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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 2190–2196
Bioorganic & Medicinal Chemistry

Novel α-aminophosphonic acids. Design, characterization, and biological activity

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Received 28 July 2005; revised 26 October 2005; accepted 2 November 2005
Available online 2 December 2005

Abstract—Novel α-aminophosphonic acids are synthesized reacting 1,3-oxazolidin-2-one derivatives with formaldehyde and phosphorus trichloride. Treatment of *N*-(phosphonomethyl)oxazolidinones with aqueous NaOH gave the expected α-aminophosphonic acids. The oxidation of (2-hydroxy-1,1-dimethylethylamino)methyl phosphonic acid in the presence of CdO and water resulted in *N*-phosphonomethyl-2-methyl-1-propanoic acid. Their structures were proved by means of IR, ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy. The genotoxic, clastogenic, and antiproliferative effects of newly synthesized original aminophosphonic acids were investigated for the first time.

1. Introduction

Aminophosphonic acids constitute an important class of biologically active compounds, and their synthesis has been a focus of considerable attention in synthetic organic chemistry as well as in medicinal chemistry. These acids are considered to be structural analogues of the corresponding amino acids, thus acting as competitive inhibitors and they can act as false substrates during the course of amino acid metabolism. In addition, aminophosphonic acids express other forms of biological activity including antibiotics and enzyme inhibiting effects.[1] Numerous applications have been found for aminophosphonic acids: in the design of enzyme inhibitors,[2–5] as plant growth regulators,[6] as antibacterial agents,[7] as neuroactive compounds,[8] and as herbicides, antifungal agents,[9–13] and anti-HIV agents.[14] The herbicidal activity of *N*-(phosphonomethyl) glycine is reported by Baird et al.[15] Another study[16] demonstrated that the *N*-(phosphonomethyl) glycine is effective in inhibiting test-tube growth of *Plasmodium falciparum*, the parasite that causes malaria. Additionally, it has the same effect on related types of single-celled parasites such as *Toxoplasma* and *Cryptosporidium* which cause opportunistic infections in AIDS patients.[17] Moreover, it has been found that the *N*-(phosphonomethyl) glycines are especially effective in suppressing the growth of cancer, tumor, virus, or bacteria. A pharmaceutical composition for treatment of mammals, warm-blooded animals, and humans, comprising a pharmaceutical carrier and an effective amount of a chemotherapeutic agent and anticancer compound selected from the group consisting of *N*-(phosphonomethyl) glycine derivatives.[18]

Consequently, development of new methods for the synthesis of α-aminophosphonic acids is an active area of research and many methods are now available: Kabachnik and Medved[19] and Fields[20] have discovered the first method for the preparation of α-aminophosphonic acids reacting ammonia or amine, dialkyl H-phosphonate, and aldehydes; the reaction of dialkyl H-phosphonates[21] or phosphorous acid[22] with imines afforded also α-aminophosphonic acids. α-Aminophosphonic acids are obtained reacting amides,[23] or 2-oxazolidinone derivatives,[24] with formaldehyde and phosphorus trichloride.

Keywords: Aminophosphonic acids; *N*-(Phosphonomethyl) glycine; 2-Oxazolidinone derivatives; Chromosome aberrations; Cell proliferation; Clastogenic effects.
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doi:10.1016/j.bmc.2005.11.002

α-Aminophosphonic acids bearing heterocyclic, aromatic rings such as furane, anthracene,[25,26] thiophene, pyrazole, imidazole, and pyridine are described.[27]

There are no available data on the genotoxic and antiproliferative effects of the investigated aminophosphonic acids, but many studies concerning the genotoxic effects of *N*-phosphonomethyl glycine (glyphosate) are carried out. The obtained results are rather contradictory.

