Synthesis and in vitro antioxidant evaluation of new bis(\(\alpha\)-aminoalkyl)phosphinic acid derivatives

Kamiran Sarac\textsuperscript{a}, Cahit Orek\textsuperscript{b}, Ahmet Cetin\textsuperscript{c}, Taner Dastan\textsuperscript{c}, Pelin Koparir\textsuperscript{d}, Sevgi Durna Dastan\textsuperscript{e}, and Metin Koparir\textsuperscript{b}

\textsuperscript{a}Department of Metallurgy and Material Engineering, Faculty of Architecture Engineering, Bitlis Eren University, Bitlis, Turkey; \textsuperscript{b}Department of Chemistry, Faculty of Science, Firat University, Elazığ, Turkey; \textsuperscript{c}Department of Chemistry, Faculty of Arts and Science, Bingol University, Bingol, Turkey; \textsuperscript{d}Department of Chemistry, Forensic Medicine Institute, Malatya, Turkey; \textsuperscript{e}Department of Zootechanical and Animal Nutrition, Faculty of Veterinary, Cumhuriyet University, Sivas, Turkey

ABSTRACT
Diamines were added to arylaldehydes in ethanol, which resulted in corresponding diimines. Novel bis-1-amino phosphinic acid compounds were synthesized through the interaction of diimines and hypophosphorus acid. The new compounds were characterized by elemental analyses, FT-IR and \(^1\)H, \(^{13}\)C and \(^{31}\)P NMR techniques. The in vitro antioxidant activity of the newly synthesized compounds were measured and found to exhibit significantly higher antioxidant activity than the standard.

GRAPHICAL ABSTRACT

Introduction
It is well known that amino acids are the main components of various proteins and that they generally play an important physiological role in the life process. 1-Aminophosphinic acids are phosphorus analogues of natural amino acids and are selective inhibitors of various proteolytic enzymes, particularly metallo-proteases.\textsuperscript{1-3} For this reason, aminophosphinic acids have been researched and developed as potential antibacterial, antitumor, and antivirus materials in recent years.\textsuperscript{4-6} Much consideration has been given to aminophosphinic acid ligands and their complexes because of their novel structures and properties.\textsuperscript{7-13} Aminophosphonic acids are also found as constituents of natural products.\textsuperscript{14} In contrast to the widely studied 1-aminophosphonic acid derivatives,\textsuperscript{15-18} relatively few papers have reported the chemistry of \(\alpha\)-aminophosphonic acid derivatives.

Reactive oxygen species (ROS) such as \(\mathrm{O}_2^-\), \(\mathrm{H}_2\mathrm{O}_2\), and \(^{1}\mathrm{OH}\) are generated in cells through aerobic metabolic processes or as a result of interaction with exogenous agents. Low ROS levels are essential for the proper cell function, but excess levels lead to “oxidative stress,” which has been linked to the progression of ageing and many human diseases, e.g., neurogenerative, cardiovascular, and cancer. Superoxide dismutases (SODs), catalase (CAT), and glutathione peroxidase (GPx) are enzymes which act as a primary cellular defence system against oxidative damage in living organisms. Organophosphorus compounds and P-heterocycles, in particular, have been recognized as antioxidant drugs.\textsuperscript{19,20} Their mechanism and structure activity relationships (SAR) have been extensively studied.\textsuperscript{21} Depending on their structure and scavenging properties, phosphites and phosphonates may act as both primary and secondary antioxidants. In general, phosphites are considered to be hydroperoxide decomposing (secondary antioxidants), but certain aryl phosphites should also be capable of acting as radical chain terminating (primary antioxidants) by trapping peroxy radicals to produce aroxyl radicals. Recent studies have elucidated...
reaction modes and the relationship between structure, reaction mechanism, and antioxidant activity.\textsuperscript{20,21}

Over the past several years, our laboratories have reported the synthesis of 1-amino-$H$-phosphinic acids.\textsuperscript{22,23} We have reported the synthesis and \textit{in vitro} antimicrobial activity of novelaminophosphinic acids containing cyclobutane and 1.3-thiazole. Building on previous studies, we have prepared a series of derivatives of new $\alpha$-aminophosphinic acids (Figure 1). In view of the importance of phosphinic acid derivatives, this study’s goal was to find new biologically active molecules. Here, we report a new series of bis-1-amino-$H$-phosphinic acids and a preliminary biological study of their antioxidative activity.

