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**Ultrasound-promoted solvent-free synthesis of some new α-aminophosphonates as potential antioxidants**

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**Abstract:** Ultrasonic irradiation has been adopted in order to achieve an efficient synthesis of some novel α-aminophosphonates by Pudovik reaction. Major benefits of this method are as follows: eco-friendly, free of catalyst, high yielding, uncomplicated work-up procedure, short reaction time, and solvent free condition. Spectral characterisation and elemental analysis of the synthesized samples was carried out. In vitro antioxidant activity of the title compounds was screened by DPPH·, O2·− and NO· scavenging methods. Good activity was shown by most of the compounds as compared with the standards.

**Keywords:** α-aminophosphonates; ultrasonic irradiation; antioxidant activity

**1 Introduction**

α-Aminophosphonates (AAPs) are most important group of organophosphorus compounds that are structural analogues of amino acids where a carboxylic substituent is substituted by phosphonic acid or related groups (Merino et al., 2008). Due to their outstanding biological and physical characteristics together with their usefulness as intermediates in synthesis; AAPs have found a wide variety of applications in industry, agriculture and medicine (Moonen et al., 2004; Palacios et al., 2004; Schug and Lindner, 2005). They are discovered to be antibacterial (Subramanyam et al., 2017), antifungal (Yang et al., 2006), antiviral (Xu et al., 2006), anti-inflammatory agents (Sujatha et al., 2017), anti-HIV (Bhattacharya et al., 2012), anticancer (Bahrami et al., 2016), anti-proliferative and apoptosis inducing (Huang et al., 2016a, 2016b; Li et al., 2015), antitumor (Liu et al., 2017), herbicidal activity (Che et al., 2016) and insecticidal activity (Jiang et al., 2013; Liu et al., 2012). Addition of trialkyl or dialkylphosphite to imines is a successful technique to obtain them (Azizi et al., 2004; Heydari et al., 2009; Kassaee et al., 2009; Manjula et al., 2013; Yadav et al., 2001). Many reviews were published using various Bronsted acids, Lewis acids, heteropoly acids, heterogeneous and nano catalysts to accomplish this transformation (Ambica et al., 2008; Anastas and Eghbali, 2010; Bhattacharya et al., 2007, 2008; Chandrasekhar et al., 2001; Heydari et al., 2007; Mitragotri et al., 2008; Sobhani et al., 2008; Yadav et al., 2003; Vahdat et al., 2008). Nevertheless, most of them are highly-expensive, corrosive and occupy tedious separation processes and long reaction times. So that, establishment of an environmental friendly protocol would expand the scope of the synthesis of AAPs. In this regard, solvent free synthesis is one of the best option especially when the solvents are toxic and flammable (Hung et al., 2016a).

Alternatively, synthesis of organic compounds by means of ultrasound irradiation is a successful protocol which is an alternate energy source for organic reactions typically accomplished by normal heating (Juarez et al., 2009, 2010). One advantage of using cavitation as an energy source to promote organic reactions includes shorter reaction times (Srivastava et al., 2009). In the cavitation process, the molecules of the liquid are split, produces bubbles which further collapse in the compression cycle during the rarefaction cycle. These quick and aggressive implosions produce tiny-lived regions by means of temperatures of approximately 5000°C, pressures of about 1000 atm and heating and cooling rates above 10⁷°C per second. Such localized hot spots can be considered as micro reactors where the sound energy is distorted into...
2 Results and discussion

2.1 Chemistry

A series of AAPs, 7a-e and 8a-e were synthesized in two steps by Pudovik reaction. At first, a series of imines (4a-e and 5a-e) were prepared by reacting equimolar quantities of amines (1a-e) and aldehydes (2 and 3) in ethanol at 40°C for approximately 1 h. Then, the corresponding imine was treated with diethylphosphite (6) to give AAPs, 7a-e and 8a-e using both conventional approach as well as ultrasonication methods. Table 1 and Scheme 1 represent the results and the synthetic protocol of this process.

