

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 Et₂O 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. (8 β)-*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 Et₃N, 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, (8 β)-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 NH₄OH in 150 mL of H₂O. 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; CaCl₂·H₂O, 1.6; KH₂PO₄, 1.2; MgSO₄, 1.2; dextrose, 10.0; and NaHCO₃, 24.8. Tissue bath solutions were maintained at 37 °C and aerated with 95% O₂/5% CO₂. 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 5HT₂ 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 ED₅₀ of the agonist in the presence of the antagonist divided by the control ED₅₀. These results were then expressed as the negative logarithm of the K_B (i.e., -log K_B).

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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 CS₂ and Et₃N 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-dithioxoimidazo[4,5-*b*:5,4-*c*]pyridin-7-ylcarbamate (**16**). Cyclization of the benzylidenehydrazino derivative (**6**) of **10** with either CS₂-Et₃N 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-

Scheme I

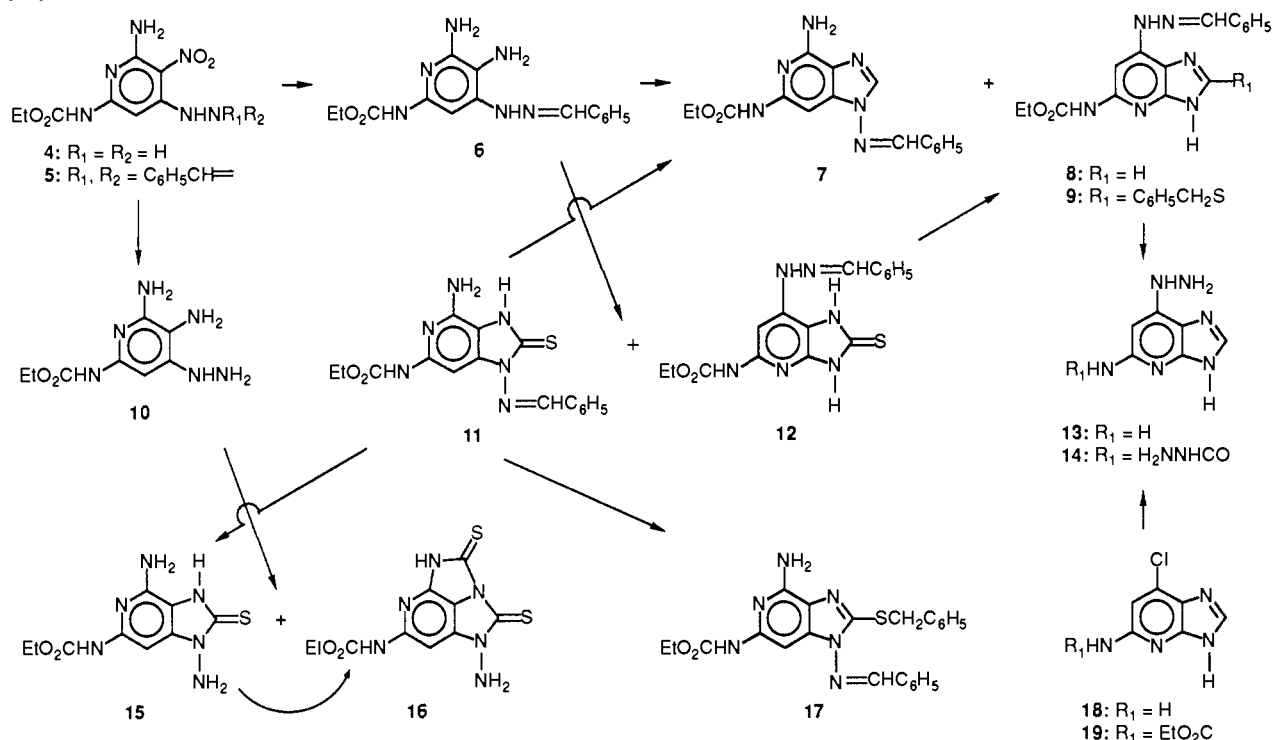
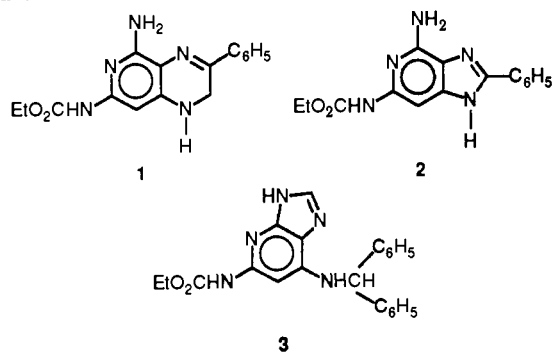


