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Synthesis, characterization and screening of biological activity of Zn(II), Fe(II) and Mn(II) complexes with trithiocyanuric acid

Pavel Kopel [a,*], Karel Doležal [b], Libor Machala [c], Vratislav Langer [d]

[a] Department of Inorganic Chemistry, Palacky University, Křížkovského 10, CZ-771 47 Olomouc, Czech Republic
[b] Laboratory of Growth Regulators, Institute of Experimental Botany ASCR and Palacky University, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic
[c] Department of Experimental Physics, Palacky University, Tr. Svobody 26, CZ-771 46 Olomouc, Czech Republic
[d] Environmental Inorganic Chemistry, Department of Chemical and Biological Engineering, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

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Abstract

New Zn(II), Fe(II) and Mn(II) complexes with a combination of nitrogen-donor ligands and trithiocyanuric acid ($ttcH_3$) were prepared and characterized by elemental analysis, IR and UV–Vis spectroscopies. The antitumor activity of the prepared complexes, together with already known Ni(II) species, were assayed in vitro against G-361 (human malignant melanoma), HOS (human osteogenic sarcoma), K-562 (human chronic myelogenous leukaemia) and MCF-7 (human breast adenocarcinoma) tumor cell lines. The IC_{50} values of the Fe(II) and Mn(II) compounds turned out to be lower than those of cisplatin and oxaliplatin. The antimicrobial activities were evaluated by MIC against bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*). The molecular structure of $[Zn(taa)(ttcH)] \cdot H_2O$ (taa = tris(2-aminoethyl)amine) was determined by X-ray diffraction. The central atom is pentacoordinated by four N atoms of taa and one N atom of the ttcH dianion.

Keywords: Complexes; Trithiocyanuric acid; Cytotoxicity; Crystal structure; Mössbauer spectra

1. Introduction

The highly water soluble compound $ttcNa_3 \cdot 9H_2O$ ($ttcH_3$ = trithiocyanuric acid, also referred to as 2,4,6-trimercapto-1,3,5-triazine) readily forms precipitates with heavy metal ions like Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Hg^{+}, Ag^{+} and Tl^{+}, and that is why it is used for the removal of heavy metal ions from industrial waste water [1–5]. It is also very efficient for the removal of residual palladium and its compounds from reaction mixtures, in which it is used as a catalyst where the residues are undesirable, especially in pharmaceutically active ingredients [6–8]. $TtcH_3$ and its salts have found a wide range of applications, for example the use as silver and nickel plating agents, the production of composite materials with metals and rubbers and rewritable image formation by photopolymerization of the acid [9–13].

The presence of nitrogen and sulfur atoms and the analogy to the pyrimidine nucleobase makes $ttcH_3$ a very interesting compound from a biological point of view. The compound itself was evaluated as a ligand of *Toxoplasma gondii orotate phosphoribosyltransferase* [14–16]. This enzyme is necessary for replication of parasitic protozoan *T. gondii*, which causes the disease toxoplasmosis. Trithiocyanuric acid is a better ligand for the enzyme than 5-fluorouracil and emimycin, i.e. compounds used for clinical treatment of toxoplasmosis. Kar et al. prepared a series of trinuclear Ru(II) complexes of the composition $[\{Ru(bpy)_2\}_3(ttc)](ClO_4)_3$, $[\{Ru(phen)_2\}_3(ttc)](ClO_4)_3$ and $[\{Ru(L)_2\}_3(ttc)](ClO_4)_3$, where bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline and L = arylazopyridine, which contain trithiocyanurate(3-) bridge bound Ru(II) centers by S, N donor sets of the anion [17,18]. The ruthenium atoms are further coordinated by bidentate nitrogen

* Corresponding author. Tel.: +420 58 563 4354; fax: +420 58 522 5737.
E-mail address: pavel.kopel@upol.cz (P. Kopel).

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ligands to give a deformed octahedral surrounding. In addition to the structural, electrochemical and spectral study, the interaction of the complexes with the circular and linear forms of p-Bluescript DNA has been reported. The three tested complexes reduce the fluorescence intensity of both circular and linear DNA, but the nature of these interactions remains unknown.

Aoki et al. prepared and studied the zinc supramolecular complex $[(Zn_3L)_4(ttc)_4](NO_3)_{12} \cdot 22H_2O$ (L = 1,3,5-tris-(1,4,7,10-tetraazacyclododecan-1-ylmethyl)benzene), where the ttc anion links three Zn-cyclen moieties through Zn–S coordination bonds [19]. The complex forms a twisted cub-octahedral exterior in which various hydrophobic guest molecules, such as adamantine, can be encapsulated. In another work of this group, zinc supramolecules have been reported with other spacers connecting the cyclene derivatives [20]. In the presented X-ray structure of $[(Zn_3L_1)_3(ttc)_3](NO_3)_9 \cdot 21.5H_2O$, ($L_1$ = tris-cyclen), there are two kinds of ttc bonding i.e. by S atoms only and a tris bidentate chelating mode. In work published by our group we prepared and solved the molecular structure of the complex [Zn(bapen)(ttcH)] · EtOH (bapen = *N*,*N*′-bis(3-amino propyl)ethylenediamine) [21]. The compound is mononuclear only and the zinc atom is coordinated by four N atoms of bapen and by the chelating S, N set of the ttcH dianion.

