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Studies on hydrazone derivatives as antifungal agents

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Abstract

The increasing clinical importance of drug-resistant fungal pathogens has urged additional need to fungal research and new antifungal compound development. For this purpose, some N-(1-benzyl-2-phenylethylidene)-N′-[4-(aryl)thiazol-2-yl]hydrazone **(1a-e)** and N-(1-phenylbutylidene)-N′-[4-(aryl)thiazol-2-yl]hydrazone **(2a-e)** derivatives were synthesised and evaluated for antifungal activity. Their antifungal activities against standard and clinical strands of *Candida albicans*, *Candida glabrata*, *Candida utilis*, *Candida tropicalis*, *Candida krusei*, *Candida zeylanoides*, and *Candida parapsilosis* were investigated. A significant level of activity was observed.

Keywords: *Thiazolylhydrazone, antifungal activity, Candida* spp.

Introduction

In recent years, fungal infections have increased markedly and one of the main agents is the opportunistic pathogen *Candida albicans*. A major obstacle in the treatment of *C. albicans* infections is the spread of antifungal drug resistance mainly in patients chronically subjected to antimycotic therapy, *i.e.*, those treated with broad-spectrum antibiotics, immunosuppressive agents, anticancer, and anti-AIDS drugs [1].

Azole and non-azole antifungal agents are usually used to treat *Candida* infections, but despite the good antifungal activities observed *in vitro*, candidemia is still a major cause of death [1]. Many valid therapy programs fail because of widespread secondary *C. albicans* infections. New effective anti-*Candida* agents are needed to combat the drug-resistant strains and widespread diffusion of *C. albicans*.

Sulfur and/or nitrogen heterocycles have acquired an enormous importance among the heterocycles, possessing pharmaceutical activities and widely occur in nature in the form of alkaloids, vitamins, pigments and as constituents of plant and animal cells. Thiazoles and their derivatives are found to be associated with various biological activities such as antimicrobial [2–8], antituberculosis [9], anti-HIV [10] activities.

On the other hand, the heterocyclic hydrazones constitute an important class of biologically active drug molecules which have attracted attention of medicinal chemists due to their wide ranging pharmacological properties. Literature survey revealed that hydrazones possess diverse chemotherapeutic activities including antimicrobial [11–13], antituberculosis [14–15], anti-HIV [16], anticonvulsant [17], antiinflammatory [18], antimalarial activities [19].

In view of the above observations, we have designed and synthesised some new hydrazone derivatives, bearing thiazole, alkylidene and phenyl functionality in the same molecule, as potential antifungal agents.

Experimental

Chemistry

All reagents were used as purchased from commercial suppliers without further purification. Melting points

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were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected. Thin-layer chromatography (TLC) was performed with glass plates (0.25 mm) precoated with Merck silica gel 60 F_{254}, and flash chromatography separations (FC) were carried out with Merck silica gel 60 (200–450 mesh), using 60/40 EtOAc/petroleum benzine as eluents. Spectroscopic data were recorded by the following instruments. IR: Shimadzu IR-435 spectrophotometer; ^{1}H-NMR Bruker 400 MHz spectrometer; MS: fast atom bombardment mass spectra (FAB-MS) were obtained by VG Quattro mass spectrometer. Microanalytical data were obtained by the Microanalytical Section of Service Center (CNRS, Ecole Normale de Chimie de Montpellier, France).

General procedure for synthesis of the compounds

1-(1-benzyl-2-phenylethylidene)thiosemicarbazone and 1-(1-arylbutylidene)thiosemicarbazone. In a flask equipped with a reflux condenser, a mixture of thiosemicarbazide (40 mmol) and the appropriate ketone derivatives (40 mmol) are reacted in 80 mL isopropylalcohol in the presence of a catalytic amount of acetic acid. The mixture was then refluxed for 1 h. and the obtained solid was filtered and used without further purification.

