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New thiopyridine complexes: design, electrochemical preparation and biological assessment

Osama I. El-Sabbagh[1*], Yamany B. Yamany[1], Hany A. Eldeab[1]

[1]*Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, P.O. Box: 888 Al-Haweiah, Zip Code 21974, Taif, Saudi Arabia.*

[*]*Corresponding author: e-mail: osamaelsabbagh@yahoo.com*

Novel complexes of Ru (III), Cu (II) and Au (III) (**2–4**) were prepared using 6-phenyl-2-thioxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (HL,**1**) adopting either electrochemical or traditional chemical methods. The electrochemical method is preferred in the synthesis of the complexes than the chemical one because it affords pure products with higher yields in shorter reaction time. The novel thiopyridine complexes were characterized by elemental analyses, IR, ^{1}H, ^{19}F-NMR, TGA and DTA measurements. The antimicrobial activity evaluation revealed that the complex bearing copper metal **3** has nearly the same activity as the reference drug ciprofloxacin. Anti-inflammatory activity evaluation showed that complex **4** containing gold displayed anti-inflammatory activity higher than the reference drug celecoxib upon using carrageenan rat hind paw edema method.

Keywords: Thiopyridine, complexes, electrochemical, antimicrobial, anti-inflammatory.

INTRODUCTION

The emergence of bacterial resistance to existing antibiotics is a worldwide issue. Morbidity and mortality due to enteric bacterial infections caused important healthy problems worldwide mainly in developing countries[1]. New classes of antimicrobial compounds are therefore urgently needed to control the virulence of the multidrug resistant pathogens. Electrochemistry is defined as the branch of chemistry that examines the phenomena resulting from combined chemical and electrical effects[2]. Studies reported that some sulphur containing ligands showed anticarcinogenic, antibacterial, tuberculostatic, and antifungal activities. Moreover, 2-mercapto pyridine carboxylic acids are versatile ligands that containing exocyclic sulphur and endocyclic nitrogen atoms which act as a bidentate ligand through the pyridine N of the heterocycle and thiolate S atoms coordinated to metal ion[3, 4]. Other study found that 3-substituted thiopyridines exhibited antibacterial, antimalarials and anticancer activities[5].

Ruthenium is widely studied due to its versatility and potential applications in several fields of the science[6, 7]. It was reported that three complexes containing ruthenium exhibited *in vitro* antitumor activity which is better than cisplatin at the same concentration. These complexes showed also antimicrobial activity against *M. tuberculosis*[8]. A study reported that some metal complexes containing copper were found to be possess potentially antibacterial against *S. aureus* and *B. subtitles*[9]. Gold compounds have been used since ancient times, particularly in traditional Chinese, Egyptian and Indian medicines, where they were found to be effective in treating inflammation, infection and tuberculosis[10, 11]. Others reported that gold compounds not only limited to inflammatory diseases but also were beneficial as anticancer[12]. Jumaa *et al* found that gold nanoparticles possess antibacterial activity against Gram-positive (*Staphylococcus aureus*) and negative bacteria *(Pseudomonas aeroginosa*) using agar well diffusion method[13].

In this work, we aim to synthesize some metal complexes of Ru (III), Cu (II) and Au(III) utilizing the ligand 6-phenyl-2-thioxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile by electrochemical and traditional chemical methods hoping to possess superior antimicrobial and anti-inflammatory activities.

EXPERIMENTAL PART

Chemistry

***General*:**

Infrared spectra were recorded with a Thermo Nicolet Nexus 470 FT-IR spectrometer in the range 4000–400 (υ, cm^{-1}) on samples in potassium bromide disks.^{1}H-NMR spectra, was obtained on Varian Gemini 400 MHz FT NMR spectrometer in DMSO-d_6; chemical shifts were recorded in δ (ppm) units, relative to Me_4Si as an internal standard. Shimadzu LCMS-QP 800 LC-MS and AB-4000 Q-trap LC-MS/MS utilized to determine the mass spectra. Elemental analysis data were obtained using a 2400 II series CHN Analyzer (Perkin Elmer, Waltham, MA, USA). The reagents were purchased from Aldrich Chemical Co. and used without further purification.

