Published in final edited form as:

Inorg Chem Commun. 2016 February ; 64: 45–49. doi:10.1016/j.inoche.2015.12.013.

A novel copper(II) complex identified as a potent drug against colorectal and breast cancer cells and as a poison inhibitor for human topoisomerase II α

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Abstract

A novel complex, [Cu(acetylenethioacetate)Cl]Cl•0.25C₂H₅OH **1** (where acetylenethioacetate = (*E*)-*N*-ethyl-2-[1-(thiazol-2-yl)ethylidene]hydrazinecarbothioamide), was shown to have anti-proliferative activity against various colon and aggressive breast cancer cell lines. *In vitro* studies showed that

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Electronic Supplementary Information (ESI) available: Experimental details and figures featuring HRMS (Fig. S1), UV-visible spectra (Fig. S2), FT IR spectra (Fig. S3), X-band ESR spectra (Fig. S4), cyclic voltammograms (Fig. S5), and *in vitro* figures (S6–S9), and an *in vitro* table (Table S1) are collated here.

complex **1** acted as a poison inhibitor of human topoisomerase II α , which may account for the observed anti-cancer effects.

Graphical abstract

Keywords

thiosemicarbazones; copper(II); human topoisomerase II α ; breast cancer; ESR spectroscopy; cyclic voltammetry

The limited efficacy of current treatments for advanced breast and colon cancers has served as an impetus for a concerted effort to identify chemo-preventive agents for treatment. This process has often involved the use of metal complexes.[1] Cisplatin is widely used for the treatment of many cancers[2] despite its high toxicity, undesirable side effects, and problems with drug resistance in primary and metastatic cancers.[3] These limitations have spurred a growing interest in novel non-platinum metal complexes that can show anti-cancer properties.[4] Ruthenium-containing complexes have been reported to possess several favourable properties suited to rational anti-cancer drug design,[5] and ruthenium-containing complexes of various types are actively studied as metallodrugs, as they are believed to have low toxicity and good selectivity for tumours.[6] Recently, we reported the effect of ruthenium(II) complexes with new chelating thiosemicarbazones on growth inhibition of MCF-7 and MDA-MB-231 (breast adenocarcinoma) as well as HCT 116 and HT-29 (colorectal carcinoma) cell lines.[7] Thiosemicarbazones and their metal complexes are used in many applications, ranging from pharmacology to nuclear medicine.[8] We have expanded our efforts by searching for non-ruthenium systems, for example; the use of gallium(III)- and vanadium(IV)-containing complexes with thiosemicarbazones as ligands, as potential anti-cancer agents.[9, 10] Copper(II) thiosemicarbazone complexes in particular have been the focus of investigation as metallodrugs for various medical applications for a long period of time. These applications include use as anti-cancer agents.[11, 12]

A number of copper(II) complexes have been shown to have anti-tumour activity, and their mechanism of action is believed to be inhibition of human topoisomerase II α . [13, 14] Topoisomerases are attractive targets for anti-cancer drugs. They are ubiquitous enzymes that are required for untangling DNA and restoring the native DNA topology after processes such as replication, transcription, and other events that distort the DNA topology.[15] Topoisomerases are essential for cell viability. These enzymes employ an active site tyrosine residue to attack the phosphodiester backbone of the DNA, causing the strand to break. The enzyme then passes the other strand through the break, religates it, and releases the now untangled DNA.[15, 16] An important feature of this mechanism is the ensuing covalent intermediate where the enzyme is covalently linked to the cleaved DNA. While this intermediate persists, the cell is in danger, as accumulation of DNA breaks can cause DNA

damage response in cells, and even apoptosis.[16] Topoisomerase poison inhibitors that can stabilize this covalent intermediate can be extremely effective anti-bacterial or anti-cancer drugs.[17]

Human topoisomerase II α (hTop2 α) is highly expressed in many cancer cells, which makes it an attractive target for anti-cancer drugs.[18] There have been many reports of metal-based complexes inhibiting human topoisomerase II α , including some platinum complexes and other copper complexes as well, as mentioned above.[13, 14, 19, 20] However, many of these previous findings with copper(II) complexes have not shown poison inhibition of hTop2 α , but rather catalytic inhibition, or have not determined the mechanism of topoisomerase inhibition at all. The identification of a novel poison inhibitor is significant, because topoisomerase poisons are highly effective anti-cancer agents. Poison inhibitors are highly effective because trapping of the covalent intermediates formed by only a small percentage of the target topoisomerases on cleaved chromosomal DNA by the drugs is sufficient to initiate cancer cell death. In contrast, near complete inhibition of the catalytic activity of the target enzyme may be needed for a catalytic inhibitor of an essential enzyme to be effective in cancer cell growth inhibition.[21]

Here, our research efforts have been extended to a novel copper(II) complex, which bears the thiosemicarbazone, *E*-*N*-ethyl-2-[1-(thiazol-2-yl)ethylidene]hydrazinecarbothioamide[10] (acetyletHTSC). As such, we now report the characterization of a novel copper(II) complex, complex **1**, which can act as an anti-tumour agent and as an hTop2 α poison inhibitor. To our knowledge, this complex is the first copper(II)-based complex to show measurable quantitative increases in the linear DNA cleavage product from trapped topoisomerase complex, with specificity to hTop2 α over the hTop1. The poisoning of hTop2 α is likely to lead to cell death, and may contribute significantly to the anti-cancer mode of action.

