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Synthesis, characterization and biological evaluation of octyltrimethylammonium tetrathiotungstate

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Abstract Octyltrimethylammonium tetrathiotungstate salt (ATT-C8) was synthesized and its ability to chelate copper was evaluated. The biological and toxic aspects were evaluated by in vitro and in vivo assays, using bovine aorta endothelial cells (BAEC) and zebrafish (*Danio rerio*) embryos. The obtained results suggest that ATT-C8 has better biocompatibility, showing a significantly lower lethal concentration 50 (LC_{50}) value in comparison to ammonium tetrathiotungstate (ATT). Zebrafish embryos assay results indicate that both tetrathiotungstate salts at the studied concentrations increase the hatching time. Even more, an in vivo assay showed that synthesized materials behave as copper antagonists and have the ability to inhibit its toxicological effects. Also, both materials were found to be active for the in vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The characterization of the materials was carried out using the following spectroscopic techniques: Ultraviolet–Visible (UV–Vis), Fourier Transform Infrared (FTIR) and proton nuclear magnetic resonance (^{1}H-NRM).

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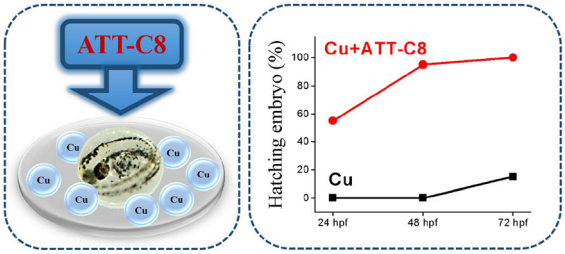
Graphic abstract



Keywords Tetrathiotungstate salts · Zebrafish embryo · Antioxidant activity · Anticopper therapy · Toxicity

Introduction

Diverse studies suggest that, as ammonium tetrathiomolybdate (ATM), ammonium tetrathiotungstate (ATT) presents excellent chelating properties (McQuaid et al. 1994; Young et al. 1982). ATM´s chelating properties have been thoroughly studied in the treatment of Wilson's disease (WD) (Brewer 2009; Brewer et al. 1994, 1991). ATM forms complexes with copper and albumin that decrease the bioavailability of copper in the blood. Nowadays, ATM is in phase II and phase III of multinational clinical trials as an anticopper therapy for the treatment of neurological symptoms in patients with WD (Aggarwal and Bhatt 2018; Brewer et al. 1996). More recently, it was found that ATM has excellent efficacy in the treatment of animal models of fibrotic, inflammatory, and autoimmune diseases, as well cancer (Brewer 2003, 2005, 2016; Brewer et al. 2000). Nevertheless, research of the potential of ATT treatments against degenerative diseases is scarce, compared to ATM.

The catalytic activity in hydrotreatment processes of the sulfides obtained from the decomposition of these thiometallates, MoS_2 and WS_2, shows an increase when presenting superficial carbon available from the synthesis method, generating the $MoS_{2-X}C_X$ and $WS_{2-X}C_X$ species (Berhault et al. 2001; Chianelli and Pecoraro 1981; Kelty et al. 2007; Pecoraro and Chianelli 1985). With this knowledge, new synthesis routes have been developed, where the carbon is dosed during the process, in the form of alkyl chains, generating the tetraalkylammonium thiometallate precursors, $(NR_4)_2MS_4$, (where R = alkyl radical and M = Mo, W). There was a notable advance in the synthesis of these materials when Alonso et al. prepared the $(NR_4)_2MS_4$ precursors (where R = H, CH_3, C_4H_9 and M = Mo, W) in aqueous media, which afterwards were decomposed into MoS_2 and WS_2 (Alonso et al. 1998, 2000). In this sense, it would be of interest to evaluate the influence of superficial carbons dosed by alkyl chains in the chelation properties of salts derived from these compounds when administered to animal models.

Zebrafish embryo is becoming a new model of choice for preclinical studies. Several interesting tests that use these embryos have been proposed as rapid, high-throughput, and cost-effective drug and chemical screens (Dooley and Zon 2000; García-Caballero et al. 2018; Tobia et al. 2011; Tran et al. 2007; Wilkinson and Van Eeden 2014). A number of different biological aspects such as toxicity, inflammation, organ regeneration or angiogenesis are studied in these screens to test drugs and biomaterials as chemical modulators. Recent studies with zebrafish embryos have evaluated the pro-angiogenic modulation of copper either directly administrated to water or lixiviated from biomaterials (Romero-Sánchez et al. 2018).

The objective of the present work is to explore the biological aspects and the copper chelating ability of a new tetrathiotungstate salt. The study includes the antioxidant activity and toxic effects of a modification of ATT with an alkyl group (octyltrimethylammonium tetrathiotungstate, ATT-C8). With this objective, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, in vitro endothelial cell viability and migration assays, and an in vivo toxicity zebrafish embryo assay were used.

