co-glycolide) (PLGA) with a D,L-lactide : glycolide molar ratio of 50 : 50 was supplied by Boehringer Ingelheim (Ingelheim, Germany). Isopropylantipyrine (1,2-dihydro-1,5-dimethyl-4-(1-methylethyl)-2-phenyl, 3 *H*-pyrazol-3-one) was obtained from Sopharma (Sofia, Bulgaria). Polyvinyl alcohol (PVA 15 000 and PVA 80 000), (Fluca). All other reagents were of analytical grade (Fluca).

2. Methods

2.1. Preparation of microspheres

Isopropylantipyrine-loaded microspheres were prepared using a solvent-evaporation method. Briefly, given amounts of model drug (1.0 g) and polymer (PMM or PLGA, respectively) were dissolved in 10 ml CH_2Cl_2 and emulsified in 100 ml PVA aqueous solution (2% wt.) by sonication (Ultrasonic disintegrator, UD-20, 22 ± 1.65 kHz). The emulsion was agitated at room temperature until CH_2Cl_2 was evaporated completely. The microspheres were collected by centrifugation conducted at 10 000 rpm and 0 °C for 10 min, washed with distilled H_2O and lyophilized or vacuum dried.

2.2. Determination of surface morphology and microsphere size

The shape and surface characteristics of the formulations were examined by scanning electron microscopy (SEM) (Stereoscan, Cambridge Instruments, Cambridge, UK).

2.3. Drug loading

A weighed quantity of microspheres was incubated in 10 ml of 1 N NaOH solution at room temperature for 24 h. After suitable dilution with 0.1 N NaOH solution the absorbance was measured spectrophotometrically at $\lambda = 266$ nm (Hewllett Packard 8452 A diode array spectrophotometer equipped with a HP Vectra 386/25 computer). The amount of drug was determined from a standard plot of isopropylantipyrine prepared under similar conditions.

2.4. In vitro drug release

In vitro drug release was conducted in a shaker bath at 37 °C (150 rpm), in phosphate buffer (ph = 7.4). The amount of isopropylantipyrine was measured spectrophotometrically ($\lambda = 266$ nm) (Hewllet Packard 8452 A).

References

1 Wada, R.; Hyon, S.-H.; Ikada, Y.: J. Biomater. Sci. Polymer Edn. **8**, 715 (1996)
2 Lambov, N.; Peikov, P.; Belcheva, N.; Gesheva, K.; Zlatkov, A.; Danchev, N.; Smid, J.: J. Controlled Rel. **45**, 193 (1997)
3 Braud, C.; Bunel, C.; Vert, M.: Polym. Bull. **13**, 293 (1985)
4 Belcheva, N.; Tsvetanov, Ch.; Panayotov, I.; Lazarova, St.: Macromol. Chem. **191**, 213 (1990)
5 Wada, R.; Hyon, S.-H.; Ikada, Y.: J. Pharm. Sci. **10**, 919 (1990)
6 Haeckel, S.; Sachse, A.; Albayrak, C.; Muller, R.: Proc. 2nd World Meeting APGI/APV, Paris, 507 (1988)

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Antiinflammatory activity of diaquabis(cresoxyacetato)zinc(II) complexes

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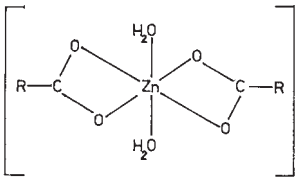
In numeros groups of (carboxylato)zinc(II) complexes their antiinflammatory, antiradical and antimicrobial activities have been studied [1–4]. It is known, that low molecular complexes of the $[Zn(H_2O)_2(ROCH_2COO)_2]$ type may be beneficial in affecting the inflammation process in animal models [1, 3]. On the basis of these findings and the study of antiphlogistic activity of uncomplexed cresoxyacetic acids as well as their copper(II) salts [5], this paper is focused on the study of antiflammatory activities of the mononuclear diaquabis(cresoxyacetato)zinc(II) complexes with the general formula $[Zn(H_2O)_2(ROCH_2COO)_2]$, where R = 2-methylphenyl (complex **1**), 3-methylphenyl (complex **2**) and 4-methylphenyl (complex **3**), including the corresponding isomeric cresoxyacetic acids **1a**–**3a**.
All studied Zn(II) complexes belong to the group of mononuclear (carboxylato)zinc(II) complexes with tetragonally-distorted octahedral stereochemistry [6], as it was found for the structure of the diaquabis(phenoxyacetato)zinc(II) complex by X-ray analysis [7]. Thus, their polyhedron is formed by aryloxyacetate ion chelate coordination via two oxygen atoms of $RCOO^-$ anions to the Zn(II) central atom and by axial water molecule coordination.



	R
1	2-methylphenyl
2	3-methylphenyl
3	4-methylphenyl

Using a routine plethysmometric method, the evaluation of antiinflammatory activity of all compounds was carried out in rat paw carrageenan-induced edema (Table). The effects of the complexes **1**–**3** were compared to those of the free isomeric cresoxyacetic acids **1a**–**3a**. In general, all diaquabis(cresoxyacetato)zinc(II) complexes are more effective then the acids. The average antiphlogistic activities of the Zn(II) complexes expressed as a measure of edema volume reduction decreased in the following order: **1/1a** (70/26%), **2/2a** (68/50%), **3/3a** (58/48%). The results obtained indicate the involvement of Zn^{2+} ions (administered in the form of complexes) in the modulation of the animal inflammation model. Because the activity of complexes has not correlated with their metal content, it is very likely that the bioavailability of these ions is closely related to the aquation processes of $[Zn(H_2O)_2(ROCH_2COO)_2]$ species and to the formation of low molecular complexes under *in vivo* conditions. Collectively, the results support the idea that Zn(II) complexes can be used to create the pharmacoative forms containing Zn(II) [1, 8]. From this point of view the complexes **1** and **2** might appear as potential antiphlogistics.

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

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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