Bioorganic & Medicinal Chemistry 22 (2014) 2844–2854

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Exploring 5-nitrofuran derivatives against nosocomial pathogens: Synthesis, antimicrobial activity and chemometric analysis

Rodrigo Rocha Zorzi [a,*], Salomão Dória Jorge [a,*], Fanny Palace-Berl [a], Kerly Fernanda Mesquita Pasqualoto [b], Leandro de Sá Bortolozzo [a], André Murillo de Castro Siqueira [a], Leoberto Costa Tavares [a]

[a] Department of Biochemical and Pharmaceutical Technology, Faculty of Pharmacy, University of São Paulo, Av. Prof Lineu Prestes, 580, São Paulo, SP 05508-900, Brazil
[b] Laboratory of Biochemistry and Biophysics, Butantan Institute, São Paulo, SP 05503-900, Brazil

ARTICLE INFO

Article history:
Received 28 January 2014
Revised 19 March 2014
Accepted 29 March 2014
Available online 8 April 2014

Keywords:
5-Nitrofuran derivatives
Molecular modification
Nosocomial infection
Bacterial resistance
Exploratory data analysis

ABSTRACT

The burden of nosocomial or health care-associated infection (HCAI) is increasing worldwide. According to the World Health Organization (WHO), it is several fold higher in low- and middle-income countries. Considering the multidrug-resistant infections, the development of new and more effective drugs is crucial. Herein, two series (**I** and **II**) of 5-nitrofuran derivatives were designed, synthesized and assayed against microorganisms, including Gram-positive and -negative bacteria, and fungi. The pathogens screened was directly related to either the most currently relevant HCAI, or to multidrug-resistant infection caused by MRSA/VRSA strains, for instance. The sets **I** and **II** were composed by substituted-[*N*′-(5-nitrofuran-2-yl)methylene]benzhydrazide and 3-acetyl-5-(substituted-phenyl)-2-(5-nitro-furan-2-yl)-2,3-dihydro-1,3,4-oxadiazole compounds, respectively. The selection of the substituent groups was based upon physicochemical properties, such as hydrophobicity and electronic effect. The compounds have showed better activity against *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis*. The findings from *S. aureus* strain, which was more susceptible, were used to investigate the intersamples and intervariables relationships by applying chemometric methods. It is noteworthy that the compound 4-butyl-[*N*′-(5-nitrofuran-2-yl)methylene]benzhydrazide has showed similar MIC value to vancomycin, which is the reference drug for multidrug-resistant *S. aureus* infections. Taken the findings together, the 5-nitrofuran derivatives might be indeed considered as promising hits to develop novel antimicrobial drugs to fight against nosocomial infection.

1. Introduction

Nosocomial or health care-associated infection (HCAI) caused by multidrug-resistant microorganisms comprises one of the most concerning problems pointed out by the World Health Organization (WHO). It is several fold higher in low- and middle-income than high income countries. It was estimated that around nine percent of hospitalized patients exhibits HCAI,[1] resulting in 4 million hospitalizations and, at least, 140,000 deaths every year in the United States and Europe.[2,3] The situation is getting even worse due to the acquired resistance by microorganisms to conventional therapy, being about 50–60% of total HCAI currently caused by multidrug-resistant microorganisms.[1,4–6] The improper use and/or abuse of antibiotics can be pointed out as quite important to the development of resistance.[1]

The Gram-positive bacteria *Staphylococcus aureus* is commonly associated with HCAI, specially the MRSA (Methicillin-resistant *S. aureus*), VISA (Vancomycin-intermediate *S. aureus*), and VRSA (Vancomycin-resistant *S. aureus*) strains.[1,4–7] The treatment for infections caused by these pathogens are extremely limited, since the strains are commonly non-susceptible to β-lactams, quinolones, tetracycline, and aminoglycosides. There are also several strains resistant to the new treatments, which have used Daptomycin and Linezolid, for instance.[5–11] The therapeutic available options, then, would be Telavancin (Vancomycin derivative), Ceftaroline fosamil (5th generation cephalosporin), and Tigecycline (glycylcycline class).[6,7]

