

Spectral changes which accompanied the oxidation employed 0.20 mM 5,6-DHT in pH 7.2 buffer at room temperature in a 0.5-cm quartz cell.

Unless otherwise noted, the pH 7.2 phosphate buffer employed had an ionic strength (μ) of 0.1.

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University of Oklahoma is gratefully acknowledged. We also thank Dr. R. N. Goyal for some of the electrochemical experiments which were performed.

Registry No. 1, 129176-09-6; 5, 129176-12-1; 6, 129176-08-5; 7, 129176-10-9; 8 (isomer 1), 129176-11-0; 8 (isomer 2), 55206-15-0; 9a, 129176-13-2; 5,6-DHT, 5090-36-8; H₂O₂, 7722-84-1; SOD, 9054-89-1; Fe, 7439-89-6; Cu, 7440-50-8; OH, 3352-57-6; O₂^{••}, 11062-77-4; creatinine, 60-27-5; catalase, 9001-05-2; peroxidase, 9003-99-0; xanthine oxidase, 9002-17-9; tyrosinase, 9002-10-2; xanthine, 68-89-6; ceruloplasmin, 9031-37-2.

Potential Antimitotic Agents. Synthesis of Some Ethyl Benzopyrazin-7-ylcarbamates, Ethyl Pyrido[3,4-*b*]pyrazin-7-ylcarbamates, and Ethyl Pyrido[3,4-*e*]-*as*-triazin-7-ylcarbamates

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Ring analogues and derivatives of the 1,2-dihydropyrido[3,4-*b*]pyrazin-7-ylcarbamates (e.g., **29**), antimitotic agents with antitumor activity, were prepared in the search for compounds with greater selectivity. Methods were developed for the conversion of substituted benzoic acids (1-4) to give benzopyrazines (12-16 and 21) and of substituted pyridin-2-carbamates (**23**, **38**, and **41**) to give 2-aminopyrido[3,4-*b*]pyrazin-7-ylcarbamates (**32** and **36**) and pyrido[3,4-*e*]-*as*-triazin-7-ylcarbamates (**47** and **50**). In vitro evaluation indicated that activity was reduced by removal of the pyridine ring nitrogen of **29** to give **14** and was destroyed by increasing the basicity of the pyrazine ring of **29** to give **32** and **47**.

The 1,2-dihydropyrido[3,4-*b*]pyrazin-7-ylcarbamates (e.g., **29**) are potent inhibitors of the in vitro polymerization of tubulin to give microtubules.¹ These compounds show anticancer activity and are cytotoxic to cultured L1210 cells at nanomolar concentrations.^{2,3} The preparation of compounds with greater cytotoxicity, however, has not provided compounds with greater antitumor activity. In work directed toward the identification of agents with greater selectivity, ring analogues and derivatives of the pyrido[3,4-*b*]pyrazines were synthesized. The effect on activity of removing the pyridine ring nitrogen was determined by the preparation of benzopyrazines, and the effect on activity of increasing the basicity of the pyrazine ring was determined by the preparation of pyrido[3,4-*e*]-*as*-triazines and 2-aminopyrido[3,4-*b*]pyrazines.

Chemistry

The commercially available 4-chloro-3,5-dinitrobenzoic acid (**1**) was converted to the corresponding acid chloride (**2**), ethyl ester (**3**), and acid azide (**4**) by the reported methods.⁴ The amination of **3** with the diethyl acetal of 2-amino-2-phenylacetaldehyde⁵ gave **6**, which was hydrogenated over Raney nickel to give **9** (Scheme I). Without isolation **9** was treated with acid, and the cyclization product was allowed to undergo air oxidation to afford **12**. Reduction of **12** with NaBH₄-Al₂O₃ gave **13**, which was isolated as a 4:1 mixture of **13** and **12**. A similar route was

attempted for the preparation of **14**. The isocyanate formed from **4** in hot toluene was reacted with methanol to give **5**. Also the preparation of **5** was attempted from **2** and excess sodium azide. Under these conditions, the ring chloro group was displaced by an azido group, which underwent loss of N₂ with formation of **17**.⁶ Amination of **5** with the diethyl acetal of 2-amino-2-phenylacetaldehyde⁵ gave **7**, which was hydrogenated over Raney nickel in ethanol to give crude **10**. The presence of **10** in this sample was supported by the mass spectrum and the absence in the ¹H NMR spectrum of **14** (or tautomer) and its oxidation product **15**. Cyclization of **10** to **14** was attempted with acid, however, these conditions resulted in the loss of the acetal moiety with the formation of the benzimidazole **20**. This reaction probably proceeds by the addition of either of the adjacent amino groups to the α -carbon of the enol form of the aldehyde followed by loss of formaldehyde and oxidation of the resulting dihydrobenzimidazole. The structure of **20** was confirmed by an unambiguous synthesis. The amination of **5** with ammonia gave **18**, which was hydrogenated over Raney nickel to give **19**. The oxidative cyclization of **19** with the sodium bisulfite addition product of benzaldehyde gave **20**, a reaction that also proceeds via a dihydrobenzimidazole intermediate.⁷

