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Original Scientific Article

4-Hydroxy-2-methyl-*N*-(2-thiazole)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide (EX15) and its Cu(II) Complex as New Oxicam Selective Cyclooxygenase-2 Inhibitors

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Abstract. 4-Hydroxy-2-methyl-*N*-(2-thiazole)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide (EX15) as nonsteroidal antiinflammatory drug (NSAIDs) of oxicam family has been synthesized bearing high selectivity for cyclooxygenase-2 (COX-2) inhibition and high ability to chelate with Cu(II) ions. The EX15-Cu(II) complex, and $[Cu(EX15)(OAc)(H_2O)_2]$, were synthesized and characterized by using elemental analysis, spectral (UV-Vis, IR), conductance, thermal and magnetic studies. Two equations were predicted using quantitative structure activity relationship (QSAR) and regression analysis for the COX-2 and COX-1 selectivity (microsomal assay) with a regression correlation (*R*) close to unity. Two techniques were used to investigate the validity of these equations; macrophage cell line (*in vitro*) selectivity and collagen-adjuvant arthritis model in rats (*in vivo*) which showed a significant antioxidant, analgesic and antirheumatic effect for 4-hydroxy-2-methyl-*N*-(2-thiazole)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide and its Cu(II) complex, $[Cu(EX15)(OAc)(H_2O)_2]$. (doi: 10.5562/cca1802)

Keywords: Oxicam; metal complexes; analgesic; rheumatoid; anti-inflammatory

INTRODUCTION

Copper induces an inhibition of PGE2 synthetase leading to a shift in synthesis from the inflammatory PGE2 to anti-inflammatory PGF series.[1] Free radical scavenging is also mediated by the copper containing superoxide dismutase that can protect joint synovial tissue from hydroxyl and superoxide radical anion $O_2^{\bullet -}$ attack.[2] The superoxide radical anion $O_2^{\bullet -}$ combining with NO to form peroxinitrate which is an important modulator of cyclooxygenase activity by transformation of arachidonic acid into inflammatory prostaglandin E2.[3] Copper acetate was found more active than hydrocortisone in the carragenan induced paw edema model. The presence of copper chelate *in vivo* was responsible for the observed anti-inflammatory activity.[4] Similarly, the anti-inflammatory activity of the clinically used antiarthritic agents could be attributed to the formation of copper complex in blood carrying it to the site of inflammation.[5] Walker and Smith[6] have separated plasma into different molecular weight fractions and showed that the species of molecular weight below 500 contain active chemicals against rheumatoid arthritis. These fractions are bonded to serum albumin and their activity is due to a mechanism depending on the release of albumin bound copper in the blood which facilitates its availability at the site of inflammation.

Two anti-inflammatory drugs (piroxicam and meloxicam) are available commercially in the market worldwide. The emergence of selective cyclooxygenase-2 enzyme inhibitors for treatment of inflammatory diseases had motivate us to synthesize a new compound having a high selectivity for cyclooxygenase-2 inhibition and ability to form stable complex with Cu(II) ions. The cyclooxygenase-2 selectivity of the new compound is predicted through QSAR using Hyperchem software.[7] Quantitative observations concerning the anti-inflammatory role of copper drug compounds have been made previously by Sorenson[4] who reported that copper salts of many acidic anti-inflammatory drugs are more

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effective for reducing inflammation. Other trace elements which include zinc and gold were used in treatment of rheumatoid arthritis. 4-Hydroxy-2-methyl-*N*-(2-thiazole)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide (EX15) and its Cu(II) complex with the acetate salt have been selected.

EXPERIMENTAL

All chemicals used were of analytical grade (Sigma, MO, USA) and used as supplied without further purification. Carbon and hydrogen contents were determined at the Microanalytical Unit of Cairo University. Metal analysis was carried out by standard methods.[8] Molar conductance value of the complex ($c = 10^{-3}$ mol dm^{-3}) in DMSO was carried out with YSI model 32 Conductivity Bridge. Infrared spectra were recorded using KBr discs on a Mattson 5000 FTIR spectrometer. Electronic spectra were recorded on a UV_2 Unicam UV/Vis spectrophotometer using one cm stoppard silica cells. A Shimadzu TGA-50H thermal analyzer was used to record the TG and DTG curves. The experiments were carried out in dynamic nitrogen (20 mL min^{-1}) with a heating rate of 10 °C min^{-1} in the temperature range 25 – 1000 °C using platinum crucibles. The sample sizes ranged from 4.65 to 10.52 mg, highly sintered Al_2O_3 was used as a reference.

