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Quinoline-2-carboxaldehyde thiosemicarbazones and their Cu(II) and Ni(II) complexes as topoisomerase IIa inhibitors

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ABSTRACT: A series of quinoline-2-carboxaldehyde thiosemicarbazones and their copper(II) and nickel(II) complexes were synthesized and characterized. In all complexes the ligands are in the *E* configuration with respect to the imino bond and behave as terdentate. The copper(II) complexes form square planar derivatives with one molecule of terdentate ligand and chloride ion. A further non coordinated chloride ion compensate the overall charge. Nickel(II) ions form instead octahedral complexes with two ligands for each metal ion, independently from the stoichiometric metal:ligand ratio used in the synthesis. Ligands and complexes were tested for their antiproliferative properties on histiocytic lymphoma cell line U937. Copper(II) derivatives are systematically more active than the ligands and the nickel complexes. All copper derivatives result to inhibit topoisomerase IIa in vitro. Computational methods were used to propose a model to explain the different extent of inhibition presented by these compounds. The positive charge of the dissociated form of the copper complexes may play a key role in their action.

KEYWORDS: Topoisomerase IIa, Quinoline, Thiosemicarbazone, Copper, Nickel

1. Introduction

Thiosemicarbazones are a class of compounds extensively studied for their biological properties [1-6]. Two fundamental steps in their development as drugs were made in the Sixties with the discovery of their activity as antitumor agents [7] and of their antiviral properties; in particular, these latter were successfully exploited to treat smallpox [8]. Since then, their anticancer properties have received increasing attention and in recent years one of them, Triapine® (3-aminopyridine-2-carboxaldehyde thiosemicarbazone), has been developed as an anticancer drug [5]. For some types of tumors, clinical trials for this molecule have now reached clinical phase II [9].

In contrast with the large quantity of data from preliminary antiproliferative assays carried out with these molecules on several cell lines *in vitro* found in the literature [4], the amount of information on specific targets (DNA, enzymes or proteins) is still wanting. In the majority of cases, the biological activity of thiosemicarbazones is strongly dependent on the presence of metal ions, since their coordination compounds are systematically more efficient than the parent ligands. According to our experience, another common feature is that, when thiosemicarbazones act as antiproliferating agents, a cell cycle block in phase G₂/M is observed [10-12]. Among putative targets consistent with this fact, a prominent place is certainly occupied by topoisomerase IIa (TopoIIa). This homodimeric enzyme, in fact, plays an essential role in all eukaryotic cells and it is particularly active in cancerous cells because they present a higher growth rate [13]. The main role of TopoIIa is the regulation of the topological states of DNA through transient cleavage strand passing and re-ligation of double-stranded DNA, resulting in decatenation of intertwined DNA molecules and relaxation of supercoiled DNA. In addition, TopoIIa plays a key role in DNA replication and is required for condensation and segregation of chromosomes. The expression of TopoIIa is cell-cycle dependent with both

protein levels and catalytic activity peaking at G₂/M. As said above, this protein is therefore a plausible candidate as a target for thiosemicarbazones, since its phosphorylation/dephosphorylation is part of the regulatory checkpoints at the entry and progression of mitosis. For this reason, molecules able to inhibit TopoIIa are valuable compounds as anticancer drugs thanks to their capacity to stop proliferation and to lead cells to apoptosis. Known TopoIIa inhibitors are extremely varied and act through different mechanisms. The target for most of these molecules is the DNA-protein complex, rather than the protein itself or DNA.[14] Some of the compounds interact predominantly with the protein (quinones), while others with DNA (ellipticine). DNA-intercalating drugs (etoposide)[15] probably bind to both DNA and the enzyme through intercalation of the aromatic part at the enzyme–DNA interface. Some drugs work by inhibiting the re-ligation of cleaved DNA, whereas others, such as ellipticine, genistein, and quinolones, are presumed to accelerate the forward rate of complex formation.

In this paper, we report the synthesis of a series of quinoline-2-carboxaldehyde thiosemicarbazones variously substituted on the terminal nitrogen (Figure 1), of the corresponding copper(II) complexes, square planar of stoichiometry M:L 1:1, and of the nickel(II) derivatives, prepared with the same stoichiometry, but which spontaneously resolve in compounds with a M:L 1:2 ratio, with octahedral geometry.

All compounds have been systematically tested for their capacity of inhibiting proliferation of a human histiocytic lymphoma, cell line U937, and their activity has been determined by evaluating their IC₅₀. Subsequently, the inhibition activity on TopoIIa was assayed in vitro in order to evaluate the capacity of the compounds to inhibit directly the enzyme. Several docking simulations of the compounds were eventually performed on TopoIIa in an attempt to understand the inhibition mechanisms.

2. Experimental

2.1 General experimental conditions

Infrared spectra were recorded in the range 4000-400 cm^{-1} using a Nicolet 5PC FTIR spectrophotometer. Elemental analyses (C, H, N) were carried out using a flash 1112 Series CHNS-O Analyser by CE Instruments. ^1H NMR spectra were recorded using a Bruker Avance 300 spectrometer.

2.2 Syntheses of the ligands

2.2.1 Quinoline-2-carboxyaldehyde thiosemicarbazone (HL1)

Thiosemicarbazide (337.6 mg, 3.7 mmol) was dissolved in 80 mL of methanol at room temperature under stirring, and the solution was added of an equimolar amount of quinoline-2-carboxaldehyde (582.1 mg, 3.7 mmol). A clear orange solution was obtained. This reaction mixture was kept under stirring and placed in an ice bath. The reaction was left standing for 20 hrs and the white precipitate afforded was filtered on a Buchner funnel and washed using ethanol. Yield: 77%. Elemental analysis: $\text{C}_{11}\text{H}_{10}\text{N}_4\text{S}$ Calc.: C 57.37%, H 4.38%, N 24.33%; Exp.: C 57.38%, H 4.22%, N 24.55%. ^1H NMR (300 MHz, DMSO- d_6) 11.79 ppm (1H, s, C=N-NH), 8.45 ppm (2H, d, $J = 8.7$ Hz, NH_2), 8.35 ppm (2H, m, CH arom.), 8.23 ppm (1H, s, CH=N), 8.00 ppm (2H, t, $J = 8.7$ Hz, CH arom.), 7.78 ppm (1H, t, $J = 8.4$ Hz, CH arom.), 7.62 ppm (1H, t, $J = 8.1$ Hz, CH arom.). IR: 3395 cm^{-1} , 3262 cm^{-1} and 3152 cm^{-1} ν N-H, 3066 cm^{-1} ν C-H arom., 2986 cm^{-1} ν C-H aliph., 1609 cm^{-1} ν C=N, 1533 cm^{-1} and 1503 cm^{-1} ν C=C, 1110 cm^{-1} and 839 cm^{-1} ν C=S