**Results and discussion**

**Chemistry**

Diimines are good precursors for synthesizing numerous organic compounds, especially heterocyclic compounds.\textsuperscript{24,25} These easily accessible precursors can be produced by reacting aromatic aldehydes in ammonia.

Schiff bases 3a–i were prepared following the published procedure\textsuperscript{22,23} by condensing the corresponding diamines (1) with arylaldehyde (2) in ethanol at room temperature. The corresponding imines were obtained in quantitative yields. The preparation of bis-aminophosphinic acids 4a–h was performed following Baylis et al.\textsuperscript{26} Reactions were carried out in boiling ethanol for 24 hr, after which mixtures were left overnight to stir at room temperature (Figure 1).

Because two stereogenic carbons bonded to a phosphorus atom, and due to the prototopic transfer of the acidic proton between the phosphoryl ($P = O$) and acidic ($P–OH$) sites, these compounds exist as two diastereomeric forms: one meso-compound ($R^*, S^*(-4)$) and one racemic pair ($S^*, S^* \text{ or } R^*, R^*(-4)$), as shown in Figure 2.

The structures of 4a–h were determined by spectroscopy (\textit{\textsuperscript{1}H}, $\text{\textsuperscript{13}C}$ and $\text{\textsuperscript{31}P}$ NMR, and FT-IR) and by comparison to data in the literature of similar compounds.\textsuperscript{26–28}
The $^1$H NMR spectra of 4a–h showed characteristic doublets at $\delta$ 6.5–7.0 ppm with $J_{\text{P-H}}=520$ Hz constants, responsible for the P–H coupling. The $^{31}$P NMR spectrum exhibited two major peaks at $\delta$ 28.6 and 24.4 due to the 4g diastereoisomers. In the infrared (IR) spectrum, the most characteristic absorptions were at 3461–3533 cm$^{-1}$ (OH), 3270–3309 cm$^{-1}$ (NH), 1163–1182 cm$^{-1}$ (P = O), 1010–1080 cm$^{-1}$ (P–O), and 2307–2390 cm$^{-1}$ (P–H).

The proposed mechanism of formation of 4a–h is illustrated in Figure 3. The addition of hypophosphorous acid to the azomethine bond of Schiff bases 3a–i led to the formation of meso-compounds and racemic pairs.

**Antioxidant activity**

All phosphinic acid derivatives 4a–h and standards were prepared at a range of concentrations from 50 to 500 µg/mL. Table S1 (Supplemental Materials) shows the results of antioxidant activity. Various methods were used to compare the antioxidant activity of the new compounds against the standards. Experimental details are given in the supplemental materials available online.

Hydroxyl radical ($\cdot$OH) scavenging activity. All compounds, BHT and ascorbic acid standards showed similar hydroxyl radical scavenging activity. Nonetheless, compounds 4c and 4f exhibited the highest values. The hydroxyl radical
Superoxide radical scavenging activity. Superoxide anions are oxygen-centred radicals and indicators of active free radicals with the potential of reacting with biological macromolecules and thus causing tissue damage. 29 All compounds showed nearly equal activity levels. Compounds 4c, 4e, and 4f exhibited the greatest values, which were higher than α-tocopherol. Compound 4b exhibited the lowest activity. The superoxide radical scavenging activity of the standards and test compounds were measured calorimeter (DSC). The IR spectra were measured with a spectrometer operating at 400 MHz for $^1$H, 100 MHz for $^{13}$C, and 162 MHz for $^{31}$P. Compounds were dissolved in NaOD/D$_2$O and chemical shifts were referenced to TMS ($^1$H and $^{13}$C NMR) and 85% H$_3$PO$_4$ ($^{31}$P NMR). Elemental analyses were performed on a LECO-CHNS-938. The Supplemental Material contains $^1$H, $^{13}$C, and $^{31}$P NMR spectra for compounds 4a–h (Figures S1–S21).

General procedure for the synthesis of 3α–h

A solution of diamine (20 mmol) in absolute ethanol (30 mL) was slowly added to a solution of aldehyde (40 mmol) in absolute ethanol (20 mL). The stirred reaction mixture was refluxed for 4 hr. After cooling, a precipitate was formed which was collected by filtration, washed with cold ethanol, and recrystallized from a 9:1 mixture of ethylene and water.

