ORIGINAL PAPER



Synthesis and crystal structure of phosphonic acid and bisphosphoramidate derivatives: QSAR studies of their anti-fungal potential on *Macrophomina Phaseolina* (Tassi) Goid

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Abstract

A series of phosphonic acid and bisphosphoramidate derivatives were synthesized and characterized. The bioactivities against the fungal pathogen *Macrophomina phaseolina* and human acetylcholinesterase AChE enzyme were studied using QSAR based on multiple linear regression. L17, with (*p*-Cl–C₆H₄–NH) (*p*-Cl–C₆H₄)C(H)P(O)(OC₂H₅)₂ skeleton, demonstrated a great mortality on the *M. phaseolina* mycelial growth by 83% inhibition at 150 mg/L; the other tested derivative showed moderate to weak antifungal activity against the fungus. QSAR model based on the GA-MLR method revealed the importance of 3D descriptors (De, Mor18e, H8m, and Mor30p) on the antifungal activity. It showed good capability in predicting the fungicidal activity of the studied molecules. Another derivative, L5, with (*m*-CH₃–NC₅H₄–NH)(*m*-CH₃–C₆H₄)C(H) P(O)(OCH₃)₂ skeleton displays the most potent anti-AChE activity. The electronic parameters, ΔE_{L-H} , and E_{LUMO} , have the highest contribution of human AChE. The authors suggest that these models could be usefully employed in designing more effective crop protection compounds without side effects on non-target organisms.

Keywords Bisphosphoramidate · Phosphonic acid · Anti-fungal agents · Anti-AchE · Structure-activity relationships

Introduction

Old methods of pesticide development like random synthesis are no longer used due to their high consumption of time and money [1]. Nowadays, efforts have been made to find new and alternative chemicals for pest control with minimum

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hazards on the environment and non-target organisms [1]. The excessive use of traditional pesticides leads to the emergence of new strains of pests difficult to control [1–4]. Since the 1950s, there has been a rapid rise in using phosphoric acid ester derivatives in insecticide chemistry [5]. In contrast, a few of organophosphorus compounds were developed to be used as fungicides [5] compared to organic compounds used to control the plant diseases [6]. In terms of the mode of action, acetylcholinesterase enzyme does not constitute a target for fungicides like within the organophosphorus insecticides. This may help develop new organophosphorus fungicides that do not interact with human cholinesterase leading to more safe agrochemicals [1].

It is well known that the development of a new pesticide is a costly process, and the recent main concern is to minimize the cost and time of these procedures [7, 8]. Computer-aided drug design (CADD) technique potentially offers a further means to probe structure–bioactivity relationships [9]. The quantitative structure–activity/property relationships (QSAR/QSPR) approaches were employed to explore the most effective physio-chemical parameters in binding interactions between drugs and their target sites [10, 11]. Descriptors used in QSAR have been classified into different categories, including constitutional, geometrical, topological, quantum chemical, and so on [12–14]. Also, several variable selection methods, including multiple linear regression (MLR) and genetic algorithm (GA), were used [15]. These approaches were previously applied to explore the binding interactions between the phosphonic acid and bisphosphoramidate derivatives and the AChE and fungal enzymes [16].

In this work, several phosphonic acids and bisphosphoramidate derivatives were synthesized and characterized. The fungicidal activity of synthesized compounds was determined on *Macrophomina phaseolina* (Tassi) Goid, a plant pathogenic fungus. Also, they were tested against acetylcholinesterase (AChE) enzyme. The results were then used to generate QSAR models to describe the relationship between these activities and the physicochemical properties of the synthesized compounds within mathematical equations.

Materials and methods

Chemicals and instruments

Triton X-100, bovine serum albumin, acetylthiocholine iodide (ATCH), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), alpha-naphthyl acetate, beta-naphthyl acetate, fast blue RR, DMSO, Na₂HPO₄ (99%), NaH₂PO₄ (99%), sodium dodecyl sulfate (SDS) were all bought from Sigma-Aldrich and Methanol form Merck. ¹H, and, ³¹P NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer. ¹H and ¹³C chemical shifts were determined relative to internal TMS, and ³¹P chemical shifts relative to 85% H₃PO₄ as an external standard. Infrared (IR) spectra were recorded on a Shimadzu model IR-60 spectrometer using KBr pellets. Melting points were obtained with an electrothermal instrument. UV/Visible Spectrophotometer was performed using a PerkinElmer Lambda 25. The fluorescence quenching studies were carried out using the PerkinElmer LS 45 fluorescence spectrophotometer.

Software

The three-dimensional structures of molecules were drawn using the HyperChem software [17]. Dragon (version 3.0) was used for calculation of molecular descriptors [18]. Gaussian 09 suite of programs was used for the calculation of quantum descriptors [19]. Windows-based SPSS software was utilized for the MLR analysis [20]. GA and MLR regression, and other calculations were performed in the MAT-LAB 7.10.0 environment [21].

Crystal structure determination

Single-crystal X-ray data of L_1 and L_{11} were collected on a Bruker APEX-II diffractometer with CCD area detector [22] using graphite monochromated Mo-Ka radiations $(\lambda = 0.71073 \text{ Å})$. Xcalibur, Eos, Gemini ultra and Xcalibur, Atlas, Gemini ultra diffractometers were used for L_1 and L_{11} . The CCD detector, software package CrysAlis171 (L_1) and CrysAlisPRO (L11) were also applied. [23] The structures were refined by full-matrix least-squares on F^2 using the Jana2006 package (L_{11}) and SHELXL-2014 (L_1) [24]. CCDC numbers: 1564405 (L₁) and 1,560,706 (L₁₁) contain the supplementary crystallographic data for these two compounds. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html. or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (+44)1223-336-033; or email: deposit@ccdc.cam.ac.uk.

Synthesis

General procedure for compounds L_1-L_{17} Compounds L_1-L_8 were prepared by 1 mmol benzaldehyde, 1.1 mmol of the primary amine, and trimethyl phosphite in the presence of 2 mmol of LiClO₄. The expected aminophosphonate was produced in 92% yield after 24 h at 50 °C in dichloromethane (Scheme 1). Compounds L_9-L_{17} were prepared by the reaction of 1 mmol aldehyde and 1.1 mmol of the primary amine in glycerol at 80 °C. After 4 h of stirring 1 mmol of diethyl phosphite was added and after 12 h the product was washed with water and ethyl acetate (Scheme 1).

(C₆H₅)(NC₅H₄–NH)C(H)P(O)(OCH₃)₂ (L₁) Mp: 138 °C. ¹H NMR (250.13 MHz, *d*-DMSO, ppm): δ=3.48 (d, ³J_{P-H}=10.5 Hz, 3H, Me), 3.61 (d, ³J_{P-H}=10.5 Hz, 3H, Me), 5.87 (dd, ²J_{P-H}=22.5 Hz, ³J_{H-H}=8.3 Hz, 1H, CH), 6.51 (t, ³J_{H-H}=6.3 Hz, 1H, H_b-py), 6.78 (d, ³J_{H-H}=8.5 Hz, 1H, H_d-py), 7.25–7.51 (m, 7H, Ph), 7.93 (d, ³J_{H-H}=4.5 Hz, 1H, H_a-py). ³¹P NMR (121.49 MHz, *d*-DMSO, ppm): δ=26.5 ppm. IR data (KBr, cm⁻¹): 3299 s (*v*_{N-H}); 3026w, 2945w, 2848w (*v*_{Aliph}); 1601 s (*v*_{py}); 1482 s (*v*_{Ar}); 1230 s (*v*_{P=O}); 1031 s (*v*_{P-O}).

(*p*-Cl-C₆H₄)(NC₅H₄-NH)C(H)P(O)(OCH₃)₂ (L₂) Mp: 171 °C. ¹H NMR (500.13 MHz, d-DMSO, ppm): δ =3.51 (d, ³J_{P-H}=10.6 Hz, 3H, Me), 3.62 (d, ³J_{P-H}=10.6 Hz, 3H, Me), 5.85 (dd, ²J_{P-H}=22.9 Hz, ³J_{P-H}=9.9 Hz, 1H, CH), 6.52 (t, ³J_{P-H}=5.9 Hz, 1H, H_b-py), 6.77 (d, ³J_{P-H}=8.4 Hz, 1H, H_d-py), 7.39 (m, 3H, Ph-py), 7.50 (d, ³J_{P-H}=7.5 Hz, 1H, C₆H₄),7.54 (dd, ³J_{P-H}=8.9 Hz, ³J_{P-H}=3.1 Hz, 1H, NH), 7.92 (d, ³J_{P-H}=4.4 Hz, 1H, H_a-py). ³¹P NMR (202.45 MHz, d-DMSO, ppm): δ =25.5 ppm. IR data



Scheme 1 Reactions diagram for the synthesis of compounds L_1-L_{22} , L_{28} and L_{29}

(KBr, cm⁻¹): 3299 s (v_{N-H}); 2951w, 2854w (v_{Aliph}); 1605 s (v_{py}); 1525w, 1483 s (v_{Ar}); 1233 s ($v_{P=O}$); 1029 s (v_{P-O}).

