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Synthesis and antifungal properties of *N*-[(1,1′-biphenyl)-4-ylmethyl]-1*H*-imidazol-1-amine derivatives

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Abstract

In the course of a study on 1*H*-imidazol-1-amine derivatives as antifungal agents, we found that *N*-[(1,1′-biphenyl)-4-ylmethyl]-*N*-[(2,4-dichlorophenyl)methyl]-1*H*-imidazol-1-amine (**1a**) exhibited promising activities. In order to explore more in detail the structure–activity relationship of this new class of antifungal agents, we report now the synthesis and the biological activity of new analogues (**1b**–**k**) of compound **1a**. The synthesis was performed using *N*-[(1,1′-biphenyl)-4-ylmethyl]-1*H*-imidazol-1-amine as starting material which was reacted with the proper arylmethyl halide. Most of the newly synthesized imidazolamines exhibited both fungal growth inhibition activity and cellular selectivity.

Keywords: Azole antifungal agents; Aminoimidazole derivatives

1. Introduction

Medical advances have led to increased numbers of immunocompromised patients living longer. Coinciding with this increase in the immunocompromised patient population is an increase in the number of clinically significant fungal infections. Unfortunately, widespread use of antifungal agents to treat these infections has led to the development of drug resistance. Several strategies to overcome resistance have been identified, but the development of new antifungal drugs is likely to have the most significant future impact on the management of drug resistance in fungal infections [1,2]. The oxidative metabolism of lanosterol, that is included in the biosynthetic pathway of ergosterol, is catalyzed by P450-14α-demethylase (CYP51) [3]. Many azole antifungal agents inhibit the oxidative removal of C-32 of lanosterol and this inhibition causes depletion of ergosterol and accumulation of 14-methylsterols in the membrane of fungal cells. Such change in sterol composition disturbs membrane function and results in growth inhibition and death of the fungal cells. Accordingly, P450-14α-demethylase is considered as the primary target for azole antifungal agents. Mammalian cells contain various species of cytochrome P450 which are responsible for many important cellular metabolic functions. If azole antifungal agents inhibit mammalian cytochrome P450 too, their systemic use may result in potentially significant adverse reactions. Binding selectivity of an azole antifungal agent to P450-14α-demethylase is predominantly determined by the substituent at N-1 of the azole group, and the substituent must interact with the substrate site of the cytochrome [4]. Extensive modification of the N-1 substituents and the screening of newly synthesized compounds may represent a tool for the development of new antifungal drugs.

Pursuing our research in this field, we focused our attention on 1*H*-imidazol-1-amine derivatives [5] and found compound *N*-[(1,1′-biphenyl)-4-ylmethyl]-*N*-[(2,4-dichlorophenyl)methyl]-1*H*-imidazol-1-amine (**1a**) to be active against yeasts and dermatophytes, with potency and selectivity comparable to those of miconazole [6]. To explore more in detail the structure–activity relationship of this new class of antifungal agents, we report now the synthesis and the biological activity of new analogues (**1b**–**k**) of compound **1a**. In this series of

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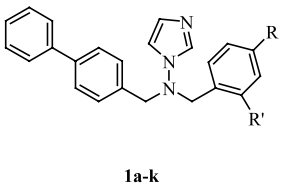


1a-k

1	R	R'
a	Cl	Cl
b	F	F
c	H	H
d	H	Cl
e	H	F
f	H	Me
g	H	OMe
h	Cl	H
i	F	H
j	Me	H
k	OMe	H

Fig. 1. Chemical structures of the *N*-[(1,1′-biphenyl)-4-ylmethyl]-1*H*-imidazol-1-amine derivatives.

derivatives the *N*-biphenylmethyl portion is kept constant while the second substituent on the imidazolamino moiety is the variable parameter (Fig. 1).

2. Chemistry

Disubstututed 1*H*-imidazol-1-amines (**1b**–**k**) were prepared by alkylation of the *N*-[(1,1′-biphenyl)-4-ylmethyl)]-1*H*-imidazol-1-amine (**2**) [6] with the proper arylmethyl halide, using two alternative routes depending on the alkylating agent: (i) anhydrous diethyl ether in the presence of potassium *tert*-butoxide and 18-crown-6; and (ii) dichloromethane/50% sodium hydroxide aqueous solution in the presence of tetrabuthylammonium hydrogen sulfate (Scheme 1).

