Chemico-Biological Interactions 272 (2017) 172–181

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

# Synthesis, cytotoxicity and antifungal activity of 5-nitro-thiophene-thiosemicarbazones derivatives

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ARTICLE INFO

*Article history:*
Received 6 February 2017
Accepted 3 May 2017
Available online 4 May 2017

*Keywords:*
*Candida*
*Cryptococcus neoformans*
Thiosemicarbazones
Citotoxicity
Antifungal

ABSTRACT

In the present work, twelve *N*-substituted 2-(5-nitro-thiophene)-thiosemicarbazones derivatives (**L1-12**) were synthesized, characterized and their *in vitro* cytotoxic and antifungal activities were evaluated against *Candida* sp. and *Cryptococcus neoformans*. The probable mechanisms of action have been investigated by sorbitol and ergosterol assays. Additionally, ultrastructural study by Scanning Electron Microscopy was performed with the **L10** compound. All compounds were obtained in good yield and their chemical structures were characterized on basis of their physico-chemical and Nuclear Magnetic Resonance - NMR, Spectrophotometric Absorption in the Infrared - IR and High-resolution Mass Spectrometry - HRMS data. The results showed that all strains were more sensitive to the compound **L10** except *Candida tropicalis* URM 6551. On the other hand, the cytotoxicity assay by incorporation of tritiated thymidine showed moderate cytotoxic activity on **L8** of the 50 μg/mLat which had the best MIC-cytotoxicity relationship. Concerning the study of the possible mechanism of action, the compounds were not able to bind to ergosterol in the membrane, do not act by inhibiting the synthesis of fungal cell wall (sorbitol assay). However, the Scanning Electron Microscopy - SEM analysis shows significant morphological changes in shape, size, number of cells and hyphae, and cell wall indicating a possible mechanism of action by inhibition of enzymes related to the ergosterol biosynthesis pathway. Our results demonstrate that *N*-substituted 2-(5-nitro-thiophene)-thiosemicarbazones derivatives are potential antifungal agents with activity associated with inhibition of enzymes related to biosynthesis of ergosterol.



## 1. Introduction

The development of research in the field of mycology shows an alarming increase in the frequency of opportunistic fungal infections, particularly those caused by *Candida* sp. and *Cryptococcus* sp. The high incidence of fungal infections caused by these yeasts presents a challenge for clinicians of different specialties due to difficulties in the diagnosis and treatment of these infections [1]. In addition, infection of the bloodstream caused by *Candida* species is associated with substantial morbidity and mortality, as well as showing an incidence rate with great geographical variation. In Europe, invasive candidiasis accounts for 2–3% of all nosocomial infections - four times less than in the United States. Incidence rates in patients in intensive care units are 5–10 times higher than in patients in medical or surgical wards. The average mortality rate for candidaemia is 43%. This is substantially higher than for any other

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http://dx.doi.org/10.1016/j.cbi.2017.05.005
0009-2797/

blood flow infection. In parallel, cryptococcosis is the most prevalent fatal fungal disease in the world with one million cases and about 600,000 deaths per year and is largely associated with HIV infection [2,3].

The invasive infection caused by species of resistant *Candida* to antifungals is an emerging problem. *Candida albicans* shows a 0–5% fluconazole resistance rate with higher values in South Africa. However, the appearance of fluconazole resistant *Candida* non-*albicans* species is even more alarming with about 5–65% with the highest rate in Denmark. This scenario is serious because this triazole is the only antifungal available for the treatment of candidiasis in many parts of the world. Resistance to echinocandin has also been reported in some contexts. In the United States of America (USA), approximately 6% of *Candida glabrata* isolates are resistant to echinocandins [4].

Although there are several classes of antifungal agents currently available in therapy, such as azoles in particular triazoles, polyenes and echinocandins, the discovery of new active drugs against *Candida* species has been of great importance since those available have a related toxicity such as hepatotoxicity of azole [5]. Cardiopathy of the echinocandins [6] and nephrotoxicity of amphotericin B [7]. At the same time, cases of resistance to these three classes of drugs have been demonstrated by several studies in the USA [8,9], indicating the increased incidence of *Candida* species of non-*albicans* resistant and consequently failure of available therapeutic [10].

In view of the need for new antifungal drugs, thiosemicarbazones have received prominence in the field of medicinal chemistry because of their great chemical versatility and promising biological applications, associated to its important pharmacological potential [11], among which it is already reported: antiproliferative [12], antiparasitic [13], antioxidant [14] and antimicrobial activities [15,16], especially antifungal [17–21]. Additionally, chemotherapeutic activity of nitro heterocycles is almost always associated with compounds having the nitro group attached to C-5 of the five membered heterocycles containing appropriate substituents at C-2 [22]. Compounds bearing 5-nitrothiophene moiety into various heterocyclic systems have been demonstrated improved biological activities in special as potent antifungal agents [23].

