Applied Organometallic Chemistry (1989) 3 537–543

0268–2605/89/03609537/$03.50

Structural effects on the antitumour activity of a series of di(4-substituted phenyl)tin dichloride complexes with nitrogen-donor ligands

Brian N Biddle and John S Gray
Faculty of Applied Sciences, Luton College of Higher Education, Park Square, Luton, Bedfordshire LU1 3JU, UK

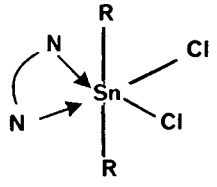
Received 24 May 1989 *Accepted 19 August 1989*

Investigation of the antitumour activity of a series of diorganotin dichloride complexes $(4\text{-}ZC_6H_4)_2SnCl_2.L_2$, where Z = OMe, Me, F, Cl, and CF_3 and L_2 = 2,2′-bipyridyl (bipy), 1,10-phenanthroline (phen) and 2-aminomethylpyridine (amp) is reported. A number of these complexes are shown to exhibit reproducible activity *in vivo* towards P388 lymphocytic leukaemia in mice. ^1H NMR data are reported for an extended series of $(4\text{-}ZC_6H_4)_4Sn$ and the parent dichlorides $(4\text{-}ZC_6H_4)_2SnCl_2$ of the above-mentioned complexes. A correlation is reported between Hammett substituent constants and ^1H chemical shift data. Attempts are made to relate the antitumour activity of the complexes to various structural factors. The dependence of antitumour activity on the electronic effect of group Z and the nature of the ligand L_2 is demonstrated.

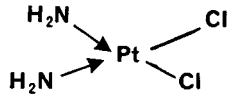
Keywords: Organotin complexes, antitumour agents, ^1H NMR spectra, aryltin chlorides, correction analysis, murine P388 leukaemia

INTRODUCTION

Following the introduction and subsequent development of the clinically important platinum complexes[1] in the treatment of certain forms of cancer, much work has been directed to compounds containing other metals[2,3] and especially to tin(IV) complexes.[4] A large number of organotin compounds has now been tested and many show reproducible antitumour activity in mice. Several activity-relationship studies in this field have been published.[5–7] Many of the active compounds previously reported are octahedral complexes of tin having *cis* halogen atoms or leaving groups (**I**) and a



I



II

partial structure analogous to the square planar platinum(II) complexes, exemplified here by the drug Cisplatin (**II**). Although activity appears to be appreciably lower for these tin compounds than for the analogous platinum compounds, their lower toxicity may indicate future potential use as drugs. The mode of action of the tin complexes may be similar to that of the platinum analogues, involving loss of both the ligand (L) and the chlorine atoms or other leaving groups.[4] It has been suggested that activity is determined principally by the dialkyltin (R_2Sn) moiety[4,8] and, in the large number of compounds tested, activity is found to be at its maximum when R = Et or Ph (T/C values 170–190%, where T/C is the ratio of test evaluation (T) to control evaluation (C) expressed as a percentage). This paper describes the effect of introducing substituents in the phenyl ring in a series of complexes $Ar_2SnCl_2L_2$ in an attempt to study the effect of electron density at the tin atom on the P388 antitumour activity of the compound.

EXPERIMENTAL

Preparation of tetra-aryltins

All eight previously reported tetra(4-substituted phenyl)tins[9–12] described in this paper (Table 1) were

Table 1 ^{1}H NMR data[a] for tetra-aryltins (4-$ZC_6H_4)_4Sn$

Z	$\delta(H_a)$	$\delta(H_b)$	$\Delta\delta$	δ_{ab}	σ_p	σ_p^+	σ_m
NMe_2	6.75	7.50	0.75	7.13	−0.83	−1.70	−0.21
OMe	6.97	7.54	0.57	7.26	−0.27	−0.78	+0.11
Me	7.23	7.56	0.33	7.40	−0.17	−0.31	−0.07
Me_3C	7.46	7.62	0.16	7.54	−0.20	−0.26	−0.10
Ph	7.39	7.61	0.22	7.50	−0.01	−0.18	+0.06
F	7.19	7.60	0.41	7.41	+0.06	−0.07	+0.34
Cl	7.48	7.52	0.04	7.50	+0.23	+0.12	+0.37
CF_3	7.76	7.76	0.00	7.76	+0.54	+0.65	+0.43

[a]$\Delta\delta$, Chemical shift difference; δ_{ab}, mean chemical shift; σ_p, σ_p^+ and σ_m, substituent constants.

prepared using standard Grignard procedures.[13] A solution of anhydrous tin tetrachloride in a hydrocarbon solvent was added to the appropriate aryl magnesium chloride or bromide prepared in tetrahydrofuran solution.

