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Synthesis and biological evaluation of [α-(1,5-disubstituted 1*H*-pyrazol-4-yl)benzyl]azoles, analogues of bifonazole

Giulia Menozzi [a,*], Luisa Mosti [a], Olga Bruno [a], Eleonora Lo Presti [a], Chiara Musiu [b], Silvia Longu [b], Paolo La Colla [b], Walter Filippelli [c], Giuseppe Falcone [c], Brunella Piucci [c]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV 3, I-16132 Genoa, Italy*
[b] *Dipartimento di Biologia Sperimentale, Università di Cagliari, Viale Regina Margherita 45, I-09124 Cagliari, Italy*
[c] *Istituto di Farmacologia e Tossicologia, Facoltà di Medicina e Chirurgia, II Università di Napoli, Via S. Andrea delle Dame 8, I-80138 Naples, Italy*

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Abstract

A series of pyrazole analogues of bifonazole, an antifungal drug used in clinical practice, **2a–h** and **4a–h** were synthesized and tested in vitro against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, with no significant results. Imidazoles **2a–h** were also tested in vivo for antiarrhythmic and antihypertensive activities; two of these compounds showed moderate activity against ventricular fibrillation caused by aconitine in rats. The above compounds were prepared by reaction of phenyl-[5 substituted 1-phenyl (or 1-methyl)-1*H*-pyrazol-4-yl]methanols with *N*,*N*′-carbonyldiimidazole (**2a–h**) or of the respective chloro derivatives with 1*H*-1,2,4-triazole (**4a–h**). © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Azole derivatives; Antifungal agents; Bifonazole analogues; Antiarrhythmic agents

1. Introduction

Since the discovery of bifonazole, an antifungal imidazole derivative developed by Bayer AG and currently used for topic therapy of mycoses and dermatomycoses, various attempts have been made to modify its structure in order to improve its antifungal potency and selectivity. In particular, the biphenyl portion of the molecule (Fig. 1) has been subjected to modifications consisting of bioisosteric replacements of either or both benzene rings with different aromatic or heteroaromatic moieties. Among the various derivatives, bifonazole analogues, in which one of the phenyl rings had been replaced with a pyrrole nucleus, turned out to be endowed with high antifungal activity in vitro [1–4].

In order to develop new active bifonazole derivatives and to contribute to the SAR studies of this class, we synthesized a number of compounds containing a phenylpyrazolyl moiety with aliphatic or aromatic substituents on the heterocyclic ring (**2a–h**) (Fig. 1). Further structural modifications involved the replacement of the imidazole by a triazole ring (**4a–h**).

In this paper we present the synthesis and biological evaluation of antimycotic, antibacterial and anti-HIV-1 activities of the above bifonazole analogues as well as some cardiovascular properties elicited by imidazoles **2a–h**.

2. Chemistry

Imidazoles **2a–h** and triazoles **4a–h** were prepared from carbinols **1b–g**, recently used by us as intermediates for the synthesis of pyrazole aminoethers with antinociceptive activity [5], and **1a**,**h**, as described in Scheme 1.

Compound **1a** was synthesized in the same way as the 5-substituted analogues **1b–g**, starting from 4-formyl-1-phenylpyrazole and phenylmagnesium bro-

* Corresponding author. Tel.: +39-010-353 8361; fax: +39-010-353 358.
E-mail address: menozzi@unige.it (G. Menozzi)

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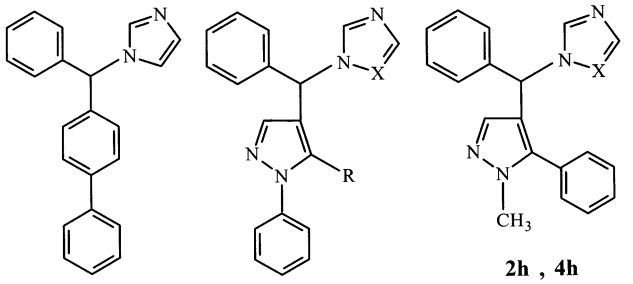


Fig. 1. Bifonazole and new pyrazole analogues (**2a–h**: X = CH; **4a–h**: X = N; R = H, alkyl, phenyl).

mide [6]. **1h** was obtained by sodium borohydride reduction of ketone **5a** [7]; similarly the reduction of **5b** [7] provided the same carbinol **1g** already prepared by Grignard's reaction [5], following an alternative route (Scheme 1). The correspondence of chemical and physical characteristics and IR and ^{1}H NMR spectral data of the two compounds, prepared in two different ways, gave further confirmation of the supposed structures.

