PURINES, PYRIMIDINES, PTERIDINES, AND CANCER

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ABSTRACT

The haloadenine nucleosides 9-β-D-arabinofuranosyl-2-fluoroadenine, 2-bromo-, 2-chloro-, and 2-fluoro-2'-deoxyadenosine are examples of second generation drugs resulting from structural modification of presently used drugs. These nucleosides are cytotoxic to a number of cell lines in culture and are highly active against both the L-1210 and P388 leukemias in mice, having been shown to be curative in the L-1210 system.

A new kind of agent has resulted from the attachment of a chemically reactive group to the 5'-position of nucleosides. 5'-Bromoacetamido-5'-deoxythymidine, the most active agent of this type prepared so far, is cytotoxic to both H.Ep.-2 and L-1210 cells in culture and has shown good activity against the P388 leukemia in mice. Biochemical studies have shown that this compound interferes with DNA synthesis perhaps by the inactivation of nucleoside diphosphate reductase.

Another new class of agents, the 1-deaza-7,8-dihydropteridines, has been found to be extremely cytotoxic and to be active against the P388 leukemia in mice. These agents are mitotic inhibitors and may prove superior to the standard clinical antimitotic agent, vincristine, in that they are active against a strain of the P388 leukemia resistant to vincristine and are more accessible synthetically. Based on the results to date, it would appear that further improvement in activity is possible via structural modification.

Although the mechanism by which any anticancer agent, even the early drugs such as methotrexate and nitrogen mustard, selectively kills cancer cells has not been clearly established (indeed only limited selectivity is achieved causing the ever-present problem of drug toxicity with which both patient and doctor are all too

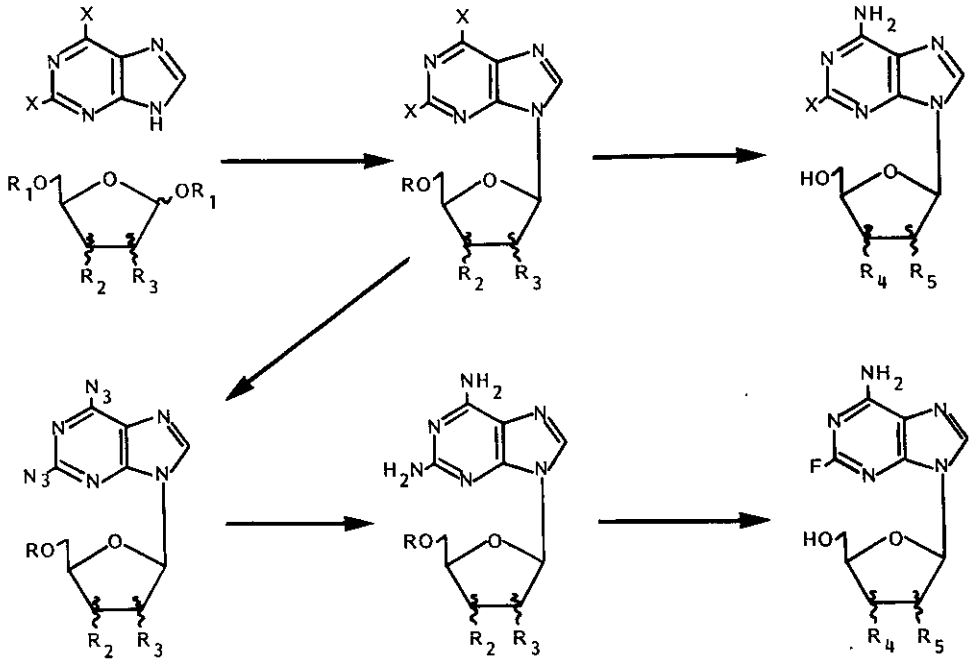
familiar), and although definitive studies on some of the newer agents are not complete, the overwhelming body of evidence points to interference with the synthesis or function of nucleic acids, or with the mitotic process itself, as being the ultimate mechanism by which most, if not all, agents kill cancer (and certain host) cells. That this is true may be self-evident, since cancer is clearly a disease of uncontrolled proliferation of cells that have mutated from normal body cells.

