In vitro Anti-acetylcholinesterase Evaluation and Molecular Docking Modeling of Some α-Aminophosphonate Derivatives

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Abstract

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The overuse of organophosphorus insecticides has resulted in the rise of pest resistance to these chemicals, thus making it necessary to develop new insecticides for global agriculture. In this investigation, we examined the anti-acetylcholinesterase (AChE) activity of certain α-aminophosphonate derivatives via both *in vitro* experiments and molecular docking modeling. The tested compounds demonstrated notable efficacy against the *Drosophila melanogaster* AChE enzyme. The compound Diethyl (2,4-dichlorophenyl) (2,4-dichlorophenylamino) methylphosphonate (M2), which contained two Cl- substituents on each ring, exhibited a significant concentration-dependent inhibition of the enzyme with an LC_{50} of 220 µg/ml. Additionally, at concentrations of 500 and 1000 µg/ml, Diethyl (2,4-dichloropheny l) (2-hydroxyophenylamino) methylphosphonate (M4) reduced the enzyme activity by 45.7 and 84.6%, respectively. Moreover, the four molecules displayed *in silico* affinity towards *Dm*-AChE as observed in the experimental assay. Notably, M2 demonstrated the highest affinity towards the enzyme, with two oxygen atoms in the molecule forming hydrogen bonds with SER-238 and HIS-480. The estimated free binding energy and inhibition constant for M2 were determined to be –10.04 kcal/mol and *Ki* = 43.83 nM, respectively. In addition, M4 exhibited an affinity of –9.36 kcal/mol and *Ki* = 137.7 nM. Further studies on the formulation and efficacy of these α-aminophosphonate derivatives are needed to serve as novel high-performance pesticides.

Keywords: α-Aminophosphonate, AChE, *in silico*, molecular docking.

Introduction

Organophosphorus (OP.) insecticides have served as one of the major classes of insecticides worldwide for the past decades; however, the excessive utility of this class of chemicals led to the emergence of resistance strains of pests that are difficult to control by the traditional OP (Fest & Schmidt, 2012). OP insecticides target the AChE enzyme, the key enzyme in the central nervous system of the insect (Eto, 1990; Chambers *et al*., 2010; Fest & Schmidt, 2012). The primary function of acetylcholinesterase (AChE) is to terminate the transmission of electrical signals by breaking down acetylcholine (ACh), thereby ensuring the regular propagation of nerve impulses (Eto, 1990; Fukuto, 1990; Kralj *et al.,* 2007).

α-Aminophosphonate derivatives like Phosfolan and Glyphosate are members of OP compounds wildly used as pesticides (Abdou *et al.,* 2012; Chen *et al.,* 2015; Che *et al.,* 2016). Several studies reported the pesticidal properties of a-aminophosphonate derivatives. *α*-aminophosphonates containing a benzothiophene moiety exhibited antiviral activity against tobacco mosaic virus (TMV) (Zhang *et al.,* 2014). Abbod *et al.* (2021) reported the fungicidal activity of α-aminophosphonate derivatives against *Macrophomina phaseolina* and *Pythium aphanidermatum.*

In this investigation, we have documented the *in vitro* anti-AChE activity of α-aminophosphonate derivatives that had previously been synthesized, characterized, and assessed for their fungicidal activity (Abbod *et al.,* 2021). Consequently, we have explored their insecticidal activity in an endeavor to introduce innovative molecules with dual activity against both insects and plant pathogens. In addition, a molecular docking analysis was conducted to elucidate the interactions between the compounds and the AChE enzyme.

Materials and Methods

Chemicals used and the tested compounds

All chemicals and solvents were from Merck, acetylcholine, acetylthiocholine iodide (ATCI), kojic acid, and 5,5′-Dithiobis (2-nitrobenzoic acid) were purchased from Sigma (Sigma-Aldrich, Steinheim, Germany). *D. melanogaster* AChE enzyme (1500 U/mL) was obtained from TAHA biotech company, Tehran, Iran.

Four aminophosphonate derivatives used in this test were previously synthesized and fully characterized by Abbod *et al*. (2021). The names and structures of these molecules are shown in Figure 1.

