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Synthesis, characterization and biological evaluation of Pd(II), Cu(II), Re(I) and $^{99m}\text{Tc(I)}$ thiazole-based complexes†

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A new thiazole-containing multidentate ligand 2-((2-phenylthiazol-4-yl)methylthio)ethanamine, L, was synthesized and used to prepare new complexes of the formula $\text{Pd}^{\text{II}}\text{LCl}_2$ (Pd-L), $\text{Cu}^{\text{II}}\text{L}_2\text{Cl}_2$ (Cu-L) and *fac*-[Re/ $^{99m}\text{Tc}(\text{CO})_3(\text{L})]^+$ (Re/ ^{99m}Tc -L). The ligand L and the metal complexes were characterized spectroscopically. Furthermore, the structures of Re-L and Cu-L were elucidated by X-ray crystallography. Ligand L acts as a bidentate (N_{th} , S) chelator in Pd-L, as a bidentate (N, S) chelator in Cu-L and as a tridentate (N_{th} , S, N) chelator in Re-L. The radiotracer ^{99m}Tc -L was synthesized in high yield and characterised by HPLC comparison with the Re-L analog. The synthesized compounds were evaluated for their anti-inflammatory and cytotoxic properties. The compounds exhibited low anti-inflammatory activity with Pd-L showing the highest activity among them. The cytotoxic activity of the ligand and the complexes against several human cancer cell lines (cervical adenocarcinoma HeLa, colorectal adenocarcinoma LS-174T, lung adenocarcinoma A549, breast adenocarcinoma MDA-MB-231 and normal human lung fibroblast cell line MRC-5) was examined using the MTT assay. The complex Cu-L exhibited the highest cytotoxicity and the complex Pd-L showed the best tumor selectivity. The changes in the cell cycle phase distribution were determined by flow cytometry and it was found that ligand L shows the highest apoptotic activity. The biodistribution studies of ^{99m}Tc -L in mice showed fast tissue clearance. Of all the thiazole-containing compounds, the palladium complex appears to be more promising for future efforts.

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1. Introduction

Metals have been used in medicine to treat various conditions since ancient times. Their use in the development of modern therapeutics increased tremendously after the discovery of the anticancer drug cisplatin. The advantages of metal complexes as synthons of drug molecules include the great chemical diversity of transition metals that, in combination with a variety

of ligands available, can create a palette of compounds with a plethora of properties and offers the possibility to discover novel drugs with new mechanisms of action. Furthermore, due to the physical properties that a number of metals possess, their coordination compounds find application as imaging agents (radiodiagnostic) as well as contrast agents for magnetic resonance imaging.¹

The search for new antitumor coordination compounds with increased tumor selectivity, broad spectrum of action and limited side-effects compared to the cisplatin prototype remains a challenging area of research and many efforts have focused on the development of either new improved derivatives of cisplatin or other non-platinum metal complexes as well as on the development of combinations of active organic drugs with metals.^{2,3} In this context, palladium analogs have been developed, generating new compounds with antitumor properties, since palladium has many chemical similarities with platinum. However, initial studies showed that the palladium compounds exchange ligands faster than the platinum analogs,⁴ thus limiting their access to the biological target. In the field of non-platinum compounds showing antitumor potential, copper-based complexes have been investigated on

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the assumption that endogenous metals may be less toxic.⁵ Organometallic complexes are attracting interest due to their kinetic stability and flexibility as building blocks for pharmaceutical applications. In particular, organometallic rhenium(i) tricarbonyl compounds have been shown to exhibit anti-tumor properties.⁶ In addition, rhenium radionuclides ¹⁸⁶Re/¹⁸⁸Re are both beta-emitters and are used for cancer radiotherapy.⁷ Furthermore, the radioactive surrogate metal of rhenium, technetium-99m, is a gamma-emitter and a protagonist in nuclear medicine imaging. Currently, research involving technetium-99m focuses on the development of tumor-targeted agents.⁸

Thiazole-containing compounds not only exist naturally as part of biologically important molecules, such as thiamine (vitamin B1) and bleomycin, but also include clinically useful drugs, such as abafungin, cefotaxime,⁹ meloxicam,¹⁰ thiabendazole,¹¹ nizatidine, ritonavir,¹² niridazole¹³ etc. Thiazole derivatives exhibit a variety of pharmacological activities, such as antibacterial,^{14,15} fungicidal,^{16,17} antioxidant,¹⁸ anti-inflammatory,^{19,20} anticancer,^{21–24} anti-HIV,^{25,26} antibiotic,²⁷ local anaesthetic,²⁸ analgesic and antipyretic,²⁹ against schizophrenia³⁰ among others. For the above reasons, thiazole-containing pharmacophores are promising and are actively used in the development of new therapeutics.³¹

As a part of our continuing research on the coordination chemistry of multidentate ligands, this work focuses on the development of a new thiazole-containing metal chelator, 2-((2-phenylthiazol-4-yl)methylthio)ethanamine, for the construction of new palladium(II), copper(II) and rhenium(I) tricarbonyl complexes. Given the fact that this ligand contains three different donor atoms, a thiazole-N (N_{th}), a thioether-S and an amine-N in combination with the different nature of first row transition metal copper, second row palladium and third row rhenium, three different coordination compounds were obtained. This diverse series of metal complexes was evaluated for cytotoxicity in a number of human adenocarcinoma cell lines as well as in a non-tumorous cell line. Furthermore, the anti-inflammatory properties of these compounds were evaluated. Finally, the technetium-99m radiotracer analog was also prepared and its bio-distribution was studied *in vivo* (Fig. 1).