(*m*-Br-C₆H₄)(NC₅H₄-NH)C(H)P(O)(OCH₃)₂ (L₃) Mp: 112 °C. ¹H NMR (500.13 MHz, d-DMSO, ppm): δ =3.53 (d, ³J_{P-H}=10.6 Hz, 3H, Me), 3.63 (d, ³J_{P-H}=10.6 Hz, 3H, Me), 5.87 (dd, ²J_{P-H}=22.9 Hz, ³J_{H-H}=10.0 Hz, 1H, CH), 6.53 (t, ³J_{P-H}=5.8 Hz, 1H, H_b-py), 6.77 (d, ³J_{P-H}=8.5 Hz, 1H, H_d-py), 7.29 (t, ³J_{P-H}=7.9 Hz, 1H, H_c-py), 7.40 (pst, ³J_{P-H}=7.7 Hz, 1H, H_f-C₆H₄), 7.47 (m, 2H, H_{e,g}-C₆H₄), 7.56 (dd, ³J_{P-H}=9.9 Hz, ³J_{P-H}=3.5 Hz, 1H, NH), 7.71 (s, 1H, H_h-C₆H₄), 7.93 (d, ³J_{P-H}=3.8 Hz, 1H, H_a-py). ³¹P NMR (202.45 MHz, d-DMSO, ppm): δ =24.7 ppm. IR data (KBr, cm⁻¹): 3390 m; 3298 s (v_{N-H}); 2943w (v_{Aliph}); 1596 s (v_{py}); 1474 s (v_{Ar}); 1234 s ($v_{P=O}$); 1034 s (v_{P-O}). (**o**-NC₅H₄–NH)(*m*-CH₃–C₆H₄)C(H)P(O)(OCH₃)₂ (L₄) Mp: 117 °C. ¹H NMR (500.13 MHz, d-DMSO, ppm): δ =2.28 (s, 3H, Me''), 3.48 (d, ³J_{P-H}=10.5 Hz, 3H, Me), 3.61 (d, ³J_{P-H}=10.5 Hz, 3H, Me), 5.83 (dd, ²J_{P-H}=22.6 Hz,, ³J_{P-H}=10.1 Hz, 1H, CH), 6.50 (t, ³J_{P-H}=6.2 Hz, 1H, H_b-py), 6.77 (d, ³J_{P-H}=8.4 Hz, 1H, H_d-py), 7.06 (d, ³J_{P-H}=7.4 Hz, 1H, H_g-C₆H₄), 7.21 (ps-t, ³J_{P-H}=7.6 Hz, 1H, H_f-C₆H₄), 7.28 (d, ³J_{P-H}=7.6 Hz, 1H, H_e-C₆H₄), 7.30 (s, 1H, H_h-C₆H₄), 7.37 (t, ³J_{P-H}=7.7 Hz, 1H, H_c-py), 7.47 (dd, ³J_{P-H}=9.8 Hz, ³J_{P-H}=2.7 Hz, 1H, NH), 7.93 (d, ³J_{P-H}=4.9 Hz, 1H, H_a-py). ³¹P NMR (202.45 MHz, d-DMSO, ppm): δ =25.4 ppm. IR data (KBr, cm⁻¹): 3390 m; 3286 s (*v*_{N-H}); 3018w, 2941w, 2853w (*v*_{Aliph}); 1601 s (*v*_{py}); 1481 s (*v*_{Ar}); 1230 s (*v*_{P=O}); 1062 s, 1035 (*v*_{P-O}). (m-CH₃-NC₅H₄-NH)(m-CH₃-C₆H₄)C(H)P(O)(OCH₃)₂ (L₅) Mp: 142 °C. ¹H NMR (500.13 MHz, d-DMSO, ppm): δ =2.30 (s, 3H, Me'), 3.50 (d, ³J_{P-H}=10.6 Hz, 3H, Me), 3.67 (d, ³J_{P-H}=10.7 Hz, 3H, Me),4.16 (s, 3H, Me"), 5.34 (dd, ³J_{P-H}=23.9 Hz, ³J_{P-H}=9.8 Hz, 1H, CH), 7.12 (d, ³J_{P-H}=7.6 Hz, 1H, H_g-C₆H₄), 7.26 (ps-t, ³J_{P-H}=7.6 Hz, 1H, H_f-C₆H₄), 7.33 (d, 1H, ³J_{P-H}=10 Hz, H_e-C₆H₄), 7.34 (s, 1H, H_h-C₆H₄), 7.73 (dd, ³J_{P-H}=8.8 Hz, ³J_{P-H}=5.7 Hz, 1H, H_c-py), 7.83 (d, ³J_{P-H}=8.5 Hz, 1H, H_d-py), 8.04 (dd, ³J_{P-H}=9.7 Hz, ³J_{P-H}=5.4 Hz, 1H, NH), 8.12 (d, ³J_{P-H}=5.5 Hz, 1H, H_b-py), 8.34 (s, 1H, H_a-py). ³¹P NMR (202.45 MHz, d-DMSO, ppm): δ =23.5 ppm. IR data (KBr, cm⁻¹): 3356w; 3275 m (v_{N-H}); 3103w, 2957w, 2851w; 1628 m, 1597 (v_{py}); 1516 m, 1458ws (v_{Ar}); 1231 s (v_{P-Q}); 1062 s, 1043 s, 1016 s (v_{P-Q}).

(NC₅H₄-NH)P(O)(OCH₃)₂C(H)(C₆H₄)C(H)-(NC₅H₄-NH) P(O)(OCH₃)₂ (L₆) Mp: 196 °C. ¹H NMR (250.13 MHz, d-DMSO, ppm): δ =3.47 (d, ³J_{P-H}=10.3 Hz, 6H, Me), 3.60 (d, ³J_{P-H}=10.3 Hz, 6H, Me), 5.87 (dd, ²J_{P-H}=24.0 Hz, ³J_{P-H}=7.5 Hz, 1H, CH), 6.49 (t, ³J_{P-H}=6.3 Hz, 2H, H_b-py), 6.75 (d, ³J_{P-H}=8.3 Hz, 2H, H_d-py), 7.34–7.45 (m, 6H, H_c-py), 7.91 (d, ³J_{P-H}=4.3 Hz, 1H, H_a-py). ³¹P NMR (121.49 MHz, d-DMSO, ppm): δ =26.5 ppm. IR data (KBr, cm⁻¹): 3294 m (v_{N-H}); 2953w, 2926w, 2851w (v_{Aliph}); 1602 s (v_{py}); 1481 s (v_{Ar}); 1238 s (v_{P=O}); 1060 s, 1030 s (v_{P-O}).

(C₆H₅NH)P(O)(OCH₃)₂C(H)(C₆H₄)C(H)−(C₆H₅NH)P(O)(OCH₃)₂ (L₇) Mp: 120 °C. ¹H NMR (500.13 MHz, d-DMSO, ppm): δ =3.38 (m, 6H, Me + solvent), 3.62 (d, ³J_{P-H}=10.4 Hz, 6H, Me), 5.07 (dd, ²J_{P-H}=24.2 Hz, ³J_{H-H}=10.1 Hz, 1H, CH), 6.32 (dd, ³J_{P-H}=9.9 Hz, ³J_{P-H}=6.0 Hz, 2H, NH), 6.50 (t, ³J_{P-H}=7.2 Hz, 2H, *p*-Ph), 6.75 (d, ³J_{P-H}=8.1 Hz, 4H, *o*-Ph), 6.97 (t, ³J_{P-H}=7.5 Hz, 4H, *m*-Ph), 7.46 (s, 4H, C₆H₄). ³¹P NMR (202.45 MHz, d-DMSO, ppm): δ =25.1 ppm. IR data (KBr, cm⁻¹): 3377 m (*v*_{N-H}); 3055w, 2955w, 2853w (*v*_{Aliph}); 1599 s; 1508 s, 1438w (*v*_{Ar}); 1248 s (*v*_{P=O}); 1055 s, 1020 s (*v*_{P-O}).

(C₆H₅)(3,5–(CH₃)₂–N₂C₄H–NH)C(H)P(O)(OCH₃)₂ (L₈) Mp: 142 °C. ¹H NMR (250.13 MHz, *d*-DMSO, ppm): δ =3.51 (ps-t, ³J_{P-H}=11.3 Hz, 6H, Me), 5.84 (dd, ²J_{P-H}=22.5 Hz, ³J_{H-H}=7.0 Hz, 1H, CH), 6.42 (s, 1H, H_b), 7.26–7.35 (m, 3H, *p*,*m*-Ph), 7.54 (d, ³J_{H-H}=6.7 Hz, 2H, *o*-Ph), 7.76 (d, ³J_{P-H}=10.0 Hz, 1H, NH). ³¹P NMR (121.49 MHz, d-DMSO, ppm): δ =25.9 ppm. IR data (KBr, cm⁻¹): 3294 m (v_{N-H}); 2952w, 2852w (v_{Aliph}); 1566 s (v_{py}); 1450 s (v_{Ar}); 1245 s ($v_{P=O}$); 1027 s (v_{P-O}).

