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trans-Bis(salicylaldoximato)copper(II) and its Derivatives as Antiproliferative and Antineoplastic Agents: a Review

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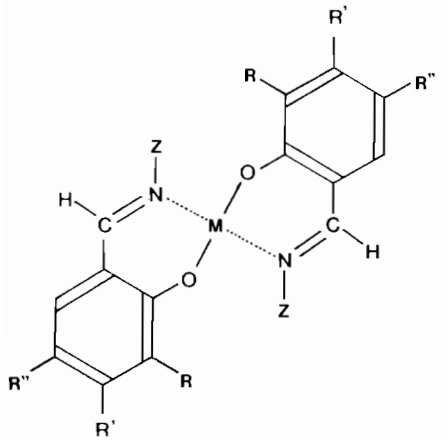
Abstract

The antineoplastic, antiproliferative and other biological properties of the copper(II) chelates of salicylaldoxime and related ligands are reviewed. Original results are reported on the toxicity and some other properties of the compounds, and possible mechanisms of action are discussed.

Introduction

Recently, a novel group of exceptionally powerful antiproliferative agents was reported, the parent compound of which is *trans*-bis(salicylaldoximato)-



I

copper(II) ($CuSAO_2$, **I**: R = R′ = R″ = H, Z = OH, M = Cu) [1]. This planar metal chelate was also found to have a powerful and often even curative antitumor activity against Ehrlich ascites carcinoma in mice *in vivo* [2]. After the original findings, several derivatives of $CuSAO_2$ have been tested *in vitro* and *in vivo* [3]. Toxicological [4–6] and immunological [3, 7, 8] studies have also been performed, and structure–activity relationships have been investigated [3]. The results obtained show that $CuSAO_2$ and related chelates have very potent biological activities and bear some interesting similarities with other metal complex antitumor agents. This short review – the first one on the present group of antitumor agents – attempts to give an overview of the results so far accumulated on $CuSAO_2$-type compounds. Some unpublished material is also included, and possible mechanisms of action are outlined and discussed.

Results and Discussion

Antiproliferative Activity in Vitro

$CuSAO_2$ and some closely related copper(II) chelates almost completely inhibit the division of tumor cells in a concentration as low as *ca.* 5–6 ppm. The original studies were performed using the murine Ehrlich ascites carcinoma and leukemia L1210 cell lines [1] but similar results have also been obtained against other cells, including human tumor cells [9]. Those derivatives of $CuSAO_2$ that differ from it only in the sense that *one* of the ring substituents, R, R′ or R″, is a hydroxyl group, appear to behave much like the parent compound. When the chelate concentration falls below *ca.* 2.5–3 ppm, the antiproliferative activity is almost totally lost. Thus, the *in vitro* dose-response curve rises very steeply between 3 and 5 ppm (see Figs. 1 and 2). This result is wholly repeatable.

According to our unpublished results [8], $CuSAO_2$ and its 4-hydroxy derivative (**I**: R = R″ = H, R′ = Z = OH, M = Cu) exert their antiproliferative effects on tumor cells very rapidly (in less than 1 h). In addition, it has been shown that only intact $CuSAO_2$ has significant activity (see Figs. 1 and 2), corresponding concentrations of cupric ions having only a low activity and even high concentrations of the free ligand being totally inactive [1].

Structure–Activity Relationships

In order to find new active derivatives and, especially, to discover possible structure–activity relationships, several derivatives of $CuSAO_2$ were synthesized and tested for antiproliferative activity against tumor cells *in vitro* [1, 3, 9].

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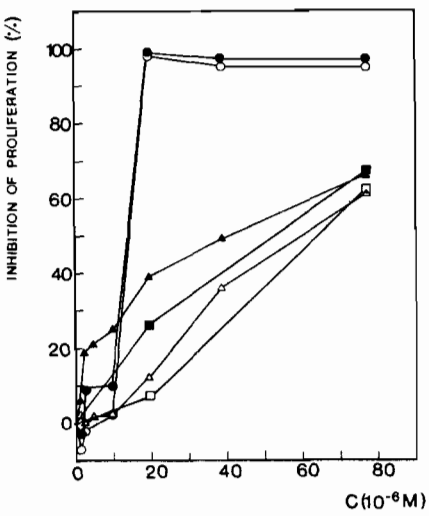


Fig. 1. The effects of $CuSAO_2$ and cupric chloride on the proliferation of leukemia L1210 cells in cell culture. $CuSAO_2$ was dissolved in dimethyl sulfoxide (○ = day 1 values, ● = day 2 values) and cupric chloride either in dimethyl sulfoxide (△ = day 1 values, ▲ = day 2 values) or in water (□ = day 1 values, ■ = day 2 values). Based on data from ref. 1.

