Antimicrobial activity of quinoxaline 1,4-dioxide and three derivatives

M. Vieira[1,2], R. Fernandes[1,3], J. P. Noronha[2], M. D. M. C. Ribeiro da Silva[4] and C. Prudêncio[1,3]

[1] Ciências Químicas e das Biomoléculas, Escola Superior de Tecnologia da Saúde, Instituto Politécnico do Porto, Portugal

[2] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal

(3) Centro de Farmacologia e Biopatologia Química (U38-FCT), Laboratório de Bioquímica, Faculdade de Medicina do Porto, Portugal

[4] CIQ – Departamento de Química, Faculdade de Ciências, Universidade do Porto, Portugal

Corresponding Author: Cristina Prudêncio, Ciências Químicas e das Biomoléculas, Rua Valente Perfeito, nº 322. 4400-330 Vila Nova de Gaia, Portugal.
Tel: 00351222061000; Fax: 00351222061001; E-mail: cps@estsp.ipp.pt.

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Resumo

O presente trabalho descreve o estudo da actividade antimicrobiana de quarto derivados da quinoxalina *N*,*N*-dióxido: quinoxalina 1,4-dióxido, 2-metilquinoxalina 1,4-dióxido, 6-cloro-2,3-dimetilquinoxalina 1,4-dióxido e 3-benzoil-2-metilquinoxalina 1,4-dióxido contra as estirpes bacterianas *Geobacillus stearothermophilus* ATCC 10149, *Escherichia coli* ATCC 25922, *Escherichia coli* HB101, *Escherichia coli* (*bla*$_{\text{TEM}}$, *bla*$_{\text{CTX-M}}$) e *Salmonella* (*bla*$_{\text{CTX-M}}$), assim como contra a estirpe de levedura *Saccharomyces cerevisiae* PYCC 4072. A determinação da concentração mínima inibitória (MIC) foi realizada pelo método de diluição. Os valores de MIC's foram estimados para cada composto e estirpe. Os resultados obtidos sugerem potenciais novas drogas para quimioterapia.

Abstract

The present work reports the study of the antimicrobial activity of four quinoxaline *N*,*N*-dioxide: quinoxaline 1,4-dioxide, 2-methylquinoxaline 1,4-dioxide, 6-chloro-2,3-dimethylquinoxaline 1,4-dioxide and 3-benzoyl-2-methylquinoxaline 1,4-dioxide against *Geobacillus stearothermophilus* ATCC 10149, *Escherichia coli* ATCC 25922, *Escherichia coli* HB101, *Escherichia coli* (*bla*$_{\text{TEM}}$, *bla*$_{\text{CTX-M}}$) and *Salmonella* (*bla*$_{\text{CTX-M}}$) bacterial strains and also against the yeast *Saccharomyces cerevisiae* PYCC 4072. The determination of the minimal inhibitory concentration (MIC) was performed by the dilution method. The MIC values were estimated for each compound and each microorganism. The results obtained suggest potential new drugs for antimicrobial chemotherapy.

1-INTRODUCTION

Antimicrobial agents are largely used in treatment and prevention of microorganism infections. The misuse and the abusive use of this kind of drugs, in human health, veterinary and animal production [1,2], led to the development, of drug-resistant and multidrug-resistant (MDR) microorganisms. In addition, the permanent contact with some antimicrobial drugs allows the development of allergies and respiratory complications, which are affecting the human population worldwide [3 – 6]. These conditions are becoming emergent public health issues in the sense that they compromise pharmacological activity and the use of these antimicrobial agents [7, 8].

Because MDR bacteria are increasing worldwide [9, 10] human kind deals with the urgent need of development of new drugs with enhanced antimicrobial activity able to fight pathogens with no adverse effects.

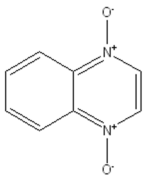
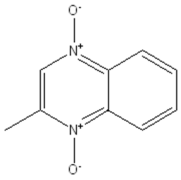
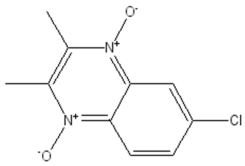
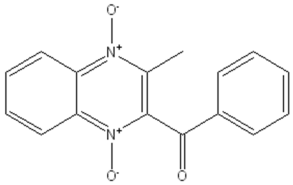
Quinoxalines and their poly-functional derivatives have been largely studied, in the latest years, namely in what regards to its medical and pharmacological applications [11 – 18]. These studies point to chemotherapeutical interests regarding the anti-tumor, anti-bacterial, anti-fungal, and anti-viral including anti-HIV [19-26] applications of these compounds. The quinoxaline derivatives with *N*-oxide and *N*,*N*-dioxide have particular interest once they present relevant anti-oxidant activity.

