

New Anticancer Agents: Synthesis of 1,2-Dihydropyrido[3,4-*b*]pyrazines (1-Deaza-7,8-dihydropteridines)

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Reaction of α -aminoacetophenone oximes (**2**) with ethyl 6-amino-4-chloro-5-nitropyridine-2-carbamate (**1**) gave ethyl 6-amino-5-nitro-4-[(2-oxo-2-phenylethyl)amino]pyridine-2-carbamate oximes (**3**), which were hydrolyzed under acidic conditions to give the corresponding ketones (**4**). Related pyridines substituted with a keto side chain were prepared from **1** and 1,3-diaminopropanone oximes and by oxidation of the side-chain hydroxy group of ethyl 6-amino-4-[[3-(*N*-methyl-*N*-phenylamino)-2-hydroxypropyl]amino]-5-nitropyridine-7-carbamates (**6**). Catalytic hydrogenation of the nitro group of **4** over Raney nickel in a large volume of ethanol gave the 1-deaza-7,8-dihydropteridines (**7**). Several of the oximes **3** were successfully hydrogenated to give **7** directly. The resulting 1-deaza-7,8-dihydropteridines showed potent cytotoxicity against cultured L1210 cells and significant anticancer activity against lymphocytic leukemia P-388 in mice. These biological activities are attributed to the accumulation of cells at mitosis.

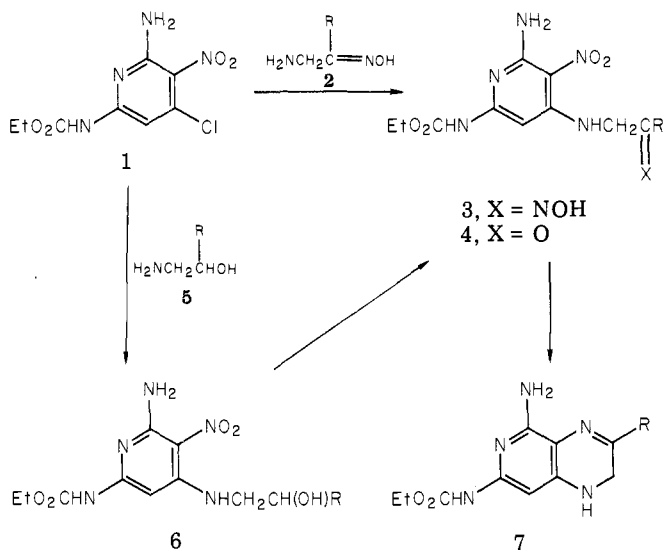
Steady progress has been made in cancer chemotherapy primarily because of the development of new agents, combinations of agents, and new approaches for the treatment of cancers. Despite those advances, some cancers do not respond to drug therapy, and some cancers that respond initially lose their responsiveness after prolonged drug therapy. The principal cause for these conditions has been attributed, respectively, to "natural" resistance and to the development of acquired resistance. The latter has been demonstrated in animal tumor systems in which the most resistant cells are steadily increasing.¹ One approach to meet the challenge of drug resistance is the development of new classes of agents, which can be used alone or in combination with conventional agents.

Recently, we reported that ethyl 5-amino-1,2-dihydro-3-[(*N*-methylanilino)methyl]pyrido[3,4-*b*]pyrazine-7-carbamate [**7**, R = C₆H₅N(Me)CH₂] was active against experimental neoplasms, including L1210 leukemia, P-388 leukemia, and intraperitoneally implanted murine colon tumor 26 and had borderline activity against a number of other tumors.² The mechanism of action of this compound was identified as the accumulation of cells at mitosis with both cultured cells and ascites cancer cells in vivo. In this paper we report the synthesis and biological activity of **7** [R = C₆H₅N(Me)CH₂] and related 1,2-dihydropyrido[3,4-*b*]pyrazines (1-deaza-7,8-dihydropteridines).

The development of procedures for the preparation of 1-deazapteridines has been carried out in these laboratories for a number of years.³ These processes are modifications of the Boon and Leigh procedure for the synthesis of pteridines from chloropyrimidines and α -amino ketones.⁴

The oximes of α -amino ketones (Table I) were prepared by several routes. Reaction of α -bromoacetophenones with hexamethylenetetramine gave the corresponding iminium salts, which were hydrolyzed by ethanolic hydrochloric acid to give α -aminoacetophenone hydrochlorides.^{5,6} The

Scheme I



condensation of the latter with hydroxylamine hydrochloride in a refluxing mixture of ethanol and pyridine gave the α -aminoacetophenone oximes.⁷ Several α -aminoacetophenone oximes were prepared by alkylation of phthalimide with α -bromoacetophenones, followed by conversion of the product to the oxime with hydroxylamine and removal of the phthaloyl protecting group with hydrazine.^{7,8} In another approach, 1-bromo-3-phthalimidopropanone was reacted with *N*-methylanilines to give 1-(*N*-methyl-*N*-phenylamino)-3-phthalimidopropanones. The latter were condensed with hydroxylamine to give the corresponding oximes from which the phthaloyl blocking group was removed with hydrazine to give 1-amino-3-(*N*-methyl-*N*-phenylamino)propanone oximes.⁷ Several 1-amino-3-(*N*-methyl-*N*-phenylamino)-2-propanols were also prepared by alkylation of *N*-methylanilines with epichlorohydrin and amination of the resulting epoxide with ammonia.¹¹

Alkylation of α -aminoacetophenone oximes (**2**) (Table I) with ethyl 6-amino-4-chloro-5-nitropyridine-2-carbamate

