

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SOME α -AMINOPHOSPHONATES

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Abstract

A series of 12 aminophosphonates (AMPP) were synthesized, characterized and tested for their potential biological activity. The antioxidant activity was tested in experimental assays with DPPH• (1,1-diphenyl-2-picryl-hydrazyl) and the potential to function as herbicides, in red blood cells (RBC) membrane destruction experiments. Regarding the reaction with DPPH• at least 3 aminophosphonates are better antioxidants than 2,6-di-tert-butyl-4-methylphenol (BHT). Aminophosphonates' capacity to function as herbicide (demonstrated through their hemolytic activity) is given by the presence in their structure of some shorter aliphatic substituents and / or more benzenic nuclei.

Rezumat

O serie de 12 acizi aminofosfonici și derivați (denumiți generic aminofosfonați, AMPP) au fost sintetizați, caracterizați și testați pentru posibila lor activitate biologică. Activitatea antioxidantă a fost testată în determinări experimentale cu DPPH• (1,1-difenil-2-picril-hidrazil) și evaluarea potențialului de a funcționa ca erbicide, în experimente de hemoliză a membranei eritrocitare. Activitatea anti-radicalică testată în reacția cu DPPH•, a dus la concluzia că cel puțin 3 aminofosfonați sunt mai buni antioxidanți decât 2,6-di-tert-butyl-4-methylphenol (BHT). Probabilitatea de a funcționa ca erbicid a aminofosfonaților (demonstrată prin activitatea hemolitică) este dată de existența unor substituenți alifatici de lungime mică și/sau a mai multor nuclee benzenice în structura lor.

Keywords: α -aminophosphonates, antioxidant activity, DPPH•, erythrocytes, hemolytic activity.

Materials and methods

1. Chemicals: salicylaldehyde, (> 98%), benzaldehyde, (> 90%), 4-dimethylaminobenzaldehyde, (> 98%), nicotinaldehyde (> 98%), n-octylamine, (> 99%), 1-amino-2-propanol, (> 98%), diphenylamine, (> 98%), diisopropylamine, (-)-1-phenylethylamine (> 98%), ethylenediamine (> 98%), dimethylphosphite, (> 98%), diethylphosphite, (> 98%), diphenylphosphite, (> 98%), phosphonic acid (> 97%), methanol (> 99%); aldehydes and amines were purchased from Fluka and Merck, and phosphites and phosphonic acid from Aldrich; 1,1-diphenyl-2-picrylhydrazyl, (DPPH•), (Merck, > 90%), 2,6-di-*tert*-butyl-4-methyl-phenol, (BHT), (Merck, > 99%), polyoxyethylene sorbitan monolaurate (Polysorbate 20) (Merck, > 99%), methanol (Chimopar, > 99%). Fresh methanol solutions of DPPH•, BHT, aminophosphonates **1-12** were prepared and used in the experiments.

2. Instruments and equipments: The microwave energy in the reaction was obtained using a domestic microwave oven, Whirlpool of 850 W power and 2450 MHz working frequency. The plates with fluorescence indicator Kieselgel F254 - TLC, were obtained from Merck. Melting points were determined on a digital apparatus Electrothermal IA9100. Infrared spectra were recorded using KBr pellets on a Carl Zeiss-Jena SPECORD M-80 IR spectrometer. UV-VIS spectra were recorded on a Pharmacia LKB Biochrom Ultrospec III UV-VIS (England). Electron impact mass (EI-MS) spectra were obtained on a Varian Finnigan MAT 212 apparatus. Electrospray ionization mass (ESI-MS) spectra in positive mode were obtained using a High Capacity Ion Trap Ultra (HCT Ultra, PTM Discovery) mass spectrometer from Bruker Daltonics, Bremen, Germany. ¹H-NMR spectra were obtained using a VARIAN GEMINI spectrometer at 400 MHz. Chemical shifts are expressed in ppm and the coupling constants in Hz.

3. General procedure for aminophosphonates synthesis: The classical aminophosphonate synthesis method is a typical two step reaction: first, an aldehyde ($R_1\text{-CH=O}$) is reacted with an amine ($R_2\text{-NH}_2$) to yield an imine ($R_1\text{-CH=N-R}_2$); second, the obtained imine is used as it is, in the reaction with dialkyl phosphite ($\text{HP(O)(OR}_3)_2$, R = Alkyl), diaryl phosphite ($\text{HP(O)(OAr}_3)_2$, Ar = Aryl), or phosphonic acid (HP(O)(OH)_2), to yield the desired aminophosphonate. Because it is time consuming (6-24 hours), we used a modern - one pot synthesis ("click synthesis"), which uses the microwave energy (MW, 2-6 minutes). [13]

In the microwave synthesis method, the reactants were stirred, first the aldehyde and the amine (because in the majority of the cases the

reaction is instantaneous exothermic), and then the obtained mixture reacts with dialkyl phosphite, diaryl phosphite or phosphonic acid. In a 50 mL Erlenmeyer glass were stirred 10 mmol aldehyde, 10.05 mmol amine and 10.10 mmol phosphite or phosphonic acid. The reaction flask was placed into the microwave oven, and heated 6 - 12 times for 30 seconds, at 100 W. The content was slowly cooled at the room temperature every time, and the final product, was filtered and repeatedly washed with ethanol and chloroform until a powder is obtained (generally white or yellowish).

