was combined with 0.44 g (3.79 mmol) of maleic acid in 25 mL of MeOH to give a solution. The slow addition of 250 mL of Et_2O produced crystals which were collected and dried in vacuo to yield 1.70 g of **40** (87.2%) as an off-white solid.

Method H. (8β)-*N*-[6-Methyl-1-(1-methylethyl)ergolin-8-yl]-4-methoxycyclohexanecarboxamide (41). A solution of 1.67 g (10.54 mmol) of **10**, 1.07 g (10.54 mmol) of Et_3N, and 30 mL of DMF under a nitrogen atmosphere was chilled to −20 °C. The addition of 1.44 g (10.54 mmol) of isobutyl chloroformate produced an immediate precipitate. After 10 min, (8β)-1-(methylethyl)-6-methylergoline-8-amine (**8**, 2.00 g, 7.06 mmol) was added and the mixture was stirred at −20 °C for 30 min, and then allowed to warm to ambient temperature. It was then added to a cold solution of 7 mL of concentrated NH_4OH in 150 mL of H_2O. The resulting precipitate was collected by filtration. The filter cake was slurried in refluxing methanol, cooled to 0 °C, filtered, and dried in vacuo to give 1.62 g of **41** (54.2%).

Isolation of Tissue for Receptor Antagonist Studies. Male Wistar rats (150–300 g) (Harlan Sprague–Dawley, Inc.) were killed by cervical dislocation. External jugular veins from the rats were dissected free of connective tissue, cannulated in situ with polyethylene tubing (PE-50, o.d. = 0.97 mm), and placed in petri dishes containing Krebs' bicarbonate buffer (see below). The tips of two 30-gauge stainless steel hypodermic needles bent into an L shape were slipped into the polyethylene tubing. Vessels were gently pushed from the cannula onto the needles. The needles were then separated so that the lower one was tied with thread to a transducer. This procedure for ring preparations (circular smooth muscle) of blood vessels has been described previously.[16]

Tissues were mounted in organ baths containing 10 mL of modified Krebs solution of the following composition (millimolar concentrations): NaCl, 118.2; KCl, 4.6; $CaCl_2 \cdot H_2O$, 1.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 10.0; and $NaHCO_3$, 24.8. Tissue bath solutions were maintained at 37 °C and aerated with 95% O_2/5% CO_2. An initial optimum resting force of 1 g was applied to the jugular vein. Isometric contractions were recorded as changes in grams of force on a Beckman Dynograph with Statham UC-3 transducers and a microscale accessory attachment. Tissues were allowed to equilibrate for 1–2 h before exposure to drugs.

Determination of Apparent $5HT_2$ Receptor Antagonist Dissociation Constants. After control cumulative contractile responses to serotonin in the jugular vein were obtained, vessels were incubated with an appropriate concentration of antagonist for 1 h. Contractile responses to serotonin were then repeated in the presence of the antagonist. Contraction to serotonin was evaluated in the jugular vein as this tissue produced marked responses to serotonin in the absence of α receptors.[17] Only one antagonist concentration was examined in each tissue. Apparent antagonist dissociation constants (K_B) were determined for each concentration of antagonist according to the following equation:

$$K_B = [B]/(\text{dose ratio} - 1)$$

where [B] is the concentration of the antagonist and dose ratio is the ED_{50} of the agonist in the presence of the antagonist divided by the control ED_{50}. These results were then expressed as the negative logarithm of the K_B (i.e., $-\log K_B$).

Acknowledgment. We thank Kathryn Schenck for her expert technical assistance in generating the $5HT_2$ receptor antagonist data.

Registry No. **3**, 18051-15-5; **4**, 18051-22-4; **5**, 123700-80-1; **6**, 123700-81-2; **7**, 123700-82-3; **8**, 119154-51-7; **9**, 100-09-4; *cis*-**10**, 73873-59-3; *trans*-**10**, 73873-61-7; **11**, 2410-19-7; **11** acid, 5878-43-3; **12**, 2312-51-8; **12** acid, 35470-52-1; **13**, 123700-83-4; **13** acid, 41710-25-2; **13** acid hydrazide, 1752-43-8; **14**, 123700-84-5; **14** acid, 41710-27-4; **15**, 101655-79-2; **16**, 123805-55-0; **17**, 123700-85-6; **18**, 121588-81-6; **19**, 123700-86-7; **19** acid, 109839-85-2; **20**, 123700-87-8; **20**·maleate, 123701-06-4; **20** acid, 109839-86-3; **21**, 2300-80-3; **21**·maleate, 50798-40-8; **22**, 121588-80-5; **23**, 121588-82-7; **24**, 123700-88-9; **24** acid, 41710-28-5; **25**, 123700-89-0; **25** acid, 41710-26-3; **26**, 121588-75-8; **27**, 123700-90-3; **27** acid, 123701-05-3; **28**, 123700-91-4; **29**, 123700-92-5; **30**, 123700-93-6; **30**·maleate, 123701-10-0; **30** acid, 109839-87-4; **31**, 123700-94-7; **31**·maleate, 123701-11-1; **31** acid, 109839-89-6; **32**, 123700-95-8; **32** acid, 2618-03-3; **33**, 123700-96-9; **33**·maleate, 123701-07-5; **34**, 123700-97-0; **34**·mesylate, 123701-08-6; **35**, 123700-98-1; **35**·maleate, 123701-09-7; **36**, 123700-99-2; **37**, 123701-00-8; **38**, 123701-01-9; *cis*-**39**, 123701-02-0; *trans*-**39**, 123805-57-2; *cis*-**39**·maleate, 123805-60-7; *trans*-**39**·maleate, 123877-28-1; *cis*-**40**, 123701-03-1; *trans*-**40**, 123805-58-3; *cis*-**40**·maleate, 123805-56-1; *trans*-**40**·maleate, 123877-29-2; *cis*-**41**, 123701-04-2; *trans*-**41**, 123805-59-4; cyclopentylamine, 1003-03-8; cyclohexylamine, 108-91-8.