Many other complexes containing trithiocyanurate anions have been prepared and structurally studied. These complexes can be mononuclear as well as polynuclear, with a chelating S, N donor set or bound by S or N atoms only [22–27]. In our previous works we prepared and structurally characterized a series of nickel(II) and iron(II) complexes containing the trithiocyanurate anion and nitrogen-donor ligands [28–30].

Herein, we present the results of the synthesis and characterization of a series of mixed-ligand complexes involving trithiocyanuric acid and nitrogen-donor ligands of the following composition: [Zn(pmdien)(ttcH)] (**1**), [Zn(taa)-(ttcH)] · H_2O (**2**), $[Fe(nphen)_2(ttcH)] \cdot H_2O$ (**3**), $[Fe(dmbpy)_3](ttcH_2)_2 \cdot 4H_2O$ (**4**), $[Mn(phen)_2(ttcH)] \cdot H_2O$ (**5**), Mn(bpy)(ttcH) · H_2O (**6**), [Ni(taa)(ttcH)] · H_2O (**7**) and $[Ni(phen)_3](ttcH) \cdot 5H_2O$ (**8**), where pmdien = *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine, $ttcH_3$ = trithiocyanuric acid, taa = tris(2-aminoethyl)amine, nphen = 5-nitro-1,10-phenanthroline, dmbpy = 4,4′-dimethyl-2,2′-bipyridine, phen = 1,10-phenanthroline and bpy = 2,2′-bipyridine. Compounds **3**, [30] **7** [31] and **8** [32] were prepared before by our group and in that work they were tested for biological activity. According to what we know, manganese complexes with a combination of N ligands and the trithiocyanurate anion are reported for the first time. We were also interested in finding out if the newly prepared complexes show any biological activity. In vitro cytotoxic activity of the complexes against G-361 (human malignant melanoma), HOS (human osteogenic sarcoma), K-562 (human chronic myelogenous leukaemia) and MCF-7 (human breast adenocarcinoma) cancer cell lines was determined. Antimicrobial activity was tested on *Escherichia coli* 3954, 3988, 4225, *Pseudomonas aeruginosa* 3955, *Staphylococcus aureus* 3953, 4223 and *Enterococcus faecalis* 4224.

2. Experimental

2.1. Materials and methods

The chemicals and solvents were purchased from Aldrich Co. and Lachema Co. and were used without further purification. The complexes $[Fe(nphen)_2(ttcH)] \cdot H_2O$ (**3**), [Ni(taa)(ttcH)] · H_2O (**7**) and $[Ni(phen)_3](ttcH) \cdot 5H_2O$ (**8**) were prepared according to the literature methods [30–32].

The C, H, N, S analyses were carried out on an EA 1108 instrument (FISONS). IR spectra (400–4000 cm^{-1}) were recorded on a Perkin–Elmer Spectrum One FT-IR spectrometer using KBr pellets. The absorption spectra in MeCN and the diffuse-reflectance spectra in nujol (11 000–40 000 cm^{-1}) were obtained on a Lambda 35 UV/Vis spectrometer (Perkin–Elmer). Magnetochemical data were obtained by the Faraday method at 293 K using a Sartorius M-25D electrobalance. $Hg[Co(SCN)_4]$ was used as a calibrant. The correction for diamagnetism was calculated using Pascal's constants. The transmission Mössbauer spectrum was recorded using a Mössbauer spectrometer in constant acceleration mode with a $^{57}Co(Rh)$ source. Isomer shift parameters are related to metallic iron (with a calibration temperature of 300 K).

2.2. Preparation of the complexes

2.2.1. [Zn(pmdien)(ttcH)] (1)

N,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (pmdien) (0.2 ml, 1 mmol) was added to an aqueous solution (70 ml) of $Zn(NO_3)_2 \cdot 6H_2O$ (0.3 g, 1 mmol). The mixture was stirred at room temperature for 30 min. A solution of $ttcNa_3 \cdot 9H_2O$ (0.14 g, 0.33 mmol) in water (5 ml) was added in the form of drops. The formation of a white precipitate was observed. The pH of the solution was adjusted to 8 by addition of aqueous NaOH and the rest of the precipitate was removed by filtration. After a week light yellow crystals were separated by filtration, washed with water and dried under an infrared lamp at 40 °C. Yield: 72%. *Anal.* Calc.: C, 34.8; H, 5.8; N, 20.3; S, 23.2. Found: C, 34.8; H, 6.0; N, 20.5; S, 23.7%. IR (cm^{-1}): 456w, 488m, 750w, 776m, 798m, 857s, 906w, 933m, 984m, 1022m, 1043w, 1120w, 1175m, 1215s, 1243m, 1264w, 1432vs, 1631m, 2897w, 2970w, 3436s.