Anti-Acetylcholinesterase (AChE) activity

The Anti-AChE activity was measured using a modified 96-well microplate assay (Ingkaninan *et al.,* 2003) based on Ellman's method (Ellman *et al.,* 1961). The enzyme hydrolyses acetylthiocholine to thiocholine which reacts with Ellman's reagent (DTNB) producing 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate that can

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be detected spectrophotometrically at 412 nm. The enzyme stock solution (1500 U/mL) was used to prepare a 5 U/mL dilution of AChE in Phosphate buffer (0.1 M, pH 8.0), then kept at –80°C for further use. DTNB was dissolved in a buffer containing 2mM $MgCl_2$, and ATCI was dissolved in deionized water. Various concentrations (50, 125, 250, 500 and 1000 µg/mL) of the tested compounds dissolved in buffer containing not more than 5% DMSO. 100 µl of 3 mM DTNB, 20 µl of (5 U/mL of AChE), 40 µL of buffer, and 20 µL of the tested compound were added to each well. After mixing, the plate was incubated for 15 min at 25°C, and then the absorbance was measured at 412 nm using BioTek (Epoch Microplate Spectrophotometer), and the readings were used as blank. The enzymatic reaction was initiated by the addition of 20 mL of 15 mM ATCI and the hydrolysis of acetylthiocholine was monitored by reading the absorbance every 5 min for 20 min. All the reactions were performed in triplicate. Inhibitory activity was calculated using this formula.

$$\text{Inhibition rate (I \%)} = \frac{A_{absorbance\ of\ control} - A_{absorbance\ of\ sample}}{A_{absorbance\ of\ control}} \times 100$$

Statistical analysis

Means comparison and the variance analysis were conducted on SPSS software using One-way ANOVA and, the Tukey method at P= 0.05.

Molecular docking modeling

Molecular docking studies were performed using AutoDock 4.2 software (Morris *et al.,* 2009). The crystal structure of the *Drosophila melanogaster Dm*-AChE enzyme (accession number: 1DX4) (Houghton *et al.,* 2006) was retrieved from the RCSB Protein Data Bank (Berman *et al*., 2003). The structures of the inhibitors and enzyme were prepared and the Kollman charges were added to the protein (Weiner *et al.,* 1984) using AutoDock tools. The docking analyses were carried out by 250 runs of AutoDock with the Lamarckian genetic algorithm method (LGA) (Morris *et al.,* 1998). The population size, maximum number of evaluation (medium), and maximum number of generations were set at 200, 2,500,000 and 27,000, respectively. The grid box was centered on the active site of the enzymes (Houghton *et al*., 2006; Liao *et al.*, 2001). Discovery Studio visualizer (Biovia, 2016) was used to show the interactions between the ligand and the enzyme active site.

Results and discussion

Anti-AChE inhibitory activity (*in vitro* assay)

Ellman's colorimetric method was used in a 96-welled microplate to evaluate the anti- Acetylcholinesterase activity of the compounds. M1 displayed a weak inhibitory activity against the enzyme with 24% at 1000 µg/ml, in contrast, the rest of the compounds were significantly more effective. M4 reduced the acetylcholinesterase activity by 45.7 % and 84.6 % at 500 and 1000 µg/ml, respectively. Moreover, M2 with two Cl-substituents on each ring significantly inhibited the enzyme in a concentration-dependent manner. It inhibited the enzyme function at 1000 µg/mL with LC_{50} of 220 µg/mL. M3 with one Cl-substituent on the benzene ring was less effective by 42 % inhibition at 1000 µg/mL. (Table 1).

Molecular docking modeling

The interaction between the synthesized compounds and *Dm*-AChE was studied using molecular docking modeling. M2, M3, and M4 compounds were docked into the active site of *D. melanogaster*-AChE enzyme (accession codes 1dx4). The three molecules occupied similar positions near the top section of the active site gorge surrounded by residues (TRP-83, THR-154, GLY-155, SER-238, GLU-80, TRP-472, TYR-370, HIS-480, TYR-374, TYR-71, and PHE-371) (Figure 2).

Similar to the experimental assay results, M2 showed the best affinity toward the enzyme, two oxygen atoms of this molecule shared hydrogen bonds with SER-238 and HIS-480 as mentioned in Figure 2a, the estimated free binding energy and inhibition constant were –10.04 kcal/mol, and K_i = 43.83 nM, respectively. Moreover, the four chlorine atoms showed hydrophobic interactions with PHE-330, MET-153, TYR-370, LEU-479, TRP-83, and TRP-472 residues.