Methods and materials

Spectroscopy characterization and molecular modeling

The chemical structures of ATT and ATT-C8 compounds were characterized using the spectroscopic techniques of Ultraviolet–Visible (UV–Vis), Fourier Transform Infrared (FTIR) and proton nuclear magnetic resonance (^{1}H-NRM). UV–Vis data was obtained using a HACH DR-6000 UV–Vis

Spectrometer in the λ = 200–700 nm range. FTIR spectra were acquired in transmission configuration with a Perkin-Elmer Spectrum GX over the range of 4000–400 cm^{-1}. ^{1}H-NMR spectra were recorded on a Bruker Avance III spectrometer 400 MHz. The chemical shifts (δ) are presented with tetramethylsilane (TMS) (δ: 0.00) as the internal standard. The 2D and 3D-structure molecular modeling of the synthesized compound was plotted using ChemDraw Professional 16. The UV–Vis, FT-IR and RMN spectra with their corresponding data, as well as the 3-D molecular structure of the obtained tungsten salts are available in the supplementary information section.

Synthesis of materials

Synthesis of ATT

ATT ($(NH_4)_2WS_4$) was prepared according to the method described by Ramanathan and Weller (1985). Ammonium metatungstate ($(NH_4)_6H_2W_{12}O_{40} \times 18H_2O$; Sigma-Aldrich) (15.0 g, 5.1 mmol) is dissolved in 75.0 mL of distilled water containing 52.5 mL of an ammonium hydroxide solution (NH_4OH (20%); Sigma-Aldrich). The resulting ammonia solution was heated in a temperature bath to 60 °C for 6 h, while hydrogen sulfide was bubbled (H_2S (g)) into the solution. The reaction mixture was then cooled in an ice bath and allowed to stand for 12 h, yielding yellow crystals. The product was vacuum-filtered, washed with isopropyl alcohol (C_3H_8O; Sigma-Aldrich) stored under N_2 atmosphere at 15 °C and it was named as ATT. The molecular structure of ATT is shown in Fig. 1a. UV–Vis λ_{max} (nm): 216, 276, 393. FTIR (cm^{-1}): 460 (ν Mo-S); 3128, 1395 (ν N–H). ^{1}H-NMR ($CDCl_3$): δ 7.14 (s, 8H, $(N\mathbf{H}_4)_2$).

Synthesis of ATT-C8

ATT-C8 was prepared by the reaction between ATT (1 equivalent) and octyltrimethylammonium bromide (2 equivalent) in aqueous solution following the method reported by Alonso et al. (1998, 2000). Freshly prepared $(NH_4)_2WS_4$ ATT (2.0 g, 5.7 mmol) was dissolved in 25.0 mL of distilled water. While stirring, 25.0 mL of a second aqueous solution was added, containing octyltrimethylammonium bromide ($CH_3(CH_2)_7$ N (Br) $(CH_3)_3$; Sigma-Aldrich) (2.9 g, 11.5 mmol). The reaction mixture is kept at rest for 12 h and the resulting precipitate is recovered by sedimentation using a centrifuge at 2000 rpm. Finally, the yellow product ATT-C8 ($(CH_3(CH_2)_7$ N $(CH_3)_3)_2WS_4$) was dried in an oven at 30 °C and stored, being named as ATT-C8. The molecular structure of ATT-C8 is show in Fig. 1b. UV–Vis λ_{max} (nm): 216, 276, 393. FTIR (cm^{-1}): 460 (ν Mo-S); 3128, 1395 (ν N–H); 3005, 2922, 2852 (ν C-H); 1468 (δ C-H). ^{1}H-NMR ($CDCl_3$): δ 3.34 (m, 11H, $\mathbf{CH_2}$-N), 3.08 (s, 18H, $\mathbf{CH_3}$-N), 1.68 (m, 4H, $\mathbf{CH_2}$-CH_2-N), 1.30 (m, 20H, $(\mathbf{CH_2})_5$-CH_2-CH_2-N), 0.87 (t, 6H, $\mathbf{CH_3}$terminal).

Antioxidant activity

The antioxidant capacity of the synthetized materials was evaluated using the DPPH radical scavenging method proposed by Blois (1958). In the method, the DPPH ($C_{18}H_{12}N_5O_6$; Sigma-Aldrich) free radical is

