Solvent-free Microwave Synthesis of Bis-pyrazolo[3,4-*b*:4',3'-*e*]-pyridines and Study of Their Antifungal Properties

J. Quiroga*, J. Portilla, B. Insuasty, R. Abonía

Grupo de Investigación de Compuestos Heterocíclicos, Departamento de Química, Universidad del Valle, A. A. 25360, Cali, Colombia

M. Nogueras*

Departamento de Química Inorgánica y Orgánica, Universidad de Jaén, 23071 Jaén, España

M. Sortino and S. Zacchino

Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, (2000), Rosario, Argentina.

Received June 3, 2004

The synthesis of a series of bis-pyrazolo[3,4-*b*:4',3'-*e*]pyridines (**3**) in the reaction of 5-amino-3-methyl-1-phenylpyrazole (**1**) with aldehydes (**2**) under microwave irradiation and solvent-free conditions is described. The structure elucidation of the products is based on detailed nmr analysis of experiments such as ^{1}H-COSY, NOESY, DEPT, HSQC and HMBC. These compounds showed moderate antifungal *in vitro* activity against dermatophytes.

J. Heterocyclic Chem., **42**, 61 (2005).

Introduction.

Microwave enhanced synthesis has attracted substantial attention in recent years, enabling many organic reactions to proceed much faster and with higher yields than when conventional heating is employed [1]. Microwave-assisted reactions have attracted substantial attention in recent years, because of the simplicity in operation, milder reaction conditions, increasing reaction rates and formation of cleaner products. In particular, solvent-free microwave-assisted reactions have gained more popularity as they provide an opportunity to work with open vessels [2].

On the other hand, the pyrazole nucleus [3] has long shown pharmacological interest as antianxiety [4], antypiretic, analgesic and anti-inflammatory agents [5], as well as for their antimicrobial properties [6], especially antibacterial and antifungal activities [6a,c][7]. Some structures containing the pyrazole skeleton, such as the pyrazolo[3,4-*b*]pyridine system have proven to be interesting classes of heterocycles due to diverse biological properties, including antitubercular and antibacterial activities [8].

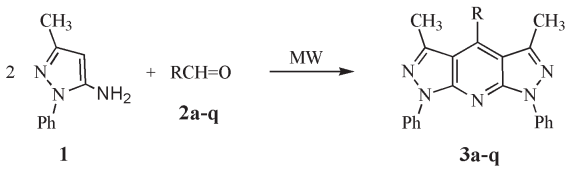
In this work we describe the novel microwave enhanced preparation of tricyclic bis-pyrazolo[3,4-*b*:4',3'-*e*]pyridines, which have received little attention in the biological field. Nevertheless, they have been attractive for physicochemical applications since they exhibit a high fluorescence in both solution and solid state under exposure to the white light [9], which make them appropriates in the design of electroluminescent materials, like organic light-emitting diodes (OLEDs) [9e-g].

As part of our ongoing project devoted to the search for new antifungal agents [10], we tested these tricyclic bis-pyrazolo[3,4-*b*:4',3'-*e*]pyridines against a panel of opportunistic pathogenic fungi including yeasts, hialohyphomycetes as well as dermatophytes. As it is very well known [11] that different azole derivatives are presently the drugs of choice for the treatment of deep mycoses, which constitute one of the major causes of morbi-mortality for immunocompromised patients. However, resistance to many currently available antifungal agents continues growing at a high rate and considerable effort is still concentrated in the azole area in order to find new, and more potent analogues, which overcome the fungal diseases. The new azole structures reported here will surely add new interesting information about this important class of antifungal compounds.

Results and Discussion.

Our approach to the target heterocyclic compounds was achieved by the synthesis of bis-pyrazolo[3,4-*b*:4',3'-*e*]-pyridines (**3a-q**) from 5-amino-3-methyl-1-phenylpyrazole (**1**) and aromatic or heteroaromatic aldehydes (**2**). Thus, the microwave irradiation during 1-5 min of aminopyrazole (**1**) and aldehydes (**2**) (in ratio 2:1) under solvent-free conditions afforded the corresponding bis-pyrazolo-[3,4-*b*:4',3'-*e*]pyridines (**3a-q**) in high yields (Scheme 1 and Table 1). A similar synthesis of this kind of compounds by microwave irradiation has previously been

Scheme 1



reported by Esteves-Souza *et al*, in which they described the preparation of one bis-pyrazolo[3,4-*b*:4',3'-*e*]pyridine along with other three products by the reaction of 1,3-dimethyl-5-aminopyrazol and *p*-methylbenzaldehyde both adsorbed on solid support (silica o sand) [12e].

