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GENOTOXIC AND ANTIPROLIFERATIVE EFFECTS OF RECENT SYNTHESIZED HYDANTOIN-PHOSPHONIC DERIVATIVES

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ABSTRACT

*The variety of biological activities expressed by aminophosphonic acids bearing hydantoin moiety is surprisingly high. Several applications, in quite different fields such as agriculture and human health, have been reported. This report refers to the investigation of genotoxic and antiproliferative effect of recently synthesized aminophosphonic acids with hydantoin moiety. These effects of the newly synthesized [(5,5-dimethyl-2,4-dioxoimidazolidine-1,3-diyl)dimethyl]diphosphonic acid (**2**), dimethyl[(5,5-dimethyl-2,4-dioxoimidazolidin-3-yl)aminomethyl]phosphonate and dimethyl[(3-[(dimethoxy-phosphoryl)methyl]amino-5,5-dimethyl-2,4-dioxoimidazolidin-1-yl)methyl]phosphonate **(4)** were investigated for the first time. They exhibited moderate clastogenicity, and high antiproliferative activity on ICR mice bone marrow cells.*

Keywords: aminophosphonic acids; hydantoin derivatives; chromosomal aberrations; cell proliferation; clastogenic effects.

INTRODUCTION

Aminophosphonates are valuable intermediates for the preparation of medicinal compounds and synthetic intermediates. In the past decades there has been an increasing interest in the study of phosphonic acids derivatives as stable mimetics of natural phosphates and substrates in the study of biochemical processes. In particular, aminophosphonic acids derivatives are isosteres of the corresponding amino acids and exhibit a variety of important biological properties. Among these phosphorus-containing compounds α-aminophosphonic acids are the most attractive substances which possess a variety of biological activities. Furthermore, aminophosphonates have found a multitude of applications in medicinal, agricultural, and industrial chemistry. The established antiproliferative effects, together with the low mammalian toxicity of these agents, have conditioned tremendous interest towards designing novel antineoplastic agents [1-6]. α-Aminophosphonates are chief substrates also in the synthesis of phosphonopeptides. Due to their structural analogy with α-amino acids, these types of organophosphorus compounds are widely used for the development of new inhibitors of enzymes, neuroactive compounds, and plant growth regulators. Numerous phosphonopeptides possess antibacterial and effective inhibitor activity against different kind of tumors, leukemia, multiple sclerosis and autoimmune diseases [1,2].

Synthesis of aminophosphonic acids is an active area of research, and many methods are now available [7-12]. Among the number of synthetic approaches to α-aminophosphonates, one of the most powerful methods is the Kabachnik-Fields reaction [13-15]. This reliable method for the synthesis of aminophosphonates is the three component reaction that occurs when a car-

bonyl compound, a primary or secondary amine, and a dialkyl- or trialkyl phosphites are reacted. The reaction is known since 1952, and has been widely applied; in most of the cases it requires an electrophilic activation brought about by a Lewis or a Broensted acid. Novel α-aminophosphonic acids with moderate clastogenic effect and significant antiproliferative activity were synthesized reacting 1,3-oxazolidin-2-one derivatives with formaldehyde and phosphorus trichloride [16]. Cyclic or heterocyclic rings, introduced into the molecular skeleton, increase its rigidity and modify the electronic effects. Thus in recent years, many cyclic a-aminophosphonic acids or aminophosphonates have been prepared [17]. A series of α,α-disubstituted cyclic derivatives of *N*-(phosphonomethyl)glycine were obtained from cycloalkaneaminocarboxylic acids, and their biological activity was studied [11]. Hence, the synthesis of novel derivatives of α- aminophosphonic acid is currently of high importance [10-12,16]. Hydantoins, substituted at C-5, are important medicinal compounds. Numerous applications have been found for hydantoin derivatives due to their antidepressant [18] and antiviral activities [19], the inhibition of binding of HIV to lymphocytes [20], as well as their anti-convulsant and cardiac anti-arrhythmic effects [21].

This work is a continuation of our previous study on the synthesis and biological effects of these interesting and perspective class biologically active compounds [22, 23]. The investigated compounds (**2** and **4**) used in this study are presented in Scheme 1. For the total characterization of these newly synthesized aminophosphonic acids bearing hydantoin moiety 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 the [(5,5-dimethyl-2,4-dioxoimidazolidine-1,3-diyl)dimethyl]diphosphonic acid (**2**), dimethyl[(5,5-dimethyl-2,4-dioxoimidazolidin-3-yl)aminomethyl] phosphonate and dimethyl[(3-[(dimethoxy phosphoryl) methyl]amino-5,5-dimethyl-2,4-dioxoimidazolidin-1-yl)methyl] phosphonate **(4)**. Chromosome aberration test was applied to solve the main task. This test allows detection of the possible genotoxic effect, the type of the chromosome aberrations and the specific chromosomes of the mouse karyotype, which are subjected to the effects.