Method A: Electrochemical synthesis of complexes

The electrochemical technique was performed according to the previously reported procedure[14, 15]. A cell unit consisted of a 100 mL beaker containing anhydrous acetone solution of the thiopyridine derivative, **1** with a platinum cathode and a sacrificial anode (Cu or Au or Ru) immersed in the liquid phase. Electrolysis of copper or gold into 60 mL of anhydrous acetone solution of the organic ligand (**1**) (0.49 g, 2 mmol), 2.5 mg Et_4NClO_4 dissolved in two drops of water and 40 mA current led to dissolution of 34 mg of metal electrode during 30 min. (E_f = 0.5 mol. F^{-1}). Since, most of the products are insoluble in the reaction mixture, the collection procedure involved filtration, after which the solid was washed with diethyl ether.

Method B, Traditional chemical synthesis:

A mixture of metal (Ru, Cu or Au) chloride (0.16 mmol) and 6-phenyl-2-thioxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (HL,**1**, 0.16 mmol) in 40 ml acetone was degassed and then heated to reflux for

10–12 h. The reaction mixtures were cooled, and the solvent was removed under vacuum. The residue was washed by hot benzene followed by petroleum ether. The complexes were left to dry under vacuum.

6-phenyl-2-thioxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (1). Yellowish brown, mp = 256°C.[16] IR (KBr, cm^{-1}) 3493 (NH), 2226 (CN) ; ^{1}H--NMR (400 MHz, DMSO-d_6) δ = 7.22 (s, pyridine H-5), 7.63–7.97 (m, Ar-H) ppm;^{19}F-NMR (376 MHz, DMSO-d_6) δ =(–62.47) (s, F, CF_3) ppm. Anal. Calcd for $C_{13}H_7F_3N_2S$: C, 55.71; H, 2.52; N, 10.00. Found: C, 55.74; H, 2.50; N, 10.03.

Tris((3-cyano-6-phenyl-4-(trifluoromethyl)pyridin-2-yl) thio)ruthenium (2). Dark brown, yield: method A, 89% (method B: 61%), mp > 300°C; IR (KBr, cm^{-1}) 2226 (CN); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.22 (s, pyridine H-5), 7.61–7.89 (m, Ar-H) ppm; ^{19}F-NMR (376 MHz, DMSO-d_6) δ = (–62.48) (s, F, CF_3) ppm ; LC-MS (ionization method): m/z 939 (M+1); Anal. Calcd for $C_{39}H_{18}F_9N_6RuS_3$: C, 49.89; H, 1.93; N, 8.95. Found: C, 49.91; H, 1.95; N, 8.98.

Bis-((3-cyano-6-phenyl-4-(trifluoromethyl)pyridin-2-yl) thio)copper diacetone (3). Previously prepared by classical method[17]. Brown, yield: method A, 96% (method B: 74%), mp > 300°C; IR (KBr, cm^{-1}) 2226 (CN); ^{1}H-NMR (400 MHz, DMSO-d_6) δ = 7.24 (s, pyridine H-5), 7.59–8.04 (m, H, Ar-H) ppm; ^{19}F-NMR (376 MHz, DMSO-d_6) δ = (–62.51) (s, F, CF_3) ppm; LC-MS (ionization method): m/z 739 (M+1); Anal. Calcd for $C_{32}H_{24}CuF_6N_4O_2S_2$: C, 52.06; H, 3.28; N, 7.59. Found: C, 52.09; H, 3.21; N, 7.61.

Tris-((3-cyano-6-phenyl-4-(trifluoromethyl)pyridin--2-yl)thio)gold (4). Light brown, yield: method A, 91% (method B: 64%), mp > 300°C; IR (KBr, cm^{-1} 2226 (CN) ; ^{1}H-NMR (400 MHz, DMSO-d_6) δ = 7.27 (s, H, pyridine H-5), 7.53–7.98 (m, H, Ar-H) ppm, ^{19}F-NMR (376 MHz, DMSO-d_6) δ = (–62.48) (s, 3F, CF_3) ppm; LC-MS (ionization method): m/z 1035 (M+1); Anal. Calcd for $C_{39}H_{18}AuF_9N_6S_3$: C, 45.27; H, 1.75; N, 8.12. Found: C, 45.28; H, 1.77; N, 8.14.