In each case the reaction mixture was boiled under reflex for about 2 h and then poured into water. The tetra-aryltin was isolated by extraction with hot petroleum ether or toluene. The combined extracts were then evaporated to yield crude solid product which was crystallized from 60–80° petroleum ether or toluene before the next stage.

Preparation of diaryltin dichlorides

The five diaryltin dichlorides used in this work (Table 2) were reported previously[9] and were made by a modification of the standard Kocheshkov procedure described therein.

Table 2 ^{1}H NMR data[a] for diaryltin dichlorides (4-$ZC_6H_4)_2SnCl_2$

Z	$\delta(H_a)$	$\delta(H_b)$	$\Delta\delta$	δ_{ab}
OMe	7.10	7.69	0.59	7.39
Me	7.40	7.68	0.28	7.54
F	7.33	7.79	0.46	7.56
Cl	7.64	7.69	0.05	7.66
CF_3	7.91	7.93	0.02	7.92

[a]$\Delta\delta$, Chemical shift difference; δ_{ab}, mean chemical shift.

Preparation of diaryltin dichloride complexes

The 15 complexes (Table 3) examined for antitumour activity and described here, were reported, together with analytical and Mössbauer data, in a previous paper.[9] All complexes were prepared by standard procedures in which the diaryltin dichloride in methanol or diethyl ether was added at room temperature to a solution of the Lewis base in the same solvent.

^{1}H NMR spectra

^{1}H NMR spectra for the tetra-aryltins (Table 1) and diaryltin dichlorides (Table 2) were recorded on a Hitachi spectrometer Model R-24B operating at 60 MHz. Chemical shifts for aryl protons were measured relative to dichloromethane, used as an internal standard (1%). Shifts obtained by locking on to the CH_2Cl_2 signal at δ 5.31 are reported as chemical shifts $\delta(^1H)$ from $(CH_3)_4Si$. All spectra were obtained using approximately 1% solutions in tetrachloroethene. Results are accurate to ±0.02 ppm.

Antitumour bioassay

The activity of all 15 complexes [$(ZC_6H_4)_2SnCl_2 . L_2$] was tested *in vivo* towards P388 lymphocytic leukaemia in mice. The activity was determined in accordance with the US National Cancer Institute standard protocol for primary screening. The complexes are generally of low solubility and were administered as suspensions in saline and Tween 80. The test evaluation is expressed as a T/C value providing a measure of effectiveness for the compound being tested. The murine P388 prescreen is a survival system and when T/C values exceed 120 further study is indicated. Results are given in Tables 3 and 4.

Six of the complexes were selected for further examination using the transplanted mouse tumour adenocarcinoma SC Colon 38. Again the complexes

Table 3 The activity of $(4\text{-}ZC_6H_4)_2SnCl_2.L_2$ complexes towards P388 lymphocytic leukaemia (T/C values, %)[a]

Complex			Dose (mg kg^{-1})							
Z	L_2		240	120	60	15	7.5	3.75	1.87	0.93
OMe	bipy	a	t	t	t	t	158	130	–	–
		b	–	–	–	–	151	150	135	112
	phen	a	t	t	t	152	142	131	–	–
		b	–	–	–	t	142	122	125	–
	amp	a	t	t	t	158	130	122	–	–
		b	–	–	–	122	109	102	102	–
Me	bipy	a	t	t	t	t	t	93	103	95
		b	–	–	–	–	–	105	105	106
	phen	a	t	t	t	t	t	95	117	93
		b	–	–	–	–	–	102	98	100
	amp	a	t	t	t	t	t	138	–	–
		b	–	–	–	–	–	122	122	119
F	bipy	a	t	t	t	111	103	96	–	–
	phen	a	t	t	t	97	104	101	–	–
	amp	a	t	t	t	t	t	116	–	–
		b	–	–	–	–	–	104	101	101
Cl	bipy	a	t	t	t	106	106	97	–	–
	phen	a	t	t	t	t	91	94	–	–
	amp	a	136	115	96	–	–	–	–	–
		b	146	135	116	–	–	–	–	–
CF_3	bipy	a	t	t	t	106	100	93	–	–
	phen	a	t	131	126	–	–	–	–	–
		b	t	131	119	101	–	–	–	–
	amp	a	137	133	112	–	–	–	–	–
		b	150	128	107	–	–	–	–	–

[a] t indicates that the complex is toxic at this concentration; –, test not carried out; a, b are separate tests.

