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Comparative studies of oxindolimine-metal complexes as inhibitors of human DNA topoisomerase IB

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ABSTRACT

Copper(II) and zinc(II) complexes with oxindolimine ligands, obtained by a condensation reaction between isatin (1H-indole-2,3-dione) and 2-(2-aminoethyl)pyridine, have been previously shown to activate apoptosis very efficiently in different cancer cell lines. Here, we show that these compounds inhibit human DNA topoisomerase IB (Topo I) activity, and this characteristic may be implicated in its pro-apoptotic and potential antitumor properties. The studied metal complexes prevent DNA relaxation and cleavage reaction, whilst they do not have any effect on the religation process. The protein inhibition occurs in two different ways since the copper compound does not permit the enzyme-DNA complex formation, while the zinc analogue permits this complex formation but inhibits the catalytic cleavage reaction. Other related copper and zinc complexes, one with an asymmetric imine ligand derived from isatin, 1,3-diaminopropane, and salicyladehyde, and another one previously reported, derived from isatin and 1,3-diaminopropane, had their corresponding inhibition results towards Topo I compared. These data demonstrated that such complexes can act as good catalytic inhibitors of Topo I, in a process modulated by the ligand features and the nature of the metal ion. Computational studies complemented and supported experimental data, showing three different characteristics of the metal complexes that influence its interaction and consequent inhibition of Topo I. The ligand planarity when bound to the protein increases the occurring interactions in different binding sites, the total charge of the complex modulates the preferential region of interaction, and the copper(II) complexes are expected to be more efficient inhibitors compared to analogous zinc(II), as verified experimentally.

1. Introduction

Topoisomerases are enzymes that control the topological state of DNA. They are involved in the relaxation of supercoiled DNA necessary for the occurrence of essential processes such as recombination, integration, repair, chromatin assembly and chromosomal segregation [1]. All the topoisomerases act cutting at least one DNA strand through a nucleophilic attack driven by a tyrosine residue of the catalytic site. This event culminates in the formation of a covalent DNA-enzyme linkage and a hydroxyl group on the end of the cleaved DNA that, after relaxation has occurred, undergoes the religation reaction that restores intact DNA [2]. Topoisomerases are divided in two classes depending on the number of DNA strands they transiently cleave: type I cuts only one DNA strand, while type II acts on both DNA strands [3,4].

Human DNA topoisomerase IB (Topo I) is a monomeric enzyme consisting of 765 amino acids, organized in four domains: N-terminal domain (1–214), core domain (215–635), linker domain (635–712), and C-terminal domain (713–765) [5–7]. The protein clamps around the DNA during the catalytic cycle changing the linking number of the supercoiled DNA [8]. Topo I is an enzyme of clinical interest, since it is the only cellular target of a family of compounds, named camptothecin (CPT), that converts the enzyme into a cellular poison by reversibly stabilizing the Topo I-DNA covalent complex [9–13]. The cytotoxic action of this drug has been attributed to the collision of advancing polymerase complex with the drug-stabilized enzyme-DNA intermediate resulting in an irreversible break of the double stranded DNA, leading to cell death [9,10,14,15]. Two water-soluble CPT derivatives, topotecan and irinotecan, have been approved by the Food and Drug Agency (FDA) for clinical use. Their use has however

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some limits due to low solubility, high cytotoxicity and the occurrence of a chemical equilibrium between a lactonic and carboxylate form, the latter one being inactive against Topo I [16–19]. Due to these reasons, the field of research for the identification of new Topo I inhibitors is very active. Topo I is actually inhibited by several compounds that are divided into two classes depending on the diverse way they interact with the enzyme [20–22]. Poisons inhibit the enzyme at the level of the religation stabilizing the DNA-Topo I cleavage complex [23–25], while catalytic inhibitors prevent the DNA binding or inhibit the cleavage reaction [26–29]. Some of them inhibit both the cleavage and religation reaction [30,31]. Different metal compounds have been developed to target this enzyme, including complexes of gold [29], platinum [32,33], copper [34] and ruthenium [35,36], since the ability of the metal to modulate the coordination geometry can be exploited to improve interactions with the enzyme at different levels of the catalytic mechanism.

