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In vivo assessment of newly synthesized achiral copper(II) and zinc(II) complexes of benzimidazole derived scaffold as a potential analgesic, antipyretic and anti-inflammatory

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Abstract

Two new complexes of copper(II), [$\mathbf{CuL_2}$] and zinc(II), [$\mathbf{ZnL_2}$] with tridentate –ONN′– Schiff base ligand (L), a bioactive scaffold derived from 2-aminobenzimidazole and 2-hydroxy-3-methoxybenzaldehyde were synthesized and characterized by using various spectroscopic techniques viz, IR, ^{1}H, ^{13}C NMR, EPR, HRMS, elemental analysis and purity by UPLC studies. Both the complexes are non- electrolyte in nature. The newly synthesized compounds were screened for acetic acid-induced analgesic and yeast-induced antipyretic activities in mice and carrageenan-induced paw edema in rats (anti-inflammatory). The results showed that [$\mathbf{CuL_2}$] compound (at 100 mg/kg b.w) possessed potent anti-inflammatory activity whereas [$\mathbf{ZnL_2}$] (at 50 mg/kg and 100 mg/kg b.w) exhibited significant analgesic activity when compared with standard drugs. Both the complexes have apparently moderate and nearly akin antipyretic activity.

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Introduction

Inflammation is a complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.[1] Inflammation is classified into two *acute* and *chronic*. Acute inflammation can lead to progressive tissue damage by the noxious stimulus (e.g. pathogens, chemical irritants). While, chronic inflammation can lead to a multitude of diseases, viz, hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, osteoarthritis, gout, Alzheimer's disease and obesity and even can cause cancer (e.g., gallbladder carcinoma).[2,3] Therefore, the treatment of inflammation is of paramount importance. Non-steroidal anti-inflammatory drugs (NSAIDs) viz. Indomethacin, ibuprofen, and naproxen have frequently used for the cure of the first streak of various chronic inflammatory disease.[4] The classical NSAIDs demonstrate their action by confining the biosynthesis of prostaglandin, some of them are pro-inflammatory, which is fetched by inhibiting the rate-limiting cyclooxygenase (COX) enzyme involved in the inflammatory cascade[5] and are responsible for the transformation of arachidonic acid to prostaglandins.[6,7]

Among NSAIDS, imidazole and fused imidazole with six-membered rings,[8] possess central position due to its significant potential as therapeutics in clinical applications.[9–12] One of the most privileged classes of heterocyclic molecules are benzimidazoles in medicinal chemistry, encompassing a diverse range of biological activity.[13] Benzimidazoles derivatives displayed potential antimicrobial, anti-inflammatory, antituberculosis, antioxidant, antihypertensive, and anticancer, activities.[14-21] Benzimidazole-5-carboxylic acid and its derivatives have been ascended as potent agents for hepatitis C virus infections also.[22] Various derivatives of benzimidazole are used as drugs, few amongst them are Omeprazole (proton pump inhibitor), Pimobendan

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(ionodilator), Albendazole (inhibitor of Encephalitozoon Intestinal infection in AIDS patients) and Mebendazole. The presence of benzimidazole ring provides an active structural motif, which possesses peculiar structural features viz, steric, electronic as well as brings planarity to the drug candidate that makes the molecule active on different targets via hydrogen bonding and π-π* stacking. Also, benzimidazoles have the advantage to get easily functionalized for synthesis purpose and lead to quite stable and biologically privileged molecules.

Schiff base compounds are privileged ligand in medicinal chemistry and have proven therapeutic potential, due to –CH=N– functionality giving rise to strong activity and plays an essential role.[23] The remarkable biological activities, including antitumor, antibacterial, antifungal, antimalarial, antiviral, antioxidant, anti-inflammatory, analgesic, anticonvulsant, antiglycation, antihypertensive, antidepressant and lipid lowering properties.[24-27]

The designing and synthesis of metal complexes with biologically active scaffold are an imperative and lively research area in bioinorganic chemistry.[28,29] Metal chelation is one of the excellent routes to increase the lipophilic character of the organic moiety that may facilitate the absorption of the drug from the GI (gastrointestinal) tract.[30] In fact, on coordination, drugs/ligands exhibited significantly improved bioactivity profiles, while some inactive ligands may acquire pharmacological properties.[31,32] Among metal salts, Cu(II) and Zn(II) are the most important biometals, Cu(II) is present in all living organisms and plays a crucial role in redox chemistry, growth and development.[33] It is a pre-requisite for the functioning of various enzymes and proteins, which are occupied in respiration, energy metabolism, and DNA synthesis, especially cytochrome oxidase, superoxide dismutase (SOD), ascorbate oxidase and tyrosinase.[34] Moreover, Cu(II) and

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Zn(II) are co-factors in metabolic processes involved in collagen and bone metabolism via articular/connective tissue and the immune system[34] and play a crucial role in the PG synthesis.[35] In particular, copper carboxylates drugs belong to an important class of anti-inflammatory and anticancer agents, some of them are commercially available drugs.[36] Thus, in the present work, we have described the synthesis and characterization of 2-aminobenzimidazole and o-vanillin derived Schiff base ligand and their achiral copper(II), [**CuL_2**] and zinc(II), [**ZnL_2**] complexes. Those above plentiful pharmacological activities of benzimidazoles and derivatives encouraged us to study the in-vivo analgesic, antipyretic and anti-inflammatory activities of synthesized complexes.