Table 4 Summary of activity towards P388 lymphocytic leukaemia

Z	L_2	T/C (%) (two best values)	Activity[a]	Structure[b]
OMe	bipy	158, 151	Active	*Trans* (1)
	phen	152, 142	Active	*Trans* (1)
	amp	158, 130	Active	*Cis* (2 or 3)
Me	bipy	106, 105	Inactive	*Cis* (3)
	phen	117, 102	Inactive	*Trans* (1)
	amp	138, 122	Active	*Cis* (2 or 3)
F	bipy	111, 103	Inactive	*Trans* (1)
	phen	104, 101	Inactive	*Trans* (1)
	amp	116, 104	Inactive	Cis (2 or 3)
Cl	bipy	106, 106	Inactive	*Trans* (1)
	phen	94, 91	Inactive	*Trans* (1)
	amp	146, 136	Active	*Cis* (2 or 3)
CF_3	bipy	106, 100	Inactive	*Trans* (1)
	phen	131, 131	Active	*Cis* (2 or 3)
	amp	150, 137	Active	*Cis* (2 or 3)
H	bipy	– –	Inactive	*Trans* (1)
	phen	– –	Inactive	*Trans* (1)
	amp	153, 150	Active	*Cis* (2 or 3)

[a]A compound is termed active if it has a T/C > 120%. [b]*Cis* organo compounds have alternative structures (**IV** and **V**).

were administered as suspensions in saline and Tween 80. The tumour system SC Colon 38 is a tumour inhibition system and indicates a degree of success when the T/C values do not exceed 42. Results for this system are presented in Table 5. They were inactive by this criterion. All antitumour assays were carried out at the Institut Jules Bordet, Brussels, Belgium.

DISCUSSION

The series of complexes $(ZC_6H_4)_2SnCl_2.L_2$ described in this paper was chosen to allow a wide variation in electronic and steric requirements which might influence antitumour activity. Group Z was varied in the aryl substituent to give a range from the very electron-withdrawing perfluoromethyl (CF_3) to the electron-donating methoxy (OMe) group. The ligand L_2 was varied substantially in both Lewis base strength and steric properties.

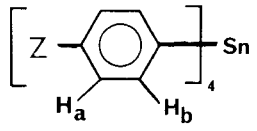
Table 5 The activity of ($4\text{-}ZC_6H_4 + SnCl_2 . L_2$) complexes towards the mouse adenocarcinoma SC Colon 38: T/C values (%)

		Dose (mg kg$_{-1}$)				
Z	L_2	30	15	7.5	3.75	Activity[a]
OMe	bipy		47	67	81	Inactive
	phen		94	133	–	Inactive
	amp	80	101	57	–	Inactive
			Dose (mg kg^{-1})			
			8	4	2	
Me	bipy		70	99	72	Inactive
	phen		76	60	111	Inactive
	amp		61	55	–	Inactive

[a]A compound is termed active if it has a T/C < 42%.

As far as the aromatic ring is concerned, some observations made by us and other workers[9,14] during the synthesis and handling of these and related compounds indicate remarkable substituent effects on the stability and reactivity of both the dihalides and their complexes. For example, when Z = OMe, the dichloride chlorine atoms are readily displaced as is shown by ready decomposition in moist air. Complex formation with this dihalide stabilizes it to some extent but the ligand L_2 is only weakly held, and particularly so in the cae of the weak Lewis base 2-aminomethyl-pyridine. The preparation of the dihalide from the tetra-aryltin by the electrophilic attack of tin tetrachloride in the Kocheshkov equilibration is very easy, confirming the substantial donating effect of the methoxy group. The reverse situation is found when electron-withdrawing groups are in the 4-position of the aryl ring. For example, the perfluoromethyl group stabilizes the halide, decreases the reaction rate in the Kocheshkov equilibration and substantially increases the stability of the complexes, presumably by an increase in the acceptor properties of the tin atom for the Lewis base. These observations are further supported by work on the preparation of the highly unreactive pentafluorophenyltin.[14] We believe, however, that no detailed study of the effect of substituents in the aromatic ring on the reactivity of groups at the tin atom has hitherto been reported.

