Synthesis and Anticancer Activity of 2-Substituted 2,3-Dihydro-1,3,2-benzoxazaphosphorin-4-one and its 2-Oxide Derivatives

Krzysztof Kostka[a)*], Marek Porada[a)+)], Julita Graczyk[b)]

a) Institute of Chemistry, Faculty of Pharmacy, Medical University, 90-151 Lodz, Poland

b) Chair of Pharmacology, Medical University, 90-151 Lodz, Poland

Received January 25, 1993; revised form received May 27, 1993

As a result of the reaction of salicylic amide with PCl_3 and $POCl_3$ 2-chloro-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-one (**1**) and its 2-oxide **2** are obtained. Compounds **1** and **2** form amides with amines in 2-position. Antineoplastic action of the derivatives containing the bis(2-chloroethyl)amide group in 2-position was found.

Synthese und zytostatische Wirkung einiger 2-substituierter 2,3-Dihydro-1,3,2-benzoxazaphosphorin-4-one und ihrer 2-Oxid-Derivate

Salicylsäureamid reagiert mit PCl_3 bzw. $POCl_3$ zu 2-Chlor-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-on (**1**) und dessen 2-Oxid **2**. Diese bilden mit Aminen die entspr. Amide **3-8**. Die 2-Bis(2-chlorethyl)amid-derivate **5** und **8** wirken zytostatisch.

For many years investigations have been carried out aiming at finding optimum antineoplastic drugs, *i.e.*, drugs revealing specific action and low toxicity, at the same time. A large group of antineoplastic drugs is formed by alkylating substances exerting non-specific effects on the DNA. The basic alkylating drug is 2-[bis(2-chloroethyl)]amino-tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-oxide(cyclophosphamide). *In vivo* the compound undergoes oxygenation to 4-hydroxy- and then 4-ketocyclophosphamide[1-3].

In the lit. there is no straightforward information as to which of the initial metabolites of cyclophosphamide is responsible for the antineoplastic action[1-8]. The metabolic decomposition leads, in the final stage, among others to acroleine, an aldehyde responsible for the toxicity of cyclophosphamide. The toxicity is abolished by administering, at the same time, compounds containing a sulfhydryl group, *e.g.* MESNA[9,10].

In our previous investigations weak antineoplastic activity and low toxicity of the derivatives of 2-phenyl-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-on-2-oxide[11] was found. This made us look for other benzene analogues of 4-ketocyclophosphamide with groups known as pharmacophoric groups. The condensed structure of the 4-ketocyclophosphamide rest with benzene makes the *in vivo* formation of acrylic aldehyde impossible.

The reactions of salicylic acid with PCl_3 and $POCl_3$ have been investigated to a low extent only. Part of our results has been published previously[12].

For the synthesis we used salicylic amide, $POCl_3$ or PCl_3, and proper amines: bis(2-chloroethyl)amine, bis(2-hydroxyethyl)amine, and morpholine.

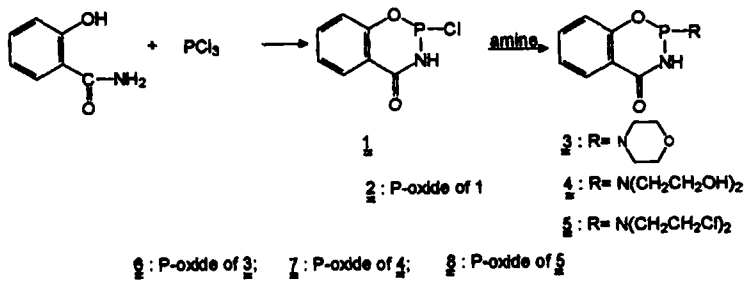
The structure of the compounds as well as their homogeneity was established by means of spectral analysis (IR, ^{1}H-NMR, ^{31}P-NMR), (Table 2), tlc, and elemental analysis (Table 1).

Compounds **3-8** were investigated for their antineoplastic activity on the development of L-1210 leukemia in mice.

Experimental Part

a) Chemistry

Melting temp.: Mikro-Heiztisch "Boetius" (non-corrected).- Elemental analysis: (N, C, H): apparatus for microanalysis "Heraeus".- IR spectra: Unicam SP 200G (Pay-Unicam) spectrophotometer.- ^{1}H-NMR spectra: 60 MHz Varian apparatus.- ^{31}P-NMR: Joel-60 (24.3 MHz).- The characteristics of the compounds are presented in Tables 1 and 2.



+) Part of the dissertation M. Porada, Med. Univ. Lodz, 1993.

