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Cytotoxicity of Tantalum(V) and Niobium(V) Small Carborane Complexes and Mode of Action in P388 Lymphocytic Leukemia Cells

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The metallacarboranes $(Et_2C_2B_4H_4)TaCl_2(C_5H_5)$ (1), $(Et_2C_2B_4H_4)NbCl_2(C_5H_5)$ (2), $(Et_2C_2B_4H_4)TaCl_2(C_5Me_5)$ (3), $[(Me_3Si)_2C_2B_4H_4]TaCl_2(C_5H_5)$ (4) and $(Me_2C_2B_4H_4)TaCl_2(C_5H_5)$ (5) are potent cytotoxic agents against suspended tumors, producing cell death in several tissue culture lines; for example, all were effective against murine L1210 lymphoid leukemia, and all except 5 against murine P388 lymphocytic growth. Human leukemic growth is also retarded since 1–4 were effective against $Tmolt_3$ cell leukemia, all except 4 against $Tmolt_4$ leukemia, and 1, 2, and 5 against HI-60 leukemia. Cytotoxicity was found towards HuT-8 lymphoma, THP-1 acute monocytic leukemia and suspended HeLa-S[3] uterine carcinoma. Some but not all of the complexes were active against Sk-2 melanoma and Mcf-7 breast effusion growth. Mode-of-action studies in P388 lymphocytic leukemia cells showed that *de novo* purine synthesis was inhibited; this inhibition reduces DNA and RNA syntheses. Purine synthesis was reduced by compounds 3 and 4 at the regulatory enzymes, i.e. phosphoribosyl pyrophosphate (PRPP) amidotransferase and dihydrofolate reductase. The agents lowered d[ATP] and d[CTP] pools, further reducing DNA synthesis. The complexes afforded DNA fragmentation leading to apoptosis, but this was not by a mechanism of nucleoside alkylation, intercalation between base-pairs or cross-linking of the DNA strands. Copyright © 2000 John Wiley & Sons, Ltd.

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INTRODUCTION

The well-documented antitumor activity[1] of certain metallocene sandwich complexes, including neutral $(\eta^5\text{-}C_5H_5)_2MX_2$ complexes as well as salts of the ferrocenium ion $\{[(\eta)^5\text{-}C_5H_5)_2Fe^+]X^-\}$, which have demonstrated effectiveness against a range of solid tumors and/or tumor cell lines, suggests the possibility that cytotoxic behavior might be exhibited by other types of organometallic sandwich species. Recently, a collaborative study by Hall, Sneddon and their co-workers[2] showed that large ferratricarbaborane clusters $(\eta^5\text{-}C_5H_5)Fe(MeC_3B_7H_9)$ and $[(\eta^5\text{-}C_5H_5)Fe(MeC_3B_7H_9)]^+X^-$, which incorporate 11-vertex FeC_3B_7 polyhedral cages, cause cell death in several tissue culture lines, including L-1210, $Tmolt_3$, HL-60, and HeLa-S[3]. These findings are particularly intriguing when one considers the substantial differences in size and structure between the ferratricarboranes and metallocene complexes, and suggest that an investigation of possible antitumor properties in small metallacarborane complexes was in order. The pentagonal-pyramidal small-carborane *nido*-$R_2C_2B_4H_4^{2-}$ ligand is comparable in size to $\eta^5\text{-}C_5H_5^-$ and isoelectronic with it, and the steric properties of its mononuclear sandwich complexes are similar to those of $(\eta^5\text{-}C_5H_5)_2MX_2$ metallocenes.[3] Moreover, their aerobic stability and ease of introduction of