Other microorganisms which can be commonly associated to multidrug-resistant HCAI are Gram-negative bacteria, including ESBL-producing (Extended-Spectrum Beta-lactamase) strains. The available treatment for these strains is based upon carbapenems (e.g., Imipenem, Meropenem, and Doripenem).[8] However, mainly *Enterobacteriaceae*, which is able to produce *Klebsiella pneumoniae* carbapenemase (KPC), can develop resistance to those antibiotics,

* Corresponding authors. Tel.: +55 11 30913693; fax: +55 11 38156386.
E-mail addresses: rodrigo.zorzi@gmail.com (R.R. Zorzi), sdjorge@gmail.com (S.D. Jorge).

http://dx.doi.org/10.1016/j.bmc.2014.03.044
0968-0896/

reducing the treatment options to the use of polimyxins (e.g., Colistin) and Tigecycline.[8–21] The resistance of Gram-negative bacteria strains to Tigecycline has already been reported.[9,10]

In addition, cases of sepsis especially associated with fungal infections can also be a serious concern for healthcare systems. The number of fungal infections has been increased more than 200% in the last two decades, being the third most common cause of bloodstream infections.[11,12] *Candida* spp. is responsible for 75% of all nosocomial infections caused by fungus, and *Candida albicans* causes 45% of the total cases primarily in immunosuppressed patients.[13] The treatment available comprises polyenes (Amphoterecin B), azoles (Fluconazole and Itraconazole), and, more recently, echinocandins. However, similar to bacteria, several strains have already presented resistance to those drugs.[14,15]

In this regard, it is important to adopt urgent measures to prevent the inappropriate impact caused by multidrug-resistant microorganisms on healthcare systems. Among this measures are the correct prescription of antibiotics; the development and implementation of protocols for cleaning and disinfecting patient rooms, surfaces, equipment, and common areas in hospitals environments; and, also the discovery of new drugs capable of treating the multidrug-resistant bacterial infections.

Nitrofurans are a class of nitro compounds, which have been used to treat bacterial infections since 1940s.[16] Several studies involving this chemical class have been carried out regarding different therapeutic uses, such as tuberculostatic,[17,18] antileishmanial,[19,20] trypanocidal activity,[21] and anti-proliferative effect on cancer cells lines.[22] Nifuroxazide (**NF**), for instance, is a nitro compound, which was widely used as antibacterial drug in 1970s and 80s. Recently, **NF** has been reported as a potential Quorum-Sensing inhibitor on *Pseudomonas aerugionosa*, even though it seems not be able to stop the bacterial growth.[23] **NF** is also considered an excellent lead compound due to its chemical structure, which benefits molecular modifications by rational design strategy. One of these modifications is the synthesis of 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazole ring system by cyclization reaction of *N*-acylhydrazone compounds, which was reviewed by Rollas and Karakuş.[24] Briefly, these structures were investigated as monoamine oxidase inhibitors,[25] antifungals,[26] anticonvulsants,[27] anti-inflammatory agents,[28] antibactericidals,[29,30] and trypanocidal agents.[29] The findings have emphasized the potential of these molecular structures for the development of novel drugs.

Herein, two series of compounds structurally analogous of **NF** (Fig. 1A) were designed, synthesized, and experimentally tested. Series **I** presents a *N*-acylhydrazone structure (azomethine derivatives), and series **II** has a heterocyclic ring system 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazole (oxadiazoline series) (see Fig. 1B). Antimicrobial activity was evaluated against microorganisms reported as HCAI pathogens (*Candida albicans*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterobacter clocae*, *Escherichia coli*, *Serratia marcescens*, and *Staphylococcus aureus*). In addition, derivatives that have showed promising activity in *S. aureus* were evaluated against multidrug-resistant *S. aureus* VISA3. The molecular properties were investigated by applying exploratory data analysis, a chemometric procedure, which comprises the principal component analysis (PCA) and hierarchical cluster analysis (HCA).[31,32]