Synthesis of 4-Hydroxy-2-methyl-*N*-(2-thiazole)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide (EX15)

EX15 was prepared by amidation method of Manjarrez *et al.*[9] as shown in Scheme 1. Brown crystals were precipitated, recrystallized from absolute ethanol and dried in a vacuum desiccator over anhydrous calcium chloride (melting point = 248 °C). The compound is characterized by a mass spectrum which showed a molecular ion peak at $m/z = 337$ $(M–1)^+$.

Preparation of Cu(II)–EX15 Complex

It was prepared by reacting 0.0338 g of EX15 (10 mmol) with 0.02 g of $Cu_2(CH_3COO)_4 \cdot 2H_2O$ (10 mmol) in 30 mL of water-ethanol mixture (volume ratio = 1 : 1). The mixture was heated for five hrs on a water bath. The resulting green precipitate was washed with ethanol and dried (melting point 276 °C).

Quantitative Structure Activity Relationship (QSAR)

The physicochemical properties (descriptors) of the investigated chemical compounds obtained from Hyperchem version 8 program at the semi-empirical theoretical method using AM1 method.[10] These descriptors include the volume, hydration energy, logarithm of partition coefficient and dipole moment on X–Y directions dmx and dmy. Multiregression statistical equations are based on the chemical descriptors data obtained from QSAR investigation.

Semi-empirical Method

The calculation method for commands was placed on the compute menu to semi-empirical quantum mechanics rather than molecular mechanics or *ab initio* quantum mechanics. These calculations solve the Schrödinger equation, with certain approximations, to describe the electron properties of atoms and molecules. In semi-empirical method, the calculations can be simplified by calculating the valence electrons only, neglecting the integrals for certain interactions using standard, non-optimized, and electron orbital basis functions. Experimental parameters eliminate the need to calculate certain quantities and to correct for errors resulting from approximations. This method is applicable and appropriate for all atoms in the periodic table, where the variables are saved in the parameter files. The choice remains until one chooses the molecular mechanics or *ab initio* module. If a file is saved after a semi-empirical calculation, the HIN file will contain the calculated atomic charges.[11]

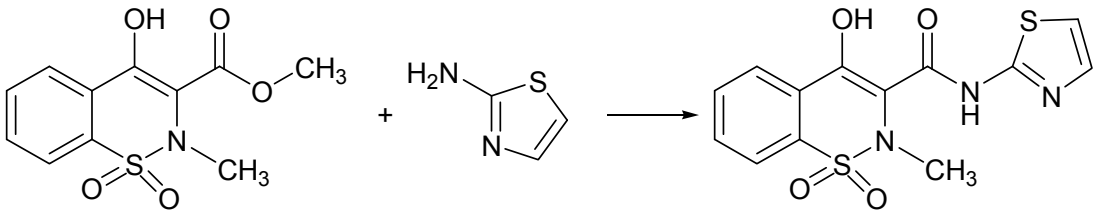
AM1

AM1 is a semi-empirical SCF and a developed MNDO method for chemical calculations.[12] It is useful for molecules containing elements from long rows 1 and 2 of the periodic table, but not transition metals. Together with PM3, AM1 is generally the most accurate semi-empirical method included in Hyperchem, it calculates the electronic properties, optimized geometries, total energy, and heat of formation.

Identification the Biological Activity of the Newly Synthesized Compounds

PGE2 Estimation

The new compound, EX15, copper acetate, piroxicam, and Cu (II) - EX15 complex were tested for their anti-inflammatory effects against COX-2 using the *in vitro* macrophage-like cells (P388Dl). Cells were washed with fresh media and the tested compounds were added in different concentrations to their respective wells in triplicate and incubated for one hour prior to LPS priming. Then, LPS was added to the wells in a final concentration of l00 ng ml^{-1}. then, it was incubated for one hour. After that, the incubated cells were removed and washed with fresh media. Then, 1.0 ml of media containing platelet-activating factor (10 nM ml^{-1}) and cytochalasin B (l μM ml^{-1}) were added. The cells were incubated for four hours and then removed and the supernatant was collected for PGE2 measurement and stored supernatant at –70 °C, till use. The assay was performed using the Titerzy1me Prostaglandin PGE ELISA Kit (Sigma). The procedure and instruction of