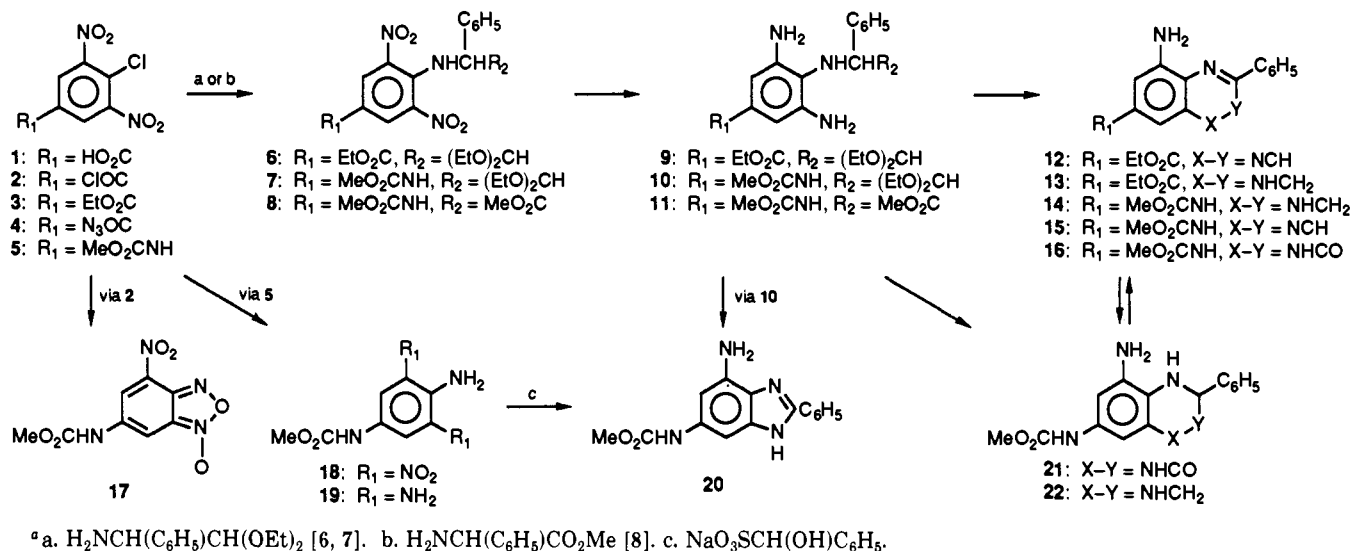
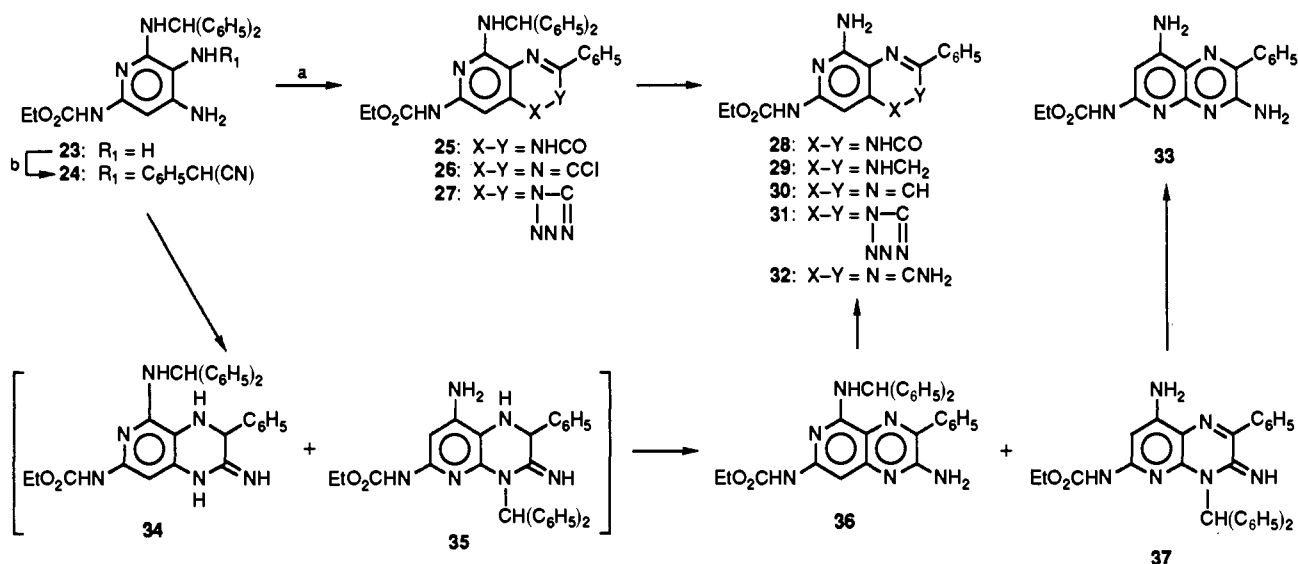
In another approach to **14**, **5** was reacted with methyl 2-phenylglycinate⁸ to give **8**. Hydrogenation of **8** over Raney nickel gave **11**, which was cyclized to give a mixture of **21** (86%) and its oxidized product **16** (14%). Also, the reductive cyclization of **8** was effected with Zn-HOAc, but in this reaction the product (**21**) was allowed to undergo air oxidation to give **16**. Reduction of the amide group of

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Scheme I^aScheme II^a

16 was effected with the borane-methyl sulfide complex to give 22 (mass spectrum), which on purification underwent air oxidation to give 15. In a trial experiment, reduction of 15 with NaBH₄-Al₂O₃ gave a 9:1 mixture of 14-15. In a repeat of this reaction the product was isolated in the presence of ascorbic acid, but these conditions gave a 1:2 mixture of 14-15.

For the preparation of 32 (Scheme II), the known 23⁹ was condensed with ethyl benzoylformate to provide 25. The structure of 25 was supported by its ¹H NMR spectrum and was confirmed by its conversion via 28 and 29 to 30. An authentic sample of 30 was prepared by oxidation of the known 29.¹⁰ Chlorohydroxylation of 25 with POCl₃ at 95 °C over 3 h gave 26. The introduction of the amino group by amination of 26 with ammonia at high temperatures, which might either react with or cleave the carbamate group,^{3a} was circumvented by the reaction of 26 with sodium azide. This reaction provided the tet-

razolo (27) rather than its azido tautomer.¹¹ The diphenylmethyl group of 27 was easily removed with CF₃CO₂H at room temperature to give 31. Because the azido tautomer of 27 should be favored under acidic conditions,¹¹ the hydrogenation of 27 over Raney nickel in acetic acid was expected to result in cleavage of the (diphenylmethyl)amino group with simultaneous reduction of the azido group to give 32. Under these conditions only the diphenylmethyl group was removed to give 31. Further hydrogenation of 31 in CF₃CO₂H in the presence of platinum gave a complex mixture from which only a low yield of 32 was isolated. In a more successful approach to 32, 23⁹ was reacted with α-cyanobenzyl 4-methylphenylsulfonate¹² to afford 24 (Scheme II). The cyclization of crude 24 with methoxide gave a mixture of 34 and 35, which underwent air oxidation to give a mixture of 36 and 37. These isomeric compounds were separated by column chromatography to eventually give a pure sample of 37 and an essentially homogeneous sample (TLC) of 36. The ¹H

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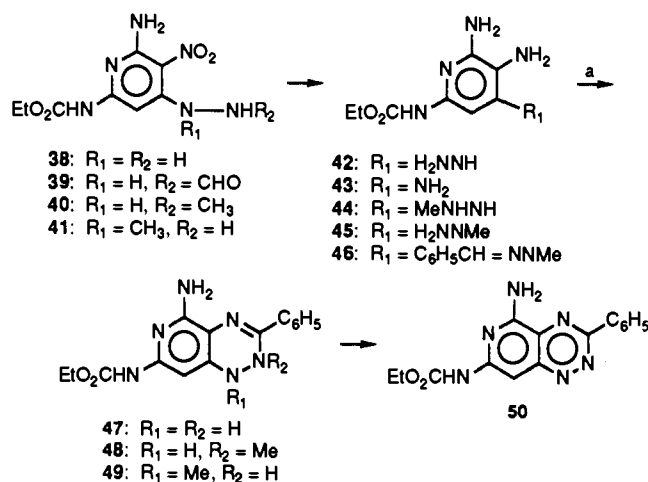
Table I. Properties of Compounds

