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Assessment of the *in vitro* broad-spectrum antiviral activity of some selected antitumor organotin complexes

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Eleven antitumor-active octahedral organotin complexes of the type $R_2SnX_2L_2$, where R = methyl, ethyl or phenyl, X = chloride or bromide, and L_2 = *o*-phenantholine (phen), 2-)2-pyridyl)-benzimidazole (PBI) or two dimethylsulfoxides (2DMSO), were examined for their broad-spectrum *in vitro* antiviral activity against a number of DNA and RNA viruses. The DNA viruses included in this study were herpes simplex virus type 1 and type 2, a TK − (thymidine kinase deficient) strain of herpes simplex virus type 1, and vaccinia virus. The RNA viruses were vesicular stomatitis virus, Coxsackie virus type B4, Sindbis virus, Semliki forest virus, parainfluenza virus type 3, and human immunodeficiency virus (HIV). Overall, the complexes showed weak antiviral activity and low selectivity. With the exception of $(CH_3)_2SnBr_2 \cdot$PBI and $(C_6H_5)_2SnCl_2 \cdot$2DMSO, all of the complexes were active against one or more of the three strains of herpes simplex viruses. On the other hand, only three complexes, $(CH_3)_2SnBr_2 \cdot$PBI, $(CH_3)_2SnBr_2 \cdot$phen, and $(C_6H_5)_5SnBr_2 \cdot$PBI, exhibited marginal activity against some of the RNA viruses. None of the complexes was active against vesicular stomatitis or parainfluenza virus. Similarly, there was no inhibitory activity towards HIV-1-associated reverse transcriptase or HIV-1-induced cytopathogenicity in human T-lymphocyte MT4 cell cultures at subtoxic concentrations.

Keywords: Organotin complexes, octahedral, antitumor, DNA viruses, RNA viruses, HIV

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INTRODUCTION

Important advances in the field of antiviral chemotherapy have been made during the past few years. A number of potent and selective as well as broad-spectrum antiviral compounds have been synthesized. Perhaps the most important successes have been the discovery of antiviral nucleosides which more or less selectively affect viral macromolecular synthesis.[1–3] However, interference with other viral processes such as virus attachment, cell penetration, uncoating, etc., presents alternative approaches to antiviral chemotherapy.

In an effort to ascertain other ways of inhibiting viral infections, we have been investigating the effect of various inorganic and organometallic compounds primarily on herpes simplex virus (HSV) infections both *in vitro* and *in vivo*.[4,5] Although metal chelating agents have been shown to possess antiviral activity,[6,7] few reports have appeared in which inorganic complexes or organometallic compounds have been examined for antiviral efficacy.[8–10]

In our previous paper, we reported that a number of antitumor-active octahedral organotin complexes of the type $R_2SnX_2L_2$, where R = methyl, ethyl or phenyl, X = chloride or bromide, and L_2 = *o*-phenanthroline or 2-(2-pyridyl)benzimidazole, exhibited weak *in vitro* antiherpes activity towards herpes simplex virus types 1 and 2, HSV-1 (F strain) and HSV-2 (MS strain). The antiviral assay employed in this study involved the simultaneous inoculation of confluent human foreskin fibroblast (HFF) cell cultures with virus and test compounds. Because of the nature of the assay, the observed antiviral activity was associated with interference by these compounds with

an early stage of the viral replication cycle. In the present study, we have altered the assay to study the effect of addition of the compounds after virus adsorption to the cells. In addition we have examined the broad-spectrum antiviral efficacy of these complexes against a number of DNA and RNA viruses including the retrovirus (HIV-1), HTLV-III_B, the etiologic agent of AIDS.[11–13] The DNA viruses included herpes simplex virus type 1 (HSV-1 KOS) herpes simplex virus type 2 (HSV-2 G strain), HSV-1 thymidine kinase deficient (TK^-) mutant strain B2006, and vaccinia virus. The RNA viruses included vesicular stomatitis virus, Coxsackie virus type B4, Sindbis virus, Semliki forest virus, and parainfluenza virus type 3. Finally, the complexes were examined for their ability to inhibit both HIV-1-associated reverse transcriptase activity and cytopathogenicity of HIV-1 for human T-lymphocytes (MT4).

