Eur J Med Chem (1992) 27, 717–722

Short communication

Research on antibacterial and antifungal agents. VIII. synthesis and antimicrobial activity of 1,4-diarylpyrroles

GC Porretta[1], M Biava[1], R Fioravanti[1], M Fischetti[2], C Melino[2], F Venza[2], P Bolle[3], B Tita[3]

[1]*Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive;*
[2]*Istituto di Igiene, Facoltà di Farmacia;*
[3]*Istituto di Farmacologia, Facoltà di Farmacia, Università degli Studi La Sapienza di Roma, place Aldo Moro 5, 00185 Rome, Italy*

(Received 24 July 1991; accepted 16 April 1992)

Summary — The synthesis and the antimicrobial activity of 1,4-diarylpyrroles are reported. The obtained data in comparison with pyrrolnitrin show that many acid derivatives **4** exhibit a selective activity against some strains of *Candida spp* and poor activity against strains of *Candida albicans*. All ester derivatives **3** are inactive. The results obtained are discussed on the basis of structure-activity relationships.

antifungal activity / 1,4-diarylpyrrole derivatives

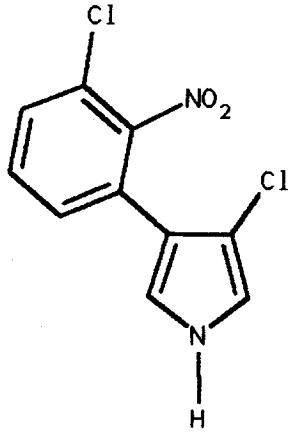
Introduction

In our previous works on natural antibiotic pyrrolnitrin 1 analogues, we have reported the synthesis of many 1,4-diarylpyrrole derivatives and their 1,5 isomers [1–7]. We also reported antimicrobial activity and pointed out some structure-activity relationships [3–5, 7] for 1,5-diarylpyrrole derivatives.

Several recent reports on antifungal activity of 1-substituted-4-phenyl-1H-pyrrole-3-carboxy derivatives [8–10] and on 1-aryl methyl-4-aryl-1H-pyrrole-3-carboxylic acids [11] led us to carry out further studies on 1,4-diarylpyrrole derivatives. We therefore decided to synthesize compounds **3** and **4** to investigate their antibacterial and antifungal activities. Moreover, compounds **4** have been tested as potential anti-inflammatory agents, bearing in mind their structural likeness to pirazolac and the anti-inflammatory activity showed by some pyrrole derivatives [12].

Chemistry

The preparation of compounds **3** was accomplished by condensation between N-(4-substituted phenacyl)arylamines **2** and ethylacetoacetate in dry benzene in the presence of anhydrous zinc chloride and of a catalytic amount of the corresponding arylamine hydrobromide. These experimental conditions are essential, as we have reported [13] to obtain 1,4-diarylpyrroles exclusively. Hydrolysis of esters **3** furnished the corresponding acids **4**.



1

Scheme 1.