2.2.2. [Zn(taa)(ttcH)] · H_2O (2)

Tris(2-aminoethyl)amine (taa) (0.15 ml, 1 mmol) was added to an EtOH solution (80 ml) of zinc(II) acetate dihydrate (0.22 g, 1 mmol). The mixture was stirred at room temperature for 30 min. A solution of $ttcNa_3 \cdot 9H_2O$ (0.14 g, 0.33 mmol) in water (5 ml) was added in the form of drops. The white precipitate that formed was dissolved

by addition of aqueous NaOH (resulting pH 11). Light yellow crystals suitable for X-ray analysis were obtained after a week. They were filtered, washed with a small amount of EtOH and dried in air. Yield: 67%. *Anal.* Calc.: C, 26.7; H, 5.2; N, 24.2; S, 23.8. Found: C, 26.9; H, 4.9; N, 24.1; S, 23.0%. IR (cm^{-1}): 422w, 469m, 545w, 632w, 650m, 742m, 781w, 858s, 872s, 978s, 994w, 1072s, 1124m, 1194vs, 1248m, 1283w, 1329s, 1360w, 1435vs, 1489vs, 1576m, 2852w, 2932w, 3297m, 3411m.

2.2.3. $[Fe(dmbpy)_3](ttcH_2)_2 \cdot 4H_2O$ (**4**)

4,4′-Dimethyl-2,2′-bipyridine (dmbpy) (0.36 g, 2 mmol) in EtOH (50 ml) was added with stirring to an aqueous solution (50 ml) of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (0.39 g, 1 mmol). The mixture was heated at 60 °C for 45 min. After cooling, a small amount of precipitate was filtered off. Then, $ttcNa_3 \cdot 9H_2O$ (0.4 g, 1 mmol) in water (10 ml) was added dropwise to the solution. A dark red substance, which appeared during addition of the solution of $ttcNa_3$, was filtered off, washed with EtOH and dried under an infrared lamp at 40 °C. Yield: 54%. *Anal.* Calc.: C, 48.8; H, 4.7; N, 16.3; S, 18.6. Found: C, 49.1; H, 4.5; N, 15.9; S, 17.8%. IR (cm^{-1}): 455s, 482m, 530m, 622w, 719w, 824m, 873s, 1038w, 1129vs, 1228vs, 1339m, 1480vs, 1521vs, 1618s, 2888w, 3068m, 3417s. UV–Vis (MeCN/cm^{-1}) ($\varepsilon \times 10^{-4}$/$dm^3\ mol^{-1}\ cm^{-1}$): 18900(1.8), 25200(1.5), 36800(10.3). $\mu_{eff} = 2.44$ BM.

Mössbauer spectrum (300 K): doublet I with isomer shift (i.s.) $= 0.29 \pm 0.01$ mm s^{-1}, quadrupole splitting (q.s.) $= 0.29 \pm 0.01$ mm s^{-1}, half-width of the spectral line (Γ) $= 0.30 \pm 0.01$ mm s^{-1} and relative spectrum area (A) = 61.5%; doublet II with i.s. $= 0.35 \pm 0.01$ mm s^{-1}, q.s. $= 0.81 \pm 0.01$ mm s^{-1}, $\Gamma = 0.43 \pm 0.01$ mm s^{-1}, $A =$ 38.5%.

2.2.4. $[Mn(phen)_2(ttcH)] \cdot H_2O$ (**5**)

1,10-Phenanthroline (phen) (0.4 g, 2 mmol) was added to a water solution (80 ml) of manganese(II) acetate tetrahydrate (0.25 g, 1 mmol). Then $ttcNa_3 \cdot 9H_2O$ (0.4 g, 1 mmol) in water (10 ml) was added in drops to the solution. The yellow precipitate which appeared immediately was filtered off, washed several times with water and EtOH and dried under an infrared lamp at 40 °C. Yield: 76%. *Anal.* Calc.: C, 53.3; H, 3.2; N, 16.1; S, 15.8. Found: C, 53.1; H, 3.0; N, 15.7; S, 15.6%. IR (cm^{-1}): 421w, 639m, 725s, 845s, 865s, 1101m, 1129m, 1206s, 1342s, 1424vs, 1460s, 1516m, 1589w, 1622m, 3429s. UV–Vis (cm^{-1}): 25150, 26200, 29400. $\mu_{eff} = 6.20$ BM.

