Researches on Antibacterial and Antifungal Agents, X[1)]

Synthesis and Antifungal Activities of 1-{p-Methyl-α-[4-(1H-pyrrol-1-yl)phenyl]benzyl}azoles and Some Related Products

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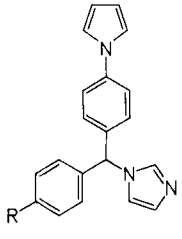
The synthesis and antifungal activities against *Candida albicans* and *Candida* spp. of some pyrrole analogues of bifonazole are reported. 1-{p-Methyl-α-[4-(1H-pyrrol-1-yl)phenyl]benzyl}imidazole was found to be equipotent or sometimes superior to bifonazole and ketoconazole, and lightly inferior to miconazole. Substitution of the imidazole moiety with other azoles retained some activities. No activity was shown when the azole aromatic rings were replaced by the heteroalicyclic ones.

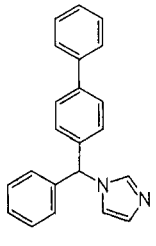
Über antibakterielle und antimykotische Wirkstoffe, 10. Mitt.:

Synthese und antimykotische Eigenschaften von 1-{p-Methyl-α-[4-(1H-pyrrol-1-yl)phenyl]benzyl}azole und einiger verwandter Verbindungen

Synthese und antimykotische Eigenschaften einiger Pyrrol-Analoge des Bifonazols gegen *Candida albicans* und *Candida* spp. werden beschrieben. 1-{p-Methyl-α-[4-(1H-pyrrol-1-yl)phenyl]benzyl}imidazol ist gleichermaßen oder stärker wirksam als Bifonazol oder Ketoconazol und etwas schwächer wirksam als Miconazol. Bei Substitution des Imidazol-Systems durch andere Azole blieb die Aktivität teilweise erhalten. Wurde dagegen der aromatische Azolring durch heteroalicyclische Gruppen ersetzt, so war keine Aktivität mehr nachzuweisen.

In a previous work[1)] we reported the synthesis and the antifungal activities against *Candida albicans* and *Candida* spp. of 1-{α-[4-(1H-pyrrol-1-yl)phenyl]benzyl}imidazole (**1**), a pyrrole isoster of the powerful antifungal chemotherapeutic agent bifonazole (**2**).



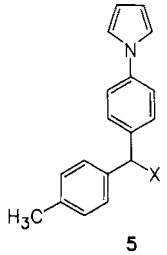


1 R = H
3 R = Cl
4 R = CH_3

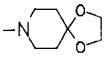
2

Derivative **1** was found to be equipotent to bifonazole as regard to antifungal profile, thus proving that substitution of phenyl by the pyrrole ring did not affect the antimicrobial activity spectrum. Surprisingly, antifungal activities were decreasing when an electron withdrawing chlorine atom was introduced into **1** to give the chloro analogue **3**. This suggested that replacement of chlorine with an electron donating group like methyl would led to more active compounds.

We therefore, synthesized and tested as antifungal agents the methyl derivative **4** and the related derivatives **5a-f** obtained by bonding azoles and heteroalicyclic rings to the p-methyl-α-[4-(1H-pyrrol-1-yl)phenyl]benzyl moiety.



5

5	X	5	X
a		e	
b		f	
c		g	–OH
d		h	–Br

The amides **10** and **11** were also prepared in an attempt to establish some structure-activity relationships.

Chemistry

$NaBH_4$-reduction of 4-nitro-4'-methylbenzophenone[2)] afforded the related carbinol **6**[3)], which was treated with PBr_3 to give the unstable bromo derivative **7**. Reaction of crude **7** with imidazole let to 1-[p-methyl-α-(4-nitrophenyl)benzyl]imidazole (**8**), which was then hydrogenated to the aminoderivative **9** (H_2, Pd/C).

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Tab. 1: Antimycotic Activities of Imidazole Derivatives

Tested substance	Fungi (n° of tested strains)					
	Candida albicans (37)			*Candida* spp. (10)		
	R (%)	$n\bar{X}$	Range	R(%)	$n\bar{X}$	Range
Miconazole	0	4.59	<0.2-12.5	0	4.22	<0.2-25
Ketoconazole	0	10.31	<0.2-25	0	4.18	<0.2-25
Bifonazole, **2**	0	35.89	1.56-100	0	45.78	<0.2-100
1	0	7.89	1.56-50	0	11.51	<0.2-50
3	5	15.44	1.56->200	30	36.05	<0.2->200
4	0	5.38	0.8-12.5	0	18.76	0.2-50
8	0	26.18	12.5-200	20	74.85	<0.4-200
9	86	200.00	200->200	70	153.12	12.5->200
10	67	45.83	12.5->200	90	50.00	50->200
11	92	6.25	6.25->200	90	50.00	50->200