The major metabolites involved in the synthesis of nucleic acids are purines, pyrimidines, and pteridines. Purines and pyrimidines — adenine, guanine, cytosine, uracil, and thymine — become integral parts of the nucleic acids whereas pteridines participate primarily as the various forms or derivatives of [6S]-tetrahydrofolate that take part in the *de novo* biosynthetic pathways of both the purine and pyrimidine nucleotides. Because of the importance of these heterocyclic ring systems in nucleic acid biosynthesis, most of the antimetabolites used today in cancer chemotherapy — the 6-thiopurines, methotrexate, 5-fluorouracil, and arabinofuranosylcytosine — belong to them. These drugs exert their effects by the inhibition of the enzymes of the biosynthetic pathway to nucleic acids or by incorporation into the nucleic acids or by both mechanisms. In any event, except for methotrexate, the parent drug, be it a purine, pyrimidine, or nucleoside thereof, must be converted to the nucleoside mono-, di-, or triphosphate-, a metabolic activation normally accomplished by one of the so-called salvage enzymes, and as a kinase or phosphoribosyltransferase. Despite their utility, these drugs have certain drawbacks. Insufficient selective toxicity and the selection, by treatment, of mutant cell populations resistant to them are major ones that often cause failure, at tolerated doses, to eradicate cancer cell populations and effect cures.

Second generation drugs have resulted from structural modification of presently used drugs, based on an understanding of metabolism, mechanism of action, and other properties such as drug transport.[1] The haloadenine nucleosides represent one such type of second generation drug. Our methods for the preparation of nucleosides of 2- and 8-haloadenines have been adequately described[2] (Charts I and II). The discovery of the resistance of 2-fluoroadenosine to deamination and of its potent cytotoxicity led to studies on other nucleosides of 2-fluoroadenine[3] and, later, of other haloadenines.[4] We have systematically studied the ribo-, 2'-deoxyribo-, arabino-, xylo-, and certain other nucleoside derivatives of most of the haloadenines and have been able to relate their biologic activity to their metabolism and to establish the probable mechanism of their cytotoxicity and anticancer activity.

Table I presents the cytotoxicity of the haloadenines and some of their nucleosides to H.Ep.-2 cells in culture. Note that the only adenine that is a potent cytotoxin is 2-fluoroadenine, although 2-chloro- and even 2-bromoadenine show some activity. These results probably reflect the ability of these compounds to serve as

Chart I.



X = Cl or Br

D-Sugars: ribose, 2'- and 3'-deoxyribose, xylose, and arabinose.

Chart II.

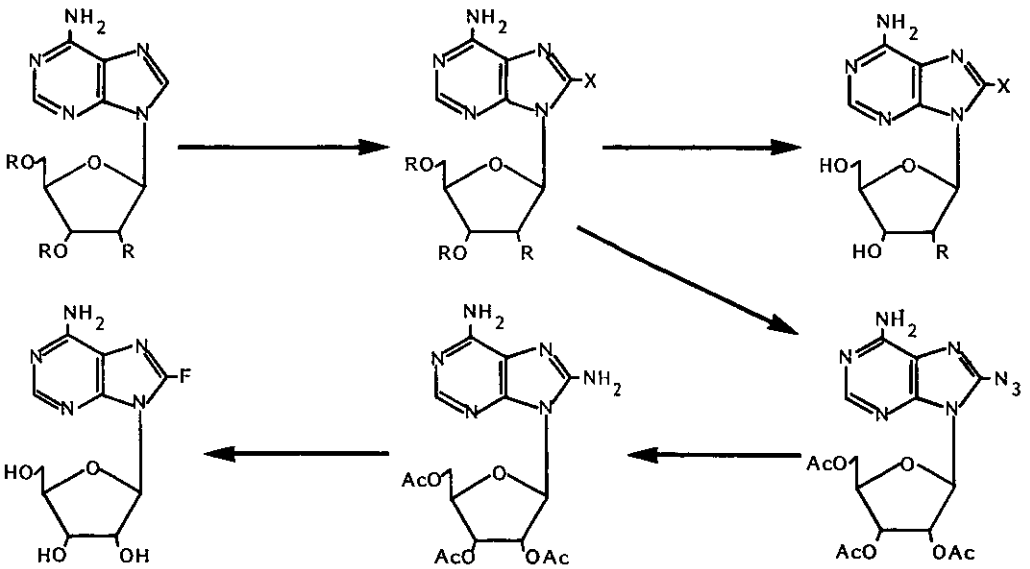
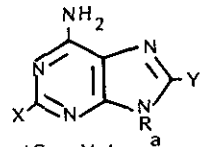
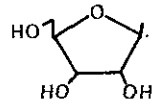
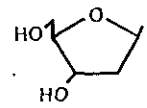
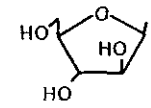


