(1.26 mol) of 7) was dissolved at room temperature in neat trifluoroacetic acid (220 mL). After 1 h the reaction mixture was concentrated, and CH_2Cl_2 and 1 N $KHCO_3$ were added to the oily residue. After extraction, the organic layers were dried and evaporated. The residue (11.5 g) was submitted to medium-pressure chromatography on silica to yield 2.3 g (0.7% referring to 7) of **11a** (R_f 0.18) and 2.5 g (0.8% referring to 7) of **11b** (R_f 0.14) [R_f values are on silica, CH_2Cl_2/CH_3OH (95:5).] Both isomers failed to crystallize.

11a: NMR ($CDCl_3$ + D_2O, 360 MHz) δ 1.87–1.98 (m, 1 H), 2.37–2.46 (m, 1 H), 2.96 (ddd, J = 13/10/3 Hz, 1 H), 3.33 (dt, J = 13/5 Hz, 1 H), 3.43 (dd, J = 15/3 Hz, 1 H), 3.71 (dd, J = 15/3 Hz, 1 H), 3.97 (q, J = 8 Hz, 1 H), 4.83 (dt, J = 8/3 Hz, 1 H), 7.5–7.8 (m, 3 H), 7.9–8.1 (m, 2 H).

11b: NMR ($CDCl_3$ + D_2O, 360 MHz) δ 1.88–2.06 (m, 2 H), 2.73–2.90 (m, 3 H), 3.26 (dd, J = 13/7 Hz, 1 H)8 3.48 (dt, J = 7/8 Hz, 1 H), 4.74 (dt, J = 8/4 Hz, 1 H), 7.45–7.78 (m, 3 H), 7.9–8.12 (m, 2 H).

***cis*-2,3,3a,4,5,6,7,7a-Octahydro-3-oxoisoxazolo[5,4-*c*]pyridine (*cis*-4).** Solid barium hydroxide octahydrate (1.76 g) was added to a solution of **11a** (1.5 g, 5.6 mmol) in dioxane/H_2O (1:1) (120 mL). The heterogeneous reaction mixture was stirred at 50 °C for 24 h. By addition of 2 N H_2SO_4, the pH was adjusted to 1 and the precipitated barium sulfate removed by filtration through talcum powder. The filtrate was extracted thoroughly with CH_2Cl_2 to remove phenylsulfonic acid. Barium hydroxide (0.1 N) was added to the water layer to adjust to pH 9, and barium sulfate was filtered off again. The filtrate was extracted once more with CH_2Cl_2 and CO_2 was bubbled through the water layer until pH 6 was reached. Precipitated barium carbonate was filtered off and the filtrate evaporated to dryness at high vacuum. A yellow amorphous residue was the result that crystallized from CH_3OH to yield 542 mg (68%) of *cis*-**4** as a white powder. For final purification this material, combined with analoguous batches, was recrystallized three times from CH_3OH; 203–204 °C. NMR (Me_2SO, 360 MHz) δ 1.55-1.75 (m, 2 H), 2.4–2.6 (m, 2 H), 2.68 (dd, J = 13/6 Hz, 1 H), 2.79 (q, J = 6 Hz, 1 H), 2.90 (dd, J = 13/5 Hz, 1 H), 4.38 (dt, J = 5/6 Hz, 1 H), 5.7–6.7 (b, 2 H). Anal. ($C_6H_{10}N_2O_2$) C, H, N; O: calcd, 22.5; found, 22.0.

***cis*-2,3,3a,4,5,6,7,7a-Octahydro-3-oxoisoxazolo[4,5-*c*]pyridine (12).** Hydrolysis of **11b** and the subsequent workup were done analogously to the hydrolysis of **11a** described above, but **12** could be isolated only as an amorphous powder. The ^1H NMR spectrum showed signals only of **12**, but according to elemental analysis the content of **12** was only about 60%, presumably due to contamination by inorganic material. NMR (D_2O, 90 MHz): δ 1.95–2.3 (m, 2 H), 2.95–3.7 (m, 6 H), 4.5–5.0 (b, 2 H + H_2O). Anal. Calcd for $C_6H_{10}N_2O_2$: C, 50.5; H, 7.1; N, 19.7; O, 22.5. Found: C, 32.1; H, 5.1; N, 10.9; O 36.6; S, 7.3.

