Cell culture and in vitro cytotoxicity testing were performed as described.[18]

General Procedures. Method A. Coupling Reaction with Dicyclohexylcarbodiimide/1-Hydroxybenzotriazole. To a solution of amino acid ester hydrochloride (13 mmol) in dry pyridine (10 mL) were added *N*-methylmorpholine (1.64 mL, 15 mmol), Cbz-protected amino acid (13 mmol), HOBT (20 mmol), and slowly a solution of DCC (20 mmol) in dry pyridine (20 mL), at 0 °C. The reaction mixture was stirred at 0 °C for 4 h and then 36 h at room temperature. The urea formed was removed by filtration, and the filtrate was evaporated. The oily residue was dissolved in ethyl acetate (200 mL) and washed successively with 5% $NaHCO_3$, water, 0.1 M HCl, and water, dried ($MgSO_4$), and evaporated. The crude product was obtained as a solid. The final purification was achieved by silica gel column chromatography and recrystallization.

Method B. Saponification of Peptide Esters in Aqueous Dioxane. To a solution of peptide esters (10 mmol) in a mixture of dioxane (90 mL) and water (10 mL) was added 1 M NaOH (13 mmol), and the mixture was stirred at room temperature overnight. The aqueous phase was acidified with solid citric acid while being chilled. Solvent was removed under reduced pressure at 45 °C. The residue was dissolved in ethyl acetate (200 mL) and washed with 0.01 M HCl and water. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to yield crude product. In most cases, the products obtained needed no further purification.

L-Leucine Methyl Ester Hydrochloride (4). The title compound was prepared from L-leucine (5 g, 0.03 mol) according to the method used to prepare N^6-Cbz-lysine methyl ester hydrochloride:[15] yield 11 g (93%); mp 153–154 °C; R_f (D) 0.45.

***N*-(Benzyloxycarbonyl)-D-valine (5).** D-Valine (6 g, 0.05 mol) was dissolved in a mixture of 2 M NaOH (117 mL) and ether (26 mL) and stirred at 5 °C, and benzyl chloroformate (11.7 mL, 0.08 mol) was added drop by drop. The reaction mixture was stirred for 3 h at 5 °C, and 25 °C for an additional 30 h. The aqueous phase was separated from ether. The pH was then adjusted to 2.0 with 2 M HCl, and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with water, dried over Na_2SO_4, and evaporated under reduced pressure: yield 11 g (87%); mp 58–60 °C; R_f (A) 0.67.

***N*-[*N*-(Benzyloxycarbonyl)-D-valylleucyl-N^6-(benzyloxylcarbonyl)lysyl]-1-β-D-arabinofuranosylcytosine (2a).** A solution of **9** (1.3 g, 2.1 mmol), *N*-hydroxysuccinimide (0.35 g, 3.0 mmol), and EDCI (0.4 g, 3.0 mmol) in dry DMF (50 mL) was stirred for 2 h at room temperature. To the solution was added **1** (0.5 g, 2.1 mmol). The mixture was stirred at 45 °C for 20 h. The solvent was removed in vacuum, and the oily residue was dissolved in ethyl acetate, washed successively with 0.01 M HCl and water, dried ($MgSO_4$), and concentrated under vacuum. The product was purified by column chromatography on silica gel 60 with solvent B to give 1.44 g (82%) of **2a** (after crystallization from ethyl acetate–ether): mp 136–138 °C; $[\alpha]^{20}_D$ = +16.7° (*c* = 0.7, MeOH); R_f (B) 0.33; ^{1}H NMR (DMSO-d_6) δ 0.85 (q, *J* = 5 Hz, 12 H, 2 C(CH_3)$_2$), 1.00–2.05 (m, 10 H, 4 CH_2, 2 CH), 2.97 (m, 2 H, CH_2), 3.32 (s, 1 H, NH), 3.62 (m, *J* = 5 Hz, 2 H, 5′-CH_2), 3.75–4.00 (m, 3 H, 3 OH), 4.06 (m, 1 H, CH), 4.37 (m, 2 H, 2CH), 4.90–5.15 (m, 5 H, 2 OCH_2, H-4′), 5.50 (m, 2 H, H-2′, H-3′), 6.05 (d, *J* = 4 Hz, 1 H, H-1′), 7.15 (d, *J* = 8 Hz, 1 H, CH), 7.35 (m, 11 H, 2 C_6H_5, NH), 7.90 (m, 1 H, NH), 8.05 (d, *J* = 8 Hz, 1 H, CH), 8.18 (m, 1 H, NH), 10.90 (d, 1 H, NH). Anal. ($C_{42}H_{57}N_7O_{12}$) C, H, N.

