Synthesis and Antitumor Activity of Cyclophosphamide Analogs. 1. Benzo Annulated Cyclophosphamide and Related Systems

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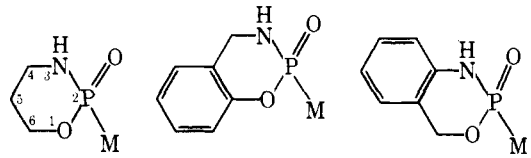
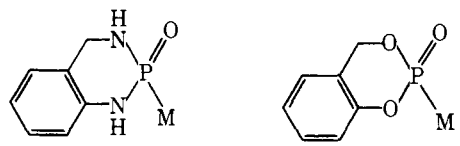
Synthesis of 2-[bis(2-chloroethyl)amino]-3,4-dihydro-2*H*-1,3,2-benzoxazaphosphorin 2-oxide (**2**), which is a benzo annulated analog of cyclophosphamide [2-bis(2-chloroethyl)aminotetrahydro-2*H*-1,3,2-oxazaphosphorin 2-oxide (**1**)], was carried out in order to test for possible increased antitumor activity relative to **1** due to the presence of an oxidatively reactive C-4 benzylic site in **2**. A structural isomer of **2**, 2-[bis(2-chloroethyl)amino]-1,4-dihydro-2*H*-3,1,2-benzoxazaphosphorin 2-oxide (**3**), and cognate systems 2-[bis(2-chloroethyl)amino]-1,2,3,4-tetrahydro-1,3,2-benzodiazaphosphorin 2-oxide (**4**) and 2-[bis(2-chloroethyl)amino]-4*H*-1,3,2-benzodioxaphosphorin 2-oxide (**5**) were also prepared for comparative purposes. In vivo antitumor evaluation in mice against L1210 lymphoid leukemia indicated no significant activity for compound **2**. Compounds **3** and **4** were likewise found to be inactive and only marginal activity was exhibited by **5**.

A considerable body of evidence is now available which supports the hypothesis that the chemotherapeutic cytotoxicity of cyclophosphamide, 2-bis(2-chloroethyl)aminotetrahydro-2*H*-1,3,2-oxazaphosphorin 2-oxide (**1**), is triggered by enzymatic oxidation of the C-4 position to yield 4-hydroxycyclophosphamide.[1] We therefore undertook the presently reported study of a cyclophosphamide analog wherein this key oxidation site is chemically activated by virtue of adjacent benzene ring fusion.[2] At an heuristic level, it was reasoned that if "benzocyclophosphamide" **2**[3] undergoes accelerated enzymatic C-4 hydroxylation, increased antitumor activity relative to **1** might obtain, granted that other metabolic and biochemical factors between **1** and **2** are roughly equal. For the sake of comparison, isomeric "benzocyclophosphamide" **3** was also prepared, together with 1,3-diaza and 1,3-dioxa systems **4** and **5**.[3]



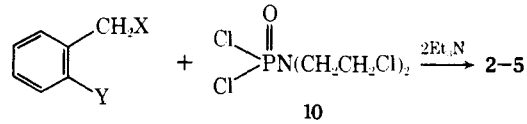
1 **2** **3**



4 **5**

M = $N(CH_2CH_2Cl)_2$

The straightforward synthetic route used to construct **2–5** involved triethylamine-mediated intermolecular cyclization of bis(2-chloroethyl)phosphoramidic dichloride (**10**) with corresponding precursors **6–9**. Compounds **6** and **8** were obtained by $LiAlH_4$ reduction of *o*-cyanophenol and *o*-aminobenzamide, respectively. Structural assignments for **2–5** follow unambiguously from their characteristic ^1H NMR spectra.[4]



10

6. X = NH_2; Y = OH
7. X = OH; Y = NH_2
8. X = NH_2; Y = NH_2
9. X = OH; Y = OH

Incorporation of a benzenoid nucleus into cyclophosphamide as in **2** must incur changes in various physicochemical properties which are in addition to the desired effect of oxidatively activating the C-4 position. For instance, and as might be expected, the change in solubility characteristics is such that **2** was found to be virtually insoluble in water whereas **1** has an aqueous solubility of 4 g/100 ml. Compound **2** also exhibited increased susceptibility toward alkaline hydrolysis when compared to **1**, presumably due to the presence of an incipient resonance-stabilized aryloxy leaving group (see Experimental Section).[5] However, **2** and **1** were both stable toward decomposition over a period of 3.5 hr while dissolved in human blood plasma at 37°.