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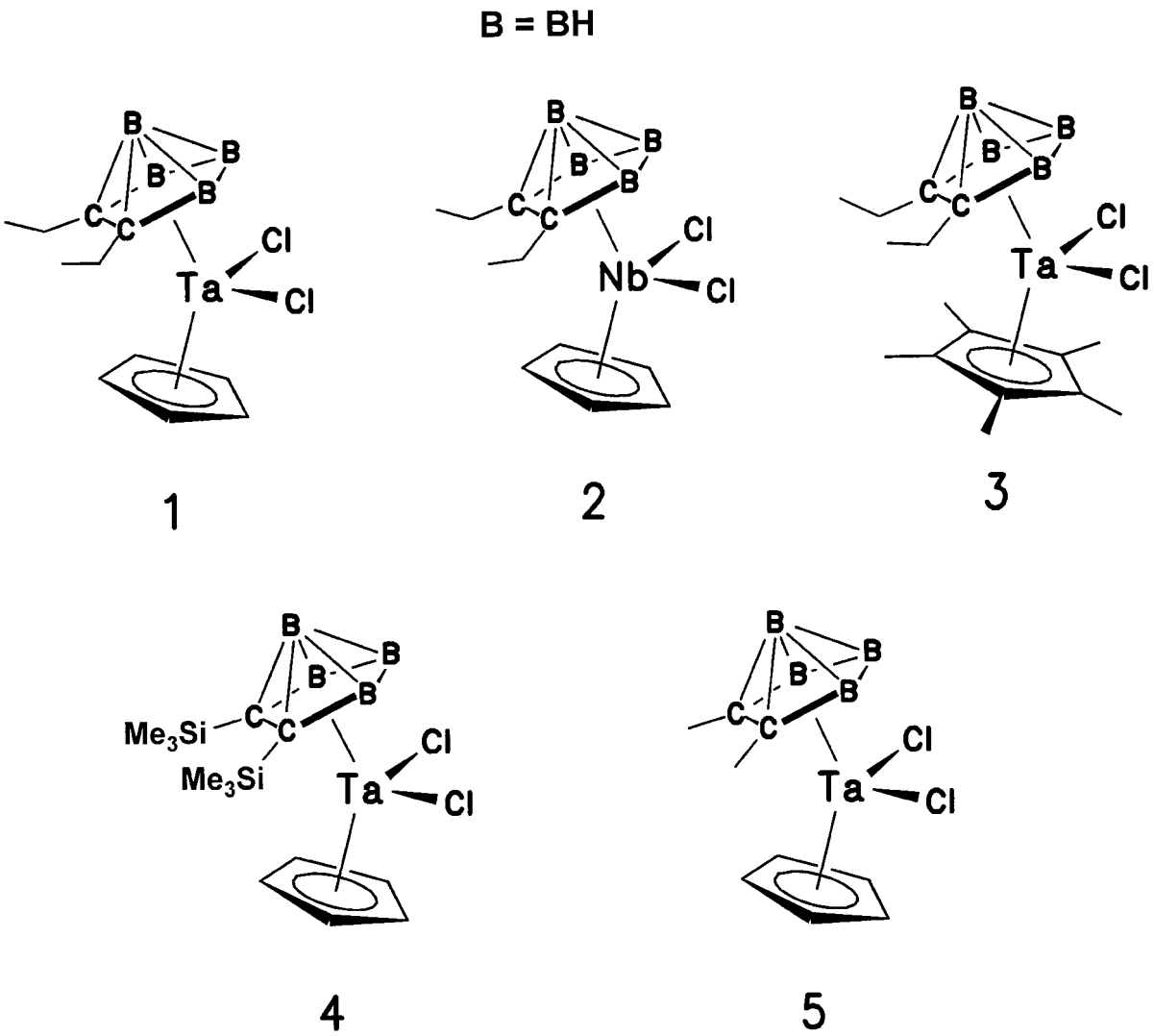

Figure 1 Structures of $(Et_2C_2B_4H_4)TaCl_2(C_5H_5)$ (**1**), $(Et_2C_2B_4H_4)NbCl_2(C_5H_5)$ (**2**), $(Et_2C_2B_4H_4)TaCl_2(C_5Me_5)$ (**3**), $[(Me_3Si)_2C_2B_4H_4]TaCl_2(C_5H_5)$ (**4**) and $(Me_2C_2B_4H_4)TaCl_2(C_5H_5)$ (**5**).

different metals and substituents[4] affords considerable versatility in the electronic fine-tuning of such species. As candidates for an initial screening we selected the early transition-metal species shown in Fig. 1, which had been prepared and characterized previously.[5]

MATERIALS AND METHODS

Source of compounds

Samples of the five metallacarboranes **1**–**5** were prepared and purified by the methods previously described.[5]

Cytotoxicity

Compounds **1**–**5** (Tables 1 and 2) were tested for cytotoxic activity by homogenizing the drugs as 1 mg ml^{-1} solutions in 0.05% Tween 80/H_2O, which were sterilized by passing them through an Acrodisc (0.45 μm). Different histological types of murine and human tumors were selected for cytotoxic testing of the agents to evaluate their potency in a wide range of tumors. Included in this

Table 1 Cytotoxicity (ED_{50}, $\mu g\,ml^{-1}$) of complexes **1**–**5** against growth of suspended tumor cells[a]

Compound	L1210 leukemia	P388 leukemia	$Tmolt_3$ leukemia	$Tmolt_4$ leukemia	HI-60 leukemia	HuT-78 lymphoma	THP-1 acute monocytic leukemia	HeLa-S^3 uterine carcinoma
1	3.33	3.71	3.37	3.51	2.97	1.38	3.67	2.95
2	2.36	2.69	2.65	3.06	3.46	2.88	2.67	3.47
3	3.27	3.86	3.33	2.17	4.56	3.82	2.71	2.72
4	2.12	3.89	2.59	4.52	4.27	4.99	4.87	2.34
5	1.51	4.07	4.59	3.57	3.41	2.34	4.87	2.95
Standards								
6-MP	2.43	2.04	0.43	2.67	6.36	1.63	3.34	2.12
Ara-C	2.07	0.79	1.29	2.36	3.90	2.50	2.54	2.13
HU	2.67	1.30	4.47	6.68	5.22	3.87		1.96

[a] ED_{50} values of less than 4 $\mu g\,ml^{-1}$ are required for significant activity (Ref. 6). These calculations were derived from four determinations at each concentration of the agent, averaged to obtain the ED_{50} value by plotting the percentage inhibition vs the logarithm of the concentration. The four determinations in each case were within 3.6% of each other.

series were a number of tumors for which there are no effective drugs to inhibit their cell growth.

