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Synthesis and inhibitory activity of ureidophosphonates, against acetylcholinesterase: Pharmacological assay and molecular modeling

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ABSTRACT

A novel method has been developed for the synthesis of 1-ureidophosphonates through a three components condensation of aldehyde with amine and diethylphosphite in the presence of sulfanilic acid as catalyst followed by subsequent reaction of the product with isocyanate. This method is easy, rapid, and good yielding. The anticholinesterase (AChE) activities (inhibition potency through IC_{50}) of newly synthesized 1-ureidophosphonates were also investigated. The activities of the synthesized compounds toward the enzyme AChE were determined and compared in terms of their molecular structures and it was found, through molecular docking simulations, that the most potent derivative (compound **3i**) inhibited the enzyme through binding to the peripheral anionic site (PAS) and not to its acylation site (A site).

1. Introduction

Phosphonic acids are of growing importance in understanding and modulating biological processes [1]. The synthesis of α-substituted phosphoryl derivatives (phosphonic and phosphinic acids) has attracted significant attention due to their biological activities with broad applications as enzyme inhibitors, antimetabolites and antibiotics [2]. Among α-functionalized phosphonic acids, α-aminoalkylphosphonic derivatives have biological activities such as anti-bacterial [3], herbicidal [4] and fungicidal [5].

While the synthesis and properties of many types of aminophosphonic acid derivatives have been widely investigated [6–9], relatively few papers describe the chemistry and bioassay of 1-ureidophosphonates. 1-Ureidophosphonates have been shown powerful antiviral activities against TMV [10]. They also display moderate activity against a number of fungal pathogens [11]. In addition, these compounds are used as active ingredients in pesticides especially insecticides and acaricides [12].

Acetylcholinesterase (AChE) is the most widely used target enzyme in studies with the purpose to synthesize new and more effective therapeutic agents to treat patients with diseases such as Alzheimer's disease or myasthenia gravis [13–16]. Also, acetylcholinesterase is used widely as a target in the studies of compounds with insecticidal activities, as all commercially available organophosphate compounds are believed to exert their effects through inhibition of this enzyme [17].

Despite wide range of biological activities of 1-aminophosphonate derivatives, the synthesis and studies of anticholinesterase activities of novel 1-ureidophosphonates have received little attention. Synthetic routes to 1-ureidophosphonates involve prolonged heating of three component reaction of trialkylphosphite with urea and aldehydes at 50 °C in the presence of BF_3 as a catalyst under nitrogen described by Birum [18,19], and the reaction of aminophosphonates with isocyanates in the presence of LDA at −30 °C [20] or in the absence of any base [21]. However, these methods have associated problems, including harsh reaction conditions, long reaction times and side reactions. As part of our efforts to explore the novel methods for the synthesis of organophosphorus compounds [22], we report a new method for the synthesis of 1-ureidophosphonates via a three components condensation of aldehyde with amine and diethylphosphite in the presence of sulfanilic acid as catalyst followed by subsequent reaction of the product with isocyanate, producing good to high yields of 1-ureidophosphonates. In the continuation of our studies on the synthesis and biological activities of novel organophosphates [23,24], we decided to investigate to what extent the inhibitory effect of the synthesized 1-ureidophosphonates toward electric eel AChE would be affected upon the inclusion of different substitutions.

In recent years, the development of more economical and environmental friendly conversion processes is gaining interest. Sulfanilic acid is inexpensive, commercially available catalyst and recently shown as a very good reagent in the organic reactions [25]. In continuation of our studies on the synthesis of 1-aminophosphonates, we now describe the synthesis and characterization of 1-ureidophosphonates via a three components condensation followed by subsequent reaction of the product with isocyanate.

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2. Materials and methods

2.1. General

Chemicals were either prepared in our laboratories or purchased from Merck, Fluka, and Aldrich Chemical Companies. All yields refer to isolated products. NMR spectra were taken by a 400 Brucker with the chemical shifts being reported as δ ppm and couplings expressed in Hertz. Silica gel column chromatography was carried out with Silica gel 100 (Merck No. 10184). UV spectra were determined on a Varian Cary 100 instrument.

2.2. General procedure for the preparation of α-ureidophosphonates

Sulfamic acid (0.097 g, 1 mmol) was added to a stirred mixture of diethyl phosphite (5 mmol), amine (5 mmol) and aldehyde (5 mmol) at room temperature and the mixture stirred for 3 h. A mixture of isocyanate (5 mmol) in dichloromethane (3 mL) was added to the reaction mixture under argon. The mixture was stirred for 5–8 h at room temperature. The mixture was washed with dichloromethane (2 × 50 mL) and solvent evaporated to give crude product. The crude product was chromatographed on silica gel (*n*-hexane:EtOAc = 80/20) to give the pure product in 74–87% isolated yield. All products gave satisfactory spectral data in accord with the assigned structures.

