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New products

Synthesis and microbiological activity of new 1,5-diarylpyrroles

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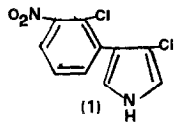
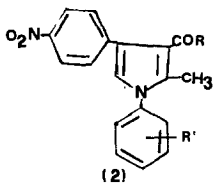
1,5-diarylpyrroles / anti-microbiological activity

Introduction

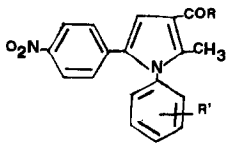
In our previous papers [1—4], we reported the synthesis of analogues of anti-fungal antibiotic pyrrolnitrin **1** [5—9] and we also suggested a general method to prepare 1,4- or 1,5-diarylpyrroles starting from ethylacetoacetate and *N*-(4-nitrophenacyl)arylamines. Finally, we have described [4] the synthesis and anti-microbial activity of **2** and **3**.



(1)



(2)

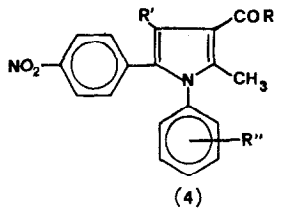


(3)

a) R=OH R'=4-Cl
b) R=OH R'=2,4-Cl
c) R=morpholyl R'=4-Cl
d) R=morpholyl R'=2,4-Cl
e) R=pyrrolidyl R'=4-Cl
f) R=pyrrolidyl R'=2,4-Cl
g) R=piperidyl R'=4-Cl
h) R=piperidyl R'=2,4-Cl
i) R=methylpiperazinyl R'=4-Cl
j) R=methylpiperazinyl R'=2,4-Cl

The microbiological data showed that **2** exhibited only a moderate anti-mycotic activity but **3**, on the contrary, showed unexpected anti-bacterial and anti-mycotic activities.

In particular, **3i** showed a very interesting microbiological activity. These results and the small amount of published data on the anti-bacterial and anti-mycotic activities shown by some natural pyrrole derivatives suggested that we should undertake research on 1,5-diarylpyrroles to improve their microbiological activity. Then we decided to study many derivatives of general formula **4** with the following structural modifications: 1) the introduction in the *N*-phenyl ring of various substituents which could enhance anti-microbial activity, *e.g.*, CF_3 group, bromine or fluorine atoms; 2) amide groups from *N*-methylpiperazine again, long chain alkyl amines and arylalkyl amines to study if the lipophilic character of the compounds influences markedly the anti-microbial activity as reported by Hansch [10].

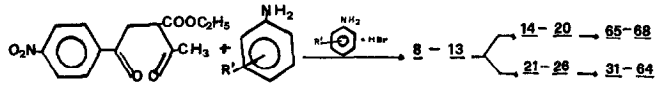


(4)

Chemistry

Ethyl 1-aryl-2-methyl-5-(4-nitrophenyl)pyrrole-3-carboxylates **8**—**13** (Scheme 1), prepared as in [1], were used as starting material. Compounds **8**—**13** were transformed by sulfuryl chloride [1] into corresponding chloroderivative **14**—**20**. Hydrolysis of esters **8**—**20** [1] furnished the corresponding acids **21**—**30**. Reaction of corresponding acid chlorides with the appropriate amines [4] gave the amide **31**—**68** in quite good yield.

The structures of derivatives **8**—**68** were supported by elemental analyses and spectral data. The IR and NMR spectra are in agreement with the proposed structures.



Scheme 1.

Microbiological assays

Derivatives **21**—**68** were tested for their *in vitro* anti-fungal activities against *Candida albicans* and various strains of *Candida sp.* Derivatives **21**—**30** were also tested for the

in vitro anti-bacterial activities against Gram-positive and Gram-negative bacteria. Pyrrolnitrin was used as positive control. The minimum inhibitory concentration *(MIC)* was determined using the method of progressive double dilutions in solid media [11]. Data were recorded after 36 h (fungi) or 18 h (bacteria) of incubation at 37°C.

For the experiments, the substances were dissolved in dimethyl sulfoxide (DMSO) (5 mg/ml); further dilution in the test medium furnished the required concentration, generally in the range of 0.1—200 μg/ml.

The cultures were obtained on sabouraud (B.B.L.) for fungi and on BHI (B.B.L.) for bacteria after 18 h of incubation at 37°C. Tests were carried out using sabouraud agar (B.B.L.) and Muller—Hinton agar (B.B.L.). Inocula

Table I. Anti-mycotic activity of pyrrolnitrin and compounds **21—68** against 30 strains of *Candida albicans* and 18 strains of *Candida* sp. at pH = 7.2.