Ideally, for structure–activity relationship studies of the type described in this paper, comparative measurements on ligand stability constants or reaction rates would be made and it is intended to carry out such work. Successful linear correlations have been made for many aromatic series between ^{1}H NMR parameters and various substituent constants and we report here ^{1}H NMR chemical shifts for a series of tetra-aryltins $(4\text{-}ZC_6H_4)_4Sn$ and for the parent dihalides $(4\text{-}ZC_6H_4)_2SnCl_2$ of our complexes. The very low solubility of the complexes themselves did not permit our obtaining ^{1}H NMR data. Neither series is sufficient in number[15] to permit quantitative correlation. Inspection of the chemical shift differences $\Delta\delta$ and the mean chemical shifts δ_{ab} (Table 1) for the two protons H_a and H_b (**III**) in the aromatic ring



III

shows the anticipated general relationship with substituent parameters σ_p and σ_p^+. The chemical shift difference $\Delta\delta$ can be shown to be related to the electron-donating or -withdrawing properties of the group Z and ranges from a maximum donating effect for Z = OMe to the powerfully electron-withdrawing Z = CF_3. Figure 1 presents a graph of the chemical shift difference $\Delta\delta$ against $\sigma_p - \sigma_m$ or against $\sigma_p^+ - \sigma_m$ for the eight substituents. The difference $\sigma_p - \sigma_m$ is an estimate of the resonance interaction of the substituent Z and for these values a reasonable linear relationship is seen; $\sigma_p^+ - \sigma_m$ is an estimate of an enhanced through-resonance contribution of the substituent which is presumably absent in these compounds since considerable departure from linearity is seen. This agrees with a previous ^{1}H NMR study by Barbieri and Taddei[16] which reports little $(d-p)\,\pi$ interaction between aromatic rings and the tin atom in a series of four related compounds.

Table 2 presents the limited set of ^{1}H NMR data for the diaryltin dichlorides used in this activity-relationship study. Again the expected substituent effect is observed and the δ_{ab} values are exactly linearly related to the δ_{ab} values for the tetra-aryltin series. The chlorine atom relays its withdrawing effect to the ring via the tin atom, resulting in a downfield shift for all H_a and H_b protons.

Application of the ^{1}H NMR additivity rules for ring protons[17] to the tetra-aryltin and diaryltin dichloride series gives good agreement with the observed