In this context, this study aimed to evaluate the antifungal potential of 5-nitrothiophen-thiosemicarbazone derivatives by *in vitro* assays against strains of *Candida* sp. and *Cryptococcus neoformans*, associated with the study of potential mechanisms of action and ultrastructural evaluation of the fungal strain treated with the 5-nitro-thiophene-thiosemicarbazone derivative.

## 2. Results and discussion

### 2.1. Synthesis of thiosemicarbazone derivatives

The synthesis of the *N*-substituted 2-(5-nitro-thiophene)-thiosemicarbazones derivatives was performed in a two-steps synthetic route according depicted in Fig. 1. In short, thiosemicarbazides were obtained by reaction of isothiocyanates with hydrazine (step 1), and the thiosemicarbazones by condensation of thiosemicarbazides with 5-nitro-thiophene-2-carboxyaldehyde (step 2).

All 2-(5-nitro-thiophene)-thiosemicarbazones derivatives (**L1-12**) were obtained in satisfactory yield (up than 50%), and the chemical structures were characterized based on their physicochemical (Table 1) and spectral data (Supporting data), and were in full agreement with the proposed structures.

The structural elucidation was performed by $^{1}$H NMR, $^{13}$C NMR, DEPT, Infrared (IR) and High Resolution Mass Spectrometry (HRMS). Since all chemical shifts data, absorption bands and other spectral data follow the same pattern, we present the data of compound **L9** as example.

The $^{1}$H NMR of **L9** showed a triplet at 1.19 ppm and a quartet at 2.60 ppm corresponding to the hydrogens of the radicals methyl and methylene of the 4-ethyl group, respectively. The signals representing the phenyl protons appears as doublets at 7.39 and 7.22 ppm. Since the protons of the thiophene ring were obtained as well as doublets, but with integration to one hydrogen each at 7.59 and 8.08 ppm. In addition, three singlets at 8.29, 10.09 and 12.13 ppm representing the hydrogens of imine (HC=N) and amine (HN-C and HN-N), respectively. The imine formation characterizes that the reaction was successful [18,28].

The possibility of thiol-thione tautomerism (H-N-C=S/N=C-SH) in these compounds was discarded because there were no signals between 2500 and 2650 cm$^{-1}$ in the IR spectra that are characteristic of the thiol group. Additionally, the stretch observed in 1498 cm$^{-1}$ in which represents thiocarbonyl (C=S) preferably shows the thione form [29,30]. Moreover, the spectra of $^{13}$C NMR and DEPT show five carbon sp$^{2}$ type and does not bind hydrogens. In addition, the signal in 176.25 ppm represents the carbon from thiocarbonyl.

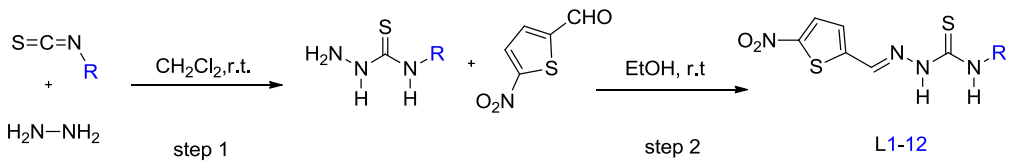
### 2.2. Biological studies

#### 2.2.1. Antifungal sensitivity, ergosterol assay and cytotoxicity of thiosemicarbazones derivatives

Results of the antifungal sensitivity, ergosterol assay and cytotoxicity of compounds *in vitro* in spleen cells from BALB/c mouse are presented in Table 2. The minimum fungicidal concentrations (MFC) are those which are capable of causing death of all yeast cells verified by re-cultivation.

All the synthesized compounds present in their chemical structure an aromatic thiophene ring, substituted at C-5 with the nitro grouping on the $N^{4}$ position of the thiosemicarbazone forming the molecular structure of the studied series.

The *Candida* strains tested showed high sensitivity to structural




R = *p*-methoxyphenyl (1), *p*-bromophenyl (2), 1-naphthyl (3), allyl (4), phenyl (5), *p*-chlorophenyl (6), 2-phenylethyl (7), *p*-tolyl (8), *p*-ethylphenyl (9), 3-pyridinyl (10), *p*-fluorphenyl (11), *p*-nitrophenyl (12).

**Fig. 1.** Synthetic route of 5-nitro-thiophene-thiosemicarbazones derivatives.