Result and Discussion

Chemistry

Heterocyclic Schiff base ligand viz, (2-[(1H-Benzoimidazol-2-ylimino)-methyl]-6-methoxy- phenol) has been synthesized with high purities and acceptable yields. The orange-yellow compound obtained is stable to air and moisture and was characterized by elemental analysis, HRMS (LC-QTOF) spectrometry and IR and NMR (^1H and ^{13}C) spectroscopy. Reaction of Schiff base viz, 2-[(1H-Benzoimidazol-2-ylimino)-methyl]-6-methoxy-phenol ligand (L) with $M(NO_3)_2{\cdot}n(H_2O)$, where M = Cu(II) or Zn(II) in 2:1 stoichiometry, led to the isolation of neutral mononuclear complexes [**CuL_2**] and [**ZnL_2**] in which the ligand behaves as anionic with deprotonation of phenolic (OH) proton and tridentate with [-ONN′-] donor set (Scheme 1).

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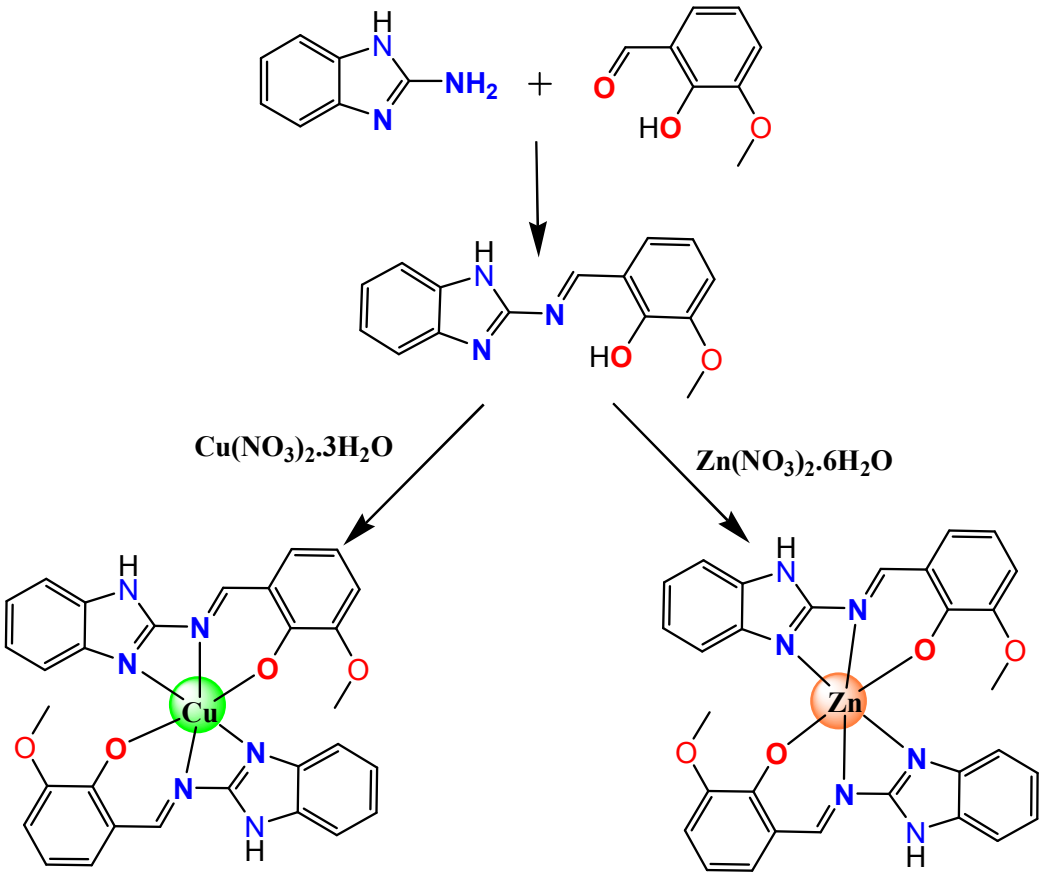

Scheme 1: Schematic representation of the synthesized ligand (**L**) and its [**CuL_2**] and [**ZnL_2**] complexes.