 $\begin{array}{l} ({\sf C_6H_5-NH})({\sf C_6H_5}){\sf C(H)P(O)(OC_2H_5)_2} \ ({\sf L_9}) \ {\sf Mp: 80-85 \ ^\circ C; \ ^1 H} \\ {\sf NMR} \ (500.13 \ {\sf MHz}, \ {\rm CDCl}_3, \ {\sf ppm}){:} \ \delta{=}7.48 \ ({\sf d}, \ ^3J_{\rm H-H}{=}7.2 \\ {\sf Hz}, \ 2{\rm H}, \ o{\rm -Ph'}), \ 7.35{-}7.26 \ ({\sf m}, \ 3{\rm H}, \ m{\rm -Ph'}, \ p{\rm -Ph'}), \ 7.12 \ ({\sf t}, \ ^3J_{\rm H-H}{=}7.3 \ {\sf Hz}, \ 2{\rm H}, \ m{\rm -Ph}), \ 6.73 \ ({\sf t}, \ ^3J_{\rm H-H}{=}7.2 \ {\sf Hz}, \ 2{\rm H}, \ p{\rm -Ph}), \ 6.66 \ ({\sf d}, \ ^3J_{\rm H-H}{=}7.1 \ {\sf Hz}, \ 2{\rm H}, \ o{\rm -Ph}), \ 4.76 \ ({\sf d}, \ ^2J_{\rm P-H}{=}23.3 \end{array}$

Hz, 1H, CH), 4.12 (q, ${}^{3}J_{H-H}$ =7.0 Hz, 2H, CH₂–OEt₁), 3.93 (q, ${}^{3}J_{H-H}$ =10.3 Hz, 1H, CH₂–OEt₂), 3.69 (q, ${}^{3}J_{H-H}$ =8.4 Hz, 1H, CH₂–OEt₂), 1.29 (t, ${}^{3}J_{H-H}$ =6.5 Hz, 3H, CH₃–OEt₁), 1.11 (t, ${}^{3}J_{H-H}$ =7.0 Hz, 3H, CH₃–OEt₂). 31 P NMR (202.45 MHz, CDCl₃, ppm): δ =22.1 ppm. IR data (KBr, cm⁻¹): *v*=3325 m (N–H); 2950w, 2900w (C–H_{alph}); 1500 m (C=C_{arm}); 1239 s (P=O); 1055 m;1030 s (P–O).

 $\begin{array}{ll} (p\mbox{-}F\mbox{-}C_6\mbox{H}_4\mbox{-}N\mbox{H})(C_6\mbox{H}_5)\mbox{C}(\mbox{H})\mbox{P}(0)(\mbox{OC}_2\mbox{H}_5)_2\mbox{(L}_{10})\mbox{ Mp: }100\mbox{-}105\mbox{}^\circ\mbox{C};\mbox{}^1\mbox{H}\mbox{NMR}\mbox{(250.13}\mbox{MHz},\mbox{CDCl}_3,\mbox{ppm}):\mbox{δ=7.48}\mbox{(d},\mbox{${}^3J_{\rm H-H}\mbox{=}6.3\mbox{ Hz},\mbox{2H},\mbox{o-Ph}),\mbox{$7.35\mbox{-}7.26\mbox{ (m},\mbox{3H},\mbox{m-Ph},\mbox{p-ph}),\mbox{6-7.48}\mbox{(d},\mbox{${}^3J_{\rm H-H}\mbox{=}6.3\mbox{ Hz},\mbox{$2H},\mbox{$o$-Ph}),\mbox{$7.35\mbox{-}7.26\mbox{ (m},\mbox{$3H},\mbox{$m$-Ph},\mbox{$p$-ph}),\mbox{$6$-7.48}\mbox{(d},\mbox{$3J_{\rm H-H}\mbox{=}6.3\mbox{ Hz},\mbox{$2H},\mbox{$o$-Ph}),\mbox{$7.35\mbox{-}7.26\mbox{ (m},\mbox{$3H},\mbox{$m$-Ph}),\mbox{$6$-7.48}\mbox{(d},\mbox{$3J_{\rm H-H}\mbox{=}8.4\mbox{ Hz},\mbox{$2H},\mbox{$0$-6.44},\mbox{$0$, 6.67\mbox{ (m},\mbox{$2H},\mbox{$C_6\mbox{H}_4$),\mbox{4, 10\mbox{ (m},\mbox{$2H},\mbox{$CH_2\mbox{-}-OEt_1$),\mbox{$3.97\mbox{ (m},\mbox{$3J_{\rm H-H}\mbox{=}6.9\mbox{ Hz},\mbox{$1H},\mbox{$CH_2\mbox{-}-OEt_1$),\mbox{$1.26\mbox{ (m},\mbox{$3J_{\rm H-H}\mbox{=}7.2\mbox{ Hz},\mbox{$3H$,\mbox{m-H}\mbox{=}7.2\mbox{ Hz},\mbox{$3H$,\mbox{m-H}\mbox{=}7.2\mbox{ Hz},\mbox{$3H$,\mbox{m-H}\mbox{=}7.2\mbox{ Hz},\mbox{$3H$,\mbox{m-H}\mbox{-}-0Et_2$),\mbox{$1.26\mbox{ (m},\mbox{$1-H}\mbox{-}-1$);\mbox{m-111\mbox{ (m},\mbox{$1-H}\mbox{-}-1$);\mbox{m-25.2\mbox{m-Ph}\mbox{-},\mbox{$1233\mbox{ s}\mbox{(P=O)};\mbox{$1029\mbox{ s},\mbox{$973\mbox{ s},\mbox{$973\mbox{ s},\mbox{$1233\mbox{ s}\mbox{-},\mbox{$1233\mbox{ s}\mbox{-},\mbox{$1233\mbox{ s},\mbox{-},\mbox{-},\mbox{$1233\mbox{ s}\mbox{-},\mbox{-},\mbox{-},\mbox{-},\mbox{$1233\mbox{ s}\mbox{-},\m$

(*p*-Cl-C₆H₄-NH)(C₆H₅)C(H)P(O)(OC₂H₅)₂ (L₁₁) Mp: 110–115 °C; ¹H NMR (500.13 MHz, CDCl₃, ppm): δ =7.49 (d, ³J_{H-H}=7.4 Hz, 2H, *o*-Ph), 7.30 (t, ³J_{H-H}=7.1 Hz, 2H, *m*-Ph), 7.23 (d, ³J_{H-H}=7.2 Hz, 1H, *p*-Ph), 7.00 (d, ³J_{H-H}=8.5 Hz, 2H, C₆H₄), 6.79 (d, 2H, ³J_{H-H}=8.7 Hz, C₆H₄), 6.59 (m, 1H, NH), 5.00 (dd, ²J_{P-H}=25.0 Hz, ³J_{H-H}=9.9 Hz, 1H, CH), 4.02 (q, ³J_{H-H}=8.2 Hz, 2H, CH₂-OEt₁), 3.95 (q, ³J_{H-H}=7.2 Hz, 1H, CH₂-OEt₂), 1.16 (t, ³J_{H-H}=7.0 Hz, 3H, CH₃-OEt₁), 1.02 (t, ³J_{H-H}=7.0 Hz, 3H, CH₃-OEt₂). ³¹P NMR (202.45 MHz, CDCl₃, ppm): δ =22.7 ppm. IR data (KBr, cm⁻¹): *v*=3291 s (N–H); 2980 s, 2905w (C–H_{alph}-); 1597 s; 1496 s, 1448 m (C=C_{arm}); 1239 s (P=O); 1027 s; 971 s.

(*p*-Br-C₆H₄-NH)(C₆H₅)C(H)P(O)(OC₂H₅)₂ (L₁₂) Mp: 120–123 °C; ¹H NMR (250.13 MHz, CDCl₃, ppm): δ =7.49 (d, ³J_{H-H}=6.9 Hz, 2H, *o*-Ph), 7.36–7.27 (m, 3H, *m*-Ph, *p*-Ph), 7.14 (d, ³J_{H-H}=8.4 Hz, 2H, C₆H₄), 6.63 (d, 2H, ³J_{H-H}=8.4 Hz, C₆H₄), 4.10 (m, 2H, CH₂-OEt₁), 3.95 (q, ³J_{H-H}=7.8 Hz, 1H, CH₂-OEt₂), 3.81 (q, ³J_{H-H}=7.2 Hz, 1H, CH₂-OEt₂), 1.26 (t, ³J_{H-H}=6.9 Hz, 3H, CH₃-OEt₁), 1.15 (t, ³J_{H-H}=6.9 Hz, 3H, CH₃-OEt₂). ³¹P NMR (121.49 MHz, CDCl₃, ppm): δ =23.4 ppm. IR data (KBr, cm⁻¹): *v*=3291 s (N-H); 2922 m (C-H_{alph}.); 1593 m; 1490 s (C=C_{arm}.); 1239 s (P=O); 1027 s; 973 s.

CH₃-OEt_{Inpurity}), 1.10 (t, 3H, CH₃-OEt₂). ³¹P NMR (202.45 MHz, CDCl₃, ppm): δ =21.4, -1.2 (Inpurity) ppm. IR data (KBr, cm⁻¹): v=3293 s (N–H); 2975 s, 29185w (C–H_{alph}.); 1496 s, 1445 m (C=C_{arm}.); 1235 s (P=O); 1028 s; 973 s.