Table 1

Microwave-assisted Synthesis of Bis-pyrazolo[3,4-*b*:4',3'-*e*]pyridines

Entry	Aldehyde (**2**) R	MW Irradiation Time (min)	MW Irradiation Yield (%)	Conventional heating Time (h)	Conventional heating Yield (%)
a	C_6H_5	1.5	65	3 [12] [a]	21 [12a], 50 [12b], 34 [12e]
b	4-FC_6H_4	2.5	73		
c	4-ClC_6H_4	1.5	92	3 [12] [a]	24 [12a], 65 [12b]
d	4-BrC_6H_4	1.5	65		
e	2-FC_6H_4	2.5	81		
f	4-$CF_3C_6H_4$	3	57		
g	2-$CF_3C_6H_4$	3	80		
h	2-$O_2NC_6H_4$	2	79		
i	3-$O_2NC_6H_4$	3	75		
j	4-$O_2NC_6H_4$	2.5	80	3 [12b] [b]	42 [12b]
k	4-$CH_3C_6H_4$	1	70		
l	4-$CH_3OC_6H_4$	2	72	3 [12] [a]	15 [12a], 47 [12b], 54 [12e]
m	2-pyridyl	1.5	68		
n	3-pyridyl	1.5	73		
o	4-pyridyl	1.5	77	3 [12a,d] [a]	34 [12a]
p	3,4-$OCH_2OC_6H_3$	1.5	67		
q	3,4,5-tri-$CH_3OC_6H_2$	5	73		

[a] Reaction temperature 220-260 °C; [b] in refluxing ethanol with 1 ml. of piperidine.

The formation of the bis-pyrazolo[3,4-*b*:4',3'-*e*]pyridines **3a-q** was confirmed by their spectral characteristics (Tables 2 and 3). The ^{1}H NMR spectra of compounds **3a-q** show a singlet for CH_3 groups of pyrazole rings at δ= 1.91-2.07 ppm with integration for six protons: a multiplet for aromatic protons of phenyl groups and the signals of a substituent at position 4 on the pyridine moiety (Table 2).

In the ^{13}C-nmr spectra (DEPT) all signals belonging to tertiary, secondary and primary carbon atoms could be determined for **3a-q** compounds (Table 3).

Table 2

^{1}H-NMR Chemical Shifts (δ) for Compounds **3a-q**, (300 MHz)

Comp.	3,5-CH_3	1,7-C_6H_5	4-R
3a	2.02	7.32, 7.56, 8.32	7.54-7.57 (m)
3b	2.07	7.45, 7.52, 8.39	7.24 (d), 7.29 (d)
3c	2.06	7.31, 7,57, 8.32	7.26 (d), 7.67 (d)
3d	2.03	7.30, 7.55, 8.31	7.77 (d), 7.80 (d)
3e	2.06	7.35, 7.57, 8.32	7.04, 7.52 (q), 7.70 (m)
3f	1.96	7.32, 7.58, 8.30	7.85-7.96 (dd)
3g	1.91	7.35, 7.62, 8.34	7.76, 7.93 (m), 8.08
3h	2.00	7. 34, 7.60, 8.34	7.99-7.81 (m), 8.39
3i	2.04	7.32, 7.57, 8.32	7.94, 8.08, 8.46
3j	2.03	7.32, 7.58, 8.32	7.92 (d), 8.44 (d)
3k[a]	2.12	7.31, 7.54, 8.42	7.83 (d), 7.84 (d)
3l[a]	2.06	7.28, 7.55, 8.32	7.14 (d), 7.48(d)
3m	2.00	7.32, 7.59, 8.33	7.66, 7.80, 8.07, 8.86
3n	2.04	7.31, 7.56, 8.31	7.63, 8.05, 8.81, 8.83
3o	1.99	7.32, 7.57, 8.30	7.65 (d), 8.82 (d)
3p[a]	2.14	7.31, 7.57, 8.33	7.03, 7.12, 7.16
3q[a]	2.10	7.31, 7.54, 8.28	6.94 (s)

[a]For **3k** CH_3 2.51 ppm; **3l** CH_3O 3.90 ppm; **3p** –OCH_2O- 6.16 ppm; **3q** tri-CH_3O 3.82 and 3.86 ppm.

Assignment of the ^{1}H- and ^{13}C- resonances of compounds **3** was deduced from the concerted application of both direct and long range heteronuclear chemical shift correlation experiments. One-bond and two and three bonds proton-carbon chemical shift correlations were established using the HSQC [13a] and the HMBC [13b] techniques.

Molecular structures of compounds **3a** and **3m** were confirmed by X-ray diffraction analysis and have recently been reported by us [14].

It is important to remark that when the reaction was carried out with 3,4,5-trimethoxybenzaldehyde **2q** under the same conditions (*i.e.*, microwave irradiation for 1.5 min) a new compound was formed, which did not show fluorescence. Elemental analysis, nmr and mass spectrum data (see experimental) suggest that compound **4** resulted from the reaction of two molecules of amine **1** with one of the aldehyde **2q**, accompanied by loss of the water molecule only, but not the loss of ammonia (Scheme 2). The subsequent microwave irradiation of compound **4** for 3.5 min additionally leads to the formation of the fluorescent compound **3q**. This experimental fact confirmed that the studied reaction proceeds through the intermediate of type **4**, with subsequent loss of water, ammonia and hydrogen molecules, such as was proposed in previous works [12].