N-(1-benzyl-2-phenylethylidene)-N′-[4-(aryl)thiazol-2-yl]hydrazones ***(1a-e)*** *and N-(1-phenylbutylidene)-N′-[4-(aryl)thiazol-2-yl]hydrazone* ***(2a-e)***. The thiosemicarbazone (20 mmol) and appropriate phenacyl halide (20 mmol) were stirred in refluxing isopropyl alcohol (80 ml) to complete dissolution, and then until a white foaming product was formed. The mixture was then allowed to cool and the solid filtered, dried, and crystallised from ethanol.

Some characteristics of the synthesized compounds are shown in Table I. Analytical and spectral data (IR, ^{1}H-NMR, FAB$^+$-MS) confirmed the structures of the new compounds.

N-(1-benzyl-2-phenylethylidene)-N′-[4-(phenyl)thiazol-2-yl]hydrazone ***(1a)***. Yield: 56%. M.p.: 154°C. IR (KBr) υ_{max}(cm^{-1}): 3180 (NH), 1600 (C=N), 1534 (C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.51 (2H, s, CH_2), 3.75 (2H, s, CH_2), 7.27 (1H, s, C_5H-thiazole), 7.30–7.89 (15H, m, aromatic protons), 11.51 (1H, s, NH, D_2O exchangeable). For $C_{24}H_{21}N_3S$ calculated: 75.16% C, 5.52% H, 10.96% N; found: 75.41% C, 5.56% H, 10.93% N. MS-FAB$^+$: m/z: 384 [M + 1].

Table I. Some characteristics of the compounds.

Comp.	R	M.P. (°C)	Yield (%)	Mol. For.	MW
1a	H	154	56	$C_{24}H_{21}N_3S$	383
1b	OCH_3	180	60	$C_{25}H_{23}N_3OS$	412
1c	NO_2	176	68	$C_{24}H_{20}N_4O_2S$	428
1d	Cl	185	62	$C_{24}H_{20}ClN_3S$	417
1e	2,5-diOCH_3	162	58	$C_{26}H_{25}N_3O_2S$	443
2a	CH_3	212	53	$C_{20}H_{21}N_3S$	335
2b	OCH_3	166	57	$C_{20}H_{21}N_3OS$	351
2c	NO_2	214	68	$C_{19}H_{18}N_4O_2S$	366
2d	Cl	213	59	$C_{19}H_{18}ClN_3S$	355
2e	2,5-diOCH_3	191	61	$C_{21}H_{23}N_3O_2S$	381

N-(1-benzyl-2-phenylethylidene)-N′-[4-(4-methoxyphenyl)thiazol-2-yl]hydrazone ***(1b)***. Yield: 60%. M.p.: 180°C. IR (KBr) υ_{max}(cm^{-1}): 3141 (NH), 1610 (C=N), 1525 (C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.53 (2H, s, CH_2), 3.77 (2H, s, CH_2), 3.81 (3H, s, 4-OCH_3-phenyl), 7.11 (1H, s, C_5H-thiazole), 7.17-7.81 (14H, m, aromatic protons), 11.56 (1H, s, NH, D_2O exchangeable). For $C_{25}H_{23}N_3OS$ calculated: 72.61% C, 5.61% H, 10.16% N; found: 72.46% C, 5.64% H, 10.20% N. MS-FAB$^+$: m/z: 413 [M + 1], 414 [M + 2].

N-(1-benzyl-2-phenylethylidene)-N′-[4-(4-nitrophenyl)thiazol-2-yl]hydrazone ***(1c)***. Yield: 68%. M.p.: 176°C. IR (KBr) υ_{max}(cm^{-1}): 3192 (NH), 1594 (C=N), 1517 (C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.50 (2H, s, CH_2), 3.79 (2H, s, CH_2), 7.70 (1H, s, C_5H-thiazole), 7.10–7.40 (10H, m, aromatic protons), 8.10 and 8.28 (4H, two d [$\mathcal{J}$ = 7.00 and 7.05 Hz] 1,4-disubstituted phenyl protons), 11.50 (1H, s, NH, D_2O exchangeable). For $C_{24}H_{20}N_4O_2S$ calculated: 67.27% C, 4.70% H, 13.07% N; found: 67.37% C, 4.68% H, 13.01% N. MS-FAB$^+$: m/z: 428 [M], 429 [M + 1].

