Potential Antineoplastics. IV. 2-Amino-4-methyl-6-phenyl-5-arylazopyrimidines

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2,4-Diamino-6-ethyl-5-(4-chlorophenyl)pyrimidine is a highly selective inhibitor of dihydrofolate reductase isolated either from *Plasmodium berghei* or from Erlich Ascites carcinoma cells.[1,2] The folic acid reductase and tumor inhibitory potencies determinations on other 2,4-diamino-6-substituted-5-arylazopyrimidines also indicate a structure–activity relationship.[3]

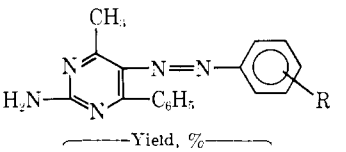
These observations and our work on pyrimidines[4] led us to synthesize some more congeners. Essentially this approach is one of "latentiation" which has been defined as "the chemical modification of a biologically active compound to form a new compound, which upon *invivo* enzymatic attack will liberate the parent compound."[5,6] Our views have been rather broader and are in agreement with those of others[7] that an active moiety of a latentiated drug might react directly at a biologically important site without liberation of the parent compound as such.

The present investigation reports (a) the synthesis of 2-amino-4-methyl-6-phenyl-5-arylazopyrimidines (I) and (b) the antineoplastic potency and host toxicity of 4,6-dimethyl[4]-and 4-methyl-6-phenyl-2-amino-5-arylazopyrimidines against L-1210 lymphoid leukemia.

The synthesis of 2-amino-4-methyl-6-phenyl-5-arylazopyrimidines, listed in Table I, involved either the coupling of diazotized anilines with 2-amino-4-methyl-6-phenylpyrimidine under modified conditions used earlier[8,9] or the condensation of 1-phenyl-2-arylhydra-

TABLE I

CHARACTERISTICS OF 2-AMINO-4-METHYL-6-PHENYL-5-ARYLAZOPYRIMIDINES



No.	R	Mp, °C	Yield, % A	Yield, % B	Color[a]	Formula	Analyses
1	H	156–157	68	58	ORN	$C_{17}H_{15}N_5$	C, H, N
2	2-Me	183–184	65	60	ORN	$C_{18}H_{17}N_5$	C, H, N
3	3-Cl	158–159	70	65	ON	$C_{17}H_{14}ClN_5$	N, Cl
4	4-Cl	172–173	65		ON	$C_{17}H_{14}ClN_5$	N, Cl
5	2-Br	226–227	66	56	ORN	$C_{17}H_{14}BrN_5$	Br, N
6	4-Br	166–167	66		ORN	$C_{17}H_{14}BrN_5$	Br, N
7	2-OMe	130–131	72	64	ON	$C_{18}H_{17}N_5O$	C, H, N
8	3-OMe	169–170	63		RN	$C_{18}H_{17}N_5O$	C, H, N
9	4-OMe	147–148	68	62	DRN	$C_{18}H_{17}N_5O$	C, H, N
10	2-NO_2	208–209	61		RN	$C_{17}H_{14}N_6O_2$	C, H, N
11	3-NO_2	176–177		64	ORN	$C_{17}H_{14}N_6O_2$	C, H, N
12	4-OEt	154–155		60	ORN	$C_{19}H_{19}N_5O$	C, H, N
13	2,3-Me_2	178–179	65	62	ORN	$C_{19}H_{19}N_5$	C, H, N
14	2,4-Me_2	151–152	66	61	RN	$C_{19}H_{19}N_5$	C, H, N
15	2,5-Me_2	190–191	64		RN	$C_{19}H_{19}N_5$	C, H, N
16	2,6-Me_2	123–124	66	55	ON	$C_{19}H_{19}N_5$	C, H, N
17	3,4-Me_2	190–191	60		BN	$C_{19}H_{19}N_5$	C, H, N
18	2,3-Cl_2	252–253	58	51	RN	$C_{17}H_{13}Cl_2N_5$	N, Cl
19	2,4-Cl_2	225–226	61		OF	$C_{17}H_{13}Cl_2N_5$	N, Cl
20	3,5-Cl_2	248–249		60	OP	$C_{17}H_{13}Cl_2N_5$	N, Cl
21	2,4-Br_2	249–250	65	59	RN	$C_{17}H_{13}Br_2N_5$	Br, N
22	2,5-Br_2	268–269	68		ON	$C_{17}H_{13}Br_2N_5$	Br, N
23	4-N,N-Et_2	120–121	62	57	DRN	$C_{21}H_{24}N_6$	C, H, N
24	2-Cl-6-Me	173–174	70	65	ORN	$C_{18}H_{16}ClN_5$	Cl, N
25	4-Cl-2,5-$(MeO)_2$	201–202	68	62	ORN	$C_{19}H_{18}ClN_5O_2$	Cl, N
26	2,5-Cl_2-4-NO_2	181–182	65	61	OP	$C_{17}H_{12}Cl_2N_6O_2$	Cl, N
27	5-Cl-2,4-$(MeO)_2$	183–184	62		ON	$C_{19}H_{18}ClN_5O_2$	Cl, N
28	4-SO_2NH_2	241–242 dec	60	54	ORN	$C_{17}H_{16}N_6O_2S$	N, S
29	2,5-$(MeO)_2$	232–233		62	ON	$C_{19}H_{19}N_5O_2$	C, H, N

