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The *in vitro* antiherpes activity of some selected antitumor organotin compounds*

Sarah G. Wardt, R. Craig Taylort and Alan J. Crowe‡
†Department of Chemistry, Oakland University, Rochester, Michigan 48309-4401, USA and
‡International Tin Research Institute, Kingston Lane, Uxbridge, Middlesex UB8 3PJ, UK

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A number of antitumor-active octahedral organotin complexes of the type $R_2SnX_2L_2$, where R = ethyl or phenyl, X = chloride or bromide, and L_2 = *o*-phenanthroline or 2-(2-pyridyl)benzimidazole, have been shown to exhibit *in vitro* antiherpes activity towards both *herpes simplex* virus types 1 and 2 (HSV-1 and HSV-2). In addition, a series of mono-, di-, and tri-organotin halides (alkyl and phenyl) demonstrated weak antiherpes activity in the same viral assay system. Selectivity indexes for the tin compounds were calculated and compared with those available in the literature for a number of well-characterized and commercially important antivirals, e.g. adenine-9-β-D-arabinofuranoside (ara-A), cytosine-β-D-arabinofuranoside (ara-C), 5-iodo-2′-deoxyuridine (IDU) and 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACY). Although the organotin complexes are less effective *in vitro* than either ACY or IDU, as determined by their selectivity indexes, they are comparable in activity with both ara-A and ara-C in this particular assay. With few exceptions, most notably $(C_2H_5)_2SnBr_2$(*o*-phen), the organotin compounds examined in this study are more active against HSV-1 (F strain) than HSV-2 (MS strain). The results presented here represent the first study of the potential antiviral properties of organotin compounds.

Keywords: Antiviral, organotin compounds, antiherpes, antitumor, HSV-1 and HSV-2 strains

INTRODUCTION

Viral diseases in man have been estimated to be responsible for more than 60% of the illnesses that occur in the developed countries of the world. Those well-defined infections include the common cold, bronchitis, hepatitis, rabies, poliomyelitis, gastroenteritis, influenza, chicken pox, measles, mumps, herpes and AIDS. In addition, viruses have been implicated in a variety of other diseases ranging from rheumatoid arthritis, diabetes, multiple sclerosis, and cervical cancer to congenital heart disease, atherosclerosis, and many other chronic and degenerative processes.[1]

One of the most common viral diseases to infect man is that caused by two members of the Herpesviridae family, in particular, *herpes simplex* virus types 1 and 2 (HSV-1 and HSV-2). At any given time 0.65–15% of the adult population of the world may be actively shedding HSV.[2–5] Although precise figures for the incidence of infection are not available, it has been estimated that recurrent labial herpes infections affect 20–40% of the population.[6,7] The problem of genital HSV infection has also grown to alarming proportions. Data collected by the US Center for Disease Control on the rate of patient consultations with private physicians for genital herpes has shown almost a nine-fold increase during the period from 1966 to 1979: 3.4/100 000 population in 1966 to 29.2/100 000 in 1979 in the USA.[8] The actual numbers of patients with genital HSV are much greater than those seeking medical attention. True figures are difficult to obtain because of uncontrollable variables such as under-reporting of communicable diseases. Overall estimates indicate that there may be as many as 300 000 initial and 9 000 000 recurrent cases in the United States per year.[9]

The increase in genital HSV infection is alarming, not only because of the large proportion of society afflicted, but also because of features that distinguish this venereal disease from others, i.e. (1) there is no known cure for HSV infections, (2) once within the ganglion, the viral DNA can become localized in neurons permitting reactivation of complete viral forms at

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some later time, (3) infection in women has been associated with cervical cancer, and (4) the consequences of vertical transmission may be associated with a 50–60% mortality rate while the majority of survivors are left with serious sequelae.[10]

To date, the development of chemical antiviral agents has paralleled that of the anticancer agents in the period prior to 1969, i.e. virtually all compounds tested have been organic in nature with few, if any, being inorganic or organometallics. The discovery of the antitumor activity of cisplatin [*cis*-$PtCl_2(NH_3)_2$] in 1969 by Rosenberg and co-workers[11] and the subsequent demonstration that this compound and other platinum-based complexes are potent, clinically useful anticancer agents have altered the bias against inorganic compounds as chemotherapeutic agents.