Compounds **2–5** were screened in mice against L1210 lymphoid leukemia according to standard procedures[6] of the National Cancer Institute. Test samples were administered intraperitoneally in a distilled water–Tween 80 (polysorbate) vehicle on day 1 only at doses of 500, 250, 125, 62.5, and 31.8 mg/kg and results were evaluated at day 30. Mean survival time was utilized as the evaluation parameter and compounds exhibiting a test/control percentage (T/C) ≥ 120 are considered to be active in this test system.

Compounds **2–4** were found to be inactive in this test, showing maximum T/C values (at 31.8 mg/kg) of 100, 104, and 102, respectively. Marginal activity for **5** was indicated by a maximum T/C of 122 (at 125 mg/kg). Toxicity differences were indicated by finding that 500 mg/kg doses of **3** and **4** were accompanied by 100% survival (6/6), while 62.5 and 250 mg/kg doses of **2** and **5**, respectively, were the highest tolerable in terms of complete survival.

The inefficacy of **2** may be rationalized in conjunction with any one of a number of factors associated with transport and metabolism of **1**. In addition to the aforementioned solubility and stability differences between **2** and **1**, it should be noted that unlike **1**, the 4-hydroxy derivative of **2** is incapable of fragmentation into phosphoramide mustard and acrolein.[7] The extent to which in vivo enzymatic hydroxylation of C-4 in **2** takes place is unknown at this time; however, competitive hydroxylation of the aryl moiety[8] in **2** and/or obvious steric inequities between **2** and **1** are factors which possibly militate against this event. Studies directed toward gauging the importance of these points will be reported in the future.

Experimental Section

Melting points were obtained with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Chemalytics, Inc., and were within ±0.4% of the theoretical values. Ir spectra were recorded with Perkin-Elmer Model 337 and Model 137 spectrophotometers and ^1H NMR spectra with Varian A-60 and HR-220 instruments using ca. 10% w/v solutions in $CDCl_3$ with tetramethylsilane as an internal reference,

except as noted. Compounds 7 and 9 were purchased from Aldrich Chemical Co. and used without additional purification. Anhydrous Et_2O was used throughout and THF was distilled from $LiAlH_4$. All reactions were run with protection from atmospheric water. Frozen human blood plasma was obtained from the Washington Regional Red Cross Blood Center.

***o*-Hydroxybenzylamine (6).** A solution of o-cyanophenol (20.8 g, 0.17 mol) in Et_2O (75 ml)–THF (75 ml) was added (45 min) to a mechanically stirred and refluxing suspension of $LiAlH_4$ (12.5 g, 0.33 mol) in Et_2O (750 ml). After additional reflux (24 hr) and standard[9] alkaline work-up, the mixture was suction filtered and the collected gray solid was then stirred with portions of 1 *N* HCl to achieve pH ~1. Undissolved solid material was discarded and the acidic extract was rapidly stirred with an equal volume of $CHCl_3$ while adding concentrated NH_4OH until pH ~13. Suction filtration of the resultant emulsion through a pad of Celite and then separation of the $CHCl_3$ layer were followed by reacidification of the aqueous layer, which was again extracted with $CHCl_3$ during basification as above. Removal of solvent from the combined $CHCl_3$ extracts gave crude product which was recrystallized from absolute EtOH–low boiling petroleum ether to yield (14%) 6 as white leaflets: mp 125.5–127° (lit.[10] mp 126–129°).