Scheme 1. Preparation of EX15.

the manufacturer was followed. Samples were read at 405 nm using a microplate reader. The absorbance of the standard (y axis) versus the log concentration of PGE2 for the standard curve was plotted. The PGE2 concentrations were calculated using the standard curve.[13,14]

Measurement of Joint Inflammation and Pain Tolerance

Thirty six Sprague-Dawley rats (200–250 g) were fed on a standard rat chow and water ad labium. Animal care and experiments were performed in accordance with NIH guide to the care and use of laboratory animals. The local ethical committee approved the study. The animals were divided into two main groups (non arthritic control and arthritic group). In the arthritic group, all rats had been inoculated by the reagent of collagen adjuvant arthritis into the left paw pad. Rats which developed right paw arthritic manifestations after 45 days were divided into five groups as follow, arthritic control, piroxicam treated, EX15 treated, copper acetate treated and copper complex treated group. The compounds were given orally *via* gastric tube once daily in a dose which calculated according to Paget's table for seven days.[15] Most of the previous compounds are insoluble in water, thus it were suspended in 0.5 % sodium carboxymethyl cellulose (CMC) mg per 200 g of rat weight.[15] This model of Collagen–Adjuvant Arthritis[16–18] is considered to be a representative of rheumatoid arthritis or ankylosing spondylitis in humans. Collagen II – Freund's adjuvant emulsion (0.1 mL) was injected intradermally into the left hind foot paw-pad of each rat (if no arthritis developed within four weeks, some of the animals were challenged by a second inoculation). After 45 days, the systemic arthritis developed in both hind paws.[19]

Pain tolerance measurement right paw pad pressure tolerance was determined for assessment of the analgesic activity of the used drugs, pressure was applied by the analgesimeter (Ugo Basile, Italy) on the rat pad of the right paw. The pressure was increased gradually (a certain number of grams per second until the rat either squeaks or tries to withdraw its limb). The force of pressure was continuously monitored by a pointer moving along a linear scale. Increased pressure tolerance of drug treated rats indicates analgesic activity of the administered drug.[20] This measurement of pressure tolerance was done at the 7th day of drug treatment (45 days after complete Freund's adjuvant injection).

Proximal joint (right ankle) mobilization tolerance (pain scoring) was graded from one to four. Grade one corresponds to tolerance of complete flexion 90°; degrees two, three and four correspond to increasing degree of maltolerance according to a rat hind limb withdrawal, squeaking and when the flexion becomes painful. Degree four corresponds to squeaking with just initiation of flexion. Each of the six non-arthritic, non-treated rats had a score of one.[21] This measurement of mobilization tolerance was done at the 7th day of drug treatment (52 days after complete Freund's adjuvant injection).

Measurement of Oxidant Stress and Rheumatoid Factor (RF)

Serum malondialdehyde (MDA) as lipid peroxidation was measured by the thiobarbituric acid (TBA) test.[22] The sample is treated with TBA at low pH, and a pink chromogen is measured. In the TBA reaction, one molecule of MDA reacts with two molecules of TBA with production of a pink pigment with an absorption maximum at λ = 532–535 nm.[23] Rheumatoid Factor (RF) was estimated using sheep erythrocytes sensitized with rabbit gamma-globulin.

Statistical Analysis

The results are expressed as mean ± SE. Multiregression analysis (one way ANOVA, Newman-Keuls and F-test) was used for correlating physicochemical descriptors to the edema inhibition through QSAR and analysis of the pharmacological data. Mann-Whitney test was for comparison purposes between two groups, whereas Kruskal–Wallis test was used to compare more than two groups. P values $\leq$ 0.05 were considered statistically significant.[24]

RESULTS AND DISCUSSION

In this work, QSAR equations have been elaborated to select compounds containing oxicam nucleus that are having high selectivity towards COX-2. Previous investigation of Lazer *et al.*[25] showed 76 derivatives of 1,2-benzothiazines (Figure 1) in which their biological