compd	yield, %	mp, °C	mass spectra ^a	¹ H NMR spectra ^a selected peaks, δ	formula	anal.
5	74	176–179 ^b	275 (M ⁺)	8.35 s (2-CH, 6-CH)	C ₈ H ₆ ClN ₃ O ₆	CHN
6	99	c	448 [(M + 1) ⁺]	8.55 s (2-CH, 6-CH)		
7	81	c	448 (M ⁺)	8.32 s (2-CH, 6-CH)	C ₂₀ H ₂₄ N ₄ O ₈	CHN
8	41	100–105	405 [(M + 1) ⁺]	5.09 d (CHC ₆ H ₅), 8.36 s (2-CH, 6-CH) ^d	C ₁₇ H ₁₆ N ₄ O ₈ ·0.1CH ₃ CH ₂ OH	CHN
12	33	161–163	294 [(M + 1) ⁺]	9.61 s (2-CH)	C ₁₇ H ₁₆ N ₃ O ₂	CHN
13 ^e	64	145–153	296 [(M + 1) ⁺] ^f	4.33 s (2-CH ₂) ^g	C ₁₇ H ₁₇ N ₃ O ₂ ·0.25C ₁₇ H ₁₅ N ₃ O ₂ ·0.4H ₂ O	CHN
			294 [(M + 1) ⁺] ^h	9.62 s (2-CH)		
14 ⁱ	71	222–225	297 [(M + 1) ⁺] ^j	4.23 s (2-CH ₂) ^k	C ₁₆ H ₁₆ N ₄ O ₂ ·2C ₁₆ H ₁₄ N ₄ O ₂ ·0.3C ₆ H ₆ O ₆	CHN
			295 [(M + 1) ⁺] ^l	9.39 s (2-CH)		
15	27	226–227 ^m	294 (M ⁺)	9.40 s (2-CH) ^g	C ₁₆ H ₁₄ N ₄ O ₂ ·0.2H ₂ O	CHN
16	74	>300	311 [(M + 1) ⁺]	6.65 d, 6.80 d (6-CH, 8-CH) ⁿ	C ₁₆ H ₁₄ N ₄ O ₃ ·0.5CH ₃ OH	CHN
17	59	165–175 ^m	254 (M ⁺)	7.85 d, 8.54 d (5-CH, 7-CH)	C ₈ H ₆ N ₄ O ₆	CHN
18	49	198–201	256 (M ⁺)	8.66 s (2-CH, 6-CH)	C ₈ H ₈ N ₄ O ₆	CHN
19	77	>300 ^m	196 (M ⁺)	6.09 s (2-CH, 6-CH) ^g	C ₈ H ₁₂ N ₄ O ₂ ·0.4H ₂ O	CHN
20 ^o	33	195–198	283 [(M + 1) ⁺]	6.79 d (7-CH), 7.29 d (5-CH) ^d	C ₁₅ H ₁₄ N ₄ O ₂ ·2.7HCl·0.7CH ₃ CH ₂ OH	CHN
21 ^p	26	201–220 ^q	313 [(M + 1) ⁺]	4.81 d (4-NH), 5.41 d (3-CH), ^{g,r} 6.77 d (6-CH, 8-CH) ^s	C ₁₆ H ₁₆ N ₄ O ₃ ·0.16C ₁₆ H ₁₄ N ₄ O ₃ ·0.8H ₂ O	CHN
24	17		493 [(M + 1) ⁺]	4.88 d, 5.17 d (NHCHCN), 6.25 br s, 6.35 d [NHCH(C ₆ H ₅) ₂]		
25	62	300–305 ^m	492 [(M + 1) ⁺]	6.77 d, 7.55 d (CHNH)	C ₂₉ H ₂₅ N ₅ O ₃	CHN
26	66	174–175 ^t	510 [(M + 1) ⁺]	6.85 d, 8.45 d (CHNH) ^u	C ₂₉ H ₂₄ ClN ₅ O ₂ ·0.1CHCl ₃	CHN
27	76	210–233 ^m	517 [(M + 1) ⁺]	7.00 d, 8.76 d (CHNH) ^v	C ₂₉ H ₂₄ N ₅ O ₂ ·C ₄ H ₈ O ₂	CHN
28	97	>300 ^m	326 [(M + 1) ⁺]	6.72 s (8-CH)	C ₁₆ H ₁₅ N ₅ O ₃ ·1.2HCl	CHN
30 ^o	60	203–205	310 [(M + 1) ⁺]	7.48 s (8-CH), 9.53 s (2-CH) ^u	C ₁₆ H ₁₅ N ₅ O ₂ ·0.35CHCl ₃	CHN
31	75	202–206 ^m	351 [(M + 1) ⁺]	8.02 s (9-CH) ^u	C ₁₆ H ₁₄ N ₅ O ₂ ·1.1CF ₃ CO ₂ H·0.06C ₁₃ H ₁₂ O	CHN
32 ^o	39 ^x	>300 ^{m,x}	325 [(M + 1) ⁺]	6.61 s (8-CH), ^x 7.09 s (8-CH)	C ₁₆ H ₁₆ N ₆ O ₂ ·1.06CF ₃ CO ₂ H	CHN
				6.68 s (8-CH) ^v	C ₁₆ H ₁₆ N ₆ O ₂	CHN
33	10	210–215 ^m	325 [(M + 1) ⁺]	6.68 s (8-CH) ^v	C ₁₆ H ₁₆ N ₆ O ₂ ·1.03CF ₃ CO ₂ H·0.2C ₄ H ₈ O ₂	CHN
36	21		491 [(M + 1) ⁺]	6.51 d, 6.99 d (CHNH) ^y		
37	15		491 [(M + 1) ⁺]	7.04 (7-CH) ^d	C ₂₉ H ₂₆ N ₆ O ₂ ·0.4CH ₃ CH ₂ OH	CHN
39	96	220–222	285 [(M + 1) ⁺]		C ₉ H ₁₂ N ₆ O ₅	CHN
40	49	178–180 ^m	271 [(M + 1) ⁺]	2.51 d, 4.98 q (CH ₃ NH)	C ₉ H ₁₄ N ₆ O ₄	CHN
47 ⁿ	15	~250 ^m	313 [(M + 1) ⁺]	5.55 (8-CH) ^d	C ₁₅ H ₁₆ N ₆ O ₂ ·HCl·0.5CH ₃ CH ₂ OH	CHN
50	17	190–192 ^m	311 [(M + 1) ⁺]	7.78 (8-CH)	C ₁₅ H ₁₄ N ₆ O ₂ ·HCl	CHN

^a See introduction to Experimental Section. ^b Presoftening from 160 °C. ^c Oil. ^d Ethanol observed, δ 1.06 t, 3.45 q. ^e ¹H NMR spectrum indicated a 4:1 ratio of 13–12. ^f 13. ^g H₂O observed, δ 3.32. ^h 12. ⁱ ¹H NMR spectrum indicated a 1:2 ratio of 14–15. ^j 14. ^k Ascorbic acid observed, δ 3.44 d. ^l 15. ^m With decomposition. ⁿ Methanol observed, δ 3.18. ^o See Experimental Section. ^p ¹H NMR spectrum indicated a 6:1 ratio of 21–16. ^q With reddening and presoftening from 150 °C. ^r 21. ^s 16. ^t With presoftening from 80 °C and crystallization at 115 °C. ^u Chloroform observed, δ 8.31. ^v Dioxane observed, δ 3.57. ^w Diphenylmethanol observed, δ 7.32. ^x Trifluoroacetate. ^y ¹H NMR spectrum determined in CDCl₃.

NMR spectrum of **36** showed the CH group of the diphenylmethyl group as a doublet, whereas, the corresponding CH group of **37** was observed as an overlapping singlet with the amino group. Treatment of both **36** and **37** with CF₃CO₂H removed the diphenylmethyl group to give **32** and **33**, respectively.