Results and discussion

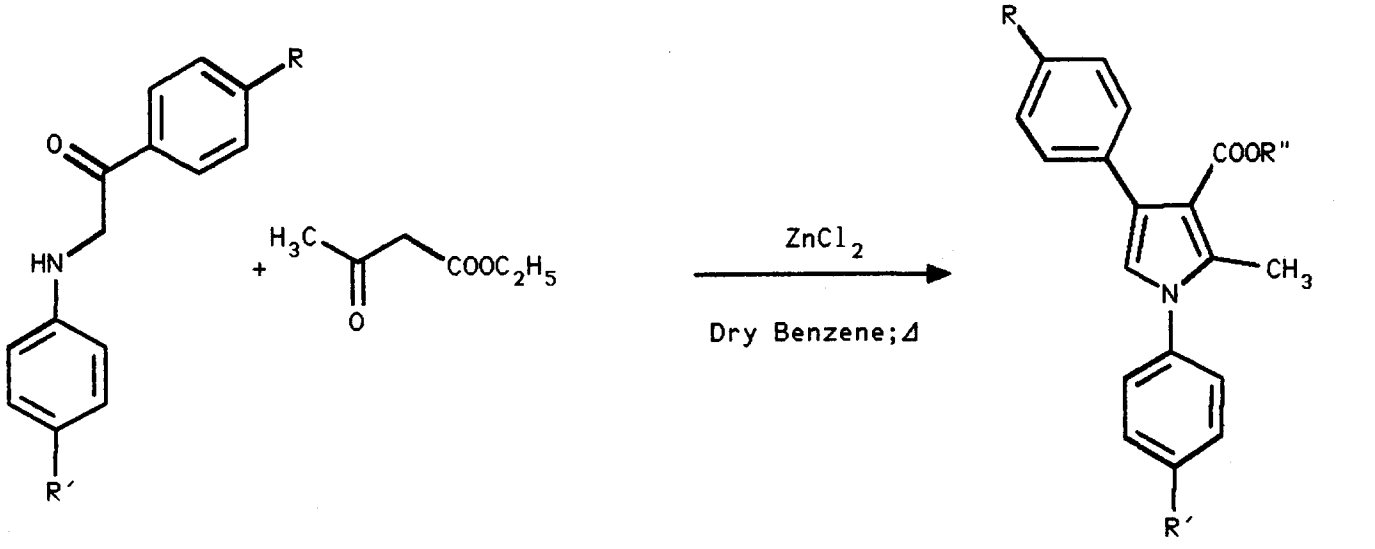
Derivatives **3** and **4** were tested *in vitro* against *Candida albicans* and some strains of *Candida* spp to investigate the antimycotic activity and against Gram-positive and Gram-negative bacteria to investigate the antibacterial activity. All tested compounds showed no antibacterial activity and therefore the biological data have not been reported. Compounds **3** were found practically inactive against *Mycetes* but acid derivatives 4 exhibited an interesting activity against *Candida albicans* and a good selective activity against *Candida* spp (tables IV–V). From the microbiological data the following remarks on structure-activity relationships can be drawn:

a) The antifungal activity is noticeably affected by the presence of the methyl group. In fact, if the methyl group is placed in *para* position of the N_1 phenyl ring, the activity decreases only against *Candida albicans*, but if the methyl group is placed in *para* position of both N_1 and C_4 phenyl rings the activity also decreases against *Candida* spp.

b) The presence of halogen atoms in *para* position of the N_1 phenyl ring increases the activity in spite of the presence of the methyl group in *para* position of the phenyl ring in C_4. Therefore the halogen atoms in *para* position of the N_1 phenyl ring are necessary for the activity as previously reported for parent 1,5-diaryl pyrroles [7].

c) The phenyl ring in N_1 must be directly bound to the pyrrole. In fact the 1-arylmethyl-4-aryl-1H-pyrrole-3-carboxylic acid derivatives, as reported by Massa *et al* [11], were found inactive against the mycetes. On the other hand, for parent 1,5-diarylpyrroles, Scalzo *et al* (submitted for publication) also pointed out that the replacement of the phenyl ring in N_1 with alkylic chains led to inactive compounds.

d) With regard to antibacterial activity, the replacement of the nitro group in the *para* position of the phenyl ring in C_4 and the presence of fluorine or bromine atoms in N_1 phenyl ring do not affect the activity. 1,4-Diarylpyrrole derivatives previously prepared [1, 2] were more active than **4**.



2

R = R′= CH_3 ; Cl ; Br ; F

3 R"= Et .

4 R"= H.

Scheme 2.

Finally, none of the compounds **4** have shown analgesic or anti-inflammatory activity. It probably depends on the fact that our compounds only have some of the chemical moieties indispensable for the activity, that are, as reported in the literature [14, 15], the pyrrole ring with acetic chain in C_3, a methyl group in C_2 and the presence of a lipophilic group (*eg* 4-chlorophenyl).

Experimental protocols

Chemistry

Melting points were taken on a Fisher-Johns apparatus and were not corrected. IR spectra were run (nujol mulls) on a Perkin-Elmer spectrophotometer model 297. NMR spectra are in agreement with proposed structures and they were recorded on a Varian EM 390 spectrometer using (DMSO) as solvent and TMS as an internal standard. Satisfactory analytical data (± 0.4%) were obtained for compounds **4**. Microanalyses were performed by A Pietrogrande, Padova (Italy). Precoated aluminium oxide plates with fluorescent indicator (Carlo Erba) were used from TLC controls. Column chromatography was performed using Merck aluminium oxide (II-III acc to Brockmann) and benzene as solvent (Carlo Erba RPE-ACS). Chemical and physical data of compounds **2–4** are reported in tables I–III.

N-(4-substituted phenacyl)arylamines 2
A solution of suitable aniline (0.16 mol) in ethanol was added by dropping onto a well stirred cooled solution in appropriate phenacyl bromide (0.08 mol) in ethanol. The mixture was stirred in an ice-bath for 4 h (**2a–i, k–p**) and 12 h (**2j**). The solid obtained was filtered, washed with water, dried and crystallized from suitable solvent.

