

## Novel, quinone-thiosemicarbazone 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

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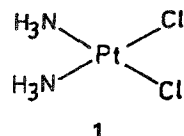
Received 22 October 1991, accepted for publication 18 November 1991

Coordinately unsaturated Cu(II) and Fe(III) complexes of the stoichiometry [Cu(L)Cl] and [Fe(L)Cl<sub>2</sub>], where L = tridentate anion of 2-hydroxy-1,4-naphthoquinone 1-thiosemicarbazone (2HNQTSC) and its 3-methyl derivative (3M2HNQTSC), were screened *in vitro* against P388 lymphocytic leukemia cells. Copper complexes were found to be more effective inhibitors of DNA synthesis than analogous Fe(III) compounds. The inhibitory activities are suggested to be related to Cu(II)–Cu(I) redox couple or nitrogen adduct formation.

**Keywords:** cyclic voltammetry; DNA biosynthesis, hybrid antitumor compounds, quinone thiosemicarbazone.

### Introduction

A large number of transition metal complexes have been screened for antitumor properties in the past decade. Amongst these platinum complexes were found to be the most potent antitumor compounds and are currently being widely used in combination with other antitumor agents. For example, *cis*-dichloro diammine Pt(II) (**1**) compound commonly



known as 'cisplatin' has been successfully used in combination therapy with adriamycin and vinblastine for the treatment of a variety of cancers (Rosenberg & Vancamp 1969, Dabrowaik &

Bradner 1987, Reedjik 1987). Several analogs of 'cisplatin' such as *cis*-sulfato-1,1-diaminocyclohexane Pt(II) (spiroplatin) and *cis*-diamine-1,1-cyclobutano dicarboxylate Pt(II) (carboplatin) are also found to be quite potent with improved renal clearance and have recently been introduced into clinical practice (Vermorken *et al.* 1985, Pasini & Zunino 1987, Krakoff 1988). Although cisplatin is highly active against some tumors it does not show any or only little effect on tumors that are very common and which account for the major share of cancer mortality today, e.g. lung tumors and tumors of the gastrointestinal tract. Experience has also shown that derivatives of cisplatin do not differ much from the parent compound in therapeutic efficacy, probably due to a similar mode of antitumor action. However, new metal complexes that do not have platinum as their central metal are more likely to act on tumors other than those affected by cisplatin (Keppler 1990).

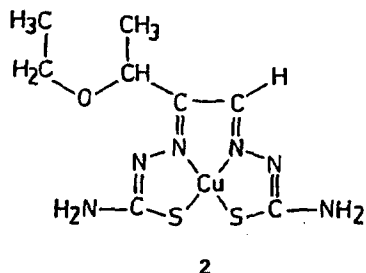
Thiosemicarbazone derivatives are emerging as a new class of non-platinum experimental anticancer chemotherapeutic compounds (Subizyrski *et al.* 1987, Petering 1980) which not only show inhibitory activities against common cancers but which are also

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found to be potent inhibitors of a crucial enzyme, ribonucleotide diphosphate reductase (RDR), which is obligatory for DNA biosynthesis and cell division. The most active compounds of this class include copper complexes of 3-ethoxy-2-oxobutraldehyde thiosemicarbazone (2), commonly known as CuKTS



(Petering 1980) and those of <sup>4</sup>N-heterocyclic thiosemicarbazones (Moore *et al.* 1970).

Quinones constitute yet another class of potential antitumor agents and some members belonging to this group of compounds are presently in use in clinical practise (Lin *et al.* 1974, Paull *et al.* 1976). We were thus motivated to create quinone-thiosemicarbazone hybrid molecules (QTSCHY) by combining structural features of both the groups with retention of their antitumor properties which may be more effective. Such 'hybrid' antitumor agents involving cisplatin and doxorubicin moieties have been found to possess lower therapeutic dosages, minimal cytotoxicities and reasonable kidney clearance (Yolles *et al.* 1982). In the present investigation we report on the *in vitro* antitumor activities of the Cu(II) and Fe(III) complexes of QTSCHY ligands prepared from the condensation reactions of lawsonone (2-hydroxy-1,4-naphthoquinone) and phthiocol (3-methyl-2-hydroxy-1,4-naphthoquinone) with a thiosemicarbazide molecule. The observed activities are then correlated with the structure and electrochemical parameters of these compounds.