Complex **1** was prepared by reacting the thiosemicarbazone, (*E*)-*N*-ethyl-2-[1-(thiazol-2-yl)ethylidene]hydrazinecarbothioamide (acetyletHTSC) in ethanol (Scheme 1). The complex was characterised by elemental analysis, HRMS, FTIR, UV-visible, and ESR spectroscopies. ICP-MS was also used to determine the percentage of Cu in the sample. The elemental analysis data for the percentage of H is not fully consistent with the calculated value; this could be due to the fact that the discrepancy is only in the percentage of H, and is more likely due to an error in the analysis process. Based on this discrepancy, it was necessary to show the HRMS data which showed an *m/z* value of 614.912544 (Figure S1) of which the proposed binuclear complex **2** was detected from a methanolic solution. In complex **2** (as a binuclear species), the acetylEtTSC ligand was found to coordinate as a thiolate anion while being detected in the positive mode while in the chamber of the mass spectrometer. This is due to the fact that thiosemicarbazones ligands can exist as thione–thiol tautomers (although the proton lost to form the anion formally belongs to the hydrazinic -NH group).[11] Such thiosemicarbazones can undergo tautomerisation and subsequent deprotonation of the thiol form allowing for a mono-anionic ligand.[11]

Chemical Formula: $C_{16}H_{22}ClCu_2N_8S_4$
Exact Mass: 614.91

The complex was soluble in DMSO as revealed by its UV-visible spectrum in DMSO (Figure S2, ESI). It is really clear that there are differences between the UV-visible spectra of the ligand and the complex (Figure S2, ESI). The UV-visible spectrum of complex **1** shows a d–d transition, which has a molar extinction coefficient value of $182\text{ M}^{-1}\text{ cm}^{-1}$ at 624 nm. Complex **1** also showed other bands at 324 and 425, with molar extinction values of 1.7×10^4 and $1.6 \times 10^4\text{ M}^{-1}\text{ cm}^{-1}$, respectively. In DMSO, the “free” ligand has a wavelength of 340 nm, with a molar extinction coefficient value of $2.8 \times 10^4\text{ M}^{-1}\text{ cm}^{-1}$. Conductivity measurements of a 1.0 mM DMSO solution of complex **1**, where $\Lambda_m = 31.0 \pm 2.0\text{ }\mu\text{S cm}^{-1}$ proved the existence of complex **1** as a 1:1 electrolyte based on the use of conductivity measurements to ascertain types of electrolytes in various solvents as reported in a review as written by Geary.[22] Complex **1** as a 1:1 electrolyte accounts for the presence of a mononuclear cation in DMSO.

The infrared spectrum of complex **1** (Figure S3, ESI) shows a band in the $\nu(\text{N-H})$ region which may be attributed to the hydrazinic nitrogen, thus suggesting that the ligands are coordinated as the thione form.[10] The ligand is reported to have stretching frequencies of 3164, 3054, and 1059, as designated for $\nu(\text{N}^1\text{H})$, $\nu(\text{N}^2\text{H})$, and $\nu(\text{N}^2\text{-N}^3)$, respectively.[10] On the other hand, complex **1** exhibited stretching frequencies of 3190, 2973, and 1166, as designated for $\nu(\text{N}^1\text{H})$, $\nu(\text{N}^2\text{H})$, and $\nu(\text{N}^2\text{-N}^3)$, respectively. The presence of a medium stretching frequency for $\text{C}=\text{S}$ at 813 cm^{-1} was determined for the “free” ligand,[10] but upon coordination, this stretching frequency is decreased to 787 cm^{-1} for the presence of $\text{C}=\text{S}$ in the thione form of the coordinated thiosemicarbazone.[10] Also, the strong stretching frequency assigned to the $\nu(\text{C}=\text{N})$ in the spectrum occurs at 1543 cm^{-1} ,[10] but is shifted to 1567 cm^{-1} , thus implying that there is coordination of the azomethine ($\text{C}=\text{N}$) to the copper(II) metal centre.[23] The stretching frequency at 1296 ($\nu(\text{C}=\text{S}) + \nu(\text{C}=\text{N})$) is shifted to 1228 cm^{-1} in the FTIR spectrum for complex **1**.

