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Evaluation of anti-inflammatory and antioxidant activities of Copper (II) Schiff mono-base and Copper(II) Schiff base coordination compounds of dien with heterocyclic aldehydes and 2-amino-5-methyl-thiazole

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Abstract

Highly reactive radicals are implicated in many pathological conditions. The quest for radical scavengers or antioxidants, spans the previous decades. A new series of complexes of the type [Cu (dien) (2a-2tn) Y₂] and [Cu (dienXXY₂) (2a-5mt)] and of the type [Cu (dptaS) Cl₂] and [Cu (dptaS) Br₂] (dptaS = 1, 3-propanediamine) or Schiff mono-base of dipropylenetriamine with 2-thiophene-carboxaldehyde, has been tested for anti-inflammatory and antioxidant activity. The tested compounds inhibit the carrageenin-induced rat paw edema (52.0–82.6%) and present important scavenging activity. Compound **6** is the most potent (82.6%) in the in vivo experiment. Lipophilicity-as R_M values – has been determined. The results support that in general, adducts of the type [Cu (dienXXY₂) (2a-5mt)] exhibit increased activity compared to the starting material of type [Cu (dienXXY₂)]. An attempt to correlate the biological results with their structural characteristics and physicochemical parameters has been made

Keywords: Copper complexes, Schiff mono-bases, adducts, Lipophilicity, anti-inflammatory activity, antioxidant activity

Introduction

In recent years, considerable interest has been shown in the chemistry of transition metal complexes of Schiff bases [1–3]. Specifically the linear or cyclic Schiff bases have attracted much attention since most of their compounds prepared to date, exhibit noteworthy bioactivity, which is due to the participation of the specific metal atom and ligands. Their use as analgesic, anti-inflammatory, antibiotic, antimicrobial [4] and especially as anticancer [5] agents is well known. Recently, a new series of Schiff mono/dibase coordination compounds with 2-amino-5-methyl-thiazole (2a-5mt), with promising anticancer and antibacterial activities, has been reported [6,7].

Highly reactive hydroxyl radicals are implicated in many pathological conditions, including cancer,

diabetes, Alzheimer's and Parkinson's diseases, heart diseases, and aging [8]. Harmful hydroxyl radicals attack any component in living organisms, including proteins, DNA, and lipids. These deleterious species are generated from the less damaging reactive oxygen species—superoxide radical anion and hydrogen peroxide—in a Fenton reaction. The quest for Fenton inhibitors, either radical scavengers or metal-ion chelators, spans the last three decades. Nowadays, antioxidants that exhibit 1, 1-diphenyl-picrylhydrazyl (DPPH) radical scavenging activity are increasingly receiving attention. They have been reported to have interesting anticancer, anti-aging and anti-inflammatory activities. Consequently, compounds with antioxidant properties could be expected to offer protection against rheumatoid arthritis and inflammation and to lead to potentially effective drugs.

