Anticancer Activity of P,P-Bis(2-methyl-1-aziridinyl)-N-2-pyrimidinylphosphinic Amide (Methylphosphazine) and Related Compounds[1]

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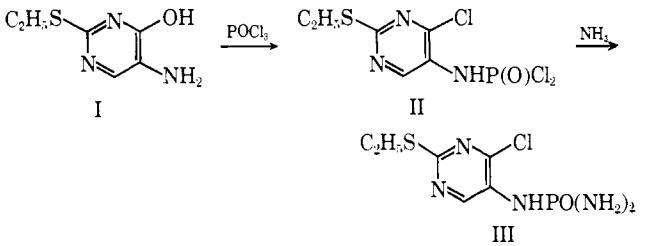
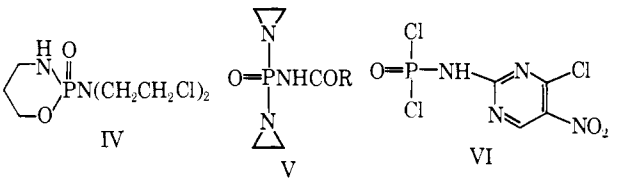
Received July 5, 1967

Methylphosphazine [P,P-bis(2-methyl-1-aziridinyl)-N-2-pyrimidinylphosphinic amide] has been prepared by chlorination of 2-aminopyrimidine with phosphorus oxychloride followed by treatment with propylenimine. This compound was found to be more active and less toxic than cytoxan, thio-TEPA, or phosphazine in preliminary screening tests against leukemia L1210 in mice and Walker carcinosarcoma 256 in rats.

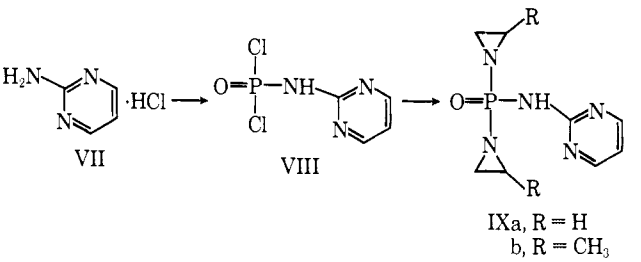
Johnson in 1905[2] chlorinated 2-ethylthio-4-hydroxy-5-aminopyrimidine (I) with phosphorus oxychloride and isolated a very stable phosphorus-containing intermediate II. This intermediate, as he reported, could be warmed with water without noticeable decomposition. Treatment of II with ethanolic ammonia at 160–165° gave a phosphoric triamide III.



The $\geq CNP(=O)N<$ system in compound III is of special interest, since cytoxan (cyclophosphamide, IV)[3], one of the many important anticancer agents which is being used clinically,[4] contains the same type of arrangement. In addition to cytoxan, Friedman, *et al.*,[5] have studied a number of phosphorodiamidic acid mustards and found that they exhibit an unusual degree of biological activity in experimental assay systems. Some alkyl N-[bis(1-aziridinyl)phosphoro]-carbamates[6] (V) also possess antitumor activity.[7] With compounds of this category, the cytotoxic moieties are usually liberated by the action of phosphamidases or phosphatases (greater amounts of phosphatases have been detected in cancerous than in normal tissues[8]). This probably explains the lack of cytotoxicity of compounds of this type *in vitro*.



The chlorination of 2-amino-4-hydroxy-5-nitropyrimidine with $POCl_3$ to yield 4-chloro-5-nitro-2-pyrimidinylphosphoramidic dichloride (VI) has been reported in a previous communication from this laboratory.[9] When 2-aminopyrimidine hydrochloride (VII) was refluxed with $POCl_3$, and the resulting 2-pyrimidinylphosphoramidic dichloride (VIII) was treated with ethylenimine by the procedure of Kropacheva and Sazonov,[10] P,P-bis(1-aziridinyl)-N-2-pyrimidinylphosphinic amide (IXa) was isolated as a hemihydrate. Compound IXa ("phosphazine"), as reported by Chernov, *et al.*,[11] demonstrated high antitumor activity against transplanted carcinoma in mice, rats, and rabbits. According to these investigators, phosphazine



