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The Synthesis and Antitumor Activity of Novel 1,3,5,2-Triazaphosphorines Linked With Nitrogen Mustards

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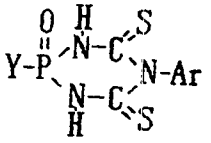
ABSTRACT

A series of novel derivatives of 2,5-dihydro-1,3,5,2-triazaphosphorine has been synthesized and the structures of the most stable tautomers have been confirmed by ^1H NMR and IR spectroscopy, MS, elemental analyses, and quantum chemistry calculations. We suggest that the thioenol form is more stable than the thione form which has been thought to be more stable up to the present time. The bioassay tests indicated that most of the new products have good antitumor activity.

INTRODUCTION

The synthesis of dihydro-1,3,5,2-triazaphosphorines has been reported in the literature [1–4], but most of these were prepared from diisothiocyanatodialkyl (or diaryl) phosphates and aromatic amines. All these products were thought to have the more stable molecular structures as dithiones with the general formular (I):



(I) Y=R, Ph, OR, OAr.

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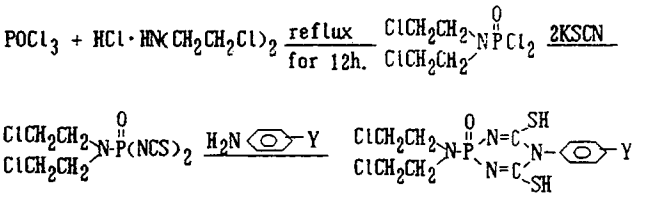

(II)

SCHEME 1 Y = p-Cl, m-Cl, o-Cl, p-Me, m-Me, o-Me, p-Br, H, p-NO_2, m-NO_2

To look for new effective antitumor drugs, we designed and synthesized a novel type of compounds II by attaching a nitrogen mustard group to the phosphorus atom of the dihydro-1,3,5,2-triazaphosphorines which were then tested for antitumor activities. The results showed that some of them are effective both in vivo and in vitro.

Since the electron-donor effect of R_2N- to P=O is stronger than that of the RO– group, the reactivity in addition reactions of the phosphorus compounds obviously decreased. As a result, the reaction conditions would be different with different substituents on the aniline. When electron-donor groups were at the *meta-* or *para*-position of the aniline, the addition proceeded smoothly and in good yield. On the other hand, when an electron-donor group was ortho- to the $-NH_2$ group or an electron-acceptor group was present at the *meta-* or *para*-position, the addition was more difficult and the yield was relatively low.

The synthetic route is shown in Scheme 1.

On studying the spectra of the products, we found that the most stable tautomeric form of each 2,5-dihydro-4,6-dithio-1,3,5,2-triazaphosphorine is

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the dithioenol, not the dithione, which has been regarded as the most stable tautomer up to the present time. Since the product is in an amorphous solid state, it was difficult to obtain a single crystal. Therefore, in order to provide evidence in support of the preceding conclusion, we used quantum chemistry calculations by means of the methods of MNDO and PM3. In fact, all the calculated results indicate that the dithioenol is the most stable form in comparison with the dithione and the thioenol-thione ones.

RESULTS AND DISCUSSION

Conditions of the Addition Reaction

According to the literature [1], the expected product was not obtained as long as the reaction mixture depicted in Scheme 1 was cooled with Dry-Ice. This was probably due to the stronger electron-donor effect of R_2N- adjacent to P=O than that of RO (or –OPh), since the phosphates undergo the reactions at low temperatures. To raise the reactivity of the corresponding dialkylamine phosphorodiisocyanato compound, the reaction temperature was raised to −20–0°C. As a result, the addition of the *meta-* or *para*-substituted anilines to the phosphorodiisocyanate proceeded smoothly and with high yields. The anilines with electron-donor groups at the *o*-position or with electron-acceptor groups at the *o-*, *m-*, or *p*-position could hardly react under similar conditions. Besides the electronic effects, steric hindrance may also play an important role. By changing reaction temperatures and times, 10 different substituted compounds were obtained (see Table 1). Unfortunately, anilines with electron-acceptor groups at the *ortho*-position failed to give any products, no matter how the reaction conditions were changed.

Structures of the Stable Tautomeric Products

The molecular structures of compounds II were confirmed by ^{1}H NMR, and IR, spectra, MS and elemental analyses. Results are given in Table 2.