MATERIALS AND METHODS

Organotin compounds

The diorganotin dihalide complexes, $R_2SnX_2L_2$ [where R = CH_3, C_2H_5, C_6H_5; X = Cl, Br; and L_2 = *o*-phenanthroline (phen), 2-(2-pyridyl)benzimidazole (PBI) and L = dimethylsulfoxide (DMSO)] were synthesized and characterized as discussed previously.[14] They were obtained from the International Tin Research Institute and used without further purification. For the viral assays (see below), the compounds were dissolved in DMSO (20 mg cm^{-3}) and diluted with culture medium to give a series of final concentrations in the range of 400–0.004 $\mu g\ cm^{-3}$. The use of DMSO in the same dilutions had no effect on normal cell growth or on viral cytopathogenicity as determined in separate experiments.

Viruses

The origin of the viruses was as follows: herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G) and the thymidine kinase deficient (TK^-) mutant of HSV-1 (strain B2006) — (see Ref. 15); vaccinia virus, vesicular stomatitis virus, Coxsackie virus type B4, and Sindbis virus — (see Ref. 16); Semliki forest virus (ATCC VR-67) and parainfluenza virus type 3 (ATCC VR-63) were derived from the American Type Culture Collection, Rockville, MD. The virus stocks were grown in primary rabbit kidney (PRK) cells (herpes simplex virus types 1,2,TK^-, vaccinia virus, and vesicular stomatitis virus), Vero cells (Coxsackie virus and Semliki forest virus), chicken embryo cells (Sindbis virus), or human embryonic lung cells (parainfluenza virus). HIV-1 was obtained from the culture supernatant of a H9 cell line persistently infected with HTLV-III_B[17] which was kindly provided by Dr R C Gallo (National Cancer Institute, Bethesda, MD). The Vero and HeLa cell lines were regularly examined for mycoplasma contamination and were found to be mycoplasma-free.

Antiviral assays

Confluent cell cultures were grown in 96-well microtiter trays and were inoculated with 100 $CCID_{50}$ (1 $CCID_{50}$ corresponds to the virus stock dilution that was infective for 50% of the cell cultures). After one hour adsorption of the virus to the cells at 37°C, the residual virus was removed and replaced by cell culture medium (Eagle's minimum essential medium) containing 3% fetal calf serum and various concentratioins of the organotin compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the untreated virus infected cell cultures, i.e. at one to two days for vesicular stomatitis virus; at two days for Semliki forest virus and Coxsackie virus; at two to three days for vaccinia virus, herpes simplex virus (all types), and Sindbis virus; at five days for HIV-1, and at six to seven days for parainfluenza virus. The antiviral activity of the compounds was expressed as the minimum (antiviral) inhibitory concentration (MIC) ($\mu g\ cm^{-3}$) required to inhibit viral cytopathogenicity by 50%. For HIV-1-induced cytopathogenicity in MT-4 cells, this activity was expressed as ED_{50} (50% effective dose, or dose required to reduce virus-induced cytopathogenicity by 50%).

Cytotoxicity

Cytotoxicity experiments were based on alterations of normal cell morphology. Confluent cell cultures which had not been infected but were treated with various concentrations of the organotin compounds were incubated in parallel with the virus-infected cell cultures and examined microscopically at the same time as viral cytopathogenicity was recorded for the virus-

infected cells. Disruption of the cell monolayer, e.g. rounding up or detachment of the cells, was considered as evidence for cytotoxicity. Cytotoxicity of the compounds against PRK and Vero cell monolayers was expressed as the minimum cytotoxic concentration (MTC) ($\mu g\ cm^{-3}$) required to cause a microscopically detectable alteration of normal cell morphology. Cytotoxicity of the compounds against MT-4 cells was expressed as the dose required to reduce the viability of the cells by 50% (CD_{50}).