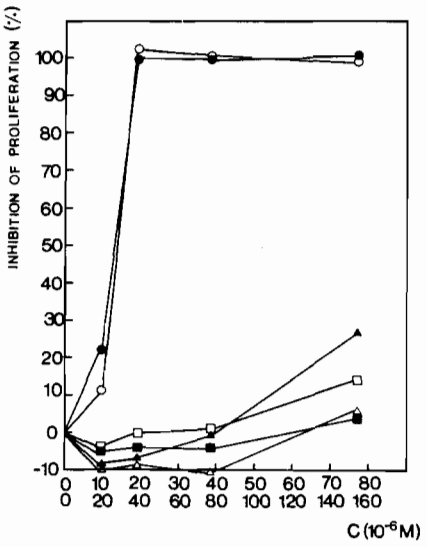
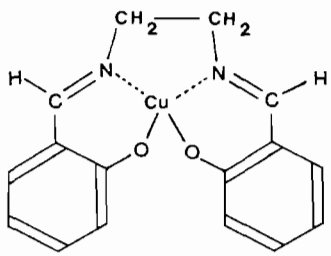


Fig. 2. The effects of $CuSAO_2$ (○ = day 1 values, ● = day 2 values), salicylaldoxime (□ = day 1 values, ■ = day 2 values) and the monosodium salt of salicylaldoxime (△ = day 1 values, ▲ = day 2 values) on the proliferation of leukemia L1210 cells in cell culture. The monosodium salt was dissolved in water and the other compounds in dimethyl sulfoxide. The lower figures on the concentration axis refer to the free ligand and its sodium salt, the upper ones to $CuSAO_2$. Based on data from ref. 1.

When tested against leukemia L1210 in cell culture, the nickel(II) analog of $CuSAO_2$ had only a very low antiproliferative activity, while the cobalt(II) analog had intermediate activity [1]. The zinc(II) analog was almost inactive [3]. As copper(II) and cobalt(II) compounds are generally more prone to redox reactions than are zinc(II) and nickel(II) compounds, the results suggest that redox reactions might be involved in the mechanism of action of the compounds. The salicylaldimine analog (I: R = R′ = R″ = Z = H, M = Cu) of $CuSAO_2$ was found to be highly active, the IC_{50} value on day 1 being only 3–4 μg/ml and the value on day 2 being even lower [3]. In spite of its instability in solution, this compound might deserve *in vivo* testing. The corresponding zinc(II) complex had only a weak antiproliferative activity [3]. When the –OH moiety of the oxime group of the ligand was replaced by either $-(CH_2)_2OH$ or $-(CH_2)_3OH$, the antiproliferative activity of the chelates was drastically decreased as compared to $CuSAO_2$ [3].

The effects of hydroxylation of the benzene rings of $CuSAO_2$ were also studied [3, 9]. As mentioned above, the addition of one hydroxyl group at position 3 or 4 in each of the benzene rings does not significantly alter the *in vitro* dose-response curve of the chelate. If the hydroxyls are added at position 5, the dose-response curve appears to rise somewhat less steeply, and higher concentrations are needed to achieve essentially total inhibition of proliferation. When two hydroxyls are added to each benzene ring at positions 3 and 4, the antiproliferative activity is drastically weakened, and even at the concentration of 20 μg/ml only 60% inhibition is achieved [3]. The ring-hydroxylated compounds have been tested against several tumor cell lines, including human, in addition to leukemia L1210, and essentially similar results have been obtained [9]. In the case of one tumor cell line, however, the chelate of 2,3,4-trihydroxybenzaldoxime was equally as active as the other compounds. Further studies are needed on this point, since as is shown below the compound is far less toxic than the less hydroxylated ones and might thus be given in much higher doses.



II

When compound II, the copper(II) chelate of *N*,*N*′-bis(salicylidene)ethylenediamine, was tested against leukemia L1210 *in vitro*, it had no measurable antiproliferative activity [3]. This compound cannot exist in the *trans*-configuration typical of most $CuSAO_2$-type compounds. Studies on further deriv-

atives are needed to reveal the possible biological significance of this configurational restriction. As shown by Hall and Waters [10], the configuration about the copper atom in **II** is pyramidal in the solid state. Whether the compound has this configuration also in solution, remains to be studied. Such non-planarity might also lie behind the lack of biological activity. One further explanation for the lack of activity may be the very low reactivity of **II** with thiols [11]. This point will be discussed below.