EXPERIMENTAL

5,5-Dimetylhydantoin, dimethyl-H-phosphonate, and paraformaldehyde were purchased from Fluka and Merck, and used without further purification. All other reagents and solvents were analytical or HPLC grade and were bought from Merck (Germany). The compounds [(5,5-dimethyl-2,4-dioxoimidazolidine-1,3-diyl)dimethyl]diphosphonic acid (**2**), dimethyl[(5,5-dimethyl-2,4-dioxoimidazolidin-3-yl)aminomethyl] phosphonate and dimethyl [(3-[(dimethoxyphosphoryl) methyl]amino-5,5-dimethyl-2,4-dioxoimidazolidin-1-yl)methyl] phosphonate **(4)** respectively were prepared according to procedure described by us [22-24].

Cytogenetical method

The cytogenetical investigation was conducted as described by Preston et al. [25]. Male and female ICR mice, weighing 20g ± 1.5g were kept under standard conditions - temperature 20°C, photoperiod 7am to 7pm, free access to standard animal food and water. [(5,5-Dimethyl-2,4-dioxoimidazolidine-1,3-diyl)dimethyl]diphosphonic acid (**2**), dimethyl [(5,5-dimethyl-2,4-dioxoimidazolidin-3-yl)aminomethyl] phosphonate and dimethyl[(3-[(dimethoxy-phosphoryl) methyl]amino-5,5-dimethyl-2,4-dioxoimidazolidin-1-yl)methyl] phosphonate **(4)** were administered i.p. at doses of 10 mg kg^{-1} and 100 mg kg^{-1} Mitomycin C (Kyowa) (3.5 mg kg^{-1}) was used as a positive control. The control animals were injected only with 0.9 % NaCl.

Bone marrow chromosome aberration assay was performed on groups of animals each one consisted of 8 males treated with the compound studied, and 10 pure control animals. All animals were injected intraperitoneally with colchicine at a dose of 0.04 mg g^{-1}, 24 hours after the administration of newly synthesized aminophosphonic acids with hydantoin moiety (**2** and **4**), Mitomycin C or 0.9 % NaCl solution and one hour before isolation of the bone marrow cells. All mice were euthanized by anaesthesia of diethylether. Bone marrow cells were flushed from the femur and hypotonized in a 0.075 M KCl at 37°C for 20 min. Thereafter the cells were fixed in methanol - acetic acid (3:1), dropped on cold slides and air dried. To examine 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.

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 [26]. 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.

RESULTS AND DISCUSSION

Chemistry

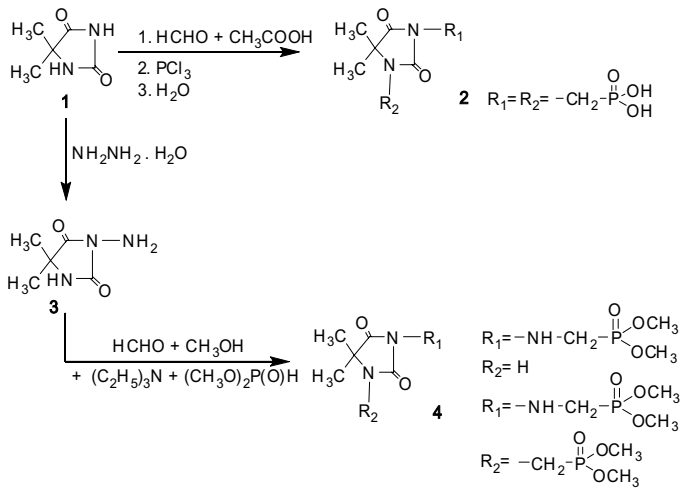
The present study is a continuation of our attempt to synthesize, characterization and biological investigation of new α-aminophosphonic acids derivatives as potential biologically active compounds. The novel aminophosphonic acids bearing hydantoin moiety have been synthesized by two different methods (see Scheme 1). For the synthesis of [(5,5-dimethyl-2,4-dioxoimidazolidine-1,3-diyl)dimethyl]diphosphonic acid (**2**) we used Engelmann and Pikl's procedure [22-24]. The interaction between 5,5-dimetylhydantoin (**1**) and formaldehyde involves the formation of bis-hydroxymethylhydantoin which further reacts with phosphorus trichloride. The subsequent hydrolysis resulted in the formation of [(5,5-dimethyl-2,4-dioxoimidazolidine-1,3-diyl)dimethyl]diphosphonic acid (**2**).