During the past few years, we have been investigating the effect of various inorganic and organometallic compounds on HSV infections both *in vitro* and *in vivo*. In addition to the demonstrated antiherpetic activity of cisplatin and several other second-generation platinum-based antitumor agents,[12] we have observed similar behavior[13] among the transition-metal cyclopentadienyl compounds (e.g. $\eta C_5H_5)_2MCl_2$ (where M = Ti, V, Mo, Zr and Hf), whose antitumour properties were discovered by Kopf-Maier and Kopf,[14] and certain dithiocarbamate derivatives of Pd(II) and Pt(II).[15]

It has been known for some time that organotin compounds possess a wide range of biological activities, e.g. as fungicides, bactericides and acaricides. It is only recently that one of us has shown that a number of octahedral diorganotin dihalide complexes of the type $R_2SnX_2L_2$ (where $R = CH_3$, C_2H_5, i-C_3H_7, n-C_4H_9 or C_6H_5; X = F, Cl, Br, I or NCS; and L = O- or N-donor organic ligand) exhibit reproducible activity towards P-388 lymphocytic leukemia in mice.[16–18] Since the publication of that work, a number of other diorganotin compounds have been reported to be active towards the same tumour system.[19–23]

The antitumor-active diorganotin complexes of the type $R_2SnX_2L_2$ all possess a *cis* arrangement of the halide or pseudohalide ligands, which has been shown in the case of the platinum complexes to be an essential requirement for biological activity. It has been postulated that the mode of antitumor action of the organotin complexes might be similar to that of the active platinum complexes, i.e. the loss of the halide ligands and subsequent coordination of the remaining metal-containing moiety to suitably oriented nitrogenous bases on DNA. In fact, examples of organotin derivatives of DNA bases, e.g. $R_2Sn(adenine)_2$, are known.[20]

Because of the demonstrated biological activity of organotin compounds, it was of interest to investigate the antiherpes properties of several mono-, di- and tri-organotin halides as well as the aforementioned diorganotin dihalide complexes. The results of this initial investigation are reported here.

MATERIALS AND METHODS

Organotin compounds

All of the organotin halides investigated in this study were obtained from the International Tin Research Institute and used without further purification. The diorganotin dihalide complexes, $R_2SnX_2L_2$ [where $R_2 = C_2H_5$, C_6H_5; X = Cl, Br; and L = *o*-phenanthroline or 2-(2-pyridyl)-benzimidazole] were synthesized and characterized as described previously.[24]

Viruses

The HSV-1 (F strain) and HSV-2 (MS strain) were purchased from the American Type Culture Collection, Rockville, MD, USA. Viral stock solutions were prepared on human foreskin fibroblast (HFF) cells and passaged at least twice before using in the viral assays.

Cell line

The HFF cells were obtained either from Professor James Varani, Department of Pathology, University of Michigan, Ann Arbor, MI, USA or from the Earl-Clay Laboratories, Novato, CA, USA. They were used beginning with the 11th passage and discarded after the 24th passage. They were maintained on Eagle's minimum essential medium with Earle's balanced salt solution (MEM) and 2 mmol dm^{-3} L-glutamine fortified with 10% fetal bovine serum and containing 100 units of penicillin, 0.25 mg fungizone, and 100 mg of streptomycin per cm^3.

Antiviral assay

The antiherpes potencies of the organotin compounds were determined by a standard