For the preparation of the pyridotriazines the known **42** (contaminated with **43**) was prepared from **38** (Scheme III).¹³ The condensation of **42** with an aqueous solution of methyl benzimidate hydrochloride in the presence of Et₃N provided a mixture of **47** and **50**.¹⁴ Extraction of this mixture with EtOH gave an insoluble product, which was identified by its ¹H NMR spectrum as a 5:1 mixture of **47–50**. In the reaction of **42** with an aqueous solution of the hydrochloride of methyl benzimidate, only the hydrochloride of **50** was isolated. The stabilization of the 1,2-dihydro moiety in the pyridotriazines was attempted by the preparation of the *N*-methyl derivatives **48** and **49**. The 4-(2-methylhydrazino)pyridine **40** was prepared by formylation of **38** with butyl formate to give **39** followed by reduction of the amide moiety with the borane–methyl sulfide complex. Hydrogenation of **40** in MeOH gave **44**, which was condensed in situ with methyl benzimidate hydrochloride to give crude **48**. Purification of this product by column chromatography resulted in the oxidative removal of the *N*-methyl group to give only **50**. Also, dif-

Scheme III^a

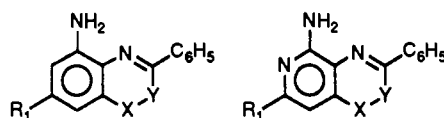
^a (a) MeOC(NH)C₆H₅ (**47**, **48**).

iculties were encountered in the preparation of **49**. The hydrogenation of the known **41**¹³ with Raney nickel in 1:1 EtOH–H₂O gave a mixture of **45** and the corresponding hydrazino cleavage product. No pyridotriazine, either **49** or **50**, was isolated from the condensation of this mixture with methyl benzimidate. In another approach the oxidative cyclization of **46**¹³ to **49** was attempted, but this reaction gave only **50** as the major product. The oxidative demethylation of both **48** and **49** are similar to demeth-

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Table II. Biological Data



compd (X-Y)	L1210			
	IC ₅₀ , μM ^a	MI _{0.5} , μM ^b	IC ₅₀ , μM ^a	MI _{0.5} , μM ^b
-(NHCH ₂), R ₁ = H ^c			2.8	>10
-(NHCH ₂), R ₁ = NH ₂ ^c			5. × 10 ⁻¹	8.5 × 10 ⁻¹
12 (N = CH), R ₁ = EtO ₂ C	>100	>10		
13 (NHCH), R ₁ = EtO ₂ C ^d	>100	>10		
30 (N = CH), R ₁ = EtO ₂ CHN			3. × 10 ⁻¹	-
15 (N = CH), R ₁ = MeO ₂ CHN	>100	-		
14 (NHCH ₂), R ₁ = MeO ₂ CHN ^d	24	-		
29 (NHCH ₂), R ₁ = EtO ₂ CHN ^e			4.7 × 10 ⁻³	2.8 × 10 ⁻³
16 (NHCO), R ₁ = MeO ₂ CHN ^f	7.5 × 10 ⁻¹	1.9		
28 (NHCO), R ₁ = EtO ₂ CHN ^f			4.2 × 10 ⁻²	2.2 × 10 ⁻²
32 (N = CNH ₂), R ₁ = EtO ₂ CHN			>30	-
47 (NHNH), R ₁ = EtO ₂ CHN ^d			>10	
50 (N = N), R ₁ = EtO ₂ CHN			>10	-

^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 (fraction 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 Reference 3a. ^d The compound was evaluated as a mixture containing the corresponding aromatic ring system. The 1,2-dihydro compound was subject to air oxidation to the aromatic ring system and the biological data might be unreliable. ^e Reference 10. ^f These compounds were evaluated for antitumor activity against lymphocytic leukemia P388 (ref 17): % ILS 21 (100 mg/kg) for 16; 38 (50 mg/kg) for 28. ^g A mixture of 21-16 (86:14) gave IC₅₀ 2.7 μM.

ylations that have been observed in the pyrimido[5,4-*e*]-*as*-triazines.¹⁵

The properties of the new compounds are listed in Table I.

Biological Evaluation

The evaluation of the new compounds was based primarily on the IC₅₀ values observed for the inhibition of proliferation of lymphoid leukemia L1210 cells (Table II).¹⁶ Selected compounds were evaluated also for the inhibition of mitosis in L1210 cells.¹⁶ Because of the instability of the 1,2-dihydro moiety in most of the target compounds, these compounds were evaluated as mixtures containing the corresponding aromatic ring system.

Previous work with (1,2-dihydropyrido[3,4-*b*]pyrazin-7-yl)carbamates showed that cleavage of the carbamate moiety to give an amino group and replacement of the carbamate by hydrogen reduced in vitro activity significantly (Table II).^{3a} Similarly no activity was observed when the carbamate nitrogen was removed to give the benzopyrazines 12 and 13. The aromatic pyrido[3,4-*b*]pyrazine 30 showed cytotoxicity and no in vivo activity, but the cytotoxicity was destroyed when the pyridine ring nitrogen was removed to give 15. Although some cytotoxicity was regained when 15 was converted to the 1,2-dihydro derivative 14, the latter was considerably less cytotoxic than the corresponding 1,2-dihydropyrido[3,4-*b*]pyrazine 29. The loss in activity when the pyridine ring nitrogen was removed was supported by the relative activities of the stable compounds 16 and 28. The magnitude of the differences in the activities of 14 and 29 as compared with 16 and 28 suggested that 14 underwent partial air oxidation to 15 during the evaluation.

The target compounds 32 and 47 were prepared to determine if increasing the basicity of the pyrazine ring might promote either an increase or a more selectively binding

of agent to tubulin. Protonation at N-1 of 47 should be aided by the ring 2-NH and in the absence of steric hindrance, protonation at N-1 of 32 should be stabilized by resonance with the 2-amino group. The inactivity of both 32 and 47 relative to 30 and 29 indicated that N-1 is unprotonated on binding to tubulin. However, the data for 47 is considered unreliable because of the facile conversion of 47 to 50, which is inactive.

In conclusion, this and previous work show that the carbamate, both the carbonyl and amino components and the pyridine ring nitrogen of the (1,2-dihydropyrido[3,4-*b*]pyrazin-7-yl)carbamates are required for potent antimetabolic activity. Presumably this region of the active agents plays a significant role in the binding of these compounds to tubulin. In contrast, the role of the 1,2-dihydro moiety of the 1,2-dihydropyrido[3,4-*b*]pyrazines is unclear. The decrease in activity with 30, 32, 47, and 50 and the retention of some activity with 16 and 28 suggest that the hydrogen donating ability of the 1-NH and the pucker of the 1,2-dihydropyrazine ring in 29 might be more important in binding to tubulin than protonation at N-1.