2.2.2 Quinoline-2-carboxyaldehyde N⁴,N⁴-dimethylthiosemicarbazone (HL2)

N⁴,N⁴-dimethyl-3-thiosemicarbazide (271 mg, 2.16 mmol) was dissolved in 120 mL of ethanol at room temperature under stirring. An equimolar amount of quinoline-2-carboxyaldehyde (351 mg, 2.16 mmol) was then added. The solution obtained was clear and deep yellow. The reaction mixture was then kept under stirring and placed in an ice bath. After an hour a light yellow precipitate began to form, but the reaction was left running for 24 hrs. The precipitate was dried on a Buchner funnel, rinsed with ethanol, allowed to dry and eventually weighed. The product was recrystallized from acetonitrile and the crystals so formed allowed to solve the structure by X-ray diffraction. Yield: 39%. Elemental analysis: C₁₃H₁₄N₄S Calc: 60.44%, H 5.46% N 21.69% Exp.: C 60.68%, H 5.40%, N 21.84%. ¹H NMR (300 MHz, DMSO-d₆) 11.36 ppm (¹H, s, NH), 8.37 ppm (¹H, s, CH=N), 8.03 ppm (4H, m, CH₄ arom.), 7.78 ppm (¹H, t, J = 5.7 Hz, CH arom.), 7.62 ppm (¹H, t, J = 5.7 Hz, CH arom.), 3.36 ppm (6H, s, N-(CH₃)₂). IR: 3066 cm⁻¹ ν N-H, 3008 cm⁻¹ ν C-H arom., 2921 cm⁻¹ ν C-H aliph., 1619 cm⁻¹ ν C=N, 1576 cm⁻¹, 1545 cm⁻¹ and 1499 cm⁻¹ ν C=C, 1104 cm⁻¹ and 822 cm⁻¹ ν C=S.

2.2.3 Quinoline-2-carboxyaldehyde N⁴-methylthiosemicarbazone (HL3)

N⁴-methylthiosemicarbazide (234.2 mg, 2.16 mmol) was dissolved in 40 mL of ethanol at room temperature and under stirring. An equimolar amount of quinoline-2-carboxyaldehyde (355 mg, 2.16 mmol). The resulting solution was clear and yellow-orange. The reaction mixture was maintained in an ice bath under stirring. After about half an hour a white precipitate began to form. The reaction was left standing for 24 h and the white precipitate was filtered on a Buchner funnel, washed with ethanol, dried on air and weighed. The product was recrystallized from acetonitrile and the crystals obtained were suitable for X-ray diffraction analysis. Yield: 83%. Elem. Anal.: C₁₂H₁₂N₄S calc. C 58.99%, H 4.95%, N 22.93%, exp. C 59.80%, H 4.92%, N 22.82%. ¹H NMR (300 MHz, DMSO-d₆) 11.87 ppm (1H, s, C=N-NH), 8.81 ppm (1H, q, J

= 4.2 Hz, S=C-NH), 8.45 ppm (1H, d, J = 8.7 Hz, CH arom.), 8.39 ppm (1H, d, J = 8.7 Hz, CH arom.), 8.23 ppm (1H, s, CH=N), 8.00 ppm (2H, t, J = 7.5 Hz, CH arom.), 7.78 ppm (1H, t, J = 7.8 Hz, CH arom.), 7.62 ppm (1H, t, J = 7.5 Hz, CH arom.), 3.07 ppm (3H, d, J = 4.5 Hz, NH-CH₃). IR: 3322 cm⁻¹ and 3117 cm⁻¹ v N-H, 3069 cm⁻¹ v C-H arom. , 2937 cm⁻¹ v C-H aliph., 1602 cm⁻¹ v C=N, 1536 cm⁻¹ and 1502 cm⁻¹ v C=C. 1112 cm⁻¹ and 846 cm⁻¹ v C=S.

2.2.4 Quinoline-2-carboxyaldehyde N⁴-phenylthiosemicarbazone (HL4)

N⁴-phenylthiosemicarbazide (391 mg, 2.3 mmol) was dissolved in 60 mL of ethanol at room temperature and under stirring. An equimolar amount of quinoline-2-carboxyaldehyde (375.3 mg, 2.3 mmol) was added. The clear dark-yellow solution was kept in an ice bath under stirring. After 24 hours, a white precipitate was filtered and washed with ethanol. Yield: 52%. Elem. Anal.: C₁₇H₁₄N₄S calc. C 66.64%, H 4.61%, N 18.29%; exp. C 66.82%, H 4.74%, N 18.06%. ¹H NMR (300 MHz, DMSO-d₆) 12.20 ppm (1H, s, C=N-NH), 10.39 ppm (1H, s, S=C-NHPhe), 8.62 ppm (1H, d, J = 8.4 Hz, CH arom._{Quin}), 8.40 ppm (1H, d, J = 8.7 Hz, CH arom._{Quin}), 8.35 ppm (1H, s, CH=N), 8.02 ppm (2H, t, J = 9 Hz, CH arom._{Quin}), 7.79 ppm (1H, t, J = 7.5 Hz, CH arom._{Quin}), 7.64 ppm (1H, t, J = 7.5 Hz, CH arom._{Quin}), 7.57 ppm (2H, d, J = 8.1 Hz, CH arom._{Phe}), 7.41 ppm (2H, t, J = 7.5 Hz, CH arom._{Phe}), 7.26 ppm (1H, t, J = 7.5 Hz, CH arom._{Phe}). IR: 3313 cm⁻¹ and 3119 cm⁻¹ v N-H, 3054 cm⁻¹ and 3041 cm⁻¹ v C-H arom., 2945 cm⁻¹ v C-H aliph., 1598 cm⁻¹ v C=N, 1537 cm⁻¹ and 1503 cm⁻¹ v C=C, 1096 cm⁻¹ and 825 cm⁻¹ v C=S.

2.2.5 Quinoline-2-carboxyaldehyde N²-methylthiosemicarbazone (L5)

N²-methylthiosemicarbazide (412.5 mg, 3.9 mmol) was dissolved in 60 mL of methanol at room temperature and under stirring. An equimolar amount of quinoline-2-carboxyaldehyde

(616.5 mg, 3.9 mmol) and a few drops of glacial acetic acid were added to obtain a clear, yellow solution. The solution was left standing for 20 h and the white precipitate that formed was filtered and washed with ethanol. The product was recrystallized from ethanol and the crystals obtained were suitable for X-ray diffraction analysis. Yield: 76%. Elem. Anal.: C₁₂H₁₂N₄S calc. C 58.99%, H 4.95%, N 22.93%; exp. C 58.86%, H 4.98%, N 23.08%. ¹H NMR (300 MHz, DMSO-d₆) 8.67 ppm (1H, m, CH arom.), 8.60 ppm (2H, d, J = 8.7 Hz, NH₂), 8.39 ppm (1H, d, J = 8.7 Hz, CH arom.), 8.02 ppm (2H, m, CH arom.), 7.96 ppm (1H, s, CH=N), 7.79 ppm (1H, t, J = 7.5 Hz, CH arom.), 7.64 ppm (1H, t, J = 7.5 Hz, CH arom.), 3.87 ppm (3H, s, N-CH₃). IR: 3403 cm⁻¹ and 3230 cm⁻¹ v N-H, 3060 cm⁻¹ v C-H arom., 2890 cm⁻¹ v C-H aliph., 1595 cm⁻¹ v C=N, 1561 cm⁻¹ v C=C, 1118 cm⁻¹ and 838 cm⁻¹ v C=S.