The chemical structures of all the title compounds 7a-e and 8a-e were characterized by IR, ¹H, ¹³C, ³¹P NMR, mass spectral data and elemental analyses and their data were presented in the experimental section. IR absorptions in the regions 3345-3308, 1236-1210 and 1018-1012 cm⁻¹ were assigned to NH, P=O and P–O–C stretching vibrations respectively for the title compounds (Subramanyam et al., 2013). The ¹H NMR spectra gave signals due to Ar-H in the range of δ 8.13-6.82 ppm. The proton signal at δ 4.55 was assigned to -NH stretching vibrations of all the title compounds. The proton signal in the range δ 4.05 and 1.18 cm⁻¹ as doublet was assigned to P–CH stretching vibrations of all the title compounds. The methylene and methyl protons of P–O–CH₂CH₃ resonated as multiplet and triplet respectively at δ 4.05 and 1.18 cm⁻¹. ¹³C NMR chemical shift for methylene and methyl carbons were observed at 61.9 ppm and 16.2 ppm respectively for the title compounds 7a-e and 8a-e. The chemical shift for P–CH carbons was observed at δ 57.5 ppm respectively for the title compounds. ³¹P NMR signals were observed in the region 20.3-15.2 ppm for 5a-j (Subramanyam et al., 2017). In their mass spectra, M⁺ ions were observed in the expected m/z values. The representative spectra of compound 7a were provided in Supplementary material as Figures S1-S6.

2.2 Antioxidant activity

Antioxidant activity of newly prepared compounds become screened at concentrations 50 μg/mL and 100 μg/mL by DPPH, Superoxide and Nitric oxide (NO) radical scavenging activities. The investigational results be obtainable in Table 2.

Out of all, the compounds; 7b and 7c showed the highest DPPH radical scavenging activity with % inhibition 64.2, 79.5 μg/mL, and 63.6, 78.4 μg/mL, respectively at 50 and 100 μg/mL. The remaining compounds showed moderate activity while compared with the antioxidant, Ascorbic acid.

In superoxide radical scavenging activity assay, the compounds 8a, 7c, and 8c showed the highest DPPH radical scavenging activity with % inhibition 64.2, 81.5 μg/mL, and 63.6, 80.3 μg/mL, respectively at 50 and 100 μg/mL. The remaining compounds showed moderate activity while compared with the antioxidant, BHT.

In NO radical scavenging activity assay, the compounds 7c and 7b showed the highest % inhibition...
Table 2: DPPH, superoxide and NO radical scavenging activities of compounds 7a-e and 8a-e.

| Compound | DPPH radical scavenging activity | Superoxide radical scavenging activity | NO radical scavenging activity |
|----------|----------------------------------|----------------------------------------|--------------------------------|
|          | % inhibition 50 μg/mL 100 μg/mL | % inhibition 50 μg/mL 100 μg/mL | % inhibition 50 μg/mL 100 μg/mL |
| 7a       | 56.4 75.5                          | 62.2 72.1                              | 43.2 63.8                        |
| 8a       | 55.6 67.6                          | 58.5 79.5                              | 50.3 78.6                        |
| 7b       | 63.6 80.3                          | 54.3 73.1                              | 64.2 90.2                        |
| 8b       | 52.1 74.2                          | 42.4 62.6                              | 31.3 62.5                        |
| 7c       | 64.2 81.5                          | 57.6 78.4                              | 67.3 93.9                        |
| 8c       | 60.7 77.2                          | 56.3 77.1                              | 55.5 83.4                        |
| 7d       | 40.4 63.6                          | 40.9 60.7                              | 56.3 85.5                        |
| 8d       | 54.2 71.5                          | 51.2 72.1                              | 52.2 79.9                        |
| 7e       | 34.5 50.8                          | 26.2 43.2                              | 39.8 65.7                        |
| 8e       | 58.6 77.3                          | 31.4 51.6                              | 58.5 87.7                        |
| *Standard| 68.4 84.2                          | 64.6 78.4                              | 68.56 96.07                      |
| Blank    | - -                                | - -                                    | - -                              |

Scheme 1: Synthesis of AAPs (7a-e and 8a-e).
67.3, 93.9 μg/mL and 64.2, 90.2 μg/mL, respectively at 50 and 100 μg/mL. The remaining exhibited considerable activity with reference to the usual antioxidant, BHT.

3 Conclusion

In conclusion, we demonstrated right here an efficient, cheaper, environmentally benign protocol for the formation of AAPs by Pudovik reaction through an intermediate imine using solvent and catalyst free condition. The antioxidant activity of the synthesized compounds was evaluated by DPPH, O₂⁻ and NO methods. Compound 7b, bearing with 4-fluorophenyl)(thiazol-2-ylamino moiety; 7c incorporated with 4-fluorophenyl) (1-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-ylamino moiety and 8c bearing 4-chlorophenyl)(1-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-ylamino motif exhibited potent antioxidant activity when in comparison with the standard antioxidants.

