European Journal of Medicinal Chemistry 46 (2011) 378–382

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and biological evaluation of novel 2,4-disubstituted-1,3-thiazoles as anti-*Candida* spp. agents

Franco Chimenti [a], Bruna Bizzarri [a,*], Adriana Bolasco [a], Daniela Secci [a], Paola Chimenti [a], Arianna Granese [a], Simone Carradori [a], Melissa D'Ascenzio [a], Daniela Lilli [b], Daniela Rivanera [b]

[a] Dipartimento di Chimica e Tecnologie del Farmaco, Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy
[b] Dipartimento di Scienze di Sanità Pubblica e Malattie Infettive, Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy

ARTICLE INFO

Article history:
Received 23 July 2010
Received in revised form
19 October 2010
Accepted 22 October 2010
Available online 29 October 2010

Keywords:
Thiazole
Hydrazone
Candida spp.
Hantzsch reaction

ABSTRACT

A new series of [4-(4′-substituted-phenyl)thiazol-2-yl]hydrazine derivatives were synthesized in good yield (86–99%) and characterized by elemental analysis, IR, ^{1}H NMR, and mass spectral studies. The compounds were assayed for their *in vitro* broad-spectrum antifungal activity, compared to clotrimazole and fluconazole, against 20 clinical isolates of pathogenic *Candida* spp., representing five different species. The results showed that the presence of heterocyclic or bicyclic rings on hydrazone moiety in position C2 of thiazole revealed a promising selective inhibitory activity especially against *Candida albicans* and *Candida glabrata*.

1. Introduction

In recent years, epidemiological studies confirm the significant impact upon human health by infections caused by pathogenic fungi [1,2]. In fact, although the genus *Candida* is present as commensal flora in the majority of healthy people, at the same time it is a common fungus responsible for opportunistic infections and it can become pathogenous because of predisposing conditions related to the host, like immunological compromise (AIDS, anti-cancer therapy, and transplants), excessive prophylaxis with antimicrobial agents, and extended use of invasive catheters [3]. Large-scale surveillance for fungal infections has demonstrated an increasing incidence of drug-resistant fungal pathogens. As a matter of fact, a significant number of fungi species (especially *Candida glabrata* and *Candida krusei*) exhibited primary resistance to amphotericin B or was less susceptible to triazoles [4]. Furthermore, as a consequence of the toxicity of the currently used polyene antifungal drugs, which leads to interrupt the therapy, and the emergence of candidal species resistant to azole-based agents, there is an urgent need for developing alternative drug therapies [5].

In the recent literature [6–11], (4-aryl-thiazol-2-yl)hydrazines have been investigated for their significant antimicrobial activity against a variety of clinically relevant fungal strains. In particular, these studies confirmed that thiazole derivatives are good pharmacophores for the design of bioactive molecules [12,13] as bioisoster of the imidazole ring, and it emerged a different mode of action, inhibiting the fungal biofilm growth, in relation to azoles and ampothericin B, a low toxicity profile, and a broad fungicidal spectrum [14]. For these reasons and pursuing our research in the field [15,16], in this paper we report on the synthesis and the biological evaluation (*in vitro* antifungal activity and synergy with clotrimazole) of a new series of 2,4-disubstituted-1,3-thiazoles bearing a bicyclic or heterocyclic ring on double bond C=N and a 4′-substituted (CH_3, OCH_3) phenyl in position C2 and C4 of thiazole nucleus, respectively (Table 1). The choice of these electron-releasing substituents on the aryl ring derived from the structure-activity relationships we extrapolated in our previous work [15]. According to the established guidelines of Clinical and Laboratory Standards Institute (CLSI) and the European Committe for Antimicrobial Susceptibility Testing (EUCAST), we assayed the susceptibility of clinical *Candida* spp. strains to our compounds by determining their minimum inhibitory concentration (MIC) [17].