## Materials and methods

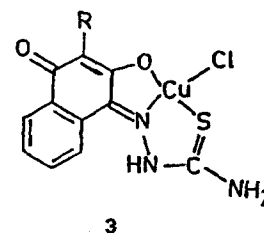
The ligands 2-hydroxy-1,4-naphthoquinone 1-thiosemicarbazone (2HNQTSC) and 3-methyl-2-hydroxy-1,4-naphthoquinone 1-thiosemicarbazone (3M2HNQTSC) were prepared according to literature methods (Dudley *et al.* 1969) with slight modifications (Padhye *et al.* 1987). Cu(II) and Fe(III) complexes of these ligands were prepared by interacting corresponding metal chlorides with 2HNQTSC and 3M2HNQTSC in the molar ratio 1:1 in ethanol solvent at 50 °C for 3 h. The reaction mixture was stored in a refrigerator overnight, after which the solvent was stripped off on a Rotavapor machine and the microcrystalline product separated out. It was washed with cold water and ether, and then dried in vacuum at room temperature. The

compounds analyzed as follows: [Fe(III)(2HNQTSC)(Cl<sub>2</sub>)] = FeC<sub>11</sub>H<sub>7</sub>O<sub>2</sub>N<sub>3</sub>SCl<sub>2</sub>: calculated: C: 32.30, H: 2.95, S: 7.84, Cl: 17.33, Fe: 13.65; found: C: 31.86, H: 3.12, S: 9.35, Cl: 15.56, Fe: 14.91. [Fe(III)(3M2HNQTSC)(Cl<sub>2</sub>)] = FeC<sub>12</sub>H<sub>10</sub>O<sub>2</sub>N<sub>3</sub>SCl<sub>2</sub>: calculated: C: 33.99, H: 3.32, S: 7.56, Cl: 16.72, Fe: 13.16; found: C: 34.71, H: 3.28, S: 8.43, Cl: 17.48, Fe: 12.94. [Cu(II)(2HNQTSC)Cl] = CuC<sub>11</sub>H<sub>7</sub>O<sub>2</sub>N<sub>3</sub>SCl: calculated: C: 36.37, H: 2.77, S: 8.82, Cl: 9.76, Cu: 17.49; found: C: 37.74, H: 2.98, S: 8.16, Cl: 9.52, Cu: 18.32. [Cu(II)(3M2HNQTSC)Cl] = CuC<sub>12</sub>H<sub>10</sub>O<sub>2</sub>N<sub>3</sub>SCl: calculated: C: 38.20, H: 3.20, S: 8.50, Cl: 9.40, Cu: 16.84; found: C: 39.13, H: 3.17, S: 9.76, Cl: 10.17, Cu: 15.12.

Cyclic voltammetric (CV) profiles of the synthesized complexes were obtained on a Bioanalytical electrochemical system CV-27 as reported previously (Kumbhar *et al.* 1991). The ligands and their Cu(II) and Fe(III) complexes were screened for *in vitro* antitumor activity against P388/S lymphocytic leukemia cells sensitive to adriamycin (P388/S) by the procedure of Chitnis *et al.* (1987). Briefly, 7 day old P388 lymphocytic leukemia cells were harvested from BDF mice. These cells were freed from red blood cells (RBC) by lysing with tris-ammonium chloride solution. In a test run, 2 × 10<sup>6</sup> cells/ml were incubated in minimum essential medium with different concentrations of both the ligands and their metal complexes in dimethylsulfoxide (DMSO) (1%) and 0.5 μCi/ml of <sup>3</sup>H-labelled thymidine ([<sup>3</sup>H]TdR) along with untreated control for a period of 4 h at 37 °C. At the end of a 4 h incubation, 1 ml aliquots in duplicate from each flask were applied to a microfiber filter disc on a millipore. The DNA was precipitated by 10% trichloroacetic acid, washed with water and, finally, washed with methanol. The discs were transferred into scintillation vials and radioactivity was measured by scintillation-counting spectrophotometry through the use of toluene-based flour.

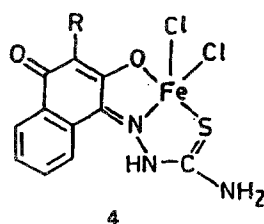
## Results and discussion

The structural characterization of the compounds based on spectral diagnostics has been recently described (Chikate *et al.* 1991), which has shown copper complexes (3) to possess square planar



geometries while square pyramidal configurations are indicated for the ferric compounds (4).

