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Original article

Synthesis and antifungal activity of a new series of 2-(1*H*-imidazol-1-yl)-1-phenylethanol derivatives

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ABSTRACT

A new series of aromatic ester and carbamate derivatives of 2-(1*H*-imidazol-1-yl)-1-phenylethanol were synthesized and evaluated for their antifungal activity towards *Candida albicans* and non-*albicans Candida* species strains. The aromatic biphenyl ester derivatives **6a**–**c** were more active than the reference compound fluconazole. **6c** possesses a MIC mean values of 1.7 ± 1.4 μg mL^{-1} *vs C. albicans* and 1.9 ± 2.0 μg mL^{-1} *vs* non-*albicans Candida* species strains. The racemic mixtures of **6a, b** were purified to afford the pure enantiomers. The (−) isomers were up to 500 times more active than (+) isomers. (−)-**6a** and (−)-**6b** were thirty and ninety times more active than fluconazole towards *C. krusei* strain respectively.

The racemates of **6a**–**c** showed low cytotoxicity against human monocytic cell line (U937) with **6a** demonstrating a CC_{50} greater than 128 μg mL^{-1}.

1. Introduction

The increasing incidence of serious fungal infections is a well-recognized problem. Such infections, predominantly affecting immunocompromised individuals, including HIV-1 infected, organ-transplanted, and those undergoing cancer chemotherapy, require prompt and appropriate therapy. *Candida* species are the main agent responsible for nosocomial fungal infections [1]. *Candida albicans*, which is commensal in healthy individuals [2], is the most common pathogen isolated in invasive candidiasis with about 30–40% of mortality. Furthermore, an increasing rates of invasive candidiasis caused by non-*albicans Candida* species have been reported worldwide; these species, typically less sensitive to the principal antifungal drugs, include *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, and *Candida krusei* [3–5]. For these reasons, the development of new and more potent antifungal drugs becomes even more urgent.

The main drugs used for the treatment of invasive candidiasis belong to the classes of azoles, polyenes (amphotericin B), echinocandines (caspofungin) and fluoropyrimidines (5-flucytosine), used alone or associated in combination therapy [6,7] (Fig. 1). The azole derivatives including triazoles (*e.g.* fluconazole) and imidazoles (*e.g.* econazole, miconazole, clotrimazole, and ketoconazole) inhibit the biosynthesis of fungal sterols through the inhibition of CYP51 and are commonly used as first line drugs to treat *Candida* infections.

In fungal species ergosterol plays an important role in the maintenance of membrane organization and functions. 14α-Lanosterol demethylase (CYP51, $P450_{14DM}$) is a key enzyme in the pathway of ergosterol biosynthesis. It is a member of the cytochrome P450 superfamily containing an iron protoporphyrin unit located in its active site catalyzing the oxidative removal of the 14α-methyl group of lanosterol to give $\Delta^{14,15}$-desaturated intermediates.

Azole drugs bind the CYP51 active site through coordination to the heme iron by the *N*-3 imidazole nitrogen (or *N*-4 triazole) thus preventing the binding of steroidic substrates [8,9]. The depletion of ergosterol and accumulation of 14α-methylated sterols alter membrane fluidity which reduce the activity of the enzymes associated with membrane and in turn increase the permeability.

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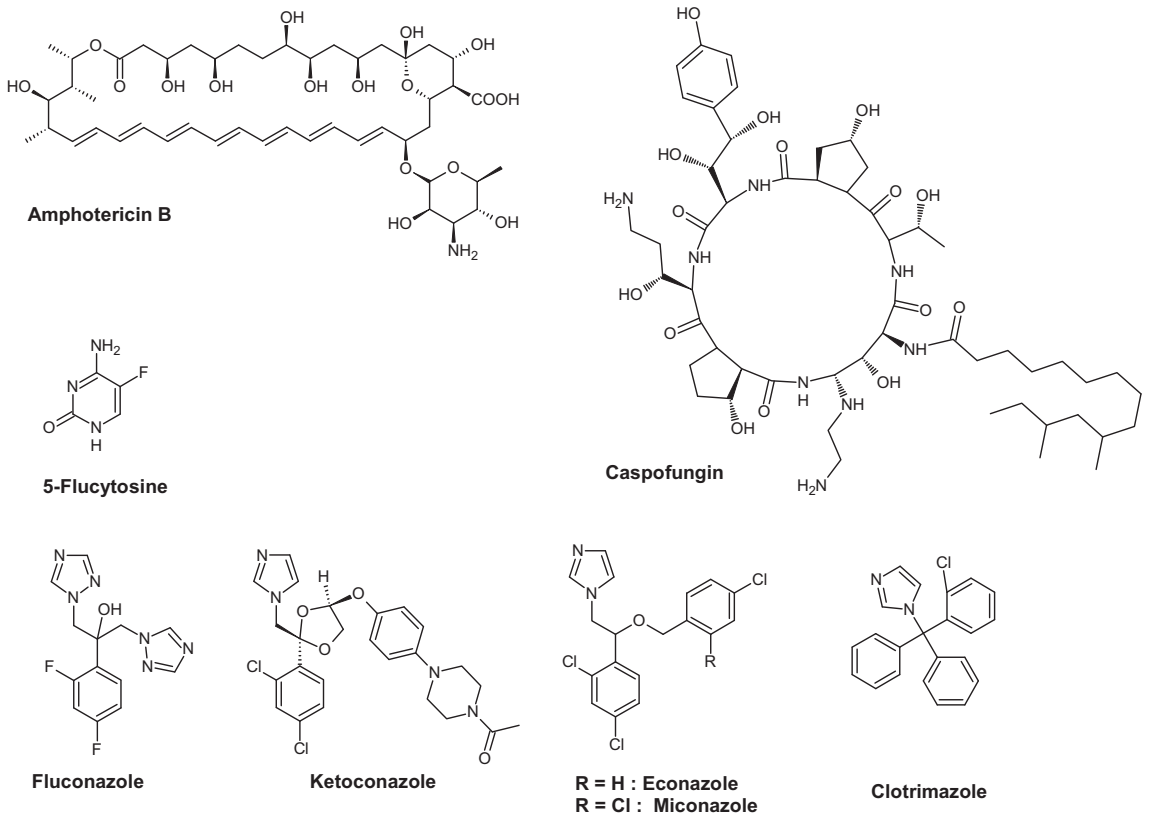


Fig. 1. Main drugs used for the treatment of invasive candidiasis.

Ultimately resulting inhibition of fungal growth and replication [10,11].

Fluconazole is the first line azole-antifungal drug and is considered the gold standard in the treatment of infections by *C. albicans* and *Cryptococcus neoformans*, but its extensive clinical use has resulted in resistance especially with respect to *C. albicans* [12].

The increasing incidence of *Candida* species infections coupled with the emergence of azole-resistant fungal strains have stimulated the search for alternative antifungal drugs.

The crystal structure of several bacterial CYP51 cytochromes, such as *Mycobacterium tuberculosis* [13] and *Trypanosoma brucei* [14] have been published. Unfortunately, there is no three-dimensional structural information available on *C. albicans* (CA-CYP51) or other fungal CYP51 enzymes. However, the rational design of new antifungal molecules can take advantage of the homology modeling and pharmacophore modeling techniques [15–17].

According to these models, schematically depicted in Fig. 2, the main azole drugs possess at least three principal pharmacophoric groups: (A) iron coordinating group, consisting of imidazole or triazole ring, able to interact with the heme iron, (B) a first hydrophobic moiety (typically aromatic) near the iron coordinating group and (C) a second aromatic region. Certain active compounds present an additional hydrophobic area referred as region (D).

In this paper, we report the synthesis and *in vitro* evaluation of antifungal activity of a new series of 2-(1*H*-imidazol-1-yl)-1-phenylethanol derivatives. We started from the observation that some phenyl-substituted 2-(1*H*-imidazol-1-yl)-1-phenylethanols described in literature are characterized by a weak antifungal activity [18]. Such compounds represent an interesting starting point to develop new antifungal drugs since they possess two specific features of the pharmacophore described in the above theoretical models: the iron-binding azole group (A) and the aromatic group adjacent to it (B). The data reported in literature encouraged our efforts to synthesize new derivatives, precisely aromatic esters and carbamates, introducing a second hydrophobic or aromatic moiety thus satisfying the required interaction features to allow a more effective interaction with the active site of CYP51.