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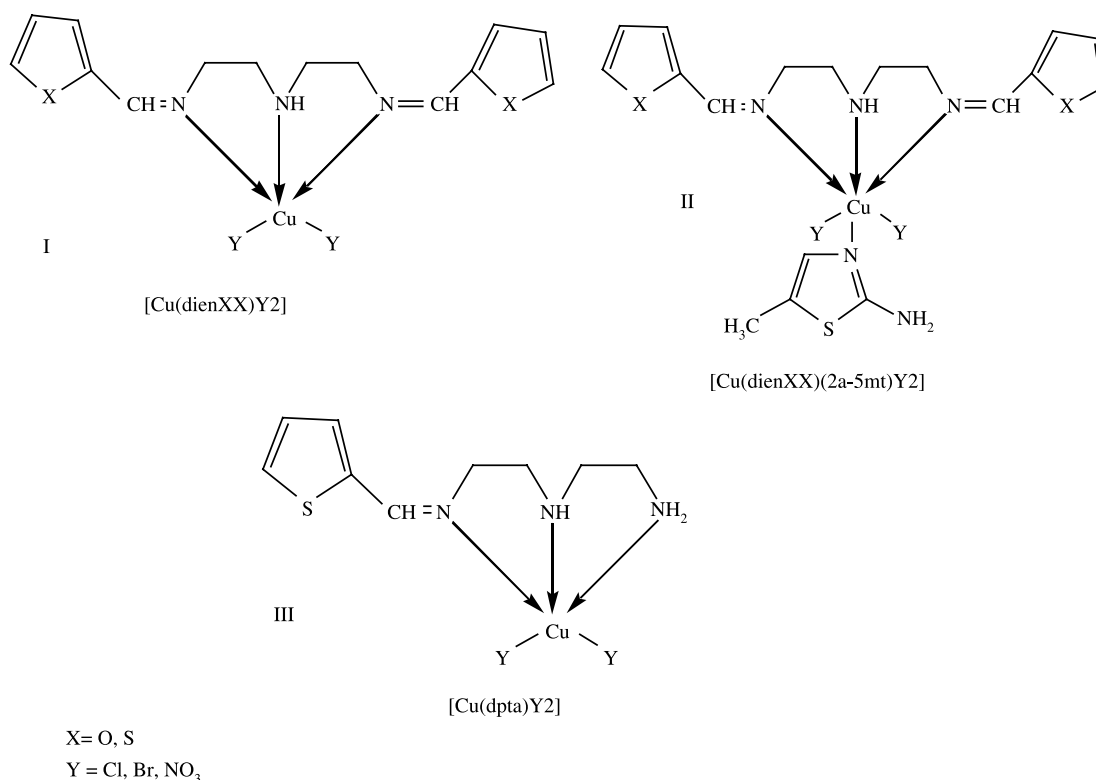


Figure 1. Structures of the tested complexes [6,7,34]

In fact, many non-steroidal anti-inflammatory drugs have been reported to act either as inhibitors of free radical production or as radical scavengers.

Copper complexes of several ligands have been prepared and evaluated for anti-inflammatory [9–14] or antioxidant [15–18] activity. Other compounds known for their anti-inflammatory properties are the S, N-heterocyclic ligands, e.g. thiazoline and its derivatives [12–13]. The bioactivity of S, N-thiazoles is largely due to their structural similarities with the imidazolyl entities of proteins [19], as well as biological features [20–21]. Their participation in the formation of compounds can modify [20–29] the bioactive and pharmaceutical characteristics of the adducts. Among the thiazoles employed to date, the 2-amino-5-methylthiazole is most appropriate for the enhancement of the bioactivity of already synthesized compounds. This unique characteristic of the 2a-5mt molecule could be attributed to its electronic distribution resulting from the positive inductive (+I) effect of the lipophilic methyl, the positive resonance (+R) effect of the hydrophilic amino group and the negative resonance (–R) effect of the thiazole ring. Furthermore, the position of the methyl group on the fifth carbon atom is essential due to steric or other reasons, since the 2a-4mt molecule where the methyl group is located on the fourth C atom, does not exhibit any activity.

Continuing our work [6,7] on the biological activity of Schiff mono/dibase compounds with S,N-heterocyclic

adducts we report here the anti-inflammatory and antioxidant activities of a new series of Schiff mono/dibase coordination compounds with 2-amino-5-methyl-thiazole (2a-5mt) [6]. (Figure 1). Non-Steroidal anti-inflammatory Drugs (NSAIDs) have a broad spectrum 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 penetrate and enter the interior of membranes [30,31]. Thus, partition coefficients such as R_M values are performed [32,33]

Materials and methods

Materials

All the chemicals used were of analytical grade and commercially available by Merck. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), nordihydroguaiaretic acid (NDGA) are purchased from the Aldrich Chemical Co. Milwaukee, WI, (USA). Soybean Lipoxygenase, linoleic acid sodium salt, NADH, Nitroterazolium Blue (NBT) and indomethacin were obtained from Sigma Chemical, Co. (St. Louis, MO, USA) and carrageenin, type K, was commercially available. For the in vivo experiments, male and female Fischer-344 rats (180–240 g) were used. N-Methylphenazonium-methyl sulfate (PMS) was purchased by Fluka.