Displacment of [^{3}H]Muscimol.[10,11] **Tissue.** Cerebellar tissue from male rats (Ivanovas, 350–400 g) was homogenized in 20 vol of ice-cold 0.32 M sucrose with a polytron homogenizer and centrifuged at 1000 *g* for 10 min at 4 °C. The supernatant was centrifuged for an additional 20 min at 20000*g* and the resulting pellet resuspended in Tris-citrate buffer (0.05 M, pH 6.7). To further remove endogenous GABA the suspension was dialyzed for 1 day at 4 °C in the resuspension buffer with three buffer changes.

Assay. Triplicate samples were incubated for 30 min at room temperature (2-mL assay volume) in the presence of 2 nM [^{3}H]muscimol (NEN 12.9 Ci/mM) alone or in the presence of varying concentrations of unlabeled displacer. Nonspecific binding was determined in the presence of 10^{-6} M GABA.

Measurement of Spontaneous Firing Rate.[12] **Tissue.** Explants from the cerebellum of 2–4 day old rats (Wistar) were grown on glass cover slips in a plasma clot in a medium consisting of 25% fetal calf serum, 25% Hanks' BSS, and 50% basal medium Eagle. Cultures were rotated by means of a roller drum, the medium was exchanged weekly and the pH maintained close to 7.0.

Electrophysiology. All electrophysiological experiments were carried out in a temperature-controlled microchamber in Hanks' BSS at 36 °C. Drugs were superfused at a rate between 30 and 60 mL/h.

Antagonism of Bicuculline-Induced Convulsions. Treatment. Female mice (OF1 strain) weighing between 22 and 27 g were given the test treatment intraperitoneally 30 min or orally 60 min before receiving a subcutaneous injection of bicuculline base (5 mg/kg). For each mouse, the time in seconds was recorded from the bicuculline injection to the occurrence of the first clonic convulsions and the mean time calculated for each dose of compound. Four to six doses were used for each test treatment and five to six mice used per dose. The ID_{150}, estimated by regression analysis, was taken to be the dose of drug that prolonged the time to the occurrence of clonic convulsions by 50% compared to the control group.

Registry No. *cis*-**4**, 96666-84-1; **5**, 694-05-3; **6**, 85838-94-4; **7**, 21272-85-5; **8**, 57359-33-8; **10a**, 96666-85-2; **10b**, 96666-86-3; **11a**, 96666-87-4; **11b**, 96666-88-5; **12**, 96666-89-6.

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Stereoselective Antitumor Properties in the Lewis Lung Carcinoma Model Using Bis(morpholinomethyl) Derivatives of Tricyclic Bis(dioxopiperazines)

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Geometric isomers of 2,11-bis(morpholinomethyl)tetrahydrodipyrazino[1,2-*a*:2′,1′-c]pyrazine-1,3,10,12-(2*H*,4*H*,9*H*,11*H*)-tetrone (**3** and **4**) and the parent bisimides (**1** and **2**) were studied for their stereoselective antimetastatic activity in the Lewis Lung carcinoma model. The morpholinomethyl cis-syn-trans isomer **4** was more effective as an inhibitor of metastasis than the other three analogues. Using a postamputation protocol, the order of decreasing activity was cis morpholinomethyl analogue **4** > trans morpholinomethyl analogue **3** > parent cis imide **2** > parent trans imide **1**. Increased activity observed for the morpholinomethyl derivatives may reflect differences in solubility and delivery (prodrug) or an intrinsic antitumor activity of the morpholinomethyl–N functionality.