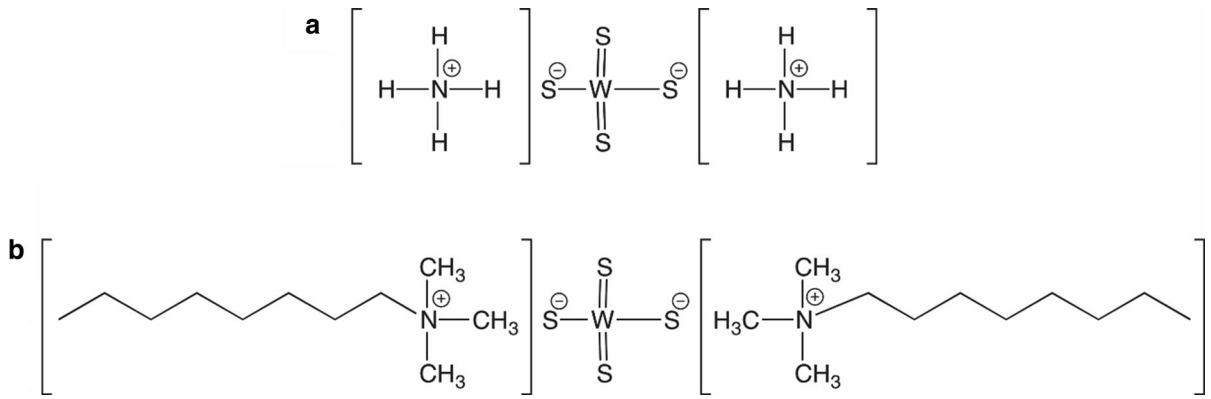


Fig. 1 Structure of the synthesized compounds **a** ATT and **b** ATT-C8

dissolved showing a purple coloration, that upon reduction by an antioxidant or a radical species, changes to yellow due to an electron or proton transfer. The change in absorbance is spectrophotometrically monitored at a wavelength of 517 nm. The DPPH radical scavenging effect was calculated according the following equation:

$$DPPH\text{-}scavenging\ effect\ (\%) = \left[1 - \left(\frac{Absorbance\ sample}{Absorbance\ control}\right)\right] \times 100$$

The half maximal inhibitory concentration (IC_{50}) value was interpreted as the concentration of antioxidant material necessary to scavenge 50% of the initial DPPH radicals. The IC_{50} values were calculated by linear regression resulting from plotting the % of DPPH-scavenging activity versus the concentration of the test samples. Analyses were performed in three independent assays.

Cell assays

Cell culture

Bovine aortic endothelial cells (BAEC) were isolated from bovine aortic arches as previously described (Cárdenas et al. 2006) and maintained in Dulbecco's modified Eagle's medium (DMEM) containing glucose (1 g/L) and supplemented with glutamine (2 mM), penicillin (50 U/mL), streptomycin (50 U/mL) and 10% FBS. Cells were maintained at 37 °C under a humidified 5% CO_2 atmosphere.

Cell viability assay

2.5×10^3 cells were seeded in 96-well microplates using a total volume of 100 µl of culture medium containing different concentrations of the ATT and ATT-C8 materials. Cells were incubated for 72 h (37 °C, 5% CO_2 in a humid atmosphere). Then, 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were added to the wells and incubated an additional 4 h in the dark. HCl isopropanol was used for resuspension of formazan crystals and asbsorbance data (550 nm) was collected using an Eon Microplate Spectrophotometer from Bio-Tek Instruments (Winooski, VT, USA). Data was collected by Gen5 software from the same manufacturers. Half-maximal inhibitory concentration (IC_{50}) values were calculated as the concentrations of compound yielding 50% cell survival, taking the values obtained for control as 100%.

Cell migration assay

Migration of BAEC was assessed using the so-called "wound-healing" assay. Cells were grown, and once they reached confluence, different concentrations of ATT and ATT-C8 were added with fresh medium and a cross-shaped scratch was done using a pipette tip. Wounded areas were observed under a microscope after 4, 7 and 24 h of incubation, photographs were taken from the same areas as those recorded at zero time. Images were analyzed with NIH Image J 1.6 software. The regrowth of BAEC into the cell-free area was expressed as the percentage of the initial wounded area (time 0) recovered by cells migration to different incubation times.

Zebrafish embryo assays

Zebrafish collection

Zebrafish (Danio rerio) embryos were obtained from fish facilities at the University of Malaga following standard procedures (Kimmel et al. 1995; Truong et al. 2011).

A breeding stock of adult zebrafish was kept on a recirculating system at 27 ± 1 °C. Fishes were maintained with a standard 12:12-h light–dark photoperiod. Embryos were produced by one-to-one or one-to-two female-male mating in fish hatch box. Fertilized eggs are collected and disinfected using a 0.5% bleach solution during 1 min and washed several times with E3 embryo media (5.00 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, 0.00003% $C_{16}H_{18}ClN_3S$; Sigma-Aldrich). Clean eggs were incubated in E3 medium in a Petri dish at 28 ± 0.1 °C.

Fishes were handled under National Laws (Law 9/2003, April 25, and Law 31/1995, November 8) by notification A/ES/12/I-22 and activity A/ES/12/24. The experiments were carried out under the procedures established and approved by the UMA Bioethics Commission under the BIO2014-56092-R grant.