2. Experimental

2.1. General

All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). [NEt₄]₂[ReBr₃(CO)₃] was prepared from

Fig. 1 Structures of the rhenium(I) tricarbonyl, copper(II) and palladium(II) complexes synthesized with the new ligand L.

Re₂CO₁₀ according to the literature³² which was then converted to [Re(H₂O)₃(CO)₃]⁺.³³ Precursor *fac*-[^{99m}Tc(CO)₃(H₂O)₃]⁺ was prepared by a standard method where 1 mL of [^{99m}TcO₄][−] ⁹⁹Mo/^{99m}Tc generator eluate was added to a kit containing 5.5 mg NaBH₄, 4 mg Na₂CO₃ and 15 mg Na–K tartrate, purged with CO gas, and the mixture was heated in boiling water for 30 min.³⁴ The synthesis of 2-phenyl-4-chloromethyl thiazole was performed with a method similar to the one described in the literature.³⁵ ESI-MS data were obtained on an MSQ Plus™ LC/MS (Thermo Scientific, Waltham, MA, USA). IR spectra were recorded as KBr pellets on a Perkin-Elmer FT-IR Spectrum BX spectrophotometer in the region 500–4000 cm^{−1}. ¹H and ¹³C NMR spectra were recorded on an Agilent DD2 500 MHz spectrometer and a Varian “Gemini 2000” (200 MHz) spectrometer. HPLC analysis was performed on an Agilent HP 1100 series pump, connected to a Gabi gamma detector (Raytest) and an HP 1100 multiple wavelength detector. Separations were achieved on an Agilent Eclipse XDB-C18 column (25 cm × 4.6 mm, 5 μm) eluted with a binary gradient system of solvent A: 0.1% TFA in water and solvent B: methanol at 1 mL min^{−1} flow rate. Initial composition consisted of 100% A–0% B which linearly converted to 25% A–75% B over 15 min and to 5% A–95% B from 15 to 20 min. The composition remained constant from 20 to 25 min at 95% B.

2.2. Chemical synthesis

2.2.1. Synthesis of 2-((2-phenylthiazol-4-yl)methylthio)ethanamine (L). 4-Chloromethyl-2-phenylthiazole (1 mmol, 209 mg) and 2-aminoethanethiol hydrochloride (1.5 mmol, 170.4 mg) were dissolved in a mixture of ethanol (8 mL)–water (5 mL), and then a solution of NaOH (2 M, 1.5 mL) was added dropwise. The reaction mixture was stirred at room temperature under N₂ for 18 h (Scheme 1). Then, the solvent was evaporated *in vacuo* to dryness. The residue was redissolved in ethanol and the solid formed was filtered. The filtrate was condensed to dryness and the product was purified by silica gel column chromatography with dichloromethane–methanol–ammonium hydroxide (9:0.85:0.15) as the eluent. Yield: 210 mg (84%); *t*_R = 15.29 min. IR (KBr): *v*_{max}/cm^{−1} 3346.3, 3047.1, 2918.9, 1640.9, 1555.4, 1517.0, 1504.1, 1478.5, 1470.0, 1431.5, 1388.7, 1341.7, 1247.4, 1239.1, 1222.1, 1175.0, 1076.7, 1034.0, 1008.3, 996.9, 871.6, 824.6, 764.7, 696.3, 649.3, 585.2, 572.4, 400.1, 314.5. ¹H NMR *δ* (500 MHz; CDCl₃) 7.99–7.84 (m, 2H, Ph), 7.45–7.35 (m, 3H, Ph), 7.09 (s, 1H, H-9), 3.86 (s, 1H, H-10), 2.87 (t, *J* = 6.3 Hz, 2H, H-12), 2.66 (t, *J* = 6.3 Hz, 2H, H-11). ¹³C NMR *δ* (126 MHz; CDCl₃) 168.32 (C-7), 154.78 (C-8), 133.47 (C-6), 128.87 (C-2/C-4), 126.5 (C-1/C-5), 115.15

Scheme 1 Synthesis of 2-((2-phenylthiazol-4-yl)methylthio)ethanamine, L.

(C-9), 40.88 (C-12), 36.10 (C-10), 31.35 (C-11). ESI(+)-MS (m/z): ($C_{12}H_{14}N_2S_2$) 250.06 calc. for $[M + H]^+$, 251.12 found.

2.2.2. Synthesis of the rhenium complex, *fac*-[Re(CO)₃(L)] (Re-L). A solution of $[Re(H_2O)_3(CO)_3]^+$ (0.15 mmol) in methanol (2 mL) was added a solution of ligand L (39.01 mg, 0.15 mmol) in methanol (5 mL) and the mixture was heated under reflux for 4 h. The solvent was evaporated to dryness, and the residue was crystallized by slow evaporation from methanol/water. Yield: 46 mg (51.1%); t_R = 17.48 min. IR (KBr): ν_{max}/cm^{-1} 3431.2, 3215.0, 3112.9, 3068.5, 2965.3, 2024.2 (CO), 1915.7 (CO), 1602.6, 1383.3, 1243.1, 1171.9, 1047.5, 992.9, 926.6, 745.0, 696.2, 638.0, 528.6, 494.0. 1H NMR δ (500 MHz; CD_3OD) 7.94 (s, 1H, H-9), 7.70–7.57 (m, 5H, Ph), 5.53 (br, 1H, NH), 4.78 (d, J = 16.9 Hz, 1H, H-10), 4.28 (d, J = 16.9 Hz, 1H, H-10), 3.91 (br, 1H, NH), 3.11 (ddd, J = 12.9, 5.9, 4.7 Hz, 1H, H-12), 2.84 (tdd, J = 10.0, 9.2, 5.4 Hz, 1H, H-11), 2.69 (tdd, J = 12.8, 8.3, 4.1 Hz, 1H, H-11), 2.55 (ddd, J = 13.1, 8.9, 4.1 Hz, 1H, H-12). ^{13}C NMR δ (126 MHz; CD_3OD) 175.15 (C-7), 154.86 (C-8), 133.16 (C-6), 131.50 (C-3), 129.28 (C-2/C-4), 129.02 (C-1/C-5), 118.73 (C-9), 48.08 (C-12), 36.76 (C-10), 35.55 (C-11). ESI(+)-MS (m/z): ($C_{15}H_{14}N_2S_2O_3Re$) 521.00 calc. for $[M]^+$, 521.11 found.