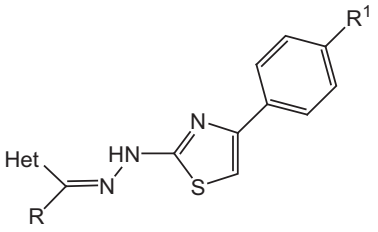
2. Chemistry

The new derivatives were synthesized successfully as reported in our previous communications [15,16,18]. Different carbonyl compounds were reacted directly with thiosemicarbazide with catalytic amounts of acetic acid in 2-propanol and the obtained

* Corresponding author. Tel.: +39 06 49913259; fax: +39 06 49913772.
E-mail address: bruna.bizzarri@uniroma1.it (B. Bizzarri).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.10.027

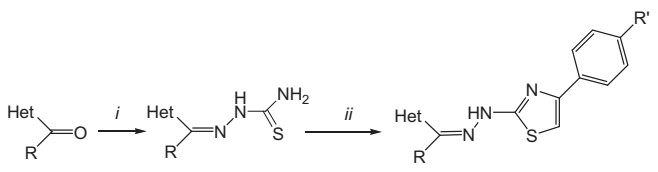
Table 1
Chemical and physical data of derivatives **1**–**28**.



Comp	Het	R	R^1	Mp (°C)	% Yield	ClogP[a]	Formula	*m/z*
1	Fur-2-yl	H	CH_3	193–194	99	4.47	$C_{15}H_{13}N_3OS$	283.34
2	Fur-2-yl	H	OCH_3	198–199	99	4.00	$C_{15}H_{13}N_3O_2S$	299.34
3	Fur-2-yl	CH_3	CH_3	249–250	87	4.36	$C_{16}H_{15}N_3OS$	297.37
4	Fur-2-yl	CH_3	OCH_3	229–230	99	3.88	$C_{16}H_{15}N_3O_2S$	313.37
5	Thiophen-2-yl	H	CH_3	240–241	99	5.04	$C_{15}H_{13}N_3S_2$	299.41
6	Thiophen-2-yl	H	OCH_3	211–212	99	4.56	$C_{15}H_{13}N_3OS_2$	315.41
7	Thiophen-2-yl	CH_3	CH_3	252–253	89	4.83	$C_{16}H_{15}N_3S_2$	313.44
8	Thiophen-2-yl	CH_3	OCH_3	249–250	86	4.35	$C_{16}H_{15}N_3OS_2$	329.44
9	Pyridin-2-yl	CH_3	CH_3	210–211	89	3.69	$C_{17}H_{16}N_4S$	308.40
10	Pyridin-2-yl	CH_3	OCH_3	219–220	89	3.21	$C_{17}H_{16}N_4OS$	324.40
11	Pyridin-3-yl	H	CH_3	221–222	86	4.06	$C_{16}H_{14}N_4S$	294.37
12	Pyridin-3-yl	H	OCH_3	210–211	99	3.58	$C_{16}H_{14}N_4OS$	310.37
13	Pyridin-3-yl	CH_3	CH_3	230–231	99	3.69	$C_{17}H_{16}N_4S$	308.40
14	Pyridin-3-yl	CH_3	OCH_3	257–258	86	3.21	$C_{17}H_{16}N_4OS$	324.40
15	Pyridin-4-yl	H	CH_3	245–247	90	4.06	$C_{16}H_{14}N_4S$	294.37
16	Pyridin-4-yl	H	OCH_3	265–267	99	3.58	$C_{16}H_{14}N_4OS$	310.37
17	Pyridin-4-yl	CH_3	CH_3	256–258	88	3.69	$C_{17}H_{16}N_4S$	308.40
18	Pyridin-4-yl	CH_3	OCH_3	243–245	86	3.21	$C_{17}H_{16}N_4OS$	324.40
19	Naphthalen-1-yl	H	CH_3	209–210	86	6.47	$C_{21}H_{17}N_3S$	343.44
20	Naphthalen-1-yl	H	OCH_3	207–208	87	5.99	$C_{21}H_{17}N_3OS$	359.44
21	Naphthalen-2-yl	CH_3	CH_3	245–246	88	6.36	$C_{22}H_{19}N_3S$	357.47
22	Naphthalen-2-yl	CH_3	OCH_3	233–234	99	3.99	$C_{22}H_{19}N_3OS$	373.47
23	Benzodioxol-5-yl	H	CH_3	245–247	88	5.54	$C_{18}H_{15}N_3O_2S$	337.39
24	Benzodioxol-5-yl	H	OCH_3	219–220	99	5.06	$C_{18}H_{15}N_3O_3S$	353.39
25	Indol-3-yl	H	CH_3	226–227	90	5.76	$C_{19}H_{16}N_4S$	332.42
26	Indol-3-yl	H	OCH_3	231–232	99	5.29	$C_{19}H_{16}N_4OS$	348.42
27	Coumarin-3-yl	CH_3	CH_3	205–206	90	4.45	$C_{21}H_{17}N_3O_2S$	375.44
28	Coumarin-3-yl	CH_3	OCH_3	235–236	99	3.98	$C_{21}H_{17}N_3O_3S$	391.44