Zebrafish embryo exposure assay

At 24 h after fertilization (hpf) 15 zebrafish embryos were transferred to wells of a 6-well plate. The embryos were incubated in 3 mL of culture medium with different concentrations of the ATT and ATT-C8 materials (0.1, 10, 100, 250, 750, 1000, 10,000 μg/ml) for 96 h at 28 °C. All solutions with different material concentrations were prepared in E3 medium. The survival rate of the embryos from each treatment group was determined. Zebrafish were observed directly on the 6-well plate using a Nikon AZ100 multizoom microscope. The hatching rate, the changes in morphology throughout development and the tetrathiotungstates effect in the copper induced embryo toxicity were also evaluated. Zebrafish embryos were exposed to control (10 μg/mL $CuCl_2$) and two experimental (10 μg/mL $CuCl_2$, 250 μg/mL ATT; and 10 μg/mL $CuCl_2$, 250 μg/mL ATT-C8) conditions and their hatching rate studied. All assays were repeated three times and at least 60 embryos were used per concentration. Mortality curves were generated using MS Excel 2016 and the lethal concentration 50 (LC_{50}) was calculated applying a nonlinear regression test (sigmoidal dose–response curve). LC_{50} indicates the concentration that kills half of tested population in zebrafish embryo model.

Statistical analysis

Data are shown as mean ± standard deviation (SD; 99% confidence interval) resulting from triplicate evaluations. Statistical significance was calculated using a Student t-test of control-experimental group pairs using the Statistical Package for the Social Sciences (SPSS) version 25 software (IBM, SPSS). Differences were considered significant when p-values are $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Results and discussion

Antioxidant activity

The DPPH scavenging activity percentage of tetrathiotungstates at varying concentrations was measured and the results are displayed in Fig. 2a. Significant DPPH radical scavenging activity was evident at all the tested concentrations of ATT (1.25–50 μg/mL) and ATT-C8 (0.63–30 μg/mL). It was found that the DPPH scavenging effect of tetrathiotungstates was dose dependent.

Figure 2b displays the DPPH IC_{50} values of ATT and ATT-C8 calculated by the regression equation of the calibration curve. The lower the IC_{50} value means higher DPPH radical scavenging of tetrathiotungstates, implying a higher antioxidant activity. ATT-C8 ($p < 0.001$) was found to have better free radical scavenging ability in comparison with ATT, having an IC_{50} value of 15.04 ± 0.01 μg/mL and 46.64 ± 0.36 μg/mL, respectively. The capacity of materials to scavenging free radicals could be attributed to the electron donor nature of the substituents -NH and $-CH_3$ present in the samples, since they are known to be good hydrogen donors (Schubert et al. 1962).

Antioxidants are molecules with the ability to eliminate or neutralize the free radicals responsible for the oxidative stress associated with cancer, arteriosclerosis, inflammatory processes and degenerative diseases (Goodman et al. 2011). In fact, bioactive materials studies use antioxidant capacity as an indicator of biological activity. However, in the knowledge of the authors, there are not existent reports of the antioxidant properties of this type of coordination compounds. Thus, although the results cannot be compared with other bioinorganic materials, the IC_{50} values for ATT and ATT-C8 are important compared to some DPPH assay reference antioxidants, such as the butyrated hydroxyl toluene (BHT) (19.4–86.6 μg/mL), vitamin C (5.8–110.7 μg/mL) and tocopherol (27.1–96.0 μg/mL) (Mishra et al. 2012).

Cell assays

In this study, BAEC was incubated in the presence of ATT and ATT-C8 materials to determine their influence on cell growth and cell migration capacity. Figure 3 displays the effects in cellular growth measured up to 72 h-culture, in the presence of ATT and ATT-C8 solutions with concentrations of 0.1–200 μg/mL. The IC_{50} values of 1.02 ± 0.24 μg/mL and 4.27 ± 0.95 μg/mL were obtained for ATT and ATT-C8 respectively, showing that both materials have cytotoxic effects over BAEC cells; with ATT