The ESR spectra in a frozen solution of DMSO shows the presence of a paramagnetic Cu(II) metal centre, where $g_{\perp}$, $g_{\parallel}$, $A_{\perp}$, and $A_{\parallel}$ were determined to be 2.0525, 2.1888, 12 G, and 168.9 G, respectively (Figure S4, ESI). The ESR spectrum of the complex indicates an axial

symmetry. As $g_{\parallel} > g_{\perp} > 2.002$, it can be inferred that the complex possesses an approximately square-planar geometry. This trend is also consistent with a $d_{x^2-y^2}$ ground state.[24, 25] Sakaguchi and Addison[25] have shown that the $g_{\parallel}/A_{\parallel}$ ratio can be used as a convenient empirical index of tetrahedral distortion in CuN_4 units. This value ranges from ca. 105 to 135 cm for the square planar structure, and this quotient increases on the introduction of tetrahedral distortion to the chromophore. Furthermore, tetrahedral distortion of a square-planar chromophore is observed when any (N, O, S) donors reduce $A_{\parallel}$ and increase $g_{\parallel}$. Using that relationship and the $g_{\parallel}/A_{\parallel}$ ratio of 139 cm (where $g_{\parallel}/A_{\parallel} = (2.1888/0.01579)$ cm) results in complex **1** having a tetrahedral distortion from the square planar geometry, and this is reflected in the structure for complex **1** as shown in Scheme 1. Such structure is also based on the fact that the ligand can be tridentate in nature.

Electrochemical studies were also carried out on the ligand and complex **1**, where some interesting features were observed in each cyclic voltammogram (Figure S5, ESI) for the metal centre and the thiosemicarbazone ligand (both coordinated and “free”). Recently, electrochemical studies of thiosemicarbazones has shown the existence of an irreversible cathodic redox peak in the region -1.26 V to -1.67 V, corresponding to the reduction of the imine moiety of the thiosemicarbazone functional group.[26] Such a case was observed of the “free” ligand, where $E_{pc} = -1.62$ V is believed to be due to the reduction of the imine moiety. The redox potential, $E_{pa} = +0.704$ V, is believed to be arise from the oxidation of the “free” ligand to a highly reactive radical cation followed by the formation of a dimeric species containing a disulphide bond.[27] The reduction of the thione functional group of a thiosemicarbazone moiety, have previously been reported to produce an irreversible cathodic peak at -1.06 V.[28] Previously reported electrochemical studies on thiosemicarbazone ligands have also shown the existence of an irreversible cathodic peak at -1.06 V.[28] We believe that the redox couple of $E_{1/2} = -0.668$ V is due to the reduction of the thione functional group. The cyclic voltammogram for complex **1** shows redox couples that are ligand- and metal-based. The irreversible $E_{pa} = +0.539$ V is believed to be arise from the oxidation of the coordinated ligand to a highly reactive radical cation; while the irreversible $E_{pc} = -1.52$ V is believed to be due to the reduction of the imine moiety. On the other hand the redox couple ($\text{Cu}^{\text{II/I}}$) is reversible at $E_{1/2} = -0.207$ V.

Once characterized, the anti-proliferative activity of complex **1** was carried out against colon cancer cell lines (HTC-116, Caco-2, and HT-29), and also compared to the anti-proliferative activity against one non-cancerous colon cell line (CCD-18Co). Complex **1** and etoposide (as positive control) were evaluated for their cytotoxicity against HCT-116, Caco-2, and HT-29 by a colorimetric assay (MTS), as described by Lewis *et al.*[10] Table S1 (ESI) summarizes the data from this evaluation. The effects of the compounds on the viability of these cells were evaluated after continuous incubation (24, 48, and 72 hours). In all cases, it was found that complex **1** had better efficacy in inhibiting cell growth of the colorectal cancer cells when compared to etoposide as shown in Table S1. Complex **1**, however, was found to be very toxic to the non-cancerous colon cell line (CCD-18Co) when compared to etoposide, with IC_{50} values after 72 hours of 0.83 ± 0.80 and 41.2 ± 2.3 μM , respectively.

Based on the results from the above study, where it was found that complex **1** was very aggressive in inhibiting cell growth of these colorectal cancer cell lines, we decided to carry

out an *in vitro* study on very aggressive breast cancer lines that are highly malignant and demonstrate increased rates of cell growth, migration and invasion. As such, to extend the potential anti-oncogenic of complex **1**, we examined its ability to prevent breast cancer cell proliferation in a preliminary study. In this preliminary study, we demonstrated a significant decrease in cell proliferation in four (4) different aggressive breast cancer cell lines, viz., HCC 1500, HCC 70, HCC 1806, and HCC 1395. The results (Figures S6–S9, ESI) that were obtained when HCC1500 cells were treated with the complex **1** at the concentrations indicated in each figure (Figures S6–S9, ESI) before incubation for 24 and 48 hours. The rate of cell proliferation was determined using an MTT cell proliferation assay. Each value is presented as the mean of the SEM of three independent determinations. The columns in each graph are presented as relative values in comparison to serum-starved cells. Bars labelled with different letters are significantly different from one another ($P < 0.05$). The black horizontal line denotes the level at which the rate of cell proliferation has been decreased by 50% of the growth factor control (positive control, yellow bar). The results (Figures S6–S9, ESI) demonstrated that complex **1** is able to prevent growth factor-stimulated cell proliferation at concentrations ranging from 1 μM to 20 μM in all the cell lines assayed. Complex **1** was tested for its ability to prevent cell growth after being exposed to the cells for 24 and 48 hours, respectively. The exposure to complex **1** for 48 hours did not result in dramatic decreases in cell growth, suggesting that a 24 hour exposure to this complex was significant enough to prevent breast cancer cell proliferation.