2.2.3. Synthesis of the copper(II) complex, $CuCl_2L_2$ (Cu-L). Copper(II) chloride (13.4 mg, 0.1 mmol) was dissolved in methanol (5 mL) and a solution of ligand L (50.12 mg, 0.2 mmol) in methanol (10 mL) was added. The reaction mixture was stirred for 5 h. The greenish solid obtained was isolated by filtration, washed with water to remove any free copper and dried at room temperature. Single crystals of Cu-L suitable for X-ray measurements were obtained by slow recrystallization from a mixture of ether/methanol by evaporation. A deep green solid powder was obtained. Yield: 34.5 mg (54.33%); t_R = 15.97 min. IR (KBr): ν_{max}/cm^{-1} 3448.61, 3280.67, 3233.89, 3113.87, 2921.14, 2651.08, 2177.65, 1623.54, 1576.25, 1514.44, 1498.85, 1459.71, 1438.01, 1409.54, 1304.01, 1235.56, 1113.64, 1096.68, 1050.10, 1001.87, 976.43, 926.86, 764.56, 727.60, 708.57, 691.18, 631.95, 599.41.

2.2.4. Synthesis of the palladium(II) complex, $PdCl_2L$ (Pd-L). An aqueous solution of $K_2[PdCl_4]$ (25.01 mg, 0.1 mmol) was mixed with a methanolic solution of L (32.64 mg, 0.1 mmol) and the mixture reacted for 2 h at room temperature. A pale yellow precipitate of the complex Pd-L was obtained, filtered off, washed with cold water to remove any free palladium, and air-dried. Yield: 31.5 mg (80.36%); t_R = 14.55 min. IR (KBr): ν_{max}/cm^{-1} 3444.54, 3190.00, 3109.44, 3090.44, 2978.04, 2933.41, 1618.76, 1577.52, 1495.83, 1459.64, 1404.80, 1322.99, 1236.67, 1136.19, 1074.33, 1001.96, 929.78, 843.47, 766.82, 712.83, 690.62. 1H NMR δ (200 MHz; $DMSO-d_6$) 8.02–7.93 (m, 2H, Ph), 7.89 (s, 1H, H-9), 7.57–7.46 (m, 3H, Ph), 5.16 (s, 2H, NH_2), 4.62 (d, J = 13.8 Hz, 1H, H-10), 4.34 (d, J = 13.9 Hz, 1H, H-10), 2.96–2.89 (m, 1H, H-11), 2.85–2.74 (m, 2H, H-12), 2.71–2.60 (m, 1H, H-11). ^{13}C NMR δ (50 MHz; $DMSO-d_6$) 168.09 (C-7), 149.59 (C-8), 132.80 (C-6), 130.67 (C-3), 129.40 (C-2/C-4), 126.40 (C-1/C-5), 120.24 (C-9), 47.70 (C-12), 37.22 (C-10), 35.83 (C-11). ESI(+)-MS (m/z): ($C_{12}H_{14}N_2S_2Cl_2Pd$) 390.91 calc. for $[M - Cl]^+$, 391.05 found.

2.3. X-Ray crystal structure determination

Crystals of both compounds were taken from the mother liquor and mounted at room temperature on a Bruker Kappa APEX 2 diffractometer equipped with a Triumph monochromator using Mo $K\alpha$ radiation. Calculation of cell dimensions and crystal system determination were performed using at least 182 high θ reflections with $I > 10\sigma(I)$. Data collection (ϕ and ω scans) and processing (cell refinement, data reduction and numerical absorption correction based on dimensions) were performed using the SAINT and SADABS programs.^{36,37} The structure was solved by means of the SUPERFLIP package.³⁸ The CRYSTALS version 14.40 program package was used for structure refinement by full-matrix least-squares methods on F^2 and the rest of all subsequent calculations.³⁹ Molecular illustrations with 50% ellipsoid probability were drawn using CAMERON incorporated into crystals.⁴⁰ All non-disordered non-hydrogen atoms have been anisotropically refined. All hydrogen atoms were found at their expected positions and were refined using appropriate riding constraints to the pivot atoms. Crystallographic details for both compounds are summarized in Table 1. Further details on the crystallographic studies as well as atomic displacement parameters are given as ESI† in the form of cif files.