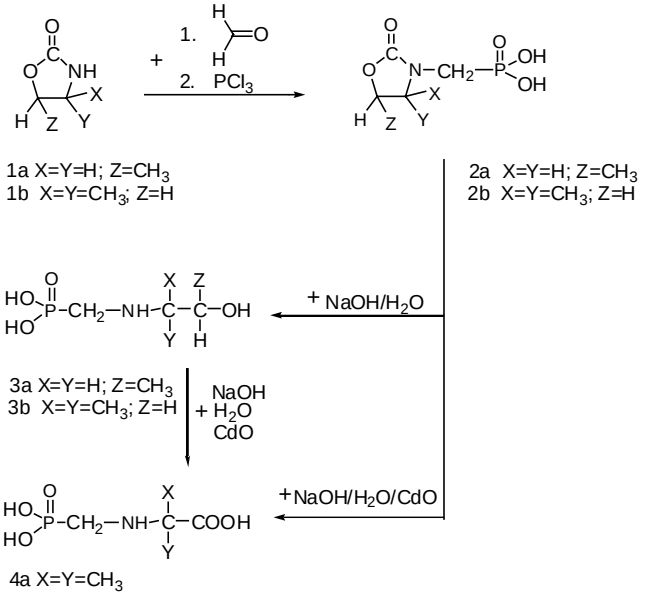
This article is a continuation of our study on the synthesis of α-aminophosphonic acids. Herein we describe the synthesis of novel α-aminophosphonic acids reacting phosphorus trichloride with formaldehyde and 1,3-oxazolidin-2-one derivatives. For the total characterization of these newly synthesized derivates of *N*-phosphonomethyl glycine it is very important and appropriate to investigate their genotoxic and antiproliferative effects. The present study is the first proof of genotoxic and antiproliferative effects of some original aminophosphonates.

2. Results and discussion

2.1. Chemistry

In our present study, we used the procedure, described by Pikl and Engelmann,[23] according to which as starting compounds for the preparation of the aminophosphonic acids are used phosphorus trichloride, formaldehyde, and 2-oxazolidinone derivatives **1a** and **1b**. The synthetic route is shown in Scheme 1.

In the first step of the interaction, *N*-(phosphonomethyl)oxazolidinones **2** was obtained in good yield and their formation were confirmed by ^{1}H, $^{13}C\{H\}$, ^{31}P NMR, and IR spectroscopy. The doublet in the ^{1}H NMR spectrum of **2a** at 3.48 ppm with $^{2}J_{P,H} = 10.4$ Hz and at 3.32 ppm with $^{2}J_{P,H} = 11.2$ Hz for **2b** can be assigned



Scheme 1. Synthesis of aminophosphonic acids.

for the P-CH_2 protons. These data confirm the formation of P–C bond. The data from the $^{13}C\{H\}$ NMR spectrum also confirm this conclusion. The doublet for **2a** at 42.45 ppm with $^{1}J_{P,H} = 154.0$ Hz and at 38.96 ppm with $^{1}J_{P,H} = 157.57$ Hz for **2b** has to be assigned to the carbon atom connected to phosphorus P-*C*H_2. The triplet in the ^{31}P NMR spectrum at 17.32 ppm with $^{2}J_{P,H} = 12.20$ Hz and that at 17.70 ppm with $^{2}J_{P,H} = 11.17$ Hz can be assigned to the phosphorus atoms of **2a** and **2b**, respectively. Treatment of **2** with aqueous NaOH gave the expected α-aminophosphonic acids **3**. The hydrolysis of oxazolidinone ring is accompanied by decarboxylation. Assignment of structures **3a** and **b** was made on the basis of ^{1}H and ^{13}C NMR spectral data reported in the Experimental part. The oxidation[28] of **3b** in the presence of CdO and water[29] results in *N*-phosphonomethyl-2-methyl-1-propanoic acid **4**. In the $^{13}C\{H\}$ NMR spectrum of **4**, there is no signal at 64.26 ppm for the carbon atom of the *C*H_2-OH group. The new signal at 173.40 ppm can be assigned to the carbonyl carbon atom of the carboxylic group, which is formed as a result of the oxidation of CH_2OH group. IR spectroscopy study confirms the structure of the above-mentioned compounds. Further studies for the preparation of *N*-(phosphonomethyl) glycine derivatives are in progress.

2.2. Biological activity

The obtained results of the induced chromosome aberrations, frequency in bone marrow mice cells after **2a**, **2b**, **3a**, **3b**, and **4** treatments are presented consequently in Table 1. (5-Methyl-2-oxo-1,3-oxazolidin-3-yl)methylphosphonic acid (**2a**), 10 mg/kg (24th hour) provoked 4.33% chromosome aberrations, predominantly centromer/centromeric fusions (c/c). On the 48th hour the clastogenic effect of the injected compound slightly decreased. In the experimental mice groups treated with 100 mg/kg, the percentages of aberrant bone marrow cells were higher than those injected with **2a**, 10 mg/kg, especially on the 48th hour groups ($p < 0.01$). In the higher concentration group, there was no significant difference between the samples on the 24th and 48th hour. Hundred milligrams per kilogram **2a** concentration ensures enough amounts of active molecules and provokes the same percentage of aberrations on the 48th hour too.