is toxic, but its toxicity is much less than that of thiophosphamide (triethylenethiophosphamide or thio-TEPA) or Dipin [N,N′-bis(diaziridinylphosphinylidyne)piperazine]. Compound IXa has now been shown to have confirmed activity against Sarcoma 180, Adenocarcinoma 755, and leukemia L1210 tumor systems in mice, and the Walker (intramuscular) carcinosarcoma 256 tumor system in rats[12] (see Table I). When both aziridinyl substituents were replaced by 2-methylaziridinyl groups, the resulting P,P-bis(2-methyl-

(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, the National Institutes of Health, U. S. Public Health Service, Contract No. PH-43-65-94.

(2) T. B. Johnson, *Am. Chem. J.*, **34**, 191 (1905).

(3) H. Arnold and F. Bourseaux, *Angew. Chem.*, **70**, 539 (1958); H. Arnold, F. Bourseaux, and N. Brock, *Naturwissenschaften*, **45**, 64 (1958).

(4) See, for example, H. Haar, G. J. Marshall, H. R. Bierman, and J. L. Steinfeld, *Cancer Chemotherapy Rept.*, **No. 6**, 41 (1960); D. R. Korst, F. D. Johnson, E. P. Frenkel, and W. L. Challener, III, *ibid.*, **No. 7**, 1 (1960); P. R. Coggins, R. G. Ravdin, and S. H. Eisman, *Cancer*, **13**, 1254 (1960); B. Hoogstraten, *Cancer Chemotherapy Rept.*, **No. 16**, 167 (1962); D. J. Fernback, W. W. Sutow, W. G. Therman, and T. J. Vietti, *ibid.*, **No. 16**, 173 (1962); R. W. Rundles, J. Laszlo, F. E. Garrison, Jr., and J. B. Hobson, *ibid.*, **No. 16**, 407 (1962); W. Snyder, P. Rodensky, and B. Lieberman, *ibid.*, **No. 41**, 37 (1964), and references cited therein.

(5) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 655 (1954); O. M. Friedman, E. Boger, V. Grubliauskas, and H. Sommer, *J. Med. Chem.*, **6**, 50 (1963); O. M. Friedman, V. Grauliauskas, and I. Wodinsky, *Proc. Am. Assoc. Cancer Res.*, **4**, 21 (1963); C. L. Maddock, A. H. Handler, O. M. Friedman, G. E. Foley, and S. Farber, *Cancer Chemotherapy Rept.*, **50**, 629 (1966); L. Nathanson, T. C. Hall, A. Rutenberg, and R. K. Shadduck, *ibid.*, **51**, 35 (1967).

(6) T. J. Bardos, Z. B. Papanastassiou, A. Segaloff, and J. L. Ambrus, *Nature*, **183**, 399 (1959); Z. B. Papanastassiou and T. J. Bardos, *J. Med. Pharm. Chem.*, **5**, 1000 (1962).

(7) S. McCracken and J. Wolf, *Cancer Chemotherapy Rept.*, **No. 6**, 52 (1960).

(8) G. Gomeri, *Proc. Soc. Exptl. Biol. Med.*, **69**, 407 (1948).

(9) D. E. O'Brien, C. W. Noell, R. K. Robins, and C. C. Cheng, *J. Med. Chem.*, **9**, 121 (1966).

(10) A. A. Kropacheva and N. V. Sazonov [*Zh. Obshch. Khim.*, **11**, 3601 (1961); *J. Gen. Chem. USSR*, **31**, 3357 (1961)] have erroneously claimed that they synthesized the pyrimidine phosphoramidic amide type compounds for the first time. See ref 2.

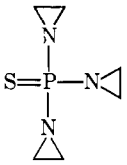
(11) V. A. Chernov, A. A. Grushina, and L. T. Lytkina, *Farmakol. i Toksikol.*, **26**, 102 (1963).