Table I. Cytotoxicity of the Haloadenines and Some of their Nucleosides



IC_{50} Values[a]

		R = H			
Y = H, X = H		100	1.0[b]	2.6[b]	ca. 5[b]
	F	0.03	0.02[c]	0.2	8
	Cl	10	7.0	0.01	ca. 3
	Br	70	120	2.0	5
	CF_3		I	14	--
X = H, Y = H		100	1.0[b]	2.6[b]	ca. 5[b]
	F	--	2.0	--	--
	Cl	>60	0.03	>100	--
	Br	>60	<2.0	400	--
	CF_3	I	35	--	--

[a] μMolar concentration of compound required to inhibit growth of treated H.Ep. #2 cells to 50% of controls. [b] In the presence of 2'-deoxycoformycin. [c] Line AK^- and $APRT^-$ >2000 resistant.

substrates for adenine phosphoribosyl transferase, but this has not been established. 2-Fluoroadenosine, which shows the same degree of cytotoxicity as 2-fluoroadenine, is about 400 times as cytotoxic as 2-chloro and 6,000 times as cytotoxic as 2-bromoadenosine, and the difference appears to relate to their ability to serve as substrates for adenosine kinase (Table II);[5] since, with the exception of 8-fluoroadenosine, all of these compounds are highly resistant to deamination (Table III). In sharp contrast to loss of potency with increasing size of the halogen atom at C-2 of the purine ring, both 8-chloro- and 8-bromoadenosine appear to be more cytotoxic than the 8-fluoro compound. Even 8-trifluoromethyladenosine is more potent than 2-bromoadenosine. Studies are in progress on the relative rates of phosphorylation of these compounds by adenosine kinase. Activity in the 2'-deoxyribonucleoside series is clearly different in that the size of the substituent at C-2 is not nearly so critical to cytotoxicity and, in fact, 2-chloro-2'-deoxyadenosine is significantly more potent than the 2-fluoro compound and the 2-bromo compound only one order of magnitude less. Even the 2-trifluoromethyl compound has significant activity, in contrast to the inactivity of the corresponding compound in the ribo series. The opposite situation exists with substitution at C-8, since both the 8-chloro- and 8-bromo-2'-deoxyadenosines are essentially inactive. Isolated enzyme studies,[6,7,8] coupled with cytotoxicity

Table II. Phosphorylation of the Haloadenine Nucleosides

Adenosine Kinase[a]			Deoxycytidine Kinase[b]		
Compound	K_m	Rel. V_{max}	Compound	K_m	Rel. V_{max}
Adenosine	0.4	100	2'-Deoxycytidine	3	50
2-Fluoroadenosine	134	206	2'-Deoxyadenosine	600	100
2-Chloroadenosine	134	0.4	2'-Deoxy-2-fluoroadenosine	50	40
2-Bromoadenosine		N.D.[c,d]	2-Chloro-2'-deoxyadenosine	50	40
8-Bromoadenosine	11	31	2-Bromo-2'-deoxyadenosine	40	40
3'-Deoxyadenosine	254	138	9-β-D-Arabinofuranosyl-2-fluoroadenine	500	30
9-β-D-Xylofuranosyladenine	86	21	8-Chloro-2'-deoxyadenosine		N.D.[d]
2'-Deoxy-2-fluoroadenosine		<0.05	8-Bromo-2'-deoxyadenosine		N.D.[d]
9-β-D-Arabinofuranosyl-2-fluoroadenine		<0.05			

[a]From rabbit liver, Miller et al., J. Biol. Chem., 254, 2346 (1979) except where noted. [b]From L1210 cells, Chang et al., unpublished. [c]From H.Ep. 2 cells, Schnebli et al., J. Biol. Chem., 242, 1997 (1967). [d]Not detectable.

studies in kinase deficient cell lines,[3,9] have shown that the 2'-deoxyribonucleosides are phosphorylated by deoxycytidine kinase and not by adenosine kinase. Furthermore, the cytotoxicity of these compounds can be correlated with their rate of phosphorylation (Table II), but not with their rate of deamination (Table III). The arabinonucleosides, mimicking the 2'-deoxyribonucleosides, are also phosphorylated by deoxycytidine kinase but are significantly less cytotoxic; whereas the xylonucleosides appear to be phosphorylated by adenosine kinase[7] but are made less potent as cytotoxic agents.[3]