Compound	Microorganism					
	Candida albicans			*Candida* sp.		
	R %	$n\overline{X}$ (μg/ml)	range (μg/ml)	*R* %	$n\overline{X}$ (μg/ml)	range (μg/ml)
Pyrrolnitrin		47.04	0.4—100		60.2	0.2—100
21	90	116.66	50—>200	100		>200
22	66	95.45	25—>200	67	150	100—>200
23	74	68.75	25—>200	83	225	25—>200
24	93	37.5	25—>200	100		>200
25	93	75	50—>200	100		>200
26	77	98.21	12.5—>200	100		>200
27	90	150	50—>200	100		>200
28	63	123.86	12.5—>200	50	200	200—>200
29	80	120.85	25—>200	100		>200
30	84	137.5	50—>200	100		>200
31		84.37	25—200		137.5	25—200
32	90	200	200—>200	100		>200
33	63	185.71	100—>200	60	100	100—>200
34	78	200	200—>200	100		>200
35	87	72.9	6.25—>200	100		>200
36		48.97	3.12—100		80.31	1.56—100
37	100		>200	100		>200
38	100		>200	100		>200
39	74	83.33	25—>200	100		>200
40	17	157.33	50—>200	83	200	200—>200
41		114.84	3.12—200		150.52	3.12—200
42	87	68.74	3.12—>200	100		>200
43	84	104.16	12.5—>200	100		>200
44	70	100	100—>200	100		>200
45	100		>200	100		>200
46		101.08	25—100		116.6	50—200
47	57	141.25	12.5—>200	100		>200
48	95	100	100—>200	100		>200
49	65	200	200—>200	83	200	200—>200
50	17	200	200—>200	83	200	200—>200
51	22	118.40	6.25—>200	66	200	200—>200
52	91	100	100—>200	100		>200
53	84	200	200—>200	100		>200
54	87	50	50—>200	100		>200
55	87	133.3	100—>200	100		>200
56	4	64.28	25—200	100	162.5	25—200
57	30	162.5	100—>200	83	200	200—>200
58	91	200	200—>200	100		>200
59	84	67.84	0.4—>200	100		>200
60	90	150	100—>200	100		>200
61	13	154.16	12.5—>200	83	200	200—>200
62	74	140.08	0.4—>200	100		200—>200
63	100		>200	100		>200
64	87	133.33	100—>200	100		>200
65		150	25—200	20	200	200—>200
66	5	175	25—>200	20	56.25	25—>200
67	14	170	50—>200	100		>200
68	24	45.62	3.12—>200	100		>200

were 10^4 for bacteria and 10^3 for *Candida*. Media *MIC* values ($n\bar{X}$) and $R\%$ were calculated as previously reported [12].

The following species of fungi and bacteria isolated from various clinical specimens were tested: 30 *Candida albicans*, 2 *Candida parapsilosis*, 2 *Candida glabra*, 2 *Candida pseudotropicalis*, 1 *Candida krusei*, 5 *Candida guillermondii*, 1 *Candida macedoniensis*, 1 *Candida melinii*, 2 *Candida langeronii*, 2 *Candida wiswanathii*, 3 *Enterobacter sp.*, 13 *Staphylococcus sp.*, 6 *E. coli*, 2 *Salmonella typhi*, 2 *Salmonella infantis*, 1 *Salmonella paratyphi A*, 1 *Salmonella paratyphi B*, 1 *Salmonella enteritis*, 1 *Serratia sp.*, 3 *Providencia*, 3 *Proteus mirabilis*, 1 *Corynebacterium sp.*, 9 *Klebsiella*.

Results and Discussion

Anti-mycotic activity

The results of anti-fungal screening are presented in Table I. Compounds **51**, **68** and **67** exhibited moderate activity against *Candida albicans* and **66** showed an interesting activity against the same strains. A good activity only against *Candida albicans* was found for **65**, while **56** showed a moderate activity against *Candida albicans* but a good activity against *Candida sp.* The most active derivatives against *Candida albicans* and *Candida sp.* were **31**, **36**, **41** and **46**. The remaining treated compounds showed a poor anti-fungal activity.

Table II.