The reaction of carbinols **1a–h** with *N,N′*-carbonyldiimidazole afforded imidazole derivatives **2a–h**, whereas the synthesis of triazoles **4a–h** was achieved by chlorination of **1a–h** to **3a–h** and subsequent reaction of the latter with 1*H*-1,2,4-triazole.

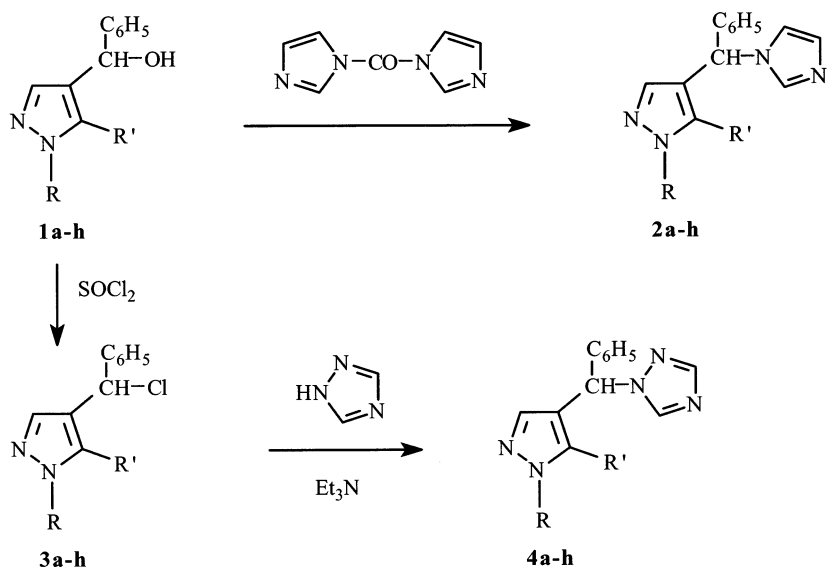
3. Experimental

3.1. Chemistry

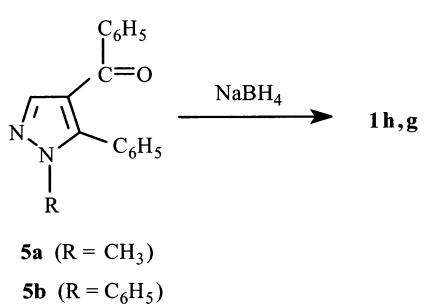
Melting points were determined with a Fisher–Johns apparatus and are uncorrected. The IR spectra were registered on a Perkin–Elmer 398 spectrophotometer in $CHCl_3$ as solvent. The ^{1}H NMR spectra were registered on a Hitachi Perkin–Elmer R-600 instrument (60 MHz); chemical shifts are reported as δ (ppm) relative to TMS as internal standard; J in Hz; $CDCl_3$ as solvent. The elemental analyses were performed using a Carlo Erba elemental analyser Model 1106 and the results were within $\pm 0.3\%$ of the calculated values.

3.1.1. Intermediates

Most of the carbinols used as intermediates for the synthesis of the title compounds were known (**1a**) [6], (**1b–g**) [5].



	R	R'
a	C_6H_5	H
b	C_6H_5	CH_3
c	C_6H_5	C_2H_5
d	C_6H_5	$(CH_2)_2CH_3$
e	C_6H_5	$CH(CH_3)_2$
f	C_6H_5	$C(CH_3)_3$
g	C_6H_5	C_6H_5
h	CH_3	C_6H_5



Scheme 1.