2.3. Syntheses of the copper(II) complexes

2.3.1 Quinoline-2-carboxyaldehyde thiosemicarbazonato copper(II) chloride dihydrate



Ligand HL1 (104 mg, 0.45 mmol) was dissolved in 200 mL of warm acetonitrile and under stirring. An equimolar amount of copper(II) chloride dihydrate (77 mg, 0.45 mmol) was added. The solution became immediately dark and a green precipitate separated. The reaction mixture was left under reflux and stirring for about 4 hours. It was then left cooling at room temperature and filtered on a Buchner funnel. The light green precipitate was washed with acetonitrile and dried. Yield: 58%. Elem. Anal.: C₁₁H₁₃ClCuN₄O₂S calc. C 36.16%, H 3.86%, N 15.33%; exp. C 35.78%, H 2.83%, N 14.50%. IR: 3267 cm⁻¹ v N-H, 3057 cm⁻¹ v C-H arom., 2970 cm⁻¹ v C-H aliph., 1573 cm⁻¹ v C=N, 1508 cm⁻¹ v C=C, 1141 cm⁻¹ and 831 cm⁻¹ v C=S.

2.3.2 Quinoline-2-carboxyaldehyde N⁴,N⁴-dimethylthiosemicarbazonato copper(II) chloride ([Cu(L2)Cl])

To a warm solution obtained dissolving ligand HL2 (62.7 mg, 0.23 mmol) in 20 mL of ethanol, an equimolar amount of copper(II) chloride dihydrate (40.2 mg, 0.23 mmol) was added, and the solution immediately turned dark and a precipitate appeared. The reaction mixture was kept under reflux and under stirring for 2 hours. It was then left to cool down and filtered. The green product was washed with ethanol and allowed to dry. From the ethanol solution crystal suitable for X-ray structure determination were afforded. Yield: 46%. Elem. Anal.: C₁₃H₁₃ClCuN₄S calc. C 43.78%, H 3.68%, N 15.72%; exp. C 43.41%, H 3.43%, N 15.35%. IR: 3011 cm⁻¹ ν C-H arom., 2924 cm⁻¹ ν C-H aliph., 1596 cm⁻¹ ν C=N, 1576 cm⁻¹ and 1510 cm⁻¹ ν C=C, 1119 cm⁻¹ and 821 cm⁻¹ ν C=S.

2.3.3 Quinoline-2-carboxyaldehyde N⁴-methylthiosemicarbazonato copper(II) chloride dihydrate ([Cu(L3)Cl]·2H₂O)

Ligand HL3 (45.6 mg, 0.19 mmol) was dissolved in 100 mL of warm acetonitrile and kept under stirring. An equimolar amount of copper(II) chloride dihydrate (31.8 mg, 0.19 mmol) was added and the reaction mixture was kept under stirring for 1 hr and a half. The mixture was cooled at room temperature and filtered. The green product was washed with acetonitrile. The compound was crystallized using a mixture acetonitrile/methanol (2:1) and crystals suitable for an X-ray analysis were obtained. Yield: 39%. Elem. Anal.: C₁₂H₁₅ClCuN₄O₂S, calc. C 37.99%, H 3.98%, N 14.77%; exp. C 38.21%, H 3.84%, N 14.64%. IR: 3137 cm⁻¹ ν N-H, 3072 cm⁻¹ ν C-H arom., 2952 cm⁻¹ ν C-H aliph., 1582 cm⁻¹ ν C=N, 1530 cm⁻¹ and 1508 cm⁻¹ ν C=C, 1124 cm⁻¹ and 835 cm⁻¹ ν C=S.

2.3.4 Quinoline-2-carboxyaldehyde N⁴-phenylthiosemicarbazonato copper(II) chloride

([Cu(L4)Cl])

An equimolar amount of copper(II) chloride dihydrate (41.8 mg, 0.25 mmol) was added to 40 mL of a methanol solution of ligand HL4 (45.1 mg, 0.15 mmol). The reaction mixture became immediately dark, but, as soon as the precipitate began to form, it turned light green. The flask was left under reflux and stirring for 4 h and then was left cooling to room temperature. The red precipitate was washed with methanol. Yield: 68%. Elem. anal.: C₁₇H₁₃ClCuN₄S calc. C 50.49%, H 3.24%, N 13.85%; exp. C 49.89%, H 3.22%, N 13.67%. IR: 3199 cm⁻¹ ν N-H, 3104 cm⁻¹ and 3059 cm⁻¹ ν C-H arom., 2951 cm⁻¹ ν C-H aliph., 1597 cm⁻¹ ν C=N, 1535 cm⁻¹ and 1504 cm⁻¹ ν C=C, 1100 cm⁻¹ and 825 cm⁻¹ ν C=S.

2.3.5 Quinoline-2-carboxyaldehyde N²-methylthiosemicarbazone copper(II) chloride

([Cu(L5)Cl₂])

Ligand L5 (80.8 mg, 0.33 mmol) was dissolved in 70 mL of acetonitrile at room temperature and under stirring. An equimolar amount of copper chloride dihydrate (56.4 mg, 0.33 mmol) was added and the mixture was left under reflux and stirring for 6 hours. The light green precipitate was filtered and washed with methanol. Yield: 88%. Elem. anal. C₁₂H₁₂Cl₂CuN₄S : calc. C 38.05%, H 3.19%, N 14.79%; C 38.21%, H 3.12%, N 14.56%. IR: 3304 cm⁻¹ and 3189 cm⁻¹ ν N-H, 2998 cm⁻¹ ν C-H, 1592 cm⁻¹ ν C=N, 1560cm⁻¹ ν C=C, 1145 cm⁻¹ and 843 cm⁻¹ ν C=S.

