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Synthesis And Antiinflamation Activity Of Bis(Diacetylmonoxime) Thiocarbohydrazone And Its Cu(II) Complex

Corresponding Author

Ahmed A.El-Asmy
Chemistry Department, Faculty of Science, Mansoura University, Mansoura, (EGYPT)
E-mail: aelasmy@yahoo.com

Co-Authors

Gamil A.Al-Hazmi[1], Yousery E.Sherif[2], Sami A.Gabr[2]
[1]Chemistry Department, Faculty of Science, Mansoura University, Mansoura, (EGYPT)
[2]Chemistry Department, Faculty of Science, Taiz University, Taiz, (YEMEN)

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ABSTRACT

Some thiosemicarbazone derivatives have been prepared, characterized and predicted through quantitative structure activity relationship (QSAR) to show their inhibition of cyclooxygenase-2-enzyme(COX-2). Bis(diacetylmonoxime) thicarbohydrazone[H_3DMT] was found more analgesic and ulceroggenic. The descriptors are mainly derived from molecular orbital calculations by the semiemperical AM1 method. The inhibition activity was found to depend on polarizability of the molecule. The study herein is focused on testing the inflammatory activity of Cu(II) acetate, H_3DMT and [$Cu_2(HDMT)_2(H_2O)$] using collagen–adjuvant arthritis model in rats. The observed anti-inflammatory of H_3DMT and [$Cu_2(HDMT)_2(H_2O)$] makes an importance for future profiting due to their ability to COX-2 inhibition and the high metal legibility.

INTRODUCTION

Rheumatoid arthritis(RA) is a chronic multi system disease of unknown etiology[1]. Although, there are a variety of systemic manifestations, the characteristic feature of RA is persistent inflammatory synovitis, usually involving peripheral joints in a symmetric distribution[1,2]. The discovery of a new isoform of cyclooxygenase(COX-2) has stimulated renewed interest in the field of nonsteroidal anti-inflammatory drugs(NSAIDs) due to their ability to inhibit the enzyme cyclooxygenase, which catalyzes the transformation of arachidonic acid to prostagalandin(PGH_2), the key step in the biosynthesis of prostaglandins(PGs)[3]. Because of the prominent situation of PGs in the inflammatory process and

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the greater understanding of the mechanism of NSAIDs, the development of selective COX-2 inhibitors gets an interest[4]. The identification and molecular biological characterization of COX-2 in inflammatory cells led to the hypothesis that a selective inhibition of COX-2 would result in relief of inflammation and pain without causing the COX-1 dependent side effects(gastrointestinal ulceration, platelet dysfunction, kidney damage) of conventional NS AIDs[5]. Many important drugs are possible to use the knowledge of the three-dimensional structures as a good basis for COX-2 inhibitor drug design[6]. Thiosemicarbazones and their metal complexes are well interesting because of their anticarcinogenic, antibacterial, and antifungal properties[7,8]. Bis (salicylato) copper(II) protects the synovial fluid from hydroxyl and superoxide radical attack[9-12]. Even the widespread hypotheses based upon prostaglandins can encompass a role for copper[13], quantitative observations concerning the anti-inflammatory role of copper drug compounds indicated that salts of many anti-inflammatory drugs are more effective in reducing inflammation than the acids. Also, the Cu(II) complexes have antiulcer activity than that of the acid[14-16]. The biological activity of certain thiosemicarbazones is due to their ability to form chelates with transition metal ions[17]. Biological activities may also related to the redox properties including oxidation and reduction of the central metal ion and the ligands[18]. The QSAR study based on molecular orbital calculations for a series of thiosemi- carbazones has performed to predict the biological activity for the promising COX-2 inhibitors. In a group of thiosemicarbazones, it was shown that COX-2 inhibitory and other reactivity indices from a COSOMO AM1 calculation[19].

EXPERIMENTAL

All chemicals used were of analytical reagent grade(Sigma) and used as supplied. Piroxicam (Sigma), H_3DMT, copper acetate and $[Cu_2(HDMT)_2(H_2O)]$ were suspended in 0.5% sodium carboxy methyl cellulose and sonicated for 20 min. The determined doses were injected intraperitoneally.

Synthesis of ligand

H_3DMT was prepared by mixing 6.05g of diacetylmonoxime with 3.18g of thiocarbohydrazide (2:1 ratio) dissolved in 10ml glacial acetic acid. The reaction mixture was heated under reflux on a water bath for 10h. After cooling, a brown precipitate was separated, filtered off, crystallized from ethanol and dried in a desiccator; its melting point is 130^0C. The proposed formula of the prepared ligand is in good agreement with the stoichiometry concluded from its analytical data and mass spectrum and confirmed through its IR spectrum.