  0365-6233/94/0404-0233 $ 5.00 + .25/0

2-Chloro-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-one (**1**)[13]

13.7 g (0.1 M) salicylic amide were dissolved in 150 cm^3 anhydrous toluene. Then 15.7 g (10 cm^3) (0.12 M) PCl_3 was added at boiling temp. under reflux with a pipe filled with $CaCl_2$ for 6 h and filtered when hot. On the following day crystals of **1** were filtered off; melting temp.: 91-92°C (according to lit.[13]: 156°C), 17.3 g, yield 86%.

2-Chloro-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-on-2-oxide (**2**)

1.37 g (0.01 M) salicylic amide were dissolved in 20 cm^3 anhydrous ethyl ether, heated with 1.7 g $POCl_3$ under reflux for 10 h and filtered when being hot. The solvent was distilled off in vacuo. The remaining oil was dissolved in THF (3 x 10 cm^3). The solution was condensed to half its volume and left overnight. The deposit of **2** was filtered off and crystallized from cyclohexane: 1.1 g (52%) **2**, m.p. 110-111°C.

2-Morpholine-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-one (**3**)

2.0 g (0.01 M) chloride **1** were dissolved in 20 cm^3 of anhydrous THF. Under argon 0.2 g (0.02 M) morpholine in 10 cm^3 THF was pipetted in at 0-10°C. Then the reaction mixture was stirred for 6 h at room temp. Morpholine-HCl was filtered off. The solvent was distilled off *in vacuo*. The oily residue was extracted with ethyl ether (3 x 20 cm^3). After evaporation the deposit was crystallized from nitromethane: 1.8 g (76%) **3**; m.p. 196-198°C.

2-[Bis(2-hydroxyethyl)amino]-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-one (**4**)

2 g (0.01 M) chloride **1** were dissolved in 30 cm^3 CH_2Cl_2. 0.2 g (0.02 M) diethanolamine in 20 cm^3 CH_2Cl_2 were pipetted in under argon at room temp. After stirring for 5 h at room temp. the deposit of diethanolamine-HCl was filtered off. The solvent was distilled off in vacuo. The remaining thick oil was crystallized from diethyl ether: acetone 4:1. 2.1 g (88%) **4**; m.p. = 176-177°C.

2-[Bis(2-chloroethyl)amino]-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-one (**5**)

2 g (0.01 M) of chloride **1** were dissolved in 50 cm^3 THF. 1.8 g (0.01 M) bis(2-chloroethyl)amine-HCl and 2.0 g triethylamine were added. The reaction mixture was heated under reflux for 12 h under argon. The amine hydrochloride deposits were filtered off, and after evaporation the remaining oil was crystallized from cyclohexane-acetone 10:1: 1.7 g (38%) **5**; m.p. 201-202°C.- With the free very toxic bis(2-chloroethyl)amine base the reaction gives a yield of 85% of **5**.

2-Morpholine-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-on-2-oxide (**6**)

2.2 g (0.01 M) oxide-chloride **2** were dissolved in 40 cm^3 THF. 0.2 g (0.02 M) morpholine in 10 cm^3 THF were pipetted in. The reaction mixture was stirred for 9 h. Morpholine-HCl deposit was filtered off. The solvent was distilled off *in vacuo*. The remaining oil was purified by cc (silica gel, 50 g (Kapsigel 60, 230 mesh)) with ethyl acetate-benzene 1:3. Fractions were collected by 5 cm^3 and chromatographically controlled: 1.5 g (56%) **6**, m.p. 152-153°C.

2-[Bis(2-hydroxyethyl)amino]-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-on-2-oxide (**7**)

To 2.2 g (0.01 M) oxide chloride **2** in 40 cm^3 THF 0.2 g (0.02 M) diethanolamine were added dropwise at -5 - +5°C. Then the reaction mixture was brought to room temp. After 3 h diethanolamine-HCl was filtered off. The solvent was distilled off from the filtrate. The remaining thick oil was crystallized from n-propanol: 1.8 g (62%) **7**, m.p. 182-183°C.

2-[Bis(2-chloroethyl)amino]-2,3-dihydro-1,3,2-benzoxazaphosphorin-4-on-2-oxide (**8**)

To 2.2 g (0.01 M) oxide chloride **2** in 40 cm^3 THF were added 1.8 g (0.01 M) bis(2-chloroethyl)amine-HCl and 2.0 g (0.02 M) triethylamine. The mixture was heated to reflux for 12 h. Hydrochloride deposits were filtered off. The filtrate was evaporated in vacuo. The remaining thick oil was purified by cc (silica gel (Kapsigel 60, 40 mesh)) with $CHCl_3$-acetone (3:1). Fractions of 5 cm^3 were collected: 1.1 g (32%) **8**, m.p. 210-212°C.

b) Pharmacology

Experiments were carried out on 60 Swiss mice for toxicity studies (18-22 g), female and on 79 BDF_1 mice (20-22 g) female. Animals were maintained on laboratory diet and given water *ad libitum*.