Experimental Section

Melting and decomposition temperatures were determined in capillary tubes in a Mel-Temp apparatus. The ¹H NMR spectra were determined on Me₂SO-*d*₆ solutions with either a Varian XL-100-15 or a Nicolet NB 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. HPLC chromatograms were determined on an ALC-242 liquid chromatograph equipped with an UV detector (254 nm), an M-6000 pump, and a μC₁₈ Bondapak ODS column (10 μm). Flash chromatography was performed with silica gel 60 (230-400 mesh) from E. Merck. Raney nickel no. 2800 was obtained from Davison Speciality Chemical Co. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical value.

Methyl (4-Chloro-3,5-dinitrophenyl)carbamate (5). A solution of 4-chloro-3,5-dinitrobenzoyl azide (4, 8.5 g, 31 mmol)⁴ in dry toluene (100 mL) was heated slowly to reflux over 30 min and maintained at reflux for 2 h. After cooling and adding dry

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MeOH (300 mL), the clear solution was stirred for 18 h at room temperature and evaporated to dryness in vacuo. The resulting yellow solid was recrystallized from 2-propanol (60 mL) to afford 5: yield, 6.3 g.

Ethyl 4-[(2,2-Diethoxy-1-phenylethyl)amino]-3,5-dinitrobenzenecarboxylate (6) and Ethyl 5-Amino-3-phenylbenzopyrazine-7-carboxylate (12). A mixture of ethyl 4-chloro-3,5-dinitrobenzoate (3, 270 mg, 1.00 mmol)⁴ and the diethyl acetal of 2-amino-2-phenylacetaldehyde (420 mg, 2.00 mmol)⁵ in EtOH (10 mL) was stirred at room temperature for 72 h, and the insoluble material was removed by filtration. The filtrate was evaporated to dryness, the residue was extracted with Et₂O (80 mL), and additional insoluble material was removed by filtration. The filtrate was washed with water (3 × 20 mL), dried (Na₂SO₄), and evaporated to dryness to afford 6 as a brownish-orange oil: yield, 440 mg.

From another preparation, a solution of 6 (2.03 g, 4.54 mmol) in EtOH (150 mL) was hydrogenated in the presence of Raney nickel (6.1 g, weighed wet, washed 2 × H₂O and 2 × EtOH). After the uptake (102% of theoretical in 2 h) had ceased, the catalyst was removed by filtration (Celite). The filtrate containing 9 was concentrated in vacuo to 100 mL and treated with 1 N HCl (100 mL). The resulting clear yellow solution was stirred at room temperature for 140 h to deposit 12 as an orange solid: yield, 437 mg.

Methyl [4-[(2,2-Diethoxy-1-phenylethyl)amino]-3,5-dinitrophenyl]carbamate (7). A solution of the diethyl acetal of 2-amino-2-phenylacetaldehyde (760 mg, 3.60 mmol),⁵ Et₃N (2.0 mL, 14 mmol), and 5 (830 mg, 3.00 mmol) in EtOH (15 mL) was refluxed for 18 h and evaporated to dryness, and the residue was partitioned between CHCl₃ (50 mL) and H₂O (50 mL). The organic layer was evaporated in vacuo, and the residue was purified by column chromatography (100 g silica gel 60, cyclohexane-EtOAc, 4:1) to afford 7 as a red oil: yield 1.1 g.

Methyl [4-[[α-(Methoxycarbonyl)benzyl]amino]-3,5-dinitrophenyl]carbamate (8). A hot solution of 5 (7.24 g, 26.3 mmol) and methyl 2-phenylglycinate (13.0 g, 78.7 mmol)⁸ in toluene (220 mL) was refluxed for 23 h and cooled at room temperature for 18 h. The precipitate of the hydrochloride of the glycinate was removed by filtration, the filtrate was evaporated to dryness in vacuo, and the residue was dissolved in Et₂O (250 mL). The excess glycinate was precipitated by addition of 12.5 N methanolic HCl (3.2 mL) and removed by filtration. Evaporation of the filtrate in vacuo followed by column chromatography (200 g, CHCl₃) afforded 8 as an orange foam: yield, 4.36 g.

Ethyl 5-Amino-1,2-dihydro-3-phenylbenzopyrazine-7-carboxylate (13). To a stirred solution of 12 (75 mg, 0.26 mmol) in dioxane (5 mL) was added NaBH₄ on alumina (800 mg, 10% NaBH₄). Additional portions of NaBH₄ (2 × 800 mg) were added at 2-h intervals. After a total of 5 h, the alumina was removed by filtration and washed with dioxane (3 × 5 mL), and the combined filtrate and washes were evaporated to dryness. The resulting semisolid was dissolved in deoxygenated (N₂) MeOH (20 mL) and stirred under N₂ overnight. The solution was evaporated to dryness in vacuo, the residue was dissolved in deoxygenated (N₂) CHCl₃, and the solution was filtered through a pad of silica gel 60 (4 g) to remove boron salts. The filtrate was evaporated in vacuo to afford 13 contaminated with 12 (20%): yield, 49 mg.

Methyl (5-Amino-1,2-dihydro-3-phenylbenzopyrazin-7-yl)carbamate (14). A mixture of 15 (206 mg, 0.69 mmol) and NaBH₄ on alumina (2.0 g) in dioxane (20 mL) was stirred under N₂ for 22 h. The residue was removed by filtration under Ar (Celite) and washed with deoxygenated (Ar) dioxane (10 mL), and the filtrate was evaporated to dryness in vacuo. To the residue was added a solution of ascorbic acid (1.25 g, 7.1 mmol) in deoxygenated (Ar) H₂O (30 mL). The mixture was chilled for 18 h under Ar to afford a 1:2 mixture of 14 and 15 as a partial ascorbate salt: yield, 154 mg.

Methyl (5-Amino-3-phenylbenzopyrazin-7-yl)carbamate (15). To a deoxygenated (N₂) solution of 16 (1.19 g, 3.83 mmol) in dry THF (190 mL) was added borane-methyl sulfide complex (19.5 mL of 2 N solution in THF), and the resulting solution was stirred under N₂ at room temperature for 72 h. The slightly cloudy solution was evaporated to dryness to afford the crude 22 as an amber foam: yield, 1.36 g (120%); MS (FAB) *m/e* 299 (M + 1)⁺. This residue was dissolved in MeOH (50 mL) and evaporated to

dryness. The dissolution and evaporation was repeated twice, and the resulting residue was dissolved in MeOH and chilled at -20 °C. The flask was opened and air was admitted after 24 h and 48 h, and chilling was continued for an additional 144 h. The resulting mixture was evaporated to dryness in vacuo and the residue was purified by silica gel chromatography (75 g, CHCl₃) to afford 15 as an amber solid: yield, 300 mg.

Methyl (5-Amino-1,2-dihydro-2-oxo-3-phenylbenzopyrazin-7-yl)carbamate (16). A solution of 8 (3.96 g, 9.79 mmol) in HOAc (100 mL) was heated at 80 °C under N₂ as zinc dust (16 g, 240 mmol) was added portionwise over 0.25 h. The mixture was stirred an additional 0.4 h at 80 °C, cooled to room temperature, and filtered. The insoluble material was washed with HOAc (3 × 25 mL), and the combined filtrate and washes were evaporated to dryness in vacuo. The residue was suspended in water, the pH was adjusted to 7.5, and the oil was extracted into EtOAc (200 mL). The aqueous layer was made basic (pH 9-10) and extracted again with EtOAc (200 mL). The combined extracts were evaporated to dryness in vacuo, and the residue was stirred 24 h in MeOH (60 mL) to deposit 16: yield, 2.25 g.