(12) Test results were provided by the Cancer Chemotherapy National Service Center of the National Cancer Institute.

TABLE I
COMPARISON OF ANTICANCER ACTIVITIES OF CYTOXAN AND THIO-TEPA WITH METHYLPHOSPHAZINE AND RELATED COMPOUNDS[a]

Compd	Test system[b]	Dose	Survivors	Animal wt diff (T − C)	Tumor wt Test	Tumor wt Control	Survival, days Test	Survival, days Control	T/C, %
IXb	LE	400.0	4/4	−6.2			10.0	8.2	121
		200.0	4/4	−5.3			12.3	8.2	150
		100.0	4/4	−2.7			11.0	8.2	134
		80.0	6/6	−2.6			12.3	8.4	146
		40.0	6/6	−3.0			12.8	8.5	150
		24.0	6/6	−2.5			10.8	8.5	127
		14.0	6/6	−1.9			10.3	8.5	121
	WM	67.0	6/6	−12	0.0	8.1			0
		67.0	6/6	−11	0.1	8.5			1
		67.0	6/6	−10	0.3	9.0			3
		67.0	6/6	−9	0.1	8.7			1
		67.0	6/6	−11	0.1	8.5			1
		100.0	6/6	−25	0.1	9.4			1
		67.0	6/6	−15	0.3	9.4			3
		40.0	6/6	−13	0.3	9.4			3
		24.0	6/6	−7	0.1	9.4			1
		14.0	6/6	−2	0.1	9.4			1
IXa	SA	500.0	0/7	...	...	...			...
		125.0	0/7	...	...	...			...
		31.0	3/6	−8.2	0	512			...
		15.0	6/6	−1.3	242	949			25
		15.0	6/6	−5.4	243	870			27
		15.0	6/6	−6.4	184	1101			16
		10.0	6/6	−2.9	655	1057			61
		7.0	6/6	−1.1	705	1057			66
		23.0	6/6	−4.3	312	1181			26
		15.0	5/6	−4.8	330	1181			27
		10.0	6/6	−3.5	343	1181			29
		7.0	6/6	0.1	908	1181			76
	CA	15.0	10/10	−5.9	0	604			0
		15.0	9/10	−6.3	0	1699			0
		7.5	10/10	−4.3	365	1470			24
		7.5	10/10	−4.1	181	1676			10
		3.8	10/10	−0.7	890	1676			53
	LE	15.0	6/6	−3.3			14.3	8.7	164
		15.0	6/6	−2.5			13.0	9.2	141
		10.0	6/6	−2.1			15.6	9.2	169
		7.0	6/6	−1.5			16.5	9.2	179
		23.0	6/6	−2.8			13.3	8.5	156
		15.0	6/6	−2.5			15.6	8.5	183
		10.0	6/6	−2.3			19.0	8.5	223
		7.0	6/6	−2.8			16.2	8.5	190
		5.0	6/6	−2.1			13.7	8.5	161
		3.0	6/6	−1.2			10.5	8.5	123
		2.0	6/6	−0.8			9.2	8.5	108
	WM	30.0	1/6	−26	0.5	11.3			...
		15.0	6/6	−24	0.8	11.3			7
		7.5	6/6	−17	1.6	11.3			14
		3.7	6/6	−8	1.8	11.3			15
		1.0	4/6	−35	0.9	12.0			7
		0.5	6/6	−24	1.5	12.0			12
		0.5	6/6	−10	0.8	11.3			7
		0.25	6/6	−2	1.5	11.3			13
		0.12	6/6	2	3.8	11.3			33
		0.06	5/6	4	6.8	11.3			60
		120.0	0/6	...	...	...			...
		3.7	6/6	−1	0.7	6.6			10
		2.0	6/6	3	3.4	7.7			44
XI	LE	24.0	4/4	−5.2			13.5	8.6	156
		12.0	4/4	−2.7			11.3	8.6	131
		6.0	4/4	−2.8			10.5	8.6	122
	WM	12.0	6/6	−20	0.5	7.5			6
		6.0	6/6	−9	0.1	7.5			1
		3.0	6/6	−6	0.9	7.5			12
		1.5	6/6	6	0.6	7.5			8
		24.0	1/6	−27	0.5	7.4			...