[a] ChemDraw Ultra 8.0.

thiosemicarbazones subsequently were condensed with α-bromo-4-methyl/4-methoxyacetophenone to 2,4-disubstituted-1,3-thiazoles (Hantzsch reaction) as shown in Scheme 1. Our choice of 2-propanol as solvent let the final products precipitate without further purification. All synthesized compounds were fully characterized by analytical and spectral data and were listed in Table 1. In general, they showed in the IR spectrum strong bands at about 3100 and 1590 cm^{-1} due to the presence of N–H and C=N group, respectively. In MS spectra, the fragment peaks which correspond to loss of $-CH_3$ or $-OCH_3$ from the molecular ion are consistent with the postulated structure. Characteristic M + 2 isotope peaks relative to sulphur atom are generally observed in the mass spectra. Furthermore, because of the hindered rotation of the double bond around the C=N group, the synthesized products could exist as couple of conformers *E*/*Z*, each interacting in different way with the biological target. Diastereoselective dynamic HPLC and theoretical studies about the *E*/*Z* interconversion and the configurational stability of the thiosemicarbazone derivatives showed that (*E*)-form dominated on (*Z*)-form [19]. The synthesis of some compounds (**5**–**7**, **23**, **24**, and **26**) has been described in previous references [11,20–23] and was performed with slight changes; their analytical and spectral data were in full agreement with those reported in the literature.



Scheme 1. Synthesis of compounds **1**–**28**. Reagents: (i) thiosemicarbazide, 2-propanol, acetic acid (cat.), rt; (ii) α-bromo-4-substituted-acetophenone, 2-propanol, rt. For substituents see Table 1.

3. Pharmacology

Compounds **1**–**28** were first evaluated for their antifungal activity against 20 clinical isolates of *Candida* spp. and compared with the reference drugs clotrimazole and fluconazole (Table 2). The used clinical isolates were recently collected from specimens of patients at the 'Azienda Policlinico Umberto I°' of Rome 'La Sapienza' University and were obtained from haematology/oncology and surgery departments, which also included an intensive care unit. In particular, the samples were isolated from the upper and lower respiratory tract, blood, and indwelling venous catheters; the isolates were identified by conventional methodologies. Prior to testing, each isolate was subcultered on a qualified medium to ensure purity and optimal growth.

Table 2
Minimal inhibitory concentration of compounds **1**–**28**, clotrimazole (C), and fluconazole (F) against 20 clinical strains of *Candida* species. Antifungal agents were distributed in the wells of microtiter plates, giving final drug concentrations between 0.125/256 μg/ml.

Comp	Tested fungi (MIC values μg/mL)				
	C. glabrata (4 strains)	*C. albicans* (8 strains)	*C. kruzei* (3 strains)	*C. sakè* (2 strains)	*C. tropicalis* (3 strains)
1	4–16	0.50–2	32–64	16	64
2	4–16	0.50–2	16–64	32	64–128
3	4–16	0.50–2	64–128	32	64–128
4	4–16	0.50–2	16–64	16	128
5	0.50–2	4–32	16–64	8–16	64–128
6	0.50–2	0.125–2	64–128	32	128
7	2–4	0.50–2	32–64	32	128
8	2–4	0.125–2	64	32	8
9	4–16	2–8	64	32	64–128
10	8–16	0.50–4	16–32	32	64–128
11	0.50–2	0.125–4	32–64	16	128
12	16–64	4–32	32–64	16	64–128
13	2–8	0.50–2	64	16–32	16
14	8–32	8–64	128–256	32	128
15	4–16	0.50–8	128–256	32	64
16	0.50–2	0.125–2	16	32	16
17	4–16	0.50–4	32	32	32–64
18	4–8	0.50–4	128–256	16–32	32–64
19	16–64	2–8	16	32	16–64
20	2–8	4–16	32–64	16	64
21	0.50–2	2–8	32–64	16	4–8
22	2–4	2–8	256	16	64
23	4–16	0.125–2	64–128	8–16	16–64
24	4–16	0.50–2	64–128	8–16	64
25	4–16	0.125–2	32–64	32	64–128
26	0.50–2	32	64	8–16	64–128
27	0.50–2	0.125–2	128	32	128
28	2–4	2	32–64	32	2–8
C	2–8	2–8	4–16	16	4–8
F	4–16	4–64	8–32	32	4–16

Furthermore, the most active compounds **6**, **7**, **8**, **11**, **16**, and **27** (drug concentration range 0.5–4 μg/mL) were tested against a number of clinical isolates of *C. albicans* and *C. glabrata* in combination with the most active reference azole (clotrimazole at concentration range 0.125–256 μg/mL), to evaluate the possible interaction between the substances on the fungal strains' growth [24] and to have in this way a preliminary insight of the possible mechanism of action of this scaffold (Table 3).