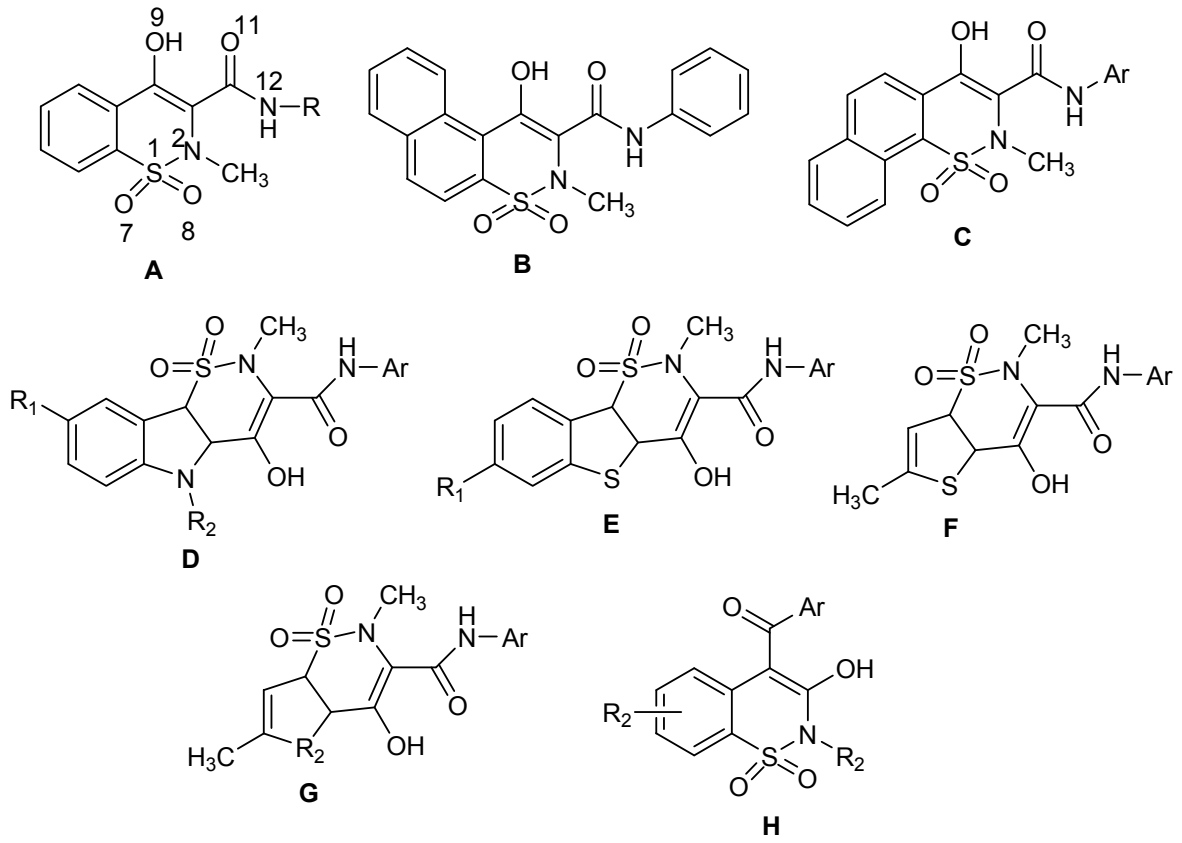


Figure 1. Thiozoline derivatives.

activities were monitored at 1.0 μgmL^{-1}. These chemical compounds were treated individually as NSAID.[25] The present QSAR data based on the chemical structures [25] that are concerned with physicochemical properties (descriptors) of the investigated chemical compounds. These descriptors were acquired using multi-regression statistical calculations together with the known biological activities. It is noted that the data include equations (Table 1) used for calculating the activity (inhibition percentage for COX-1 and COX-2) of the compounds and focusing on the degree of the validity of the two equations and the most descriptors affecting the biological activity (Table 1). These descriptors include the area (*A*), volume (*V*), hydration energy (HE), logarithm of partition coefficient (log *P*), high occupied molecular orbital (HOMO), low unoccupied molecular orbital (LUMO), difference between HOMO and LUMO, dipole moment on X-Y-Z directions (dmx, dmy, dmz), net dipole moment, charge on sulfur [$S_{(1)}$] atom, charge on nitrogen atom (N–CH_3), sum of charges of oxygen atoms on SO_2 [$O_{(7)}$+$O_{(8)}$], charge on phenolate oxygen atom, [$O_{(9)}$], charge on keto oxygen [$O_{(11)}$], and charge on nitrogen atom [$N_{(12)}$] (Scheme 2).