Specifically, 5 μM of complex **1** was sufficient to reduce cell proliferation by 50% in all the cell lines after 24 hours (note black horizontal line on each figure (Figures S6–S9, ESI)). Additionally, with a longer exposure to the complex (48 hours), the amount of complex **1** needed to reduce 50% of cell proliferation was only 2 μM for the HCC 70, HCC 1500, and HCC 1806 cell lines, however it remained at 5 μM for the HCC 1395 cell lines. This is interesting, since the HCC 1395 cell line is the only cell line that is derived from a Caucasian-American female, while the other three cell lines are derived from African-American females.

To understand the complex's mechanism for exerting toxicity on the cancer cell lines, the effect of the complex on hTOP2 α activity was evaluated, since many anticancer drugs are known to target this enzyme. The complex's effects on htop2 α were determined *in vitro*. A standard assay for detecting topoisomerase inhibition involves gel electrophoresis; the enzyme's ability to relax supercoiled DNA is determined in the presence and absence of the compound of interest.[29] When complex **1** was incubated with hTop2 α , it prevented the enzyme's ability to relax DNA. It was shown to inhibit the relaxation activity of human topoisomerase II α with an IC₅₀ value of 12.5–25 μM (Figure 1). The ligand alone has no effect on the enzyme activity.

Complex **1**, in addition to inhibiting the relaxation activity of hTop2 α , also produced an increase in the linear DNA cleavage product. The presence of linear DNA product indicates that the complex can act as a poison inhibitor of human topoisomerase II α . As mentioned earlier, poison inhibitors prevent DNA religation following cleavage by the topoisomerase enzyme. The DNA-enzyme covalent intermediate persists, and the DNA is trapped with a double stranded break.[17] Figure 2 shows the increase in cleavage product observed for

complex **1**. Free copper in the form of the copper(II) salt, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, did not result in any significant increase in topoisomerase cleavage product. Densitometry analysis of the bands shows a 3.6-fold increase in linear DNA product when compared to the DMSO lane (negative control) for complex **1** at 100 μM , and a 2.8-fold increase at 25 μM .

These results are significant, because a quantitative increase in linear DNA cleavage product was not observed for other copper(II)-containing complexes reported in previous studies, for example, 1-((5(or 6)-carboxy-1*H*-benzo[d]imidazol-2-yl)methyl)pyridinium copper(II) chloride hydrate,[13] copper(II) complexes with α -heterocyclic thiosemicarbazones as ligands,[14] and an *R*- and *S*-enantiomeric copper(II) complex derived from *N,N*-bis(1-benzyl-2-ethoxyethane),[19] all of which showed either little or no poisoning effect. In our study, this novel effect is significant, since the accumulation of topoisomerase cleavage intermediates would be an important mode of action for anti-cancer activity. Additionally, “free” Cu(II) ions and the acetylenic TSC ligand were not able to poison the topoisomerase on their own, but it is the complex that is formed from the copper(II) metal centre and the ligand that exerted the poisoning effect.

To determine specificity, complex **1** was tested against human topoisomerase **I**. It exhibited little to no effect on the enzyme relaxation activity, supporting the hypothesis that complex **1** can act as a specific inhibitor of hTop2 α . Its effect on the type I enzyme is shown in Figure 3.

This copper(II) complex is the first of its kind to show measurable quantitative increases in the linear DNA cleavage product from hTop2 α . The quantitative increase in linear DNA (as shown in Figure 2) is indicative of a poison mechanism of inhibition, as an hTop2 α poison would cause an increase in permanent double-stranded breaks. This mechanism of action is particularly lethal, and may account for the anti-proliferative effects exerted on the various cancer cell lines studied in this work reported within. Only a few stabilized htop2 α covalent complex trapped by complex **1** on chromosomal DNA may be sufficient to initiate the apoptosis pathway. At 12.5 μM complex **1**, the linear DNA product formed by htop2 α on plasmid DNA increased by 30% as determined by densitometry analysis. Cytotoxicity from complex **1** could be observed at submicromolar concentrations. The level of htop2 α covalent complex that can be trapped by complex **1** on chromosomal DNA in tumour cells might be further enhanced by the involvement of htop2 α in DNA replication complexes. It is also possible that there may be additional mode of actions involved in the anti-tumour activity of complex **1**. The *in vivo* effect of complex **1** and similar copper complexes should be investigated further in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Mississippi INBRE (P20RR016476) funded by the National Center for Research Resources, National Institutes of Health, and grant R01AI069313 from the National Institutes of Health to YT. Funding was also provided by The University of Southern Mississippi Lucas Endowment Grant and ExxonMobil Research and Engineering Company. We are also grateful for the use of an EMX^{micro} ESR spectrometer, which