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Antimitotic Agents: Synthesis of Imidazo[4,5-*c*]pyridin-6-ylcarbamates and Imidazo[4,5-*b*]pyridin-5-ylcarbamates

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Cyclization of ethyl 5,6-diamino-4-hydrazinopyridin-2-ylcarbamate (**10**) with a mixture of CS_2 and Et_3N in dimethylacetamide gave mainly ethyl 1,4-diamino-2(3*H*)-thioxoimidazo[4,5-*c*]pyridin-6-ylcarbamate (**15**), whereas, in the absence of dimethylacetamide, a double cyclization gave mainly ethyl 5-amino-2(1*H*)-4-dithioxodiimidazo-[4,5-*b*:5,4-*c*]pyridin-7-ylcarbamate (**16**). Cyclization of the benzylidenehydrazino derivative (**6**) of **10** with either CS_2–Et_3N or $(EtO)_3CH$–HCl gave 1-(benzylideneamino)imidazo[4,5-*c*]pyridines **11** and **7** as major products and 7-(benzylidenehydrazino)imidazo[4,5-*b*]pyridines **12** and **8** as minor products. Dethiolation of **11** to give **7** and of **12** to give **8** was effected with excess Raney nickel in refluxing ethanol. The benzylidene group of **11** was removed with hydrazine in ethanolic HCl to give **15**. This key compound was condensed with benzaldehydes to give 1-benzylideneamino derivatives (**20**, **21**) and alkylated with benzyl halides to give 2-benzylthio derivatives (**24**–**26**). In addition, cyclization of ethyl 5,6-diamino-4-(benzylidene-1-methylhydrazino)pyridin-2-ylcarbamate (**30**) with $(EtO)_3CH$ provided a method for the synthesis of an imidazo[4,5-*b*]pyridine (**23**) uncontaminated with isomeric products. The presence of an aryl group in both the imidazo[4,5-*c*]- and -[4,5-*b*]pyridines gave compounds that inhibited proliferation of growth and caused mitotic arrest against lymphoid leukemia L1210 at micromolar concentrations. However, the more active in vitro compounds (**7**, **8**, **24**–**26**) gave only borderline activity in mice against lymphocytic leukemia P388.

The *Vinca* alkaloids (e.g., vincristine), clinically used anticancer agents, prevent the polymerization of tubulin to give microtubules and as a result inhibit cell division.[1] A new class of antimitotic agents, 1,2-dihydropyrido[3,4-

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Scheme I

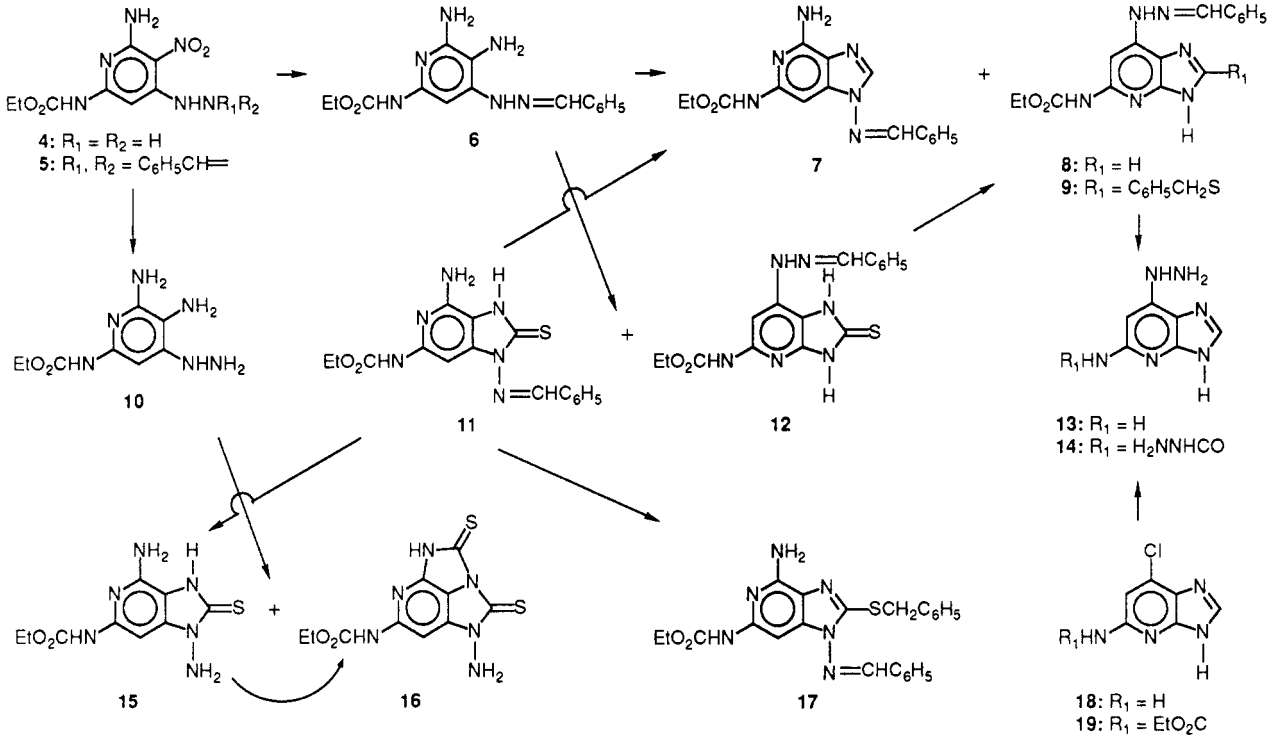
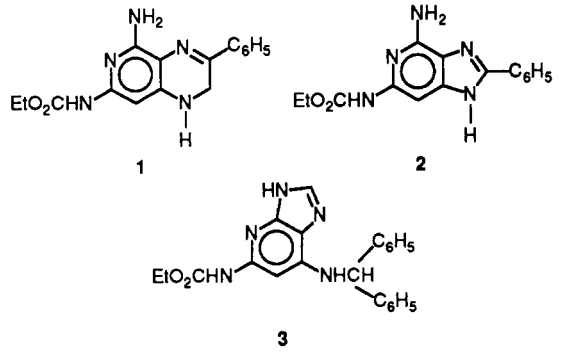