plaque reduction method.[25] All assays were carried out in confluent HFF cell cultures in 96-well microtiter plates (Corning Cell Wells). The cell cultures were grown to confluency in MEM supplemented with 2% fetal bovine serum. They were inoculated with 100 $CCID_{50}$ ($CCID_{50}$ is the 50% cell-culture infective dose; the concentration of virus necessary to infect 50% of the confluent monolayer as determined in separate plaque reduction assay experiments), and immediately thereafter exposed to eight different concentrations of the organotin compounds (ranging from about 600 to about 0.1 $\mu g\,cm^{-3}$) dissolved in $(CH_3)_2SO$:MEM (DMSO:2% MEM, 1:10 by volume). (The use of DMSO had no effect on normal cell growth or on the degree of viral infection as determined in separate experiments.) The microtiter plates were incubated for 4 h at 37°C in a 5% CO_2 atmosphere. The medium was removed by vacuum aspiration and replaced with a soft overlay of 0.3% sea plaque agarose in 2% MEM. After refrigeration for 10 min at 4°C in order to allow the agarose to gel, the plates were incubated at 37°C for 72 h. The resultant plaque counts obtained after this incubation period were expressed as percentages of the counts obtained with untreated virus control cultures. These values were plotted against the logarithm of the concentration to give dose–response lines, from which ID_{50} values were determined. Antiviral activity was expressed as ID_{50}, the 50% inhibitory dose, i.e. the concentration of compound required to reduce the plaque count by 50% compared with the control cell cultures.

Cytotoxicity

In tests which were run in parallel with the antiviral assays, the compounds were also examined for their effect on normal cell morphology in confluent HFF cell cultures. LD_{50} values were determined after the compounds had been in contact with the cells for 4 h under the same conditions as described above. The LD_{50} value corresponded to the dose required to cause a microscopically visible disruption of normal cell morphology in 50% of the cells.

RESULTS AND DISCUSSION

The data, which are presented in Tables 1–4, represent average values for three separate experiments for HSV-2 (three wells/concentration/experiment) and two experiments (three wells/concentration/experiment) for HSV-1. Following DeClerq,[26–28] we have calculated a selectivity index (S.I.) for each compound. This parameter is a ratio of LD_{50}/ID_{50} and represents one measure of the antiviral potency of a given compound. We have also compared the relative efficacy of these compounds towards both types of *herpes simplex* virus. Representative data (taken from Ref. 26), for some well-known organic antivirals are given in Table 5. It should be noted that the values listed in Table 5 are the averages for seven strains of HSV-2 (not

Table 1 Mono-, di-, and tri-organotin halides ($R_{4-m}SnX_m$) against HSV-1 (F strain)

Compound	LD_{50} ($\mu g\,cm^{-3}$)	ID_{50} ($\mu g\,CM^{-3}$)	S.I.
$R=C_2H_5$; $m=2$; X=Cl	11	3.1	3.6
$R=(CH_3)_2CH$; $m=2$; X=Cl	8	2.4	3.3
$R=n\text{-}C_4H_9$; X=Cl			
$m=1$	<1	<1	n.d.[a]
$m=2$	1.2	2.0	0.6
$R=n\text{-}C_5H_{11}$; $m=2$; X=Cl	<1.4	<1.4	n.d.
$R=n\text{-}C_7H_{15}$; $m=2$; X=Cl	26	15	1.7
$R=n\text{-}C_8H_{17}$; X=Cl			
$m=2$	75	48	1.6
$m=3$	245	110	2.2
$R=C_6H_5$; X=Cl			
$m=1$	2.3	<0.5	>4.6
$m=2$	1.1	0.3	3.7
$m=3$	1.6	0.6	2.0
$m=2$; X=Br	2.0	0.45	4.4

[a]n.d., Not determined.

Table 2 Diorganotin dihalide complexes ($R_2SnX_2L_2$) against HSV-1 (F strain)

Compound	LD_{50} ($\mu g\,cm^{-3}$)	ID_{50} ($\mu g\,CM^{-3}$)	S.I.
$R=C_2H_5$			
X=Cl; L_2=phen[a]	23	7.6	3.0
X=Cl; L_2=PBI	19	8.1	2.3
X=Br; L_2=phen	31	19	1.6
X=Br; L_2=PBI	13	13	1.0
$R=C_6H_5$			
X=Cl; L_2=phen	0.9	0.35	2.6
X=Cl; L_2=PBI	6.0	3.9	1.5
X=Br; L_2=phen	0.9	0.23	3.9
X=Br; L_2=PBI	1.8	0.70	2.6
X=Cl; L_2=2 DMSO	<1.0	1.0	<1.0

[a]phen = 1,10-phenanthroline; PBI = 2-(2-pyridyl)benzimidazole.