E. coli bacteria is a commensally inhabitant of the vertebrates gastrointestinal gut and may also be found in common urinary infections, in neonatal meningitis, diarrhea and

dysentery caused by pathogenic *E. coli* strains, especially in third world countries [27, 28]. Resistant *E. coli* is also a concerning problem in the more developed countries in the sense that they have developed mechanisms by which they are able to destroy the antimicrobial agent by enzymatic action. The acquisition of β-lactamases with extended spectrum (ESBL) activity genes (*bla*) is a worldwide concerning problem [7, 8]. Infections caused by Enterobacteria may be asymptomatic but generally they cause small complications or symptoms and, in extreme situations involving virulent strains, may be fatal [29].

In the present study four quinoxaline derivatives were tested (Table 1) for their antibacterial and antifungal activity: quinoxaline 1,4-dioxide (QNX), 2-methylquinoxaline 1,4-dioxide (2METQNX), 6-chloro-2,3-dimethylquinoxaline 1,4-dioxide (2,3METCLQNX) and 3-benzoyl-2-methylquinoxaline 1,4-dioxide (2METBQNX).

Table 1. Quinoxaline derivatives *N*,*N*-dioxide

Quinoxaline 1,4-dioxide	2-methylquinoxaline 1,4-dioxide	6-chloro-2,3-dimethylquinoxaline 1,4-dioxide	3-benzoyl-2-methylquinoxaline 1,4-dioxide
			

The activity of these compounds was tested against bacteria and yeast in order to understand the biological activity in both prokaryotic and eukaryotic models. Bacteria standard strains used in this study included Gram-negative and Gram-positive microorganisms *E. coli* ATCC 25922 and *G. stearothermophilus* ATCC 1014 respectively.

Regarding *E. coli*, besides the standard strain ATCC 25922 it was also included in this study two genetic variants: *E. coli* HB101, strain widely used for biotechnology and academic proposes exhibiting genetic resistance to streptomycin, and *E. coli* (bla_{TEM}, $bla_{\text{CTX-M}}$), a genetically modified resistant strain harbouring two distinct ESBL genes TEM and CTX-M enzymes genes, bla_{TEM} and $bla_{\text{CTX-M}}$ respectivlly. It was also used a clinical isolate, *Salmonella* ($bla_{\text{CTX-M}}$).

2 – RESULTS AND DISCUSSION:

2.1 –Gram-positive Bacteria

The four quinoxaline derivatives were tested against *G. stearothermophilus* ATCC 10149. The results presented in Table 2 show that QNX and 2METQNX exhibit inhibition for concentrations between 1 and 3μg/μL. For 2,3METCLQNX, no inhibition was observed in concentrations under 6μg/μL. 2METBQNX presents inhibition at concentrations under 1 μg/μL. These results evidence the antibacterial activity of the four quinoxaline derivatives against this standard strain.

Table 2. Antimicrobial activity of the quinoxaline derivatives against Gram-positive *G. stearothermophilus* ATCC 10149

			Concentration (µg/µL)				
			24	**12**	**6**	**3**	**1**
G. stearothermophilus ATCC 10149	**Inhibition zone (mm)**	QNX	24	12	6	3	1
		2METQNX	42	50	36	22	NI
		2,3METCLQNX	24	26	12	10	NI
		2METBQNX	34	32	30	28	22

2.2 –Gram-negative Bacteria

The results obtained for the Gram-negative bacteria studied are presented in Table 3. For Gram-negative microorganisms, 2,3METCLQNX and 2METBQNX have no influence on normal bacteria growth. In fact, only 2,3METCLQNX exhibited growth inhibition against *E. coli* HB101. For QNX and 2METQNX, growth inhibition was observed only for concentrations under 3µg/µL, except for QNX tested against *E. coli* ATCC 25922 and *E. coli* HB 101, that revealed inhibition of growth for concentrations under 1µg/µL. Analysing the results, we may assume that three of the quinoxaline derivatives tested induct Gram-negative growth inhibition.