The results showing *in vitro* percentage inhibition of DNA synthesis with increasing concentration of thiosemicarbazone ligands and their Cu(II) and Fe(III) complexes are shown in Table 1. It can be

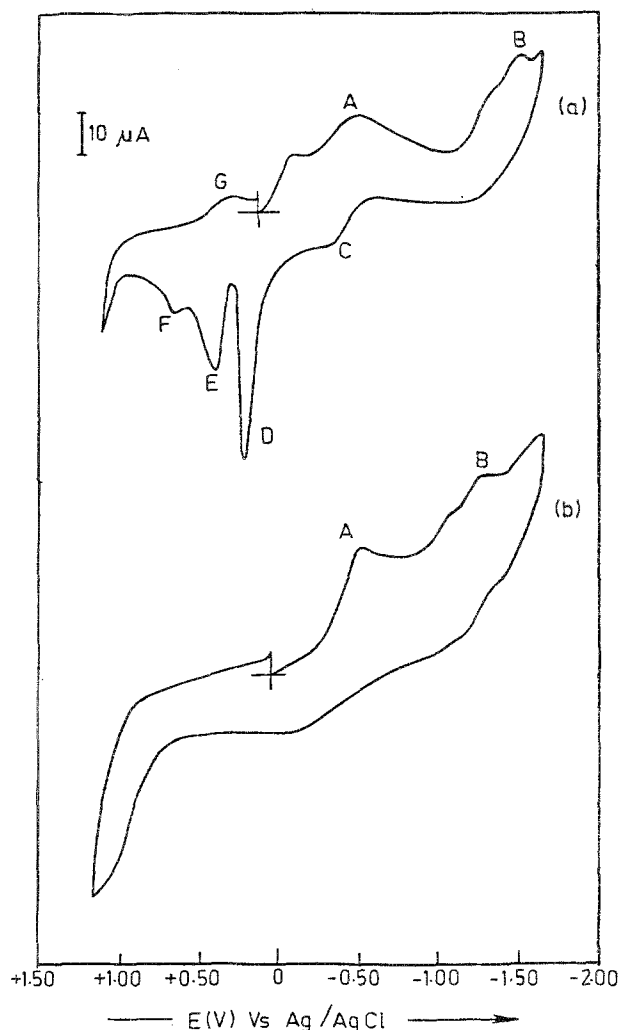


clearly seen from the results of [ $^3\text{H}$ ]TdR incorporation into P388 leukemia cells that the uncomplexed ligands do not exhibit any inhibitory activities while their metal complexes do. On the whole, Cu(II) complexes are found to be more active than analogous Fe(III) compounds. The metal salts employed for the preparation of the present complexes were also screened to ensure proper control and no inhibitory effects were observed for them even at concentrations 10 times higher (200  $\mu\text{g}/\text{ml}$ ) than the test experiments. The present results, therefore, indicate that intact metal complexes are the pharmacologically active species in our studies. The fact that Cu(II) are more active than Fe(III) complexes suggests that redox reactions may perhaps have a role in the antiproliferative activities of this class of compounds. CV studies were, therefore, performed on the present complexes (Figure 1), results of which are summarized in Table 2.

To facilitate discussion on the CV profiles of the test compounds the observed peaks are denoted by

**Table 1.** Effect of QTSCHY ligands and their metal complexes on [ $^3\text{H}$ ]TdR incorporation in P388/S tumor cells (*in vitro*)

Compound	Concentration ( $\mu\text{g}/\text{ml}$ )	Inhibition of [ $^3\text{H}$ ]TdR incorporation(%)
2HNQTSC	1	0
	5	0
	10	0
3M2HNQTSC	1	0
	5	0
	10	0
[Fe(2HNQTSC)Cl <sub>2</sub> ]	1	12.8
	5	28.0
	10	21.2
[Fe(3M2HNQTSC)Cl <sub>2</sub> ]	1	12.2
	5	27.8
	10	35.3
[Cu(2HNQTSC)Cl]	1	39.2
	5	69.0
	10	92.4
[Cu(3M2HNQTSC)Cl]	1	56.6
	5	88.1
	10	90.1



**Figure 1.** Cyclic voltammogram (scan rate 100  $\text{mV s}^{-1}$ ) of  $10^{-3}$  M solutions in DMSO (0.1 M  $\text{Et}_4\text{NCIO}_4$ ) at 25  $^{\circ}\text{C}$  of (a) [Cu(2HNQTSC)Cl] and (b) [Fe(2HNQTSC)Cl<sub>2</sub>].