HIV-1 reverse transcriptase assay

The procedure employed to measure reverse transciptase activity was a slight modification of that reported by Balzarini *et al.*[18] Exogenous poly(rA):oligo(dT)$_{12-18}$ served as the template:primer for the HIV-1 reverse transcriptase assay. The reaction mixture (50 μL) contained 5 mmol dm^{-3} dithiothreitol, 300 μmol dm^{-3} glutathione, 50 mmol dm^{-3} Tris–HCl (pH 7.8), 5 mmol dm^{-3} $MgCl_2$, 150 μmol dm^{-3} KCl, 1.25 μg of bovine serum albumin, 1 μmol dm^{-3} [*methyl*-^{3}H]dTTP (specific radioactivity 30 Ci $mmol^{-1}$, 5 μCi), 0.01 unit of poly(rA):oligo(dT)$_{12-18}$, 0.03% Triton X-100, 10 μL of the compound solution (containing varying concentrations of the test compounds), and 10 μL of the HIV-1 reverse transcriptase preparation (partially purified by low centrifugation of the supernatant of a H9/HTLV-III$_B$ cell suspension, followed by filtration (0.45 μm) and ultracentrifugation (100 000*g*, 2h)). The reaction mixtures were incubated for 60 min at 37 °C, at which time 200 μL of ice-cold 5% trichloroacetic acid was added to stop the reaction. After keeping the samples at 0–4 °C for an additional 30 min, the acid-insoluble material was filtered, washed with water, dried and analyzed for radioactivity. Suramin, a potent inhibitor of HIV reverse transcriptase[19] was included as a standard.

RESULTS AND DISCUSSION

The data shown in Tables 1, 2 and 3 represent average values for three separate experiments in PRK cell cultures (Table 1) and two separate experiments in Vero cells (Table 2) and MT-4 cells (Table 3). Following the criteria of De Clercq[20,21] we have estimated a selectivity index (SI) for each compound and virus (Tables 3 and 4). This parameter is based on the ratio of minimum cytotoxic concentration (or cytotoxic dose), MTC, to the minimum antiviral concentration (or effective dose), MIC, and represents a measure of the antiviral selectivity of a given compound. In general, the $R_2SnX_2L_2$ comounds are quite toxic for both the PRK and Vero cells, the order of increasing toxicity varying with the hydrocarbon substituent on the tin, $C_6H_5 > C_2H_5 > CH_3$. This same

Table 1 Antiviral and cytotoxic effects of $R_2SnX_2L_2$ derivatives in PRK cell cultures

Compound	MIC ($\mu g\ cm^{-3}$)[a]					MTC ($\mu g\ cm^{-3}$)[b]
	HSV-1(KOS)	TK$^-$HSV-1 (B2006)	HSV-2(G)	Vaccinia virus	Vesicular stomatitis virus	
$(CH_3)_2SnBr_2 \cdot PBI$	40	40	40	40	40	≥40
$(CH_3)_2SnBr_2 \cdot phen$	4	4	4	4	40	≥40
$(C_2H_5)_2SnCl_2 \cdot PBI$	0.4	4	4	4	4	≥4
$(C_2H_5)_2SnCl_2 \cdot phen$	0.4	0.4	1	4	4	≥4
$(C_2H_5)_2SnBr_2 \cdot PBI$	1	≥0.4	4	4	4	≥4
$(C_2H_5)_2SnBr_2 \cdot phen$	1	0.2	0.2	4	4	4
$(C_6H_5)_2SnCl_2 \cdot PBI$	1	1	0.4	1	1	≥1
$(C_6H_5)_2SnCl_2 \cdot phen$	1	1	1	1	1	≥1
$(C_6H_5)_2SnBr_2 \cdot PBI$	0.2	0.4	0.2	0.4	0.4	0.4
$(C_6H_5)_2SnBr_2 \cdot phen$	0.2	>0.4	0.2	1	1	1
$(C_6H_5)_2SnCl_2 \cdot 2DMSO$	0.4	0.4	0.4	0.4	0.4	≥0.4

[a] Minimum (antiviral) inhibitory concentration required to inhibit virus-induced cytopathogenicity by 50%; average of three experiments.
[b] Minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology; average of three experiments.

Table 2 Antiviral and cytotoxic effects of $R_2SnX_2L_2$ derivatives in Vero cell cultures

Compound	MIC ($\mu g\ cm^{-3}$)[a]				MTC ($\mu g\ cm^{-3}$)[b]
	Coxsackie virus type B4	Sindbis virus	Semliki forest virus	Parainfluenza virus type 3	
$(CH_3)_2SnBr_2 \cdot PBI$	4	≥40	>40	>40	≥40
$(CH_3)_2SnBr_2 \cdot phen$	4	10	>40	>40	≥40
$(C_2H_5)_2SnCl_2 \cdot PBI$	>1	>1	1	>1	≥1
$(C_2H_5)_2SnCl_2 \cdot phen$	1	>1	>1	>1	≥1
$(C_2H_5)_2SnBr_2 \cdot PBI$	>1	>1	>1	>1	≥1
$(C_2H_5)_2SnBr_2 \cdot phen$	0.4	>1	>1	>1	≥1
$(C_6H_5)_2SnCl_2 \cdot PBI$	>1	>1	>1	>1	≥1
$(C_6H_5)_2SnCl_2 \cdot phen$	1	>1	>1	>1	1
$(C_6H_5)_2SnBr_2 \cdot PBI$	0.4	>0.4	0.1	>0.4	0.4
$(C_6H_5)_2SnBr_2 \cdot phen$	>1	>1	>1	>1	1
$(C_6H_5)_2SnCl_2 \cdot 2DMSO$	>0.4	>0.4	>0.4	>0.4	0.4