4. Characterization of the prepared aminophosphonates. The purity of the compounds was checked by thin layer chromatography. They were characterized by melting points (m.p.), infrared spectroscopy - IR, UV-VIS, EI-MS, (+)ESI-MS, and $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) [14], [15].

5. Biological activity. a) Radical scavenging activity. Aminophosphonates radical scavenging activity was tested in reaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH•) and the reference reaction was DPPH• and antioxidant – 2,6-di-tert-butyl-4-methylphenol (BHT). The DPPH• molecule is a stable free radical, the spare electron being delocalized over the entire molecule. When in a solution of DPPH•, a hydrogen atom donor compound is introduced, the stable non-radical form of DPPH is formed (Figure 2), accompanied by changing the violet color of the medium to a residual pale yellow color. The process can be monitored by absorbance measurements at $\lambda_{\text{max}} = 517 \text{ nm}$.

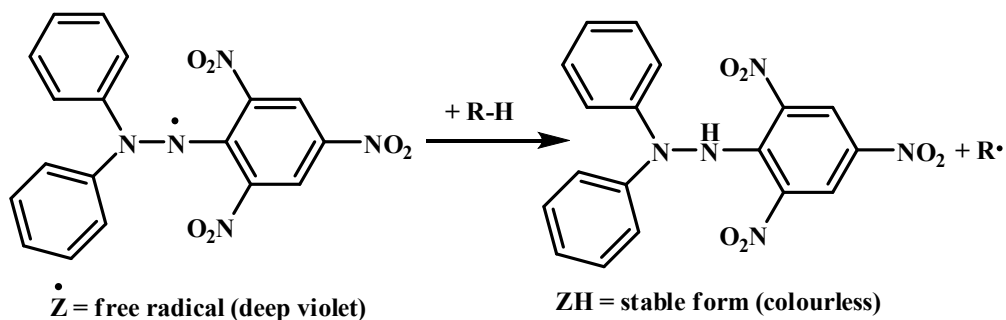


Figure 2.

Structural formula of 1,1-diphenyl-2-picryl-hydrazine (DPPH) as free radical and stable form

Absorbance variation was registered spectrometrically for 40 minutes, beginning from the preparation of the mixtures. To 0.1 mL methanol solution of 0.1 mmol/L DPPH•, a volume of 2.9 mL methanol solution of 0.1 mmol/L of BHT (as a standard hydrogen donor), or

aminophosphonate **1 – 12**, was added [16,17,18]. Thus in the final mixtures (of 3 mL volume) the concentrations were: 3.33 $\mu\text{mol/L}$ for DPPH• and, respectively, 96.67 $\mu\text{mol/L}$ for the antioxidant. The experiments were repeated three times and the data variation was under 5%.

b) Preliminary screening referring to aminophosphonates' functionality as herbicides was made in experiments related to the human RBC membrane. At high aminophosphonate concentrations the destruction of RBC double layer membrane may result (hemolysis) [4]. As revealed by the literature, the herbicidal activity of chemical compounds can be correlated with the hemolytic activity in experiments on the RBC membrane [15]. Thus possible functionality as herbicides was expressed by their hemolytic capacity, using the following formula [19]:

$$\text{Hemolysis (\%)} = \frac{A_{540\text{AMPP}} - A_{540\text{Buffer}}}{A_{540\text{Tween20}} - A_{540\text{Buffer}}} \cdot 100 \quad (1)$$

where: $A_{540\text{AMPP}}$ – aminophosphonate sample, $A_{540\text{Buffer}}$ - buffer sample and $A_{540\text{Tween20}}$ - Tween 20 sample absorbances measured at 540 nm.

Tween 20, or polyoxyethylene sorbitan monolaurate (Polysorbate 20), is a non-ionic surfactant that effectively suppresses unspecific reactions between antibodies, antigens and other molecules. It is also used as a solubilizer in membrane chemistry and, in our experiments a solution 4% of Tween 20, was used as a RBC 100% hemolyzing agent.

These experiments were made taking into account the limits of RBC destruction: the initial reference point (the blank) with no aminophosphonate added and the RBC total destruction, when treated with a 4% Tween 20 solution.

Fresh EDTA treated human erythrocytes (RBC), were washed four times in an isoosmotic phosphate buffer solution – PBS (100 mL Na_2HPO_4 0.05 M + 20 mL KH_2PO_4 0.05 M + 8.311g NaCl for 1000 mL) of pH 7.4. [1], [11] To RBC suspension volumes were added calculated volumes of aminophosphonates (concentration 10 mM/L), to give a final solution of 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5 mM/L with a final hematocrit 2%. This mixture was incubated for few hours at 37°C. The percentage of hemolysis was correlated with the measured absorbance of supernatant samples taken after 1.5 hours of incubation. Absorbance' measurements (after dilution 1:4 with PBS solution) were realized with a spectrophotometer (Helyos, England) at 540 nm.

