Synthesis of novel thiazolyl-phenyl-thiazole derivatives as promising anti-Candida agents

Anca-Maria Borcea1, Gabriel Marc1*, Dan C Vodnar2, Laurian Vlase3 and Ovidiu Oniga1
1Department of Pharmaceutical Chemistry, “Iuliu Hațieganu” University of Medicine and Pharmacy, 41 Victor Babes Street, Cluj-Napoca, Romania
2Department of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, 3-5 Manastur Street, Cluj-Napoca, Romania
3Department of Pharmaceutical Technology and Biopharmaceutics, “Iuliu Hațieganu” University of Medicine and Pharmacy, 41 Victor Babes Street, Cluj-Napoca, Romania

Abstract: New imine derivatives, that contain the thiazolyl-phenyl-thiazole scaffold, were synthesized and evaluated as anti-Candida agents. Elemental analysis and FT-IR, MS, 1H-NMR and 13C-NMR spectroscopic methods confirmed the structure of the newly synthesized compounds. The in vitro antifungal activity was investigated using the broth microdilution method against different Candida spp, including C. albicans, C. krusei and C. parapsilosis. All synthesized compounds exhibited good antifungal activity. Compound 4f showed the highest inhibitory effect against all tested Candida strains, being more potent than fluconazole. The results revealed that the new compounds have promising antifungal activity, with MIC values, ranging from 3.9 to 31.25µg/mL and MFC values between 7.81 and 62.5 µg/mL and could be considered for further development as anti-Candida agents.

Keywords: Thiazolyl-phenyl-thiazole, imine derivative, fungicidal activity, anti-Candida.

INTRODUCTION

Fungal strains of the genus Candida live commensally in the human body (Leite et al., 2014). Conversion of Candida species from a commensal fungus to an invasive pathogen is linked to defective host immune system responses, caused by clinically significant immunosuppression or illness and exposure to broad spectrum antibiotics (Paulovicova et al., 2016). A major concern nowadays is that Candida species are not only responsible for life-threatening infections in immunocompromised patients, but also for nosocomial bloodstream infections in healthy individuals. Moreover, the widespread incidence of infections represents a global economic challenge, due to increased care costs and length of hospitalization (Lv et al., 2016). Azole antifungals are the most frequently prescribed drugs used for Candida infections, although, latest studies have proved that there are Candida strains that have intrinsic or developed resistance to these antifungal agents (Whaley et al., 2016).

Notable anti-infective activity has been described for new compounds bearing thiazole scaffold, which strongly confirm that thiazoles are an important class of biologically active heterocyclic compounds. Recently published studies revealed that substituted thiazoles and bisthiazoles exhibit antifungal activity, being active against a broad spectrum of fungal strains, including different species of Candida (Bikobo et al., 2017, Chimenti et al., 2011, Desai et al., 2016, Maillard et al., 2013). Furthermore, new series of Schiff bases, thiosemicarbazones or hydrazones, which contain the imine group (C=Н) have been synthesized and evaluated for their anti-Candida activity (Kamal et al., 2015, Kaplancikli et al., 2016, Stana et al., 2016). The association of different pharmacophores in the same molecule could be an interesting approach to obtain new compounds with increased biological activity.

Considering the lack of efficiency of the authorized antimicrobials for multidrug-resistant fungal infections, herein we report the synthesis and in vitro biological evaluation of new thiazolyl-phenyl-thiazole imine derivatives as potential anti-Candida agents.

MATERIALS AND METHODS

Chemistry

Reagents and solvents used for synthesis were purchased from Sigma-Aldrich and Alfa Aesar. All chemicals were of analytical grade purity. The progress of all reactions and the purity of the newly synthesized compounds were verified by thin layer chromatography (TLC), performed on Merck precoated Silica Gel 60F254 sheets, using ethylacetate - heptane 7:3 as mobile phase and UV light for visualization (254 nm). Melting points (m.p.) were determined using the open glass capillary method, on an Electrothermal 9100 melting point meter and are uncorrected. Elemental analysis was carried out on a Vario El CHNS instrument. IR spectra were recorded on a JASCO FT/IR 6100 spectrometer, after compression under vacuum in anhydrous KBr pellets. Water and carbon dioxide signals were removed from IR spectrum.
using Spectra Manager software and assignment of signals was assisted by Know It All 7.8 by Bio-Rad Laboratories. Mass spectra were recorded on an Agilent 1100 series and an Agilent Ion Trap SL mass spectrometer. Analyses were performed at 70 eV and electrospray ionization was carried out in the positive ion mode. 1H-NMR analyses were performed at room temperature, on a Bruker Avance NMR spectrometer operating at 500 MHz, using tetramethylsilane (TMS) as internal standard. Chemical shifts (δ values) are expressed in parts per million (ppm). The synthesized compounds were dissolved in DMSO-d6 (δH = 2.51 ppm). 13C NMR spectra were recorded on a Bruker Avance NMR spectrometer operating at 125 MHz, in DMSO-d6, using a waltz-16 decoupling scheme, with TMS as internal standard. The chemical synthesis of compounds 1-3 has been previously reported (Borcea et al., 2017).

