ELSEVIER

www.elsevier.com/locate/farmac

Il Farmaco 56 (2001) 633–640

IL FARMACO

Synthesis and biological evaluation of azole derivatives, analogues of bifonazole, with a phenylisoxazolyl or phenylpyrimidinyl moiety

Giulia Menozzi [a,*], Luisa Mosti [a], Paola Fossa [a], Chiara Musiu [b], Chiara Murgioni [b], Paolo La Colla [b]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV 3, I-16132 Genova, Italy*
[b] *Dipartimento di Biologia Sperimentale, Università di Cagliari, Viale Regina Margherita 45, I-09124 Cagliari, Italy*

Received 13 February 2001; accepted 8 March 2001

Abstract

A series of azole derivatives, isoxazole or pyrimidine analogues of the antifungal drug bifonazole, were synthesized and tested in vitro against representative human pathogenic fungi (*Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*). They were also evaluated as antibacterial agents against *Staphylococcus aureus* and *Salmonella* spp. Only 5-(imidazol-1-yl-phenylmethyl)-2,4-diphenyl-pyrimidine **7c** showed weak antimicrobial activity (MIC = 66 μM) against *C. albicans*, *C. neoformans* and *S. aureus*. Results of biological tests proved, therefore, that replacement of the biphenyl portion of the bifonazole with a phenylisoxazolyl or phenylpyrimidinyl moiety is not profitable for antimicrobial properties.

Keywords: Azole derivatives; Antifungal agents; Bifonazole analogues

1. Introduction

The continuously changing epidemiology of invasive fungal infections results in the need for an expanded armamentarium of antifungal therapies. On the other hand, the increased use of antimicrobial agents in recent years has caused the development of resistance to available drugs. For these reasons, a constant effort toward the synthesis of new antifungal agents has been made in the last few years.

In particular, the class of azoles (imidazole and triazole derivatives) has supplied many effective antifungal drugs currently in clinical use, and newer azoles with expanded spectrum of activity are at the moment in continuous development [1].

After the discovery of bifonazole (**I**), an imidazole derivative used currently for topic therapy of mycoses and dermatomycoses, many modifications were carried out on its structure so as to improve its antifungal potency and selectivity. In particular, bioisosteric replacements of either or both benzene rings, constituting the biphenyl portion of the molecule, have been studied [2].

In a previous paper [3], we reported the synthesis and biological activity of pyrazole analogues (**II**) of bifonazole, in which the biphenyl portion was replaced with a phenylpyrazolyl moiety, bearing an aliphatic or aromatic substituent at C-5 of the heterocyclic ring (Fig. 1).

In pursuing our research in the area of heterocyclic analogues of bifonazole, we planned the synthesis of new azole derivatives, containing an isoxazole (**III**) or a pyrimidine nucleus (**IV**).

In support of our approach, the literature reported some isoxazolecarboxanilides [4] and phenylisoxazole derivatives [5] endowed with fungicidal activity. Moreover, pyrimidine nucleus is a basic component of well-known chemotherapeutic drugs, like sulfanylamides, pyrimethamine, trimethoprim and 5-fluorocytosine, and it is also present in the structure of voriconazole [6], a new azole drug active against resistant yeasts and

* Corresponding author.
E-mail address: menozzi@unige.it (G. Menozzi).

0014-827X/01/$ - see front matter
PII: S0014-827X(01)01117-X

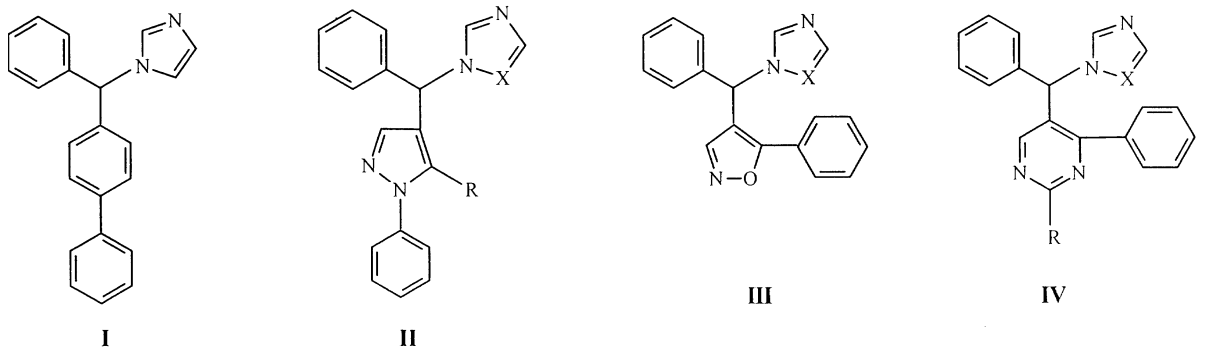


Fig. 1. Bifonazole (**I**), pyrazole (**II**), isoxazole (**III**) and pyrimidine (**IV**) analogues; X = H, N.

molds, which is in phase III of clinical trials. Finally, natural compounds with antifungal properties, bacimethrin [7] and blasticidin S [8], are pyrimidine derivatives.

In this paper we present the synthesis and biological evaluation of some bifonazole-like azole derivatives (**3**, **4**, **7b**–**g**, **10b**,**c**,**e**): besides the antifungal activity, both antibacterial and anti-human immunodeficiency virus-1 (HIV-1) properties were tested.