2.2.5. $Mn(bpy)(ttcH) \cdot H_2O$ (**6**)

A preparation similar to that for the synthesis of (**5**) was applied, only 2,2′-bipyridine (bpy) (0.32 g, 2 mmol) was used instead of 1,10-phenanthroline. The substance is also yellow. Yield: 66%. *Anal.* Calc.: C, 38.6; H, 2.7; N, 17.3; S, 23.8. Found: C, 38.4; H, 2.8; N, 16.9; S, 22.9%. IR (cm^{-1}): 411w, 627m, 649m, 736m, 763s, 873s, 1016m, 1042w, 1059w, 1153 m, 1224s, 1315w, 1439vs, 1473s, 1574w, 1595s, 3090w, 3413s. UV–Vis (cm^{-1}): 25450, 27400, 28900, 36360. $\mu_{eff} = 5.90$ BM.

2.3. X-ray crystallography

X-ray data of (**2**) were collected on a Siemens SMART CCD diffractometer with Mo Kα radiation ($\lambda =$ 0.71073 Å, graphite monochromator). The crystal was cooled to 173(2) K by a flow of nitrogen gas using the LT-2A device. A full sphere of reciprocal space was scanned by 0.3 steps in ω with a crystal-to-detector distance of 3.97 cm. A preliminary orientation matrix was obtained from the first frames using SMART [33]. The collected frames were integrated using the preliminary orientation matrix which was updated every 100 frames. Final cell parameters were obtained by refinement of the positions of reflections with $I > 10\sigma(I)$ after integration of all the frames using the SAINT software [33]. The data were empirically corrected for absorption and other effects using the SADABS program [34]. The structures were solved by direct methods and refined by full-matrix least squares on all $|F^2|$ data using SHELXTL software [35].

The largest peak and hole on the final difference map were 0.427 and −0.251 e $Å^{-3}$. Important crystallographic parameters are as follows: $C_9H_{21}N_7OS_3Zn$, wavelength 0.71073 Å, monoclinic, space group $P2_1/c$, $a =$ 11.8044(3) Å, $b = 12.7373(4)$ Å, $c = 10.7776(3)$ Å, $\beta =$ 95.051(1)°, volume 1614.19(8) $Å^3$, $Z = 4$, density (calc.) 1.666 Mg/m^3, absorption coefficient 1.919 mm^{-1}, $F(000) = 840$, crystal size $0.31 \times 0.19 \times 0.11$ mm, index ranges $-18 \leqslant h \leqslant 17$, $-19 \leqslant k \leqslant 18$, $-16 \leqslant l \leqslant 16$, reflections collected/independent 29364/5883 ($R_{int} = 0.0213$), refinement method full-matrix least-squares on F^2, data/restraints/parameters 5883/0/224, goodness-of-fit on $F^2 = 1.024$, final R_1 (2σ data) = 0.0207, $wR_2 = 0.0550$, final R_1 (all data) = 0.0250, $wR_2 = 0.0571$.

2.4. Biological activity testing

K-562, MCF7, G-361 and HOS were used for a cytotoxicity determination of the complexes by a calcein AM assay. The tumor cells were maintained in 75 ml plastic tissue culture flasks (TPP) and grown on Dulbecco's modified Eagle's cell culture medium (DMEM) containing 1 g l^{-1} glucose, 4 mM glutamine, 100 IU ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 10% foetal bovine serum and 3.7 g l^{-1} sodium bicarbonate. The cell suspension of approximate density 1.25×10^{-5} cells/ml was redistributed into 96-well microtitre plates (Nunc, 80 μl per well). After preincubation (12 h at 37 °C, 5% CO_2), the test compounds, in sixfold dilutions, were added (20 μl per well). Incubation of the cells with the tested compounds (in the 0.2–100 μM range) lasted for 72 h at 37 °C in 5% CO_2 atmosphere, 100% humidity. At the end of this period, the cells were incubated for 1 h with calcein AM and the fluorescence of the live cells was measured at 485 nm/538 nm (ex/em) with a Fluoroscan Ascent (Labsystems,

Finland). IC_{50} values, the drug concentrations lethal to 50% of the tumor cells, were estimated.

The antimicrobial activities of $ttcNa_3$ and its metal complexes were estimated by MIC ($\mu g\ ml^{-1}$). The bacteria were inoculated into 5 ml of a liquid medium (Mueller-Hinton broth), and cultured for 2 h at 37 °C, then diluted with distilled water in a 1:10 ratio. The cultured fluids were adjusted to the cell concentration of 100 μl and used for inoculation in the MIC test. The test ligand and complexes were dissolved in water. Solutions with concentrations 100 $\mu g\ ml^{-1}$ for $ttcNa_3$ and 357 $\mu g\ ml^{-1}$ for the complexes were prepared. Each 1 ml of culture medium containing various concentrations of the test materials was inoculated with 0.1 ml of the microorganism suspension prepared above. Bacteria were cultured for 18 h at 37 °C, then the growth of microorganisms was observed. When no growth of microorganisms was observed in the medium containing the lowest concentration of the test materials, the MIC of the test material was defined at this point of dilution. All compounds were tested three times and results are given as an average of the three independent experiments.