Synthesis, analytical data and structural information of the tested coordination compounds are given in references [6,7,34]

Physical measurements

Determination of lipophilicity as R_M values: Reversed phase TLC (RPTLC) was performed on silica gel plates impregnated with 55% (v/v) liquid paraffin in light petroleum ether. For the R_M determination glass plates 20 × 20 cm Kieselgel 60F254 (Merck 5715) were used.

The mobile phase was a methanol/water mixture (75/25, v/v) containing 4% aqueous ammonia (27%) [34]. The plates were developed in closed chromatography tanks saturated with the mobile phase at 24°C. Spots were detected under UV light or by iodine vapours. R_M values were determined from the corresponding R_f values (from 10 individual measurements) using the equation $R_M = \log [(1/R_f) - 1]$. (Table I)

Biological assays

In vitro assays. In the *in vitro* assays each experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean. For the *in vitro* tests a Lambda 20 (Perkin–Elmer) UV–Vis double beam spectrophotometer was used.

Determination of the reducing activity of the stable radical 1,1-diphenyl-picrylhydrazyl (DPPH) [32,33]. Various concentrations (0.1–0.2 mM) of the test compound in DMSO were added to an ethanolic solution of DPPH radical (0.1 mM). The mixture was shaken vigorously and allowed to stand for 20 min or 60 min; absorbance at 517 nm was determined spectrophotometrically and the percentage of activity

was calculated. All tests were undertaken on three replicates and the results were averaged. (Table II)

Competition of the tested compounds with DMSO for hydroxyl radicals [32,33]. The hydroxyl radicals generated by the Fe^{3+} + ascorbic acid system, were detected by the determination of formaldehyde produced from the oxidation of DMSO. The reaction mixture contained EDTA (0.1 mM), Fe^{3+} (167 μ M), DMSO (33 mM) in phosphate buffer (50 mM, pH 7.4), the tested compounds (concentration 0.1 mM) and ascorbic acid (10 mM). After 30 min of incubation (37°C) the reaction was stopped with CCl_3COOH (17% w/v). (Table II)

Soybean lipoxygenase inhibition study in vitro [32,33]. The tested compounds dissolved in DMSO were incubated at room temperature with sodium linoleate (0.1 ml) and 0.2 ml of enzyme solution ($1/9 \times 10^{-4}$ w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor. (Table II)

Non-enzymatic assay of superoxide radicals measurement of superoxide radical scavenging activity [32,33]. The superoxide producing system was set-up by mixing phenazine methylsulfate (PMS), NADH and air – oxygen. The production of superoxide was estimated by the nitroblue tetrazolium method. The reaction mixture containing compounds, 3 μ M PMS, 78 μ M NADH, and 25 μ M NBT in 19 μ M phosphate buffer pH 7.4 was incubated for 2 min at room temperature and the absorption measured at 560 nm against a blank containing PMS. The tested compounds were preincubated for 2 min before adding NADH. (Table II)

In vivo assays

Inhibition of the carrageenin-induced edema [32,33]. Edema was induced in the right hind paw of Fisher 344 rats (150–200 g) by the intradermal injection of 0.1 ml 2% carrageenin in water. Both sexes were used. Females pregnant were excluded. Each group was composed of 6–15 animals. The animals, which have been bred in our laboratory, were housed under standard conditions and received a diet of commercial food pellets and water ad libitum during the maintenance but they were entirely fasted during the experiment period. Our studies were in accordance with recognised guidelines on animal experimentation. The tested complexes 0.01 mmol/kg body weight, were diluted in water and they were given intraperitoneally simultaneously with the carrageenin injection. The rats were euthanized 3.5 h after carrageenin injection. The difference between the weight of the injected and uninjected paws was calculated for each animal. The change in paw weight

Table I. Lipophilicity values: Experimentally determined R_M values of the Cu (II) complexes.