Biological activity evaluation

Antimicrobial activity

Methodology:

The ligand (**1**) and complexes (**2–4**) which were previously prepared by method A, were dissolved in *DMF* at concentration 10 mg/mL. The antibacterial ciprofloxacin and the antifungal nystatin were used as references drugs at the same dose level while DMF was used as a negative control[18]. Nutrient agar (Oxoid, England) was inoculated with one bacterial strain. Bacterial strains used were Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Esherichia coli*). Saburaud's dextrose agar (Oxoid, England) was seeded with *Candida albicans* as representative of fungi. After solidification, cups were made by cork borer, and then filled with 50 μl from compounds solution. Each compound was assessed in triplicate. The plates were incubated overnight at 37°C and then the inhibition zones were measured in mm. The results of antimicrobial activity for the new compounds were recorded in Table 2. Bacterial and fungal strains were isolated and identified by Department of Microbiology, Faculty of Pharmacy, Zagazig University, Zagazig Egypt.

Statistical analysis.

Data were analyzed using computer program SPSS. The differences in mean values were determined by analysis of variance (one-way ANOVA) followed by Least Significant Difference (LSD).

Anti-inflammatory activity evaluation

Materials

Carrageenan (carrageenan kappa-type III) and all other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The ligand (**1**), the test compounds (**2–4**) prepared by method A and reference drug celecoxib were used in the following assays. Housing and management of the animals and the experimental protocols were made as stipulated in the Guide for Care and Use of Laboratory Animals Guidelines of the National Institutes of Health (NIH), and accepted by the local authorities of Zagazig University, Zagazig, Egypt.

Animals

Mature male albino rats weighing 150–200 g were used. All experimental animals were provided from the Faculty of Veterinary Medicine, Zagazig University, Egypt. All animals were held under standard laboratory conditions in the animal house (temperature 27°C) with a 12/12 light-dark cycle. Animals were fed laboratory diet and water ad libitum. The animal experiments were performed in accordance with international guidelines.

Method:

The rat hind paw edema method[19] by Winter *et al.,* 1962 was applied to determine the anti-inflammatory activity of the ligand (**1**) and the test complexes (**2–4**) using celecoxib as a standard. The animals were divided into 6 equal groups (each of six). The first group was left as a control group while the second group was injected i.p. with celecoxib at a dose of 18 mg/Kg body weight. The ligand and the test compounds were injected i.p. in the remaining groups at the same dose level. After 1 h, edema in the right hind paw was induced by injecting 0.1 mL of 10% carrageenin. The thickness of the paw was measured using a skin caliber 1, 2, 3, and 4 h after the carrageenin injection to determine the anti-inflammatory activity of the test compounds (Table 3).

RESULTS AND DISCUSSION

Chemistry

In the present work, the targets metal complexes of Ru (III) ,Cu (II) and Au(III) (**2–4**) were prepared starting from the ligand the 6-phenyl-2-thioxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile (**1**). The latter was prepared adopting the reported procedure[16]. This ligand (HL,1) exists in the following tautomeric forms (Fig. 1).

The targets metal complexes of Ru (III) Cu (II) and Au (III) (**2–4**) were prepared adopting two methods. The first, electrochemical method depends on direct oxidation of the metals in the presence of a ligand solution. It is a one-step process which represents a convenient