3. Results and discussion

New Zn(II), Fe(II) and Mn(II) complexes were prepared by the reaction of metal salts with the appropriate amines or imines followed by addition of the sodium salt of trithiocyanuric acid in aqueous or ethanol media. Their composition was proved by elemental analysis and they were characterized by spectroscopic methods and magnetochemical measurements. The results of elemental analysis together with results of physical techniques are given in Section 2. Moreover, our findings dealing with the composition of the complexes were confirmed by complete X-ray analysis of complexes **1**, **2** and **4**. In this work we present the X-ray structure of **2** only, whereas structures of complexes **1** and **4** will be published elsewhere. Briefly, the zinc atom in complex **1** is in a deformed trigonal bipyramidal arrangement formed by three N atoms of pmdien and the N,S donor set of the $ttcH^{2-}$ anion. The structure is similar to that of [Ni(pmdien)(ttcH)], which we reported earlier [29]. The iron atom in **4** is in a deformed octahedral arrangement of three N–N ligands (dmbpy), whereas the $ttcH_2^-$ anions and water molecules are outside the coordination sphere.

3.1. Spectral and magnetochemical study

The vibrational frequencies with their relative intensities are given in the experimental part. In our previous work we used density-functional theory calculations for the geometry optimization and IR frequencies calculations of differently protonated forms of $ttcH_3$ [30]. On the base of DFT calculations, we can assign bands which are connected with the ν(C–N) and ν(C–S) vibrations of the 2,4,6-trimercapto-1,3,5-triazine ring. These are observed in the IR spectra of the complexes in the 1515–1576 and 1175–1264 cm^{-1}, and 845–873 and 411–422 cm^{-1} regions, respectively. The bands observed in the 3068–3436 cm^{-1} region can be attributed to ν(C–H) and ν(N–H) vibrations [36]. In the spectra of complexes **1**, **2** and **4** the bands in the region 2852–2970 cm^{-1} can be connected with $\nu_s(CH_2)$ and $\nu_{as}(CH_2)$ vibrations. IR spectra of complexes **4**–**6**, with coordinated aromatic heterocycles (bpy, phen), contain several bands in the regions 700–900 and 1400–1600 cm^{-1} which are attributed to the ν(C–C) and ν(C–N) ring modes and the ring deformation vibrations, respectively. Most of the peaks of the free ligands are shifted to higher frequencies upon coordination.

There are three bands in the electronic absorption spectrum of **4**. The weak bands at 18 900 and 25 200 cm^{-1} can be attributed to d–d and metal to ligand charge transfer (MLCT) transitions, respectively [37]. The third strong absorption band observed at 36 800 cm^{-1} is probably due to a CT or π–π* transition associated with the ligands. In the spectra of the Mn(II) complexes **5** and **6** we can observe bands expected for octahedral high spin species at 25 150, 26 200 and 29 400 cm^{-1} for **5**, and 25 450, 27 400 and 28 900 cm^{-1} for **6**. These bands can be attributed to the d–d transitions. Very intensive bands in the UV range are probably due to CT or π–π* transitions.

The magnetic moment value for **4** ($\mu_{eff} = 2.44$ BM) is higher than that expected for a low spin deformed octahedral Fe(II) complex. A similar value of magnetic moment ($\mu_{eff} = 2.38$ BM) was calculated for $[Fe(phen)_2(NCS)_2]$ [38]. The authors explained a higher value of magnetic moment by residual paramagnetism due to polymorphism of the complex. Another explanation of a high magnetic moment value is deformation of the octahedral iron environment caused by the presence of methyl groups and the influence of water in the crystal lattice. This suggestion seems to be in agreement with the results of Mössbauer spectroscopy. The room temperature Mössbauer spectrum of **4** is composed of two doublets with the values of isomer shift (0.29 and 0.35 mm s^{-1}) typical for octahedral low-spin iron(II) complexes [39,40]. The doublet I with relative spectrum area $A = 61.5\%$ has a lower value of the quadrupole splitting parameter (q.s. = 0.29 mm s^{-1}) than the doublet II ($A = 38.5\%$, q.s. = 0.81 mm s^{-1}). The two different values of quadrupole splitting show that there are two octahedrally coordinated iron centers with lower and higher distortion from ideal octahedral arrangement found in the polycrystalline material. In our previous work, similar spectra with two doublets were also obtained and interpreted as a result of distortion of the octahedral arrangement of the central atoms in iron(II) complexes [30].

The manganese complexes **5** and **6** have magnetic moments 6.20 and 5.90 μ_{eff}/BM, respectively. These values are very close to the spin only value (5.93 μ_{eff}/BM) expected for five unpaired electrons of high spin deformed octahedrally coordinated manganese(II) atoms. From the composition of **6** we can propose a polymeric structure for the complex, where ttcH can form a bridge between the central atoms.