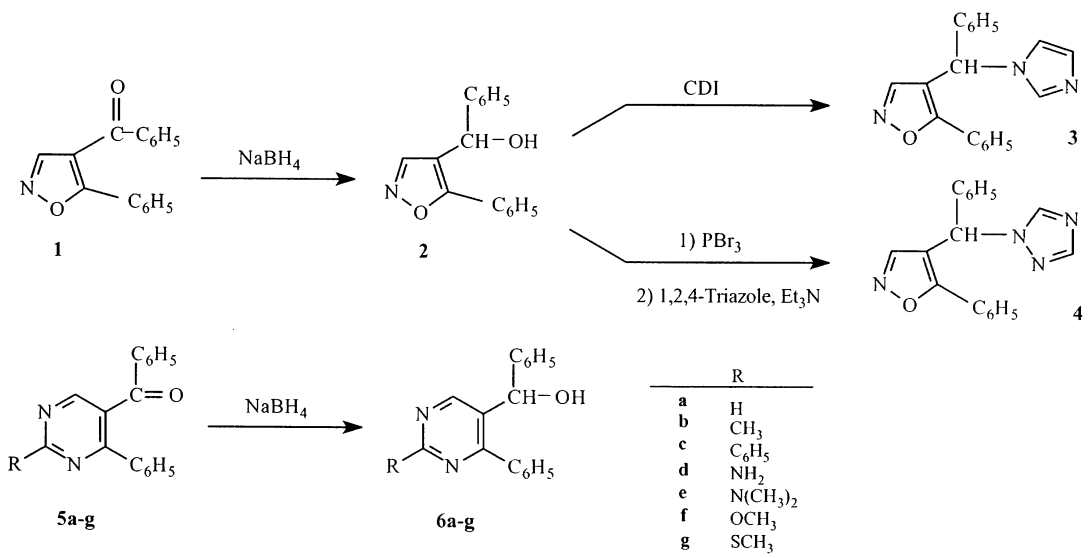
2. Chemistry

The title azole derivatives were prepared from isoxazole carbinol **2** or pyrimidine carbinols **6a**–**g**, as depicted in Schemes 1 and 2. **2** and **6a**–**g** were obtained by a sodium borohydride reduction of the known ketones **1** [9], **5a**–**d** [10] and new ketones **5e**–**g**, which were prepared similar to **5a**–**d**.

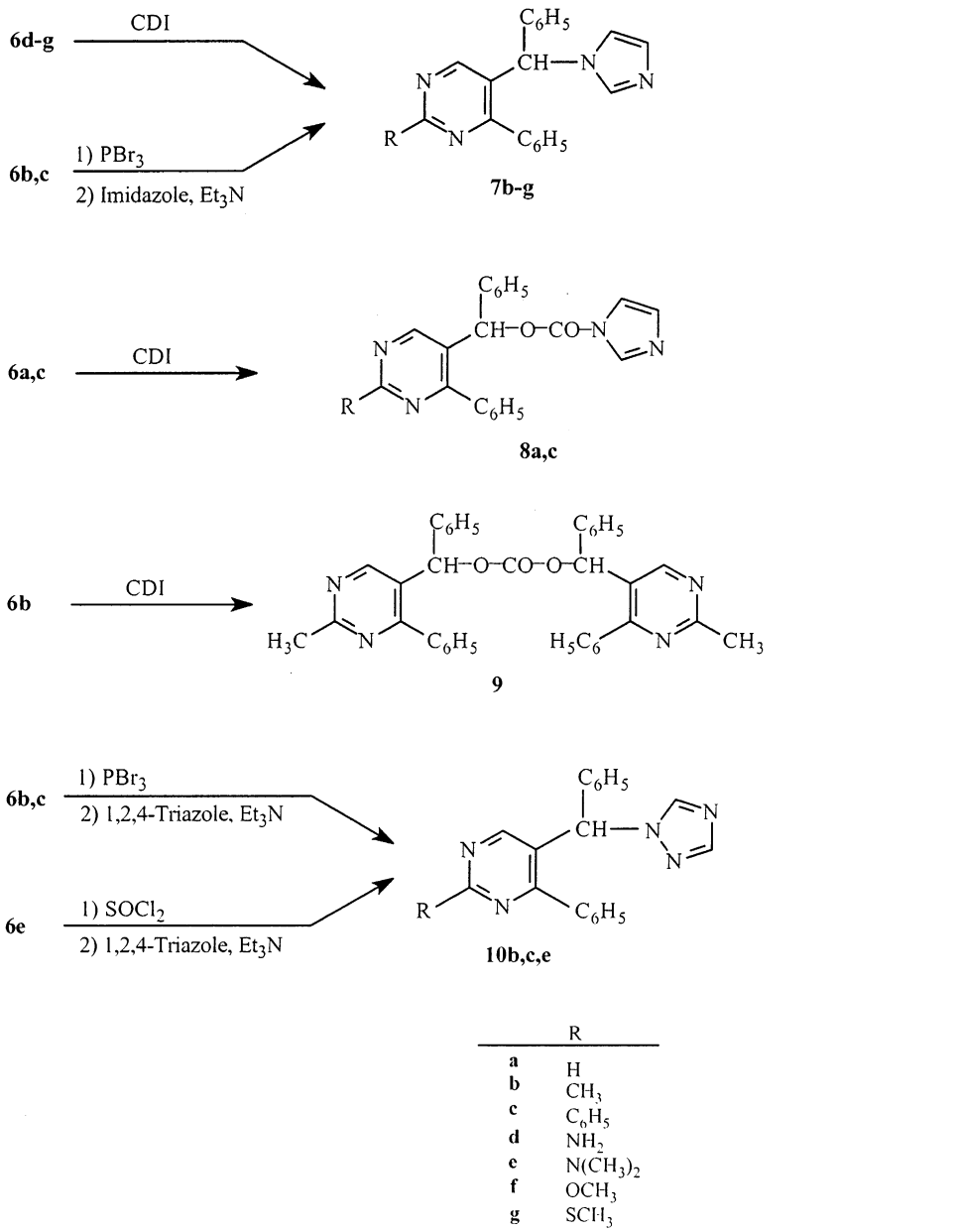
The reaction of carbinols **2** and **6d**–**g** with *N*,*N*′-carbonyldiimidazole (CDI) afforded, with variable yields (38–82%), imidazole derivatives **3** and **7d**–**g**, respectively; **6a**–**c**, in the same reaction conditions, did not give the desired compounds, but gave intermediate imidazole-*N*-carboxylic esters (**8a**,**c**) or a carbonic diester (**9**), not behaving as benzyl alcohols should [11]. Therefore, in order to obtain the required imidazole derivatives from **6a**–**c**, we followed another route: treatment of carbinols with PBr_3 and successive reaction of crude bromo derivatives with imidazole in the presence of triethylamine. Finally **6b**,**c** gave, although in low yields (17 and 25%, respectively), the imidazole derivatives **7b**,**c**, whereas **6a** did not afford the desired compound.