Wahbi and coll. reported that some alkyl esters of 2-(1*H*-imidazol-1-yl)-1-phenylethanols were characterized by lower antifungal activity than reference drugs [19]. While Walker and coll. reported that some aryl and arylalkyl esters are slightly more active than Miconazole towards some *C. albicans* strains [20], indicating the importance of the second aromatic region (C). In this context, it is relevant to point out that some azole compounds containing biphenyl moiety inhibit *Trypanosoma cruzi* and *T. brucei* CYP51 reducing parasite cells growth [21,22]. These results encouraged us

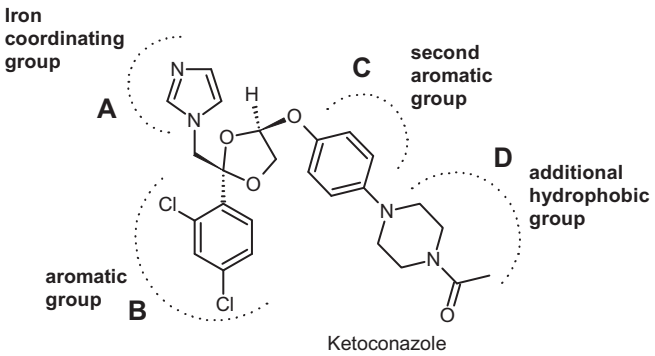


Fig. 2. Schematic representation of the pharmacophore of CA-CYP51 binding azoles.

to synthesize the biphenyl esters derivatives of 2-(1*H*-imidazol-1-yl)-1-phenylethanol.

Karakurt and coworkers reported the lack of antifungal activity, against *C. albicans* and non-*albicans Candida* species, of some 2-(1*H*-imidazol-1-yl)-1-(naphthalen-2-yl)ethanol alkyl and aryl esters [23]. These results suggest to us not to increase the size of the aromatic ring (B), since this could lead to a reduction in antifungal activity.

The literature data [19,20] also showed that a double substitution of hydrogen atom at positions 2 and 4 of the aromatic ring (B) with chlorines does not appear to be determinant for activity. For this reason, in the first instance, we decided to investigate only the replacement of H in position 4 with a halogen atom, *i.e.* fluorine and chlorine.

2. Results and discussion

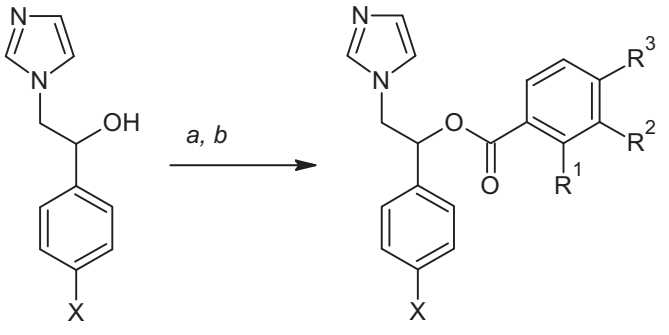
2.1. Chemistry

2-(1*H*-imidazol-1-yl)-1-phenylethanols (**2a**–**d**) were prepared, in two steps as described in literature [18]. Treatment of substituted 2-bromo-1-phenylethanones with 1*H*-imidazole in dimethylformamide afforded 2-(1*H*-imidazol-1-yl)-1-phenylethanones, which were subsequently reduced by methanolic sodium borohydride to give rise to the alcohols **2** (**2a**–**d**).

The racemic alcohols **2a**–**d** were treated with sodium hydride in anhydrous acetonitrile followed by the addition of the appropriate acylchloride to afford the final desired products (Schemes 1 and 2). Likewise, carbamates **8a**–**c** were prepared by adding 4-(2-propyl)-phenylisocyanate to an acetonitrile suspension of the alcoholates obtained from **2a**–**c** (Scheme 3).

The isocyanate of 2,6-dichloro-4-aminopyridine was generated using triphosgene and TEA in anhydrous benzene and reacted with alcohol **2c** to afford the carbamate **9c** (Scheme 4).

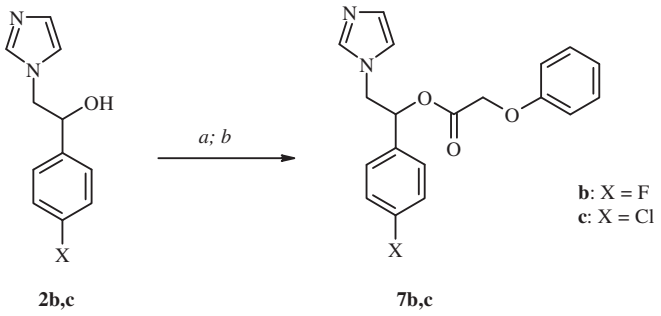
All compounds were tested as racemic mixtures to evaluate the antifungal and cytotoxic activities, and only active racemates were resolved. Racemates **6a**,**b** were separated to generate (−)-**6a**,**b** and (+)-**6a**,**b** which were tested for the antifungal activity.



2a : X=H
2b : X=F
2c : X=Cl
2d : X=CF_3

3a: R^1=R^3=H, R^2=CF_3, X=H
3b: R^1=R^3=H, R^2=CF_3, X=F
3c: R^1=R^3=H, R^2=CF_3, X=Cl
3d: R^1=R^3=H, R^2=CF_3, X=CF_3

Scheme 1. (a) NaH, CH_3CN, rt, 2 h; (b) **3a**–**c**: 3-(trifluoromethyl)benzoyl chloride, 24 h, rt. **3d**: 3-(trifluoromethyl)benzoyl chloride, 48 h, rt. **5a**: 4-(trifluoromethyl)benzoyl chloride, 24 h, rt. **6a**–**c**: biphenyl-4-carbonyl chloride, 48 h, rt.



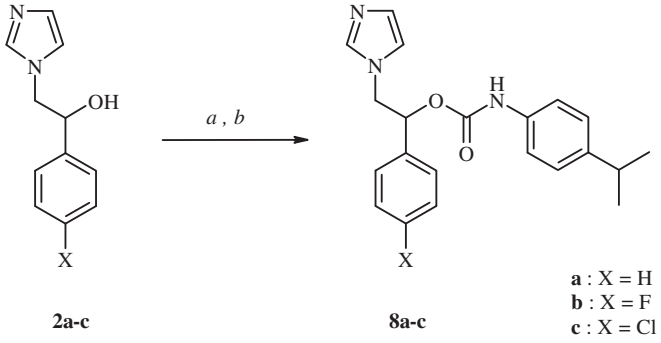
Scheme 2. (a) NaH, CH_3CN, rt, 2 h; (b) phenoxyacetyl chloride, over night, rt.

The separation was performed by HPLC on the Chiralcel OD chiral stationary phase (CSP) using the binary mixture *n*-hexane-ethanol 50:50 (v/v) as eluent (Fig. 3). The optimized analytical conditions were easily scaled-up to semipreparative level employing a 1 cm I.D. Chiralcel OD column. Multi-mg amounts of enantiomerically pure samples (ee > 99%) were isolated and submitted to polarimetric measurements. The first eluting enantiomers were dextrorotatory in ethanol solution at 589 nm wavelength and the more retained ones were levorotatory in the same experimental conditions.

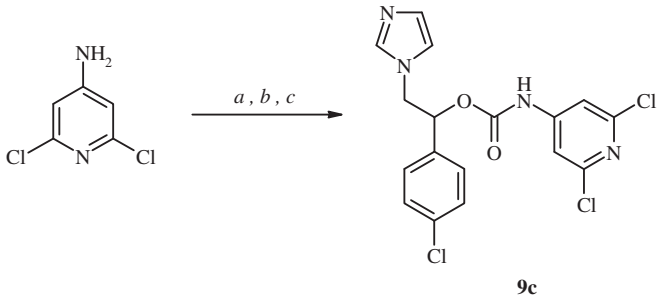
2.2. In vitro antifungal activity

The *in vitro* antifungal activity of imidazole derivatives **2a**–**c**, **3a**–**d**, **4b**–**c**, **5a**, **6a**–**c**, **7b**–**c**, **8a**–**c** and **9c** was evaluated against four strains of *C. albicans* and seven strains of non-*albicans Candida* species. The obtained data, expressed as the mean of minimal inhibitory concentration (MIC) values, MIC_{50}, MIC_{90} and MIC range are reported in Table 1 for *C. albicans* and in Table 2 for non-*albicans Candida* species.