was funded by the NSF CRIF:MU Award # 0741991; also a 400 MHz NMR spectrometer, which was also funded by the NSF CRIF:MU Award # 0840390. AAH would also like to thank Old Dominion University's Faculty Proposal Preparation Program (FP3) and also for the Old Dominion University start-up package that allowed for the successful completion of this work. AAH would also like to thank his graduate student, Mr. Michael Celestine for acquiring all cyclic voltammograms; and also Professor Floyd Beckford, The University of Virginia's College at Wise, for carrying out the conductivity measurements. The authors are also very grateful for the helpful comments that were provided by the reviewers.

References

1. Banerjee S, Chakravarty AR. Metal Complexes of Curcumin for Cellular Imaging, Targeting, and Photoinduced Anticancer Activity. *Acc Chem Res.* 2015; 48:2075–2083. [PubMed: 26158541] Patil SA, Patil SA, Patil R, Keri RS, Budagumpi S, Balakrishna GR, Tacke M. N-heterocyclic carbene metal complexes as bio-organometallic antimicrobial and anticancer drugs. *Future Med Chem.* 2015; 7:1305–1333. [PubMed: 26144266] Yamada M, Foote M, Prow TW. Therapeutic gold, silver, and platinum nanoparticles, Wiley Interdiscip. Rev: Nanomed Nanobiotechnol. 2015; 7:428–445. Johnstone TC, Suntharalingam K, Lippard SJ. Third row transition metals for the treatment of cancer, *Philos. Trans R Soc, A.* 2015; 373:1–12. Tsirul'nikova NV, Podmareva ON. Metal Complexes with Ethylenediaminedicarboxylic Acids and Their Derivatives, Promising Pharmacological and Diagnostic Agents (Review). *Pharm Chem J.* 2015; 48:738–743. Yang Y, Ouyang R, Xu L, Guo N, Li W, Feng K, Ouyang L, Yang Z, Zhou S, Miao Y. Review: Bismuth complexes: synthesis and applications in biomedicine. *J Coord Chem.* 2015; 68:379–397.
2. Wong E, Giandomenico CM. Current Status of Platinum-Based Antitumor Drugs. *Chem Rev.* 1999; 99:2451. [PubMed: 11749486]
3. Galanski M, Arion VB, Jakupc MA, Keppler BK. Recent developments in the field of tumor-inhibiting metal complexes. *Curr Pharm Des.* 2003; 9:2078. [PubMed: 14529417] Jakupc MA, Galanski M, Keppler BK. Tumour-inhibiting platinum complexes—state of the art and future perspectives. *Rev Physiol, Biochem Pharm.* 2003; 146:1.
4. Ott I, Gust R. Non Platinum Metal Complexes as Anti-cancer Drugs. *Archiv der Pharmazie - Chemistry in Life Sciences.* 2007; 340:117–126.
5. Clarke MJ. Ruthenium metallopharmaceuticals. *Coord Chem Rev.* 2003; 203:209. Clarke MJ, Zhu F, Frasca DR. Non-Platinum Chemotherapeutic Metallopharmaceuticals. *Chem Rev.* 1999; 99:2511. [PubMed: 11749489] Armitage B. Photocleavage of nucleic acids. *Chem Rev.* 1998; 98:1171. [PubMed: 11848929]
6. Silva, DdO. Perspectives for novel mixed diruthenium-organic drugs as metallopharmaceuticals in cancer therapy. *Anti-Cancer Agents Med Chem.* 2010; 10:312.
7. Beckford FA, Shaloski M Jr, Leblanc G, Thessing J, Lewis-Alleyne LC, Holder AA, Li L, Seeram NP. Microwave synthesis of mixed ligand diimine-thiosemicarbazone complexes of ruthenium(II): biophysical reactivity and cytotoxicity. *Dalton Trans.* 2009:10757–10764. [PubMed: 20023905]
8. Tarasconi P, Capacchi S, Pelosi G, Cornia M, Albertini R, Bonati A, Dall'Aglio PP, Lunghi P, Pinelli S. Synthesis, spectroscopic characterization and biological properties of new natural aldehydes thiosemicarbazones. *Bioorg Med Chem.* 2000; 8:157–162. [PubMed: 10968274] West DX, Swearingen JK, Valdes-Martinez J, Hernandez-Ortega S, El-Sawaf AK, Van Meurs F, Castineiras A, Garcia I, Bermejo E. Spectral and structural studies of iron(III), cobalt(II,III) and nickel(II) complexes of 2-pyridineformamide N(4)-methylthiosemicarbazone. *Polyhedron.* 1999; 18:2919–2929.
9. Kumar K, Schniper S, González-Sarrías A, Holder AA, Sanders N, Sullivan D, Jarrett WL, Davis K, Bai F, Seeram NP, Kumar V. Highly potent anti-proliferative effects of a gallium(III) complex with 7-chloroquinoline thiosemicarbazone as a ligand: Synthesis, cytotoxic and antimalarial evaluation. *Eur J Med Chem.* 2014; 86:81–86. [PubMed: 25147149]
10. Lewis NA, Liu F, Seymour L, Magnusen A, Erves TR, Arca JF, Beckford FA, Venkatraman R, González-Sarrías A, Fronczek FR, VanDerveer DG, Seeram NP, Liu A, Jarrett WL, Holder AA. Synthesis, Characterisation, and Preliminary In Vitro Studies of Vanadium(IV) Complexes with a Schiff Base and Thiosemicarbazones as Mixed Ligands. *Eur J Inorg Chem.* 2012; 2012:664–677. [PubMed: 23904789]