Previously, we have shown that metal complexes with oxindolimine ligands, obtained by a condensation reaction between isatin (1H-indole-2,3-dione) and 2-(2-aminoethyl)pyridine followed by metallation, from hereafter called $[Cu(isaepy)]^+$ and $[Zn(isaepy)]^+$, where *isaepy* = (E)-3-((2-(pyridin-2-yl)ethyl)imino)-1λ^2-indolin-2-one, are able to activate apoptosis in different cancer cell lines [37–39]. These studies also indicated that DNA and mitochondria are important intracellular targets of this class of compounds [40]. More recently, we reported the efficient inhibition of Topo I by a copper(II) and an analogous zinc(II) complex with another oxindolimine ligand, *isapn* or (3E,3′E)-3,3′-(propane-1,3-diylbis(azaneylylidene))bis(indolin-2-one) [41]. Herein, we investigated the influence of ligand features in modulating the interactions of these metal complexes with Topo I. Copper and zinc complexes with three different ligands (Fig. 1), *isaepy*, *isapn*, and *isapnsal* (or (E)-3-((3-(((E)-1-(2-hydroxyphenyl)ethylidene)amino)propyl)imino)indolin-2-one, were tested, and their inhibition data were compared. Additionally, rationalization of the experimental results was provided by molecular docking.

2. Methods and materials

2.1. Syntheses of metal complexes

2.1.1. Synthesis of compounds *[Zn(isaepy)Cl$_2$]* **1** and *[Cu(isaepy)H$_2$O]ClO$_4$* **2**

These complexes have been already prepared in our laboratory [39]. Briefly, the oxindolimine ligand (*isaepy*) was prepared in solution by dissolving isatin (0.736 g, 5 mmol) in 15 mL ethanol, and adding 2-(2-aminoethyl)pyridine (0.600 µL, 5 mmol) to it. The pH was then adjusted to 5.5 with a few drops of aqueous HCl 1 M, and the final solution was stirred during several hours. The metallation of the formed ligand was carried out by addition of an aqueous solution of zinc(II) chloride (0.682 g, 5 mmol), or copper(II) perchlorate (1.85 g or 5 mmol dissolved in 2 mL water). The pH was re-adjusted to 7.5–8.0 by addition of a few drops of NaOH solution. After cooling the mixture in an iced bath, an orange solid (for zinc complex) or a brown solid (for copper complex) was collected, filtered off, washed with cooled ethanol and ethyl ether, and dried in vacuum.

[Zn(isaepy)Cl$_2$], Yield: 90% (1.74 g, 4.50 mmol), MW 387.56 g/mol. Found: C, 46.45; H, 3.41; N, 11.01%. Calc. for $C_{15}H_{13}N_3OCl_2Zn$: C, 46.49; H, 3.38; N, 10.84%. FT-IR (cm^{-1}, KBr): 3469 m, ν(O—H); 3205 s, ν(N—H); 1735 s, ν(C═O), and 1639 s, 1575 ν(C═N).

[Cu(isaepy)H$_2$O]ClO$_4$, Yield 66%, MW 431.29 g/mol. Anal. Found: C, 42.16%; H, 3.64%; N, 9.72%. Calcd. for $C_{15}H_{14}N_3O_2Cu(ClO_4)$: C, 41.77%; H, 3.47%; N, 9.74%. EPR parameters (frozen solution C_2H_5OH/H_2O): $g_\perp = 2.075$; $g_{//} = 2.309$; $A_{//} = 154 \times 10^{-4}$ cm^{-1}; $g_{//}/A_{//} = 150$ cm. This complex has been previously characterized by MS (ESI+): *m/z* = 314.1 [MW = 431.29; in CH_3OH/H_2O, fragment $C_{15}H_{12}N_3OCu$]; 316.1 [isotopic pattern ($Cu^{63/65}$) monocation]; 564.1 [keto-form, fragment $C_{30}H_{24}N_6O_2Cu$ (*[Cu(isaepy)$_2$]*), MW = 566.11); 566.2 [isotopic pattern ($Cu^{63/65}$) monocation] [39]. FTIR (cm^{-1}, KBr): 3575 m, ν (O—H); 1731 s, ν (C═O); 1647 s, ν (C═N); 1120 and 625 s, ν (ClO_4 non-coordinated).