***N*-(D-Valylleucyllysyl)-1-β-D-arabinofuranosylcytosine (2).** Compound **2a** (1.27 g, 1.5 mmol) was hydrogenolyzed over 10% Pd/C (0.4 g) in dry methanol (100 mL) in the presence of 2% HCOOH. The catalyst was removed by filtration, the filtrate was evaporated in vacuum, and the residue was recrystallized from methanol–ether to afford the title compound: yield 0.7 g (87%); mp 126–130 °C; $[\alpha]^{20}_D$ = +14.6° (*c* = 0.2, MeOH); ^{1}H NMR (DMSO-d_6) δ 0.80 (q, *J* = 5 Hz, 12 H, 2 C(CH_3)$_2$), 1.07–2.05 (m, 10 H, 4 CH_2, 2 CH), 2.75 (m, 2 H, CH_2), 3.22 (d, 1 H, CH), 3.62 (d, *J* = 5 Hz, 2 H, 5′-CH_2), 3.80–4.10 (m, 3 H, 3 OH), 4.40 (m, 2 H, 2 CH), 4.50–6.00 (m, 7 H, 2 NH_2, 3 H, H-3′, H-2′, H-4′), 6.05 (d, *J* = 5 Hz, 1 H, H-1′), 7.15 (d, *J* = 8 Hz, 1 H, CH), 8.10 (d, *J* = 8 Hz, 1 H, CH), 8.35 (m, 2 H, NH). Anal. ($C_{28}H_{45}N_7O_8$) C, H, N.

cis-Bis(pyridine)platinum(II) Organoamides with Unexpected Growth Inhibition Properties and Antitumor Activity

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The platinum(II) organoamides [Pt(NRCH$_2$)$_2$L$_2$] (L = pyridine (py), R = *p*-HC_6F_4, C_6F_5, *p*-IC_6F_4, *p*-ClC_6F_4, *p*-$C_6F_5C_6F_4$; L = 4-methylpyridine, R = *p*-HC_6F_4) and [Pt(NRCH$_2$CH$_2$NR′)(py)$_2$] (R = *p*-HC_6F_4, R′ = C_6F_5, *p*-BrC_6F_4, or *p*-MeC_6F_4) inhibit the growth of murine L1210 leukemia cells in culture with ID_{50} values for continuous exposure in the range 0.6–2.7 μM. Representative complexes are also active against L1210 cells in 2-h pulse exposures, as well as against the cisplatin-resistant variant L1210/DDP and human colonic carcinoma cell lines HT 29 and BE. Three complexes [Pt(NRCH$_2$)$_2$L$_2$] (R = *p*-HC_6F_4, C_6F_5, or *p*-IC_6F_4) have good activity ($T/C \geq 180\%$) against P388 leukemia in mice, and all other compounds tested are active except when R = *p*-$C_6F_5C_6F_4$, L = py. Although the molecular basis of the biological activity of these complexes is not known, the observation of good activity for amineplatinum(II) compounds with no hydrogen substitutents on the nitrogen donor atoms introduces a new factor in the anticancer behavior of platinum(II) complexes.

Introduction

Initial studies of the antitumor effects of *cis*-[$PtCl_2(py)_2$] (py = pyridine) revealed moderate activity against Ehrlich ascites carcinoma in vivo[1] but none against the sarcoma 180 tumor model.[2] More recently, the compound has been shown to be cytostatic against murine L1210 leukemia cells in culture, though with substantially less potency than either *cis*-[$PtCl_2(NH_3)_2$] (cisplatin) or, surprisingly,