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Table I. α -Amino Ketone Oximes (2) and α -Amino Alcohols (5)

no.	R	method	yield, ^a %	mp, °C	formula	anal.
2	C ₆ H ₅ N(Me)CH ₂	I	56	<i>b</i>	C ₁₀ H ₁₅ N ₂ O · 0.33H ₂ O	C, H, N
2	4-MeOC ₆ H ₄ N(Me)CH ₂	I	28	<i>b</i>	C ₁₁ H ₁₇ N ₂ O · 0.37H ₂ O	C, H, N
2	C ₆ H ₅ ^c	III	40	96-110	C ₈ H ₁₀ N ₂ O	C, H, N
2	4-MeC ₆ H ₄ ^d	II	34	127-129	C ₉ H ₁₂ N ₂ O · 0.13C ₂ H ₆ O	C, H, N
2	2,4-Me ₂ C ₆ H ₃	II	64	<i>e</i>	C ₁₀ H ₁₄ N ₂ O	<i>f</i>
2	3,4-Me ₂ C ₆ H ₃ ^g	II	34	115-118	C ₁₀ H ₁₄ N ₂ O · 0.64HCl	C, H, N
2	4-FC ₆ H ₄	III	70	142-143	C ₈ H ₉ FN ₂ O	<i>h</i>
2	4-ClC ₆ H ₄ ⁱ	III	37	129-130 ^j	C ₈ H ₉ ClN ₂ O · HCl	C, H, N
2	2,4-Cl ₂ C ₆ H ₃	II	22	115-120 ^k	C ₈ H ₈ Cl ₂ N ₂ O · 0.17H ₂ O	C, H, N
2	3,4-Cl ₂ C ₆ H ₃	II	91	123-127	C ₈ H ₈ Cl ₂ N ₂ O	C, H, N
2	3-MeOC ₆ H ₄	II	77	<i>e</i>	C ₉ H ₁₂ N ₂ O ₂	<i>l</i>
2	4-MeOC ₆ H ₄ ^d	III	6	137-139	C ₉ H ₁₂ N ₂ O · HCl · 0.31H ₂ O	C, H, N
2	3,4,5-(MeO) ₃ C ₆ H ₂	II	70	134-137	C ₁₁ H ₁₆ N ₂ O ₄ · 0.16C ₂ H ₆ O	C, H, N
2	4-O ₂ NC ₆ H ₄ ^m	III	20	124-125	C ₈ H ₉ N ₂ O ₃ · 0.28HCl	C, H, N
2	4-CF ₃ C ₆ H ₄ ⁿ	II	31	129-130	C ₉ H ₉ F ₃ N ₂ O	C, H, N
2	4-C ₆ H ₅ C ₆ H ₄	III	73	<i>b</i>	C ₁₄ H ₁₄ N ₂ O · 0.33HCl	C, H, N
5	C ₆ H ₅ ^p	IV	23	71-72		
5	4-ClC ₆ H ₄ N(Me)CH ₂	IV	32	117-119 ^q	C ₁₀ H ₁₁ ClN ₂ O	C, H, N
5	4-MeOC ₆ H ₄ N(Me)CH ₂	IV	17	163	C ₁₁ H ₁₈ N ₂ O ₂	C, H, N

^a Overall yield. ^b Indefinite melting point. ^c Reference 9. ^d Reference 7b. ^e Oil. ^f *m/e* 178 (M⁺). ^g Phthalimide intermediate. ^h *m/e* 168 (M⁺). ⁱ Hexamethylenetetramine salt. ^j Free base. ^k Presoftening at 76-81 °C. ^l *m/e* 180 (M⁺). ^m Amino ketone intermediate. ⁿ Phthalimide intermediate. ^p Reference 11. ^q Resolidified and remelted 123 °C.

Table II. Ethyl 4-(Substituted-amino)-6-amino-5-nitro-2-pyridinecarbamates (3-6)