The degree of validity of the two equations is based on calculating the inhibition percentage of COX-1 and COX-2 at 1.0 μg mL^{-1}. The data obtained are monitored with the data of Lazer *et al*;[25] one can notice a great coincidence with the previous results.[25] From calculations, the most important descriptors affecting the percentage of inhibition of COX-1 and COX-2 are

Table 1. Regression analysis reflecting the validity of the proposed two equations

Equation	*F*-Value[(a)]	*P*-Value[(b)]	*R*[(c)]
At 1.0 μg Calcd. % inh. COX-1 = 0.140A + 743HO + 796LU + 700(LU-HO) + 6.40dmx + 6.73dmy – 2.44DMz. + 11DM + 0.14ΔH + 225Q[S(1)] – 1232Q[N(2)] – 2.4Q[O(7) + O(8)] – 99.5Q[O(9)] + 151.8Q[O(11)] – 258.03Q[N(12)] – 1376.62	13.71	< 0.017	0.88
At 1.0 μg Calcd. % inh. COX-2 = 1.17A – 0.518V + 3.53H.E + 6.93dmx + 5.93dmy + 7.38dmz – 0.218DM + 0.05ΔH – 598.9Q[S(1)] – 409.16Q[N(2)] + 3.17[O(7) + O(8)] + 498.3Q[O(11)] + 1494.1	9.22	< 0.001	0.87

[(a)] The degree of freedom.
[(b)] The degree of significance.
[(c)] Regression coefficient.

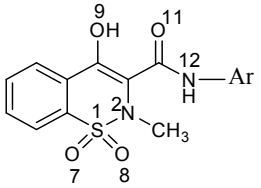
the area of the chemical compounds (Table 1). Such foundations are highly coincident with the reported x-ray data.[26] The calculated data confirm that the area and volume of the active site of COX-2 are larger than that of COX-1. This fact is the backbone of our thinking in constructing a chemical compound occupying the active site of COX-2 at the expense of COX-1.

The descriptors of seventeen postulated structures (1,2-benzothiazine moiety) are examined (Table 2). Also, their inhibition % on COX-2 and COX-1; EX15 was found the best at 1.0 μg, accordingly its preparation and structure elucidation will be fulfilled. EX15 is characterized by elemental analysis; *Anal*. *Calcd*. mass fractions of elements, *w*/%: C = (46.1); H = (3.3), IR spectra: 3400; 3117; 1622; 1601; 1525; 1353 and 1178 [*ν*(OH); *ν*(NH); *ν*(C=O); *ν*(C=C); *ν*(C=N); *δ*(OH) and *ν*(C–O)], and the mass spectrum showed a molecular ion peak at $m/z = 337\ (M-1)^+$.

The reaction between Cu (II) acetate and EX15 gave dark green crystals with melting point of 276 °C. The elemental analysis [*Anal*. *Calcd*. mass fractions of elements, *w*/%: C = 36.7 (36.2); H = 2.5 (2.6); Cu = 18.2 (18.2)] showed a good agreement with the formula [Cu (EX15)(OAc)$(H_2O)_2$]. The complex is stable in air, insoluble in common organic solvents and partially soluble in DMF and DMSO. The IR data reveal that EX15 acts as a monobasic bidentate coordinating through the C=O and the deprotonated C–OH. The coordination was illustrated by the disappearance of the broad band at $\tilde{\nu} = 3400\ cm^{-1}$ due to *ν*(OH) and the observable lower shifts of the *ν*(C=O) and *ν*(C–O) bands, at 1622 and 1178 cm^{-1} in the EX15 spectrum, to 1535 and 1159 cm^{-1} in the complex with complete obscure of *ν*(OH) band at 1353 cm^{-1} (Ref. 27). The acetate group acts as a bidentate ligand by the appearance of two bands at 1513 and 1400 cm^{-1} with a difference of 130 cm^{-1} (Ref. 28). The appearance of new bands assigned to *ν*(M←OH_2) at 580 cm^{-1} and *ν*(M–O) at 440 cm^{-1} supports the suggested coordination sites.[29] The *ν*(C=N) observed at 1525 cm^{-1} in the EX15 spectrum still at the same position in the complex spectrum, but with high intensity. Previous work reported its participation.[30]