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trans-[$PtCl_2L_2$] (L = py or 4-methylpyridine (mepy)), and inactive against P388 leukemia in mice.[3] The poor biological activity of *cis*-[$PtCl_2(py)_2$] may be attributable to the fact that the complex lacks an important structural characteristic of active amineplatinum(II) complexes,[4,5] viz. a hydrogen substituent on a N-donor atom. We now report that novel fluorocarbon-substituted organoamidoplatinum(II) complexes [$Pt(NRCH_2)_2L_2$] and [$Pt(NRCH_2CH_2NR')(py)_2$] (R ≠ R' = polyfluorophenyl; L = py or mepy) are potent inhibitors of the growth of murine leukemia (L1210) and human colonic carcinoma cells (HT 29 and BE) in culture and that representative examples have high activity against the P388 tumor in mice despite the fact that they also lack hydrogen substituents on the N-donor atoms. In addition, the compounds studied show activity toward cisplatin-resistant L1210 cells in vitro but not against cisplatin-resistant P388 in mice.

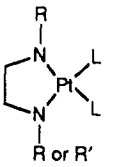
Results

Chemistry. The fluorocarbon-stabilized organoamidoplatinum(II) complexes [$Pt(NRCH_2)_2L_2$] and [$Pt(NRCH_2CH_2NR')(py)_2$] (R ≠ R' = polyfluorophenyl) have recently been prepared by decarboxylation,[6-8] a synthetic method unprecedented in organoamidometallic chemistry,[9-12] and their structures have been established by X-ray crystallography[6] and spectroscopic methods.[7,8] They are stable to atmospheric moisture and can be recovered from pyridine by addition of water, in contrast with the extreme moisture-sensitivity of simple (ethane-1,2-diaminato)-platinum(II) complexes, e.g. [$Pt(NRCH_2)_2bpy$] (R = H or Me; bpy = 2,2'-bipyridine).[13,14] *N,N'*-Bis(2,3,5,6-tetrafluorophenyl)ethane-1,2-diamine, needed for comparison of biological properties with those of the platinum organoamides, was prepared by reaction between *N*-(2,3,5,6-tetrafluorophenyl)ethane-1,2-diamine[8] with pentafluorobenzene: $p\text{-}HC_6F_4NHCH_2CH_2NH_2 + C_6F_5H + Et_3N \rightarrow (p\text{-}HC_6F_4NHCH_2)_2 + Et_3NHF$.

It is surprising that the synthesis requires much more severe conditions (180 °C/86 h) than those (refluxing EtOH/7 h) for the preparation of $p\text{-}HC_6F_4NHCH_2CH_2NH_2$ from ethane-1,2-diamine, since the two-carbon chain would be expected to insulate the NH_2 group from the electron withdrawing $p\text{-}HC_6F_4$ group.

Biological Testing Results. Initially, we investigated the growth inhibitory effects of a wide range of complexes, [$Pt(NRCH_2)_2L_2$] (L = py, R = $p\text{-}HC_6F_4$, C_6F_5, $p\text{-}ClC_6F_4$, $p\text{-}BrC_6F_4$, $p\text{-}IC_6F_4$, $p\text{-}MeC_6F_4$, $p\text{-}C_6F_5C_6F_4$, or $2,3,5\text{-}F_3C_6H_2$; L = mepy, R = $p\text{-}HC_6F_4$ or C_6F_5) and [$Pt(NR(CH_2)_3NR)(py)_2$] (R = $p\text{-}HC_6F_4$ or C_6F_5), against murine L1210 leukemia cells in culture. All were found to be active with ID_{50} values for continuous exposure in the range 0.6–3.0 μM, and several (**1a–f**, Table I) were chosen for more intensive examination together with the unsymmetrical complexes [$Pt(NRCH_2CH_2NR')(py)_2$] (**2a–c**) (Table I). The selected examples inhibit the growth of L1210 cells in culture (Table II) with similar potencies to that[15] of cisplatin when assessed by both cell counting and MTT dye assays. It is noteworthy the [$Pt(NRCH_2)_2L_2$] complexes are significantly more active than *cis*-[$PtCl_2(py)_2$][3,16] (**3**), whereas the protonated organoamide ligand ($p\text{-}HC_6F_4NHCH_2)_2$ (**4**) was found to be essentially inactive (Table II). Activity was preserved at much the same levels for the representative complexes **1a**, **1c**, and **1f** in exper-

