In the Search for New Anticancer Drugs, I Antitumor Activity of Various Nitroxyl- and Aziridine-Containing Phosphorus Compounds

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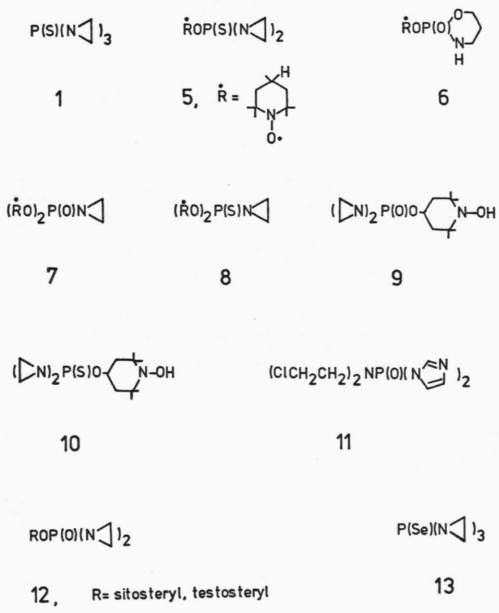
Antitumor Activity, Phosphorus Compounds

A series of pentavalent phosphorus compounds containing the nitroxyl and/or aziridinyl moieties was evaluated for antitumor activity against P 388 and L 1210 lymphocytic leukemias, and compared to the clinical agent thio-TEPA (**1**). The compounds with the structures **5**–**13** were found to be active, with a T/C value greater than 125. The percent ILS was also determined.



1 — 5, R = — 6

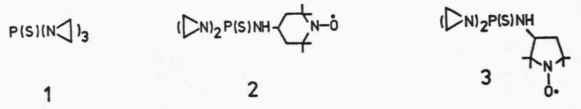
7 — 8 — 9

10 — 11

12, R = sitosteryl, testosteryl — 13

The biological activity of derivatives of pentavalent phosphorus containing the aziridine moiety has been known for many years [1–7]. In fact, thio-TEPA (**1**, triethyleneimine thiophosphoramide) has been used clinically against ovarian carcinoma, Hodgkin's disease, carcinoma of the breast and other malignancies [8].

Since 1971, when Rozantsev and coworkers [9] reported the synthesis of the spin-labeled phosphorus-containing aziridine derivatives **2** and **3**, we have been intrigued by the potential of spin labeled phosphorus compounds which could perhaps mimic

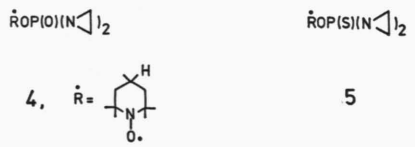


1 — 2 — 3

the biological activity of clinical alkylating agents, such as, thio-TEPA (**1**) and at the same time contain a reporter group which might be detectable by EPR [10, 11]. The usefulness of this approach for the study of anticancer drugs *in vivo* was subsequently demonstrated by Dodd and coworkers [12].

Furthermore, it was hoped that the introduction of a long lived and comparatively non-toxic radical moiety, such as the nitroxyl radical, might result in a drug which is less toxic and/or more active than the parent compound **1**.

In 1973, Sosnovsky and coworkers reported [13] in this journal the preparation of nitroxyl labeled thio-TEPA analogs **4** and **5**, which are more



4, R = — 5

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Table. Results on the anti-tumor activity of selected pentavalent phosphorus compounds.

Compound	Tumor[a]	Frequency of dose[b]	Dose mg/kg	T/C	ILS [%]
1[c,d]	P	A	5.7	253	153
		A	1.8	125	25
5[d,e]	L	A	100	149, 104	49
		A	50	244, 119	144
		A	25	185, 200	100
		A	12.5	161	61
7[d,e]	L	A	100	112, 128	41
		A	50	130, 110	31
		A	25	113, 104	65
		A	12.5	102	2
8[f,g]	P	A	200	168	96
		A	100	147	48
		A	50	124	24
11[g,h]	P	A	200	209	109
		A	100	181	82
		A	50	130	30
12, R = sitosteryl[g,i]	P	A	200	171, 163	71
		A	100	149, 152	49.5
		A	50	130, 134	31
		A	25	126	26
5[d,e]	L	B	200	150	50
		B	100	122	22
		B	50	112	12
6[d,h]	P	B	55	165	52
		B	27.5	150	50
		B	13.7	133	34
7[d,e]	L	B	400	165	65
10[d,j]	P	B	400	187	88
		B	200	142	43
		B	100	160	61
13[d,j]	P	B	27.5	155	56
		B	13.7	119	19
5[d,e]	P	C	200	146, 198	98
		C	100	206, 169	107
		C	50	192, 133	92
12, R = testosteryl[d,i]	P	C	400	144	45
		C	200	128	28
		C	100	110	10
9[g,h]	P	D	200	88	0
		D	100	105	6
		D	50	194	94