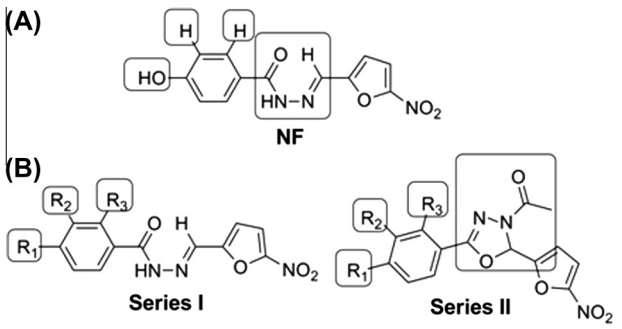

Figure 1. (A) Chemical structure of nifuroxazide (**NF**), pointing out the regions where molecular modifications were carried out on this study. (B) General chemical structures of series **I** and **II**.

2. Results and discussion

2.1. Chemistry

The designed compounds were obtained as show in Scheme 1. The compounds of series **I** (substituted-((5-nitrofuran-2-yl)methylene)benzohydrazide, **2a**–**v**) were obtained from the reaction of substituted benzohydrazides (**1a**–**v**) with 5-nitrofuran-2-carbaldehyde.[33] The compounds of series **II** (3-acetyl-5-(substituted-phenyl)-2-(5-nitro-furan-2-yl)-2,3-dihydro-1,3,4-oxadiazole, **3a**–**v**) were obtained by cyclization reaction of **2a**–**v** with acetic anhydride.[29,34,35] The substituent groups attached at benzene moiety were chosen based on their physicochemical properties, such as hydrophobicity and electronic effect.[36]

In this study, 41 compounds (22 compounds of series **I** and 19 compounds of series **II**) were synthesized and identified. All compounds were synthesized in two steps, starting from the corresponding benzohydrazides. The first step was based on classical Schiff's base formation, whose synthesis and mechanism of reaction have been well reported and discussed.[29,37] Satisfactory yields (around 90%) were obtained in this step. The second step was performed by a cyclization reaction of Schiff's base, and presented 66% yield.[35] The compounds were structurally identified (see the Supplementary information section, p. S2–S49).

Compounds **3h** ($R_1 = SO_2NH_2$), **3j** ($R_1 = N(CH_3)_2$), **3k** ($R_1 = NH_2$), and the respective oxadiazole analogue of **NF** ($R_1 = OH$) were not obtained probably due to a reaction of the substituent groups with acetic anhydride. The presence of unbound electrons seems to provide stronger nucleophiles, as the amidic nitrogen from Schiff's base, for instance. This observation was confirmed by ^1H NMR and ^{13}C NMR spectra (Fig. 2), considering the chemical deviation (δ) related to the internal standard reference (tetrametilsilane). Regarding the ^1H NMR spectra of **NF** (Fig. 2A), it can be noticed the presence of a singlet signal around δ 12 ppm, which is related to the proton of amidic nitrogen (H8). Also, a singlet signal at δ 8.40 ppm indicated the azomethine hydrogen atom (H6). After a cyclization reaction, the absence of the signal corresponding to the amidic nitrogen and a singlet signal at δ 7.35 ppm (H2), which indicates the 2,3-dihydro-1,3,4-oxadiazoline ring group formation, were observed in ^1H NMR spectra of the product (Fig. 2B). Furthermore, a singlet with six protons integration at δ 2.29 ppm was observed, confirming the three protons related to the acetyl group and to the acetoxylation of hydroxyl group. Analyzing the ^{13}C NMR spectra (Fig. 2C), there are signals at 167.1 and 20.9 ppm, which indicate the presence of carbonyl (C20) and alkyl groups (C22), respectively. Also, the signals at 153.4 (C5) and 84.6 ppm (C2) are related to the oxadiazole ring system. The presence of the acetoxy group attached to benzene moiety can be observed through the signals at 168.5 and 20.7 ppm.

2.2. Biological activity

The minimal inhibitory concentration (MIC) was determined by broth microdilution method against the following strains: *Candida albicans* 537Y, *Klebsiella pneumoniae* ATCC 700603, *Enterococcus faecalis* ATCC 29212, *Enterobacter clocae* ATCC 23355, *Escherichia coli* ATCC 25922, *Serratia marcescens* ATCC 14576, and