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.

Acknowledgment. The support of this work through grants from the National Institutes of Health through Grant No. GM-32367, the Oklahoma Center for the Advancement of Science and Technology through Contract No. 3745 HR8-072, and by the Research Council of the 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; H_2O_2, 7722-84-1; SOD, 9054-89-1; Fe, 7439-89-6; Cu, 7440-50-8; OH, 3352-57-6; $O_2^{\bullet-}$, 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

Carroll Temple, Jr.* and Gregory A. Rener

Southern Research Institute, P.O. Box 55305, Birmingham, Alabama 35255-5305. *Received March 7, 1990*

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-phenylacetaldehyde[5] 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 $NaBH_4$-Al_2O_3 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 N_2 with formation of **17**.[6] Amination of **5** with the diethyl acetal of 2-amino-2-phenylacetaldehyde[5] 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 oxidization 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-phenylglycinate[8] 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

(1) Bowdon, B. J.; Waud, W. R.; Wheeler, G. P.; Hain, R.; Dansby, L.; Temple, C., Jr. *Cancer Res.* **1987**, *47*, 1621.
(2) Griswold, D. P., Jr.; Temple, C. G., Jr.; Troder, M. W.; Leopold, R. H., III; Laster, W. R., Jr.; Dykes, D. J. *Proc. of the AACR* **1986**, *27*, 306 (abstract 1215).
(3) (a) Temple, C., Jr.; Rener, G. A.; Comber, R. N. *J. Med. Chem.* **1989**, *32*, 2363. (b) Temple, C., Jr.; Rener, G. A. *J. Med. Chem.* **1989**, *32*, 2089.
(4) Lindeman, H.; Wessel, W. *Chem. Ber.* **1925**, *58*, 1221-1230.
(5) Carothers, W. H.; McEwen, W. L. *Organic Syntheses*; Wiley: New York, 1943; Collect. Vol. II, p 319.
(6) Temple, C., Jr.; Kussner, C. L.; Montgomery, J. A. *J. Org. Chem.* **1968**, *33*, 2086.
(7) Temple, C., Jr.; Rose, J. D.; Comber, R. N.; Rener, G. A. *J. Med. Chem.* **1987**, *30*, 1746.
(8) Brenner, M.; Huber, W. *Helv. Chim. Acta* **1953**, *36*, 1109.