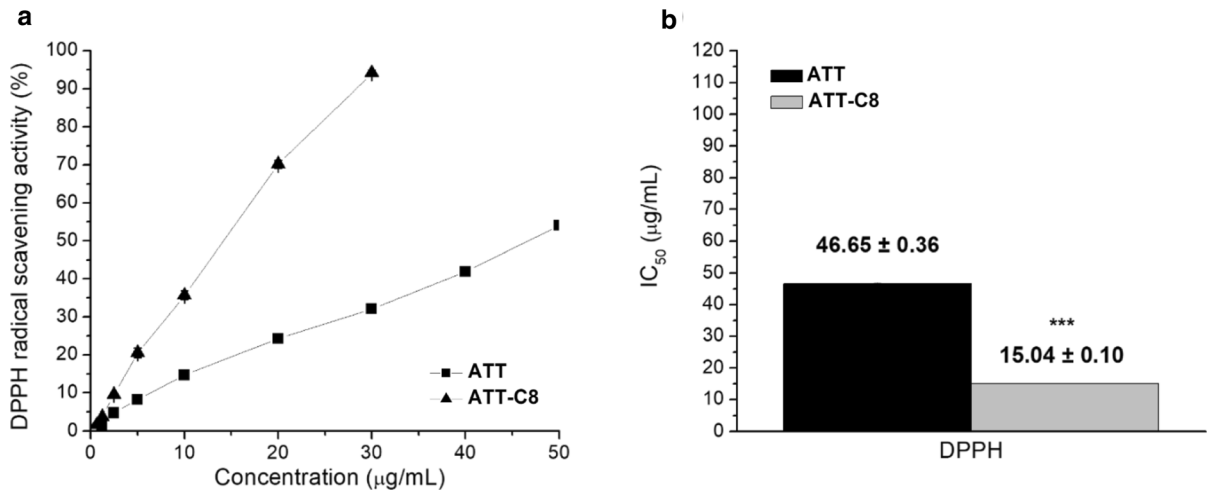


Fig. 2 DPPH free radical scavenging assay results: **a** Concentration–response curve of antioxidant activity and **b** Bar graph with IC_{50} (μg/mL) values of synthesized materials. Values represent mean ± SD of three replicates. The figure includes the statistical significance of the ATT-C8 group measured with respect to the control group (ATT); ***$p < 0.001$ by Student's t test

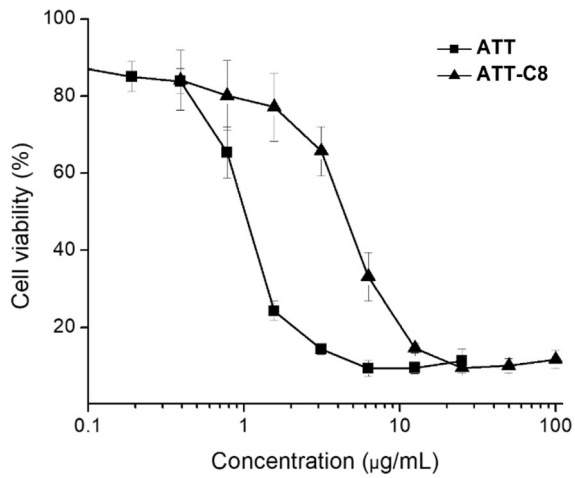


Fig. 3 In vitro BAEC cell growth assay obtained with the MTT assay after exposure to ATT and ATT-C8 materials at different concentrations for a 72 h incubation period. The plots represent the mean ± SD of three independent experiments. Dose–response curves were calculated using a nonlinear regression test

presenting more acute toxic effects with respect to ATT-C8.

The wound healing assay with BAEC was used to evaluate the inhibitory or stimulating effects on angiogenesis. At short periods (4 and 7 h), cell wound filling area is due exclusively to its migration and not to its proliferation. Figure 4 shows cell migration after 4, 7 and 24 h of the wound healing assay compared to the control group. After 4 h, a slight promotion in cellular migration was observed for the ATT-C8 culture at low concentration (2 μg/mL), in comparison to the bare medium control culture. However, there were no significant differences between the experimental BAEC cultures, suggesting that the studied materials have no angiogenic influence at the studied concentrations (Fig. 4).

Zebrafish embryo assays

Developmental toxicity and biological responses of zebrafish embryos exposed to an ATT and ATT-C8 dissolution were evaluated (Fig. 5). Figure 6 shows the survival rate of zebrafish embryos treated with different concentrations of tetrathiotungstates for several days. The control group, without the addition of tetrathiotungstate salts, presented a normal behavior with an overall mortality rate < 1%. The tetrathiotungstate salts induced lower than 5% mortality zebrafish embryos incubated at 0.1–100 μg/mL concentrations.

At 72 hpf, embryos under these concentrations were anatomically indistinguishable from control (Fig. 6a, b). The absence of sublethal effects, such as the lack of instant motility, depigmentation and development of edemas or clots, indicate that at the studied concentrations (1–100 μg/mL), ATT and ATT C-8 do not have any toxic or teratogenic effects in zebrafish