Table 3. Antimicrobial activity of the quinoxaline derivatives against the Gram-negative Bacteria

			Concentration (µg/µL)				
			24	**12**	**6**	**3**	**1**
E. coli ATCC 25922	**Inhibition zone (mm)**	QNX	30	36	18	16	12
		2METQNX	22	28	16	12	NI
		2,3METCLQNX			NI		
		2METBQNX			NI		
E. Coli HB 101		QNX	52	46	42	--	40
		2METQNX	42	36	36	28	--
		2,3METCLQNX	28	26	18	14	NI
		2METBQNX			NI		
E. coli (bla_{TEM}, bla_{CTX-M})		QNX	24	28	22	18	NI
		2METQNX	22	22	15	12	NI
		2,3METCLQNX			NI		
		2METBQNX			NI		
Salmonella (bla_{CTX-M})		QNX	26	34	18	14	NI
		2METQNX	16	28	18	12	NI
		2,3METCLQNX			NI		
		2METBQNX			NI		

2.3 –Yeast strain

According to the results obtained, the four quinoxaline derivatives tested do not present growth inhibition against *S. cerevisiae* for the concentrations tested in this study. The higher concentration was of 24µg/µL.

2.4 –Ampicilin

Ampicilin (AMP) was used as a control, once it is a recurrent antibacterial drug

prescribed for bacterial infections. The inhibition zone obtained for a solution with 25μg/μL is represented Table 4.

Table 4. Antibacterial activity of ampicilin (AMP) at a concentration of 25μg/μL.

Bacteria	Inhibition Zone/mm
G. Stearothermophilus ATCC 10149	54
Escherichia Coli ATCC 25922	24
E. Coli HB101	26
E. coli (*bla*$_{TEM}$, *bla*$_{CTX-M}$)	N I
Salmonella (*bla*$_{CTX-M}$)	N I

2.5 – MIC determination

There was no growth of the strains studied at concentrations over 3 μg/μL (Table 5), except for 2,3METCLQNX tested against *G. stearothermophilus*, in agreement with the results obtained by the plate diffusion method.

Table 5. Minimal inhibitory concentration (MIC) obtained by dilution method

	QNX	2-METQNX	2,3-METCLQNX	2-MET-3-BQNX
G. Stearothermophilus ATCC 10149	< 1.5 μg/μL	< 3 μg/μL	< 7,5 μg/μL	< 1.5 μg/μL
E. Coli ATCC 25922	< 1.5 μg/μL	< 1.5 μg/μL	N I	N I
E. Coli HB101	< 3 μg/μL	< 1.5 μg/μL	< 1.5 μg/μL	N I
E. coli (*bla*$_{TEM}$, *bla*$_{CTX-M}$)	< 3 μg/μL	< 1.5 μg/μL	N I	N I
Salmonella (*bla*$_{CTX-M}$)	< 1.5 μg/μL	< 1.5 μg/μL	N I	N I

Quinoxaline and its derivatives are an interesting family of heterocyclic compounds, once they present a structure with similarity with quinolones, which present anti-microbial activity [30]. In this study, it was patent a selectivity between bacteria and yeast. The quinoxaline derivatives studied have presented activity against bacteria, but no activity against the eukaryotic model, *S. cerevisiea*, at the concentrations used. Also, there was observed a relevant growth inhibition in resistant strains of bacteria *E. coli* (*bla*$_{TEM}$, *bla*$_{CTX-M}$) and *Salmonella* (*bla*$_{CTX-M}$). These results might suggest the performance of more studies, in order to determine their potential use as anti-bacterial agents, of human and veterinary interest.

Different quinoxaline derivatives revealed selective activity among the bacterial strains studied. This fact strength us to believe that the mechanism involved in anti-bacterial activity of the quinoxaline derivatives studies may be related to the cell wall structures. The substituting groups presented in the quinoxaline structure reveal different activity against bacteria, that might be related to their electron donor or electron withdrawing comportment.

3 - CONCLUSION:

The compounds studied presented growth inhibition against Gram-positive bacteria (Figure 1). 2METQNX is the compound with a smaller inhibition zone, and the only one with electron donor characteristics. The two electron acceptors 2,3METCLQNX and 2METBQNX present greater inhibition zones. It was observed an increase in the inhibition zone as we have compounds with withdrawing characteristics. All quinoxaline derivatives present a smaller inhibition zone, for the same concentration, when compared with ampicilin, so it may indicate that these compounds present no advantage of use against Gram-positive strains.