The TG thermogram of [Cu(EX15)(OAc)$(H_2O)_2$] shows a thermal stability till 221 °C. A decomposition stage at 222–276 °C with weight loss of 5.5 (*Anal*. *Calcd*. 7.3 %) is attributed to the removal of the coordi-

Table 2. The calculated biological activity by the predictable two equations which are concerned with the descriptors of the speculated chemical compounds in Table 4

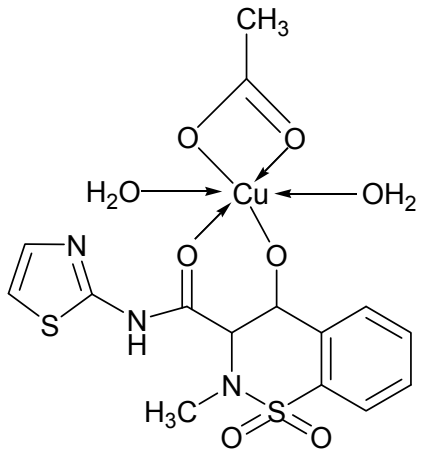


Compound No.	Speculated molecule Ar	1.0 μg COX-2	1.0 μg COX-1
1	2-Aminobenzothiozolyl	12.21	54.07
2	3-Amino-5-methylthio-1*H*-1,2,4-triazolyl	37.73	54.01
3	2-Aminopicoline	–41	3.9
4	4-Ethylthiosemicarbazide	32.69	17.74
5	4-Phenylthiosemicarbazide	11.56	17.3
6	5-Aminotetrazolol	–34.8	123
7	4-Aminophenylalcohol	–17.7	6.78
8	2-Aminoacetophenone	3.164	18.65
9	3-Amino-1,2,4-triazole-5-carboxyl	–16.6	1.56
10	2,4-Diamio-1,3,4-thiadiazolol	–4.31	19.81
11	2-(2-Aminophenyl)indole	–20.5	46.25
12	2-Amino-1,3,4-thiadizole	–10.4	17.35
13	2-Amino-4-phenylthiazole	51.52	–73.2
14	7-Amino-4-(trifluoromethyl)coumarin	0.31	38.55
15	**4-Hydroxy-2-methyl-*N*-(2-thiazole)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide**	**39.3**	**13.4**
16	1-Amino-4-metyl-piperazyl	15.59	31.01
17	5-Amino-1,3,4 thiadiazole-2-thiol	–11.8	19.4

nated water ($2H_2O$). The electronic spectrum of [Cu(EX15)(OAc) $(H_2O)_2$] exhibits two bands , in nujol, at 12 980 and 20 000 cm^{-1} assigned to $^2T_{2g} \rightarrow {}^2E_g$ and ligand-metal charge transfer. The broadness of the first band may be due to Jahn-Teller effect which enhances the distortion in octahedral geometry.[31] The magnetic moment value (1.85 BM) is consistent with the presence of one unpaired electron in an octahedral geometry.[32]

P388D1 cell line was selected because of its ability to induce COX-2 and to produce PGE2 upon stimulation with bacterial lipopolysaccharide (LPS) and platelet-activating factor (PAF). Moreover, it has a short doubling time (28 h). Arachidonic acid must first be released from phospholipid stores by the action of phospholipases (PLA).[13] Macrophages deacetylate arachidonic acid *via* the action of phospholipase A_2. The macrophage cell line used in this experiment is beneficial because it contains several PLA2 enzymes.[14] The data in Table 3 show that EX15 treated macrophage culture has an inhibitory effect on PGE2 production. This inhibitory effect was maintained at high concentration like the medium concentration; a pattern of response is similar to that obtained with meloxicam which is indicative of COX-2 selectivity.[33] The pharmacological COX-2 selectivity of EX15 is in accordance with the suggested chemicals through QSAR descriptors especially molecular area (Table 4).[26,34] It is suggested that the EX15 molecule is more powerful to be a selective COX-2 inhibitory than the meloxicam (the standard COX-2 inhibitor). The more descriptors for COX-2 selectivity were the molecular area and log *P* (as indicator of lipid solubility) (Table 4). The larger the molecular area and the higher the lipid solubility the greater will be the COX-2 selectivity. Also, EX15 was found selective than meloxicam in accordance with the *in vitro* data (Table 5).