Methyl (4-Nitrobenzofurazan-6-yl)carbamate 1-Oxide (17). A hot solution of 4-chloro-3,5-dinitrobenzoic acid (1, 1.0 g, 4.1 mmol) in SOCl₂ (10 mL) was refluxed for 1.5 h, cooled to room temperature, and evaporated to dryness at reduced pressure. Dry benzene (3 × 10 mL) was evaporated from the residue, which was then dissolved in dioxane (8 mL). To the resulting solution (0-5 °C) was added a solution of NaN₃ (0.50 g, 7.7 mmol) in H₂O (2 mL), and the ice bath was removed. After the solution was stirred for 0.25 h, H₂O (25 mL) was added, and the oily material which separated was extracted into Et₂O (3 × 50 mL). The combined organic layers were dried (Na₂SO₄) and evaporated in vacuo to a light brown oil. A solution of this oil in dry toluene (25 mL) was heated slowly to boiling, refluxed for 2 h, cooled at 20 °C, and diluted with dry MeOH (30 mL). After stirring for 16 h at room temperature, the red solution was evaporated to dryness, and the residue was stirred with Et₂O (20 mL) to afford 17 as a brownish orange solid: yield, 620 mg.

Methyl (4-Amino-3,5-dinitrophenyl)carbamate (18). A solution of 5 (2.0 g, 7.3 mmol) in methanolic ammonia (140 mL, ca. 8 N), was heated at 50 °C in a glass-lined bomb for 17 h, and the red solution was evaporated to dryness. The residue was triturated with 0.05 N aqueous NH₃ (100 mL), collected by filtration, and recrystallized from benzene (150 mL) to afford 18 as a brick-red solid: yield, 924 mg.

Methyl (3,4,5-Triaminophenyl)carbamate (19). A suspension of 18 (102 mg, 0.398 mmol) in H₂O-EtOH (1:1, 26 mL) was hydrogenated in the presence of Raney nickel (200 mg, weighed wet; washed 2 × H₂O and 2 × EtOH). After hydrogen uptake had ceased (104% of theoretical in 1.5 h), the catalyst was removed by filtration under N₂ (Celite) and the filtrate was evaporated to dryness in vacuo to give 19 as a brownish semisolid: yield, 62 mg.

Methyl (4-Amino-2-phenyl-1H-benzimidazol-6-yl)carbamate (20). **Method A.** A solution of 7 (420 mg, 0.93 mmol) in EtOH (85 mL) was hydrogenated in the presence of Raney nickel (850 mg, weighed wet, washed 2 × H₂O and 2 × EtOH). After the uptake had ceased (4 h), the catalyst was removed by filtration (Celite) under N₂, and the filtrate was evaporated to dryness in vacuo. The resulting yellow-green semisolid of crude 10 [mass spectrum, 388 (M)⁺] was triturated with a solution of 5 mL concentrated HCl in deoxygenated (N₂) EtOH (25 mL) to deposit 20: yield, 86 mg; HPLC [MeCN-OAc (0.1 M, pH 3.6), gradient 1:9 → 9:1] single peak at 11.07 min, which was identical (co-injection) with 20 prepared by method B.

Method B. A suspension of 18 (180 mg, 0.70 mmol) in 1:1 H₂O-EtOH (46 mL) was hydrogenated under atmospheric pressure in the presence of Raney nickel. After the uptake had ceased (103% of theoretical in 3 h), the catalyst was removed by filtration under N₂ (Celite) and washed with 1:1 H₂O-EtOH (2 × 25 mL). The filtrate containing 19 was treated with the sodium bisulfite addition product of benzaldehyde (150 mg, 0.70 mmol). After the solution was stirred for 44 h at room temperature under N₂, a trace amount of insoluble material was removed by filtration and the filtrate was evaporated to dryness in vacuo. The residue was washed with water (10 mL), dried in vacuo (P₂O₅), and dissolved in EtOH (5 mL), and the resulting solution was diluted

with ether (50 mL). A small amount (19 mg) of precipitate was removed by filtration, and the filtrate was evaporated to dryness. The resulting greenish-amber glass was triturated with a solution of concentrated HCl (0.6 mL) in EtOH (3 mL) to deposit **20**: yield, 76 mg. This material gave the correct mass ion [m/e 283, ($M + 1$)⁺], and the samples prepared by both methods were identical by TLC and ¹H NMR, including the ¹H NMR spectrum of a mixture of the two.

Methyl (5-Amino-1,2,3,4-tetrahydro-2-oxo-3-phenylbenzopyrazin-7-yl)carbamate (21). A solution of **8** (0.48 g, 1.2 mmol) in MeOH (50 mL) was hydrogenated for 3 h at room temperature and 3 h in a 48 °C oil bath in the presence of Raney nickel (1.5 g, weighed wet, washed 2 × H₂O and 2 × MeOH). The resulting yellow-green suspension was clarified by the addition of THF (50 mL) followed by refluxing for 0.3 h. The catalyst was removed by filtration (Celite) and the light orange filtrate was evaporated to dryness in vacuo. The semisolid residue was chromatographed (20 g silica gel 60, CHCl₃-MeOH 97:3) to afford **21** contaminated with **16**: yield, 97 mg.

Ethyl [4-Amino-5-[(cyanophenylmethyl)amino]-6-[(diphenylmethyl)amino]pyridin-2-yl]carbamate (24). To a suspension of ethyl [4,5-diamino-6-[(diphenylmethyl)amino]pyridin-2-yl]carbamate dihydrochloride (**23**, 102 mg, 0.226 mmol)⁹ in deoxygenated (N₂) EtOH (5 mL) was added Et₃N (45 mg, 0.45 mmol), α -cyanobenzyl 4-methylbenzenesulfonate (66 mg, 0.23 mmol),¹² and additional Et₃N (23 mg, 0.23 mmol). After stirring under N₂ for 72 h, more α -cyanobenzyl 4-methylbenzenesulfonate (15 mg, 0.052 mmol) was added, and stirring under N₂ was continued for an additional 96 h. The brown solution was evaporated to dryness in vacuo, and the residue was triturated with H₂O to give a light brown solid, which was purified by column chromatography (20 g, CHCl₃) to afford **24** as a brown semisolid: yield, 19.1 mg. The structure of **24** was supported by the mass spectrum, which showed a fragment ion at 376 (492 - C₆H₅CHCN).

Ethyl [5-[(Diphenylmethyl)amino]-1,2-dihydro-2-oxo-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (25). To a stirred suspension of 23·2HCl (4.02 g, 8.93 mmol)⁹ in EtOH (170 mL) was added sodium ethoxide (1.22 g, 17.9 mmol) and, after 0.3 h, ethyl benzoylformate (1.59 g, 8.94 mmol). After stirring for 18 h at room temperature, the mixture was heated at reflux under N₂ for 12 h and cooled at room temperature for 72 h. The resulting precipitate was collected by filtration and washed with EtOH (20 mL) and H₂O (2 × 50 mL) to afford **25**: yield, 2.72 g.