By analogous reaction of carbinols **2**, **6b**,**c** with 1,2,4-triazole via bromo derivatives, were obtained, in very low yields (17, 7 and 15%, respectively), triazoles **4**, **10b**,**c**; chlorination of **6e** with $SOCl_2$ and successive reaction of crude chloro derivative with 1,2,4-triazole in the presence of triethylamine afforded **10e** in 29% yield.

No effort was carried out to transform carbinols **6f**,**g** into the corresponding triazole derivatives owing to the



Scheme 1.



Scheme 2.

easy cleavage of the ether function by the required halogenation agents, while all attempts made starting from **6a**,**d** were unsuccessful.

3. Experimental

3.1. Chemistry

Melting points were determined with a Fisher–Johns apparatus and are uncorrected. IR spectra were registered on a Perkin–Elmer 398 spectrophotometer and are expressed in cm^{-1}. ^1H NMR spectra were registered on a Hitachi Perkin–Elmer R-600 instrument (60 MHz); chemical shifts are reported as δ (ppm) relative to TMS as internal standard (J in Hz). Elemental analyses were performed using a Carlo Erba Elemental Analyzer Model 1106 and results were within $\pm 0.3\%$ of the calculated values.

3.1.1. Phenyl(2-substituted 4-phenylpyrimidin-5-yl)methanones (5e–g)

5e–**g** were prepared as described previously for **5a**–**d** [10], namely by cyclization of 2-dimethylaminomethylene-1,3-diphenylpropane-1,3-dione with 1,1-dimethylguanidine or *O*-methylisourea or *S*-methylisothiourea, respectively.

5e: Yield 88%, m.p. 138–139 °C from 95% ethanol. IR ($CHCl_3$, cm^{-1}): ν(CO) 1642. ^1H NMR ($CDCl_3$): δ 3.34 (s, 6H, $(CH_3)_2N$), 7.1–7.85 (m, 10H, $2C_6H_5$), 8.64 (s, 1H, H-6). *Anal.* $C_{19}H_{17}N_3O$ (C, H, N).

5f: Yield 52%, m.p. 97–98 °C from anhydrous diethyl ether–petroleum ether (b.p. 40–70 °C). IR ($CHCl_3$, cm^{-1}): ν(CO) 1660. ^1H NMR ($CDCl_3$): δ 4.19 (s, 3H, CH_3O), 7.1–7.85 (m, 10H, $2C_6H_5$), 8.74 (s, 1H, H-6). *Anal.* $C_{18}H_{14}N_2O_2$ (C, H, N).

5g: Yield 77%, m.p. 99–100 °C from anhydrous diethyl ether. IR ($CHCl_3$, cm^{-1}): ν(CO) 1658. ^1H NMR ($CDCl_3$): δ 2.69 (s, 3H, CH_3S), 7.15–7.9 (m, 10H, $2C_6H_5$), 8.70 (s, 1H, H-6). *Anal.* $C_{18}H_{14}N_2OS$ (C, H, N).