N-(1-benzyl-2-phenylethylidene)-N'-[4-(4-chlorophenyl)thiazol-2-yl]hydrazone ***(1d)***. Yield: 62%. M.p.: 185°C. IR (KBr) υ_{max}(cm^{-1}): 3220 (NH), 1562 (C=N), 1513 (C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.53 (2H, s, CH_2), 3.72 (2H, s, CH_2), 7.35 (1H, s, C_5H-thiazole), 7.15–7.35 (10H, m, aromatic protons), 7.47 and 7.87 (4H, two d [$\mathcal{J}$ = 6.78 and 8.54 Hz] 1,4-disubstituted phenyl protons), 11.30 (1H, s, NH, D_2O exchangeable). For $C_{24}H_{20}ClN_3S$ calculated: 68.97% C, 4.82% H, 10.05% N; found: 68.83% C, 4.78% H, 10.07% N. MS-FAB$^+$: m/z: 417 [M], 418 [M + 1], 419 [M + 2].

N-(1-benzyl-2-phenylethylidene)-N′-[4-(2,5-dimethoxyphenyl)thiazol-2-yl]hydrazone ***(1e)***. Yield: 58%. M.p.: 162°C. IR (KBr) υ_{max} (cm^{-1}): 3200 (NH), 1573 (C=N), 1529 (C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.54 (2H, s, CH_2), 3.62 (2H, s, CH_2), 3.75 (3H, s, 2,5-diOCH_3-phenyl), 3.85 (3H, s, 2,5-diOCH_3-phenyl), 7.42 (1H, s, C_5H-thiazole), 6.83-7.58 (13H, m, aromatic protons), 11.75 (1H, s, NH, D_2O exchangeable). For $C_{26}H_{25}N_3O_2S$ calculated: 70.40% C, 5.68% H, 9.47% N; found: 70.37% C, 5.67% H, 9.39% N. MS-FAB$^+$: m/z: 444 [M + 1].

N-(1-phenylbutylidene)-N′-[4-(4-methylphenyl)thiazol-2-yl]hydrazone ***(2a)***. Yield: 53%. M.p.: 212°C. IR (KBr) υ_{max}(cm^{-1}): 3131 (NH), 1620 (C═N), 1555 (C═C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 1.04 (3H, t, CH_3), 1.50 (2H, m, CH_2), 2.82 (2H, t, CH_2), 3.02 (3H, s, 4-CH_3-phenyl), 7.21 (1H, s, C_5H-thiazole), 7.24-7.42 (9H, m, aromatic protons), 11.56 (1H, s, NH, D_2O exchangeable). For $C_{20}H_{21}N_3S$ calculated: 71.61% C, 6.31% H, 12.53% N; found: 71.56% C, 6.44% H, 12.50% N. MS-FAB$^+$: m/z: 336 [M + 1].

N-(1-phenylbutylidene)-N′-[4-(4-methoxyphenyl)thiazol-2-yl]hydrazone ***(2b)***. Yield: 57%. M.p.: 166°C. IR (KBr) υ_{max}(cm^{-1}): 3175 (NH), 1581 (C═N), 1519 (C═C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 1.07 (3H, t, CH_3), 1.59 (2H, m, CH_2), 2.92 (2H, t, CH_2), 3.72 (3H, s, 4-OCH_3-phenyl), 7.56 (1H, s, C_5H-thiazole), 7.58-7.88 (9H, m, aromatic protons), 12.55 (1H, s, NH, D_2O exchangeable). For $C_{20}H_{21}N_3OS$ calculated: 68.35% C, 6.02% H, 11.96% N; found: 68.00% C, 6.09% H, 11.50% N. MS-FAB$^+$: m/z: 351 [M], 352 [M + 1].