General procedure for the synthesis of 4α–h

Hyrophosphoric acid (50%, 2 mol equivalent) in ethanol was added to the imine in ethanol (50 mL for 20 mmol). The mixture was refluxed for 24 hr and then cooled. The solid product was filtered off and washed with a solvent mixture of ethanol and water and air dried at room temperature.

$($Ethane-1,2-diylbis[imino][2-hydroxyphenyl]methanediyli)]bis(phosphonic acid)$(4a)(C$_{15}$H$_{24}$N$_4$O$_5$P$_2$)

White solid, yield 60%, m.p. 250–252°C. IR (KBr, ν, cm$^{-1}$): 3400 (−OH), 3215 (−NH), 3041 (−Ar-H), 2825–2971 (−C-H), 2384 (−PH), 1597 (−C = C), 1182 (−P = O), 1017–1042 (−P-O) cm$^{-1}$. $^1$H-NMR (400 MHz, NaOD/D$_2$O, δ, ppm): 6.92 (m, 4H, Ar-H), 6.60 (d, 2H, J = 514.8 Hz, PH), 6.40 (m, 4H, Ar-H), 3.99 (m, 2H, PCH), 2.71 (m, 4H, N-CH$_2$). $^{13}$C-NMR (100 MHz, NaOD/D$_2$O, δ, ppm): 163.3, 128.2, 127.7, 124.4, 118.6, 113.6, 55.9 46.1. Elem. Anal.: Calculated: C, 50.01; H, 5.54; N, 7.00. Found: C, 48.50; H, 5.20; N, 6.85.

$($Ethane-1,2-diylbis[imino][2-oxoxyphenyl]methanediyli)]bis(phosphonic acid)$(4b)(C$_{15}$H$_{24}$N$_4$O$_5$P$_2$)

White solid, yield 55%, m.p. 200–203°C. IR (KBr, ν, cm$^{-1}$): 3411 (−OH), 3266 (−NH), 3078 (−Ar-H), 2836–2971 (−C-H), 2324 (−PH), 1612 (C = C), 1165 (−P = O), 1046–1069 (−P-O) cm$^{-1}$. $^1$H-NMR (400 MHz, NaOD/D$_2$O, δ, ppm): 6.88 (m, 4H, Ar-H), 6.57 (d, 2H, J = 516.4 Hz PH), 6.28 (m, 4H, Ar-H), 3.01 (m, 2H, PCH), 2.87 (s, 6H, O-CH$_3$), 2.02 (m, 4H, N-CH$_2$). $^{13}$C-NMR (100 MHz, NaOD/D$_2$O, δ, ppm): 158.1, 129.6, 127.9, 114.0, 63.1, 62.3, 55.3, 44.8. Elem. Anal.: Calculated: C, 50.47; H, 6.12; N, 6.54. Found: C, 50.05; H, 6.50; N, 6.93.

$($Ethane-1,2-diylbis[imino][3-thiophen-2-ylmethanediyli)]bis(phosphonic acid)$(4c)(C$_{17}$H$_{27}$N$_4$O$_5$P$_2$)

White solid, yield 43%, m.p. 220–222°C. IR (KBr, ν, cm$^{-1}$): 3437 (−OH), 3241 (−NH), 3082–3095 (−Ar-H), 2940–2963 (−C-H), 2318 (−PH), 1617 (−C = C), 1180 (−P = O), 1066 (−P-O) cm$^{-1}$. $^1$H-NMR (400 MHz, NaOD/D$_2$O, δ, ppm): 7.10 (s, 2H, Ar-H), 6.75 (s, 2H, Ar-H), 6.71(d, 2H, J = 518.4 Hz PH), 6.68 (s, 2H, Ar-H), 3.67 (m, 2H, PCH), 2.22 (m, 4H, N-CH$_2$).
In this study, we synthesized a new series of bis-1-amino-\(\text{H}\)phosphonic acid derivatives and used different methods to compare their antioxidative activity. The novel phosphonous acids were obtained in moderate yields varying from 40 to 60%, which was more than expected, since several authors [40-42] reported much lower conversion rates for additions to two azomethine groups. Among various bis-1-amino-\(\text{H}\)-phosphonic acids derivatives, compounds 4c and 4f showed higher antioxidative activity than the other derivatives and the standard BHT compound. The lowest activity values were generally from compounds 4a and 4b.