Experimental

Materials and methods

The chemicals were purchased from Sd. Fine Chem. Ltd., India and some of them were purified using standard procedures. The purity of the compounds was checked by TLC on silica gel coated Al sheet. In conventional technique, the reaction was carried out on magnetic stirrer cum hot plate. Ultrasonicator, BANDELIN SONOREXR (Germany) (35 kHz, 200 W) with inbuilt heating 30°C-80°C was used for ultrasonic irradiation technique. J (coupling constants) and δ (chemical shift) values were reported in Hz and ppm, respectively. Bruker AMX spectrometer was used to record 31P (161.9 MHz), 1H (400 MHz) and 13C (100 MHz) NMR spectra. The symbols ‘s’ for singlet, ‘d’ for doublet, ‘t’ for triplet and ‘m’ for multiplet were used to represent peaks in NMR spectra. L.C. MS were recorded on SHIMADZU 2010A. Thermo Finnigan Flash 1112 apparatus was used for CHN analysis. Bruker IFS 55 (Equinox) FTIR spectrometer in KBr was used to record IR spectra.

Procedure

Synthesis of imines (4a-e and 5a-e)

The reaction of equimolar amounts of numerous amines (1a-e) (0.02 mole) with aldehydes (2 and 3) (0.02 mole) in ethanol at 40°C for about 1 h produced the respective imines (4a-e and 5a-e).

Synthesis of AAPs, 7a-e and 8a-e by conventional heating method

The imine (0.01 mole) become reacted with diethylphosphite (6) (0.015 mole) in EtOH using tetramethylguanidine (TMG) at 50°C-60°C for 3.5 h to yield the corresponding AAPs (7a-e and 8a-e). TLC (ethylacetic: n-hexane, 6:4) was used for verifying the development of the process. As soon as the reaction was finished, as confirmed via TLC, the aggregate became chilled to room temperature. The pure compounds (7a-e and 8a-e) were obtained by column chromatography by means of n-hexane:ethyl acetate (2:3) as eluent. The yield become located in the range of 68-78%.

Ultrasonication procedure for the synthesis of AAPs, 7a-e and 8a-e

The imines (4a-e and 5a-e) (0.01 mole) which received formerly and diethylphosphite (6) (0.015 mole) were positioned in a RB Flask and the reactants become irradiated in ultrasonicator at room temperature for about 15-40 min to obtain respective AAPs (7a-e and 8a-e). TLC (ethyl acetate: n-hexane, 6:4) was used for verifying the progress of the reaction. Once the reaction was completed, as tested by TLC, the combination turned into chilled to room temperature. The pure compounds (7a-e and 8a-e) were acquired through column chromatography using ethyl acetate: n-hexane (3:2) as eluent. The yield of the synthesized compounds was observed within the range of 85-95%.

Characterization of title compounds (7a-e and 8a-e)

**Diethyl (4-fluorophenyl)(pyridin-3-ylamino)methylphosphonate (7a).** Yield: 90%; semi solid. δH (DMSO-d6): 8.13-7.28 (m, 7H, Ar-H), 4.55 (s, 1H, -NH), 4.05 (p, 4H, O-CH2CH3), 3.86 (d, 1H, P-CH), 1.18 (t, J = 7.6 Hz, 6H, O-CH2CH3); δC (DMSO-d6): 162.3 (C-13), 147.8 (C-16), 139.5 (C-19), 136.4 (C-21), 132.9 (C-10), 127.3 (C-11, C-15), 125.9 (C-18), 119.1 (C-17), 116.6 (C-12, C-14), 61.9 (C-5, C-8), 57.5 (C-1), 16.2 (C-6, C-9); δP (DMSO-d6): 16.7 ppm; IR (KBr) (νmax cm⁻¹): 3326 (NH), 1233 (P=O), 1017 (P-O-C alip); LCMS (m/z, %): 339 (M+H +, 100); For C16H20FN2O3P; calcd: C, 56.80; H, 5.96; N, 8.28%; found: C, 56.86; H, 5.91; N, 8.32%.
Diethyl (4-chlorophenyl)(pyridin-3-ylamino)methylphosphonate (8a). Yield: 89%; semi solid. δH (DMSO-d6): 7.35-6.82 (m, 7H, Ar-H), 4.05 (p, 4H, O-CH2CH3), 3.86 (d, 1H, -NH), 1.24 (s, 3H, -CH3); δC (DMSO-d6): 164.8 (C-16), 162.3 (C-13), 138.3 (C-18), 132.9 (C-10), 127.3 (C-11, C-15), 116.6 (C-12, C-14), 61.9 (C-5, C-8), 58.9 (C-17, C-21), 51.2 (C-18), 46.6 (C-22); IR (KBr) (νmax cm-1): 3345 (NH), 1235 (P=O), 1018 (P-O-Calip); LCMS (m/z, %): 386 (M+H+, 100) 388 (M+2+, 32.7); For C15H24FN3O5P; calcd: C, 49.71; H, 5.49; N, 10.91%; found: C, 49.93; H, 5.42; N, 10.98%.