Ethyl [2-Chloro-5-[(diphenylmethyl)amino]-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (26). A mixture of **25** (1.42 g, 2.89 mmol) and POCl₃ (30 mL) was heated at 95 °C for 3 h, and most of the POCl₃ was removed by evaporation at reduced pressure (50 °C). The resulting brown syrup was added dropwise to cold H₂O (100 mL) to give a yellow solid, which was purified by flash chromatography (80 g, CHCl₃) to afford **26** as a yellow foam: yield, 996 mg.

Ethyl [6-[(Diphenylmethyl)amino]-4-phenyltetrazolo[1,5-*d*]pyrido[3,4-*b*]pyrazin-8-yl]carbamate (27). To a solution of **26** (498 mg, 0.977 mmol) in dioxane (11 mL) was added a solution of NaN₃ (83 mg, 1.3 mmol) in H₂O (2 mL), and the resulting mixture was heated at reflux for 8 h. The reaction mixture was cooled at room temperature to deposit **27** as a bright yellow solid: yield, 452 mg.

Ethyl (5-Amino-1,2-dihydro-2-oxo-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (28). A suspension of **25** (2.0 g, 4.1 mmol) in concentrated HCl (100 mL) was vigorously stirred for 18 h and evaporated to dryness at reduced pressure. After drying in vacuo (P₂O₅), the yellow solid was triturated for 18 h with Et₂O (100 mL) to afford **28**: yield, 1.48 g.

Ethyl (5-Amino-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (30). **Method A**. To a solution of ethyl (5-amino-1,2-dihydro-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (**29**, 1.0 g, 3.2 mmol)¹⁰ in acetone (500 mL) was added anhydrous MgSO₄ (2.0 g), followed by dropwise addition over 1 h of a solution of KMnO₄ (324 mg, 2.16 mmol) in acetone (150 mL). After the solution was stirred an additional 0.5 h, solid KMnO₄ (75 mg, 0.47 mmol) was added and the reaction mixture was stirred another 0.5 h to complete the oxidation. The insoluble material was removed by filtration and the filtrate was evaporated to dryness in vacuo. The resulting brown residue was extracted with CHCl₃ (100 mL), the insoluble material was removed by filtration, and

the clear yellow filtrate was washed with H₂O (50 mL). The organic layer was dried (Na₂SO₄) and evaporated in vacuo to afford **30** as a yellow solid: yield, 592 mg.

Method B. To a suspension of 28·1.2HCl (102 mg, 0.276 mmol) in dry THF (17 mL) was added a solution of Me₂S·BH₃ in THF (2 N, 1.5 mL, 3.0 mmol). After stirring for 20 h, the amber solution was evaporated to dryness (N₂), and the residue was dissolved in boiling EtOH (10 mL). The solution was cooled to room temperature, and 1 N HCl (0.3 mL) was added in an unsuccessful attempt to precipitate **29** or the corresponding tetrahydro derivative. After the solution was evaporated to dryness, a portion of EtOH (10 mL) was evaporated from the sample, which was then dissolved in EtOH-H₂O (4:1, 10 mL) and neutralized to pH 8.5 with 0.1 N NaOH. The EtOH was evaporated at reduced pressure to deposit a gummy residue, which was separated by decantation and dissolved in acetone. The resulting solution was treated with excess KMnO₄, stirred for 0.8 h, and evaporated to dryness (N₂). The residue was triturated with water, and the brown solid was collected by filtration, dried in vacuo and extracted with CHCl₃ (3 × 20 mL). Removal of the CHCl₃ in vacuo afforded **30** as a yellow solid: yield, 13.2 mg. The TLC and ¹H NMR spectrum of this sample were identical with those of **30** prepared by method A. The ¹H NMR spectrum on a mixture of the products made by the two methods indicated that both preparations gave the same product.

Ethyl (6-Amino-4-phenyltetrazolo[1,5-*d*]pyrido[3,4-*b*]pyrazin-8-yl)carbamate (31). A solution of 27-dioxanate (79 mg, 0.131 mmol) in CF₃CO₂H (4 mL) was stirred for 18 h and evaporated to dryness (N₂). Trituration of the residue with Et₂O gave the trifluoroacetate of **31** as a yellow solid: yield, 46 mg.

Ethyl (2,5-Diamino-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (32). **Method A**. A solution of **27** (92 mg, 0.18 mmol) in HOAc (10 mL) was hydrogenated for 48 h at atmospheric pressure in the presence of Raney nickel (200 mg, weighed wet, washed 3 × H₂O and 2 × HOAc). The catalyst was removed by filtration (Celite), and the filtrate was evaporated to dryness in vacuo. TLC indicated that **31** was recovered. A solution of the semisolid residue in CF₃CO₂H (5 mL) was added to a suspension of pre-reduced (0.5 h) PtO₂ (9.5 mg) in CF₃CO₂H (5 mL), and the hydrogenation was continued for an additional 24 h. After a second addition of PtO₂ (20 mg), the hydrogenation was continued for 18 h. The catalyst was filtered off (Celite) and the filtrate was evaporated to a dark semisolid, which was purified by column chromatography [25 g silica gel, cyclohexane-EtOAc (gradient, 50% EtOAc → 100% EtOAc, then EtOAc-MeOH 9:1)]. Further purification was carried out by flash chromatography [20 g, CHCl₃, then CHCl₃-MeOH (99:1)] to afford **32** as a yellow glass: yield, 3.1 mg.

Method B. A solution of crude **36** (0.54 g) in CF₃CO₂H (10 mL) was stirred at room temperature for 144 h and evaporated to dryness at reduced pressure. Ethanol (10 mL) was evaporated from the residue, which was triturated with Et₂O (10 mL) to deposit the reddish-orange trifluoroacetate of **32**: yield, 191 mg.

A sample (52 mg) of this salt was converted to the free base with aqueous NaOH and recrystallized from MeOH to give **32**: yield, 13.2 mg. The ¹H NMR spectrum was identical with that of **32** prepared under method A.

Ethyl (2,5-Diamino-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (33). A solution of crude **37** (638 mg) in CF₃CO₂H (10 mL) was stirred at room temperature for 120 h and evaporated to dryness at reduced pressure. Ethanol (2 × 20 mL) was evaporated from the residue, which was stirred for 18 h with Et₂O (20 mL). The resulting solid was collected and recrystallized from dioxane (15 mL) to afford **33** as the trifluoroacetate: yield, 63 mg.