3.1.2. Phenyl(5-phenylisoxazol-4-yl)methanol (2)

A solution of sodium borohydride (0.38 g, 10 mmol) in water (6 ml) was added dropwise to a cooled (0 °C) solution of phenyl(5-phenylisoxazol-4-yl)methanone (**1**) (2.50 g, 10 mmol) in tetrahydrofuran (180 ml). The mixture was stirred at 0 °C for 1 h and, then, at room temperature (r.t.) for 3 h. After cooling at 0 °C, water (60 ml) was added and the solution was stirred for few minutes, concentrated to a small volume under reduced pressure and extracted with chloroform (3×20 ml). Organic extracts were washed with water, dried ($MgSO_4$) and evaporated under reduced pressure to give a liquid residue which was purified by bulb-to-bulb distillation in vacuo (b.p. 177–180 °C (0.2)), followed by crystallization from anhydrous diethyl ether–petroleum ether (b.p. 40–70 °C), m.p. 69–70 °C, yield 1.85 g (74%). IR ($CHCl_3$, cm^{-1}): ν(OH) 3580 and 3370. ^1H NMR ($CDCl_3$): δ 3.36 (d, 1H, $J = 3.5$ Hz, OH; disappears with D_2O), 5.97 (d, 1H, $J = 3.5$ Hz, CHO; becames s with D_2O), 7.2–7.95 (m, 10H, $2C_6H_5$), 8.10 (s, 1H, H-3). *Anal.* $C_{16}H_{13}NO_2$ (C, H, N).

3.1.3. General procedure for the preparation of phenyl(2-substituted 4-phenylpyrimidin-5-yl)methanols (6a–g)

A solution of sodium borohydride (0.38 g, 10 mmol) in water (6 ml) was added dropwise to a solution of **5a–g** (10 mmol) in tetrahydrofuran (220 ml). The mixture was refluxed for 1 h (3 h in the case of **6e**), cooled at r.t. and diluted with water (70 ml). After concentration to a small volume under reduced pressure, the solution was extracted with chloroform (3×20 ml). Organic extracts were washed with water, dried ($MgSO_4$) and evaporated under reduced pressure to give a residue, which was purified by chromatography on Florisil (diethyl ether as eluent), followed by a recrystallization from a suitable solvent.

Yields, m.p. values, recrystallization solvents and ^1H NMR spectral data are reported in Table 1. IR spectral

Table 1
Yields, physical, analytical and ^1H NMR data of methanols **6a–g**

Comp.	R	Yield (%)	m.p. (°C)	Molecular formula	Analyses	^1H NMR ($CDCl_3$), δ (ppm)
6a	H	78	181–182 [a]	$C_{17}H_{14}N_2O$	C, H, N	~3.6 (very br s, 1H, OH, disappears with D_2O), 6.03 (s, 1H, CH–Ph), 7.1–7.35 (m, 5H, C_6H_5), 7.47 (s, 5H, C_6H_5), 8.94 (s, 1H, H-6), 9.10 (s, 1H, H-2)
6b	CH_3	76	98–99 [b]	$C_{18}H_{16}N_2O$	C, H, N	2.63 (s, 3H, CH_3), 4.58 (d, J~3 Hz, 1H, OH, disappears with D_2O), 5.89 (d, J~3 Hz, 1H, CH–Ph, became s with D_2O), 6.95–7.3 (m, 5H, C_6H_5), 7.39 (s, 5H, C_6H_5), 8.71 (s, 1H, H-6)
6c	C_6H_5	98	138–139 [b]	$C_{23}H_{18}N_2O$	C, H, N	3.32 (br s, 1H, OH, disappears with D_2O), 6.03 (s, 1H, CH–Ph), 7.27 (s, 5H, C_6H_5), 7.35–7.8 (m, 8H, ArH), 8.35–8.75 (m, 2H, ArH), 8.97 (s, 1H, H-6)
6d	NH_2	60	186–187 [a]	$C_{17}H_{15}N_3O$	C, H, N	5.3–5.55 (m, 1H, OH, disappears with D_2O), 5.6–5.95 (m, 3H, CH–Ph + NH_2, 2H, disappear with D_2O), 7.27 (s, 5H, C_6H_5), 7.35–7.75 (m, 5H, C_6H_5), 8.41 (s, 1H, H-6)
6e	$N(CH_3)_2$	72	95–96 [b]	$C_{19}H_{19}N_3O$	C, H, N	2.26 (d, J~4 Hz, 1H, OH, disappears with D_2O), 3.23 (s, 6H, $(CH_3)_2N$), 5.96 (d, J~4 Hz, 1H, CH–Ph, became s with D_2O), 7.31 (s, 5H, C_6H_5), 7.4–7.8 (m, 5H, C_6H_5), 8.40 (s, 1H, H-6)
6f	OCH_3	86	155–156 [a]	$C_{18}H_{16}N_2O_2$	C, H, N	3.20 (d, J~4 Hz, 1H, OH, disappears with D_2O), 4.02 (s, 3H, CH_3O), 6.00 (d, J~4 Hz, 1H, CH–Ph, became s with D_2O), 7.1–7.35 (m, 5H, C_6H_5), 7.4–7.6 (m, 5H, C_6H_5), 8.63 (s, 1H, H-6)
6g	SCH_3	81	134–135 [b]	$C_{18}H_{16}N_2OS$	C, H, N	2.56 (s, 3H, CH_3S), 2.95 (br s, 1H, OH, disappears with D_2O), 5.97 (s, 1H, CH–Ph), 7.0–7.7 (m, 10H, $2C_6H_5$), 8.67 (s, 1H, H-6)

[a] From ethyl acetate.
[b] From anhydrous diethyl ether–petroleum ether (b.p. 40–70 °C).