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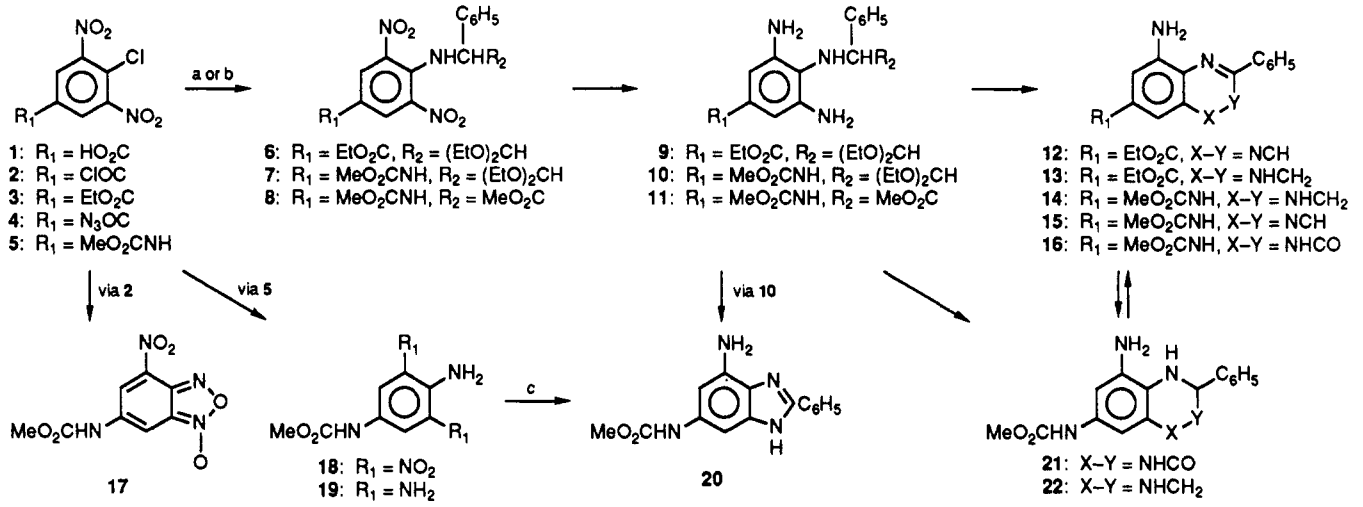
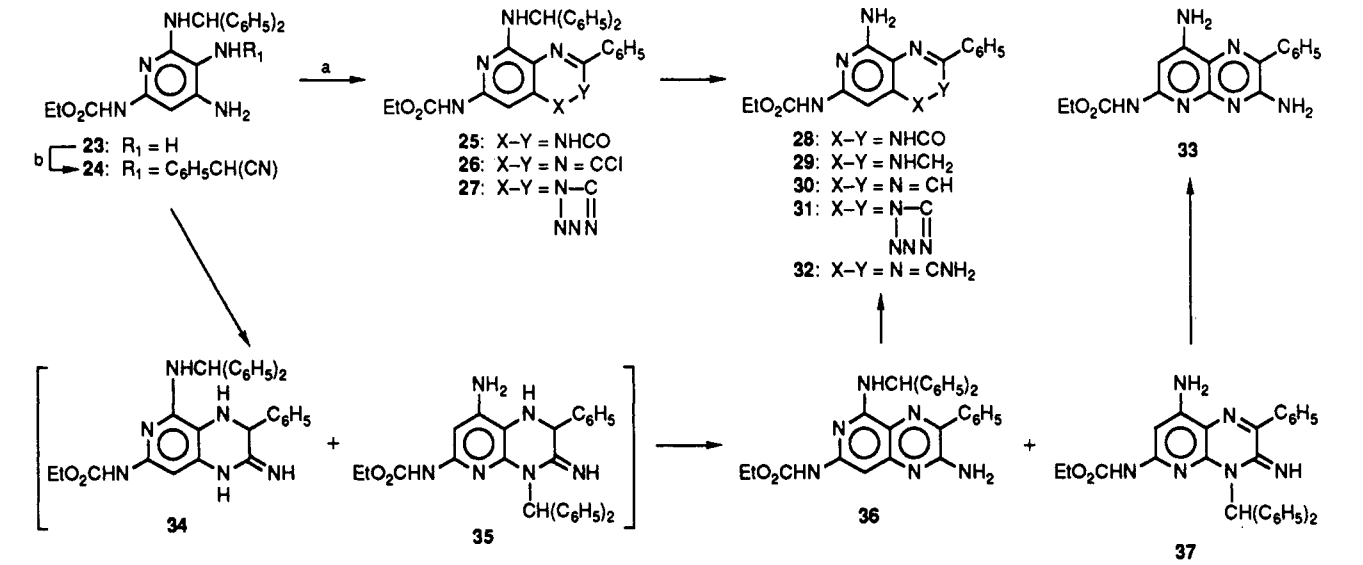
Scheme I[a]



[a] a. $H_2NCH(C_6H_5)CH(OEt)_2$ [6, 7]. b. $H_2NCH(C_6H_5)CO_2Me$ [8]. c. $NaO_3SCH(OH)C_6H_5$.

Scheme II[a]



[a] a. $EtO_2CCOC_6H_5$. b. $4\text{-}MeC_6H_4SO_2OCH(CN)C_6H_5$.