Synthesis of $[Cu_2(HDMT)_2(H_2O)]$

The complex was prepared as previously mentioned[20] by mixing equimolar amounts(3 mmol) of H_3DMT and the acetate salt of Cu(II) in aqueous-ethanol solution. The solid complex was formed after 3h of heating under reflux, directly. The isolated complex was filtered off, washed several times with hot ethanol, dried and preserved.

Animals

Thirty six rats(Albino), weighting around 200gm each, were housed under similar standard laboratory conditions. The animals were divided into three groups(control, arthritic and arthritic treated). In 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 seven groups and subjected for drug treatments.

Arthritis model

This is a modified form of the previous models[21]. This model of collagen-adjuvant arthritis[22,23] is considered a nearby representative of rheumatoid arthritis or ankylosing spondylitis in human. Collagen II Freund's adjuvant emulsion(0.1ml) was injected intradermally into the left hind foot paw-pad of each rat (if no arthritis developed within 4 weeks, some of the animals were challenged by a second inoculation). After 45 days, the systemic arthritis developed in both hind paws[24].

Measurement of joint inflammation and pain tolerance

Scoring of joint inflammation and pain tolerance

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were based on severity and extent of the erythema and edema of the periarticular tissue, and the enlargement, distortion or ankylosis of the joints. Its inflammation was graded from 1 to 4[25].

Measurement of antioxidant activity

Serum malondialdehyde(MDA) as lipid peroxidation was measured by the thiobarbituric acid(TBA) test[26,27]. The sample under test 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 the production of a pink pigment with an absorption maximum at 532-535 nm[27].

The copper content in serum is estimated by atomic absorption spectroscopy(AAS) without pretreatment[28].

Measurement of rheumatoid factor(RF)

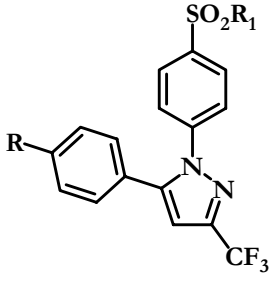
It was estimated using sheep erythrocytes sensitized with rabbit gamma-globulin[29].

Statistical analysis

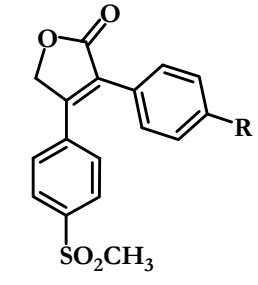
The results are expressed as mean standard errors. Multiregression analysis was used for correlating the descriptors to the COX inhibition through QSAR[30] and analysis of the pharmacological data.

RESULTS AND DISCUSSION

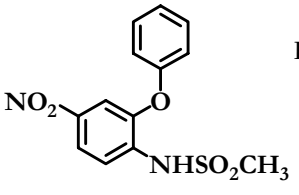
Quantitative structure activity relationship (QSAR)



1 R= Br , R_1= NH_2



2 R=CH_3 , R_1= NH_2



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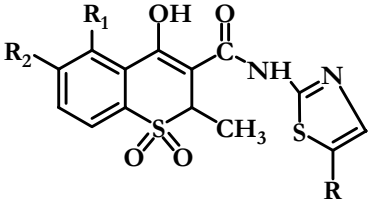
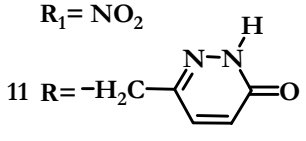
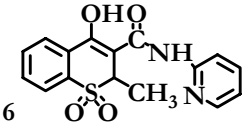


4

5 R=CH_3 , R_1= R_2 = H
10 R = R_2 = -$CH(CH_3)_2$
R_1= NO_2

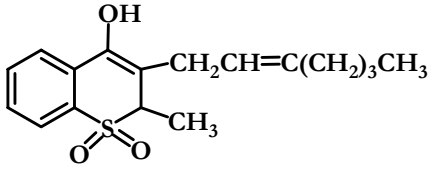


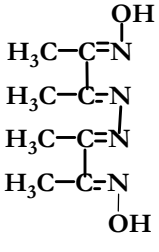
11 R= $-H_2C$ O R_1 = R_2 = H



6

7 R = $(CH_2)_2COCH_3$
8 R = $CO(CH_2)_2CH_3$
9 R = -$CH(CH_3)COOH$





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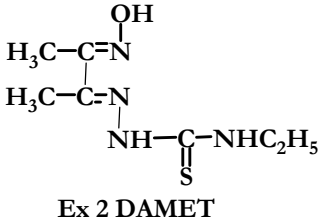

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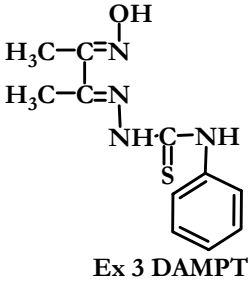

