method was exactly as for **29d** except using acid **10b**. Data was as for **29e** except $[\alpha]^{20}_D = -63.4°$ (c = 0.21, Me_2CO).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*R**)]-3-(1*H*-Indol-3-ylmethyl)-3-methyl-4,9,11-trioxo-7-phenyl-12-oxa-2,5,8-triazatridecanoate (30).** The method was exactly as for **22** except using methyl (chloroformyl)acetate and amine **25d**: yield 43 mg, 43%; $[\alpha]^{20}_D = -11.5°$ (c = 0.27, MeOH); IR film 1740, 1700, and 1660 cm^{-1}; NMR ($CDCl_3$) δ 1.49 (3 H, s), 1.52–1.60 (3 H + H_2O, m), 1.73–2.01 (11 H, m), 3.30–3.46 (5 H, m), 3.73 (3 H, s), 3.89 (1 H, m), 4.84 (1 H, br s), 5.13 (2 H, br s), 6.48 (1 H, m), 6.98 (1 H, d, J = 2 Hz), 7.07–7.37 (8 H, m), 7.57 (1 H, d, J = 8 Hz), 7.68 (1 H, d, J = 8 Hz), 8.23 (1 H, s).

[*R*-(*R,*R**)]-3-[[2-[[3-(1*H*-Indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-3-oxopropanoic Acid (31).** The method was as for **18** except using ester **30**: yield 81 mg, 69%; $[\alpha]^{20}_D = -8.4°$ (c = 0.17, $CHCl_3$); IR (film) 3310, 1707, and 1663 cm^{-1}; NMR (DMSO-d_6) δ 1.23 (3 H, br s), 1.48–1.62 (2 H, m), 1.72–2.07 (12 H, m), 3.14–3.55 (6 H + H_2O, m), 4.72 (1 H, br s), 5.02 (1 H, s), 6.72 (1 H, br s), 6.92–6.97 (2 H, m), 7.02 (1 H, t, J = 7 Hz), 7.24–7.36 (6 H, m), 7.81 (1 H, m), 8.96 (1 H, m), 10.91 (1 H, br s).

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Registry No. **5a**, 130406-30-3; **5b**, 129397-83-7; **6a**, 130406-33-6; **6b**, 130406-34-7; **6b** (*N*-Fmoc derivative), 130406-32-5; **7a**, 120666-53-7; **7a** (*O*-tosylate derivative), 130406-37-0; **7b**, 130406-31-4; **8a**, 130406-35-8; **8b**, 130406-36-9; **9a**, 96551-27-8; **9a** (*N*-2Adoc derivative), 130406-39-2; **10a**, 130406-40-5; **10b**, 130406-41-6; **10c**, 130406-42-7; **11a**, 130406-43-8; **11b**, 130406-44-9; **11c**, 130466-73-8; (*R*,*R*)-**12**, 130406-48-3; (*R*,*R*)-**12** (free alcohol), 130406-45-0; (*R*,*S*)-**12**, 130406-49-4; (*R*,*S*)-**12** (free alcohol), 130406-46-1; **13**, 130406-47-2; **14**, 130406-50-7; **15e**, 130406-51-8; **16e**, 130406-52-9; **17**, 130406-53-0; **18**, 130406-56-3; **19**, 130406-54-1; **20**, 130406-57-4; **21d**, 130406-58-5; **21e**, 130406-59-6; **21f**, 130406-60-9; **21g**, 130406-61-0; **22**, 130406-55-2; **23**, 130406-62-1; **24d**, 130406-63-2; **25d**, 130406-64-3; **26**, 130406-65-4; **27**, 130406-67-6; **28** (free base), 130406-68-7; **28**·HOAc, 130406-73-4; **29d**, 130332-27-3; **29e**, 130406-69-8; **29f**, 130406-70-1; **29g**, 130406-71-2; **30**, 130406-66-5; **31**, 130406-72-3; (*R*)-(+)-$H_2NCH(CH_2Ph)CH_2OH$, 5267-64-1; (*S*)-(−)-$H_2NCH(CH_2Ph)CH_2OH$, 3182-95-4; (*R*)-$H_2NCHPhCH_2OH$, 56613-80-0; (*S*)-$H_2NCHPhCH_2OH$, 20989-17-7; (*R*)-$BzO_2CNHCHPhCH_2N_3$, 130406-38-1; $PhCH_2CH_2NH_2$, 64-04-0; (*RS*)-$H_2NCH_2CHPhOH$, 1936-63-6; (*E*)-MeO_2CCH=CHCOOH, 2756-87-8; $MeO_2C(CH_2)_3COCl$, 1501-26-4; MeO_2CCH_2COCl, 37517-81-0; α-methyl-(*S*)-tryptophan, 16709-25-4; α-methyl-(*RS*)-tryptophan, 153-91-3; 2-adamantanol, 700-57-2; succinic anhydride, 108-30-5; glutaric anhydride, 108-55-4.

Preparation, Characterization, and Anticancer Activity of a Series of *cis*-$PtCl_2$ Complexes Linked to Anthraquinone Intercalators

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A new series of complexes of the type *cis*-PtL_2X_2 [where L is a monodentate AQ-$Y(CH_2)_nNH_2$ and L_2 is a bidentate AQ-$Y(CH_2)_nNH(CH_2)_2NH_2$; AQ = anthraquinone, X = Cl, I, Y = NH, O] in which anthraquinone intercalators are tethered to the *cis*-$PtCl_2$ unit via an (aminoalkyl)amino, (oxyalkyl)amino, or polyethylene glycol (aminoethyl)amino linker chains was prepared and screened in vitro against P388 leukemia. In vivo toxicity studies were carried out on selected complexes. All complexes were characterized by means of elemental analysis, ^{195}Pt NMR spectroscopy, and FTIR. The 1:1 Pt–intercalator complexes displayed much higher in vitro cytotoxic activities than the 1:2 Pt–intercalator complexes. The dichloride complexes were consistently more active than their diiodide counterparts. Among the 1:1 Pt–intercalator complexes those with the shorter linker chains (n = 2, 3) exhibited the highest cytotoxic activities. Three compounds, [[2-[[2-(anthraquinon-1-ylamino)ethyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II), [[2-[[3-(anthraquinon-1-ylamino)propyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II), and [[2-[[3-(anthraquinon-1-yloxy)propyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II), were as active in vitro as cisplatin (ED_{50} = 2–4 × 10^{-7} M) while on a molar basis their acute in vivo toxicity was significantly lower than that of cisplatin. In vivo screening against P388 leukemia indicated that these complexes have activity comparable to cisplatin.