After the (4,4-dimethyl-2-oxo-1,3-oxazolidin-3-yl)methylphosphonic acid (**2b**) administration, the percentages of cells with aberration for the two applied concentrations and for the samples from 24th and 48th hours were slightly different but the differences in the calculated values were not significant ($p > 0.05$).

A more detailed analysis has showed that **2b** (10 mg/kg, 24th and 48th hour) induced predominantly c/c fusions (4.33 ± 0.61 and 4 ± 0.52%, respectively). The percentage of breaks and fragments was significantly lower (1 ± 0.43%) (24th hour) and 0.6 ± 0.27 (48th hour).

There was no difference in the percentage of chromosome aberrations between 24th and 48th hour after **2b**, 100 mg/kg injection. On the 24th hour highly predominated c/c fusions ($p < 0.001$).

Table 1. Frequencies of chromosome aberrations and mitotic activity in affected C57Bl mouse bone marrow cells after ip treatment with novel α-aminophosphonic acids

Compound and doses	Interval (h)	Analyzed metaphases	Type of aberrations					Metaphases with more than two aberrations	Cells with aberrations, % ($\bar{X} \pm SE$)	Mitotic index, ‰ ($\bar{X} \pm SE$)
			Breaks	Fragments	Exchanges					
					c/c	t/t	c/t			
2a, 10 mg/kg	24	220	2	0	7	2	0	0	4.33 ± 0.97	9.42 ± 1.02
	48	300	0	0	9	1	0	0	2.67 ± 0.42	7.09 ± 0.50
2a, 100 mg/kg	24	300	2	0	8	1	0	0	6.25 ± 0.62	6.69 ± 0.50
	48	250	2	1	10	0	0	0	5.20 ± 0.48	5.12 ± 0.34
2b, 10 mg/kg	24	300	2	1	13	0	0	0	6.00 ± 1.03	5.13 ± 0.76
	48	300	2	0	12	0	0	0	4.67 ± 0.67	5.99 ± 0.41
2b, 100 mg/kg	24	300	0	0	12	0	0	0	4.00 ± 0.52	5.76 ± 0.56
	48	300	1	1	14	0	0	0	5.33 ± 0.84	6.15 ± 0.73
3a, 10 mg/kg	24	300	3	2	11	0	0	2	6.00 ± 0.73	5.43 ± 0.31
	48	300	0	0	17	0	0	2	5.67 ± 0.80	7.10 ± 0.47
3a, 100 mg/kg	24	300	2	5	16	0	0	0	6.64 ± 0.86	5.21 ± 0.49
	48	300	3	3	14	0	0	0	6.67 ± 0.67	5.91 ± 0.54
3b, 10 mg/kg	24	300	2	0	7	0	0	0	3.00 ± 0.45	11.92 ± 0.39
	48	300	2	0	6	0	0	0	2.67 ± 0.42	12.35 ± 0.66
3b, 100 mg/kg	24	300	3	4	11	0	0	0	5.00 ± 0.68	8.53 ± 0.91
	48	300	3	0	6	0	0	0	3.00 ± 0.45	5.2 ± 0.54
4, 10 mg/kg	24	300	3	0	6	0	0	0	3.00 ± 0.45	10.39 ± 0.65
	48	300	2	1	5	0	0	2	2.67 ± 0.42	10.96 ± 0.19
4, 100 mg/kg	24	300	4	4	4	0	0	0	4.00 ± 0.52	5.18 ± 0.42
	48	300	1	0	7	0	0	0	2.67 ± 0.42	5.25 ± 0.96
Mitomycin C, 3.5 mg/kg	24	150	10	13	11	0	0	36	46.67 ± 3.03	5.49 ± 0.19
0.9% NaCl	24	200	0	0	2	0	0	0	1.00 ± 0.57	17.3 ± 1.00

c/c, centromer/centromeric fusions; t/t, telomer/telomeric fusions; c/t, centromer/telomeric fusions.