[a] P = P-388 lymphocytic leukemia, L = L-1210 lymphoid leukemia; [b] A = daily for 9 days, except for thio-TEPA (**1**) which as injected daily for 10 days; B = one injection; C = every fourth day for a total of three injections; D = every fourth day for a total of two injections; [c] vehicle used: saline, 3% ethanol; [d] data evaluated be mean survival time; [e] vehicle used: water; [f] vehicle used: saline plus alcohol; [g] data evaluated by median survival time; [h] vehicle used: saline; [i] vehicle used: saline plus tween 80; [j] vehicle used: hydroxypropyl cellulose.

economically obtained than compounds **2** and **3** [9]. During the subsequent years, additional nitroxyl- and/or aziridine-containing compounds were prepared, and submitted to the National Cancer Institute for evaluation as antitumor agents. In the present communication, our interpretations of these results are presented (Table). In addition, we have independently evaluated the clinical agent thio-TEPA (**1**) [14] for comparison.

The criterion of T/C, where T represents the mean survival time of the treated group, and C the mean survival time of the tumor bearing control group,

is used in evaluating the chemotherapeutic potency of a given compound. A value of T/C ≥ 125 is considered as a minimum requirement for a compound to be considered as active [14]. The percent increase life span (ILS) parameter was calculated using the formula $[(T-C)/C] \times 100$. Clearly, the larger the value for the ILS, the more promising is the compound as an anticancer drug.

The spin-labeled derivatives **5** [13, 15, 16], **6** [17], **7** [15, 16], and **8** [15, 16] proved to be active. The

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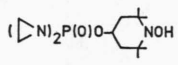
corresponding hydroxylamine derivatives **9** [18] and **10** [18] also were active. This result is somewhat surprising, in view of the fact that compound **4** [13, 15, 16], the nitroxyl parent of the hydroxylamine **9**, appears to be inactive. As can be derived from the data, several derivatives appear to be superior to thio-TEPA with respect to the doses required for a given T/C to be obtained.



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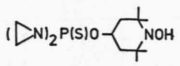

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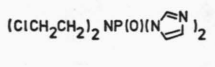
The compounds **11**, **12**, R = testosteryl and sitosteryl [19], and **13** [20] likewise are active. Surprisingly, the sulfur derivative corresponding to **11**, *i.e.* compound **14** [21] and the tocopheryl derivative **12**, R = α-tocopheryl [19] were inactive, perhaps due to the instability of these compounds.



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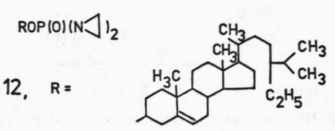
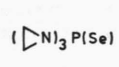


12, R =



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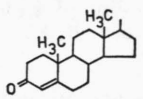
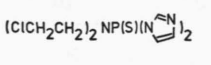


During the course of our studies, there appeared a report by Emanuel and coworkers [22] on the antitumor activity of compounds **2** and **15**. Compound **2** was reported [22] to be less toxic than thio-TEPA (**1**) towards certain carcinomas. Compound **2** is claimed to have a lower toxicity and a higher antitumor activity than thio-TEPA.



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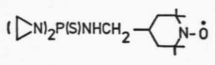

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In summary, although the potential for antitumor agents containing aziridine rings appeared to be exhausted, promising little new insights, on the basis of our present results, a revitalization of this field seems to be possible. It is conceivable that some of the analogs of thio-TEPA discussed here will present certain advantages over the clinical agent, perhaps by having lower toxicity and/or a more favorable therapeutic index. In addition the spin labeled analogs are particularly attractive since they have high activity, possess a group easily detectable by EPR spectroscopy, and thus be utilized for metabolic studies. The full details of our investigations with the more promising compounds presented in this communication will be published in forthcoming papers [23].