Chart I



b]pyrazines (**1**), also have shown anticancer activity.[1-3] The 1,2-NHCH moiety of **1** is unstable and oxidation provides inactive heteroaromatic compounds. In a previous paper we reported the synthesis of some 2-aryl-1*H*-imidazo[4,5-*c*]pyridin-6-ylcarbamates (e.g., **2**) as more stable ring analogues of **1** (Chart I).[4] Although many of the structural features necessary for activity in **1** were incorporated in **2**, the imidazopyridines showed only minimal activity. The lower activity was attributed to the lack of basicity at the ring NH group, and possibly to the differences in orientation of the aryl groups of **1** and **2**. In this paper we report the preparation of 1-amino-1*H*-imidazo[4,5-*c*]pyridines substituted at the 2-position with the more flexible benzylthio moiety. Also, this work provided some new derivatives of the imidazo[4,5-*b*]-pyridine ring system.

Chemistry

In the initial approach to the target compounds, **4** was hydrogenated in the presence of Raney nickel to give **10**, which was contaminated with the amino compound resulting from cleavage of the hydrazino group.[4] The crude **10** was treated with CS_2 and Et_3N with the expectation that the NH_2 of the hydrazino group and 5,6-diamino groups would react with CS_2 to generate blocking groups at these positions and that cyclization would provide **15** or a CS_2 derivative. Under the conditions of the reaction, however, a double cyclization occurred to give a product that was later identified as **16** (see below).

To block one mode of cyclization, **4** was condensed with benzaldehyde to give **5**, which was hydrogenated over Raney nickel to give **6**. Treatment of a solution of **6** in dimethylacetamide with CS_2 and Et_3N gave both **11** and **12** with **11** being formed in greater yield. During this reaction or workup, a portion of **11** was hydrolyzed to give **15**. Both **11** and **12** were dethiolated in the presence of excess Raney nickel to give **7** and **8**, respectively. Of interest, the benzylidene group protected the hydrazino group from cleavage during the preparation of **6**–**8**.

To confirm the structure of **8**, the latter was reacted with aqueous hydrazine to cleave the carbamate group and remove the benzylidene group to give **13**. An authentic sample of **13** was prepared from **18**[5] and aqueous hydrazine by the reported procedure.[6] Although the reaction with **8** gave a small amount of **13**, which was established by its ^1H NMR spectrum, the major product was identified as **14**. An authentic sample of **14** was prepared from **19**[5] and hydrazine. With the establishment of the structure of **8**, it follows that the structures of **11** and **12** were correctly assigned. In addition, cyclization of **6** with the $(EtO)_3CH$–HCl reagent[7] provided a mixture of **7** and **8** with