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 $NaBH_4$–Al_2O_3 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 23[9] 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] Chlorodehydroxylation of 25 with $POCl_3$ 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 tetrazolo (27) rather than its azido tautomer.[11] The diphenylmethyl group of 27 was easily removed with CF_3CO_2H 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 CF_3CO_2H 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, 23[9] was reacted with α-cyanobenzyl 4-methylphenylsulfonate[12] 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

(9) Temple, C., Jr.; Smith, B. H., Jr.; Montgomery, J. A. *J. Org. Chem.* **1973**, *38*, 1095.
(10) Temple, C., Jr.; Wheeler, G. P.; Elliott, R. D.; Rose, J. D.; Kussner, C. L.; Comber, R. N.; Montgomery, J. A. *J. Med. Chem.* **1982**, *25*, 1045.
(11) Temple, C., Jr.; Kussner, C. L.; Montgomery, J. A. *J. Org. Chem.* **1966**, *31*, 2210.
(12) Sisdo, K.; Nozaki, H.; Nozaki, M.; Okano, K. *J. Org. Chem.* **1954**, *19*, 1699–1703.

Table I. Properties of Compounds

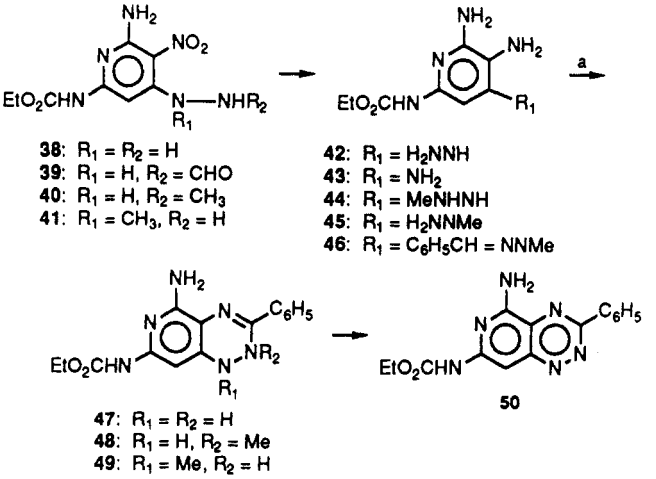
compd	yield, %	mp, °C	mass spectra[a]	^{1}H NMR spectra[a] selected peaks, δ	formula	anal.
5	74	176–179[b]	275 (M^+)	8.35 s (2-CH, 6-CH)	$C_8H_6ClN_3O_6$	CHN
6	99	c	448 [(M + 1)$^+$]	8.55 s (2-CH, 6-CH)		
7	81	c	448 (M^+)	8.32 s (2-CH, 6-CH)	$C_{20}H_{24}N_4O_8$	CHN
8	41	100–105	405 [(M + 1)$^+$]	5.09 d (CHC_6H_5), 8.36 s (2-CH, 6-CH)[d]	$C_{17}H_{16}N_4O_8 \cdot 0.1CH_3CH_2OH$	CHN
12	33	161–163	294 [(M + 1)$^+$]	9.61 s (2-CH)	$C_{17}H_{15}N_3O_2$	CHN
13[e]	64	145–153	296 [(M + 1)$^+$][f]	4.33 s ($2\text{-}CH_2$)[g]	$C_{17}H_{17}N_3O_2 \cdot 0.25C_{17}H_{15}N_3O_2 \cdot 0.4H_2O$	CHN
			294 [(M + 1)$^+$][h]	9.62 s (2-CH)		
14[i]	71	222–225	297 [(M + 1)$^+$][j]	4.23 s ($2\text{-}CH_2$)[k]	$C_{16}H_{16}N_4O_2 \cdot 2C_{16}H_{14}N_4O_2 \cdot 0.3C_6H_8O_6$	CHN
			295 [(M + 1)$^+$][l]	9.39 s (2-CH)		
15	27	226–227[m]	294 (M^+)	9.40 s (2-CH)[g]	$C_{16}H_{14}N_4O_2 \cdot 0.2H_2O$	CHN
16	74	>300	311 [(M + 1)$^+$]	6.65 d, 6.80 d (6-CH, 8-CH)[n]	$C_{16}H_{14}N_4O_3 \cdot 0.5CH_3OH$	CHN