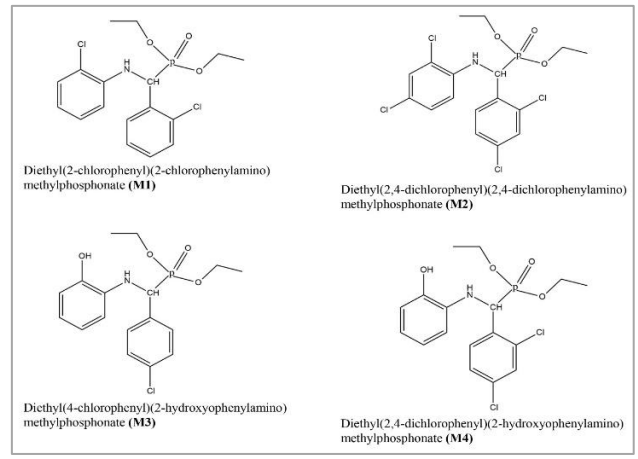

Figure 1. The structures of the tested compounds (Abbod *et al*., 2021).

Table 1. The AChE inhibitory activity for the tested compounds

Tested concent. µg/mL	Inhibition ratio %			
	M1	M2	M3	M4
50	2.0±1.15 b	9.6±2.1 a	4.5±0.83 ab	10.2±1.0 a
125	5.6±1.2 c	22±1.15 a	13.0±0.6 b	15.3±1.87 b
250	12.0±0.58 c	54±3.78 a	17.9±0.94 bc	24.07±1.16 b
500	15.3±0.9 a	75.9±3.5 b	34.02±1.15 c	45.7±2.4 d
1000	*24.0±1.0 a	100.0±0 b	42.0±1.73 c	84.6±1.35 d

* Inhibition was measured experimentally (mean ± SE), replicate number *n*=3. Means followed by the same letter in the same row are not significantly different at P= 0.05.

In the case of M4 the oxygen atoms of P=O and hydroxyl group created two hydrogen bonds with TYR-374 and THR-154 (Figure 2c), the estimated free binding energy and inhibition constant were –9.36 kcal/mol, and K_i = 137.7 nM. Furthermore, M3 was less effective than M2 and M4 either *in vitro* or by molecular modeling study, it shared two hydrogen bonds with TYR-370 and HIS-480 (Figure 2b) with estimated free binding energy and inhibition constant –8.6 kcal/mol and K_i = 495.3 nM. It can be seen from Figure 2d that the three molecules shared almost the same orientation into the active-site *gorge*.

The organophosphorus compounds can be considered traditional inhibitors of the enzyme acetylcholinesterase (Fest & Schmidt, 2012). Several derivatives of α-aminophosphonate have demonstrated inhibitory effects on acetylcholinesterase as well as other biological activities (Naydenova *et al.,* 2010; Ren *et al.,* 2016). Our molecules have displayed notable inhibitory activity against the enzyme, via both *in vitro* experiments and computational simulations. It is possible that these molecules block the entrance of the active site gorge or interact with the active site residues, leading to a modification in their conformation. This, in turn, may result in the blocking or reduction of the enzyme's functionality. Based on the findings presented by Abbod *et al.* (2021), the examined compounds, particularly M4, have the potential to act as dual-purpose pesticide, effectively targeting both insects and plant pathogens.

It can be concluded that the compounds investigated in this study exhibited a detected level of AChE inhibitory activity. M2, possessing four Cl-substituents on its rings, displayed the highest level of activity among the molecules, followed by M4 and M3, respectively. Further experimentation is still required to thoroughly investigate the efficacy of these molecules and their formulation, to enhance their bioactivity against pests.