The following cell lines were maintained by literature techniques:[6] murine L1210 lymphoid leukemia and P388 lymphocytic leukemia, human $Tmolt_3$ and $Tmolt_4$ T-cell leukemia, HI-60 leukemia, Hut-78 cutaneous lymphoma, THP-1 monocytic leukemia, SW480 colorectal adenocarcinoma, HCT-8 ileocecal adenocarcinoma, MB-9812 lung bronchogenic, A-549 lung carcinoma, Soas-2 osteosarcoma, KB epidermoid nasopharynx, A431 skin epidermoid, HeLa-S^3 suspended and HeLa solid cervical carcinomas, Mck-7 breast effusion, clear cell renal Caki, Sk-MEL-2 malignant melanoma and U-87-MG glioma. The NCl protocol was used to assess the cytotoxicity of the test compounds and standards in each cell line.[6] Values of cytotoxicity were expressed as the ED_{50} [$\mu g\,ml^{-1}$], i.e. the concentration of the compound inhibiting 50% of cell growth. ED_{50} values were determined by the Trypan Blue exclusion technique.[6] A value of less than 4 $\mu g\,ml^{-1}$ was required for significant activity of growth inhibition. Solid tumor cytotoxicity was determined utilizing Crystal Violet/MeOH and read at 580 nm (Molecular Devices).[7]

Incorporation studies

The effects of drugs on the incorporation of radiolabeled precursors thymidine (72 Ci $mmol^{-1}$), uracil (12 Ci $mmol^{-1}$) or leucine (120 Ci $mmol^{-1}$) into [^{3}H]DNA, [^{3}H]RNA and [^{3}H]protein, respectively for 10^6 P388 cells at 25, 50 and 100 μM was determined over 60 min.[8] The acid-insoluble labeled DNA, RNA or protein was collected on GF/A disks which were counted in a Packard beta counter. The incorporation of [^{14}C]glycine (53.0 mCi $mmol^{-1}$) into purines was obtained by the method of Cadman *et al.*[9] Incorporation of [^{14}C]formate (53.0 mCi $mmol^{-1}$) into pyrimidines was determined by the method of Christopherson *et al.*[10] The final purines or pyrimidines were separated by TLC from the starting components using the appropriate R_f for standard nucleoside bases, and counted.

Enzyme assays

Since the tantalum(V) carborane complexes **1**–**5** effectively inhibited RNA and DNA syntheses in P388 cells, their effects on a number of enzymes involved in nucleic acid metabolism were determined at 25, 50 and 100 μM after 60 min incubations. DNA polymerase α activity was determined in cytoplasmic extracts isolated by Eichler *et al.*'s method.[11,12] The DNA polymerase α assay was described by Sawada *et al.*[13] with 2-deoxy [^{3}H] ribothymidine-5′-triphosphate (TTP) (53 Ci $mmol^{-1}$). Messenger, ribosomal and transfer RNA polymerase enzymes were isolated with different concentrations of ammonium sulfate; individual RNA polymerase activities were determined using [^{3}H]uridine-5′-triphosphate (UTP) (35 Ci $mmol^{-1}$).[14,15] The following enzyme activities were determined using P388 homogenates. Ribonucleoside reductase activity was measured using [^{14}C] cytidine-5′-diphosphate (CDP) (19.4 Ci $mmol^{-1}$) with dithioerythritol.[16] 2′-Deoxy

Table 2 Cytotoxicity (ED_{50}, $\mu g\,ml^{-1}$) of complexes **1**–**5** against growth of solid tumors[a]

Compound	KB Naso-pharynx	Colon SW480	Lung A549	Lung MB9812	HeLa solid uterine	Melanoma Sk-Mel2	HCT-8 ileum	MCF7 breast	Caki kidney	Saos-2 bone	A431 skin	Glioma U-87-MG
1	9.56	8.94	7.99	7.16	7.06	7.53	7.81	11.54	7.65	9.09	6.77	5.48
2	10.11	5.35	5.85	7.32	7.29	10.20	6.94	8.63	7.04	6.88	7.68	4.82
3	8.00	8.91	7.13	7.09	7.62	3.94	5.59	5.60	6.29	6.46	10.48	6.32
4	6.61	8.38	7.11	5.28	6.51	4.27	6.04	2.88	5.82	5.51	9.39	6.59
5	9.02	9.90	7.12	9.53	7.91	3.78	7.61	2.99	6.37	11.90	10.13	7.30
Standards												
6-MP	5.74	3.61	4.71	4.29	5.61	6.86	1.15	8.84	9.35	7.16	9.13	4.46
Ara-C	2.84	3.42	5.62	6.16	4.74	10.53	2.54	12.45	1.38	13.97	0.86	1.88
HU	5.27	7.33	8.89	7.18	8.12		1.77				2.87	2.27

[a] See footnote to Table 1.
ED_{50} value $\leq 4\ \mu g/ml$ required for significant activity.

[^{14}C] ribocytidine-5′-diphosphate was separated from the [^{14}C] CDP by TLC on polyethyleneimine cellulose (PEI) plates.