3. Results and discussion

The title compounds were evaluated in vitro against several pathogenic fungi responsible for human disease. Test pathogens included representatives of yeasts (*Candida albicans*, *Candida parapsilosis*, *Criptococcus neoformans*), dermathophytes (*Tricophyton verrucosum*, *Tricophyton rubrum*, *Microsporum gypseum*) and molds (*Aspergillus fumigatus*). Miconazole was used as reference drugs.

As shown in Table 1, substitution of 2,4-dichlorophenyl moiety in compound **1a** lowered the activities against yeasts of the newly synthesized imidazolamines. Strangely, replacing in **1a** the 2,4-dichlorophenyl moiety with 2,4-difluorophenyl one (**1b**) led to a dramatic decrease in activity. The presence of substituents on phenyl group seems to be necessary being compound **1c** devoid of activity. 2-Substituted phenyl compounds display better results than 4-substituted phenyl ones, especially against *C. neoformans*. Inhibition of dermatophytes was less influenced by the aryl portion. Also in this case compound **1a** displayed the best performance, even if 2-substituted phenyl compounds **1f** and **1g** exhibited an equivalent minimum inhibitory concentration against *T. verrucosum* when compared to miconazole. All compounds were inactive when used against the mold strain tested (*A. fumigatus*).

Title compounds were also examined for antibacterial activity and all compounds show no activity against representative Gram negative bacteria (*Salmonella* spp.). Conversely, some derivatives are active against representative Gram positive bacteria (*Staphylococcus aureus*). The minimum inhibitory concentrations of 4-substituted phenyl derivatives **1j**,**k** were found comparable with that of Streptomicine (Table 1).

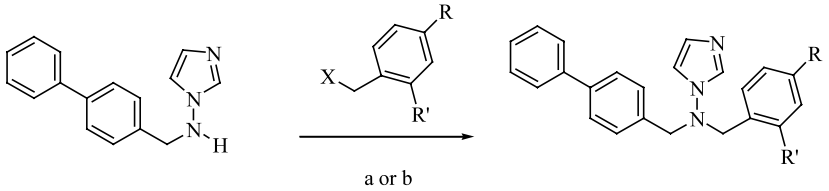
Title compounds were tested for in vitro cytotoxicity in a lymphoid cell line (MT-4). Cytotoxicity evaluation was performed in order to determine whether test compounds were endowed with selective antimicrobial activity. As show in Table 1, all the derivatives are less toxic than miconazole.

In conclusion, although most of the new synthesized compounds resulted active against pathogenic fungi at concentrations lower than those cytotoxic, the modification of the 2,4-dichlorophenyl portion did not enhance the antifungal activity of compound **1a**. These data help us to plan further chemical modifications in order to gain more information on this new class of antifungal agents. These studies are currently underway.

4. Experimental

4.1. Chemistry

M.p.s were determined in open capillary tubes with a Büchi apparatus and are uncorrected. Elemental analyses (C, H, N) were performed by Dr Emilio Cebulec at the Chemistry Department of the University of Trieste and are within ±0.4% of the calculated values. The ^{1}H NMR spectra were determined on a Varian 200 instru-



2 **1b-k**

Scheme 1. X = Cl or Br; (a) 18-crown-6, diethyl ether, *t*-BuOK; (b) dichloromethane/water, NaOH, TBAHS.

Table 1
In vitro antifungal and antibacterial activity of compounds **1a**–**k**

No.	CC_{50} MT-4 [a]	MIC [b]/MBC [c] (MFC)						
		S. aureus	*C. parapsilosis*	*C. neoformans*	*C. albicans*	*T. verrucosum*	*M. gypseum*	*T. rubrum*
a	48	> 200	6.2/6.2	2.5/7.4	6.2/6.2	2.4/7.4	7.4/22	0.8/2.5
b	41.5	200/200	200/200	33/33	200/200	5.5/33	33/33	200/200
c	42	200/ > 200	200/200	200/200	200/200	33/33	33/33	200/200
d	> 200	200/200	33/33	33/33	33/33	5.5/33	33/33	5.5/5.5
e	67.6	200/200	200/200	33/33	200/200	33/33	33/33	200/200
f	> 200	40/200	33/200	5.5/5.5	200/200	0.9/33	33/33	5.5/5.5
g	> 200	40/200	33/33	5.5/5.5	33/33	0.9/33	5.5/33	33/33
h	> 200	200/200	33/200	200/200	33/200	33/33	33/33	5.5/33
i	45.3	> 200	33/200	200/200	33/200	33/33	33/200	200/200
j	68.1	8/40	33/200	200/200	33/200	5.5/33	33/33	33/33
k	> 200	8/ > 200	200/ > 200	200/ > 200	200/200	33/200	200/200	33/200
M [d]	18		12.5/12.5	0.8/1.56	12.5/12.5	0.8/0.8	3.1/3.1	0.4/0.8
S [e]	> 200	6.2/6.2						