Bis(dioxopiperazines) are of considerable importance owing to their antimetastatic properties and their actions ameliorating anthracycline-induced toxicity in animals.[1] Cyclopropylbis(dioxopiperazines)[2,3] and related tetraazaperhydrophenanthrenes (**1** and **2**)[4] have been employed to assess stereoselective antimetastatic properties. Although

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Table I. Lewis Lung Carcinoma (LL) Metastasis Study[a]

	survival data				autopsy data[b]					
					% mice with no metast	av no. metast/mouse	av no. (and %) metast in each size range			av lung wt/mouse, mg
	survival days	MST[c]	% ILS[d]	N/T[e]			<1 mm	1–2 mm	>2 mm	
control	30, 30, 31, 32, 33, 34, 37, 37, 41, 44	33.5		0/10	0	17.5	0.2 (1)	5.4 (31)	11.9 (68)	942
				Preamputation Schedule[f]						
trans-3	30, 31, 37, 38, 38, 39, 41, >50, >50, >50	38.5	15	3/10	30	15.0	0.2 (1)	5 (33)	9.8 (66)	754
cis-4	30, 32, 33, 36, 37, 41, >50, >50, >50, >50	39	16	4/10	40	12.8	1.0 (8)	3.5 (27)	8.3 (65)	641
				Postamputation Schedule[g]						
trans-3	30, 32, 33, 33, 35, 35, 40, 43, >50, >50	35	4	2/10	20	13.8	0.6 (4)	3.8 (28)	9.4 (68)	752
cis-4	30, 34, 40, 43, >50, >50, >50, >50, >50, >50	>50	>49	6/10[h]	40	7.7	0.3 (4)	2.6 (34)	4.8 (62)	538

[a] For these studies 10 BDF_1 mice weighing 19–21 g were employed. Implantation was on day 0. Amputation was on day 10. [b] Survivors killed and autopsied on day 50. Data is the average of 10 mice (dying and killed mice included). [c] Median survival time (days). [d] Percent ILS of ≥25 indicates activity. [e] Number of 50-day survivors/total mice. [f] Preamputation schedule: 160 mg/kg ip from −1 h on day 0 q2d×5. Solutions were prepared daily in 0.9% NaCl solutions. [g] Postamputation schedule: 160 mg/kg ip from day 11 q2d×4. Solutions were prepared daily in 0.9% NaCl solutions. [h] 2/6 mice killed had each one metastasis (5 mm i.d.).

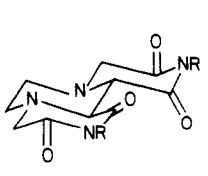
Table II. Comparison of Analogues 1–4 in the Lewis Lung Carcinoma (LL) Metastasis Study Using the Postamputation Schedule[a]

	survival data				autopsy data[b]				
					av body wt, g	av lung wt, mg	av no of metastasis		
compd	survival days	MST[c]	% ILS[d]	N/T[e]			<2 mm	>2 mm	M/T[f]
control	28, 32, 32, 33, 34, 34, 39, >50	33.5		1/8	17.6	765	0	14	7/8
1	30, 31, 33, 34, 39, 39, 41, >50, >50	39.0	16	2/9	17.7	749	2	13	7/9
2	29, 33, 34, 41, 49, >50, >50, >50	45.0	34	3/8	17.8	813	1	14	5/8
3	26, 32, 34, 39, >50, >50, >50, >50, >50	>50	>49	5/9	19.4	564	0	9	4/9
4	34, 34, >50, >50, >50 >50, >50, >50, >50	>50	>49	7/9	19.9	369	0	3	1/9