Thymidine, thymidine-5′-monophosphate (TMP) and thymidine-5′-diphosphate (TDP) kinase activities were determined using [^{3}H] thymidine (58.3 mCi mmol^{-1}) in the medium of Maley and Ochoa[17] and separated by TLC. Carbamyl phosphate synthetase activity was determined by the method of Kalman *et al.*[18] and the product citrulline was determined colorimetrically.[19] Aspartate transcarbamylase activity was measured using the incubation method of Kalman *et al.*,[18] the product carbamyl aspartate was determined colorimetrically by the method of Koritz *et al.*[20] Thymidylate synthetase activity was analyzed by the method of Kampf *et al.*[21] The $^{3}H_2O$ separated by charcoal was proportional to the amount of TMP formed from 2′-deoxy[^{3}H] ribouridine-5′-monophosphate (UMP) (20 Ci mmol^{-1}). Dihydrofolate reductase activity was determined by the NADH disappearance spectrophotometric method of Ho *et al.*[22] at 340 nm. Phosphoribosyl pyrophosphate (PRPP) amidotransferase activity was determined by the method of Spassova *et al.* as the generation of NADH[23] and inosine-5′-monophosphate (IMP) dehydrogenase activity was analyzed with 8-[^{14}C] IMP (54 mCi mmol^{-1}) (Amersham, Arlington Heights, IL, USA) after separating [^{14}C] xanthosine-5′-monophosphate (XMP) on PEI plates (Fisher Scientific) by TLC,[24] then counting it. Protein content was determined for the enzymic assays by the Lowry technique.[25]

DNA studies

After deoxyribonucleoside triphosphates were extracted[26] from P388 cells, d[NTP] levels were determined by the method of Hunting and Henderson[27] with calf thymus DNA, *E. coli* DNA polymerase I non-limiting amounts of the three deoxyribonucleoside triphosphates not being assayed, and either 0.4 mCi of [^{3}*H-methyl*]dTTP (53.1 Ci mmol^{-1}) or [5-^{3}H]dCTP (30 Ci mmol^{-1}). Thus, 2′-deoxyriboadenosine-5′-triphosphate (d[ATP], 2′-deoxyriboguanisine-5′-triphosphate (d[GTP]), 2′-deoxyribocytidine-5′-triphosphate (d[CTP]), and thymidine-5′-triphosphate (d[TTP]) levels were determined after incubation with the drugs for 60 min at 100 μM. The effects of compounds **1**–**5** on DNA strand scission were determined by the methods of Suzuki *et al.*,[28] Pera *et al.*[29] and Woynarowski *et al.*[30] P388 lymphocytic leukemia cells were incubated with 10 μCi [*methyl-*^{3}*H*]thymidine (84.0 mCi mmol^{-1}) for 24 h at 37 °C. P388 cells (10^7) were harvested and then centrifuged at 600 g $\times$ 10 min in phosphate-buffered saline (PBS). They were later washed and suspended in 1 ml of PBS. Lysis buffer (0.5 ml; 0.5 M NaOH, 0.02 M EDTA, 0.01% Triton X-100 and 2.5% sucrose) was layered onto a 5–20% alkaline-sucrose gradient (5 ml; 0.3 M NaOH, 0.7 M KCl and 0.01 M EDTA); this was followed by 0.2 ml of the cell preparation. After the gradient was incubated for 2.5 h at room temperature, it was centrifuged at 12 000 g $\times$ 17 h at 8 °C. Fractions (0.2 ml) were collected from the bottom of the gradient, neutralized with 0.2 ml of 0.3 M HCl, and measured for radioactivity. Calf thymus DNA thermal denaturation studies were conducted from 37 to 100 °C in order to determine the T_m values for the control and drugs at 100 μM for 24 h. Changes in DNA UV absorption between 220 and 340 nm, and DNA viscosity studies were conducted after incubation of compounds **1**–**5** at 100 μM at 37 °C for 24 h.[31]

Statistics

The mean and standard deviations are designated by $x \pm$ SD. The probable level of significance (P) between test and control samples was determined by Student's t-test with the raw data.

RESULTS

Cytotoxicity

The tantalum(V) carboranes proved to be potent cytotoxic agents against suspended tumor cells, e.g. all of the compounds were active in the murine L1210 lymphoid leukemia screen, with complex **5** affording the best activity of 1.51 μg ml^{-1} (Table 1). All of the compounds were active in the murine P388 lymphocytic screen except **5**. Human leukemic growth was also retarded by the compounds: **1**–**4** were effective against $Tmolt_3$ T-cell leukemia, **1**–**3** and **5** were effective against $Tmolt_4$ leukemia, and compounds **1**, **2** and **5** were effective against HI-60 leukemia growth. HuT-8 lymphoma growth was inhibited by compounds **1**–**3** and **5** and THP-1 acute monocytic leukemia growth was reduced by **1**–**3**. Suspended HeLa-S^3 uterine carcinoma growth was reduced significantly by all of the compounds. The tantalum(V) carborane complexes were as potent as the standard clinical drugs in the inhibition of suspended leukemia, lymphomas, and HeLa-S^3 uterine carcinoma cell growth after three days.