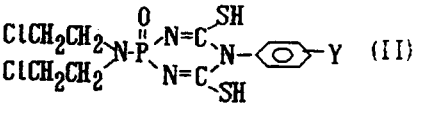
^{1}H NMR spectra of the compounds have been determined in deutero-acetone by use of a 90 MHz-NMR Instrument. The *bis* (2-chloroethyl)-amino group of the titled phosphorus heterocyclic compounds showed the absorption bands of eight protons of the four methylene groups at δ = 3–4 (which appeared as double-multiple peaks) and the protons of the phenyl groups gave peaks above δ = 7. However, if the cyclic structure of the products was of the dithione type, the two protons on nitrogen atoms at δ = 5–8 should be observed, but no signal was found there. To find out the chemical shift of these two protons, the ^{1}H NMR spectrum of II6 was taken repeatedly in deuterated chloroform on a 200 MHz instrument, and the peaks of the two protons were displayed at around δ = 2.6 as a single sharp peak which is consistent with the dithioenol structure.

The FAB and EI mass spectra of all of the II series of the titled phosphorus heterocyclic compounds gave the anticipated molecular ion peaks.

Both FAB and EI mass spectra advantageously lost a small molecule, N≡CSH, giving fragment peaks $M^+ - 59$.

The IR spectra of each II compound displayed the normal absorption bands except for one at 1800–2000 cm^{-1}, a weak broad absorption band which could not be explained by the dithione structure (see Table 2), but could by the dithioenol structure. The absorption of "–N=C–SH" appeared exactly in the range of 1800–2000 cm^{-1}. By comparing the structure of the dithione with that of the tautomeric dithioenol, it has been found that the

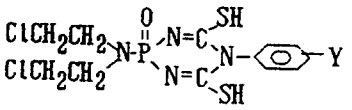
TABLE 1 Data of the Reaction



(II)

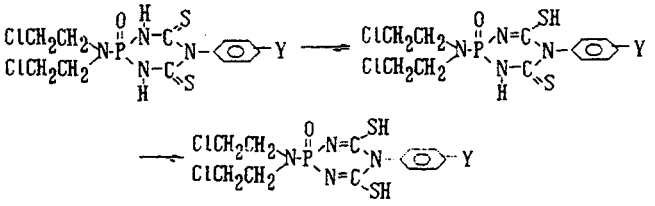
Number	Substituent (Y)	Temperature (°C)	Time (h)	Yield (%)	Mp (°C)
II1	p-Cl	−20	3.5	81.4	118–120
II2	m-Cl	−20–0	4.0	78.1	117–119
II3	o-Cl	−10–20	6.0	51.6	96–97
II4	p-Me	−20	3.0	85.6	114–116
II5	m-Me	−20	4.0	80.3	108–109
II6	o-Me	−20–10	5.5	59.4	107–109
II7	p-Br	−20	4.0	80.9	111–113
II8	H	−20	4.0	78.2	110–111
II9	o-NO_2	−15–20	4.0	63.8	124–126
II10	m-NO_2	−10–40	4.5	56.3	109–110