(2-Hydroxypropylamino)methylphosphonic acid (**3a**) at a concentration of 10 mg/kg caused the presence of 6 ± 0.73% (24th hour) and 5.67 ± 0.8% (48th hour) bone marrow cells with aberrant metaphases. Significant differences between treated groups injected with 10–100 mg/kg at the two time span intervals were not detected ($p > 0.05$). The values of c/c fusions were close to those obtained for **2a**. The number of cells with breaks and fragments was significantly lower. Metaphases with more than two aberrant chromosomes were also observed.

In the experimental mice samples treated with (2-Hydroxy-1,1-dimethylethylamino)methylphosphonic acid (**3b**), 10 mg/kg (24th hour), a very low percentage of chromosome aberrations was obtained. In the groups, treated with 100 mg/kg **3b**, the value of aberrant mitoses on the 24th hour significantly increased ($p < 0.05$).

N-Phosphonomethyl-2-methyl-1-propanoic acid (**4**) at 10 mg/kg induced 3.0 ± 0.45% chromosome aberrations at the 24th hour and 2.67 ± 0.42% at the 48th hour ($p>0.05$). A tendency to higher incidences of chromosome changes—c/c fusions, specific for **2b** and **3a**, was also followed.

There were no significant differences between the percentage of chromosome aberrations in the experimental groups, treated with 10 and 100 mg/kg. The higher dose applied induced only 4 ± 0.52% on the 24th hour and 2.67 ± 0.42% on the 48th hour.

Generally, in the most treated groups the percentage of aberrant metaphases differed significantly from that of the control groups, except for **3b** and **4**, 10 mg/kg. This percentage was significantly higher than that of negative control and significantly lower than that of positive control—Mitomycin C ($p < 0.01$). These results are evidence for some of the clastogenic effects of the studied aminophosphonic acids. Furthermore, no dispersed metaphases were observed on the slides of the aminophosphonic acid experimental groups in comparison with those of the Mitomycin C positive control.

Some other conclusions about the relatively low genotoxicity of the studied aminophosphonic acids could be drawn using the data of the correlation between the different types of aberrations. The investigated aminophosphonic acids in the two applied concentrations induced c/c fusions predominantly. The number of metaphase plates with chromosome breaks and fragments was significantly lower. For a comparison, in the negative control group cells with breaks and fragments were not found, but in the positive Mitomycin C control the percentage was very high up to 71.3% (or 29.33% of the total number of analyzed cells).

It can be summarized that all the five investigated aminophosphonic acids possess moderate clastogenic effects. The analysis of the type of chromosome aberrations obtained after the treatment of the investigated compounds showed that in all the experimental groups centromeric fusions predominated, with the exception of the **4** 100 mg/kg (24th hour) group, where the values of chromosome breaks were significantly higher in all variants. These results allowed a hypothesis that newly synthesized aminophosphonic acids damage predomi-

nantly the centromeric chromosome regions. This might be facilitated by the telocentric nature of mice chromosomes, which allows centromer/centromeric recombinations between non-homologous chromosomes without altering the normal mice genome.

The effect of the newly synthesized aminophosphonic acids on the proliferative activity of bone marrow cells was evaluated by the mitotic index parameter. The obtained results are presented in Table 1.

The mitotic index values (MI) of bone marrow cells in the groups, treated with **2a** in a dose of 10 mg/kg, slightly decreased especially on the 24th hour after treatment. Compound **2a** at a dose of 100 mg/kg had a strong antiproliferative affect and the values of MI were 6.69 ± 0.50 and 5.12 ± 0.34‰, respectively.

After a 10 mg/kg **2b** treatment, the mitotic activity on the 24th hour (5.13 ± 0.76‰) was close to that on the 48th hour (5.99 ± 0.41‰). The data about the effect of **2b** 100 mg/kg were closely similar to those after a 10 mg/kg treatment.

Significant differences between the mitotic index values obtained for the investigated bone marrow cell populations at the 24th and 48th hours, treated with the two applied **3a** concentrations, were not found. MI varied from 5.21 ± 0.49 to 7.10 ± 0.47‰.

Compounds **4** and **3b** in dose 10 mg/kg had a comparatively low expressed antiproliferative effect—from 10.39 to 12.35‰. The higher concentration in both applied compounds (**4** and **3b**) suppressed significantly the cell division in bone marrow cells (8.53‰ for **3b** on the 24th hour to 5.18‰ for **4** on the 24th hour).