2.4. Syntheses of the nickel(II) complexes

2.4.1 (Quinoline-2-carboxyaldehyde thiosemicarbazonato) (quinoline-2-carboxyaldehyde thiosemicarbazone) nickel(II) chloride methanol solvate ([Ni(L1)(HL1)]Cl·CH₃OH)

To 70 mL of a warm methanol solution of ligand HL1 (88.2 mg, 0.38 mmol) were added 3 drops of NaOH 1N and an equimolar amount of nickel chloride hexahydrate (91 mg, 0.38 mmol). The solution immediately turned dark red. The reaction mixture was left under stirring for about 5 h and then cooled to room temperature. The precipitate was a brick-red microcrystalline powder. The product was recrystallized from methanol and crystals apt to and XRD analysis were obtained. Yield: 66%. Elem. Anal. C₂₃H₂₃ClN₈NiO₂S₂ Calc. C 47.16%, H 3.96%, N 19.13%; exp. C 47.46%, H 3.70%, N 19.27%. ¹H NMR (300 MHz, DMSO-d₆): 11.79 ppm (1H, s, C=N-NH), 8.45 ppm (2H, d, J = 8.7 Hz, NH₂), 8.35 ppm (2H, m, CH arom.), 8.23 ppm (1H, s, CH=N), 8.00 ppm (2H, t, J = 8.7 Hz, CH arom.), 7.78 ppm (1H, t, J = 8.4 Hz, CH arom.), 7.62 ppm (1H, t, J = 8.1 Hz, CH arom.). IR: 3329 cm⁻¹, 3242 cm⁻¹, 3134 cm⁻¹ v C-H arom.; 2938 cm⁻¹ v C-H aliph.; 1575 cm⁻¹ v C=N; 1539 cm⁻¹ and 1505 cm⁻¹ v C=C; 1116 cm⁻¹ and 817 cm⁻¹ v C=S.

2.4.2 Bis(quinoline-2-carboxyaldehyde N⁴,N⁴-dimethylthiosemicarbazone)nickel(II) dichloride ([Ni(HL2)₂]Cl₂·2H₂O)

Nickel chloride hexahydrate (41.4 mg, 0.17 mmol) was added to 35 mL of a warm ethanol solution of ligand HL2 (44.5 mg, 0.17 mmol). The solution turned red-orange. The mixture was kept under reflux and stirring for 5 h and was then left to cool down to room temperature. The product was a red-orange powder. Yield: 56%. Elem. Anal, C₂₆H₂₈Cl₂N₈NiS₂ calc. C 48.32%, H 4.38%, N 17.33%; exp. C 48.24%, H 4.86%, N 17.46%. IR: 3080-2800 cm⁻¹ v C-H, 1606 cm⁻¹ v C=N, 1570 cm⁻¹ v C=C, 1118 cm⁻¹ and 798 cm⁻¹ v C=S.

2.4.3 Bis(quinoline-2-carboxyaldehyde N⁴-methylthiosemicarbazonato)nickel(II) ([Ni(L3)₂])

Ligand HL3 (65.2 mg, 0.26 mmol) was dissolved in 50 mL of warm ethanol under stirring. An equimolar quantity of nickel chloride hexahydrate (59.8 mg, 0.26 mmol) was then added and the solution immediately turned dark red. The reaction mixture was kept under reflux and stirring for 2 h and a half. The solution was left cooling down to room temperature and poured into a crystallizer. The product was recrystallized in a mixture methanol/ethanol (1:1) and crystals suitable for X-ray diffraction studies were obtained. Yield: 51%. Elem. Anal. C₂₄H₂₂N₈NiS₂ Calc. C 52.84%, H 4.06%, N 20.54%, exp. C 52.64%, H 3.84%, N 20.36%. IR: 3174 cm⁻¹ ν N-H; 3038 cm⁻¹ ν C-H arom.; 2961 cm⁻¹ ν C-H aliph.; 1574 cm⁻¹ ν C=N; 1531 cm⁻¹ and 1505 cm⁻¹ ν C=C; 1123 cm⁻¹ and 834 cm⁻¹ ν C=S

2.4.4 Bis(quinoline-2-carboxyaldehyde N⁴-phenylthiosemicarbazonato)nickel(II) ([Ni(L4)₂])

Ligand HL4 (58.7 mg, 0.19 mmol) was dissolved in 80 mL of warm methanol. To this mixture, an equimolar amount of nickel chloride hexahydrate (45.5 mg, 0.19 mmol) was added and the solution became dark red. The mixture was kept 5 h under stirring and then left cooling to room temperature. The product obtained by slow evaporation of the solvent was an orange-brown microcrystalline powder. Yield: 48%. Elem. Anal. C₃₄H₂₆N₈NiS₂ Calc. C 61.00%, H 3.91%, N 16.74%; exp. C 59.66%, H 3.13%, N 16.55%. IR: 3403-3270 cm⁻¹ ν N-H, 3057 cm⁻¹ ν C-H arom., 1596 cm⁻¹ ν C=N, 1541 cm⁻¹ and 1507 cm⁻¹ ν C=C 1096 cm⁻¹ and 825 cm⁻¹ ν C=S.

2.4.5 Bis(quinoline-2-carboxyaldehyde N²-methylthiosemicarbazone)nickel(II) chloride tetrahemihydrate ([Ni(L5)₂]Cl₂·4.5H₂O)

Ligand L5 (88.8 mg, 0.36 mmol) was dissolved in 70 mL of methanol at room temperature and an equimolar amount of nickel chloride hexahydrate (86.4 mg, 0.36 mmol) was added under

stirring. The solution immediately turned dark red. After 20 h under stirring the mixture was left cooling down to room temperature and then poured into a crystallizer. The slow evaporation afforded a microcrystalline precipitate and the yield was 75%. The product was recrystallized from methanol and crystal suitable for an XRD structure determination were obtained. Yield: 75%. Elem. Anal. C₂₄H₃₃Cl₂N₈NiO_{4.5}S₂ Calc. C 41.22%, H 4.76%, N 16.02%; exp. C 40.34%, H 4.82%, N 15.63%. ¹H NMR (300 MHz, DMSO-d₆): 8.67 ppm (1H, m, CH arom.); 8.60 ppm (2H, d, J = 8.7 Hz, NH₂); 8.39 ppm (1H, d, J = 8.7 Hz, CH arom.); 8.02 ppm (2H, m, CH arom.); 7.96 ppm (1H, s, CH=N); 7.79 ppm (1H, t, J = 7.5 Hz, CH arom.); 7.64 ppm (1H, t, J = 7.5 Hz, CH arom.); 3.87 ppm (3H, s, N-CH₃). IR: 3400-3200 cm⁻¹ ν N-H, 3180-2950 cm⁻¹ ν C-H, 1585 cm⁻¹ ν C=N, 1555 cm⁻¹ ν C=C, 1140 cm⁻¹ and 829 cm⁻¹ ν C=S

2.5 Crystal structure determination

The X-ray structures of ligands HL2, HL3 and HL5 were collected on a Siemens AED diffractometer with Cu-K α radiation ($\lambda = 1.54178 \text{ \AA}$) and those of compounds [Cu(L2)Cl], [Cu(L3)Cl], [Ni(L1)(HL1)]Cl·CH₃OH, [Ni(L3)₂] and [Ni(L5)₂]Cl₂·4.5H₂O were collected on a Bruker SMART APEX2 with Mo-K α radiation ($\lambda = 0.71069 \text{ \AA}$). The structures were solved through direct methods with SIR97[16] and refined with SHELXL97[17] implemented in WinGX [18]. The drawing were obtained using the ORTEPIII program.[19] The relevant crystal data are reported in the Supplementary Material file as Table 1S. CCDC 1062429-1062436 contain the supplementary crystallographic data. 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.