Ethyl 1,4-diaryl-2-methyl-1H-pyrrole-3-carboxylates 3
A mixture of the suitable (**2**) (0.02 mol), ethylacetoacetate (0.04 mol), fused zinc chloride (0.036 mol) and corresponding arylamine hydrobromide (0.001 mol) as catalyst, in 100 ml of dry benzene was refluxed for 20 h. After cooling the solution was poured onto water, acidified to pH = 5 with hydrochloric acid (12 M) and extracted with ethylacetate. The organic layer was separated, dried on anhydrous sodium sulphate and removed under vacuum. The residue was dissolved in benzene and passed through an aluminium oxide column. The eluates were collected after TLC control and the solvent was removed to give (**3a–p**) which was recrystallized from ethanol. Compound **3a** showed characteristic IR bands at 1680 (C = O) cm^{-1}, ^1H NMR (**3a**, DMSO) δ: 7.2–7.5 (unr m, 9H, Ar protons); 6.7 (s, Ha pyrrole); 4.3 (q, 2H, CH_2 -CH_3) J = 6 Hz; 2.5 (s, 3H, CH_3 Ar); 2.4 (s, 3H, CH_3 pyrrole); 1.2 (t, 3H, CH_3 - CH_2-). J = 6 Hz.

1,4-Diaryl-2-methyl-1H-pyrrole-3-carboxylic acids 4
A solution of the suitable (**3**) (0.05 mol) and sodium hydroxide (0.1 mol) in ethanol-water (4:2) was refuxed for 10 h. The reaction was controlled by TLC. After cooling, the solution was reduced and acidified to pH = 3 with hydrochloric acid (12 M). The precipitate stored in a cool place overnight was filtered, washed with water, dried and recrystallized from suitable solvent. Compound **4a** showed characteristic IR bands at 2900 (OH) cm^{-1}, 1650 (C = O) cm^{-1}, ^1H NMR (**4a**, DMSO) δ: 9.7 (s, broad, COOH); 7.3–7.5 (unr, m, 9H, Ar protons); 6.7 (s, Ha pyrrole); 2.5 (s, 3H, CH_3 pyrrole); 2.4 (s, 3H, CH_3 Ar).

Microbiology

Derivatives (**3–4**) were tested *in vitro* against various strains of *Candida albicans* and various strains of different *Candida* spp. Pyrrolnitrin was used as the reference compound. The minimum inhibitory concentration (MIC) was determined using the method of progressive double dilution in solid media [18]. Data were recorded after 36 h of incubation at 37°C. The test

Table I.

Compound	*R*	*R'*	*mp (°C)*	*Yield %*	*Crystn solvent*	*Formula*	*MW*	*Analysis*
2a[a,b]	Cl	CH_3	142–6	70	b	$C_{15}H_{14}ClNO$	259.59	C, H, Cl, N
2b[a,b]	Cl	Cl	155–7	70	a	$C_{14}H_{11}Cl_2NO$	280.04	C, H, Cl, N
2c	Cl	F	127–31	65	b	$C_{14}H_{11}ClFNO$	263.58	C, H, Cl, F, N
2d	Cl	Br	165–8	77	b	$C_{14}H_{11}BrClNO$	324.48	C, H, Br, Cl, N
2e[b]	Br	CH_3	147–51	90	b	$C_{15}H_{14}BrNO$	304.04	C, H, Br, N
2f[b]	Br	Cl	156–60	60	b	$C_{14}H_{11}BrClNO$	324.48	C, H, Br, Cl, N
2g	Br	F	132–5	55	b	$C_{14}H_{11}BrFNO$	308.02	C, H, Br, F, N
2h	Br	Br	158–62	65	b	$C_{14}H_{11}Br_2NO$	368.93	C, H, Br, N
2i	F	CH_3	130–4	65	b	$C_{15}H_{14}FNO$	243.13	C, H, F, N
2j	F	Cl	160–4	58	b	$C_{14}H_{11}ClFNO$	263.58	C, H, Cl, F, N
2k	F	F	120–4	70	b	$C_{14}H_{11}F_2NO$	247.11	C, H, F, N
2l	F	Br	162–6	78	b	$C_{14}H_{11}BrFNO$	308.02	C, H, Br, F, N
2m	CH_3	CH_3	150–2	80	b	$C_{16}H_{17}NO$	239.15	C, H, N
2n[a]	CH_3	Cl	168–70	65	b	$C_{15}H_{14}ClNO$	259.59	C, H, Cl, N
2o	CH_3	F	145–6	80	b	$C_{15}H_{14}FNO$	243.13	C, H, F, N
2p	CH_3	Br	175–7	70	b	$C_{15}H_{14}BrNO$	304.04	C, H, Br, N

[a]lit [17]; [b]lit [18]; a = benzene-cyclohexane (1:1); b = benzene

Table II. a = ethanol.