[a] Compound dose (μM) required to reduce the viability of MT-4 cells by 50%, as determined by the MTT method.
[b] Minimum inhibitory concentration (μM).
[c] Minimum bactericidal (fungicidal) concentration (μM).
[d] Miconazole.
[e] Streptomicine.

ment. Chemical shifts are given in δ values downfield from TMS as internal standard. Mass spectra data were determined on a V6-Micromass 7070H mass spectrometer. Silica gel chromatography was performed using Merck silica gel 60 (0.015–0.040 mm). Petroleum ether refers to petroleum ether (40–60 °C).

4.2. General procedure for the preparation of N-arylmethyl-N-[(1,1′-biphenyl)-4-ylmethyl])-1H-imidazol-1-amines (**1b**–**k**)

4.2.1. Method A

A solution of *N*-[(1,1′-biphenyl)-4-ylmethyl]-1*H*-imidazol-1-amine (2.47 g, 10.0 mmol) and tetrabutylammonium hydrogen sulfate (3.39 g, 10.0 mmol) in CH_2Cl_2 (50 ml) was treated with the appropriate halide (15.0 mmol) and 50% NaOH aqueous solution (30 ml). After 15 h of stirring at room temperature (r.t.), CH_2Cl_2 (60 ml) was added and the mixture was poured into ice-water (60 ml) and extracted with CH_2Cl_2 (3 × 50 ml). The combined organic extracts were evaporated and the residue was taken up with EtOAc (150 ml), washed with brine, dried over Na_2SO_4 and evaporated. The crude product was purified by silica gel column chromatography using EtOAc as eluent.

4.2.2. Method B

Potassium *tert*-butoxide (2.24 g, 20.0 mmol) was added to a solution of 18-crown-6 (0.53 g, 2.00 mmol) in Et_2O (50 ml), then *N*-[(1,1′-biphenyl)-4-ylmethyl]-1*H*-imidazol-1-amine (4.94 g, 20.0 mmol) was added in a single portion. After stirring for 15 min at r.t. under nitrogen, a solution of the appropriate halide (20.0 mmol) in Et_2O (30 ml) was added dropwise to the cooled reaction mixture over a period of 20 min.

After 4 h of stirring at r.t., water (60 ml) was added and the mixture extracted with Et_2O (3 × 50 ml). The combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated. The crude product purified by silica gel column chromatography using EtOAc as eluent.

Compounds **1b**–**k** were obtained as colorless glasses. Trituration with petroleum ether followed by recrystallization (C_6H_6–ligroin), gave compounds **1b**–**f**,**h**–**k** as white solids. Compound **1g** was crystallized (EtOH–Et_2O) as hydrochloride salt. Yields, m.p.s, analytical and spetroscopic data are reported in Table 2.

4.3. Microbiology

4.3.1. Compounds

Test compounds were dissolved in DMSO at an initial concentration of 200 mM and then were serially diluted in culture medium.

4.3.2. Cells

Cell line were from American Type Culture Collection (ATCC); bacterial and fungal strains were either clinical isolates (obtained from Clinica Dermosifilopatica, University of Cagliari) or collection strains from ATCC.

4.3.3. Antibacterial assays

S. aureus, group D *Streptococcus*, *Shighella* and *Salmonella* spp. were recent clinical isolates. Tests were carried out in nutrient broth, pH 7.2, with an inoculum of 10^3 cells/tube. MICs were determined after

Table 2
Yields, physical, analytical and spectroscopic data of compounds **1b**–**k**