N-(1-phenylbutylidene)-N′-[4-(4-nitrophenyl)thiazol-2-yl]hydrazone ***(2c)***. Yield: 68%. M.p.: 214°C. IR (KBr) υ_{max}(cm^{-1}): 3198 (NH), 1600 (C═N), 1572 (C═C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 1.06 (3H, t, CH_3), 1.57 (2H, m, CH_2), 2.88 (2H, t, CH_2), 7.72 (1H, s, C_5H-thiazole), 7.13-7.45 (5H, m, aromatic protons), 8.06 and 8.12 (4H, two d [$\mathcal{J}$ = 7.02 and 7.05 Hz] 1,4-disubstituted phenyl protons), 11.45 (1H, s, NH, D_2O exchangeable). For $C_{19}H_{18}N_4O_2S$ calculated: 62.28% C, 4.95% H, 15.29% N; found: 62.37% C, 4.69% H, 15.26% N. MS-FAB$^+$: m/z: 366 [M], 367 [M + 1].

N-(1-phenylbutylidene)-N′-[4-(4-chlorophenyl)thiazol-2-yl]hydrazone ***(2d)***. Yield: 59%. M.p.: 213 °C. IR (KBr) υ_{max}(cm^{-1}): 3167 (NH), 1544 (C═N), 1522 (C═C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 0.99 (3H, t, CH_3), 1.50 (2H, m, CH_2), 2.82 (2H, t, CH_2), 7.39 (1H, s, C_5H-thiazole), 7.35-7.60 (5H, m, aromatic protons), 7.78 and 7.92 (4H, two d [$\mathcal{J}$ = 7.68 and 7.46 Hz] 1,4-disubstituted phenyl protons), 11.42 (1H, s, NH, D_2O exchangeable). For $C_{19}H_{18}ClN_3S$ calculated: 64.12% C, 5.10% H, 11.81% N; found: 64.36% C, 5.07% H, 11.80% N. MS-FAB$^+$: m/z: 355 [M], 356 [M + 1], 357 [M + 2], 358 [M + 3].

N-(1-phenylbutylidene)-N′-[4-(2,5-dimethoxyphenyl)thiazol-2-yl]hydrazone ***(2e)***. Yield: 61%. M.p.: 191 °C. IR (KBr) υ_{max}(cm^{-1}): 3187 (NH), 1584 (C═N), 1510 (C═C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 1.11 (3H, t, CH_3), 1.52 (2H, m, CH_2), 2.90 (2H, t, CH_2), 3.78 (3H, s, OCH_3-phenyl), 3.87 (3H, s, OCH_3-phenyl), 7.60 (1H, s, C_5H-thiazole), 6.91–7.85 (8H, m, aromatic protons), 12.35 (1H, s, NH, D_2O exchangeable). For $C_{21}H_{23}N_3O_2S$ calculated: 66.12% C, 6.08% H, 11.01% N; found: 66.36% C, 6.07% H, 11.00% N. MS-FAB$^+$: m/z: 381 [M], 382 [M + 1].

Microbiology

Microorganisms. Microorganisms were obtained from ATCC, NRRL and clinical isolates (Faculty of Medicine, Eskisehir Osmangazi University, Turkey) and were stored in 15% glycerol containing micro-test tubes at −86°C (strain numbers of microorganisms are given in Table II). All *Candida strains* were inoculated on Sabouraud Dextrose Agar (SDA) prior to the experiments at 37°C. After sufficient growth *Candida* spp. were then transferred to Mueller Hinton Broth (MHB) for further incubation under the same conditions for another 24 h.