Results and discussion

Characterization of the prepared aminophosphonates

(1) Dimethyl (2-hydroxyphenyl)(octylamino)methylphosphonate, $C_{17}H_{30}NO_4P$, $M = 343.398 \text{ g}\cdot\text{mol}^{-1}$; fine white powder; **m.p.** = 300 - 302°C; **IR (KBr, cm^{-1})** ν : 3428iw (Ar–OH) intense wide band; 3296i (N–H), 1608i (aromatic nucleus), 1458 (benzenic nucleus vibrations), 1212 (P=O), 1056 (P–O–C), 756m (P–C); **UV-VIS** (λ_{max} , cm^{-1}): 229, 280; **EI-MS (56 eV) [m/z , Da (rel. ab., %)]**: 41 (45.6), 43 (40.9), 55 (20.4), 65 (13.3), 78 (46.4), 80 (22.8), 108 (100, B. P.), 121 (36.2), 134 (69.2), 135 (72.4), 148 (87.4), 169 (74.0), 198 (26.7), 233 (78.7), 244 (37.0), 343 (2, M. P.); **(+)ESI-MS [m/z , Da]**: 344.2 ($[\text{M}+\text{H}]^+$), 327.2 ($[(\text{M}+\text{H})-\text{OH}]^+$), 274.4 ($[(\text{M}+\text{H})-\text{C}_5\text{H}_{10}]^+$), 234.2 ($[\text{M}-\text{P}(\text{O})(\text{OCH}_3)_2]^+$), 169.0 ($[(\text{M}+\text{H})-\text{CH}_3(\text{CH}_2)_7\text{NH}_2-\text{CH}_3\text{OH}-\text{CH}_3]^+$); **$^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , ppm)**: 7.1 – 7.3 (bp, 2H, Ar), 6.7 – 6.8 (bp, 2H, Ar), 4.1 – 4.2 (m, 7H, P–CH₂, (O–CH₂)₂), 2.7 (m, 2H, NH–CH₂–(CH₂)₆–CH₃), 1.55, 1.18, 0.83 (3m, 15H, NH–CH₂–(CH₂)₆–CH₃);

(2) Diethyl (2-hydroxyphenyl)(octylamino)methylphosphonate, $C_{19}H_{34}NO_4P$, $M = 371.451 \text{ g}\cdot\text{mol}^{-1}$; fine white powder; **m.p.** = 296 - 298°C; **IR (KBr, cm^{-1})** ν : 3408iw (Ar–OH), 3292 (N–H), 1608(Ar), 1460(Ar), 1212 (P=O), 1132 (P–O–C), 754m (P–C); **UV-VIS** (λ_{max} , cm^{-1}): 222, 280; **EI-MS (56 eV) [m/z , Da (rel. ab., %)]**: 41 (42.3), 43 (39.2), 55 (21.6), 65 (14.6), 78 (44.5), 80 (20.3), 108 (100, B.P.), 121 (34.6), 134 (56.6), 135 (61.2), 137 (62.4), 169 (65.0), 198 (23.8), 233 (70.2), 244 (37.0), 371 (3, M.P.); **(+)ESI-MS [m/z , Da]**: 372.2 ($[\text{M}+\text{H}]^+$), 356.2 ($[(\text{M}+\text{H})-\text{OH}]^+$), 284.2 ($[\text{M}-\text{C}_3\text{H}_6-\text{C}_2\text{H}_5\text{OH}]^+$), 260.2 ($[(\text{M}+\text{H})-\text{C}_8\text{H}_{16}]^+$), 234.2 ($[\text{M}-\text{P}(\text{O})(\text{OC}_2\text{H}_5)_2]^+$); **$^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , ppm)**: 7.1-7.4 (m, 2H, Ar), 6.7-6.8 (bp, 2H, Ar), 4.3 (bp, 5H, P–CH₂, 2 x (O–CH₂–CH₃)), 2.7 (s, 2H, NH–CH₂–(CH₂)₆–CH₃), 1.59, 1.15, 0.96, 0.82 (4s, 21H, 2 x (O–CH₂–CH₃), CH₂–(CH₂)₆–CH₃);

(3) Diphenyl (2-hydroxyphenyl)(octylamino) methylphosphonate, $C_{27}H_{34}NO_4P$, $M = 467.537 \text{ g}\cdot\text{mol}^{-1}$; fine white powder; **m.p.** = 286-288°C; **IR (KBr, cm^{-1})** ν : 3428iw (Ar–OH), 1610i, 1460, 1214i (P=O), 1085i (P–O–C), 1056i ($\nu_{\text{aliph}}(\text{C}-\text{C})$), 958i (P–O–C_{Ar}), 892i, 766i, 744m (P–C); **UV-VIS** (λ_{max} , cm^{-1}): 225, 281; **EI-MS (56 eV) [m/z , Da (rel. ab., %)]**: 41(39.3), 43(36.2), 44(29.1), 55(17.3), 65(12.5), 78(37.3), 80(19.6), 106(11.0), 108(91.3), 121(35.4), 134(65.3), 135(86.6), 233 (100, B.P.), 254(2.3), 296(3.1) 297(11.0), 148(3.1); **(+)ESI-MS [m/z , Da]**: 468.2 ($[\text{M}+\text{H}]^+$), 451.0 ($[\text{M}-\text{OH}]^+$), 330.31 ($[\text{M}-\text{C}_6\text{H}_6-\text{C}_3\text{H}_6-\text{OH}]^+$), 316.28 ($[(\text{M}+\text{H})-2\times\text{C}_6\text{H}_5+2\text{H}]^+$), 234.2 ($[(\text{M}+\text{H})-\text{HP}(\text{O})(\text{OC}_6\text{H}_5)_2]^+$); **$^1\text{H-NMR}$ (400 MHz, DMSO, d_6 , ppm)**: 7.1 – 7.3 (m, 4H, Ar), 6.8 – 6.9 (m, 10H, Ar), 4.2 (d, 2H, P–CH₂, CH–NH–(CH₂)₇–CH₃), 2.6 (bp, 2H, CH–NH–CH₂–(CH₂)₆–CH₃), 1.5, 1.2, 0.8 (3s, 15H, NH–CH₂–(CH₂)₆–CH₃);