MIC values obtained for the alcohols **2a**–**c** confirm their previously observed weak antifungal activity [18] and are in agreement with above mentioned pharmacophoric models, which suggest that azole drugs need at least three points of binding to have an optimal interaction with CA-CYP51. **2a**–**c** possess only the iron-binding imidazole and the aromatic group (B) adjacent to it. According to this observation, the esterification of alcoholic function of **2a**–**c** with aromatic groups produced, in most cases, an enhancement in antifungal activity. The presence of a second single aromatic moiety produced a noticeable, but not particularly pronounced effect, as indicated from the observed activity values of the 3-trifluorobenzoyl ester **3b**. **3b** is one of the most active benzoyl derivatives, with MIC mean values of 27.7 ± 49.4 and 52.9 ± 61.7 μg mL^{-1} *vs C. albicans* and non-*albicans Candida* species



Scheme 3. (a) NaH, CH_3CN, rt, 2 h; (b): 1-Isocyanato-4-(propan-2-yl)benzene, 48 h, rt.



Scheme 4. (a) TEA, benzene, 35 °C; (b) triphosgene, 12 h, rt; (c) **2c**, 48 h, rt.

strains respectively. The corresponding hydrochloride **4b** showed MIC mean values of 34.3 ± 46.1 μg mL^{-1} *vs C. albicans* and 22.6 ± 20.1 μg mL^{-1} *vs* non-*albicans Candida* species strains, indicating that the protonation of imidazole ring do not produce a significant modification in antifungal activity.

Replacement of the ester function with a carbamate moiety led to compounds **8a**–**c** and **9c**. Despite the presence of a second aromatic moiety the compounds of this sub-series were not very active. In particular, the most active carbamate **8b** showed MIC mean values of 39.6 ± 48.1 μg mL^{-1} *vs C. albicans* strains and 68.4 ± 59.2 μg mL^{-1} *vs* non-*albicans Candida* species strains, only slightly better than those found for alcohols **2a**–**c**. Compound **9c**, in which a 2,6-dichloropyridine moiety was introduced as the second aromatic moiety, was found to be the least active of the carbamate sub-series.

The esterification of alcoholic function of **2a**–**c** with biphenyl-4-carbonyl chloride gave rise to the most active compounds of this series (**6a**–**c**). These analogs were found to be more active than fluconazole towards both *C. albicans* and non-*albicans Candida* species in 90% of strains tested. Compounds **6a**, **6b** and **6c** showed MIC$_{90}$ values of 8, 8 and 4 μg mL^{-1} towards *C. albicans* strains and 4, 8 and 4 μg mL^{-1} towards non-*albicans Candida* strains compared to 16 μg mL^{-1} for fluconazole (Tables 1 and 2). Furthermore, **6c** possesses a MIC mean values of 1.7 ± 1.4 μg mL^{-1} *vs C. albicans* and 1.9 ± 2.0 μg mL^{-1} *vs* non-*albicans Candida* species strains, lower than fluconazole which exhibited MIC values of 4.9 ± 6.9 μg mL^{-1} and 7.9 ± 15.8 μg mL^{-1} *vs C. albicans* and non-*albicans Candida* species, respectively (Tables 1 and 2).

The biphenyl esters **6a**–**c** were found to be active towards *C. krusei*, a *Candida* species intrinsically resistant to fluconazole. In

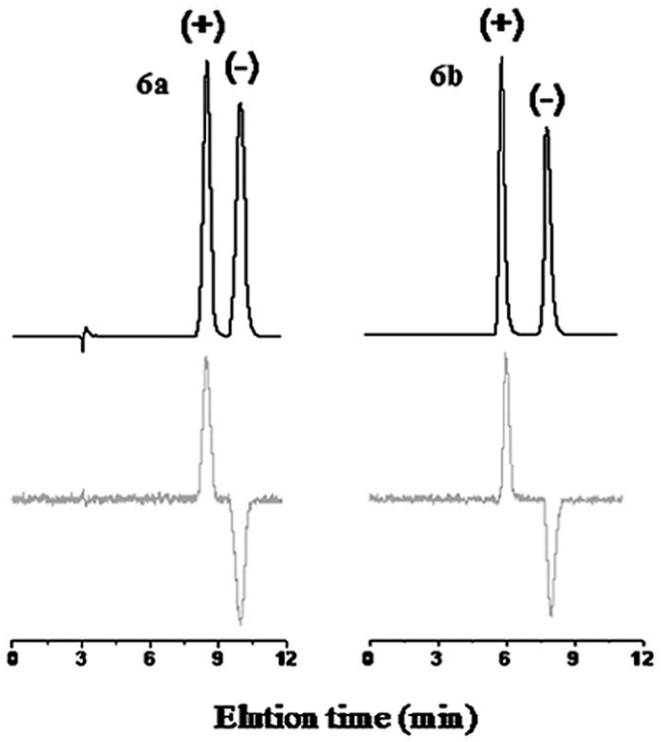


Fig. 3. Typical chromatograms illustrating the resolution of **6a,b**. Column, Chiralcel OD (250 mm × 4.6 mm I.D.); eluent, *n*-hexane/ethanol 50:50 (v/v); flow-rate, 1 mL min^{-1}; detector, UV (black) and CD (gray) at 254 nm; temperature, 40 °C.

Table 1
In vitro antifungal activity for studied compounds against *Candida albicans* strains.

Compound	MIC± SD (μg mL^{-1})	MIC$_{50}$ (μg mL^{-1})	MIC$_{90}$ (μg mL^{-1})	Range (μg mL^{-1})
2a	106.7 ± 33.0	128	128	64–128
2b	101.3 ± 42.5	128	128	32–128
2c	112.0 ± 39.2	128	128	32–128
3a	91.4 ± 34.2	64	128	64–128
3b	27.7 ± 49.4	8	16	2–128
3d	91.4 ± 34.2	64	128	64–128
4b	34.3 ± 46.1	8	64	8–128
4c	46.3 ± 56.5	8	16	4–128
5a	33.1 ± 46.7	8	64	8–128
6a	3.7 ± 5.1	1	8	0.125–16
6b	4.4 ± 3.1	4	8	0.25–8
6c	1.7 ± 1.4	1	4	0.125–4
7b	76.6 ± 52.6	64	128	8–128
7c	67.7 ± 59.8	64	128	2–128
8a	77.7 ± 36.3	64	128	32–128
8b	39.6 ± 48.1	16	64	1–128
8c	60.0 ± 52.1	64	128	4–128
9c	118.8 ± 24.2	128	128	64–128
Fluconazole	4.9 ± 6.9	1	16	0.25–16

Antifungal activity was determined according to CLSI guidelines (CLSI document M27-A3,2008). MIC = arithmetic mean of minimal inhibitory concentration; SD = standard deviation. MIC$_{50}$ and MIC$_{90}$: MICs at which 50% and 90% of strains, respectively, are inhibited. Data represent MIC values of three separate experiments in triplicate. Additional data are given in supplemental Table S1.

Table 2
In vitro antifungal activity of studied compounds against non-*albicans Candida* species.

Compound	MIC± SD (μg mL^{-1})	MIC$_{50}$ (μg mL^{-1})	MIC$_{90}$ (μg mL^{-1})	Range (μg mL^{-1})
2a	98.5 ± 33.2	64	128	64–128
2b	104.0 ± 45.0	128	128	16–128
2c	108.0 ± 38.1	128	128	16–128
3a	85.0 ± 49.0	64	128	8–128
3b	52.9 ± 61.7	8	128	4–128
3d	100.0 ± 39.8	128	128	32–128
4b	22.6 ± 20.1	16	32	1–64
4c	43.8 ± 49.5	32	128	0.5–128
5a	55.5 ± 50.2	32	128	4–128
6a	2.1 ± 3.9	0.5	4	0.25–16
6b	2.5 ± 2.9	1	8	1–8
6c	1.9 ± 2.0	1	4	0.125–8
7b	61.1 ± 63.7	4	128	1–128
7c	51.3 ± 58.1	16	128	2–128
8a	106.7 ± 32.0	128	128	64–128
8b	68.4 ± 59.2	4	128	4–128
8c	114.7 ± 40.0	128	128	8–128
9c	120.9 ± 21.3	128	128	64–128
Fluconazole	7.9 ± 15.8	2	16	0.5–64

Antifungal activity was determined according to CLSI guidelines (CLSI document M27-A3,2008). MIC = arithmetic mean of minimal inhibitory concentration; SD = standard deviation. MIC$_{50}$ and MIC$_{90}$: MICs at which 50% and 90% of strains, respectively, are inhibited. Data represent MIC values of three separate experiments in triplicate. Additional data are given in supplemental table S1.