Table III. Kinetic Constants of Haloadenine Nucleosides as Substrates of Adenosine Deaminase[a]

Compound	K_m (μM)	V_{max} (μmoles/min/mg)
Adenosine	29	435
2-Fluoroadenosine	81	0.78
2-Chloroadenosine	110	0.06
2-Bromoadenosine	63	0.01
8-Fluoroadenosine	1,000	230
8-Chloroadenosine	830	7.6
8-Bromoadenosine	250	0.04

[a]Sigma, calf intestinal.

9-β-D-Arabinofuranosyl-2-fluoroadenine (2-fluoro-ara-A) has received the most attention of the haloadenine nucleosides, because it was the first to be shown to be curative in the L-1210 leukemia system. In

addition, its maximum effectiveness can be demonstrated on a daily dose schedule, in contrast to the combination of 9-β-D-arabinofuranosyl adenine and 2-deoxycoformycin, which must be administered every three hours for 8 courses on days 1, 5, and 9 in order to achieve cures[8] (Table IV). This latter schedule is also optimal for the 2'-deoxy-2-haloadenosines.[2,9] To date, these compounds have shown no advantage over 2-fluoro-ara-A, although the 5'-O-nonanoate of 2-chloro-2'-deoxyadenosine, a depot form, does show comparable activity on the daily schedule (Table V).

Table IV. Comparison of the Response of L-1210 Leukemia to Ara-A, the 2-Halo-ara-A's and Some Soluble Derivatives

Agent	Dosage (mg/kg/dose)[a]	Optimal Schedule	% ILS[b]	"Cures"[c]
Ara-A·H_2O[d]	240	q3hx8; Days 1, 5, 9	38	0/19
Ara-A+2'-dCF[e]	80 + 0.1	q3hx8; Days 1, 5, 9	152	4/20
Ara-AMP+2'-dCF	125 + 0.1	q3hx8; Days 1, 5, 9	185	7/20
2-F-ara-A	130 - 150	qd 1-9	125	4/15
2-F-ara-AMP·2NH_4	220	qd 1-9	137	3/10
2-Cl-ara-A[f]	100	qd 1-9	56	0/6
2-Br-ara-A	100	qd 1-9[g]	18	0/6

[a] LD_{10} or MTD. [b] Median % increase in lifespan of dying animals only. [c] 60-Day survivors are considered cures. [d] Ara-A or ara-AMP alone is inactive given qd 1-9. [e] 2'-dCF = 2'-deoxycoformycin (pentostatin). This combination shows low activity given qd 1-9. Except where indicated, data from Brockman et al., Cancer Res., 40, 3610 (1980). [f] Data supplied by Division of Cancer Treatment, NCI. [g] Only schedule used.

The decay of 2-fluoro-ara-ATP levels in L-1210 cells harvested from leukemic mice treated with 2-fluoro-ara-A correlates well with the effectiveness of the daily schedule, but determinations of the levels of the 2'-deoxy-2-halo ATP's have not yet been made. 2-Fluoro-ara-A has also shown good activity against the P388 leukemia in mice and against some solid tumors, leading to a Phase I clinical trial. Responses in patients with adenocarcinoma of the lung, colorectal carcinoma, and head and neck cancer have been noted.[10]

The 2-chloro derivative of ara-A has shown moderately good activity against the P388 and L-1210 leukemias,[11] whereas the 2-bromo-ara-A was inactive against leukemia L-1210 (Table IV). The ribonucleosides and the xylonucleosides that have been evaluated showed little or no in vivo activity.

5'-Deoxy-2-fluoroadenosine[12] and 5'-deoxy-5'-ethylthio-2-fluoroadenosine[13] were found to be quite toxic to H.Ep.-2 cells in culture (Table VI) but were inactive against L-1210 leukemia in mice, although they were tolerated at 50-100 mg/kg daily, whereas the maximum tolerated dose of 2-fluoroadenosine is 1 mg/kg. These