***o*-Aminobenzylamine (8).** A solution of o-aminobenzamide (6.8 g, 0.05 mol) in Et_2O (50 ml)–1,2-dimethoxyethane (50 ml) was added (1 hr) to a mechanically stirred and refluxing suspension of $LiAlH_4$ (3.8 g, 0.1 mol) in Et_2O (100 ml), during which time there was formation of a gummy solid mass. Continued reflux (4 days) was followed by standard[9] alkaline work-up. The brown solid hydrolysate collected by suction filtration was washed with CH_2Cl_2 (2 × 200 ml) and the solvent from the combined filtrate plus washings was then removed under reduced pressure. The residue was dissolved in a minimum volume of boiling CH_2Cl_2 and unreacted starting material was removed after inducing its crystallization with low boiling petroleum ether. Kugelrohr distillation (110–130°, 1 mm) of the concentrated mother liquor gave crude 8 (50%, corrected), mp 46–49° (lit.[11] 59–59.5°), which was used without further purification.

Bis(2-chloroethyl)phosphoramidic Dichloride (10). Reaction of $POCl_3$ with bis(2-chloroethyl)amine hydrochloride was carried out according to the procedure of Friedman and Seligman.[12] After removing ca. 90% of the excess $POCl_3$ by atmospheric distillation, the pot residue was subjected to rapid Kugelrohr distillation (120–130°, 0.5 mm) to afford a near quantitative yield of 10, mp 54–56° (lit.[12] mp 54–56°). *Caution!* Vigorous decomposition with copious gas evolution was once encountered during removal of the unreacted $POCl_3$.

2-[Bis(2-chloroethyl)amino]-3,4-dihydro-2*H*-1,3,2-benzoxazaphosphorin 2-Oxide (2). A solution of 10 (4.14 g, 16 mmol) in EtOAc (20 ml) was added (15 min) at ambient temperature to a magnetically stirred solution of 6 (1.97 g, 16 mmol) and Et_3N (4.45 ml, 32 mmol) in EtOAc (25 ml). After 48 hr, Et_3N·HCl was removed by suction filtration and solvent from the filtrate was removed on a rotary evaporator. The residual solid was slowly recrystallized from CH_2Cl_2–Et_2O to give (48%) pure 2: mp 90.5–92.5°; δ (220 MHz) 7.30–6.98 (m, 4, aromatic), 4.46–4.11 (AB part of ABX, 2, benzylic; δ_A = 4.36, δ_B = 4.21, J_{AB} = 15 Hz, J_{AX} = 15 Hz, J_{BX} = 20 Hz), 3.66–3.57 (m, 4), and 3.50–3.34 (m, 4); ir (Nujol) 3160, 1235, 1036, 1090, 985, 935, 910, 750, and 745 cm^{-1}. Anal. ($C_{11}H_{15}Cl_2N_2O_2P$) C, H, N, Cl, P.

2-[Bis(2-chloroethyl)amino]-1,4-dihydro-2*H*-3,1,2-benzoxazaphosphorin 2-Oxide (3). Incorporation of *o*-aminobenzyl alcohol (7) into the above procedure for 2 gave 3 (25%) as small white needles: mp 131.5–132.5°; NMR δ (220 MHz) 7.27–6.87 (m, 4, aromatic), 5.37 (doubled d, $^2J_{HH}$ = 13.5 Hz, $^3J_{HP}$ = 7 Hz, 1, benzylic), 4.98 (doubled d, $^2J_{HH}$ = 13.5 Hz, $^3J_{HP}$ = 24.5 Hz, 1, benzylic), 3.67 (t, $^3J_{HH}$ = 7 Hz, 4, CH_2Cl), and 3.43–3.27 (m, 4, NCH_2); ir (Nujol) 1610, 1225 (broad), 1090, 1020, 985, 950, 935, and 830 cm^{-1}. Anal. ($C_{11}H_{15}Cl_2N_2O_2P$) C, H, N, Cl, P.

2-[Bis(2-chloroethyl)amino]-1,2,3,4-tetrahydro-1,3,2-benzodiazaphosphorin 2-Oxide (4). Reaction of 8 and 10 according to the method used for preparation of 2 afforded 4 (13.5%) as small white needles: mp 154.5–155.5°; NMR δ (60 MHz, Me_2SO-d_6) 7.65 (broadened d, $^2J_{HP}$ = 5 Hz, 1, NH, exchangeable with D_2O), 7.50–6.40 (m, 4, aromatic), 5.20–4.75 (m, 1, NH, exchangeable with D_2O), 4.30–2.80 (m, 10); ir (Nujol) 3125, 1600, 1275, 1215, 1180, 1035, 985, 945, 925, and 755 cm^{-1}. Anal. ($C_{11}H_{16}Cl_2N_3OP$) C, H, N, Cl, P.