The achiral nature of the complexes was studied first by the help of polarimeter, the specific rotation $[\alpha]^{25}$ value (1 x 10^{-3} M solution of the compound in DMSO) was found zero. To ascertain the achirality, we also checked with circular dichroism, which confirms the polarimeter results by giving no spectra. The stability of the complexes was evaluated by UV–Vis spectral analysis at different times in a mixture containing H_2O/DMSO in 95%: 5% ratio and the absorbance, as well as wavelength, were remain unaffected, even after 12 h. Thus, results indicate that the complexes are quite stable in solution. Both complexes were characterized by analytical and spectroscopic techniques and the purity of ligand, and the complexes are checked with the help of

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UPLC technique, which confirmed the purity of the synthesized materials to ~96 ± 2% of retention time 7.827 min [**L**], 5.633 min [**CuL_2**] and 6.747 min **[ZnL_2].** Further, the HR-MS exhibited molecular ion peak at m/z = 595.1 for **[CuL_2]** and m/z = 597.1 for **[ZnL_2]** which corresponded to the predicted molecular weight of the $[M+2H]^+$ and $[M+H]^+$ ions, respectively. The isotopic patterns of these signals fits well with the theoretical isotopic distributions).

The significant IR vibrational bands and the ^1H chemical shift values of the free ligand and its complexes are listed in the experimental section (Fig. S1-S8, see electronic supplementary material). As shown, during metal complexation, the ligand behaves as tridentate anionic forming two four-membered and one six-membered chelate rings around the metal center. The stretching vibration υ(C=N) and the in-plane imidazole deformation bands are shifted to higher wavenumbers that are consistent with the implication of imine and imidazole nitrogen atoms in the coordination. In the ligand (**L**), ^1H NMR spectrum, a broad singlet at δ = 12.98 ppm is observed for NH protons. A characteristic signal of aldimine proton is found at δ = 9.61 ppm as a sharp singlet. A multiplet at δ = 7.28-7.25 ppm, two doublets at δ = 7.18-7.17 and 7.10-7.08 ppm and a triplet at δ = 6.98-6.94 ppm is assigned to aromatic ring protons. A sharp singlet at δ= 3.91 ppm is assigned to the methyl protons. In [**ZnL_2**] complex, the characteristic signal for NH protons on complexation got de-shielded and shifted to the higher value of 14.51 ppm. Further, the coordination of the ligand to the metal center is evidenced by the significant change of the aldimine signal to δ= 9.41 ppm. The aromatic ring protons appear as multiplets between δ=7.58–6.94 ppm, the assigning of the respective peak is difficult due to the merging of the aromatic peaks and at 6.63-6.61 observed triplet. The

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rest of the signals associated with the remaining protons appeared as expected. ^{13}C NMR spectra of the free ligand and the [**ZnL_2**] compound exhibited carbon signals supporting the ^{1}H NMR assignments and by the proposed structures.

The EPR spectrum of [**CuL_2**] in DMSO at LNT was recorded in the X-band region (Fig S9, see electronic supplementary material). The g factor is in respect to the standard marker TCNE (g = 2.00277). The EPR spectrum of copper complex [**CuL_2**] exhibited $g_{\parallel}$= 2.11 and $g_{\perp}$ = 2.06, values, respectively. EPR spectrum of the [**CuL_2**] revealed axial features ($g_{\parallel} > g_{\perp} > 2.0023$) and suggest a dx^2-y^2 ground state. The measure of exchange interaction between the copper centers in the polycrystalline compound can be given by the geometric parameter "G" using the relation: $G = (g_{\parallel} - 2.0023) / (g_{\perp} - 2.0023)$. The exchange interaction may be negligible if G is greater than 4. A considerable exchange interaction is indicated in the solid complex if G is less than 4.[37] The value $g_{\parallel} > g_{\perp} > 2.0023$ indicates d_{x-y} or d_z ground state are consistent with octahedral geometry. The magnetic moment obtained for [**CuL_2**] was 1.88 BM, consistent with values expected for copper(II) with the spin S = 1/2.

Evaluation of Biological Activity in vivo

Tissue injury leads inflammation after a prolonged period of infection characterized by edema, pain, tenderness and redness. These signs of inflammation are mediated by various chemical mediators including prostaglandins (PGs) such as leukotrienes (LTs) and hydroxy-eicosatetraenoic acids HETEs). Generally, these inflammatory mediators are produced in two successive steps after incurring of any tissue injury in biological systems. The first step involves the production of arachidonic acid by the action of phospholipase A2 followed by the production of the individual inflammatory mediators via the action of several enzymes as the second step. For example, generation of

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prostaglandins by action of Cyclooxygenase 1 (COX1) and cyclooxygenase 2 (COX2) while production of leukotrienes by 5-lipoxygenase and/or 12-lipoxygenase enzymes.38 It is documented that prostaglandins are responsible for vasodilation, capillary permeability and pain during inflammatory response whereas LTs triggers capillary permeability, chemotaxis of inflammatory mediators and extravasation of white blood cells leading to sustenance of inflammation.[39] Furthermore, these PGs are also the major mediators in pain sensitization while the LTs are the primary mediators of inflammation development.[38] Thus all the chemical compounds or substances that inhibit inflammation, pain and lower body temperature to the optimum, most probably show these effects by inhibiting lipoxygenase enzyme.