Table 2
Yields, physical, analytical and ^{1}H NMR data of imidazoles **7b–g**

Comp.	R	Yield (%)	m.p. (°C)	Molecular formula	Analyses	^{1}H NMR ($CDCl_3$), δ (ppm)
7b	CH_3	17	137–138 [a]	$C_{21}H_{18}N_4$	C, H, N	2.82 (s, 3H, CH_3), 6.60 (s, 1H, CH–Ph), 6.75–7.7 (m, 13H, $2C_6H_5$ + 3H imidazole), 8.43 (s, 1H, H-6 pyrimidine)
7c	C_6H_5	25	162–163 [a]	$C_{26}H_{20}N_4$	C, H, N	6.67 (s, 1H, CH–Ph), 6.75–7.7 (m, 16H, 13ArH + 3H imidazole), 8.45–8.75 (m, 3H, 2ArH + H-6 pyrimidine)
7d	NH_2	66	196–197 [b]	$C_{20}H_{17}N_5$	C, H, N	5.78 (s, 2H, NH_2, disappears with D_2O), 6.43 (s, 1H, CH–Ph), 6.75–7.7 (m, 13H, $2C_6H_5$ + 3H imidazole), 8.06 (s, 1H, H-6 pyrimidine)
7e	$N(CH_3)_2$	82	176–177 [c]	$C_{22}H_{21}N_5$	C, H, N	3.24 (s, 6H, $(CH_3)_2N$), 6.46 (s, 1H, CH–Ph), 6.8–7.65 (m, 13H, $2C_6H_5$ + 3H imidazole), 8.10 (s, 1H, H-6 pyrimidine)
7f	OCH_3	60	175–176 [b]	$C_{21}H_{18}N_4O$	C, H, N	4.09 (s, 3H, CH_3O), 6.60 (s, 1H, CH–Ph), 6.8–7.7 (m, 13H, $2C_6H_5$ + 3H imidazole), 8.31 (s, 1H, H-6 pyrimidine)
7g	SCH_3	38	172–173 [a]	$C_{21}H_{18}N_4S$	C, H, N	2.60 (s, 3H, CH_3S), 6.57 (s, 1H, CH–Ph), 6.8–7.7 (m, 13H, $2C_6H_5$ + 3H imidazole), 8.29 (s, 1H, H-6 pyrimidine)

[a] From anhydrous diethyl ether.
[b] From ethyl acetate.
[c] From ethyl acetate–petroleum ether (b.p. 40–70 °C).

data are consistent with the proposed structures and are not reported.

3.1.4. General procedure for the preparation of 4-[1H-imidazol-1-yl-(phenyl)methyl]-5-phenylisoxazole (3) and 2-substituted 5-[1H-imidazol-1-yl-(phenyl)methyl]-4-phenylpyrimidines (7d–g)

N,*N*′-Carbonyldiimidazole (1.78 g, 11 mmol) was added to a solution of the proper carbinol **2** or **6a–g** (10 mmol) in dry toluene (120 ml) and the mixture was refluxed for 4 h. After being cooled, the supernatant solution was decanted and evaporated under reduced pressure and the residue was dissolved in chloroform (100 ml). The organic solution was washed with water (3×20 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a crude oil, which was purified by chromatography on Florisil, using diethyl ether (**3**, **7f**,**g**) or ethyl acetate (**7d**,**e**) as eluent. The solid residue, obtained after evaporation of the solvent, was recrystallized from a suitable solvent.

3: Yield 1.43 g (48%), m.p. 109–110 °C from anhydrous diethyl ether. ^{1}H NMR ($CDCl_3$): δ 6.63 (s, 1H, CH–Ph), 6.9–7.7 (m, 13H, $2C_6H_5$ + 3H imidazole), 8.03 (s, 1H, H-3 isoxazole). *Anal.* $C_{19}H_{15}N_3O$ (C, H, N).

Yields, m.p. values, recrystallization solvents and ^{1}H NMR spectral data of compounds **7d–g** are reported in Table 2. IR spectral data are consistent with the proposed structures and are not reported.

In the case of carbinols **6a–c**, instead of expected imidazole derivatives, from the reaction mixture were isolated side compounds which, by analytical and spectral data, resulted to be the imidazole *N*-carboxylic esters **8a**,**c** and the carbonic diester **9** (Scheme 2).

8a: Yield 2.08 g (59%), m.p. 107–108 °C from anhydrous diethyl ether–petroleum ether (b.p. 40–70 °C). IR ($CHCl_3$, cm^{-1}): ν(CO) 1760. ^{1}H NMR ($CDCl_3$): δ 6.8–7.6 (m, 13H, $2C_6H_5$ + 3H imidazole), 8.15 (s, 1H, CH–O), 9.02 (s, 1H, H-6 pyrimidine), 9.34 (s, 1H, H-2 pyrimidine). *Anal.* $C_{21}H_{16}N_4O_2$ (C, H, N).

8c: Yield 3.35 g (78%), m.p. 167–168 °C from anhydrous diethyl ether–petroleum ether (b.p. 40–70 °C). IR ($CHCl_3$, cm^{-1}): ν(CO) 1760. ^{1}H NMR ($CDCl_3$): δ 7.0–7.8 (m, 16H, 13 ArH + 3H imidazole), 8.16 (s, 1H, CH–O), 8.4–8.8 (m, 2H, ArH), 9.03 (s, 1H, H-6 pyrimidine). *Anal.* $C_{27}H_{20}N_4O_2$ (C, H, N).