(4) Diethyl (2-hydroxyphenyl)(2-hydroxypropylamino)methylphosphonate, $C_{14}H_{24}NO_5P$, $M = 317.318 \text{ g}\cdot\text{mol}^{-1}$; white powder; **m.p.** = 254 - 257°C; **IR (KBr, cm^{-1})**: 3384i (Ar–OH); 3288 (N–H), 1604i, 1460i, 1194 (P=O), 1194 (P=O), 1124 (P–O–C), 756m (P–C); **UV-VIS** (λ_{max} , cm^{-1}): 219, 277; **EI-MS (56 eV) [m/z , Da (rel. ab., %)]**: 45 (69.2), 46 (22.0), 51 (17.3), 65 (40.9), 78 (50.3), 80 (24.4), 85

(29.1), 106 (14.9), 108 (100, B.P.), 121 (40.1), 134 (90.5), 135 (20.4), 174 (32.2), 180 (26.7), 213 (7.0), 243 (5.5), 271 (6.2); **(+)ESI-MS** ($[m/z, Da]$: 318.39 ($[M+H]^+$), 300.36 ($[(M+H)-H_2O]^+$), 244.06 ($[(M+H)-2 \times C_2H_4-H_2O]^+$); **1H -NMR (400 MHz, DMSO- d_6 , ppm)**: 7.2 – 7.4 (m, 2H, Ar), 6.8 – 6.9 (m, 2H, Ar), 3.9 – 4.0 (m, 1H, P-CH, CH-OH), 3.7 – 3.8 (m, 2H, P-O-CH₂-CH₃), 2.9-3.1 (m, 3H, Ar-CH-NH-CH₂-CH), 1.0-1.3 (m, 9H, 2 x O-CH₂-CH₃, CH₂-CH(OH)-CH₃);

(5) (diphenylamino)(2-hydroxyphenyl)methylphosphonic acid, C₁₉H₁₈NO₄P, M = 355.324 g·mol⁻¹; green powder; **m.p.** = 243 - 246°C; **IR (KBr, cm⁻¹)**: 1602, 1420, 1312 (N-CH-Ar), 1284 (P=O), 1168 (P-O-C), 754m (P-C); **UV-VIS** (λ_{max} , cm⁻¹): 322, 458, 689; **EI-MS (56 eV) $[m/z, Da]$ (rel. ab., %)**: 45 (72), 46 (22), 51 (20), 65 (41), 78 (50), 106 (18), 108 (100, B.P.), 134 (91), 179 (43), 180 (38), 243 (8), 317 (4, M.P.); **(+)ESI-MS** ($[m/z, Da]$: **(+)ESI-MS** ($[m/z, Da]$: 356.45 ($[M+H]^+$), 338.16 ($[(M+H)-H_2O]^+$), 244.00 ($[(M+H)-H_2O-C_6H_5OH]^+$), 171.00 ($[(M+H)-OH-(C_6H_5)_2NH+H]^+$); **1H -NMR (400 MHz, DMSO- d_6 , ppm)**: 11.9-12.1 (m, 2H, C-P(O)(OH)₂), 7.2-7.4 (m, 4H+4H, Ar_{2,3}); 7.0-7.1 (m, 2H, Ar₁); 6.8-6.9 (m, 2H+H+H, Ar_{1,2,3}); 3.9-4.0 (d, HO-C₆H₄-CH[P(O)(OH)₂]-N(C₆H₅)₂);

(6) (diisopropylamino)(2-hydroxyphenyl)methylphosphonic acid, C₁₃H₂₂NO₄P, M = 287.292 g·mol⁻¹; fine white powder; **m.p.** = 158 - 160°C; **IR (KBr, cm⁻¹)**: 3120 (N-H), 1596, 1468, 1208 (P=O), 1132 (P-O-C), 670m (P-C); **UV-VIS** (λ_{max} , cm⁻¹): 213, 258; **EI-MS (56 eV) $[m/z, Da]$ (rel. ab., %)**: 60 (24), 64 (50), 83 (18), 87 (48), 124 (5), 173 (6), 236 (5), 287 (5, M. P.); **(+)ESI-MS** ($[m/z, Da]$: 288.00 ($[M+H]^+$), 270.00 ($[(M+H)-H_2O]^+$), 244.00 ($[(M+H)-H_2O-2 \times C_3H_6]^+$); **1H -NMR (400 MHz, DMSO, d_6 , ppm)**: 11.9-12.1 (m, 2H, C-P(O)(OH)₂), 7.0-7.1 (m, 2H, Ar), 6.8-6.9 (m, 2H, Ar), 3.9-4.0 (d, 1H, =CH-), 2.6-2.7 (m, 2H, 2 x -N[CH-(CH₃)₂]₂), 1.0-1.2 (m, 12H, 2 x CH(CH₃)₂);