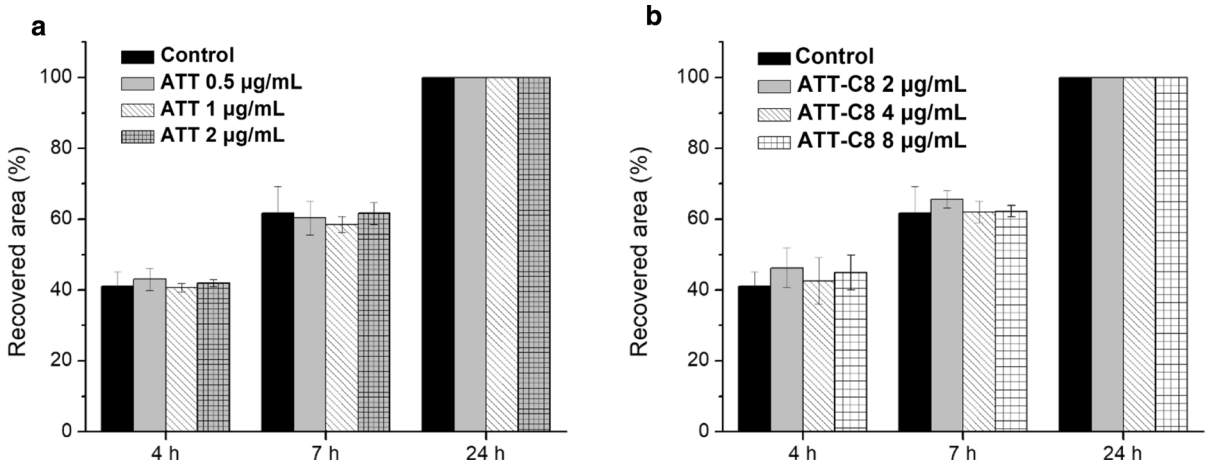


Fig. 4 Wound assay was used to assess in vitro cell migration of BAEC. The graphics present frequency of wound recovery in **a** ATT and **b** ATT-C8 culture cells. The values are presented as the mean ± SD of three independent experiments

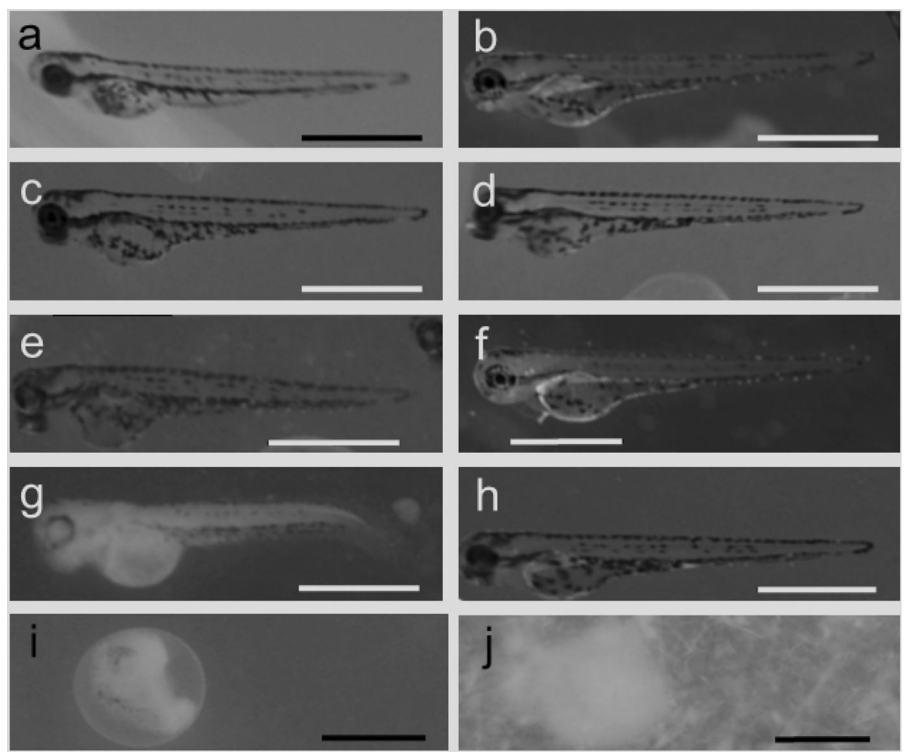


Fig. 5 Representative images of zebrafish larvae (72 hpf) after 48 h exposure to **a** bare medium; **b** 100 µg/mL ATT; **c** 250 µg/mL ATT; **d** 250 µg/mL ATT-C8; **e** 500 µg/mL ATT; **f** 500 µg/mL ATT-C8; **g** 750 µg/mL ATT; **h** 750 µg/mL ATT-C8; **i** 1000 µg/mL ATT and **j** 1000 µg/mL ATT-C8. Scale bar: 10 µm

embryos. A significantly higher mortality rate than the one measured in the control group was initially observed at a concentration of 250 µg/mL of tetrathiotungstate salts (Fig. 6), and there was a 0% survival rate when the embryos were exposed to concentrations above 1000 µg/mL of ATT for more than 48 h and above 10,000 µg/mL ATT-C8 for more than 24 h.