[a] B, Brown; D, deep; F, fibers; G, golden; N, needles; O, orange; Pe, pale; P, plates; R, red; Y, yellow.

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(2) J. R. Bertino, J. P. Perkins, and D. G. Johns, *Biochemistry*, **4**, 839 (1965).
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(4) H. G. Garg and R. A. Sharma, *ibid.*, **12**, 1122 (1969).
(5) N. J. Harper, *ibid.*, **12**, 467 (1969).
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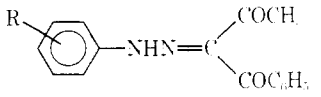
(9) H. G. Garg and R. A. Sharma, *J. Med. Chem.*, **13**, 579 (1970).

TABLE II

CHARACTERISTICS OF 1-PHENYL-2-ARYLHYDRAZONO-1,2,3-BUTANETRIONES



No.	R	Mp, °C	Color[a]	Formula	Analyses
1	2-Br	108–109	YN	$C_{16}H_{13}BrN_2O_2$	Br, N
2	4-Br	103–104	PeYN	$C_{16}H_{13}BrN_2O_2$	Br, N
3	2,3-Me_2	92–93	YN	$C_{18}H_{18}N_2O_2$	C, H, N
4	2,6-Me_2	90–91	YN	$C_{18}H_{18}N_2O_2$	C, H, N
5	3,4-Me_2	98–99	BN	$C_{18}H_{18}N_2O_2$	C, H, N
6	2,3-Cl_2	103–104	YN	$C_{16}H_{12}Cl_2N_2O_2$	Cl, N
7	2,4-Cl_2	135–136	ON	$C_{16}H_{12}Cl_2N_2O_2$	Cl, N
8	2,4-Br_2	162–163	YN	$C_{16}H_{12}Br_2N_2O_2$	Br, N
9	2,5-Br_2	135–136	ON	$C_{16}H_{12}Br_2N_2O_2$	Br, N
10	4-N,N-Et_2	98–99	RBN	$C_{20}H_{23}N_3O_2$	C, H, N
11	2-Cl-6-Me	101–102	GYP	$C_{17}H_{15}ClN_2O_2$	Cl, N
12	4-Cl-2,5-Me_2	170–171	OF	$C_{18}H_{17}ClN_2O_4$	Cl, N
13	5-Cl-2,4-4-$(MeO)_2$	178–179	ON	$C_{18}H_{17}ClN_2O_4$	Cl, N
14	2,5-Cl_2-4-NO_2	130–131	BF	$C_{16}H_{11}Cl_2N_3O_4$	Cl, N

[a] See footnote *a* of Table I.