4. Results and discussion

The data reported in Table 3 showed that all compounds have a lower or similar antifungal activity with respect to clotrimazole or fluconazole against *C. kruzei*, *C. sakè*, and *C. tropicalis*. On the other hand, in the case of *C. albicans* and *glabrata*, most of the tested agents (**6**–**8**, **11**, **16**, **27**, and **28**) revealed a better selectivity toward these strains exhibiting an anti-mycotic activity comparable or higher than that of the reference compounds. As regards the relationships between the structure of the heterocyclic scaffold and the detected anti-*Candida* activity, thiophene (with R=H or CH_3), pyridine (with R=H), and coumarin (with R=CH_3) are endowed with the best *in vitro* inhibitory activity. As far as the para position of the aromatic ring (R′) is concerned, the substitution with a methyl or methoxy group did not influence the activity but there is an evidence that derivatives bearing a 4′-OCH_3 moiety could improve the inhibitory activity.

Table 3
Effects of the most active compounds on *C. albicans*[a] and *C. glabrata*[b] to sensitivity to clotrimazole (C).

Comp	18 h, MIC (μg/ml)		24 h, MIC (μg/ml)		48 h, MIC (μg/ml)	
	C. albicans (4 strains)	*C. glabrata* (2 strains)	*C. albicans* (4 strains)	*C. glabrata* (2 strains)	*C. albicans* (4 strains)	*C. glabrata* (2 strains)
C	2	4	4	4	4	8
C + **6**	0.5	2	2	2	2	2
C + **7**	1	4	2	4	2	4
C + **8**	2	4	2	4	2	4
C + **11**	0.5	2	2	2	2	2
C + **16**	1	2	2	2	2	2
C + **27**	1	2	2	2	2	2

[a] *C. albicans* strains isolated from bronchial washing of transplant patient and from blood culture of surgery unit's patient.
[b] *C. glabrata* strains isolated from venous catheter of coronary unit's patient and from wound's swab of surgery unit's patient. Data are reported at the best concentrations of selected compounds and reference drug shown in Table 2.

Furthermore, as depicted in Table 3, the addition of **6**, **7**, **8**, **11**, **16**, and **27** derivatives to clotrimazole demonstrated to enhance the susceptibility of clinical isolates of *C. albicans* and *C. glabrata* to treatment. The best concentrations of the selected compounds and clotrimazole resulted the lowest MIC values reported in Table 2. In particular, compounds **6** and **11** gave a 2-fold reduction of the MIC values against *C. albicans* with respect to clotrimazole alone at 18 h. When the MIC end points were determined following 24 and 48 h of incubation, the observed anti-*Candida* effect was slightly lower but it remained constant in time. The reported data suggested that the combined treatment could lead to an encouraging synergic effect on the fungal strains' growth which might be useful to limit the induction of resistance to clotrimazole and its toxicity.

5. Conclusion

The substitution of the nucleus linked to the hydrazone moiety had a deep impact on the antifungal activity on this scaffold of derivatives. Based on the reported results, derivatives **8**, **16**, **21**, and **28** could represent good lead compounds for the development of novel broad-spectrum anti-*Candida* spp. agents and they could be used in association with azole derivatives to enhance their antifungal activity.

6. Experimental protocols

The chemicals, solvents for synthesis and spectral grade solvents were purchased from Aldrich (Italy) and used without further purification. Melting points (uncorrected) were determined automatically on an FP62 apparatus (Mettler-Toledo). ^{1}H NMR spectra were recorded at 400 MHz on a Bruker spectrometer using DMSO-d_6 as solvent. Chemical shifts are expressed as δ units (parts per millions) relative to the solvent peak. Coupling constants J are valued in Hertz (Hz). IR spectra were registered on a Perkin–Elmer FT-IR Spectrometer Spectrum 1000 in KBr. Elemental analysis for C, H, and N were recorded on a Perkin–Elmer 240 B microanalyzer and the analytical results were within ±0.4% of the theoretical values for all compounds. All reactions were monitored by TLC performed on 0.2 mm thick silica gel plates (60 F_{254} Merck). Electron ionization (EI) mass spectra were obtained by a Fisons QMD 1000 mass spectrometer (70 eV, 200 μA, ion source temperature of 200 °C). The samples were introduced directly into the ion source.