Tab. 1: Elementary analysis of 2-substituted 2,3-dihydro-1,3,2-benzoxazaphosphorin-4-ones and their 2-oxides

Compound No.	Summary formula (Molecular mass)	Analyses % calculated C	H	N	obtained C	H	N
1	$C_7H_5O_2PClN$ (201.5)	41.7	2.50	7.0	41.5	2.36	6.8
2	$C_7H_5O_3PClN$ (217.5	38.7	2.32	6.4	38.6	2.12	6.2
3	$C_{11}H_{13}N_2O_3P$ (252.2)	52.4	5.20	11.1	52.2	5.38	10.8
4	$C_{11}H_{15}N_2O_4P$ (270.2)	48.9	5.60	10.4	48.8	5.48	10.2
5	$C_{11}H_{13}N_2O_2PCl_2$ (307.1)	43.0	4.27	9.1	42.8	4.11	9.0
6	$C_{11}H_{13}N_2O_4P$ (268.2)	49.3	4.85	10.5	49.1	4.63	10.4
7	$C_{11}H_{15}N_2O_5P$ (286.2)	46.2	5.28	9.8	46.1	4.98	9.7
8	$C_{11}H_{13}N_2O_3PCl_2$ (323.1)	40.9	4.06	8.7	40.7	3.98	8.6

Tab. 2: Spectroscopic data of 2-substituted 2,3-dihydro-1,3,2-benzoxazaphosphorin-4-ones and their 2-oxides

Compound No.	IR cm^{-1}	^{31}P NMR ppm	^{1}H NMR ppm
1		(CD_3COCD_3): 159.6	(CD_3COCD_3): 7.1-8.3(m;4H arom.), 6.15(s;NH)
2		($CDCl_3$): 11.2	($CDCl_3$): 7.3-8.8(m;4H arom.), 6.4(s;NH)
3	1695(C=O), 3042(NH)	($CDCl_3$): 151.8	($CDCl_3$): 2.9-3.8(m;8H, CH_2), 7.0-8.2(m;4H arom.), 6.4(s;NH)
4	1702(C=O), 3055(NH)	(CD_3COCD_3): 156.7	(CD_3COCD_3): 3.1-4.1(m;8H, CH_2), 5.1(s;2H OH), 7.25-8.42(m;4H arom.), 6.8(s;NH)
5	1700(C=O), 3050(NH)	($CDCl_3$): 158.3	($CDCl_3$): 3.7-4.4(m;8H, CH_2), 6.9(s;NH), 7.8-8.6(m;4H arom.)
6	1695(C=O), 3042(NH)	($CDCl_3$): 14.6	($CDCl_3$): 3.4-4.4(m;8H, CH_2), 6.6(s; NH), 7.3-8.1(m; 4H arom.)
7	1698(C=O), 3046(NH), 1249(P=O)	($CDCl_3$): 16.6	($CDCl_3$): 3.3-4.3(m;8H, CH_2), 5.3(s;2H, OH), 6.6(s;NH), 7.4-8.5(m;4H arom.)
8	1705(C=O), 3050(NH), 1252(P=O)	($CDCl_3$): 18.1	($CDCl_3$): 3.6-4.1(m;9H, CH_2), 6.2(s; NH), 7.6-8.4(m;4H arom.)

Group	Compound	LD_{50} mg/kg i.p.	Dose mg/kg	ILS %	Evaluation according Geran
1	Not treated				
2	Cyclophosphamide	600	60 x 5	330	+
3	3	1000	100 x 5	0	-
4	4	750	75 x 5	13	-
5	5	750	75 x 5	100	+
6	6	620	62 x 5	0	-
7	7	750	75 x 5	13	-
8	8	750	75 x 5	100	+

Group	Compound	Dose mg/kg	ILS %	Evaluation according
1	Not treated			-
2	Cyclophosphamide	100 x 3	330	+
3	3	167 x 2	0	-
4	4	125 x 2	0	-
5	5	125 x 3	100	+
6	6	103 x 2	13	-
7	7	125 x 2	13	-
8	8	125 x 3	100	+

Compounds 5, 8, and cyclophosphamide were given three times. The remaining compounds were given twice, as the animals died before day 9.