2.2.1. Diethyl [(phenylcarbamoyl)(phenyl)amino](phenyl)methylphosphonate (**3a**)

Mp 87–89 °C (*n*-hexane–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.14 (t, 3H, J = 6.8 Hz), 1.38 (t, 3H, J = 6.8 Hz), 3.94–4.00 (m, 1H), 4.04–4.11 (m, 1H), 4.25–4.32 (m, 2H), 6.12 (s, 1H, NH), 6.51 (d, 1H, J_{HP} = 22.4 Hz), 7.01–7.43 (m, 15H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 16.2 (d, J_{PC} = 6.0 Hz), 16.5 (d, J_{PC} = 6.0 Hz), 55.7 (d, J_{PC} = 159.9 Hz), 62.7 (d, J_{PC} = 6.0 Hz), 62.9 (d, J_{PC} = 6.0 Hz), 119.4, 123.2, 128.3, 128.4, 128.8, 129.2, 129.5, 131.0 (d, J_{PC} = 8.0 Hz), 131.5, 133.8 (d, J_{PC} = 5.0 Hz), 137.6, 138.5, 154.2 (d, J_{PC} = 5.0 Hz); ^{31}P NMR (162.0 MHz, $CDCl_3$, H_3PO_4): δ = 20.88 ppm. Anal. Calcd for $C_{24}H_{27}N_2O_4P$: C, 65.74; H, 6.21; N, 6.39. Found: C, 65.55; H, 6.25; N, 6.21%.

2.2.2. Diethyl [(phenylcarbamoyl)(phenyl)amino](4-isopropylphenyl) methylphosphonate (**3b**)

Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.14 (t, 3H, J = 7.2 Hz), 1.23 (d, 6H, J = 5.6 Hz),1.36 (t, 3H, J = 7.2 Hz), 2.89 (sep, 1H, J = 6.8 Hz), 3.96–4.00 (m, 1H), 4.05–4.09 (m, 1H), 4.23–4.29 (m, 2H), 6.12 (s, 1H, NH), 6.47 (d, 1H, J_{HP} = 22.8 Hz), 7.00–7.41 (m, 14H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 16.2 (d, J_{PC} = 6.0 Hz), 16.5 (d, J_{PC} = 6.0 Hz), 23.8, 23.9, 33.8, 55.5 (d, J_{PC} = 159.9 Hz), 62.5 (d, J_{PC} = 7.0 Hz), 62.8 (d, J_{PC} = 7.0 Hz), 119.4, 123.1, 126.3, 128.8, 129.0, 129.3, 130.9 (d, J_{PC} = 9.0 Hz), 131.1 (d, J_{PC} = 4.0 Hz), 131.5, 137.8, 138.6, 149.1, 154.3 (d, J_{PC} = 5.0 Hz); ^{31}P NMR (162.0 MHz, $CDCl_3$, H_3PO_4): δ = 21.27 ppm. Anal. Calcd for $C_{27}H_{33}N_2O_4P$: C, 67.48; H, 6.92; N, 5.83%. Found: C, 67.58; H, 6.83; N, 5.72%.

2.2.3. Diethyl [(phenylcarbamoyl)(phenyl)amino](3-methoxyphenyl) methylphosphonate (**3c**)

Mp 122–124 °C (*n*-hexane–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.16 (t, 3H, J = 7.2 Hz), 1.37 (t, 3H, J = 7.2 Hz), 3.68 (s, 3H), 3.97–4.01 (m, 1H), 4.05–4.12 (m, 1H), 4.24–4.30 (m, 2H), 6.12 (s, 1H, NH), 6.48 (d, 1H, J_{HP} = 22.4 Hz), 6.83–7.41 (m, 14H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 16.3 (d, J_{PC} = 6.0 Hz), 16.5 (d, J_{PC} = 6.0 Hz), 55.2, 55.7 (d, J_{PC} = 161.0 Hz), 62.6 (d, J_{PC} = 7.0 Hz), 62.9 (d, J_{PC} = 7.0 Hz), 114.8, 115.8 (d, J_{PC} = 9.0 Hz), 119.4, 119.5, 123.3, 123.4 (d, J_{PC} = 9.0 Hz), 128.8, 129.2 (d, J_{PC} = 12.1 Hz), 129.4, 131.5, 135.0 (d, J_{PC} = 5.0 Hz), 137.7, 138.6, 154.2 (d, J_{PC} = 4.0 Hz), 159.3; Anal. Calcd for $C_{25}H_{29}N_2O_5P$: C, 64.09; H, 6.24; N, 5.98%. Found: C, 64.31; H, 6.28; N, 5.72%.