Table 1 Crystallographic data for Re-L and Cu-L

Crystal data		
CCDC deposition number	CCDC 1821097	CCDC 1821098
Chemical formula	$C_{15}H_{14}N_2O_6ReS_2$	$C_{24}H_{28}Cl_2CuN_4S_4$
M_r	582.62	635.23
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Temperature (K)	295	295
a (Å)	26.3056(13)	19.911(11)
b (Å)	9.4798(4)	8.771(4)
c (Å)	16.1346(6)	8.088(4)
β (°)	106.786(2)	93.416(15)
V (Å ³)	3852.1(3)	1409.9(12)
Z	8	2
Radiation type	Mo $K\alpha$	Mo $K\alpha$
μ (mm ⁻¹)	6.56	1.28
Crystal size (mm)	0.43 × 0.19 × 0.16	0.17 × 0.16 × 0.06
Absorption correction	Numerical	Numerical
T_{min} , T_{max}	0.29, 0.35	0.81, 0.93
Reflections		
Measured	38 228	23 119
Independent	9952	2636
Observed	6648	1881
$[I > 2.0\sigma(I)]$		
R_{int}	0.021	0.034
$(\sin \theta/\lambda)_{max}$ (Å ⁻¹)	0.682	0.613
Refinement		
$R[F^2 > 2\sigma(F^2)]$	0.036	0.047
$wR(F^2)$	0.054	0.101
S	1.00	1.00
No. of reflections	6648	1881
No. of parameters	487	160
H-Atom treatment	H-Atom parameters constrained	H-Atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å ⁻³)	1.68, -2.74	0.65, -0.57

2.4. Synthesis and stability of $^{99m}\text{Tc-L}$

The precursor $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ (5 mCi/185 MBq, 0.5 mL) (pH 6) was added *via* a syringe to a crimped vial with L (50 μL , 0.01 M) and the mixture was heated at 95 $^\circ\text{C}$ for 30 min. Complex formation was verified by HPLC. The HPLC-purified ^{99m}Tc complex (50 μL , approx. 10 MBq) was incubated with 0.5 mL of 0.1 M pH 7.4 PBS at 37 $^\circ\text{C}$ for 18 h. The mixtures were analyzed by HPLC at 1 and 18 h.

2.5. *In vitro* cell studies

2.5.1. *In vitro* cytotoxicity. The examined compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM. Human cancer cell lines, cervical adenocarcinoma HeLa, colorectal adenocarcinoma LS-174T, lung adenocarcinoma A549 and breast adenocarcinoma MDA-MB-231, and a normal cell line, embryonic lung fibroblast MRC-5, were grown in RPMI-1640 medium and seeded into 96-well microtiter plates (cell densities: 2000, 7000, 5000, 5000 and 5000 cells per well for each cell line, respectively). 24 hours later, five concentrations of complexes (compounds) (concentrations ranged from 12.5 μM to 200 μM for L and Pd-L and from 3.1 μM to 50 μM for Cu-L) were added to the cells, except the control cells, according to the standardized procedure.⁴¹ Control cells were grown only in nutrient medium. After 72 hours of treatment with the compounds, cytotoxic effects on cells were determined by the MTT assay, according to the method of Mosmann, Ohno and Abe.^{42,43} Cisplatin was used as a positive control.

To calculate the cell survival (%), the absorbance (*A*) of cells treated with various concentrations of the complexes was divided by the absorbance of the control and multiplied by 100. The calculated IC_{50} was the concentration of the compound that inhibited cell survival by 50% compared with the control. The results were obtained from three independent experiments and presented with standard deviations.

The conditions for cell culture growth were described earlier.⁴¹ The cell lines were received from the American Type Culture Collection (Manassas, VA, USA) and the reagents were products of Sigma-Aldrich, St. Louis, MO.

2.5.2. Cell cycle analysis. 200 000 HeLa cells per well were seeded into 6-well plates overnight and then treated with IC_{50} and $2 \times \text{IC}_{50}$ concentrations of the examined compounds for 24 hours. Collected cells were fixed in 70% ethanol and stored at $-20\text{ }^\circ\text{C}$ for one week. The cells were washed, treated with RNase A (100 $\mu\text{g mL}^{-1}$) at 37 $^\circ\text{C}$ for 30 min and stained with propidium iodide (PI) (40 $\mu\text{g mL}^{-1}$).⁴¹ The results were obtained using a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) and CELLQuest software (BD Biosciences), for 10 000 cells per sample.

2.6. Animal studies

The animal studies were approved by the Aristotle University Committee for animal experimentation and were performed according to the EU guidelines. The animals were housed un-

der standard conditions and received a diet of commercial food pellets and water *ad libitum* prior to experimentation.

2.6.1. Inhibition of the carrageenin-induced edema.

Edema was induced in the right hind paw of mice (AKR) by the intradermal injection of 0.05 mL of 2% carrageenin in water. Both sexes were used, but pregnant females were excluded. Each group was composed of 6–10 animals. The tested compounds (0.01 mmol per kg body weight) were suspended in water with a few drops of Tween-80 and ground in a mortar before use and were given intraperitoneally simultaneously with the carrageenin injection. The mice were euthanized 3.5 h after the carrageenin injection. The difference between the weight of the injected and uninjected paws was calculated for each animal. It was compared with that in control animals (treated with water) and expressed as percent inhibition of the edema (CPE% values). Each experiment was performed in duplicate, and the standard deviation was less than 10%.