Zinc (Zn) and Copper (Cu) are second and the third most important transition bio-metals after iron in all forms of the life.[40,41] Both the bio-metals participate in over 100 enzymes involved in metabolism, DNA-transcription, stress management, immunity, reproduction and nervous system.[42] They are also essential for the expression of important antioxidant molecules like superoxide dismutase, ceruplasmin, and metallothionein that maintain redox homeostasis in living organisms.[43] However, its excessiveness, as well as deficiency both, have an adverse impact on the immune and antioxidant response exacerbating any infection or inflammation of the living organisms.[44,45] Thus, the present study is aimed to investigate if the synthesized metal [**CuL_2**] and [**ZnL_2**] complex have any anti-inflammatory, antipyretic and analgesic properties in vivo.

Effects of [CuL_2] and [ZnL_2] on yeast-induced hyperthermia in mice

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Administration of [**CuL_2**] in a dose of 100 mg/kg (but not 50mg/kg) i.p. to mice significantly decreased body temperature at 60, 90 and 120 min after injection ($p < 0.001$, 0.001 and 0.01, respectively, n=6). Administration of [**ZnL_2**] in a dose of 100 mg/kg (but not 50 mg/kg) i.p. to mice also significantly decreased body temperature at 60, 90 and 120 min after injection ($p< 0.05$ and 0.001, respectively, n=6). (Figure 1). On the other hand, [**CuL_2**] 100 mg/kg produced four folds the antipyretic activity of [**CuL_2**] 50 mg/kg at 60 and 90 min while two folds at 120 min. However, statistical analysis of these results revealed that they are not significantly different (Table 1).

Table 1: Percentage inhibition of hyperpyrexia, algesia, and inflammation induced by each dose of the compounds relative to its zero time (pretreatment readings i.e. control). Paracetamol was used as a standard (positive control) for both antipyretic and analgesic experiments while diclofenac was used as a standard for the anti-inflammatory experiment.

Test	Time (min)	% inhibition of pyresis, algesia, and inflammation					
		Standard	Predrug (control)	[**CuL_2**] 50 mg/kg	[**CuL_2**] 100 mg/kg	[**ZnL_2**] 50 mg/kg	[**ZnL_2**] 100mg/kg
Antipyretic activity	60	8.6	0	1.1	4.2	1.9	2.8
	90	10.5	0	1	4	1.9	3.1
	120	10.2	0	1.2	2.9	0.8	2.9
Analgesic activity	30	51.8	0	13.1	13.0	11.9	37.6
	60	62.4	0	23.2	36.8	29.8	53.5
	120	65.9	0	26.4	25.9	26.3	48.5
Anti-inflammatory activity	180	65.4	0	36.3	56.1	30.6	46

The compound [**ZnL_2**] produced two folds the activity in 100 mg/kg then 50 mg/kg, but none of these differences were statistically significant. Comparison of all the doses for

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[**CuL_2**] with all the doses for [**ZnL_2**] revealed that [**CuL_2**] 100 mg/kg appears to be more efficient in all the other treatments as antipyretic. However, the results are not statistically significant.