2.2.4. Diethyl [(phenylcarbamoyl)(phenyl)amino](4-nitrophenyl) methylphosphonate (**3d**)

Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 (t, 3H, J = 7.2 Hz), 1.36 (t, 3H, J = 7.2 Hz), 4.02–4.09 (m, 1H), 4.10–4.17 (m, 1H), 4.24–4.31 (m, 2H), 6.13 (s, 1H, NH), 6.46 (d, 1H, J_{HP} = 23.2 Hz), 7.04–7.46 (m, 10H), 7.66 (d, 2H, J = 8.4 Hz), 8.15 (d, 2H, J = 8.4 Hz); ^{113}C NMR (100.6 MHz, $CDCl_3$): δ = 16.3 (d, J_{PC} = 6.0 Hz), 16.4 (d, J_{PC} = 6.0 Hz), 56.0 (d, J_{PC} = 161.0 Hz), 62.9 (d, J_{PC} = 7.0 Hz), 63.2 (d, J_{PC} = 7.0 Hz), 119.6, 123.5, 123.6, 128.9, 129.6, 129.9, 131.0, 131.6 (d, J_{PC} = 8.0 Hz), 137.6, 138.1, 141.6 (d, J_{PC} = 5.0 Hz), 147.7, 154.2 (d, J_{PC} = 4.0 Hz); ^{31}P NMR (162.0 MHz, $CDCl_3$, H_3PO_4): δ = 19.60 ppm. Anal. Calcd for $C_{24}H_{26}N_3O_6P$: C, 59.62; H, 5.42; N, 8.69%. Found: C, 59.61; H, 5.38; N, 5.32%.

2.2.5. Diethyl [(phenylcarbamoyl)(phenyl)amino](4-methylphenyl) methylphosphonate (**3e**)

Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.15 (t, 3H, J = 7.2 Hz), 1.37 (t, 3H, J = 7.2 Hz), 2.33 (s, 3H), 3.94–3.98 (m,1H), 4.04–4.08 (m, 1H), 4.24–4.34 (m, 2H), 6.11 (s, 1H, NH), 6.48 (d, 1H, J_{HP} = 22.8 Hz), 7.01–7.43 (m, 14H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 16.3 (d, J_{PC} = 6.0 Hz), 16.5 (d, J_{PC} = 6.0 Hz), 21.2, 55.4 (d, J_{PC} = 161.0 Hz), 62.6 (d, J_{PC} = 8.0 Hz), 62.8 (d, J_{PC} = 8.0 Hz), 119.5, 123.2, 128.8, 129.0, 129.1, 129.4, 130.7 (d, J_{PC} = 5.0 Hz), 130.9 (d, J_{PC} = 9.0 Hz), 131.6, 137.7, 138.2, 138.6, 154.3 (d, J_{PC} = 5.0 Hz); ^{31}P NMR (162.0 MHz, $CDCl_3$, H_3PO_4): δ = 21.11 ppm. Anal. Calcd for $C_{25}H_{29}N_2O_4P$: C, 66.36; H, 6.46; N, 6.19%. Found: C, 66.61; H, 6.38; N, 6.02%.

2.2.6. Diethyl [(phenylcarbamoyl)(4-methoxyphenyl)amino](phenyl) methylphosphonate (**3f**)

Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.13 (t, 3H, J = 7.2 Hz), 1.39 (t, 3H, J = 7.2 Hz), 3.84 (s, 3H), 3.93–4.03 (m, 1H), 4.05–4.07 (m, 1H), 4.25–4.31 (m, 2H), 6.15 (s, 1H, NH), 6.52 (d, 1H, J_{HP} = 22.4 Hz), 6.86 (br, 2H), 7.01–7.33 (m, 10H), 7.41 (d, 2H, J = 6.8 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 16.2 (d, J_{PC} = 6.0 Hz), 16.5 (d, J_{PC} = 6.0 Hz), 55.3 (d, J_{PC} = 161.0 Hz), 55.5, 62.7 (d, J_{PC} = 7.0 Hz), 62.9 (d, J_{PC} = 7.0 Hz), 114.5, 119.3, 123.1, 128.3, 128.4, 128.8, 129.6, 131.1 (d, J_{PC} = 8.0 Hz), 132.7, 133.8 (d, J_{PC} = 4.0 Hz), 138.6, 154.5 (d, J_{PC} = 5.0 Hz), 159.8; ^{31}P NMR (162.0 MHz, $CDCl_3$, H_3PO_4): δ = 20.93 ppm. Anal. Calcd for $C_{25}H_{29}N_2O_5P$: C, 64.09; H, 6.24; N, 5.98%. Found: C, 64.15; H, 6.18; N, 5.83%.