Introduction

cis-Diamminedichloroplatinum(II) (*cis*-DDP) is a clinically effective widely used anticancer agent which has been studied extensively.[1,2] The drug is believed to effect cytotoxicity by covalently modifying the DNA, its putative biological target, and arresting DNA replication.[3-5] The major adduct of *cis*-DDP with DNA, both in vitro and in vivo, is the covalent chelate formed between $Pt(NH_3)_2^{2+}$ and the d(GpG) fragment.[6-8] The detailed geometry as well as the metrical parameters of this adduct have been recently described in a single crystal X-ray diffraction study.[9,10] Despite its clinical success, *cis*-DDP suffers from

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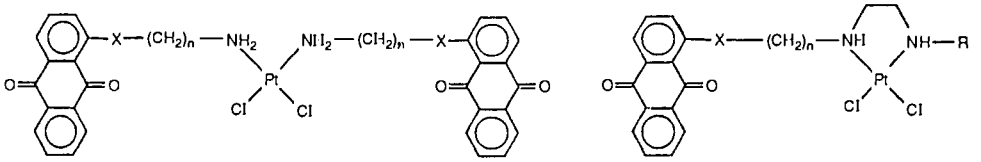

1:2 Pt:intercalator

X=NH, n=3,4,6,12
X=O, n=3

1:1 Pt:intercalator

X=NH	n=2,3,6	R=H
X=O	n=3	R=H
X=NH	n=3	R=H
X=OCH$_2$CH$_2$O	n=2	R=H

Figure 1. Pt complexes of anthraquinones.

a narrow range of activity, severe side effects, and the phenomenon of acquired resistance.[11] Thus, the search for Pt-based compounds with improved therapeutic properties still goes on.

cis-DDP is often administered to cancer patients in combination with intercalative drugs such as adriamycin and actinomycin.[12,13] In an attempt to understand the nature of this clinical synergism, Tullius et al.[14,15] demonstrated that ethidium bromide (EthBr) affected the DNA-binding properties of *cis*-DDP while neither acridine orange (AO) nor adriamycin exhibited such effects. Bowler and Lippard[16-18] have tethered AO to Pt(en)Cl_2 via a tri- and hexamethylene linker chain and compared the modulation of the DNA binding properties of *cis*-DDP by the free and linked intercalator. They rationalized the interaction in terms of sequence preferences, stereochemistry, and relative residence times. The formation of metastable ternary complexes among intercalators, DNA, and *cis*-DDP has been reported by Malinge et al.,[19,20] and subsequently Sundquist et al.[21] prepared such compounds and demonstrated that DNA facilitates the reaction between intercalators and *cis*-DDP.

To date, most of the Pt intercalator work has focused on the affects of intercalation on the DNA-binding properties of platinum complexes. We have not encountered any studies designed to probe the antitumor activities of Pt–intercalator systems by performing systematic studies of the structure–activity relationships. We have decided to perform such a study on a novel series of compounds where the platinum moiety is tethered to various anthraquinones (see Figure 1). We have selected the anthraquinones as the intercalators for three main reasons: (a) the anthraquinones are closely related to the intercalative moiety in the antitumor drug adriamycin,[22,23] (b) simple bis[(aminoalkyl)amino] derivatives of 9,10-anthracenedione have demonstrated remarkable antitumor properties,[24-26] and (c) this system lends itself to flexible preparative work, allowing variation of structural and electronic parameters.[27,28]

In this paper we report the preparation, characterization, and the biological activities of two novel series of complexes where PtX_2 (X = Cl, I) is tethered to functionalized anthraquinones.

Results

Chemical Studies. Preparation of Ligands. The synthesis of most of the ligands used in this study has been described recently.[29] We have prepared seven new ligands for this specific study and used the preparative routes outlined in Figure 2.[29] Since all the ligands consist of an anthraquinone moiety bearing a side chain containing primary and/or secondary amines, purification of the ligands was effected by extraction into acidified aqueous solutions and their subsequent isolation as the hydrochloride salts.

Preparation of *cis*-PtL_2I_2 Complexes. Initially we have attempted to prepare these complexes by the reaction of aqueous tetraiodoplatinate (generated in situ by addition of 4.1 equiv of KI to an aqueous solution of K_2PtCl_4) with a methanolic solution of the free amine form of the various ligands. This method proved unsatisfactory since it often resulted in the precipitation of unreacted salts. This obstacle was overcome by generating the K_2PtI_4 in a minimal amount of water in DMF using a 2-fold excess of iodide. The ensuing reaction with 2 equiv of the ligands proceeded for long periods of time without occurrence of precipitation. Evaporation of the DMF followed by addition of water precipitated out the water-insoluble neutral diaminediodoplatinum complexes. The solid obtained was first triturated with water in order to remove any unreacted Pt compounds and inorganic salts and then rinsed

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Table I. ^{195}Pt NMR Spectral Data for the *cis*-PtL_2X_2 Complexes

no.	compound	δ, ppm
1	*cis*-Pt(1C3)$_2$Cl$_2$	−2230
2	*cis*-Pt(1C4)$_2$Cl$_2$	−2255
3	*cis*-Pt(1C6)$_2$Cl$_2$	−2218
4	*cis*-Pt(1C12)$_2$Cl$_2$	−2218
5	*cis*-Pt(1OC3)$_2$Cl$_2$	−2223
6	*cis*-Pt(1C3)$_2$I$_2$	−3346
7	*cis*-Pt(1C4)$_2$I$_2$	−3334
8	*cis*-Pt(1C6)$_2$I$_2$	−3346
9	*cis*-Pt(1C12)$_2$I$_2$	−3324
10	*cis*-Pt(1OC3)$_2$I$_2$	−3330
11	Pt(1C2C2)I$_2$	−3455
12	Pt(1C3C2)I$_2$	−3455
13	Pt(1C2C2)Cl$_2$	−2342
14	Pt(1C3C2)Cl$_2$	−2342
15	Pt(1C6C2)Cl$_2$	−2348
16	Pt(1C3C2NHE)Cl$_2$	−2345
17	Pt(1OC3C2)Cl$_2$	−2321
18	Pt(1OC2OC2C2)Cl$_2$	−2343

with ethanol. This was followed by trituration with chloroform to remove any unreacted ligands and finally the products were dried with ether.