Table 3
MIC mean values for compounds **6a**–**c** towards various non-*albicans* *Candida* species.

Compound	MIC±SD (μg mL^{-1})			
	C. parapsilosis	*C. tropicalis*	*C. krusei*	*C. glabrata*
6a	0.70 ± 0.74	0.50 ± 0.39	4.70 ± 5.70	4 ± 0
6b	1.30 ± 0.96	4.08 ± 3.47	2.25 ± 2.02	1 ± 0
6c	1.05 ± 0.62	0.89 ± 0.67	3.70 ± 2.60	1 ± 0
Fluconazole	1.60 ± 0.55	3.25 ± 1.4	26.0 ± 23.71	0.87 ± 0.25

fact, compounds **6a**, **6b** and **6c** showed MIC mean values for *C. krusei* of 4.70 ± 5.70, 2.25 ± 2.02 and 3.70 ± 2.60 μg mL^{-1}. Five to eleven times lower than that observed for fluconazole (26.0 ± 23.71 μg mL^{-1}, Table 3).

The presence of a halogen atom at 4 position of the aromatic ring of the phenylethyl moiety does not markedly affect the antifungal activity, as evidenced by MIC mean values of compounds **6a**–**c**.

The antifungal activity of the pure separated enantiomers of esters **6a**, **b** was preliminarily assessed against strains of *C. albicans* (ATCC24433), *C. parapsilosis* (DSM11224), *C. krusei* (PMC0613) and *C. tropicalis* (DSM11953). The data are summarized in Table 4, which also reports the activities of the corresponding racemates and fluconazole.

These data indicate that the levorotatory enantiomers were more active than the dextrorotatory ones. (−)-**6b** was found to be about 500 times more active than (+)-**6b** towards *C. albicans*, *C. parapsilosis* and *C. krusei* strains and about 120 times towards *C. tropicalis*.

(−)-**6a** and (−)-**6b** were found to be more active than fluconazole towards strains of *C. albicans*, *C. parapsilosis*, *C. tropicalis* and up to 30–90 times towards strains of *C. krusei*.

2.3. In vitro cytotoxic activity

The *in vitro* toxicity of compounds **6a**–**c** were assessed analyzing the dose related effects towards the growth of cultured human monocytic cells (U937) and calculating the corresponding CC_{50}, i.e. the concentration (μg mL^{-1}) required to reduce cell viability by 50% (Table 5, Fig. S1 Supplementary material); the selectivity index (SI), *i.e.* the ratio between CC_{50} and mean value of MIC observed towards all *Candida* species, was also reported. The toxicity test showed that compound **6a** presents the highest value of CC_{50} (>128 μg mL^{-1}) and good selectivity towards fungi. In this context, it can be pointed out that the presence of a halogen atom, at 4 position of the aromatic ring of the phenylethyl moiety, influenced the toxicity of compounds **6a**–**c** towards cultured human cells (U937), as indicated by CC_{50} values of fluoro derivative **6b** (41.5 ± 5.1 μg mL^{-1}) and chloro derivative **6c** (88.3 ± 10.8 μg mL^{-1}).

Table 4
MIC mean values of racemates **6a**, **b** and their pure enantiomers against four strains of *Candida* species.

Compound	MIC±SD (μg mL^{-1})			
	C. albicans ATCC24433	*C. parapsilosis* DSM11224	*C. krusei* PMC0613	*C. tropicalis* DSM11953
(±) **6a**	0.37 ± 0.22	0.33 ± 0.14	0.67 ± 0.29	0.58 ± 0.38
(+) **6a**	33.70 ± 12.50	26.67 ± 9.24	21.33 ± 9.24	26.67 ± 9.24
(−) **6a**	0.15 ± 0.09	0.125 ± 0	0.37 ± 0.22	0.15 ± 0.09
(±) **6b**	1.5 ± 0.87	0.42 ± 0.14	0.83 ± 0.29	2.17 ± 1.76
(+) **6b**	64 ± 0	35.55 ± 17.50	64 ± 0	32 ± 0
(−) **6b**	0.125 ± 0	0.08 ± 0.04	0.125 ± 0	0.27 ± 0.22
Fluconazole	1 ± 0	1 ± 0	12 ± 4.4	2.5 ± 1.6

Antifungal activity was determined according to CLSI guidelines (CLSI document M27-A3,2008). MIC = arithmetic mean of minimal inhibitory concentration; SD = standard deviation.

Table 5
Effect of **6a**, **6b** and **6c** on the human monocytic cells (U937) growth and their calculated selectivity index values (SI).

Compound	CC_{50} (μg mL^{-1})	SI
6a	> 128	> 44.1
6b	41.5 ± 5.1	12.0
6c	88.3 ± 10.8	49.1

CC_{50}: concentration (μg mL^{-1}) required to reduce cell viability by 50%; SI: defined as the ratio between CC_{50} and mean value of MIC (the concentration of the drug needed to reduced fungal cell viability to 50%) observed towards all *Candida* species. Data represent means ± standard deviation from three independent experiments, each performed in triplicate.

3. Conclusions

The results obtained for this series of 2-(1*H*-imidazol-1-yl)-1-phenylethanolesters and are in agreement with the previously developed theoretical pharmacophoric models. The presence of an additional extended aromatic biphenyl moiety was essential to enhance the activity as demonstrated by compounds **6a**–**c**. The presence of a halogen atom at 4 position of the aromatic ring of the phenylethyl moiety proved to be a minor factor.

In the 90% of tested strains of *C. albicans* and non-*albicans* *Candida* species, compounds **6a**–**c** were more active than fluconazole. These compounds showed an interesting activity against *Candida* species strains with lower sensitivity to fluconazole (>8 μg mL^{-1}). (Table S1, Supplementary material).

Cytotoxicity studies showed that compounds **6a**–**c** presented a low toxicity on cultured human monocytic cell line (U937). This activity was negatively influenced by the presence of a halogen atom at 4 position of the aromatic ring of the phenylethyl moiety.

Preliminary data on pure enantiomers showed a markedly higher antifungal activity of (−)-**6a** and (−)-**6b** with respect to dextrorotatory isomers, thus suggesting that the former are able to better satisfy the pharmacophore features to establish optimal interactions with CA-CYP51. Further studies are in progress to determine the absolute configuration of the (−)-isomers and to assess how these compounds overlap with computational pharmacophoric models.

In conclusion, the antifungal activity and preliminary toxicity data obtained for esters **6a**–**c**, in particular for their levorotatory enantiomers, may suggest that these compounds represent an interesting starting point to develop new antifungal azole drugs characterized by higher potency and an improved safety profile.

4. Experimental section

4.1. Chemistry

4.1.1. Materials and methods

All reagents and solvents were of high analytical grade and were purchased from Sigma–Aldrich (Milano, Italy). Substituted 2-(1*H*-imidazol-1-yl)-1-phenylethanols (**2a**–**d**) were prepared according to literature procedure [18]. Melting points were determined on Tottoli apparatus (Buchi) and are uncorrected. Infrared spectra were recorded on a Spectrum One ATR Perkin–Elmer FT-IR spectrometer. ^{1}H NMR spectra were acquired on a Bruker AVANCE-400 spectrometer at 9.4 T, in DMSO-d_6 or $CDCl_3$ at 27 °C; chemical shift values are given in δ (ppm) with respect to TMS as internal reference. Coupling constants are given in Hz.

Mass spectra were recorded on an API-TOF Mariner by Perspective Biosystem (Straford, Texas, USA), samples were

injected by an Harvard pump using a flow-rate of 5–10 μl min^{-1}, infused in the electrospray system.