Tab. 2: Antimycotic Activities of Other than Imidazole Derivatives

Tested substance	Fungi (n° of tested strains)					
	Candida albicans (19)			*Candida* spp. (15)		
	R(%)	$n\bar{X}$	Range	R(%)	$n\bar{X}$	Range
Bifonazole, **2**	0	4.31	0.2-25	0	18.36	0.4-50
5a	0	42.74	0.2->200	100		>200
5b	100		>200	100		>200
5c	0	10.12	0.2-50	0	32.91	3.12-200
5d	100		>200	100		>200
5e	100		>200	100		>200
5f	63	111.60	6.25->200	100		>200

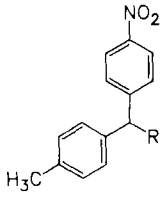
Tab. 3: Preparative and Analytical Data

Nr.	Yield (%)	M.p.(°C) (Solvent)	Formula (Mol.Weight)	Analysis(%): Found Calcd.		
				C	H	N
4	23[a], 48[b]	thick oil[c]	$C_{21}H_{19}N_3$	79.8	6.06	13.0
			(313.4)	80.5	6.11	13.4
5a	39	96-99	$C_{20}H_{18}N_4$	76.1	5.77	17.6
		(Et_2O)	(314.4)	76.4	5.77	17.8
5b	38	132-136	$C_{19}H_{17}N_5$	72.6	5.70	21.9
		(C_6H_6/C_6H_{12})	(315.4)	72.4	5.43	22.2
5c	29	thick oil[d]	$C_{19}H_{17}N_5$	72.7	5.75	22.0
			(315.4)	72.4	5.43	22.2
5d	48	thick oil[d]	$C_{22}H_{24}N_2$	83.4	7.66	8.4
			(316.4)	83.5	7.64	8.8
5e	72	thick oil[d]	$C_{22}H_{24}N_2S$[e]	75.5	7.12	8.0
			(348.5)	75.8	6.94	8.0
5f	49	thick oil[d]	$C_{25}H_{28}N_2O_2$	77.0	7.47	7.0
			(388.5)	77.3	7.27	7.2
5g	97	93-96	$C_{18}H_{17}NO$	82.1	6.56	5.3
		(ligroin)	(263.3)	82.1	6.51	5.3
8	70[f]	thick oil[d]	$C_{17}H_{15}N_3O_2$	69.9	5.01	14.0
			(293.3)	69.6	5.15	14.3
9	73	133-135	$C_{17}H_{17}N_3$	77.4	6.59	16.2
		(C_6H_6/C_6H_{12})	(263.3)	77.5	6.51	16.0
10	60	glassy material[g]	$C_{24}H_{20}Cl_3N_3O$	61.2	4.49	8.7
			(472.8)	61.0	4.26	8.9
11	72	glassy material[h]	$C_{24}H_{21}ClFN_3O$	68.0	5.20	9.8
			(421.9)	68.3	5.01	10.0
12	77	171-175	$C_{18}H_{15}NO$	83.0	5.70	5.1
		(C_6H_6/petr.ether)	(261.3)	82.7	5.79	5.4

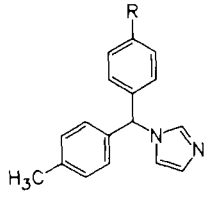
a) From **9**; b) From **5g**; c) Purity of **4** was confirmed by RP-HPLC; d) Homogeneous by TLC; e) Sulfur(%): found 8.9, calcd. 9.2; f) From **6**; g) Analyzed as crude hydrochloride. Chlorine(%): found 22.7, calcd. 22.5; h) Analyzed as crude hydrochloride. Chlorine(%): found 8.4, calcd. 8.4; fluorine(%): found 4.4, calcd. 4.5.

