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Antimicrobial activity of quinoxaline 1,4-dioxide with 2- and 3-substituted derivatives☆

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ABSTRACT

Quinoxaline is a chemical compound that presents a structure that is similar to quinolone antibiotics. The present work reports the study of the antimicrobial activity of quinoxaline *N*,*N*-dioxide and some derivatives against bacterial and yeast strains. The compounds studied were quinoxaline-1,4-dioxide (QNX), 2-methylquinoxaline-1,4-dioxide (2MQNX), 2-methyl-3-benzoylquinoxaline-1,4-dioxide (2M3BenzoylQNX), 2-methyl-3-benzylquinoxaline-1,4-dioxide (2M3BQNX), 2-amino-3-cyanoquinoxaline-1,4-dioxide (2A3CQNX), 3-methyl-2-quinoxalinecarboxamide-1,4-dioxide (3M2QNXC), 2-hydroxyphenazine-*N*,*N*-dioxide (2HF) and 3-methyl-*N*-(2-methylphenyl)quinoxalinecarboxamide-1,4-dioxide (3MN(2MF)QNXC). The prokaryotic strains used were *Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 6538P, *S. aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *E. coli* S3R9, *E. coli* S3R22, *E. coli* TEM-1 CTX-M9, *E. coli* TEM-1, *E. coli* AmpC Mox-2, *E. coli* CTX-M2 e *E. coli* CTX-M9. The *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* PYCC 4072 were used as eukaryotic strains. For the compounds that presented activity using the disk diffusion method, the minimum inhibitory concentration (MIC) was determined. The alterations of cellular viability were evaluated in a time-course assay. Death curves for bacteria and growth curves for *S. cerevisiae* PYCC 4072 were also accessed. The results obtained suggest potential new drugs for antimicrobial activity chemotherapy since the MIC's determined present low values and cellular viability tests show the complete elimination of the bacterial strain. Also, the cellular viability tests for the eukaryotic model, *S. cerevisiae*, indicate low toxicity for the compounds tested.

1. Introduction

Antimicrobial agents are largely used in treatment and prevention of microorganism infections. Among others, the misuse and, especially, the abusive use of this kind of drugs, in human health, veterinary and animal production, led to the development of drug-resistant and multidrug-resistant (MDR) microorganisms (Roe 2008; Moreno et al. 2008). In addition, the permanent contact with some antimicrobial drugs, besides the resistance development, allows the increase of allergies and respiratory complications which are affecting the human population worldwide (Santos et al. 2007; Vollaard and Clasener 1994; Butaye et al. 2001; Witte et al. 2008). Resistant bacteria are increasing and the interval between the appearances of new and multi-drug resistant species is happening in short periods of time (Alanis 2005). These conditions are becoming emergent public health issues in the sense that they compromise pharmacological activity and the efficacy of these antimicrobial agents (Fernandes et al. 2008, 2009) and thus the heath of the population.

Because MDR bacteria are increasing worldwide human kind deals with the urgent need of development of new drugs with enhanced antimicrobial activity able to fight pathogens with no adverse effects (Fernandes et al. 2013). It is also expected to develop drugs that can reverse the resistance observed overturning the actual bacterial profile. Some approaches have been developed in order to evaluate the bioactivity of numerous compound families against several strains of microorganisms (Gradelski et al. 2001; Moellering 2011).

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Quinoxaline is an organic heterocyclic compound that has been used as base of synthesis of bioactive derivatives and several investigation groups have demonstrated their potential in medical and pharmacological applications (Zanetti et al. 2005; Carta et al. 2002; Sanna et al. 1999). These studies point to chemotherapeutical interests regarding the anti-tumor, anti-bacterial, anti-fungal, and anti-viral including anti-HIV (De Clercq 1997; Waring et al. 2002; Haykal et al. 2008; Harakeh et al. 2004) applications of these compounds. Relevant bioactivity has been reported in *Mycobacterium* spp. strains (Zanetti et al. 2005). No studies were found reporting biological activity for the quinoxaline derivatives in the present study with the microbial strains used.

The quinoxaline derivatives with *N*-oxide and *N*,*N*-dioxide have particular interest since they present relevant anti-oxidant activity. Many compounds with nitrogen—oxygen bonds play important biological roles by releasing NO groups or by cellular deoxygenating (Burguete et al. 2011; Hossain et al. 2012).

The present study pretends to be a contribution to the characterization of antibacterial and antifungal activity some *N*,*N*-quinoxaline derivatives (Table 1).

The activity of these compounds was tested against bacteria and yeast in order to understand the biological activity in both eukaryotic and prokaryotic microbial models. In the present work *Saccharomyces cerevisiae* and *Candida albicans* were used as representative models of eukaryotic microorganisms. Likewise, several strains of *Staphylococcus aureus* and *Escherichia coli* were used as prokaryotic representative models of Gram-positive and Gram-negative respectively.

