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

Anca-Maria Borcea[1], Gabriel Marc[1*], Dan C Vodnar[2], Laurian Vlase[3] and Ovidiu Oniga[1]

[1]Department of Pharmaceutical Chemistry, "Iuliu Haţieganu" University of Medicine and Pharmacy, 41 Victor Babes Street, Cluj-Napoca, Romania

[2]Department of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, 3-5 Manastur Street, Cluj-Napoca, Romania

[3]Department of Pharmaceutical Technology and Biopharmaceutics, "Iuliu Haţieganu" 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, ^{1}H-NMR and ^{13}C-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=N) have been synthesized and evaluated for their anti-*Candida* activity (Kamal *et al.*, 2015, Kaplancıklı *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 $60F_{254}$ sheets, using ethyl-acetate - 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

**Corresponding author:* e-mail: marc.gabriel@umfcluj.ro

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. ^{1}H-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-d_6 (δ_H =2.51 ppm). ^{13}C NMR spectra were recorded on a Bruker Avance NMR spectrometer operating at 125 MHz, in DMSO-d_6, 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).

General procedure for the synthesis of thiazolyl-phenyl-thiazole imine derivatives 4a-g

To a solution of 1-(4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-5-yl)ethanone 3 (0.314 g, 1 mmol) in absolute ethanol (10 mL), an appropriate N-nucleophile R-NH_2 (1 mmol) was added. For the reactions involving amine hydrochloride compounds, 1 mmol of anhydrous sodium acetate was added. For the synthesis in which amine bases were involved, glacial acetic acid was used as catalyst. The resulting mixture was refluxed until TLC indicated completed consumption of compound 3. The thiazolyl-phenyl-thiazole imine derivatives were obtained pure after filtering and washing with distilled water.

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) (Ionu *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.

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, ^{1}H-NMR and ^{13}C-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

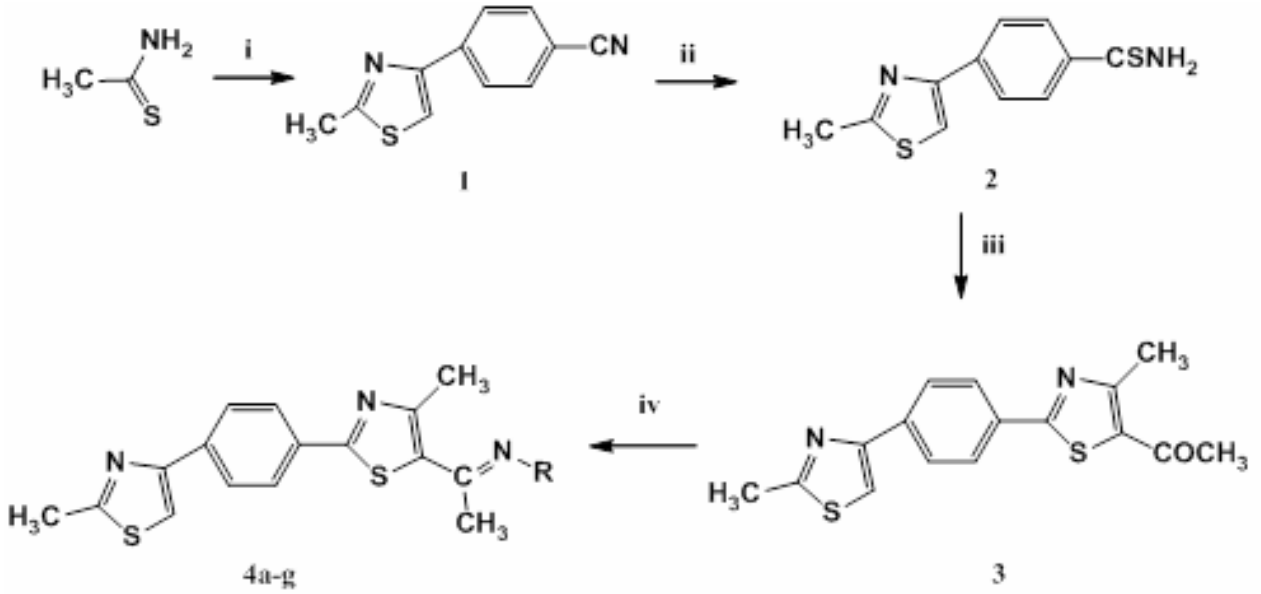
1-(4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-5-yl)ethanone oxime (4a) Yellow solid. Yield 71%. M.p. 215-6°C. ^{1}H NMR (DMSO-d_6, 500MHz) δ ppm: 10.76 (s, 1H, -OH), 8.09 (s, 1H, thiazole-C_5H), 8.07 (dd, 2H, phenyl), 7.97 (dd, 2H, phenyl), 2.75 (s, 3H, -CH_3), 2.59 (s, 3H, -CH_3), 2.22 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125MHz) δ ppm: 166.90 (C), 166.26 (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 (CH_3), 18.47 (CH_3), 16.79 (CH_3). FT IR (KBr) ν cm^{-1}: 3147 (O-H str), 3109 (C-H thiazole str) 3051 (C-H str arom), 2919 (C-H str CH_3), 1652 (C=N str), 1600, 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*. Calcd. for $C_{16}H_{15}N_3OS_2$ (%): C, 58.33; H, 4.59; N, 12.76; S, 19.47. Found (%): C, 58.51; H, 4.48; N, 12.84; S, 19.69.