no.	R	reaction yield,		mp, °C	formula	anal.	
		method	time, h				
3	C ₆ H ₅ N(Me)CH ₂	V	24	84	192-193 dec	C ₁₈ H ₂₁ N ₃ O ₅	C, H, N
3	4-MeOC ₆ H ₄ N(Me)CH ₂	V	15 ^a	75	190	C ₁₉ H ₂₃ N ₃ O ₆	C, H, N
3	C ₆ H ₅	V	8	52	245-246 dec	C ₁₆ H ₁₈ N ₃ O ₅	C, H, N
3	4-MeC ₆ H ₄	V	2	87	234-235 dec	C ₁₇ H ₂₀ N ₃ O ₅ · 0.22HCl	C, H, N
3	2,4-(Me) ₂ C ₆ H ₃	V	2	18	198-200	C ₁₈ H ₂₂ N ₃ O ₅	C, H, N
3	3,4-(Me) ₂ C ₆ H ₃	V	1.5	82	236-237 dec	C ₁₈ H ₂₂ N ₃ O ₅ · 0.25H ₂ O	C, H, N
3	4-FC ₆ H ₄	V	2	86	240-242	C ₁₆ H ₁₇ FN ₃ O ₅ · 0.13HCl	C, H, N
3	4-ClC ₆ H ₄	V	3	73	224-225 dec	C ₁₆ H ₁₇ ClN ₃ O ₅ · 1.1H ₂ O	C, H, N
3	2,4-Cl ₂ C ₆ H ₃		2.5	45	177-179 dec	C ₁₆ H ₁₆ Cl ₂ N ₃ O ₅	C, H, N
3	3,4-Cl ₂ C ₆ H ₃	V	1.5	64	214-216	C ₁₆ H ₁₆ Cl ₂ N ₃ O ₅ · 0.54C ₂ H ₆ O	C, N; H ^b
3	3-MeOC ₆ H ₄	V	3	56	223-227 dec	C ₁₇ H ₂₀ N ₃ O ₆	C, H, N
3	4-MeOC ₆ H ₄	V	4	55	218-219 dec	C ₁₇ H ₂₀ N ₃ O ₆ · 0.12HCl	C, H, N
3	3,4,5-(MeO) ₃ C ₆ H ₂	V	1	37	205-208 dec	C ₁₉ H ₂₄ N ₃ O ₈ · 0.2HCl	C, H, N
3	4-O ₂ NC ₆ H ₄	V	2	44	207-208 dec	C ₁₆ H ₁₇ N ₃ O ₇	C, H, N
3	4-CF ₃ C ₆ H ₄	V	2	47	224-225 dec	C ₁₇ H ₁₇ F ₃ N ₃ O ₅	C, H, N
3	4-C ₆ H ₅ C ₆ H ₄	V	48	48 ^c	~130 dec		
4	C ₆ H ₅ N(Me)CH ₂	VII	2.5	80	~80 ^d	C ₁₈ H ₂₂ N ₃ O ₅ · H ₂ O · 0.5(CH ₃) ₂ SO	C, H, N
4	4-ClC ₆ H ₄ N(Me)CH ₂	VII	20	20	123-125	C ₁₈ H ₁₇ ClN ₃ O ₅ · 0.5H ₂ O	C, H, N
4	C ₆ H ₅	VI	2	59	177-178	C ₁₆ H ₁₇ N ₃ O ₅ · 0.66C ₄ H ₈ O ₂ · 0.16H ₂ O	C, H, N
4	4-MeC ₆ H ₄	VI	3	73	184-185	C ₁₇ H ₁₉ N ₃ O ₅	H, N; C ^e
4	3,4-Me ₂ C ₆ H ₃	VI	4	80	196-197 dec	C ₁₈ H ₂₁ N ₃ O ₅	C, H, N
4	4-FC ₆ H ₄	VI	24	48	204-205	C ₁₆ H ₁₆ FN ₃ O ₅	C, H, N
4	4-ClC ₆ H ₄	VI	18	59	180-181 dec	C ₁₆ H ₁₆ ClN ₃ O ₅	C, H, N
4	3,4-Cl ₂ C ₆ H ₃	VI	6	69	191-192	C ₁₆ H ₁₆ Cl ₂ N ₃ O ₅ · 0.25C ₄ H ₈ O ₂	C, H, N
4	3-MeOC ₆ H ₄	VI	5	81	>171-172 dec	C ₁₇ H ₁₉ N ₃ O ₆ · 0.8H ₂ O	C, H, N
4	4-MeOC ₆ H ₄	VI	20	53	194 dec	C ₁₇ H ₁₉ N ₃ O ₆	C, H, N
4	3,4,5-(MeO) ₃ C ₆ H ₂	VI	2.5	80	192-193 dec	C ₁₉ H ₂₃ N ₃ O ₈ · 2.2H ₂ O · 0.1C ₄ H ₈ O ₂	C, H, N
4	4-O ₂ NC ₆ H ₄	VI	24	65	204-205 dec	C ₁₆ H ₁₇ N ₃ O ₇ · HCl · H ₂ O	C, H, N
4	4-CF ₃ C ₆ H ₄	VI	2	31	166.5-167.5	C ₁₇ H ₁₆ F ₃ N ₃ O ₅ · 0.75C ₄ H ₈ O ₂	C, H, N
4	4-C ₆ H ₅ C ₆ H ₄	VI	1	80	<i>f</i>	C ₂₂ H ₂₁ N ₃ O ₅ · HCl · H ₂ O	C, H, N
6	C ₆ H ₅	VIII	18	73	88-90	C ₁₈ H ₂₄ N ₃ O ₅	C, H, N
6	4-ClC ₆ H ₄ N(Me)CH ₂	VIII	18	64	181	C ₁₈ H ₂₃ ClN ₃ O ₅	C, H, N
6	4-MeOC ₆ H ₄ N(Me)CH ₂	VIII	18	79	108 dec	C ₁₉ H ₂₆ N ₃ O ₆	C, H, N

^a Solvent, methanol. ^b H: calcd, 4.10; found, 3.59. ^c Crude yield, used directly to prepare ketone. ^d With pre-softening. ^e C: calcd, 54.69; found, 54.26. ^f Indefinite.

(1)¹² under nitrogen in refluxing ethanol containing triethylamine as an acid acceptor gave the ethyl 6-amino-5-nitro-4-[(2-oxo-2-phenylethyl)amino]pyridine-2-carbamate oximes (3) listed in Table II. (Scheme I) Treatment of these oximes with a 1:1 mixture of 1 N hydrochloric acid and dioxane at 60 °C hydrolyzed the oxime function to give

the ethyl 6-amino-5-nitro-4-[(2-oxo-2-phenylethyl)amino]pyridine-2-carbamates (4) listed in Table II. Similarly, reaction of 1 with the 1,3-diaminopropanone oximes (Table I), followed by hydrolysis of the resulting oximes (Table II), gave the ethyl 6-amino-4-[[3-(*N*-methyl-*N*-phenylamino)-2-oxopropyl]amino]-5-nitropyridine-2-carbamates listed in Table II. Another approach for the preparation of the pyridines containing the keto side chain involved the alkylation of 1-amino-3-(*N*-methyl-*N*-

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Table III. Ethyl 3-Substituted-5-amino-1,2-dihydropyrido[3,4-*b*]pyrazine-7-carbamates (7)