No.	Formula*	Yield % (method)	M.p. (°C)	MS m/z $[M^+]$	^{1}H NMR: δ (ppm)
1b	$C_{23}H_{19}F_2N_3$	21 (A)	98–100	375	4.21 (s, 2H, CH_2), 4.25 (s, 2H, CH_2), 6.71–6.85 (m, 2H, Ar–H), 6.96 (s, 1H, imidazole–H5), 7.06–7.65 (m, 12H, imidazole–H2, imidazole–H4, Ar–H)
1c	$C_{23}H_{21}N_3$	49 (A)	76–78	339	4.19 (s, 2H, CH_2), 4.21 (s, 2H, CH_2), 6.95 (s, 1H, imidazole–H5), 7.14–7.62 (m, 16H, imidazole–H2, imidazole–H4, Ar–H)
1d	$C_{23}H_{20}ClN_3$	51 (A)	83–84	373	4.26 (s, 2H, CH_2), 4.35 (s, 2H, CH_2), 6.94 (s, 1H, imidazole–H5), 7.18–7.60 (m, 15H, imidazole–H2, Ar–H)
1e	$C_{23}H_{20}FN_3$	30 (A)	64–67	357	4.25 (s, 2H, CH_2), 4.26 (s, 2H, CH_2), 6.92–7.60 (m, 16H, imidazole–H, Ar–H)
1f	$C_{24}H_{23}N_3$	47 (A)	101–103	353	2.39 (s, 3H, CH_3), 4.30 (s, 4H, CH_2 and CH_2), 6.94 (s, 1H, imidazole–H5), 7.03–7.60 (m, 15H, imidazole–H2, imidazole–H4, Ar–H)
1g	$C_{24}H_{23}N_3O\cdot HCl$	35 (B)	164–168	369 $[M^+ - (HCl)]$	3.78 (s, 3H, CH_3), 4.24 (s, 2H, CH_2), 4.27 (s, 2H, CH_2), 6.82–6.95 (m, 3H, imidazole–H5, Ar–H), 7.15–7.60 (m, 13H, imidazole–H2, imidazole–H4, Ar–H)
1h	$C_{23}H_{20}ClN_3$	21 (A)	68–70	373	4.14 (s, 2H, CH_2), 4.21 (s, 2H, CH_2), 6.94 (s, 1H, imidazole–H5), 7.12–7.60 (m, 15H, imidazole–H2, imidazole–H4, Ar–H)
1i	$C_{23}H_{20}FN_3$	32 (A)	85	357	4.15 (s, 2H, CH_2), 4.22 (s, 2H, CH_2), 6.90–7.02 (m, 3H, imidazole–H5, Ar–H), 7.10–7.60 (m, 13H, imidazole–H2, imidazole–H4, Ar–H)
1j	$C_{24}H_{23}N_3$	48 (A)	80–82	353	2.31 (s, 3H, CH_3), 4.15 (s, 2H, CH_2), 4.19 (s, 2H, CH_2), 6.93 (s, 1H, imidazole–H5), 7.05–7.60 (m, 15H, imidazole–H2, imidazole–H4, Ar–H)
1k	$C_{24}H_{23}N_3O$	39 (B)	77–81	369	3.77 (s, 3H, CH_3), 4.11 (s, 2H, CH_2), 4.19 (s, 2H, CH_2), 6.82 (d, $J = 8.5$ Hz, 2H, Ar–H), 6.95 (s, 1H, imidazole–H5), 7.10–7.60 (m, 13H, imidazole–H2, imidazole–H4, Ar–H)

*Analytical results for C, H, N were within $\pm 0.4\%$ of the calculated values.

18 h incubation at 37 °C in the presence of serial dilutions of the test compounds. The minimal bactericidal concentration (MBC) was determined by subcultivating in Triptosio agar samples from cultures with no apparent growth.

4.3.4. Antimycotic assays

Yeast inocula were obtained by properly diluting cultures incubated at 37 °C for 30 h in Sabouraud dextrose broth to obtain 5×10^3 cells/ml. On the contrary, dermatophyte and mold inocula were obtained from cultures grown at 37 °C for 5 days in Sabouraud broth by finely dispersing clumps with a glass homogenizer before diluting to 0.05 OD (590 nm). Then, 20 μl of the above suspensions were added to each well of flat-bottomed microtiter trays containing 80 μl of medium with serial diluitions of test compounds and were incubated at 37 °C. Growth controls were visually determined after 1 (yeasts and molds) or 3 days (dermatophites). MIC was defined as the compound concentration at which no macroscopic sign of fungal growth was detected. The minimal fungicidal concentration (MFC) was determined by subcultivating in Sabouraud dextrose agar samples from cultures with no apparent growth.

The cytotoxicity evaluation of compounds was based on the viability of MT-4 cells, as monitored by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [7].

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