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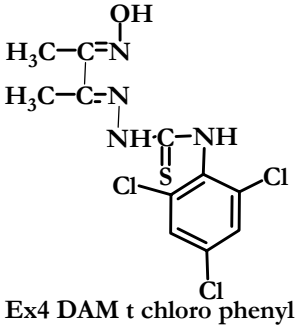
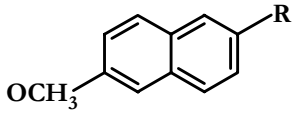
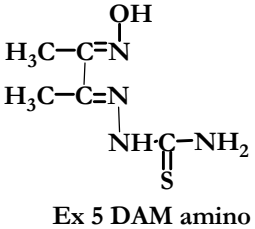
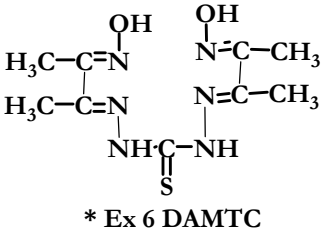


Ex4 DAM t chloro phenyl

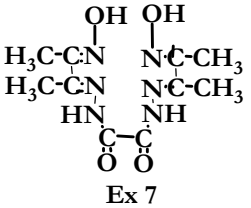




Ex 5 DAM amino



* Ex 6 DAMTC



Ex 7

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New selective inhibitor for COX-2 was developed through QSAR molecular modeling studies[31] which are the preferential COX-2 inhibitory activity of some nonsteroidal anti-inflammatory agents in terms of intermolecular energy using biosystem programs. However, once the drug has passed through the gate of COX-2 or COX-1, intermolecular energy can clearly explain the binding efficiency and selectivity of COX inhibitors. A favorable complex is one which does not disturb the native protein to a less stable form. A more negative intermolecular energy signifies that the attractive force is more than the repulsive force and the molecule is in a minimum energy conformation. Based upon these considerations, Fabiola et al[31] proposed analogues compounds to meloxicam (Figure 1 and TABLE 1) likely having better COX-2 selectivity.

TABLE 1: Intermolecular energies(kcal/mol) between inhibitor and COX[12]

Fabiola et al Compounds	COX-1	COX-2
1	-53	-57
2	-50	-56
3	-47	-47
4	-35	-38
5	-40	-44
6	-46	-41
7	-34	-35
8	-32	-33
9	-29	-36
10	-48	-56
11	-46	-56
12	-41	-46
13	-46	-57
14	-42	-48

TABLE 2: Calculated descriptors for the investigated compounds by hyperchem

Fibiola et al compounds	Surface energy	Volume	Hydration ion energy	Log P	Refractive index	Polarization	Mass
1	571	875	-14.40	0.55	101.70	29.6	437.0
2	573	861	-13.60	0.66	98.40	28.8	372.0
3	597	799	-7.70	3.27	86.60	25.7	304.0
4	614	776	-12.50	-2.86	82.15	23.8	301.0
5	559	835	-1.67	-1.41	89.27	29.4	346.0
6	589	789	-9.50	1.63	85.00	26.7	323.0
7	592	702	-1.62	0.22	68.00	22.7	218.0
8	590	697	-1.62	0.92	68.50	23.0	218.2
9	504	672	-8.17	0.02	61.70	22.5	223.0
10	700	1118	-10.00	-3.00	111.50	41.0	461.5
11	608	944	-16.30	-0.85	107.00	36.3	426.0
12	487	959	-13.00	-1.34	98.40	34.0	414.0
13	663	1158	- 9.61	-0.12	119.00	43.3	473.0
14	609	769	-6.35	-6.72	74.30	223.0	278.0

In the present work, the data obtained from QSAR based on the chemical structures delivered from TABLE 1 have been used to calculate the physicochemical properties of the investigated compounds (TABLE 2). The descriptors include the area, volume, hydration energy, logarithm of partition coefficient and polarizability which are obtained from hyperchem version 7 calculated programs[32] at the semiemperical theoretical method. Fruitful descriptions are gained in using multi-regression statistical calculations in winks program 4.65[33] feeding with intramolecular energy, previously mentioned in TABLE 1 and 2. It is noted that, the data obtained (TABLE 3) include equations used for calculating intramolecular energy(corresponding to inhibition percentage for COX-1 and COX-2) of the compounds as well as focusing on the most chief descriptors(TABLE 4). Accordingly, two equations had been obtained.