2. Material and methods

2.1. Quinoxaline N,N-dioxide and quinoxaline derivatives

The compounds used in the present study were previously used by some of our collaborators and were gently provided by the Center of Investigation in Chemistry of the University of Porto. Synthesis, spectra and thermochemical properties were already studied for the quinoxaline derivatives used in the present study (Table 1) (Acree et al. 1997; Ribeiro da Silva et al. 2004; Gomes et al. 2005, 2007).

Stock solutions of the compounds were prepared in a 500 mL volume at a 500 μg/L final concentration. Since the compounds are thermally stable, the solutions were sterilized in an autoclave (AJC Uniclave 88) for 20 min at 120 °C. From these solutions, standards were prepared at the final concentrations 500, 100, 50, 20, and 5 μg/L.

2.2. Bacterial strains

The strains used in this study were stored deep frozen at −80 °C. The selected strains, in order to evaluate the susceptibility of a bacterial cell model to the proposed compounds, included *S. aureus* ATCC 6538, *S. aureus* ATCC 6538P, *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *E. coli* S3R9 and *E. coli* S3R22 (a penicillin resistant strain and a multidrug resistant strain, respectively). It was included some *E. coli* strains harboring extended spectrum β-lactamases (ESBL) such as TEM-1, TEM-1 + CTX-M9, CTX-M2, CTX-M9 and the AmpC β-lactamase MOX-2.

2.3. Yeast strains

The strains used in this study were *S. cerevisiae* PYCC 4072 (UNL, Portugal) and *C. albicans* ATCC 10231 and were also stored deep-frozen at −80 °C.

2.4. Microorganisms culture and zone inhibition

In order to assess the potential microbial activity of the compounds presented, disk diffusion method was used with two purposes. The first one was to determine the sensibility of the strains to known antibiotics, cefoxitin (FOX) and ciprofloxacin (CIP). The second was to assess the inhibition zone for new compounds. Bacteria and yeast cells were sub-cultured in broth agar (tryptic soy broth – TSB) and incubated for 24 h at 37 °C. Freshly prepared bacterial cells were transferred into a saline solution (NaCl 0.9%, Carlo Erba Reactifs, France) and density was settled in the interval 0.09 and 0.10, corresponding to 0.5 McFarland ($1–2 \times 10^8$ CFU/mL). The density of the solutions was measured at 625 nm using a spectrophotometer (Thermo Scientific Genesys 20). Solutions were spread onto a Trypticase Soy Agar (TSA; Cultimed, Spain) nutrient plate in a laminar flow cabinet. Yeast strains were spread onto Yeast Extract Peptone Dextrose (YEPD; Oxoid, Basingstoke, UK) nutrient plate, in laminar flow cabinet. Blank sterile disks were immersed in the standard solutions, with the final concentration of 500, 100, 50, 20, and 5 μg/L for each compound. Plates were incubated for 24 h at 37 °C and zone inhibition diameters were measured in millimeters. Each one of these bacteria was tested with cefoxitin disks (FOX) 30 μg and ciprofloxacin (CIP) 5 μg. Cefoxitin is a β-lactam and ciprofloxacin is a quinolone that has a similar structure of quinoxaline. The compounds studied have no established reference values regarding the sensitive/resistant behavior for the quinoxaline derivatives, so microdilution method was employed in order to determine de minimum inhibitory concentration. Strains studied were classified (Table 2) as susceptible (S) or resistant (R) according to Clinical Laboratory Standard Institute (CLSI) guidelines, by disk diffusion, considering the values of CLSI to the β-lactam and quinolone used in the present study. All results were confirmed by replica.

2.5. Minimum inhibitory assays

The minimum inhibitory concentration (MIC) for each quinoxaline derivative was estimated using the microdilution method, according to the CLSI (Rex, 2009; Wilder 2005, 2006). The MIC's were determined for each strain/compound pairs that presented antimicrobial activity.

The microplates used consisted of 96 wells. The TSB was dispensed into several wells, 8 for each chemical compound. One of these 8 wells corresponded to the positive control (containing the culture medium and bacterial suspension) and another to the negative control (containing only the culture medium). The remaining wells were used to prepare volumetrically diluted in series from stock solution (1:1, 1:2, 1:4, 1:8, 1:16 and 1:32) for each compound and considering the concentrations that presented inhibition halo. Fresh prepared cultures of the microorganisms were suspended in a saline solution to a density of 0.100 at 625 nm. Each well was prepared to a final volume of 200 μL. The microplates were closed and incubated at 37 °C, for 16–20 h. The presence or absence of turbidity was verified and rechecked by inoculating a fraction of the wells in solid culture medium (Mueller-Hinton broth, Cultimed, Spain). The plates were incubated for 24 h at 37 °C. The results obtained in the wells and plates were compared. All results were confirmed by replica.

2.6. Cellular viability of bacteria

In order to evaluate the growing or death performance cellular viability was analyzed for all strain/quinoxaline derivative pairs that presented growth inhibition and for which MIC's were determined. Microbial suspensions were prepared at 0.5 McFarland density with TSB medium for bacterial strains. The solutions of the studied compounds were colored and the optical density (OD)