All the five investigated aminophosphonic acids on bone marrow mice test system induced a statistically significant ($p < 0.05$) decrease of the proliferative activity.

3. Conclusion

We describe the synthesis of novel α-aminophosphonic acids reacting 1,3-oxazolidin-2-one derivatives with formaldehyde and phosphorus trichloride.

The clastogenic and antiproliferative effects of the newly synthesized original aminophosphonic acids were investigated for the first time.

The studied compounds did not possess clear expressed relationship 'dose–effect' (high percentage of aberrations after higher dose applied) in their clastogenic effects as this relationship is specific for the alkylating agent Mitomycin C.

Comparatively, low percentage of bone marrow metaphases with chromosome aberrations and the lack of aberrant metaphase plates with disintegrated chromosomes and dispersed chromatin are evidence of the moderate clastogenic effect of the newly synthesized aminophosphonic acids.

Metaphase analysis showed that the changes of the chromosome structure in the bone marrow cells of the treated animals were predominantly c/c fusions and rarely breaks and fragments. These results suggest that the investigated compounds affect the centromeric chromosome regions, which allow centromer/centromeric recombinations between non-homologous chromosomes without altering the normal mice genome.

The correlation between the moderate clastogenic effect and the low values of mitotic index (MI), obtained after the treatment with **2a** and **2b**, 100 mg/kg, **3a** and **4** 100 mg/kg is an interesting fact. We consider further detailed investigations on the experimental tumor models in vivo and in vitro as appropriate.

4. Experimental

Melting points (mp) were determined on a Koffler microscope and are uncorrected. The infrared (IR) spectra in KBr were recorded on a Perkin-Elmer Model 1600 Series FTIR instrument. The purity of the products was checked by TLC on precoated plates of Silica gel 60 F_{254} (Merck) using a mobile phase *n*-BuOH/AcOH/H_2O, 3:1:1. Spots on TLS chromatograms were detected by chlorine/*o*-toluidine reaction. The microanalyses were performed on a Perkin-Elmer elemental analyzer. All compounds' characterization with ^1H, ^{13}C, and ^{31}P NMR spectra was recorded on a Bruker DRX 250 spectrometer.

4.1. General methods

Phosphorus trichloride, paraformaldehyde, 1-amino-2-propanol, 2-amino-2-methyl-1-propanol, and 1,3-dioxolan-2-one (ethylene carbonate) were purchased from Aldrich and used without further purification. 2-Oxazolidinone derivatives were synthesized by reacting an equimolar amount of ethylene carbonate with amino alcohol at temperatures below 10 °C[29]. After that, the reaction mixture allowed to stand at 60 °C for 6 h. The resulting substituted 2-oxazolidinones were isolated by vacuum distillation. All compound characterization with ^1H, ^{13}C, and ^{31}P NMR spectra was recorded on a Bruker DRX 250 spectrometer.

4.2. 5-Methyl-1,3-oxazolidin-2-one (1a)

The titled compound was obtained reacting 1,3-dioxolan-2-on with 1-amino-2-propanol. Bp 75–77 °C/0.05 mmHg. Yield 87%; solid product. ^1H NMR (250 MHz, CD_3Cl): $\delta = 1.45$ (d, $^3J_{H,H} = 3.8$ Hz, 3H, CH_3), 3.65–3.71 (m, 2H, *N*-CH_2), 4.73–4.81 (m, 1H, CH), 6.8 (br s, 1H, NH) ppm. $^{13}C\{H\}$ NMR (250 MHz, $CDCl_3$): $\delta = 20.84$ (CH_3), 47.78 (*N*-CH_2), 73.90 (CH), 160.91 (C=O) ppm.

4.3. 4,4-Dimethyl-1,3-oxazolidin-2-one (1b)

The titled compound was obtained reacting 1,3-dioxolan-2-on with 2-amino-2-methyl-1-propanol. Bp 95 °C/0.05 mmHg, mp 48–51 °C. Yield 85%; solid product.

^{1}H NMR (250 MHz, CD_3Cl): δ = 1.23 (s, 6H, CH_3), 3.9 (s, 2H, O-CH_2), 6.8 (br s, 1H, NH) ppm. ^{13}C{H} NMR (250 MHz, $CDCl_3$): δ = 24.82 (CH_3), 59.3 C4 atom, 73.90 (O-CH_2), 159.91 (C=O) ppm.

