrations ranging from 512 to 8 μg/mL and were used as controls. The plates were incubated at 35 °C for 24 h, after which the readings were performed with the aid of resazurin. The MICs were recorded as the least concentration necessary to the growth inhibition. For the assessment of the substance as a modulator of antibiotic and antifungal action, the MIC of antibiotics and antifungals was evaluated in the presence and absence of the substance in sterile microplates. The antibiotics and antifungals were analyzed at concentrations ranging from 512 to 0.5 μg/mL. All antibiotics tested were obtained from Sigma–Aldrich (St. Louis, USA).

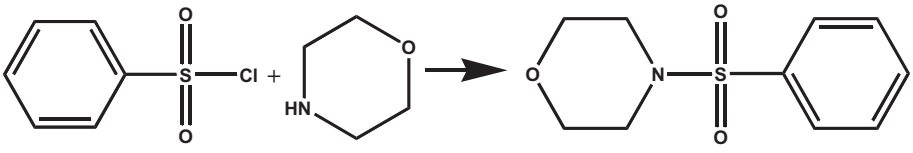

Figure 1 Synthesis of the compound 4-(Phenylsulfonyl) morpholine ($C_{10}H_{13}NO_3S$).