2.2.7. Diethyl [(phenylcarbamoyl)(4-methoxyphenyl)amino](4-fluorophenyl) methylphosphonate (**3g**)

Mp 104–106 °C (*n*-hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.14 (t, 3H, J = 7.2 Hz), 1.39 (t, 3H, J = 7.2 Hz), 3.85 (s, 3H), 3.94–4.03 (m, 1H), 4.04–4.07 (m, 1H), 4.25–4.31 (m, 2H), 6.14 (s, 1H, NH), 6.50 (d, 1H, J_{HP} = 22.4 Hz), 6.88 (br, 2H), 6.96–7.39 (m, 11H); ^{113}C NMR (100.6 MHz, $CDCl_3$): δ = 16.2 (d, J_{PC} = 6.0 Hz), 16.5 (d, J_{PC} = 6.0 Hz), 54.4 (d, J_{PC} = 162.0 Hz), 55.5, 62.7 (d, J_{PC} = 7.0 Hz), 62.8 (d, J_{PC} = 7.0 Hz), 114.6, 115.2, 115.4, 119.4, 128.8, 129.4, 129.8 (dd, J_{PC} = 5.0, J_{FC} = 3.0), 132.9 (d, J_{FC} = 24.1 Hz), 133.0 (d, J_{FC} = 17.1 Hz), 138.6, 154.5 (d, J_{PC} = 5.0 Hz), 159.9, 162.7 (d, J_{FC} = 247.5 Hz); ^{31}P NMR (162.0 MHz, $CDCl_3$, H_3PO_4): δ = 20.69 ppm. Anal. Calcd for $C_{25}H_{28}N_2FO_5P$: C, 61.72; H, 5.80; N, 5.76%. Found: C, 61.06; H, 5.98; N, 5.52%.

2.2.8. Diethyl [(phenylcarbamoyl)(4-methoxyphenyl)amino](4-methoxyphenyl) methylphosphonate (**3h**)

Mp 123–125 °C (*n*-hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.13 (t, 3H, J = 7.2 Hz), 1.37 (t, 3H, J = 7.2 Hz), 3.79 (s, 3H),

3.83 (s, 3H), 3.9–3.95 (m, 1H), 4.01–4.05 (m, 1H), 4.23–4.29 (m, 2H), 6.13 (s, 1H, NH), 6.47 (d, 1H, J_{HP} = 22.4 Hz), 6.77 (d, 2H, J = 8.4 Hz), 6.86 (br, 2H), 6.99–7.28 (m, 7H), 7.30 (d, 2H, J = 8.4 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 16.2 (d, J_{PC} = 6.0 Hz), 16.5 (d, J_{PC} = 6.0 Hz), 54.5 (d, J_{PC} = 161.0 Hz), 55.2, 55.4, 62.6 (d, J_{PC} = 7.0 Hz), 62.8 (d, J_{PC}7.0 Hz), 113.6, 114.4, 119.4, 123.1, 125.8 (d, J_{PC} = 5.0 Hz), 128.8, 129.6, 132.5 (d, J_{PC} = 9.0 Hz), 132.8, 138.7, 154.5 (d, J_{PC} = 5.0 Hz), 159.6, 159.8; 31P NMR (162.0 MHz, $CDCl_3$, H_3PO_4): δ = 21.18 ppm. Anal. Calcd for $C_{26}H_{31}N_2O_6P$: C, 62.64; H, 6.27; N, 5.62%. Found: C, 62.52; H, 6.36; N, 5.62%.