4.4. (5-Methyl-2-oxo-1,3-oxazolidin-3-yl)methylphosphonic acid (2a)

5-Methyl-1,3-oxazolidin-2-one (8.66 g, 0.085 mol) and paraformaldehyde (2.57 g, 0.085 mol) were added in a four-necked round-bottomed flask equipped with a magnetic stirrer, reflux condenser, thermometer, dropping funnel, and argon inert. At vigorous stirring glacial acetic acid 42.6 mL was added dropwise. A white suspension formed. The reaction mixture was refluxed (approximately 115 °C) for 45 min, after which it became a clear solution. Then the temperature was lowered to 20 °C and 7.5 mL phosphorus trichloride was added dropwise. During and after the addition, hydrogen chloride evolved. The reaction mixture was refluxed (approximately 118 °C). After 2 h refluxing, 48.5 mL water (distilled) was added. After 1 h and 30 min refluxing, the reaction mixture was concentrated under reduced pressure. The crude mixture was purified by crystallization from the mixture ethyl acetate/ethyl alcohol 2:1. Yield 9.62 g (57.5%); mp 160–162 °C; R_f (*n*-BuOH/AcOH/H_2O, 3:1:1) = 0.37.

IR (KBr) 3400–3000 (NH^+ and OH, br), 1718 (C=O), 1309, 1268 (P=O), 1074, 975 (C–O–C) cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.32 (d, $^3J_{H,H}$ = 6.2 Hz, 3H, C*H*$_3$), 3.24 (t, 1H, $^3J_{H,H}$ = 7.8 Hz, *N*-C*H*$_2$) and 3.78 (t, 1H, $^3J_{H,H}$ = 8.4 Hz, *N*-C*H*$_2$), 3.48 (d, $^2J_{P,H}$ = 10.4 Hz, 2H, P-C*H*$_2$), 4.60 (sex, 1H, $^3J_{H,H}$ = 7.8 Hz, O-C*H*), 9.23 (br s, 2H, P-OH) ppm. ^{13}C{H} NMR (250 MHz, $CDCl_3$): δ = 21.17 (*C*H$_3$), 42.45 (d, $^1J_{P,H}$ = 154.0 Hz, P-*C*H$_2$), 52.21 (*N*-*C*H$_2$), 70.72 (O-*C*H), 158.15 (C=O) ppm. ^{31}P NMR (250 MHz, DMSO): δ = 17.32 (t, $^2J_{P,H}$ = 12.2 Hz) ppm. ^{31}P NMR (250 MHz, D_2O): δ = 20.65 (t, $^2J_{P,H}$ = 10.47 Hz) ppm. Anal. Calcd for $C_5H_{10}NO_5P$ (195.11): C, 30.78%; H, 5.17%; N, 7.18%; P, 15.87%. Found: C, 30.48%; H, 5.02%; N, 7.05%; P, 15.23%.

4.5. (4,4-Dimethyl-2-oxo-1,3-oxazolidin-3-yl)methylphosphonic acid (2b)

4,4-Dimethyl-1,3-oxazolidin-2-one (9.00 g, 0.078 mol) and paraformaldehyde (2.34 g, 0.078 mol) were added in a four-necked round-bottomed flask equipped with a magnetic stirrer, reflux condenser, thermometer, dropping funnel, and argon inert. At vigorous stirring glacial acetic acid 38.76 mL was added dropwise. The reaction mixture was refluxed (approximately 115 °C) for 45 min, after which it became a clear solution. Then the temperature was lowered to 20 °C and 6.8 mL phosphorus trichloride was added dropwise. During and after the addition, hydrogen chloride evolved. The reaction mixture was refluxed (approximately 118 °C). After 2 h refluxing, 44 mL water (distilled) was added. After 1 h and 30 min refluxing, the reaction mixture was concentrated under reduced pressure. The crude mixture was purified by crystallization from ethyl alcohol. Yield 11.5 g (70.3%); mp 157–158 °C; R_f (*n*-BuOH/AcOH/H_2O, 3:1:1) = 0.40. IR (KBr) 2986–2920 (C–H), 2315 (NH^+) 1687 (C=O), 1304, 1246 (P=O), 1158, 1064, 1012 (C–O–C) cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.23 (s, 6H, C*H*$_3$), 3.30 (d, 2H, $^2J_{H,H}$ = 11.2 Hz, P-C*H*$_2$), 3.90 (s, 2H, O-C*H*$_2$), 7.90 (br s, 2H, P-OH) ppm. ^{13}C{H} NMR (250 MHz, $CDCl_3$): δ = 24.82 (*C*H$_3$), 38.96 (d, $^1J_{P,H}$ = 154.0 Hz, P-*C*H$_2$), 59.30 (C4), 75.12 (C5), 157.86 (C=O) ppm. ^{31}P NMR (250 MHz, DMSO): δ = 17.70 (t, $^2J_{P,H}$ = 11.17 Hz) ppm. ^{31}P NMR (250 MHz, D_2O): δ = 21.53 (t, $^2J_{P,H}$ = 11.10 Hz) ppm. Anal. Calcd for $C_6H_{12}NO_5P$ (209.14): C, 34.46%; H, 5.78%; N, 6.70%; P, 14.81%. Found: C, 34.18%; H, 5.65%; N, 6.48%; P, 14.20%.