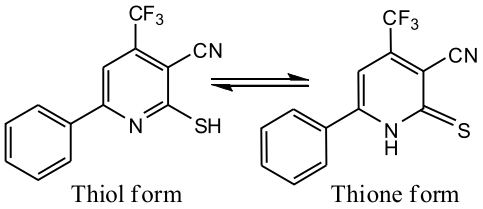


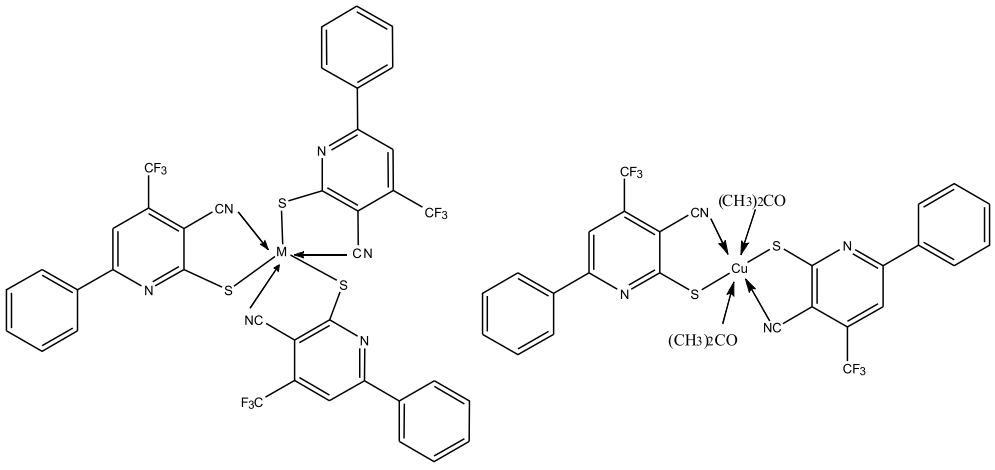
Figure 1. Tautomeric forms of ligand (HL,**1**)

and simple route to a variety of transition metal complexes. Measurements of the electrochemical efficiency, Ef, defined as moles of metal dissolved per Faraday of electricity, for the M/L system (where M = Ru, Cu, and Au and L = ligand used) gave Ef = 0.5 ± 0.05 mol F^{-1}. The second, traditional chemical method, depends on reaction of equimolar amounts of metal (Ru, Cu or Au) chloride with the thiopyridine derivative (HL,**1**) in acetone by heating the reactants at reflux for 10–12h. It was noted that the first, electrochemical method is preferred in the synthesis of the complexes than the second chemical method because it affords pure products with higher yields in shorter reaction time.

The isolated thiopyridine complexes (Fig. 2) were characterized by their elemental analyses, TGA, DTA measurements and different spectroscopic methods. The results of the elemental analyses are in good agreement with the calculated values.

IR for thiopyridine ligand (**1**) revealed the appearance of strong absorption band at ν = 3493 cm^{-1} due to NH group as well as another band at ν = 2226 cm^{-1} due to CN group. Moreover, IR for thiopyridine complexes showed the disappearance of NH band at ν = 3493 cm^{-1} in addition to a decrease in the intensity of the absorption band due to CN group at 2226 cm^{-1}confirming the formation of metal complexes **2–4** (Fig. 3).

Furthermore, thermoanalytical methods, such as thermogravimetry (TG), are excellent tools to follow the thermal decomposition of the complexes. The thermogravimetric analysis results of thiopyridine (HL,**1**) and its metal complexes proved the formation of metal complexes as indicated in table (1). The thermal behavior of the Au (III) complex as an example of pyridinethione metal complexes was studied by thermogravimetric (TG) and differential thermal analysis (DTA) as indicated in figure 4.



2:M = Ru **4**: M = Au **3**

Figure 2. Suggested structures of the complexes (**2–4**)

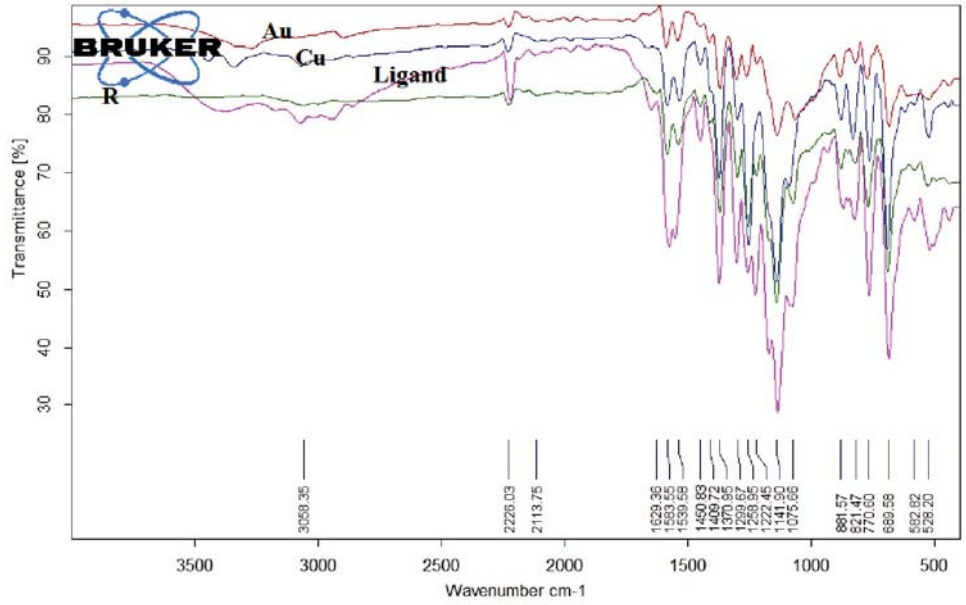


Figure 3. IR spectrum for ligand (**1**) and metal complexes (**2–4**); Ligand: compound **1**, Au: complex **4** , Cu: complex **3**, R: complex **2**