TABLE 2 Spectral Data and Elemental Analyses



Number	Formula	^1H NMR (δ)	Elemental Analysis (%)			
			IR(cm^{-1})	C	H	N
II1	$C_{12}H_{14}Cl_3N_4OPS_2$ (p-Cl)	2.65, (ds, 2H, 2SH), 3.4–3.9, (dm, 8H, 2Cl CH_2CH_2N), 7.3–7.5 (dd, 4H)	3092, 2861, 1990 (wb) 1664 (m), 1425, 1396, 1316 (s, P=O) .1163, 1000	3.22 (3.39)	3.40 (3.25)	12.80 (12.97)
II2	$C_{12}H_{14}Cl_{13}N_4OPS_2$ (m-Cl)	3.4–4.0 (dm, 8H), 7.2–9 (tm, 4H)	3090, 2977, 2816, 1970 (wb), 1602 (m) 1570, 1471, 1309 (m) 1160, 980	33.64 (33.39)	3.25 (3.25)	12.88 (12.97)
II3	$C_{12}H_{14}Cl_{13}N_4OPS_2$ (o-Cl)	3.4–3.9 (dm, 8H), 7.4–7.7 (sb, 4H)	3068, 2449, 1968 (wb), 1480, 1439, 1223 (m), 1078, 793	33.08 (33.39)	3.19 (3.25)	12.92 (12.97)
II4	$C_{13}H_{17}Cl_2N_4OPS_2$ (p-Me)	2.3 (s, 3H), 3.5–3.9 (dm, 8H), 7.1–7.3 (d, 2H), 7.4–7.6 (d, 2H)	3090, 2977, 2820, 1985, (wb), 1608 (m), 1429, 1381, 1228 (m), 1167, 1002	37.90 (37.84)	4.01 (4.16)	13.76 (13.62)
II5	$C_{13}H_{17}Cl_2N_4OPS_2$ (m-Me)	2.3 (s, 3H), 3.4–4.1 (dm, 8H), 7.0–7.9 (m, 4H)	3061 (w), 2986, 2820, 1980 (wb), 1608 (m), 1429, 1381, 1228 (m), 1167, 1002	37.00 (37.84)	4.11 (4.16)	13.64 (13.62)
II6	$C_{13}H_{17}Cl_2N_4OPS_2$ (o-Me)	2.2–2.3 (d, 3H), 2.61 (s, 2H, 2SH), 3.3–3.9 (dm, 8H), 7.2–7.5 (sb, 4H)	3053, 2986, 2825, 1981 (wb), 1608 (m), 1429, 1390, 1229 (m), 1002	38.01 (37.84)	4.25 (4.16)	13.87 (13.62)
II7	$C_{12}H_{14}BrCl_2N_4OPS_2$ (p-Br)	3.3–3.9 (dm, 8H), 7.1–7.8 (mb, 4H)	3097, 2974, 2820, 1970 (wb), 1425, 1382, 1314 (m), 1164, 980	30.05 (30.27)	2.90 (2.96)	11.51 (11.76)
II8	$C_{12}H_{15}Cl_2N_4POS_2$ (H)	3.2–4.1 (dm, 8H), 7.1–7.5 (bm, 3H), 7.5–7.8 (mb, 2H)	3090, 2990, 2820, 1970 (wb), 1605 (m), 1420, 1310 (m), 1190, 1161, 985	35.97 (36.28)	3.73 (3.81)	14.18 (14.09)
II9	$C_{12}H_{14}Cl_2N_5PO_3S_2$ (p-NO_2)	3.4–4.0 (dm, 8H), 6.7–6.8 (d, H), 7.9–8.4 (mb, 3H)	3053, 2971, 1980 (wb), 1422, 1342, 1268, (m), 1112, 988	32.20 (32.59)	3.06 (3.19)	15.56 (15.82)
II10	$C_{12}H_{14}Cl_2N_5PO_3S_2$ (m-NO_2)	3.5–4.1 (dm, 8H), 7.2–9.0 (tm, 4H)	3156, 2984, 2810, 2023 (wb), 1517, 1427, 1205 (m), 1169, 1096, 999	32.72 (32.59)	3.22 (3.19)	15.69 (15.82)

empty 3d-orbital of the phosphorus atom, a lone pair of electrons of the nitrogen atom on position-5, and two "C=N" double bonds formed a Hückel aromatic six-membered ring. It is thought that there might exist an equilibrium between the dithione and dithioenol.



In contrast, for the structure of the dithione, the stereoelectronic effect is very strong for the three lone pair electrons of the three nitrogen atoms repelling one another [6]. Therefore, this might be one of the causes leading to the instability of the dithione. In addition, the radius of the sulfur atom is much larger than that of the carbon atom, so that the double bond formed between them is unstable. The results from analysis of the spectra are consistent with these conclusions.

In order to provide additional evidence for the accuracy of the conclusions concerning the stability of the tautomers, calculations by quantum chemistry methods, MNDO and PM3, have been employed.

The results of the two methods are consistent with each other, indicating that the most stable structure among the tautomers is that of the dithioenol (see Table 3).

According to the MNDO method, ΔET between the dithioenol and dithione is −0.2468 ev, which is equal to about −6 kcal/mol. Similarly, ΔET between the dithioenol and dithione by the PM3 method is −0.841 ev, equal to about −20 kcal/mol.

All the data in Table 3 suggest that, at room temperature, the three tautomers exist simultaneously and are in equilibrium with one another, but the main tautomer is the dithioenol, not the dithione.

Antitumor Activities

The results of preliminary antitumor tests indicated that most of the II series of the phosphorus heterocyclic compounds inhibited Leukemia of L1210 tumor cell selectively. However, they were not effective against the Bel-7402 and BGC-823 cells. The data from the bioassays are listed in Table 4.

EXPERIMENTAL

Instruments

The elemental analyses were performed with a CHN CORDERD MT-3 elementary analyzer. ^{1}H NMR spectra were recorded with JEOL-FX-90Q and BRUKER AC-P200 spectrometers. TMS was used as an internal standard for ^{1}H NMR spectra. The IR spectra were measured by using a SHIMADZU-435 instrument. Melting points were determined with a Thomas–Hoover melting point apparatus and the thermometer was not standardized.