2-[Bis(2-chloroethyl)amino]-4*H*-1,3,2-benzodioxaphosphorin 2-Oxide (5). Substitution of o-hydroxybenzyl alcohol (9) in the procedure detailed above for 2 gave 5, which was isolated (33%) as a viscous pale yellow oil following chromatography through silica gel using $CHCl_3$ (50%)–Et_2O (50%) as solvent: NMR δ (60 MHz) 7.70–6.70 (m, 4, aromatic), 5.78–4.86 (AB part of ABX, 2, benzylic; δ_A = 5.57, δ_B = 5.19, J_{AB} = 14 Hz, J_{AX} = 5 Hz, J_{BX} = 20 Hz), 4.15–3.15 (m, 8); ir (neat) 3075, 3040, 2965, 1620, 1590, 1275 (broad), 1190, 1110, 915, 830, 755, 680, and 575 cm^{-1}. Anal. ($C_{11}H_{14}Cl_2NO_3P$) C, H, N.

Blood Plasma Stability of 2 and 1. Human blood plasma (20 ml) was added to 2 (10 mg) and the mixture was placed in a heater–shaker bath at 37°. After dissolution of 2 (ca. 3.5 hr), the sample was heated for an additional 3.5 hr and then extracted with $CHCl_3$ (10 ml). Following centrifugation, a portion (5 ml) of the separated $CHCl_3$ layer was concentrated in vacuo, diluted with $CHCl_3$ (0.25 ml), and then compared by TLC (silica gel; $CHCl_3$ (50%)–Et_2O (50%); I_2 visualization) with a standard solution of 2 (10 mg/0.25 ml). Relative spot intensities indicated that no significant decomposition of 2 occurred while dissolved in the plasma. An identical procedure using 1, which immediately dissolved in the plasma, gave the same results.

Alkaline Stability of 2 and 1. A sample of 2 (4.2 mg, 1.15×10^{-2} mmol) was treated at room temperature with 1 equiv (1.15 ml) of 10^{-2} *M* NaOH in H_2O (50%)–EtOH (50%). TLC analysis (silica gel; $CHCl_3$ (90%)–MeOH (10%); I_2 visualization), which was performed 10 min after mixing, indicated that essentially all of 2 had reacted to give a major hydrolysis product (R_f 0.75) and two minor products (R_f 0.32 and 0.23); 6 and bis(2-chloroethyl)amine were not detected. A similar experiment performed with 1 revealed that little if any hydrolysis took place.

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References and Notes

(1) (a) K. Norpoth, E. Golovinsky, and H. M. Rauen, *Hoppe-Seyler's Z. Physiol. Chem.*, **351**, 377 (1970); H.-J. Hohorst, A. Ziemann, and N. Brock, *Arzneim.-Forsch.*, **21**, 1251 (1971); D. L. Hill, W. R. Laster, Jr., and R. F. Struck, *Cancer Res.*, **32**, 658 (1972); N. E. Sladek, *ibid.*, **33**, 651 (1973); T. A. Connors, P. J. Cox, P. B. Farmer, A. B. Foster, and M. Jarman, *Biochem. Pharmacol.*, **23**, 115 (1974); R. F. Struck, *Cancer Res.*, **34**, 2933 (1974); A. Takamizawa, S. Matsumoto, T. Iwata, Y. Tochino, K. Katagiri, K. Yamaguchi, and O. Shiratori, *J. Med. Chem.*, **18**, 376 (1975).

(2) A. Korolkovas, "Essentials of Molecular Pharmacology", Wiley-Interscience, New York, N.Y., 1970, Chapter 4.