Table 1. The thermal data of 6-phenyl-2-thioxo-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile ligand (**1**) and its metal complexes (**2–4**)

Compound	steps	Temperature ring [ºC]	TG weight loss [%]		Assignments	T_{max} / ºC
			Calc.	Found		
HL (1)	1	25–380	72.60	73.10	3/2F_2, N_2, H_2CS and 3C_2 fragments	337
	2	More than 380	27.40	26.90	(C_6H_5)	573
Ru(L)$_3$ (2)	1	25–344	22.05	21.84	3/2(C_2F_6) fragments	243
	2	344–440	16.62	16.35	3(NCCN) fragments	332
	3	440–1000	42.81	43.22	3/2(HCCH), 3/2(SCCS) and three phenyl rings fragments.	449
	4	More than 1000	18.44	18.90	3C_2 and Ru metal residual	
Cu (L)$_2$ (3)	1	25–425	51.90	51.98	Two acetone, 2(CF_3), 2(NC-CN) and HCCH molecules	394
	2	425–526	18.40	18.96	SCCS and 2C_2 fragments	501
	3	526 – more than100	29.50	29.00	phenyl ring and Cu(II)	622
Au (L)$_3$ (4)	1	25–316	35.07	35.16	2/3(C_2F_6) and 3(NCCN).	240
	2	316–405	38.34	37.80	3/2(HCCH), 3/2(SCCS) and three phenyl rings fragment	340
	3	405 – more than 1000	26.59	27.04	Residue metal of Au and 3C_2 fragment	482

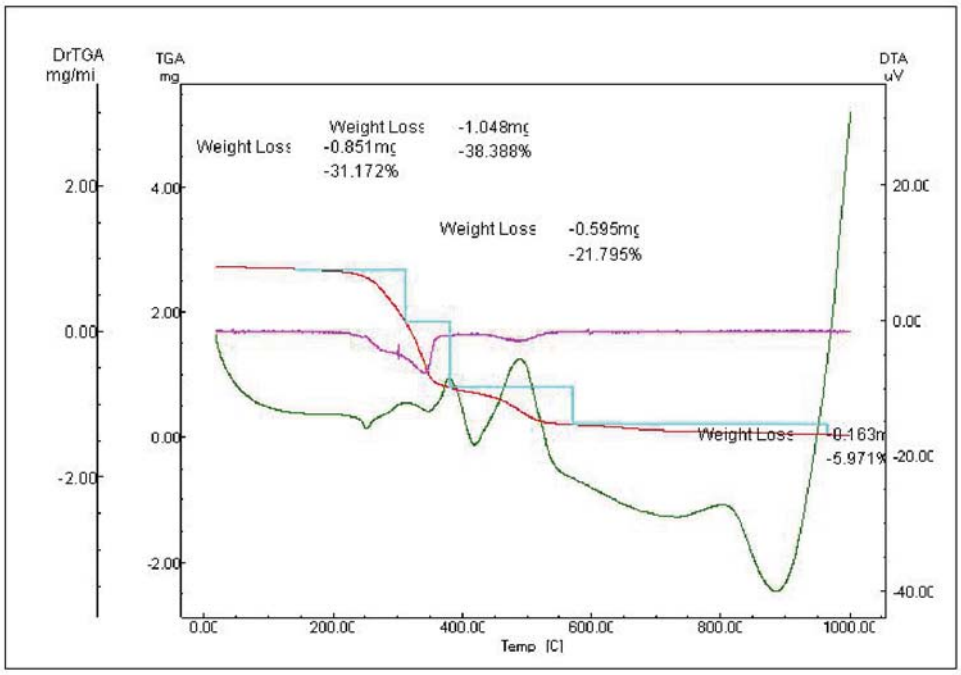


Figure 4. TGA and DTA thermogram for Au(L)3

Biological Activities

The ligand (**1**) and the novel derivatives (**2–4**) were subjected to evaluate their *in vitro* antimicrobial as well as *in vivo* anti-inflammatory activities.