(7) phenyl(1-phenylethylamino)methylphosphonic acid, C₁₅H₁₈NO₃P, M = 291.282 g·mol⁻¹; fine white-yellowish crystalline powder; **m.p.** = 156 - 158°C; **IR (KBr, cm⁻¹)**: 3292 (N-H), 1632, 1454, 1132 (P=O), 1112 (P-O-C); **UV-VIS** (λ_{max} , cm⁻¹): 217, 257; **EI-MS (56 eV) $[m/z, Da]$ (rel. ab., %)**: 78 (18), 81 (29), 92 (26), 106 (100, B.P.), 121 (7), 196 (20), 286 (4); **(+)ESI-MS** ($[m/z, Da]$: 292.30 ($[M+H]^+$), 212.13 ($[(M+H)-P(O)(OH)_2+H]^+$), 186.12 ($[(M+H)-C_6H_5CH=CH_2+H]^+$), 122.30 ($[C_6H_5CH(CH_3)(NH_3)]^+$); **1H -NMR (400 MHz, DMSO, d_6 , ppm)**: 11.9-12.1 (m, 2H, C-P(O)(OH)₂), 7.1-7.4, (m, 10H, C₆H₅-CH[P(O)(OH)₂]-NH-CH(C₆H₅)-CH₃), 4.0-4.1 (m, 1H, NH-CH(C₆H₅)-CH₃), 3.8-3.9 (m, 1H, (HO)₂P(O)-CH[C₆H₄(OH)]-NH), 1.1-1.2 (m, 3H, NH-CH(C₆H₅)-CH₃);

(8) (2-hydroxyphenyl)(1-phenylethylamino)methylphosphonic acid, C₁₅H₁₈NO₄P, M = 307.281 g·mol⁻¹; fine white - yellowish crystalline powder; **m.p.** = 179 - 180°C; **IR (KBr, cm⁻¹)**: 3304 (N-H), 1632, 1454, 1232 (P=O), 1112 (P-O-C), 724m (P-C); **UV-VIS** (λ_{max} , cm⁻¹): 221, 257; **EI-MS (56 eV) $[m/z, Da]$ (rel. ab., %)**: 63 (12), 64 (38), 65 (2), 78 (44), 81 (43), 83 (13), 103 (6), 104 (12), 106 (100, B.P.), 202 (14), 307 (5, M.P.); **(+)ESI-MS** ($[m/z, Da]$: 308.25 ($[M+H]^+$), 232.11 ($[(M+H)-C_6H_5+H]^+$), 226.10 ($[M-P(O)(OH)_2]^+$), 214.08 ($[M-C_6H_5OH]^+$), 204.12 ($[(M+H)-C_6H_5CH=CH_2]^+$), 122.27 ($[HOC_6H_4CHNH_2]^+$); **1H -NMR (400**

MHz, DMSO, d_6 , ppm): 11.9-12.1 (m, 2H, C-P(O)(OH)₂), 6.9-7.4, (m, 9H, HO-C₆H₄-CH[(P=O)(OH)₂]-NH-CH(C₆H₅)-CH₃), 5.3 (s, 1H, C_{Ar}-OH), 4.0-4.1 (m, 1H, NH-CH(C₆H₅)-CH₃), 3.8-3.9 (m, 1H, (HO)₂P(O)-CH[C₆H₄(OH)]-NH-(C₆H₅)-CH₃), 1.1-1.2 (m, 3H, NH-CH(C₆H₅)-CH₃);

(9) (4-(dimethylamino) phenyl) (octylamino) methylphosphonic acid, C₁₇H₃₁N₂O₃P, M = 342.413 g·mol⁻¹; fine yellowish crystalline powder; **m.p.** = 78-80°C; **IR (KBr, cm⁻¹):** 3148 (N-H), 1636, 1434, 1326 (N-C-Ar), 1208 (P=O), 1134 (P-O-C), 738m (P-C); **UV-VIS ($\lambda_{\max}$, cm⁻¹):** 275, 294; **EI-MS (56 eV) [m/z , Da (rel. ab., %)]:** 44 (9), 81 (25), 89 (19), 114 (23), 133 (47), 247 (100, B.P.), 328 (4, M.P.); **(+)ESI-MS ([m/z , Da]:** 343.27 ([M+H]⁺), 324.37 ([M-H₂O]⁺), 255.22 ([M+H]-H₂O-C₅H₁₀)⁺, 240.24 ([M+H]-H₂O-C₆H₁₂)⁺, 222.10 ([M+H]-C₆H₄N(CH₃)₂)⁺, 214.20 ([M+H]-C₆H₄N(CH₃)₂CHP(O)(OH)₂)⁺, 187.08 ([M-C₈H₁₆]⁺), 130.27 ([CH₃(CH₂)₇NH₃]⁺); **¹H-NMR (400 MHz, DMSO, d_6 , ppm):** 11.9-12.1 (m, 2H, C-P(O)(OH)₂), 7.1 - 7.3 (bp, 2H, Ar), 6.7 - 6.8 (bp, 2H, Ar), 3.8-3.9 (m, 1H, (HO)₂P(O)-CH[C₆H₄-N(CH₃)₂]-NH-(CH₂)₇CH₃), 3.0-3.1 (m, 6H, C₆H₄-N(CH₃)₂), 2.66 (m, 2H, NH-CH₂-(CH₂)₆-CH₃), 1.58, 1.16, 0.84 (3m, 15H, NH-CH₂-(CH₂)₆-CH₃);