The pharmacological activity of [Cu(EX15)(OAc) $(H_2O)_2$] has been tested (Table 6). The distinguished copper associability and dissociability of EX15 as indicated chemically ($O_{(9)}$ and $O_{(11)}$ charges) could be encouraging to study the effect of copper on the degree of



Scheme 2. [Cu(EX15)(OAc)$(H_2O)_2$].

COX-2 selectivity of EX15 together with its anti-inflammatory effect evaluation. Also, the pharmacologically observed potency of copper acetate and [Cu(EX15)(OAc)$(H_2O)_2$] complex could be of an additional encouragement to extend the study of EX15 to cupper complex. This distinguished copper associability of EX15 was chemically expected early through QSAR (Table 4) which indicates that Q[$O_{(9)}$] and Q[$O_{(11)}$] electrical charge of oxygen number 9 and 11 could be considered theoretically as indicator of metal legibility; the greater charge the stronger the legibility. This theoretical suggestion has been confirmed through the infrared spectral characterization of [Cu (EX15)(OAc)$(H_2O)_2$]. The electrical charges on $O_{(9)}$ and $O_{(11)}$ of EX15 molecule, are of sufficient strength which can permit high coordination and dissociability.

The anti-inflammatory action of Cu(II) complex may result from the redox activity of copper(II), in particular its ability to scavenge the highly reactive pro-inflammatory superoxide radical anion $O_2^{\cdot -}$ (Refs. 35 and 36). [Cu (EX15) (OAc)$(H_2O)_2$] has a concomitant near normal degree of big joint manipulation tolerance and normal numeric pad pressure tolerance (Table 6). The results are in accordance with the reported re-

Table 3. Effect of three doses of piroxicam, and EX15 (oxicam derivative) at three doses on the PGE-2 levels in macrophage like cell line (M ± SD, $n = 3$)

Non Prime	Prime	Dose	Copper acetate	Piroxicam (Market)	Meloxicam	EX15
66.87 ± 99.1	1704.99 ± 99.1[(a)]	1 ug	312 ± 63[(b)]	1127 ± 400	51.18 ± 15.2[(b)]	63.16 ± 17.15[(b)]
		10 ug	287 ± 33[(b)]	173 ± 41[(b),(c)]	21.28 ± 15.2[(b)]	16.69 ± 17.15[(b)]
		100 ug	345 ± 79[(b)]	53 ± 7[(b),(c),(d)]	35.5 ± 15.2[(b)]	34.72 ± 17.15[(b)]

Prime Macrophage; stimulation by lipopolysaccheride (LPS):
[(a)] Significant from the non-prime $P < 0.05$.
[(b)] Significant from the prime $P < 0.05$.
[(c)] Significant from 1μg/mL effect $P < 0.05$.
[(d)] Significant from 10μg/mL effect $P < 0.05$.

Table 4. Regression analysis reflecting the validity of the proposed two equations

Descriptor	EX15	Melox.	Pirox.
Area	503	531.3	486.4
Volume	826	882.3	882.6
H.E.	–13	–11.81	11.07
Log *P*	–3	–2.67	–2.51
HOMO	–9.1	–9.06	–8.86
LUMO	–1.2	–1.39	–0.90
HOMO-LUMO	7.92	7.67	7.96
dmx	3.97	4.35	2.29
dmy	–2.4	–2.31	0.60
dmz	–3.9	–4.08	0.37
dm	6.06	6.40	2.39
Δ*H*	–34	41.42	–43.03
QS1	2.86	2.86	2.86
QN2	–0.72	–0.73	–0.73
QO7 + QO8	1.87	–1.84	–1.86
QO9	–0.26	–0.25	–0.25
QO11	–0.28	–0.25	–0.37
QN12	–0.29	0.00	–0.31