TABLE I (*Continued*)

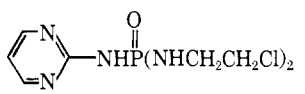
Compd	Test system[b]	Dose	Survivors	Animal wt dif (T − C)	Tumor wt: Test	Tumor wt: Control	Survival, days: Test	Survival, days: Control	T/C, %
XI	WM	1.0	6/6	5	1.1	7.4			14
		0.5	6/6	3	3.6	7.4			48
		0.25	6/6	−2	5.7	7.4			77
	WA	1.0	6/6	4	12.5	15.8			79
X	LE	400.0	4/4	0.3			9.8	9.5	103
		200.0	4/4	0.4			9.8	9.5	103
		100.0	4/4	0.2			10.0	9.5	105
	WM	400.0	6/6	−35	1.8	5.2			34
		400.0	6/6	−20	3.9	6.0			65
IV	LE	100.0	4/4	−3.2			14.8	9.9	149
		100.0	4/4	−1.8			15.0	9.1	164
		100.0	4/4	−3.4			14.0	9.1	153
		100.0	6/6	−2.8			14.7	9.2	159
	WM	5.0	6/6	2	1.0	6.1			16
		5.0	6/6	7	0.3	4.7			6
		5.0	6/6	0	0.3	5.3			5
		5.0	6/6	7	1.9	6.1			31
		2.5	4/4	6	0.7	7.5			9
		2.5	6/6	3	1.5	11.4			13
		2.5	6/6	2	1.0	8.5			11
		2.5	6/6	3	1.2	8.1			14
		2.5	6/6	8	0.2	6.4			3
	SA	16.0	0/6	...	...	...			...
		8.0	1/6	0	140	846			...
		4.0	6/6	−1.9	258	672			38
		2.0	5/6	−0.1	347	672			51
	CA	6.0	7/10	−6.9	84	1356			6
		3.0	8/10	−4.5	194	1356			14
		1.5	10/10	−2.3	514	1356			37
		0.6	9/10	−1.5	1013	1617			62
	LE	10.0	6/6	−2.0			11.0	9.5	115
		5.0	6/6	−1.6			10.7	9.5	112
		3.13	6/6	−3.2			14.5	8.7	166
		1.57	6/6	−2.2			10.7	8.7	122
		2.4	10/10	−1.7			10.1	8.2	123
		2.4	10/10	−0.4			11.2	7.7	145
	WM	10.0	6/6	−5	0.5	10.5			4
		5.0	6/6	−13	1.9	10.5			18
		2.5	6/6	−12	1.6	10.5			15
		1.25	6/6	−9	5.3	10.5			50
	WA	2.4	6/6	−14	0.0	4.9			0
		0.6	6/6	0	0.0	4.9			0
		0.3	6/6	2	1.8	4.9			36

[a] All test results presented in this table were provided by the Cancer Chemotherapy National Service Center of the National Cancer Institute. [b] SA = Sarcoma 180, implanted subcutaneously in axillary region of Swiss mice. CA = Adenocarcinoma 755, implanted subcutaneously in axillary region of BDF_1 mice. LE = lymphoid leukemia L1210, ascitic fluid implanted intraperitoneally in BDF_1 mice. WM = Walker 256, implanted intramuscularly in thigh of noninbred albino rats. WA = Walker 256, implanted subcutaneously in axillary region of noninbred albino rats (for alkylating agents only).

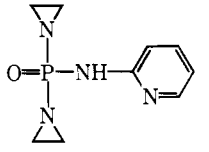
1-aziridinyl)-N-2-pyrimidinylphosphinic amide (IXb, methylphosphazine) demonstrated excellent activity against both the leukemia L1210 and Walker 256. In the case of the latter test system, compound IXb was found to be more active and less toxic than the clinical drugs, thio-TEPA or cytoxan, or phosphazine (see Table I). Furthermore, unlike most alkylating agents, compound IXb is very stable under ordinary storage conditions.