Table 3 Effects (% control) of compound **1** on P388 leukemia cell metabolism after 60 min incubation

Assay (N = 6)	Control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 5[a]	53 ± 4*	43 ± 4*	31 ± 3*
RNA synthesis	100 ± 6[b]	69 ± 5*	64 ± 4*	62 ± 4*
Protein synthesis	100 ± 5[c]	100 ± 6	71 ± 5	65 ± 4*
DNA polymerase α	100 ± 6[d]	95 ± 5	70 ± 4*	59 ± 4*
m-RNA polymerase	100 ± 7[e]	92 ± 5	86 ± 5	71 ± 5*
r-RNA polymerase	100 ± 4[f]	132 ± 6*	116 ± 5	82 ± 5
t-RNA polymerase	100 ± 7[g]	113 ± 6	111 ± 5	108 ± 6
Ribonucleoside reductase	100 ± 5[h]	69 ± 4*	20 ± 3*	12 ± 2*
Dihydrofolate reductase	100 ± 5[i]	44 ± 3*	33 ± 2*	25 ± 3*
Purine synthesis	100 ± 5[j]	100 ± 5	81 ± 4*	69 ± 4*
PRPP amidotransferase	100 ± 6[k]	139 ± 6*	$76 + 5$*	76 ± 4*
IMP dehydrogenase	100 ± 5[l]	85 ± 4	$73 + 4$*	30 ± 3*
Pyrimidine synthesis	100 ± 6[m]	106 ± 5	89 ± 5	87 ± 6
Carbamyl phosphate synthetase	100 ± 7[n]	89 ± 3	85 ± 5	73 ± 5*
Aspartate transcarbamylase	100 ± 6[o]	$77 + 5$*	65 ± 4*	46 ± 4*
Thymidylate synthetase	100 ± 5[p]	112 ± 5	92 ± 6	84 ± 5
Thymidine kinase	100 ± 6[q]	87 ± 6	52 ± 5*	46 ± 4*
Thymidine monophosphate kinase	100 ± 7[r]	66 ± 5*	58 ± 6*	55 ± 5*
Thymidine diphosphate kinase	100 ± 6[s]	48 ± 5*	47 ± 4*	41 ± 3*
d[ATP]	100 ± 5[t]			68 ± 5*
d[GTP]	100 ± 6[u]			82 ± 4*
d[CTP]	100 ± 5[v]			92 ± 5
d[TTP]	100 ± 4[w]			93 ± 5

* $P \leq 0.001$; control values based on 10^6 P388 cells.

[a] 26 152 dpm
[b] 4 851 dpm
[c] 7 461 dpm
[d] 47 804 dpm
[e] 4 239 dpm
[f] 1 502 dpm
[g] 6 400 dpm
[h] 2 744 dpm
[i] 0.868 OD unit
[i] 0.868 OD unit
[j] 92 551 dpm
[k] 0.121 OD unit
[l] 76 058 dpm
[m] 19 758 dpm
[n] 0.392 mol citrulline
[o] 1.064 mol *N*-carbamyl aspartate
[p] 18 463 dpm
[q] 1 317 dpm
[r] 1 179 dpm
[s] 1 891 dpm
[t] 6.17 pmol
[u] 5.27 pmol
[v] 6.87 pmol
[w] 6.94 pmol

Only certain compounds demonstrated significant activity against the growth of cultured cells derived from solid tumors (Table 2). Complexes **3** and **5** demonstrated marginal activity against the growth of Sk-2 melanoma, and **4** and **5** were active against Mcf-7 breast effusion growth. It should be noted that the tantalum(V) carborane complexes were more potent against the growth of melanoma SK-Mel-2 and breast than the standard clinical drugs.

Effects on nucleic acid metabolism

A mode-of-action study in P388 lymphocytic leukemia cells demonstrated that compounds **1**–**4** significantly suppressed DNA synthesis in a concentration-dependent manner to 55–71% after 60 min at 100 μM (Tables 3–6).

RNA and protein syntheses were reduced by complexes **1**–**3**, but in a less dramatic manner, under the same conditions. DNA synthesis appeared to be reduced by a number of mechanisms which were probably additive to produce cell death. DNA polymerase α activity was reduced by 25–44% by compounds **1**–**4** at 100 μM after 60 min. m-RNA polymerase activity was reduced by 25–29% by compounds **4** and **1**. r-RNA polymerase activity was reduced by 43% by compound **3** and by 57% by compound **4**. t-RNA polymerase activity was not affected by the agents. Ribonucleoside reductase activity was significantly reduced by 39–91% and dihydrofolate reductase activity by 57–81% by the four compounds at 100 μM after 60 min. Purine *de novo* synthesis was reduced by 23–42% after 60 min at 100 μM. PRPP amidotransferase activity was reduced by 24% by compound **1** and by 40% by

Table 4 Effects (% control) of compound **2** on P388 Leukemia Cell Metabolism after 60 min incubation