Compound	*R*	*R'*	*mp (°C)*	*Yield %*	*Crystn solvent*	*Formula*	*MW*	*Analysis*
3a	Cl	CH_3	77–8	65	a	$C_{21}H_{20}ClNO_2$	353.64	C, H, Cl, N
3b	Cl	Cl	108–10	60	a	$C_{20}H_{17}Cl_2NO_2$	374.08	C, H, Cl, N
3c	Cl	F	117–8	50	a	$C_{20}H_{17}ClFNO_2$	357.62	C, H, Cl, F, N
3d	Cl	Br	110–2	60	a	$C_{20}H_{17}BrClNO_2$	418.53	C, H, Br, Cl, N
3e	Br	CH_3	122–4	55	a	$C_{21}H_{20}BrNO_2$	398.09	C, H, Br, N
3f	Br	Cl	112–5	70	a	$C_{20}H_{17}BrClNO_2$	418.53	C, H, Br, Cl, N
3g	Br	F	131–3	60	a	$C_{20}H_{17}BrFNO_2$	402.07	C, H, Br, F, N
3h	Br	Br	114–5	50	a	$C_{20}H_{17}Br_2NO_2$	462.98	C, H, Br, N
3i	F	CH_3	90–3	60	a	$C_{21}H_{20}FNO_2$	337.18	C, H, F, N
3j	F	Cl	88–90	55	a	$C_{20}H_{17}ClFNO_2$	357.62	C, H, Cl, F, N
3k	F	F	74–6	70	a	$C_{20}H_{17}F_2NO_2$	341.16	C, H, F, N
3l	F	Br	91–2	60	a	$C_{20}H_{17}BrFNO_2$	402.07	C, H, Br, F, N
3m	CH_3	CH_3	90–1	50	a	$C_{22}H_{23}NO_2$	333.20	C, H, N
3n	CH_3	Cl	102–3	73	a	$C_{21}H_{20}ClNO_2$	353.64	C, H, Cl, N
3o	CH_3	F	107–8	80	a	$C_{21}H_{20}FNO_2$	337.18	C, H, F, N
3p	CH_3	Br	108–9	70	a	$C_{21}H_{20}BrNO_2$	398.09	C, H, Br, N

Table III. a = benzene-cyclohexane (1:1); b = benzene.

Compound	*R*	*R'*	*mp (°C)*	*Yield %*	*Crystn solvent*	*Formula*	*MW*	*Analysis*
4a	Cl	CH_3	201–5	60	a	$C_{19}H_{16}ClNO_2$	325.62	C, H, Cl, N
4b	Cl	Cl	203–5	70	a	$C_{18}H_{13}Cl_2NO_2$	346.06	C, H, Cl, N
4c	Cl	F	200–1	90	b	$C_{18}H_{13}ClFNO_2$	329.60	C, H, Cl, F, N
4d	Cl	Br	202–4	65	b	$C_{18}H_{13}BrClNO_2$	390.51	C, H, Br, Cl, N
4e	Br	CH_3	205–8	65	a	$C_{19}H_{16}BrNO_2$	370.07	C, H, Br, N
4f	Br	Cl	199–201	70	b	$C_{18}H_{13}BrClNO_2$	390.51	C, H, Br, Cl, N
4g	Br	F	203–5	90	b	$C_{18}H_{13}BrFNO_2$	374.05	C, H, Br, F, N
4h	Br	Br	199–200	60	b	$C_{18}H_{13}Br_2NO_2$	435.09	C, H, Br, N
4i	F	CH_3	189–90	60	b	$C_{19}H_{16}FNO_2$	309.16	C, H, F, N
4j	F	Cl	211–2	80	a	$C_{18}H_{13}ClFNO_2$	329.60	C, H, Cl, F, N
4k	F	F	194–6	66	b	$C_{18}H_{13}F_2NO_2$	313.14	C, H, F, N
4l	F	Br	207–8	78	b	$C_{18}H_{13}BrFNO_2$	374.05	C, H, Br, F, N
4m	CH_3	CH_3	210–3	60	a	$C_{20}H_{19}NO_2$	305.18	C, H, N
4n	CH_3	Cl	200–2	70	a	$C_{19}H_{16}ClNO_2$	325.62	C, H, Cl, N
4o	CH_3	F	170–2	75	a	$C_{19}H_{16}FNO_2$	309.16	C, H, F, N
4p	CH_3	Br	197–200	70	a	$C_{19}H_{16}BrNO_2$	370.07	C, H, Br, N