Statistical evaluation

Data analyses were done using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Linear regression was conducted to provide mathematical models of determinism between dependency (concentration) and independency (inhibition percentage). IC50 values were calculated with both linear and cubical models. Data were evaluated at 95% confidence intervals.

References

1. Latajka, R.; Krezal, A.; Mucha, A.; Jewginski, M.; Kafarski, P. J. Mol. Struct. 2008, 877, 64–71.
2. Gattes, L. A.; Li, V. S. Pharm. Res. 1995, 2, 135–136.
3. Ye, Y.; Liu, M.; Kao, J.-L.; Marshall, G.R. Biopolymer 2008, 89, 72–85.
4. Gittens, S. A.; Bansal, G.; Zernicke, R. F.; Uludag, H. Adv. Drug Deliv. Rev. 2005, 57, 1011–1036.
5. Sanders, J. M.; Gómez, A. O.; Mao, J.; Meints, G. A.; Van Brussel, E. M.; Burzynska, A.; Kafarski, P.; Gonzales-Paconowska, D.; Oldfield, E. J. Med. Chem. 2000, 43, 1985–1992.
6. Collinsona, M.; Jiracek, J. Curr. Med. Chem. 2005, 12, 57, 1011–1036.
7. Kafarski, P.; Lejczak, B.; Tyka, R.; Koba, L.; Pliszczak, E.; Wieczorek, P. J. Plant Growth Reg. 1995, 14, 199–203.
8. Luckman, S. P.; Coxon, F. P.; Ebertin, F. H.; Russell, G. G.; Rogers, M. J. Bone Miner. Res. 1998, 13, 1668–1678.
9. Katoh, M.; Hiratake, J.; Kato, H.; Oda, J. Bioorg. Med. Chem. Lett. 1996, 6, 1437–1442.
10. Kaboudin, B.; As-Habei, N. Tetrahedron Lett. 2003, 44, 4243–4245.
11. Kaboudin, B.; Haruki, T.; Yamagishi, T.; Yokomatsu, T. Tetrahedron 2007, 63, 8199–8205.
12. Kaboudin, B.; Haruki, T.; Yamagishi, T.; Yokomatsu, T. Synthesis 2007, 20, 3226–3232.
13. Kaboudin, B.; Jafari, J. Iran. Chem. Soc. 2008, 5, 597–5102.
14. Kukhar, V. P.; Hudson, H. R. *Aminophosphonic and Aminophosphinic Acids*, Wiley: Chichester, 2000.

15. Hyun-Joon, H.; Gong-Shil, N. *Synth. Commun.* 1992, 22, 1143.

16. Ganczarz, R.; Wieczorek, J. S. *Synthesis* 1978, 625.

17. Seyferth, D.; Marmor, R. S.; Hilbert, P. J. *Org. Chem.* 1971, 36, 1379–1386.

18. Worms, K. H.; Schmidt-Dunker, M. In: G. M. Kosolapoff; L. Maier (Eds.), *Organic Phosphorus Compounds*, Vol. 7; Wiley: New York, 1976, p. 1.

19. Nurulain, S. M.; Szegi, P.; Tekes, K.; Naqvi S. N. *Arb Hig Rada Toksikol*, 2013, 64, 169–177. doi: 10.2478/10004-1254-64-2013-2294