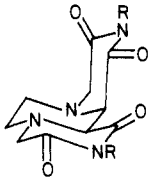
[a] BDF_1 female (19–21) g) mice; implantation on day 0; amputation on day 9. Postamputational schedule: 160 mg/kg from day 9, q2d×4 (ip). Drugs 1 and 2 were administered in suspension (0.9% NaCl solution), whereas drugs 3 and 4 were soluble in saline solution. [b] Autopsy data of dying mice and mice killed day 50. [c] Median survival time (days). [d] Increase in life span of 25% or greater indicates activity. [e] Number of 50-day survivors/total mice. [f] Number of mice with metastasis/total.

a "cisoid" relationship of dioxopiperazine rings seems to be important for antimetastatic activty, pretreatment of B16-F10 cells for 24 h with *trans-anti-trans*-**1**, but not *cis-syn-trans*-**2**, significantly inhibited metastases following their injection into the tail vein of C57B1/6J mice.[4]

During the course of these studies a morpholinomethyl analogue of ICRF-154, an open-chain compound related to **1** and **2**, namely 1,2-bis[4-(morpholinomethyl)-3,5-di-



1, R = H



2, R = H

3, R = CH_2N O

4, R = CH_2N O

oxopiperazinyl]ethane, was reported to be active, by both the oral and ip routes, against various experimental tumors.[5] Results of clinical investigations in China indicate that this compound may be useful in the treatment of malignant lymphomas, uveitis, sympathetic ophthalmitis, and psoriasis.[5] Since morpholinomethyl–N groups may impart antineoplastic properties to a molecule owing to possible alkylating activities not unlike those proposed[6] for certain hydroxymethyl–N metabolites of therapeutically useful drugs, we prepared morpholinomethyl derivatives **3** and **4** to investigate their stereoselectivity in the Lewis Lung carcinoma (LL) model.

For our initial studies, 50 BDF_1 female mice (19–21 g) were sorted at random into five groups of 10 mice and treatment was as summarized in Table I. LL (10^5 cells in 0.05 mL) was implanted in the right hind leg on day 0. At day 10 the tumor-bearing legs were amputated. Mice showed no weight loss due to drug toxicity in any of the treatment groups. Dying mice were autopsied. No mouse had regrowth of primary tumor at the amputation site and all developed extensive metastasis in the lungs, the only organ in which they were found. Mice surviving at 50 days following implantation were killed and autopsied. All but two appeared free of tumor and these are noted in the footnote to Table I.

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By either the pre- or postamputation schedules, both morpholinomethyl analogues inhibited metastases but *cis-syn-trans*-**4** was the more effective stereoisomer. Furthermore, in this study **4** significantly increased life span when employed in the postamputation schedule. Since the reverse stereoselective activity was observed for **1** and **2** when B16-F10 melanoma cells were pretreated in vitro, it may be that **4** is not primarily exerting its action following metabolic transformation to **2** but rather exhibits intrinsic antineoplastic properties possibly reflecting macromolecular alkylation by a compound possessing an appropriate geometry different from that required for the parent antimetastatic bis(dioxopiperazines).[1-4,7] This possibility requires further research, however, since morpholinomethyl derivatives are predictably unstable and undergo hydrolysis to the parent dioxopiperazines.[8]

To further investigate this effect, we compared the morpholinomethyl geometric isomers (**3** and **4**) with the parent compounds (**1** and **2**), using the postamputation schedule. Results are summarized in Table II. Again, the cis morpholinomethyl analogue **4** was the most effective inhibitor of metastasis and provided the greatest number of 50-day survivors. However, in this study the trans morpholinomethyl analogue **3** also exhibited significant activity, which was clearly better than that observed for parent trans-**1**. Interestingly, parent *cis*-**2** provided for a significant increase in life span of treated mice. Thus, the stereoselective effect in the LL model was reversed from that observed in the B16-F10 study.[4] These results are not in agreement with sructure–activity interpretations based on X-ray diffraction analyses of **1** and **2**.[7] Increased activity observed for the morpholinomethyl derivatives may reflect differences in solubility and delivery (prodrug) or an intrinsic antitumor activity of the morpholinomethyl–N functionality.