2.2.9. Diethyl [(phenylcarbamoyl)benzyl amino](4-methylphenyl) methylphosphonate (**3i**)

Mp 89–91 °C (*n*-hexane–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.15 (t, 3H, J = 7.2 Hz), 1.37 (t, 3H, J = 7.2 Hz), 2.33 (s, 3H), 3.94–3.98 (m,1H), 4.04–4.08 (m, 1H), 4.24–4.34 (m, 2H), 6.11 (s, 1H, NH), 6.48 (d, 1H, J_{HP} = 22.8 Hz), 7.01–7.43 (m, 14H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 16.3 (d, J_{PC} = 6.0 Hz), 16.5 (d, J_{PC} = 6.0 Hz), 21.2, 55.4 (d, J_{PC} = 161.0 Hz), 62.6 (d, J_{PC} = 8.0 Hz), 62.8 (d, J_{PC} = 8.0 Hz), 119.5, 123.2, 128.8, 129.0, 129.1, 129.4, 130.7 (d, J_{PC} = 5.0 Hz), 130.9 (d, J_{PC} = 9.0 Hz), 131.6, 137.7, 138.2, 138.6, 154.3 (d, J_{PC} = 5.0 Hz); ^{31}P NMR (162.0 MHz, $CDCl_3$, H_3PO_4): δ = 21.11 ppm. Anal. Calcd for $C_{25}H_{29}N_2O_4P$: C, 66.36; H, 6.46; N, 6.19%. Found: C, 66.48; H, 6.28; N, 5.96%.

2.2.10. Diethyl [(phenylcarbamoyl)n-butylamino](phenyl) methylphosphonate (**3j**)

Mp 88–90 °C (*n*-hexane–EtOAc). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.81 (t, 3H, J = 7.2 Hz), 1.15–1.25 (m, 2H), 1.21 (t, 3H, J = 7.2 Hz), 1.31 (t, 3H, J = 7.2 Hz), 1.54–1.62 (m, 2H), 3.36–3.59 (m, 2H), 4.01–4.28 (m, 4H), 5.80 (d, 1H, J_{HP} = 23.5 Hz), 6.99 (t, 1H, J = 7.2 Hz), 7.21–7.67 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.7, 16.3 (d, J_{PC} = 5.7 Hz), 16.5 (d, J_{PC} = 5.7 Hz), 20.1, 30.9, 46.5, 55.6 (d, J_{PC} = 158.5 Hz), 62.8 (d, J_{PC} = 7.5 Hz), 63.2 (d, J_{PC} = 6.9 Hz), 119.7 (d, J_{PC} = 7.5 Hz), 123.0, 128.4, 128.7, 128.8, 129.6 (d, J_{PC} = 8.8 Hz), 134.2 (d, J_{PC} = 6.9 Hz), 139.0 (d, J_{PC} = 4.4 Hz), 155.1 (d, J_{PC} = 3.1 Hz); 31P NMR (101.2 MHz, $CDCl_3$, H_3PO_4): δ = 22.50 ppm. Anal. Calcd for $C_{22}H_{31}N_2O_4P$: C, 63.14; H, 7.47; N, 6.69%. Found: C, 63.26; H, 7.37; N, 6.53%.

2.2.11. Diethyl [(phenylcarbamoyl)-cyclohexylamino](phenyl) methylphosphonate (**3k**)

Mp 140–142 °C (*n*-hexane–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.00–2.05 (m, 16H), 4.14–4.40 (m, 5H), 5.23 (d, 1H, J_{HP} = 29.6 Hz), 7.01 (t, 1H, J = 7.6 Hz), 7.26 (t, 1H, J = 8.4 Hz), 7.29–7.38 (m, 4H), 7.46 (d, 2H, J = 7.6), 7.60 (d, 2H, J = 7.6), 8.25 (s, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 16.4 (d, J_{PC} = 6.0 Hz), 16.5 (d, J_{PC} = 6.0 Hz), 25.5, 26.1, 26.2, 31.3, 31.5, 55.8 (d, J_{PC} = 153.9 Hz), 56.7 (d, J_{PC} = 3.0 Hz), 63.0 (d, J_{PC} = 8.0 Hz), 63.9 (d, J_{PC} = 8.0 Hz), 119.4, 122.6, 128.1, 128.2, 128.7, 128.8, 134.7 (d, J_{PC} = 6.0 Hz), 139.4, 155.3 (d, J_{PC} = 2.0 Hz); ^{31}P NMR (162.0 MHz, $CDCl_3$, H_3PO_4): δ = 24.04 ppm. Anal. Calcd for $C_{24}H_{33}N_2O_4P$: C, 64.85; H, 7.48; N, 6.30%. Found: C, 64.72; H, 7.53; N, 6.40%.