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Scheme II

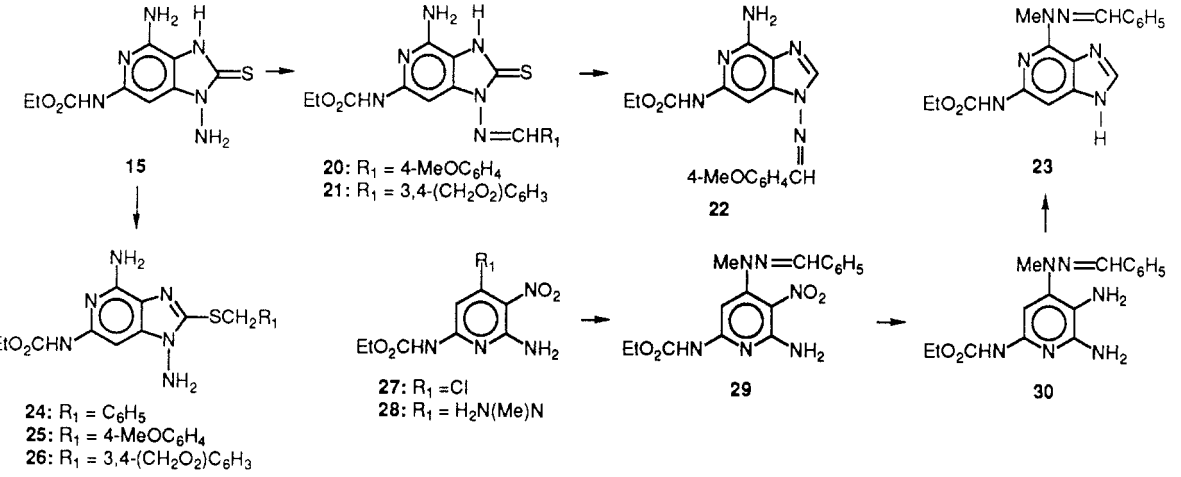

Table I. Properties of Compounds

compd	% yield	mp, °C	mass[a] spectrum	^{1}H NMR[b] CH peaks	formula	anal.
5	93	285–6	345	7.69, 8.53	$C_{15}H_{16}N_6O_4$	C, H, N
6	83	>260[c]	315	7.19, 8.01	$C_{15}H_{18}N_6O_2$	C, H, N
7	34[d]	195 dec	324[e]	7.40, 8.75, 9.15	$C_{16}H_{16}N_6O_2$	C, H, N
8	38	>267 dec	325	7.74, 8.04, 8.33	$C_{16}H_{16}N_6O_2$[f]·0.9EtOH	C, H, N
9	22	280 dec	447	7.71, 8.33	$C_{23}H_{22}N_6O_2S$	C, H, N
11	36	>300	357	7.20, 9.88	$C_{16}H_{16}N_6O_2S$	C, H, N
12	15	>300	357	7.63, 8.00	$C_{16}H_{16}N_6O_2S$[f]·Me_2NCOMe	C, H, N
13[g]			165	9.10[h]		
14	76	262 dec		6.91, 9.20	$C_7H_{10}N_8O$	H, N, C[i]
15	12[d]	>275 dec	269	7.11	$C_9H_{12}N_6O_2S$[f]·0.9EtOH	C, H, N
16	~93[j]	>300	311	6.69	$C_{10}H_{10}N_6O_2S_2$·0.5H_2O	C, H, N
17	95	107–8	447	7.74, 8.94	$C_{23}H_{22}N_6O_2S$·0.8H_2O	C, H, N
20	29	299–300 dec	387	7.13, 9.62	$C_{17}H_{18}N_6O_3S$	H, N, C[k]
21	38	>320	401	7.14, 9.62	$C_{17}H_{16}N_6O_4S$[f]·0.8Me_2NCOMe	C, H, N
22	14	195–6	355	7.36, 8.69, 9.06	$C_{17}H_{18}N_6O_3$	C, H, N
23	38	251–2 dec	339	7.72, 8.16, 8.98	$C_{17}H_{18}N_6O_2$[f]·0.8Me_2CHOH·HCl·0.3H_2O	C, H, N
24	86	129 dec	359	7.17	$C_{16}H_{18}N_6O_2S$	C, H, N
25	29	256–8 dec	389	7.16	$C_{17}H_{20}N_6O_3S$[f]·0.27PrOH	C, H, N
26	44	161–2 dec		7.14	$C_{17}H_{18}N_6O_4S$[f]·0.8PrOH	C, H, N
28	89	169–70	270[e]	6.73	$C_9H_{14}N_6O_4$	C, H, N
29	94	191–3 dec	358[e]		$C_{16}H_{18}N_6O_4$	C, H, N

[a] Fast atom bombardment mode $(M + 1)^+$ unless otherwise indicated. [b] Chemical shifts (δ) were determined in Me_2SO-d_6 unless otherwise indicated. [c] Shrinkage and discoloration from 182 °C. [d] See the Experimental Section. [e] Electron-impact mode $(M)^+$. [f] The presence of solvent in these compounds was confirmed by the ^{1}H NMR spectrum: EtOH, δ 1.06, 3.44; Me_2NCOMe, δ 1.97, 2.80, 2.96; Me_2CHOH, δ 1.03, 3.77; PrOH, δ 0.83, 1.41, 3.39. [g] See ref 5. [h] Spectrum determined in CF_3CO_2D. Apparently the pyridine ring CH underwent deuterium exchange. [i] C: calcd, 37.83; found, 38.40. [j] Crude yield calculated from **1**. [k] C: calcd, 52.84; found, 52.31.

7 being the major product. Thus, in the reaction of **6** either with CS_2 or $(EtO)_3CH$, the major product was formed via cyclization at the 1-nitrogen of the hydrazino group.

At room temperature, **11** was hydrolyzed to **15** with aqueous hydrazine or faster with hydrazine in ethanolic hydrogen chloride. This product was identical with the material that resulted from hydrolysis of **11** in the CS_2 cyclization reaction. Also, **15** was the major product when a solution of **10** in dimethylacetamide was treated with CS_2–Et_3N. In addition, reaction of **15** with CS_2 under heterogeneous conditions gave **16**, identical with the product isolated from **10** and CS_2 under heterogeneous conditions. Alkylation of **12** with benzyl chloride gave **9** and similarly **11** gave **17** (Scheme I).

The key compound **15** was condensed with benzaldehydes to give **20** and **21** and alkylated with benzyl halides to give **24–26** (Scheme II). In addition, **20** was dethiolated to afford **22**. To develop a shorter route to compounds like **8**, **27**[8] was reacted with methyl hydrazine to give **28**. Condensation of **28** with benzaldehyde provided **29**, which was hydrogenated to give **30**. The latter was cyclized with $(EtO)_3CH$–HCl to give **23**. The chemical properties of the new compounds are listed in Table I.

Biological Evaluation

In a preliminary screen for inhibition of proliferation against lymphoid leukemia L1210, compounds **4**, **5**, **13**, **14**, **16** and **20–22** gave IC_{50} values greater than 100 μM and were eliminated from further consideration.[9] Most of the compounds that exhibited an IC_{50} of less than 5 μM were evaluated for antimitotic activity (Table II).[9] From these studies, selected compounds were evaluated for antileukemic activity in mice implanted with lymphocytic leukemia P388.[10]

The intermediate **6** showed in vitro activity but insignificant in vivo activity. In the imidazo[4,5-*b*]pyridines

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Table II. Biological Activity of Compounds

	L1210		P388,[c] 10^6 tumor cell implant, ip, qd 1–5	
compd	IC_{50},[a] μM	$MI_{0.5}$,[b] μM	dose, mg/kg	% ILS[d]
1	4.7×10^{-3}	2.8×10^{-3}	2	51
2	0.9	8	200	0
6	4.6	15	50	4
7	0.15	0.61	100	39[e]
8	0.25	0.94	100	46[e]
9	46			
11	0.47	11	200	6
12	1.5	5.5	200	10
15	>30			
17	50			
23	3.1			
24	0.6	1.6	200	39[e]
25	0.06		100	24[f]
26	0.17		100	25[f]

[a] Micromolar concentration of agent that inhibits proliferation of cultured lymphoid leukemia L1210 cells to 50% control growth during 48 h. [b] Micromolar concentration of agent that causes a mitotic index (number of cells in mitosis divided by total cells) of 0.5 for cultured lymphoid leukemia L1210 cells during an exposure period of 12 h. [c] Lymphocytic leukemia P388. [d] Increase in life span at a nontoxic dose. [e] Toxic dose by weight loss when repeated. [f] Highest dose tested.