17	59	165–175[m]	254 (M^+)	7.85 d, 8.54 d (5-CH, 7-CH)	$C_8H_6N_4O_6$	CHN
18	49	198–201	256 (M^+)	8.66 s (2-CH, 6-CH)	$C_8H_8N_4O_6$	CHN
19	77	>300[m]	196 (M^+)	6.09 s (2-CH, 6-CH)[g]	$C_8H_{12}N_4O_2 \cdot 0.4H_2O$	CHN
20[o]	33	195–198	283 [(M + 1)$^+$]	6.79 d (7-CH), 7.29 d (5-CH)[d]	$C_{15}H_{14}N_4O_2 \cdot 2.7HCl \cdot 0.7CH_3CH_2OH$	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]	$C_{16}H_{16}N_4O_3 \cdot 0.16C_{16}H_{14}N_4O_3 \cdot 0.8H_2O$	CHN
24	17		493 [(M + 1)$^+$]	4.88 d, 5.17 d (NHCHCN), 6.25 br s, 6.35 d [$NHCH(C_6H_5)_2$]		
25	62	300–305[m]	492 [(M + 1)$^+$]	6.77 d, 7.55 d (CHNH)	$C_{29}H_{25}N_5O_3$	CHN
26	66	174–175[t]	510 [(M + 1)$^+$]	6.85 d, 8.45 d (CHNH)[u]	$C_{29}H_{24}ClN_5O_2 \cdot 0.1CHCl_3$	CHN
27	76	210–233[m]	517 [(M + 1)$^+$]	7.00 d, 8.76 d (CHNH)[v]	$C_{29}H_{24}N_8O_2 \cdot C_4H_8O_2$	CHN
28	97	>300[m]	326 [(M + 1)$^+$]	6.72 s (8-CH)	$C_{16}H_{15}N_5O_3 \cdot 1.2HCl$	CHN
30[o]	60	203–205	310 [(M + 1)$^+$]	7.48 s (8-CH), 9.53 s (2-CH)[u]	$C_{16}H_{15}N_5O_2 \cdot 0.35CHCl_3$	CHN
31	75	202–206[m]	351 [(M + 1)$^+$]	8.02 s (9-CH)[w]	$C_{16}H_{14}N_8O_2 \cdot 1.1CF_3CO_2H \cdot 0.06C_{13}H_{12}O$	CHN
32[o]	39[x]	>300[m,x]	325 [(M + 1)$^+$]	6.61 s (8-CH),[x] 7.09 s (8-CH)	$C_{16}H_{16}N_6O_2 \cdot 1.06CF_3CO_2H$ / $C_{16}H_{16}N_6O_2$	CHN / CHN
33	10	210–215[m]	325 [(M + 1)$^+$]	6.68 s (8-CH)[v]	$C_{16}H_{16}N_6O_2 \cdot 1.03CF_3CO_2H \cdot 0.2C_4H_8O_2$	CHN
36	21		491 [(M + 1)$^+$]	6.51 d, 6.99 d (CHNH)[y]		
37	15		491 [(M + 1)$^+$]	7.04 (7-CH)[d]	$C_{29}H_{26}N_6O_2 \cdot 0.4CH_3CH_2OH$	CHN
39	96	220–222	285 [(M + 1)$^+$]		$C_9H_{12}N_6O_5$	CHN
40	49	178–180[m]	271 [(M + 1)$^+$]	2.51 d, 4.98 q (CH_3NH)	$C_9H_{14}N_6O_4$	CHN
47[o]	15	~250[m]	313 [(M + 1)$^+$]	5.55 (8-CH)[d]	$C_{15}H_{16}N_6O_2 \cdot HCl \cdot 0.5CH_3CH_2OH$	CHN
50	17	190–192[m]	311 [(M + 1)$^+$]	7.78 (8-CH)	$C_{15}H_{14}N_6O_2 \cdot HCl$	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] ^{1}H NMR spectrum indicated a 4:1 ratio of **13**–**12**. [f] **13**. [g] H_2O observed, δ 3.32. [h] **12**. [i] ^{1}H 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] ^{1}H 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] ^{1}H NMR spectrum determined in $CDCl_3$.