No	Compounds	R_M^a
1*	Cu(dienSS)Cl ₂	– 0.691 (0.062)
2**	Cu(dienSS)(2a5mt)Cl ₂	– 0.332 (0.018)
3*	Cu(dienSS)Br ₂	– 0.630 (0.000)
4**	Cu(dienSS)(2a5mt) Br ₂	– 0.356 (0.007)
5*	Cu(dienOO)Br ₂	– 0.595 (0.038)
6**	Cu(dienOO)(2a5mt) Br ₂	– 0.581 (0.042)
7*	Cu(dienOO)Cl ₂	– 0.590 (0.040)
8**	Cu(dienOO)(2a5mt) Cl ₂	– 0.553 (0.055)
9*	Cu(dienOO)(NO ₃) ₂	– 0.562 (0.043)
10**	Cu(dienOO)(2a5mt) (NO ₃) ₂	– 0.536 (0.042)
11***	Cu(dptaS)Cl ₂	– 0.463 (0.033)
12***	Cu(dptaS)Br ₂	– 0.489 (0.012)
13***[34]	Cu(dptaS)(NO ₃) ₂	– 0.827 (0.039)

^a R_M values are the average of at least 10 measurements; * corresponds to structure I; ** corresponds to structure II; ***corresponds to structure III.

Table II. Antioxidant and antiinflammatory activities of Cu(II) complexes.

Comp	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	LO ^a IC ₅₀ mM % mM	PMS ^b %	%ICPE ^{c,d} (± SEM)
1	31.6	26.9	37.3	44.6	No	0.55	No	nt
2 ^g	78.2	78.9	89.9	88.2	No	0.21	7.8	68.3* (2.03)
3	9.7	15.9	6.0	26.4	No	83% (1 mM) ^e	45.6	nt
4 ^h	87.6	78.6	86.9	77.0	99	0.6	67.4	65.6* (4.10)
5	10.4	16.6	3.8	21.8	No	0.675	78.3	nt
6 ^j	89.2	80.9	92.1	89.6	99.7	0.43	45.6	82.6** (0.60)
7 ⁱ	92.6	85.6	90.8	90.7	No	0.5	9.0	71.7** (1.6)
8 ^k	90.6	85.4	86.9	89.3	No	IC ₅₀ ^e	56.5	58.2** (1.48)
9	15.2	26.8	18.4	30.0	No	0.7	78.3	nt
10	11.8	13.7	15	17.5	No	0.465	46.6	nt
11 ^l	77.1	78.5	90.6	87.8	93.7	0.525	45.6	52.0* (4.10)
12 ^m	86.1	96.3	87.5	81.6	96.1	IC ₅₀ ^f	8.7	59.7** (1.82)
13	39.0	45.4	44.3	40.4	no	0.47	78.3	nt
NDGA	81	80	82.6	80	nt	nt	nt	nt
Trolox	Nt	nt	Nt	nt	88.2	nt	nt	nt
BHT	31.3	52.7	60	78	nt	nt	nt	nt

% Interaction with DPPH (RA %); % Competition with DMSO for hydroxyl radical (HO[•] %); *In vitro* inhibition of soybean lipoxygenase (LO) (IC₅₀); Superoxide anion scavenging activity PMS % (0.1 mM); *In vivo* % inhibition of induced carrageenin rat paw edema (%ICPE) at 0.01 mmol/Kg body weight.

NDGA (Nordihydroguaiaretic acid); BHT (butylated hydroxytoluene) nt: not tested; no: no action under the experimental conditions;

^a caffeic acid 0.6 mM; ^b caffeic acid 86% (1 mM); ^c statistical studies were done with student's T-test, ** p < 0.01, * p < 0.05;

^d Indomethacin as a standard 47% (0.01 mM/Kg = 3.58 mg/Kg); SEM standard error of the mean; ^e 14% (0.1 mM)/38% (1 mM) IC₅₀ values could not be possible to be determined for these complexes; ^f 25% (0.1 mM)/38% (1 mM) IC₅₀ values could not be possible to be determined for these complexes; ^g 5.4 mg/Kg for the *in vivo* experiment; ^h 6.28 mg/kg for the *in vivo* experiment; ⁱ 5.96 mg/kg for the *in vivo* experiment;

^j 3.93 mg/kg for the *in vivo* experiment; ^k 5.08 mg/kg for the *In vivo* experiment; ^l 3.6 mg/kg for the *in vivo* experiment; ^m 4.48 mg/kg for the *in vivo* experiment.

was compared with that in control animals (treated with water) and expressed as a percent inhibition of the edema %ICPE values (Table II and Figure 2). Indomethacin was tested as a reference compound in 0.01 mmol/kg (47%).