3.2. X-ray structure of [Zn(taa)(ttcH)] · H_2O (2)

The molecular structure of [Zn(taa)(ttcH)] · H_2O (**2**) is depicted in Fig. 1, while selected bond lengths and angles are listed in Table 1. The crystal structure is stabilized by hydrogen bonds (see Table 2, Fig. 2). The molecular structure of **2** consists of an electroneutral zinc complex and one crystal water molecule, which is hydrogen-bonded to the hydrogen atom of $ttcH^{2-}$. The central zinc atom is coordinated by four N atoms of taa and one N atom of the trithiocyanurate(2^-) dianion. The coordination polyhedron around the zinc atom is deformed trigonal-bipyramid with terminal nitrogen atoms N3A, N3B and N3C of taa in the basal plane while the central (tertiary) nitrogen atom N1 of taa and N2 atom of $ttcH^{2-}$ are coordinated in apical positions. The axial Zn–N1 bond length (2.3490(8) Å) is significantly longer than the equatorial Zn–N bond lengths, which are in the range 2.0618(9)–2.0887(9) Å. The Zn–N2 bond length of 2.1272(8) Å is longer than that found in Ni–N(trithiocyanurate) of [Ni(taa)(ttcH)] (2.040(2) Å),

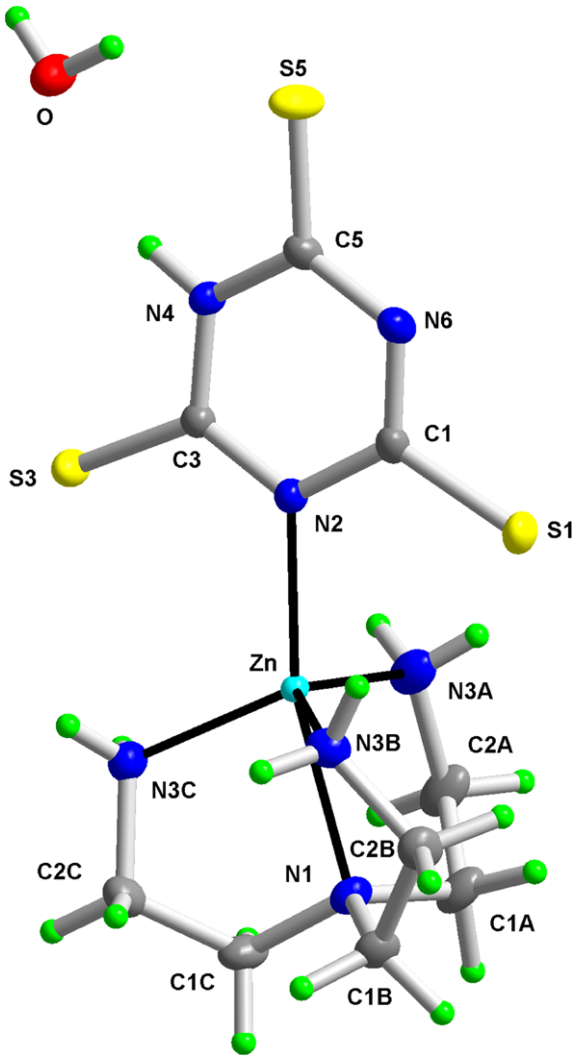

Fig. 1. The molecular structure of **2** with the numbering scheme and atomic displacement ellipsoids drawn at the 50% probability level.

Table 1
Selected bond lengths (Å) and angles (°) for [Zn(taa)(ttcH)] · H_2O (**2**)

Zn–N3C	2.0618(9)
Zn–N3A	2.0689(10)
Zn–N3B	2.0887(9)
Zn–N2	2.1272(8)
Zn–N1	2.3490(8)
N3C–Zn–N3A	110.30(4)
N3C–Zn–N3B	107.82(4)
N3A–Zn–N3B	130.52(4)
N3C–Zn–N2	114.60(3)
N3A–Zn–N2	94.39(4)
N3B–Zn–N2	97.33(3)
N3C–Zn–N1	79.62(3)
N3A–Zn–N1	79.48(3)
N3B–Zn–N1	77.59(3)
N2–Zn–N1	165.77(3)

Table 2
Hydrogen bonds for [Zn(taa)(ttcH)] · H_2O (**2**) (Å, °)

D–H...A	*d*(D–H)	*d*(H...A)	*d*(D...A)	∠(DHA)
O–H1...S5	0.85(2)	2.45(2)	3.1777(10)	144.5(17)
O–H2...S3^{i}	0.785(19)	2.55(2)	3.3361(10)	176.1(18)
N4–H4...O	0.88	2.16	2.9891(12)	157.6
N3A–H31A...S1^{ii}	0.867(19)	2.805(19)	3.5978(10)	152.9(16)
N3A–H31A...N6^{ii}	0.867(19)	2.408(19)	3.1727(14)	147.4(16)
N3A–H32A...S1	0.850(18)	2.878(18)	3.4827(12)	129.7(15)
N3B–H31B...S1	0.839(15)	2.651(15)	3.1906(10)	123.4(13)
N3B–H32B...O^{iii}	0.880(16)	2.449(16)	3.1209(14)	133.6(13)
N3B–H32B...O^{iv}	0.880(16)	2.425(16)	3.1810(14)	144.3(14)
N3C–H31C...S5^{ii}	0.874(17)	2.645(17)	3.4527(10)	154.1(14)
N3C–H32C...S3	0.847(17)	2.853(16)	3.2457(9)	110.3(13)
C2A–H2A1...N6^{v}	0.99	2.55	3.4061(14)	145.3
C2C–H2C1...S3^{vi}	0.99	2.85	3.8339(11)	175.5