LUMO, lowest unoccupied molecular orbital; HOMO, highest occupied molecular orbital; H.E, hydration energy; Log *P*, Log of calculated octanol-water partition coefficient; dmx (dipole x), dipole moment in *X* direction; dmz, dipole moment in *Z* direction; dm (total dipole), polarization magnitude of dipole moment; Δ*H*, difference between heats of formation COSMO and vacuum.

sults for complexation of NSAIDs with Cu(II), Zn(II), Cd(II) and Pt(II) ions to produce safer NSAIDs.[37–39]

EX15 and its Cu (II) complex have antioxidant activity as indicated by their lowering effect on serum malonaldehyde level (Table 6) as compared to copper acetate treated groups of rats subjected to collagen adjuvant. The antioxidant activity of piroxicam had been proved by Pipe *et al*.[40] The mechanism operating for the antioxidant activity of the generated copper acetate complex and piroxicam in the body involves redox cycling at the metal center. The anti-inflammatory improvement of Cu (II) complex was clearly observed from the data obtained when RF was measured in the treated group (Table 6). Copper complex has mobilization tolerance as indicated by its lowering effect as compared to arthritic non treated group of rats subjected to collagen adjuvant. The RF for the Cu (II) complex has lower effect when compared with arthritic non treated group (Table 6).

Table 5. Cyclooxogenase inhibition power for meloxicam, piroxicam, and EX15 as theoretically determined through our QSAR equations and experimentally as reported earlier.[25]

	% Inh. COX at 1ug drugs			
	COX-2		COX-1	
	Found	Calcd.	Found	Calcd.
Meloxicam	72	69.61	–1	7.12
Piroxicam	58	20.92	10	10.07
EX15	23	39	17	13.4

CONCLUSION

EX15 has been synthesized bearing two advantages; high selectivity for COX-2 inhibition and high ability to chelate with Cu (II) ions. The experimental testing of these compounds (EX15 and the copper complex [Cu(EX15)(OAc)(H_2O)$_2$]) revealed a significant antioxidant, analgesic and anti-rheumatic effects.

Table 6. Influence of copper acetate, piroxicam and [Cu(EX15)(OAc)(H_2O)$_2$] complex on MDA, rheumatoid factor, pain tolerance, and mobilization tolerance in adjuvant induced rheumatoid arthritis model in rats (M ± SE)

Group	Dose (expressed in mg 200 g^{-1}) according to Paget's Table[30]	Pain tolerance *n* = 6	Proximal joint mobilization tolerance *n* = 6	MDA / nmol mL^{-1} *n* = 6	RF (expressed in IU mL^{-1}) *n* = 6
Non-arthritic non-treated	[SCMC] Solvent	1.0 ± 0.00	1.48 ± 1.18	2.74 ± 2.1	178 ± 2.1$^{(c)}$
Arthritic non-treated	[SCMC] Solvent	3.9 ± 0.10$^{(a),(c)}$	3.15 ± 0.55^{+}	4.0 ± 0.39$^{(a)}$	528 ± 71.0$^{(a),(c)}$
$Cu(Ac)_2 \cdot 2H_2O$	0.22	2.5 ± 0.09$^{(a),(b),(c)}$	2.58 ± 0.15	2.8 ± 0.33	385 ± 21.0$^{(a),(b),(c)}$
Piroxicam	0.36	2.1 ± 0.12$^{(a),(b)}$	2.07 ± 0.28$^{(b)}$	2.8 ± 0.2	325 ± 10.0$^{(a),(b)}$
EX15	0.36	2.8 ± 0.14$^{(a),(b),(c)}$	2.58 ± 0.6	1.5 ± 0.42$^{(a),(b),(c)}$	258 ± 31.0$^{(a),(b),(c)}$
Cu complex	0.96	1.7 ± 0.09$^{(a),(b),(c)}$	1.67 ± 0.11$^{(b)}$	1.8 ± 0.42$^{(a),(b),(c)}$	190 ± 9.0$^{(a),(b),(c)}$

n, number of rats; SCMC, 0.5 % sodium carboxymethyl cellulose.
(a) $P < 0.001$ *vs*. non-arthritic control.
(b) $P < 0.01$ *vs*. arthritic control.
(c) $P < 0.01$ *vs*. piroxicam.

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