(10) (4-(dimethylamino)phenyl)(1-phenylethylamino)methylphosphonic acid, C₁₇H₂₃N₂O₃P, M = 334.145 g·mol⁻¹; fine white - yellowish crystalline powder; **m.p.** = 116 - 118°C; **IR (KBr, cm⁻¹):** 3116 (N-H); 1642, 1458, 1326 (N-C), 1202 (P=O), 1132 (P-O-C), 752m (P-C); **UV-VIS ($\lambda_{\max}$, cm⁻¹):** 380, 433; **EI-MS (56 eV) [m/z , Da (rel. ab., %)]:** 44 (23), 81 (42), 89 (25), 120 (67), 133 (26), 253 (100, B.P.), 334 (4, M.P.); **(+)ESI-MS ([m/z , Da]:** 335.26 [M+H]⁺, 317.20 ([M+H]-H₂O)⁺, 290.01 ([M-(CH₃)₂N]⁺), 278.00 ([M+H]-(CH₃)₂N-CH₃+2H)⁺, 195.07 ([M-H₂O-(C₆H₅CH(CH₃)NH₂+H)⁺, 186.00 ([M+H]-C₆H₅CH=CH₂-(CH₃)₂N)⁺, 122.20 ([M+H]-C₆H₅CH(CH₃)NH₂)⁺; **¹H-NMR (400 MHz, DMSO, d_6 , ppm):** 12-12.2 (m, 2H, C-P(O)(OH)₂), 6.7 - 7.4 (4m, 9H, Ar), 3.8-3.9 (m, 1H, (HO)₂(O)P-CH[C₆H₄-N(CH₃)₂]-NH-(C₆H₅)-CH₃), 3.0-3.1 (m, 6H, C₆H₄-N(CH₃)₂), 1.2-1.3 (m, 3H, NH-CH(C₆H₅)-CH₃);

(11) (ethane-1,2-diylbis(azanediyl))bis((2-hydroxyphenyl)methylene) diphosphonic acid, C₁₆H₂₂N₂O₈P₂, M = 432.302 g·mol⁻¹; fine white crystalline powder; **m.p.** = 250-252°C; **IR (KBr, cm⁻¹):** 3316 (N-H), 1605, 1437, 1201 (P=O), 1110 (P-O-C), 807, 710m (P-C); **UV-VIS ($\lambda_{\max}$, cm⁻¹):** 226, 276; **EI-MS (56 eV) [m/z , Da (rel. ab., %)]:** 58 (14), 81 (55), 106 (100, B.P.), 164 (21), 270 (32), 351 (18), 432 (6, M.P.); **(+)ESI-MS ([m/z , Da]:** 433.32 [M+H]⁺, 415.30 ([M+H]-H₂O)⁺, 335.28 ([M+H]-H₂O-P(O)(OH)₂+H)⁺, 256.00 ([M-2×P(O)(OH)₂-H₂O+2H]⁺, 243.07 ([M+H]-P(O)(OH)₂-C₆H₄OH + 2H)⁺; **¹H-NMR (400 MHz, DMSO, d_6 , ppm):** 12-12.2 (m, 4H, 2 x C-P(O)(OH)₂), 7.1-7.2 (m, 4H, Ar), 6.8-6.9 (m, 4H, Ar), 5.4 (s, 2H, 2 x HO-C₆H₄-), 3.9-4.0 (m, 2H, 2 x P-CH(C₆H₄OH)-NH), 2.7 (s, 4H, HN-(CH₂)₂-NH), 2.0 (s, 2H, =CH-NH-(CH₂)₂-NH-CH=);

(12) (ethane-1,2-diylbis(azanediyl))bis(pyridin-3-ylmethylene)diphosphonic acid, C₁₄H₂₀N₄O₆P₂, M = 402.279 g·mol⁻¹; fine white powder; **m.p.** = 223-225°C; **IR (KBr, cm⁻¹):** 3317 (N-H), 1605, 1438, 1305 (P=O), 1115 (P-O-C), 807, 711m

(P-C);; UV-VIS ($\lambda_{\max}$, cm^{-1}): 231, 256; EI-MS (56 eV) [m/z , Da (rel. ab., %)]: 58 (21), 78 (12), 81 (29), 91 (100, B.P.), 149 (56), 240 (9), 321 (22), 402 (5, M.P.); (+)ESI-MS ([m/z , Da]: 403.30 ([$M+H$] $^+$), 310.40 ([$(M+H)-C_5H_3N-OH+H$] $^+$), 217.00 ([$(HO)_2(O)PCH(NH_2CH_2CH_3)(C_5H_3N)$] $^+$), 171.00 ([$(HO)(O)PCH(NH_2)(C_5H_3N)$] $^+$); $^1\text{H-NMR}$ (400 MHz, DMSO, d_6 , ppm): 12-12.2 (m, 4H, 2 x C-P(O)(OH) $_2$), 7.3, 7.5, 8.3, 8.5 (4m, 8H, Ar), 4.0-4.1 (m, 2H, 2 x P-CH(C_5H_4N)-NH), 2.7-3.3 (m, 4H, HN-(CH $_2$) $_2$ -NH), 2.0 (s, 2H, CH-NH-(CH $_2$) $_2$ -NH-CH).

Biological activity

a) Antiradical (radical scavenging) activity. As criterion for the radical scavenging capacity, was considered, the half-life, $t_{1/2}$, the time it takes for one-half of the original amount of material (proportional with the absorbance) to react. The experimental results for the aminophosphonates **1** – **12**, and BHT are presented in Figure 3. The compounds having shorter half life than BHT can be considered better antioxidants than this reference compound. Aminophosphonates **6** > **8** > **9** > **12**, present a greater antioxidant potential than BHT.