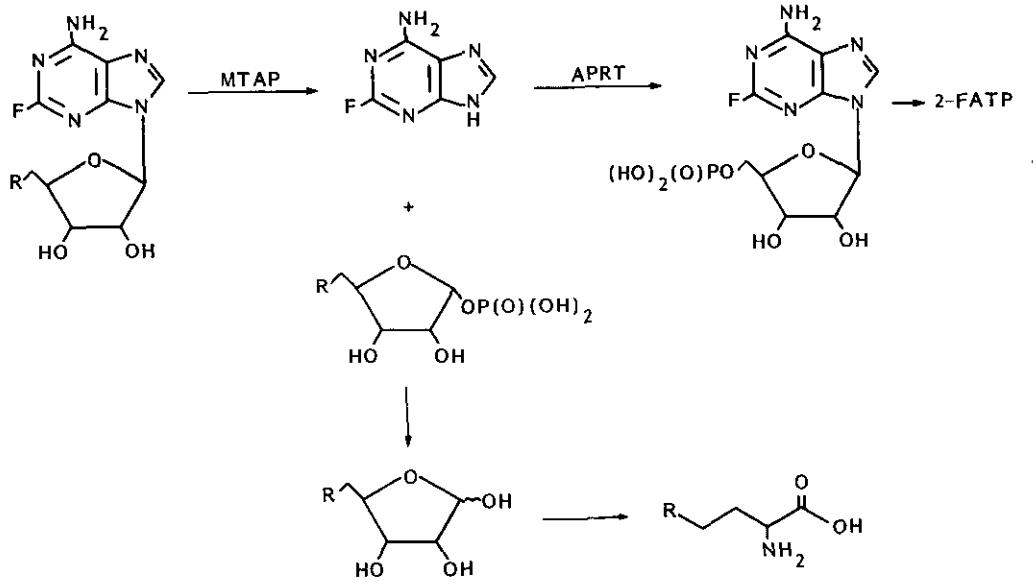
Table V. In Vivo Activity of the 2-Halo-2'-deoxyadenosines vs. Leukemia L-1210

Nucleoside	Dosage (mg/kg/dose)[a]	Schedule[b]	% ILS[c]	Cures/ Treated
2'-Deoxyadenosine	660	A	10	0/8
2'-Deoxyadenosine + 2'-Deoxycoformycin	150 + 0.05	A	32	0/10
2-Chloro-2'-deoxyadenosine	50	B	81	0/6
	15	A	175	5/10
2-Chloro-2'-deoxyadenosine 5'-nonanoate	50	C	244	4/6
2'-Deoxy-2-fluoroadenosine	40	A	100	3/10
2-Bromo-2'-deoxyadenosine	40	A	125	3/6

[a]Mg/kg/dose (at less than or equal to the lethal dose for 10% of the animals (autopsied for cause of death)). [b]A = every three hours for 24 hours on days 1, 5, and 9; B = daily, days 1-6; C = daily, days 1-9. [c]Median % increase in lifespan of dying animals only.

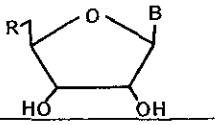
compounds were also found to be inactive in H.Ep.-2 cells deficient in adenine phosphoribosyltransferase, suggesting that they were cleaved in H.Ep.-2 cells to 2-fluoroadenine which, in cells containing the transferase, was converted to 2-fluoro-AMP (Chart III). Indeed, L-1210 cells were later found to be deficient in 5'-deoxy-5'-(methylthio)adenosine phosphorylase,[14,15] an enzyme which was shown to cleave the 5'-ethylthio

Chart III.



compound (Table VII), and presumably the 5'-deoxy compound, to 2-fluoroadenine. H.Ep.-2 and a number of other cell lines contain this enzyme and are quite sensitive to compounds of this type.[16] In vivo evaluations of 5'-deoxy-2-fluoroadenosine are underway.

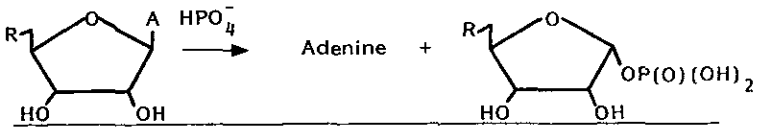
Table VI. Cytotoxicity of 5'-Substituted-5'-Deoxy-2-Fluoroadenosines



	IC_{50} (μM)			
	H.Ep.-2 Epidermoid Carcinoma	HCT-15 Colon Carcinoma	L-1210 Leukemia[a]	CCRF-CEM Leukemia[a]
B = 2-Fluoroaden-9-yl				
R				
H	<0.3	--	>30	--
I	0.04	0.2	>40	13
MeS	0.4	--	ca. 120	34
EtS	<1	0.4	--	>15
2-Fluoroadenine	0.02	0.7	--	0.1
B = Aden-9-yl				
R				
I	--	146	--	--
EtS	--	85	--	--
B = 4-Aminopyrazolo-[3,4-d]pyrimidin-1-yl				
R				
EtS	>60	>200	--	>200
4-Aminopyrazolo[3,4-d] pyrimidine	0.3	0.6	--	1-7

[a]Deficient in MTAase.