(*p*-NO₂-C₆H₄-NH)(C₆H₅)C(H)P(O)(OC₂H₅)₂ (L₁₄) Mp: 111 °C; ¹H NMR (500.13 MHz, CDCl₃, ppm): δ =8.01 (d, ³J_{H-H}=9.1 Hz, 2 H, C₆H₄), 7.45 (d, ³J_{H-H}=7.4 Hz, 2H, *o*-Ph), 7.37 (t, ³J_{H-H}=7.7 Hz, 2H, *m*-Ph), 7.32 (d, ³J_{H-H}=6.8 Hz, 1H, *p*-Ph), 6.58 (d, ³J_{H-H}=9.2 Hz, 2H, C₆H₄), 6.79 (d, 2H, ³J_{H-H}=8.7 Hz, C₆H₄), 6.58 (m, 1H, NH), 4.80 (dd, ²J_{P-H}=24.1 Hz, 1H, CH), 4.14 (q, ³J_{H-H}=8.3 Hz, 2H, CH₂-OEt₁), 3.90 (q, ³J_{H-H}=9.82 Hz, 1H, CH₂-OEt₂), 3.61 (q, 1H, CH₂-OEt₂), 1.31 (t, ³J_{H-H}=5.6 Hz, 3H, CH₃-OEt₁), 1.10 (t, ³J_{H-H}=7.0 Hz, 3H, CH₃-OEt₂). ³¹P NMR (202.45 MHz, CDCl₃, ppm): δ =21.3 ppm. IR data (KBr, cm⁻¹): *v*=3285 m (N-H); 2977w, 2922w, 1530 s; 1348 s, (C=C_{arm.}); 1232 s (P=O); 1016 s; 971 m.

(*p*-Cl-C₆H₄-NH)(*p*-F-C₆H₄)C(H)P(O)(OC₂H₅)₂ (L₁₅) Mp: 78–82 °C; ¹H NMR (500.13 MHz, CDCl₃, ppm): δ =7.84 (d, Ar), 7.53–7.03 (m, Ar), 6.51 (d, Ar), 4.70 (d, ²J_{P-H}=23.6 Hz, 1H, CH),6.72(br 4.11 (m, 2H, CH₂–OEt₁), 3.95 (q, ³J_{H-H}=10.1 Hz, 1H, CH₂–OEt₂), 3.65 (q, ³J_{H-H}=8.5 Hz, 1H, CH₂–OEt₂), 1.29 (t, ³J_{H-H}=6.2 Hz, 1H, CH₃–OEt₁), 1.11 (t, ³J_{H-H}=7.0 Hz, 1H, CH₃–OEt₂). ³¹P NMR (202.45 MHz, CDCl₃, ppm): δ =22.3 s, 21.6w ppm. IR data (KBr, cm⁻¹): *v*=3290 m (N–H); 2983w, 2909w (C–H_{alph}-); 1520 s; 1440 s, (C=C_{arm}); 1236 s (P=O); 10125 s; 9673 m.

(*p*-Cl-C₆H₄-NH)(NC₅H₄)C(H)P(O)(OC₂H₅)₂ (L₁₆) Mp: 106–108 °C; ¹H NMR (500.13 MHz, CDCl₃, ppm): δ =8.59 (d, ³J_{H-H}=4.5 Hz, 1H, py), 7.70 (t, ³J_{H-H}=7.5 Hz, 1H, py), 7.50 (d, ³J_{H-H}=7.6 Hz, 1H, py), 7.24 (t-br, 1H, py), 7.06 (d, ³J_{H-H}=8.6 Hz, 1H, C₆H₄), 6.61 (d, ³J_{H-H}=8.6 Hz, 1H, C₆H₄), 5.35 (br, 1 H, NH), 4.98 (d, ²J_{P-H}=21.6 Hz, 1 H, CH), 4.12 (m, 2H, CH₂-OEt₁), 4.03 (m, 1H, CH₂-OEt₂), 3.905 (m, 1H, CH₂-OEt₂), 1.27 (t, ³J_{H-H}=6.2 Hz, 1H, CH₃-OEt₁), 1.15 (t, ³J_{H-H}=7.0 Hz, 1H, CH₃-OEt₂). ³¹P NMR (202.45 MHz, CDCl₃, ppm): δ =20.7 s, 21.6w ppm. IR data (KBr, cm⁻¹): *v*=3285 s (N–H); 2985w, 2911w (C–H_{alph}.); 1493 s; 1434w, (C=C_{arm}.); 1239 s (P=O); 1029 s; 968 m.

(*p*-Cl-C₆H₄-NH)(*p*-Cl-C₆H₄)C(H)P(O)(OC₂H₅)₂ (L₁₇) Mp: 103–106 °C; ¹H NMR (500.13 MHz, CDCl₃, ppm): δ =7.40 (d, ³J_{H-H}=6.6 Hz, 2H, C₆H₄`), 7.30 (d, ³J_{H-H}=8.1 Hz, 2H, C₆H₄`), 6.82 (st, ³J_{H-H}=8.5 Hz, 2H, C₆H₄`), 7.24 (t-br, 1H, py), 6.57 (m, 2H, C₆H₄`), 4.66 (d, ²J_{P-H}=23.8 Hz, 1H, CH), 4.12 (q, 2H, ³J_{H-H}=7.2 Hz, CH₂-OEt₁), 3.98 (q, ³J_{H-H}=9.8 Hz, 2H, CH₂-OEt₂), 3.80 (q, ³J_{H-H}=8.0 Hz, 1H, CH₂.OEt₂), 1.29 (t, 2H, ³J_{H-H}=7.0 Hz, 1H, CH₃.OEt₁), 1.16 (t, ³J_{H-H}=7.0 Hz, 1 H, CH₃-OEt₂), ³¹P NMR (202.45 MHz, CDCl₃, ppm): δ =21.4 s, 21.6w ppm. IR data (KBr, cm⁻¹):

v = 3288 m (N–H); 2997w, 2923w (C–H_{alph}.); 1514 s; 1444 s, (C=C_{arm}); 1232 s (P=O); 1020 s; 980 m.

General procedure for compound L₁₈-L₂₂, L₂₈, and L₂₉

Compounds $L_{18}-L_{22}$, L_{28} , and L_{29} were prepared by a Mannich-type reaction according to the procedure previously described [25]. The corresponding amine (1 mmol) was mixed with 37% hydrochloric acid (5 mL), deionized water (5 mL) and phosphorous acid (3 mmol). The mixture was allowed to reflux at 100–120 °C for 1.5 h, then paraformal-dehyde (4 mmol) was added in small portions over a period of 1 h, and the mixture was refluxed for an additional hour, a white powder was yielded after removing the solvents.

(C₆H₅)₂−CH−N−[(CH₂)P(0)(OH)₂]₂ (L₁₈) Mp: 204 °C. ³¹P NMR (202.46 MHz, D₂O): δ =7.48 ppm. ¹H NMR(500.13 MHz, D₂O): δ =3.19 ppm (CH, s, 1H), 3.35 (CH₂, d, 4H, ²J_{P,H}=12.23 Hz), 7.36−7.40 (Ar−H, m). IR (KBr, cm⁻¹): v = 3419 (w,NH⁺), 2926 (s, CH), 2856 (s, CH), 2358 (s), 1636 (m), 1081−1176 (s, PO₃), 921 (s, PO₃), 783 (s, P−C), 553 (s,p=o), 481 (m).

(*m*-NC₅H₄)(CH₂)-N-[(CH₂)P(O)(OH)₂]₂ (L₁₉) Mp: 248 °C. ³¹P NMR (202.46 MHz, D₂O): δ =8.11 ppm. ¹H NMR (500.13 MHz, D₂O): δ =3.37 ppm (CH₂, d, 4H, ²J_{P,H}=12.35 Hz), 4.85 (-CH₂, s, 2H), 8.05–9.01 (m, 4H). IR (KBr, cm⁻¹): *v* = 3405 (w, NH⁺), 2965 (s, CH), 2845 (s, CH), 2720 (s, P-OH), 2545 (s), 1284 (m, C-N), 1166–1229 (s, PO₃), 935– 1008 (s, PO₃), 746 (s, P-C), 573 (s, p=o), 485 (m), 421 (m).

(*p*-F−C₆H₄)−(CH₂)−N−[(CH₂)P(O)(OH)₂]₂(L₂₀) Mp: 227 °C. ³¹P NMR (202.46 MHz, D₂O): δ = 7.62 ppm. ¹H NMR (500.13 MHz, D₂O): δ = 3.36 (CH₂, d, 4H, ²J_{P,H} = 12.66 Hz), 4.60 (CH₂, s, 2H), 7.29−7.53 (Ar−H, m, 4H). IR (KBr, cm⁻¹): *v* = 3415 (w, NH⁺), 3040 (m, CH), 2870 (m, CH), 2775 (m, P−OH), 2590 (m), 1450 (w), 1237 (m, C−N), 1161−1200 (s, PO₃), 940−1004 (s, PO₃), 744 (w, P−C), 575 (m), 493 (m), 405 (m).