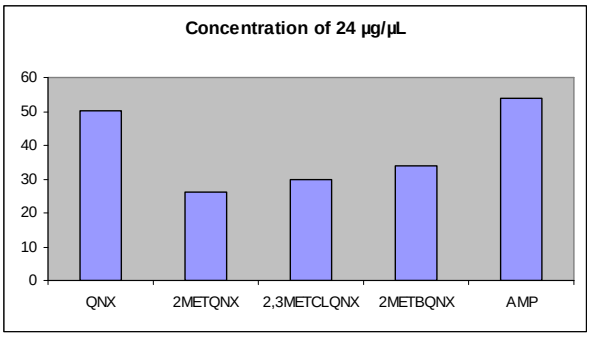


Figure 1. Inhibition zone of the four quinoxalines tested and of ampicillin (AMP) against *G. Stearothermophilus*

The activity against Gram-negative bacteria seems more selective. QNX and 2METQNX present growth inhibition for all the bacteria tested (Figure 2). *E. coli* HB 101 seems more sensible to these drugs and, in all species, the activity of 2METQNX is smaller than for QNX. These facts may be related with the presence of one $-CH_3$ electron donor group in this compound. On the other hand, 2,3METCLQNX and 2METBQNX have groups with antagonistic characteristics and, curiously, they have no influence on the growth of Gram-negative bacteria, except for *E. coli* HB 101, that is inhibited by 2,3METCLQNX. When compared with AMP, Gram-negative bacteria *E. coli* ATCC 25922 and *E. coli* HB 101 present larger inhibition zones with the same drug concentration, for QNX and 2METQNX.

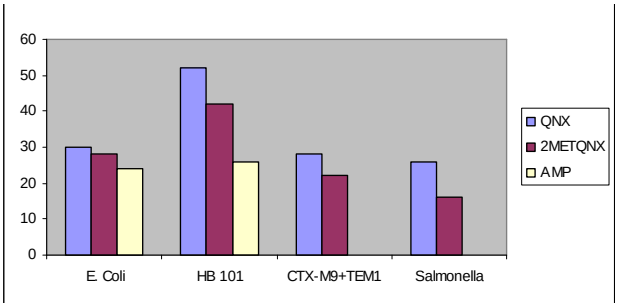


Figure 2. QNX, 2METQNX and AMP at a concentration of 24 µg/µL activity against Gram-negative *G. stearothermophilus*

All quinoxaline derivatives tested revealed no activity against *S. cerevisiae* at the concentration range tested, suggesting a potential use as antibacterial agents in humans since no eukaryotic damage appears to be observed, using this yeast as a reference

eukaryotic model. Naturally, further studies are needed to test the hypothesis.

4 - EXPERIMENTAL:

4.1 – Materials

The culture media used were Trypton Soy Broth (Ref: 610058, LiofilChem, Italy), for liquid bacteria cultures, and Mueller-Hinton Agar (Ref: 422765, Oxoid, Basingstoke, UK), for solid bacteria cultures. The culture medium used for yeast cultures was YEPD (Oxoid, Basingstoke, UK). Ampicilin was obtained by Applichem (Ref: A0839,0100, AppliChem, Germany). *E. Coli* ATCC 25922 (Ref: 84204, LiofilChem, Italy) and *G. stearothermophilus* ATCC 10149 (Ref: 84248, LiofilChem, Italy) were obtained in pellet form. *E. coli* HB 101 was obtained from Bio – Rad Laboratories, CA, USA, *E. coli* (*bla*$_{\text{TEM}}$, *bla*$_{\text{CTX-M}}$) was transformed in our laboratories and results were confirmed by PCR technique [8] and *Salmonella* (*bla*$_{\text{CTX-M}}$) is a clinical isolate. *S. cerevisiae* PYCC 4072 was genteelly offered by Universidade Católica Portuguesa. All tests were performed at least three times, with 14 days interval between, in duplicate. Sterile blank disks with 6 mm diameter were used in every assay.

4.2 – Quinoxaline compounds

The four quinoxalines derivatives were synthesised and purified in previous thermochemical studies [22,31-36]. All the compounds were sublimed under reduced pressure for additional purification, before the tests performance.

4.3 – Microorganisms culture and zone inhibition. MIC determination

The bacteria and the yeast were sub-cultured according to Fernandes *et al* [7] and Prudêncio *et al* [10].Stock solutions with different concentrations (1, 3, 6, 12 and 24 μg/μL) of the compounds were prepared and sterilised. Blank sterile disks were emerged in these solutions and poured over the prepared plates. Ampicilin (AMP) was used as standard drug.

The MIC for each compounds/strain was estimated using the dilution method, according to the Clinical and Laboratory Standards Institute (CLSI, formely NCCLS) [37, 38].

5 - ACNOWLEDGMENT:

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