2.6.2. Biodistribution studies of $^{99m}\text{Tc-L}$. Inflammation edema was induced in ten-week-old BALB/c mice of approximately 25 g weight by intramuscular injection of 2% carrageenin (0.1 mL) into their right hind limb 2 h prior to the experiment. Each animal was then injected intravenously with 370 kBq of the purified $^{99m}\text{Tc-L}$ in 0.1 mL saline. The animals were sacrificed at 5 min and 1 h post-injection (p.i.) by cervical dislocation followed by blood withdrawal and cardiectomy. Organs and tissues of interest were excised rapidly and weighed, and their radioactivity was determined using a γ -counter. The activity of the tissue samples was decay-corrected and calibrated by comparing the counts in the tissue with the counts of a standard solution corresponding to 1% of the injected dose. Counts of the sample and calibration aliquots were measured in the γ -counter at the same time. The amount of activity in the selected tissues and organs is expressed as a percent of the injected dose per gram tissue (% ID per g). Values are quoted as the mean % ID $\pm$ standard deviation (SD) of four mice per group. Blood volume and muscle mass were estimated at 7 and 43% of body weight, respectively.

3. Results and discussion

3.1. Synthesis and characterization

Ligand L, 2-((2-phenylthiazol-4-yl)methylthio)ethanamine, was prepared in high yield (Scheme 1) and was used to prepare the new Re(I), Cu(II), Pd(II) and Tc(I) complexes. Ligand L is soluble in water, methanol, chloroform, dichloromethane and dimethyl sulfoxide. The reaction of $[\text{Re}(\text{H}_2\text{O})_3(\text{CO})_3]^+$ with an equimolar amount of ligand L in methanol led to the formation of complex *fac*- $[\text{Re}(\text{L})(\text{CO})_3]$, Re-L (Fig. 1). Re-L was crystallized by slow evaporation from methanol/water. A new copper(II) complex with ligand L, having the formula CuCl_2L_2 (Fig. 1), was synthesized by the reaction of copper(II) chloride and L in methanol in a Cu:L molar ratio of 1:2. Single crystals of Cu-L were obtained by slow recrystallization from a mixture of ether/methanol by evaporation. The complex Pd-L

(Fig. 1) was obtained by mixing an aqueous solution of $K_2[PdCl_4]$ and an equimolar amount of L in methanol.

The infrared spectra of the complexes were compared with that of the free ligand in order to determine the involvement of the coordination sites in chelation. The IR spectrum of the free ligand shows two bands at 1640.9 and 1555.4 cm^{-1} that correspond to the stretching vibrations of the thiazole ring, $\nu(-C=N)$ and $\nu(-C=C)$.⁴⁴ A shift in the stretching frequency of these bands to a lower wavenumber was observed in the IR spectra of Re-L and Pd-L, which indicates the involvement of the thiazole nitrogen atom in the coordination, while the thiazole is not involved in the coordination in Cu-L.⁴⁵ Strong bands in the region 2024–1915 cm^{-1} in the IR spectrum of the complex Re-L indicate the presence of three carbonyl ligands in a facial arrangement.^{46–48} The C–S–C band of the free ligand, which appears at 764.7 cm^{-1} , showed a very little effect by complexation indicating that the sulfur atom is not involved in the chelation.^{49–51} Asymmetric $\nu(-CH_2)$ stretching vibrations of moderate intensities were recorded around 2900 cm^{-1} . Another band observed at 1455–1476 cm^{-1} was assigned to $\nu_{asym}(C_6H_5)$, while the band at 1220 cm^{-1} was assigned to $\nu(C-N)$ stretching. The coordination of nitrogen to the metal is further supported by the appearance of a new band of medium intensity in the region 440–490 cm^{-1} due to $\nu(M-N)$ vibration.⁵²

The 1H NMR spectrum of L was recorded in $CDCl_3$, that of complex Re-L in CD_3OD and that of complex Pd-L in $DMSO-d_6$. The 1H NMR spectra of the ligand and the complexes showed signals in the aromatic region at δ 7.10–7.95 ppm that correspond to protons H-1 to H-9. The downfield shift of H-9 in the Re and Pd complexes signifies the coordination of the thiazole ring with the metals. In addition, the signals that correspond to the aliphatic protons H-10, H-11 and H-12 were shifted indicating the coordination mode of L with $fac-[Re(CO)_3]^+$ and $PdCl_2$, respectively. In particular, for the Re-L complex where L has coordinated as a tridentate (N_{th} , S, N) chelator, each methylene proton exhibits different shifts due to the different chemical environments upon coordination with the metal, at 4.78 and 4.28 ppm for H-10, 2.84 and 2.69 ppm for H-11, and 3.11 and 2.55 ppm for H-12 and, in addition, two visible signals of the two NH_2 protons at 5.53 and 3.91 ppm, respectively, indicating the coordination of the terminal nitrogen. In the palladium complex, the H-10 signals appear at 4.62 and 4.34 ppm and the H-11 signals are observed at 2.96–2.89 (m) and 2.71–2.60 (m) ppm, respectively, while the H-12 and NH_2 protons show only one signal at 2.85–2.74 (m) ppm and 5.16 ppm, respectively, indicating that the terminal nitrogen has not coordinated with the metal. Therefore, from the NMR study, it was concluded that in Pd-L, L has coordinated as a bidentate (N_{th} , S) chelator. The ^{13}C NMR spectrum of the ligand showed the carbon signals of the thiazole ring at 115, 155 and 168 ppm (ref. 53) as well as the signals due to the phenyl ring in the range of 126.0–133.43 ppm. Analogous signals were observed for the Re and Pd complexes as well.