Table 3

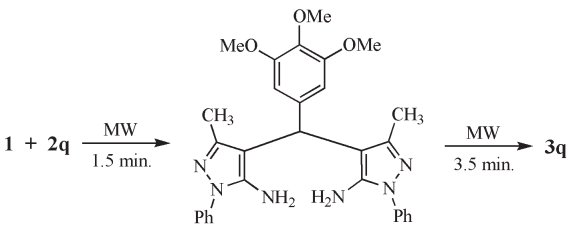
^{13}C-NMR Chemical Shift (δ) for compounds **3a-q** (75 MHz)

Comp.	3,5-CH_3	1,7-C_6H_5	C-3, 3a, 4, 8a	4-R
3a	13.6	138.8, 119.4, 128.3, 124.6	143.7, 112.6, 141.4, 150.4	128.6, 128.4, 127.5, 138.8
3b	14.9	139.6, 120.2, 129.0, 125.2	144.2, 113.6, 140.2, 150.4	130.3, 130.8, 115.5, 164.9
3c	13.8	138.7, 119.5, 128.4, 124.7	143.5. 112.5, 140.0, 149.6	133.8, 130.3, 127.6, 131.9
3d	13.8	138.8, 119.5, 128.4, 124.7	143.5, 112.5, 139.9, 149.7	132.3, 130.6, 130.6, 122.3
3e	13.7	138.8, 120.0, 129.1, 125.4	143.8, 113.2, 139.0, 149.8	149.8, 115.6, 115.9, 124.6, 131.5, 132.2
3f [a]	14.1	138.8, 119.6, 128.8, 124.6	143.6, 112.5, 139.7, 149.6	129.8. 125.1, 129.8, 137.4
3g [a]	13.4	138.8, 119.8. 129.2, 125.4	143.9, 113.3, 137.6, 149.5	127.0, 127.4, 130.3, 131.0, 131.6, 132.5
3h	12.8	138.6, 119.5, 128.4, 124.9	143.0, 112.2, 136.2, 149.6	127.5, 147.8, 124.0, 131.2, 133.1, 130.8
3i	13.6	138.6, 119.5, 128.4, 124.8	143.3, 112.2, 138.2, 149.8	134.8, 123.2, 123.5, 129.3, 134.9
3j	13.0	138.7, 118.7, 127.6, 124.0	143.3, 112.2, 139.7, 149.7	129.1, 121.7, 129.3, 131.3
3k [a]	13.6	139.0, 120.3, 129.0, 125.1	144.7, 113.3, 139.8, 150.6	128.9, 128.5, 128.0, 139.0
3l [a]	13.8	138.8, 119.4, 128.3, 124.5	143.7, 112.9, 141.6, 149.7	125.0, 129.8, 113.2, 159.7,
3m	14.0	138.8, 119.6, 128.8, 124.8	143.6, 112.3, 139.7, 149.9	151.8, 125.0, 136.2, 124.0, 149.0
3n	13.6	138.7, 119.5, 128.4, 124.8	143.4, 112.8, 143.3, 149.8	129.1, 149.8, 148.3, 122.3, 136.0
3o	13.6	138.7, 119.6, 128.8, 125.1	143.5, 112.0, 141.2, 149.6	138.0, 123.6, 149.1
3p [a]	13.6	138.8, 119.4, 128.3, 124.6	143.7, 112.9, 141.2, 149.7	126.4, 107.4, 146.8, 147.6, 109.0, 122.4
3q [a]	14.5	139.0, 119.6, 128.6, 125.2	144.4, 113.0, 141.9, 149.8	152.5, 137.9, 128.6, 106.7

[a]For **3f** CF_3 124.6 ppm; **3g** CF_3 126.3 ppm; **3k** CH_3 15.3 ppm; **3l** CH_3O 54.9 ppm; **3p** –OCH_2O- 100.9; **3q** tri-CH_3O 56.2 and 60.4 ppm

The structure of the intermediate was also completely characterised by spectroscopic (see experimental), analytical methods and confirmed by X-ray diffraction analysis [14c].

Scheme 2



4

These new compounds were assayed *in vitro* for antifungal properties against a panel of fungal strains comprising human opportunistic pathogenic yeasts, hialohyphomycetes as well as dermatophytes with the microbroth dilution method (Table 4).

To carry out the antifungal evaluation, concentrations of compounds up to 250 μg/mL were incorporated to growth media according with reported procedures [15]. Results showed that none of the compounds tested, was active against the yeasts *C. albicans*, *C. tropicalis*, *Saccharomyces cerevisiae* or *Cryptococcus neoformans* nor against the filamentous fungi *Aspergillus niger*, *A. fumigatus* or *A. flavus* (results not shown). In contrast, moderate antifungal effects were obtained for the compounds of the series against dermatophytes. These results are shown in Table 4. In particular, compounds **3g, 3k, 3n** and **3o** showed antifungal activities with MICs between 100-200 μg/ml possessing the three species tested a similar sensibility.

