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Evaluation of anti-inflammatory and antioxidant activities of mixed-ligand Cu(II) complexes of dien and its Schiff dibases with heterocyclic aldehydes and 2-amino-2-thiazoline

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Abstract—A new series of complexes of the type [Cu(dien)(2a-2tzn)Y_2] and [Cu(dienXX)(2a-2tzn)Y_2] has been tested for anti-inflammatory and antioxidant activity. The tested compounds inhibit significantly the carrageenin induced paw edema (36.4–55.8%) and present important scavenging activities. Although their interaction with the free stable radical DPPH is not high they proxide anions. Compound **7** is the most potent (55.8%) in the in vivo experiment. Lipophilicity—as R_M values and theoretically calculated log *P* values—has been determined. An attempt to correlate the biological results with their structural characteristics and physicochemical parameters has been done.

The synthesis of a new series of copper complexes of the type [Cu(dien)(2a-2tzn)Y_2] and [Cu(dienXX)(2a-2tzn)Y_2] has been recently reported.[1] The compounds tested were six-coordinate Cu(II) chelate complexes with the anions of halogens and dien or dien's Schiff base complexes with heterocyclic aldehydes, of the general types given in Figure 1. Results regarding toxicity and antitumor activities of the investigated compounds are promising. Copper complexes of many several ligands have been prepared and evaluated for anti-inflammatory,[2–7] antioxidant[8–11] activities and irritancy after oral, subcutaneous, and local administration in rats and guinea pigs. Other compounds known for their anti-inflammatory properties are the S,N-heterocyclic ligands, e.g., thiazoline and its derivatives.[5,6] The Cu(II) thiazoline complexes are anticipated to yield agents with enhanced anti-inflammatory activity and reduced gastrointestinal (GI) toxicity, compared to the uncomplexed ligands.

The reported derivatives were tested for their antioxidant and anti-inflammatory activities. Non-steroidal anti-inflammatory drugs (NSAIDs) have a broad spectrum of effects and it has been suggested that the variations in both efficacy and their tolerability are partly due to differences in their physicochemical properties, which determine their distribution in the body and their ability to pass through and to enter the interior of membranes.[12,13] Thus, partition coefficients such as R_M values are performed[14,15] and compared with the corresponding theoretically calculated[16] log *P* values. In acute toxicity experiments, the in vivo examined compounds were endowed with a 50% lethal dose[1] of 28–114 mg/kg body weight. To access the anti-inflammatory activity of the complexes the rat carrageenin

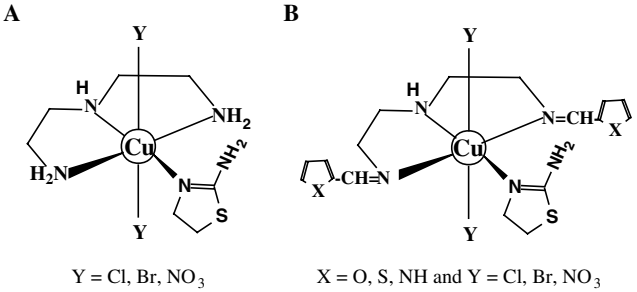

Figure 1. Structures of [Cu(dien)(2a-2tzn)Y_2] (A) and [Cu(dienXX)(2a-2tzn)Y_2] (B).

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induced paw edema assay was employed as a model for acute inflammation. Indomethacin was included as a reference drug. The development of the edema induced by carrageenin has been described as a biphasic event. The first phase of the inflammatory response is mediated by histamine and serotonin, the second phase mediated by kinins and presumably by prostaglandins. Since edemas of this type are highly sensitive to NSAIDs, carrageenin has been accepted as a useful agent for studying new NSAIDs. This model reliably predicts the anti-inflammatory efficacy of the NSAIDs during the second phase. It detects compounds that are anti-inflammatory agents, as a result of inhibition of prostaglandin amplification. Edema was induced in the right hind paw of Fisher 344 rats (150–200 g) by the intradermal injection of 0.1 ml of 2% carrageenin in water.[17] The tested compounds, 0.01 mmol/kg body weight, were dissolved in water and were given intraperitoneally simultaneously. It is of interest that from the tested compounds especially **3**, **7**, **8**, and **11** possess significant protection. The protection ranges from 36.4 to 55.8%. Compound **4** has the lowest effect (36.4%). Lipophilicity does not seem to affect the biological responses (**3**, **7**, **8**, and **11** Table 1). Several findings concerning the correlation of antiinflammatory activity and the structures of these complexes emerge from Table 1: (a) a factor that should be taken into consideration is the nature of the heterocyclic atom of the Schiff dibases, dien vs. dienSS, dienOO and dienNN, since the activity of the substituted complexes differs depending on the presence of S, O, or N atom in the heterocyclic ring, thiophene, furyl, or pyrrole. (b) The observed differences in activity seem to depend partly on the nature of the counteranion; Cl, Br, or NO_3 since these can be regarded preferentially as leaving groups.