Preparation of *cis*-PtL_2Cl_2 Complexes. These complexes have been prepared by two routes. The first method involved the reaction of PtL_2I_2 with 2 equiv of $AgNO_3$, removal of the AgI precipitate, and subsequent addition of dilute HCl to precipitate out the dichloro complex. The second method involved the direct reaction between the free amines and K_2PtCl_4 in water/DMF. These reactions were allowed to proceed for 4–8 days at room temperature and then a purification procedure similar to the one discussed above was employed. The latter procedure seemed more effective and resulted in better yields and purer products.

Preparation of $PtLCl_2$ Complexes. The dichloro complexes containing a 1:1 ratio of Pt–intercalator was prepared by reaction of K_2PtCl_4 with 1 equiv of the free diamine ligands in a mixture of methanol and water. The product precipitated out of the reaction mixture but no precipitation of unreacted salts was observed. The high solubilities of the reagents coupled with the low solubility of the product in the water/methanol system helped to drive the reaction toward completion, resulting in relatively high yields as well as pure complexes. As this method seemed satisfactory, no attempts were made to utilize the corresponding diiodo complexes as precursors for a Dhara type synthesis.

All the platinum complexes that were analyzed satisfactorily for C, H, N, X were examined by ^{195}Pt NMR spectroscopy and FTIR. The results of the ^{195}Pt NMR studies are listed in Table I. As the chemical shift range for Pt(II) compounds in ^{195}Pt NMR is rather broad and since the chemical shift is sensitive to the nature of the donor atoms, this technique provides an extremely useful tool for the characterization of platinum complexes. The resonances observed in these complexes are well in agreement with literature values.[30] Since all complexes of this type are insoluble in aqueous solution, all the NMR spectra were measured in DMF solutions. The combination of an acceptable elemental analysis with a clean ^{195}Pt NMR spectrum served as the criteria for the characterization of the complexes.

Since all the compounds are neutral diaminedihalo complexes, they are insoluble in water, but dissolve readily in DMF and DMSO. The high reactivity of DMSO toward Pt complexes can produce undesirable effects.[31] Thus, DMF was used as the solvent for the NMR and ED_{50} studies. By employing surfactants (6% TWEEN-80) we were able to significantly increase the aqueous solubility of most of the complexes (ca. 1.6 mg/mL).

Biological Studies. All the compounds were tested for in vitro growth inhibitory activity against P388 murine leukemia cells and against their multidrug resistant subline (P388/ADR). The in vivo toxicity of the compounds that have shown the highest activities in these tests was also evaluated. Compounds that fared well in the in vitro and toxicity studies were screened for in vivo activity against P388 leukemia.

The results of the biological studies of the complexes are given in Table II. For comparison, the results obtained with *cis*-DDP, Pt(en)Cl$_2$, the free ligands and mixtures of the free ligands, and the platinum complexes which were measured under the same experimental conditions are shown in Table III. The structure–activity relationships observed in these studies can be summarized as follows (see

Table II. Biological Data for the *cis*-PtL_2X_2 Complexes

		ED_{50} (P388), μM			
no.	compound	S/ADR	R/ADR	LD_{50}[a]	T/C[b]
1	*cis*-Pt(1C3)$_2$Cl$_2$	6	>30		
2	*cis*-Pt(1C4)$_2$Cl$_2$	8	>60		
3	*cis*-Pt(1C6)$_2$Cl$_2$	>60	>60		
4	*cis*-Pt(1C12)$_2$Cl$_2$	60	>60		
5	*cis*-Pt(1OC3)$_2$Cl$_2$	>10	>10		
6	*cis*-Pt(1C3)$_2$I$_2$	40	>60		
7	*cis*-Pt(1C4)$_2$I$_2$	40	>60		
8	*cis*-Pt(1C6)$_2$I$_2$	>60	>60		
9	*cis*-Pt(1C12)$_2$I$_2$	60	>60		
10	*cis*-Pt(10C3)$_2$I$_2$	>10	>10		
11	Pt(1C2C2)I$_2$	4.5	12		
12	Pt(1C3C2)I$_2$	1.2	>10		
13	Pt(1C2C2)Cl$_2$	0.2	>60	>28	126 (10)
14	Pt(1C3C2)Cl$_2$	0.45	>60	>28	130 (20)
15	Pt(1C6C2)Cl$_2$	45	>60		
16	Pt(1C3C2NHE)Cl$_2$	>30	>60		
17	Pt(1OC3C2)Cl$_2$	0.45	12	'>28	120 (10)
18	Pt(1OC2OC2C2)Cl$_2$	0.8	>60		127 (10)

[a] The LD_{50} values are given in mg/kg. [b] The optimal dose in units of mg/kg is given in parentheses.

Table III. Biological Data for the Pt Standards, Free Ligands, and 1:1 Molar Mixtures

		ED_{50} (P388), μM			
no.	compound	S/ADR	R/ADR	LD_{50}[a]	T/C[b]
	cis-Pt(NH$_3$)$_2$Cl$_2$	0.19	1.4	6.6	134
	Pt(en)Cl$_2$	6	20		
19	1C3	20	45		
20	1C4	40	50	200	
21	1C6	45	45	133	
22	1C12	60	20		
23	1OC3	10	>10		
24	1C2C2	4.5	8		
25	1C3C2	4.5	8		
26	1C6C2	4.5	8	200	
27	1OC3C2	6	60		
28	1C3C2NHE	20	>10		
29	1OC2OC2C2	0.2	>60		
	Pt(en)Cl$_2$ + 1C2	8	12		
	Pt(en)Cl$_2$ + 1C3	4.5	12		
	Pt(en)Cl$_2$ + 1C6	4.5	12		
	Pt(en)Cl$_2$ + 1C3C2	3	4.5		
	cis-DDP + 1C3C2	0.8	6		

[a] The LD_{50} values are given in mg/Kg. [b] The optimal dose in units of mg/Kg is given in parenthesis.