Assay (N = 6)	Control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 5^{a}	72 ± 4	48 ± 4*	44 ± 3*
RNA synthesis	100 ± 6^{b}	87 ± 5	73 ± 4*	63 ± 5*
Protein synthesis	100 ± 5^{c}	100 ± 5	90 ± 6	79 ± 5*
DNA polymerase α	100 ± 6^{d}	135 ± 6*	86 ± 5	63 ± 4*
m-RNA polymerase	100 ± 7^{e}	147 ± 5*	120 ± 5	101 ± 6
r-RNA polymerase	100 ± 4^{f}	117 ± 5	98 ± 6	91 ± 5
t-RNA polymerase	100 ± 7^{g}	163 ± 5*	140 ± 5*	120 ± 5
Ribonucleoside reductase	100 ± 5^{h}	25 ± 4*	22 ± 4*	9 ± 1*
Dihydrofolate reductase	100 ± 5^{i}	68 ± 4*	48 ± 4*	43 ± 5*
Purine synthesis	100 ± 5^{j}	75 ± 5*	73 ± 4*	58 ± 4*
PRPP amidotransferase	100 ± 6^{k}	138 ± 6*	121 ± 5	93 ± 5
IMP dehydrogenase	100 ± 5^{l}	52 ± 4*	41 ± 4*	31 ± 4*
Pyrimidine synthesis	100 ± 6^{m}	112 ± 5	89 ± 5	69 ± 4*
Carbamyl phosphate synthetase	100 ± 7^{n}	67 ± 4*	63 ± 4*	63 ± 4*
Aspartate transcarbamylase	100 ± 6^{o}	62 ± 4*	50 ± 4*	40 ± 3*
Thymidylate synthetase	100 ± 5^{p}	143 ± 6*	116 ± 5	104 ± 6
Thymidine kinase	100 ± 6^{q}	74 ± 5*	46 ± 4*	37 ± 3*
Thymidine monophosphate kinase	100 ± 7^{r}	60 ± 4*	59 ± 4*	51 ± 4*
Thymidine diphosphate kinase	100 ± 6^{s}	47 ± 2*	37 ± 3*	36 ± 3*
d[ATP]	100 ± 5^{t}			92 ± 4
d[GTP]	100 ± 6^{u}			64 ± 4*
d[CTP]	100 ± 5^{v}			86 ± 5
d[TTP]	100 ± 4^{w}			107 ± 5

* $P \leq 0.001$; control values based on 10^6 L1210 cells.
[a–w] For control values, see footnote to Table 3.

compound **3**. IMP dehydrogenase activity was reduced by 69–70% by compounds **2** and **1** but only by 24% for compound **4**. *De novo* pyrimidine synthesis was inhibited by 31% by compound **2** but was actually elevated by 43% by compound **4**. Carbamyl phosphate synthetase activity was suppressed by 27%, 37% and 39% by compounds **1, 2** and **3**, respectively. Asparate transcarbamylase activity was reduced by 42%, 54% and 60% by compounds **4, 1** and **2**, respectively. Thymidylate synthetase activity was not significantly reduced by any of the compounds but thymidine kinase activity was reduced by 44–63% by the four agents. TMP kinase activity was reduced by 48–59% and TDP kinase activity was inhibited by 59–64% by the four agents over 60 min at 100 μM.

DNA studies

d[ATP] pools were reduced by 32% by compound **1** and by 38% by compound **4**, and d[GTP] pools were reduced 18% by compound **1**, 36% by compound **2**, 23% by compound **3** and 41% by compound **4** after incubation for 60 min. d[TTP] pools were elevated by 54% by compound **3** and d[CTP] pools were reduced by 18% by compound **4** after incubation for 60 min at 100 μM. After incubation of the drugs for 24 h at 100 μM P388 DNA, strand scission or fragmentation was evident with smaller-molecular-weight DNA in the gradient (Fig. 2). ct-DNA studies *in vitro* demonstrated that after 24 h of incubation at 100 μM there was no hyperchromic shift in the UV absorption of the bases from 220 to 340 nm. Therefore, the agents were not causing S_N1 or S_N2 reactions with the bases. There was a 2–6% decrease in ct-DNA viscosity but no change was observed in the T_m value for ct-DNA thermal denaturation after incubation for 24 h at 100 μM. Thus, it appears that the agents do not alkylate the bases of DNA or intercalate between the base-pairs of DNA. The reduction in DNA viscosity was consistent with the ability of the agents to cause DNA fragmentation.

DISCUSSION

The tantalum(V) and niobium(V) carborane complexes are effective cytotoxic agents against

Table 5 Effects (% control) of compound **3** on P388 leukemia cell metabolism after 60 min incubation