11. Beckford FA, Thessing J, Stott A, Holder AA, Poluektov OG, Li L, Seeram NP. Anticancer activity and biophysical reactivity of copper complexes of 2-(benzo[d][1,3]dioxol-5-ylmethylene)-N-alkylhydrazinecarbothioamides. *Inorg Chem Commun.* 2012; 15:225–229. [PubMed: 23440300]
12. Qi J, Liang S, Gou Y, Zhang Z, Zhou Z, Yang F, Liang H. Synthesis of four binuclear copper(II) complexes: Structure, anticancer properties and anticancer mechanism. *Eur J Med Chem.* 2015; 96:360–368. [PubMed: 25899339] Bacher F, Doemoetoer O, Chugunova A, Nagy NV, Filipovic L, Radulovic S, Enyedy EA, Arion VB. Strong effect of copper(II) coordination on antiproliferative activity of thiosemicarbazone-piperazine and thiosemicarbazone-morpholine hybrids. *Dalton Trans.* 2015; 44:9071–9090. [PubMed: 25896351] Huetting R, Kersemans V, Tredwell M, Cornelissen B, Christlieb M, Gee AD, Passchier J, Smart SC, Gouverneur V, Muschel RJ, Dilworth JR. A dual radiolabelling approach for tracking metal complexes: investigating the speciation of copper bis(thiosemicarbazones) in vitro and in vivo. *Metallomics.* 2015; 7:795–804. [PubMed: 25768310] Chakraborty, Saswati A.; Dash, SP.; Panda, AK.; Acharyya, R.; Biswas, A.; Mukhopadhyay, S.; Bhutia, SK.; Crochet, A.; Patil, YP.; Nethaji, M.; Dinda, R. Synthesis, X-ray structure and in vitro cytotoxicity studies of Cu(I/II) complexes of thiosemicarbazone: special emphasis on their interactions with DNA. *Dalton Trans.* 2015; 44:6140–6157. [PubMed: 25736331] Sharma B, Kothari R. Synthesis, characterization, anticancer, antibacterial and antioxidant evaluation of macrocyclic copper (II) complexes derived from thiosemicarbazide. *Int J Pharma Bio Sci.* 2015; 6:1154–1169. Gutierrez E, Richardson DR, Jansson PJ. The Anticancer Agent Di-2-pyridylketone 4,4-Dimethyl-3-thiosemicarbazone (Dp44mT) Overcomes Prosurvival Autophagy by Two Mechanisms. *J Biol Chem.* 2014; 289:33568–33589. [PubMed: 25301941] Ma Z-Y, Shao J, Bao W-G, Qiang Z-Y, Xu J-Y. A thiosemicarbazone copper(II) complex as a potential anticancer agent. *J Coord Chem.* 2015; 68:277–294. Bacher F, Doemoetoer O, Kaltenbrunner M, Mojovic M, Popovic-Bijelic A, Graeslund A, Ozarowski A, Filipovic L, Radulovic S, Enyedy EA, Arion VB. Effects of Terminal Dimethylation and Metal Coordination of Proline-2-formylpyridine Thiosemicarbazone Hybrids on Lipophilicity, Antiproliferative Activity, and hR2 RNR Inhibition. *Inorg Chem.* 2014; 53:12595–12609. [PubMed: 25391085] Kumar, S Mathan; Dhahagani, K.; Rajesh, J.; Anitha, K.; Chakkaravarthi, G.; Kanakachalam, N.; Marappan, M.; Rajagopal, G. Synthesis, structural analysis and cytotoxic effect of copper(II)-thiosemicarbazone complexes having heterocyclic bases: A selective naked eye sensor for F- and CN. *Polyhedron.* 2015; 85:830–840. Bisceglie F, Pinelli S, Alinovi R, Goldoni M, Mutti A, Camerini A, Piola L, Tarasconi P, Pelosi G. Cinnamaldehyde and cuminaldehyde thiosemicarbazones and their copper(II) and nickel(II) complexes: A study to understand their biological activity. *J Inorg Biochem.* 2014; 140:111–125. [PubMed: 25108184] Paterson BM, Donnelly PS. Copper complexes of bis(thiosemicarbazones): from chemotherapeutics to diagnostic and therapeutic radiopharmaceuticals. *Chem Soc Rev.* 2011; 40:3005–3018. [PubMed: 21409228] Donnelly PS. The role of coordination chemistry in the development of copper and rhenium radiopharmaceuticals. *Dalton Trans.* 2011; 40:999–1010. [PubMed: 21203624] Yu Y, Kalinowski DS, Kovacevic Z, Siafakas AR, Jansson PJ, Stefani C, Lovejoy DB, Sharpe PC, Bernhardt PV, Richardson DR. Thiosemicarbazones from the Old to New: Iron Chelators That Are More Than Just Ribonucleotide Reductase Inhibitors. *J Med Chem.* 2009; 52:5271–5294. [PubMed: 19601577] Wood KA, Wong WL, Saunders MI. [⁶⁴Cu]diacetylbis(N 4-methyl-thiosemicarbazone) - a radiotracer for tumor hypoxia. *Nucl Med Biol.* 2008; 35:393–400. [PubMed: 18482676] Blower PJ, Dilworth JR, Maurer RI, Mullen GD, Reynolds CA, Zheng Y. Towards new transition metal-based hypoxic selective agents for therapy and imaging. *J Inorg Biochem.* 2001; 85:15–22. [PubMed: 11377691]
13. Galal SA, Hegab KH, Hashem AM, Youssef NS. Synthesis and antitumor activity of novel benzimidazole-5-carboxylic acid derivatives and their transition metal complexes as topoisomerase II inhibitors. *Eur J Med Chem.* 2010; 45:5685–5691. [PubMed: 20884089]
14. Zeglisi BM, Divilov V, Lewis JS. Role of metalation in the topoisomerase II α inhibition and antiproliferation activity of a series of α -heterocyclic-N4-substituted thiosemicarbazones and their Cu(II) complexes. *J Med Chem.* 2011; 54:2391–2398. [PubMed: 21391686]
15. Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. *Nat Rev Mol Cell Biol.* 2002; 3:430–440. [PubMed: 12042765]