%ICPE values are the mean from two different experiments with a standard error of the mean less than 10%.

Results and discussion

The reported compounds were tested for their antioxidant and anti-inflammatory activities.

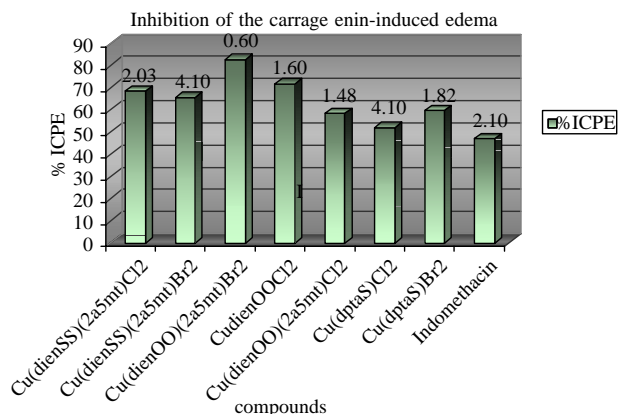


Figure 2. Inhibition of the carrageenin-induced edema.

The model of the scavenging of the stable DPPH radical is widely 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, 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. All compounds were tested for their interaction with the stable free radical DPPH. Butylated hydroxytoluene (BHT) and nordihydroguaiaretic acid (NDGA) were used as reference compounds. This interaction indicates their radical scavenging activity in an iron-free system and expresses their reducing activity. Compounds 2, 4, 6, 7, 8 showed high interactions, whereas compounds 1, 3, 5, 9 and 10 were found to have very low activity. Compound 9 presents the highest activity at 0.1 mM. In general, no changes are observed among the interaction values, concerning time and concentration of the compounds (Table II). Further investigations are in progress in order to attain a detailed study on their interaction with DPPH. The competition of complexes with dimethylsulfoxide (DMSO) for hydroxyl radicals, generated by the Fe³⁺/ascorbic acid system

expressed as the inhibition of formaldehyde production was used for the evaluation of their hydroxyl radical scavenging activity. Compounds **1**, **2**, **3**, **5**, **7**–**10** and **13** did not show any inhibition, whereas compounds **4**, **6**, **11** and **12** inhibited significantly high the DMSO oxidation in concentration 0.1 mM. (Table II)

Non-enzymatic superoxide anion radicals were generated. The superoxide-producing system was set up by mixing phenazine methosulfate (PMS), nicotinamide-adenine-dinucleotide (NADH) and air–oxygen. The production of superoxide was estimated by the nitroblue tetrazolium method. Compound **1** did not show any result whereas compounds **2** and **7** present low activity. All the other compounds exhibit high scavenging activity (45.6–78.3% Table II). Taking under consideration the R_M values, lipophilicity does not positively influence 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. Several findings concerning the correlation of antioxidant activity and the structures of these complexes emerge from Table I. a) For compounds **1**–**10** a factor that should be taken under consideration is the nature of the heterocyclic atom of the Schiff dibases, dien vs. dienSS, dienOO, since the activity of the substituted complexes differs depending on the presence of S or O, atom in the heterocyclic ring, thiophene, or furyl and (b) on the presence of 2a-5mt in the adducts. In general, the latter exhibit higher scavenging activity compared to the starting materials with some exceptions. (c) The observed differences in activity seem to depend partly on the nature of the counter anion, Cl or Br or NO_3 since these can be regarded preferentially as leaving groups. The latter seems to be important for the monobases **11**–**13**.

Compounds **1**–**13** were further evaluated for the inhibition of soybean lipoxygenase (LO) by the UV absorbance-based enzyme assay [33] (Table II). Perusal of % inhibition values or IC_{50} values shows that compound **2** is the most active within the set, followed by compounds **6**, **10** and **13**.