2.1.2. Synthesis of compound *[Zn(isapnsal)]ClO$_4$* **3**

To 1.0 mL salicylaldehyde dissolved in 20 mL ethanol, 830 µL propylenediamine was added. After some hours of reaction under stirring, the solution became yellow, indicating the formation of corresponding imine, that was metalated by addition of 3.759 g (10.09 mmol) Zn $(ClO_4)_2{\cdot}6H_2O$ dissolved in a few mL of water. The pH of the solution was adjusted to 5.5 with propylenediamine. A solution of 1.476 g (10.03 mmol) isatin dissolved in 30 mL hot ethanol was then added, under mild stirring and the pH was adjusted to 4.5. After 30 min, it was observed the formation of an orange precipitate that was completed after 4 h reaction. This product was collected, washed with cold ethanol, and dried in a desiccator under low pressure. Yield: 40%

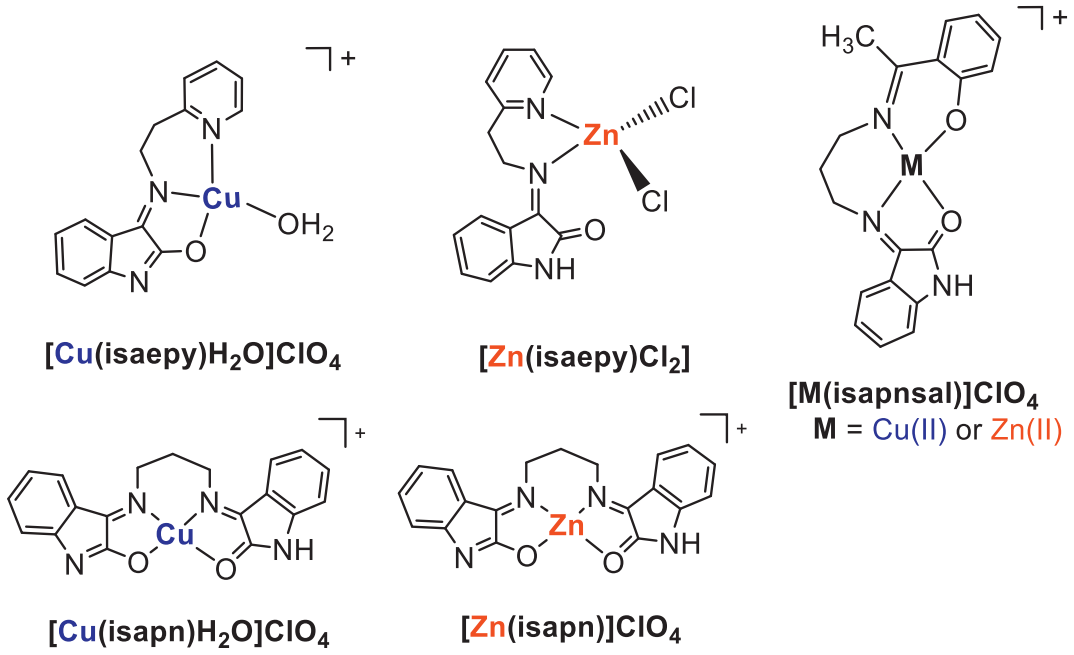

Fig. 1. Structure of oxindole-metal complexes, as isolated in solid state.