compounds were dissolved in dimethylsulfoxide (5 mg/ml); further dilution in the test medium furnished the required concentration ranging from 0.2–200 μg/ml. The cultures were obtained on Sabouraud (BBL) for fungi and BHI (BBL) for bacteria after 18 h of incubation at 37°C. Tests were carried out using Sabouraud agar (BBL) and Muller Hinton agar (BBL); inocula were 10^3 for *Candida* and 10^4 for bacteria; $n\bar{X}$ (mean MIC of sensitive strains) and R% (percentage of resistant strains) values were calculated as previously reported [19]. The following species of fungi and bacteria and their different strains isolated from various clinical specimens were tested: 40 *Candida albicans*, 20 *Candida* spp (2 *C wiswanathii*, 1 *C tropicalis*, 2 *C claussenii*, 4 *C guilliermondii*, 1 *C krusei*, 1 *C pseudotropicalis*, 1 *C lipolytica*, 2 *C macedoniensis*, 4 *C parapsilosis*, 1 *C melinii*, 1 *C utilis*), 12 *Klebsiella pneumoniae*, 9 *Escherichia coli*, 22 *Salmonella* sp (6 *S typhi*, 3 *S enteritidis*, 3 *S paratyphi*, 2 *S paratyphi B*, 2 *S infantis*, 3 *S typhimurium*, 1 *S anatum*, 2 *S wien*), 5 *Enterobacter aeruginosa*, 6 *Pseudomonas aeruginosa*, 11 *Staphylococcus aureus*.

Table V. MIC values (μg/ml) of pyrrolnitrin and compounds **4a–p** against 20 strains of *Candida* spp at pH = 7.2.

Compound / *Microorganisms*	*Pyrrol*	*4a*	*4b*	*4c*	*4d*	*4e*	*4f*	*4g*	*4h*	*4i*	*4j*	*4k*	*4l*	*4m*	*4n*	*4o*	*4p*
C wiswanathii S_1	> 200	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	100	25	> 200	25
C wiswanathii S_2	25	> 200	6.25	12.5	25	6.25	6.25	> 200	25	25	6.25	25	12.5	100	25	25	25
C tropicalis cdc	25	12.5	6.25	12.5	25	> 200	6.25	25	> 200	25	6.25	25	12.5	100	25	25	25
C claussenii S_2	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	100	25	25	25
C claussenii S_{15}	25	12.5	6.25	12.5	> 200	> 200	> 200	25	> 200	25	6.25	25	> 200	> 200	25	> 200	25
C guilliermondii S_1	25	12.5	6.25	12.5	25	6.25	6.25	25	> 200	25	6.25	25	12.5	> 200	25	25	25
C guilliermondii S_2	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	> 200	25	25	25
C guilliermondii S_3	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	> 200	25	25	25
C guilliermondii 238	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	> 200	25	12.5	> 200	25	25	25
C krusei 234	25	> 200	6.25	> 200	25	6.25	6.25	25	> 200	25	> 200	> 200	12.5	> 200	25	25	25
C pseudotropicalis	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	> 200	25	25	25
C lipolytica	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	> 200	25	25	25
C macedoniensis 1	25	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	6.25	> 200	12.5	> 200	> 200	> 200	> 200
C macedoniensis 2	25	> 200	> 200	> 200	> 200	6.25	> 200	> 200	> 200	> 200	6.25	> 200	> 200	> 200	> 200	> 200	> 200
C melinii	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	100	25	25	25
C parapsilosis cdc	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	> 200	25	25	25
C parapsilosis 25	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	> 200	25	25	25
C parapsilosis 225	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	> 200	25	25	25
C parapsilosis 747	25	12.5	6.25	12.5	25	6.25	6.25	25	25	25	6.25	25	12.5	> 200	25	25	25
C utilis	25	12.5	6.25	12.5	25	> 200	6.25	25	> 200	25	6.25	25	12.5	> 200	25	25	25

Table IV. Antifungal activity of pyrrolnitrin and compounds **4a–p** against 40 strains of *Candida albicans* at pH = 7.2.