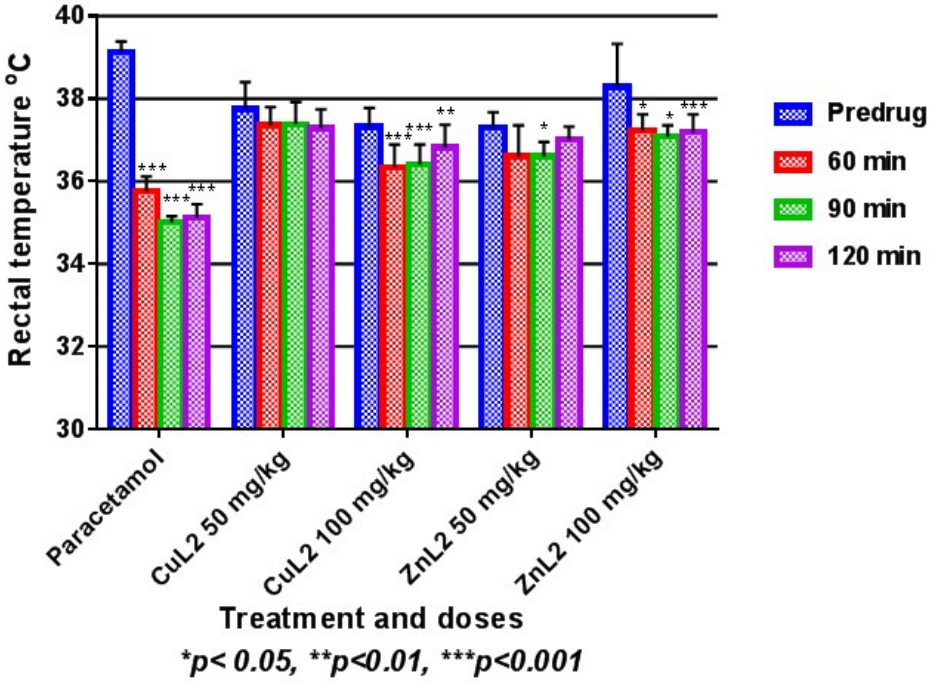

Figure 1. Antipyretic effects of [**CuL_2**] and [**ZnL_2**] complexes at 60 min, 90 min, and 120 min after yeast-induced hyperpyrexia. Rectal temperature after 60, 90 and 120 min were compared to the rectal temperature before the administration of the compounds (pre-drug temp.) i.e. [**CuL_2**] and [**ZnL_2**] in doses of 50 and 100 mg/kg i.p. Results have been expressed in mean ±SD. ANOVA with Dunnett's test as post Hoc test was used to check for any significance (n=6)

Effects of [CuL_2] and [ZnL_2] on hot plate-induced analgesia:

The synthesized molecule [**CuL_2**] reduced analgesic activity significantly only after 120 min of the administration ($p<0.05$, n=6). Whereas, [**ZnL_2**] (injected in doses of 50 and 100 mg/kg, i.p.) produced dose- dependent analgesia at 30, 60 and 120 min after injection ($p<0.001$, n=6). In the results, Figure 2 shows a comparison of different doses of [**CuL_2**] with those of [**ZnL_2**]. The current findings revealed that [**ZnL_2**] 100 mg/kg is significantly more potent analgesic than 50 mg/kg and more potent than CuL_2 all doses

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(p<0.001, table 1). [**CuL_2**] 100 mg/kg in turn is significantly more potent than 50 mg/kg at 60 min (p<0.01, table 1).