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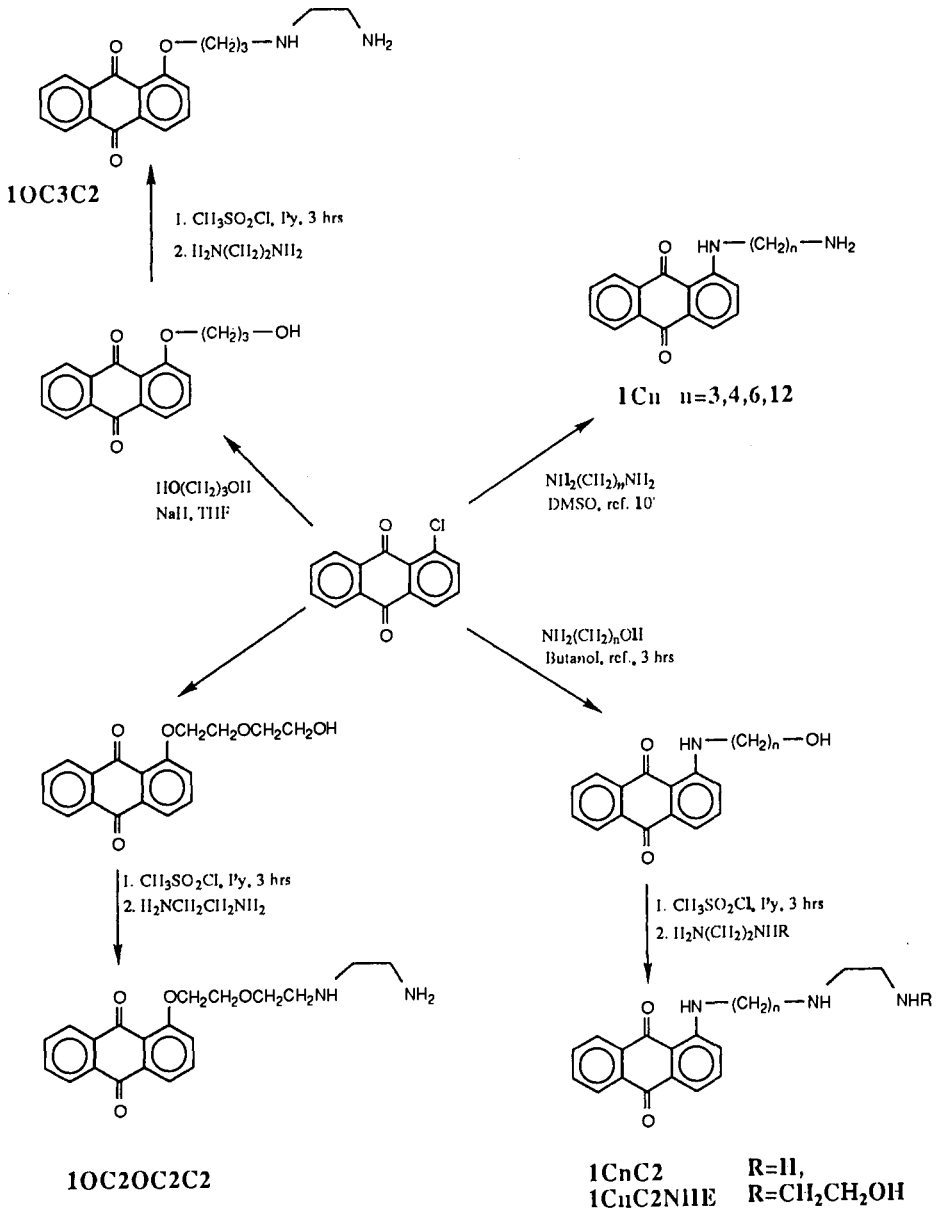

Figure 2. Ligand synthesis and nomenclature.

the Experimental Section for abbreviations):

1. In the *cis*-Pt(1C*n*)$_2$Cl$_2$ series, the activity seems to decrease with increasing chain length.
2. The diiodo complexes are less active than their dichloro counterparts.
3. Changing the exocyclic linking heteroatom in position 1 (an ether linkage instead of an NH group) does not significantly alter the activity.
4. The 1:1 Pt–intercalator complexes, Pt(1C*n*C2)Cl$_2$, displayed higher activity than the 1:2 complexes.
5. The highest activities for the 1:1 Pt–intercalator complexes were obtained with the shorter linker chain lengths (n = 2, 3).

The 1:2 Pt–intercalator complexes were not very active in the in vitro studies performed. Variation of the chain length did have some effect on the activity. We thought that a medium-length linker chain (n = 5, 6) should facilitate simultaneous intercalation and covalent binding, yet the complexes with the shorter linker chains were more active. The iodide complexes were less active than their chloride counterparts. Changing the electronic properties of the anthraquinone unit by substituting an oxygen atom for the NH linking group in the 1 position of the anthraquinone did not significantly affect the activity.

In contrast to the 1:2 Pt–intercalator complexes, the 1:1 complexes displayed much higher activities and it is interesting to note that the 1:1 diiodo complexes were more active than the 1:2 dichloro complexes. The results listed in Table II clearly show that three compounds, [Pt(1C2C2)Cl$_2$, Pt(1C3C2)Cl$_2$, and Pt(1OC3C2)Cl$_2$], have ED$_{50}$ values of approximately 10^{-7} M, which are the same order of magnitude as that of *cis*-DDP and significantly less than that of Pt(en)Cl$_2$. Also, these complexes are significantly more active than the free ligands themselves. As the chain length increases from two methylene groups to three, there is a slight decrease in activity, and upon extending the linker chain to six methylene groups, there is significant loss in antitumor activity. The decrease in activity resulting from elongation of the linker chain is more pronounced in the 1:1 complexes (displaying a sharp cutoff point) than in the 1:2 complexes. This phenomenon might be related to the ability of these compounds to bind DNA.

While some of the compounds displayed in vitro cytotoxic activities similar to that of *cis*-DDP, evaluation of the therapeutic potential necessitates examination of the in vivo toxicities of these compounds relative to that of

cisplatin. Thus we have determined the median lethal dose (LD_{50}) in mice. To enhance solubility in water, a surfactant was added. The low concentration of the surfactant which was used to increase the aqueous solubility (6% TWEEN 80) has been shown to have no adverse effects,[32] and the ED_{50} values of the compounds dissolved in a solution of 6% TWEEN 80 in water compared well with those obtained with DMF as the solvent. We were unable to determine the LD_{50} values for most of the compounds since even with saturated solutions (26.6 mg/kg) no mortalities were observed. Therefore, it is clear that on a molar basis *cis*-DDP (LD_{50} = 6.6 mg/kg) is at least 2 times more toxic than these compounds, while displaying similar in vitro cytotoxic activity.