16. Vos SM, Tretter EM, Schmidt BH, Berger JM. All tangled up: how cells direct, manage and exploit topoisomerase function. *Nat Rev Mol Cell Biol.* 2011; 12:827–841. [PubMed: 22108601]
17. Pommier Y. Drugging Topoisomerases: Lessons and Challenges. *ACS Chem Biol.* 2013; 8:82–95. [PubMed: 23259582]
18. Jarvinen TA, Liu ET. Simultaneous amplification of HER-2 (ERBB2) and topoisomerase IIa (TOP2A) genes—molecular basis for combination chemotherapy in cancer. *Current Cancer Drug Targets.* 2006; 6:579–602. [PubMed: 17100565]
19. Arjmand F, Sharma GC, Muddassir M, Tabassum S. Synthesis and enantiopreferential DNA-binding profile of late 3d transition metal R- and S-enantiomeric complexes derived from N,N-bis-(1-benzyl-2-ethoxyethane): Validation of R-enantiomer of copper(II) complex as a human topoisomerase II inhibitor. *Chirality.* 2011; 23:557–567. [PubMed: 21695735]
20. Das P, Jain CK, Dey SK, Saha R, Chowdhury AD, Roychoudhury S, Kumar S, Majumder HK, Das S. Synthesis, crystal structure, DNA interaction and in vitro anticancer activity of a Cu(II) complex of purpurin: dual poison for human DNA topoisomerase I and II. *RSC Advances.* 2014; 4:59344–59357. Liu J, Leung CH, Chow AL-F, Sun RW-Y, Yan S-C, Che C-M. Cyclometalated platinum(II) complexes as topoisomerase II[small alpha] poisons, *Chem. Commun.* 2011; 47:719–721.
21. Nitiss JL. Targeting DNA topoisomerase II in cancer chemotherapy. *Nat Rev Cancer.* 2009; 9:338–350. [PubMed: 19377506]
22. Geary WJ. The use of conductivity measurements in organic solvents for the characterisation of coordination compounds. *Coord Chem Rev.* 1971; 7:81–122.
23. Ilies D-C, Shova S, Radulescu V, Pahontu E, Rosu T. Synthesis, characterization, crystal structure and antioxidant activity of Ni(II) and Cu(II) complexes with 2-formilpyridine N(4)-phenylthiosemicarbazone. *Polyhedron.* 2015; 97:157–166.
24. Rapheal PF, Manoj E, Kurup MRP. Copper(II) complexes of N(4)-substituted thiosemicarbazones derived from pyridine-2-carbaldehyde: Crystal structure of a binuclear complex. *Polyhedron.* 2007; 26:818–828.
25. Sakaguchi U, Addison AW. Spectroscopic and redox studies of some copper(II) complexes with biomimetic donor atoms: implications for protein copper centers. *J Chem Soc, Dalton Trans.* 1979:600–608.
26. Raposo MMM, Garcia-Acosta B, Abalos T, Calero P, Martinez-Manez R, Ros-Lis JV, Soto J. Synthesis and Study of the Use of Heterocyclic Thiosemicarbazones As Signaling Scaffolding for the Recognition of Anions. *J Org Chem.* 2010; 75:2922–2933. [PubMed: 20373768] Bollo S, Soto-Bustamante E, Nunez-Vergara LJ, Squella JA. Electrochemical study of nitrostilbene derivatives: nitro group as a probe of the push-pull effect. *J Electroanal Chem.* 2000; 492:54–62. Cakir S, Odabasoglu M, Bicer E, Yazar Z. Synthesis, spectroscopic and voltammetric studies of a novel Schiff-base of cysteine and saccharin. *J Mol Struct.* 2009; 918:81–87.
27. Blankespoor RL, Doyle MP, Hedstrand WH, Tamblyn WH, Van Dyke DA. Formation and reactions of dithiodicarbocation salts. *J Am Chem Soc.* 1981; 103:7096–7101.
28. Sonawane P, Kumbhar A, Padhye S, Butcher RJ. Synthesis, spectroscopic and structural characterization of the mer isomer of ammonium bis(phenylpyruvic acid thiosemicarbazone)-cobalt(III) hemihydrate. *Transition Met Chem.* 1994; 19:277–282. Padhye S, Chikate R, Kumbhar A, Shallom JM, Chitnis MP. Novel, quinonethiosemicarbazone hybrid (QTSCHY) non-platinum antitumor agents: inhibition of DNA biosynthesis in P388 lymphocytic cells by coordinatively unsaturated copper(II) and iron(III) complexes of naphthoquinone thiosemicarbazones. *BioMetals.* 1992; 5:67–71. [PubMed: 1525479] Murugkar A, Padhye S, Guha-Roy S, Wagh U. Metal complexes of taxol precursor. 1. Synthesis, characterization and antitumor activity of the copper complex of 10-deacetylbaccatin thiosemicarbazone. *Inorg Chem Commun.* 1999; 2:545–548.
29. Zhu C-X, Tse-Dinh Y-C. The Acidic Triad Conserved in Type IA DNA Topoisomerases Is Required for Binding of Mg(II) and Subsequent Conformational Change. *J Biol Chem.* 2000; 275:5318–5322. [PubMed: 10681504]