(OH)₂(O)P(CH₂)NC₅H₉(CH₂)₃C₅H₉N(CH₂)P(O)(OH)₂ (L₂₁) Mp: 257 °C. ³¹P NMR (202.46 MHz, D₂O): δ =7.33 ppm. ¹H NMR (500.13 MHz, D2O): δ =1.14–1.544 (CH₂, m, 2H), 1.82 (CH₂, m, 2H), 2.92 (CH₂, m, 2H), 3.11 (CH₂, d, 4H,²J_{P,H}=11.0 Hz), 3.23 (CH₂, 2H), 3.57 (CH₂, m, 2H). IR (KBr, cm⁻¹): υ = 3415 (w, NH⁺), 3040 (m, CH), 2870 (m, CH), 2775 (m, P–OH), 2590 (m), 1450 (w), 1237 (m, C–N), 1161–1200 (s, PO₃), 940–1004 (s, PO₃), 744 (w, P–C), 575 (m), 493 (m), 405 (m).

C₆H₅-CH₂-N-[(CH₂)P(O)(OH)₂]₂ (L₂₂) Mp: 209 °C. ³¹P NMR (202.46 MHz, D₂O): δ =6.20 ppm. ¹H NMR (500.13 MHz, D₂O): δ =281 3.06 ppm (CH₂, d, 4H, ²J_{PH}=12.1), 4.20

(CH₂, s, 2H), 7.31–7.48 (Ar–H, m, 5H). IR (KBr, cm⁻¹): $v = 3405(w, NH^+)$, 2965 (s, CH), 2876 (282 s, CH), 2348 (s), 1633 (m), 1071–1186 (s, PO₃), 283 930 (s, PO₃), 756 (s, P–C), 553 (s, p=o), 476 (m).

(o-CO₂H)-C₅H₉N)(CH₂)P(O)(OH)₂(L₂₈) Mp: 221 °C. ³¹P NMR (202.46 MHz, D₂O): δ =7.36 ppm. ¹H NMR (500.13 MHz, D₂O): δ =1.87-2.40 (CH₂, m), 3.18 (CH₂, d, 2H, ²J_{P,H}=11.9 Hz), 3.39 (CH, m) 3.77 (CH, s) 4.26 (CH, m). IR (KBr, cm⁻¹): v = 3435(w, NH⁺), 2980 (m, CH), 2755 (m, P-OH), 2295 (m), 1274 (m, C-N), 1078-1192 (s, PO₃), 913-1015 (s, PO₃), 759 (w, P-C), 521 (s, p=o), 461 (m), 411 (m).

[(*p*-OH)(*o*-CO₂H)C₅H₈N](CH₂)P(O)(OH)₂(L₂₉) Mp: 237 °C. ³¹P NMR(202.46 MHz, D₂O): δ =7.21 ppm. ¹H NMR(500.13 MHz, D₂O): δ =1.99–2.40 (CH₂, m), 3.18 (CH₂, d, 2H, ²J_{P,H}=12.3 Hz), 3.54 (CH, m) 3.66 (CH, s) 4.16 (CH, m). IR (KBr, cm⁻¹): *v* = 3395 (w, NH⁺), 3000 (m, CH), 2870 (m, CH), 2735 (m, P–OH), 2570 (m), 1420 (w), 1259 (m, C–N), 1173–1231 (s, PO₃), 928–1000 (s, PO₃), 784 (w, P–C), 595 (m), 552 (m), 509 (m).

General procedure for compound L₃₂

This compound was prepared by adding a solution of the (thio)hydrazide compound (1 mmol) and triethylamine (1 mmol) in THF at 0 °C to a solution of $(C_6H_5-NH)_2P(O)$ Cl (1 mmol) in THF. After stirring for 4 h, the solvent was removed under vacuum, and the resulting product was washed with distilled water.

(o-S-C₄H₃)C(O)NH–NHP(O)(NH–C₆H₅)₂ (L₃₂) Mp: 224 °C. ¹H NMR (500.13 MHz, MeOD, ppm): 7.04 (dd, ²J_{H–H}=3.8 Hz, 1H, H_{Thio}), 7.13 (d, ²J_{PNH}=8.54 Hz, 1H, N–H_α), 7.3–7.49 (br, m, 4H, H_{Ph}), 7.52 (d, ²J_{H–H}=2.7 Hz, 1H, H_{Thio}), 7.55 (m, 2H, H_{Ph}), 7.67–7.8 (br, m,4H, H_{Ph}); 7.84 (d, ²J_{H–H}=4.8 Hz,1H, H_{Thio}); 7.99 (d, ²J_{PNH}=6.1 Hz, 2H, N–H_{aniline}); 8.27 (s, 1H, NH_β); ³¹P NMR (202.45 MHz, MeOD, ppm): δ =26.61 ppm. IR data (KBr, cm⁻¹): 3273 s, 3229 s (v_{N-H}); 3054 m; 1647 s ($v_{C=O}$); 1497 s; 1409 m, 1295 m, 1212 s (vs, $v_{P=O}$); 1071 s (v_{N-N}); 956 s (v_{P-N}), 905 s, 724 m.

General procedure for compounds L₂₃-L₂₇, and L₃₃

A solution of amine or diamine (1 mmol) and triethylamine (2 mmol) in hydrated THF were added at 50 °C to a solution of $(R)_2P(X=O, S)Cl$ (2 mmol) in hydrated THF. After 8 h refluxing, the solvent was removed in vacuum and the resulting white powder was washed with distilled water and recrystallized at room temperature.

(C₆H₅)₂P(O)–NH–CH₂–C₆H₁₀–CH₂–NH–P(O)(C₆H₅)₂ (L₂₃) Mp: 209 °C. ¹H NMR (500.13 MHz, *d*-DMSO, ppm): 1.22–1.36

(m, 8H, CH₂); 1.54 (s, 2H, CH); 2.48 (t, ${}^{3}J_{H-H}$ =1.65 Hz, 4H, CH₂); 5.18 (m, 2H, NH), 7.42–7.49 (m, 8H, C₆H₅), 7.6–7.7 (m, 4H, C₆H₅), 7.71–7.77 (m, 8H, C₆H₅) ppm. ³¹P NMR (202.45 MHz, *d*-DMSO, ppm): δ =21.47 ppm. IR data (KBr, cm⁻¹): 3432 s, 3198 (v_{N-H}); 2914 m ($v_{C-Halph}$.); 2852; 1435 s (v_{Ar}); 1185 s ($v_{P=O}$); 1067 s (v_{P-N}); 723; 697.

(C₂H₅O)₂P(S)(NH)₂C(O)(NH)₂P(S)(OC₂H₅)₂ (L₂₄) Mp: 187 °C. ¹H NMR (500.13 MHz, CDCl₃, ppm): δ = 1.33 (t, ²J_{H-H} = 7 Hz, 12H, Me), 4.13–4.19 (m, 8H, CH₂), 6.86 (br, 2H, N– H_β), 7.26 (s, 2H, NH_{amide}). ³¹P NMR (202.45 MHz, CDCl₃, ppm): δ = 69.33 ppm. IR data (KBr, cm⁻¹): 3294 s (v_{N-H}); 1667 s (v_{C=O}); 2983w (v_{C-Halph}); 1521 s (v_{Aliphatic}); 1023 s (v_{N-N}); 966 s (v_{P-N}); 811 s (v_{P=S}).

 $\begin{array}{l} ({\sf C_6H_5O})_2{\sf P(O)-NH-NH-C(O)-NH-NH-P(O)}({\sf OC_6H_5})_2\\ ({\sf L_{25}}) \ {\rm Mp:} \ 190\ ^\circ{\rm C.}\ ^1{\rm H}\ {\rm NMR}\ (250.13\ {\rm MHz},\ d\text{-}{\rm DMSO},\ {\rm ppm}):\\ \delta\!=\!5.89\ ({\rm dd},\ ^2J_{\rm P-H}\!=\!23\ {\rm Hz},\ ^3J_{\rm H-H}\!=\!8.8\ {\rm Hz},\ 2{\rm H},\ {\rm NH}_{\alpha}),\ 7.18\\ ({\rm m,}\ 2{\rm H},\ {\rm NH}_{\beta}),\ 7.39\ ({\rm d},\ ^2J_{\rm P-H}\!=\!7\ {\rm Hz},\ 4{\rm H},\ {\rm N-H_{aniline}}),\ 7.46\\ ({\rm m,}\ 8{\rm H},\ {\rm C_6H_5}).\ 7.52\ ({\rm m,}\ 4{\rm H},\ {\rm C_6H_5}),\ 7.8({\rm m,}\ 8{\rm H},\ {\rm C_6H_5}).\ ^{31}{\rm P}\\ {\rm NMR}\ (121.49\ {\rm MHz},\ d\text{-}{\rm DMSO},\ {\rm ppm}):\ \delta\!=\!22.16\ {\rm ppm}.\ {\rm IR}\\ {\rm data}\ ({\rm KBr},\ {\rm cm}^{-1}):\ 3223\ {\rm s},\ 3061\ (v_{\rm N-H});\ 1685\ {\rm s}\ (v_{\rm C=O});\ 1529\\ {\rm s}\ (v_{\rm Ar});\ 1207\ {\rm s}\ (v_{\rm P=O});\ 1030\ {\rm s}\ (v_{\rm P-O});\ 1051\ {\rm s}\ (v_{\rm N-N});\ 944\ {\rm m}\\ (v_{\rm P=N});\ 749\ {\rm m}. \end{array}$

(C₆H₅)₂P(O)−HN−(C₆H₁₀)−CH₂−(C₆H₁₀)−HN−P(O)(C₆H₅)₂ (L₂₆) Mp: 122 °C. ¹H NMR (250.13 MHz, *d*-DMSO, ppm): 0.77 (m, 2H, CH₂); 0.89 (m, 2H, CH); 1.3−1.4 (m, 8H, CH₂), 1.62−2.01 (m, 8H, CH₂), 2.76 (m, 2H, CH); 7.35 (m, 2H, NH), 7.45−7.52 (m, 8H, C₆H₅), 7.54−7.56 (m, 4H,C₆H₅), 7.76−7.87 (m, 8H, C₆H₅) ppm. ³¹P NMR (121.49 MHz, MeOD, ppm): δ =25.24 ppm. IR data (KBr, cm⁻¹): 3399w, 3197 (*v*_{N−H}); 2926 (*v*_{C−Halph}); 2856 m (*v*_{Aliphatic}); 1442 s (*v*_Ar); 1262 m, 1189vs (*v*_{P=O}); 1115vs (*v*_{P−O}); 898 m (*v*_{P−N}); 697.