All the complexes presented in this study are comprised of two separate moieties, each a cytotoxic agent in its own right. One of the underlying ideas of this undertaking was to try and obtain compounds which are more active than the sum of their components. We tested this hypothesis by measuring the ED_{50} values of the 1:1 molar mixture of *cis*-$Pt(NH_3)_2Cl_2$ or $Pt(en)Cl_2$ with the free ligands. The results of these studies, which appear in Table III, indicate that the activities measured for the various mixtures (which were chosen in an attempt to best emulate the active complexes) were significantly lower than those of the complexes. This indicates that the high activity of the complexes, which far exceeds the additive effect of the two components, is imparted by their unique structure.

In vivo screening against P388 leukemia cells was carried out for the four most promising compounds. The T/C values measured for the four compounds were around 120 while the T/C for cisplatin was measured at 134 (see Table II and III).

Discussion

To date, only one system of platinum-linked intercalators has been reported. The potency of $Pt(en)Cl_2$ tethered by a hexamethylene linker chain to AO has been found to be somewhat less than that of cisplatin on in vivo model systems.[16-18] The Pt–AO work focused on the interaction of the complexes with DNA but does not represent a systematic study of structure–activity relationships. To the best of our knowledge no 1:2 Pt–intercalator complexes have ever been prepared and characterized.

While the 1:2 complexes, whether dichloro or diiodo, were not very active, some of the 1:1 complexes displayed high in vitro and in vivo activities. The 1:2 complexes have four possible DNA binding sites (two intercalative and two covalent) versus three for the 1:1 complexes, and seemingly the former might have a higher affinity for DNA. Even if these complexes have the same mode of action as cis-DDP and effect cytotoxicity by binding to the DNA and arresting normal replication and transcription processes, DNA binding affinities need not be the sole criterion for cytotoxic activity. The lower solubility and the higher hydrophobicity of the 1:2 complexes might interfere with their ability to penetrate the membrane and react with DNA.

The high activity of $Pt(1C2C2)Cl_2$, $Pt(1C3C2)Cl_2$, and $Pt(1OC3C2)Cl_2$ and the inactivity of $Pt(1C6C2)Cl_2$ prove the strong dependence of activity on chain length. Bowler et al. reported that a flexible linker chain of six methylene groups allows covalent binding of the Pt(en) unit to the DNA with the AO intercalating one or two base pairs away. Yet, in the Pt–anthraquinone system, linker chains of di- and trimethylene groups, which appear to be too short to allow both intercalation and covalent chelation, displayed the highest activities. Geometrically, the functionalized AO and anthraquinone systems differ from each other in that the linker chain in the former is attached in the middle of the planar three-fused ring system (position 10) while the latter is attached in position 1. Also, the anthraquinone lacks the two NMe_2 substituents of the AO, facilitating a side-on intercalation similar to the one observed for daunomycin.[33] If platinum binding to DNA results in a 40° kinking of the DNA toward the major groove as suggested by Rice et al.,[34] then the shorter linker chain which protrudes into the major groove might aid intercalation. We are currently in the midst of DNA binding studies which are designed to answer some of these questions.

$Pt(1C2C2)Cl_2$, $Pt(1C3C2)Cl_2$, and $Pt(1OC3C2)Cl_2$ are more active than the closest platinum analogue, $Pt(en)Cl_2$, and more active than the free ligands themselves. Moreover, they are more active than the equimolar mixture of $Pt(en)Cl_2$ and the free ligands. This clearly demonstrates that chemically linking the intercalator to the Pt moiety enhances the activity of the complex and is preferable to a simple physical combination of the drugs in an attempt to obtain clinical synergism.

Conclusions

The systematic study of complexes where $Pt(en)Cl_2$ is linked to anthraquinones showed that 1:2 Pt–intercalators were not very active against P388 cell lines. Among the 1:1 Pt–intercalator complexes, the complexes with the shorter linker chains (n = 2, 3) were active while the complexes with the longer linker chains were not as active. The active compounds displayed higher activity than each of the components (the analogous $Pt(en)Cl_2$ and the free ligands) and higher than a 1:1 molar mixture of the two, demonstrating the usefulness of thethering the two units.

Experimental Section

Physical Methods. ^1H and ^{13}C NMR spectra were measured with a Bruker WH-300 spectrometer using a 5-mm dual probehead. ^{195}Pt NMR spectra (42.935 MHz) were measured on a Bruker WP-200 spectrometer using a 10-mm broadband probehead. Typical aquisition parameters included an 8-μs pulse with a spectral width of 125 000 Hz. Most spectra were processed with a 200-Hz line broadening. The ^{195}Pt chemical shifts were referenced externally to K_2PtCl_4 in D_2O at −1624 ppm. IR spectra were recorded on a Bruker IFS 113V spectrometer using a Ge-coated KBr beam splitter and an MCD detector. Typical spectra were recorded with 0.5% KBr pellets or polystyrene using 32 pulses and a 2 cm^{-1} resolution. Elemental analyses were performed by the Microanalytical Laboratory at the Hebrew University. Melting points were determined with a Thomas-Hoover capillary melting point apparatus.

Compound Preparation. All the reagents were purchased commercially. The ethylenediamine was distilled and the THF was distilled from sodium. All other reagents were used without further purification. K_2PtCl_4 was purchased from Aldrich.

The preparation and purification of 1C3, 1C4, 1C3C2, and 1C2C2 have already been detailed elsewhere.[29] We report the preparation of seven new anthraquinone derivatives used as ligands for the Pt complexes. All the ligands were characterized by ^1H NMR spectroscopy and mass spectrometry and their purity was verified by elemental analysis, melting point, and TLC. All the Pt complexes were characterized by elemental analysis, ^{195}Pt NMR, and FTIR.

Ligand Synthesis. 1-C6. 1-[(6-Aminohexyl)amino]-anthraquinone Hydrochloride (21). 1-Chloroanthraquinone

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(5 g, 0.0206 mol) was dissolved in 50 mL of DMSO and heated to reflux. 1,6-Diaminohexane (10 g, 0.086 mol) was added and kept under reflux for 5–10 min, and the solvent was evaporated to dryness. The dark red residue was then dissolved in chloroform, washed twice with water, and extracted with a 10% aqueous solution of HCl. The acidic solution was then evaporated at low pressure, and the residue was recrystallized from ethanol (yield 5.5 g, 74%). Anal. ($C_{20}H_{22}N_2O_2$·HCl) C, H, N, Cl.