(8, 9, and 12), differences in potency were observed in vitro. This activity increased as the size of the substituent at the 2-position of the ring decreased and only 8, with no substituent at this position, exhibited in vivo activity. The structure of 8 is similar to that of 3,[4] which was previously reported to have in vivo activity. Imidazo[4,5-*b*]pyridine 23 with a methyl group at the 1-nitrogen of the hydrazino group required a higher concentration than 8 to inhibit the growth of L1210 cells.

Also inhibition of growth and antimitotic activity were observed in the imidazo[4,5-*c*]pyridines (7, 11, 15, 17, 24–26). As anticipated the 1-amino compounds 24–26 showed in vitro activity but were disappointing in that only borderline in vivo activity was observed. Surprisingly, in the 1-benzylideneamino compounds, 11 exhibited in vivo activity. In this series, activity was reduced by the absence of an aryl moiety (15) and by the presence of two aryl moieties (17).

In addition to the above studies, compounds 6–9, 11–13, 15–17, and 24 were tested for in vitro antiviral activity. None of these compounds showed activity against herpes simplex virus type 1 and human influenza virus type Ao/PR-8/34.[11]

In conclusion, the 1-amino-2-[(arylmethyl)thio]-imidazo[4,5-*c*]pyridines were more active than the corresponding 2-aryl compounds (2) but considerably less potent than the 1,2-dihydro-3-arylpyrido[3,4-*b*]pyrazines (1). In the latter the 1,2-NHCH and 3-aryl moieties appear to be necessary for activity. The results with 24–26, however, suggested that the conformation of the 1,2-dihydropyrazine ring rather than basicity is more important to activity. In the compounds (e.g., 7, 8, 24) that show in vivo activity, the position in space of the aryl group would appear to be variable, indicating that the aryl group might bind at different sites with tubulin. However, superimposing the pyridine ring nitrogen of 8 and 24 with that of 2 and allowing for the flexibility of the side chains would orient the aryl groups to approximately the same position (see 3). Currently the preparation of 2-aminopyrido[3,4-*b*]-pyrazines is expected to provide additional information on the structural features of 1 that are necessary for activity.

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Experimental Section

Melting and decomposition temperatures were determined in capillary tubes in a Mel-Temp apparatus. The ^1H NMR spectra were determined on DMSO-d_6 solutions with either a Varian XL-100-15 or a Nicolet NT300NB spectrometer with tetramethylsilane as internal standard. Mass spectra were taken with a Varian Mat 311A spectrometer operating in either the electron-impact or fast atom bombardment mode to provide the M^+ and $(M + 1)^+$ molecular ion, respectively. The progress of reactions was followed by thin-layer chromatography (TLC) on plates of silica gel from Analtech, Inc. Raney nickel no. 2800 was obtained from Davison Specialty Chemical Co. All samples were dried in vacuo at room temperature over P_2O_5. *N,N*-Dimethylacetamide is abbreviated as DMAC.

Ethyl *N*-[6-Amino-4-(2-benzylidenehydrazino)-5-nitropyridin-2-yl]carbamate (5). Benzaldehyde (1.16 g, 10.9 mmol) was added with stirring to a solution of 4 (2.56 g, 10.0 mmol)[1] in DMAC (20 mL). After 17 h at room temperature the mixture was diluted with H_2O (200 mL) and the product was collected by filtration and washed with H_2O: yield, 3.20 g.

Ethyl *N*-[5,6-Diamino-4-(2-benzylidenehydrazino)-pyridin-2-yl]carbamate (6). A solution of 5 (1.38 g, 4.01 mmol) in DMAC (40 mL) containing Raney nickel [4 g, weighed wet, washed with H_2O (2×) and DMAC (2×)] was hydrogenated at room temperature and atmospheric pressure. The uptake was stopped after the theoretical amount of H_2 (293 mL) was absorbed within 110 min. The flask was flushed with N_2, and the catalyst was removed by filtration through Celite into a flask containing deoxygenated (N_2) H_2O (250 mL). The product that separated from the cooled filtrate was collected by filtration: yield, 1.04 g.

Ethyl *N*-[4-Amino-1-(2-benzylideneamino)imidazo[4,5-*c*]pyridin-6-yl]carbamate (7). Method A. A solution of 11 (1.50 g, 4.21 mmol) in EtOH (250 mL) containing Raney nickel [11.5 g, weighed wet, washed with H_2O and EtOH (2×)] was refluxed with stirring for 2.5 h. The catalyst was removed from the hot reaction mixture by filtration through Celite and washed with hot EtOH (100 mL). The combined filtrate and wash was evaporated in vacuo to 100 mL, and the resulting gel was heated to boiling, diluted with hot H_2O (250 mL), and cooled: yield, 466 mg.

Method B. A suspension of 6 (628 mg, 2.00 mmol) in ethyl orthoformate (10 mL) was stirred as concentrated HCl (0.17 mL, 2.0 mmol) was added to give a clear but dark solution. A precipitate appeared within 15 min and after 18 h the solid was collected by filtration: yield, 553 mg. TLC (silica gel, 40:2.1 $CHCl_3$–MeOH–HOAc) and ^1H NMR spectra data showed that this material was about a 7:1 mixture of 7 and 8. A suspension of the product in H_2O (25 mL) was adjusted to pH ~10 with 0.1 N NaOH, and the solid was collected by filtration and recrystallized from EtOH–H_2O to give a homogeneous (TLC) sample of 7: yield, 245 mg (39%).