TABLE III

SUMMARY OF THE SCREENING RESULTS AGAINST L-1210 LYMPHOID LEUKEMIA, ASCITIC FLUID IMPLANTED INTRAPERITONEALLY IN BDF_1 MICE.

2-AMINO-4-METHYL-6-PHENYL-5-ARYLAZOPYRIMIDINES

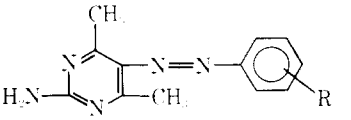
No.[a]	Survivors[b]	T/C,[c] %
1	6/6	94
2	6/6	91
3	6/6	94
4	6/6	92
5	6/6	88
6	5/6	95
7	6/6	94
8	6/6	89
9	6/6	91
10	6/6	102
11	6/6	98
12	6/6	94
13	6/6	98
14	6/6	96
15	6/6	95
16	6/6	92
17	6/6	94
18	5/6	93
19	6/6	94
20	6/6	96
21	6/6	88
22	6/6	100
23	0/6	
24	6/6	102
25	6/6	88
26	5/6	86
27	6/6	100
28	6/6	92
29	6/6	94

[a] Numbers same as in Table I. [b] All doses were 400 mg/kg. [c] Ratio of mean survival time of test animals (T) to control animals (C). Mean survival time of control is 30 days.

zono-1,2,3-butanetriones (Table II) with guanidine nitrate in MeOH containing NaOH at 60–70°. The synthesis of the precursors 2-amino-4-methyl-6-phenylpyrimidine and 1-phenyl-2-arylhydrazono-1,2,3-butanetriones have been achieved by adopting procedures of Rose[10] and Garg, *et al.*,[11,12] respectively.

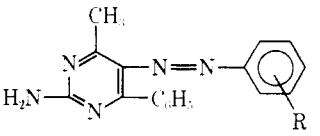
TABLE IV

2-AMINO-4,6-DIMETHYL-5-ARYLAZOPYRIMIDINES[a]



No.	R	Survivors	T/C,[b] %
1	H	6/6[c]	96
2	2-Me	6/6[c]	90
3	4-Me	5/6[e]	91
		6/6[d]	97
4	3-Cl	6/6[c]	90
5	4-Cl	6/6[c]	90
6	2-Br	6/6[c]	103
7	4-Br	6/6[c]	93
8	2-NO_2	5/6[c]	92
9	3-MeO	6/6[c]	93
10	4-MeO	6/6[c]	88
11	4-EtO	6/6[c]	91
12	4-SO_2NH_2	6/6[c]	101
13	2,3-Me_2	6/6[c]	90
14	2,4-Me_2	4/6[c]	100
15	2,5-Me_2	6/6[c]	93
16	2,6-Me_2	6/6[c]	98
17	2-Cl-6-Me	6/6[c]	93
18	2-Cl-4-NO_2	6/6[c]	98
19	2,3-Cl_2	6/6[c]	91
20	2,4-Cl_2	5/6[c]	104
21	2,5-Cl_2	6/6[c]	101
22	3,5-Cl_2	6/6[c]	98
23	2,4-Br_2	6/6[c]	107
24	2,5-Br_2	6/6[c]	95
25	2,5-$(MeO)_2$	6/6[c]	98
26	4-Cl-2,5-$(MeO)_2$	6/6[c]	97
27	2-MeO	6/6[c]	96

[a] Reported earlier (ref 4). [b] Dose 400 mg/kg. [c] Dose 150 mg/kg. [d] Dose 75 mg/kg. [e] See footnote c, Table III.