Chart I



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-yl carbamates (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 deprotected 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 18⁵ 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 19⁵ 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 $(EtO)_3CH-HCl$ reagent⁷ provided a mixture of 7 and 8 with

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Scheme II

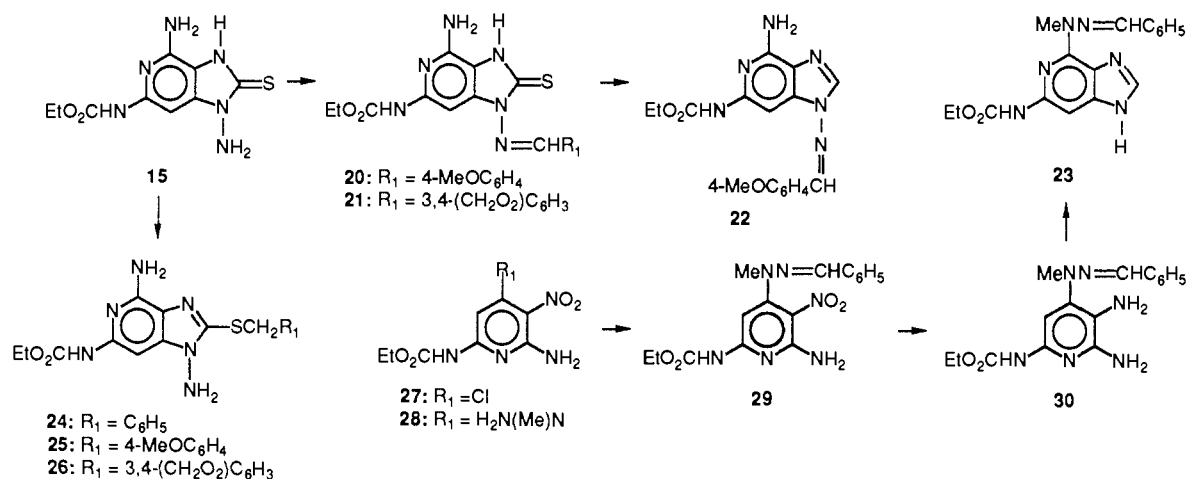


Table I. Properties of Compounds

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 ₁₆ H ₁₆ N ₆ O ₄	C, H, N
6	83	>260 ^c	315	7.19, 8.01	C ₁₅ H ₁₃ N ₆ O ₂	C, H, N
7	34 ^d	195 dec	324 ^e	7.40, 8.75, 9.15	C ₁₆ H ₁₆ N ₆ O ₂	C, H, N
8	38	>267 dec	325	7.74, 8.04, 8.33	C ₁₆ H ₁₆ N ₆ O ₂ ·0.9EtOH	C, H, N
9	22	280 dec	447	7.71, 8.33	C ₂₃ H ₂₂ N ₆ O ₂ S	C, H, N
11	36	>300	357	7.20, 9.88	C ₁₆ H ₁₆ N ₆ O ₂ S	C, H, N
12	15	>300	357	7.63, 8.00	C ₁₆ H ₁₆ N ₆ O ₂ S ^f ·Me ₂ NCOMe	C, H, N
13 ^g			165	9.10 ^h		
14	76	262 dec		6.91, 9.20	C ₇ H ₁₀ N ₆ O	H, N, C ⁱ
15	12 ^d	>275 dec	269	7.11	C ₉ H ₁₂ N ₆ O ₂ S ^f ·0.9EtOH	C, H, N
16	~93 ^j	>300	311	6.69	C ₁₀ H ₁₀ N ₆ O ₂ S ₂ ·0.5H ₂ O	C, H, N
17	95	107-8	447	7.74, 8.94	C ₂₃ H ₂₂ N ₆ O ₂ S ^f ·0.8H ₂ O	C, H, N
20	29	299-300 dec	387	7.13, 9.62	C ₁₇ H ₁₈ N ₆ O ₃ S	H, N, C ^k
21	38	>320	401	7.14, 9.62	C ₁₇ H ₁₈ N ₆ O ₄ S ^f ·0.8Me ₂ NCOMe	C, H, N
22	14	195-6	355	7.36, 8.69, 9.06	C ₁₇ H ₁₈ N ₆ O ₃	C, H, N
23	38	251-2 dec	339	7.72, 8.16, 8.98	C ₁₇ H ₁₈ N ₆ O ₂ S ^f ·0.8Me ₂ CHOH·HCl·0.3H ₂ O	C, H, N
24	86	129 dec	359	7.17	C ₁₆ H ₁₆ N ₆ O ₂ S	C, H, N
25	29	256-8 dec	389	7.16	C ₁₇ H ₂₀ N ₆ O ₃ S ^f ·0.27PrOH	C, H, N
26	44	161-2 dec		7.14	C ₁₇ H ₁₈ N ₆ O ₄ S ^f ·0.8PrOH	C, H, N
28	89	169-70	270 ^e	6.73	C ₉ H ₁₄ N ₆ O ₄	C, H, N
29	94	191-3 dec	358 ^e		C ₁₆ H ₁₈ N ₆ O ₄	C, H, N

^aFast atom bombardment mode (M + 1)⁺ unless otherwise indicated. ^bChemical shifts (δ) were determined in Me₂SO-d₆ unless otherwise indicated. ^cShrinkage and discoloration from 182 °C. ^dSee the Experimental Section. ^eElectron-impact mode (M)⁺. ^fThe presence of solvent in these compounds was confirmed by the ¹H NMR spectrum: EtOH, δ 1.06, 3.44; Me₂NCOMe, δ 1.97, 2.80, 2.96; Me₂CHOH, δ 1.03, 3.77; PrOH, δ 0.83, 1.41, 3.39. ^gSee ref 5. ^hSpectrum determined in CF₃CO₂D. Apparently the pyridine ring CH underwent deuterium exchange. ⁱC: calcd, 37.83; found, 38.40. ^jCrude yield calculated from 1. ^kC: calcd, 52.84; found, 52.31.