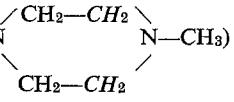
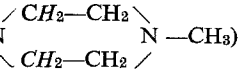
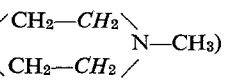
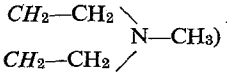
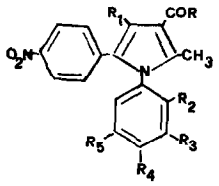
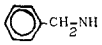
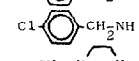
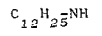
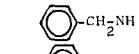
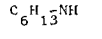
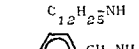
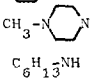
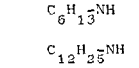
Compounds	NMR δ ppm	IR cm^{-1}
31, 36, 41, 46, 51, 56	2.13—2.23 (s, 3H, N—*CH*$_3$) 2.36—2.43 (s, 3H, *CH*$_3$ pyrrole) 2.50—2.60 (m, 4H,  3.78—3.88 (m, 4H,  6.63—6.70 (s, 1H, pyrrole) 7.10—8.30 (m, Ar protons)	3300—3320 (NH); 1600—1620 (C=O)
32, 37, 42, 47, 52, 57, 61	0.80—0.90 (m, 3H, $(CH_2)_4$—*CH*$_3$) 1.33—1.40 (m, 8H, CH_2—(*CH*$_2$)$_4$—) 2.40—2.46 (s, 3H, *CH*$_3$ pyrrole) 3.46—3.60 (m, 2H, NH—*CH*$_2$—) 6.01—6.13 (t, J = 5 cps, 1H, *NH*—CH_2—) 6.80—7.04 (s, 1H, pyrrole) 7.10—8.30 (m, Ar protons)	3300—3320 (NH); 1600—1620 (C=O)
33, 38, 43, 48, 53, 58, 62	0.80—0.90 (m, 3H, $(CH_2)_{10}$—*CH*$_3$) 1.25—1.30 (m, 20H, CH_2—(*CH*$_2$)$_{10}$—) 2.40—2.46 (s, 3H, *CH*$_3$ pyrrole) 3.40—3.46 (m, 2H, NH—*CH*$_2$—) 5.95—6.00 (t, J = 5 cps, 1H, *NH*—CH_2—) 6.80—7.04 (s, 1H, pyrrole) 7.10—8.30 (m, Ar protons)	3300—3320 (NH); 1600—1620 (C=O)
34, 35, 39, 40, 44, 45, 49, 50, 54, 55, 59, 60, 63, 64	2.33—2.46 (s, 3H, *CH*$_3$ pyrrole) 4.50—4.66 (d, J = 6 cps, 2H, NH—*CH*$_2$—) 6.33—6.76 (t, J = 5 cps, 1H, *NH*—CH_2—) 6.67—6.80 (s, 1H, pyrrole) 7.10—8.30 (m, Ar protons)	3300—3320 (NH); 1600—1620 (C=O)
65, 68	2.13—2.23 (s, 3H, N—*CH*$_3$) 2.36—2.43 (s, 3H, *CH*$_3$ pyrrole) 2.50—2.60 (m, 4H,  3.78—3.88 (m, 4H,  7.10—8.30 (m, Ar protons)	3300—3320 (NH); 1600—1620 (C=O)

Table III.