no.	R	method	reac- tion time, h	yield, %	mp, °C	formula	anal.	<i>m/e</i> ^a
7	C ₆ H ₅ N(Me)CH ₂	IX	12	63	165 ^b	C ₁₈ H ₂₂ N ₆ O ₂ · 0.3H ₂ O·0.15C ₂ H ₆ O ^c	C, N; H ^d	354 (M ⁺)
7	C ₆ H ₅ N(Me)CH ₂	X	42	32	80 ^e	C ₁₈ H ₂₂ N ₆ O ₂ ·0.5C ₂ H ₆ O ^c	C, H, N	354 (M ⁺)
7	4-ClC ₆ H ₄ N(Me)CH ₂	X	20	16	<i>f</i>	C ₁₈ H ₁₇ ClN ₆ O ₂ · 0.5H ₂ O·0.5C ₂ H ₆ O ^c	C, H, N	388 (M ⁺)
7	4-MeOC ₆ H ₄ N(Me)CH ₂	IX	3	73	187	C ₁₉ H ₂₄ N ₆ O ₃	C, H, N	384 (M ⁺)
7	C ₆ H ₅	X	6	89	>180 dec	C ₁₆ H ₁₇ N ₅ O ₂	C, H, N	311 (M ⁺)
7	4-MeC ₆ H ₄	X	5	83	270-280 dec	C ₁₇ H ₁₉ N ₅ O ₂	C, H, N	325 (M ⁺)
7	2,4-Me ₂ C ₆ H ₃	IX	5	87	158-160	C ₁₈ H ₂₁ N ₅ O ₂ · 0.1C ₂ H ₆ O·H ₂ O ^c	C, H, N	339 (M ⁺)
7	3,4-Me ₂ C ₆ H ₃	X	5	83	190-192 dec	C ₁₈ H ₂₁ N ₅ O ₂ ·0.7C ₂ H ₆ O ^c	C, H, N	339 (M ⁺)
7	4-FC ₆ H ₄	X	12	78	296-300 dec	C ₁₆ H ₁₆ FN ₅ O ₂	C, H, N	329 (M ⁺)
7	4-ClC ₆ H ₄	X	19	77	>270 dec	C ₁₆ H ₁₆ ClN ₅ O ₂	H, N; C ^g	345 (M ⁺)
7	2,4-Cl ₂ C ₆ H ₃	IX	24	64	203-205 dec	C ₁₆ H ₁₅ Cl ₂ N ₅ O ₂ ·0.8H ₂ O ^c	C, H, N	379 (M ⁺)
7	3,4-Cl ₂ C ₆ H ₃	X ⁱ	40	57	299-302 dec	C ₁₆ H ₁₅ Cl ₂ N ₅ O ₂ ·0.6H ₂ O· C ₂ H ₆ O ^c	C, H, N	379 (M ⁺)
7	3-MeOC ₆ H ₄	X	15	70	174-176	C ₁₇ H ₁₉ N ₅ O ₂ · 0.3C ₂ H ₆ O·0.2H ₂ O ^c	C, H, N	341 (M ⁺)
7	4-MeOC ₆ H ₄	X	6	71	>170 dec	C ₁₇ H ₁₉ N ₅ O ₂ · 2.1H ₂ O·0.64C ₂ H ₆ O ^c	C, N; H ^h	341 (M ⁺)
7	3,4,5-(MeO) ₃ C ₆ H ₂	X	24	70	250-255 dec	C ₁₉ H ₂₃ N ₅ O ₅ ·HCl ⁱ · 0.5H ₂ O ^c	C, H, N	401 (M ⁺)
7	4-H ₂ NC ₆ H ₄	X	6	17	>300 dec	C ₁₆ H ₁₈ N ₆ O ₂ · 2.5HCl·0.5C ₂ H ₆ O ^c	C, H, N	326 (M ⁺)
7	4-CF ₃ C ₆ H ₄	X	48	56	>300 dec	C ₁₇ H ₁₆ F ₃ N ₅ O ₂ · 0.2H ₂ O·0.15HCl ⁱ	C, H, N	379 (M ⁺)
7	4-C ₆ H ₅ C ₆ H ₄	X ^j	48	86	<i>d</i>	C ₂₂ H ₂₁ N ₅ O ₂ ·HCl· 0.23C ₄ H ₉ NO ^c	C, H, N	387 (M ⁺)

^a Mass spectral data were determined with a Varian MAT 311A instrument. ^b Prior sintering. ^c The presence of reaction solvent and water of hydration in these compounds was confirmed by the ¹H NMR spectra, which were determined in (CD₃)₂SO solutions with a Varian XL-100-15 spectrometer operating at 100 MHz [internal (CH₃)₄Si]: H₂O, δ 3.33-3.35 (br s); CH₂CH₂OH, δ 1.06 t, 3.45 q; (CH₃)₂NCOCH₃, 1.96, 2.79, 2.94 (3 s). ^d H: calcd, 6.46; found, 7.05. ^e Sintering. ^f Indefinite. ^g C: calcd, 55.57; found, 56.11. ^h H: calcd, 6.68; found, 6.16. ⁱ Cl: calcd, 1.41; found, 1.41. ^j Solvent, *N,N*-dimethylacetamide.

phenylamino)-2-propanols with 1 to give ethyl 6-amino-4-[[3-(*N*-methyl-*N*-phenylamino)-2-hydroxypropyl]-amino]-5-nitropyridine-2-carbamates (6). Oxidation of the side-chain alcohol function of 6 was successful with 6 [R = C₆H₅N(Me)CH₂ and 4-ClC₆H₄N(Me)CH₂], but unsuccessful for the preparation of 6 [R = 4-MeOC₆H₄N(Me)CH₂].

The catalytic hydrogenation of the α-amino ketones 4 with a 2- to 3-fold amount of Raney nickel in a large volume of ethanol at atmospheric pressure at room temperature or with intermittent warming with a water bath gave the intermediate 5-aminopyridines, which were cyclized in situ with the elimination of water to give the ethyl 3-substituted-5-amino-1,2-dihydropyrido[3,4-*b*]pyrazine-7-carbamates 7 listed in Table III. Some of the pyridines containing oxime functions were unsuccessfully hydrolyzed to the ketones, and these oximes were hydrogenated directly to give the desired 1-deaza-7,8-dihydropteridines.

Biological Evaluation Previously, both 1-deaza- and 3-deazamethotrexate were prepared in our laboratory.^{3c} Although 3-deazamethotrexate was active against leukemia L1210 in mice and inhibited dihydrofolic reductase, 1-deazamethotrexate showed only borderline activity, indicating that the 1-nitrogen of methotrexate was necessary for activity. However, an intermediate in the synthesis of 1-deazamethotrexate, ethyl 5-amino-1,2-dihydro-3-[[*p*-(methoxycarbonyl)-*N*-methylanilino]methyl]pyrido[3,4-*b*]pyrazine-7-carbamate,^{3c} showed cytotoxicity in the KB cell culture screen and activity against leukemia L1210 in mice. In contrast, this intermediate was a weak inhibitor of both *Streptococcus faecium* and dihydrofolic reductase (pigeon liver), and preliminary data from equilibrium dialysis experiments indicated that the compound was not

binding to DNA. These results led to the synthesis of a series of 1-deaza-7,8-dihydropteridines and investigations into their mechanism of action. Recently, we reported that treatment of cultured L1210 cells with 7 [R = C₆H₅N(Me)CH₂] at a concentration that prevented proliferation and colony formation of the cells resulted in a progressive increase with time of exposure in the number of cells in mitosis.² This compound was as effective as vincristine in promoting the accumulation in mitosis of cultured L1210 cells. In addition, this compound caused the accumulation of L1210 cells in mitosis in mice bearing ascitic L1210 leukemia.