2.6 Biological experiments

2.6.1 Materials.

For cell cultures, RPMI 1640 medium, fetal bovine serum (FBS), penicillin/streptomycin, L-glutamine, phosphate buffered saline (PBS) were purchased from Euroclone (Milan, Italy) and flasks/plates from Costar, Corning (Amsterdam, The Netherlands).

2.6.2 Cell culture.

U937, a monoblastoid line, was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin, 100µg/mL streptomycin, and 2mM L-glutamine and maintained at 37°C in a humidified atmosphere with 5% CO₂.

2.6.3 Cell treatment.

Ligand and complex solutions were prepared from a concentrated 10 mg/mL stock solution in DMSO. U937 at a mid-log phase were seeded at a density of 1.5×10^5 cells/mL in RPMI 1640 medium in plates or flasks. After 24h the cells in the exponential phase of growth were treated with DMSO 0.1% (control) and all the compounds for different periods.

2.6.4 Proliferation assay.

Cell proliferation was measured using the Cell-Titer 96® AQueous One Solution Cell Proliferation Assay (MTS) kit. U937 cells were seeded into 96-well plates in triplicate. One hour prior to the end of each treatment MTS reagent (20 µL) was added to each well. At the end of exposure time, the absorbance of the formazan product was measured at 490 nm by a

microwell plate reader (Multiskan Ascent, Thermo Labsystems, Helsinki, Finland). Untreated cells were used for the calibration curve. Medium containing MTS and different concentrations of complexes, but without cells, was included to detect possible interaction between the MTS and the test compounds. Each experiment was repeated on three separate occasions.

Cell viability was monitored also by counting viable cells in a hemocytometer (trypan blue exclusion); after incubation with copper complexes, cells collected from eight wells were pooled and placed in trypan blue for 5 min.

2.6.5 TopoIIa inhibition assay

Inhibition assays on TopoIIa were carried out using a kit by TopoGen (Port Orange, FL, USA). The standard procedure was followed [20]: 14 μl di H₂O, 4 μl of buffer solution, 1 μl supercoiled DNA and 1 μl of eukaryotic topoisomerase II α (~ 2 A.U. as described by the producer) are mixed in a microcentrifuge tube (1.7 ml). In the experiments which require the presence of our compounds we added 2 μl of a stock solution of the compound to be tested dissolved in DMSO and the initial volume of water was corrected to reach a final volume of 20 μl . The reaction mixture was gently shaken, centrifuged for a short time and incubated at 37° for 30 minutes. After 30 minutes 2 μl of a 10% SDS solution was added to quench the reaction, 2 μl of proteinase K to digest the remaining proteins and the reaction mixture was incubated at 37° for 15 minutes. After this, 2.5 μl of loading dye (10X) was added to the reaction mixture and the tube was subsequently gently shaken and centrifuged. The sample was then loaded on a 1% agarose gel and an electrophoresis was run for 5 hrs at 5 V/cm. The resulting gel was then dyed in a TAE solution containing 5 $\mu\text{g/L}$ of ethidium bromide. The resulting gels were visualized with a BioRad Fluor-STM MultiImager.

2.7 Docking simulations

The crystal structures of both the ATPase and the cleavage domains of human TopoIIa were retrieved from the Protein Data Bank (PDB) [21] and used after deletion of all water molecules and other ligands, but keeping the DNA chains. The structure of the ATP binding domain was used in the dimeric form (PDB ID: 1zxm [22]), while the structure of cleavage domain is available only in monomeric form (PDB ID: 4fm9[23]).

For ligand HL2, HL3, HL5 and complexes $[\text{Cu}(\text{L}2)]^+$ and $[\text{Cu}(\text{L}3)]^+$ the crystal structures determined from our structural studies were used. The structure of ANP (phosphoaminophosphonic acid-adenylate ester), used as a control, was extracted from the ATP binding domain crystal structure. ANP is an ATP molecule in which the oxygen between $\text{P}\beta$ and $\text{P}\gamma$ is substituted by a nitrogen to avoid the hydrolysis of the molecule and to make possible the crystallization of the complex, The structures of the other compounds were built by means of Hyperchem8 [24] and then refined by minimization with the Avogadro v. 1.1.0 [25] software. Both the *E* and the *Z* configurations of the ligands were built and used in the simulations.

The docking simulations were performed with the Autodock4 software package, with the aid of the AutoDockTools (ADT) interface [26]. The Kollmann partial charges [27] were added to the receptors by means of ADT. The partial charges were added to the compounds by means of the UCSF Chimera package [28] using the Antechamber module [29] and the Amber ff12SB force field. After the deletion of chlorine, the copper atom was replaced with iron, because Autodock4 does not contains the docking potential parameters for copper; we exploited the similar ionic radius presented by the two ions in the oxidation state of +2. The charge on the deprotonated ligand was -1 (delocalized partly on the nitrogen and partly on the sulfur, as suggested by the experimental results), therefore giving a net charge of +1 to the complex. The only exception in the series was complex $[\text{Cu}(\text{L}5)]^{2+}$, in which the presence of a methyl group

bound to the hydrazinic nitrogen prevents the deprotonation of the ligand and confers a net charge of +2 to the whole complex.

After an initial check, all torsions of both the receptors and the compounds were kept fixed. The grid maps centered on the ATP binding pocket had a box size of 72 x 66 x 64 points, spaced 0.375 Å and the one at the cleavage site had a box size of 62 x 68 x 72 points, with the same spacing. For the docking calculations, the Lamarckian Genetic Algorithm [30] was used, performing 100 runs with 150 individuals in the population, 27000 generations and 2.5×10^6 energy evaluations. A cluster analysis was performed and the ligand-receptor complexes with the lowest binding energy and/or in the more populated cluster were chosen for the analysis. Structural analysis of the binding modes was made using ADT, VMD [31] and Swiss-PdbViewer [32] softwares and the web tool for macromolecular visualization FirstGlance in Jmol (<http://bioinformatics.org/firstglance/fgij/index.htm>).

3. Results and discussion

3.1 Synthesis and characterization.

In a paper published by Huang *et al.* in 2010, [33] among a variety of compounds assayed in search for TopoIIa inhibitors, quinoline-2-carboxaldehyde N⁴-N⁴dimethylthiosemicarbazone resulted to possess the highest activity, with IC₅₀ between 0.02 and 0.08 μM, depending on the tumor cell line. In this study we have synthesized derivatives modified on the thiosemicarbazide nitrogens and their coordination compounds with copper(II) and nickel(II) in order to explore the influence of the substituents, of the metal ions and of the molecular shape on the overall biological behavior. More specifically, we have tested the capacity of these molecules to inhibit one of the putative targets of thiosemicarbazones in cells, TopoIIa, and to propose a model that could explain their different activity.