Acute toxicity of compounds was calculated by *Deichmann, Le Blanck* method[14] to choose doses to investigate antineoplastic activity.

Antineoplastic effect on the L-1210 leukemia development *in vivo* was examined according to *Geran et al.*[15].

The L-1210 leukemia was propagated in DBA/2 mice, and transplanted into BDF_1 mice. All the animals were inoculated intraperitoneally with 3 x 10^5 cell/mouse. They were divided into 8 groups. The 1st, untreated, control group contained 30 mice. The 2nd control group contained the cyclophosphamide treated mice - positive control. The animals of groups 3 to 8 were given the investigated compounds. Each group consisted of 7 mice.

The following schemes of injection of the investigated compounds were planned: 1) everyday injection for 5 days in the total dose 1/2 LD_{50}, 2) the 1st, 5th, 9th days of the experiment in the total dose of 1/2 LD_{50}. The compounds were injected intraperitoneally starting the treatment 24 h after neoplasm implantation. The increase of the life span (ILS%) of the treated mice in comparison with untreated animals was assumed as the antineo-

plastic activity. The increase of the life span of at least 25% in comparison to the survival of the untreated group testifies the antineoplastic activity of the medicine dose or the dose of the investigated compound. The observation of the animals was conducted for 30 days; results are shown in Tables 3 and 4.

Discussion

Six new compounds - derivatives of 2-substituted 2,3-dihydro-1,3,2-benzoxazaphosphorin-4-on-2-oxide - were obtained, with pharmacophoric groups, *i.e.* bis(2-chloroethyl)amine, bis(2-hydroxyethyl)amine and morpholine at 2-position.

The acute toxicity of **4-8** is similar to that of cyclophosphamide. Compound **3** is less toxic than cyclophosphamide. Compounds **5** and **8** given twice, in two therapeutic patterns, at a total dose of 1/2 LD_{50} reveal antineoplastic action against L-1210 leukemia. The treated animals inoculated with L-1210 leukemia lived twice as long as the untreated ones (control animals). The effect is weaker than that of cyclophosphamide which caused life span increase by 330%. The remaining four compounds do not reveal antineoplastic activity against L-1210 leukemia.

Out of the investigated compounds only those show antineoplastic activity which have the bis(2-chloroethyl)amine group in their molecule. This group is also present in cyclophosphamide.

References

1 S.M. Ludeman, V.L. Boyd, J.B. Regan, K.A. Gallo, G. Zon, *J. Med. Chem.* **1986**, *29*, 716-736.
2 M. Colvin, J. Hilton, *Cancer Treatm. Rep.* **1981**, *65*, 89-93.
3 R.A.V. Milsted, M. Jarman, *Cancer Chemother. Pharmacol.* **1982**, *8*, 311-313.
4 G. Zon, S.M. Ludeman, J.A. Brant, V.L. Boyd, G. Ozkan, W. Egan, *J. Med. Chem.* **1984**, *27*, 466-472.
5 W. Boyd, J.D. Rabins, W. Egan, S.M. Ludeman, *J. Med. Chem.* **1986**, *29*, 1206-1213.
6 D.L. Hill, C. Kirk, R.F. Struck, *J. Am. Chem. Soc.* **1970**, *92*, 3207-3209.
7 R. Miller, *Eur. J. Med. Chem.-Chim. Ther.* **1974**, *9*, 301-305.
8 K. Misiura, K. Pankiewicz, *J. Med. Chem.* **1983**, *26*, 674-678.
9 L.W. Guito, J.A. Hipkens, S.P. Sharma, *Cancer Res.* **1981**, *41*, 3584-3591
10 J. Lähdetie, R. Räty, M. Sorsa, *Mutation Res.* **1990**, *27*, 245-247.
11 M. Porada, E. Budzisz, J. Graczyk, W. Pakulska, *Acta Polon. Pharm.*, in print.
12 K. Kostka, M. Porada, *Arch. Pharm. (Weinheim)* **1992**, *325*, 325-328.
13 A.K. Kuliev, V.V. Moskva, D. A. Ahmiebzabie, E. B. Sahnovskaja, T. W. Zykova, F. Sz. Szagbaliejev, M. M. Guciejnova, *Zh. Obshch. Khim.* **1985**, *55*, 936-937; *Chem. Abstr.* **1985**, *103*, 196163t.
14 W.B. Deichmann, T.J. Le Blanc, *J. Ind. Hyg. Toxicol.* **1943**, *25*, 415-417; *Chem. Abstr.* **1945**, *39*, 4979. [Ph159]