Table VII.



R	K_m (μM)	Rel. V_{max}
MeS	4	100
HO	1400	115
H	23	180
$(Me)_2CHCH_2S$	8	89
EtS(FA)*	20	73
Cl	21	46
Br	8	37
I	10	25

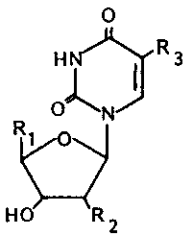
*A = 2-Fluoroadenine.

Another, more difficult, approach to the development of new anticancer agents is that of ab initio design. This approach is difficult primarily because of the lack of an identifiable, exploitable biochemical difference between normal and malignant tumor cells be they of animal or human origin. At the same time, it is clear that the anticancer agents with proven clinical utility, except perhaps for the hormones, interfere in one way or another with cell division, and since cancer cells must divide or eventually die, these drugs are cytotoxic agents with some degree of specificity for neoplastic cells. It would thus seem logical that the search for new lead compounds should focus on new structural types that will also interfere with one or another of the processes of cell division. The most approachable of these is the design of enzyme inhibitors. At least 85 enzymatic reactions are involved in the *de novo* synthesis of purine and pyrimidine nucleotides, in their interconversions, in their polymerization to nucleic acids, and in the so-called salvage pathways. Approximately 14 of these 85 enzymes are known to be inhibited by metabolite analogs or anabolites thereof, and these inhibitions are thought to be responsible for, or at least to contribute to, the anticancer activity of these compounds.

Except for the early reactions of the biosynthetic pathway to uridylic acid, all of the enzymatic reactions leading to nucleotides and involved in their polymerization take place at the nucleoside mono-, di-, and triphosphate levels, and most of the known inhibitors are in situ generated nucleoside mono-, di-, and triphosphates, which are not themselves practical drugs because of their lability and inability to penetrate cells intact. Furthermore, resistance to the purine and pyrimidine antimetabolites that are administered as the free bases or nucleosides thereof normally results from the selection of a mutant cell population deficient in the enzymes that convert the analogs to the nucleoside monophosphates.[17] Attempts, so far largely unsuccessful, to circumvent this problem have until now consisted mostly of efforts to find, and attach to nucleosides, groups that might simulate the binding properties of the phosphate group and yet allow compounds containing them to enter cells intact[18] or to prepare nucleotide derivatives that can enter cells and be cleaved intracellularly to the active nucleotides.[19-21] There is another possible approach that attempts to turn this liability of the nucleotide analog approach into an asset. The mode of binding of nucleotides to certain enzymes is known precisely from X-ray data.[22] This knowledge of the nature of the groups in the enzyme active site that bind nucleotides and the precise position of these groups relative to the phosphate moiety of the substrates allows the design of nucleosides containing the proper kind of chemically reactive function properly positioned to react with the phosphate binding group and thereby inactivate the enzyme. The nucleosides selected for modification are ones where phosphate derivatives are known to participate in the biosynthetic pathways to nucleic acids.

Activity has been observed with certain nitrosoureidonucleosides, halomethylketo nucleosides, and α-haloacyl nucleosides (Tables VIII and IX). The most active compounds against the P388 leukemia in mice are

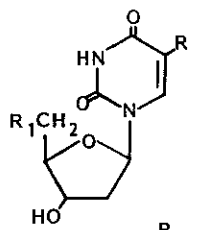
Table VIII.