*3.1.1.1. Phenyl-(1-methyl-5-phenyl-1H-pyrazol-4-yl)-methanol (**1h**).* A solution of 4-benzoyl-1-methyl-5-phenyl-1*H*-pyrazole (2.62 g, 10 mmol) in THF (220 ml) and water (8 ml) was treated with sodium borohydride (0.38 g, 10 mmol) and refluxed for 1 h. After cooling to room temperature, water (60 ml) was added to the solution and the mixture was stirred for some minutes, concentrated to a small volume and extracted with chloroform (3 × 20 ml). The organic layer was washed with water, dried ($MgSO_4$), filtered and evaporated to give a crude residue, which was purified by recrystallization from anhydrous diethyl ether/petroleum ether (b.p. 40–70°C), m.p. 110–111°C, yield 1.35 g (51%). IR ($CHCl_3$): ν_{max} 3585 and 3270 (OH) cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.47 (d, $J = 4$, 1H, OH; disappears with D_2O), 3.65 (s, 3H, CH_3), 5.60 (d, $J = 4$, 1H, CHO; becomes s with D_2O), 7.1–7.6 (m, 11H, $2C_6H_5$ + H-3). *Anal.* (C, H, N) for $C_{17}H_{16}N_2O$.

3.1.2. General procedure for the synthesis of imidazoles **2a–h**

N,*N*′-Carbonyldiimidazole (1.78 g, 11 mmol) was added to a solution of the proper carbinol **1b–g** (10 mmol) in dry toluene (130 ml) or **1a**,**h** (10 mmol) in dry benzene (130 ml) and the mixture was refluxed for 4 h. The solvent was removed under reduced pressure and the residue was dissolved in chloroform (200 ml). The organic solution was washed with water (3 × 50 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give crude **2a–h**, which were purified by chromatography on Florisil (diethyl ether as eluent) and recrystallized from a suitable solvent.

Yields, m.p. values, recrystallization solvents and ^1H NMR spectral data are reported in Table 1. IR spectral data are consistent with the proposed structures and are not reported.

3.1.3. General procedure for the synthesis of triazoles **4a–h**

A solution of the proper carbinol **1a–h** (10 mmol) in thionyl chloride (20 ml) was stirred at room temperature for 24 h and then evaporated under reduced pressure. The reddish oily residue obtained was chromatographed on Florisil, eluting with diethyl ether/chloroform (1:1) to give a yellow, sticky oil, which was dissolved in dry acetonitrile (20 ml) and added dropwise into a solution of 1*H*-1,2,4-triazole (0.69 g, 10 mmol) and triethylamine (2.02 g, 20 mmol) in the same solvent (20 ml). The mixture was refluxed for 24 h and then evaporated under reduced pressure to give a brownish oil, which was dissolved in chloroform (200 ml). The organic solution was washed with saturated NaCl solution (3 × 30 ml), dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue obtained was purified by recrystallization from a suitable solvent.