20. Schwetlick, K.; Pionteck, J.; Habicher, T. *Eur. Polym.* 1987, 23, 383–388.

21. Földes, E.; Maloscik, E.; Kriston, I.; Staniek, P.; Pukánszky, B. *Polym. Degrad. Stabil.* 2006, 91, 479–487.

22. Koparir, P.; Karaarslan, M.; Orek, C.; Koparir, M. *Phosphorus Sulfur Silicon Relat. Elem.* 2011, 186, 2368–2376.

23. Koparir, P.; Karaarslan, M.; Orek, C.; Koparir, M. *Phosphorus Sulfur Silicon Relat. Elem.* 2012, 187, 864–870.

24. (a) Saigo, K.; Kubota, N.; Takebayashi, S.; Hasegawa, M. *Bull. Chem. Soc. Jpn.* 1986, 59, 931–932; (b) Corey, E. J.; Kuhnle, F. N. M. *Tetrahedron Lett.* 1997, 38, 8631–8634; (c) Larter, M. L.; Phillips, M.; Ortega, F.; Aguirre, G.; Somanathan, R.; Walsh, P. J. *Tetrahedron Lett.* 1998, 39, 4785–4788; (d) Nishiyama, K.; Saito, M.; Oba, M. *Bull. Chem. Soc. Jpn.* 1998, 61, 609–611; (f) Uchida, H.; Shimizu, T.; Reddy, P. Y.; Nakamura, S.; Toru, T. *Synthesis* 2003, 8, 1236–1240; (g) Uchida, H.; Tanikoshi, H.; Nakamura, S.; Reddy, P. Y.; Toru, T. *Synlett* 2003, 1117–1120; (h) Kaboudin, B.; Saadati, F. *Heteroatom Chem.* 2005, 65, 353–357.

25. (a) Corey, E. J.; Grogan, M. *J. Org. Lett.* 1999, 1, 157–160; (b) Isobe, T.; Fukuda, K.; Araki, Y.; Ishikawa, T. *Chem. Commun.* 2001, 3, 243–244; (c) Anastassiadou, M.; Baziard-Mouysset, G.; Payard, M. *Synthesis* 2000, 13, 1814–1816; (d) Corey, E. J.; Huang, H. C. *Tetrahedron Lett.* 1989, 30, 5235; (e) Corey, E. J.; Imwinkelried, R.; Pikul, S. B. *J. Am. Chem. Soc.* 1989, 111, 5493–5495; (f) Corey, E. J.; Kim, S. S. *J. Am. Chem. Soc.* 1990, 112, 4976–4977.

26. Lewkowski, J.; Rybaczuk, M. *Heterocycles* 2008, 19, 283–287.

27. Boyd, E. A.; Chan, W. C.; Loh Jr., V. M. *Tetrahedron Lett.* 1996, 37, 1647–1650.

28. Schmidt, H. *Chem. Ber.* 1948, 81, 477–483.

29. Halliwell, B.; Gutteridge, J. M. C.; Aruoma O. I. *Anal. Biochem.* 1987, 165, 215–219.

30. Wright, J. S.; Johnson, E. R.; Dilabio, G. A. J. *Am. Chem. Soc.* 2001, 123, 1173–1183.

31. Orhani, I.; Ozelci˘k, B.; Sener, B. *Turk. J. Biol.* 2011, 35, 251–258.

32. Yamaguchi, F.; Ariga, T.; Yoshimura, Y.; Nakazawa, H. *J. Agric. Food Chem.* 2000, 48, 180–185.

33. Finefrock, A. E.; Bush, A. I.; Doraissyamy, P. M. *J. Am. Geriatr. Soc.* 2003, 51, 1143–1148.

34. Kilic, I.; Yesiloglu, Y.; Bayrak, Y. *Spectrochim. Acta A.* 2014, 130, 447–452.

35. Liu, F.; Ooi, V. E. C.; Chang, S. T. *Life Sci.* 1997, 60, 763–771.

36. Nguyen, M. A. T.; Munagara, A. K.; Kim, J. A.; Lee, K. D.; Park, S. *Phosphorus Sulfur Silicon Relat. Elem.* 2015, 190, 191–199

37. Cuendet, M.; Hostettmann, K.; Poterat, O. H. *Helv. Chim. Acta.* 1997, 80, 1144–1152.

38. Dinis, T. C. P.; Madeira, V. C.; Almeida, L. M. *Arch. Biochem. Biophys.* 1994, 315, 161–169.

39. Ruch, R. J.; Cheng, S. J.; Klaunig, J. E. *Carcinogenesis* 1989, 10, 1003–1008.

40. Fallla, S.; Finocchiaro, P. *Phosphorus Sulfur* 1993, 85, 65–72.

41. Fallla, S.; Finocchiaro, P.; Hagele, G.; Rapisardi, R. *Phosphorus Sulfur* 1993, 82, 79–90.

42. Fallla, S.; Finocchiaro, P. *Phosphorus Sulfur* 1995, 107, 79–86.