The radio-tracer $fac-[^{99m}Tc(CO)_3L]^+$, $^{99m}Tc-L$, was synthesized in high radiochemical yield (>95%) and was character-

ized by radio-HPLC comparison of its elution time ($^{99m}Tc-L$ t_R = 17.24 min) with that of its analog Re-L (t_R = 17.47 min) (Fig. 2). The same elution time of the two compounds indicates that they share the same structure.^{46,47}

3.2. Description of the structures

The monoclinic unit cell of the compound Re-L comprises eight independent monocationic complexes and eight nitrate counter ions. Each asymmetric unit contains two complexes with very slight differences between them. The ligand 2-((2-phenylthiazol-4-yl)methylthio)ethanamine, L, acts as a tridentate chelating agent as it is coordinated to the Re(I) cation through the thiazole nitrogen atom, the methylthio sulfur atom and the terminal ethanamine nitrogen atom. This coordination mode forms two stable five-membered chelate rings as shown in Fig. 3. Three carbon atoms from three carbonyl molecules are also connected to the Re(I) cation fulfilling the final coordination number of six. The geometry around the metal is distorted octahedral. As the most axial vector passes through C14 and S1, these atoms lie on the octahedral axial positions while the equatorial plane is formed from N1, N2, C13 and C15 atoms (Fig. 2). The interatomic distances and angles of the compound are similar to those of analogous complexes^{32–34,46–48} found in the literature and are given in Table 2. Slight to moderate hydrogen bonding interactions arise between the nitrate oxygen atoms and the protons of the primary amine group.

In the monoclinic unit cell of Cu-L, two independent neutral centrosymmetric complexes are present. Each complex comprises one Cu(II) cation placed on the center of symmetry and symmetrically coordinated to two ligand molecules and two chlorine anions. Each ligand 2-((2-phenylthiazol-4-yl)methylthio)ethanamine, L, acts in this case as a bidentate chelating agent. The Cu(II) cation is coordinated to the methylthio sulfur atoms and the terminal ethanamine nitrogen atoms forming stable five-membered rings. The final distorted octahedral geometry around copper and the coordination number of six are fulfilled by the two chlorine anions (Fig. 4). The selected geometrical parameters of the complex

Fig. 2 HPLC chromatograms of $^{99m}Tc-L$ (blue, front trace) and its analog Re-L (red, back trace).

Fig. 3 Molecular structure of one of the two Re-L complexes present in the asymmetric unit together with the nitrate counter ion.

are given in Table 3 and are in accordance with similar complexes found in the literature.^{54–56}

3.3. Anti-inflammatory activity

The compounds were evaluated for their anti-inflammatory activity *in vivo*. The results are shown in Fig. 5. As can be seen from these data, our compounds showed low to moderate anti-inflammatory activity. The best activity was observed for Pd-L (40.9% CPE), where the indomethacin standard exhibited 47% CPE, followed by Cu-L (35.1% CPE), L (30.1% CPE) and Re-L (21.4% CPE) which showed the lowest anti-inflammatory activity. The study of the structure–activity relationship revealed that the activity of these complexes de-

pends on the metal. Thus, introduction of Cu to the complex increases the anti-inflammatory activity of the initial ligand. The replacement of Cu with Pd furnished the most active compound, while introduction of Re diminished the anti-inflammatory activity compared to the initial ligand. Since none of the compounds tested exhibited significant activity in this *in vivo* test, they were not studied further (*e.g.* to determine anti-COX properties that would allow us to explain their mode of action).

3.4. *In vitro* cytotoxicity

The viability of a series of adenocarcinoma cell lines as well as a normal cell line was evaluated by the MTT assay after treatment of the cells for 72 h with all the compounds. Our compounds showed good to moderate and dose-dependent cytotoxic effects against various adenocarcinoma cell lines (Table 4). Ligand L and palladium complex Pd-L exhibited moderate to low cytotoxicity, with L showing higher cytotoxicity against the HeLa, LS-174T and A549 cancer cell lines, while the cytotoxicity had an inverse trend in the case of the MDA-MB-231 cancer cell line, against which Pd-L showed higher cytotoxicity compared to L. The complex Pd-L shows the highest activity against HeLa cells, among the tested cell lines, and furthermore it exhibited the lowest toxicity, two to three times lower, in normal MRC-5 cells compared to the cancer cells. In general, Pd-L shows good selectivity. Therefore, Pd-L shows better selectivity compared to L with selectivity indexes of 3.04 for Pd-L and 2.79 for L, respectively, for HeLa cells. The complex Cu-L exhibited the highest cytotoxicity among the other investigated compounds. In particular, it was found to be more cytotoxic against the A549 and MDA-MB-231 cell lines compared to cisplatin. Nevertheless, it was

Table 2 Selected bond lengths [Å] and angles [°] for Re-L

Distances			
Re1–N1	2.204(5)	C1–C6	1.390(9)
Re1–N2	2.206(5)	C2–C3	1.386(9)
Re1–S1	2.4627(16)	C3–C4	1.361(11)
Re1–C13	1.889(6)	C4–C5	1.407(11)
Re1–C14	1.940(7)	C5–C6	1.369(8)
Re1–C15	1.874(6)	C6–C7	1.468(8)
N1–C12	1.488(7)	C8–C9	1.410(9)
N2–C7	1.300(8)	C8–C10	1.445(8)
N2–C8	1.370(7)	C11–C12	1.517(8)
S1–C10	1.778(6)	C13–O1	1.171(7)
S1–C11	1.830(6)	C14–O2	1.135(7)
S2–C7	1.737(6)	C15–O3	1.170(7)
S2–C9	1.654(8)		
Angles			
N1–Re1–N2	84.33(18)	S1–Re1–C14	177.13(19)
N1–Re1–S1	80.56(13)	C13–Re1–C14	89.5(3)
N2–Re1–S1	79.57(13)	N1–Re1–C15	91.6(2)
N1–Re1–C13	173.8(2)	N2–Re1–C15	173.6(2)
N2–Re1–C13	93.4(2)	S1–Re1–C15	94.9(2)
S1–Re1–C13	93.40(19)	C13–Re1–C15	90.1(3)
N1–Re1–C14	96.6(2)	C14–Re1–C15	85.4(3)
N2–Re1–C14	99.9(2)	C14–Re1–C15	85.4(3)

Fig. 4 Molecular structure of the Cu-L complex.