(1-(4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-5-yl)ethylidene)sulfamic acid (4b)

Yellow solid. Yield 68%. M.p. 197°C. ^{1}H NMR (DMSO-d_6, 500MHz) δ ppm: 11.35 (s, 1H, -OH), 8.11 (s, 1H, thiazole-C_5H), 8.08 (dd, 2H, phenyl), 7.98 (dd, 2H, phenyl), 2.74 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 2.38 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125MHz) δ ppm: 167.51 (C=N), 166.94 (C), 166.39 (C), 163.25 (C), 155.98 (C), 137.95 (C), 136.35 (C), 132.41 (C), 127.07 (2CH), 124.71 (2CH), 115.67 (CH), 19.43 (CH_3), 18.72 (CH_3), 17.48 (CH_3). FT IR (KBr) ν cm^{-1}: 3161 (O-H str), 3110 (C-H thiazole str) 3054 (C-H str arom), 2920 (C-H str CH_3), 1649 (C=N str), 1600, 1527, 1500, 1442 (Ar ring str), 1348 (S=O str), 981 (C-H bend arom), 853 (C-H def arom). MS (ESI, 70eV): *m/z* 394.07 (M+H$^+$). *Anal*. Calcd. for $C_{16}H_{15}N_3O_3S_3$ (%): C, 48.84; H, 3.84; N, 10.68; S, 24.45. Found (%): C, 48.69; H, 3.91; N, 10.83; S, 24.57.

2-(1-(4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-5-yl)ethylidene)hydrazinecarboxamide (4c)

Yellow solid. Yield 84%. M.p. 375°C. ^{1}HNMR (DMSO-d_6, 500MHz) δ ppm: 8.93 (s, 1H, -NH-), 8.09 (s, 1H,



Compound	R	Compound	R
4a	-OH	4e	-NHPh
4b	$-OSO_3H$	4f	-NHPh-pBr
4c	$-NHC(=O)NH_2$	4g	-NHC(=O)Ph-pOH
4d	$-NHC(=S)NH_2$		

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-NH_2

thiazole-C_5H), 8.05 (dd, 2H, phenyl), 7.97 (dd, 2H, phenyl), 6.31 (s, 2H, -NH_2), 2.75 (s, 3H, -CH_3), 2.60 (s, 3H, -CH_3), 2.27 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125MHz) δ ppm: 166.49 (C), 166.36 (C), 163.50 (C), 153.48 (C=O), 150.81 (C), 139.92 (C=N), 136.17 (C), 132.87 (C), 127.16 (2CH), 126.97 (C), 124.03 (2CH), 115.58 (CH), 19.41 (CH_3), 17.19 (CH_3), 16.82 (CH_3). FT IR (KBr) ν cm^{-1} : 3470, 3286 (N-H str unsubstituted amide), 3347 (N-H amide), 3128 (C-H thiazole str) 3046 (C-H str arom), 2918 (C-H str CH_3), 1690 (C=O str), 1623 (C=N str), 1577, 1536, 1505, 1431 (Ar ring str), 980 (C-H bend arom), 849 (C-H def arom). MS (ESI, 70eV): *m/z* 372.3 ($M+H^+$). *Anal*.Calcd.for $C_{17}H_{17}N_5OS_2$ (%): C, 54.96; H, 4.61; N, 18.85; S, 17.26. Found (%): C, 54.87; H, 4.75; N, 18.99; S, 17.51.

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

Yellow solid. Yield 69%. M.p. 261°C.^{1}H NMR (DMSO-d_6, 500MHz) δ ppm: 9.55 (s, 1H, -NH-), 8.13 (s, 1H, thiazole-C_5H), 8.09 (dd, 2H, phenyl), 7.94 (dd, 2H, phenyl), 6.78 (s, 2H, -NH_2), 2.78 (s, 3H, -CH_3), 2.64 (s, 3H, -CH_3), 2.29 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125MHz) δ ppm: 171.29 (C=S), 166.86 (C), 166.53 (C), 163.38 (C), 153.82 (C), 139.99 (C=N), 136.75 (C), 132.22 (C), 126.96 (2CH), 126.58 (C), 124.81 (2CH), 115.12 (CH), 19.39 (CH_3), 17.21 (CH_3), 16.89 (CH_3). FT IR (KBr) ν cm^{-1} : 3298, 3145 (N-H str unsubstituted thioamide), 3252 (N-H str thioamide), 3116 (C-H thiazole str), 3019 (C-H str arom), 2920 (C-H str CH_3), 1617 (C=N str), 1582, 1518, 1505, 1430 (Ar ring str), 1267 (C=S str), 996 (C-H bend arom), 835 (C-H def arom). MS (ESI, 70eV): *m/z* 388.4 ($M+H^+$). *Anal*.Calcd.for $C_{17}H_{17}N_5S_3$ (%): 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.