Table 3 Mono-, di-, and tri-organotin halides ($R_{4-m}SnX_m$) against HSV-2 (MS strain)

Compound	LD_{50} ($\mu g\,cm^{-3}$)	ID_{50} ($\mu g\,CM^{-3}$)	S.I.
$R=CH_3$; $m=2$; X=Br	72	74	1.0
$R=C_2H_5$; $m=2$			
X=Cl	4.4	3.7	1.2
X=Br	19	13	1.5
$R=(CH_3)_2CH$; $m=2$; X=Cl	6.8	6.7	1.0
R=n-C_4H_9; X=Cl			
$m=1$	<1.0	<1.0	n.d.[a]
$m=2$	1.1	<1.0	>1.0
$m=3$	39	59	0.7
R=n-C_5H_{11}; $m=2$; X=Cl	<1.0	<1.0	n.d.
R=n-C_7H_{15}; $m=2$; X=Cl	29	17	1.7
R=n-C_8H_{17}; X=Cl			
$m=2$	33	45	0.7
$m=3$	370	390	0.9
$R=C_6H_5$; X=Cl			
$m=1$	<1.0	<1.0	n.d.
$m=2$	1.2	0.9	1.3
$m=3$	1.1	<1.0	>1.0
$m=2$; X=Br	1.2	1.1	1.1

[a]n.d., Not determined.

Table 4 Diorganotin dihalide complexes ($R_2SnX_2L_2$) against HSV-2 (MS strain)

Compound	LD_{50} ($\mu g\,cm^{-3}$)	ID_{50} ($\mu g\,CM^{-3}$)	S.I.
$R=CH_3$			
X=Br; L_2=phen[a]	75	72	1.0
X=Br; L_2=PBI	83	56	1.5
$R=C_2H_5$			
X=Cl; L_2=phen	19	9.0	2.1
X=Cl; L_2=PBI	11	8.2	1.3
X=Br; L_2=phen	17	2.7	6.3
X=Br; L_2=PBI	14	16	0.9
$R=C_6H_5$			
X=Cl; L_2=phen	5.2	2.4	2.2
X=Cl; L_2=PBI	1.4	0.7	2.0
X=Br; L_2=phen	2.6	2.0	1.3
X=Br; L_2=PBI	4.2	2.4	1.8
X=Cl; L_2=2 DMSO	<1.0	<1.0	n.d.

[a]Abbreviations as in Tables 1 and 2.

including the MS strain) and 11 strains of HSV-1 (including the F strain). These values were determined in primary rabbit kidney cell cultures. Although a direct quantitative comparison

Table 5 Selectivity indices for some organic antivirals in PRK (primary rabbit kidney) cell cultures[a]

Compound	HSV-1, S.I.[b]	HSV-1 (F strain)	HSV-2, S.I.[c]	HSV-2/ HSV-1[d]
PAA[e]	15	25	12	0.48
PFA	15	28	9	0.32
ara-C	10	10	12	1.2
ara-A	15	14	10.5	0.75
IDU	1540	1300	3540	2.7
ara-FC	0.8	1.3	0.2	0.15
EHNA	1.1	1.5	0.9	0.60
TFT	57	57	57	1.0
ACY	5000	5000	5000	1.0

[a]From Ref. 26. [b]S.I. for 11 strains of HSV-1. [c]S.I. for seven strains of HSV-2. [d]Ratio of HSV-2 to HSV-1 (F strain only). [e]PAA = phosphonoacetic acid; PFA = trisodiumphosphonoformate; ara-C = Cytarabine; ara-A = Vidarabine; IDU = 5-iodo-2'-deoxyuridine; ara-FC = 1-β-D-arabinofuranosyl-5-fluorocytosine; EHNA = erythro-9(2-hydroxy-3-nonyl)adenine; TFT = trifluorothymidine; ACY = acyclovir.