The residue (193 mg) from evaporation of the reaction filtration was shown by TLC to be a crude mixture containing both 7 and 8 as major components.

Ethyl *N*-[7-(2-Benzylidenehydrazino)-3*H*-imidazo[4,5-*b*]pyridin-5-yl]carbamate (8). A suspension of 12·DMAC (400 mg, 0.902 mmol) and Raney nickel [5 g, weighed wet, washed with H_2O (3×)] in EtOH (50 mL) was refluxed with stirring for 4 h. The hot mixture was filtered through Celite and the filtrate was cooled to deposit 8: yield, 126 mg.

The catalyst was washed with HOAc (50 mL) and the wash was combined with the ethanol filtrate from above to give an additional 94 mg of crude 8.

Ethyl *N*-[7-(2-Benzylidenehydrazino)-2-(benzylthio)-3*H*-imidazo[4,5-*b*]pyridin-5-yl]carbamate (9). A mixture of 12·DMAC (877 mg, 2.00 mmol) in DMSO (10 mL) containing Et_3N (0.28 mL) and benzyl chloride (0.23 mL, ~2 mmol) was stirred at room temperature for 20 h and a small amount of insoluble material was removed by filtration. The filtrate was diluted with H_2O (50 mL), and the solid (758 mg) that deposited was collected

by filtration and recrystallized first from EtOH and then $CHCl_3$ to give **9** contaminated with **12**: yield, 396 mg. A solution of this sample in $CHCl_3$ (100 mL) was applied to a silica gel 60 (230-400 mesh) pad (20 g) and eluted with hot 99:1 $CHCl_3$–MeOH (300 mL). The solid isolated from this fraction was recrystallized from $CHCl_3$ (charcoal) to give pure **9**: yield, 193 mg.

Ethyl *N*-(5,6-Diamino-4-hydrazinopyridin-2-yl)carbamate (10). A mixture of **4** (2.45 g, 9.57 mmol)[4] and Raney nickel [5 g, weighed wet, washed with H_2O (3×)] in 1:1 EtOH–H_2O (500 mL) was hydrogenated at 23 °C and atmospheric pressure. The theoretical amount (697 mL) of H_2 was absorbed in 1 h. The catalyst was removed by filtration and washed with EtOH. After the addition of concentrated HCl (1.6 mL), the filtrate was evaporated to dryness in vacuo to give a dark residue of crude hydrochloride: yield, 2.75 g; mass spectrum (FAB) *m/e* 212 $(M + 1)^+$ and 227 $(M + 1)^+$. The former was identified as ethyl *N*-(4,5,6-triaminopyridin-2-yl)carbamate (~10%)[4] and the latter as **10** (~80%) by HPLC chromatograms [μ Bondapak C_{18}, 9:1 NH_4OAc (pH 6)–MeCN)]. A greater amount of the cleavage product was formed when the hydrogenation was carried out in either H_2O or EtOH.

Ethyl *N*-[4-Amino-1-(benzylideneamino)-2(3*H*)-thioxoimidazo[4,5-*c*]pyridin-6-yl]carbamate (11) and Ethyl *N*-[7-(2-Benzylidenehydrazino)-2(3*H*)-thioxo-1*H*-imidazo[4,5-*b*]pyridin-5-yl]carbamate (12). A mixture of **6** (16.5 g, 53.5 mmol), Et_3N (5.40 g, 53.5 mmol), and CS_2 (4.07 g, 53.5 mmol) in DMAC (500 mL) was stirred at room temperature for 23 h. The insoluble material was collected by filtration, washed with Et_2O (2 × 100 mL), and dried to give **12**·DMAC: yield, 3.48 g.

The reaction filtrate was added with stirring to H_2O (2 L) at 70 °C, and the resulting mixture was allowed to stand at room temperature for 18 h and then chilled in an ice bath. The precipitate was collected by filtration, washed with Et_2O (450 mL), and reprecipitated from hot MeOH (3.2 L) with an equal volume of H_2O to give **11**: yield, 6.9 g.

The cloudy DMAC–H_2O filtrate from above gave **15** as described below.

7-Hydrazino-5-[(hydrazinocarbonyl)amino]-3*H*-imidazo[4,5-*b*]pyridine (14). Method A. A suspension of **19** (122 mg, 0.507 mmol)[5] in 55% aqueous hydrazine (2.7 mL) was refluxed with stirring for 2 h and cooled to room temperature. The precipitate was collected by filtration and washed with water: yield, 86 mg. The ^1H NMR spectrum in CF_3CO_2D showed that this sample contained a trace amount of **13**.[6]

Method B. A suspension of **8** (100 mg, 0.273 mmol) in 55% aqueous hydrazine (2.7 mL) was refluxed with stirring for 2 h, and the resulting cloudy solution was filtered hot. The filtrate was allowed to stand overnight at room temperature, and the precipitate was collected by filtration and washed with water: yield, 38 mg. The ^1H NMR spectrum (CF_3CO_2D) showed that this sample was a 2:1 mixture of **14** and **13**, which was confirmed by repeating the spectrum after the addition of **13**. HPLC chromatograms indicated that increased amounts of **13** were observed when samples containing **14** were dissolved by heating in DMAC.

Ethyl *N*-[1,4-Diamino-2(3*H*)-thioxoimidazo[4,5-*c*]pyridin-6-yl]carbamate (15). Method A. The DMAC–H_2O filtrate from the preparation of **11** and **12** was concentrated in vacuo to ~200 mL to deposit **15** (4.40 g), which was reprecipitated from hot MeOH (300 mL) with H_2O (350 mL) and then recrystallized from EtOH to give **15**·0.9 EtOH: yield, 2.03 g. An additional amount (0.73 g) of impure **15** was isolated from the ethanol filtrate.