9: Yield 1.0 g (35%), m.p. 188–189 °C from anhydrous diethyl ether. IR ($CHCl_3$, cm^{-1}): ν(CO) 1743. ^{1}H NMR ($CDCl_3$): δ 2.79 (s, 6H, $2CH_3$), 6.80 (s, 2H, 2CH–O), 6.95–7.6 (m, 20H, $4C_6H_5$), 8.82 (s, 2H, 2H-6 pyrimidine). *Anal.* $C_{37}H_{30}N_4O_3$ (C, H, N).

3.1.5. General procedure for the preparation of 2-substituted 5-[1H-imidazol-1-yl-(phenyl)methyl]-4-phenylpyrimidines (7b,c) and of 2-substituted 4-phenyl-5-[phenyl-(1H-1,2,4-triazol-1-yl)methyl] pyrimidines (10b,c)

A solution of phosphorous tribromide (1.9 g, 7 mmol) in anhydrous diethyl ether (30 ml) was added dropwise to a stirred solution of **6b**,**c** (10 mmol) in the same solvent (200 ml). The mixture was stirred at r.t. for 2 h, then diluted with chloroform (80 ml) and further stirred for 2 h. The clear solution so obtained was washed with 5% CH_3COONa and with saturated NaCl solution, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was dissolved in dry acetonitrile (50 ml) and added dropwise into a stirred solution of the proper azole (10 mmol) and triethylamine (1.0 g, 10 mmol) in the same solvent (20 ml).

The mixture was heated at 60 °C for 24 h, concentrated to a small volume under reduced pressure, diluted with toluene and washed with saturated NaCl solution. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give a sticky oil, which was chromatographed on silica gel, using first a mixture of ethyl acetate–petroleum ether (b.p. 40–70 °C) 1:1 and, then, ethyl acetate, as eluents. The solid residue, obtained from ethyl acetate eluate, was recrystallized from a suitable solvent.

Yields, m.p. values, recrystallization solvents and ^1H NMR spectral data of compounds **7b**,**c** and **10b**,**c** are reported in Tables 2 and 3, respectively. IR spectral data are consistent with the proposed structures and are not reported.

*3.1.6. 1-[Phenyl(5-phenylisoxazol-4-yl)methyl]-1H-1,2,4-triazole (**4**)*

A solution of phosphorous tribromide (1.9 g, 7 mmol) in anhydrous diethyl ether (30 ml) was added dropwise to a stirred solution of **2** (2.51 g, 10 mmol) in the same solvent (80 ml). The mixture was stirred at 0 °C for 1 h and at r.t. for 3 h. The solution was then washed with 5% CH_3COONa and with saturated NaCl solution, dried ($MgSO_4$) and evaporated under reduced pressure. The white solid residue was dissolved in dry acetonitrile (30 ml) and added dropwise into a cooled (0 °C) solution of 1,2,4-triazole (0.69 g, 10 mmol) and triethylamine (1.0 g, 10 mmol) in the same solvent (20 ml). The mixture was stirred at 0 °C for 3 h and at r.t. overnight, then concentrated to a small volume under reduced pressure, diluted with chloroform (50 ml) and washed with saturated NaCl solution. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow oil, which was chromatographed on silica gel (ethyl acetate–petroleum ether, (b.p. 40–70 °C), 1:1 as eluent). The solid product, isolated from the last fractions, was recrystallized from anhydrous diethyl ether, m.p. 139–140 °C, yield 0.51 g (17%). ^1H NMR ($CDCl_3$): δ 6.88 (s, 1H, CH-Ph), 7.0–7.7 (m, 10H, $2C_6H_5$), 8.10 (s, 1H, H-3 triazole), 8.15 (s, 1H, H-5 triazole), 8.29 (s, 1H, H-3 isoxazole). *Anal.* $C_{19}H_{15}N_3O$ (C, H, N).

*3.1.7. N,N-dimethyl-4-phenyl-5-[phenyl(1H-1,2,4-triazol-1-yl)methyl]pyrimidin-2-amine (**10e**)*

A solution of **6e** (3.04 g, 10 mmol) and $SOCl_2$ (11 ml) was stirred at r.t. for 20 h and then evaporated to dryness, under reduced pressure. The viscous oily residue was dissolved in dry acetonitrile (20 ml) and added dropwise into a stirred solution of 1,2,4-triazole (0.69 g, 10 mmol) and triethylamine (3.03 g, 30 mmol) in the same solvent (10 ml). The mixture was stirred at reflux temperature for 24 h and evaporated under reduced pressure to give a residue, which was dissolved in chloroform. The organic solution was washed with saturated NaCl solution, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was chromatographed on Florisil column (diethyl ether as eluent). Evaporation of the eluates gave a solid, which was recrystallized from anhydrous diethyl ether.

Yield, m.p. value and ^1H NMR spectral data are reported in Table 3.

3.2. Biological assays

All compounds were evaluated in vitro for antimicrobial activity against representative human pathogenic fungi (*Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*), Gram positive (*Staphylococcus aureus*) and Gram negative (*Salmonella* spp.) bacteria. Bifonazole and streptomycin were used as reference drugs in antifungal and antibacterial assays, respectively. Test compounds were also evaluated for antiretroviral activity in MT-4 cells infected with HIV-1.

Cytotoxicity against MT-4 cells, carried out in parallel with anti-HIV-1 activity, was evaluated to determine whether the compounds were endowed with selective antimicrobial/antiviral activity.