Table I. Structure of the [$Pt(NRCH_2)_2L_2$] and [$Pt(NRCH_2CH_2NR')L_2$] Complexes[a]



compd	R	L
1a	$p\text{-}HC_6F_4$	py
1b	C_6F_5	py
1c	$p\text{-}ClC_6F_4$	py
1d	$p\text{-}IC_6F_4$	py
1e	$p\text{-}HC_6F_4$	mepy
1f	$p\text{-}C_6F_5C_6F_4$	py

compd	R	R'	L
2a	$p\text{-}HC_6F_4$	$p\text{-}BrC_6F_4$	py
2b	$p\text{-}HC_6F_4$	$p\text{-}MeC_6F_4$	py
2c	$p\text{-}HC_6F_4$	C_6F_5	py

[a] Other compounds: *cis*-[$PtCl_2(py)_2$] (**3**), ($p\text{-}HC_6F_4NHCH_2)_2$ (**4**).

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Table II. In Vitro Growth Inhibition Properties of $[Pt(NRCH_2)_2L_2]$ and $[Pt(NRCH_2CH_2NR')L_2]$ Complexes

	in vitro ID_{50} values[a]					
	L1210 leukemia		HT-29 and BE human colon carcinoma		L1210/DDP leukemia	
compd	CC[b]	MTT[c]	CC[b]	MTT[c]	CC[b]	MTT[c]
1a	1.2 ± 0.2 (1.6 ± 0.1)	1.6 ± 0.4 (1.8 ± 0.2)	1.0–1.4	1.1–1.7	1.0 ± 0.2 (1.7)	1.4 ± 0.1 (2.0)
1b	0.9 ± 0.1 [0.3][d]	1.5 ± 0.4	0.9–1.0	1.1–1.2	0.9 ± 0.1 [0.4][d]	1.5 ± 0.2
1c	0.8 ± 0.1 (1.5 ± 0.1)	1.1 ± 0.1 (1.7 ± 0.1)	0.9–1.1	1.1–1.3	0.5 (1.5)	0.8 (1.7)
1d	0.6 ± 0.1	0.7 ± 0.2	–	–	0.4 ± 0.1	0.7 ± 0.1
1e	2.0 ± 0.8 [0.3][d]	2.7 ± 0.2	0.8–1.0	1.0–1.1	0.7 [0.3][d]	1.1
1f	1.2 ± 0.1 (2.1 ± 0.1)	1.1 ± 0.1 (2.1 ± 0.1)	– –	– –	0.5 (2.0)	0.7 (2.0)
2a	0.7 ± 0.1	0.9 ± 0.1	–	–	0.6 ± 0.1	0.7 ± 0.1
2b	1.0 ± 0.2	1.6 ± 0.8	–	–	0.7 ± 0.2	0.7 ± 0.1
2c	1.7 ± 0.1	1.6 ± 0.3	–	–	0.9 ± 0.1	–
3	5.1 ± 1.5[e]	5.6 ± 1.4	–	–	3.0 ± 1.0[f]	6.2 ± 2.0
CisPt[g]	0.6 ± 0.2 (5.0)	1.1 ± 0.6	0.7–3.0	–	6.7 ± 0.2	20.3 ± 4.5
4	37.0	–	–	–	–	–

[a] ID_{50}, concentration of drug in μM to inhibit by 50% the growth in culture of murine L1210 leukemia or its cisplatin-resistant variant (L1210/DDP) or human colon carcinoma cells HT-29 and BE. [b] Coulter counter (CC). [c] Tetrazolium (MTT) assay. Results are after continuous exposure (48 h) or after 2-h pulse testing (in parentheses) and are single determinations, ranges, or mean ± range of two to four determinations. [d] Tested against P388 (first column) or P388/DDP (fifth coulumn) for 48 h of continuous exposure. [e] Reported values: ID_{50} = 7.3 ± 0.7 (ref 3a); 4.4 (ref 3b). [f] Reported value: 3.3 (ref 3b). [g] Cisplatin.