Most of the LO inhibitors are antioxidants or free radical scavengers, since lipoxygenation occurs via a carbon-centered radical. Although lipophilicity is referred to [35] as an important physicochemical property for LO inhibitors, all the above tested derivatives do not follow this concept with the exception of complex **2**. Taking under consideration the R_M values, as an expression of lipophilicity it seems that compound **2** with $R_M = -0.332$ is more potent compared to the rest molecules (Table II). It seems that LO inhibition ($\log 1/\text{IC}_{50}$) *in vitro* is correlated with the superoxide anion scavenging activity $\log \% \text{PMS}$.

$$\log 1/\text{IC}_{50} = -0.464(\pm 0.171) \log \% \text{PMS} \\ + 4.094(\pm 0.291)$$

$$n = 8, r = 0.938, r^2 = 0.881, q^2 = 0.823, \\ s = 0.062, F_{1,6} = 43.82, \alpha = 0.01$$

To assess the anti-inflammatory activity of the complexes, the rat carrageenin 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 is 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 2% carrageenin in water. Complexes **2**, **4**, **6**, **7**, **8**, **11** and **12** presenting the higher DPPH interaction values were tested (0.01 mmol/ml/kg body weight). They were dissolved in water and were given intraperitoneally simultaneously. Compounds **2**, **4**, **6**, **7**, and **12** were found to possess significant protection. The protection ranges from 52.0 to 82.6%. Compound **11** has the lowest effect (52.0%). Lipophilicity, as R_M values, does not seem to affect the biological responses. From our results an attempt has been made to delineate the role of specific structural characteristics correlated with the anti-inflammatory activity. The main differences in terms of structural changes for compounds **1**–**10** are: a) the nature of the heterocyclic aldehyde, b) the nature of the anionic group Y_2 , c) the presence of a dien or dien's Schiff base adducts in the structure. The complexes derived from 2-furaldehyde (compounds **6**, **7**) seem to be more potent. The nature of $\text{Y} = \text{Cl} / \text{Br}$ does not influence the *in vivo* results (compound **2** = 68.5%, compound **4** = 65.6%). However the presence of $\text{Y} = \text{Cl} / \text{Br}$ in the case of the furyl complexes causes a significant change in carrageenin paw edema inhibition (compound **6** = 82.6%, compound **8** = 58.2%). The presence of $\text{Y} = \text{Cl}$ is responsible for this decrease. Perusal of Table II shows that adduct Cu (dienOO) 2a5mtBr₂ is more potent than the corresponding adduct of the type Cu (dienSS) 2a5mtBr₂. Not many changes are observed among the %ICPE protection values of the monobases Schiff complexes formed with the presence of 2-thiophene-carboxaldehyde (compounds **11**, **12**).

Attempts to correlate these expressions of activity with R_M values in a linear or non-linear regression analysis, gave statistically non-significant correlations.

Unfortunately the number of compounds is not enough to calculate a combination of all the effects. The *in vitro/in vivo* activities have not succeeded in providing a clear correlation among all the physico-chemical parameters in a QSAR analysis.

Conclusion

Both antibacterial and antiproliferative studies [6] as well as the anti-inflammatory and antioxidant results showed that adducts exhibit high bioactivity. This means that the insertion of 2a-5mt affects the aggregation of electronic, physicochemical and steric properties of the resulting compounds thus moderating their biological activity. The Cu (II) Schiff mono-bases coordination compounds also exhibit anti-inflammatory activities.

It is of interest that all the tested *in vivo* compounds possess higher protection compared to the reference drug indomethacin in equipotent dose. Taking also under consideration the *in vivo* results, compound 7 as a starting material emerges as new potential prototype whereas compound 6 is a very potent anti-inflammatory agent representing a new lead. The antiradical activity of the tested complexes supports, at least in part, the *in vivo* anti-inflammatory activity.

To study the physiological relevance of our findings, we are currently investigating the most potent inhibitors identified here, in various biological systems. These results will be published in due course.

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