All five ligands were synthesized and characterized according to the procedures reported in the experimental section and for three of them the XRD structure has also been determined. All these molecules are planar due to an extended system of conjugated double bonds and to a thione-thiol tautomerism of the thiosemicarbazone fragment. The configuration of the molecules with respect to the imino double bond in solution easily undergo *E* and *Z* interconversion [34], but in the solid state only one of the two conformation is observed. The determining factor seems to be, besides possible packing interactions, the pattern of intramolecular hydrogen bonds that the molecule can form. For instance, if at least one hydrogen atom is present on the terminal nitrogen, an intramolecular hydrogen bond with the imino nitrogen is usually present. This pattern is usually associated with a sterically more favorable *E* configuration. When the terminal nitrogen is completely substituted and becomes a tertiary amine, as in compound HL2, it is the hydrazinic hydrogen that forms a hydrogen bond with the quinoline nitrogen, forcing the C=N bond to a *Z* configuration. (Figure 3) This trend is corroborated also by the structure of the quinoline-2-carboxaldehyde thiosemicarbazone reported in the literature [35].

As far as the packing of the above mentioned ligands are concerned, it is interesting to observe how each one presents a peculiar hydrogen bond that characterizes the packing. Ligand HL2 shows an intramolecular H-bond between the hydrazinic NH and the quinoline nitrogen, in ligand HL3 the H-bond involves the same atoms but belonging to two different adjacent molecules, while L5 uses the NH of the terminal amino group to form the H-bond with the quinoline nitrogen of a nearby molecule. Weak van der Waals interactions between adjacent molecules contribute to the packing and only molecules HL3 and L5 show intermolecular stacking interactions between the aromatic system of the quinoline and the thiosemicarbazide moiety.

The introduction of a coordinating metal ion affects dramatically the configuration of these ligands, since the energy implied in coordination prevails on that of the hydrogen bonds found in the ligand molecules and complexation imposes an *E* configuration on the ligands by forming three coordinative bonds, i.e. with the quinoline nitrogen, the imino nitrogen and the sulfur.

In the case of the copper derivatives the syntheses afforded complexes of stoichiometry M:L 1:1. The ligand chelates the metal with its three donor atoms which are sterically more favored and the fourth position is occupied by a chloride ion that completes the square planar geometry.

In complexes [Cu(L1)Cl], [Cu(L2)Cl], [Cu(L3)Cl] and [Cu(L4)Cl], the deprotonation causes the negative charge to delocalize partly on the nitrogen and partly on the sulfur, as can be inferred by the bond lengths observed in the two structures characterized through XRD. The distances C1-N2 becomes shorter passing from 1.359Å and 1.356Å in the ligands to 1.342Å and 1.324Å in the complexes, and contemporarily the C1-S1 bond changes from 1.687Å and 1.677Å in the ligands to 1.750Å and 1.746Å in the complexes. The crystal structures of compounds [Cu(L2)Cl] and [Cu(L3)Cl] are reported in Figure 4. In the structures, the two copper derivatives are characterized by a distorted square planar coordination geometry where the chlorine atom is markedly out of the plane (the Cu-Cl bond forms angles of 21.05° and 28.91° with the coordination plane, in [CuL2Cl] and [CuL3Cl] respectively). Moreover, the complex molecules present, pairwise, copper-sulfur interactions of 2.82 and 2.86Å, respectively in which the sulfur occupies the apical position of a square pyramid centered on the metal ion. Neither stacking interactions nor hydrogen bonds are present in the packing. In [Cu(L5)Cl₂], where the ligand cannot undergo deprotonation, the compound contains a further chloride ion to compensate the missing negative charge.

Differently from copper, solutions of nickel(II) chloride added of each of the ligands have afforded complexes with stoichiometry M:L 1:2. The complexes present a nickel ion in an

octahedral environment with two terdentate ligands perpendicular to each other. The molecular structures of compounds $[\text{Ni}(\text{L1})(\text{HL1})]\text{Cl}$, $[\text{Ni}(\text{L3})_2]$ and $[\text{Ni}(\text{L5})_2]\text{Cl}_2$ are reported in Figure 5. The nickel derivatives present the most diverse packing systems. The crystals of $[\text{Ni}(\text{L1})(\text{HL1})]\text{Cl}\cdot\text{CH}_3\text{OH}$ contain a chloride ion and a methanol. The chloride plays a relevant role in determining the packing through a complex H-bond system. It is involved in three hydrogen bonds: two with the terminal NH of two different complexes and a third with the methanol OH. One of the terminal NH also binds the methanol oxygen. In addition, the aromatic rings form loose dimers through parallel and perpendicular interactions of the pi-systems. Compound $[\text{Ni}(\text{L3})_2]$ contains neither counterions nor solvent and its structure is characterized by helices formed by rototranslation of the asymmetric unit, where the molecules are joined together by hydrogen bonds between the terminal NH, which acts as a donor, and the non coordinated hydrazinic nitrogen. Another peculiar feature presented by these molecules is the short contact between the terminal methyl and the pi-system of the quinoline moiety of an adjacent molecule. The packing of $[\text{Ni}(\text{L5})_2]\text{Cl}_2\cdot 4.5\text{H}_2\text{O}$ is the most complex one, as can be predicted by the presence of two chloride ions and by the large number of water molecules. The structure is characterized by an extensive system of hydrogen bonds connecting the water molecules, the chloride ions and the terminal NH_2 of the ligand. The pi-pi interactions are limited to a single weak contact of two aromatic systems at 3.599 and 3.593 Å. Figure 1S to 6S in the Supplementary material show the described interactions.

3.2 Biology

All the compounds reported, ligands and complexes, were tested for their capacity to inhibit proliferation in cell line U937 and assayed on TopoIIa. The difference in the substitution on the ligands and the nature of the metals, together with the shape of their complexes, are the

main variables that can influence the biological activity of these compounds. It is worth noting here that equivalent amounts of copper(II) and nickel(II) salts do not have influence on cell proliferation [36] and copper(II) on topoisomeraseIIa inhibition [20].