4.6. (2-Hydroxypropylamino)methylphosphonic acid (3a)

(5-Methyl-2-oxo-1,3-oxazolidin-3-yl)methylphosphonic acid (**2a**) (5.0 g, 0.025 mol), NaOH (10.12 g, 0.25 mol) in 85.6 mL H_2O were placed in a three-necked round-bottomed flask equipped with a magnetic stirrer, reflux condenser, and thermometer. The reaction mixture was heated at 140 °C for 7.30 h. Subsequently, the reaction mixture was treated with Dowex 50WX8-200 in order to exchange the sodium cations by hydrogen-ion exchange. The crude mixture was purified by crystallization from water. Yield, 2.75 g (63%); mp 198–200 °C; R_f (*n*-BuOH/AcOH/H_2O, 3:1:1) = 0.21.

IR (KBr) 3413–3000 (NH and OH, br), 1579 (NH), 1312 (P=O), 1097, 1064 (C–O, CH-OH) cm^{-1}. ^{1}H NMR (250 MHz, D_2O): δ = 1.18 (d, $^3J_{H,H}$ = 6.4 Hz, 6H, C*H*$_3$), 2.61 (d, $^3J_{P,H}$ = 13.0 Hz, 2H, P-C*H*$_2$), 3.95 (sex, $^3J_{H,H}$ = 6.3 Hz, 1H, C*H*-OH) ppm.^{13}C{H} NMR (250 MHz, D_2O): δ = 20.0 (*C*H$_3$), 47.50 (d, $^1J_{P,C}$ = 136.30 Hz, P-*C*H$_2$) 57.25 (*N*-*C*H$_2$), 66.03 (*C*H-OH) ppm. ^{31}P NMR (250 MHz, D_2O): δ = 9.90 ppm. Anal. Calcd for $C_4H_{12}NO_4P$ (169.12): C, 28.41%; H, 7.15%; N, 8.28%; P, 18.32%. Found: C, 28.31%; H, 7.09%; N, 8.19%; P, 18.17%.

4.7. (2-Hydroxy-1,1-dimethylethylamino)methylphosphonic acid (3b)

(4,4-Dimethyl-2-oxo-1,3-oxazolidin-3-yl)methylphosphonic acid (**2b**) (1.5 g, 0.007 mol), NaOH (4.72 g, 0.118 mol), and 23.6 mL H_2O were placed in a three-necked round-bottomed flask equipped with a magnetic stirrer, reflux condenser, and thermometer. The reaction mixture was heated at 130 °C. Subsequently, the reaction mixture was treated with Dowex 50WX8-200 in order to exchange the sodium cations by hydrogen-ion exchange. The crude mixture was purified by crystallization from water. Yield 1.15 g (88%) mp = 130–132 °C; R_f (*n*-BuOH/AcOH/H_2O, 3:1:1) = 0.50.

IR (KBr) 3413–3000 (NH and OH, br), 1631 (NH), 1269 (P=O), 1080 (C–O, CH_2-OH) cm^{-1}. ^{1}H NMR (250 MHz, D_2O): δ = 1.34 (s, 6H, C*H*$_3$), 3.05 (d, $^2J_{P,H}$ = 13.5 Hz, 2H, P-C*H*$_2$), 3.64 (s, 2H, C*H*$_2$-OH) ppm. ^{13}C{H} NMR (250 MHz, D_2O): δ = 19.72 (*C*H$_3$), 38.20 (d, $^1J_{P,C}$ = 132.23 Hz, P-*C*H$_2$), 44.7 (s, tert C), 64.26 (*C*H$_2$-OH) ppm. ^{31}P NMR (250 MHz, D_2O): δ = 9.81 ppm. Anal. Calcd for $C_5H_{14}NO_4P$ (183.14): C,

32.79%; H, 7.71%; N, 7.65%; P, 16.91%. Found: C, 32.54%; H, 7.69%; N, 7.22%; P, 16.47%.