Materials and Methods

Thio-TEPA was obtained from Lederle Laboratories of Pearl River, New York, and was used without further purification. The synthesis of the derivatives listed in the Table have been previously described [13, 15–21]. The results for these derivatives were provided by the National Cancer Institute. The results for thio-TEPA were obtained in our laboratory. The detailed description of our methodology will be published in subsequent publications [23].

Preparation of 2-[Bis(2-chloroethyl)amino] N,N'-diimidazolyl phosphoramide (**11**)

A solution of bis(2-chloroethyl)phosphoramide dichloride [24] (2.55 g, 1 mmol) in benzene (50 ml) was added dropwise at 8–10 °C to a suspension of imidazole (1.36 g, 2 mmol) and triethylamine (2.5 g, 2.5 mmol) in benzene (25 ml). Following the addition, the reaction mixture was stirred at 15–20 °C for 15 h, the filtered. The filtrate was concentrated on a rotating evaporator at 20 °C/15 torr to an oil, which set to a solid on prolonged (several weeks) storage at 0° C, 3.15 g (99%), m.p. 60–62 °C (dec.).

The microanalysis was within the limits of C ± 0.2%, H ± 0.3%, N ± 0.2%.

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[1] O. M. Friedman, Can. Chem. Rep. **51 (6),** 347 (1967).
[2] O. M. Friedman, R. S. Levi, Z. B. Papanastassiou, and W. M. Whaley, J. Med. Chem. **6,** 449 (1963).
[3] J. Cheymol, P. Chabrier, G. Deysson, and A. Carayon-Gentil, Biol. Med. (Paris) **56 (6),** 519 (1967).
[4] M. D. Dowling, I. H. Krakoff, and D. A. Karnofsky, Mechanism of Action of Anti-Cancer Drugs in Chemotherapy of Cancer, pp. 1–74, W. H. Cole (Ed.), Lea and Febiger, Philadelphia 1970.
[5] Z. B. Papanastassiou and T. J. Bardos, J. Med. Chem. **5,** 1000 (1962).
[6] M. Sykes, D. Karnofsky, F. Phillips, and J. Burchenal, Cancer **6,** 142 (1953).
[7] K. Sigiura and C. Stock, Can. Res. **15,** 38 (1955).
[8] R. Silver, R. D. Lauper, and C. Jarowski, A Synopsis of Cancer Chemotherapy, p. 35, Dun-Donnelley Publishing Corp. New York 1977.
[9] A. B. Shapiro, A. A. Kropacheva, V. I. Suskina, B. V. Rozynov, and E. G. Rozantsev, Izv. Akad. Nauk SSSR, Ser. Khim. **1971,** 864.
[10] E. G. Rozantsev, Free Nitroxyl Radicals, Plenum Press, N. Y. 1970, and references therein.
[11] L. Berliner (Ed.), Spin Labeling I and II: Theory and Applications. Academic Press, N. Y. 1976, 1979, and references therein.
[12] N. J. F. Dodd, R. G. Harcus, and P. N. Preston, Z. Naturforsch. **31c,** 328 (1976).
[13] G. Sosnovsky, Y.-I. Yeh, and G. Karas, Z. Naturforsch. **28c,** 781 (1973).
[14] R. Geran, N. Greenberg, M. MacDonald, A. Schumacher, and B. Abbott, Can. Chem. Rep. Part III **3 (2),** 1 (1972).
[15] G. Sosnovsky and G. Karas, Phosphorus **6,** 123 (1976).
[16] G. Sosnovsky and M. Konieczny, Z. Naturforsch. **32b,** 870 (1977).
[17] G. Sosnovsky and M. Konieczny, Z. Naturforsch. **32b,** 1048 (1977).
[18] G. Sosnovsky and M. Konieczny, Synthesis **1977,** 619.
[19] G. Sosnovsky and M. Konieczny, Z. Naturforsch. **31b,** 1379 (1976).
[20] G. Sosnovsky and M. Konieczny, Synthesis **1978,** 583.
[21] G. Sosnovsky and M. Konieczny, unpublished results.
[22] N. M. Emanuel, N. P. Konovalova, and R. F. Djachkovskaya, Can. Treat Rep. **60,** 1605 (1976).
[23] P. L. Gutierrez, M. Konieczny, and G. Sosnovsky, in preparation.
[24] O. M. Friedman and A. M. Seligman, J. Am. Chem. Soc. **76,** 655 (1954).