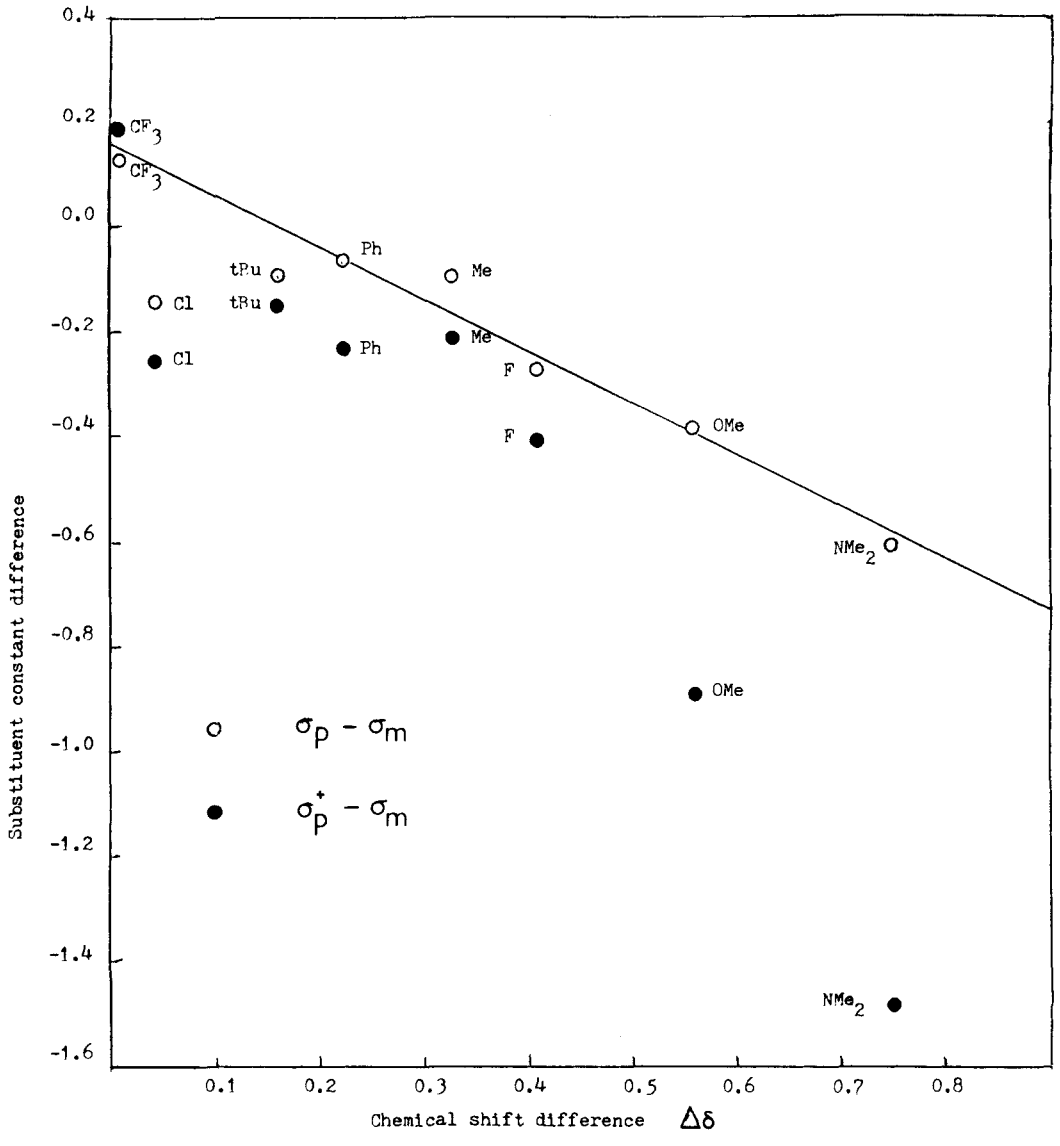


Figure 1 Hammett plots of chemical shift differences ($\Delta\delta$) from ^{1}H NMR data versus substituent constant differences ($\sigma_p - \sigma_m$).

chemical shifts and supports the argument that tin and the substituents are behaving as groups with weak conjugative effects. This has already been reported by Barbieri and Taddei,[16] who calculated tin parameters for the ortho (*o*) and meta (*m*) ring protons ($\delta_{Sn,o} = -0.37$; $\delta_{Sn,m} = -0.12$) in some tetraaryltins. Similar calculations using our data for the diaryltin dichlorides gives average values for the $SnCl_2$ group of $\delta_{Sn,o} = -0.50$ and $\delta_{Sn,m} = -0.33$ (± 0.02). The differences between the two sets of values (0.13 for H_b and 0.21 for H_a) give a measure of the mainly inductive withdrawal due to chlorine transmitted through the tin atom. However, the lower value for H_b may indicate a small mesomeric back-donation from chlorine operating via the tin atom.

Tables 3 and 4 present the full experimental data on the activity and toxicity of the series of complexes using mouse P388 lymphocytic leukaemia as the test system. Antitumour activity and toxicity can be determined by a variety of factors: water solubility and lipophilic properties of the agent, the geometrical distribution of groups around the tin atom, the ease of ligand

displacement and the ease of nucleophilic displacement of the chlorine atom.[4] In the series we describe here two structural features are varied: (1) the space-occupying power and Lewis base strength of the bidentate ligand and (2) the electronic effect of the aryl substituent. All the complexes we have prepared are highly insoluble in water (and in most other solvents) and this presents a major problem in transporting the agent to the tumour.