2.3. Biochemical methods: AChE inhibition assay

Inhibitory activities of synthesized α-ureidophosphonates were determined at 25 °C by the colorimetric method of Ellman et al. [26]. The assay solution contained 0.1 M phosphate buffer, pH 8, 0.3 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 0.125 units of AChE (Sigma Chemical Co. from electric eel (*Electrophorous electricus*), and 0.2 mM acetylthiocholine iodide as the substrate in a total volume of 2.5 mL. Synthesized compounds were added to the assay solution and preincubated with the enzyme for 10 min at 25 °C. In order to solubilize the synthesized compounds the reaction mixture contained 2% ethanol (50 μL in 2.5 mL reaction mixture volume). Control experiments were run and it was seen that no inhibitory effect was exerted on the enzyme by ethanol. The changes in absorbance at 412 nm were recorded for 5 min with a Camspec 501 Single Beam Scanning UV/Vis Spectrophotometer. Percent inhibitions were calculated and the concentration of the compounds that produced 50% inhibition of AChE (IC50) was determined through the use of Excel-Solver program. The dose response curves were fit by nonlinear regression. The results have been shown as the averages of the results of three independent experiments together with SEM's.

2.4. Computer-aided molecular modeling

Molecular Docking was carried out using AutoDock 4.2 and AutoDockTools version 1.5.4 with standard parameters [27]. Crystal structure of EeAChE was obtained from protein data bank (pdb code 1C2O) and ligand structure was constructed and energy minimized using PRODRG online server [28]. All calculations were performed with the use of a PC with Intel Core 2 Quad 2.66 GHz Processor. Images were generated with PMV [27].

3. Results and discussion

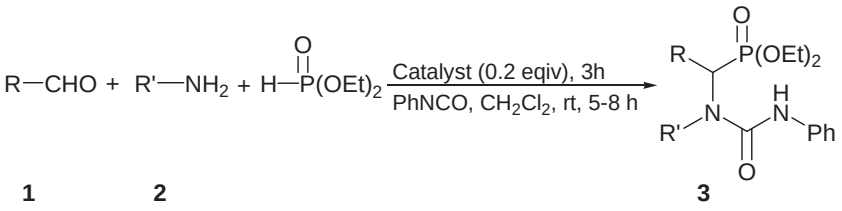
First, benzaldehyde (**1a**) was treated with diethyl phosphite and aniline (**2a**) in the presence of 20 mol% of sulfanilic acid under solvent-free conditions at room temperature (Scheme 1). The reaction proceeded smoothly to afford the corresponding 1-aminophosphonate. When phenylisocyanate was added to the reaction mixture in dichloromethane, it afforded the corresponding 1-ureidophosphonate **3a** in 74% isolated yield at room temperature for 8 h. The reaction gave 33% of 1-ureidophosphonate **4a** after 48 h without any catalyst. It should be noted that the reaction of **1a** with a mixture of **2a** and diethylphosphite followed by reaction with phenylisocyanate in THF, DMF, CH_3CN, and toluene as a solvent gave lower yields of **3a** than CH_2Cl_2. When the reaction was carried out using methanesulfonic acid and benzenesulfonic acid as catalyst gave **3a** in moderate yields of 67 and 51%, respectively (Table 1). According to these results, the reaction of various aldehydes and anilines was examined using sulfanilic acid as catalyst in dichloromethane at room temperature.

Interestingly, different substituted benzaldehydes reacted smoothly with aniline and diethylphosphite followed by reaction with phenylisocyanates to give the corresponding 1-ureidophosphonates in good yields (Table 2 and Scheme 1). Treatment of *p*-methoxyaniline (**2b**) with aldehydes and diethylphosphite followed by reaction with phenylisocyanate afforded the desired compounds **3d–3f** in 72–83% yields. The reaction of aliphatic amines with a mixture of diethylphosphite and aldehydes followed by reaction with phenylisocyanate in the presence of sulfanilic acid gave the desired1-ureidophosphonates in good yields. In all the reactions we have reported in this paper cleavage of Et-O-P bond were not detected and the conversion of the substrates to their corresponding 1-ureidophosphonates was clean.

Newly synthesized 1-ureidophosphonates were assayed for AChE (*Electrophorus electricus*) inhibition potency by the Ellman method [26]. The results are summarized in Table 2.

Except for compound **3i** (IC_{50} = 2.23 μM) and compound **3g** (IC_{50} = 69 μM), other synthetic inhibitor compounds did not show enough potency to be considered as a lead compound for the design of potentially effective inhibitors, with a medicinal chemistry point of view.

The structural differences between the compound **3g** (IC_{50} = 69 μM) and the compound **3i** (IC_{50} = 2.23 μM) are in the aromatic ring proximal to the phosphonate moiety and the other aromatic ring proximal to the central nitrogen. In **3i** there is methyl group



Scheme 1. Synthesis of α-ureidophosphonates (**3**).

Table 1
Reaction of benzaldehyde with a mixture of diethylphosphite and aniline in the presence of sulfanilic acid followed by reaction with phenylisocyanate at room temperature.