[a] Minimum (antiviral) inhibitory concentration required to inhibit virus-induced cytopathogenicity by 50%; average of two experiments. [b] Minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology; average of three experiments.

Table 3 Anti-HIV-1 and cytotoxic effects of $R_2SnX_2L_2$ deriviatives in MT-4 cells

Compound	ED_{50}[a] ($\mu g\ cm^{-3}$)	CD_{50}[b] ($\mu g\ cm^{-3}$)
$(CH_3)_2SnBr_2 \cdot PBI$	>8	12.3
$(CH_3)_2SnBr_2 \cdot phen$	>0.32	0.70
$(C_2H_5)_2SnCl_2 \cdot PBI$	>0.32	0.64
$(C_2H_5)_2SnCl_2 \cdot phen$	>0.32	0.62
$(C_2H_5)_2SnBr_2 \cdot PBI$	>0.32	0.61
$(C_2H_5)_2SnBr_2 \cdot phen$	>0.32	0.59
$(C_6H_5)_2SnCl_2 \cdot PBI$	>0.32	0.25
$(C_6H_5)_2SnCl_2 \cdot phen$	>0.06	0.14
$(C_6H_5)_2SnBr_2 \cdot PBI$	>0.32	0.29
$(C_6H_5)_2SnBr_2 \cdot phen$	>0.06	0.15
$(C_6H_5)_2SnCl_2 \cdot 2DMSO$	>0.06	0.15

[a] 50% effective dose required to inhibit HIV-1 induced cytopathogenicity in MT-4 cells by 50%. [b] 50% cytotoxic dose required to reduce the viability of MT-4 cells by 50%.

relative order was observed by us in a previous study in which short term (4 h) toxicity was determined on human foreskin fibroblasts (HFF).[5] In the present study, the compounds were kept in contact with the cell lines during the entire assay period (up to six or seven days in the case of parainfluenza type 3 virus). This resulted in somewhat greater cytotoxicity, i.e. lower values of MTC than previously reported. In spite of this, very little difference was noted in the toxicity of the compounds for the three cell lines employed in the two studies (HFF, PRK and Vero).

Activity towards DNA viruses

In a previous study, the $R_2SnX_2L_2$ complexes exhibited weak antiherpes activity against two particular strains of herpes simplex virus, i.e. HSV-1 (F strain) and HSV-2 (G strain).[5] The selectivity indexes were in the range of 1–6.3. These compounds appeared to be slightly more active towards HSV-1 than towards HSV-2. The current study tends to support the previous one with about the same level of antiherpes effectiveness (SI within the range 1–10) towards two additional virus strains, HSV-1(KOS) and HSV-2(G). No apparent trend was observed as a function of organic substituent or halide. In general, the *o*-phenanthroline derivatives were slightly more active than the PBI-containing derivatives, a trend which was also noted previously. In the case of the TK^- HSV-1, a thymidine kinase deficient mutant strain, there was no appreciable difference in antiviral effectiveness as compared with the HSV-1(KOS) (Table 4). Whatever is the mechanism of activity upon which these compounds are based, the presence or absence of the virus-specified TK enzyme makes no