Table III. In Vivo Antitumor Activity of $[Pt(NRCH_2)_2L_2]$ and $[Pt(NRCH_2CH_2NR')L_2$ Complexes

	L1210[a]		P388[a]		P388/DDP[a]	
compd	%T/C[b]	μmol/kg[c]	%T/C	μmol/kg	%T/C	μmol/kg
1a	172	113	206	113	115	113
1b	105	538	207	269	91	538
1c	102	259	165	97	117	258
1d			180	104		
1e			126	136		
1f			112	385		
2a			158	93	114	93
2b			165	69	131	110
3			96	75	94	150
CisPt			248	20	122	20
4			108	140		

[a] Cell lines tested were murine leukemias L1210, P388 and cisplatin-resistant P388 (P388/DDP). [b] Results are mean survival time of treated animals compared with those of control animals expressed as a percentage (%T/C). Activity is indicated if $T/C > 125\%$ for L1210 and P388, and >150% for P388/DDP. [c] The doses represent the optimum dose for active compounds, or the maximum tolerated dose or highest dose used for inactive compounds.

iments where the compounds were incubated with the cells for only 2 h (Table II). By contrast 2-h pulse treatments with cisplatin resulted in an approximate 8-fold loss in potency (Table II). High activity was also observed for representative compounds tested against two human colon cancer cell lines, BE and HT-29, again with ID_{50} values comparable with those of cisplatin. Testing against the cisplatin-resistant L1210 cell line (L1210/DDP) in vitro revealed that complexes **1a–f** and **2b** were at least as active as they were against the sensitive cells (Table II) and again they were more active than **3**. This activity against cisplatin-resistant cells was maintained in 2-h pulse treatments (Table II). The responses of murine P388 and cisplatin-resistant P388 cells to the organoamides **1b** and **1e** in culture were similar to those of the L1210 and L1210/DDP cell lines.

The organoamidoplatinum(II) complexes except **1f** are active ($T/C > 125\%$) against P388 leukemia in the mouse model and three (**1a**, **1b**, and **1d**) have good activity ($T/C \geq 180\%$) (Table III). This markedly contrasts the inactivity of **3** in vivo, ($T/C = 109\%$),[3] which we have confirmed (Table III). Optimum activity for **1a–e**, **2a**, and **2b** was lower and was observed at much higher doses than for cisplatin. This lower efficacy and potency may arise from the lack of solubility of the organoamides in aqueous media. Initial in vivo testing against L1210 leukemia has shown that **1a** is active ($T/C = 172\%$), whereas **1b**, **1c**, and **3**[3] are not. T/C values in testing against B16 melanoma were 130% (113 μmol/kg), 130% (269 μmol/kg), and 134% (259 μmol/kg) for **1a**, **1b**, and **1c**, respectively. These do not reach the value ($T/C \geq 150\%$) considered active against this tumor type. By contrast with the cell culture results for behavior against cisplatin-resistant lines L1210/DDP and P388/DDP, **1a–c**, **2a**, and **2b** were inactive against P388/DDP in the mouse model.

Discussion

The most noteworthy feature of the cell culture testing results is the pronounced activity against the cisplatin-resistant lines. In both these tests and against normal L1210 cells, the activity of the [*N*,*N*'-bis(polyfluorophenyl)ethane-1,2-diaminato(2–)]bis(pyridine)platinum(II) complexes **1a–f** and **2a–c** is significantly greater than that of **3**[3,16] (Table II). In general, the ID_{50} values for L1210 are similar to or less than those of the anomalously active *trans*-$PtCl_2$(py or mepy)$_2$.[3] A further interesting feature is the maintenance of high activity in 2-h pulse testing whereas cisplatin's declines 8-fold. This suggests that

sustained exposure to the compounds is not necessary for activity. Variation in the fluorocarbon groups including the presence of two different groups (**2a–c**) has little effect on ID_{50} values, nor does the change of neutral ligand from pyridine to 4-methylpyridine. Some structure–activity variation may emerge with examination of a wider range of polyfluorophenyl groups, but this requires development of new synthetic methods. The currently used decarboxylation reactions[6-8] lead to p-XC_6F_4 substituents on nitrogen.