Entry	Catalyst	Solvent	Reaction time (h)	Yield%[a]
1	H_2N–SO_3H	CH_2Cl_2	8	74
2	–	CH_2Cl_2	48	33
3	H_2N–SO_3H	THF	8	63
4	H_2N–SO_3H	DMF	8	49
5	H_2N–SO_3H	CH_3CN	8	47
6	H_2N–SO_3H	Toluene	8	51
7	CH_3SO_3H	CH_2Cl_2	8	67
8	$PhSO_3H$	CH_2Cl_2	8	51

[a] Isolated yields of **3a**.

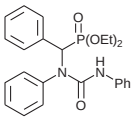
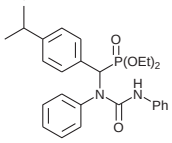
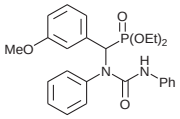
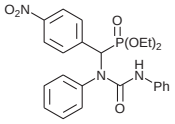
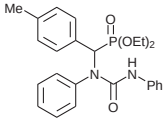
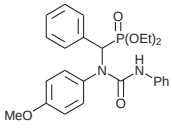
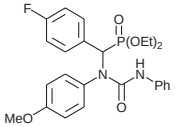
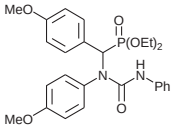
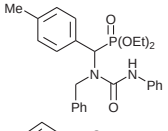
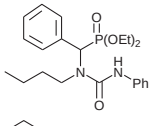
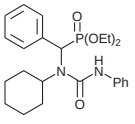
in the *ortho* position of the aromatic ring proximal to the phosphonate moiety and in **3g** there is a fluorine atom at the same position.

Here the non-bonding electrons of oxygen in methoxy group can easily resonate with the π electron system in its vicinity because of their orbital. Consequently the delocalization of the electrons makes the probability of H-bonding and π–π interactions with candidate side chains of the enzyme minimal. Hence it seems that π–π interactions between this inhibitor and aromatic side chains in the enzyme could be a determining factor. Instead there is no methoxy group in the same position in compound **3i**, and the aromatic ring is separated from the central nitrogen with a methylene group which may have improved the potency of the compound **3i** through an orientational effect. Considering the presence of a potent electron withdrawing fluoride group on the aromatic ring proximal to the phosphonate moiety in compound **3g** and a methyl group at the same position in compound **3i** we may say that the non-bonding electrons of fluorine atom has had the same delocalizing effect as discussed about the oxygen in the methoxy group and hence had made the probability of H-bonding and π–π interactions with candidate side chains of the enzyme minimal compared with the methyl group in the compound **3i**.

The only difference in the structures of compound **3i** (IC_{50} = 2.23 μM) and compound **3e** (IC_{50} = 441 μM) is that in compound **3i** the proximal aromatic ring is connected to the central nitrogen atom via a methylene group. This observation again emphasizes the orientational effect of the group attached to central nitrogen.

Since there are two major binding sites in AChE, a narrow active site gorge, at the base of which is located the catalytic triad or acylation site (A-site) and another binding site named peripheral anionic site (PAS), and since the inhibitors of AChE bind to either the A-site (tacrine [29], organophosphates [30], rivastigmine [31]) or PAS (propidium iodide [32]) or to both of them (donepezil [33]) in AChE, we decided to examine the interaction of the most potent inhibitor (compound **3i**) with the whole enzyme AChE through molecular docking simulations. To the best of our knowledge the study of interactions between ureidophosphonates and AChE, as potential inhibitors, through molecular docking experimentation, has not been reported before, although there have been increasing number of reports, particularly in recent years, about the

Table 2
Synthesis of α-ureidophosphonates (**3**) and their anticholinesterase activities.

Entry	Product **3**	Time (h)	Yield[a]%	AChE IC_{50} (μM)
a		8	74	172.07 ± 0.34
b		8	79	230.01 ± 1.09
c		8	85	–[b]
d		6	81	811.33 ± 4.01
e		7	77	441.39 ± 2.18
f		8	72	–[b]
g		6	82	69.31 ± 0.01
h		6	83	–[b]
i		6	85	2.23 ± 0.00
j		5	82	–[b]
k		6	87	148.14 ± 0.21

[a] Yields refer to the isolated pure products after column chromatography.
[b] No inhibition was detected.