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 CF_3CO_2H 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 Et_3N provided a mixture of **47** and **50**.[14] Extraction of this mixture with EtOH gave an insoluble product, which was identified by its ^{1}H 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, difficulties were encountered in the preparation of **49**. The hydrogenation of the known **41**[13] with Raney nickel in 1:1 EtOH–H_2O 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-

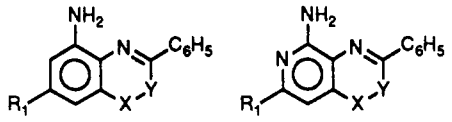
Scheme III[a]



[a] (a) $MeOC(NH)C_6H_5$ (**47**, **48**).

(13) Temple, C., Jr. *J. Med. Chem.* **1990**, *33*, 656.
(14) Temple, C., Jr.; Kussner, C. L.; Montgomery, J. A. *J. Org. Chem.* **1974**, *39*, 2866.

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]			$5. \times 10^{-1}$	8.5×10^{-1}
12 (N = CH), R_1 = EtO_2C	>100	>10		
13 (NHCH), R_1 = EtO_2C[d]	>100	>10		
30 (N = CH), R_1 = EtO_2CHN			$3. \times 10^{-1}$	-
15 (N = CH), R_1 = MeO_2CHN	>100	-		
14 ($NHCH_2$), R_1 = MeO_2CHN[d]	24	-		
29 ($NHCH_2$), R_1 = EtO_2CHN[e]			4.7×10^{-3}	2.8×10^{-3}
16 (NHCO), R_1 = MeO_2CHN[f,g]	7.5×10^{-1}	1.9		
28 (NHCO), R_1 = EtO_2CHN[f]			4.2×10^{-2}	2.2×10^{-2}
32 (N = CNH_2), R_1 = EtO_2CHN			>30	-
47 (NHNH), R_1 = EtO_2CHN[d]			>10	
50 (N = N), R_1 = EtO_2CHN			>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 IC_{50} 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 IC_{50} 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 antimitotic 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 Me_2SO-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 μC_{18} 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

(15) Taylor, E. C.; Cocuzza, A. J. *J. Org. Chem.* **1979**, *44*, 1125.
(16) Wheeler, G. P.; Bowdon, B. J.; Werline, J. A.; Adamson, D. J.; Temple, C., Jr. *Cancer Res.* **1982**, *42*, 791.
(17) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Resp., Part 3* **1972**, *3*, 9.

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 Et_2O (80 mL), and additional insoluble material was removed by filtration. The filtrate was washed with water (3 × 20 mL), dried (Na_2SO_4), 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 × H_2O 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] Et_3N (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 $CHCl_3$ (50 mL) and H_2O (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)[6] 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 Et_2O (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, $CHCl_3$) 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 $NaBH_4$ on alumina (800 mg, 10% $NaBH_4$). Additional portions of $NaBH_4$ (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 (N_2) MeOH (20 mL) and stirred under N_2 overnight. The solution was evaporated to dryness in vacuo, the residue was dissolved in deoxygenated (N_2) $CHCl_3$, 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 $NaBH_4$ on alumina (2.0 g) in dioxane (20 mL) was stirred under N_2 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) H_2O (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 (N_2) 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 N_2 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, $CHCl_3$) 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 N_2 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 $SOCl_2$ (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 NaN_3 (0.50 g, 7.7 mmol) in H_2O (2 mL), and the ice bath was removed. After the solution was stirred for 0.25 h, H_2O (25 mL) was added, and the oily material which separated was extracted into Et_2O (3 × 50 mL). The combined organic layers were dried (Na_2SO_4) 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 Et_2O (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 NH_3 (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 H_2O–EtOH (1:1, 26 mL) was hydrogenated in the presence of Raney nickel (200 mg, weighed wet; washed 2 × H_2O and 2 × EtOH). After hydrogen uptake had ceased (104% of theoretical in 1.5 h), the catalyst was removed by filtration under N_2 (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-1*H*-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 × H_2O and 2 × EtOH). After the uptake had ceased (4 h), the catalyst was removed by filtration (Celite) under N_2, 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 (N_2) 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 (coinjection) with **20** prepared by method B.