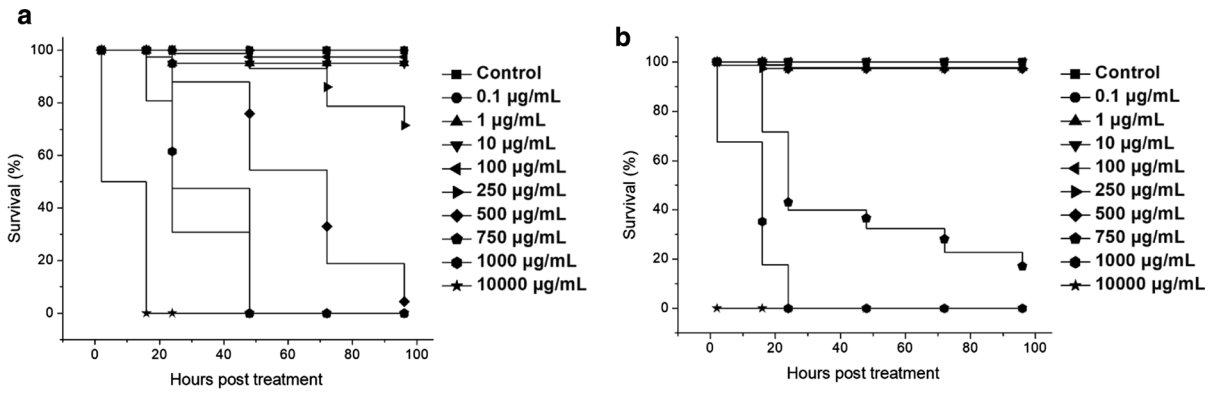


Fig. 6 Survival rate of zebrafish embryos exposed to different concentrations of **a** ATT and **b** ATT-C8 during 24, 48 and 72 h. All results are representative of three independent experiments

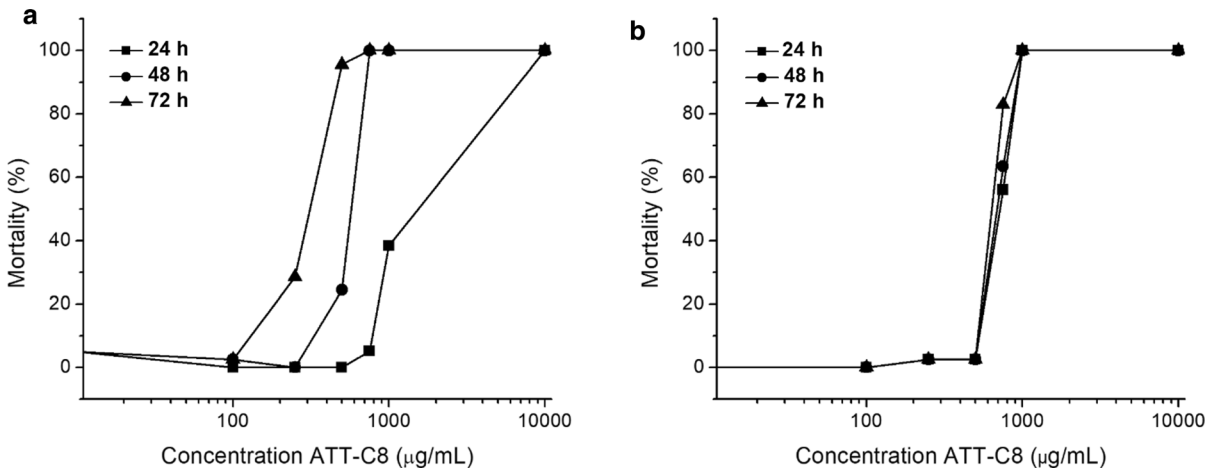


Fig. 7 Mortality curves of zebrafish embryos exposed to **a** ATT and **b** ATT-C8 materials at different concentrations during 24, 48 and 72 h exposure. All the experiments were done in triplicate. Dose–response curves were calculated using a nonlinear regression test

Figure 7 shows the mortality rate plotted for embryos exposed to ATT and ATT-C8 materials for 24, 48 and 72 h. The LC_{50} values of ATT and ATT-C8 (Table 1) were estimated based on these mortality curves. ATT shows a concentration and time dependent toxic profile with an LC_{50} of 1211 ± 84, 542 ± 57 and 316 ± 68 µg/mL at 24, 48 and 72 h of exposure, respectively. Consistent with the obtained IC_{50} values in the cell viability assay, zebrafish is more sensitive to the ATT sample at 72 h of exposure. In the case of ATT-C8, constant IC_{50} values were observed at around 700 µg/mL ($p < 0.001$) at 24, 48 and 72 h of exposure. In this regard, the results suggest that the incorporation of carbon into the structure improves ATT biocompatibility.

Table 1 Results of LC_{50} at to 24, 48 and 72 h

Material	LC_{50} (µg/mL)		
	24 h	48 h	72 h
ATT	1211 ± 84	542 ± 57	316 ± 68
ATT-C8	735 ± 25	722 ± 31	689 ± 29

Values represent the mean ± SD of three independent experiments

Figure 8 displays the hatching rate of zebrafish embryos incubated for 72 h to the 250 µg/mL solutions of tetrathiotungstate salts. The results show an increase in the hatching rate of zebrafish embryos of the groups exposed to tetrathiotungstate salts, compared to the control group. All of the embryos exposed

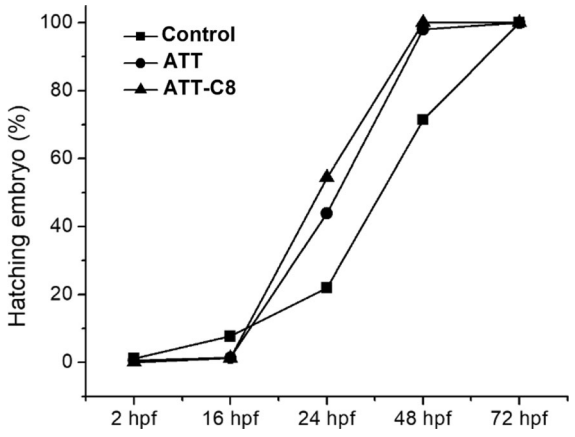


Fig. 8 Hatching rate of zebrafish embryos exposed to 250 µg/mL ATT and ATT-C8 materials during 72 h. All the experiments were done in triplicate