The complexes derived from 2-furaldehyde (compounds **7**, **8**) seem to be more potent. The nature of Y = Cl/Br does not influence the in vivo results (compound **7** = 55.8%, compound **8** = 54.5%). However, the presence of Y = NO_3 causes a significant decrease in carrageenin paw edema inhibition (compound **9** = 38.9%). Among the 2-pyrrol-carboxaldehyde complexes (compounds **10**, **11**, and **12**) compound **10** is the less potent. The presence of Y = Cl is responsible for this decrease. Not many changes are observed among the CPE % protection values of the complexes formed with the presence of 2-thiophene-carboxaldehyde (compounds **4**, **5**, and **6**) with the exception of compound 4 (36.4%, Y = Cl). Perusal of Table 1 shows that complexes CudienCl_2·2a2tzn are more potent than the corresponding complexes of the type Cu(dienXX)Cl_2 where XX = SS, NN. Taking under consideration the in vivo results compounds **7** and **8** emerge as new potential prototypes/leads.

Nowadays, antioxidants that exhibit DPPH radical scavenging activity are increasingly receiving attention. They have been reported to have interesting anticancer, antiaging, and anti-inflammatory activities. Consequently, compounds with antioxidant properties could be expected to offer protection in rheumatoid arthritis and inflammation, and to lead to potentially effective drugs. In fact, many non-steroidal anti-inflammatory drugs have been reported to act either as inhibitors of free radical production or as radical scavengers. The model of the scavenging of the stable DPPH radical is extensively used to evaluate antioxidant activities in less time than other methods. DPPH is a stable free radical that can accept an electron or hydrogen radical and thus be converted into a stable, diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 517 nm. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons taken up. Such a change in the absorbance produced in this reaction has been widely applied to test the capacity of numerous molecules to act as free radical scavengers. The scavenging effect of the synthesized compounds on the DPPH radical was evaluated according to the methods of Hadjipavlou et al.[14,18] All compounds were tested for their interaction with the stable free radical DPPH. BHT and NDGA were used as reference compounds. This interaction indicates their radical scavenging activity in an iron-free system and expresses their reducing activity. Compounds **1**, **2**, **3**, **7**, **8**, **9**, and **12** were found to have very low activity, whereas compounds **4**, **5**, **9**, and **10** showed high interactions. Compound **11** presents the

Table 1. Lipophilicity values: Experimentally determined R_M and theoretically calculated Clog *P* values and dipole moment (Debye) of the Cu(II) thiazoline complexes; inhibition % of induced carrageenin rat paw edema (CPE %) at 0.01 mmol/kg body weight

No.	Compound	%CPE[a,b]	R_M[c]	Clog *P*	μ (D)
1	CudienCl_2·2a2tzn	40*	−0.517 (0.010)	0.944	8.416
2	CudienBr_2·2a2tzn	41.5*	−0.556 (0.014)	0.944	1.192
3	Cudien$(NO_3)_2$·2a2tzn	52.6*	−0.520 (0.007)	4.350	4.289
4	CudienSSCl_2·2a2tzn	36.4**	−0.453 (0.010)	5.286	9.330
5	CudienSSBr_2·2a2tzn	44.1*	−0.443 (0.005)	5.286	6.158
6	CudienSS$(NO_3)_2$·2a2tzn	49.4*	−0.472 (0.005)	5.028	6.127
7	CudienOOCl_2·2a2tzn[1]	55.8*	−0.486 (0.002)	4.346	6.902
8	CudienOOBr_2·2a2tzn	54.5**	−0.574 (0.001)	4.346	4.095
9	CudienOO$(NO_3)_2$·2a2tzn	38.9*	−0.376 (0.003)	4.088	5.161
10	CudienNNCl_2·2a2tzn	38.5**	−0.419 (0.003)	3.206	8.186
11	CudienNNBr_2·2a2tzn	51.6	−0.371 (0.0030)	3.206	5.615
12	CudienNN$(NO_3)_2$·2a2tzn	48.9**	−0.382 (0.002)	2.948	7.192

[a] Statistical studies were done with Student's *t* test, *$p < 0.01$, **$p < 0.05$.
[b] Indomethacin as a standard 47% (0.01 mM).
[c] R_M values are the average of at least 10 measurements.