(3) Names for the new heterocyclic derivatives 2–5 preferred by the IUPAC rules (pre-1972 *Chem. Abstr.*) are, respectively, 2-[bis(2-chloroethyl)amino]-3,4-dihydro-2*H*-1,3,2-benzoxazaphosphorin 2-oxide, 2-[bis(2-chloroethyl)amino]-1,4-dihydro-2*H*-3,1,2-benzoxazaphosphorin 2-oxide, 2-[bis(2-chloroethyl)amino]-1,2,3,4-tetrahydro-1,3,2-benzodiazaphosphorin 2-oxide, and 2-[bis(2-chloroethyl)amino]-4*H*-1,3,2-benzodioxaphosphorin 2-oxide. However, the currently preferred Chemical Abstracts names for 2–5 are, respectively, *N*,*N*-bis(2-chloroethyl)-3,4-dihydro-2*H*-1,3,2-benzoxazaphosphorin-2-amine 2-oxide, *N*,*N*-bis(2-chloroethyl)-1,4-dihydro-2*H*-3,1,2-benzoxazaphosphorin-2-amine 2-oxide, *N*,*N*-bis(2-chloroethyl)-3,4-dihydro-1,3,2-benzodiazaphosphorin-2(1*H*)-amine 2-oxide, and *N*,*N*-bis(2-chloroethyl)-4*H*-1,3,2-benzodioxaphosphorin-2-amine 2-oxide.

(4) For comparative spectral data see J. Durrieu, R. Kraemer, and J. Navech, *Org. Magn. Reson.*, **5**, 407 (1973).

(5) C. Brown, J. A. Boudreau, B. Hewitson, and R. F. Hudson, *J. Chem. Soc., Chem. Commun.*, 504 (1975).

(6) Instruction 14, "Screening Data Summary Interpretation and Outline of Current Screen", Drug Evaluation Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., 1973.

(7) R. A. Alarcon and J. Meienhofer, *Nature (London), New Biol.*, **233**, 250 (1971); M. Colvin, C. A. Padgett, and C. Fenselau, *Cancer Res.*, **33**, 915 (1973); M. Thomson and M. Colvin,

ibid., **34**, 981 (1974). Two-step fragmentation of the 4-hydroxy derivative of **2** into phosphoramide mustard [$H_2N(HO)P(O)N(CH_2CH_2Cl)_2$] and o-hydroxybenzaldehyde is conceivable. The importance of phosphoramide mustard production in the metabolism of 1 has been recently discussed: P. J. Cox, P. B. Farmer, and M. Jarman, *Biochem. Pharmacol.*, **24**, 599 (1975).
(8) J. R. Gillette, *Adv. Pharmacol.*, **4**, 234 (1966).
(9) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol. 1, Wiley, New York, N.Y., 1967, p 584.
(10) L. C. Raiford and E. P. Clark, *J. Am. Chem. Soc.*, **45**, 1738 (1923).
(11) N. Kornblum and D. C. Iffland, *J. Am. Chem. Soc.*, **71**, 2137 (1949).
(12) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 655 (1954).

Synthesis and Antimicrobial Activity of Certain Imidazo[1,2-*a*]pyrimidines

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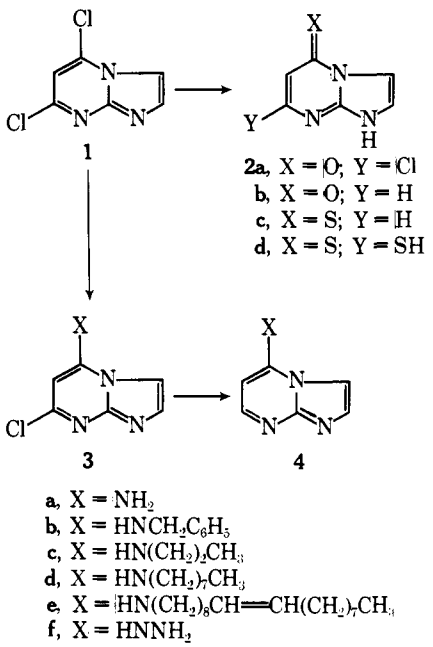
A series of 5-substituted and 5,7-disubstituted imidazo[1,2-*a*]pyrimidines has been prepared. The in vitro antimicrobial activity of these compounds against a variety of microorganisms is reported. 5-*n*-Octylaminoimidazo[1,2-*a*]pyrimidine exhibited significant activity against all the microorganisms studied.