Ethyl [8-Amino-4-(diphenylmethyl)-3-imino-2-phenylpyrido[2,3-*b*]pyrazin-6-yl]carbamate (37) and Ethyl [2-Amino-5-[(diphenylmethyl)amino]-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (36). To a solution of crude **24** [8.0 g, ca. 76% purity, 12.4 mmol] in dry MeOH (250 mL) under N₂ was added a solution of NaOMe (0.51 g, 9.4 mmol) in dry MeOH (200 mL). The resulting solution, protected with a KOH drying tube, was stirred at room temperature for 192 h. Additional NaOMe (170 mg, 3.1 mmol) was then added, and stirring was continued for an additional 96 h. After removing a trace amount of insoluble material by filtration, the dark reddish filtrate was

evaporated to dryness and the semisolid residue was eluted from a flash column (250 g, cyclohexane-EtOAc, gradient, 3:1 → 1:1) to afford crude **37**: yield, 929 mg. A portion (102 mg) of this material was further purified on a silica gel thick plate (2000 μ m, CHCl₃-EtOAc, 95:5) to afford **37** as an amber glass: yield, 29 mg.

Further development of the above flash column with cyclohexane-EtOAc (1:1) afforded crude **36** (1.56 g), which after an additional flash column [125 g, CHCl₃ → CHCl₃-MeOH (98:2)] gave **36** as an amber semisolid: yield, 1.28 g. This material was essentially homogeneous by TLC, but the ¹H NMR spectrum indicated the presence of trace amounts of unidentified phenyl containing impurities.

Ethyl [6-Amino-4-(2-formylhydrazino)-5-nitropyridin-7-yl]carbamate (39). A suspension of **38** (2.5 g, 9.8 mmol)⁷ in butyl formate (250 mL) was heated at reflux with stirring. A clear solution was formed after 1 h, and after 2 h the resulting mixture was cooled to room temperature. The product was collected by filtration and dried in vacuo over P₂O₅: yield, 2.67 g.

Ethyl [6-Amino-4-(2-methylhydrazino)-5-nitropyridin-2-yl]carbamate (40). To a partial solution of **39** (4.3 g, 15 mmol) in anhydrous THF (850 mL) at room temperature was added 2 M BH₃-SMe₂ in THF (35 mL) with stirring. After 2.5 h the dark mixture was filtered (Celite), and the filtrate was evaporated to dryness in vacuo. This residue was dissolved in MeOH (100 mL) and reevaporated to remove some of the borane reagent. This residue was dissolved in preheated 2-propanol (50 mL), cooled to room temperature, and then refrigerated. The solid was collected by filtration, washed with hexane (150 mL), and dried in vacuo over P₂O₅: yield, 2.0 g.

The combined filtrate and wash from above gave another crop of impure **40**: yield, 1.0 g (24%). Further recrystallization from 2-propanol resulted in a low recovery of **40**. The solid recovered from alcoholic solutions appeared to contain the corresponding 4-(methylazo)pyridine [mass spectrum, 269 (M + 1)⁺].

Ethyl (5-Amino-1,2-dihydro-3-phenylpyrido[3,4-*e*]-as-triazin-7-yl)carbamates (47-49) and Ethyl (5-Amino-3-phenylpyrido[3,4-*e*]-as-triazin-7-yl)carbamate (50). **Method A**. The hydrogenation of ethyl (6-amino-4-hydrazino-5-nitropyridin-2-yl)carbamate (**38**, 570 mg, 2.23 mmol)⁷ was performed as previously reported to give a product containing **43** and the hydrochloride of **42** (670 mg, 82% purity by HPLC).¹³ This sample and methyl benzimidate hydrochloride (430 mg, 2.51 mmol) were dissolved in H₂O (10 mL) containing Et₃N (0.5 mL) and stirred at room temperature for 19 h. The red precipitate was collected by filtration, washed with H₂O (10 mL), and extracted with hot EtOH (50 mL): yield, 132 mg. The ¹H NMR

spectrum in DMSO-*d*₆ showed a 5:1 ratio of **47-50**. The EtOH filtrate was cooled to afford a second crop (234 mg), which contained a 1:7 ratio of **47-50**. In another experiment an aqueous solution of the hydrochloride of **42** (from hydrogenation of 1.0 g of **38**) was reacted with methyl benzimidate hydrochloride (700 mg) in the absence of Et₃N. The red product was washed well with H₂O, which gave an essentially pure sample of the hydrochloride of **50**: yield, 237 mg.

Method B. A solution of **40** (135 mg, 0.500 mmol) in MeOH (20 mL) containing Raney nickel (0.5 g, weighed wet, washed 1 × H₂O and 2 × MeOH) was hydrogenated at room temperature and atmospheric pressure for 1 h. The catalyst was removed by filtration (Celite) and washed with MeOH (5 mL), and to the combined filtrate and wash containing **44** was added methyl benzimidate hydrochloride (118 mg, 0.690 mmol). After stirring for 16 h the dark reaction mixture was evaporated to dryness and the residue was washed with Et₂O: yield, 142 mg. This sample showed multiple spots by TLC, but the mass spectrum [327 (M + 1)⁺] indicated the presence of **48**. Column chromatography (silica gel, 10 g; 95:5 and 9:1 CHCl₃-MeOH) gave multiple red bands, which were combined and recolumned (silica gel, 3 g; CHCl₃) to give one major red band: yield, 41 mg. This product was identified as **50** by TLC and its mass spectrum.

Method C. The hydrogenation of ethyl (6-amino-4-(1-methylhydrazino)-5-nitropyridin-2-yl)carbamate (**41**, 1.0 g, 3.7 mmol)¹³ in 1:1 H₂O-EtOH (200 mL) gave a mixture of **45** [mass spectrum, 241 (M + 1)⁺] and the hydrazine cleavage product [mass spectrum, 226 (M + 1)⁺]. Reaction of a solution of this mixture in H₂O with methyl benzimidate hydrochloride gave only a low yield of recovered cleavage product. In another approach the preparation of **46** from **41** was performed as previously reported.¹³ A sample of crude **46** (80 mg) was dissolved in hot EtOH (10 mL) and maintained at the temperature for 1 h. After the addition of H₂O (5 mL), the solution was cooled for 16 h to deposit a mixture containing **50** rather than **49** as a major component [TLC; mass spectrum, 311 (M + 1)⁺].

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Inhibition of Enzymes of Estrogen and Androgen Biosynthesis by Esters of 4-Pyridylacetic Acid

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A variety of esters of 4-pyridylacetic acid have been prepared by base mediated exchange from the methyl ester. Several of the esters of alcohols that contained a cyclohexyl ring were potent inhibitors of human placental aromatase and of the rat testicular 17 α -hydroxylase/C₁₇₋₂₀lyase complex. The most potent agents found against both enzyme complexes were the borneyl, isopinocampheyl, and 1-adamantyl esters. These were over 100 times more potent than aminoglutethimide against aromatase and of greater potency than ketoconazole against hydroxylase/lyase. Potency against either enzyme complex was reduced if the ester function was borne on the cyclohexyl ring in an axial rather than an equatorial position. Some differential selectivity could be introduced since whereas methyl substitution adjacent to the carbonyl group reduced the inhibition of aromatase, it increased that against hydroxylase/lyase.

The aromatase enzyme complex performs the last steps in the biosynthesis of estrogens. Since a proportion of breast tumors are dependent upon estradiol for their

growth, depletion of circulating estradiol levels by inhibition of this enzyme provides an approach to the treatment of hormone-dependent breast cancer.¹ For this purpose,