I

Biological Results.—During a screening study in BDF_1 mice for antitumor activity against L-1210 lymphoid leukemia (Tables III, IV) the compounds are found essentially inactive.

Experimental Section

All melting points were determined using a Kofler hot-stage-type apparatus.

1-Phenyl-2-(2,4-dimethoxyphenyl)hydrazono-1,2,3-butanetrione.—2,4-Dimethoxyaniline (3.06 g, 0.02 mol) was dissolved in 3 *N* HCl (3.0 ml.) and cooled to 0°. $NaNO_2$ (1.5 g, 0.02 mol) dissolved in H_2O (20.0 ml) was gradually added and the diazonium salt solution so obtained was filtered into a well-cooled, stirred mixture of NaOAc (5.0 g) and 1-phenyl-1,3-butanedione (3.2 g, 0.02 mol) containing EtOH (50 ml). The butanetrione started precipitating almost immediately. After keeping for 4 hr the precipitate was filtered, washed well with H_2O, and recrystallized from EtOH (6.0 g, 83%) as orange needless, mp 150°. *Anal.* ($C_{18}H_{18}N_2O_4$) C, H, N.

(10) F. L. Rose, *J. Chem. Soc.*, 3448 (1952).
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Characteristics of the new 1-phenyl-2-arylhydrazono-1,2,3-butanetriones are summarized in Table II.

2-Amino-4-methyl-6-phenylpyrimidine.—Guanidine nitrate (2.5 g, 0.02 mol) was added to 1-phenyl-1,3-butanedione (3.2 g, 0.02 mol) containing 10 *N* NaOH (10.0 ml) and MeOH (20.0 ml). The mixture was stirred for 10 hr at 50–60° and left for another 12 hr at room temperature. The precipitated 2-amino-4-methyl-6-phenylpyrimidine was collected and washed with MeOH and hot H_2O. It was recrystallized from MeOH (2.3 g, 65%), mp 171°. *Anal.* ($C_{11}H_{11}N_3$)C, H, N.

2-Amino-4-methyl-6-phenyl-5-(2,4-dimethoxyphenylazo)-pyrimidine. Method A.—The diazotized 2,4-dimethoxyaniline, free from HNO_2 was added to a well-cooled and stirred solution of 2-amino-4-methyl-6-phenylpyrimidine (3.7 g, 0.02 mol) in AcOH (45.0 ml) containing sufficient NaOAc so as to maintain the reaction mixture at pH 6–7. The mixture was stirred for another 6 hr at 0–5° and left for 24 hr at room temperature. The product thus precipitated was collected, washed well with H_2O, and recrystallized from DMF–EtOH (3.6 g, 66%) as golden yellow plates, mp 160°. *Anal.* ($C_{19}H_{19}N_5O_2$) C, H, N.

Method B.—Guanidine nitrate (2.5 g, 0.02 mol) was added to 1-phenyl-2-(2,4-dimethoxyphenyl)hydrazono-1,2,3-butanetrione (6.5 g, 0.02 mol) which in turn was prepared by coupling diazotized 2,4-dimethoxyaniline with 1-phenyl-1,3-butanedione, containing 10 *N* NaOH (10.0 ml) and MeOH (20.0 ml). The mixture was stirred for 12 hr at 60–70° and left for another 12 hr at room temperature. 2-Amino-4-methyl-6-phenyl-5-(2,4-dimethoxyphenylazo)pyrimidine precipitated, was collected, and was washed successively with MeOH and hot H_2O. It was recrystallized from DMF-EtOH (2.7 g., 51%) as golden yellow plates, mp 160°.

By similar procedures, several 2-amino-4-methyl-6-phenyl-5-arylazopyrimidines were prepared; they are summarized in Table I.

Acknowledgment.—The authors are greatly indebted to Drs. H. B. Wood and H. W. Bond of the Cancer Chemotherapy National Service Center for their cooperation and for making the screening data available. We are also thankful to Professor W. U. Malik, Head of the Chemistry Department, for providing the necessary facilities for this work and the C.S.I.R., New Delhi (India) for a Junior Research Fellowship (held by R.A.S.).