Table 4

In vitro Evaluation of the Antifungal Activity of Bis-pyrazolo[3,4-*b*:4',3'-*e*]pyridines using Broth Microdilution Methods (MIC values are given in μg/ml)

Compound	M.g	T.r	T.m
3a	>250	>250	>250
3b	>250	>250	>250
3c	>250	>250	>250
3d	>250	>250	>250
3e	>250	>250	>250
3f	>250	>250	>250
3g	200	200	200
3h	>250	>250	>250
3i	>250	>250	>250
3j	>250	>250	>250
3k	200	100	100
3l	>250	>250	>250
3m	>250	>250	>250
3n	200	200	100
3o	200	100	200
3p	>250	>250	>250
3q	>250	>250	>250
Amphotericin B	0.125	0.075	0.075
Ketoconazole	0.05	0.025	0.025
Terbinafine	0.04	0.01	0.04

M.g: Microsporum gypseum C 115, *T.r: Trichophyton rubrum* C 113, *T.m: Trichophyton mentagrophytes* ATCC 9972.

Since dermatophytes are a group of fungi which characteristically infect the keratinized areas of the body and dermatomycoses are very difficult to eradicate, it is interesting to point out that these derivatives showed activity against

dermatophytes and not against another type of fungi and, although not very active, they can open new avenues for the design and synthesis of more potent structures of the bis-pyrazolo[3,4-*b*:4',3'-*e*]pyridines type, easily synthesized by the convenient microwave methodology presented here. In addition, this work adds valuable data to the structure-activity relationships for azole compounds and avoids unnecessary duplication of work.

Conclusions.

In summary, we have developed a simple, convenient and rapid method for the synthesis of bis-pyrazolopyridines under microwave irradiation. The present method avoids the use of solvent and extended reaction times, being very useful for the preparation of the title compounds from aldehydes especially, which typically give low yields under conventional conditions. The reduced reaction times along with the minimization of thermal decomposition of the products are the main advantages of microwave heating. Regarding their antifungal behavior, they display moderate activity against dermatophytes, adding an interesting knowledge to the azole area devoted to the finding of new and more potent analogues which overcome the toxicity and increasing resistance to the existing azole antifungal drugs.

EXPERIMENTAL

Melting points were determined in a Büchi Melting Point Apparatus and are uncorrected. The ^{1}H- and ^{13}C nmr spectra were run on a Bruker DPX 300 spectrometer operating at 300 MHz and 75 MHz respectively, using dimethyl sulfoxide-d_6 as solvent and tetramethylsilane as internal standard. The mass spectra were recorded on a Fisons-Platform interface APCI in methanol and recorded on a Hewlett Packard HP Engine-5989 spectrometer (equipped with a direct inlet probe) and operating at 70 eV. The elemental analysis has been obtained using a LECO CHNS-900 elemental analyzer.

General Procedure for the Synthesis of 4-Aryl-3,5-dimethyl-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridines **3a-q**.

A mixture of aminopyrazole **1** (2.0 mmoles) and the corresponding aldehyde **2** (1.0 mmoles) was placed into pyrex-glass open vessels and irradiated in a domestic microwave oven for 1-5 min (at 600 watts) (tlc control). The reaction mixture was treated with ethanol. The products **3a-q** were collected by filtration, washed with ethanol and recrystallized from ethanol or dimethyl formamide.

3,5-Dimethyl-1,4,7-triphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3a**).

This compound was obtained according to general procedure as pale yellow crystals, mp 241-243 °C (244 °C[9c][12a], 242 °C[12b]). Ms: EI m/z (relative abundance) = 416 (36), 415 (100, M^+), 414 (10), 77 (30), 51 (12).

Anal. Calcd. for $C_{27}H_{21}N_5$: C, 78.05; H, 5.09; N, 16.86. Found: C, 78.08; H, 5.03; N, 16.81.

4-(4-Fluorophenyl)-3,5-dimethyl-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3b**).

This compound was obtained according to general procedure as white crystals, mp 216-218 °C. Ms: EI m/z (relative abundance) = 434 (24), 433 (79, M^+), 77 (100), 51 (49).

Anal. Calcd. for $C_{27}H_{20}FN_5$: C, 74.81; H, 4.65; N, 16.16. Found: C, 74.84; H, 4.72; N, 16.23.

4-(4-Chlorophenyl)-3,5-dimethyl-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3c**).

This compound was obtained according to general procedure as white crystals, mp 249-251 °C (243 °C[12a], 241 °C[12b]). Ms: EI m/z (relative abundance) = 451/449 (39/100, M^+), 450 (34), 77 (37), 51 (13).

Anal. Calcd. for $C_{27}H_{20}ClN_5$: C, 72.08; H, 4.48; N, 15.56. Found: C, 72.13; H, 4.52; N, 15.51.

4-(4-Bromophenyl)-3,5-dimethyl-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3d**).

This compound was obtained according to general procedure as white crystals, mp 227-228 °C. Ms: EI m/z (relative abundance) = 495/493 (100/98, M^+), 494 (38), 77 (85), 51 (31).

Anal. Calcd. for $C_{27}H_{20}BrN_5$: C, 65.60; H, 4.08; N, 14.17. Found: C, 65.67; H, 4.02; N, 14.21.