By contrast with the in vitro data, the in vivo results show considerable sensitivity to change of ligand. Activity against P388 increases in the sequence: R, R′ = p-$C_6F_5C_6F_4$ < p-HC_6F_4, p-BrC_6F_4 < p-HC_6F_4, p-MeC_6F_4 ≃ p-ClC_6F_4 < p-IC_6F_4 < C_6F_5 ≃ p-HC_6F_4 for L = py, and L = mepy < py for R = p-HC_6F_4 (Table III). These relationships unfortunately do not provide a clear lead in the design of more active drugs of the types **1** or **2**. The consistent P388 in vivo activity of these complexes, and the activity of **1a** against L1210, highlight the inactivity of *cis*-$PtCl_2(py)_2$, the difference being far more marked than that observed in cell culture testing. There are unpublished NIH data[17] for four *cis*-$[PtX_2L_2]$ (L = pyridine or substituted pyridine) complexes. Two are inactive in the P388 screen, while *cis*-$[PtCl_2(4\text{-Pr-py})_2]$ and *cis*-$[Pt(C_2O_4)(py)_2]$ have moderate activity (optimum T/C = 140%), though even the latter is inactive on our testing schedule. Thus, the high activity of **1a**, **1b**, and **1d** is unexpected, as is the consistent activity of the complexes (except **1f**). The activity is of particular interest since the organoamides do not have a hydrogen substituent on the nitrogen donor atoms of the amine ligands (or the amide ligands), a characteristic feature of active *cis*-diamineplatinum(II) complexes.[4,5] Indeed, it is also unusual for active compounds to have four nitrogen donor atoms.[5]

Although the molecular basis of the biological activity of complexes **1a–f** is not known, some possibilities can be excluded. For example, the cytostatic and antitumor behavior does not derive from the possible hydrolysis products, $(RNHCH_2)_2$ (R as in Table I), since (p-$HC_6F_4NHCH_2)_2$ has poor cytostatic behavior against L1210 cells (Table II) and is inactive against P388 in vivo (Table III). Similarly, cytostasis is not due to pyridine, which has ID_{50} > 100 μM. The compounds are unlikely to be prodrugs for *cis*-$[PtCl_2(py)_2]$, since this complex is less effective than **1a–f** in vitro and inactive in vivo. The ^1H and ^{19}F NMR spectra of **1a** in MeOH/D_2O (9:1, v/v) and the ^{19}F NMR spectrum in EtOH/MEM (9:1, v/v) (MEM = minimum essential medium) are unchanged after 2 h, hence the undissociated complex may be the extracellular active agent in 2-h pulse testing against L1210 leukemia. However, the concentrations needed for NMR measurements are significantly higher than those in biological testing.

It is possible that the organoamide ligands increase the biological effectiveness of the $Pt(py)_2^{2+}$ units; alternatively the $Pt(NRCH_2)_2^{2+}$ group may itself contribute to biological activity. In any case, observation of good activity for amineplatinum(II) compounds with no hydrogen substituents on the nitrogen donor atoms introduces a new factor in the anticancer behavior of platinum(II) complexes. A detailed study of breakdown products of $[Pt(NRCH_2)_2L_2]$ complexes under biologically relevant conditions has begun.

Experimental Section

(a) Reagents and Synthesis. Spectroscopic and analytical methods are as in earlier papers.[7,8,18] Cisplatin was obtained from the Institute of Drug Technology, Melbourne, Australia. *cis*-dichlorobis(pyridine)platinum(II) was prepared by the reported method,[19] and the identity was confirmed by indexation of the X-ray powder photograph against parameters derived from the X-ray crystal structure.[20] Preparations of [*N*,*N*′-bis(polyfluorophenyl)ethane-1,2-diaminato(2−)]platinum(II) complexes were carried out by the reported methods[7,8] and the compounds had spectroscopic properties identical with those reported.

***N*,*N*′-Bis(2,3,5,6-tetrafluorophenyl)ethane-1,2-diamine (4).** *N*-(2,3,5,6-Tetrafluorophenyl)ethane-1,2-diamine[8] (9.1 mmol), pentafluorobenzene (9.0 mmol), and triethylamine (72 mmol) were heated in a sealed Carius tube at 180 °C for 86 h. The resulting light yellow suspension was evaporated to dryness under vacuum. Extraction with ether, filtration, drying over $MgSO_4$, and evaporation under vacuum gave the crude title compound, which was purified by column chromatography (silica, Kieselgel 60, 70–230 mesh, Merck) with CH_2Cl_2/petroleum ether (bp 60–65 °C) (2:3 v/v) as eluant. Evaporation to crystallization gave a colorless solid: yield 41%; mp 90–91 °C; ^{19}F NMR spectrum (acetone-d_6) 142.2 (m, 4 F, F3,5), 160.4 (m, 4 F, F2,6); ^1H NMR spectrum (acetone-d_6) 3.71 (m, 4 H, CH_2), 5.47 (br, 2 H, NH), 6.66 (tt, $^3J_{H,F}$ = 10 Hz, $^4J_{H,F}$ = 7 Hz, 2 H, HC_6F_4); infrared spectrum [intense features] 3448 (s) [ν(NH)], 1648 (vs), 1528 and 1509 (vs), 1466 (s), 1456 (s), 1144 (vs) [ν(CF)], 919 (s) [ν(CF)] cm^{-1}; mass spectrum m/z 356 (13, M^+), 179 (37, $HC_6F_4NHCH_3^+$), 178 (100, $HC_6F_4NHCH_2^+$). Anal. ($C_{14}H_8F_8N_2$) C, H, F, N.

