Table: Antiinflammatory activity of compounds 1–3 and 1a–3a

Compd.	Edema volume changes ΔV (± SEM) (cm^3)						
	Time interval (min)						
	30	60	120	180	240	300	360
CG	0.15 (0.01)	0.18 (0.01)	0.19 (0.01)	0.20 (0.01)	0.18 (0.01)	0.17 (0.01)	0.16 (0.01)
1	0.05^{***} (0.01)	0.06^{***} (0.00)	0.06^{***} (0.01)	0.06^{***} (0.01)	0.05^{***} (0.01)	0.05^{***} (0.01)	0.04^{***} (0.01)
1a	0.11^{*} (0.00)	0.12^{***} (0.01)	0.14 (0.01)	0.14 (0.01)	0.15^{*} (0.01)	0.13^{*} (0.01)	0.13 (0.01)
2	0.06^{***} (0.01)	0.05^{**} (0.01)	0.06^{***} (0.01)	0.06 (0.01)	0.06^{***} (0.01)	0.05^{***} (0.01)	0.04^{***} (0.01)
2a	0.09^{**} (0.01)	0.10^{***} (0.01)	0.09^{***} (0.01)	0.09^{***} (0.01)	0.10^{***} (0.01)	0.08^{***} (0.01)	0.08^{***} (0.01)
3	0.05^{***} (0.01)	0.07^{***} (0.01)	0.08^{***} (0.01)	0.09^{***} (0.01)	0.08^{***} (0.01)	0.06^{***} (0.01)	0.06^{***} (0.01)
3a	0.09^{**} (0.01)	0.11^{***} (0.01)	0.11^{***} (0.01)	0.10^{***} (0.01)	0.10^{***} (0.01)	0.08^{***} (0.01)	0.07^{***} (0.01)

CG control group of animals (n = 18); statistical significance * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$ (n = ()

Experimental

The complexes **1**–**3** and the corresponding cresoxyacetic acids **1a**–**3a** were used for biological tests. Their preparation and basic physico-chemical characterization were published previously [9]. All compounds were dispersed in sterilized saline with concentration of 50 μmol/cm^3 (calculated for cresoxyacetate fragment) and stabilized by 0.05% Tween® 80 (Merck). Wistar male rats (Velaz Prague), weighing 230–270 g, were used. The acute antiphlogistic activity (Table) was measured by reduction of rat paw edema, induced by injection of 0.1 cm^3 of 1% carrageenan (Serva) in sterilized saline. The tested compounds were applied i.p. in a single dose of 50 μmol/kg body weight, 30 min before injecting the irritant substance. Control animals received only vehicle. The changes of edema volume were evaluated plethysmometrically [10]. Statistical significance of results was established using the Student's t-test. All differences were considered significant at $P < 0.05$.

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References

1 Sorenson, J. R. J.; Soderberg, L. S. F.; Chang, L. W.; Wilingham, W. M.; Baker, M. L.; Barnett, J. B.; Salari, H., Bond, K.: Eur. J. Med. Chem. **28**, 221 (1993)
2 Aaseth, J.; Haugen, M.; Foree, O.: Analyst **123**, 3 (1998)
3 Sokolík, J.; Tumová, I.; Havlas, J., Blahová, M., Švec, P.: Farm. Obzor **68**, 12 (1999)
4 Pohleven, F.; Petrič, M.; in: Ondrejovič, G.; Sirota, A. (Eds.): Current Trends in Coordination Chemistry, p. 379, Slovak Technical University Press, Bratislava 1995
5 Blahová, M.; Sokolík, J.; Tumová, I.; Švec, P.: Pharmazie **54**, 156 (1999)
6 Melník, M.; Györyová, K.; Skořepa, J.; Holloway, C. E.: J. Coord. Chem. **35**, 179 (1995)
7 Smith, G.; O'Reilly, E. J.; Kennard, C. H. L.; Stadnicka, K.; Oleksyn, B.: Inorg. Chem. **47**, 111 (1981)
8 Kaim, W.; Rall, J.. Angew. Chem. (Int. Ed.) **35**, 43 (1996)
9 Blahová, M.; Sokolík, J.; Tumová, I.; Gavenčiak, J.; Mišíková, E.; Švec, P.: Čes. a Slov. Farm. **48**, 175 (1999)
10 Lenfeld, J.; Marek, J.:Arzneim.-Forsch. **16**, 664 (1966)

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Doz. Dr. M. Blahová, CSc.
Faculty of Pharmacy
Comenius University
Odbojárov 10
832 32 Bratislava
Slovakia

Benzodihydrocarbazoles activity on triazole susceptible and resistant *Candida* sp.