4-(2-Fluorophenyl)-3,5-dimethyl-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3e**).

This compound was obtained according to general procedure as white crystals, mp 265-266 °C. Ms: EI m/z (relative abundance) = 434 (35), 433 (100, M^+), 432 (9), 77 (19), 51 (8).

Anal. Calcd. for $C_{27}H_{20}FN_5$: C, 74.81; H, 4.65; N, 16.16. Found: C, 74.77; H, 4.61; N, 16.13.

4-(4-Trifluoromethylphenyl)-3,5-dimethyl-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3f**).

This compound was obtained according to general procedure as white crystals, mp 211-212 °C. Ms: EI m/z (relative abundance) = 484 (31), 483 (100, M^+), 482 (10), 77 (17), 51 (7).

Anal. Calcd. for $C_{28}H_{20}F_3N_5$: C, 69.56; H, 4.17; N, 14.48. Found: C, 69.53; H, 4.12; N, 14.53.

4-(2-Trifluoromethylphenyl)-3,5-dimethyl-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3g**).

This compound was obtained according to general procedure as pale yellow crystals, mp 235-236 °C. Ms: EI m/z (relative abundance) = 484 (33), 483 (100, M^+), 482 (6), 77 (16), 51 (8).

Anal. Calcd. for $C_{28}H_{20}F_3N_5$: C, 69.56; H, 4.17; N, 14.48. Found: C, 69.60; H, 4.11; N, 14.41.

3,5-Dimethyl-4-(2-nitrophenyl)-1,7-diphenyl-1*H*,7*H*-bispyrazolo-[3,4-*b*:4',3'-*e*]pyridine (**3h**).

This compound was obtained according to general procedure as orange crystals, mp 253-255 °C. Ms: EI m/z (relative abundance) = 461 (35), 460 (100, M^+), 413 (18), 412 (10), 77 (32), 51 (8).

Anal. Calcd. for $C_{27}H_{20}N_6O_2$: C, 70.42; H, 4.38; N, 18.25. Found: C, 70.38; H, 4.31; N, 18.32.

3,5-Dimethyl-4-(3-nitrophenyl)-1,7-diphenyl-1*H*,7*H*-bispyrazolo-[3,4-*b*:4',3'-*e*]pyridine (**3i**).

This compound was obtained according to general procedure as pale yellow crystals, mp 213-215 °C. Ms: EI m/z (relative

abundance) = 461 (29), 469 (100, M^+), 77 (40), 51 (12).

Anal. Calcd. for $C_{27}H_{20}N_6O_2$: C, 70.42; H, 4.38; N, 18.25. Found: C, 70.35; H, 4.42; N, 18.21.

3,5-Dimethyl-4-(4-nitrophenyl)-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3j**).

This compound was obtained according to general procedure as yellow crystals, mp 270-272 °C (271 °C [12b]). Ms: EI m/z (relative abundance) = 461 (11), 460 (34, M^+), 77 (100), 51 (43).

Anal. Calcd. for $C_{27}H_{20}N_6O_2$: C, 70.42; H, 4.38; N, 18.25. Found: C, 70.46; H, 4.35; N, 18.29.

3,5-Dimethyl-4-(4-methylphenyl)-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3k**).

This compound was obtained according to general procedure as pale yellow crystals, mp 259-260 °C. Ms: EI m/z (relative abundance) = 430 (24), 429 (80, M^+), 78 (12), 77 (100), 51 (42).

Anal. Calcd. for $C_{28}H_{23}N_5$: C, 78.30; H, 5.40; N, 16.30. Found: C, 78.35; H, 5.32; N, 16.33.

4-(4-Methoxyphenyl)- 3,5-dimethyl-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3l**).

This compound was obtained according to general procedure as pale yellow crystals, mp 233-234 °C (232 °C [9c][12a], 222 °C [12b]). Ms: EI m/z (relative abundance) = 446 (34), 445 (100, M^+), 77 (21), 51 (6).

Anal. Calcd. for $C_{28}H_{23}N_5O$: C, 75.49; H, 5.20; N, 15.72. Found: C, 75.47; H, 5.16; N, 15.77.

3,5-Dimethyl-1,7-diphenyl-4-(2-pyridyl)-1*H*,7*H*-bispyrazolo-[3,4-*b*:4',3'-*e*]pyridine (**3m**).

This compound was obtained according to general procedure as pale yellow crystals, mp 243-244 °C. Ms: EI m/z (relative abundance) = 417 (25), 416 (100, M^+), 415 (15), 77 (32), 51 (15).

Anal. Calcd. for $C_{26}H_{20}N_6$: C, 74.98; H, 4.84; N, 20.18. Found: C, 74.93; H, 4.78; N, 20.15.

3,5-Dimethyl-1,7-diphenyl-4-(3-pyridyl)-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3n**).

This compound was obtained according to general procedure as white crystals, mp 288-290 °C. Ms: EI m/z (relative abundance) = 417 (31), 416 (91, M^+), 415 (13), 77 (100), 51 (49).