Intermolecular energy (kcal/mol) COX-1 = -0.128 mass - 0.0816 polarization - 0.0942 refractive index - 1.742 Log P - 0.0529 hydration energy + 0.0547 volume - 0.0408 surface - 10.513

Intermolecular energy (kcal mol) COX- 2 = -0.047 mass - 0.0797

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TABLE 3: Theoretical(calculated by two equations) and Biosym. Program COX-1 and COX-2 degrees of inhibition

Fabiola et al. compounds	Intra COX-1	Intra COX-2 cal	Intra COX-2	Intra COX-1cal
1	-53	-56.0	-57	-54.1
2	-50	-51.9	-56	-46.5
3	-47	-46.6	-47	-45.6
4	-35	-40.9	-38	-35.7
5	-40	-43.8	-44	-40.2
6	-46	-46.1	-41	-45.3
7	-34	-33.7	-35	-32.7
8	-32	-34.5	-33	-34.2
9	-29	-32.9	-36	-30.1
10	-48	-53.8	-56	-45.1
11	-46	-55.9	-56	-48.9
12	-41	-50.3	-47	-39.9
13	-45	-58.7	-57	-48.8
14	-42	-48.5	-48	-42.0

polarization - 0.3539 refractive index -0.86 LogP + 0.1960529 hydration energy + 0.018 volume - 0.0044 surface - 7.057

The scanning tool used to investigate the validity of the two equations is the regression value, R, and the degree of closeness of R to unity which means more precise and accurate results. As shown in TABLE 5, the value of R is near to unity reflecting more validity of the proposed two equations where the F and P values are highly close to biosystem programs. From calculations, the most important descriptors affecting the intermolecular energies between inhibitor and COX-2 are the polarizability(TABLE 4).

According to the facts obtained from applying Hyperchem programs[31], the descriptors of seven newly thiosemicarbazone compounds are examined (TABLE 6). Taking into account these data and applying the two equations, the inhibition percentage of COX-2 and COX-1 of the seven compounds is calculated(TABLE 7). Among these, H_3DMT (Ex6) was found the best. Accordingly, its preparation and structure elucidation will be fulfilled.

TABLE 4: The most important physicochemical descriptors affecting the intermolecular energies (kcal/mol) between inhibitor and COX by the predictable two equations indicated by p- and t-values according to Hyperchem & Winks programs

95% Confidence Using Intervel At 10ug Drug			
COX -1		COX -2	
Polarzability		Polarzability	
t-value	p-value	t-value	p-value
<-3.3	<0.016	-2.6	<0.041

TABLE 5: Regression analysis reflecting the validity of the proposed two equations

	F-value	P-value	R
Equation 1 COX-2	10.36	< 0.006	0.922
Equation 2 COX-1	10.44	< 0.005	0.924

F, P and R are the degree of freedom, the degree of significance and regression coefficient

Characterization of $[Cu_2(HDMT)_2(H_2O)]$

The isolated complex is stable in air, insoluble in common organic solvents and partially soluble in DMF and DMSO. The elemental analysis, color and melting points together with the formula weights obtained from mass spectra support structure 1. The IR data reveal that H_3DMT acts as a dibasic pentadentate which coordinates through the $C=N_{hydrazone}$ and $C=N_{oxime}$ groups. The thiol group is being the fifth coordination site bridged two molecules. The coordinate bonds were illustrated by the observable lower shift in $\nu(C=N_{hydrazone})$ band from 1627 to 1541 cm^{-1}, while the two covalent bonds were formed through

TABLE 6: Calculated physicochemical descriptors of 7 newly speculated chemical compounds

Compound	Surface energy	Volume	Hydration energy	Log P	Refractive index	Polarization	Mass
Ex 1	311.0	566.0	-22.80	1.88	46.73	18.46	191.00
Ex 2	362.0	574.7	-14.93	2.67	49.8	19.30	197.20
Ex 3	478.0	717.0	-15.46	2.65	72.00	25.80	245.30
Ex 4	502.8	854.5	-14.90	2.33	88.84	32.93	351.60
Ex 5	298.3	542.0	-19.20	1.74	45.00	17.82	175.23
Ex 6	446.0	806.5	-23.80	4.68	72.00	28.25	272.30
Ex 7	429.0	811.0	-15.60	2.70	69.00	27.00	284.30

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TABLE 7: The calculated intermolecular energies (kcal/mol) between inhibitor and COX by the predictable two equations which are concerned with the descriptors of the speculated chemical compounds in TABLE 6

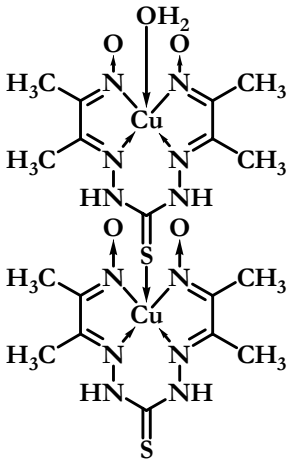
Compound	Intra COX-1cal	Intra COX-2 cal
1	-24.6669	-31.3090
2	-29.2158	-31.9585
3	-34.8803	-40.6295
4	-43.6176	-49.4027
5	-23.1744	-29.4547
6	-35.4303	-43.7224
7	-32.6263	-39.6593

the $C=N_{oxime}$ groups, which bonded after the deprotonation of oxime OH protons and supported by the lower(72 cm^{-1}) and higher(130 cm^{-1}) shifts of both $\nu(C=N_{oxime})$ and $\nu(NO)$ bands, respectively with complete obscure of $\delta(OH)$ band. The lower shift of $\nu(C=S)$ supports the coordination of the ligand in its thione form. The appearance of new bands assigned to $\nu(M-OH_2)$, $\nu(M-N)$ and $\nu(M-S)$ supports the suggested coordination sites.