Biological data on two series of 1-deaza-7,8-dihydropteridines (7) are listed in Table IV. In the 6-(*N*-methylanilino)methyl series, the concentration of drug required for the inhibition of the proliferation of cultured L1210 cells (ID₅₀) was greater than that required for vincristine but similar to that of nocodazole.² Within the series, the ID₅₀ for the 4-methoxyphenyl compound resembles that of the parent phenyl compound, whereas the 4-chlorophenyl and 4-(methoxycarbonyl)phenyl compounds had higher ID₅₀'s. The mitotic indexes (MI) listed in Table IV show that each compound caused the accumulation of cells at mitosis.² These values of the MI are the results of preliminary work but are probably minimum values (see below). Superimposition of the semilogarithmic plots of the inhibition of cell proliferation and the MI at various concentrations is shown for 7 [R = C₆H₅N(Me)CH₂] in Figure 1. The similarity of the plots suggested that cytotoxicity can be attributed primarily to mitotic arrest. The decrease in the MI at the higher concentration is caused at least in part by lysis of some of the cells. Although each of these compounds has significant activity

Table IV. Biological Data for 1-Deaza-7,8-dihydropteridines

compd or R	L1210 ID ₅₀ , ^a μM	mitotic index ^b		P-388 ^c 10 ⁶ tumor cell implant, ip	
		12 h (μM)	24 h (μM)	schedule, days	96 ILS (mg/kg) ^d
nocodazole ^e	27 × 10 ⁻³		0.19 (0.3)		
vincristine	< 1 × 10 ⁻³		0.62 (0.3)	1	100 (2.7)
C ₆ H ₅ N(Me)CH ₂	8.4 × 10 ⁻³ ^f	0.77 (0.03)	0.80 (0.3)	1-9	145 (0.33)
				1 ^g	114 (100)
4-MeOC ₆ H ₄ N(Me)CH ₂	7.9 × 10 ⁻³ ^j	0.64 (0.03)	0.49 (0.3)	1 ^g	66 (100) ^h
				1	90 (67)
				1	80 (50) ^k
4-ClC ₆ H ₄ N(Me)CH ₂	14.5 × 10 ⁻³	0.71 (0.03)	0.44 (0.1)	1	133 (25)
				1-9	150 (25)
4-MeO ₂ CC ₆ H ₄ N(Me)CH ₂ ^l	58 × 10 ⁻³	0.61 (0.3)	0.44 (0.1)	1	65 (100)
				1-9	30 (25)
C ₆ H ₅	4.7 × 10 ⁻³ ^j	0.65 (0.03)	0.54 (0.3)	1	55 (12.5) ^j
				1 ⁱ	50 (12)
				1, 5, 9	42 (4.5)
				1-9	51 (2)
3-MeOC ₆ H ₄	1.22 × 10 ⁻³	0.56 (0.003)	0.27 (0.3)	1	50 (25)
				1, 5, 9	63 (25)
4-MeOC ₆ H ₄	4.1 × 10 ⁻³			1-9	35 (3)
3,4,5-(CH ₃ O) ₃ C ₆ H ₂	78 × 10 ⁻³	0.41 (0.3)	0.19 (0.3)	1	35 (50)
				1-5	49 (3)
4-H ₂ NC ₆ H ₄	23 × 10 ⁻³ ^j			1	38 (200) ^m
4-FC ₆ H ₄	6.8 × 10 ⁻³	0.47 (0.01)	0.26 (0.3)	1, 5, 9	37 (12.5)
4-ClC ₆ H ₄	52 × 10 ⁻³			1-9	38 (6.25)
2,4-Cl ₂ C ₆ H ₃	44 × 10 ⁻³	0.53 (0.03)	0.20 (0.3)	1-5	49 (12.5)
				1-5	58 (10)
3,4-Cl ₂ C ₆ H ₃	18 × 10 ⁻³	0.69 (0.01)	0.22 (0.3)	1-5	30 (12)
				1-5	23 (20) ⁿ
4-MeC ₆ H ₄	24 × 10 ⁻³			1-5	44 (12.5)
2,4-Me ₂ C ₆ H ₃	42 × 10 ⁻³	0.66 (0.1)	0.16 (0.3)	1	0 (90) ⁿ
3,4-Me ₂ C ₆ H ₃	138 × 10 ⁻³			1, 5, 9	0 (30) ⁿ
4-CF ₃ C ₆ H ₄	150 × 10 ⁻³		0.01 (0.3)	1-9	17 (50)
4-C ₆ H ₅ C ₆ H ₄	6.1 × 10 ⁻³	0.65 (0.01)	0.42 (0.3)	1	36 (25)

^a Concentration of agent that inhibits proliferation of cultured lymphoid leukemia L1210 cells to 50% control growth during 48 h. ^b Fraction of the cell population of cultured lymphoid leukemia L1210 cells in mitosis. ^c Lymphocytic leukemia P-388. ^d Increase in life span at the highest nontoxic dose tested. ^e Methyl [5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate. ^f Average of three determinations. ^g Methotrexate-resistant line of P-388 (designated tumor P7 by the Drug Evaluation Branch, National Cancer Institute). ^h One cure. ⁱ Vincristine-resistant line of P-388. ^j Average of two determinations. ^k Two cures. ^l Reference 3c. ^m Toxic dose. ⁿ Highest dose tested.

against P-388 in mice,¹³ the phenyl and 4-methoxyphenyl compounds have the greater activity. The activity of 7 [R = C₆H₅N(Me)CH₂ and 4-MeOC₆H₄N(Me)CH₂] against the methotrexate-resistant line of P-388¹⁴ indicated that the mechanism of action of this compound was different from that of methotrexate (e.g., inhibition of dihydrofolic reductase). In addition, the activity of 7 [R = C₆H₅N(Me)CH₂] against the vincristine-resistant line of P-388¹⁶

indicated that the mechanism of mitotic arrest might be different from that of vincristine. Preliminary studies showed that 7 [R = C₆H₅N(Me)CH₂] prevented the polymerization of tubulin to give microtubules,¹⁷ which suggested that this compound forms a complex with tubulin at a site different from that of vincristine.