N,N′-Bis(2-chloroethyl)-N′′-2-pyrimidinylphosphoric triamide (X) was prepared by the acid cleavage of IXa. The 2-chloroethylamino derivative, however, is less active than the analogous aziridine derivatives against Walker 256 and inactive in the leukemia L1210 systems.

The corresponding 2-pyridyl derivative of phosphazine, P,P-bis(1-aziridinyl)-N-2-pyridylphosphinic amide[13] (deazaphosphazine, XI), was readily prepared from 2-aminopyridine. This compound also demon-



X



XI

(13) American Cyanamid Co., British Patent 885,370 (Dec 28, 1961); *Chem. Abstr.*, **58**, 7949 (1963).

strated antitumor activity against both leukemia L1210 and Walker 256 systems. As expected, compound XI was inactive in KB cell culture test system. It is of interest that, although compound XI possesses two aziridinyl groups, it failed to show activity against the Walker (subcutaneous) 256 test system designed for the evaluation of alkylating agents.

Attempts to prepare the thione analogs of phosphazine and methylphosphazine were not successful in our hands. Phosphochlorination of 2-amino-*s*-triazine and 2-amino-*as*-triazine with thiophosphoryl chloride gave only intractable materials.

Experimental Section[14]

2-Aminopyrimidine Hydrochloride (VII).—Through a suspension of 200 g (2.01 moles) of 2-aminopyrimidine[15] (Eastman) in 1600 ml of absolute EtOH was passed, without cooling, a generous stream of dry HCl. The temperature of the reaction mixture gradually rose almost to boiling while the solid slowly dissolved. After *ca.* 30 min the hydrochloride salt started to precipitate from the hot solution. The stream of HCl was continued for another 15 min, and the resulting mixture was allowed to cool to room temperature. The solid was collected by filtration, washed well with absolute EtOH, then dried at 70–80° to give 210 g (76% yield) of VII, mp 200–202°, pure enough for the next step.

2-Pyrimidinylphosphoramidic Dichloride (VIII).—A mixture of 190 g (1.445 moles) of VII and 1 l. of $POCl_3$ was refluxed for 6 hr, then cooled to room temperature. The resulting solid was collected by filtration and washed well with C_6H_6 to give 294 g (90% yield) of VIII, mp 171–173°. This product was used as such in the next preparation after drying *in vacuo* at room temperature for 5 hr in a rotary evaporator. An analytically pure sample, mp 188–190° (lit.[10] mp 190°), can be obtained by recrystallization of the crude product from a large volume of C_6H_6.

P,P-Bis(1-aziridinyl)-N-2-pyrimidinylphosphinic amide (IXa) was prepared essentially by the procedure of Kropacheva and Sazonov:[10] λ_{max}^{EtOH} 222 mμ (ε 17,500), 276 mμ (ε 2800).

P,P-Bis(2-methyl-1-aziridinyl)-N-2-pyrimidinylphosphinic Amide (IXb).—To a stirred mixture of 190 g (0.9 mole) of VIII in 2 l. of anhydrous C_6H_6 cooled in an ice bath was added dropwise 128 g (2.24 moles) of propyleneimine (Interchemical Corp., Organic Chemicals Department, Carlstadt, N. J.) and 226 g (2.24 moles) of Et_3N in 200 ml of anhydrous C_6H_6 at such a rate that the temperature of the reaction mixture did not exceed 20°. The mixture was allowed to stir for another 30 min in the ice bath and for an additional 2 hr without cooling. The solvent was removed *in vacuo* at *ca.* 50°, and the residue was swirled in 1800 ml of hot (70°) anhydrous C_6H_6. The insoluble $Et_3N \cdot HCl$ was removed by filtration and washed with 200 ml of hot C_6H_6. The combined filtrate and washings were allowed to cool, yielding the first crop of IXb. This was isolated by filtration, and the volume of the filtrate was reduced to 500 ml when another portion of IXb precipitated on cooling: total 96 g, mp 142–145°. An additional 41 g of product was isolated when the volume of the filtrate was reduced to 250 ml, mp 140–143°, total yield 60%. An analytical sample was obtained by recrystallization from C_6H_6: mp 145–147°; λ_{max}^{EtOH} 223 mμ (ε 17,000), 277 mμ (ε 2500). This compound is stable at room temperature under ordinary storage conditions.