Dimethyl[(5,5-dimethyl-2,4-dioxoimidazolidin-3-yl)aminomethyl]phosphonate (66%) and dimethyl[(3-[(dimethoxy-phosphoryl)methyl]amino-5,5-dimethyl-2,4-dioxoimidazolidin-1-yl)methyl]phosphonate (33%) (**4**) were obtained from 3-amino-5,5-dimethylimidazolidine-2,4-dione (**3**) via Kabachnik-Fields reaction [13-15] (see Scheme 1).

Biological Activity

Cytogenetical investigations were carried out on ICR murine bone marrow metaphase plates following i.p. treatment with the respective compounds in concentrations of 10 and 100 mg kg^{-1} body weight. Structural chromosome aberrations (breaks and fragments) and intrachromosomal exchanges (centromer/centromeric and telomere/telomeric fusions) were reported. The results of this analysis are presented in Table 1, Fig. 1 and Fig. 2.

Cytogenetic data on the impact of [(5,5-dimethyl-2,4-dioxoimidazolidine-1,3-diyl)dimethyl]diphosphonic acid (**2**), dimethyl[(5,5-dimethyl-2,4-dioxoimidazolidin-3-yl)aminomethyl]phosphonate and dimethyl[(3-[(dimethoxy-phosphoryl)methyl]amino-5,5-dimethyl-2,4-dioxoimidazolidin-1-yl)methyl]phosphonate **(4)** show that there was no difference in its clastogenic effect in labora-



Scheme 1. General synthetic pathway.

Table 1. Frequencies of chromosome aberrations in affected mouse bone marrow cells after i. p. treatment of α-aminophosphonic compounds.

Substance	Interval of treatment	Metaphases analysed	Type of aberrations					Polyploid cells	Cells with aberrations % (X±m)	Mitotic index ‰ (X±m)
			Breaks	Fragments	Exchanges					
					c/c	t/t	c/t			
2 10 mg kg^{-1}	24h	400	8	7	9	1	0	0	6.5 ± 0.63	6.53 ± 0.94
	48h	400	2	1	11	1	0	0	3.75 ± 0.70	9.92 ± 1.22
2 100 mg kg^{-1}	24h	400	6	2	13	0	0	0	5.25 ± 0.37	12.68 ± 1.48
	48h	400	4	1	11	0	0	0	4.0 ± 0.38	7.46 ± 1.73
4 10 mg kg^{-1}	24h	400	2	1	11	0	0	0	3.50 ± 0.63	6.54 ± 1.14
	48h	400	4	0	7	0	0	0	2.75 ± 0.37	10.09 ± 0.92
4 100 mg kg^{-1}	24h	400	7	6	13	0	0	0	6.50 ± 0.91	13.13 ± 1.52
	48h	350	3	3	13	1	0	0	5.71 ± 0.68	10.30 ± 1.73
Mit. C	24h	400	17	30	7	1	0	0	30.5 ± 2.36	7.47 ± 1.85
Control	24h	500	3	0	2	0	0	0	1.0 ± 0.33	19.30 ± 1.23

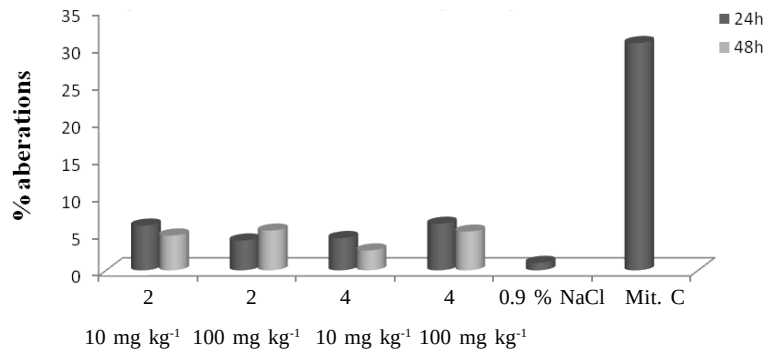


Fig. 1. Frequences of chromosome aberrations after i.p. treatment of aminophosphonic acids.

tory mice injected with 10 and 100 mg kg^{-1} substance at 24 hours after treatment (p> 0.05). Moreover in slides prepared 48 hours after injection of the substances chromosome damaging effect is smaller, reaching only 2.75 % in bone marrow cell population of mice treated with 10 mg kg^{-1} (**4**).