Table 4 Antiviral selectivity indexes for $R_2SnX_2L_2$ derivatives

Compound	Selectivity indexes, SI[a]									
	HSV-1(KOS)	TK⁻HSV-1 (B2006)	HSV-2(G)	Vaccinia virus	Vesicular stomatitis virus	Coxsackie virus type B4	Sindbis virus	Semliki forest virus	Parainfluenza virus type 3	HIV-1
$(CH_3)_2SnBr_2 \cdot PBI$	≥1	≥1	≥1	≥1	≥1	≥10	1	<1	<1	<1.5
$(CH_3)_2SnBr_2 \cdot phen$	≥10	≥10	≥10	≥10	≥1	≥10	≥4	<1	<1	<2
$(C_2H_5)_2SnCl_2 \cdot PBI$	≥10	≥1	≥1	≥1	≥1	<1	<1	≥1	<1	<2
$(C_2H_5)_2SnCl_2 \cdot phen$	≥10	≥10	≥4	≥1	≥1	≥1	<1	<1	<1	<2
$(C_2H_5)_2SnBr_2 \cdot PBI$	≥4	10	≥1	≥1	≥1	<1	<1	<1	<1	<1.9
$(C_2H_5)_2SnBr_2 \cdot phen$	4	20	20	1	1	≥2.5	<1	<1	<1	<1.9
$(C_6H_5)_2SnCl_2 \cdot PBI$	≥1	≥1	≥2.5	≥1	≥1	<1	<1	<1	<1	<0.8
$(C_6H_5)_2SnCl_2 \cdot phen$	≥1	≥1	≥1	≥1	≥1	1	<1	<1	<1	<2.3
$(C_6H_5)_2SnBr_2 \cdot PBI$	2	1	2	1	1	1	<1	4	<1	<0.9
$(C_6H_5)_2SnBr_2 \cdot phen$	5	<2.5	5	1	1	<1	<1	<1	<1	<2.5
$(C_6H_5)_2SnCl_2 \cdot 2DMSO$	≥1	≥1	≥1	≥1	≥1	<1	<1	<1	<1	<2.5

[a] SI = ratio of MTC to MIC (Tables 1 and 2) or ratio of CD_{50} to ED_{50} (Table 3).

apparent difference. In contrast, the antiherpes agent, acyclovir, which is converted to the monophosphate in herpes-virus-infected cells by a viral-encoded thymidine kinase enzyme, is about 175 times more active against HSV-1(KOS) than HSV-1(TK^-).[15]

Only $(CH_3)_2SnBr_2 \cdot phen$ demonstrated any activity towards vaccinia virus, the only other DNA virus investigated. In spite of its favorable selectivity index (SI ≥ 10) (Table 4), $(CH_3)_2SnBr_2 \cdot phen$ is much less inhibitory towards vaccinia virus in PRK cells than are the nucleoside analogs Neplanocin A (SI = 1300) and 3-deaza-aristeromycin (SI ≥ 570).[22]

Activity towards RNA viruses

Examination of Tables 2 and 4 reveals that with few exceptions, most notably the PBI and phen derivatives of dimethyltin dibromide, the organotin complexes show no activity against a number of RNA viruses such as vesicular stomatitis, Coxsackie virus type B4, Sindbis virus, Semliki forest virus, and parainfluenza virus type 3. Only $(C_2H_5)_2SnBr_2 \cdot phen$ and $(C_6H_5)_2SnBr_2 \cdot phen$ showed marginal inhibition against Sindbis and Semliki forest virus, respectively. Modest selectivity indexes were obtained for the two methyl-containing complexes against Coxsackie virus type B4, but the selectivity indexes were far surpassed by both Neplanocin A (SI = 250) and 3-deaza-aristeromycin (SI > 200).[22]

Activity towards a retrovirus, HTLV-III_B (HIV-1)

Because of the considerable urgency to develop agents that would be efficacious in the treatment of AIDS,[23] all of the organotin complexes reported in this paper were investigated for their ability to inhibit HIV-1 reverse transcriptase. None of the compounds was found to be inhibitory at the highest concentration (200 $\mu g\ cm^{-3}$) tested (data not shown). Furthermore, when these complexes were examined for their inhibitory effect on the cytopathogenicity of HIV-1 in human T-lymphocyte MT4 cells (Table 3; see Ref. 24 for details concerning experimental protocols), none proved active at subtoxic concentrations. CD_{50} values for MT4 cells (Table 3) were generally lower than the MTC values for PRK and Vero cells (Tables 1 and 2).

CONCLUSION

The $R_2SnX_2L_2$ complexes have been examined for their broad-spectrum *in vitro* antiviral activity against a number of DNA and RNA viruses, including HIV-1. With the exception of a weak activity towards herpes simplex virus, few of the complexes demonstrated effectiveness towards RNA viruses and all were non-inhibitory towards HIV-1. Because of their relatively

poor selectivity indexes, this group of antitumor organotin complexes yields little promise as potential antiviral drugs.

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