capital letters. The cyclic voltammogram of the copper complex (Figure 1a) exhibits a pair of peaks consisting of the reduction peak A and its oxidative counterpart C ( $E_{1/2} = -0.550$  V) which can be identified as the Cu(II)–Cu(I) redox couple. Considering the scan rates employed, the separation between peaks A and C is representative of a quasireversible one electron process (Nicholson & Shain 1964). Such quasireversibility observed for many square planar Cu(II) complexes has been ascribed to stereochemical reorganization from planar Cu(II) species to tetrahedral Cu(I) species (Hendrickson *et al.* 1976, Zanello 1988). A peak at a more negative potential peak (B) in the cyclic voltammogram of the Cu(II) compounds probably corresponds to the reduction of the azomethine double bond of the coordinated ligand while peaks D and E are due to coupled chemical oxidations.

**Table 2.** CV results for Cu(II) and Fe(III) complexes of QTS ligands

Complex	$E_{pa}^a$	$E_{pc}$	$E^{1/2}$
[Fe(2HNQTSC)Cl <sub>2</sub> ]	-1.250	-1.325	-1.300
Fe(3M2HNQTSC)Cl <sub>2</sub> ]	-1.380	-1.280	-1.330
Cu(2HNQTSC)Cl]	-0.625	-0.475	-0.550
Cu(3M2HNQTSC)Cl]	-0.625	-0.500	-0.550

<sup>a</sup>In volts versus Ag/AgCL.

The reduction peak F and its oxidative counterpart peak G may represent the Cu(II)–Cu(III) couple (Zanello 1988).

The cyclic voltammograms of the Fe(III) complexes (Figure 1b), however, show two irreversible reduction peaks denoted by A and B which, according to the earlier discussion, may correspond to the reduction of Fe(III) to Fe(II) species and reduction of the azomethine group, respectively. The absence of an oxidation counterpart for peak A probably indicates that the Fe(II) species formed is unstable and undergoes further mutation. It has been well-established that ferric complexes of thiosemicarbazone derivatives have enhanced thermodynamic stabilities compared with their ferrous counterparts. As a result thiosemicarbazone ligands have been suggested as potential ferric chelators for removing iron overloads in Cooley's anemia (Spigarn & Sartorelli 1979).

A possible mode of action for the inhibition of DNA synthesis by heterocyclic thiosemicarbazones has been thought to involve inhibition of RNA to DNA conversion by binding of the complex at the site of the ribonucleotide reductase enzyme which serves to bind and orient substrates undergoing further reactions (Thelander 1979, Levinson 1980). In a classic study on the antitumor action of a 3-ethoxy 2-oxobutaldehyde bis thiosemicarbazone copper compound (Petering et al. 1980) it has been demonstrated that thiols present in the tumor cells are oxidized in presence of the CuKTS complex to disulphide compounds, which are the actual cytotoxic species at the cellular level. The thiol oxidation is promoted in presence of O<sub>2</sub> with concomitant reduction of Cu(II) to Cu(I). These findings suggest that reaction with cellular thiols and consequent destruction of the chelate may have a role in the antiproliferative activity of these compounds.

It is reasonable to suggest that the mechanism of action of our compounds may be similar to that proposed for CuKTS involving the formation of catalytically active Cu(I) compounds in the reaction with cellular thiols. Quasireversible reduction processes observed at easily accessible electrode poten-

tials for the present copper compounds justify their observed inhibitory activities. Irreversible reductions in the case of Fe(III) complexes are probably responsible for the loss of their activity.

A second possible mechanism may involve the binding of the active complex to the nitrogen bases of DNA or RNA, thus hindering or blocking base replication (Antholine *et al.* 1977). Four-coordinate, planar Cu(II) complexes seem to be more suitable for such adduct formation than the corresponding five-coordinate Fe(III) compounds.

The present work has thus shown that Cu(II) complexes of the QTSCHY class can be considered as potentially useful non-platinum antitumor compounds whose synthesis can be judiciously monitored on the basis of Cu(II)–Cu(I) redox couples.

## Acknowledgements

S. P. would like to acknowledge the hospitality of Dr Wayne Frasch and support from the ASU Center for the Study of Early Events in Photosynthesis.

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