Symmetry transformations used to generate equivalent atoms: (i) $-x$, $-y+1$, $-z$, (ii) x, $-y+1/2$, $z-1/2$, (iii) $-x$, $y-1/2$, $-z+1/2$, (iv) x, $-y+1/2$, $z+1/2$, (v) $-x+1$, $y-1/2$, $-z+1/2$, (vi) $-x$, $-y$, $-z$.

whereas other Ni–N(taa) distances are within the range of 2.063(3)–2.109(3) Å, comparable with those found in **2** [31]. In [Zn(bapen)(ttcH)] · EtOH (bapen = *N*,*N*′-bis-(3-aminopropyl)ethylenediamine), where $ttcH^{2-}$ is coordinated in a chelating fashion through N and S atoms, the Zn–N(trithiocyanurate) (2.3132(18) Å) distance is significantly longer in comparison with the Zn–N2 distance in **2** [21].

When we compare the known structures of zinc complexes with the taa ligand [Zn(taa)(H_2O)](ClO_4)$_2$, [Zn(taa)(MeOH)](ClO_4)(BPh_4) and [Zn(taa)(NCS)](SCN) with **2**, we can observe that the equatorial Zn–N bond lengths are very similar, whereas there are big differences in Zn–N(axial) bonds [41–43]. The axial bond lengths are 2.193(4), 2.16(1) and 2.292(4) Å, for the above mentioned complexes, while in **2** the distance is longer (2.3490(8) Å). In comparison with Zn–N(thiocyanate), the distance (2.073(5) Å) in [Zn(taa)(NCS)](SCN) is shorter than that found in **2**, Zn–N(trithiocyanurate) (2.1272(8) Å). We can also mention big differences in the equatorial N–Zn–N angles. In the three complexes mentioned above the

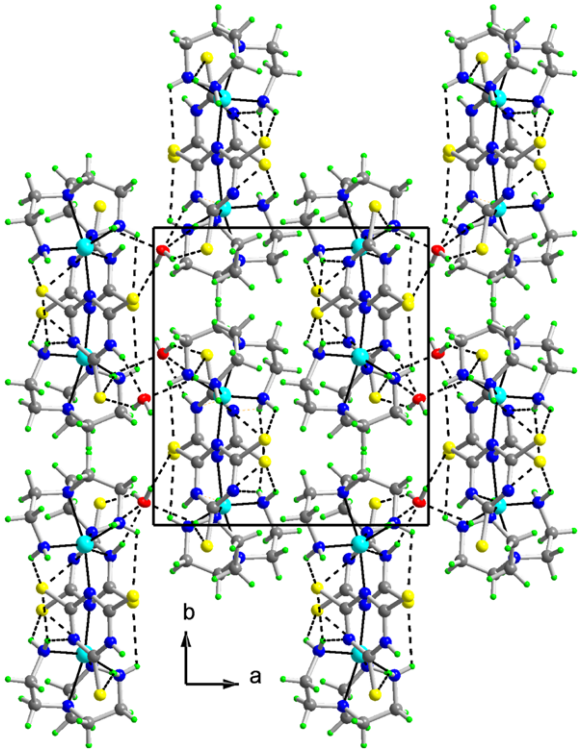

Fig. 2. Hydrogen bonding pattern in projection along the *c*-axis. Hydrogen bonds are shown as broken lines. For geometrical details see Table 2.

equatorial angles are all within an average of 118°, whilst in **2** the angles are in the range 107.82(4)–130.52(4)°.

3.3. In vitro cytotoxicity and antimicrobial activities

The cytotoxic activity of the complexes **1**–**8** and the sodium salt of trithiocyanuric acid ($ttcNa_3$) were studied on G-361, HOS, K-562 and MCF7 cancer cell lines. The results of the study are listed in Table 3. It can be clearly seen that zinc (**1**, **2**) and nickel (**7**, **8**) complexes as well as $ttcNa_3$ show no activity against K-562 and MCF7 in the concentration range used. On the other hand, very promising results were achieved for iron (**3**, **4**) and manganese (**5**, **6**) compounds. The four compounds show activity on K-562 and MCF7 cell lines. Based on average IC_{50} values, in the two cell lines compounds **3** and **5** are the most active ones and the results are comparable or mostly better than those obtained for cisplatin and oxaliplatin. Compound **4** is less active and **6** shows very low activity. The very low activity of **6** can be caused by the possible polymeric structure of the compound. The most active compounds i.e. **3** and **5** were selected for testing on G-361 and HOS cancer cell lines. The compounds are very efficient in inhibiting these cell line growths. They are comparable in activity with cisplatin and are two to four times more effective than oxaliplatin.