Table 3 Selected bond lengths [Å] and angles [°] for Cu-L

Distances			
Cu1–Cl1	2.4010(16)	C6–C7	1.454(7)
Cu1–N1	2.004(4)	C7–S2	1.701(5)
Cu1–S1	2.7163(16)	C8–C9	1.292(7)
N1–C12	1.455(6)	C8–C10	1.470(7)
N2–C7	1.316(6)	C9–S2	1.722(5)
N2–C8	1.305(6)	C10–S1	1.817(5)
C1–C2	1.397(7)	C11–C12	1.506(7)
C1–C6	1.392(7)	C11–S1	1.762(5)
C2–C3	1.391(8)		
Angles			
S1 ⁱ –Cu1–Cl1 ⁱ	84.27(6)	N1 ⁱ –Cu1–N1	180
S1 ⁱ –Cu1–N1 ⁱ	82.83(12)	Cl1–Cu1–N1	88.71(12)
Cl1 ⁱ –Cu1–N1 ⁱ	88.71(12)	S1 ⁱ –Cu1–S1	180
S1 ⁱ –Cu1–Cl1	95.73(6)	Cl1 ⁱ –Cu1–S1	95.73(4)
Cl1 ⁱ –Cu1–Cl1	180	N1 ⁱ –Cu1–S1	97.17(13)
N1 ⁱ –Cu1–Cl1	91.29(13)	Cl1–Cu1–S1	84.27(4)
S1 ⁱ –Cu1–N1	97.17(12)	N1–Cu1–S1	82.83(13)
Cl1 ⁱ –Cu1–N1	91.29(12)		

Symmetry code: (i) $-x + 1, -y + 1, -z + 2$.

found to be more cytotoxic against the normal MRC-5 cell line, which suggests poor selectivity.

3.5. Cell cycle analysis

Cell cycle analysis in the HeLa cell line (which was the most sensitive to L and Pd-L among the tested cell lines) was performed in order to obtain an early estimate of whether there is any apoptotic response of the cells after treatment with the

Fig. 5 % Inhibition of carrageenan paw edema in mice after injection of 0.01 mmol of the ligand L or the M-L complexes, where M = Re, Cu or Pd.

compounds for 24 h, at two different concentrations (IC_{50} and $2 \times IC_{50}$). Results from our experiments showed a dose-dependent increase in the percentages of HeLa cells in the subG1 phase 24 hours after exposure (Fig. 6). The cell cycle studies indicate that Pd-L as well as ligand L induced an appreciable increase in the subG1 phase (cells entering apoptosis). The highest increase in the percentage of HeLa cells in the subG1 phase was observed after treatment with ligand L (12.64% at IC_{50} and 18.14% at $2 \times IC_{50}$ for control = 5.53%), followed by Pd-L (9.37% at IC_{50} and 14.96% at $2 \times IC_{50}$). The significant increase in the percentages of treated cells in the subG1 phase after exposure to the tested compounds indicates the ability of the investigated compounds to induce apoptosis. For complex Cu-L, no accumulation of apoptotic cells was measured and therefore no conclusion as to its potential mechanism of cytotoxicity can be drawn at this point. It should be noted that the most sensitive cell lines to Cu-L were not HeLa but MDA-MB-231 and MRC-5. However, because it exhibited no tumor selectivity, it was not evaluated any further.

3.6. Biodistribution in mice

The biodistribution studies of ^{99m}Tc -L (Table 5) showed a fast blood clearance of $0.46 \pm 0.06\%$ ID per g at 1 h p.i. Its

Table 4 Concentrations of compounds that induced 50% decrease in target cell survival

Compound	HeLa	LS-174T	IC ₅₀ (μM)		
			A549	MDA-MB-231	MRC-5
L	38.67 ± 0.72	46.29 ± 3.2	103.46 ± 3.95	131.16 ± 2.22	108.14 ± 1.12
Pd-L	47.52 ± 1.53	60.28 ± 2.82	114.75 ± 2.19	76.92 ± 2.17	144.68 ± 2.67
Cu-L	6.13 ± 0.03	7.28 ± 0.8	7.38 ± 0.2	3.76 ± 0.16	4.42 ± 0.22
Cisplatin	2.24 ± 0.17	5.38 ± 1.34	10.12 ± 0.58	11.62 ± 1.73	39.85 ± 0.64

IC₅₀ values are presented as the mean ± standard deviation (SD) from three independent experiments.