Antimicrobial activity evaluation.

The preliminary antimicrobial activity for the ligand and its metal complexes (**1-4**) was carried out using cup-plate technique.[18] Among the metal complex derivatives (**2-4**), it was found that complex bearing copper metal **3** has nearly the same activity as the reference drug ciprofloxacin against both Gram-positive and negative bacteria. Other complexes (**2**,**4**) showed very weak antibacterial activity in comparison with the reference drug ciprofloxacin , so, copper metal is preferred than Ru and Au in inducing antibacterial. All the tested compounds did not show any antifungal activity (Table 2).

Anti-inflammatory activity evaluation:

Anti-inflammatory activity was evaluated by employing carrageenan rat hind paw edema method using celecoxib as a reference drug [19]. Mean changes in paw edema thickness after 1, 2, 3 and 4 h from induction of inflammation and edema inhibition given by the tested compounds and celecoxib at 18 mg/kg body weight dose level were displayed in Table 3. It was noted that complex **4** bearing gold displayed higher anti-inflammatory activity after 4 h than the reference drug celecoxib.

CONCLUSIONS

New complexes of Ru (III), Cu (II) and Au (III) (**2–4**) were prepared starting from pyridinethione ligand (HL,**1**) adopting electrochemical and traditional chemical procedures. The electrochemical method is preferred than the latter one because it affords pure products with higher yields in shorter reaction time. The preliminary antimicrobial activity evaluation revealed that complex bearing copper metal **3** has nearly the same activity as the reference drug ciprofloxacin. Anti-inflammatory ac-

Table 2. Antimicrobial activity evaluation for compounds **1–4** expressed by diameter of inhibition zone (mm)#

Compound	Diameter of inhibition zone [mm]			
	Gram- positive		Gram- negative	Fungi
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia. coli*	*Candida albicans*
1	6 ± 0.2*	7 ± 0.01*	5 ± 0.3*	–
2	9 ± 0.4*	8 ± 0.4*	7 ± 0.2*	–
3	16 ± 0.3	18 ± 0.3	12 ± 0.5	–
4	6 ± 0.2*	8 ± 0.2*	4 ± 0.4*	–
Ciprofloxacin	17 ± 0.8	19 ± 0.5	12 ± 0.3	–
Nystatin	–	–	–	20 ± 0.5

#Values were expressed as means ± SD., *P<0.001 v.s ciprofloxacin.

Table 3. Anti-inflammatory activity of the tested compounds (**1–4**) and celecoxib (18 mg/kg p.o.) against carrageenan-induced hind paw edema in rats. (Mean ± S.D; n = 6)

Compound	Initial thickness (Zero time)	Thickness of rat paw (mm) after			
		1 hour	2 hours	3 hours	4 hours
Control	0.22 ± 0.025	0.77 ± 0.02^{a}	0.88 ± 0.03^{a}	0.88 ± 0.03^{a}	0.88 ± 0.03^{a}
Celecoxib	0.22 ± 0.025	0.45 ± 0.06^{d}	0.42 ± 0.04^{d}	0.45 ± 0.04^{c}	0.50 ± 0.05^{cd}
1	0.22 ± 0.025	0.67 ± 0.02^{abc}	0.62 ± 0.02^{bc}	0.60 ± 0.04^{b}	0.62 ± 0.04^{bc}
2	0.22 ± 0.025	0.70 ± 0.07^{ab}	0.67 ± 0.04^{b}	0.60 ± 0.08^{b}	0.55 ± 0.06^{bcd}
3	0.22 ± 0.025	0.50 ± 0.04^{cd}	0.50 ± 0.04^{cd}	0.52 ± 0.04^{b}	0.67 ± 0.04^{b}
4	0.22 ± 0.025	0.55 ± 0.08^{bcd}	0.50 ± 0.06^{cd}	0.47 ± 0.03^{c}	0.45 ± 0.04^{d}

#Means within the same column having different letters were significantly different at $P \leq 0.05$, one-way ANOVA

tivity evaluation showed that complex **4** containing gold displayed higher activity than the reference drug celecoxib upon using carrageenan rat hind paw edema method.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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