Interesting (special) results are obtained in terms of changes in mitotic index. In both applied concentrations hydantion-phosphonic derivatives show a pronounced antiproliferative effect. Mitotic index values were fairly lower compared to untreated controls (p> 0.099). Lowest values for this parameter are 6.53 ±0.94 ‰ (**3**, 24 hours) versus 19.3 ‰ ± 1.23 for the untreated control. These values are close to the values for proliferation inhibitory effect of alkylating agent Mitomycin-C (Kyowa) - 7.47±1.85 ‰ (Fig. 2).

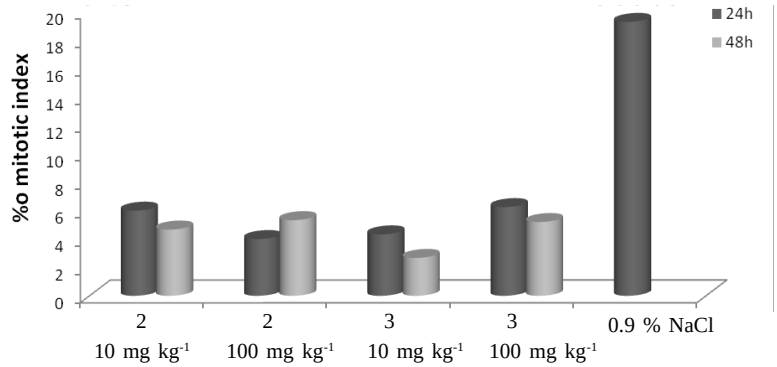


Fig. 2. Mitotic index in bone marrow cell after i.p. treatment of aminophosphonic acids.

The results obtained for the amount of dividing cells in rapidly proliferating bone marrow cell population of mice treated with a dose of 10 mg kg^{-1}, making it possible the following assumption. After intraperitoneal introduction of a greater amount hydantion-phosphonic derivatives the transition of proliferating cells during the mitotic cycle is delayed.

As a result of metabolic elimination of some of the introduced active phosphonic molecules, bone marrow cells enter into mitosis, which is manifested by an increase in mitotic index.

Established antiproliferative effect of two investigated new compounds was similar to that established in our previous studies of new series α-aminophosphonic acids derivatives [11, 16].

CONCLUSIONS

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 is an 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.

Along with the moderate clastogenic effect the two newly synthesized compounds strongly inhibit cell division. Their effect is similar to the proven cytostatic effect of antitumour antibiotic Mitomycin C. These results may serve as a real basis for the study on appropriate experimental models for possible anticancer action of those substances.

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REFERENCES

1. K. D. Troev, Chemistry and Application of H-Phosphonates, Elsevier, Amsterdam 2006.
2. P. Kafarski, B. Lejczak, Aminophosphonic Acids of Potential Medical Importance, Curr. Med. Chem. – Anti-Cancer Agents, **1**, 2001, 301-312.
3. J. G. Allen, F.R. Atherton, M. J. Hall, C.H. Hassall, S.W. Holmes, R. W. Lambert, L. J. Nisbet, P.S. Ringrose, Phosphonopeptides, a new class of synthetic antibacterial agents, Nature, **272**, 1978, 56-58.
4. W.W. Smith, P. A. Bartlett, Macrocyclic Inhibitors

of Penicillopepsin. 3. Design, Synthesis, and Evaluation of an Inhibitor Bridged Between P2 and P1, J. Am. Chem. Soc., **120**, 1998, 4622-4628.