The ESR spectrum of $[Cu_2(HDMT)_2(H_2O)]$ exhibits an axially symmetric g-tensor parameters with $g\| > g\perp > 2.0023$ indicating that $d_{x^2-y^2}$ is the ground state. The $g\| > 2.26) > g\perp$ (2.06) > g_o (2.0023) agrees with a square pyramidal geometry of CuN_4O coordination. A band corresponding to the forbidden magnetic dipolar transition is observed at half-field (ca. 1500 G, g=4.0), but the intensity is very weak, indicating that the complex is binuclear. In axial symmetry, the $G[=(g\|-2)/(g\perp-2)]$ value is 4.3 suggesting the absence of exchange coupling between the Cu(II) centers in the solid state.

The TG thermogram of $[Cu_2(HDMT)_2(H_2O)]$ shows a thermal stability till 184^0C. A decomposition stage with weight loss of 30.0(Calcd. 31.5%) is attributed to the removal of $H_2O+8(C_4H_6)$ as a ligand terminal part.

A square pyramid structure is proposed for $[Cu_2(HDMT)_2(H_2O)]$. This is based on the appearance of a distinguished band at 12230 cm^{-1} assigned to the $^2B_1 \rightarrow {}^2E$ transition. The magnetic moment value (2.1 B.M.) recorded for each copper atom in the complex lies within the range reported for the d^9 system containing one unpaired electron. This value excludes



Structure of $[Cu_2(HDMT)_2(H_2O)]$

the metal-metal interaction even the presence of two nuclear atoms in the complex sphere; this phenomenon may be due to the parallel arrangement of the two atoms in the complex.

Pharmacological evaluation of H_3DMT and $[Cu_2(HDMT)_2(H_2O)]$

The major ulcerogenic effect of acidic NSAIDs includes topical irritation of the gastric epithelium and the suppression of prostaglandins biosynthesis.

The complexation of NSAIDs with metal ions has been reported to produce safe NSAIDs[34]. Fiabane and Williams[35] studied the acidic drug-serum albumin Cu(II) interactions using visible spectrophotometry and molecular filtration. They suggested that antirheumatoid drugs may function by releasing Cu(II) from serum albumin either by a direct competitive complexing mechanism or through a remote mechanism whereby the drug becomes bonded to a site some distance from Cu(II) and facilitates its release through allosteric effects. Physicochemical studies and animal screens conclude that indomethacin, naproxen, ketoprofen and fenoprofen are capable of releasing Cu(II) remotely to a low molecular weight pharmacoactive molecules whereas phenylbutazone is incapable. The author observation suggested that copper supplementation is desirable during the treatment of rheumatoid arthritis[35]. Administered anti-inflammatory agent either being capable of complexing with Cu(II) or not increases the concentration of low molecular weight plasma protein molecules which form pharmacoactive copper complexes in plasma

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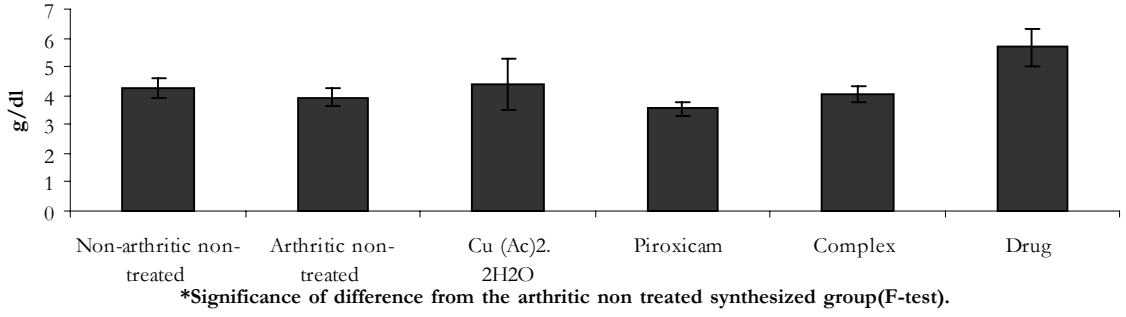
presumably from the circulating pool of serum albumin bound copper.