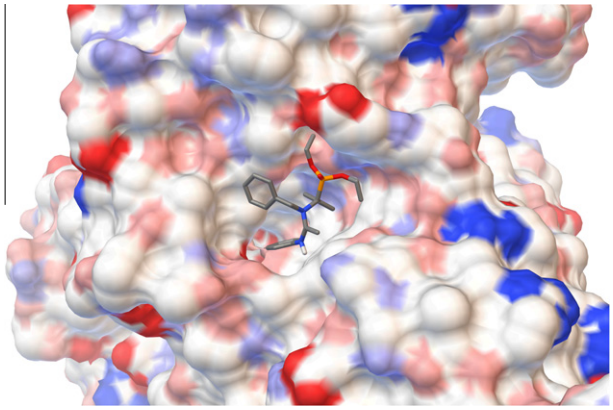


Fig. 1. The surface polarability map of AChE that shows its interactions with the compound **3i**. As it can be seen the orientation of the drug is such that its aromatic rings distal and proximal to the central nitrogen atom have interacted with the hydrophobic surfaces at the entrance of the active site gorge (i.e., PAS site). It is also indicated that the molecule has not been entered the gorge and the phosphonate group has been oriented and located favorably against the surface structural groups at the PAS site.

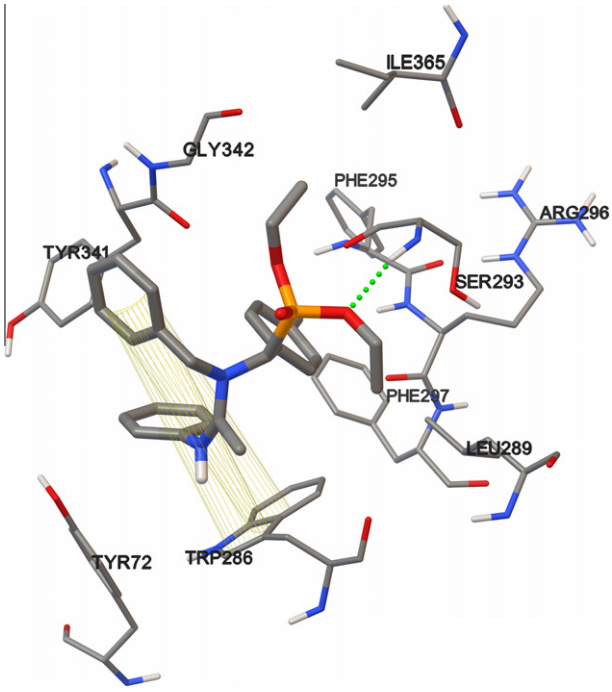


Fig. 2. Peripheral site residues of the enzyme, interacting with compound **3i** through π–π interactions. The interacting residue is Trp 286 and the two aromatic rings in the compound **3i**. As it can be seen the proximal aromatic ring to the central nitrogen is close enough to the aromatic side chain of Trp 286 to have a π–π interaction with it. The positioning of the above proximal aromatic ring has also made possible π–π interaction between Trp 286 and the proximal aromatic ring to the phosphonate moiety in the compound. There is also an H-bond between the phosphonate moiety and the main chain α-amino group of Ser 293.

molecular modeling simulations of either interactions between cholinesterases and their substrates [34] or interactions between other types of organophosphates [35] and cholinesterases [36], e.g., sarin and phosphothioates. Figs. 1 and 2 show the results. As it can be seen in Fig. 1, after providing enough time for interaction between the enzyme and the inhibitor molecule, the compound **3i** has been oriented in such a way that the two aromatic rings interacted only with the hydrophobic structures in the PAS site and there was no interaction between the inhibitor and the active site of the enzyme. The interaction is sterically favorable with respect to either the aromatic rings or the phosphonate moiety. Fig. 2 shows the π-π interaction between the distal aromatic ring of the compound and the aromatic ring of Trp 286 residue in the PAS site of the enzyme. It can be seen that the distal ring is at close proximity to Trp 286 and has also made possible a π–π interaction between Trp 286 and the proximal aromatic ring of compound **3i** through a kind of relay system provided by distal ring in the compound. There is also an H-bond between the phosphonate moiety of the compound and the α-amino group of Ser 293 which seems to be secondary to the mentioned π–π interactions, although may have had an orientational effect.

4. Conclusions

In conclusion the results of this study shows that the synthetic ureidophosphonates containing aromatic rings in their structures can exert different degrees of inhibitory effects on the enzyme AChE, through binding to the PAS site of the enzyme. It seems that the size and orientation of the chemical groups on the compounds have been such that they have not been able to enter the active site gorge to interact with the catalytic A-site of the enzyme, although the docking experiments have been performed on the whole enzyme and not just on the peripheral site. Future experimentations on the synthesized ureidophosphonates in the presence and absence of the ligands that are known to interact with the active site and/or the PAS site residues would clarify more precisely the mode of action of these ureidophosphonates.

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