Assay (N = 6)	Control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 5^{a}	78 ± 5*	51 ± 4*	29 ± 4*
RNA synthesis	100 ± 6^{b}	126 ± 5	83 ± 3	82 ± 4
Protein synthesis	100 ± 5^{c}	114 ± 7	103 ± 5	71 ± 4*
DNA polymerase α	100 ± 6^{d}	102 ± 5	80 ± 4*	56 ± 4*
m-RNA polymerase	100 ± 7^{e}	94 ± 4	88 ± 5	88 ± 4
r-RNA polymerase	100 ± 4^{f}	90 ± 5	72 ± 4*	57 ± 3*
t-RNA polymerase	100 ± 7^{g}	156 ± 6*	109 ± 5	104 ± 6
Ribonucleoside reductase	100 ± 5^{h}	100 ± 4	65 ± 4*	61 ± 3*
Dihydrofolate reductase	100 ± 5^{i}	55 ± 4*	21 ± 3*	19 ± 3*
Purine synthesis	100 ± 5^{j}	122 ± 5	119 ± 5	77 ± 4*
PRPP amidotransferase	100 ± 6^{k}	119 ± 6	72 ± 5*	60 ± 5*
IMP dehydrogenase	100 ± 5^{l}	112 ± 5	96 ± 6	82 ± 5
Pyrimidine synthesis	100 ± 6^{m}	114 ± 6	111 ± 5	87 ± 6
Carbamyl phosphate synthetase	100 ± 7^{n}	99 ± 6	78 ± 4*	61 ± 5*
Aspartate transcarbamylase	100 ± 6^{o}	105 ± 5	85 ± 6	80 ± 4*
Thymidylate synthetase	100 ± 5^{p}	140 ± 6*	132 ± 5*	121 ± 5
Thymidine kinase	100 ± 6^{q}	55 ± 5*	40 ± 5*	39 ± 4*
Thymidine monophosphate kinase	100 ± 7^{r}	46 ± 4*	43 ± 4*	41 ± 3*
Thymidine diphosphate kinase	100 ± 6^{s}	45 ± 5*	41 ± 3*	37 ± 4*
d[ATP]	100 ± 5^{t}			62 ± 4*
d[GTP]	100 ± 6^{u}			77 ± 4*
d[CTP]	100 ± 5^{v}			92 ± 5
d[TTP]	100 ± 4^{w}			154 ± 4*

* $P \leq 0.001$; control values based on 10^6 L1210 cells.
[a–w] For control values, see footnote to Table 3.

the growth of a number of leukemias, lymphomas and suspended HeLa-S[3] uterine carcinomas. Nevertheless, they were more selective in the inhibition of cell growth derived from solid tumors. This pattern is very similar to that observed for the previously reported copper complexes of 2-furaldehyde oximes,[37,38] ferratricarbadecacarboranyl complexes,[2] and the copper–, cobalt– and chromium–boron complexes,[33] in that they are significantly more potent in inhibiting the growth of suspended cells than solid tumor growth. Surprisingly, the tantalum(V) and niobium(V) carborane complexes are significantly active against the growth of breast MCF-7 and melanoma Sk-Mel-2, two human tumors for which effective drugs against cell growth are currently being sought.

The carborane complexes inhibited nucleic acid synthesis by multiple mechanisms that were probably additive. The significant magnitude of reduction by the agents of dihydrofolate reductase activity over 60 min alone would account for the observed magnitude of reduction of DNA synthesis over the same time period. Inhibition of this enzyme would block one carbon-atom transfer for *de novo* purine synthesis and to a lesser extent *de novo* pyrimidine synthesis. Indeed, *de novo* purine synthesis was inhibited by all of the agents and to a lesser extent *de novo* pyrimidine synthesis was suppressed after 60 min. Reduction of both pathways by the agents would lead to inhibition of RNA and DNA syntheses. However, since the pool level of RNA compared to DNA is 10:1 in mammalian tumor cells, it is not surprising to observe the effects of the agents in 60 min first on DNA synthesis and to a lesser extent on RNA synthesis. In addition, the activities of regulatory enzymes of both purine and pyrimidine pathways were inhibited in a concentration-dependent manner by the complexes. The magnitude of inhibition of PRPP amidotransferase and IMP dehydrogenase activities by the compounds is consistent with the observed inhibition of total *de novo* purine synthesis. The inhibition of carbamyl phosphate synthetase and aspartate transcarbamylase activities is consistent with the overall inhibition of pyrimidine synthesis. In general, the magnitude of the reduction afforded by the complexes on these two pathways is not

Table 6 Effects (% control) of compound **4** on P388 leukemia cell metabolism after 60 min incubation

Assay (N = 6)	Control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 5^{a}	55 ± 4*	37 ± 3*	36 ± 4*
RNA synthesis	100 ± 6^{b}	142 ± 7*	126 ± 5	92 ± 5
Protein synthesis	100 ± 5^{c}	100 ± 6	102 ± 5	92 ± 5
DNA polymerase α	100 ± 6^{d}	156 ± 6*	113 ± 4	75 ± 4*
m-RNA polymerase	100 ± 7^{e}	126 ± 4	102 ± 5	76 ± 5*
r-RNA polymerase	100 ± 4^{f}	82 ± 5	81 ± 3*	43 ± 4*
t-RNA polymerase	100 ± 7^{g}	161 ± 5*	114 ± 5	104 ± 6
Ribonucleoside reductase	100 ± 5^{h}	114 ± 5	55 ± 4*	20 ± 3*
Dihydrofolate reductase	100 ± 5^{i}	38 ± 4*	33 ± 4*	20 ± 3*
Purine synthesis	100 ± 5^{j}	118 ± 5	93 ± 6	73 ± 5*
PRPP amidotransferase	100 ± 6^{k}	148 ± 5*	146 ± 6*	85 ± 6
IMP dehydrogenase	100 ± 5^{l}	86 ± 5	79 ± 5*	78 ± 4*
Pyrimidine synthesis	100 ± 6^{m}	107 ± 5	110 ± 6	143 ± 6*
Carbamyl phosphate synthetase	100 ± 7^{n}	104 ± 6	103 ± 5	88 ± 5
Aspartate transcarbamylase	100 ± 6^{o}	93 ± 6	65 ± 4*	58 ± 5*
Thymidylate synthetase	100 ± 5^{p}	127 ± 7	124 ± 5	120 ± 5
Thymidine kinase	100 ± 6^{q}	106 ± 5	65 ± 4*	56 ± 3*
Thymidine monophosphate kinase	100 ± 7^{r}	81 ± 5	67 ± 5	52 ± 3*
Thymidine diphosphate kinase	100 ± 6^{s}	93 ± 4	78 ± 4*	52 ± 4*
d[ATP]	100 ± 5^{t}			85 ± 5
d[GTP]	100 ± 6^{u}			59 ± 4*
d[CTP]	100 ± 5^{v}			82 ± 4
d[TTP]	100 ± 4^{w}			102 ± 5