6.1. General procedure for the synthesis of derivatives *1*–*28*

The appropriate carbonyl compound (50 mmol) was dissolved in 100 mL of 2-propanol and stirred with an equimolar quantity of thiosemicarbazide for 24 h at room temperature with catalytic amounts of acetic acid. The desired thiosemicarbazone precipitated from reaction mixture, was filtered, crystallized from suitable solvent, and dried. Equimolar amounts of the prepared thiosemicarbazone (50 mmol) and α-bromo-4-methyl- (or 4-methoxy-) acetophenone (50 mmol), both dissolved in 2-propanol, were

reacted at room temperature under magnetic stirring for 2 h. The precipitate was filtered and dried to give compounds **1**–**28** in high yield.

*6.1.1. 1-(Furan-2-ylmethylene)-2-(4-p-tolylthiazol-2-yl)hydrazine (**1**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.31 (s, 3H, $ArCH_3$), 6.60 (s, 1H, CH=), 6.79 (s, 1H, C_5H-thiazole), 7.19–7.22 (m, 3H, furan), 7.71–7.90 (m, 4H, Ar), 12.27 (bs, 1H, NH, D_2O exch.).

*6.1.2. 1-(Furan-2-ylmethylene)-2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazine (**2**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.77 (s, 3H, OCH_3), 6.59–6.61 (m, 1H, furan), 6.79–6.80 (m, 1H, Ar), 6.94–6.97 (m, 2H, furan), 7.13 (s, 1H, CH=), 7.75–7.79 (m, 2H, Ar), 7.91–7.92 (m, 1H, Ar), 11.95 (bs, 1H, NH, D_2O exch.).

*6.1.3. 1-(1-(Furan-2-yl)ethylidene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**3**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.25 (s, 3H, $ArCH_3$), 2.52 (s, 3H, N=C–CH_3), 7.04 (s, 1H, C_5H-thiazole), 7.21–7.28 (m, 3H, furan), 7.73–7.80 (m, 4H, Ar), 11.48 (bs, 1H, NH, D_2O exch.).

*6.1.4. 1-(1-(Furan-2-yl)ethylidene)-2-(4-(4-methoxyphenyl) thiazol-2-yl)hydrazine (**4**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.23 (s, 3H, N=C–CH_3), 3.77 (s, 3H, OCH_3), 6.57–6.58 (m, 1H, furan), 6.82–6.83 (m, 1H, furan), 6.95–6.97 (m, 1H, furan), 7.12 (s, 1H, CH=), 7.76–7.79 (m, 4H, Ar), 11.95 (bs, 1H, NH, D_2O exch.).

*6.1.5. 1-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(1-(thiophen-2-yl) ethylidene)hydrazine (**8**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.33 (s, 3H, N=C–CH_3), 2.49 (s, 3H, OCH_3), 6.95–7.07 (m, 3H, thiophene), 7.12 (s, 1H, C_5H-thiazole), 7.38–7.40 (m, 2H, Ar), 7.77–7.79 (m, 2H, Ar), 11.02 (bs, 1H, NH, D_2O exch.).

*6.1.6. 1-(1-(Pyridin-2-yl)ethylidene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**9**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.34 (s, 3H, $ArCH_3$), 2.50 (s, 3H, N=C–CH_3), 7.74–7.78 (m, 4H, Ar), 7.09 (s, 1H, C_5H-thiazole), 8.40–8.42 (m, 4H, pyridine), 12.20 (bs, 1H, NH, D_2O exch.).

*6.1.7. 1-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(1-(pyridin-2-yl) ethylidene)hydrazine (**10**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.41 (s, 3H, N=C–CH_3), 3.78 (s, 3H, OCH_3), 6.97–7.00 (m, 2H, Ar), 7.23 (s, 1H, C_5H-thiazole), 7.55–7.56 (m, 1H, Ar), 7.80–7.82 (m, 2H, pyridine + Ar), 8.07–8.14 (m, 2H, pyridine), 8.63–8.64 (m, 1H, pyridine), 11.55 (bs, 1H, NH, D_2O exch.).