Elemental analyses were obtained by a PE 2400 (Perkin–Elmer) analyzer and the analytical results were within ±0.4% of the theoretical values for all compounds.

*4.1.2. General procedure for the synthesis of **3a–d**, **5a** and **6a–c***

*4.1.2.1. Example: synthesis of (±)2-(1H-imidazol-1-yl)-1-phenylethyl-3-(trifluoromethyl)benzoate (**3a**).* To a stirred suspension of 2-(1*H*-imidazol-1-yl)ethanol (**2a**) (0.53 mmol) in dry acetonitrile (5 mL) was added sodium hydride (0.53 mmol). The reaction mixture was stirred for 2 h at room temperature (rt) and then treated with 3-(trifluoromethyl)benzoyl chloride (0.75 mmol). The reaction mixture was stirred for 24 h at rt. The solvent was removed under reduced pressure and the residue was dissolved in an adequate volume of dichloromethane (5–25 mL) and washed with aqueous saturated potassium carbonate.

The separated organic layer was dried over anhydrous sodium sulphate and after filtration was evaporated under reduced pressure. The obtained crude yellow oil was purified by column chromatography on silica gel using methanol/dichloromethane (1 : 9) as eluent to give 80 mg of **3a** as colorless oil (yield 58%).

^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H), 8.21 (d, 1H, J = 7.8 Hz), 7.86 (dd, 1H, J = 7.8 Hz, J = 0.8 Hz), 7.63 (t, 1H, J = 7.8 Hz), 7.41–7.38 (m, 4H), 7.34–7.30 (m, 2H), 7.04 (s, 1H), 6.86 (s, 1H), 6.21 (dd, 1H, J = 6.6 Hz, J = 4.7 Hz), 4.49 (dd, 1H, J = 6.6 Hz, J = 16.6 Hz). 4.43 (dd, 1H, J = 4.7 Hz, J = 16.6 Hz). IR: ν 1726 cm^{-1}. MS, *m*/*z*: 361 $[M + H]^+$.

*4.1.2.2. Synthesis of (±)1-(4-fluorophenyl)-2-(1H-imidazol-1-yl) ethyl 3-(trifluoromethyl)benzoate (**3b**).* **3b** was prepared as described above for **3a** using the alcohol **2b**. Refluxing conditions were used during 2 h period. Methanol/dichloromethane (0.4 : 9.6) was used as eluent for the chromatographic purification. **3b** was obtained in 54% yield as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 8.30 (s, 1H), 8.23 (d, 1H, J = 7.8 Hz), 7.87 (d, 1H, J = 7.8 Hz), 7.63 (t, 1H, J = 7.8 Hz), 7.36 (s, 1H), 7.29–7.26 (m, 2H), 7.07–7.02 (m, 3H), 6.87 (s, 1H), 6.21 (t, 1H, J = 6.6 Hz), 4.46 (dd, 1H, J = 14.6 Hz, J = 6.6 Hz), 4.40 (dd, 1H, J = 14.6 Hz, J = 4.8 Hz). IR: ν 1729 cm^{-1}. MS, *m*/*z*: 379 $[M + H]^+$.

*4.1.2.3. Synthesis of (±)1-(4-chlorophenyl)-2-(1H-imidazol-1-yl) ethyl 3-(trifluoromethyl)benzoate (**3c**).* **3c** was prepared as described above for **3a** using the alcohol **2c**. Methanol/dichloromethane (0.4 : 9.6) was used as eluent for the chromatographic purification. **3c** was obtained in 56% yield as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H), 8.21 (d, 1H, J = 7.8 Hz), 7.87 (d, 1H, J = 7.8 Hz), 7.64 (t, 1H, J = 7.8 Hz), 7.39–7.33 (m, 3H), 7.28–7.21 (m, 2H), 7.04 (s, 1H), 6.86 (s, 1H), 6.20 (t, 1H, J = 5.3 Hz), 4.48–4.41 (m, 2H). IR: ν 1729 cm^{-1}. MS, *m*/*z*: 395 $[M + H]^+$.

*4.1.2.4. Synthesis of (±)1-(4-trifluorophenyl)-2-(1H-imidazol-1-yl) ethyl 3-(trifluoromethyl)benzoate (**3d**).* **3d** was prepared as described above for **3a** using the alcohol **2d**. After addition of 3-(trifluoromethyl)benzoyl chloride the reaction mixture was stirred for 48 h. Methanol/dichloromethane (0.4 : 9.6) was used as eluent for the chromatographic purification. **3d** was obtained in 25% yield as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 8.30 (s, 1H), 8.22 (d, 1H, J = 7.8 Hz), 7.87 (d, 1H, J = 7.8 Hz), 7.67–7.60 (m, 3H), 7.42 (d, 2H, J = 8.1 Hz), 7.37 (s, 1H), 7.04 (s, 1H), 6.86 (s, 1H), 6.27 (t, 1H, J = 5.5 Hz), 4.51 (dd, 1H, J = 13,7 Hz, J = 4.2 Hz), 4.46 (dd, 1H, J = 13,7 Hz, J = 2.9 Hz). IR: ν 1730 cm^{-1}. MS, *m*/*z*: 429 $[M + H]^+$.

*4.1.2.5. Synthesis of (±)2-(1H-imidazol-1-yl)-1-phenylethyl 4-(trifluoromethyl)benzoate (**5a**).* **5a** was prepared as described above for **3a** using the alcohol **2a** and 4-(trifluoromethyl)benzoyl chloride. Methanol/dichloromethane (0.4 : 9.6) was used as eluent for the chromatographic purification. **5a** was obtained in 48% yield as a white solid, mp 70–2 °C (from ethylacetate).^{1}H NMR ($CDCl_3$): δ 8.14 (d, 2H, J = 7.6 Hz), 7.72 (d, 2H, J = 7.6 Hz), 7.38–7.32 (m, 6H), 7.00 (s, 1H), 6.84 (s, 1H), 6.22 (dd, 1H, J = 6.7 Hz, J = 5.0 Hz), 4.44 (dd, 1H, J = 14.1 Hz, J = 6.3 Hz), 4.39 (dd, 1H, J = 14.1 Hz, J = 5.0 Hz). IR: ν 1726 cm^{-1}. MS, *m*/*z*: 361 $[M + H]^+$.

*4.1.2.6. Synthesis of (±)2-(1H-imidazol-1-yl)-1-phenylethyl biphenyl-4-carboxylate (**6a**).* **6a** was prepared as described above for **3d** using the alcohol **2a** and biphenyl-4-carbonyl chloride. Methanol/ethylacetate (0.25 : 9.75) was used as eluent for the chromatographic purification. **6a** was obtained in 40% yield as a white solid, mp 110–2 °C (from ethylacetate). ^{1}H NMR ($CDCl_3$): δ 8.15–8.08 (m, 2H), 7.55–7.68 (m, 2H), 7.69–7.60 (m, 2H), 7.50–7.29 (m, 9H), 7.02 (s, 1H), 6.84 (s, 1H), 6.24 (t, 1H, J = 5.4 Hz), 4.49–4.40 (m, 2H). IR: ν 1718 cm^{-1}. MS, *m*/*z*: 369 $[M + H]^+$.

*4.1.2.7. Synthesis of (±)1-(4-fluorophenyl)-2-(1H-imidazol-1-yl) ethyl biphenyl-4-carboxylate (**6b**).* **6b** was prepared as described above for **6a** using the alcohol **2b**. After addition of biphenyl-4-carbonyl chloride the reaction mixture was refluxed for 3 h. Methanol/ethylacetate (0.25 : 9.75) was used as eluent for the chromatographic purification, and the more retained fraction was furtherly purified by a column chromatography on alumina with the same eluent. **6b** was obtained in 75% yield as colorless waxy solid.

^{1}H NMR ($CDCl_3$): δ 8.13 (d, 2H, J = 8.3 Hz), 7.71 (d, 2H, J = 8.9 Hz), 7.69–7.61 (m, 2H), 7.55–7.46 (m, 3H), 7.44–7.40 (m, 1H), 7.33–7.25 (m, 2H), 7.09–7.01 (m, 3H), 6.88 (s, 1H), 6.25 (t, 1H, J = 5.4 Hz), 4.46 (d, 2H, J = 5.4 Hz). IR: ν 1713 cm^{-1}. MS, *m*/*z*: 387 $[M + H]^+$.