Table 2. Interaction % with DPPH (RA %); Competition % with DMSO for hydroxyl radical (HO· %); % Superoxide radical scavenging activity (PMS %)

Compound	RA %, 0.1 mM, 20 min	RA %, 0.2 mM, 20 min	RA %, 0.1 mM, 60 min	RA %, 0.2 mM, 60 min	HO· (%), 0.1 mM	HO· (%), 1 mM	PMS %, 0.1 mM
1	7.7	2.8	11.6	11	97	99	77
2	3.7	No*	8.7	1	97.7	No*	93.5
3	1.8	2.2	1.8	2.4	98.2	96.4	100
4	31	12.6	33	32.4	97.2	97	99
5	33.5	14.2	34.6	16.7	100	100	81
6	18.7	0.5	27.5	3.2	100	92	79
7	3.6	21.2	6.4	31.6	27	No*	89
8	9	No*	13	No*	97.8	94	99
9	35.6	2.2	36.4	12.2	82.7	90	No*
10	14.3	1.8	14.7	5	No*	No*	80
11	55.6	8.7	63.5	14	90.4	99	54
12	12	No*	19	2	91.8	No*	86.2
NDGA	81	80	82.6	80	nt	nt	nt
Trolox	nt	nt	nt	nt	88.2	~100	nt
BHT	31.3	52.7	60	78			
Caffeic acid	nt	nt	nt	nt	nt	nt	31

NDGA, nordihydroguaiaretic acid; BHT, butylated hydroxytoluene; nt, not tested.
* No, no action under the experimental conditions.

highest activity at 0.1 mM. In general, the results are not proceeded in parallel to the increase of time and concentration (Table 2). Further investigations are in progress in order to have a detailed study on their interaction with DPPH.

The competition of complexes with DMSO for OH radicals,[14] generated by the Fe^{3+}/ascorbic acid system expressed as the inhibition of formaldehyde production, was used for the evaluation of their hydroxyl radical scavenging activity. Trolox was used as a reference compound. Compound **10** did not show any inhibition, whereas compounds **1**–**6**, **8**–**12** inhibited significantly the DMSO oxidation in concentrations 0.1 and 1 mM, respectively. Compounds **5** and **6** were found to be the most active. Again no role was found for lipophilicity.

Non-enzymatic superoxide anion radicals were generated.[19] The superoxide producing system was set up by mixing phenazine methosulfate (PMS), NADH and air–oxygen. The production of superoxide was estimated by the nitroblue tetrazolium method. Caffeic acid was used as a reference compound. Compound **9** did not show any result. All the other compounds present high scavenging activity (54–100%, Table 2). In general lipophilicity does not influence positively the scavenging activity. Considering the structures of these compounds and their reactivity against superoxide anion we can confirm that there is a relation between this activity and the electron-donor properties of the ligands.

Although compounds **1**, **2**, **3**, **4**, **5**, **6**, **8**, **9**, **10**, and **12** present excellent inhibitory activity on OH radicals and on superoxide anions, they did not inhibit significant DPPH and this indicates selectivity of the complexes to different free radicals. The antiradical activity of the tested complexes supports at least in part, the in vivo anti-inflammatory activity. The in vitro/in vivo activities have not been able to provide a clear correlation among all the physicochemical parameters in a QSAR analysis. Poor correlation ($r < 0.2$) was obtained between C$\log P$ and R_M.

For the in vivo result the following equation was derived:

$$\mathrm{CPE}\% = -0.033(0.019)\mu + 1.8839(0.132),$$

$$N = 10, r = 0.807, r^2 = 0.651, q^2 = 0.527, s = 0.043,$$

$$F_{1,8} = 14.4, \alpha = 0.01.$$

The dipole moment[20] μ is the most significant parameter. This fact proceeds in parallel to the observation that lipophilicity is not highly involved in the above biological results.

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17. Both sexes were used. Females pregnant were excluded. Each group was composed of 6–15 animals. The rats were euthanized 3.5 h after carrageenin injection.
18. Various concentrations (0.1–0.2 mM) of the test compound in absolute ethanol were added to an ethanolic solution of DPPH radical (final concentration of DPPH was 0.1 mM). The mixture was shaken vigorously and allowed to stand for 20 or 60 min; absorbance at 517 nm was determined by a Perkin-Elmer U-2001 spectrophotometer, and the percentage of activity was calculated. All tests and results were undertaken on three replicates and the results averaged.
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