A combination of imines and the trithiocyanurate anion is not sufficient in bringing about cytotoxicity. It is seen when complexes **4** and **8** are compared. It can be clearly seen that the metal ion has a big influence on cytotoxicity of the complexes. Zinc and nickel compounds with amines or imines are inactive, whereas those containing iron or manganese ions show cytotoxicity. It seems that it is also important whether the trithiocyanurate anion is coordinated to the central atom or not. It can be demonstrated

Table 3
In vitro cytotoxicity of the complexes

	K-562	MCF7	G361	HOS
(**1**) [Zn(pmdien)(ttcH)]	>25	>25		
(**2**) $[Zn(taa)(ttcH)] \cdot H_2O$	>100	>100		
(**3**) $[Fe(nphen)_2(ttcH)] \cdot H_2O$	5.1	3.9	3.4	3.0
(**4**) $[Fe(dmbpy)_3](ttcH_2)_2 \cdot 4H_2O$	9.8	22.7		
(**5**) $[Mn(phen)_2(ttcH)] \cdot H_2O$	2.7	6.0	3.8	1.6
(**6**) $Mn(bpy)(ttcH) \cdot H_2O$	65.6	45.3		
(**7**) $[Ni(taa)(ttcH)] \cdot H_2O$	>25	>25		
(**8**) $[Ni(phen)_3](ttcH) \cdot 5H_2O$	>25	>25		
$ttcNa_3 \cdot 9H_2O$	>100	>100		
Oxaliplatin	8.8	18.2	7.1	6.8
Cisplatin	4.7	10.9	2.9	3.0

The results are given as IC_{50} values for the compounds (μM) assessed by calcein AM assay of surviving tumor cells. The tumor cell lines (K-562, MCF7, G361, HOS) were treated with a solution of the tested compound in the 0.2–25 μM (**1**, **7**, **8**) and 0.2–100 μM range (for the other compounds) for 72 h at 37 °C, 5% CO_2.

Table 4
Antimicrobial activities of the complexes evaluated by MIC (μg ml^{-1})[a]

	3953[b]	4223[b]	4224[b]	4225[b]	3988[b]	3954[b]	3955[b]
(**1**) [Zn(pmdien)(ttcH)]	>357	>357	>357	>357	>357	>357	>357
(**2**) $[Zn(taa)(ttcH)] \cdot H_2O$	>357	>357	>357	>357	>357	>357	>357
(**3**) $[Fe(nphen)_2(ttcH)] \cdot H_2O$	44.6	44.6	>357	>357	>357	>357	>357
(**4**) $[Fe(dmbpy)_3](ttcH_2)_2 \cdot 4H_2O$	89.3	89.3	>357	89.3	89.3	89.3	89.3
(**5**) $[Mn(phen)_2(ttcH)] \cdot H_2O$	>357	>357	>357	44.6	44.6	89.3	178.5
(**6**) $Mn(bpy)(ttcH) \cdot H_2O$	357	357	>357	357	357	357	357
(**7**) $[Ni(taa)(ttcH)] \cdot H_2O$	>357	>357	>357	>357	>357	>357	>357
(**8**) $[Ni(phen)_3](ttcH) \cdot 5H_2O$	178.5	178.5	>357	357	357	>357	>357
$ttcNa_3 \cdot 9H_2O$	>100	>100	>100	>100	>100	>100	>100

[a] The final concentration was 357 μg ml^{-1} for the complexes and 100 μg ml^{-1} for $ttcNa_3 \cdot 9H_2O$.
[b] The antimicrobial activities were evaluated against *Staphylococcus aureus* 3953, 4223, *Enterococcus faecalis* 4224, *Escherichia coli* 4225, 3988, 3954, *Pseudomonas aeruginosa* 3955.

on complexes **3** and **4**. Complex **3**, where the ttcH anion is coordinated, shows better activity.

The results of antimicrobial activities of $ttcNa_3$ and the complexes, estimated by MIC ($\mu g\ ml^{-1}$), are listed in Table 4. The free salt $ttcNa_3$ and complexes **1**, **2**, **6** and **7** did not inhibit the growth of the test organisms. Compound **3** showed selective and effective antimicrobial activities against Gram-positive bacteria (*S. aureus*); **4** exhibited effective activities against all tested bacteria, except for *E. faecalis*. Complex **5** showed effective activities against *Escherichia coli* and modest activity against *P. aeruginosa*, whereas **8** exhibited modest activities against *S. aureus*. It seems that compounds containing imines are active with the exception of **6**, where a polymeric structure is suggested.

4. Supplementary material

CCDC 610058 contains the supplementary crystallographic data for **2**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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