*6.1.8. 1-(Pyridin-3-ylmethylene)-2-(4-p-tolylthiazol-2-yl)hydrazine (**11**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.32 (s, 3H, $ArCH_3$), 7.23–7.26 (m, 2H, Ar), 7.33 (s, 1H, C_5H-thiazole), 7.75–7.78 (m, 2H, Ar), 7.85–7.87 (m, 1H, pyridine), 8.13–8.15 (m, 1H, CH=), 8.56–8.67 (m, 3H, pyridine), 12.65 (bs, 1H, NH, D_2O exch.).

*6.1.9. 1-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(pyridin-3-ylmethylene)hydrazine (**12**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.78 (s, 3H, OCH_3), 6.96–6.98 (m, 2H, Ar), 7.23 (s, 1H, C_5H-thiazole), 7.76–7.79 (m, 2H, Ar), 7.80 (s, 1H, CH=), 8.12–8.13 (m, 1H, pyridine), 8.48–8.50 (m, 1H, pyridine), 8.76–8.78 (m, 1H, pyridine), 9.00–9.02 (m, 1H, pyridine), 12.45 (bs, 1H, NH, D_2O exch.).

*6.1.10. 1-(1-(Pyridin-3-yl)ethylidene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**13**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.33 (s, 3H, $ArCH_3$), 2.49 (s, 3H, N=C–CH_3), 7.23–7.24 (m, 2H, Ar), 7.32 (s, 1H, C_5H-thiazole), 7.77–7.79 (m, 2H, Ar), 7.99–9.08 (m, 4H, pyridine), 11.50 (bs, 1H, NH, D_2O exch.).

*6.1.11. 1-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(1-(pyridin-3-yl) ethylidene)hydrazine (**14**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.40 (s, 3H, N=C–CH_3), 2.49 (s, 3H, OCH_3), 6.97–6.99 (d, $J_o = 8.0$ Hz, 2H, Ar), 7.37 (s, 1H, C_5H-thiazole), 7.80–7.82 (d, $J_o = 8.0$ Hz, 2H, Ar), 8.24–8.25 (m, 2H, pyridine), 8.86–8.87 (m, 2H, pyridine),12.45 (bs, 1H, NH, D_2O exch.).

*6.1.12. 1-(Pyridin-4-ylmethylene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**15**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.31 (s, 3H, $ArCH_3$), 7.23–7.24 (m, 2H, Ar), 7.40 (s, 1H, C_5H-thiazole), 7.62–7.64 (m, 2H, Ar), 7.90–7.93 (m, 2H, pyridine), 8.06–8.08 (m, 1H, CH=), 8.75–8.78 (m, 2H, pyridine), 12.00 (bs, 1H, NH, D_2O exch.).

*6.1.13. 1-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(pyridin-4-ylmethylene)hydrazine (**16**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.78 (s, 3H, OCH_3), 6.97–6.99 (m, 2H, Ar), 7.33 (s, 1H, C_5H-thiazole), 7.78–7.80 (m, 2H, Ar), 8.09–8.10 (m, 2H, pyridine), 8.12 (s, 1H, CH=), 8.80–8.81 (m, 2H, pyridine), 13.30 (bs, 1H, NH, D_2O exch.).

*6.1.14. 1-(1-(Pyridin-4-yl)ethylidene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**17**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.32 (s, 3H, $ArCH_3$), 2.37 (s, 3H, N=C–CH_3), 7.24–7.25 (m, 2H, Ar), 7.39 (s, 1H, C_5H-thiazole), 7.77–7.78 (m, 2H, Ar), 8.20–8.21 (m, 2H, pyridine), 8.85–8.86 (m, 2H, pyridine), 12.00 (bs, 1H, NH, D_2O exch.).

*6.1.15. 1-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(1-(pyridin-4-yl) ethylidene)hydrazine (**18**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.36 (s, 3H, N=C–CH_3), 3.79 (s, 3H, OCH_3), 6.97–6.99 (m, 2H, Ar), 7.38 (s, 1H, C_5H-thiazole), 7.80–7.82 (m, 2H, Ar), 8.11–8.12 (m, 2H, pyridine), 8.79–8.80 (m, 2H, pyridine), 12.01 (bs, 1H, NH, D_2O exch.).

*6.1.16. 1-(Naphthalen-1-ylmethylene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**19**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.33 (s, 3H, $ArCH_3$), 7.21–7.23 (m, 1H, C_5H-thiazole), 7.54–8.28 (m, 11H, Ar), 8.98–9.00 (m, 1H, CH=), 11.50 (bs, 1H, NH, D_2O exch.).