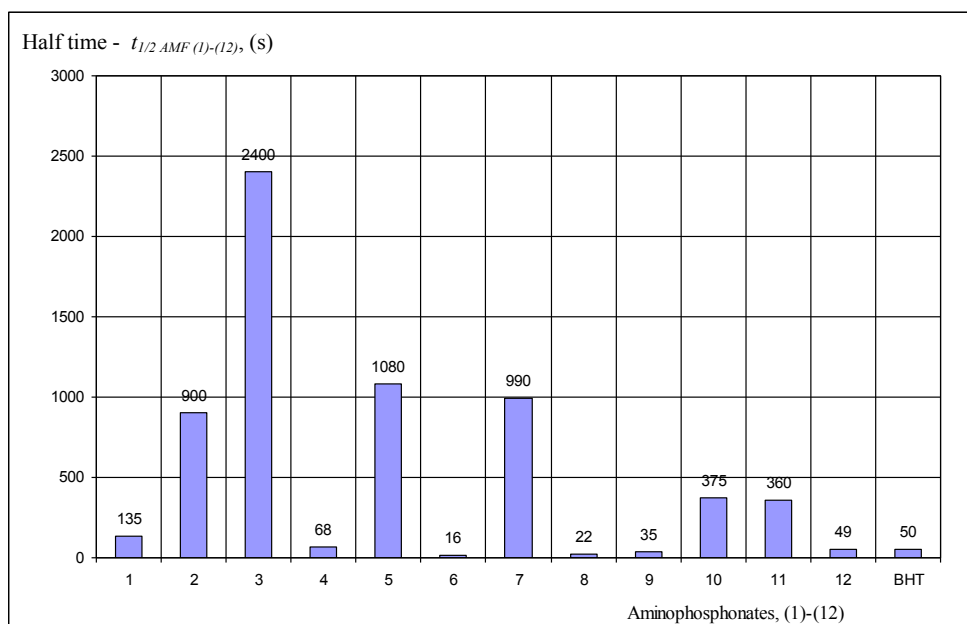


Figure 3.

DPPH• half life in the reaction with aminophosphonates (**1**) – (**12**) and BHT. Molar rapports DPPH• : antioxidant (1)-(12) = 1 : 29

b) Aminophosphonates functionality as herbicides. Table I presents the hemolysis concentrations – HC $_{50}$ (concentration of

aminophosphonates which gives 50% hemolysis of human erythrocytes compared to Tween-20 treatment) of the aminophosphonates **1** – **12** solutions, obtained by graphical representation of RBC percent hemolysis variation using formula (2), against the aminophosphonates solutions of 0 (blank), 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mM/L final concentration, after 1.5 h (90 minutes) of incubation at 37°C.

Table I.
Aminophosphonates **1** – **12**, HC_{50} (50% hemolysis) values in experimental conditions against human red blood cells

AMPP 1 – 12	General chemical formula	Molar mass, g/mol	HC_{50} , mM/L
1	$C_{17}H_{30}NO_4P$	343.191	3.60
2	$C_{19}H_{34}NO_4P$	371.223	2.70
3	$C_{27}H_{34}NO_4P$	467.223	2.10
4	$C_{14}H_{24}NO_5P$	317.318	1.30
5	$C_{19}H_{18}NO_4P$	355.097	0.20
6	$C_{13}H_{22}NO_4P$	287.129	0.13
7	$C_{15}H_{18}NO_3P$	291.102	*
8	$C_{15}H_{18}NO_4P$	307.097	2.50
9	$C_{17}H_{31}N_2O_3P$	342.207	1.00
10	$C_{17}H_{23}N_2O_3P$	334.145	*
11	$C_{16}H_{22}N_2O_8P_2$	432.085	3.10
12	$C_{14}H_{20}N_4O_6P_2$	402.086	4.40

*Note: *The HC_{50} for aminophosphonates (7) and (10), which are more inactive compared with the rest of the aminophosphonates, are outside (0÷5) mM / L concentration, by about 2-5 times fold.*

Aminophosphonates physico-chemical characteristics

The aminophosphonates IR spectra contain characteristics around values (ex. for AMPP **1**): $\nu(\text{Ar-OH})$ absorption occurs around 3400 cm^{-1} , (3428 cm^{-1} , (Ar-OH) intense wide band); $\nu(\text{N-H})$ absorption occurs around 3300 cm^{-1} (3296 cm^{-1} , (N-H) intense); $1608i$ (aromatic nucleus vibrations) intense, 1458 (aromatic nucleus vibrations). Strong absorption around 1220 cm^{-1} is considered to arise from the P=O vibrations, $\nu(\text{P=O})$ is 1212 cm^{-1} for AMPP **1**. At 1056 cm^{-1} is the absorption for P-O-C , and around 756 cm^{-1} ,

absorption for P–C. Aminophosphonates physico-chemical characterization data, which are in accordance with aminophosphonates **1** – **12** structures, and results obtained by other authors, in case of similar compounds [22].

The majority of the UV VIS spectra present at 220 nm, characteristics for a $\pi \rightarrow \pi^*$ transition, due to a conjugation in an unsaturated system, and around 280 nm, characteristics for a $n \rightarrow \pi^*$, due to a conjugation with a system containing a lone pair of electrons [23].

Due to the fact that electron impact - mass spectrometry (EI-MS) spectra are obtained in a high energy field, there are obtained fragments characteristic to benzenic nuclei fragmentation, phosphonate or phosphonic group elimination and alkyl, N-alkyl or N-(alkyl)(aryl) elimination.