In the phenyl series, 14 compounds were prepared which inhibited the proliferation of cultured L1210 cells with ID₅₀'s of 1-150 × 10⁻³ μM. Within this range, the compounds could be separated into four groups. The lowest ID₅₀'s were observed with the unsubstituted phenyl and its 4-fluoro, 3- and 4-methoxy, and 4-phenyl derivatives. Intermediate ED₅₀'s were given by the 4-methyl-, 4-amino-, and 3,4-dichlorophenyl derivatives and the 4-chloro-, 2,4-dichloro-, 2,4-dimethyl-, and 3,4,5-trimethoxyphenyl derivatives. The highest ED₅₀'s were shown by the 4-(tri-

(13) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* 1972, 3(2), 9.

(14) In mice with a 10⁶ cell implant (ip), methotrexate at a dose of 2 mg/kg on the qd 1-9 schedule gave a 2 log cell kill against the sensitive line of P-388 and a 2 log increase in cells against the methotrexate-resistant line of P-388 (ref 15).

(15) Schabel, Jr., F. M., unpublished results.

(16) Wilkoff, L. J.; Dulmage, E. A. *J. Natl. Cancer Inst.* 1978, 61, 1521.

(17) Horwitz, S. B., unpublished results.

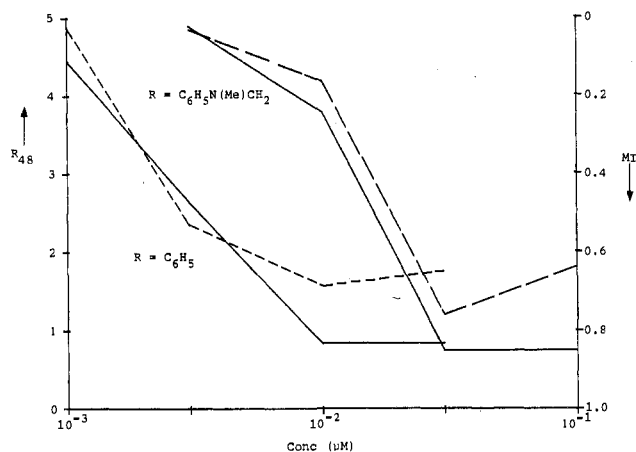


Figure 1. Effects of 1-deaza-7,8-dihydropteridines upon the proliferation (—) and the mitotic index (--) of cultured L1210 cells at the indicated concentrations. R_{48} , ratio of the final cells/milliliter to the initial cells/milliliter after 48 h.

fluoromethyl)- and 3,4-dimethylphenyl derivatives. These compounds showed potent cytotoxicities but diversity in MI values. In general, lower MI values were observed for the 6-phenyl relative to the 6-(*N*-methylanilino)methyl series. These results are attributed, at least in part, to a greater degree of cell lysis at the concentrations and time of exposure for these determinations. For 7 ($R = C_6H_5$), the inhibition of cell proliferation follows the MI as described above for 7 [$R = C_6H_5N(Me)CH_2$] (Figure 1). This compound also showed activity against the vincristine-resistant line of P-388. These 1-deaza-7,8-dihydropteridines showed significant activity against P-388 in mice, with the exception of 7 [$R = 4-CF_3C_6H_4$ and 2,4-(Me)₂C₆H₃], which were inactive.¹³

No combination of the hydrophobic (π) and electronic (σ) constants of the phenyl substituents of the 1-deaza-7,8-dihydropteridines gave a linear correlation with the ID_{50} 's.¹⁸ However, the overall biological results indicated that positive values of π and σ are undesirable. An exception is 7 ($R = C_6H_5C_6H_4$, $\pi = 1.96$), which has an ID_{50} similar to that of 7 [$R = C_6H_5N(Me)CH_2$]. This result indicated a spacer moiety between the 1-deaza-7,8-dihydropteridine and phenyl rings was not detrimental to activity.

In summary the 1-deaza-7,8-dihydropteridines are novel compounds that have shown anticancer activity in mice by causing the accumulation of cells in mitosis. Further work is in progress to determine the features of this ring system that are necessary for activity. In addition, studies on the binding of these compounds to tubulin will be initiated.

Experimental Section

Typical procedures are given for the preparation of the compounds listed in Tables I–III.

Method I. 1-Amino-3-(*N*-methyl-*N*-phenylamino)propanone Oxime. A mixture of 1-bromo-3-phthalimido-propanone (145 g, 514 mmol), *N*-methylaniline (55.1 g, 514 mmol), and NaHCO₃ (43.2 g, 514 mmol), in DMAC (1450 mL) was stirred at room temperature for 24 h, followed by heating at 40 °C for 2 h. The mixture was cooled in an ice bath and slowly diluted with cold H₂O (440 mL). The yellow solid that precipitated was collected by filtration, washed with a 1:3 mixture of H₂O–DMAC (160 mL) and cold H₂O, and dried in vacuo over P₂O₅; yield, 130 g. Dilution of the filtrate with additional H₂O (1200 mL) gave a second crop of product: yield 20.1 g. The combined crops (150

g) were recrystallized from EtOH to give the diaminopropanone in three crops: yield 145 g.

A solution of this product (474 mmol), hydroxylamine hydrochloride (49.3 g, 709 mmol), and pyridine (482 mL) in EtOH (2000 mL) was refluxed for 2.5 h and evaporated to dryness in vacuo. The residue was washed with cold H₂O (2 × 500 mL) and EtOH (250 mL) and recrystallized from EtOH to give the product in three crops: yield 143 g.