Anal. Calcd. for $C_{26}H_{20}N_6$: C, 74.98; H, 4.84; N, 20.18. Found: C, 74.88; H, 4.89; N, 20.21.

3,5-Dimethyl-1,7-diphenyl-4-(4-pyridyl)-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3o**).

This compound was obtained according to general procedure as pale yellow crystals, mp 258-260 °C (261 °C [9c][12a]). Ms: EI m/z (relative abundance) = 417 (31), 416 (100, M^+), 415 (10), 77 (19), 51 (8).

Anal. Calcd. for $C_{26}H_{20}N_6$: C, 74.98; H, 4.84; N, 20.18. Found: C, 74.96; H, 4.87; N, 20.13.

3,5-Dimethyl-4-(3,4-methylenedioxyphenyl)-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3p**).

This compound was obtained according to general procedure as white crystals, mp 260-261 °C. Ms: EI m/z (relative abundance) = 460 (35), 459 (100, M^+), 77 (53), 51 (15).

Anal. Calcd. for $C_{28}H_{21}N_5O_2$: C, 73.19; H, 4.61; N, 15.24. Found: C, 73.16; H, 4.65; N, 15.21.

4-(3,4,5-Trimethoxyphenyl)-3,5-Dimethyl-1,7-diphenyl-1*H*,7*H*-bispyrazolo[3,4-*b*:4',3'-*e*]pyridine (**3q**).

This compound was obtained according to general procedure as white crystals, mp 238-239 °C. Ms: EI m/z (relative abundance) = 509 (37), 505 (100, M^+), 77 (31), 51 (9).

Anal. Calcd. for $C_{30}H_{27}N_5O_3$: C, 71.27; H, 5.38; N, 13.85. Found: C, 71.24; H, 5.40; N, 13.80.

Obtention of α,α-Bis-(5-amino-3-methyl-1-phenylpyrazol-4-yl)-3,4,5-trimethoxytoluene (**4**).

This compound was obtained according to general procedure by microwave irradiation for 1.5 min. as white crystals, yield 84 %, mp. 219-220 °C. ^{1}H NMR: δ = 1.89 (s, 6H, CH_3); 3.66 (s, 3H, OCH_3); 3.71 (3, 6H, OCH_3); 4.49 (s, 4H, NH_2); 5.26 (s, 1H, CH); 6.56 (s, 2H, H*o* trimethoxyphenyl); 7.58 (d, 4H, H*o* of Ph); 7.44 (t, 4H, H*m*); 7.26 (d, 2H, H*p*). ^{13}C NMR: δ = 12.9 (CH_3); 35.2 (CH-methynic); 55.9 (CH_3O); 60.1 (CH_3O); 102.0 (C-4 pyrazolic ring); 105.7 (C*o* trimethoxyphenyl); 137 (C-5 pyrazolic ring); 143.4 (C-3 pyrazolic ring). The mass spectrum shows the following peaks: ms: (70 eV) m/z (%) = 526 (4), 525 (17), 524 (31, M^+), 353 (18), 352 (84), 351 (32), 350 (100), 321 (8), 320 (38), 173 (10), 77 (23).

Anal. Calcd. for $C_{30}H_{32}N_6O_3$: C, 68.68; H, 6.15; N, 16.02. Found: C, 68.61; H, 6.06; N, 16.12.

Microorganisms and Media.

For the antifungal evaluation, strains from the American Type Culture Collection (ATCC), Rockville, MD, USA and CEREMIC (C), Centro de Referencia Micológica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina were used. *Candida albicans* ATCC 10231, *C. tropicalis* C131, *Saccharomyces cerevisiae* ATCC9763, *Cryptococcus neoformans* ATCC32264, *Aspergillus flavus* ATCC9170, *A. fumigatus* ATTC26934, *A. niger* ATCC9029, *Trichophyton rubrum* C110, *T. mentagrophytes* ATCC 9972 and *Microsporum gypseum* C115.

Antifungal Susceptibility Testing.

The Minimal Inhibitory Concentration (MIC) of each extract was determined by using broth microdilution techniques following the guidelines of the National Committee for Clinical Laboratory Standards for yeasts (M27-A2) (NCCLS, 2002)[15]. MIC values were determined in RPMI 1640 (Sigma, St Louis, Mo, USA) buffered to a pH 7.0 with MOPS. The starting inocula were $1x10^3$ to $5x10^3$ CFU/ml. Microtiters trays were incubated at 35 °C for yeasts and hialophyphomycetes and at 28-30 °C for dermatophyte strains in a moist, dark chamber, and MICs were recorded at 48 h for yeasts, and at a time according to the control fungus growth, for the rest of fungi. The susceptibilities of the standard drugs Ketoconazol, Terbinafine and Amphotericin B were defined as the lowest concentration of drug which resulted in total inhibition of fungal growth. For the assay, extracts stock solutions were two-fold diluted with RPMI from 250-0.98 μg/ml (final volume = 100 μl) and a final dimethyl sulfoxide concentration ≤ 1%. A volume of 100 μl of inoculum suspension was added to each well with the exception of the sterility control where sterile water was added to the well instead. The MIC was defined as the minimum inhibitory concentration of the extract which resulted in total inhibition of the fungal growth.