Cell viability was checked after a 24 h exposure to increasing concentrations of ligands and complexes (range 0.1–100 μM). (Table 1). From these IC_{50} values it is possible to do some considerations. If we compare each ligand with the corresponding metal complexes, we can observe that the copper derivatives are systematically more active than both the ligands and the analogous nickel complexes. The IC_{50} for the copper(II) derivatives are in the range 0.48 μM (compound $[\text{Cu}(\text{L}2)\text{Cl}]$) to 16.2 μM (compound $[\text{Cu}(\text{L}5)\text{Cl}_2]$). In this latter case, it is noteworthy that the parent ligand (L5), and the analogous Ni(II) complex ($[\text{Ni}(\text{L}5)_2]\text{Cl}_2$), do not show any activity up to a 100 μM concentration suggesting that the disappearance of the thione/thiol tautomeric equilibrium plays a relevant role. Also the substituents seem to play a relevant role in the potency of the compounds. The ligands and complexes with the doubly methylated terminal nitrogen result to be the most effective of the series (ligand HL2: $\text{IC}_{50} = 0.82 \mu\text{M}$, Cu(II) complex $[\text{Cu}(\text{L}2)\text{Cl}]$: $\text{IC}_{50} = 0.48 \mu\text{M}$ and Ni(II) complex $[\text{Ni}(\text{HL}2)_2]\text{Cl}_2$: $\text{IC}_{50} = 8.69 \mu\text{M}$). The copper complexes with ligands possessing a partial substitution on the very same nitrogen either with a single methyl ($[\text{Cu}(\text{L}3)\text{Cl}]$: $\text{IC}_{50} = 2.25 \mu\text{M}$) or with a phenyl ($[\text{Cu}(\text{L}4)\text{Cl}]$: $\text{IC}_{50} = 1.8 \mu\text{M}$) do not present a significant difference in their IC_{50} values. Compound L5 do not show any activity up to a concentration of 100 μM . The related copper complex $[\text{Cu}(\text{L}5)\text{Cl}_2]$ results to be the least active among the analogous tested compounds, with an IC_{50} of 16.2 μM ; it is therefore apparent that the methyl on the N2 position seems to reduce the efficacy of this compounds. In addition, its higher positive charge could interfere with its ability to cross the cell membrane.

As far as the TopoIIa tests are concerned, the results are quite interesting. In fact, we find an overall correlation between proliferation inhibition and TopoIIa inhibition: the copper derivatives are outstandingly more active than all the other compounds (Figure 7S).

3.3 Docking simulations

The structure of TopoIIa can be divided in three distinct domains. Two of them host two functionally different active sites: the C-terminal domain with the ATP-binding site and the central domain with the catalytic DNA cleavage-religation site.[14] The ATP-binding domain possesses a lid whose opening mechanism permits not only the binding of ATP and its hydrolysis, but also the dimerization of this N-terminal region. [22, 37] Interestingly, the dimer presents a domain swapping involving a little helix, named “strap” helix, that positioning over the lid that closes the ATP-binding site, stabilizes the closed conformation.[38]

The DNA cleavage-religation domain, which is already in the dimeric form, binds DNA, cuts it and re-ligates it, after the passage of a second DNA strand. It requires for its function two Mg^{2+} ions that, binding to a cluster of acidic residues (Asp541, Asp543, Glu461) and to a phosphate, are involved in the cutting and anchoring of DNA[37, 39]

Molecular docking simulations were performed on the compounds to analyze their interactions with both the ATPasic domain, led by its structural similarity with ATP, and at the cleavage domain, led by a putative analogy with etoposide-like inhibitors.

The simulations were performed on the ligands (both in the *E* and in the *Z* conformation) and on the copper complexes, after a substitution of the copper atom with iron. (see Materials and Methods section).

3.3.1 ATPasic domain

At first, we performed a control simulation with the ANP molecule, that was present in the binding site of the crystal structure of TopoIIa (pdb id code 1ZXM, see Materials and Methods). Our control simulation retrieved for ANP the same identical position that it has in the crystal, checking the reliability of the docking software and parameters and permitting the estimation of a reference binding energy for the endogenous ligand ($E_b = -13.8$ Kcal/mol) that could be used for comparison.

The docking simulations of all the ligands in both *E* and *Z* conformations show a binding mode inside the ATP pocket, in the same place of ANP/ATP. Nevertheless, their binding energies were too high to compete with the endogenous ligand (ranging from -4.13 to -3.01 Kcal/mol). On the contrary, the complexes with metal do not find any binding site at the ATP place, but they all bind externally, in the same place at the edge of the pocket (Figure 6). In fact, they find a very favorable site for Cu^{2+} in the loop of the pocket lid, that contains four consecutive acidic residues (152-155) creating a strong negative potential region. The metal complexes $[\text{Cu}(\text{L1-L4})]^+$ link the loop of the lid with the *strap* helix, by means of their hydrophobic rings. Among them, complex $[\text{Cu}(\text{L4})]^+$ has the most favorable binding energy, due to its phenyl ring in R2 position, forming additional hydrophobic interactions with the carbons of Arg98 side chain. Complex $[\text{Cu}(\text{L5})]^{2+}$, that has the methyl group in R1 position, inverts its orientation in a way that makes the CH_3 to interact with Ile33 on the *strap* helix while the rest of the tail of the molecule can make H-bonds with Arg32 and Gln35 (Figure 6). This position and the particular charge distribution of this complex (see Materials and Methods) produces a net gain in binding energy of complex **10** in respect to all the other metal complexes (Table 2).

It is worthwhile to note that, even if ATP has a $2+$ ion (Mg^{2+}) that could also bind in the site of the metal complex, it has nevertheless a highly negative net charge, due to the phosphate groups, that fits well in its very basic binding pocket; the positive charge of the pocket is instead not favorable to accommodate our metal complexes, that have a net charge of $+1$ or $+2$.

Observing the binding mode of the metal complexes in the ATPasic domain, we can hypothesize a first inhibition mechanism that, linking the strap helix to the lid, could prevent the release of the monomeric form of this domain and the restart of the enzyme cycle.

3.3.2 Cleavage domain

The docking simulations on the cleavage domain show again for the ligands, in both *E* and *Z* conformations, spread and aspecific binding modes, with higher binding energies than the metal complexes (ranging from -5.18 to -3.09). All metal complexes, instead, adopt two equally favorable binding modes, both involving the interaction of the metal with the oxygen atoms of the last phosphate at the edge of the cut. We have to pinpoint that, since the available crystal structure of TopoIIa was monomeric, we used the superimposition with the dimeric structure of topoisomerase IIb (PDB ID code 3QX3 [15]) to check the hindrance of the other protomer containing the other DNA fragment at the opposite edge of the cut.

In the first binding mode the molecules are oriented almost parallel to the DNA bases, in an *etoposide*-like manner, with the rings in contact with the sugar of the first base and the tail in contact with the pyrimidine ring of the second base (Figure 7a). In particular, the interaction of the second base with the methyl groups of complexes $[\text{Cu}(\text{L2})]^+$ and $[\text{Cu}(\text{L3})]^+$ are favored if compared with complex $[\text{Cu}(\text{L1})]^+$; while complex $[\text{Cu}(\text{L4})]^+$, that has the phenyl ring in stacking with the base ring, shows the lower, i.e. more favorable, binding energy (Table 2). Complex $[\text{Cu}(\text{L5})]^{2+}$ is the only one for which this binding mode is not present.

The second binding mode involves the other oxygen of the last phosphate, but the conformations are at the external edge of the DNA strand, almost perpendicular to the basis plane (Figure 7b). As in the ATPasic domain, complex $[\text{Cu}(\text{L5})]^{2+}$ inverts its orientation and has a lower binding energy than the other complexes. In this case, it is complex $[\text{Cu}(\text{L1})]^+$ that does not assume this position.