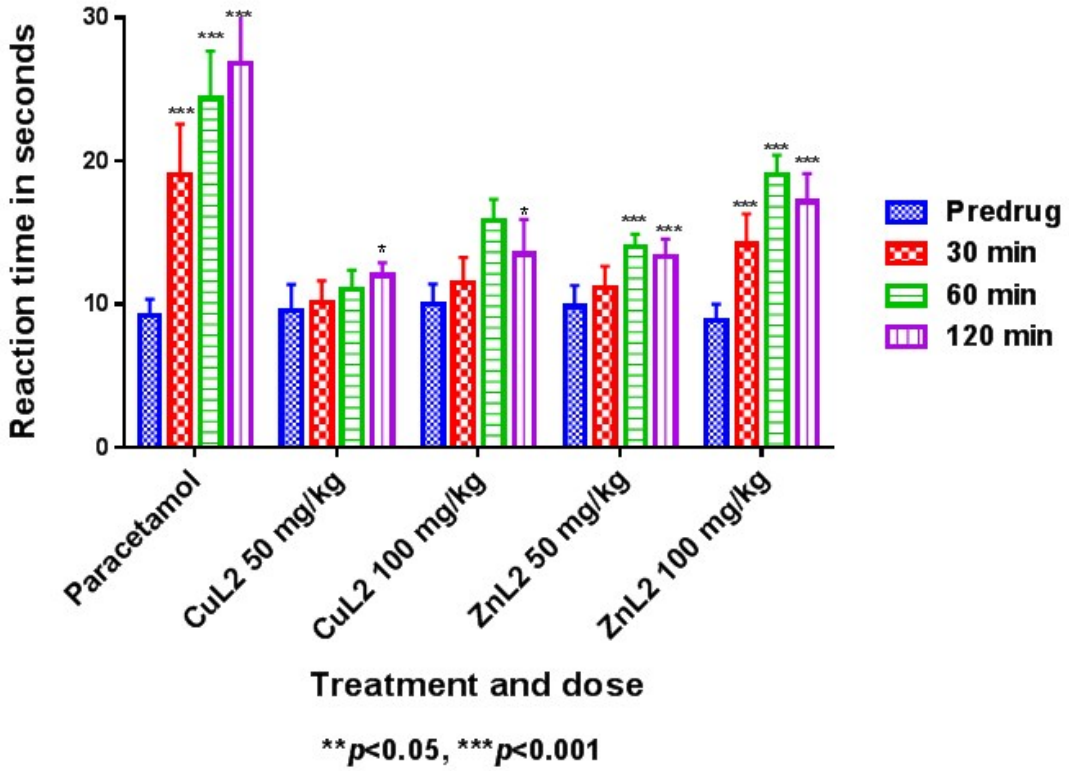

Figure 2. Analgesic effect of [**CuL_2**] and [**ZnL_2**] complexes on hot plate-induced algesia. Mice reaction time to the hot plate at 30, 60 and 120 min after compounds' treatment was compared with pre-drug reaction time (reaction time prior to the administration of the compounds). Results are plotted as mean ±SD. ANOVA with Dunnett's test as post Hoc test was used to check for any significance (n=6).

Effects of [CuL_2] and [ZnL_2] on carrageenan-induced rat paw edema:

Administration of the proposed metal complex [**CuL_2**] in doses of 50 and 100 mg/kg produced a dose-dependent decrease in rat paw edema respectively (p<0.001, p<0.0001, n=6). On the other hand, [**ZnL_2**] produced a dose-dependent decrease in paw edema when injected in doses of 50 and 100 mg/kg i.p. (p<0.05 and p<0.0001, respectively, n=6). Hence, the anti-inflammatory effect of CuL_2 at 100 mg/kg was

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significantly higher than that of 50 mg/kg and also as compared to both doses of ZnL_2 (p<0.01, Table 1).

However, [**ZnL_2**] showed stronger analgesic potential than [**CuL_2**] (Figure 2) whereas [**CuL_2**] tends to be more potent anti-inflammatory than [**ZnL_2**] (Figure 3). Hitherto, there is no apparent statistically significant difference between [**CuL_2**] and [**ZnL_2**] on the antipyretic activity. Intriguingly, [**CuL_2**] at a dose of 100 mg/kg demonstrated the antipyretic and anti-inflammatory activities but not an analgesic activity which might be due to inhibition of 5-lipoxygenase and/or 12-lipoxygenase enzymes but might not involve inhibition of cyclooxygenase enzyme due to lack of analgesic activity.

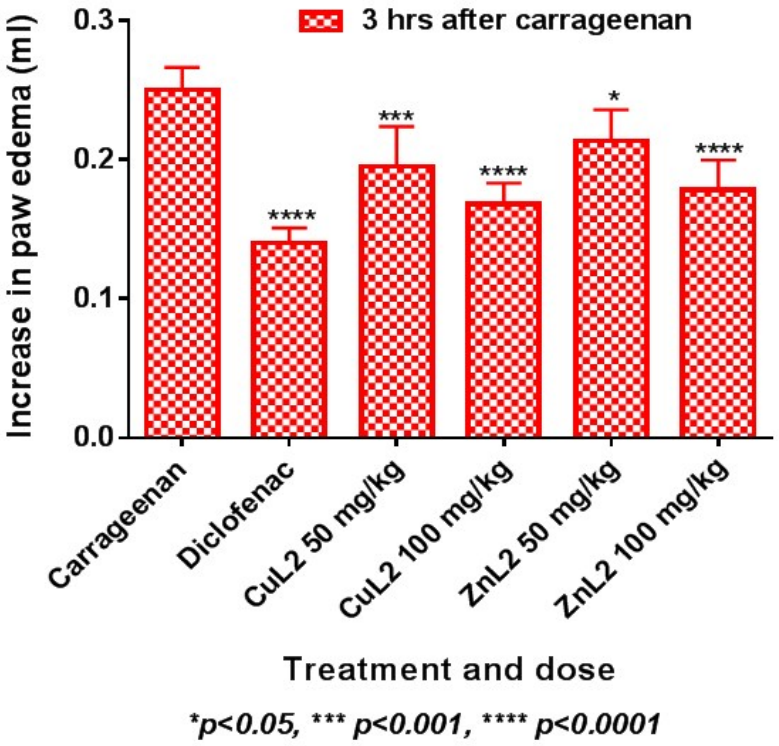

Figure 3. Effects of compounds on the carrageenan-induced increase in paw edema in rats. The increase in paw edema 3h after carrageenan administration in the compounds-treated groups was compared with the carrageenan control group (not treated with compounds). ANOVA test with Dunnett's test as post Hoc test was used to Check for any significance, n=6.

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On the other hand, [**ZnL_2**] (at doses of 50 and 100 mg/kg) possessed antipyretic, analgesic and anti-inflammatory activities. These effects might be attributed to inhibition of lipoxygenase enzyme with subsequent inhibition of arachidonic acid production or inhibition of 5-lipoxygenase, 12-lipoxygenase, and cyclooxygenases leading to inhibition of prostaglandins and leukotrienes.