(OEt)₂**(O)**P–**NH**–**C**₆**H**₄–**NH**–**P(S)(OEt)**₂ (**L**₃₃) Mp: 169 °C. ¹H NMR (500.13 MHz, *d*-DMSO, ppm): $\delta = 1.17$ (t, 12H, ³*J*_{P–H} = 7.0 Hz, 4CH3), 3.93 (m, 4H, CH₂), 3.99 (m, 4H, CH₂), 6.8–6.9 (m, 4H, C₆H₄), 7.72 (d, 1H, ²*J*_{P–H}=9.2 Hz, NH_{P=O}), 8.04 (d, 1H, ²*J*_{P–H}=15.10 Hz, NH_{P=S}). ³¹P NMR (202.45 MHz, *d*-DMSO, ppm): $\delta = 65.17$ (P=S), 3.5 (P=O) ppm. IR data (KBr, cm⁻¹): 3426 m + 3265 m (v_{N-H}),

2975+2916 ($v_{Aliphatic}$); 1509 m (v_{Ar}); 1276 ($v_{P=O}$); 1216 ($v_{P=O}$); 1034 s+961 s ($v_{P=N}$); 808 m ($v_{P=S}$).

AChE enzymes assay

Absorbance assay Human AChE activity measurements were taken essentially according to Ellman's method [26]. Reactions were carried out at 37 °C in 70 mM phosphate buffer (Na_2HPO_4/NaH_2PO_4 , pH = 7.4) containing the AChE (human erythrocyte; Sigma, Cat. No. C0663) enzyme (10 μ l volume, diluted 100 times in phosphate buffer, pH = 7.4), DTNB (concentration = 10^{-4} M and ATCh (concentration = 1.35×10^{-4} M). Each compound was dissolved in isopropanol and then added to the buffer for in vitro ChE assays. The absorbance changes at 37 °C were monitored with the spectrophotometer at 412 nm for 3 min, and three replicates were run in each experiment. In the absence of an inhibitor, the absorbance change was directly proportional to the enzyme level. The reaction mixtures for determination of IC50 values (the median inhibitory concentration) consisted of DTNB solution, 100 μ l; inhibitor, x μ l; acetylthiocholine iodide (ATCh) solution, 40 µl; phosphate buffer $(850 - x) \mu l$ and hAChE solution, 10 μl . The plot of $V_{\rm I}/V_0$ (V_I and V₀ are the activity of the enzyme in the presence and absence of inhibitors, respectively) against $\log[I]$, where [I] is the inhibitor concentration, gave the IC_{50} values of L₁-L₁₆ (Table 2).

Antifungal assay

We used Macrophomina phaseolina Mph44, which was previously isolated and identified by Mahdizadeh and Safaie 2011 [27]. The isolate was originally obtained from melons with charcoal rot disease in Khorasan. For evaluating the effect of each compound on mycelial growth of M. phaseolina, the poisoned food technique was used [28]. The synthesized compounds have been tested in vitro by transferring a specific volume of each compound into a Petri dish containing 20-25 ml melted warm PDA medium, and they were gently shaken for mixing (Compounds final concentration = 150 mg/L). The PDA plates were aseptically inoculated by transferring a 6 mm diameter agar disc of 7-dayold culture of the pathogen to the center of the Petri dish. Three replications were maintained for each treatment. The basal medium (PDA) without synthetic compounds served as control. The inoculated plates were incubated at 25 °C and colony diameter was measured and recorded after 3-7 days. The percentage of mycelial growth inhibition is given by the following equation:

Mycelial growth inhibition% $(I\%) = [(C-T)/C] \times 100$

where C is the diameter of the fungal colony (mean) in control, and T is the diameter of the fungal colony (mean) in the presence of the synthesized compound.

Descriptor calculation

A total number of 1497 descriptors of (0D), mono-dimensional (1D), bi-dimensional (2D), and three-dimensional (3D) were generated using E-Dragon software [18]. The descriptors' total was computed after removing invariable descriptors for all molecules and correlated descriptors (R > 0.9). The electronic and structural descriptors are obtained by either the quantum chemical calculations, theoretical and experimental studies. All the quantum chemical calculations were carried out by using the Gaussian 09 program package [19]. The electronic descriptors include the energy of frontier orbitals (E_{HOMO} and E_{LUMO}), hydrogen bonding energy (E_{HB}) , electrophilicity (ω) , polarizability (P_L) , and the net atomic charges (Q). Moreover, dipole moment (μ) , molar refractivity (M_r) , surface area (SA), and the molecular volume (M_{ν}) are the structural descriptors. The bioactivities of organophosphorus analogs and association constants are expressed in terms of I%, $\log(I)$, and p(1/IC₅₀) (Table 2).

Data processing and modeling

Multiple linear regression (MLR) analysis

MLR is a method used for linear relationship modeling between a dependent variable and an independent variable [29]. In our study, the dependent variable is the compound bioactivity values, and the independent variable is the molecular descriptors. The values of regression coefficients are calculated using MLR by applying the least-squares curve fitting method. Regression equation takes the form below:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + \dots + b_n X_n + e$$

where Y is the dependent variable, $X_1, X_2, ..., X_k$ are independent variables (descriptors), *e* is a random error and b_0 , $b_1, b_2, ..., b_k$ are the regression coefficients [29].

Genetic algorithm (GA)

GA is a searching procedure rooted in Darwin's theory of natural selection and evolution. It has three basic operations (selection, crossover, and mutation). Because of its simplicity and effectiveness, GA has been used as a promising method for variable selection [30]. In our study, the genetic Algorithm was used to select the main descriptors that affected the fungicidal activity of the studied compounds.

Results and discussion

Spectral study

Phosphorus chemical shifts $\delta(^{31}P)$ for L₁-L₁₆ were observed in the range of 20.7 ppm (L_{16}) to 26.5 ppm (L_6) . However, phosphorus chemical shifts for $L_{26}-L_{35}$ were appeared in a vast range (from -1.80 ppm for L_{34} to 69.83 ppm for L_{27}). This splitting pattern arises from spin couplings between the phosphorus nucleus and the NH protons. The ¹H NMR spectra of L_1-L_8 showed two signals for the CH₃O protons, while in the case of L_9-L_{16} , two triplets and two quartets were seen for OC₂H₅ protons. Protons of the C(H)–NH appeared in the range of 4.66–5.87 ppm, and CH–N(H) protons appeared from 5.14 to 8.04 ppm. The IR analysis spectra indicated that the fundamental $v_{P=0}$ stretching modes for all the derivatives appeared in 1230–1247 cm⁻¹. N–H vibration of CH–N(H) for all the compounds was seen in the range 3275-3377 cm⁻¹. Stretching vibrations of $v_{C=0}$ were in the range of 1677 (L_{24}) to 1687 cm⁻¹ (L_{25}).

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A mixture of chloroform/hexane (4:1) was used for L_1 and chloroform/methanol (2:1) for L11. The crystallographic data and the details of the X-ray analysis for these compounds are given in Table 1. Phosphorus atoms have a slightly distorted tetrahedral configuration with the bond angles in the range of 123.2° to 101.2° around the P atoms in L₁ and L₁₁ (Fig. 1). The P=O and P–O angles are 1.478 and 1.570 Å, respectively. The phosphorus atoms have a slightly distorted tetrahedral configuration with the bond angles in the range 101.94°-116.47° around the P atoms. The P=O and P-O distances are 1.467 and 1.574 Å, respectively. The $R_v^X(Z)$ graph-set notation is descriptive of a Z-membered ring produced by the X hydrogen bonds between the Y donor-acceptor units [31]. Polymeric chains formed in the crystal lattice with cyclic $R_2^2(8)$ motifs via P=O...H–N (d=3.012 Å for L_1 and d = 2.952 Å for L_{11}) hydrogen bonds (Figs. 2, 3, 4). Interestingly, each methanol molecule in the crystal network of L₁ is surrounded by six neighboring molecules via C-H... O-C hydrogen interactions. These interactions are established with different donor-acceptor distances of C-O(6)... H(7)–C (d = 2.478 Å) to C–O(6)...H(2)–C (d = 2.707 Å) (Fig. 5).