The DMAC filtrate from above was evaporated to dryness to give a mixture in which the major component appeared to be **11** (TLC): yield, 2.90 g.

Method B. A suspension of **11** (7.00 g, 19.6 mmol) in H_2O (2.4 L) containing hydrazine (8.4 mL) was stirred at room temperature for 72 h. The unreacted **11** (5.65 g) was collected by filtration and the filtrate was neutralized with concentrated HCl to deposit **15**: yield, 0.800 g (19%).

The unreacted **11** in a mixture of hydrazine (9 mL) and 1 N ethanol HCl (90 mL) was stirred at room temperature for 24 h. The insoluble material was collected by filtration, suspended in H_2O (50 mL), and acidified with HOAc. Filtration gave a solid which was recrystallized from EtOH (Celite): yield, 2.52 g (48%). The total yield from the two methods was 67%.

Method C. A solution of the crude hydrochloride of **10** (2.75 g) in DMAC (50 mL) containing CS_2 (100 mL) and Et_3N (7 mL) was stirred at room temperature for 40 h. The solid was collected by filtration, suspended in hot H_2O (200 mL), and acidified to pH 2 (paper) with concentrated HCl. After cooling, the solid (1.26 g) was collected by filtration and recrystallized from MeOH to give **15**·0.8MeOH·0.3H_2O: yield, 0.820 g. The residue (0.320 g) from evaporation of the MeOH filtrate was shown by TLC (silica gel, 4:1 $CHCl_3$–MeOH) to be about a 1:1 mixture of **15** and **16**. The reaction filtrate was evaporated to dryness in vacuo and the residue was suspended in H_2O (100 mL) and acidified with HCl to give an additional amount of slightly impure **15**: yield, 0.600 g.

Ethyl *N*-[5-Amino-2,4(1*H*,5*H*)-dithioxodiimidazo[4,5-*c*]pyridin-7-yl]carbamate (16). Method A. A suspension of the crude hydrochloride of **10** (900 mg) [prepared from 2.96 mmol of **4**] in CS_2 (40 mL) containing Et_3N (2.2 mL) was stirred vigorously at room temperature for 42 h with the addition of DMAC at 2 (1 mL), 18 (5 mL) and 24 h (16 mL). After filtration the filtrate was evaporated to dryness in vacuo, and the residue was washed with H_2O (100 mL) to give crude **16**: yield, 640 mg. The aqueous dark red filtrate was concentrated in vacuo, a small amount of insoluble was removed by filtration, and the filtrate was acidified with 1 N HCl to give an additional amount of crude **16**: yield, 218 mg. This sample was washed with CH_2Cl_2 (10 mL), extracted with hot EtOH (125 mL) and the extract evaporated under N_2 to deposit **16**: yield, 90 mg.

Method B. A suspension of **15**·0.8MeOH·0.3H_2O (500 mg, 1.67 mmol) in CS_2 (40 mL) containing Et_3N (2 mL) was stirred at room temperature for 86 h with the addition of DMAC at 24 (1 mL) and 46 h (45 mL). The resulting red solution was evaporated to dryness in vacuo and the residue was suspended in H_2O (50 mL) and acidified with concentrated HCl (0.4 mL). The solid (400 mg) was collected by filtration and recrystallized from EtOH to give slightly impure **16**: yield, 268 mg; ^1H NMR (Me_2SO-d_6) δ 6.68 (pyridine CH).

Ethyl *N*-[4-Amino-1-(benzylideneamino)-2-(benzylthio)imidazo[4,5-*c*]pyridin-6-yl]carbamate (17). A solution of **11** (713 mg, 2.00 mmol) in DMAC (10 mL) containing Et_3N (0.3 mL) and benzyl chloride (0.25 mL) was stirred at room temperature for 20 h. After filtration to remove insoluble material, the filtrate was added with stirring to hot H_2O (60 mL), and the precipitate was collected by filtration and washed with H_2O and Et_2O (10 mL): yield, 851 mg.

Ethyl *N*-[4-Amino-1-[(4-methoxybenzylidene)amino]-2-(3*H*)-thioxoimidazo[4,5-*c*]pyridin-6-yl]carbamate (20). A mixture of **15**·0.9EtOH (2.00 g, 6.45 mmol) and 2-anisaldehyde (1.03 g, 7.57 mmol) in DMAC (20 mL) containing a small drop of concentrated H_2SO_4 was heated with stirring at 93 °C for 2.5 h. The hot solution was added with stirring to hot H_2O (110 mL), and the resulting mixture was allowed to cool at room temperature. The solid was collected by filtration and extracted with hot MeCN (1.8 L), and the extract was concentrated to give slightly impure **20**: yield, 1.57 g. This sample was reprecipitated from a hot solution in 5:1 MeOH–DMAC (450 mL) with H_2O (400 mL) to give **20** contaminated with a trace amount of **15**: yield, 0.726 g.

Ethyl *N*-[4-Amino-1-[[3,4-(methylenedioxy)benzylidene]amino]-2(3*H*)-thioxoimidazo[4,5-*c*]pyridin-6-yl]carbamate (21). A mixture of **15**·9EtOH (1.32 g, 4.26 mmol) and piperonal (651 mg, 4.34 mmol) in DMAC (50 mL) containing a small drop of concentrated H_2SO_4 was heated at 90 °C for 2.5 h. The resulting solution was added to H_2O (500 mL) and after refrigeration the gel was collected by filtration. This residue was dissolved in hot EtOH (700 mL), and the solution was diluted with hot H_2O (700 mL) to give slightly impure **21**: yield, 810 mg. The sample was dissolved in hot 3:1 MeOH–DMAC and reprecipitated by the addition of H_2O (160 mL): yield, 756 mg.