A. Amoroso[1], M. Radice[1], A. Segall[2], L. Rodero[5], F. Hochenfellner[5], M. T. Pizzorno[2], J. Moretton[3], D. Garrido[4], and G. Gutkind[1]

Cátedras de Microbiología, Control de Calidad de Medicamentos[2], Higiene[3] y Bioestadística[4], Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, and Inst. Nac. de Microbiología Dr. C. Malbrán[5], Buenos Aires, Argentina.

Since the early 1970's our group has been engaged in the synthesis of tetrahydrocarbazoles, dihydrobenzocarbazoles, bis-benzylimidazoles, 3-aminomethyl indol derivatives and related compounds with trypanomicidal activity [1–5]. Some of them proved inhibitory activity when tested against a series of gram positive and gram negative bacteria, *Aspergillus niger*, *Mucor mucedo* and *C. albicans* [6]. From twenty-two new *N*-alkylated dihydro[*a*]benzocarbazoles tested in 1996, exhibiting activity on gram positive bacteria but not on gram negative bacteria [7], 6,11-dihydro-2-methoxy-5 *H*-benzo[a]carbazole (**1**) and 6,11-dihydro-2-methoxy-11-[2-(1-piperidinyl)ethyl-5 *H*-benzo[*a*]carbazole **2** were able to completely inhibit the growth of *C. albicans* below a concentration of 4 μg/ml. These previous results led us to continue with their antifungal activity evaluation.

Both compounds were tested in duplicate against the following strains: fourteen fluconazole susceptible (MIC < 6.2 μg/ml) *Candida albicans* and one *C. tropicalis*, and twelve fluconazole resistant (MIC > 50 μg/ml) *C. albicans*, three *C. tropicalis*, one *C. krusei* and one *C. glabrata*. MIC values (80% relative growth inhibition) ranged from 2.5 to 25 μg/ml for the N-substituted compound, while the non-substituted ring was slightly less active (ranging from 6.2 to 25 μg/ml, except for a single *C. albicans* that could not be inhibited up to 100 μg/ml). Data from each drug were compared to those of fluconazole using Kendall's ranges correlation coefficient Tau-b (τ_b) [8], using SPSS Base 7.5 (SPSS Inc., Chicago). No significative correlation could be detected between each set of treatments (Table).

Also two dermatophytes were inhibited by the tested drugs (data not shown). Both compounds proved to be

Table: MICs of compounds 1 and 2 on fluconazole susceptible or fluconazole resistant *Candida* sp.

Strain number	Species	MIC (μg/ml)		
		Fluconazole	**1**	**2**
R1	*Candida albicans*	100	6.2	12.5
R2	*Candida albicans*	>100	6.2	12.5
R3	*Candida albicans*	>100	25	25
R4	*Candida albicans*	100	25	12.5
R5	*Candida glabrata*	100	6.2	12.5
R6	*Candida albicans*	100	25	12.5
R7	*Candida albicans*	100	6.2	12.5
R8	*Candida albicans*	100	6.2	12.5
R9	*Candida albicans*	100	6.2	12.5
R10	*Candida albicans*	100	25	12.5
R11	*Candida tropicalis*	100	25	12.5
R12	*Candida tropicalis*	100	50	12.5
R13	*Candida krusei*	100	6.2	12.5
R14	*Candida tropicalis*	100	25	12.5
R15	*Candida albicans*	>100	6.2	6.2
R16	*Candida albicans*	>100	6.2	12.5
R17	*Candida albicans*	>100	25	12.5
S1	*Candida albicans*	<0.1	25	12.5
S2	*Candida albicans*	0.1	25	12.5
S3	*Candida albicans*	0.2	25	12.5
S4	*Candida albicans*	0.2	12.5	12.5
S5	*Candida albicans*	<0.1	25	25
S6	*Candida albicans*	<0.1	25	25
S7	*Candida albicans*	<0.1	12.5	12.5
S8	*Candida tropicalis*	0.2	12.5	6.2
S9	*Candida albicans*	0.2	12.5	6.2
S10	*Candida albicans*	0.2	6.2	6.2
S11	*Candida albicans*	0.2	6.2	6.2
S12	*Candida albicans*	0.2	25	12.5
S13	*Candida albicans*	0.2	6.2	12.5
S14	*Candida albicans*	0.2	25	12.5
S15	*Candida albicans*	0.8	>100	25

1 vs Fluconazole: $\tau_b = -0.197$ (no significative, $P = 0.20$)
2 vs Fluconazole: $\tau_b = -0.014$ (no significative, $P = 0.93$)

non-mutagenic by the Ames test. They exhibited selective antifungal activity with (at least) very little or no-antibacterial activity. Although **1** was more active than **2**, both can be considered active on fluconazole susceptible and (more interestingly) fluconazole-resistant candida.