AChE enzyme hydrolyzes acetylthiocholine to produce

thiocholine, which reacts with the DTNB (5,5'-dithiobis(2nitrobenzoic acid)) to produce 2-nitrobenzoate-5-mercap-

tothiocholine and 5-thio-2-nitrobenzoate. The inhibition

constant (log(1/IC₅₀)) values of AChE against compounds

Anti-AChE assay

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Crystal structure

Compounds L_1 and L_{11} were recrystallized at room temperature to obtain suitable crystals for the X-ray analysis.

Table 1Crystallographic dataof compounds L_1 and L_{11}

Forms	L ₁	L ₁₁
Empirical formula	$C_{15}H_{20}N_2O_3PClO_4$	C ₁₇ H ₂₁ N ₁ O ₃ PCl ₁
Formula weight	406.75	353.80
Temperature (K)	173 (2)	100 (2)
Crystal system	Monoclinic	Monoclinic
space group	P21/c	P21/c
a (Å)	8.6749 (9)	9.466 (10)
b (Å)	11.0093 (8)	23.632 (3)
c (Å)	19.5372 (16)	7.927 (7)
α (°)	90	90
β (°)	132.005 (2)	92.706 (8)
γ (°)	90	90
$V(\text{\AA}^3)$	1851.6(3)	1771.6(3)
Z, Calculated density (Mg m^{-3})	16, 1.459	4, 1.326
F(000)	848	744
Crystal size (mm)	$0.20 \times 0.30 \times 0.40$	$0.12 \times 0.13 \times 0.24$
θ range for data collection (°)	3.5–29.1	3.1–29.5
Reflections collected / unique	7340 / 3958, [R(int)=0.109]	7395/ 4119, [R(int)=0.042]
Final <i>R</i> indices	$R_1 = 0.1825, wR_2 = 0.4692$	$R_1 = 0.5, wR_2 = 0.1160$
Largest diff. peak and hole (e \AA^{-3})	1.71 and -0.57	0.46 and -0.54



Fig.1 ORTEP representation of compounds L_1 and L_{11}

 L_1-L_{16} were in the range of -0.81 (L_5) to 0.11 (L_9) (Table 2). In L_3 and L_4 with skeleton R(NC₅H₄-NH)C(H) $P(O)(OCH_3)_2$, the inhibitory activity of L₄ in which R = m- $CH_3-C_6H_4$ was higher than that in L₃ with $R = m-Br-C_6H_4$ $[p(1/IC_{50}) = -0.77 \text{ and } -0.40, \text{ respectively}]$. L₅ with the skeleton $(m-CH_3-NC_5H_4-NH)(m-CH_3-C_6H_4)C(H)P(O)(OCH_3)_2$ versus AChE displayed the most potent inhibitory activity. In L_{10} , L_{11} , and L_{12} with the skeleton (*p*-R-C₆H₄-NH)(Ph) $C(H)P(O)(OC_2H_5)_2$ and R = F, Cl, and Br, the inhibitory of L_{10} is higher than the other two ligands. The presence of fluorine electron acceptor connected to the aromatic group increases the inhibitory potential of phosphoric acid derivatives. To get a better understanding of the inhibitory potential of the synthesized compounds, QSAR method was used to examine the interaction between the phosphoric acid derivatives and the AChE structures.



Fig. 2 P–O···H–N hydrogen bonds in L_1 and L_{11}

Anti-fungal assay

Mycelial growth inhibition was measured using the poisoned food technique at 150 mg/L, in which the inhibition ratios ranged between 0 to 83%. L_{17} (p-Cl–C₆H₄–NH) (p-Cl–C₆H₄)C(H)P(O)(OC₂H₅)₂ was the most active compound against the fungus with 83% inhibition of the mycelial growth and full inhibition of the microsclerotia reproduction as compared to the control, the compound L_{16} , (*p*-Cl–C₆H₄–NH)(NC₅H₄)C(H)P(O)(OC₂H₅)₂, exhibited up to 41% mycelial growth inhibition, and the formation of the microsclerotia bodies was entirely inhibited. Both L_2 and L_3 with skeletons (*p*-Cl–C₆H₄)(NC₅H₄–NH)C(H)P(O)(OCH₃)₂ showed reasonable antifungal activity by 49% and 46%



Fig. 3 Packing diagram of L1, formed by N-H···O-P hydrogen bonds



Fig. 4 Packing diagram of L11, formed by N-H-O-P hydrogen bond

respectively, the microsclerotia formation was significantly inhibited by both compounds even after 7 days of treatment as well. Moreover, $(C_6H_5)_2$ -CH-N-[(CH₂)P(O) (OH)₂]₂ (**L**₁₈) illustrated the moderate inhibition activity with 33% growth inhibition, respectively. **L**₁₀ with fluorine substituent (*p*-F-C₆H₄-NH)(C₆H₅)C(H)P(O)(OC₂H₅)₂, showed a 22% inhibition ratio. Other compounds showed relatively weak bioactivity on both mycelial growth and microsclerotia production, as given in Table 2. The results of the fungicidal assays were used to generate the QSAR model as described below:

QSAR study

QSAR models of Anti-AChE activity

QSAR studies were done in order to recognize the effect of descriptors on the activity of AChE. The stepwise MLR procedure was used for model selection, which is a common method used in QSAR studies, where *n* is the number of compounds, *r* is the correlation coefficient, R^2 is the determination coefficient of regression, S_{reg} is the standard **Fig. 5** Weak interactions around the methanol molecule in L_1



deviation of regression, and $F_{\text{statistic}}$ is the Fisher's statistic [29] (Eq. 1):

structures were drawn and pre-optimized using molecular mechanics force fields (MM+) embedded in HyperChem

$$log(1/IC_{50}) = -9.789\Delta E_{L-H} + 8.617E_{LUMO} - 5.059Q_C - 3.077Q_N - 0.116\mu - 0.003Mv - 0.733$$
(1)
$$n = 16; R^2 = 0.937; S_{reg} = 0.298; F_{\text{statistic}} = 22.375$$

In this equation, the inhibitory potency of AChE is mainly influenced by the electronic parameters (as frontier molecular orbital energies). Compared with the structural parameters, $\Delta E_{\text{L-H}}$ and E_{LUMO} with the coefficient values of -9.789 and +8.617 have a higher contribution in log(1/IC₅₀) considerably. The positive and negative signs of $\Delta E_{\text{L-H}}$ and E_{LUMO} in log(1/IC₅₀) disclose that the compound with the higher energy difference between LUMO and HOMO (ΔE_{L-H}) and lower molecular orbital (E_{LUMO}) has higher toxicity against AChE enzyme.

QSAR models of anti-fungal activity

The dataset consists of 26 compounds from the organophosphorus derivatives that showed different fungicidal activity levels against the plant pathogenic fungus, M. *phaseolina* (Table 2). The logarithmic values of mycelial growth inhibition percentages were calculated and considered as the dependent variable for the feature selection and the QSAR analysis's endpoints. The compounds' program [17]. The three-dimensional molecular geometries were refined using AM1 semi-empirical quantumchemical method procedure with a root-mean-square gradient of 0.01 kcal mol⁻¹. For the modeling job, a wide set of theoretical molecular descriptors [18] were used to capture and magnify distinct aspects of chemical structures. 1214 descriptors were computed out of 1497 using E-Dragon 3.0 software [18] after removing invariable descriptors for all molecules and correlated descriptors (R > 0.9). The list of these descriptors, their meaning, and the calculation procedures were provided with related references by the E-Dragon [18, 32]. The genetic algorithm [30] combined with multiple linear regression (GA-MLR) was used to select the main set of descriptors from all those that perform good predictions with minimum error in comparison with the experimental data [33]. The fitness function applied in this study was the leave-one-out cross-validated correlation coefficient (Q^2_{LOO}) [34]. The GA-MLR program is implemented in MATLAB 7.10.0 software [21]. The genetic algorithm method was applied

Table 2 The experimental and predicted bioactivity of the tested compounds by QSAR study