Tab. 4: ^{1}H-NMR Data

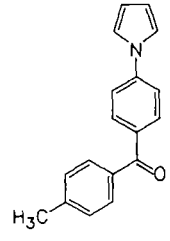
Nr.	δ (ppm)
4	2.32 (s, 3H, CH_3), 6.26 (m, 2H, pyrrole-β H), 6.45 (s, 1H, CH), 6.80 (m, 1H, imidazole), 6.9-7.5 (m, 12H, other aromatic H)
5a	2.32 (s, 3H, CH_3), 6.33 (m, 2H, pyrrole-β H), 6.77 (s, 1H, CH), 7.0-7.5 (m, 10H, benzene and pyrrole-α H), 8.00 (s, 1H, triazole-H-3), 8.07 (s, 1H, triazole-H-5)
5b	2.32 (s, 3H, CH_3), 6.33 (m, 2H, pyrrole-β H), 7.07 (m, 2H, pyrrole-α H), 7.1-7.5 (m, 9H, CH and benzene H), 8.58 (s, 1H, tetrazole)[5]
5c	2.32 (s, 3H, CH_3), 6.35 (m, 2H, pyrrole-β H), 7.0-7.6 (m, 11H, CH, pyrrole-α and benzene H), 8.50 (s, 1H, tetrazole)[5]
5d	1.63 (m, 4H, pyrrolidine), 2.13 (s, 3H, CH_3), 2.30 (m, 4H, pyrrolidine), 4.35 (s, 1H, CH), 6.18 (m, 2H, pyrrole-β H), 6.8-7.6 (m, 10H, pyrrole-α and benzene H)
5e	2.30 (s, 3H, CH_3), 2.63 (s, 8H, thiomorpholine), 4.40 (s, 1H, CH), 6.30 (m, 2H, pyrrole-β H), 7.0-7.6 (m, 10H, pyrrole-α and benzene H)
5f	1.60 (m, 4H, piperidine), 2.23 (s, 3H, CH_3), 2.46 (m, 4H, piperidine), 3.80 (s, 4H, dioxolane), 4.23 (s, 1H, CH), 6.16 (m, 2H, pyrrole-β H), 6.85-7.50 (m, 10H, pyrrole-α and benzene H)
5g	2.23 (s, 3H, CH_3), 2.60 (s, 1H, OH), 5.70 (s, 1H, CH), 6.33 (m, 2H, pyrrole-β H), 7.0-7.5 (m, 10H, pyrrole-α and benzene H)
8	2.33 (s, 3H, CH_3), 6.63 (s, 1H, CH), 6.8-8.3 (m, 11H, benzene and imidazole H)
9	2.33 (s, 3H, CH_3), 3.80 (s, 2H, NH_2), 6.38 (s, 1H, CH), 6.45-7.40 (m, 11H, aromatic H)



6 R = OH
7 R = Br



8 R = NO_2
9 R = NH_2



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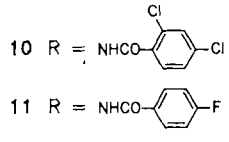

10 R = NHCO–Cl
11 R = NHCO–F

Preparation of **4** from **9** was achieved by use of 2,5-dimethoxytetrahydrofuran in glacial acetic acid according to *Clauson-Kaas*[4]. A better yield was obtained when **4** was synthesized in a similar way starting from **12**, *via* the intermediates **5g** and **5h** (crude). Compound **12** was obtained by *Clauson-Kaas* reaction on 4-amino-4'-methylbenzophenone[2]. The amides **10** and **11** were preparated by aroylation of **9** with the proper aroyl chloride.

Crude **5h** was reacted with triazole, pyrrolidine, thiomorpholine and 1,4-dioxa-8-azaspiro(4,5)decane to afford **5a**, **5d**, **5e**, and **5f**, respectively. Analogous reaction with tetrazole led to a mixture of the two expected isomers **5b** and **5c**.

The chemical structure of the azoles was attributed taking into consideration the literature data on ^{1}H-NMR spectra of related compounds[5].

Microbiological Part

Materials and Methods

The antimycotic activities against *Candida albicans* and *Candida* spp. were evaluated by means of the minimal inhibitory concentration (MIC) using the serial dilution test in a liquid nutrient medium. For the preparation of the dilution series 5 mg of active ingredient were dissolved in DMSO (1 ml) and the solution was treated on shaking with distilled water (9 ml). Further progressive double dilutions with test medium furnished the required concentrations in the range from 0.2 to 200 µg/ml; in some cases dissolution was completed by addition of a few drops of diluted HCl.

All the tested microorganisms were preliminarily incubated at 37 °C on *Sabouraud* (BBL) dextrose broth. The incubation time was 18 h.

Antimicrobial tests were performed on *Mueller-Hinton* (BBL) agar using inocula of 10^3/ml of fungi. Readings of MICs were taken after 36 h incubation at 37 °C.

The minimal growth-inhibitory concentration (MIC) was defined as the lowest concentration of substance at which there was no macroscopic colonial growth when compared with a blank experiment after the preset incubation time. Blanks were prepared in the test medium with the above reported quantities of water and DMSO but without test substances.