Table 1
Yields, physical, analytical and ^1H NMR data of imidazoles **2a–h**

Comp.	R	R′	Yield (%)	M.p. (°C)	Molecular formula	*Anal.*	^1H NMR ($CDCl_3$), δ (ppm)
2a	C_6H_5	H	67	98–99 [a]	$C_{19}H_{16}N_4$	C, H, N	6.56 (s, 1H, CH–Ph), 6.9–7.9 (m, 15H, $2C_6H_5$+3H imidazole+2H pyrazole)
2b	C_6H_5	CH_3	77	91–92 [b]	$C_{20}H_{18}N_4$	C, H, N	2.16 (s, 3H, CH_3), 6.48 (s, 1H, CH–Ph), 6.9–7.7 (m, 14H, $2C_6H_5$+3H imidazole+H-3 pyrazole)
2c	C_6H_5	C_2H_5	64	89–90 [c]	$C_{21}H_{20}N_4$	C, H, N	0.90 (t, $J = 7.2$, 3H, CH_3), 2.65 (q, $J = 7.2$, 2H, CH_2), 6.50 (s, 1H, CH–Ph), 6.9–7.7 (m, 14H, $2C_6H_5$+3H imidazole+H-3 pyrazole)
2d	C_6H_5	$(CH_2)_2CH_3$	71	89–90 [b]	$C_{22}H_{22}N_4$	C, H, N	0.70 (t, $J = 6.6$, 3H, CH_3), 0.95–1.55 (m, 2H, CH_2), 2.59 (near t, $J \sim 7.5$, 2H, CH_2), 6.50 (s, 1H, CH–Ph), 6.9–7.7 (m, 14H, $2C_6H_5$+3H imidazole+H-3 pyrazole)
2e	C_6H_5	$CH(CH_3)_2$	85	114–115 [a]	$C_{22}H_{22}N_4$	C, H, N	1.06 (d, $J = 6.6$, 3H, CH_3), 1.25 (d, $J = 6.6$, 3H, CH_3), 3.15 (h, $J = 7.2$, 1H, $CHMe_2$), 6.72 (s, 1H, CH–Ph), 6.9–7.7 (m, 14H, $2C_6H_5$+3H imidazole+H-3 pyrazole)
2f	C_6H_5	$C(CH_3)_3$	76	120–121 [c]	$C_{23}H_{24}N_4$	C, H, N	1.17 (s, 9H, $(CH_3)_3C$), 6.8–7.7 (m, 15H, CH–Ph+$2C_6H_5$+3H imidazole+H-3 pyrazole)
2g	C_6H_5	C_6H_5	87	140–141 [d]	$C_{25}H_{20}N_4$	C, H, N	6.30 (s, 1H, CH–Ph), 6.85–7.65 (m, 19H, $3C_6H_5$+3H imidazole+H-3 pyrazole)
2h	CH_3	C_6H_5	86	137–138 [c]	$C_{20}H_{18}N_4$	C, H, N	3.78 (s, 3H, CH_3), 6.20 (s, 1H, CH–Ph), 6.8–7.65 (m, 14H, $2C_6H_5$+3H imidazole+H-3 pyrazole)

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

Table 2
Yields, physical, analytical and ^{1}H NMR data of triazoles **4a–h**

Comp.	R	R′	Yield (%)	M.p. (°C)	Molecular formula	*Anal.*	^{1}H NMR ($CDCl_3$), δ (ppm)
4a	C_6H_5	H	65	121–122 [a]	$C_{18}H_{15}N_5$	C, H, N	6.81 (s, 1H, CH–Ph), 7.15–7.9 (m, 12H, $2C_6H_5$+2H pyrazole), 8.09 and 8.18 (2s, 2H, triazole)
4b	C_6H_5	CH_3	36	126–127 [a]	$C_{19}H_{17}N_5$	C, H, N	2.19 (s, 3H, CH_3), 6.74 (s, 1H, CH–Ph), 7.1–7.7 (m, 11H, $2C_6H_5$+H-3 pyrazole), 8.09 and 8.14 (2s, 2H, triazole)
4c	C_6H_5	C_2H_5	63	147–148 [a]	$C_{20}H_{19}N_5$	C, H, N	0.90 (t, J = 7.2, 3H, CH_3), 2.67 (q, J = 7.2, 2H, CH_2), 6.77 (s, 1H, CH–Ph), 7.0–7.7 (m, 11H, $2C_6H_5$+H-3 pyrazole), 8.10 and 8.16 (2s, 2H, triazole)
4d	C_6H_5	$(CH_2)_2CH_3$	48	112–113 [a]	$C_{21}H_{21}N_5$	C, H, N	0.73 (t, J = 7.2, 3H, CH_3), 0.9–1.5 (m, 2H, CH_2), 2.63 (near t, J ~7.5, 2H, CH_2), 6.76 (s, 1H, CH–Ph), 6.9–7.7 (m, 11H, $2C_6H_5$+H-3 pyrazole), 8.10 and 8.16 (2s, 2H, triazole)
4e	C_6H_5	$CH(CH_3)_2$	63	154–155 [a]	$C_{21}H_{21}N_5$	C, H, N	1.07 (d, J = 6.6, 3H, CH_3), 1.26 (d, J = 6.6, 3H, CH_3), 2.8–3.5 (m, 1H, $CHMe_2$), 7.01 (s, 1H, CH–Ph), 7.05–7.7 (m, 11H, $2C_6H_5$+H-3 pyrazole), 8.10 and 8.14 (2s, 2H, triazole)
4f	C_6H_5	$C(CH_3)_3$	55	158–159 [a]	$C_{22}H_{23}N_5$	C, H, N	1.16 (s, 9H, $(CH_3)_3C$), 7.0–7.6 (m, 12H, CH–Ph+$2C_6H_5$+H-3 pyrazole), 8.06 and 8.08 (2s, 2H, triazole)
4g	C_6H_5	C_6H_5	56	165–166 [b]	$C_{24}H_{19}N_5$	C, H, N	6.59 (s, 1H, CH–Ph), 6.9–7.6 (m, 15H, $3C_6H_5$), 7.72 (s, 1H, H-3 pyrazole), 8.06 and 8.12 (2s, 2H, triazole)
4h	CH_3	C_6H_5	84	146–147 [c]	$C_{19}H_{17}N_5$	C, H, N	3.79 (s, 3H, CH_3), 6.45 (s, 1H, CH–Ph), 7.0–7.7 (m, 11H, $2C_6H_5$+H-3 pyrazole), 8.00 and 8.05 (2s, 2H, triazole)

