The Antiproliferative Agents *trans*-Bis(resorcylaldoximato)copper(II) and *trans*-Bis(2,3,4-trihydroxybenzaldoximato)copper(II) and Cytopathic Effects of HIV

Hannu Elo

Division of Pharmacognosy, Faculty of Pharmacy,
P. O. Box 56 (Viikinkaari 5, Biocenter 2),
FIN-00014 University of Helsinki, Finland.
Fax: +358-9-19159882. E-mail: Hannu.Elo@Helsinki.Fi

Z. Naturforsch. **59 c**, 609–611 (2004);
received April 23/May 27, 2004

trans-Bis(resorcylaldoximato)copper(II) and *trans*-bis-(2,3,4-trihydroxybenzaldoximato)copper(II) ($CuRES_2$ and $CuTRI_2$, respectively) have been tested for antiviral properties against HIV, using an *in vitro* assay that measures the ability of the test compounds to prevent the killing of susceptible human cells by HIV. In the case of $CuTRI_2$, T4 lymphocytes (CEM-V and CEM-Z cell lines) were exposed to HIV at a virus to cell ratio approx. 0.05 in microtiter plates. In the case of $CuRES_2$, a human leukemia cell line (MT-2) was used instead. The tetrazolium salt XTT was added to all wells, and the cultures were incubated and analyzed spectrophotometrically to quantitate formazan production and viewed microscopically for detection of viable cells. In spite of their antiproliferative properties, neither agent had any detectable ability to prevent the cytopathic effects of HIV in cultures of the target cells used. Because the test system employed was constructed in such a way as to detect antiviral agents acting at any stage of the virus reproductive cycle, the results obtained strongly suggest that neither studied agent has any value as the direct prevention of the cell destruction caused by HIV is concerned.

Key words: trans-Bis(salicylaldoximato)copper(II) Analogues, Human Immunodeficiency Virus, Anti-HIV Drug Development

Introduction

Many antineoplastic agents (but not all) are known to have also antiviral activity. Quite recently, the value of anticancer agents for the development of anti-HIV drugs has been heavily emphasized (Sadaie *et al.*, 2004), and it has been proposed that they could suppress HIV strains that are resistant to 'traditional' inhibitors of viral enzymes, decrease proviral burden *in vivo*, or reduce reservoirs of infection via killing infected cells, and thus might be an effective adjunct therapy or perhaps result in a cure (Sadaie *et al.*, 2004). The present author and Lumme have shown (Elo and Lumme, 1985) that *trans*-bis(salicylaldoximato)copper(II) ($CuSAO_2$; R = R′ = H; see Fig. 1) is a powerful antitumour agent *in vivo*, being capable of drastically increasing the life span of mice bearing Ehrlich ascites carcinoma. In many cases, the compound has even a curative effect. This compound as well as many of its congeners, most notably the 4-hydroxylated analogue *trans*-bis(resorcylaldoximato)copper(II) ($CuRES_2$; R = H, R′ = OH; see Fig. 1), also have potent antiproliferative activity against tumour cells *in vitro* [Elo and Lumme, 1986, 1987 (for further references on the properties of these agents, see Elo and Lumme, 1987)]. Thus, also salicylaldoximato-type chelates are worth screening for antiviral properties, especially as they are also known to have immunomodulating properties (Elo and Lumme, 1986 1987; Elo, 1987; unpublished results). In this paper, I report the screening of two compounds of this family, namely $CuRES_2$ and *trans*-bis(2,3,4-trihydroxybenzaldoximato)copper(II) ($CuTRI_2$; R = R′ = OH; see Fig. 1), against the cytopathic effects of the human immunodeficiency virus (HIV).

Experimental

$CuTRI_2$ was tested against the cytopathic effects of HIV according to the standard protocols of the U. S. National Cancer Institute. The assay employed basically involves the killing of T4 lymphocytes by HIV. In brief, the procedure consisted of the following steps: T4 lymphocytes (CEM-V and CEM-Z cell lines) were exposed to HIV at a virus to cell ratio approx. 0.05, and plated along with noninfected control cells in 96-well microtiter plates. $CuTRI_2$ was then diluted to 1:200 in the cell culture medium. Further dilutions (half-$\log_{10}$) were prepared before adding to an equal volume of medium containing either infected or noninfected cells. Cultures were incubated at 37 °C in a 5% carbon dioxide atmosphere for 6 or 7 d. The tetrazolium salt XTT {*i.e.*, 2,3-bis(2-methoxy-4-nitro-5-sulphenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide, inner salt, sodium salt}, an agent whose metabolic reduction gives a coloured formazan compound (Scudiero *et al.*, 1988), was added to all wells, and the cultures were incubated

0939–5075/2004/0700–0609 $ 06.00

to allow formazan colour development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate the formazan production, and in addition were viewed microscopically for detection of viable cells. Data were reviewed in comparison with other tests done at the same time. The tests were also compared with a positive [zidovudine (azidothymidine, 'AZT')-treated] control done at the same time under identical conditions.