Experimental Section

Material and Methods

2-Aminobenzimidazole, o-vanillin, $Cu(NO_3)_2 \cdot 3H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, Acetic acid, All the solvents were used as purchased from the company Sigma-Aldrich without further purification. Infrared spectra were recorded as KBr pellets, using a Shimadzu IR Affinity-1 spectrometer with a resolution of 4 cm^{-1}. Elemental analysis (C, H, N) were performed on a PerkinElmer 2400 Series II CHNS/O system. NMR spectra were recorded at JEOL- ECP-400 spectrometer. The EPR spectrum of the copper complex was acquired on a Varian E 112 spectrometer using X-band frequency (9.1 GHz) at liquid nitrogen temperature in the solid state. Circular dichroic spectra of both complexes using Circular Dichroism (CD) Spectrometer with Stop Flow-Applied PhotoPhysics Chirascan and JASCO P-1020 (Jasco International Co., Ltd, Tokyo, Japan) digital polarimeter with a 10 cm optical length cell at 25 °C used for calculating specific rotation. Ultra-performance liquid chromatography (UPLC) experiments were performed on a gradient system of Agilent Technologies (1290 Infinity) equipped with infinity binary pump (G4220A), autosampler (G4226A), Thermostat column compartment (G1316c), fraction collector (G1364C) and a photodiode array detector (G4212A). Chem-station software programs the above configuration of UPLC. The

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separations were performed on Eclipse C18 column (Agilent) 4.6 × 100 mm, 3.5 µm by using a gradient method.

All the animal based experiments were performed in compliance with the relevant laws and institutional guidelines and have been approved and permitted by the by the ethics committee of the College of Pharmacy, King Saud University, Riyadh, KSA.

Synthesis of Schiff base (L)

Solutions of equimolar amounts of 2-aminobenzimidazole (0.133 g, 1.0 mmol) and vanillin (0.152 g, 1.0 mmol) absolute ethanol with 2-3 drops of acetic acid were refluxed for 4 h and the product obtained was filtered off and washed with hexane (3 x 5ml) and diethyl ether (3 x 5ml). Recrystallized from ethanol to obtain deep orange color crystalline product. Yield: 0.188 g, 66 %; Anal. calc. for C15H13N3O2 (267.1): C, 67.40; H, 4.90; N, 15.72, found: C, 67.36; H, 4.89; N, 15.70; HRMS m/z {in $CHCl_3$, observed (calcd)} for $C_{15}H_{13}N_3O_2$ + H^+: 268.1, (268.1). FT IR (KBr pellets) cm^{-1}: 3305, 1600, 1515, 1462, 1252, 735. ^{1}H NMR (400MHz, DMSO-d_6, ppm): 12.95 (br, NH, 1H), 9.61 (s, N=CH, 1H), 7.28-7.25 (m, Ar-H, 2H), 7.18-7.16 (d, Ar-H, 2H), 7.09-7.07 (d, Ar-H, 2H), 6.98-6.94 (t, Ar-H, 1H), 3.91(s, O(CH3), 3H), 2.16 (s, OH, 1H). ^{13}C NMR (100MHz, DMSO-d_6, ppm): 167.6 (characteristic peak of Schiff base, N=CH), 151.6, 125.5, 123.2, 119.7, 118.9, 116.4 (Ar-Cs), 56.4 (OCH3).

Synthesis of copper(II) complex [CuL_2]

To a methanolic (10 mL) solution containing Schiff base (267.1 mg, 1.0 mmol) was added slowly methanolic solution (10 mL) of $Cu(NO_3)_2 \cdot 3H_2O$ (120.5 mg, 0.5 mmol) and stirred for 5h. The precipitate filtered off and washed with hexane (3 x 5ml) diethylether (3 x 5ml) and $CHCl_3$ (3 x 5ml) and dried. Yield: 215 mg, 55%. Anal. Calcd. for ($C_{30}H_{24}N_6O_4Cu$) (595.1): C, 60.45; H, 4.06; N, 14.10. found: C, 60.39; H,

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4.04; N, 14.07. HRMS m/z {in DMSO, observed (calcd)} for $C_{30}H_{24}N_6O_4Cu$: 595.1, (595.1). FT-IR (KBr pellets) cm^{-1}: 1609, 1535, 1455, 1254, 744. μ_{eff} = 1.88 BM.