*6.1.17. 1-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(naphthalen-1-ylmethylene)hydrazine (**20**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.79 (s, 3H, OCH_3), 7.19–7.22 (m, 1H, C_5H-thiazole), 7.59–7.60 (m, 3H, Ar), 7.79–7.81 (m, 3H, Ar), 7.99–8.00 (m, 3H, Ar), 8.52–8.53 (m, 2H, Ar), 8.68–8.69 (m, 1H, CH=), 12.23 (bs, 1H, NH, D_2O exch.).

*6.1.18. 1-(1-(Naphthalen-2-yl)ethylidene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**21**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.32 (s, 3H, $ArCH_3$), 2.44 (s, 3H, N=C–CH_3), 7.30 (s, 1H, C_5H-thiazole), 7.99–8.40 (m, 11H, Ar), 11.45 (bs, 1H, NH, D_2O exch.).

*6.1.19. 1-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(1-(naphthalen-2-yl)ethylidene)hydrazine (**22**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.44 (s, 3H, N=C–CH_3), 3.78 (s, 3H, OCH_3), 6.98–7.00 (m, 2H, Ar), 7.30 (s, 1H, C_5H-thiazole),

7.51–7.52 (m, 2H, Ar), 7.75–7.85 (m, 5H, Ar), 8.10–8.15 (m, 1H, Ar), 8.27–8.32 (m, 1H, Ar), 11.45 (bs, 1H, NH, D_2O exch.).

*6.1.20. 1-((1H-Indol-3-yl)methylene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**25**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.32 (s, 3H, $ArCH_3$), 7.21–7.22 (m, 1H, Ar), 7.50 (s, 1H, C_5H-thiazole), 7.70–7.73 (m, 3H, Ar), 7.80–7.82 (m, 3H, Ar), 8.20–8.23 (m, 1H, Ar), 8.20–8.21 (m, 1H, C_2H-indole), 8.40 (s, 1H, CH═), 11.50 (bs, 1H, NH, D_2O exch.).

*6.1.21. 1-(1-(2-Oxo-2H-chromen-3-yl)ethylidene)-2-(4-p-tolylthiazol-2-yl)hydrazine (**27**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.07 (s, 3H, $ArCH_3$), 2.27 (s, 3H, N═C–CH_3), 7.40–7.42 (m, 2H, Ar), 7.50–7.52 (m, 1H, C_5H-thiazole), 7.54–7.57 (m, 2H, Ar), 7.61–7.75 (m, 4H, Ar), 8.20 (s, 1H, CH═), 11.30 (bs, 1H, NH, D_2O exch.).

*6.1.22. 1-(1-(2-Oxo-2H-chromen-3-yl)ethylidene)-2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazine (**28**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.49 (s, 3H, N═C–CH_3), 3.94 (s, 3H, OCH_3), 6.95–6.97 (m, 2H, Ar), 7.17 (s, 1H, C_5H-thiazole), 7.37–7.40 (m, 2H, Ar), 7.44–7.45 (m, 1H, Ar), 7.61–7.78 (m, 3H, Ar), 7.90–8.10 (m, 1H, CH═), 11.45 (bs, 1H, NH, D_2O exch.).

6.2. Antifungal activity

All derivatives were dissolved in dimethylsulfoxide (DMSO). The *in vitro* antifungal activities were determined with the broth microdilution method with Sabouraud dextrose broth (BBL Microbiology Systems, Cockeysville, MD) as recommended by the NCCLS [17]. Microtiter plates containing serial dilutions of each compound were inoculated with each organism to yield the appropriate density (10^3/mL) in a 100 μL final volume; each plate included positive controls (fungi without a compound) and a negative control (medium only). The plates were incubated for 24 h at 37 °C. The MIC for all isolates was defined as the lowest concentration of antifungal agents that completely inhibited the growth of the organism, as detected by unaided eye.

6.3. Broth microdilution susceptibility test method

The yeast inoculum was adjusted to a concentration of $0.5–2.5 \times 10^3$ CFU/mL in RPMI medium, and an aliquot of 0.1 mL was added to each well of the microdilution plate. The plates were incubated at 35 °C, the MIC end points were read visually following 18, 24, and 48 h of incubation and were defined as the lowest concentration that produced a prominent decrease in turbidity compared with that of the drug-free growth control.

Acknowledgments

This work was supported by grants from FIRB Project 2006 (RBIP067F9E).

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2010.10.027.