R_1	R_2	R_3	IC_{50} (μg/ml)[a] H.Ep.-2	IC_{50} (μg/ml)[a] L-1210	P388 % ILS/Dose[b]
$MeN(NO)CONHC_6H_4$-4-NHCO	H	Me	20	40	79/200
$MeNHCONHC_6H_4$-4-NHCO	H	Me	--	--	1/200[c]
$MeN(NO)CONHC_6H_4$-4-NHCO	OH	H	10	40	56/200
$MeN(NO)CONH(CH_2)_2NHCO$	OH	H	20	--	28/200
$MeN(NO)CONHCH_2$	H	Me	--	100[d]	--
$Cl(CH_2)_2N(NO)CONHCH_2$	H	Me	4	10	135/25(1)[e]
$Cl(CH)_2N(NO)CONHCH_2$	H	H	10	8	142/13(2)[e]
$ClCH_2COC_6H_4$-4-NHCO	H	Me	10	15	1/200[c]
$BrCH_2COC_6H_4$-4-NHCO	H	Me	8	3	38/200
$ICH_2COC_6H_4$-4-NHCO	H	Me	6	3	1/100[c]

[a] IC_{50} is the concentration that inhibits the proliferation of cells to 50% of untreated controls. [b] % Increase in lifespan/dose in mg/kg/day, qd 1-5. [c] I = Inactive, toxic. [d] Lin et al, J. Med. Chem., 21, 130 (1978). [e] Cures.

Table IX.



R	R_1	IC_{50} (μg/ml)[a] L-1210	IC_{50} (μg/ml)[a] H.Ep.-2	P388 (qd 1-5) % ILS/Dose (mg/kg)
CH_3	$NHCOCH_2Br$	3.4	2.7	60/100
CH_3	$NHCOCH_2I$	8.3	3.5	34/50
CH_3	$NHCOCH_2Cl$		11.6	9/200
CH_3	$CH_2NHCOCH_2Br$		>20	ND
CH_3	$NHCOCH(CH_3)Br$	>40	>20	8/50
CH_3	$OCOCH_2Br$	2.9	0.4	17/50
CH_3	NHCO-C6H4-$COCH_2Br$	>40	>20	15/50
H	$NHCOCH_2Br$	4.6	3.3	57/100
H	$NHCOCH_2I$			44/100
H	$NHCOCH(CH_3)Br$	>40	22	5/100
I	$NHCOCH_2Br$	4.8	1.2	21/50
Br	$NHCOCH_2Br$	0.2	3.5	28/50

[a] See footnotes to Table VIII.

the 2-chloroethylnitrosoureidonucleosides, but the lack of activity of a corresponding methylnitrosoureidonucleoside,[23] and studies on the effects of other active methylnitrosoureidonucleosides on macromolecular synthesis in inhibited cells, indicates that all these compounds are acting like nitrosoureas that probably owe their activity to interaction with DNA rather than specific enzyme inhibitions.

The halomethylketones have not shown significant in vivo activity, but the bromo- and iodoacetamido compounds have. A comparison of the effects of the 5'-bromoacetamido derivatives of uridine, 2'-deoxyuridine, and thymidine in macromolecular synthesis in L-1210 cells in culture shows that the 2'-deoxyribonucleosides inhibit precursor incorporation whereas the uridine derivative does not (Table X).

Table X. Effect of 5'-Bromoacetamido Derivatives of Pyrimidine Nucleosides on Macromolecular Synthesis in L-1210 Cells in Culture

	Percent of Control[1]								
	Urd Derivative[2]			dUrd Derivative[3]			dThd Derivative[4]		
Precursor	DNA	RNA	Protein	DNA	RNA	Protein	DNA	RNA	Protein
[C^3H_3]dThd	97			87			22		
[6^3H]dUrd	88			62			4		
[5^3H]dCyd	92			119			107		
[5^3H]Urd	89	141		32	118		1	102	
[5^3H]Cyd	105	107		45	109		15	91	
[8-^{14}C]Ade	83	109		35	82		14	44	
[8-^{14}C]Hyp	103	127		50	109		11	65	
[4,5-^{3}H]Leu			102			74			45

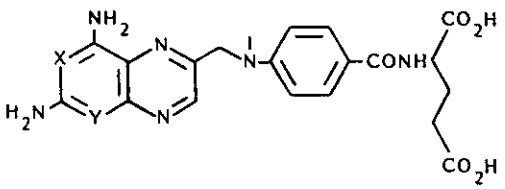
[1] Inhibitors (25 μM) were added to L-1210 cells in suspension culture prior to addition of radioactive substrate. Samples were taken for analysis 1, 2, 4, and 6 hours after addition of labeled substrate. Data presented here are based on the 4-hour sample. [2] SRI 6280. [3] SRI 6218. [4] SRI 6104.