A solution of the oxime (442 mmol) in EtOH (5300 mL) at 70 °C was treated dropwise during 20 min with a solution of 95% hydrazine (16.4 g) in EtOH (200 mL). The resulting solution was heated at 40 °C for 22 h, and the cooled reaction mixture was treated with 1 N HCl (485 mL). After stirring in an ice bath for 1 h, the precipitated phthalic acid hydrazide was removed by filtration and washed with 1:1 EtOH–H₂O (600 mL). The combined filtrate and wash was evaporated to dryness in vacuo (40 °C), the residue was stirred with warm H₂O (1500 mL), and, after cooling, the insoluble yellow solid was removed by filtration and washed with H₂O (200 mL). The clear yellow aqueous filtrate was treated with concentrated NH₄OH (35 mL), and the oil that separated was extracted with CHCl₃ (3 × 300 mL). The combined extracts were dried over Na₂SO₄ and evaporated to give a gum, which solidified on drying in vacuo over P₂O₅. The solid was pulverized, washed by vigorous stirring with cold H₂O (700 mL), and redried in vacuo over P₂O₅ to give the α -amino ketone oxime as a mixture of syn and anti isomers: yield 57.3 g.

Method II. α -Amino-2,4-dichloroacetophenone Oxime. α ,2,4-Trichloroacetophenone (15 g, 67 mmol) was added with stirring to a suspension of potassium phthalimide (16 g, 86 mmol) in *N,N*-dimethylformamide (68 mL) at 5 °C. After 5 min, the resulting solution was allowed to warm the room temperature, followed by heating at 50 °C for 15 min. The solution was mixed successively with CHCl₃ (103 mL) and H₂O (341 mL), and the H₂O phase was separated and extracted with additional CHCl₃ (3 × 46 mL). The combined CHCl₃ extracts were washed with 2% NaOH (57 mL) and H₂O (57 mL) and evaporated to a small volume in vacuo (40 °C). The residue was diluted with cold H₂O (225 mL), and the mixture was chilled to deposit a semisolid, which was separated by decantation. The residue was washed with EtOH and Et₂O and dried to give the phenacylphthalimide: yield 9.2 g.

A solution of this solid (28 mmol) and hydroxylamine hydrochloride (2.9 g, 41 mmol) in a mixture of pyridine (28 mL) and EtOH (117 mL) was stirred at reflux for 1.5 h. The solvent was evaporated in vacuo, and the resulting oily ketone oxime was washed with H₂O: mass spectrum, m/e 348 (M^+). The phthaloyl group of this product was removed by the procedure described in method I: yield 2.4 g.

Method III. α -Amino-*p*-phenylacetophenone Oxime. A solution of α -bromo-*p*-phenylacetophenone (25.0 g, 90.9 mmol) and hexamethylenetetramine (13.2 g, 94.0 mmol) in CHCl₃ (200 mL) was heated with stirring at 48 °C for 4 h. The resulting mixture was cooled to room temperature, and the quaternary salt was collected by filtration: yield 36.2 g. This salt was stirred in a mixture of EtOH (135 mL) and concentrated HCl (67 mL) for 19 h. The hydrochloride of the product was collected by filtration and washed with small portions of H₂O: yield 18.2 g.

A suspension of this product (73 mmol) and hydroxylamine hydrochloride (15.3 g, 220 mmol) in pyridine (190 mL) and EtOH (190 mL) was stirred at reflux for 0.5 h. The resulting solution was evaporated to dryness in vacuo (30 °C), and the residue was washed with water: yield 11.7 g; mp indefinite. On cooling, the filtrate gave an additional 4.1 g. The total amount obtained was 15.8 g.

Method IV. 1-Amino-3-[*N*-(4-chlorophenyl)-*N*-methylamino]-2-propanol. A solution of epichlorohydrin (11 mL) and 4-chloro-*N*-methylaniline (11 g, 78 mmol) in a mixture of EtOH (10 mL) and H₂O (7 mL) was refluxed for 2 h, diluted with H₂O (20 mL), and extracted with Et₂O (3 × 50 mL). The combined extracts were evaporated to dryness, the residue was treated with a solution of NaOH (5 g) in H₂O (10 mL) for 1 h, and the resulting mixture was extracted with Et₂O (4 × 25 mL). The combined extracts were dried (MgSO₄) and evaporated to dryness in vacuo to give 1-[*N*-(4-chlorophenyl)-*N*-methylamino]-2,3-epoxypropane: yield 11 g (72%). A solution of this sample in a mixture of EtOH (50 mL) and liquid NH₃ (20 mL) was heated in a glass-lined

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stainless-steel bomb at 100 °C for 3 h. The resulting reaction solution was evaporated to dryness, and the dried residue was recrystallized from C₆H₆; yield 5.3 g (44%).

Method V. Ethyl 6-Amino-4-[[3-(*N*-methyl-*N*-phenylamino)-2-oxopropyl]amino]-5-nitro-2-pyridinecarbamate Oxime. A solution of 1 (10.3 g, 39.5 mmol), 1-amino-3-(*N*-methyl-*N*-phenylamino)propanone oxime (7.77 g, 40.2 mmol), and triethylamine (4.27 g, 42.2 mmol) in EtOH (200 mL) was heated under N₂ at 75 °C for 24 h. After the reaction mixture was cooled, the yellow solid was collected by filtration, washed with cold EtOH, and dried in vacuo over P₂O₅ at 65 °C; yield 13.8 g.

Method VI. Ethyl 6-Amino-5-nitro-4-[(2-oxo-2-phenylethyl)amino]-2-pyridinecarbamate. A solution of ethyl 6-amino-5-nitro-4-[(2-oxo-2-phenylethyl)amino]-2-pyridinecarbamate oxime (4.72 g, 12.6 mmol) in a 1:1 mixture of 1 N HCl-dioxane (170 mL) was heated with stirring at 60 °C for 2 h. The yellow solid that deposited from the chilled solution was collected by filtration and recrystallized from a 1:1 mixture of H₂O-dioxane (1 L); yield 3.13 g.