In the present study, the data obtained for H_3DMT treated group seemed to have a higher effect on copper release in serum of treated rats than of $[Cu_2(HDMT)_2(H_2O)]$; the later showed a lower effect in serum complex which comprises a higher release of serum copper content of treated rats when compared with control group (Figure 2), However, a close similarity was observed between $[Cu_2(HDMT)_2(H_2O)]$ and piroxicam on serum copper level. Also, the drug showed a higher affinity to serum copper when compared with piroxicam (Figure 2). In group of rats treated with H_3DMT, the drug showed a higher content of serum albumin when compared with $[Cu_2(HDMT)_2(H_2O)]$ and piroxicam (Figure 3). This may be related to that serum albumin was the normal carrier of the circulating copper. Albumin has been described as a circulating metal ion blotter or reservoir that accepts or yields metal ions upon demand[36]. Serum albumin possesses 190 groups which are capable of being protonated and has a molecular weight of 67,000. Thus, it is capable of behaving as an immense ligand having more electron donor groups[36]. The data support the higher affinity of H_3DMT for copper in serum complex which facilitates copper transfer to the inflammation sites more efficiency than the $[Cu_2(HDMT)_2(H_2O)]$.

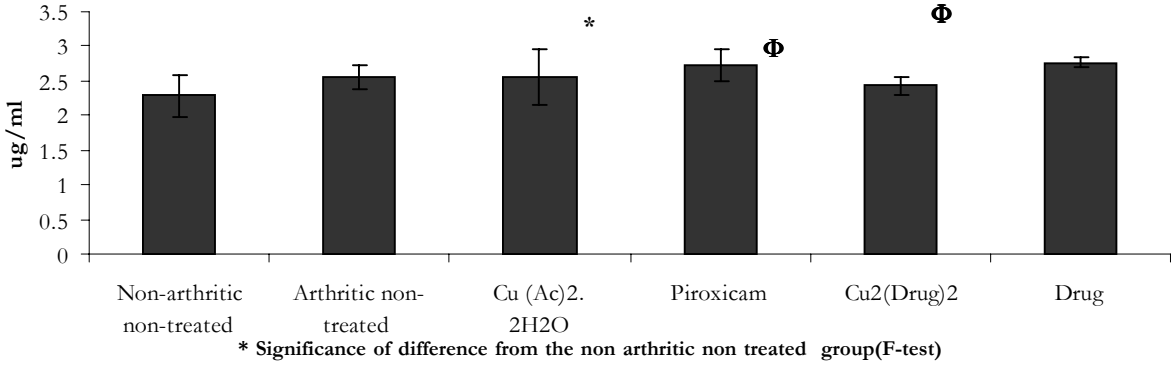
MDA was measured as a lipoperoxidation product produced to detect the antioxidant activity of compounds in treated groups(Figure 4). H_3DMT and $[Cu_2(HDMT)_2(H_2O)]$ showed a lower level of MDA when compared with control groups. However, $[Cu_2(HDMT)_2(H_2O)]$ showed a higher antioxidant affinity towards free radicals than H_3DMT. The antioxidant activity of the complex was related to its chemical structure which acts as a scavenger to free radicals.

H_3DMT and $[Cu_2(HDMT)_2(H_2O)]$ seem to have the antioxidant activity as indicated by their lowering effect on serum malonaldehyde level (Figure 4) as compared to copper acetate treated groups of rats subjected to adjuvant-collagen model. Their antioxi-



*Significance of difference from the arthritic non treated synthesized group(F-test).

Figure 2: Influence of copper acetate, H_3DMT and $[Cu_2(HDMT)_2(H_2O)]$ on copper content of serum(μg/ml) in adjuvant collagen of rheumatoid arthritis model (m ± S.E)



* Significance of difference from the non arthritic non treated group(F-test)

ΦSignificance of difference from the copper acetate group(F-test)

Figure 3: Influence of piroxicam (Sigma), copper acetate, H_3DMT and $[Cu_2(HDMT)_2(H_2O)]$ on albumin content of serum (g/dl) in adjuvant collagen of rheumatoid arthritis model(m ± S.E)