Acknowledgements.

Authors are grateful to COLCIENCIAS, to Universidad del Valle, to the Spanish "Ministerio de Educación, Cultura y Deporte (Programa de Cooperación con Iberoamérica, AECI)" and Agencia de Promociones Científicas y Tecnológicas de la Argentina (SAZ) for financial support. This research is part of Project X.7-PIBEAFUN (Iberian-American Project on Search and Development of Antifungal Agents) of the Iberian-American Program for the Development of Science and Technology (CYTED), sub-program X.

REFERENCES AND NOTES

[1a] A. Loupy, A. Petit, J. Hamelin, F. Texier-Boullet, P. Jacquault and D. Mathe, *Synthesis*, , 1213 (1998); [b] R. S. Varma, *Green Chemistry*, 43 (1999); [c] S. Deshayes, M. Liagre, A. Loupy, J.-L. Luche and A. Petit, *Tetrahedron*, **55**, 10951 (1999).

[2a] K. Tanaka and F. Toda, *Chem. Rev.*, **100**, 1025 (2000); [b] R. S. Varma, *Clean Products and Processes*, **1**, 132 (1999); [c] R. S. Varma, *Pure Appl. Chem.*, **73**, 193 (2001); [d] J. Quiroga, S. Cruz, B. Insuasty and R. Abonia, *Heterocyclic Commun.*, **6**, 275 (2000); [e] J. Quiroga, C. Cisneros, B. Insuasty, R. Abonia, M. Nogueras and A. Sánchez, *Tetrahedron Lett.*, **42**, 5625 (2001); [f] J. Quiroga, A. Rengifo, B. Insuasty, R. Abonía, M. Nogueras and A. Sánchez, *Tetrahedron Lett.*, **43**, 9061 (2002).

[3] K. Kirschke, in: E. Schaumann (Ed.), Hetarene III part 2, Georg Thieme Verlag, Stuttgart, 1994, Houben-Veyl Vol. **E8b**, pp. 399-763.

[4a] J. Haufel and E. Breitmaier, *Angew. Chem.*, **13**, 604 (1974); [b] D. J. Wustrow, T. Capiris, R. Rubin, J. A. Knobelsdorf, H. Akunne, M. D. Davis, R. MacKenzie, T. A. Pugsley, K. T. Zoski, T. G. Heffner and L. D. Wise, *Bioorg. Med. Chem. Lett.*, **8**, 2067 (1998).

[5a] A. I. Eid, M. A. Kira and H. H. Fahmy, *J. Pharm. Belg.*, **33**, 303 (1978); [b] T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, L. F. Lee, J. W. Malecha, J. M. Miyashiro, R. S. Rogers, D. J. Rogier, S. S. Yu, G. D. Anderson, E. G. Burton, J. N. Cogburn, S. A. Gregory, C. M. Koboldt, W. E. Perkins, K. Seibert, A. W. Veenhuizen, Y. Y. Zhang and P. C. Isakson, *J. Med. Chem.*, **40**, 1347 (1997).

[6a] N. S. Habib and G. C. Tawil, *Sci. Pharm.*, **49**, 42 (1981); [b] G. Daidone, B. Maggio, S. Plescia, D. Raffa, C. Musiu, C. Milia, G. Perra and M. E. Marongiu, *Eur. J. Med. Chem.*, **33**, 375 (1998); [c] T. I. El-Emary and E. A. Bakhite, *Pharmazie*, **54**, 106 (1999).

[7a] R. B. Pathak and S. C. Bahel, *J. Indian Chem. Soc.*, **57**, 1108 (1980); [b] S. Devi, P. Mitro, S. B. Mishra and A. S. Mittra, *J. Indian Chem. Soc.*, **60**, 679 (1983); [c] C. B. Vicentini, G. Forlani, M. Manfrini, C. Romagnoli and D. Mares, *J. Agric. Food Chem.*, **50**, 4839 (2002); [d] D. Mares, C. Romagnoli, B. Tosi, R. Benvegnu, A. Bruni and C. B. Vicentini, *Fungal Genet. Biol.*, **36**, 47 (2002); [e] D. Mares, C. Romagnoli, E. Andreotti, M. Manfrini and C. B. J. Vicentini, *Agric. Food Chem.*, In Press (2004) .