Anticandidal assay. The activity of the compounds (**1a-e, 2a-e**) were first screened using an agar diffusion

Table II. MIC values mg/ml of compounds **1a-e** and **2a-e**.

Compounds	A⋆	B	C⋆	D	E	F	G	H
1a	0.2500	0.2500	0.5000	0.2500	0.1250	0.5000	1.0	0.0625
1b	0.2500	0.0300	0.2500	0.2500	0.2500	0.5000	0.5000	0.1250
1c	>2.0	>2.0	–	–	>2.0	–	–	–
1d	0.1250	0.0300	0.2500	0.1250	0.2500	0.5000	0.5000	0.0625
1e	0.5000	0.5000	0.5000	1.0	2.0	1.0	1.0	1.0
2a	0.2500	0.1250	1.0	1.0	1.0	1.0	1.0	0.5000
2b	>2.0	>2.0	–	–	>2.0	–	–	–
2c	0.1250	0.0156	0.5000	0.5000	0.1250	0.0625	0.1250	0.0312
2d	0.2500	0.1250	0.0625	0.2500	0.1250	0.2500	0.2500	0.1250
2e	>2.0	>2.0	–	–	>2.0	–	–	–
Ketoconazole	0.0625	0.0625	0.1250	0.0312	0.0078	0.0312	0.2500	0.0156

A⋆: *Candida albicans* (isolates obtained from Osmangazi University, Faculty of Medicine, Department of Microbiology, Eskisehir, Turkey), B: *Candida albicans* (ATCC 90028), C⋆: *Candida glabrata* (isolates obtained from Osmangazi University, Faculty of Medicine, Department of Microbiology, Eskisehir, Turkey), D: *Candida utilis* (NRLL Y-900), E: *Candida tropicalis* (NRLL Y-12968), F: *Candida krusei* (NRLL Y-7179), G: *Candida zeylanoides* (NRLL Y-1774), H: *Candida parapsilosis* (NRLL Y-12696).

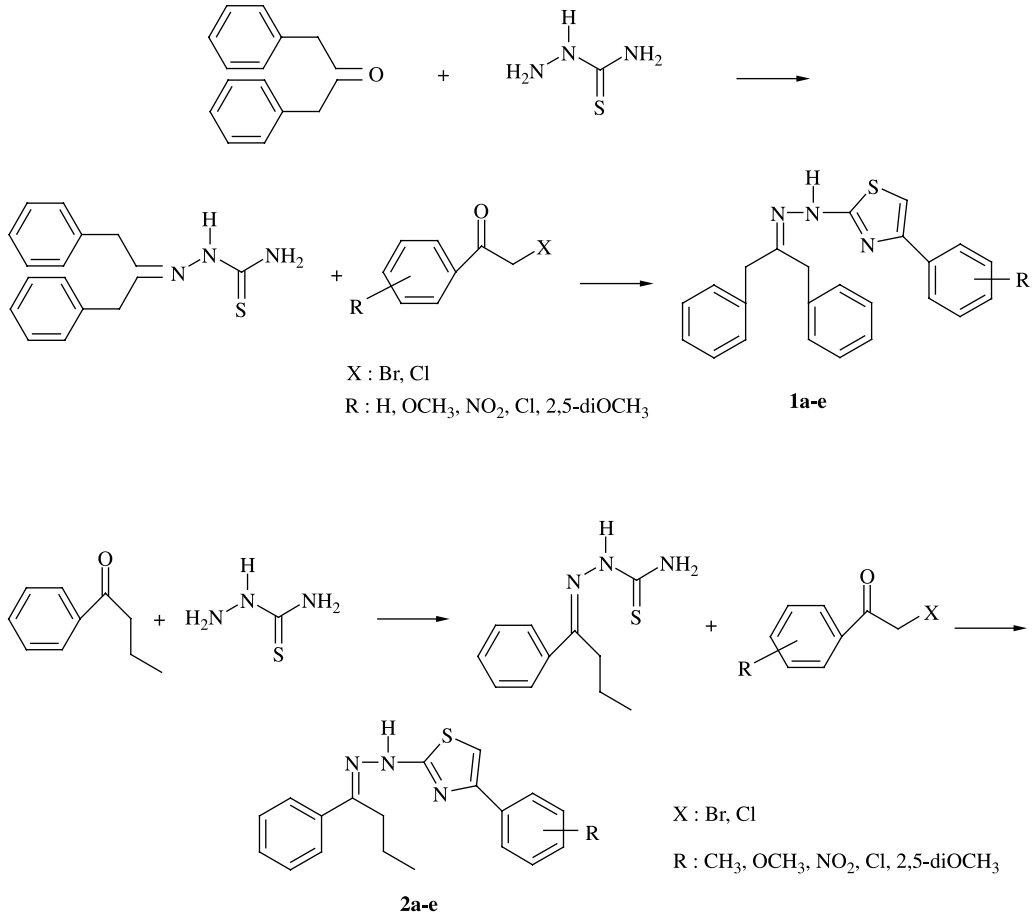
method for *C. albicans* (clinical isolate) and *C. tropicalis* and all active compounds (inhibition zones $>9-11$ mm, at 2 mg/ml concentration) were further evaluated using the microdilution broth method to identify the minimum inhibitory concentrations against all *Candida* spp. in Table II [22,23].