Anal. Calcd for $C_{10}H_{16}N_5OP$: C, 47.4; H, 6.37; N, 27.7. Found: C, 47.2; H, 6.38; N, 27.4.

N,N'-Bis(2-chloroethyl)-N''-2-pyrimidinylphosphoric Triamide (X).—Phosphazine IXa (20 g) was added portionwise to 400 ml of methanolic HCl (saturated at 5°). The resulting mixture was left overnight at room temperature and evaporated under reduced pressure to a clear viscous oil. The oil was dissolved in 150 ml of H_2O, and the pH of the solution was adjusted to 4 by careful addition of 1 *N* NaOH. After 15 hr the precipitate was filtered, washed with cold H_2O, and dried at 70° for 18 hr to give 9.2 g of X, mp 103–104°. Recrystallization from H_2O afforded an analytical sample: mp 105–106°; λ_{max}^{EtOH} 223 mμ (ε 16,700), 277 mμ (ε 27001).

Anal. Calcd for $C_8H_{14}Cl_2N_5OP$: C, 32.2; H, 4.74; N, 23.5; Cl, 23.8. Found: C, 32.2; H, 4.56; N, 23.6; Cl, 23.5.

P,P-Bis(1-aziridinyl)-N-2-pyridylphosphinic amide (XI) was prepared by the known procedure[15] from 2-aminopyridine;[16] λ_{max}^{EtOH} 226 mμ (ε 12,3001), 280 mμ (ε 3900).

Acknowledgments.—The authors wish to express their appreciation to Mrs. Margaret L. Rounds, Mr. John R. Cravatt, and Mr. Leland R. Lewis for the analytical and instrumental measurements. They are also grateful to Dr. Harry B. Wood, Jr., and Dr. Robert R. Engle of CCNSC for providing the screening results of cytoxan and thio-TEPA.

(14) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The uv absorption spectra were determined with a Beckman DK-2 spectrophotometer.

(15) S. Gabriel, *Ber.*, **34**, 3364 (1901).

(16) A. E. Tschitschibabin and O. A. Seide, *J. Russ. Phys.-Chem. Soc.*, **46**, 1216 (1914); K. Ziegler and H. Zeiser, *Ber.*, **63**, 1847 (1930).

Studies on Antiprotozoans. Synthesis and Biological Activity of Some Styrylimidazole Derivatives

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Received March 23, 1967

A series of 1-aminoalkyl- and 1-aminoalkyl-2-methyl-5(4)-nitro-4(5)-styrylimidazoles were synthesized and examined for biological activity. These compounds were tested on *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Candida albicans*. Their *in vitro* activity against *T. vaginalis* was found particularly interesting. For the 1-aminoalkyl-5(4)-nitro-4(5)-styrylimidazoles, we have separated isomers and determined their activities. Different methods used to assign positions to the nitro group in the heterocyclic ring are described.

For several years we have been carrying out in our laboratories research on heterocyclic substances with trichomonacidal activity as reported in a previous publication.[1] Continuing our study with other heterocyclic compounds, we have investigated some imidazole derivatives, since this heterocyclic system proved to have a marked trichomonacidal activity in compounds like azomycine and metronidazole.[2]

It is well known that the introduction of a styryl group into appropriate molecules gives substances highly active against trypanosomes; styrylquinolines and styrylbenzothiazoles are also active in the presence

(1) F. Lauria, V. Vecchietti, and I. de Carneri, *Farmaco* (Pavia), *Ed. Sci.*, **22**, 479 (1967).

(2) C. Cosar and L. Julou, *Ann. Inst. Pasteur*, **96**, 238 (1959).