Highlights (for review)

- A novel copper(II) complex with (*E*)-*N*-ethyl-2-[1-(thiazol-2-yl)ethylidene]hydrazinecarbothioamide) was synthesized and characterized
- Anti-proliferative activity against various colon (in one case, an IC₅₀ value of 242 nM) and aggressive breast cancer cell lines
- *In vitro* studies showed that compound acted as a poison inhibitor of human topoisomerase II α , which is unlike most copper(II) complexes

Figure 1.

The effect of inhibition of hTop2 α relaxation activity by complex **1**. Lane 1: negatively supercoiled pBAD/Thio plasmid DNA with no enzyme; Lane 2: DMSO as negative control; Lane 3: positive control *m*AMSA at 75 μ M; Lanes 4–6: 100, 50, and 25 μ M acetylenethTSC; Lanes 7–10: 100, 50, 25, and 12.5 μ M complex **1**. N = nicked, FR = fully relaxed, PR = partially relaxed, and S = supercoiled.

Figure 2.

The effect of poisoning of hTop2 α in the presence of complex **1**. Lane 1: negatively supercoiled pBAD/Thio plasmid DNA with no enzyme; Lane 2: DMSO as negative control; Lane 3: positive control mAMSA at 25 μM ; Lanes 4–6: 200, 100, and 50 μM $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; Lanes 7–9: 100, 50, and 25 μM acetylenethTSC; Lanes 10–13: 100, 50, 25, and 12.5 μM complex **1**. Electrophoresis buffer contained 0.5 $\mu\text{g ml}^{-1}$ ethidium bromide. N = nicked, L = linear, and CC = covalently closed.

Figure 3.

The effect complex **1** on human topoisomerase I activity. Lane 1: negatively supercoiled pBAD/Thio plasmid DNA; Lane 2: DMSO as negative control; Lane 3: positive control camptothecin at 100 μM ; Lanes 4–6: 100, 50, and 25 μM acetylenethTSC; Lanes 7–10: 100, 50, 25, and 12.5 μM complex **1**. N = nicked, FR = fully relaxed, PR = partially relaxed, and S = supercoiled.

Scheme 1.
Synthesis of complex **1**.