Table 3
Yields, physical, analytical and ^1H NMR data of triazoles **10b**,**c**,**e**

Comp.	R	Yield (%)	m.p. (°C)	Molecular formula	Analyses	^1H NMR ($CDCl_3$), δ (ppm)
10b	CH_3	7	122–123 [a]	$C_{20}H_{17}N_5$	C, H, N	2.81 (s, 3H, CH_3), 6.84 (s, 1H, CH–Ph), 6.9–7.7 (m, 10H, $2C_6H_5$), 7.90 (s, 1H, H-3 triazole), 8.05 (s, 1H, H-5 triazole), 8.56 (s, 1H, H-6 pyrimidine)
10c	C_6H_5	15	220–221 [b]	$C_{25}H_{19}N_5$	C, H, N	6.78 (s, 1H, CH–Ph), 6.9–7.75 (m, 13H, 13ArH), 8.06 (s, 2H, H-3 + H-5 triazole), 8.4–8.8 (m, 3H, 2ArH + H-6 pyrimidine)
10e	$N(CH_3)_2$	29	145–146 [a]	$C_{21}H_{20}N_6$	C, H, N	3.23 (s, 6H, $(CH_3)_2N$), 6.73 (s, 1H, CH–Ph), 6.9–7.65 (m, 10H, $2C_6H_5$), 7.93 (s, 1H, H-3 triazole), 8.05 (s, 1H, H-5 triazole), 8.24 (s, 1H, H-6 pyrimidine)

[a] From anhydrous diethyl ether.
[b] From ethyl acetate.

3.2.1. Materials and methods

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

3.2.1.2. Cells. Cell lines 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. H9/IIIB, MT-4 and C8166 cells (grown in RPMI 1640 containing 10% fetal calf serum (FCS), 100 UI/ml penicillin G and 100 μg/ml streptomycin) were used for anti-HIV-1 assays. Cell cultures were checked periodically for the absence of mycoplasma contamination with a Myco Tect Kit (Gibco).

3.2.1.3. Viruses. HIV-1 III_B strain was obtained from supernatants of persistently infected $H9/III_B$ cells. HIV-1 stock solutions had a titer of 5×10^7 cell culture infectious dose fifty ($CCID_{50}$)/ml.

3.2.1.4. Antiviral assays. Activity against the HIV-1 multiplication in cells infected acutely was based on inhibition of virus-induced cytopathogenicity in MT-4 cells [12]. Briefly, 50 μl of RPMI 10% FCS containing 1×10^4 cells were added to each well of flat-bottomed microtiter trays containing 50 μl of medium and serial dilutions of test compounds. Twenty microliters of an HIV-1 suspension containing 100 $CCID_{50}$ were then added. After 4 days of incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [13,14]. Cytotoxicity of compounds, based on the viability of mock-infected cells as monitored by the MTT method, was evaluated in parallel with their antiviral activity.

3.2.1.5. Antibacterial assays. *S. aureus* and *Salmonella* spp. were recent clinical isolates. Assays were carried out in nutrient broth, pH 7.2, with an inoculum of 10^3 bacterial cells/tube. Minimum inhibitory concentrations (MIC) were determined after 18 h incubation at 37 °C in the presence of serial dilutions of test compounds.

3.2.1.6. Antimycotic assays. Yeast inocula were obtained by properly diluting cultures incubated for 30 h at 37 °C in Sabouraud dextrose broth to obtain 5×10^3 cells/ml. On the contrary, dermatophyte inocula were obtained from cultures grown at 37 °C for 5 days in Sabouraud dextrose broth by finely dispersing clumps with a glass homogenizer before diluting to 0.05 OD_{590}/ml. Then, 20 μl of the above suspensions were added to each well of flat-bottomed microtiter trays containing 80 μl of medium with serial dilutions of test compounds, and were incubated at 37 °C. Growth controls were determined visually after 2 days (yeasts) or 3 days (dermatophytes). MIC was defined as the compound

Table 4
In vitro biological activity of compounds **3**, **4**, **7b**–**g**, **10b**,**c**,**e**

Comp.	CC_{50} [a]	EC_{50} [b]	MIC [c]/MGC [d]				
	MT-4	HIV-1	*C. albicans*	*C. neoformans*	*A. fumigatus*	*S. aureus*	*Salmonella* spp.
3	76	>76	In [e]	In	In	In	In
7b	>200	>200	In	In	In	In	In
7c	26	>26	66/>200	66/>200	In	66/>200	In
7d	134	>134	In	In	In	In	In
7e	58.7	>58.7	In	In	In	In	In
7f	177.5	>177.5	In	In	In	In	In
7g	61.7	>61.7	In	In	In	In	In
4	>200	>200	In	In	In	In	In
10b	>200	>200	In	In	In	In	In
10c	>200	>200	In	In	In	In	In
10e		97	>97	In	In	In	50/100
AZT	150	0.01					
Streptomycin	>200					3.1/3.1	6.2/6.2
Bifonazole	28		15/15	3.7/3.7	3.7/3.7		

[a] Compound concentration (μM) required to induce the viability of MT-4 cells by 50%, as determined by the MTT method.
[b] Compound concentration (μM) required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytopathogenicity, as determined by the MTT method.
[c] Minimum inhibitory concentration (μM).
[d] Minimum germicidal concentration (μM).
[e] Inactive up to MIC and MGC >200 μM.

concentration at which no macroscopic sign of fungal growth was detected. The minimal germicidal concentrations (MBC or MFC) were determined by subcultivating samples from cultures with no apparent growth in Sabouraud dextrose agar.