Diethyl (4-chlorophenyl)(1-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-ylamino)methylphosphonate (7c). Yield: 89%; semi solid. δH (DMSO-d6): 7.29 (d, 2H, Ar-H), 7.15 (d, 2H, Ar-H), 4.05 (p, 4H, O-CH2CH3), 3.90 (d, 1H, -CH), 2.72 (m, 4H, -CH2), 2.32 (s, 3H, -CH3); δC (DMSO-d6): 165.4 (C-16), 163.4 (C-20), 162.3 (C-13), 161.4 (C-18), 132.9 (C-10), 127.3 (C-11, C-15), 116.6 (C-12, C-14), 76.8 (C-17), 61.9 (C-5, C-8), 57.5 (C-1), 2.28 (m, 4H, -CH2), 2.12 (s, 1H, -NH), 1.18 (t, J = 7.6 Hz, 6H, O-CH2CH3); δC (DMSO-d6): 20.3 ppm; IR (KBr) (νmax cm-1): 3345 (NH), 1235 (P=O), 1018 (P-O-Calip); LCMS (m/z, %): 386 (M+H+, 100) 388 (M+2+, 32.7); For C15H24FN3O5P; calcd: C, 49.93; H, 5.42; N, 10.98%.
16.2 (C-6, C-9); δ_p (DMSO-d6): 17.7 ppm; IR (KBr) (ν_max cm⁻¹): 3313 (NH), 1212 (P=O), 1015 (P-O-Calip); LCMS (m/z, %): 376 (M+H+, 100) 378 (M+2+, 36.6); For C_{16}H_{27}FN_{3}O_{3}P; calcd: C, 53.47; H, 7.57; N, 11.69%; found: C, 53.53; H, 7.72; N, 11.75%.

Diethyl (4-chlorophenyl)(4-methylpiperazin-1-ylamino)methylphosphonate (8e). Yield: 91%; semi solid. δ_H (DMSO-d6): 7.37 (d, 2H, Ar-H), 7.17 (d, 2H, Ar-H), 4.05 (p, 4H, O-CH₂CH₃), 3.90 (d, 1H, P-CH), 2.72 (m, 4H, -CH₂), 2.32 (s, 3H, -CH₃), 2.28 (m, 4H, O-CH₂), 2.12 (s, 1H, -NH), 1.18 (t, J = 7.6 Hz, 6H, O-CH₂CH₃); δ_C (DMSO-d6): 132.8 (C-10), 132.3 (C-13), 128.9 (C-12, C-14), 128.3 (C-11, C-15), 61.9 (C-5, C-8), 57.5 (C-1), 56.1 (C-17, C-21), 51.2 (C-18, C-20), 46.6 (C-22), 16.2 (C-6, C-9); δ_p (DMSO-d6): 16.2 ppm; IR (KBr) ν_max cm⁻¹: 3308 (NH), 1210 (P=O), 1012 (P-O-Calip); LCMS (m/z, %): 376 (M+H+, 100) 378 (M+2+, 36.6); For C_{16}H_{27}ClN_{3}O_{3}P; calcd: C, 53.47; H, 7.57; N, 11.69%; found: C, 53.53; H, 7.72; N, 11.75%.

**Antioxidant activity**

**DPPH* Scavenging activity**

We followed the usual approach of Cotelle et al. (1996) with some adjustments for screening DPPH scavenging activity of synthesized compounds at concentrations 50 and 100 μg/mL. The screening was carried out in triplicate and the common values were taken as final result. The % inhibition of DPPH was tested by taking the results of the test with those of the control.

**O₂⁻ scavenging activity**

We have used the same old method of Robak and Gryglewski (1988) with minor variations in incubation period and concentration of PMS for screening O₂⁻ scavenging activity of synthesized compounds at concentrations 50 and 100 μg/mL. Butylated hydroxyl toluene (BHT) was used as standard.

**NO⁻ Scavenging activity**

NO⁻ Scavenging activity of the title compounds was carried out using standard method of modified protocol of Green et al. (1982) and Marcocci et al. (1994) with minor modifications at concentrations 50 and 100 μg/mL. BHT was used as standard drug for the study. The experiment was carried out in triplicate and the results are presented in Table 2.

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**Conflict of interest:** The authors declare that there is no conflict of interest about the eBook of this newsletter.

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