Fig. 6 Changes in the cell cycle phase distribution of HeLa cells after treatment for 24 h.

liver and kidney uptake was $14.50 \pm 0.76\%$ ID per g and $9.75 \pm 1.10\%$ ID per g at 1 h p.i., respectively. In 1 h, $23.38 \pm 2.3\%$ of the injected dose was found in the intestine and $22.51 \pm 3.63\%$ ID in the urine. No accumulation of the radiotracer was observed in the edema compared to normal muscle at 1 h p.i. with values of 0.47 ± 0.08 and $0.46 \pm 0.04\%$ ID per g, respectively. The heart uptake was $2.18 \pm 0.18\%$ ID per g at 5 min p.i. and $1.52 \pm 0.14\%$ ID per g at 1 h, which is attributed to the cationic nature of $^{99m}\text{Tc-L}$.⁵⁷

In this work, we synthesized and evaluated the cytotoxicity as well as the anti-inflammatory properties of a new thiazole-containing ligand L and its respective metal complexes M-L (M = Pd, Cu, Re). It is interesting as a starting point that the ligand itself exhibits moderate cytotoxicity and anti-inflammatory activity. Thiazoles have been shown in the liter-

ature to exhibit a variety of biological properties, and anti-inflammatory and antitumor properties are among them. The respective complexes M-L exhibit biological properties that are mostly related to the nature of the metal. The palladium complex Pd-L has structural similarities to cisplatin; it is square planar, it has a neutral bidentate ligand and two *cis* chlorine atoms that are exchangeable in aqueous solution. It is known that palladium complexes are about 105 times more reactive than their Pt(II) analogs (Pd being a second row metal vs. Pt being a third row one) leading to rapid hydrolysis of the leaving group. The hydrolysed species $[\text{Pd}(\text{L})(\text{H}_2\text{O})_2]^{2+}$, being cationic, does not diffuse through the cellular membranes and therefore it cannot interact with its targets. This would explain its lower toxicity in the cell lines in comparison to cisplatin. However, its good selectivity along with its moderate anti-inflammatory activity makes it the most promising compound in this work. In future work, a bidentate labile ligand could be used instead of the two chlorines to improve the complex's pharmacological profile.

The copper complex is octahedral and has two axial chlorines that could be exchanged in water, but since they are not in *cis* conformation as in the palladium complex, their structures cannot be directly compared. On the other hand, copper(II) is very reactive and transchelation could occur *in vivo*, in either axial or equatorial positions. Its cytotoxicity was found to be very high, comparable to that of cisplatin against all the cancer cell lines, however it was more toxic against normal cells which renders it unsuitable as an anti-cancer agent. Its anti-inflammatory properties were comparable to those of L and Pd-L.

Table 5 Biodistribution of $^{99m}\text{Tc-L}$ in mice (mean ± SD)

Organ	% ID per organ		% ID per g	
	5 min	1 h	5 min	1 h
Blood	2.83 ± 0.22	0.87 ± 0.12	1.49 ± 0.18	0.46 ± 0.06
Heart	0.25 ± 0.00	0.20 ± 0.03	2.18 ± 0.18	1.53 ± 0.14
Liver	25.69 ± 2.12	22.22 ± 1.50	17.18 ± 1.18	14.50 ± 0.76
Lungs	0.25 ± 0.01	0.15 ± 0.03	1.87 ± 0.42	1.03 ± 0.13
Muscle	7.38 ± 0.04	5.25 ± 0.10	0.63 ± 0.03	0.46 ± 0.04
Kidneys	32.62 ± 2.27	3.55 ± 0.41	93.81 ± 7.66	9.75 ± 1.10
Spleen	0.09 ± 0.01	0.05 ± 0.00	1.43 ± 0.05	0.73 ± 0.08
Intestine	16.60 ± 0.95	23.38 ± 2.30	10.37 ± 0.94	15.56 ± 1.83
Stomach	0.54 ± 0.08	1.13 ± 0.61	2.27 ± 0.17	4.91 ± 3.72
Urine	6.80 ± 1.15	22.51 ± 3.63	—	—
Edema	—	—	0.82 ± 0.11	0.47 ± 0.08

It should be noted that initially the rhenium complex Re-L was included in the cytotoxicity study, however in preliminary studies it was found to be inactive, hence it was not studied further (data not included). As far as its anti-inflammatory properties are concerned, Re-L was found to be the least active. Rhenium(I) tricarbonyl complexes with tridentate ligands are inert and this could explain the absence of biological activity observed in Re-L. Its *in vivo* inertness can be perceived by extrapolation of the biodistribution results of its radioactive analog $^{99m}\text{Tc-L}$, where fast clearance from circulation was observed *via* renal and hepatobiliary excretion.

4. Conclusions

The new tridentate thiazole-containing ligand coordinated in different modes with each of the three metals used, Cu(II), Pd(II) and Re(I). With copper(II), it formed an octahedral homodimeric (S, N) complex, with palladium(II) a bidentate (N_{th} , S) chelate and with the Re tricarbonyl core a tridentate (N_{th} , S, N) cationic complex. The Re-L compound did not exhibit significant anti-inflammatory properties, while the analogous $^{99m}\text{Tc-L}$ radiotracer did not exhibit *in vivo* accumulation in carrageenan-induced edema in the right hind limb. Compounds L and Pd-L exhibited similarly moderate anti-tumor activity against the tested adenocarcinoma cell lines where the tumor selectivity of Pd-L showed the most promise. L and Pd-L both induced apoptosis in HeLa cells. Cu-L was the most cytotoxic and exhibited poor selectivity. In terms of the anti-inflammatory properties of these compounds, an increase in their activity was observed in Cu-L and Pd-L *versus* the ligand L, while Pd-L exhibited the most significant anti-inflammatory properties among the tested compounds. Overall, Pd-L exhibited the most promising biological properties in terms of both anti-tumor and anti-inflammatory activities and future studies could focus on the development of palladium complexes with an improved pharmacological profile.

Conflicts of interest

The authors declare no competing interest.

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