(b) Biological Testing. In Vitro. L1210 or cisplatin-resistant L1210 (L1210/DDP) mouse leukemia cells were grown as suspension cultures in MEM (minimum essential medium, Eagle's) plus 1% glutamine and 15% fetal calf serum (Flow Laboratories). Drugs were dissolved in ethanol (**1a–e**, **2a–c** and **3**; final maximum solvent concentration 1.0%), ethanol/DMSO (**1f**; maximum solvent concentration each 0.5%), or DMF (**3**; maximum solvent concentration 0.5%). Cisplatin was dissolved in saline. Growth inhibition was tested in log-phase cells that were counted using a Coulter counter after 48 h in the presence of drug or solvent or after 2 h of drug exposure followed by 46 h of growth. Growth inhibition was also measured by mitochondrial metabolism of a tetrazolium salt (MTT) using an assay modified from the method of Mosmann.[21] The human colon lines HT-29 and BE were grown for 12 h in EMEM (MEM with Earle's salts) with 1% glutamine and 15% fetal calf serum. Drugs or solvents were then added as for L1210 experiments and the cells were incubated for a further 2 days. Cells were released from the surface of the culture wells with pronase and counted or used in MTT experiments. The ID_{50}, or dose causing 50% inhibition of cell growth, was determined from the curve of percentage growth versus dose. Control cultures exposed only to the vehicle were included with each test.

In Vivo.[22] Tumors for in vivo testing were obtained from the NCI and included P388 and L1210 leukemias and B16 melanoma. The cisplatin-resistant tumor (P388/DDP) was a gift from Dr. S. M. Schmid, Southern Research Institute, Birmingham, AL. The tumors were kept frozen in liquid nitrogen and were reinitiated every 20 passages (10 for the P388/DDP). The leukemias were passaged weekly by intraperitoneal (ip) inoculation of 10^6 (P388/DDP), 10^5 (P388), or 10^4 (L1210) cells in DBA/2 mice. A

(17) Private communication, Drug Synthesis and Chemistry Branch, National Institutes of Health, to B.L.H.

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1:10 brei of the B16 melanoma was prepared, and 0.5 mL was inoculated subcutaneously in C57BL/6 mice fortnightly.

For the antitumor testing, 10^6 P388 leukemia cells (or 5×10^6 for P388/DDP, 10^5 for L1210, or 0.5 mL of 1:10 brei for B16) were injected ip into groups of six BDF_1 mice on day 0. On days 1, 5, and 9, Pt compounds in 1% Tween-20/saline (1:9) were administered ip at five concentrations over a 16-fold range incorporating the maximum tolerated dose. Negative control groups were untreated or received only the vehicle, and the positive control group received 5-fluorouracil (50 mg/kg) or cisplatin (6 mg/kg, for B16 and P388/DDP). Animals were observed for 30–60 days. Data are expressed as $\%T/C$, the mean survival time of treated animals vs that of control animals expressed as a percentage.

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Registry No. **1a**, 92181-58-3; **1b**, 92181-60-7; **1c**, 108089-50-5; **1d**, 108089-52-7; **1e**, 108089-48-1; **1f**, 108110-95-8; **2a**, 135389-37-6; **2b**, 135389-39-8; **2c**, 135389-38-7; **3**, 15227-42-6; **4**, 142581-10-0; C_6HF_5, 363-72-4.