The energies of the two binding modes are comparable. As it happens in the ATPase domain, the complexes $[\text{Cu}(\text{L4})]^+$ and $[\text{Cu}(\text{L5})]^{2+}$ show the lowest binding energies. Probably, the higher net charge of the first compound and the hydrophobic phenyl ring of the latter, gives a strong contribution to the binding.

In neither of the two binding modes, the metal is in the place of Mg^{2+} (i.e. coordinated by the acidic cluster) preferring instead the DNA phosphate, probably because of the more favorable hydrophobic interactions that can be formed. This fact suggests a second putative inhibition mechanism that prevents the re-ligation of DNA, with the formation of cleavage intermediates resulting toxic for cells.

4. Conclusions

A series of quinoline-2-carboxaldehyde thiosemicarbazones, differing in the substituents of the thiosemicarbazide moiety, were synthesized and characterized. In all complexes the ligands are in the *E* configuration with respect to the imino bond and behave as terdentate. Copper(II) complexes present a metal:ligand ratio 1:1 and form square planar derivatives with one molecule of ligand and chloride ions to compensate the overall charge. Independently from the stoichiometric amounts used, nickel(II) ions form, instead, octahedral complexes with two ligands for each metal ion. Ligands and complexes were tested for their antiproliferative properties on histiocytic lymphoma cell line U937. As already observed by Zeglis et al (2011)[20] metal complexes are more active than the corresponding ligands, and copper compounds are the most active. All the compounds were tested for their specific activity on TopoII a and among them only copper derivatives resulted to inhibit the enzyme in vitro. Computational methods have been used to propose a model that explains the different extent of inhibition presented by these compounds. Both Huang and co-workers (2010) [33] and Zeglis and co-workers (2011) [20] propose a catalytic inhibition mechanism, that is to say, an

interaction of these compounds with the enzyme through the ATP pocket, but the first author tests only the ligands as probes, while the second one assumes the same mechanism for the metal complexes without exploring other possible target sites in the enzyme. A feature of these copper complexes is certainly the possibility to dissociate the chloride ion and give rise to positively charged metal ions as soon as they enter the cytoplasm, a mechanism already proposed for cisplatin.[40] We propose that the positive charge of the dissociated form of the copper complexes may play a key role in their action. The charge drives the metal complex to find its way in the enzyme structure and binds more favorably in negatively charged sites.

In our calculations we observe that the ligands, in both their *E* and *Z* forms, do enter in the ATP pocket and bind to the site, but with much lower binding energies than the endogenous ligand. What we observe with the complexes is something different: the metal derivatives tend to get stuck between the lid and the strap helix, binding to an acidic cluster. The access into the ATP pocket is made even more difficult by the presence of basic residues which are ideal for attracting a negatively charged ATP molecule. Compound [Cu(L5)Cl₂] is the least active of the copper compounds as far as the IC₅₀ is concerned but a good inhibitor of TopoII both *in vitro* and *in silico*. This peculiar behavior can be easily explained taking into account that the methylation on the N2 nitrogen prevents the deprotonation of the ligand and confers to the complex, differently from the other copper compounds which are neutral, [Cu(L)Cl], a cationic nature, [Cu(L5)Cl]⁺, and this could certainly account for a possible hindrance to the access into the cell by passive diffusion.

We docked our molecules also in the cleavage site, finding again for the ligands a spread and aspecific binding, with higher energies than the complexes and comparable with those at the ATP site. Considering the features of the binding, the ATP domain seems a more reliable target for the ligands, in agreement with what was found by Huang and co-workers (2010) [33]. The copper compounds, instead, strongly interact with the DNA phosphate group at the cut edge,

likely preventing the re-ligation to the opposite strand. Again, compound $[\text{Cu}(\text{L5})]^{2+}$ has the most favorable energy, probably due to its cationic feature that, in this case, favors the binding at the DNA negatively charged group. In both domains, compound $[\text{Cu}(\text{L1})]^+$ has the worst energy, confirming the relevant role of substitutions in enhancing the binding affinity.

The comprehension of the inhibition mechanisms, based on the structural characterization, and the relevance of the Cu role, could lead to a new approach for the synthesis of differently substituted thiosemicarbazones and for the study of their biomedical application as anticancer drugs.

ABBREVIATIONS

RPMI Roswell Park Memorial Institute medium;

FBS Fetal bovine serum;

PBS Phosphate buffered saline;

MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

SDS Sodium Dodecyl Sulfate

ANP Phosphoaminophosphonic acid-adenylate ester

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Figure and Table captions

Figure 1. Scheme of the ligands.

Figure 2. Thiosemicarbazide thione/thiol tautomerism equilibrium.

Figure 3. ORTEP drawings of compound HL2, HL3 and L5 with 50% probability ellipsoids. Intramolecular hydrogen bonds are represented with dashed lines.

Figure 4. ORTEP plots of two copper(II) derivatives, compound [Cu(L2)Cl] and [Cu(L3)Cl], with 50% probability ellipsoids.

Figure 5. ORTEP plots of three nickel(II) derivatives, compound [Ni(L1)(HL1)Cl], [Ni(L3)₂] and [Ni(L5)₂Cl₂], with 50% probability ellipsoids.

Figure 6. Best docked positions of metal complexes in the ATPasic domain. Complex [Cu(L1)]⁺ = blue, [Cu(L2)]⁺ = red, [Cu(L3)]⁺ = gray, [Cu(L4)]⁺ = yellow, [Cu(L5)]²⁺ = green. The metal is in a spacefill representation, in the same color as the ligand. The ATP pocket (orange) with the lid loop (cyan) and the strap helix of the opposite chain are in opaque cartoon representation. All the rest of the dimeric molecule (gray) is in transparent cartoon. The main interacting residues (in ball and stick) are labelled. The crystal position of ANP is shown in purple.

Figure 7. Best docked positions of metal complexes in the cleavage domain. (a) first binding mode, parallel to the bases rings; (b) second binding mode, perpendicular to the bases rings. Complex [Cu(L1)Cl] = blue, [Cu(L2)]⁺ = red, [Cu(L3)]⁺ = gray, [Cu(L4)]⁺ = yellow, [Cu(L5)]²⁺ = green. The metal atom is in a space-fill representation, in the same color as the ligand. In black is the putative position of the Mg²⁺ ion, coordinated by the acidic cluster (red sticks). The protein is in transparent cartoon, colored by secondary structure (beta strands = yellow; alpha-

helices = purple; 310-helices = blue; turns = cyan). The DNA fragment (gray) is in opaque sticks representation, with the phosphate group colored by element.

Table 1. Proliferation inhibition concentrations, IC_{50} , (the compound concentrations used in the test were 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 20 50 and 100 μ M) and TopoIIa inhibition assay outcome (the compound concentration used for the topoisomeraseIIa inhibition test following the Topogen® kit protocol is 50 μ M).

Table 2. Binding energies (Kcal/mol) of metal complexes in the ATPasic and cleavage domains. For the latter, the energies of the two preferential binding mode are reported, i.e. parallel (//) and perpendicular (\perp) to the DNA bases plane.