Experimental

1. Chemistry

The preparation of compounds **1**–**2** has been reported elsewhere [7] .

2. Antifungal susceptibility tests

2.1. Minimal Inhibitory Concentration (MIC) for yeasts

A microbroth dilution method based on the M27 methodology [9] was used to determine MICs for yeasts The culture medium employed was RPMI 1640 (Sigma Chemical Co) buffered to pH 7.0 with morphanepropanesulfonic acid (MOPS) (Gibco Laboratories) supplemented with 1.8% glucose [10]. Stock solutions were prepared as follows: both drugs were dissolved in ethanol to a concentration of 6.0 mg/ml, and frozen at −20 °C. Series of 10 twofold dilutions were prepared in ethanol for each drug, and then diluted with 19 volumes of water, bringing their concentrations to three times the desired final concentrations. These solutions (50 μl) were pipetted in each well of flat-bottom microdilution plates (Nunclon 1 67008, Nunc, Denmark). The final concentrations for each drug ranged from 0.2 to 100 μg/ml. Sterile distilled water was dispensed for serving as sterility controls and blanks for spetrophotometric assays (with sterile RPMI), and growth controls (with inoculated RPMI). Inocula were adjusted to tube 1 of Mc Farland, and 100 μl added to 10 ml 5X RPMI medium; 100 μl of the inoculated medium were added to each desired well, bringing all the reagents and the inoculum to the desired final concentration. Following incubation at 35 ºC for 48 h, the trays were shaken for 5 min and turbidity was read at 405 nm with a Labsystem Multiskan RC microplate spectrophotometer. MIC endpoints were defined as 80% of growth inhibition compared to the control [11].

2.2. Inhibitory activity on dermatophytes

Appropriated dilutions in hot ethanol were mixed with thermostatized Antibiotic Medium # 1 (Difco) to final concentrations of 100 μg/ml, 50 μg/ml and 10 μg/ml of each drug. Once solidified, the plates were inoculated with 10 μl suspensions of *Trichophyton mentagrophytes* and *Microsporum gypseum* (clinical isolates) adjusted to tube 1 of Mc Farland. Control plates (containing the same amount of ethanol) were included. Plates were incubated at 28 °C and observed at 48 h and 7 d for microbial growth.

3. Mutagenic activity

The maximum amount of drug tested (0.1 mg/ml) could be added with no inhibitory effects by a spot test. Two ml soft agar were mixed with 0.1 ml of the drug dilution and 0.1 ml of *Salmonella typhimurium* TA 98 and *S. typhimurium* TA 100, with or without microsomal fraction (S9) addition. Colonies were counted after 24 hr incubation at 35 °C [12].

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References

1 Gallo Pecca, J.; Dobrecki, J.; Albónico, S. M.: J. Pharm. Sci. **60**, 650 (1971)
2 Poliakoff, N.; Alvarez, M.; Albónico, S. M.; Vernengo, M. J.: J. Med. Chem. **16**, 1411 (1973)
3 Gallo Pecca, J.; Albónico, S. M. : J. Med. Chem. **14**, 448 (1971)
4 Albónico, S. M.; Assem, M. E.; Benages, I. A.; Montiel, A. A.; Pizzorno, M. T.; Docampo, R.; Stoppani, A. O. M.: Rev. Arg. de Microb. **19**, 121 (1987)
5 Giancaspro, G. I.; Colombari, V. D.; Albónico, S.M.; Cardoni, R.; Rivas, C; Pizzorno, M. T.: Rev. Microb. **25**, 201 (1994)
6 Pappa, H.; Segall, A.; Pizzorno, M. T.; Radice, M.; Amoroso, A.; Gutkind, G. : Il Farmaco **49**, 333 (1994)
7 Segall, A.; Pappa, H.; Pizzorno, M. T.; Radice, M.; Amoroso, A.; Gutkind, G.: Il Farmaco **51**, 513 (1996)
8 Everitt, B.S. : The analysis of contingency tables. p. 54, Chapman & Hall, London, 1992
9 National Committee for Clinical Laboratory Standards, Document M27-A. Villanova, Pa. 1997
10 Rodriguez Tudela, J. L.; Martinez Suarez, J. V.: Antimicrob. Agents Chemother. **38**, 45 (1994)
11 Odds, F. C.; Vranckx, L.; Woestenborghs, F.: Antimicrob. Agents Chemother. **39**, 2051 (1995)
12 Maron, D. M.; Ames, B. N.: Mutation Research. **113**, 173 (1983)

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Dr. Gabriel O. Gutkind
Facultad de Farmacia y Bioquímica
Junin 954
1113-Buenos Aires
Argentina
ggutkind@huemul.ffyb.uba.ar