Experimental Section

All melting points are uncorrected and were taken on a Thomas-Hoover capillary melting point apparatus. Analyses were obtained from Galbraith Laboratories Inc., Knoxville, TN.

2,11-Bis(morpholinomethyl)-*trans*-tetrahydrodipyrazino[1,2-*a*:2′,1′-*c*]pyrazine-1,3,10,12(2*H*,4*H*,9*H*,11*H*)-tetrone (3). To a suspension of *trans-anti-trans*-**1**[4] (2.5 g, 9.9 × 10^{-3} mol) in 25 mL of Me_2SO was added 3 mL of morpholine and 3 mL of 37% HCHO. The mixture was heated at 55 °C for 5 h and the Me_2SO removed under reduced pressure. The residue was triturated with 20 mL of cold EtOH and the white solid filtered, washed with 20 mL of EtOH, and dried, affording 3.29 g (71.7%) of crystals, mp 216–218 °C. Anal. C, H, N.

2,11-Bis(morpholinomethyl)-*cis*-tetrahydrodipyrazino-[1,2-*a*:2′,1′-*c*]pyrazine-1,3,10,12(2*H*,4*H*,9 *H*,11*H*)-tetrone (4). This was prepared from *cis-syn-trans*-**2**[4] accordingg to the preparation of **3** from **1**, affording 3.4 g (76%) of white crystals, mp 212–214 °C. Anal. C, H, N.

Registry No. **1**, 79751-02-3; **2**, 79744-15-3; **3**, 96728-95-9; **4**, 96728-96-0; morpholine, 110-91-8.

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Activity of Platinum(II) Intercalating Agents against Murine Leukemia L1210

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Four series of intercalating, square-planar Pt(II) complexes derived from the ligands 2,2′-bipyridine, 2,2′:6′,2″-terpyridine, 1,10-phenanthroline, and 3,4,7,8-tetramethyl-1,10-phenanthroline were synthesized and aspects of their activity against murine leukemia L1210 cells investigated. The 2,2′:6′,2″-terpyridine–thiolato complexes are growth inhibitory in culture, with IC_{50} values in the range 6–32 μM, and cause cell lysis at high concentrations. Of the remaining three series, the 2,2′-bipyridine complexes are the least potent in their effects. There is a general enhancement in activity on moving from the 1,10-phenanthroline complexes to the 3,4,7,8-tetramethyl-1,10-phenanthroline analogues. Flow cytometric analysis on representative complexes shows that they are not cell cycle specific. Alkaline elution experiments indicate no damage to DNA of cells exposed to (thiophenolato)(2,2′:6′,2″-terpyridine)platinum(II) chloride monohydrate (**2a**) and (ethylenediamine)(1,10-phenanthroline)platinum(II) dichloride dihydrate (**5a**) although (ethylenediamine)(3,4,7,8-tetramethyl-1,10-phenanthroline)platinum(II) dichloride dihydrate (**6a**) causes both single-strand breaks and DNA cross-links. Compounds **2a**, **5a**, and **6a** showed no antitumor activity against L1210 in mice.

There are numerous antitumor agents capable of intercalative binding to DNA,[1] and an intercalating moiety is a structural feature of many naturally occurring, clinically useful drugs such as dactinomycin, adriamycin, ellipticine, bleomycin, and their analogues.[2] This observation can be exploited in the design of new antitumor agents. For example, starting with the intercalating chromophore, 9-aminoacridine, Cain and his colleagues have synthesized a large number of derivatives,[3] one of which, 4′-(9-acridinylamino)methanesulfon-*m*-aniside (amsacrine) (**1**), is a potent antitumor agent effective against both acute nonlymphoblastic leukemia and acute lymphoblastic leukemia.[4]

Although metallointercalators derived from 1,10-phenanthroline, 2,2′-bipyridine, 2,2′:6′,2″-terpyridine, and

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