5. G. Lavielle, P. Hautefaye, C. Schaeffer, J. A. Boutin, C. A. Cudennec, A. Pierre, New alpha-amino phosphonic acid derivatives of vinblastine: chemistry and antitumor activity, J. Med. Chem., **34**, 1991, 1998-2003.
6. L. Maier, P. Z. Diel, Synthesis, Physical and Biological Properties of the Phosphorus Analogues Of Phenylalanine and Related Compounds, Phosphorus Sulfur Silicon, **90**, 1994, 259-279.
7. J. Huang, R. Chen, An overview of recent advances on the synthesis and biologicalactivity of α-aminophosphonic acid derivatives, Heteroatom Chem., **11**, 2000, 480-492.
8. K. M. Yager, C. M. Taylor, A. B. Smith, Asymmetric Synthesis of .alpha.-Aminophosphonates via Diastereoselective Addition of Lithium Diethyl Phosphite to Chelating Imines, J. Am. Chem. Soc., **116**, 1994, 9377-9378.
9. D. Redmore, Chemistry of phosphorous acid: new routes to phosphonic acids and phosphate esters, J. Org. Chem., **43**, 1978, 992-996.
10. E. D. Naydenova, P. T. Todorov, K. D. Troev, Resent Synthesis of Aminophosphonic Acids as Potential Biological Importance, Amino Acids, **38**, 1, 2010, 23-30.
11. E. Naydenova, P. Todorov, M. Topashka-Ancheva, G. Momekov, T. Yordanova, S. Konstantinov, K. Troev, Novel N-(phosphonomethyl) glycine derivatives. Design, characterization and biological activity, Eur. J. Med. Chem., **43**, 2008, 1199-1205.
12. P. Todorov, E. Naydenova, J. Popova, K. Troev, Synthesis and Characterization of Novel (9H-Fluoren-9-ylamino)carbonylaminomethyl phosphonic acid, Heteroatom Chem., **19**, 2008, 7, 719-722.
13. M. Kabachnik, T. Medved, New method for preparation of α-aminophosphonic acids, Dokl. Acad. Nauk. SSSR, **83**, 1952, 689, (in Russian).
14. E. K. Fields, The synthesis of esters of substituted amino phosphonic acids, J. Am. Chem. Soc., **74**, 1952, 1528-1531.
15. N. S. Zefirov, E. D. Matveeva, Catalytic Kabachnik-Fields reaction: new horizons for old reaction, Arkivoc 2008, (i) 1-17.
16. E. Naydenova, M. Topashka-Ancheva, P. Todorov, Ts. Yordanova, K. Troev, Novel α-aminophosphonic acids. Design, characterization, and biological activity, Bioorg. Med. Chem., **14**, 2006, 2190-2196.
17. N. Rabasso, N. Louaisil, A. Fadel, Asymmetric Synthesis of (R)- and (S)-Amino-3-piperidinylphosphonic Acids via Phosphite Addition to Iminium Ions, Synthesis, 2007, 289-293.
18. F. L. Wessels, T. J. Schawn, S. F. Pong, Synthesis and antidepressant activity of 5-(4-dimethylaminobenzyl)imidazolidine-2,4-dione, J. Pharm. Sci., **69**, 1980, 1102-1104.
19. A. A. El-Barbarly, A. I. Khodair, E. B. Pedersen, C. Nielsen, S-Glucosylated hydantoins as new antiviral agents, J. Med. Chem., **37**, 1994, 73-77.
20. W. M. Cloyd, S. W. Lynn, K. Ramsey, S. Baron, Inhibition of human immunodeficiency virus (HIV-1) infection by diphenylhydantoin (dilantin) implicates role of cellular calcium in virus life cycle, Virology, **173**, 1989, 581-590.
21. M. L. Brown, G. B. Brown, W. J. Brouillette, Effects of log P and Phenyl Ring Conformation on the Binding of 5-Phenylhydantoins to the Voltage-Dependent Sodium Channel, J. Med. Chem., **40**, 1997, 602-607.
22. P. Todorov, E. Naydenova, K. Troev, Synthesis of Novel Aminophosphonic Acids with Hydantoin Structure, Heteroatom Chem., **20**, 2009, 87–90.
23. E. Naydenova, P. Todorov, K. Troev, Synthesis and Characterization of Novel Cycloalkanespiro-5-hydantoin Phosphonic Acids, Phosphorus, Sulfur, and Silicon and the Relat. Elem., **185**, 2010, 1315-1320.
24. M. Engelmann, J. Pikl, Organic compound and process of preparing the same, U.S. Patent 1942, 2,304, 156.
25. R. Preston, B. Dean, S. Galloway, H. Holden, A.F Mc Fee, M. Sheldy, Mammalian *in vivo* cytogenetic assays: analysis of chromosome aberrations in bone marrow cells, Mutation Res., **189**, 1987, 157-165.
26. J. Dixon, M. Brown, L. Engelman, R. Jennrich, BMDP Statistical Software Manual, University of California Press, Berkeley, 1990.