In Vivo Activity

$CuSAO_2$ has a powerful antitumor activity against Ehrlich ascites carcinoma in NMRI mice *in vivo* [2]. Survival is markedly increased, and in some cases the treatment appears to be curative. In a multi-dose experiment, the best dosage was found to be 2 X 40 mg per kg body weight daily for 2 days. In general, administration of the drug twice daily gave better results than administration of the same daily dose as a single injection [2]. Therefore, the development of slow-release formulations of $CuSAO_2$-type compounds might be worthwhile.

In the experiments in which NMRI mice were challenged with Ehrlich ascites carcinoma and then treated with $CuSAO_2$, a few mice were apparently cured. When they were challenged again with the same tumor 101 days after the first challenge, some of them appeared to be resistant and did not take the tumor, while all animals of a large control group did [3]. These very preliminary results suggest a possible role for immunity in the *in vivo* antitumor action of $CuSAO_2$ and bear interesting similarities with the results of Kimoto and coworkers who have reported that mice cured from Ehrlich ascites carcinoma by treatment with the combination of ascorbate and the cupric chelate of glycylglycylhistidine become immune to the tumor [12].

In vitro, $CuSAO_2$ appears to be equally active against leukemia L1210 and Ehrlich ascites carcinoma [1]. Yet, it had no activity against leukemia L1210 in DBA/2J mice *in vivo* [2]. Possibly, the very low solubility of $CuSAO_2$ in aqueous systems prevents systemic spreading of the compound, thus making $CuSAO_2$ inactive against the metastasizing leukemia. The present authors have prepared several derivatives and analogs of $CuSAO_2$ [3, 9] and some of them (**I**: R = Z = OH, R′ = R″ = H, M = Cu; **I**: R = R′ = Z = OH, R″ = H, M = Cu; and **I**: R = R′ = R″ = H, Z = $CH_2CH(OH)CH_3$, M = Cu) have been tested *in vivo* against leukemia P388. None of these compounds appears to have activity against the leukemia *in vivo*[≠]. (A detailed report will be given elsewhere.) The lack of activity of $CuSAO_2$-type compounds against leukemias is in line with the results obtained with almost all types of copper complexes so far tested.

Toxicity

The most prominent toxic effects of intraperitoneally administered $CuSAO_2$ in mice are weight loss and weakness. They were dose-limiting in antitumor tests. Acrodynia (in the form of swollen noses) and the transient blindness of some animals were also encountered. Eventually, all of these side effects disappeared from survivors [2, 9]. The weight loss was very rapid but also weight recovery began rapidly [3, 9]. The toxicity of $CuSAO_2$ appears to be distinctly reduced in tumor-bearing mice, as compared to healthy ones [2, 9]. The reason for this curious feature is so far unknown.

The acute toxicity of $CuSAO_2$ has been studied also in rats, using both the intraperitoneal and oral routes of administration [4]. When given perorally as a suspension, the compound had no acute lethal toxicity even at the dose 5000 mg per kg body weight. The peroral LD_{50} value (in rats) of the corresponding free ligand, salicylaldoxime, is known [13] to be only 400 mg/kg. The decreased toxicity of $CuSAO_2$, as compared to the free ligand, may at least in part be due to its lack of absorption. $CuSAO_2$ was found to be absorbed poorly, if at all, when given perorally. When $CuSAO_2$ was given intraperitoneally, it appeared to be absorbed incompletely, and no attempt was made to determine its LD_{50} value. In any case, rats appeared to tolerate much higher doses (per kg body weight) than did mice [4, 9].

Because of the incomplete absorption of $CuSAO_2$, its 4-hydroxy derivative, *i.e.* *trans*-bis(resorcylaldoximato)copper(II) ($CuRES_2$, **I**: R = R″ = H, R′ = Z = OH, M = Cu), was chosen as a model compound for toxicity studies [5, 6]. This compound is adequately absorbed after intraperitoneal administration in spite of its low aqueous solubility [5]. It, or at least the copper derived from it, appears to be preferentially accumulated in the pancreas, giving rise to extremely high concentrations (almost three orders of magnitude higher than those found in the liver and kidneys) [5, 6]. This is in striking contrast to intoxications caused by copper salts, since in the latter, the copper is known to be stored mainly in the liver, kidneys, muscles and heart [14]. The reason for this difference is so far unknown. $CuRES_2$ also appears to rapidly destroy the normal tissue architecture of the pancreas [6]. Other features of the toxicology of $CuRES_2$ will be reported elsewhere. The preferential affinity for the pancreas possibly is not a property restricted to $CuRES_2$, since $CuSAO_2$ also appears to cause pancreatic changes at least in some cases [4]. The high affinity for the pancreas might have therapeutic implications in

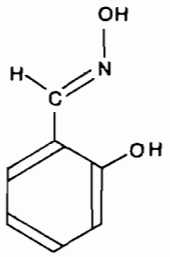
[≠]These data are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., U.S.A.

the treatment of pancreatic tumors. This point is worth further study.