Synthesis and Antibronchospastic Activity of 8-Alkoxy- and 8-(Alkylamino)imidazo[1,2-*a*]pyrazines

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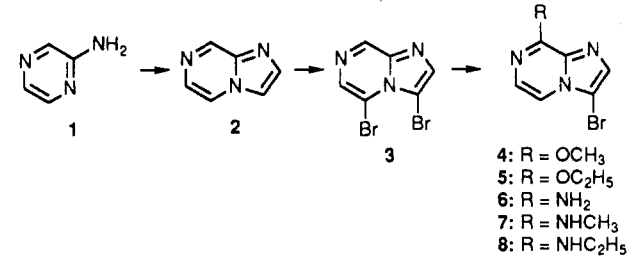
Theophylline still occupies a dominant place in asthma therapy. Unfortunatly its adverse central nervous system (CNS) stimulant effects can dramatically limit its use, and adjustments in the dosage are often needed. We have synthesized a new series of imidazo[1,2-*a*]pyrazine derivatives which are much more potent bronchodilators than theophylline in vivo and do not exhibit the CNS stimulatory profile. In vitro studies on isolated rat uterus and guinea pig trachea confirm the high potentialities of these derivatives. 6-Bromo-8-(methylamino)imidazo[1,2-*a*]-pyrazine-3-carbonitrile (**23**) is identified as the most potent compound of the series. As in the case of theophylline, phosphodiesterase inhibition appears unlikely to be the unique mechanism of action of this series of heterocycles.

Many imidazo[1,2-*a*]pyrazine derivatives have been acclaimed for their pharmacological profile, ranging from antidepressant to inotropic or antiulcer activity.[1] In an earlier paper, several 8-hydrogeno- or 8-bromoimidazo-[1,2-*a*]pyrazines were found to exhibit uterine-relaxing, antibronchospastic, and cardiac-stimulating properties.[2] In the present study, we report the synthesis of new 8-alkoxy- and 8-(alkylamino)imidazo[1,2-*a*]pyrazines (Table I) and investigate their potential as bronchodilator agents. Their pharmacological profile is compared to that of theophylline. 8-(Methylamino)imidazo[1,2-*a*]pyrazine derivatives demonstrate high antibronchospastic activity in vitro and in vivo. Interestingly, these compounds do not exhibit the adverse central nervous system (CNS) stimulant effects of theophylline.

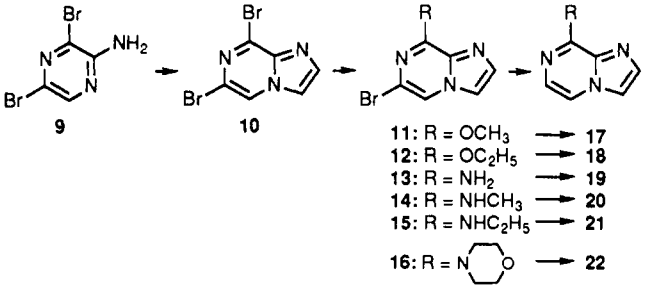
Chemistry

The classical condensation of an α-halogeno carbonyl compound with aminopyrazine (**1**) or 2-amino-3,5-dibromopyrazine (**9**) led to the imidazo[1,2-*a*]pyrazine series. Imidazo[1,2-*a*]pyrazine (**2**) yielded 3,5-dibromoimidazo-[1,2-*a*]pyrazine (**3**) via bromination with bromine in acetic acid.[3,4] The 3-bromo-8-substituted series was obtained via telesubstitution of the bromine atom in position 5 after a nucleophilic attack at the 8-position (Scheme I). This mechanism, which has already been reported for the formation of 5-bromo-8-methoxyimidazo[1,2-*a*]pyrazine[3] (**4**) and extended here to amino or alkylamino derivatives, was first described in the azoloazine series with a bridgehead nitrogen atom by Bradac et al.[4] on the triazolo[4,3-*a*]-

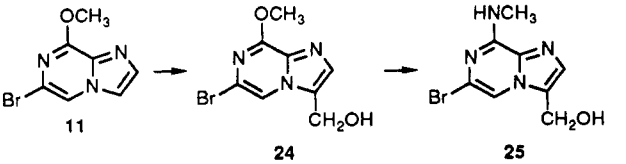
Scheme I. Synthesis of 8-Alkoxy- and 8-(Alkylamino)-3-bromoimidazo[1,2-*a*]pyrazines by Telesubstitution of 3,5-Dibromoimidazo[1,2-*a*]pyrazine



Scheme II



Scheme III



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