3.2.1.7. Linear regression analysis. Viral growth at each drug concentration was expressed as a percentage of untreated controls and the concentrations resulting in 50% (EC_{50}) growth inhibition was determined by linear regression analysis.

4. Results and discussion

As shown in Table 4, none of the compounds was capable of protecting MT-4 cells from the cytopathic effect induced by HIV-1, at least at concentrations lower than the cytotoxic ones.

When tested against yeast and mold representatives, only **7c** was active against *C. albicans* and *C. neoformans* (MIC = 66 μM). **7c** also showed activity against *S. aureus* (MIC = 66 μM) whereas **10e** showed activity only against *S. aureus* (MIC = 50 μM). All other compounds were devoid of any antimicrobial activity.

These data led us to conclude that replacement of the biphenyl portion of the bifonazole with a phenylisoxazolyl or phenylpyrimidinyl moiety afforded heterocyclic analogues which were inactive as antimycotic agents. Among all tested compounds the only one showing any antifungal activity was **7c**, which is characterized by a pyrimidine nucleus bearing a double aromatic substitution at positions 2 and 4.

Finally, the replacement of the imidazole moiety with a triazole ring (**10b**,**c**,**e**) provided inactive compounds: even **10c** resulted devoid of the weak activity showed by its imidazole analogue **7c**.

Acknowledgements

We would like to thank CNR and the Ministero dell'Università e della Ricerca Scientifica e Tecnologica for their financial support.

References

[1] T.F. Patterson, Role of newer azoles in surgical patients, J. Chemother. 11 (1999) 504–512.
[2] M. Artico, R. Di Santo, R. Costi, S. Massa, A. Retico, M. Artico, G. Apuzzo, G. Simonetti, V. Strippoli, Antifungal agents. 9. 3-Aryl-4-[α-(1*H*-imidazol-1yl)arylmethyl]pyrroles: a new class of potent anti-*Candida* agents, J. Med. Chem. 38 (1995) 4223–4233 (and references cited therein).
[3] G. Menozzi, L. Mosti, O. Bruno, E. Lo Presti, C. Musiu, S. Longu, P. La Colla, W. Filippelli, G. Falcone, B. Piucci, Synthesis and biological evaluation of [α-(1,5-disubstituted 1*H*-pyrazol-4-yl)benzyl]azoles, analogues of bifonazole, Farmaco 54 (1999) 416–422.
[4] G. Heubach, G. Hoerlein, B. Sachse, Fungicidal isoxazole derivatives, German Patent DE 2525023, 1976 (Chem. Abstr. 86 (1977) 106569h).
[5] J.D. Davenport, B.A. Dreikorn, A.F. Elsasser, Fungicidal agent containing 3-phenyl-5-(substituted methyl)isoxazole, German Patent DE 2723688, 1976 (Chem. Abstr. 88 (1978) 132015k).
[6] R.P. Dickinson, A.S. Bell, C.A. Hitchock, S. Narayanaswami, S.J. Ray, K. Richardson, P.F. Troke, Novel antifungal 2-aryl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol derivatives with high activity against *Aspergillus fumigatus*, Bioorg. Med. Chem. Lett. 6 (1996) 2031–2036.
[7] F. Tanaka, S. Takeuchi, N. Tanaka, H. Yonehara, H. Umezawa, Y. Sumiki, Bacimethrin, a new antibiotic produced by *Bacillus megaterium*, J. Antibiotics 14A (1961) 161–162 (Chem. Abstr. 56 (1962) 15958d).
[8] Y. Sumiki, H. Umezawa, Blastocidin S, an antibiotic substance. Jpn. Patent 16 449, 1960 (Chem. Abstr. 55 (1961) 21474d).
[9] G. Menozzi, P. Schenone, L. Mosti, Reaction of 2-dimethylaminomethylene-1,3-diones with dinucleophiles. II. Synthesis of 5-(alkyl)(phenyl)-4-acylisoxazoles and 6,7-dihydro-1,2-benzisoxazol-4(5*H*)-ones, J. Heterocycl. Chem. 20 (1983) 645.
[10] L. Mosti, G. Menozzi, P. Schenone, Reaction of 2-dimethylaminomethylene-1,3-diones with dinucleophiles. III. Synthesis of 5-acylpyrimidines and 7,8-dihydroquinazolin-5(6*H*)-ones, J. Heterocycl. Chem. 20 (1983) 649.
[11] H.A. Staab, New methods of preparative organic chemistry. IV. Syntheses using heterocyclic amides (azolides), Angew. Chem., Int. Ed. Engl. 1 (1962) 351–367.
[12] A. Mai, M. Artico, G. Sbardella, S. Quartarone, S. Massa, A.G. Loi, A. De Montis, F. Scintu, M. Putzolu, P. La Colla, Dihydro(alkylthio)(naphthylmethyl)oxopyrimidines: novel non-nucleoside reverse transcriptase inhibitors of the S-DABO series, J. Med. Chem. 40 (1997) 1447–1454.
[13] F. Denizot, R. Lang, Rapid colorimetric assay for cell growth and survival, J. Immunol. Methods 89 (1986) 271–277.
[14] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Scholds, P. Herdewijn, J. Desmyter, E. De Clercq, Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds, J. Virol. Methods 20 (1988) 309–321.