cannot be made between our results and those of De Clerq because the cell lines and other experimental variables differ, the *in vitro* activities of the organotin compounds with the highest selectivity indices look promising and warrant further investigation. In all instances the measure of cytotoxicity was determined by changes in cell morphology. Even though this method of determining toxicity is somewhat subjective, it has been used to evaluate the effect of antiviral agents on normal cellular processes.[26]

In general, the organotin compounds screened for antiherpes potential in this investigation exhibit selectivity indices which are considerably less than that of the current benchmark for antiherpes agents, acyclovir (ACY). It should be pointed out, however, that the relative potency and/or selectivity of compounds in cell cultures does not necessarily predict the therapeutic efficacy of a given compound. It is pertinent to note in this context that PAA and PFA were more effective and ara-C less effective in the topical treatment of cutaneous HSV-1 infections of athymic nude mice[29] than one would expect from their respective S.I. values for HSV-1 in cell cultures (Table 5). A number of the organotin compounds have selectivity indexes in the range from approximately 2 to 7 and thus are quite comparable in their antiherpes potency with PFA, PAA, ara-C, and ara-A, and more active than ara-FC and EHNA.

Organotin halides

It is worth noting that the progressive introduction of organic groups at the tin atom in any $R_{4-m}SnX_m$ series produces a maximum biological activity against all species when $m=1$. If the chain length of the n-alkyl group is increased within any trialkyltin series, the highest mammalian toxicity is attained for the triethyltin compounds. For insects, however, the trimethyltins are usually most toxic; for gram-negative bacteria the tri-n-propyltins, and for gram-positive bacteria and fungi the tri-n-butyltins, show the highest activity. Further increase in the n-alkyl chain length produces a sharp drop in biological activity (the trioctyltins are essentially non-toxic to all living species). The triphenyltin species show a high fungicidal activity. Both the dialkyltins and the monoalkyltin halides show a similar trend of decreasing toxicity with increasing length of the alkyl chain.[30] Within the limited series of organotin halides tested (Tables 1 and 3), it appears that the phenyltin halides, $(C_2H_5)_2SnCl_2$, and $((CH_3)_2CH)_2SnCl_2$ have the greatest activity towards HSV-1. However, there is no discernible trend towards HSV-2; in fact, the selectivity indices for all the organotin compounds tested against the MS strain of HSV-2 were less than 2.0. Potency against HSV-1 appears to be a better choice for determining structure/activity relationships for these compounds. In this context, the highest selectivity indices for the alkyltins were obtained for $(C_2H_5)_2SnCl_2$ and $(iso\text{-}C_3H_7)_2SnCl_2$ with values decreasing as the length of the alkyl chain increases. In the series $(C_6H_5)_{4-m}SnCl_m$, the greatest S.I. was observed for $m=1$ and the lowest S.I. for $m=3$. On the basis of only one comparison, the bromide appears to be more effective than the chloride [$(C_6H_5)_2SnBr_2$ versus $(C_6H_5)_2SnCl_2$]. Whether this is a general result remains to be determined. Obviously, a more thorough and complete survey of the organotin halides needs to be undertaken before one can make any definitive conclusions about structure/antiherpes activity relationships.

Diorganotin dihalide complexes

The diethyl- and diphenyl-tin dihalide complexes, $R_2SnX_2L_2$, were the most potent tin-containing compounds tested (Tables 2 and 4). In contrast to the antitumour data presented by Crowe,[18] and the results obtained in this current study for the organotin halides, the chlorides, in general, were found to be more active than the bromides, although marginally so. This activity appears to be a function of the complex since the organic ligands are inactive (data not shown) and the parent diorganotin dihalides have low activities. One of us has suggested that the antitumor properties of these complexes, and by inference the antiherpes activity, is related to the stability of the Sn–L interaction; those complexes with the highest activity are stable enough to allow the active R_2Sn moiety to be transported to the site of action.[31] This implies that a predissociation of the bidentate ligand may be a crucial step in the formation of a tin–DNA complex. Whether or not this mechanism is responsible for the antiherpes activity of these compounds remains to be ascertained.

Based on the data presented in this study, organotin halide complexes deserve further attention as potential antiviral agents.

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