According to our unpublished results, intraperitoneally administered bis(2,3,4-trihydroxybenzaldoximato)copper(II) (I: R = R′ = Z = OH, R″ = H, M = Cu) is drastically less toxic to mice than are the structurally closely related compounds $CuSAO_2$, $CuRES_2$ and *trans*-bis(2,3-dihydroxybenzaldoximato)copper(II) (I: R = Z = OH, R′ = R″ = H, M = Cu). Its antiproliferative activity against most cell lines tested is, however, also far lower than that of the latter compounds [3, 9]. The reasons for the decreased toxicity deserve further study.

Possible Mechanisms of Action

The ligand of $CuSAO_2$, salicylaldoxime (**III**), resembles pyridoxal oxime (**IV**), a well-known



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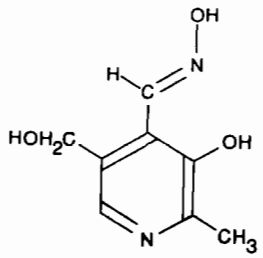

IV

vitamin B_6 analog and antagonist and an extremely powerful inhibitor of pyridoxal kinase [15–18]. Since vitamin B_6 deficiency and B_6 antagonists are known to have growth-inhibitory and antitumor activity [19, 20], and since the side effects of $CuSAO_2$ (weight loss and acrodynia) resemble those of B_6 deficiency and B_6 antagonists [19, 21], it appeared that pyridoxal antagonism might be involved in the mechanism of action of $CuSAO_2$ and might also lie behind the side-effects. However, an attempt to reduce the toxicity of $CuSAO_2$ in mice *in vivo* with pyridoxal hydrochloride led to increased toxicity [3].

In spite of the finding that pyridoxal did not reduce the toxicity of $CuSAO_2$, a possible involvement of vitamin B_6 antagonism in the mechanism of action of $CuSAO_2$ cannot be wholly excluded, since B_6 antagonists are known, whose effects cannot be reversed by B_6 vitamers [22]. However, the pyridoxal theory alone cannot explain the greatly different antiproliferative activities of $CuSAO_2$, $NiSAO_2$ and $CoSAO_2$ in any straightforward way. The well-known antineoplastic copper(II) chelates of various bis(thiosemicarbazones) are known to readily react with thiols, e.g., glutathione. This reaction occurs also in tumor cells and appears to be involved in the mechanism of action of those compounds [23]. Therefore, the reactivity of $CuSAO_2$ and related chelates with glutathione, cysteine and ascorbic acid was studied [11]. A structure–activity correlation was found: $CuSAO_2$ and other highly antiproliferative chelates reacted rapidly with 1–2 equivalents of glutathione in the test tube, losing their typical colors immediately, while under similar experimental conditions, the non-antiproliferative analog **II** retained its typical color, the first small changes in the color of the solution being observable after a few hours. The chelates with intermediate antiproliferative activity lost their colors more slowly than the highly active ones [11]. The only exception was $CoSAO_2$, which retained its typical color in spite of its antiproliferative properties [11]. These findings suggest that reaction with cellular thiols and consequent destruction of the chelate may have a role in the antiproliferative action of the compounds. Thus, the mechanism of action of the compounds might be similar to that proposed [23] for the copper(II) chelates of bis(thiosemicarbazones), involving the formation of catalytically active Cu(I) compounds in the reaction with cellular thiols and subsequent oxidation of further cellular thiols. In any case, further studies on the reactions of $CuSAO_2$ and related compounds with thiols are absolutely warranted. Also, it should be studied whether the reactions take place in tumor cells.

The finding that some mice cured from Ehrlich ascites carcinoma by treatment with $CuSAO_2$ developed immunity against the tumor [3] suggests a possible role for immunity in the *in vivo* action of $CuSAO_2$. This theory is indirectly supported also by the findings that certain $CuSAO_2$-type chelates have interesting immunological properties in mice *in vivo* [8] and appear to distinctly enhance the cytotoxicity of murine spleen effector cells to tumor cells *in vitro* [7].

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