**Results**

**Chemistry**

The thiazolyl-phenyl-thiazole imine derivatives were synthesized as shown in Scheme 1.

Physico-chemical characterization, elemental analysis and spectral data proved the identity and purity of the new compounds. The spectral data for IR, MS, 1H-NMR and 13C-NMR, given below, are in accordance with the proposed structure of the new compounds. Results obtained from C, H, N, S quantitative elemental analysis for the synthesized compounds are within ±0.4% of the theoretical values.

**Structural characterization data of compounds 4a-g**

1-(4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-5-yl)ethanone oxime (4a) Yellow solid. Yield 71%. M.p. 215-6°C. 1H NMR (DMSO-d6, 500MHz) δ ppm: 10.76 (s, 1H, -OH), 8.09 (s, 1H, thiazole-C-H), 8.07 (dd, 2H, phenyl), 7.97 (dd, 2H, phenyl), 2.75 (s, 3H, -CH3), 2.59 (s, 3H, -CH3), 2.22 (s, 3H, -CH3). 13C NMR (DMSO-d6, 125MHz) δ ppm: 160.90 (C), 160.26 (C), 163.51 (C), 153.32 (C), 140.11 (C=N), 136.28 (C), 132.39 (C), 127.21 (2CH), 126.97 (C), 124.01 (2CH), 115.59 (CH), 19.42 (CH3), 18.47 (CH3), 16.79 (CH3). FT IR (KBr) ν cm⁻¹: 3417 (O-H str), 3109 (C-H str thiazole) 3015 (C-H str arom), 2919 (C-H str CH3), 1675 (C-N str), 1626, 1526, 1499, 1442 (Ar ring str), 980 (C-H bend arom), 853 (C-H def arom). MS (ESI, 70eV): m/z 330.5 (M+H+). Anal. Caled. for C16H15N3O3S3 (%): C, 48.84; H, 3.84; N, 10.68; S, 24.57. Found (%): C, 58.31; H, 4.48; N, 12.84; S, 19.69.

Anti-candida activity

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values was carried out on several Candida strains including cultures of C. albicans ATCC 10231, C. albicans ATCC 18804, C. krusei ATCC 6258 and C. parapsilosis ATCC 22019. The yeasts used for this evaluation were obtained from the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Romania.

Stock solutions (1 mg/mL) were prepared by dissolving the synthesized compounds and fluconazole, used as reference antifungal, in sterile DMSO. Antifungal activity of the synthesized compounds was determined using the broth microdilution method, following a previous reported protocol and according to the guidelines of Clinical Laboratory Standards Institute (CLSI) (Ionuț et al., 2016, Winnicka et al., 2012). The MIC was defined as the lowest concentration without visible growth of the fungal strain (using the binocular microscope) and the MFC as the lowest concentration that killed at least 99.5% of the initial inoculum. All MIC and MFC experiments were performed in triplicate.
Scheme 1: Synthetic pathway of thiazolyl-phenyl-thiazole imine derivatives i. 4-(2-bromoacetyl)benzonitrile; ii. hydrogen sulfide gas; iii. 3-chloropentane-2,4-dione; iv. R-NH₂

2-(1-(4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-5-yl)hydrazinocarbothioamide (4d)