*4.1.2.8. Synthesis of (±)1-(4-chlorophenyl)-2-(1H-imidazol-1-yl) ethyl biphenyl-4-carboxylate (**6c**).* **6c** was prepared as described above for **6a** using the alcohol **2c**. Methanol/ethylacetate (0.25 : 9.75) was used as eluent for the chromatographic purification. **6c** was obtained in 56% yield as a colorless waxy solid. ^{1}H NMR ($CDCl_3$): δ 8.12 (d, 2H, J = 8.4 Hz), 7.70 (d, 2H, J = 8.4 Hz), 7.67–7.60 (m, 2H), 7.51–7.41 (m, 4H), 7.35 (d, 2H, J = 8.4 Hz), 7.24 (d, 2H, J = 8.4 Hz), 7.04 (s, 1H), 6.86 (s, 1H), 6.22 (t, 1H, J = 5.4 Hz), 4.43 (d, 2H, J = 5.4 Hz). IR: ν 1718 cm^{-1}. MS, *m*/*z*: 403 $[M + H]^+$.

*4.1.3. Preparation of the hydrochlorides **4b**, **c***

3b was dissolved in methanol and the solution was saturated with gaseous HCl for 1 h and cooled at 0 °C. The solvent was removed under reduced pressure to give the hydrochloride 4b as a white solid. The solid was washed with diethylether and crystallized from methanol/diethylether. 4c was prepared using the same procedure.

4b. Yield 100% as a white solid, mp 175–7 °C ^{1}H NMR (DMSO-d_6): 8.93 (s, 1H), 8.34 (d, 1H, J = 7.7 Hz), 8.27 (s, 1H), 8.09 (d, 1H, J = 7.7 Hz), 7.80 (t, 1H, J = 7.7 Hz), 7.76 (s, 1H), 7.61–7.53 (m, 3H), 7.29 (t, 2H, J = 8.9 Hz), 6.36 (dd, 1H, J = 8.9 Hz, J = 3.3 Hz), 4.87 (dd, 1H, J = 14.4 Hz, J = 8.9 Hz), 4.73 (dd, 1H, J = 14.4 Hz, J = 3.3 Hz). IR: ν 1723 cm^{-1}.

4c. Yield 100% as a white solid, mp 156–8 °C (from methanol). ^{1}H NMR (DMSO-d_6): δ 9.08 (s, 1H), 8.34 (d, 1H, J = 7.7 Hz), 8.27 (s, 1H), 8.09 (d, 1H, J = 7.7 Hz), 7.79–7.72 (m, 2H), 7.61 (s, 1H), 7.57–7.50 (m, 4H), 6.41, (dd, 1H, J = 8.6 Hz, J = 3.4 Hz), 4.88 (dd, 1H, J = 14.3 Hz, J = 8.6 Hz), 4.77 (dd, 1H, J = 14.3 Hz, J = 3.4 Hz). IR: ν 1727 cm^{-1}.

*4.1.4. General procedure for the synthesis of **7b**, **c***

*4.1.4.1. Example: Synthesis of (±)1-(4-fluorophenyl)-2-(1H-imidazol-1-yl)ethyl phenoxyacetate (**7b**).* To a stirred suspension of 1-(4-

fluorophenyl)-2-(1*H*-imidazol-1-yl)ethanol (**2b**) (0.53 mmol) in dry acetonitrile (5 mL) was added sodium hydride (0.53 mmol). The reaction mixture was stirred for 2 h at rt, then phenoxyacetyl chloride (1.59 mmol) was dropwise added and the reaction mixture was over night stirred at rt. The solvent was removed under reduced pressure and the obtained residue was dissolved in dichloromethane and washed with aqueous saturated potassium carbonate.

The separated organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The solid residue was crystallized from ethylacetate to give pure **7b** in 95% yield (mp 106–8 °C).

^{1}H NMR (DMSO-d_6): δ 7.54 (s, 1H), 7.39–7.33 (m, 2H), 7.29–7.18 (m, 4H), 7.13 (s, 1H), 6.96 (t, 1H, J = 7.4 Hz), 6.87 (s, 1H), 6.84–6.75 (m, 2H), 6.15–6.08 (m, 1H), 4.90 (d, 1H, J = 16.7 Hz), 4.81 (d, 1H, J = 16.7 Hz), 4.48–4.41 (m, 2H). IR: ν 1752 cm^{-1}. MS, *m*/*z*: 341 $[M + H]^+$.

*4.1.4.2. Synthesis of (±)1-(4-chlorophenyl)-2-(1H-imidazol-1-yl) ethyl phenoxyacetate (**7c**).* **7c** was prepared as described above for **7b** using the alcohol **2c**. **7c** was obtained in 95% yield as a white solid, mp 103–5 °C (from ethylacetate).

^{1}H NMR (DMSO-d_6): δ 7.54 (s, 1H), 7.43 (d, 2H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.5 Hz), 7.27 (dd, 2H, J = 8.5 Hz, J = 7.4 Hz), 7.13 (s, 1H), 6.97 (t, 1H, J = 7.4 Hz), 6.86–6.77 (m, 3H), 6.11 (t, 1H, J = 5.5 Hz), 4.91 (d, 1H, J = 16.7 Hz), 4.83 (d, 1H, J = 16.7 Hz), 4.42 (d, 2H, J = 5.5 Hz). IR: ν 1751 cm^{-1}. MS, *m*/*z*: 357 $[M + H]^+$.

*4.1.5. General procedure for the synthesis of **8a–c***

*4.1.5.1. Example: Synthesis of (±)2-(1H-imidazol-1-yl)-1-phenylethyl [4-(propan-2-yl)phenyl]carbamate (**8a**).* To a stirred suspension of 2-(1*H*-imidazol-1-yl)ethanol (0.53 mmol) in dry acetonitrile (10 mL), was added sodium hydride (0.53 mmol). The reaction mixture was stirred for 2 h at rt, then 1-isocyanate-4-(propan-2-yl) benzene (0.79 mmol) was added and the reaction mixture was stirred for 24 h at rt. The solvent was removed under reduced pressure and the obtained residue was washed with methanol (2 × 3 mL). The mother liquor was dried and purified by silica gel column chromatography using 9 : 1 dichloromethane/methanol as eluent, to obtain a white solid that was washed with diethylether and crystallized from ethylacetate (yield 53%, mp: 190–2 °C).

^{1}H NMR (DMSO-d_6): δ 9.74 (s, broad, 1H), 7.56 (s, 1H), 7.40–7.30 (m, 7H), 7.17 (s, 1H), 7.16–7.09 (m, 2H), 6.85 (s, 1H), 5.99–5.91 (m, 1H), 4.44–4.37 (m, 2H), 2.85–2.78 (m, 1H), 1.15 (d, 6H, J = 6.9 Hz). IR: ν 1719 cm^{-1}. MS, *m*/*z*: 350 $[M + H]^+$.

*4.1.5.2. Synthesis of (±)1-(4-fluorophenyl)-2-(1H-imidazol-1-yl)-1-phenylethyl [4-(propan-2-yl)phenyl]carbamate (**8b**).* **8b** was prepared as described for **8a** using the alcohol **2b**. Methanol/dichloromethane (1 : 9) was used as eluent for the chromatographic purification. **8b** was obtained in 45% yield as a white solid, mp 200–2 °C (from ethylacetate). ^{1}H NMR (DMSO-d_6): δ 9.74 (s, broad, 1H), 7.55 (s, 1H), 7.45–7.39 (m, 2H), 7.31 (d, 2H, J = 8.5 Hz), 7.26–7.18 (m, 2H), 7.15 (s,1H), 7.13 (d, 2H, J = 8.5 Hz) 6.84 (s, 1H), 5.94 (t, 1H, J = 5.4 Hz), 4.47–4.40 (m, 2H), 2.83–2.77 (m, 1H), 1.16 (d, 6H, J = 6.9 Hz). IR: ν 1715 cm^{-1}. MS, *m*/*z*: 368 $[M + H]^+$.