1-C12. 1-[(12-Aminododecyl)amino]anthraquinone (22). This compound was prepared in the same fashion as the previous one. 1-Chloroanthraquinone (5 g, 0.0206 mol) and 18 g (0.09 moles) of 1,12-diaminododecane were refluxed in DMSO for 5–10 min. After evaporation of the solvent the pure product was obtained by column chromatography over alumina using chloroform, chloroform/MeOH 1:1 as eluent to yield 5.2 g (65%) of the free base. Anal. ($C_{26}H_{34}N_2O_2$) C, H, N.

1-C6C2. 1-[[6-[(2-Aminoethyl)amino]hexyl]amino]-anthraquinone Dihydrochloride (26). This compound was prepared in a manner analogous to the one described in ref 29 for the preparation of 1C2C2 to yield 60% of a dark red powder. Anal. ($C_{22}H_{27}N_3O_2$·2HCl) C, H, N, Cl.

1-C3C2NHE. 1-[[3-[[2-[(2-Hydroxyethyl)amino]ethyl]-amino]propyl]amino]anthraquinone (28). To a solution of 1.0 g (28 mmol) of 1-[[3-[(methylsulfonyloxy]propyl]amino]-anthraquinone[29] in 50 mL of $CHCl_3$ was added 1.6 g (11 mmol) of 2-[(2-aminoethyl)amino]ethanol and the mixture was stirred at room temperature for 24 h. The reaction solution was then evaporated to dryness and recrystallized from methanol/ether 1:1, yielding 700 mg of the final product (70%). An analytical sample (150 mg) was passed through a column of alumina gel (chloroform, chloroform/methanol 1:1). Anal. ($C_{21}H_{25}N_3O_3$).

1-OC3. 1-(3-Aminopropoxy)anthraquinone Nitrate (23). 3-(Benzylideneamino)propanol[35] was reacted with 1-chloroanthraquinone according to the method of Krapcho and Shaw.[36] 3-(Benzylideneamino)propanol (3 g, 0.018 mol) was added to a suspension of 0.62 g (0.0185 mol) of NaH (55% oil dispersion) in 20 mL of dry THF under an N_2 atmosphere. A solution of 3 g (0.0123 mol) of 1-chloroanthraquinone in 100 mL of THF was added after 0.5 h and the reaction was stirred at room temperature for 20 h. The mixture was then concentrated and poured into iced water and the resulting precipitate was filtered, washed with water, and suspended overnight in HNO_3 (10%) at room temperature. The mixture was filtered and the yellow filtrate was evaporated to dryness in a vacuum. Recrystallization from ethanol gave 2.8 g (66.3%) of nitrate salt, mp 205 °C. Anal. ($C_{17}H_{15}N-O_3$·HNO_3) C, H, N.

1-OC3C2. 1-[3-[(2-Aminoethyl)amino]propoxy]anthraquinone (27). As can be seen in Figure 2, the synthesis of this ligand is a three stage process. Following are the details of each of the synthetic steps.

(a) 1-(3-Hydroxypropoxy)anthraquinone. 1-Chloroanthraquinone (3 g, 0.0124 mol) was dissolved in 30 mL of THF and added to a dry THF solution (70 mL) containing 9 mL (0.124 mol) of 1,3-propanediol and 0.6 g (0.0186 mol) of NaH (55% oil dispersion). The reaction mixture was brought to reflux temperature and stirred overnight. The mixture was cooled and concentrated in a vacuum. The residue was added to H_2O (2 × 50 mL) and extracted with $CHCl_3$ (100 mL). The chloroformic phase was dried ($MgSO_4$) and concentrated in a vacuum. Purification was obtained by chromatographing the sample over a column of silica gel (EtOAc/petroleum ether 1:1, chloroform). Recrystallization from EtOAc yielded 1.8 g (55%), mp 164 °C. Anal. ($C_{17}H_{14}O_4$) C, H.

(b) 1-[3-[(Methylsulfonyl)oxy]propoxy]anthraquinone. To a solution of 1.2 g (4.2 mmol) of the product of part a in 50 mL of pyridine was added 0.718 g (6.3 mmol) of methanesulfonyl chloride and the mixture was stirred at room temperature for 3 h. The mixture was then evaporated to dryness, and the residue was dissolved in $CHCl_3$ (50 mL) and washed with H_2O (2 × 50 mL), 10% HCl (3 × 50 mL), and brine (2 × 50 mL). The chloroformic phase was then dried ($MgSO_4$), evaporated, and recrystallized from EtOAc, yielding 1.45 g (96%) of final product. Anal. ($C_{18}H_{16}O_6S$) C, H, S.

(c) 1-[3-[(2-Aminoethyl)amino]propoxy]anthraquinone Dihydrochloride. To a solution of 1.45 g (0.0040 mol) of the product of part b in 50 mL of CH_3CN was added 0.9 g (0.01011 mol) of 1,2-diaminoethane and the mixture was stirred at room temperature for 24 h. The solvent was then evaporated to dryness and the residue was dissolved in 50 mL of $CHCl_3$, washed with H_2O, and extracted with an aqueous solution of 10% HCl. The acidic solution was then evaporated at low pressure. The product was recrystallized from EtOH, yielding 1.0 g (77%). Anal. ($C_{19}H_{20}N_2O_3$·HCl) C, H, N, Cl.

1OC2OC2C2. 1-[2-[2-[(2-Aminoethyl)amino]ethoxy]ethoxy]anthraquinone (29). The preparation of this compound is a three-step process (Figure 2).

(a) 1-[2-(2-Hydroxyethoxy)ethoxy]anthraquinone. 1-Chloroanthraquinone (5 g, 0.02 mol) was dissolved in 30 mL of hot THF and added to a solution containing 11 g (0.1 mol) of diethylene glycol and 1 g (0.03 mol) of NaH (55% oil dispersion) in 100 mL of dry THF. The reaction was brought to reflux temperature and stirred overnight. The mixture was then cooled and concentrated in a vacuum. The residue was then added to H_2O (2 × 100 mL) and extracted with $CHCl_3$ (200 mL). The chloroformic phase was dried ($MgSO_4$) and concentrated in a vacuum. The residue was then chromatographed over a silica gel column (chloroform, chloroform/MeOH 1:1). Recrystallization from CH_2Cl_2 gave 3 g of the desired product (yield, 48%), mp 170 °C. Anal. ($C_{18}H_{16}O_5$) C, H.