For all aminophosphonates **1-12**, (+) electrospray ionisation - mass spectrometry (ESI-MS) spectra contain their $[M+H]^+$ peaks. Characteristic fragments are obtained by elimination of hydroxyl group from *ortho* position, elimination of phosphonate group, alkenes from phosphonate group, and alcohols from phosphonate group, amino alkyl or alkenes from amino alkyl groups, acid phosphonic from aminophosphonic acids [24,25,26].

The ^1H NMR resonance assignments of aminophosphonates **1** – **12**, in DMSO- d_6 , are in agreement with their structure, and results obtained by other authors, in case of similar compounds [27,28].

Biological activity

a) Antiradical activity. Aminophosphonates **4** > **1** > **11** > **10** > **2** > **7** > **5** > **3** (decreasing order), have a greater half life period than the reference substance – BHT, therefore are less active. Aminophosphonates structure comprise three benzene nuclei for aminophosphonates **5** > **3**, two benzene nuclei for aminophosphonates **11** > **10** > **7**, and only one benzene nuclei for aminophosphonates **4** > **1** > **2**, in their structures.

With the exception of aminophosphonate **2**, we can say that their half life period is shorter with the decrease of benzene nuclei number from three to one. Probably the steric hindrance of benzene nuclei plays an important role in the reaction with DPPH•.

Aminophosphonates **6** > **8** > **9** > **12** (Figure 3) present a greater antioxidant potential than BHT. Those aminophosphonates have the possibility to mobilize 4, 2, 3, and respectively 3, active hydrogen.

As stated before, regarding the aminophosphonates structural formula, the results are conveying to other researchers conclusions, which found that DPPH• free radical can go to a stable form by reacting with active hydrogen, or an electron by going to a stable diamagnetic molecule [29].

b) Aminophosphonates functionality as herbicides. The data literature indicates a direct correlation between the aminophosphonates hemolytic capacity and their plant growth inhibition (herbicidal properties) [30].

It is reasonable to expect that the primary herbicide effects are the result of perturbations in physicochemical properties of biological membranes. This may lead to the destruction of the cellular membrane when the concentration of the lipophylic compound is high enough (% hemolysis, see HC_{50} values from Table I).

The studied aminophosphonates were classified in three classes:

b1) at least good herbicides ($6 > 5 > 9 > 4$; for aminophosphonates **6**, **5** and **9**, the only difference are the substituents at the nitrogen atom: **6** – diisopropyl, **5** – diphenyl and for **9** – n-octyl; **6**, **5** and **9**, are free phosphonic acids, only **4** is a diethylphosphonate with a 2-hydroxypropyl group attached at nitrogen; we can conclude that the hemolytic activity of the aminophosphonates $6 > 5 > 9 > 4$, is decreasing in the order, diisopropyl > diphenyl > n-octyl > (2-hydroxypropyl + diethyl).

b2) of medium biological application ($3 > 8 > 2 > 11$), which contain in their molecule 3, 2, 1 and 2×1 benzenic nuclei (2×1 notation for a bifunctional aminophosphonic acid), which means that the greater the number of benzenic nuclei, the greater is the hemolytic activity of the aminophosphonates: $3 > 8 > 2 > 11$.

b3) with less or no herbicide activity ($1 > 12 > 7 > 10$). From this series we can extract the conclusion: if the hydroxyl group is not present in the *ortho* position, the aminophosphonates activity is decreasing drastically. Aminophosphonate **1**, contains in its structure a 2-hydroxyl group, while **12**, **7** and **10**, does not. In the same manner are the aminophosphonates **11** – of medium biological application, which contains 2 groups 2-hydroxyphenyl attached to the central carbon atoms of the molecule, and aminophosphonates **12** – with less or no herbicide activity, which contains instead 2 piridin-3-yl groups.

In the case of aminophosphonates $3 > 2 > 1$, which have the same n-octyl substituent at the nitrogen atom, the unique difference being the substituents from the phosphonate group, the hemolytic activity is decreasing in the following order: phenyl > ethyl > methyl. The obtained data are in accordance with experimental data results published by other authors [5].

The above results were confirmed using an automated method by counting the total RBC number, before and after 90 minutes of the aminophosphonates addition. In this case was used the hematology analyzer

Celltak α MEK-6318K (Nihon Kohden, England). The parameter RBC – red blood cell count can be measured in the range $(0 - 14.99) \cdot 10^6 / \mu\text{L}$, with a coefficient of variation – $\text{CV} = 1.5\%$. The instrument uses the volumetric method of cell counting. The instrument is measuring twice this parameter and displays the arithmetic mean. If the measured RBC counts values are too different, the instrument counts one more time the RBC value and displays this value as final.

Conclusions

Experimental results related to aminophosphonates reaction with DPPH•, show a direct relationship between the aminophosphonates antioxidant character and the number of active hydrogen that can be theoretically mobilized, presenting an inverse dependence with the number of benzene nuclei. We found that 4 of the synthesized aminophosphonates, present a better antioxidant activity then BHT.

The optimal condition ensuring aminophosphonates high effectiveness as hemolyzing agents, and subsequently to act as good herbicides, is a combination of short branched aliphatic substituents and aromatic radicals. Activity is decreasing when in the aminophosphonates' molecule are included more benzenic nuclei ($1 > 2 > 3$).

Based on the experiments presented here (series b), and other results published before by us [31,32], and other authors [6,7,8,33], an important conclusion is that it's not important the type of RBC used (human, sheep, pig, etc.), the conclusions are similar, at least regarding the qualitative hierarchy of series members for the studied biological activity – in this case – the aminophosphonates hemolyzing activity.

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