4.8. *N*-Phosphonomethyl-2-methyl-1-propanoic acid (4)

To a 100 mL stainless steel autoclave equipped with magnetic stirrer and pressure gauge was introduced (4,4-dimethyl-2-oxo-1,3-oxazolidin-3-yl)methylphosphonic acid (**2b**) (4.0 g, 0.019 mol), NaOH (3.8 g, 0.095 mol) in 23.0 mL H_2O and 0.24 g CdO. The reaction mixture was heated at 230 °C for 8.30 h. The reaction solution was cooled to 5 °C and filtered. The filtrate was decarboxylated by adding 6.75 mL HCl. At this time copious gas evolution resulted. Subsequently, the reaction mixture was treated with Dowex 50WX8-200 in order to exchange the sodium cations by hydrogen-ion exchange. The crude mixture was purified by crystallization from water. Yield 1.95 g (52%); mp = 166–168 °C; R_f (*n*-BuOH/AcOH/H_2O, 3:1:1) = 0.44. IR (KBr) 3400–3000 (NH and OH, br), 1620, 1404 (COO^-), 1253, 1288 (P=O), cm^{-1}.

^1H NMR (250 MHz, D_2O): δ = 1.57 (s, 6H, CH_3), 3.14 (d, $^2J_{P,H}$ = 13.9 Hz, 2H, P-CH_2) ppm. $^{13}C\{H\}$ NMR (250 MHz, D_2O): δ = 20.8 (CH_3), 39.58 (d, $^1J_{P,C}$ = 138.70, P-CH_2), 48.7 (s, tert C), 173.4 (C=O) ppm.. Anal. Calcd for $C_5H_{12}NO_5P$ (197.13); C, 30.46%; H, 6.14%; N, 7.11%; P, 15.71%. Found: C, 30.33%; H, 6.10%; N, 7.02%; P, 15.32%.

4.9. Cytogenetic method

The cytogenetic investigation was conducted as described by Preston et al.[31] Male and female C57Bl mice, weighing 20 g ± 1.5 g, were kept under standard conditions—20 °C, 12 h light/dark cycle, having free access to food and water. All the five investigated aminophosphonic acids were administered ip at doses of 10 and 100 mg/kg. Mitomycin C (Kyowa) 3.5 mg/kg was used as a positive control. Animals injected with 0.9% NaCl were used as a negative control.

Bone marrow chromosome aberration assay was performed on groups of animals. Each one consists of three males and three females treated with the studied compound and five control animals. The animals were injected ip with colchicine at a dose of 0.4 mg/kg, 24, 48 h after the administration of the applied chemicals or 0.9% NaCl solution and 1h before isolation of the bone marrow cells. Bone marrow cells were flushed from femur and hypotonized in a 0.075 M KCl at 37 °C during 20 min. Thereafter the cells were fixed in methanol/acetic acid (3:1), dropped on cold slides, and air-dried. To examine the chromosome aberrations, the slides were stained with 5% Giemsa solution (Sigma Diagnostic). At least 50 well-spread metaphases were analyzed per experimental animal at random.

Mitotic indices were determined by counting the number of dividing cells among 1500 cells per animal in the bone marrow slides to score aberrations.

The frequencies of abnormalities and the mitotic index were determined for each animal and then the mean ± standard error for each group was calculated.

4.10. Statistical analysis

Three-way analysis of variance (ANOVA) with fixed effects, followed by two-group Student's *t* test and post hoc pairwise comparison test of Dunnett with a control, was performed using BMDP4V, BMDP3D, and BMDP7D programs.[30] Statistical significance is expressed as $***p < 0.001$; $**p < 0.01$; $*p < 0.05$; $p > 0.05$ (not significant). Unless otherwise stated eight animals were used per group.

Acknowledgment

This study was supported by Grant 'BYX-12' from the Ministry of Education and Science.

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