In continuation of our investigations on the purine antagonists, we recently described [1-5]the synthesis and antiviral activity of nucleosides resembling inosine and guanosine of various heterocyclic systems containing a bridgehead nitrogen atom. We now wish to report the synthesis and antimicrobial activity of some amino-substituted imidazo[1,2-*a*]pyrimidines.

Scheme I



Chemistry. The compounds described herein (Scheme I) were prepared by treating the reactive 5,7-dichloroimidazo[1,2-*a*]pyrimidine (**1**) with the appropriate amine. The synthesis of 1 has been achieved as described earlier[4] by the successful chlorination of 5,7-dihydroxyimidazo[1,2-*a*]pyrimidine.[6] Treatment of 1 with 5% aqueous sodium hydroxide solution at reflux gave 7-chloroimidazo[1,2-*a*]pyrimidin-5-one[4] (**2a**) which on subsequent catalytic dehalogenation with 10% palladium on carbon in a hydrogen atmosphere at room temperature furnished imidazo[1,2-*a*]pyrimidin-5-one (**2b**). Chlorination of **2b** with phosphorus oxychloride in the presence of *N,N*-dimethylaniline gave the intermediate 5-chloroimidazo[1,2-*a*]pyrimidine, which without isolation was treated with thiourea in ethanol to obtain the 6-mercaptopurine analog, imidazo[1,2-*a*]pyrimidine-5-thione (**2c**). There was observed an absorption band in the ir spectrum (KBr) at 1625 cm^{-1} which was assigned to C=S stretching[7] indicating that **2c** exists in the thione rather than the thiol form. Reaction of 1 with 2 molar equiv of thiourea produced the dimercapto derivative **2d**.

The difference in reactivity between the two chlorine atoms of 1 was made use to prepare a number of 5-substituted imidazo[1,2-*a*]pyrimidines. Treatment of 1 with concentrated ammonium hydroxide at room temperature gave 5-amino-7-chloroimidazo[1,2-*a*]pyrimidine (**3a**) which on catalytic dehalogenation converted to the adenine analog, 5-aminoimidazo[1,2-*a*]pyrimidine (**4a**). Similarly, treatment of 1 with benzylamine, propylamine, *n*-octylamine, and oleylamine gave the corresponding 5-substituted 7-chloroimidazo[1,2-*a*]pyrimidines (**3b–e**) which on subsequent catalytic dehalogenation with 10% palladium on carbon in a hydrogen atmosphere furnished 5-benzylamino- (**4b**), 5-propylamino- (**4c**), 5-*n*-octylamino- (**4d**), and 5-oleylamino- (**4e**) imidazo[1,2-*a*]pyrimidines, respectively. Displacement of chlorine adjacent to the bridgehead nitrogen of 1 by hydrazine at room temperature gave almost quantitative yield of 7-chloro-5-hydrazinoimidazo[1,2-*a*]pyrimidine (**3f**). Physical constants of the 16 compounds screened for antimicrobial activity are listed in Table I.

Of the 16 compounds screened in vitro, only 5-*n*-octylaminoimidazo[1,2-*a*]pyrimidine (**4d**) exhibited significant activity against all the microorganisms[8] tested [MIC (μmol/ml) *Ca*, 0.08; *Ef*, 0.08; *Mc*, 0.04; *Sa*, 0.16; *Tm*, 0.08] and is presently under further evaluation. All other compounds tested were not active.

In conclusion, a series of purine analogs in the imidazo[1,2-*a*]pyrimidine ring system has been synthesized by an unambiguous method. These compounds resemble 1-deazapurines with a bridgehead nitrogen atom in which C-5 and N-7 are interchanged. Of all the compounds examined, only **4d** exhibited significant in vitro antimicrobial activity.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H NMR) spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-20A spectrometer in Me_2SO-d_6 using DSS as an internal standard and infrared (ir) spectra on a Perkin-Elmer 257 spectrophotometer (KBr pellets). ICN-Woelm silica gel (70–230