N,N-Bis(2-chloroethyl)ammonium Chloride

48 g (0.46 mol) of diethanolamine and 70 mL chloroform were added into a 500 mL three-necked flask. A mixture of 130 mL (1.83 mol) of thionyl chloride and 80 mL of chloroform was dropped in slowly with stirring. Heat was evolved. Stirring was

TABLE 3 Data of MNDO and PM3 Methods

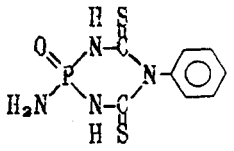
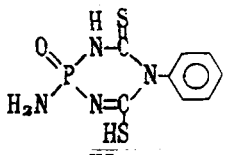
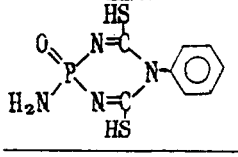
Model	*Hf (kcal/mol)*		*ET(ev)*	
	MNDO	*PM3*	*MNDO*	*PM3*
	36.77	8.67	−2913. 5203	−1522. 6223
	31.28	−3.68	−2913. 7582	−2523. 1574
	31.07	−10.72	−2913. 7631	−2523. 4633

TABLE 4 Data of Antitumor Activity

Number	Substituent (Y)	Cancer Cell	Concentration (μg/mL)	Inhibition Rate (%)
II1	p-Cl	L_{1210}	25	67.617
II2	m-Cl	L_{1210}	10	74.30
			25	92.79
II3	o-Cl	L_{1210}	25	77.15
II4	p-Me	L_{1210}	25	87.73
II6	o-Me	L_{1210}	10	80.69
			25	92.54
II7	p-Br	L_{1210}	10	73.68
			25	98.58
II8	H	L_{1210}	10	72.61
			25	92.64
II9	p-NO_2	L_{1210}	10	85.29
			25	91.93
II10	m-NO_2	L_{1210}	10	86.73
			25	97.09

maintained for 1 hour, and the reaction mixture was allowed to stand overnight. The excess of thionyl chloride was removed by distillation under vacuum. The solid residue was recrystallized from acetone giving 10.5 g (61.9%) of colorless salt, mp 214–216°C (lit. [6]: 216°C).

N,N-Bis(2-chloroethyl) Dichlorophosphoramide

10 g (0.056 mol) of N,N-*bis*(2-chloroethyl) ammonium chloride was added in portions to 100 mL of redistilled trichlorophosphorus oxide. The reaction mixture was refluxed for about 17 hours until the solid salt had disappeared. Excess $POCl_3$ was removed by evaporation, giving a brown viscous substance which was recrystallized from acetone-petroleum ether (1:5) to afford 10.8 g (75.6%) of colorless crystals, mp 52 ~ 54°C (lit. [5]: 54–56°C).

N,N-Bis(2-chloroethyl) Diisothiocyanato-phosphoramide

2 g (7.7 mmol) of N,N-bis(2-chloroethyl) dichlorophosphamide and 40 mL of anhydrous acetonitrile were added into a three-necked flask. 1.9 g (19.6 mmol) of KNCS was added portionwise at 10°C with stirring. The mixture was stirred for another 3 hours. The solvent was removed by evaporation on a rotary evaporator under reduced pressure. The crude product was purified by flash chromatography on a silica gel column using a mixture of chloroform, ether, and petroleum ether (1:1:3 V/V) as the eluent. 1.86 g (79.2%) of colorless crystals was obtained, mp 64–65°C.

Elemental Analysis

	C%	H%	N%
Found	23.45	2.48	13.71
Calculated	23.69	2.65	13.81

^{1}H NMR (δ): 3.4–3.8 (dm, 8H, 2Cl CH_2CH_2)
^{31}P NMR (δ): 28.8
IR (cm^{-1}): 2997,1966 (s, –N=C=S)
1265 (m, P=O), 1096, 1024
932
MS (EI, *m/e*): 305 (M^+ +2), 303 (M^+), 254 (base peak), 192

2-[Bis(2-chloroethyl)]amino-2,5-dihydro-4,6-dithiohydroxy-5-substituted phenyl-triazaphosphorine-2-oxide

2 g (6.6 mmol) of N,N-bis-(2-chloroethyl)diisothiocyanato phosphoramide was dissolved in 80 mL of anhydrous toluene. The same molar amount of substituted aniline in 20 mL of anhydrous toluene was added dropwise at −20 ~ (−10°C) with stirring. The reaction mixture was stirred for 2 or 3 hours, being maintained at the same temperature, and then stirred for another 4–10 hours until the solid which had precipitated gradually did not increase. The solvent was removed. The crude product was recrystallized from acetone and ether (1:3), yield 50–86%.

ACKNOWLEDGMENTS

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