Furthermore, incorporation into DNA is primarily affected, and the thymidine derivative is clearly a better inhibitor than is the 2'-deoxyuridine derivative. Inhibition of incorporation of purines into DNA indicates inhibition of the synthesis of DNA and not simply competition of inhibition with labeled precursor for entry into cells as could be the case with 2'-deoxyuridine or thymidine. The selective inhibition of incorporation of uridine and cytidine into DNA and the lack of inhibition of incorporation of 2'-deoxycytidine by the 2'-deoxyribonucleoside derivatives is of particular interest. A possible basis for such an effect would be the inhibition of ribonucleoside diphosphate reductase with pronounced suppression of the 2'-deoxycytidine triphosphate pool, but with sufficient pools of the other 2'-deoxyribonucleotides to permit some DNA synthesis. Lack of inhibition of the incorporation of 2'-deoxycytidine into DNA indicates that DNA polymerase

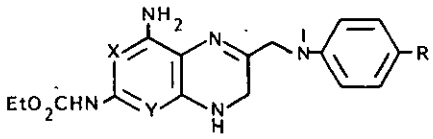
is probably not inhibited. Further investigations of the effects of the 5'-bromoacetamido derivative of thymidine are currently underway.

In 1971, we reported the synthesis and biologic activity of 1- and 3-deazamethotrexate (Chart IV).[24] 1-Deazamethotrexate had little effect on dihydrofolic reductase and was inactive against leukemia L-1210. In contrast, 3-deazamethotrexate was a potent inhibitor of dihydrofolate reductase and was about as active as methotrexate in the L-1210 leukemia system. These studies established the importance of N-1 to the binding of methotrexate to dihydrofolate reductase and, consequently, to its biologic activity. In addition, however, they led to a new type of anticancer agent. The intermediate 1- and 3-deaza-7,8-dihydropteridines (Chart IV) prepared for the synthesis of 1- and 3-deazamethotrexate were examined for their cytotoxicity: The 3-deaza compound showed no significant activity, but the 1-deaza compound was quite cytotoxic and showed some activity against leukemia P388 in vivo. Based on these results, a series of 1-deaza-7,8-dihydropteridines were prepared, and biologic studies have shown that these compounds inhibit the proliferation of L-1210 cells in culture at nanomolar concentrations, and possess significant activity against leukemia P388 (Table XI), and against its methotrexate-resistant and vincristine-resistant sublines.[25,26] These compounds cause the *accumulation of cells at mitosis, both in vivo and in vitro.*[27] *Several of these new agents may be superior to* the standard clinical antimitotic agent vincristine in that they are more accessible synthetically, and based on results to date, it would appear that further improvement in activity is possible via structural modification.

Chart IV.



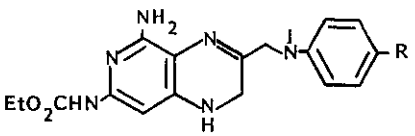
1-Deazamethotrexate (X = N, Y = CH)
3-Deazamethotrexate (X = CH, Y = N)



3-Deaza: X = CH, Y = N, R = CO_2Me
1-Deaza: X = N, Y = CH, R = CO_2Me
X = N, Y = CH, R = H
X = N, Y = CH, R = OMe

Preliminary experiments indicate that the deazadihydropteridines bind to tubulin at the colchicine rather than the vincristine site(s).[28] This result is consistent with the potentiation of cytotoxicity to cultured cells treated with combinations of vincristine and a deazapteridine. In addition, synergism was observed in vivo with methotrexate.[29]

Table XI.



R	IC_{50}, nM[a]	Mitotic Index[b] 12 h (μM)	24 h (μM)	P388 Leukemia (10^6 Cells, ip) (mg/kg/dose)[c]	Schedule	% ILS
CO_2Me	58	0.61 (0.3)		25	qd 1-9	30
H	8.4	0.77 (0.03)	0.80 (0.3)	100	Day 1	114
Cl	15	0.71 (0.03)		100	Day 1	65
OMe	7.9	0.64 (0.03)		25	Day 1	133
Vincristine	<1		0.62 (0.3)	2.7	Day 1	100
				0.33	qd 1-9	145

[a]Concentration that inhibits the proliferation of cultured L-1210 cells to 50% of control growth in 48 h. [b]Fraction of cultured L-1210 cells in mitosis. [c]Injected ip.

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