The results of screening tests on 115 complexes of general structure $R_2SnCl_2.L_2$ were reported earlier by Crowe and co-workers.[6] Although both the ligands L_2 and most of the parent dichlorides are inactive, a substantial number of the complexes were shown to be active towards the P388 test system. In general, the preferred ligands for activity had strong *N*-donor properties and included 1,10-phenanthroline. No correlation between the structure of the tin dichloride, its ligand acceptor strength and the antitumour activity could be discerned. The acceptor properties of the tin atom are influenced by the group R. When R = phenyl the stability of the complex is enhanced compared with complexes where R is alkyl and electron-donating. Antitumour results on the complexes of $Ph_2SnCl_2.L_2$ showed[6] that nine of 13 complexes tested were active, including the complex with the weak Lewis base, 2-aminomethylpyridine (amp). Both the 2,2′-bipyridyl and 1,10-phenanthroline complexes were inactive, however. Recently, and since our work was completed, further work has been recorded on the effect of ligands on antitumour activity[18] and it is proposed that TMphen (3,4,7,8-tetramethyl-1,10-phenanthroline) and PBI [2-(2-pyridyl)benzimidazole] are the preferred ligands for activity. A further series of complexes with these ligands is currently being examined for antitumour activity.

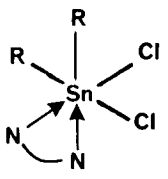
The results of antitumour testing on our series of 4-substituted phenyl complexes, together with the unsubstituted parent $Ph_2SnCl_2.L_2$, are summarized in Table 4. It can be seen that the preferred ligand is amp, for which five out of six of the complexes are active. In the case of the phenanthroline complexes, only two show activity and in the case of the bipyridyl complexes, only one is active.

A consideration of substituent effects (4-Z) on activity and toxicity shows some notable relationships. Toxicity of the complexes generally decreases as the group Z becomes increasingly electron-withdrawing (Table 2: δ_{ab} values) and the amp complexes for $Z = CF_3$ and Cl are non-toxic at doses of 240 mg kg^{-1}. In the case where Z = OMe all three complexes were found to be active at low dose rates and this may be related to the powerful electron-donating effect of the methoxy group which increases both the ease of nucleophilic replacement of the chlorine atoms at the tin atom and the loss of the bidentate ligand. Decrease in the electron-donating power of the aryl substituent is accompanied by decreasing activity and by decreasing toxicity. In the case of the 4-fluoro substituent, none of the complexes shows activity at any of the dose rates tested. Presumably the toxicity factor here outweighs any antitumour activity of the agent. However, as toxicity decreases further with increasing electron withdrawal, activity is again seen but at much greater dose rates and for $Z = CF_3$ two of the three complexes show activity. An additional factor here may be the improved membrane transport characteristics of highly fluorinated drugs.

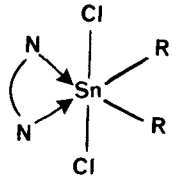
Unfortunately, none of the complexes tested has T/C values greater than tin compounds already reported[4] (T/C = 179–190%).

It is also disappointing to note that those complexes which are active against P388 leukaemia proved to be inactive when tested against the mouse adenocarcinoma SC Colon 38 (TAble 5). This is in line with the observations of other workers in the organotin antitumour field. Many complexes have been found to be active against the P388 leukaemia but very few have shown reproducible activity against other systems. It has been suggested by Atassi[18] that the low water solubility of many of the tin agents is a major hindrance to their antitumour activity. Recent attempts to produce active water-soluble agents, however, have not been very successful. It is interesting to note that the most active of these new compounds as reported by Silvestri *et al.*[19] (Ph_2Sn–L-cysteinate complex; T/C 181%), has the diaryl Ar_2Sn grouping in common with our complexes.

A factor which determines the activity of the platinum complexes **II**, and may be important in determining activity in the tin complexes, is the *cis* distribution of the halogen atoms or leaving groups. The Mössbauer data and inferred structures for the series of complexes discussed here have been published previously[9] and the structures are summarized in Table 4. In the case of all *trans* (organo) isomers represented by **I** there is a *cis* requirement for the two chlorine atoms but only two of the ten *trans* isomers reported here are active. In the case of the *cis* isomers,



IV



V

two different configurations (**IV** and **V**) are possible and one only of these has *cis* chlorine atoms. Six of the other reported *cis* isomers are active, however, and it is noteworthy that one of these inactive *cis* isomers is known to have *trans* chlorine atoms (Z = Me, L_2 = bipy).[20] The configurations of the remainder are unknown, in the absence of X-ray data. Obviously *cis* chlorine atoms may be a requirement but do not necessarily confer activity.

Acknowledgements We are very grateful to Drs A J Crowe and P J Smith of the International Tin Research Institute, London, for their active support throughout the project, and to staff at the Institut Jules Bordet, Brussels, where the antitumour work was carried out. The Royal Society of Chemistry is thanked for financial assistance. The antitumour data reported in this paper are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, USA.

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