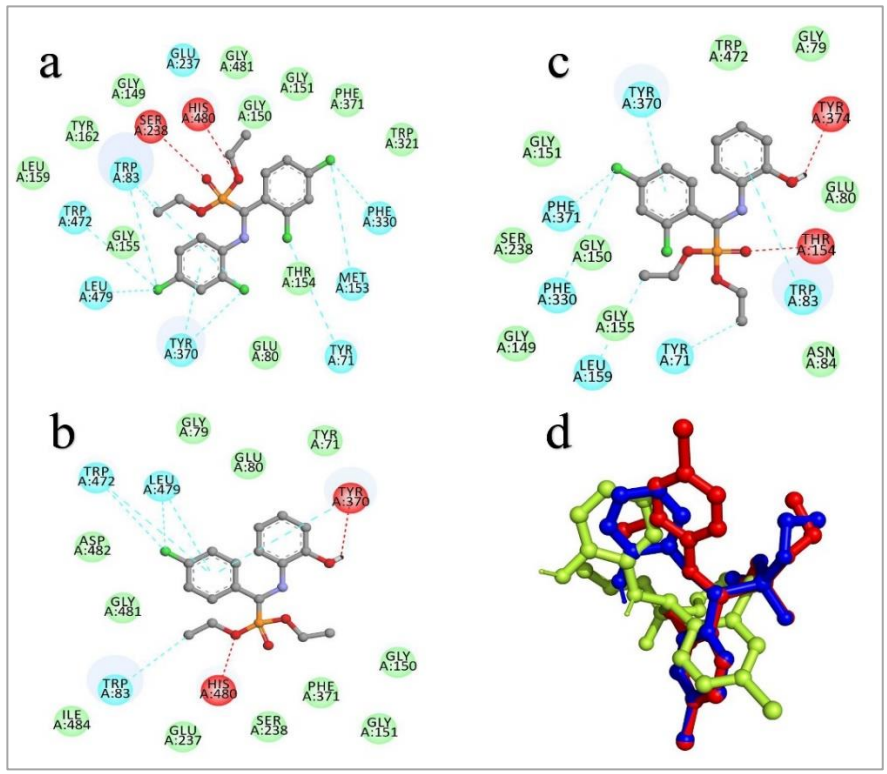

Figure 2. The interaction of *Dm*-AChE with M2, M3 and M4. (a, b and c) represent the two-dimensional view of the M4, M2 and M3 interactions with active site residues respectively, the red dashed lines represent the hydrogen bonds, the turquoise one's point to the interaction between the halogen atoms of the inhibitor with the residues and the hydrophobic interactions (d) the positioning of M2 (red), M3 (blue) and M4 (green) in the active site gorge of *Dm*-AChE.

الملخص

عبود، محسن، ناصر صفايي، خدايار قليوند ومحمد مهرأبادي. 2025. تقييم الفعالية المضادة لأنزيم الأسيتيل كولين إستيراز مختبرياً وعبر نمذجة الإرساء الجزيئي لبعض مشتقات الألفا أمينو فوسفونات. مجلة وقاية النبات العربية، 43(2): 263–267.

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أدى الاستخدام المفرط للمبيدات الحشرية الفوسفورية العضوية إلى ظهور سلالات من الآفات المقاومة لهذه المركبات، مما يجعل تطوير مبيدات جديدة أمراً ضرورياً. في هذا البحث، تمّت دراسة فعالية بعض مشتقات الألفا أمينو فوسفونات ضدّ أنزيم الأسيتيل كولين إستيراز (AChE) مختبرياً ومن خلال نمذجة الإرساء

الجزيئي (Molecular docking modelling). أظهرت المركبات التي تم اختبارها فعالية ملحوظة ضدّ الأنزيم المستهدف، حيث كان المركب ثنائي إيتيل (2،4–ثنائي كلور فينيل) (2،4–ثنائي كلور فينيل أمينو) ميثيل فوسفونات (M2)، الذي يحتوي على متبادلين من ذرات الكلور -Cl في كل حلقة من الحلقات التي يتملكها، هو الأكثر فعاليةً ضدّ الأنزيم وعلى ارتباط بزيادة التركيز المستخدم. بلغت قيمة التركيز القاتل النصفي (LC_{50}) 220 ميكروغرام/مل. علاوةً على ذلك، خفّض المركب ثنائي إيتيل (2،4–ثنائي كلور فينيل)(2–هيدروكسي فينيل أمينو)ميثيل فوسفونات (M4) نشاط الأنزيم بنسبة 45.7 و 84.6% عند التراكيز 500 و 1000 مغ/مل، على التوالي. كانت نتائج نمذجة الإرساء الجزيئي الحاسوبية متوافقة مع النتائج المختبرية، حيث أظهر المركّب M2 أعلى درجة تثبيط للأنزيم وشكّلت ذرتي الأكسجين في هذا الجزيء روابط هيدروجينية مع الأحماض الأمينية SER-238 و HIS-480، وكانت قيمة طاقة الارتباط المقدرة وثابت التثبيط –10.04 كيلو كالوري/مول و Ki = 43.83 نانومتر، على التوالي. أما بالنسبة للمركب M4 فقد كانت قيمة طاقة الارتباط المقدرة وثابت التثبيط –9.36 كيلو كالوري/مول و Ki = 137.7 نانومتر، على التوالي. أظهرت النتائج المتحصل عليها الحاجة لإجراء المزيد من التجارب حول مستحضرات مشتقات هذه المركبات وفعاليتها وصولاً لمبيدات حشرية مبتكرة ذات كفاءة عالية.

كلمات مفتاحية: ألفا أمينو فوسفونات، أسيتيل كولين إستيراز، نمذجة حاسوبية، الإرساء الجزيئي.

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