7 being the major product. Thus, in the reaction of 6 either with CS₂ 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 CS₂ cyclization reaction. Also, 15 was the major product when a solution of 10 in dimethylacetamide was treated with CS₂-Et₃N. In addition, reaction of 15 with CS₂ under heterogeneous conditions gave 16, identical with the product isolated from 10 and CS₂ 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^g 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 IC₅₀ values greater than 100 μM and were eliminated from further consideration.⁹ Most of the compounds that exhibited an IC₅₀ 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

compd	L1210		P388, ^c 10 ⁶ tumor cell implant, ip, qd 1-5	
	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-arylpseudo[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.

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 P₂O₅. *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 H₂O (200 mL) and the product was collected by filtration and washed with H₂O: 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 H₂O (2×) and DMAC (2×)] was hydrogenated at room temperature and atmospheric pressure. The uptake was stopped after the theoretical amount of H₂ (293 mL) was absorbed within 110 min. The flask was flushed with N₂, and the catalyst was removed by filtration through Celite into a flask containing deoxygenated (N₂) H₂O (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 H₂O 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 H₂O (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 CHCl₃-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 H₂O (25 mL) was adjusted to pH ~10 with 0.1 N NaOH, and the solid was collected by filtration and recrystallized from EtOH-H₂O 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 H₂O (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 Et₃N (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 H₂O (50 mL), and the solid (758 mg) that deposited was collected