Ketoconazole, miconazole and/or bifonazole were chosen as standard controls.

Mean MIC values $n\bar{X}$ (C_{max} at least 200 µg/ml) were calculated by the equation

$$n\bar{X} = \frac{\Sigma\, MIC_i}{s_t}$$

where MIC_i are the minimal inhibitory concentration values of all sensitive strains at the used concentration C_i and s_t is the total number of sensitive strains.

Strains with MIC>200 µg/ml are regarded as resistant (R) and are expressed in percentage by the equation

$$R(\%) = \frac{N_t - N_s}{N_t} \cdot 100$$

where N_t is the total number of tested strains and N_s is the number of sensitive strains.

Experiments were carried out employing two different lots of *Candida albicans* and *Candida* spp. freshly isolated from hospitalized patients. The specimens used were: 37 strains of *Candida albicans* and 10 strains of *Candida* spp (2 *C. glabrata*, 2 *C. guilliermondii*, 1 *C. tropicalis*, 1 *C. pseudotropicalis*, 1 *C. lipolytica*, 1 *C. parapsilosis*, 1 *C. krusei*, and 1 *C. viswanathii*) for imidazole derivatives (Table 1) and 19 strains of *Candida albicans* and 15 strains of *Candida* spp (3 *C. parapsilosis*, 3 *C. tropicalis*, 3 *C. guilliermondii*, 3 *C. glabrata*, 1 *C. incospicua*, 1 *C. krusei*, and 1 *C. viswanathii*) for the other tested derivatives (Table 2).

Results and Discussion

From data in Table 1 and Table 2 the best antifungal agents against all the tested strains are derivatives **4** and **5c**. Less potent but still showing no resistant strains against *Candida albicans* are **5a**, **8** and the previously reported derivative **1**[1].

The antifungal profile of **4** against *Candida albicans* is clearly superior to that of bifonazole and ketoconazole and comparable to that of miconazole. Only three derivatives, namely **4**, **1** and **5c**, retained their antifungal activity when tested against *Candida* spp. showing no resistant strains (R%=O).

The activity dramatically abated when the imidazole ring was replaced by pyrrolidine, thiomorpholine and 1,4-dioxa-8-azaspiro(4,5)decane, thus evidencing that the presence of an aromatic heterocyclic ring is absolutely required for activity. However, among the azoles described activities decrease from very high to no-activity in the order **4**, **5c**, **5a** and **5b**. It is interesting that **5b** lacks completely the good activity of the isomeric **5c**.

The scarce activity showed by the amides **10** and **11** accounts for the importance of a pyrrole ring in the bifonazole-like compounds. A large decrement of activity was also observed when the nitroderivative **8** was reduced to the corresponding amino-derivative **10**.

In conclusion, among the methyl derivatives tested here, derivative **4** is the most active compound superior to derivatives **1** and **3** and to bifonazole **2**, too. This is confirming our opinion that an alkyl chain attached to the free benzene ring of **1** would remarkably enhance the antifungal activities in our pyrrole isosteres of bifonazole.

Chemical Experimental Part

M.p.: Büchi 510 (uncorr.). - IR-spectra (nujol mulls): Perkin Elmer 297. - ^{1}H-NMR-spectra: Varian EM-390 (90 MHz, TMS, $CDCl_3$) - Column chromatography: silica gel Merck (70-230 mesh) and alumina Merck (70-230 mesh). - TLC: Stratocrom silica gel (Carlo Erba), $CHCl_3$. - Microanalyses: Laboratories of Prof. *A. Pietrogrande*, University of Padova (Italy). - Organic extracts were dried over anhydrous Na_2SO_4. - Evaporation of solvents under reduced pressure.

1-[p-Methyl-α-(4-nitrophenyl)benzyl]imidazole (**8**)

A solution of PBr_3 (0.05 mol) in anhydrous Et_2O (50 ml) was added dropwise to a solution of **6**[3] (0.075 mol) in Et_2O (200 ml). The mixture was stirred at room temp. for 4 h. The ethereal solution was washed with 5% aqueous sodium acetate, then with saturated NaCl solution and dried. Evaporation of the solvent gave an oily residue, which was dissolved in acetonitrile (50 ml) and added by dropping to a solution of imidazole (0.075 mol) and triethylamine (0.075 mol) in acetonitrile (150 ml). After addition the mixture was heated at 60 °C overnight and then concentrated to a small volume. Benzene (200 ml) and saturated NaCl solution (200 ml) were added and the org. layer was separated and dried. Removal of the solvent afforded an oily material, which was chromatographed on alumina (500 g) eluting with $CHCl_3$. The central eluates were evaporated to afford pure **8** (TLC showed a single spot).