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

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

3.2. Biological assays

All compounds were evaluated in vitro for antimicrobial activity against representative human pathogenic fungi (*C. albicans*, *C. neoformans*, *A. 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.

3.2.1. Material and methods

3.2.1.1. Compounds. Test compounds were dissolved in DMSO at an initial concentration of 200 mM and then were 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% foetal 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. Human immunodeficiency virus type-1 (HIV-1, III_B strain) was obtained from supernatants of persistently infected H9/III_B cells. HIV-1 stock solutions had a titre 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 acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 cells [8]. 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. A total of 20 μl of an HIV-1 suspension containing 100 $CCID_{50}$ was then added. After 4 days 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 [9,10]. Cytotoxicity of compounds, based on

Table 3
Activity of compounds **2a–h** against ventricular fibrillation caused by aconitine in rats [a]

Comp.	Dose (mg/kg p.o.)	Appearance time (s ± SE) of extrasystoles	Death time (s ± SE)
Control (aconitine HCl)	[b]	182 ± 14.7	658 ± 21.3
Quinidine	25	359 ± 16.3 [c]	1003 ± 11.6 [c]
2a	50	212 ± 16.1	698 ± 17.1
2b	50	224 ± 19.7	693 ± 22.5
2c	50	307 ± 11.6 [c]	760 ± 19.7 [c]
	25	251 ± 13.7 [d]	687 ± 16.5 [d]
	12.5	227 ± 18.1	661 ± 19.3
2d	50	198 ± 12.5	665 ± 15.9
2e	50	215 ± 13.8	689 ± 16.8
2f	50	321 ± 21.5 [c]	739 ± 14.7 [c]
	25	288 ± 15.9 [c]	706 ± 19.3 [c]
	12.5	241 ± 21.3 [d]	672 ± 20.4 [d]
2g	50	209 ± 15.4	685 ± 20.2
2h	50	209 ± 18.2	723 ± 18.1 [d]

[a] Groups of ten animals (250–300 g) of both sexes, pregnant females excluded.
[b] 15 mg/kg/i.v./min until death.
[c] Statistically significant value calculated in comparison with the test performed with aconitine only ($P<0.01$).
[d] Statistically significant value calculated in comparison with the test performed with aconitine only ($P<0.05$).

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 visually determined after 2 days (yeasts) or 3 days (dermatophytes). MIC was defined as the compound 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 concentration resulting in 50% (EC_{50}) growth inhibition was determined by linear regression analysis.

3.3. Pharmacology

Preliminary tests on the imidazole derivative **2b** (as hydrochloride) carried out by Panlabs Inc. (Bothell, WA, USA) have shown significant antiarrhythmic and hypotensive activities at doses of 100 mg/kg (in in vivo tests in mice) or at a concentration of 100 μM (in in vitro tests). Subsequently, the presence of these pharmacological activities was further investigated at the Istituto di Farmacologia e Tossicologia (Università di Napoli) on all the imidazoles **2a–h**.

Antihypertensive and antiarrhythmic (Table 3) activities were evaluated by standard procedures.

ED_{50} of the most active compounds were determined by the administration of three dosages: 12.5, 25 and 50 mg/kg.

4. Results and discussion

4.1. Microbiology

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 which were lower than the cytotoxic ones.