References

[1] S.K. Fridkin, D. Kaufman, J.R. Edwards, S. Shetty, T. Horan, Pediatrics 117 (2006) 1680–1687.
[2] M.D. Richardson, J. Antimicrob. Chemother. 56 (Suppl. 1) (2005) 5–11.
[3] A.C. Pasqualotto, D.W. Denning, J. Antimicrob. Chemother. 61 (2008) i19–i30.
[4] K. Gulshan, W.S. Moye-Rowley, Eukaryot. Cell 6 (2007) 1933–1942.
[5] P.M. Pinto, R. de Cássia Bothelho Weikert-Oliveira, J.P. Lyon, V.F. Cury, R.R. Arantes, C.Y. Koga-Ito, M.A. Resende, Microbiol. Res. 163 (2008) 579–585.
[6] A. Cukurovali, I. Yilmaz, S. Gur, C. Kazaz, Eur. J. Med. Chem. 41 (2006) 201–207.
[7] E. Maccioni, M.C. Cardia, L. Bonsignore, A. Plumitallo, M.L. Pellerano, A. De Logu, Il Farmaco 57 (2002) 809–817.
[8] A. Özdemir, G. Turan-Zitouni, Z.A. Kaplancikli, F. Demirci, G. Iscan, J. Enzym. Inhib. Med. Chem. 23 (2008) 470–475.
[9] P. Karegoudar, M.S. Karthikeyan, D.J. Prasad, M. Mahalinga, B.S. Holla, N.S. Kumari, Eur. J. Med. Chem. 43 (2008) 261–267.
[10] G. Turan-Zitouni, J.A. Fehrentz, P. Chevallet, J. Martinez, Z.A. Kaplancikli, A. Özdemir, M. Arslanyolu, M.T. Yildiz, Arch. Pharm. Chem. Life Sci. 340 (2007) 310–314.
[11] S.K. Bharti, G. Nath, R. Tilak, S.K Singh, Eur. J. Med. Chem. 45 (2010) 651–660.
[12] M.V. Nora De Souza, J. Sulfur Chem. 26 (2005) 429–449.
[13] P. Vicini, A. Geronikaki, M. Incerti, F. Zani, J. Dearden, M. Hewitt, Bioorg. Med. Chem. 16 (2008) 3714–3724.
[14] A. De Logu, M. Saddi, M.C. Cardia, R. Borgna, C. Sanna, B. Saddi, E. Maccioni, J. Antimicrob. Chemother. 55 (2005) 692–698.
[15] F. Chimenti, B. Bizzarri, D. Secci, A. Bolasco, R. Fioravanti, P. Chimenti, A. Granese, S. Carradori, D. Rivanera, D. Lilli, A. Zicari, S. Distinto, Bioorg. Med. Chem. Lett. 17 (2007) 4635–4640.
[16] F. Chimenti, S. Carradori, D. Secci, A. Bolasco, P. Chimenti, A. Granese, B. Bizzarri, J. Heterocycl. Chem. 3 (2009) 575–578.
[17] National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Susceptibility Testing Supplement M100-S13. National Committee for Clinical Laboratory Standards, Wayne, PA, 2003.
[18] F. Chimenti, E. Maccioni, D. Secci, A. Bolasco, P. Chimenti, A. Granese, S. Carradori, S. Alcaro, F. Ortuso, M. Yáñez, F. Orallo, R. Cirilli, R. Ferretti, F. La Torre, J. Med. Chem. 51 (2008) 4874–4880.
[19] R. Cirilli, R. Ferretti, F. La Torre, D. Secci, A. Bolasco, S. Carradori, M. Pierini, J. Chromatogr. A 1172 (2007) 160–169.
[20] S.M. Mohamed, M. Unis, H. El-Hady, Indian J. Chem. Sect B Org. Chem. Incl. Med. Chem. 45B (2006) 1453–1462.
[21] M.S. Solanki, J.P. Trivedi, J. Indian Chem. Soc. 49 (1972) 37–39.
[22] F. Castelh, M.T. Cambria, P. Mazzone, R. Pignatello, Thermochim. Acta 302 (1997) 143–150.
[23] G. Raciti, P. Mazzone, A. Raudino, G. Mazzone, A. Cambria, Bioorg. Med. Chem. 3 (1995) 1485–1491.
[24] National Committee for Clinical Laboratory Standard, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard, second ed., Document M27-A2 National Committee for Clinical Laboratory Standard, Wayne, PA.