Compound	*Candida albicans*		
	R%	*n*	*range (μg/ml)*
Pyrrolnitrin	5	47.92	25–200
4a	2.5	52.92	6.25– > 200
4b	2.5	24.90	1.56–200
4c	10	83.33	12.25– > 200
4d	2.5	24.85	6.25–200
4e	5	47.92	25– > 200
4f	2.5	17.32	1.56–200
4g	2.5	78.57	25– > 200
4h	2.5	12.29	1.56–200
4i	2.5	11.91	3.12– > 200
4j	2.5	26.04	6.25–200
4k	2.5	41.67	25– > 200
4l	7.5	16.77	3.12–200
4m	95	93.79	25– > 200
4n	65	36.50	3.12–200
4o	80	73.33	12.5– > 200
4p	85.6	158.33	100– > 200

Pharmacology

Male Long Evans rats, weighing 180–220 g were used. The anti-inflammatory and analgesic activities were studied as reported by Winter [20] and Woolfe [21].

Acknowledgments

This research was supported by a grant from Istituto Pasteur-Fondazione Cenci Bolognetti, Università degli Studi La Sapienza di Roma.

References

1 Filacchioni G, Porretta GC, Scalzo M, Giardina MC, Giardi T (1983) *Il Farmaco Ed Sc* 38, 630–639
2 Porretta GC, Chimenti F, Biava M, Bolasco A, Scalzo M, Panico S, Simonetti N, Villa A (1985) *Il Farmaco Ed Sc* 40, 589–607
3 Scalzo M, Biava M, Cerreto F, Porretta GC, Panico S, Simonetti N (1988) *Eur J Med Chem* 23, 587–591
4 Scalzo M, Porretta GC, Chimenti F, Casanova MC, Panico S, Simonetti N (1988) *Il Farmaco Ed Sc* 43, 665–676
5 Scalzo M, Porretta GC, Chimenti F, Bolasco A, Casanova MC, Simonetti N, Villa A (1988) *Il Farmaco Ed Sc* 43, 677–691
6 Porretta GC, Cerreto F, Fioravanti R, Biava M, Scalzo M, Fischetti M, Riccardi F (1989) *Il Farmaco Ed Sc* 44, 51–63
7 Porretta GC, Cerreto F, Fioravanti R, Biava M, Scalzo M, Simonetti N, D'Auria FD (1989) *Il Farmaco Ed Sc* 44, 65–76
8 *Eur Pat Appl* 182,783 (1986) CA 105, 114899s

9 *Eur Pat Appl EP* 174,910 (1986) CA 105, 114897q
10 *Eur Pat Appl* 182,737 (1986) CA 105, 114898r; *Eur Pat Appl* 111,452 (1984) CA 101, 124892r
11 Massa S, Di Santo R, Mai A, Botta M, Artico M, Panico S, Simonetti G (1990) *Il Farmaco* 45, 833–846
12 Lombardino JG (1985) *In: Non Steroidal Anti-inflammatory Drugs John Wiley and Sons Inc New York* 275–279 and 292–294
13 Filacchioni G, Cecchi L, Porretta GC, Scalzo M, Giardi T (1983) *Il Farmaco Ed Sc* 38, 619–629
14 Ross JR, Sowell JW (1987) *J Heterocyclic Chem* 24, 757–765
15 Gillet C, Dehoux E, Kestens J, Roba J, Lambelin G (1976) *Eur J Med Chem* 2, 173–181
16 Schmidt RR, Dimmler M, Hemmerich P (1976) *Chem Ber* 109, 2395–2404
17 Sharma TC, Lal A (1976) *Indian J Chem Sect B* 14B (10) 815–816, (1977) CA 86, 171021n
18 Shadomy S, Espinel A (1980) Susceptibility testing with antifungal drugs in *Manual of Clinical Microbiology 3rd edn Am Soc Microbiol Washington DC* 647
19 Porretta GC, Biava M, Cerreto F, Scalzo M, Panico S, Simonetti N, Villa A (1988) *Eur J Med Chem* 23, 311–317
20 Winter CA, Risley EA, Nuss GW (1962) *Proc Soc Exp Biol Med* 111, 544–547
21 Woolfe G, MacDonald AD (1944) *J Pharmacol Exp Ther* 80, 300–307