* $P \leq 0.001$; control values based on 10^6 L1210 cells.
[a–w] For control values, see footnote to Table 3.

sufficient to suggest that these are major targets of the carborane complexes for the inhibition of DNA synthesis.

Furthermore, the complexes reduced the activities of DNA polymerase α, nucleoside kinases and ribonucleoside reductase, which would be more selective for the suppression of DNA synthesis. The fact that the agents suppressed ribonucleoside reductase markedly in 60 min would lead to reduced synthesis of deoxyribonucleosides and their entrance into the nucleus for DNA synthesis. In mammalian cells this is the only enzymic reaction that gives rise to deoxyribonucleosides. Suppression of DNA polymerase α would lead to the accumulation of deoxyribonucleosides which were not incorporated into a new strand of DNA; thus, the observed reduction of d[GTP] and d[ATP] pools after 60 min suggests that the complexes' ability to inhibit *de novo* purine synthesis and dihydrofolate reductase activity is more important to their mechanism of action than is the inhibition of DNA polymerase α activity. The effects of the agents on the activities of the RNA polymerases are inconsistent and are probably not a major factor in the mechanism of action of these compounds. The observation that the tantalum(V) and niobium(V) metallacarboranes function at multiple sites in nucleic acid metabolism is not surprising, since a number of metal complexes have been shown to do this.[32–39]

In contrast to cisplatin and other metal complexes,[32–39] the tantalum and niobium compounds do not alkylate the bases of DNA, cause intercalation between base-pairs or produce cross-linking of the strands of DNA, but they do effect P388 DNA fragmentation and reduced DNA viscosity, which leads directly to apoptosis (cell death). Many of the previously studied metal complexes are topoisomerase II inhibitors, which would explain their ability to cause DNA fragmentation. This possibility will be explored with the current compounds and structurally related metallacarboranes.

In conclusion, these preliminary studies on tantalum and niobium carborane complexes suggest that this class of compounds has the potential for development into potent antineoplastic agents. Further studies are currently in progress to identify

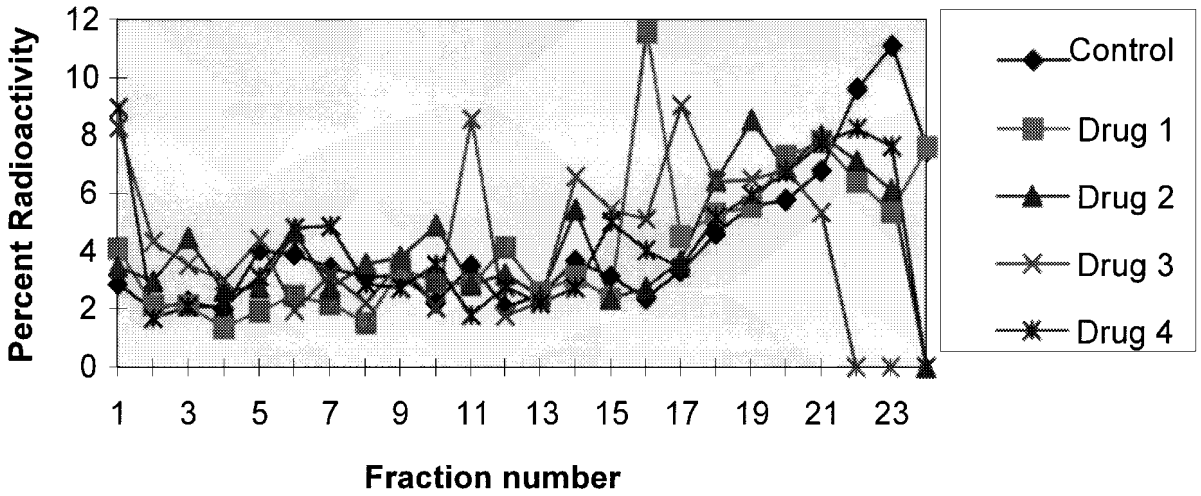

Figure 2 P388 DNA strand scission after 24 h at 100 μM.

more potent derivatives that show activity against the growth of solid tumors.

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