*4.1.5.3. Synthesis of (±)1-(4-chlorophenyl)-2-(1H-imidazol-1-yl) ethyl[(4-propan-2-yl)phenyl]carbamate (**8c**).* **8c** was prepared as described for **8a** using the alcohol **2c**. Methanol/dichloromethane (1 : 9) was used as eluent for the chromatographic purification. **8c** was obtained in 30% yield as a white solid, mp 162–4 °C (from ethylacetate). ^{1}H NMR (DMSO-d_6): δ 9.75 (s, broad, 1H), 7.55 (s, 1H), 7.43 (d, 2H, J = 8.4 Hz), 7.36 (d, 2H, J = 8.4 Hz), 7.30 (d, 2H, J = 8.4 Hz), 7.15 (s, 1H), 7.12 (d, 2H, J = 8.4 Hz), 6.84 (s, 1H), 5.98–5.90 (m, 1H), 4.45–4.37 (m, 2H), 2.84–2.76 (m, 1H), 1.14 (d, 6H, J = 6.8 Hz). IR: ν 1722 cm^{-1}. MS, *m*/*z*: 384 $[M + H]^+$.

*4.1.5.4. Synthesis of (±)1-(4-chlorophenyl)-2-(1H-imidazol-1-yl) ethyl(2,6-dichloropyridin-4-yl)carbamate (**9c**).* 2,6-Dichloro-4-aminopyridine (1.23 mmol) was dissolved in 30 mL of anhydrous benzene at 30 °C, then triethylamine (3.09 mmol) was added. The solution was stirred at rt and 5 mL of anhydrous benzene containing 0.75 mmol of triphosgene were added dropwise. The yellow suspension was stirred for 12 h at rt and then 1.23 mmol of **2c** were added. The reaction mixture was stirred for 48 h at rt. Afterward 30 mL of aqueous saturated sodium carbonate was added under vigorous stirring; the organic layer was separated and the alkaline solution was extracted with 3 portions (10 mL each) of dichloromethane. The combined organic fractions were dried over anhydrous sodium sulphate and evaporated under reduced pressure to give a residue which was purified on silica gel column chromatography using methanol/dichloromethane (1 : 9) as eluent. The purification provided a white solid that was washed with dichloromethane and crystallized from methanol. Yield 25%, mp: 227-9 °C.

^{1}H NMR (DMSO-d_6): δ 10.81 (s, broad, 1H), 7.54 (s, 1H), 7.50–7.41 (m, 4H), 7.38 (d, 2H, J = 8.5 Hz), 7.14 (s, 1H), 6.86 (s, 1H), 6.03 (t, 1H, J = 5.7 Hz), 4.48 (d, 2H, J = 5.7 Hz). IR: ν 1722 cm^{-1}. MS, *m*/*z*: 412 $[M + H]^+$.

4.1.6. Enantioselective HPLC

Analytical HPLC analysis of **6a**, **b** were performed using the commercially available 250 mm × 4.6 mm I.D. Chiralcel OD column (Chiral Technologies Europe, Illkirch, France). HPLC grade ethanol was purchased from Aldrich (St. Louis, Missouri USA). Semi-preparative HPLC enantioseparations were performed using the commercially available 250 mm × 10 mm I.D. Chiralcel OD column.

The analytical HPLC apparatus consisted of a Perkin–Elmer (Norwalk, CT, USA) 200 LC pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 20 μL sample loop, an HPLC Dionex CC-100 oven (Sunnyvale, CA, USA) and a Jasco (Jasco, Tokyo, Japan) Model CD 2095 Plus UV/CD detector.

The semipreparative separation was carried out with a Perkin–Elmer (Norwalk, CT, USA) 200 LC pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 1 mL sample loop, a Perkin–Elmer LC 101 oven and a Waters 484 detector (Waters Corporation, Milford, MA, USA). The signal was acquired and processed by Clarity software (DataApex, Prague, The Czech Republic).

4.1.7. Polarimetry

Specific rotations were measured at 589 nm by a Perkin–Elmer polarimeter model 241 equipped with a Na/Hg lamp. The volume of the cell was 1 mL and the optical path was 10 cm. The system was set to 20 °C.

The specific rotation values of the enantiomers of **6a**, **b** were: (+)-**6a**, $[\alpha]_D^{20}$ +31 (*c* = 0.2, EtOH); (−)-**6a**, $[\alpha]_D^{20}$ −32 (*c* = 0.2, EtOH); (+)-**6b**, $[\alpha]_D^{20}$ +31 (*c* = 0.2, EtOH); (−)-**6b**, $[\alpha]_D^{20}$ −31 (*c* = 0.2, EtOH).

4.2. In vitro antifungal activity

4.2.1. Organisms

For the antifungal evaluation, strains obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), from the German Collection of Microorganisms (DSMZ, Braunschweig, Germany) and from Pharmaceutical Microbiology Culture Collection (PMC, Department of Public Health and Infectious Diseases, Sapienza, Rome, Italy) were tested. The strains obtained from the ATCC were: *C. albicans* ATCC10231, *C. albicans* ATCC3153, *C. albicans* ATCC76615, *C. albicans* ATCC24433 (tested as reference strain),

C. parapsilosis ATCC22019 (tested as quality control strain). The strains obtained from the DSMZ were: *C. tropicalis* DSM11953, *C. krusei* DSM6128, *C. parapsilosis* DSM11224. The strains obtained from PMC were: *C. krusei* PMC0613, *C. tropicalis* PMC0910, *C. glabrata* PMC0805. All the strains were stored at −70 °C in 15% glycerol solution, as indicated in Clinical and Laboratory Standards Institute (CLSI) document M27-A3 [24]. Yeasts were grown on sabouraud dextrose agar (Sigma–Aldrich, St. Louis, Missouri, U.S.A) plate for 24 h at 35 °C, in accordance with the CLSI procedures [24].

4.2.2. Antifungal susceptibility testing

The tests were conducted in accordance with the CLSI M27-A3 broth microdilution methods [24]. Yeast inoculum suspensions were prepared as described in the CLSI protocols. The antifungal reference fluconazole (Sigma–Aldrich, St. Louis, Missouri, U.S.A.) was also tested (range 0.0078–128 μg mL^{-1}). The final concentrations ranged from 0.125 to 64 μg mL^{-1} for new compounds. Microdilution trays containing 100 μl of serial two-fold dilutions of each substance in RPMI 1640 medium (Sigma–Aldrich, St. Louis, Missouri, U.S.A) were inoculated with an organism suspension adjusted to attain a final inoculum concentration of 1.0×10^3–1.5×10^3 cells mL^{-1}. The panels were incubated at 35 °C and observed for the presence of growth at 48 h. Quality control was performed by testing the CLSI-recommended strain *C. parapsilosis* ATCC22019 as indicated in CLSI document M27-S3 [25]. MICs were determined three times in triplicate. The MICs of new compounds and fluconazole were read as the lowest concentration at which a significant decrease in turbidity (≥50%) was discerned compared to that of the growth control.

The minimum concentrations of the drug that inhibited 90% and 50% of the isolates were defined as the MIC_{90} and MIC_{50}, respectively.

4.3. In vitro cytotoxicity test

4.3.1. Cell viability assay

The cytotoxicity of tested new compounds was evaluated on human monocytic cell line (U937) obtained from American Type Culture Collection (ATCC CRL1593.2, Rockville, MD, USA) by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay [26]. The cells (2×10^4 cells $well^{-1}$) were seeded into 96-well plates containing 100 μL of supplemented RPMI 1640 (Invitrogen, San Diego, CA, USA) without phenolred, supplemented with 10% fetal bovine serum (Invitrogen, San Diego, CA, USA), L-glutamine (0.3 mg mL^{-1}), penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}) (EuroClone, Celbio, Milan, Italy), and they were cultured at 37 °C in 5% CO_2. The cells were exposed to the indicated compounds (dissolved in dimethyl sulfoxide, DMSO) at the final concentration ranging from 8 to 128 μg mL^{-1}. Each concentration and control was assayed in three replicates with at least five concentrations. The cells were cultured at 37 °C and 5% CO_2 for 48 h. MTT solution (Sigma–Aldrich, St. Louis, Missouri, U.S.A.) was added to each well in an amount equal to 10% of the culture volume, and the plates were incubated for 3–4 h at 37 °C in 5% CO_2. MTT solvent (Sigma–Aldrich, St. Louis, Missouri, U.S.A.) was successively added to dissolve the intracellular crystal. The plates were then incubated at 37 °C in 5% CO_2, and the optical density of each well was measured spectrophotometrically at 570 nm. The cytotoxicity of the compounds was calculated as percentage reduction in viable cells with respect to the control culture (cells treated with DMSO only). The 50% cytotoxic concentration (CC_{50}) was evaluated as the drug concentration required to reduce human cell viability by 50% compared to the drug-free control. The Selectivity Index (SI) was defined as the ratio of the concentration of the drug that reduced human cell viability to 50% (CC_{50}) to the concentration of the drug needed to reduced fungal cell viability to 50% (MIC).