4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)-5-(1-(2-phenylhydrazono)ethyl)thiazole (4e)

Orange solid. Yield 73%. M.p. 187°C. ^{1}H NMR (DMSO-d_6, 500MHz) δ ppm: 9.86 (s, 1H, -NH-), 8.10 (s, 1H, thiazole-C_5H), 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, -CH_3), 2.62 (s, 3H, -CH_3), 2.32 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 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 (CH_3), 17.44 (CH_3), 16.92 (CH_3). FT IR (KBr) ν cm^{-1}: 3232 (N-H secondary amine str), 3103 (C-H thiazole str), 3025 (C-H str arom), 2920 (C-H str CH_3), 1670 (C=N str), 1599, 1560, 1490, 1423 (Ar ring str), 1246 (C-N str arom amine), 994 (C-H bend arom), 849 (C-H def arom). MS

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

Compound	*C. albicans* ATCC 10231		*C. albicans* ATCC 18804		*C. krusei* ATCC 6258		*C. parapsilosis* ATCC 22019	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
4a	31.25	62.5	15.62	31.25	15.62	31.25	15.62	31.25
4b	31.25	62.5	15.62	31.25	15.62	31.25	15.62	31.25
4c	15.62	31.25	15.62	31.25	15.62	31.25	15.62	31.25
4d	15.62	31.25	15.62	31.25	15.62	31.25	15.62	31.25
4e	15.62	31.25	15.62	31.25	15.62	31.25	3.9	7.81
4f	3.9	7.81	7.81	15.62	7.81	15.62	3.9	7.81
4g	15.62	31.25	15.62	31.25	15.62	31.25	15.62	31.25
Fluconazole	15.62	31.25	15.62	31.25	15.62	31.25	7.81	15.62
Inoculum control	Growth in all concentrations							
Broth control	No growth							

(ESI, 70eV): *m/z* 405.7 ($M+H^+$). *Anal*. Calcd. for $C_{22}H_{20}N_4S_2$ (%):C, 65.32; H, 4.98; N, 13.85; S, 15.85. Found (%): C, 65.58; H, 4.79; N, 13.89; S, 15.74.

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. ^{1}H NMR (DMSO-d_6, 500MHz) δ ppm: 9.79 (s, 1H, -NH-), 8.12 (s, 1H, thiazole-C_5H), 8.11 (dd, 2H, phenyl), 8.07 (dd, 2H, phenyl), 8.04 (dd, 2H, phenyl), 7.69 (dd, 2H, phenyl), 2.75 (s, 3H, -CH_3), 2.58 (s, 3H, -CH_3), 2.25 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 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 (CH_3), 17.52 (CH_3), 16.81 (CH_3). 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 CH_3), 1644 (C=N str), 1593, 1556, 1486, 1428 (Ar ring str), 1250 (C-N str arom amine), 999 (C-H bend arom), 839 (C-H def arom), 497 (C-Br str). MS (ESI, 70eV): *m/z* 484.1 ($M+H^+$). *Anal*.Calcd.for $C_{22}H_{19}BrN_4S_2$ (%): 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, 11.48; 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. ^{1}H NMR (DMSO-d_6, 500MHz) δ ppm: 12.9 (s, 1H, OH), 9.65 (s, 1H, -NH-), 8.13 (s, 1H, thiazole-C_5H), 8.09 (dd, 2H, phenyl), 8.06 (dd, 2H, phenyl), 8.02 (dd, 2H, phenyl), 7.62 (dd, 2H, phenyl), 2.73 (s, 3H, -CH_3), 2.61 (s, 3H, -CH_3), 2.21 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 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 (CH_3), 16.82 (CH_3), 15.74 (CH_3). 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 CH_3), 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 $C_{23}H_{20}N_4O_2S_2$ (%): 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 C_5-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 ^{1}H 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 ^{13}C-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, ^{1}H-

NMR and ^{13}C-NMR are in agreement with the proposed structure of the new compounds.

Analysing the results obtained in the anti-*Candida* evaluation, we have observed that our tested compounds displayed significant inhibitory activity against all fungal strains. The anti-*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 *C. albicans* ATCC 10231 and a 2-fold greater activity than our reference drug, against *C. albicans* ATCC 18804, *C. krusei* and *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 *Candida albicans* and *Candida krusei* strains, with no difference in activity against *Candida parapsilosis*. With compound 4f exhibiting the most potent anti-*Candida* activity in our series, we managed to confirm that para-substitution of the phenyl ring is associated with enhanced anti-*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 *C. albicans* species and *C. krusei*, this compound proved to have a 2-fold higher activity against *C. parapsilosis,* in comparison with the reference antifungal drug. This is important because *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 *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.

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