When tested against yeast and mould representatives, **2d–g** were active against *C. neoformans* at concentrations ranging between 22 and 66 μM; only **2g** also showed activity against *C. albicans* (MIC = 22 μM) and *S. aureus* (MIC = 66 μM) whereas **4g** showed activity only against *S. aureus* (MIC = 50 μM). Triazoles **4a–f**, **h** were devoid of antimicrobial activity.

In conclusion, replacement of the biphenyl portion with a phenyl–pyrazolyl moiety afforded bifonazole

analogues with low antimycotic activity. Among these compounds the most active turned out to be **2g**, which is characterized by a pyrazole nucleus bearing a double aromatic substitution at positions 1 and 5. Among derivatives in which the second substituent on the heterocycle is an alkyl chain (**2b–f**), the antifungal activity was directly related to the presence of a bulky alkyl radical.

When the imidazole moiety was replaced by a triazole ring (**4a–h**), the weak antimycotic activity shown by **2d–g** vanished. This observation is in agreement with the results of a recent molecular modelling study carried out on azole antifungal agents active against *C. albicans* [4].

4.2. Pharmacology

Preliminary in vitro tests regarding calcium antagonism, angiotensin I inhibition, angiotensin II antagonism, performed by Panlabs Inc. on imidazole **2b** as hydrochloride at a 100 μM concentration (data not reported in this paper), suggested a possible hypotensive activity. Therefore all imidazoles **2a–h** were later evaluated in vivo on spontaneously hypertensive rats at the dose of 50 mg/kg p.o., at the laboratories of Istituto di Farmacologia e Tossicologia of the University of Napoli, but none of them showed a significant antihypertensive activity.

Preliminary in vitro (at a 100 μM concentration) and in vivo (at 100 mg/kg i.p. dose on mice) tests specific for the antiarrhythmic activity, performed on **2b** as hydrochloride by Panlabs Inc. (data not reported in this paper) pointed out significant results. Further investigation of the antiarrhythmic activity was carried out on rats at lower doses (50, 25 and 12.5 mg/kg) of all derivatives **2a–h**. The results are reported in Table 3. The most active compounds turned out to be **2c**, **f**, which showed a moderate protection against ventricular extrasystoles caused by aconitine, with an ED_{50} in delaying the appearance of extrasystoles of 40.78 (32.44–51.25) and 29.55 (24.79–35.24) mg/kg, respectively. Moreover, the ED_{50} in protracting death time resulted >50 mg/kg in both cases.

The results which emerged from the pharmacological tests performed on imidazoles **2** discouraged us from also extending the investigation to their triazole analogues **4**.

Acknowledgements

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Table 4
In vitro biological activity of compounds **2a–h** and **4a–h**

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.
2a	38.2	>38.2	In [e]	200/200	In [e]	In [e]	In [e]
2b	72.2	>72.2	In [e]	200/>200	In [e]	In [e]	In [e]
2c	53.4	>53.4	In [e]	200/200	In [e]	In [e]	In [e]
2d	40.3	>40.3	In [e]	66/66	In [e]	200/200	In [e]
2e	41.3	>41.3	In [e]	66/66	In [e]	200/>200	In [e]
2f	30	>30	In [e]	66/66	In [e]	200/200	In [e]
2g	22.2	>22.2	22/>200	22/22	In [e]	66/>200	In [e]
2h	56	>56	In [e]	In [e]	In [e]	In [e]	In [e]
4a	105.5	>105.5	In [e]	In [e]	In [e]	In [e]	In [e]
4b	162	>162	In [e]	In [e]	In [e]	In [e]	In [e]
4c	54.8	>54.8	In [e]	In [e]	In [e]	In [e]	In [e]
4d	55	>55	In [e]	In [e]	In [e]	In [e]	In [e]
4e	44	>44	In [e]	In [e]	In [e]	In [e]	In [e]
4f	43.5	>43.5	In [e]	In [e]	In [e]	In [e]	In [e]
4g	41	>41	In [e]	In [e]	In [e]	50/100	In [e]
4h	>200	>200	In [e]	In [e]	In [e]	In [e]	In [e]
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 MT4 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.

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