Yellow solid. Yield 69%. M.p. 261°C. ¹H NMR (DMSO-d₆, 500MHz) δ ppm: 9.55 (s, 1H, -NH-), 8.10 (s, 1H, thiazole-C₅H), 8.06 (dd, 2H, phenyl), 8.03 (dd, 2H, phenyl), 7.82 (dd, 2H, phenyl), 7.79 (dd, 2H, phenyl), 7.11 (s, H, phenyl), 2.76 (s, 3H, -CH₃), 2.62 (s, 3H, -CH₃), 2.32 (s, 3H, -CH₃). ¹³C NMR (DMSO-d₆, 125MHz) δ ppm: 166.99 (C), 166.82 (C), 163.51 (C), 154.02 (C), 142.24 (C), 140.11 (C=N), 134.95 (C), 133.27 (C), 133.12 (C), 130.87 (2CH), 127.11 (2CH), 126.78 (2CH), 122.45 (CH), 115.17 (CH), 114.76 (2CH), 19.42 (CH₃), 17.44 (CH₃), 16.92 (CH₃). FT IR (KBr) ν cm⁻¹: 3232 (N-H secondary amine str), 1669 (C=N str) 1617 (C=N str), 1599 (C=N str), 1560, 1490 (Ar ring str), 1246 (C-N str amine amine), 994 (C-H bend amine), 849 (C-H def amine). MS (ESI, 70eV): m/z 388.4 (M+H⁺). Anal.Calcd.for C₁₇H₁₇N₅S₃ (%): C, 52.69; H, 4.42; N, 18.07; S, 24.82. Found (%): C, 52.48; H, 4.57; N, 17.98; S, 24.97.
Synthesis of novel thiazolyl-phenyl-thiazole derivatives as promising anti-Candida agents


5-(1-(2-(4-bromophenyl)hydrazono)ethyl)-4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazole (4f)
Red solid. Yield 58%. M.p. 194°C. 1H NMR (DMSO-d6, 500MHz) δ ppm: 9.79 (s, 1H, -NH-), 8.12 (s, 1H, thiazole-C5H), 8.11 (dd, 2H, phenyl), 8.07 (dd, 2H, phenyl), 8.04 (dd, 2H, phenyl), 2.75 (s, 3H, -CH3), 2.58 (s, 3H, -CH3), 2.25 (s, 3H, -CH3). 13C NMR (DMSO-d6, 125MHz) δ ppm: 166.91 (C), 166.86 (C), 163.65 (C), 153.97 (C), 141.82 (C), 140.64 (C=N), 134.78 (C), 133.83 (C), 133.55 (C), 131.06 (2CH), 127.73 (2CH), 126.61 (2CH), 115.17 (CH), 114.12 (2CH), 112.88 (C), 19.40 (CH3), 17.52 (CH3), 16.81 (CH3). FT IR (KBr) ν cm⁻¹: 3244 (N-H secondary amine str), 3116 (C-H thiazole str), 3044 (C-H str arom), 2920 (C-H str CH3), 1644 (C=O str amide), 1642 (C=N str), 1607, 1583, 1509, 1444 (Ar ring str), 1003 (C-H bend arom), 848 (C-H def arom). MS (ESI, 70eV): m/z 484.1 (M+H +). Anal. Calcd. for C22H19BrN4S2 (%): C, 54.66; H, 3.96; Br, 16.53; N, 11.59; S, 13.27. Found (%): C, 54.29; H, 3.99; Br, 16.67; N, 12.49; S, 13.57.

4-hydroxy-N’-(1-(4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-5-yl)ethylidene)benzo-hydrazide(4g)
Yellow solid. Yield 63%. M.p. 298°C. 1H NMR (DMSO-d6, 500MHz) δ ppm: 12.9 (s, 1H, OH), 9.65 (s, 1H, -NH-), 8.13 (s, 1H, thiazole-C6H), 8.09 (dd, 2H, phenyl), 8.06 (dd, 2H, phenyl), 8.02 (dd, 2H, phenyl), 2.73 (s, 3H, -CH3), 2.61 (s, 3H, -CH3), 2.21 (s, 3H, -CH3). 13C NMR (DMSO-d6, 125MHz) δ ppm: 165.77 (C), 164.87 (C), 164.24 (C), 154.38 (C=O), 152.11 (C), 140.93 (C), 140.12 (C=N), 135.03 (C), 134.11 (C), 132.97 (C), 131.56 (2CH), 127.36 (2CH), 125.99 (2CH), 115.76 (CH), 113.93 (2CH), 113.11 (C), 19.36 (CH3), 16.82 (CH3), 15.74 (CH3). FT IR (KBr) ν cm⁻¹: 3432 (O-H str phenol), 3143 (N-H str amide), 3123 (C-H thiazole str), 3065 (C-H str arom), 2965 (C-H str CH3), 1646 (C=O str amide), 1642 (C=N str), 1607, 1583, 1509, 1444 (Ar ring str), 1003 (C-H bend arom), 848 (C-H def arom). MS (ESI, 70eV): m/z 449.4 (M+H +). Anal. Calcd. for C23H20N4O2S2 (%): C, 61.59; H, 4.49; N, 12.49; S, 14.30. Found (%): C, 61.65; H, 4.37; N, 12.62; S, 14.24.