Method B. A suspension of **18** (180 mg, 0.70 mmol) in 1:1 H_2O–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 N_2 (Celite) and washed with 1:1 H_2O–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 N_2, 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 (P_2O_5), 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 × H_2O 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, $CHCl_3$–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 (N_2) EtOH (5 mL) was added Et_3N (45 mg, 0.45 mmol), α-cyanobenzyl 4-methylbenzenesulfonate (66 mg, 0.23 mmol),[12] and additional Et_3N (23 mg, 0.23 mmol). After stirring under N_2 for 72 h, more α-cyanobenzyl 4-methylbenzenesulfonate (15 mg, 0.052 mmol) was added, and stirring under N_2 was continued for an additional 96 h. The brown solution was evaporated to dryness in vacuo, and the residue was triturated with H_2O to give a light brown solid, which was purified by column chromatography (20 g, $CHCl_3$) 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 – C_6H_5CHCN).

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 N_2 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 H_2O (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 $POCl_3$ (30 mL) was heated at 95 °C for 3 h, and most of the $POCl_3$ was removed by evaporation at reduced pressure (50 °C). The resulting brown syrup was added dropwise to cold H_2O (100 mL) to give a yellow solid, which was purified by flash chromatography (80 g, $CHCl_3$) 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 NaN_3 (83 mg, 1.3 mmol) in H_2O (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 (P_2O_5), the yellow solid was triturated for 18 h with Et_2O (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 $MgSO_4$ (2.0 g), followed by dropwise addition over 1 h of a solution of $KMnO_4$ (324 mg, 2.16 mmol) in acetone (150 mL). After the solution was stirred an additional 0.5 h, solid $KMnO_4$ (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 $CHCl_3$ (100 mL), the insoluble material was removed by filtration, and the clear yellow filtrate was washed with H_2O (50 mL). The organic layer was dried (Na_2SO_4) 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 Me_2S·BH_3 in THF (2 N, 1.5 mL, 3.0 mmol). After stirring for 20 h, the amber solution was evaporated to dryness (N_2), 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–H_2O (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 $KMnO_4$, stirred for 0.8 h, and evaporated to dryness (N_2). The residue was triturated with water, and the brown solid was collected by filtration, dried in vacuo and extracted with $CHCl_3$ (3 × 20 mL). Removal of the $CHCl_3$ 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 CF_3CO_2H (4 mL) was stirred for 18 h and evaporated to dryness (N_2). Trituration of the residue with Et_2O 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 × H_2O 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 CF_3CO_2H (5 mL) was added to a suspension of prereduced (0.5 h) PtO_2 (9.5 mg) in CF_3CO_2H (5 mL), and the hydrogenation was continued for an additional 24 h. After a second addition of PtO_2 (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, $CHCl_3$, then $CHCl_3$–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 CF_3CO_2H (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 Et_2O (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 CF_3CO_2H (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 Et_2O (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 N_2 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 μm, $CHCl_3$–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, $CHCl_3$ → $CHCl_3$–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 P_2O_5: 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 BH_3·SMe_2 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 P_2O_5: 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 H_2O (10 mL) containing Et_3N (0.5 mL) and stirred at room temperature for 19 h. The red precipitate was collected by filtration, washed with H_2O (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 Et_3N. The red product was washed well with H_2O, 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 × H_2O 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 Et_2O: 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 $CHCl_3$–MeOH) gave multiple red bands, which were combined and recolumned (silica gel, 3 g; $CHCl_3$) 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 H_2O–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 H_2O 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 H_2O (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

Raymond McCague,* Martin G. Rowlands, S. Elaine Barrie, and John Houghton

Drug Development Section, Institute of Cancer Research, Sutton, Surrey, SM2 5NG, England. *Received December 11, 1989*

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 17α-hydroxylase/C_{17-20}lyase 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,

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