$CuRES_2$ was tested essentially similarly. In the case of $CuRES_2$, the cell line used was MT-2 (a human leukemia). $CuRES_2$ was synthesized according to a modification of the method of Mukherjee (1955). The experimental details will be published elsewhere, as will also the synthesis of $CuTRI_2$.

Results and Discussion

The results obtained are shown in Tables I and II. As it is evident from the Tables, neither $CuRES_2$ nor $CuTRI_2$ (Fig. 1) had any detectable activity against the cytopathic effects of HIV in cultures of MT-2 cells and CEM cells, respectively. Neither agent increased the viability of HIV-infected cells in the concentration range employed. Instead, the highest tested concentrations of both

Table I. Effect of $CuTRI_2$ on the viability of CEM cells in the presence and absence of HIV[a,b].

Concentration of $CuTRI_2$ [M]	Response (% of uninfected untreated control culture)[c]	
	HIV-infected culture of CEM-V cells	Uninfected culture of CEM-V cells
1.08×10^{-8}	14.9	111.1
3.41×10^{-7}	13.8	116.4
1.07×10^{-6}	13.7	94.5
3.40×10^{-5}	– 3.0	– 0.4
	HIV-infected culture of CEM-Z cells	Uninfected culture of CEM-Z cells
1.08×10^{-8}	9.9	105.9
3.41×10^{-7}	11.8	100.8
1.07×10^{-6}	10.7	98.3
1.07×10^{-5}	0.1	56.9
3.40×10^{-5}	– 2.7	– 0.7

[a] In the case of uninfected target cells, the estimated IC_{50} value of $CuTRI_2$ is 1.29×10^{-5} M for CEM-V cells and 1.23×10^{-5} M for CEM-Z cells.
[b] These data are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland.
[c] The colorimetric response, as determined after incubation of the cultures with XTT, is a measure of the number of viable cells present at the end of the test.

Table II. Effect of $CuRES_2$ on the viability of MT-2 cells in the presence and absence of HIV[a,b].

Concentration of $CuRES_2$ [μg/ml]	Response (% of uninfected untreated control culture)[c] HIV-infected culture	Uninfected culture
1.56×10^{-4}	94.50	15.30
1.56×10^{-3}	100.40	14.70
1.56×10^{-1}	97.80	13.80
1.56	92.80	11.30
15.6	0.30	0.60

[a] In the case of uninfected target cells, the estimated IC_{50}, IC_{90} and IC_{100} values of $CuRES_2$ are 4.52 μg/ml (1.3×10^{-5} M), 12.2 μg/ml (3.3×10^{-5} M) and 15.6 μg/ml (4.3×10^{-5} M), respectively.
[b] These data are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland.
[c] The colorimetric response is a measure of the number of viable cells present at the end of the test.

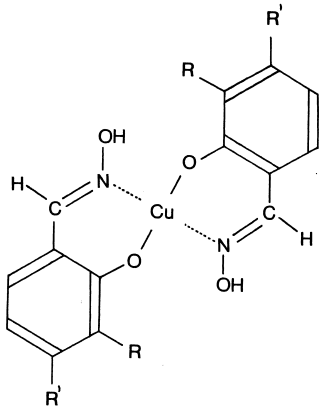

Fig. 1. The structures of $CuSAO_2$ (R = R′ = H), $CuRES_2$ (R = H, R′ = OH) and $CuTRI_2$ (R = R′ = OH).

compounds decreased the viability of uninfected cells and also that of HIV-infected cells. The last mentioned result is in line with unpublished observations of the present author and his coworkers that indicate that $CuSAO_2$ and $CuRES_2$ have strong cytotoxic effects *in vitro* against a variety of human and murine tumour cells and that those effects are essentially irreversible and occur in hours or even within one hour or less.

As the ability of copper(II) chelates of salicylaldoximates to inhibit the proliferation of mammalian cells is concerned, the present results are clearly in line with previous ones obtained under a variety of different conditions (Elo and Lumme, 1986, 1987; Elo, 2004; unpublished observations). Thus, both compounds tested were potent antiproliferative agents also in the present test system, in which the experimental details (*e.g.*, incubation time, cell lines) were different from those used in most studies previously reported. Thus, also the present results clearly suggest that copper salicylaldoximates have a very broad spectrum of activity.

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