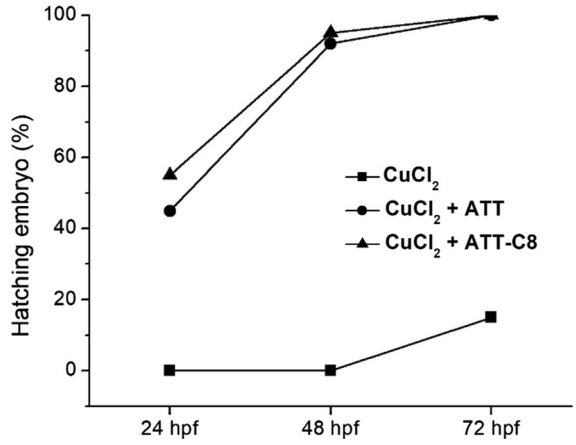


Fig. 9 Hatching rate of zebrafish embryos exposed to 10 µg/mL $CuCl_2$ solution (negative control), a mixture of 10 µg/mL $CuCl_2$ and 250 µg/mL ATT and a mixture of 10 µg/mL $CuCl_2$ and 250 µg/mL ATT-C8. All results are representative of three independent experiments

to ATT and ATT-C8 hatched at 72 hpf. It is known that the activity of chorionic hatching enzymes (ZHE) and embryo movements affect their hatching time (Nechaev and Pavlov 2004). In this case, the embryo membranes were very deteriorated just before hatching, suggesting that the studied compounds do not inactivate the involved enzymes or embryo motility. Other reports mention that the hatching of zebrafish embryos is plastic in response to potential stress changes or changes in the salinity of the environment (Ord 2019; Warkentin 2011). In this way, it could be inferred that an increase in the concentration of external ions causes moderate hypoxia that accelerates the hatching time. Overall, tetrathiotungstate salts were found to accelerate the hatching rate of zebrafish embryos at low concentrations and cause acute death or chronic lethal toxicity at high concentrations.

In order to evaluate whether tetrathiotungstate salts revert the copper-mediated embryo toxicity, embryos were treated with 10 µg/mL of $CuCl_2$ and a mixture of 10 µg/mL $CuCl_2$ and 250 µg/mL of ATT or ATT-C8 for 72 h. It is well documented that copper interferes with the enzymatic activity of the ZHE, delays hatching and induces abnormal morphology and mortality (Lin et al. 2013; Romero-Sánchez et al. 2018). The obtained results show that copper significantly delays hatching up to 96 hpf and 120 hpf, in good agreement with reported results. Tetrathiotungstate salts thus significantly inhibit the delaying effect of copper in the hatching process ($p < 0.001$). Figure 9 shows that 90% of embryos incubated into the mixture of copper and tetrathiotungstate salts hatch at 48 hpf. All these results suggest that tetrathiotungstate salts could be effective protectors against copper toxicity. As reported for ATM and ATT, ATT-C8 also acts as a copper antagonist, becoming an alternative chelating agent in anticopper therapies.

Conclusions

The copper chelating abilities of ATM and its use in clinical assays have opened new research opportunities for treatments against diseases where an increase of copper levels is involved. In this work, we have studied the bioactive potentialities of a new tetrathiotungstate salt, ATT-C8 in different in vitro and in vivo assays.

Our results show that ATT and ATT-C8 behave as copper antagonists. This copper chelating ability of the tested tetrathiotungstate salts was evaluated in a zebrafish embryo hatching assay, validating their potential use in anticopper therapies. Toxicity results suggest that the materials have toxic effects in BAEC cells and zebrafish embryos over a determined concentration, which increase at higher doses and exposure time. This effect ameliorates (72 h data) with the addition of the eight carbon residues, cell migration increases and zebrafish hatching time decreases. Further studies of these promising compounds are

needed to strictly define these dose-dependent effects and to analyze the stability of compounds. It is interesting to note that the results of the in vitro assays suggest that the tetrathiotungstate salts could neutralize free radicals (antioxidant activity), a property that is useful in the treatment of oxidative stress-induced degenerative diseases.

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Author contributions KV-G: Investigation, Writing-Reviewing and Editing. JC-R: Conceptualization, Funding acquisition. JFH-M: Methodology, Data curation. MM-B: Conceptualization, Visualization. LJD-R: Formal analysis. IC-G: Resources. DC-V: Validation. MCO: Investigation. MAM: Supervision. LBR-S: Writing-Original draft preparation, Project administration.

Compliance with ethical standards

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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