Ethyl *N*-[4-Amino-1-[(4-methoxybenzylidene)amino]imidazo[4,5-*c*]pyridin-6-yl]carbamate (22). A mixture of **20** (0.870 g, 2.25 mmol) and Raney nickel (8 g, weighed wet, washed with H_2O and EtOH) in EtOH (100 mL) was refluxed for 2.5 h and the catalyst was removed by filtration (Celite) and washed with hot EtOH (50 mL). The combined filtrate and wash was concentrated under N_2 to $^1/_3$ volume to deposit **22**, which was recrystallized from MeOH: yield, 0.11 g.

An additional amount (0.33 g) of crude **22** was isolated from the alcohol filtrates.

Ethyl *N*-[7-(2-Benzylidene-1-methylhydrazino)-3*H*-imidazo[4,5-*b*]pyridin-5-yl]carbamate (23). A solution of **29** (1.18 g, 3.30 mmol) in a 10:3 mixture of EtOH and DMAC (65 mL) containing Raney nickel (3 g, weighed wet, washed with H_2O and EtOH) was hydrogenated at room temperature and atmospheric pressure for 75 min. The catalyst was removed by filtration (Celite) and washed with DMAC (5 mL). The combined filtrate and wash was evaporated to dryness in vacuo and crude **30**, a colored oil (1.57 g), was dissolved in $(EtO)_3CH$ (10 mL). This solution was treated with concentrated HCl (0.3 mL) and the resulting mixture was stirred at room temperature for 16 h. The mixture was diluted with Et_2O, and the solid was collected by filtration and recrystallized from 2-propanol (900 mL): yield, 535 mg.

Ethyl *N*-[1,4-Diamino-2-(benzylthio)imidazo[4,5-*c*]pyridin-6-yl]carbamate (24). A solution of **15** (1.00 g, ~3.23 mmol)[12] in H_2O (50 mL) and 1 N NaOH (3.25 mL) containing excess benzyl chloride (0.415 mL) was stirred at room temperature for 1.5 h. The precipitate was collected by filtration and washed with H_2O (50 mL) and Et_2O (40 mL): yield, 1.05 g.

Ethyl *N*-[1,4-Diamino-2-[(4-methoxybenzyl)thio]imidazo[4,5-*c*]pyridin-6-yl]carbamate (25). A solution of **15** (400 mg, ~1.49 mmol)[12] in H_2O (25 mL) and 1 N NaOH (1.5 mL) containing excess 4-methoxybenzyl chloride (0.22 mL) was stirred at room temperature for 1.5 h to deposit a mixture of **15** and **25**. The precipitate (426 mg) was retreated as described above and the product was washed with 0.02 N NaOH (15 mL) and recrystallized from propanol: yield, 174 mg.

Ethyl *N*-[1,4-Diamino-2-[[3,4-(methylenedioxy)benzyl]thio]imidazo[4,5-*c*]pyridin-6-yl]carbamate (26). To a solution of **15** (388 mg, ~1.45 mmol)[12] in H_2O (25 mL) and 1 N NaOH (1.5 mL) was added portionwise with stirring a solution of 3,4-(methylenedioxy)benzyl bromide (500 mg, 2.33 mmol)[13] in dioxane (5 mL). After 1.5 h, the acidic mixture was treated with 1 N NaOH (1.5 mL), and the product was collected by filtration and recrystallized from propanol: yield, 289 mg.

Ethyl *N*-[6-Amino-4-(1-methylhydrazino)-5-nitropyridin-2-yl]carbamate (28). Methylhydrazine (7.80 g, 170 mmol) was added with stirring to a hot suspension of **27** (4.90 g, 18.8 mmol) in EtOH (150 mL). The clear solution was cooled and the precipitate was collected by filtration: yield, 4.54 g.

Ethyl *N*-[6-Amino-4-(2-benzylidene-1-methylhydrazino)-5-nitropyridin-2-yl]carbamate (29). A solution of **28** (939 mg, 3.48 mmol) in DMAC (10 mL) containing benzaldehyde (397 mg, 3.75 mmol) was stirred at room temperature for 16 h. After dilution with H_2O (40 mL), the product was collected by filtration, and washed with H_2O: yield, 1.18 g.

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(12) This sample of **12** was homogeneous by TLC, but the degree of solvation was unknown.

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Synthesis and Biological Activity of Atrial Natriuretic Factor Analogues: Effect of Modifications to the Disulfide Bridge

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A series of atrial natriuretic factor (ANF) analogues with modifications to the disulfide bridge and lacking the exocyclic N-terminal sequence was synthesized. The native cystine residue was substituted by isofunctional deamino carba, β,β-dimethyl carba and dehydro dicarba spanners that bridge residues 106 and 120. The compounds were prepared by segment condensation coupling using the base-labile (9-fluorenylmethyl)carboxyl protecting group. Biological evaluation revealed that the exocyclic N-terminal segment of ANF is not necessary for expression of high biological activity. The compounds retained high affinity for ANF receptors in bovine adrenal zona glomerulosa cells and were found to be potent antihypertensive and diuretic agents, indicating that the native disulfide bridge can be mimicked by isosteric spanning residues. It was noted that the reported analogues, unlike the endogenous hormone, show marked reduced inhibitory activity on PGE_1-stimulated aldosterone secretion from adrenal zona glomerulosa cells. This lack of inhibition may be a contributing element to the low saluresis in spite of the high level of diuresis observed with some analogues.

Atrial natriuretic factor (ANF) is an important regulatory hormone secreted by atrial myocytes[1] whose role as an endocrine, renal, and hemodynamic modulator renders it therapeutically attractive.[2,3] The pharmacological activity of ANF in normal and pathologic states has been the subject of considerable investigation. Its peripheral effects oppose the renin–angiotensin–aldosterone system[4] and indicate a primary role in the homeostatic regulation of extracellular fluid volume and electrolyte excretion. Ac-

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