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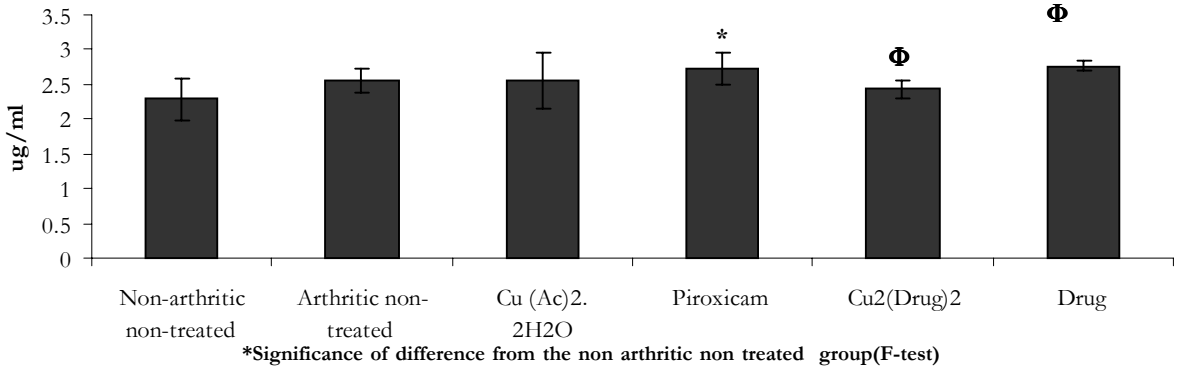
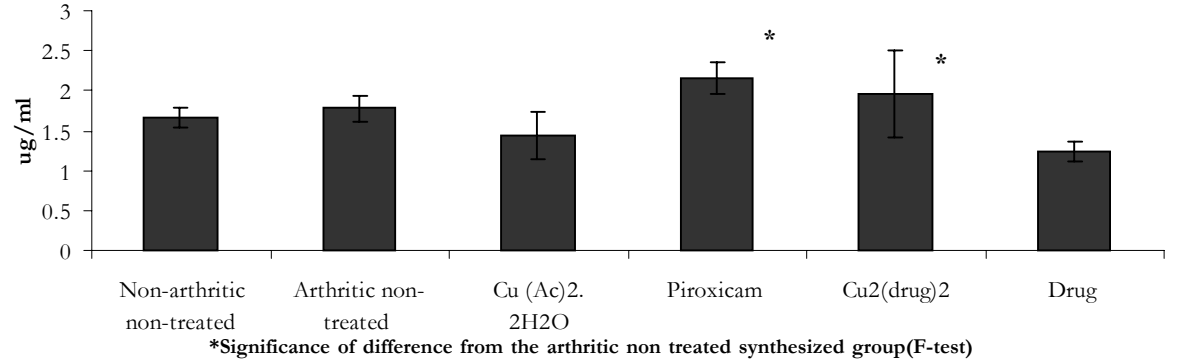


*Significance of difference from the non arthritic non treated group(F-test)
ΦSignificance of difference from the copper acetate group(F-test)

Figure 4: Influence of copper acetate, H_3DMT and $[Cu_2(HDMT)_2(H_2O)]$ on MDA content of serum (μg/ml) in collagen -adjuvant model of Rheumatoid Arthritis(m ± S.E)



*Significance of difference from the arthritic non treated synthesized group(F-test)

Figure 5: Influence of copper acetate, H_3DMT and $[Cu_2(HDMT)_2(H_2O)]$ on rheumatoid factor content of serum (iu/ml) in collagen-adjuvant of rheumatoid arthritis model(m ± S.E)

dant activity was similar to that of piroxicam which had been approved by Pipe et al[37]. They also approved the antioxidant activity of copper-piroxicam complex through measurement of the degree of inhibition of the reduction of nitro blue tetrzolium *in vitro*. The antioxidant activity of $[Cu_2(HDMT)_2(H_2O)]$ was believed to involve the redox cycling at the central metal ion.

The anti-inflammatory and analgesic effects were measured in rats treated with H_3DMT. There was an improvement in the 7th day of measurements when compared with piroxicam, which is closely similar to that in control group. The anti-inflammatory and analgesic measurements were matched with quantitative observations concerning the anti-inflammatory role of copper-drug compounds observed by Sorenson[14] who reported that salts of many anti-inflammatory drugs currently in use are more effective in reducing inflammation than their parent acids. Also, the copper complexes have antiulcer activity than that of the parent acid[14-16].

The anti-inflammatory improvement of H_3DMT and $[Cu_2(HDMT)_2(H_2O)]$ was clearly observed from the data obtained when RF was measured in the treated group(Figure 5). However, in groups treated with$[Cu_2(HDMT)_2(H_2O)]$ and copper acetate, the anti-inflammatory and analgesic effects(Figures 6 and 7) showed an improvement in copper acetate group than$[Cu_2(HDMT)_2(H_2O)]$. The normal endogenous response to inflammation has been postulated to involve low molecular weight species which become firmly bonded to serum albumin[38]. Furthermore, the administered acidic drugs also bind to albumin. The anti-arthritic activity of these endogenous and exogenous agents was suggested to be through a mechanism which encourages the release of albumin-bound copper[2]. Low molecular weight drug, D-penicillamine, has also been used in treating rheumatoid arthritis[39] and was capable of effecting remission and was suggested to labialize