(b) 1-[2-[2-[(Methylsulfonyl)oxy]ethoxy]ethoxy]anthraquinone. To a solution of 1.2 g (3.8 mmol) of the product from part a in 50 mL of pyridine was added 0.52 g (4.56 mmol) of methanesulfonyl chloride and the mixture was stirred at room temperature for 3 h. The mixture was evaporated to dryness and the residue was dissolved in $CHCl_3$ (50 mL) and washed with H_2O (2 × 50 mL), 10% HCl (2 × 50 mL), and brine (2 × 50 mL). The chloroformic layer was then dried ($MgSO_4$), evaporated, and recrystallized from ethyl acetate, yielding 1.4 g (95%) of final product. Anal. ($C_{19}H_{18}O_7S$) C, H.

(c) 1-[2-[2-[(2-Aminoethyl)amino]ethoxy]ethoxy]anthraquinone Dihydrochloride. To a solution of 1 g (2.5 mmol) of the product from part b in 50 mL of chloroform was added 0.6 mg (1 mmol) of 1,2-diaminoethane and the mixture was stirred at room temperature for 24 h. The reaction solution was then washed with H_2O and extracted with an aqueous solution of 10% HCl. The acidic solution was then evaporated at low pressure and the product was recrystallized from ethanol, yielding 0.75 g of final product (70%). Anal. ($C_{20}H_{22}N_2O_4$·2HCl) C, H, N, Cl.

Complex Preparation. General Procedure for *cis*-PtL_2I_2. K_2PtCl_4 (207.5 mg, 0.5 mmol) was dissolved in 5 mL of water and a solution of 664 mg (4 mmol) of KI in 5 mL of H_2O was added. The solution was stirred at room temperature for 30 min. This solution was then diluted to 150 mL with DMF and a solution of 1 mmol of the appropriate aminoanthraquinone either as the free amine or as the salt (adding 10 mL of 0.1 N NaOH) in 100 mL of DMF was slowly added over 1 h and stirred at 50 °C for 3 days or at room temperature for 7 days. The solution was then concentrated to a volume of 10 mL and after addition of 15 mL of water the product precipitated. This was filtered, washed with H_2O, ethanol, ether, and chloroform, and dried in vacuo at 50 °C.

***cis*-Bis[[3-(anthraquinon-1-ylamino)propyl]amine]diiodoplatinum(II) (6):** yield 70%. Anal. ($PtC_{34}H_{32}N_4O_4I_2$) C, H, N.

***cis*-Bis[[4-(anthraquinon-1-ylamino)butyl]amine]diiodoplatinum(II) (7):** yield 40%. Anal. ($PtC_{36}H_{36}N_4O_4I_2$) C, H, N.

***cis*-Bis[[6-(anthraquinon-1-ylamino)hexyl]amine]diiodoplatinum(II) (8):** yield 60%. Anal. ($PtC_{40}H_{44}N_4O_4I_2$) C, H, N.

***cis*-Bis[[12-(anthraquinon-1-ylamino)dodecyl]amine]diiodoplatinum(II) (9):** yield 68%. Anal. ($PtC_{52}H_{68}N_4O_4I_2$) C, H, N.

***cis*-Bis[[3-(anthraquinon-1-yloxy)propyl]amine]diiodoplatinum(II) (10):** yield 83%. Anal. ($PtC_{34}H_{30}N_2O_4I_2$) C, H, N, I.

General Procedure for *cis*-PtL_2Cl_2. Method 1. The PtI_2L_2 complex (0.2 mmol) was dissolved in 100 mL of DMF and 0.39 mmol of $AgNO_3$ was dissolved in 2 mL of DMF. The two solutions

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(36) Krapcho, A. P.; Shaw, K. J. *J. Org. Chem.* **1988**, *48*, 3541.

were combined, and the resulting solution was stirred in the dark for 30 min at 50 °C. The solution was then filtered through Celite to remove the AgI and 4 mL of 0.4 N HCl was added. After stirring of the solution for 48 h at 50 °C, all but 5–6 mL of the DMF was removed by rotary evaporation. The product was precipitated by adding water. The solid was collected by filtration, washed with water, EtOH, and Et_2O, and dried in vacuo.

***cis*-Bis[[3-(anthraquinon-1-ylamino)propyl]amine]dichloroplatinum(II) (1)**: yield 57% (overall yield from K_2PtCl_4 40%). Anal. ($PtC_{34}H_{32}N_4O_4Cl_2$) C, H, N.

***cis*-Bis[[4-(anthraquinon-1-ylamino)butyl]amine]dichloroplatinum(II) (2)**: yield 76% (overall yield from K_2PtCl_4 30%). Anal. ($PtC_{36}H_{36}N_4O_2Cl_2$) C, H, N.

***cis*-Bis[[6-(anthraquinon-1-ylamino)hexyl]amine]dichloroplatinum(II) (3)**: yield 49% (overall yield from K_2PtCl_4 29%). Anal. ($PtC_{40}H_{44}N_4O_4Cl_2$) C, H, N.

***cis*-Bis[[12-(anthraquinon-1-ylamino)dodecyl]amine]dichloroplatinum(II) (4)**: yield 90% (overall yield from K_2PtCl_4 60%). Anal. ($PtC_{52}H_{68}N_4O_2Cl_2$) C, H, N.

***cis*-Bis[[3-(anthraquinon-1-yloxy)propyl]amine]dichloroplatinum(II) (5)**: yield 86% (overall yield from K_2PtCl_4 70%). Anal. ($PtC_{34}H_{34}N_2O_4Cl_2$) C, H, N, Cl.

Method 2. K_2PtCl_4 (0.5 mmol) was dissolved in 3 mL of water and 50 mL of DMF was added to the solution. The appropriate aminoanthraquinone (1 mmol) as the free base or as the salt (adding 10 mL of 0.1 N NaOH) was dissolved in 50 mL and added dropwise to the K_2PtCl_4 solution over 1 h. DMF was added when necessary in order to keep everything in solution. The solution was stirred for 7 days at 50 °C. The solvent was then reduced to a small volume by rotary evaporation, followed by addition of water in order to precipitate the product, which was filtered, washed with EtOH, Et_2O, $CHCl_3$, and then dried in vacuo.

***cis*-Bis[[3-(anthraquinon-1-ylamino)propyl]amine]dichloroplatinum(II) (1)**: yield 60%. Anal. ($PtC_{34}H_{32}N_4O_4Cl_2$) C, H, N.