Antifungal activity was determined according to CLSI guidelines (CLSI document M27-A3,2008). MIC = arithmetic mean of minimal inhibitory concentration; SD = standard deviation.

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Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2012.01.034. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] M.A. Pfaller, D.J. Diekema, Epidemiology of invasive mycoses in North America, Cri. Rev. Microbiol. 36 (2010) 1–53.
[2] A. Puel, C. Picard, S. Cypowyj, D. Lilic, L. Abel, J.L. Casanova, Inborn errors of mucocutaneous immunity to *Candida albicans* in humans: a role for IL-17 cytokines? Curr. Opin. Immunol. 22 (2010) 467–474.
[3] M.A. Pfaller, D.J. Diekema, Epidemiology of invasive candidiasis: a persistent public health problem, Clin. Microbiol. Rev. 20 (2007) 133–163.
[4] E. Concia, A.M. Azzini, Epidemiology, incidence and risk factors for invasive candidiasis in hight-risk patients, Drugs 69 (Suppl.1) (2009) 5–14.
[5] M.C. Arendrup, Epidemiology of invasive candidiasis, Curr. Opin. Crit. Care 16 (2010) 445–452.
[6] M. Pennisi, M. Antonelli, Clinical aspects of invasive candidiasis in critically Ill patients, Drugs 69 (Suppl. 1) (2009) 21–28.
[7] S.C. Chen, E.G. Playford, T.C. Sorrell, Antifungal therapy in invasive fungal infection, Curr. Opin. Pharmacol. 10 (2010) 522–530.
[8] N. Strushkevich, S.A. Usanov, Structural basis of human CYP51 inhibition by antifungal azoles, J. Mol. Biol. 397 (2010) 1067–1078.
[9] F. Odds, A.J.P. Brown, N.A.R. Gow, Antifungal agents:mechanism of action, Trends Microbiol. 11 (2003) 272–279.
[10] J.L. Adams, B.W. Metcalf, Therapeutic Consequences of the inhibition of sterol metabolism, in: C. Hansch, P.G. Sammes, J.B. Taylor (Eds.), Comprehensive Medicinal Chemistry, vol. 2, Pergamon Press, Oxford, England, 1990, pp. 333–364.
[11] Y.Q. Zhang, S. Gamarra, G. Garcia-Effron, Requirement for ergosterol in V-ATPase function underlies antifungal activity of azole drugs, PLOS Pathogenes 6 (6) (June 2010) e1000939.
[12] M.A. Pfaller, D. Andes, D.J. Diekema, Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and *Candida*: time for harmonization of CLSI and EUCAST broth microdiluition methods, Drug Resist. Updates 13 (2010) 180–195.
[13] L.M. Podust, T.L. Poulos, M.R. Waterman, Crystal structure of cytochrome P450 14R-sterol demethylase (CYP51) from *Mycobacterium tuberculosis* in complex with azole inhibitors, Proc. Natl. Acad. Sci. U.S.A 98 (6) (2001) 3068–3073.
[14] G.I. Lepesheva, H. Park, T.Y. Hargrove, B. Vanhollebeke, Z. Wawrzak, J.M. Harp, M. Sundaramoorthy, W.D. Nes, E. Pays, M. Chaudhuri, F. Villalta, M.R. Waterman, Crystal structures of *Trypanosoma brucei* sterol 14-demethylase and Implications for selective treatment of human infections, J. Biol. Chem. 285 (3) (2010) 1773–1780.
[15] C. Sheng, W. Wang, X. Che, G. Dong, S. Wang, H. Ji, Z. Miao, J. Yao, W. Zhang, Improved model of lanosterol 14α-demethylase by ligand-supported homology modeling: Validation by virtual screening and azole optimization, Chem. Med. Chem. 5 (2010) 390–397.
[16] R. Di Santo, A. Tafi, R. Costi, M. Botta, M. Artico, F. Corelli, M. Forte, F. Caporuscio, L. Angiolella, A.T. Palamara, Antifungal agents. 11. N-Substituted derivatives of 1-[(aryl)(4-aryl-1H-pyrrol-3-yl)methyl]-1H-imidazole: synthesis, anti-*Candida* activity, and QSAR studies, J. Med. Chem. 48 (2005) 5140–5153.
[17] G. La Regina, F.D. D'Auria, A. Tafi, F. Piscitelli, S. Olla, F. Caporuscio, L. Nencioni, R. Cirilli, F. La Torre, N. Rodrigues De Melo, S.L. Kelly, D.C. Lamb, M. Artico, M. Botta, A.T. Palamara, R. Silvestri, 1-[(3-Aryloxy-3-aryl)propyl]-1H-imidazoles, new imidazoles with potent activity against *Candida albicans* and Dermatophytes. Synthesis, structure-activity relationship, and molecular modeling studies, J. Med. Chem. 51 (2008) 3841–3855.

[18] G.C. Porretta, R. Fioravanti, M. Biava, R. Cirilli, N. Simonetti, A. Villa, U. Bello, P. Faccendini, B. Tita, Research on antibacterial and antifungal agents. X. Synthesis and antimicrobial activity of 1-phenyl-2-(1H-azol-1-yl) ethane derivatives. Anticonvulsivant activity of 1-(4-methylphenyl)-2-(1H-imidazol-1-yl) ethanol, Eur. J. Med. Chem. 28 (1993) 749–760.
[19] Y. Wahbi, C. Tournaire, R. Caujolle, M. Payard, M.D. Lynas, J.P. Seguela, Aliphatic ethers and esters of 1-(2,4-dichlorophenyl)-2-(1H-imidazolyl)-ethanol: study of antifungal activity yeast and hydrophobic character, Eur. J. Med. Chem. 29 (1994) 701–706.
[20] K.A.M. Walker, D.R. Hirschfeld, M. Marx, Antimycotic imidazole. 2. Synthesis and antifungal properties of 1-[2-Hydroxy(mercapto)-2-phenylethyl]-1H-imidazoles, J. Med. Chem. 21 (1978) 1335–1338.
[21] G.I. Lepesheva, R.D. Ott, T.Y. Hargrove, Y.Y. Kleshchenko, I. Schuster, W.D. Nes, G.C. Hill, F. Villalta, M.R. Waterman, Sterol 14α-demethylase as a potential target for antitrypanosomal therapy: enzyme inhibition and parasite cell growth, Chem. Biol. 14 (2007) 1283–1293.
[22] G.I. Lepesheva, H.W. Park, T.Y. Hargrove, B. Vanhollebeke, Z. Wawrzak, J.M. Harp, M. Sundaramoorthy, W.D. Nes, E. Pays, M. Chaudhuri, F. Villalta, M.R. Waterman, Structural insights into inhibition of Sterol 14α-demethylase in the human pathogen *Trypanosoma cruzi*, J. Biol. Chem. 285 (2010) 25852 25590.
[23] A. Karakurt, M. Ozalp, S.I. sik, J.P. Stables, S. Dalkara, Synthesis, anti-convulsivant and antimicrobial activities of some new 2-acetylnaphtalene derivatives, Bioorg. Med. Chem. 18 (2010) 2902–2911.
[24] CLSI, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts Approved Standard-Third Edition. CLSI document M27–MA3, Clinical and Laboratory Standards Institute, Wayne, PA, 2008.
[25] CLSI, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts Third Informational Supplement. CLSI document M27–MS3, Clinical and Laboratory Standards Institute, Wayne, PA, 2008.
[26] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay, J. Immunol. Methods 65 (1983) 55–63.