Method VII. Ethyl 6-Amino-4-[[3-(*N*-methyl-*N*-phenylamino)-2-oxopropyl]amino]-5-nitro-2-pyridinecarbamate. Crystalline orthophosphoric acid (3.08 g, 31.5 mmol) was added to a stirred solution of ethyl 6-amino-4-[[2-hydroxy-3-(*N*-methyl-*N*-phenylamino)propyl]amino]-5-nitro-2-pyridinecarbamate (3.18 g, 7.87 mmol) and *N,N'*-dicyclohexylcarbodiimide (4.86 g, 23.6 mmol) in dry Me₂SO (40 mL). The mildly exothermic reaction was kept below 25 °C by water-bath cooling. After 2.5 h, the deposit of dicyclohexylurea was filtered off and washed with Me₂SO (25 mL). The filtrate was cooled in an ice bath and diluted slowly with H₂O (100 mL) to precipitate the product as a bright yellow solid, which was washed thoroughly with H₂O and dried in vacuo over P₂O₅; yield 2.89 g.

Recrystallization of the crude product from several common organic solvents was unsuccessful. In hot EtOH, the product underwent rapid degradation to a chromatographically complex red gum. Mass spectral analysis showed a prominent molecular ion at M⁺ 241, which corresponds to ethyl 4,6-diamino-5-nitro-2-pyridinecarbamate.

Method VIII. Ethyl 6-Amino-4-[[3-[*N*-(4-chlorophenyl)-*N*-methylamino]-2-hydroxypropyl]amino]-5-nitro-2-pyridinecarbamate. A solution of 1 (10.0 g), 1-amino-3-[*N*-(4-chlorophenyl)-*N*-methylamino]-2-propanol (8.25 g), and triethylamine (10.7 mL) in MeOH (120 mL) was heated at 60 °C for 18 h and evaporated to dryness in vacuo. The dark residue was washed with Et₂O (1.5 L) to give a yellow solid. This solid

was washed with H₂O (50 mL) and recrystallized twice from a mixture of ethanol and hexane: yield 4.92 g. Similar treatment of the residue obtained from the ether wash from above gave a slightly impure sample of product: yield 5.89 g (35%); mp 175 °C. The total yield was 64%.

Method IX. Ethyl 5-Amino-1,2-dihydro-3-[[*N*-methyl-*N*-phenylamino]methyl]pyrido[3,4-*b*]pyrazine-7-carbamate. A suspension of the oxime of ethyl 6-amino-4-[[3-(*N*-methyl-*N*-phenylamino)-2-oxopropyl]amino]-5-nitro-2-pyridinecarbamate (30.0 g, 71.9 mmol) and Raney nickel (60 g, weighed wet, washed with EtOH) in EtOH (3000 mL) was hydrogenated at room temperature and atmospheric pressure with vigorous stirring. At the end of 12 h, the hydrogen (7048 mL) absorbed corresponded to 134% of the theoretical for 3 molar equiv of the nitropyridine and 101% of the theoretical for 4 molar equiv. The resulting mixture was heated nearly to boiling under an atmosphere of N₂, and the catalyst was removed by filtration and washed with boiling EtOH (5 × 200 mL). The combined filtrate and wash were concentrated to about 1000 mL in vacuo and cooled in an ice bath to deposit the product as a pale yellow crystalline solid: yield 16.7 g.

Method X. Ethyl 5-Amino-1,2-dihydro-3-phenylpyrido[3,4-*b*]pyrazine-7-carbamate. A solution of ethyl 6-amino-5-nitro-4-[(2-oxo-2-phenylethyl)amino]pyridine-2-carbamate dioxanate (10:7) (3.10 g, 7.25 mmol) in EtOH (4 L) was hydrogenated in the presence of Raney nickel (9 g, weighed wet, washed with EtOH) at atmospheric pressure with intermittent warming with a water bath. After 6 h the catalyst was removed by filtration, and the filtrate was concentrated in vacuo (<40 °C) to 1/16 volume. The solid that deposited from the chilled mixture was collected by filtration and dried in vacuo over P₂O₅; yield 1.82 g. From the filtrate a second crop was obtained: yield 0.17 g. The total yield was 1.99 g.

Acknowledgment. This investigation was supported by Grant CA-25311 awarded by the National Cancer Institute, National Institute of Health. The authors are indebted to Dr. W. R. Laster for the screening of data, to B. J. Bowdon for mitotic indexes, to D. J. Adamson for the inhibition data on the proliferation of cells, and to Dr. W. C. Coburn, Jr., and other members of the Molecular Spectroscopy Section of Southern Research Institute who performed most of the microanalytical and spectral determinations.

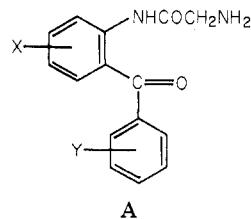
Quinazolines and 1,4-Benzodiazepines. 90.¹ Structure-Activity Relationship between Substituted 2-Amino-*N*-(2-benzoyl-4-chlorophenyl)acetamides and 1,4-Benzodiazepinones

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The syntheses of 2-amino-*N*-(2-benzoyl-4-chlorophenyl)acetamides are reported. The pharmacological properties of these compounds were compared with data obtained from the corresponding cyclized products [5-(2,6-dichlorophenyl)-1,4-benzodiazepin-2-ones]. Evidence is presented which suggests that the central nervous system activity observed for 1,4-benzodiazepines is inherent only in the closed seven-membered ring and is not due to the ring-opened form.

One of the procedures extensively employed during the course of our studies on the synthesis of 1,4-benzodiazepin-2-one derivatives involved the preparation and cyclization of aminoacetanilides of type "A".² These intermediates could be isolated either as the free base or as the hydrochloride salt.³ It was noted empirically that the substituent in the ortho position of the benzoyl group seemed to have an effect on the ease of the ring closure.



Biological evaluation of the "open amines" showed that their pharmacological profiles were qualitatively the same

[†]Deceased July 8, 1981.