Compo	unds name and	l structure			Absorbance assay(AChE)		Anti-fungal assay			Ref
	NHR 	R	R'		p(1/IC ₅₀)	% I	Log (I) _{exp}	Log (I) _{pr}	Log (I) _{pr.Loo}	
R'CH	P(O)(OMe)	2								
L		$(o-NC_5H_4)$	$(C_{\epsilon}H_{5})$		-0.60	_	_	_	_	_
L,		$(o-NC_5H_4)$	$(p-Cl-C_6H_4)$		-0.40	49.0	1.690	1.671	1.667	_
L ₃		$(o-NC_5H_4)$	$(m-Br-C_6H_4)$		-0.40	46.0	1.663	1.68	1.761	_
L ₄		$(o-NC_5H_4)$	$(m-CH_3-C_6H_4)$		-0.77	13.0	1.114	1.284	1.307	_
L ₅		$(m-CH_3-NC_5H_4)$	$(m-CH_3-C_6H_4)$		-0.81	_	_	_	_	_
L ₆		$(o-NC_5H_4)$	$(p-CHO-C_6H_4)$		-0.26	12.0	1.079	1.012	0.976	_
L ₇		(C ₆ H ₅)	$(p-CHO-C_6H_4)$		-0.70	_	_	_	_	_
L ₈		$[3,5-(CH_3)_2-2,6(N)_2C_4H]$	(C ₆ H ₅)		-0.20	7.0	0.845	0.963	0.978	-
	NHR	R	R'							
R'CH	P(O)(OEt)2								
L ₉		$(C_{6}H_{5})$	(C ₆ H ₅)		0.11	_	_		_	_
L ₁₀		$(p-F-C_6H_4)$	(C ₆ H ₅)		-0.08	22.0	1.342	1.250	1.239	_
L ₁₁		$(p-Cl-C_6H_4)$	(C ₆ H ₅)		0.02	_	_	_	_	_
L ₁₂		$(p-Br-C_6H_4)$	(C_6H_5)		0.10	_	_	_	_	_
L ₁₃		$(p-I-C_6H_4)$	(C_6H_5)		-0.74	_	_	_	_	_
L ₁₄		$(p-NO_2-C_6H_4)$	(C_6H_5)		-0.65	_	_	_	_	_
L ₁₅		$(p-Cl-C_6H_4)$	$(p-F-C_6H_4)$		-0.08	_	_	_	_	_
L ₁₆		$(p-Cl-C_6H_4)$	$(o-N-C_5H_4)$		-0.08	41.0	1.613	1.597	1.593	_
L ₁₇		$(p-Cl-C_6H_4)$	$(p-Cl-C_6H_4)$		_	83.0	1.919	1.936	1.974	_
PO(OH)) ₂ PO(OH) ₂	R								
N R										
L ₁₈		$(C_6H_5)_2$ -CH			-	33.0	1.519	1.446	1.430	-
L ₁₉		$(m-NC_5H_4)-(CH_2)$			-	16	1.204	1.020	0.988	-
L ₂₀		$(p-F-C_6H_4)-(CH_2)$			-	8.0	0.903	1.173	1.211	-
L ₂₁		C ₅ H ₉ (CH ₂) ₃ C ₅ H ₉			-	13.0	1.114	1.086	1.077	-
L ₂₂		C ₆ H ₅ -CH ₂			-	13	1.114	1.084	1.08	-
Y ∥ (Z)₂P−	Y ║ −XP(Z)₂	X	Y	Z						
L23		$\frac{\text{NH}(\text{CH}_2)(\text{C}_6\text{H}_{10})}{(\text{CH}_2)\text{NH}}$	0	C_6H_5	-	6.0	0.778	0.651	0.585	-
L24		$(NH)_{2}C(O)(NH)_{2}$	S	OC ₂ H ₅	_	14.0	1.146	1.094	1.077	_
L25		$(NH)_2C(O)(NH)_2$	0	OC ₄ H ₅	_	12.0	1.079	1.152	1.274	_
L26		$NH(C_6H_{10})-CH_2-$ (C ₆ H ₁₀)NH	0	C ₆ H ₅	-	19.0	1.279	1.297	1.308	-
L27		NH-C ₆ H ₁₀ -O-	0	OC ₆ H ₅	_	0.0	_	_	_	_
RN	P(O)(OH) ₂	R								
~ L ₂₈		(o-CO ₂ H)–C ₅ H ₉ -			_	7.0	0.845	0.979	0.999	_
L ₂₉		(<i>p</i> -OH)(<i>o</i> -CO ₂ H) C ₅ H ₈ -			-	10.0	1.00	0.888	0.848	-

Table 2 (continued)

Compounds name and structure		Absorbance assay(AChE)		Anti-fungal assay			Ref
L ₃₀		_	5.0	0.699	0.564	0.518	[48]
L ₃₁		-	11.0	1.041	0.939	0.919	[49]
L ₃₂		-	16.0	1.204	1.082	1.075	-
L ₃₃		-	4.0	0.602	0.71	0.728	-
L ₃₄		-	1.0	0.000	0.114	0.231	[49]
L ₃₅		-	5.0	0.699	0.792	0.815	[50]
L ₃₆		-	2.0	0.301	_	-	
L ₃₇		-	9.0	0.954	0.98	0.989	[51]

 $Log (I)_{exp.} = experimental log I values of the studied compounds$

 $Log (I)_{pr.} = predicted log I values of the studied compounds$

 $Log (I)_{pr.Loo.} = predicted log I after applying in the leave-one-out cross-validated correlation coefficient (Q²_{LOO})$

to select the best subset of descriptors. The GA-MLR model and its statistical parameters are presented as Eq. 2:

 R^2 is the squared correlation coefficient; RMSE is the root-mean-square error, Q^2_{LOO} shows the squared

$$Log I\% = 4.1693 + 0.0363(T(P..Cl)) - 8.3494(De) + 3.3914(H8m) + 0.3867(Mor18e) + 0.3544(GGI4) + 6.0164(R1p+) n = 26, R2 = 0.9237; RMSELOO = 0.1411; Q2LOO = 0.8718$$

(2)

cross-validation coefficients for leave-one-out. GGI4 from Galves topological charge indices [18, 32, 35], and T(P.. Cl) from the topological descriptors [18, 32, 36] showed a positive effect on the fungicidal activity. As demonstrated in Eq. 2, the topological distance between P and Cl atoms affects the studied compounds' activity.

De belongs to the VHIM descriptors [37, 38], the descriptor with the highest negative coefficient has a significant effect on the studied compounds' inhibition ratio values. Mor18e belongs to the 3D-MoRSE descriptors (3D-Molecule Representation of Structures based on Electron diffraction descriptors), which were described in 1996 [39, 40], and widely used in QSAR studies [41–43]. Other 3D-descriptors [18] are H8m and R1p + that belong to the GETAWAY descriptors [44, 45] that have a good capability in QSAR/QSPR studies [46]. The two descriptors showed a significant positive effect on the fungicidal activity of the tested molecules with a coefficient of +3.3914 and +6.0164, respectively.

3D-descriptors showed good predictive ability compared with 2D ones [47]. In our GA-MLR model, four of six selected descriptors (De, Mor18e, H8m, and Mor30p) belong to the 3D-descriptors [32], which may reveal the importance of theses parameters in prediction the fungicidal activity of our phosphoramidate derivatives. Table 3 shows the selected descriptors and their meanings. Table 2 shows the predicted values of the antifungal activity by this model compared with the experimental ones. The plot of the predicted against the experimental values is represented in Fig. 6. It is clear that the predicted values are in good agreement with the experimental ones. The cross-validation results show that the generated GA-MLR model is valid, and it can be set to calculate the inhibition ratio of these types of organophosphorus compounds.

Conclusions

Herein, QSAR has been performed using multiple linear regressions (MLR) to investigate the relationship between the phosphonic acid and bisphosphoramidate derivatives and



Fig. 6 The plot represents the predicted log (I%) [Log $(I)_{pr.Loo.}$] values by the GA-MLR modeling against the experimental ones[Log $(I)_{exp.}$]. Good correlation can be seen between the predicted and the experimental values with $Q^2_{LOO} = 0.8718$

their bioactivities against Macrophomina phaseolina fungal pathogen. Two compounds $(p-Cl-C_6H_4-NH)(p-Cl-C_6H_4)$ $C(H)P(O)(OC_2H_5)_2$ (L₁₇) and (*p*-Cl-C₆H₄)(NC₅H₄-NH) $C(H)P(O)(OCH_3)_2$ (L₂) had the most mortality on the *M*. *phaseolina*. The cyclic motifs of L_1 and L_{11} were further determined by X-ray crystallography determination. The biological activity on human acetylcholinesterase (AChE) enzyme was determined and showed that L_5 with (*m*- $CH_3-NC_5H_4-NH$)(m- $CH_3-C_6H_4$)C(H)P(O)(OCH_3)₂ skeleton displays the most potent anti-AChE activity and the electronic parameters ΔE_{L-H} and E_{LUMO} have the highest contribution of human AChE. L₁₇ was the most active compound against the fungus with 83% inhibition of the mycelial growth; the other tested compounds showed moderate to weak antifungal activity. QSAR study showed the role of 3D-descriptors, especially 3D-MoRSE, VHIM, and GET-AWAY descriptors in the fungicidal activity of the tested

 Table 3
 The descriptors selected by GA-MLR method for antifungal activity

Descriptors	Chemical meanings	Descriptor group
T(PCl)	Sum of topological distance between P.Cl	Topological descriptors
De	D total accessibility index/ weighted by atomic Sanderson electronegativities	WHIM descriptors
H8m	H autocorrelation of lag 8/ weighted by atomic Masses	GETAWAY descriptors
Mor18e	3D-MoRSE - signal18 / weighted by atomic Sanderson electronegativities	3D-MoRSE descriptors
GGI4	Topological charge index of order 4	Galves topological charge indices
R1p+	R maximal autocorrelation of lag 1/ weighted by atomic polarizabilities	GETAWAY descriptors

compounds. Further studies need to be done in terms of these compounds' bioactivities on both target and non-target organisms to use them as a start point in fungicides design.

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Authors contributions Gholivand, Valmoozi, Hosseini, Barzegar, Nasrollah Tabar, and Yaghoubi participated in the synthesis of the compounds and crystal structure analysis, Abbod provided the antifungal assay and the GA-MLR QSAR study of the fungicidal activity and wrote the related sections. Valmoozi were the major contributors in writing the manuscript, Safaie participated in the antifungal assay, Valmoozi provided the anti-AChE assay and related QSAR study. All authors read and approved the final manuscript.

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Availability of data and materials All data generated or analyzed during this study are included in article main text and supplementary material.

Compliance with Ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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