Agar diffusion assay. 1 ml of pregrown *Candida* suspension in MHB adjusted to McFarland No. 0.5 were inoculated to each Petri plate (9 cm and 20 ml MHA) under sterile conditions. With the aid of an sterile cork borer (8 mm) wells were formed in each Petri plate to accommodate the test samples (50 microlitre from a stock solution of 2 mg/ml DMSO) including the solvent and antifungal controls to incubate at 37°C for 24 hrs. Inhibition zones were visualized using a tetrazolium salt (TTC, Aldrich) where clear inhibition zones ≥ 9 mm were considered as active, for further testing.

Microdilution assay. The test compounds and the antimicrobial standards were first dissolved in dimethyl sulfoxide (DMSO) which was used to prepare the stock solutions at an initial concentration of 2 mg/ml. Serial dilution series were prepared in 100 µl MHB with an equal amount of the test samples. The last row was filled only with water as growth control for the microorganism. Overnight grown microorganism suspensions were first diluted in double strength MHB and standardized to 10^8 CFU/ml (using McFarland No: 0.5) under sterile conditions. Then each microorganism suspension was pippetted into each well and incubated at 37 °C for 24 h. Ketoconazole was used as a standard antifungal agent against *Candida* spp. Sterile distilled water and medium served as a positive growth control. The first well without turbidity was assigned as the minimum inhibitory concentration (MIC, in mg/ml). Average results of separately performed three experiments as given in Table II.

Results and discussion

In this present work, thiazolylhydrazone derivatives **(1a-e; 2a-e)** were synthesized by in accordance with the method described in the literature [20,21]. Aryl ketones reacted with thiosemicarbazide to give the required thiosemicarbazones. Compounds **(1a-e;**



Scheme 1. Synthetic route to the title compounds

2a-e) were obtained by reacting the thiosemicarbazones with phenacyl halide or its derivatives in isopropyl alcohol (Scheme 1). Substitution of the *para* position of phenacyl halide played an important role in the thiazole formation step. Namely, the phenacyl halide derivatives with a strong electron-withdrawing group like NO_2 in *para* position of the benzene ring, gave higher yield compared to the other groups.