Synthesis of zinc (II) complex [$\mathbf{ZnL_2}$]

Similar procedure was adopted as for copper complex, using $Zn(NO_3)_2{\cdot}6H_2O$ (148.7 mg, 0.5 mmol) Yield: 215 mg, 55%. Anal. Calcd. for ($C_{30}H_{24}N_6O_4Zn$) (597.1): C, 60.26; H, 4.05; N, 14.05. found: C, 60.19; H, 4.04; N, 14.03. HRMS, m/z {in DMSO, observed (calcd)} for $C_{30}H_{24}N_6O_4Zn$ + H+: 597.2, (597.9). FT IR (KBr pellets) cm^{-1}: 1608, 1535, 1449, 1256, 741. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 14.51 (br, NH, 2H), 9.40 (s, N=CH, 2H), 7.58-7.52 (dd, Ar-H, 4H), 7.19-6.94 (m, Ar-H, 4H), 7.08-6.94 (t, Ar-H, 4H), 6.65-6.61 (t, Ar-H, 2H), 3.87 (s, O(CH_3), 6H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 165.7 (characteristic peak of Schiff base, N=CH), 153.8, 150.5, 148.0, 142.6, 134.14, 122.43, 122.4, 123.5, 119.4, 119.2, 116.5, 111.2 (Ar-Cs), 55.9 (OCH_3).

In Vivo Biological Studies

Antipyretic test (yeast induced hyperpyrexia in mice)

Hyperpyrexia was induced in mice by subcutaneous injection of (20 % Aqueous suspension of brewer's yeast) of 20 ml/kg body weight (6 animals in each group) in the back below the nape of the neck (Loux et al., 1972).[46] The animals then fasted for the duration of the experiment (approximately 26 h); water was made available ad lib. Control temperatures were taken 24 h after the yeast injection to determine the pyretic response to yeast. Rectal temperatures taken 1h before drug administration in fevered animals served as a pre–drug control.

Analgesic Activity (Acetic acid induced writhing test i.e. algesia)

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Acetic acid-induced writhing in the mice-The test was carried out using the technique of Siegmund et al.,[47] as modified by Koster et al..[48] Both the complexes (50 and 100 mg/kg body weight) were administered orally, to 16 h fasten mice, and divided into groups of 6 animals each. One hour after treatment, the mice were injected intraperitoneally with 0.2 ml of 3% acetic acid solution to induce the characteristic writhing. The number of writhings occurring between 5 and 15 min after the acetic acid injection in control and treated animals was recorded. The responses of compounds-treated groups were compared with those of animals receiving in diclofenac sodium (as a standard drug), 4 mg/kg, as well as with the control group.

Anti-inflammatory activity (Carrageenan-induced paw edema in rats)

Pedal inflammation in albino rats (8 to10 weeks old) of either sex weighing180-20 0 g was carried out following the method described by Winter et al..[49] An injection was made of 0.0 5ml of 1% carrageenan sodium salt (BDH) into the right hind foot of each rat under the plant araponeurosis. The test groups of rats were treated orally with 50 and 100 mg/kg 1h before the carrageenan injection. At the same time, the control group was given 5 ml/kg of normal saline and the reference group was given 100 mg/kg of an aqueous solution of oxyphenbutazone. The measurements of foot volume were done by the displacement technique using a plethysmometer (Apelex, France) after +3 h after the injection of carrageenan. The inhibitory activity was calculated according to the following formula:

$$\text{Percent inhibition} = 100\ [1-(a-x)/(b-y)]$$

Where 'b' is the mean paw volume of control rats after carrageenan injection and 'y' before the injection; whereas 'x' is the mean paw volume of treated rats before injection and 'a' is the paw volume mean after carrageenan injection.

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Statistical analysis

Results are expressed as mean ±SEM. One-way ANOVA was used for a comparison test of significant differences among groups followed by Dunnet's multiple comparison post- tests. Level of significance ($P<0.05$) was taken for each test.

Conclusion

In this work, we have designed and synthesized new Cu(II) and Zn(II) complexes of 2-[(1H- Benzoimidazol-2-ylimino)-methyl]-6-methoxy-phenol Schiff base ligand derived from the condensation of 2-aminobenzimidazole and o-vanillin. Conventional measurements characterized the ligand and complexes. The ligand behaves as a neutral, tridentate (-ONN′-) donor yielding octahedral geometries of the metal complexes. These synthesized complexes are achiral/non-stereo-selective in nature. Therefore, these synthesize NSAIDs will not cause side effects. The *in vivo* anti-inflammatory, analgesic and antipyretic activities of both the complexes are potent. These studies suggested that a synergistic combination of ligand and metal ion was critical in the design of potential NSAIDs. However the precise molecular mechanism(s) of the anti-inflammatory, analgesic and antipyretic effects discovered in the present study warrants further investigations.

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In vivo assessment of newly synthesized achiral copper(II) and zinc(II) complexes of benzimidazole derived scaffold as a potential analgesic, antipyretic and anti-inflammatory

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Graphical Abstract

Anti-pyretic, Analgesic and Anti-inflammatory Activity (NSAIDs)

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