Anti-candida activity
The antifungal activity of the newly synthesized compounds 4a-g was evaluated against four different Candida spp.: two Candida albicans strains (C. albicans ATCC 10231 and C. albicans ATCC 18804) and two non-C. albicans species (C. krusei ATCC 6258 and C. parapsilosis ATCC 22019). The obtained results are summarized in table 1.

DISCUSSION
Starting from thioacetamide, 1-(4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-5-yl)ethanone 3 was obtained in a three-step synthesis, following the previously published procedure (Borcea et al., 2017). Subsequently, the carbonyl group of compound 3 was modulated through condensation with various N-nucleophiles, in order to obtain, in good yields, thiazolyl-phenyl-thiazole imine derivatives 4a-g.

FT-IR spectra of compounds 4a-g showed an absorption band at 3128 - 3103 cm⁻¹ due to the stretching vibration of the C5-H from the thiazole ring, as well as a characteristic band for C=N stretching in the range of 1670-1617 cm⁻¹. Mass spectra for all synthesized compounds displayed the molecular ion peak as expected from the molecular formula. The 1H NMR spectra for all compounds showed additional proton signals, when compared to thiazolyl-phenyl-thiazole ketone 3, indicating that the synthesis of the imine derivatives successfully took place. In the 13C-NMR spectra of compounds 4a-g, the number of signals equaled the number of different carbons from the chemical structure. Therefore, the data for IR, MS, 1H-

Table 1: Minimum inhibitory concentration (MIC, µg/mL) and minimum fungicidal concentration (MFC, µg/mL) of compounds 4a-g

<table>
<thead>
<tr>
<th>Compound</th>
<th>C. albicans ATCC 10231</th>
<th>C. albicans ATCC 18804</th>
<th>C. krusei ATCC 6258</th>
<th>C. parapsilosis ATCC 22019</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
</tr>
<tr>
<td>4e</td>
<td>3.9</td>
<td>7.81</td>
<td>7.81</td>
<td>15.62</td>
</tr>
</tbody>
</table>

Inoculum control Growth in all concentrations
Broth control No growth


Inoculum control Growth in all concentrations
Broth control No growth

2088 Pak. J. Pharm. Sci., Vol.31, No.5(Suppl), September 2018, pp.2085-2090
NMR and $^{13}$C-NMR are in agreement with the proposed structure of the new compounds.

Analysing the results obtained in the anti-\textit{Candida} evaluation, we have observed that our tested compounds displayed significant inhibitory activity against all fungal strains. The anti-\textit{Candida} activity of some compounds is similar to or even higher than the positive control fluconazole. Our screened compounds possessed MIC values, ranging from 3.9 to 31.25 µg/mL and MFC values between 7.81 and 62.5 µg/mL, compound 4f being the most active among tested derivatives. Compound 4f showed to have a 4-fold greater potency than fluconazole, against \textit{C. albicans} ATCC 10231 and a 2-fold greater activity than our reference drug, against \textit{C. albicans} ATCC 18804, \textit{C. krusei} and \textit{C. parapsilosis}. Moreover, compound 4f, with a para-bromine substituent on the phenyl ring, showed better inhibitory activity than the corresponding unsubstituted analog 4e, against \textit{Candida albicans} and \textit{Candida krusei} strains, with no difference in activity against \textit{Candida parapsilosis}. With compound 4f exhibiting the most potent anti-\textit{Candida} activity in our series, we managed to confirm that para-substitution of the phenyl ring is associated with enhanced anti-\textit{Candida} activity, as it was previously reported in literature (Nastasă et al., 2015, Secci et al., 2012).

Nevertheless, even if the antifungal activity of compound 4e is equal to the one of fluconazole against \textit{C. albicans} species and \textit{C. krusei}, this compound proved to have a 2-fold higher activity against \textit{C. parapsilosis}, in comparison with the reference antifungal drug. This is important because \textit{C. parapsilosis} is often intrinsically resistant or likely to gain resistance to clinically used antifungal agents (Božinović et al., 2016).

**CONCLUSION**

New thiazolyl-phenyl-thiazole imine derivatives were obtained, in good yields, by condensation of previously synthesized compound 3 with various N-nucleophiles. Physico-chemical parameters and spectral data confirmed the identity and purity of the compounds.

The antifungal activity of the newly synthesized compounds was evaluated against four different \textit{Candida} strains. The results revealed the most active derivative among the tested compounds, 4f, which can be subjected to further optimization as a lead compound.

**ACKNOWLEDGMENTS**

This study was supported by Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Romania through doctoral research project No. 7690/11/2016, 5200/10/2017 and 3067/3/2018.

**REFERENCES**


Synthesis of novel thiazolyl-phenyl-thiazole derivatives as promising anti-Candida agents