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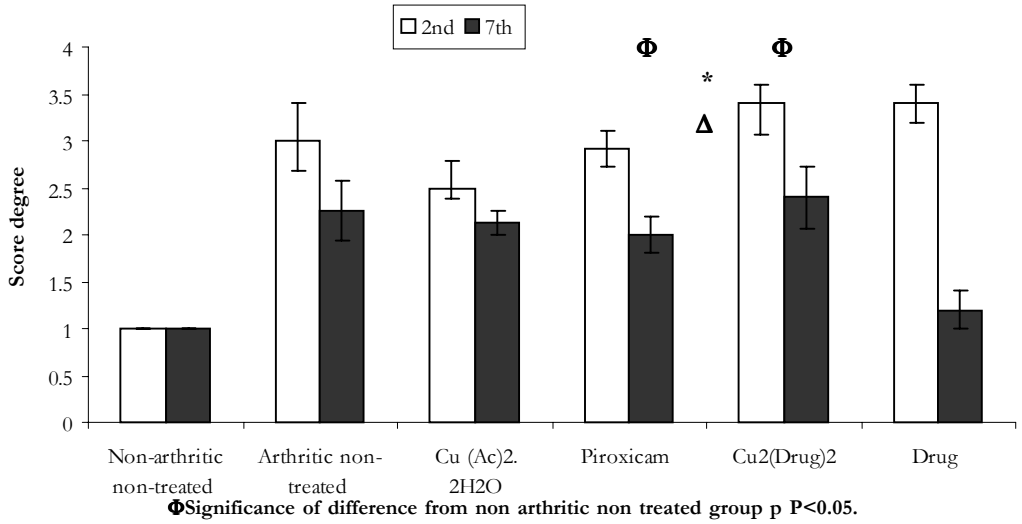
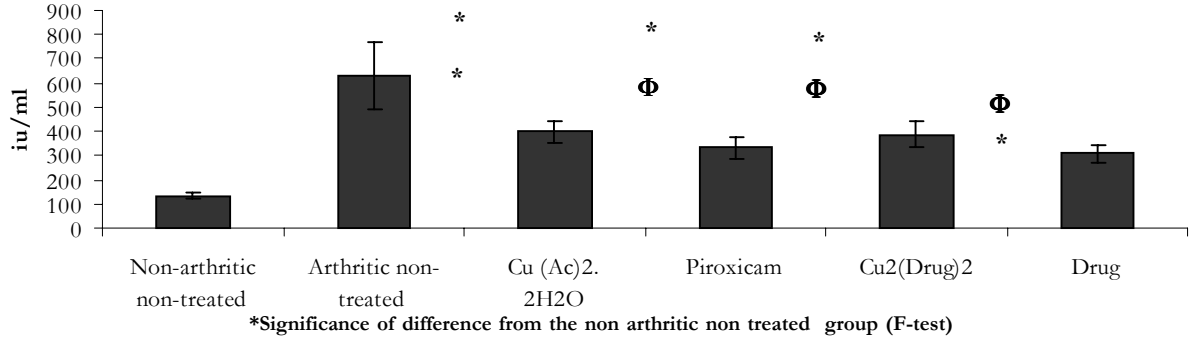


ΦSignificance of difference from non arthritic non treated group p P<0.05.
***Significance of difference from arthritic Nontreated group P <0.05).**
ΔSignificance of difference from 2nd day Anti-inflammatory effect P <0.05). (One way ANOVA).

Figure 6: Anti-inflammatory effect in score (contralateral ankle) of copper acetate, H_3DMT and [Cu_2 $(HDMT)_2(H_2O)$]



***Significance of difference from the non arthritic non treated group (F-test)**
ΦSignificance of difference from the arthritic non treated group(F-test)

Figure 7: Analgesic effect(contralateral ankle manipulation rance in score) copper acetate, H_3DMT and [$Cu_2(HDMT)_2(H_2O)$] (m±S.E)

body stores of copper into low molecular weight form, ceruloplasmin bound copper being a possible source[40].

In RF test, the results of H_3DMT and [Cu_2 (HD MT)$_2$(H_2O)] were closely related to each other when compared with control non treated groups (Figure 5). Jackson et al.[15] in their study on the justifications for copper administration in rheumatoid arthritis reported that (i) copper ions can afford protection against inflammation even without the assistance of administered organic therapeuticals, (ii) it seems relevant to monitor plasma copper, histidine and cystine contents of arthritic patients and to supplement if necessary, and (iii) low molecular weight copper in plasma occurs predominantly as ternary complexes. All of which involve histidinate and the most important having cystinate as the secondary ligand. The existence of these species accounted for the foregoing conclusions.

The observed anti-inflammatory of the used compounds especially copper salt and [Cu_2(HDMT)$_2$ (H_2O)] makes an importance for future profiting of our new molecule H_3DMT with its designed two abilities of high COX-2 inhibitory of high metal legibility and high antioxidant activity (Figure 2). The results are in accordance with the reported results of NSAIDs complexes with Zn(II), Cd(II) and Pt(II) ions to produce safer NSAIDs[31]. Jain et al[41] have reported that zinc-naproxen was found to have greater analgesic activity and comparable anti-inflammatory

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activity to naproxen(rat paw edema). The complex of naproxen with zinc markedly reduced its ulceroggenic effec[41].

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