[8a] C. R. Hardy, *Adv. Heterocyclic Chem.*, **36**, 343 (1984); [b] R. N. Misra, D. B. Rawlins, H. Xiao, W. Shan, I. Bursuker, K. A. Kellar, J. G. Mulheron, J. S. Sack, J. S. Tokarski, S. D. Kimball and K. R. Webster, *Bioorg. Med. Chem. Lett.*, **13**, 1133 (2003); [c] A. Straub, J. Benet-Buckholz, R. Fröde, A. Kern, C. Kohlsdorfer, P. Schmitt, T. Schwarz, H. Siefert and J. Stasch, *Bioorg. Med. Chem.*, **10**. 1711 (2002); [d] M. Suzuki, H. Iwasaki, Y. Fujikawa, M. Sakashita, M. Kitahara and M. Sakoda, *Bioorg. Med. Chem. Lett.*, **11**, 1285 (2001); [e] G. Elgemei, Y. Mohamed, N. Tathy and L. Faddah, *Arch. Pharm.*, **45**, 5532 (1991); [f] M. Masui, M. Kawakami, M. Nakajima, S. Hara, H. Ito and M. Ueda, *Drug. Dev. Res.*, **20**, 453 (1990).

[9a] Z. He, G. Milburn, A. Danel, A Puchala, P. Tomasik and D. Rasala, *J. Mater. Chem.*, **7**, 2323 (1997); [b] Brack, A. Dipirazolopyridine. Belg. Patent 616,472. 1961; *Chem. Abstr.* **58**, 73360, (1962); [c] A. B. J. Parusel, R. Schamschule, D. Piorun, K. Rechthaler, A. Puchala, D. Rasala, K. Rotkiewicz and G. Kohler, *J. Mol. Struct. (TEOCHEM)*, **419**, 63 (1997); [d] K. Rechthaler, R. Schamschule, A. B. J. Parusel, K. Rotkiewicz, D. Piorun and G. Kohler, *Acta Physica Polonica A*, **95**, 321-334 (1999); [e] Y. T. Tao, C. H. Chuen, C. W. Ko and J. W. Peng, *Chem. Mater.*, **14**, 4256 (2002); [f] C. H. Chuen and Y. T. Tao, *Appl. Phys. Lett.*, **81**, 4499 (2002); [g] C. W. Ko, Y. T. Tao, *Appl. Phys. Lett.*, **79**, 4234 (2001).

[10a] L. Svetaz, A. Tapia, S. López, R. Furlán, E. Petenatti, R. Pioli, G. Schmeda-Hirschmann and S. Zacchino, *J. Agric. Food Chem.*, **52**, 3297 (2004); [b] L. Vargas, M. V. Castelli, V. Kouznetsov, J. Urbina, S. López, M. Sortino, R. Enriz and J. C. Ribas, Zacchino, S. *Bioorg. Med. Chem.*, **11**, 1531 (2003); [c] B. Insuasty, H. Torres, J. Quiroga, R. Abonía, M. Nogueras, A. Sánchez, M. Sortino, S. Zacchino and J. Low, *Heterocyclic Commun.*, **9,** 153 (2003); [d] B. Insuasty, J. Quiroga, R. Abonía, H. Insuasty, M. Mosquera, S. Cruz, M. Nogueras, M. Sortino and Z. Zacchino, *Heterocyclic Commun.*, **10**, 103 (2004).

[11a] S. Arikan and J. H. Rex, *Exp. Op. Em. Drugs*, **7**, 3 (2002); [b] E. T. Burt, *Exp. Op. Em. Drugs*, **11**, 269 (2001); [c] J. B. Behr, *Curr. Med. Chem-Anti-infective Drugs*, **2**, 173 (2003).

[12a] E. Gonzalez, R. Sarlin and J. Elguero, *Tetrahedron*, **34**, 1175 (1978); [b] L. Hennig, J. Hofmann, A. Astudillo and G. Mann, *J. Prakt. Chem*., **332**, 351 (1990); [c] M. A. Abramov, E. Ceulemans, C. Jackers, M. Van der Auweraer and W. Dehaen, *Tetrahedron*, **57**, 9123 (2001); [d] A. Brack, *Ann. Chem.*, **681**, 105 (1965) and *Justus Liebigs Annalen der Chemie*, **681**, 105 (1965); [e] A. Esteves-Souza, A. Echevarría, I. Vecanto, M. L. Jimeno and J. Elguero, *Tetrahedron*, **57**, 6147 (2001); [f] A. Puchala, D. Rasala, E. Kolehmainen and M. Prokesova, *Organic Preparations and Procedures International*, **29**, 226 (1997).

[132a] A. Bax and S. Subramanian, *J. Magn. Reson.*, **65**, 565 (1986); [b] A. Bax and M. F. Summers, *J. Am. Chem. Soc*., **108**, 2093 (1986).

[14a] J. N. Low, J. Cobo, J. Portilla and J. Quiroga, *Acta Cryst.*, **E59**, 1330 (2003); [b] J. N. Low, J. Cobo, J. Portilla and J. Quiroga, *Acta Cryst.*, **E59**, 1327 (2003); [c] J. Low, J. Cobo, J. Portilla, J. Quiroga and C. Glidewell, *Acta Cryst.*, **E60**, 1034 (2004).

[15a] NCCLS, National Committe for Clinical Laboratory Standards (2002), Method M27-A2, Second Edition,Wayne, Pa Vol. **22** (15), pp 1-29; (b) NCCLS, National Committe for Clinical Laboratory Standards, (2002), Method M-38A, Second Edition,Wayne, Pa Vol. **22** (16), pp 1-27.