1-[α-(4-Aminophenyl)-p-methylbenzyl]imidazole (**9**)

A solution of **8** (0.015 mol) in ethyl acetate (100 ml) was hydrogenated over 10% Pd/C (400 mg) in a *Parr* apparatus (6 h, 3 atm). After filtration, the solvent was evaporated to give an oily residue, which was purified by passing through an alumina column (200 g, $CHCl_3$). The central eluates were collected and evaporated to afford **9**. - IR: 3425-3230 (NH_2) cm^{-1}.

1-{α-[4-(2',4'-Dichlorobenzoylamino)phenyl]-p-methylbenzyl}imidazole (**10**)

To a cooled (0-5 °C) and stirred solution of **9** (0.0038 mol) and triethylamine (0.0038 mol) in anhydrous THF (50 ml) a solution of 2,4-dichlorobenzoyl chloride (0.0038 mol) in the same solvent (10 ml) was gradually added. After stirring at room temp. for 1 h, the mixture was warmed at 60 °C for 1.5 h and then filtered. The solution was concentrated to a small volume, diluted with ethyl acetate and sequentially washed with N NaOH, saturated NaCl solution and water. The dried solution was evaporated to afford a crude residue, which was chromatographed on alumina (200 g) eluting with $CHCl_3$/ethyl acetate 2/1 to give **10**. - IR: 3215 (NH), 1620 (C=O) cm^{-1}.

1-{α-[4-(4'-Fluorobenzoylamino)phenyl]-p-methylbenzyl}imidazole (**11**)

Prepared from **9** and 4-fluorobenzoyl chloride as reported for **10**. - IR: 3210 (NH), 1630 (C=O) cm^{-1}.

4-Methyl-4'-(1H-pyrrol-1-yl)benzophenone (**12**)

A solution of 4-amino-4'-methylbenzophenone[2] (0.025 mol) and 2,5-dimethoxytetrahydrofuran (0.025 mol) in glacial acetic acid (100 ml) was heated at reflux for 30 min, then poured onto crushed ice and neutralized with solid $NaHCO_3$. The mixture was extracted with ethyl acetate, the org. solution was separated, dried and evaporated to give a residue, which was chromatographed on a silica gel column (300 g, benzene as eluent). The first eluates were discarded, the central eluates were evaporated fo furnish **12**. - IR: 1640 (C=O) cm^{-1}.

p-Methyl-α-[4-(1H-pyrrol-1-yl)phenyl]benzylalcohol (**5g**)

12 (0.02 mol) was dissolved in THF (100 ml) containing 2 ml of water and $NaBH_4$ (0.02 mol) was added portionwise. The mixture was heated at reflux for 2 h, then water (30 ml) was added. After evaporation to a small volume, the mixture was extracted with ethyl acetate and the org. solution was dried. Removal of solvent led to **5g**. - IR: 3310 (OH) cm^{-1}.

1-{p-Methyl-α-[4-(1H-pyrrol-1-yl)phenyl]benzyl}imidazole (**4**)

From **9**: A solution of **9** (0.04 mol) and 2,5-dimethoxytetrahydrofuran (0.04 mol) in glacial acetic acid (70 ml) was heated at 60 °C for 1 h. The mixture was poured onto crushed ice (300 g), basified by solid Na_2CO_3 and extracted with ethyl acetate. The org. layer was dried and evaporated to afford a residue, which was purified by passing through a silica gel column (300 g, ethyl acetate). The first eluates were discarded and the central ones evaporated to give oily **4**.

From **5g**: A solution of **5g** (0.015 mol) in anhydrous Et_2O (50 ml) was treated under stirring with PBr_3 (0.01 mol) in Et_2O (20 ml) and then stirred at room temp. for 4 h more. The ethereal solution was washed with 5% aqueous sodium acetate, then with water and dried. The solution was filtered and added dropwise to a solution of imidazole (0.015 mol) and triethylamine (0.035 mol) in acetonitrile (50 ml). The mixture was heated at 60 °C for 15 h, cooled and treated with benzene (100 ml) and saturated NaCl solution (100 ml). The org. layer was separated, dried and evaporated. The residue was passed through a silica gel column (ethyl acetate). The central eluates were evaporated to furnish **4**.

Preparation of **5a-f**

5a-f were prepared starting from **5g** as described for **4**.

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[Ph569]