***cis*-Bis[[4-(anthraquinon-1-ylamino)butyl]amine]dichloroplatinum(II) (2)**: yield 40%. Anal. ($PtC_{36}H_{36}N_4O_2Cl_2$) C, H, N.

***cis*-Bis[[6-(anthraquinon-1-ylamino)hexyl]amine]dichloroplatinum(II) (3)**: yield 60%. Anal. ($PtC_{40}H_{44}N_4O_4Cl_2$) C, H, N.

***cis*-Bis[[12-(anthraquinon-1-ylamino)dodecyl]amine]dichloroplatinum(II) (4)**: yield 42%. Anal. ($PtC_{52}H_{68}N_4O_2Cl_2$) C, H, N.

***cis*-Bis[[3-(anthraquinon-1-yloxy)propyl]amine]dichloroplatinum(II) (5)**: yield 86%. Anal. ($PtC_{34}H_{34}N_2O_4Cl_2$) C, H, N, Cl.

Preparation of $PtLI_2$. K_2PtCl_4 (207.5 mg, 0.5 mmol) were dissolved in 5 mL of water and a solution of 664 mg (4 mmol) of KI in 5 mL of H_2O was added. The solution was stirred at room temperature for 30 min. This solution was then diluted to 150 mL with DMF and a solution of 0.5 mmol of the appropriate bidentate aminoanthraquinone dihydrochloride salt in 100 mL of DMF (to which 10 mL of 0.1 N NaOH had been added) was added over 1 h and the resulting mixture was stirred at 50 °C for 3 days. The solution was then concentrated to a volume of 10 mL and after addition of 10 mL of H_2O the product precipitated. This was filtered, washed with H_2O, EtOH, Et_2O, and $CHCl_3$ and dried in vacuo.

[[2-[[2-(Anthraquinon-1-ylamino)ethyl]amino]ethyl]amine-*N*,*N*']diiodoplatinum(II) (11): yield 65%. Anal. ($PtC_{18}H_{19}N_3O_2I_2$) C, H, N.

[[2-[[3-(Anthraquinon-1-ylamino)propyl]amino]ethyl]amine-*N*,*N*']diiodoplatinum(II) (12): yield 52%. Anal. ($PtC_{19}H_{21}N_3O_2I_2$) C, H, N.

Preparation of $PtLCl_2$. K_2PtCl_4 (0.5 mmol) was dissolved in 10 mL of H_2O. The appropriate bidentate aminoanthraquinone dihydrochloride (0.5 mmol) was dissolved in 100 mL of MeOH (to which 10 mL of 0.1 N NaOH had been added) and was added to the K_2PtCl_4 solution over 1 h. MeOH was added when needed to keep everything in solution. The solution was stirred at room temperature until the product precipitated from the solution. The product was filtered, washed with water, EtOH, and Et_2O, and dried in a vacuum.

[[2-[[2-(Anthraquinon-1-ylamino)ethyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II) (13): yield 70%. Anal. ($PtC_{18}H_{19}N_3O_2Cl_2$) C, H, N, Cl.

[[2-[[3-(Anthraquinon-1-ylamino)propyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II) (14): yield 45%. Anal. ($PtC_{19}H_{21}N_3O_2Cl_2$) C, H, N, Cl.

[[2-[[6-(Anthraquinon-1-ylamino)hexyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II) (15): yield 70%. Anal. ($PtC_{22}H_{27}N_3O_2Cl_2$) C, H, N, Cl.

[[2-[[3-(Anthraquinon-1-ylamino)propyl]amino]ethyl](2-hydroxyethyl)amine-*N*,*N*']dichloroplatinum(II) (16): yield 70%. Anal. ($PtC_{21}H_{25}N_3O_3Cl_2$) C, H, N, Cl.

[[2-[[3-(Anthraquinon-1-yloxy)propyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II) (17): yield 70%. Anal. ($PtC_{19}H_{20}N_2O_3Cl_2$) C, H, N, Cl.

[[2-[[2-[2-(Anthraquinon-1-yloxy)ethoxy]ethyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II) (18): yield 70%. Anal. ($PtC_{20}H_{22}N_2O_4Cl_2$) C, H, N, Cl.

In Vitro Cytotoxic Screening. P388 murine leukemia cells and a multidrug resistant subline (P388/ADR) were propagated continuously in suspension culture. Cells were grown in Roswell Park Memorial Institute 1640 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 10 μM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO), 50 units/mL of penicillin base, and 50 μg/mL of streptomycin base (both from GIBCO). Cell growth was assessed by measuring cell density in a Coulter counter (Coulter Electronics Ltd., Harpenden, Hertfordshire, UK). An inoculum of cells was transferred to fresh medium once every 4 days, in order to maintain growth in an exponential phase. Initial cell density was 10^5 cells/mL and after 4 days in culture it reached $1–2 \times 10^6$ cells/mL. Cell growth rates were calculated from the culture densities measured once a day for 4 days.

The sensitivity of a cell line to a given compound was assessed as follows: cells were cultured in the presence of various concentrations for 4 days and the slope of the log of cell density versus time plot was calculated by linear-regression analysis. The growth rate at each drug concentration was expressed as a percentage of the control growth rate. In this manner dose–effect curves were produced and used to determine the compounds drug concentration effective at inhibiting the growth rate by 50% (ED_{50}). The adriamycin ED_{50} for the drug sensitive and the drug resistant cell lines was $2–6 \times 10^{-8}$ M and from 1×10^{-6} to 2×10^{-6} M, respectively. No change in the drug sensitivity of either cell line was observed during 8 years of continuous in vitro culture. The ED_{50} values obtained have a standard error of less than 10% of the mean.

In Vivo Toxicity Studies. Groups of five Viale mice weighing 30 ± 3 g were injected ip with increasing concentrations of each test compound dissolved in 6% Tween 80 in water in a constant volume of 0.5 mL. The control group received the same volume of dissolving solution. The mice were observed for 30 days and mortality was recorded. The median lethal dose (LD_{50}) was calculated from the 30th day survival–dose data.

In Vivo Antitumor Screening. DBA/2 mice were inoculated intraperitoneally (ip) with 10^6 P388 cells on day 0 and on day 1 groups of five mice were injected ip with increasing doses of the compound tested. The mice were observed for 30 days and mortality was recorded. The mean survival time for the control group as well as for the mice injected with the dissolving solution was ±11 days. For each of the treated groups, the ratio of the mean survival time to the mean survival time of the control group (T/C) was also calculated.

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