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by filtration and recrystallized first from EtOH and then CHCl₃ to give **9** contaminated with **12**: yield, 396 mg. A solution of this sample in CHCl₃ (100 mL) was applied to a silica gel 60 (230-400 mesh) pad (20 g) and eluted with hot 99:1 CHCl₃-MeOH (300 mL). The solid isolated from this fraction was recrystallized from CHCl₃ (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 H₂O (3×)] in 1:1 EtOH-H₂O (500 mL) was hydrogenated at 23 °C and atmospheric pressure. The theoretical amount (697 mL) of H₂ 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 C₁₈, 9:1 NH₄OAc (pH 6)-MeCN]. A greater amount of the cleavage product was formed when the hydrogenation was carried out in either H₂O 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), Et₃N (5.40 g, 53.5 mmol), and CS₂ (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 Et₂O (2 × 100 mL), and dried to give 12-DMAC: yield, 3.48 g.

The reaction filtrate was added with stirring to H₂O (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 Et₂O (450 mL), and reprecipitated from hot MeOH (3.2 L) with an equal volume of H₂O to give **11**: yield, 6.9 g.

The cloudy DMAC-H₂O 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 CF₃CO₂D 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 (CF₃CO₂D) 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-H₂O 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 H₂O (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 H₂O (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 H₂O (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 CS₂ (100 mL) and Et₃N (7 mL) was stirred at room temperature for 40 h. The solid was collected by filtration, suspended in hot H₂O (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-0.3H₂O: yield, 0.820 g. The residue (0.320 g) from evaporation of the MeOH filtrate was shown by TLC (silica gel, 4:1 CHCl₃-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 H₂O (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 CS₂ (40 mL) containing Et₃N (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 H₂O (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 CH₂Cl₂ (10 mL), extracted with hot EtOH (125 mL) and the extract evaporated under N₂ to deposit **16**: yield, 90 mg.

Method B. A suspension of 15-0.8MeOH-0.3H₂O (500 mg, 1.67 mmol) in CS₂ (40 mL) containing Et₃N (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 H₂O (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 (Me₂SO-*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 Et₃N (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 H₂O (60 mL), and the precipitate was collected by filtration and washed with H₂O and Et₂O (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 H₂SO₄ was heated with stirring at 93 °C for 2.5 h. The hot solution was added with stirring to hot H₂O (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 H₂O (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 piperone (651 mg, 4.34 mmol) in DMAC (50 mL) containing a small drop of concentrated H₂SO₄ was heated at 90 °C for 2.5 h. The resulting solution was added to H₂O (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 H₂O (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 H₂O (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 H₂O 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 N₂ 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)-3H-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 H₂O 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)₂CH (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 Et₂O, 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 H₂O (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 H₂O (50 mL) and Et₂O (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 H₂O (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 H₂O (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 H₂O (40 mL), the product was collected by filtration, and washed with H₂O: yield, 1.18 g.

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Registry No. 4, 109182-40-3; 5, 123753-53-7; 6, 123753-54-8; 7, 123753-55-9; 8, 123753-56-0; 9, 123753-57-1; 10, 123753-58-2; 10-XHCl, 123753-74-2; 11, 123753-59-3; 12, 123753-60-6; 12-DMAC, 123775-15-5; 13, 37660-66-5; 14, 123753-61-7; 15, 123753-62-8; 16, 123753-63-9; 17, 123753-64-0; 19, 37436-94-5; 20, 123753-65-1; 21, 123775-16-6; 22, 123753-66-2; 23, 123753-67-3; 24, 123753-68-4; 25, 123753-69-5; 26, 123753-70-8; 27, 6506-86-1; 28, 123753-71-9; 29, 123753-72-0; 30, 123753-73-1; PhCHO, 100-52-7; *o*-MeOC₆H₄CHO, 135-02-4; *p*-MeOC₆H₄CH₂Cl, 824-94-2; 3,4-(OCH₂)C₆H₃CH₂Br, 2606-51-1; MeNHNH₂, 60-34-4; ethyl N-(4,5,6-triaminopyridin-2-yl)carbamate, 123753-52-6; piperonal, 120-57-0.

(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 PGE₁-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 myocytes¹ 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 system⁴ and indicate a primary role in the homeostatic regulation of extracellular fluid volume and electrolyte excretion. Ac-

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