Synthesis of 5-Iodo-3-indolylphosphodiesters of 5-Fluorodeoxyuridine As Possible Chromogenic Cancer Chemotherapeutic Agents[1]

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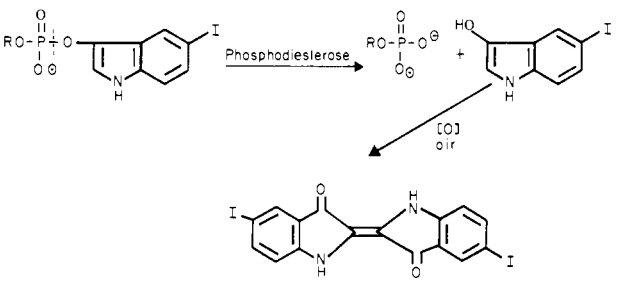
In the design and synthesis of anticancer agents based on enzyme rationale,[2] it would be desirable to have one of the enzymatically hydrolyzed products as a chromogen so that the fate of such enzyme-mediated drugs can be followed. Previously, we reported the synthesis of several tetrazolium mustards which could be reduced *in vivo* to a more toxic, colored formazan mustard.[3] The present paper reports the incorporation of such a concept to a known useful antimetabolite-type anticancer agent, utilizing the phosphodiesterase principle reported previously,[4] and as illustrated in Scheme I.

SCHEME I

Principle of Phosphodiesterose



5,5'-diiodoindigo (purple-blue dye)

Through detailed study by Heidelberger and his associates, 5-fluorodeoxyuridine 5′-phosphate (FUDRP) was shown to be the active antitumor metabolite when either 5-fluorouracil (FU) or 5-fluorodeoxyuridine (FUDR) were used in the management of several types of cancer.[5] However, the use of the nucleotide, FUDRP, itself has not been an easy task in cancer research because of the facile hydrolysis by either 5′-nucleotidase or an acid phosphatase. With the success we have had in the synthesis of 5′-iodoindolyl phosphodiesters of deoxythymidine,[6] it was considered of interest to synthesize the 5′- and the 3′-(5-iodoindolyl)phosphodiesters of 5-fluorodeoxyuridine, **1** and **2**, respectively, as possible chromogenic anticancer agents. Upon enzymatic hydrolysis, they could liberate the 5′- and 3′-nucleotides, and thus provide a known active anticancer agent, especially in the case of the 5′ derivative.

The synthesis of the 5′ and the 3′ derivative is illustrated in Scheme II.

The starting materials, 5′-*O*-trityl-5-fluorodeoxyuridine and 3′-*O*-acetyl-5-fluorodeoxyuridine, were prepared by a procedure similar to that of Remy, Sunthanker, and Heidelberger,[7] based on methods of Michelson and Todd,[8] and Gilman and Khorana[9] for deoxythymidine derivatives. 5-Iodoindolyl-*N*-acetate was prepared according to the method of Rabiger, *et al.*[10] The phosphodichloridate was prepared in dry pyridine and used directly for the reaction. The product was purified and isolated as the ammonium salt on a Sephadex column with a linear gradient of 0.02 to 0.3 *M* NH_4HCO_3. The purity of the product was checked by elemental analysis as well as R_f values with paper chromatography in three different solvent systems. The ir of the 3′ derivative differs from the 5′ only at

(1) This work was supported by AEC Research Grant AT(30-1)3784 and U. S. Public Health Service Research Grant CA-07339.

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(5) M. Umeda and C. Heidelberger, *Cancer Res.*, **28**, 2529 (1968).

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(10) D. Rabiger, M. Y. Chang, S. Matsukawa, and K. C. Tsou, *J. Heterocycl. Chem.* (in press).