Formulas of compounds **(1a-e; 2a-e)** (Table I) were found by elemental analyses and their structures were determined by IR, ^{1}H-NMR and FAB$^+$-MS spectral data. IR data were very informative and provided evidence for the formation of the expected structures. In the IR spectra, some significant stretching bands due N—H, C=N and C=C were at 3220–3131 cm^{-1}, 1620–1510 cm^{-1} respectively. The ^{1}H-NMR spectra data were also consistent with the assigned structures. In the 400 MHz ^{1}H-NMR spectrum of compounds; NH proton were observed as a singlet at 11.30–12.55 ppm., all the other aromatic and aliphatic protons were observed at expected regions. The mass spectra (MS(FAB)) of compounds showed [M + 1] peaks, in agreement with their molecular formula. All compounds gave satisfactory elemental analysis.

The antifungal activity of the compounds was studied with eight pathogenic fungi. Ketoconazole has been used as reference for inhibitory activity against fungi. MIC's were recorded as the minimum concentration of a compound that inhibits the growth of tested microorganisms. All of the compounds tested showed good antifungal activity when compared with ketoconazole. The MIC values were generally within the range of 0.0312–2 mg/ml against all evaluated strains. The results are summarized in Table II.

In comparing their MIC values with ketoconazole, compounds **1b**, **1d**, **2a**, **2d** were effective against *C. albicans* (ATCC 90028). Compounds **1b** and **1d** especially showed strong activity. Compounds **2a** and **2d** showed moderate activity when compared with the reference agent. Compounds **1b**, **1d**, **2c**, **2d** were effective against *C. zeylanoides* (NRLL Y-1774). Compound **2c** showed strong activity. Compounds **2d** showed a similar level of activity with ketoconazole and **1b,** and **1d** showed moderate activity.

In comparing their MIC values with the reference agent, ketoconazole, compounds **1b**, **1d**, **2d** were effective against *C. glabrata* (Clinical Isolate). Compound **2d** especially showed high activity. Compound **1b** and **1d** showed moderate activity. On the other hand the compounds exhibited comparable activities against *C. albicans* (Cinical Isolate). Compounds **1d** and **2c** showed moderate activity and the other compounds were found less active than the reference agent.

From the similar results obtained with *C. krusei* (NRLL Y-7179) and *C. parapsilosis* (NRLL Y-12696), compound **2c** showed moderate activity, whereas all other compounds showed less activity when compared with ketoconazole. The compounds were less active against *C. utilis* (NRRL Y-900) and *C. tropicalis* (NRLL Y-12968).

Considering all the results obtained from antifungal test, in comparison with reference agents, it is possible to say that the tested **1b**, **1d**, **2a**, **2c**, and **2d** compounds are mostly active than other compounds.

Based on the limited number of compounds evaluated, it appears that 4-chloro **(1d) (2d)**, 4-methyl **(2a)**, 4-methoxy **(1b)**, and nitro **(2c)** substitution on the phenyl ring attached to the thiazole moiety has made a good contribution to the antifungal activity in this series of thiazolyl-hydrazon combination. Meanwhile, the modification on alkylidene moiety has been played interesting role on the activity.

It is well known that azole antifungal agents act on the synthesis of the fungal ergosterol by inhibiting the cytochrome P450-dependent enzyme lanosterol demethylase which also plays an important role in cholesterol synthesis in mammals. In therapeutic concentrations, azole antifungal efficacy is attributed to their greater affinity and selectivity for fungal P-450_{DM} than for the mammalian enzyme. Generally, exposure of fungi to an active azole causes depletion of ergosterol and accumulation of 14-methylated sterols, which interferes with the important functions of ergosterol in fungal membranes. Azoles disrupt both the structure of the membrane and several of its functions such as nutrient transport and fungal chitin synthesis [24].

As the thiazole ring in the present study is a bioisoster of the imidazole ring, which also include important azole antifungal therapeutics, it can be concluded that the compounds also display remarkable antifungal activity by using same the mechanism of the current azole antifungals. It is worthwhile to extend the bioactivity evaluation from whole cells to an enzymatic level.

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