Compound	R	R_1	R_2	R_3	R_4	R_5	Yield %	M.p. °C
8	OEt	H	H	H	F	H	60	147-148 (a)
9	OEt	H	H	H	Br	H	70	186-188 (a)
10	OEt	H	H	CF_3	H	H	75	123-125 (a)
11	OEt	H	Cl	H	H	CF_3	80	122-124 (a)
12	OEt	H	H	Cl	H	Cl	78	138-140 (a)
13	OEt	H	H	Cl	Cl	H	82	161-163 (a)
14	OEt	Cl	H	H	F	H	45	127-130 (a)
15	OEt	Cl	H	H	Br	H	48	181-184 (a)
16	OEt	Cl	H	CF_3	H	H	47	54-55 (a)
17	OEt	Cl	Cl	H	H	CF_3	40	86-88 (a)
18	OEt	Cl	H	Cl	H	Cl	42	74-75 (a)
19	OEt	Cl	H	Cl	Cl	H	50	63-65 (a)
20	OEt	Cl	Cl	H	Cl	H	51	154-155 (a)
21	OH	H	H	H	F	H	51	>330 (b)
22	OH	H	H	H	Br	H	53	283-285 (b)
23	OH	H	H	CF_3	H	H	49	229-231 (a)
24	OH	H	Cl	H	H	CF_3	50	240-241 (b)
25	OH	H	H	Cl	H	Cl	55	302-304 (a)
26	OH	H	H	Cl	Cl	H	60	270-273 (a)
27	OH	Cl	H	H	F	H	47	253-254 (a)
28	OH	Cl	H	H	Br	H	51	288-290 (a)
29	OH	Cl	H	Cl	H	Cl	45	225-227 (a)
30	OH	Cl	Cl	H	Cl	H	48	242-244 (a)
31		H	H	H	F	H	50	154-155 (c)
32	$C_6H_{13}NH$	H	H	H	F	H	70	176-177 (c)
33	$C_{12}H_{25}NH$	H	H	H	F	H	40	159-160 (c)
34		H	H	H	F	H	75	209-210 (c)
35		H	H	H	F	H	45	206-207 (c)
36		H	H	H	Br	H	51	195-196 (c)
37	$C_6H_{13}NH$	H	H	H	Br	H	70	196-197 (c)
38	$C_{12}H_{25}NH$	H	H	H	Br	H	40	152-153 (c)
39		H	H	H	Br	H	75	199-200 (c)
40		H	H	H	Br	H	48	225-226 (c)
41		H	H	CF_3	H	H	50	184-185 (c)
42	$C_6H_{13}NH$	H	H	CF_3	H	H	75	151-152 (c)
43	$C_{12}H_{25}NH$	H	H	CF_3	H	H	40	119-120 (c)
44		H	H	CF_3	H	H	60	189-190 (c)
45		H	H	CF_3	H	H	45	205-207 (c)
46		H	Cl	H	H	CF_3	51	215-216 (c)
47	$C_6H_{13}NH$	H	Cl	H	H	CF_3	78	118-119 (c)
48	$C_{12}H_{25}NH$	H	Cl	H	H	CF_3	45	159-160 (c)
49		H	Cl	H	H	CF_3	40	206-207 (c)
50		H	Cl	H	H	CF_3	35	244-245 (c)
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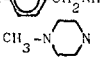
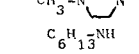
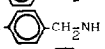
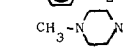
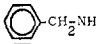
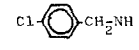
52	$C_6H_{13}NH$	H	H	Cl	H	Cl	80	179-180 (c)
53	$C_{12}H_{25}NH$	H	H	Cl	H	Cl	40	136-137 (c)
54		H	H	Cl	H	Cl	80	193-194 (c)
55		H	H	Cl	H	Cl	47	250-251 (c)
56		H	H	Cl	Cl	H	50	203-204 (c)
57	$C_6H_{13}NH$	H	H	Cl	Cl	H	75	153-154 (c)
58	$C_{12}H_{25}NH$	H	H	Cl	Cl	H	45	132-133 (c)
59		H	H	Cl	Cl	H	82	221-222 (c)
60		H	H	Cl	Cl	H	45	234-235 (c)
61	$C_6H_{13}NH$	H	Cl	H	Cl	H	60	166-167 (c)
62	$C_{12}H_{25}NH$	H	Cl	H	Cl	H	85	102-103 (c)
63		H	Cl	H	Cl	H	48	111-112 (c)
64		H	Cl	H	Cl	H	40	114-115 (c)
65		Cl	H	H	F	H	80	98-99 (c)
66		Cl	H	H	Br	H	85	184-185 (c)
67		Cl	H	Cl	H	Cl	70	215-216 (c)
68		Cl	Cl	H	Cl	H	75	79-80 (c)

Crystallization solvent: (a) = EtOH; (b) = EtOH—H_2O; (c) = benzene.

Anti-bacterial activity

The anti-bacterial screening was carried out only for compounds **21**—**30** in agreement with our previous observations [4], which showed that amide derivatives did not possess anti-bacterial activity. Only compounds **24**, **25**, **28**, **29** and **30** showed a weak activity against strains of *Staphylococcus sp.* while all tested compounds were inactive against strains of Gram-negative and Gram-positive bacteria.

From data reported in Table I and observations of activity against *Staphylococcus sp.*, we can derive the following structure—activity relationships: 1) the introduction in the *N*-phenyl ring of a CF_3 group, fluorine or bromine atoms, or a second chlorine atom does not markedly affect the anti-bacterial and anti-fungal activities; 2) the amide derivatives are more active than corresponding acid compounds but only the *N*-methylpiperazinyl substituents must be regarded as fundamental to anti-fungal activity. This is in agreement with our previous observation [4] and probably depends upon the more evident basic character that the structure undertakes when the *N*-methylpiperazinyl substituent is present. Indeed

molecular lipophilicity is strongly enhanced in exyl and dodecyl amide derivatives.

Experimental protocols

Melting points, uncorrected, were taken on a Fisher—Johns apparatus. Infrared spectra (nujol mulls) were run on a Perkin—Elmer spectrophotometer mod. 297. The NMR spectra were recorded on a Varian EM 390 (90 MHz) spectrometer using deurerochloroform as the solvent and tetramethylsilane as the internal standard. Compounds **31—68** were analyzed for C, H, N and, where present, Cl, Br and F. The analyzed values were within $\pm 0.4\%$ of the calculated amounts. Elemental analyses were performed by A. Pietrogrande, Padova, Italy. Merck aluminium oxide (II—III acc. to Brockmann) and Merck sliica gel (0.05—0.2 mm, 70—352 mesh ASTM) were used for chromatographic purifications. Chemical and physical data of compounds **8—68** are reported in Tables II and III.

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