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Interpretation of the mechanism of acetylcholinesterase inhibition ability by organophosphorus compounds through a new conformational descriptor. an experimental and theoretical study

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Abstract Organophosphorus pesticides are the most common classes involved in poisonings related to pesticides. We used inhibitory ability on enzymatic activity of acetylcholinesterase activity and molecular mechanics or *ab initio* methods of molecular modelling to perform a theoretical approach of the enzyme interaction mechanism of these compounds. Kinetic values for strong and weak inhibitors were measured in a high amplitude range for affinity (K_a) and phosphorylation constants (K_p). To quantitatively describe the conformational behaviour of these molecules, conformational descriptors of free molecules were developed. Quantitative structure activity relationships (QSARs) were constructed with inhibition kinetic values and their molecular descriptors. The conformational descriptors show a high degree of correlation with the kinetic behaviour of these molecules. A positive correlation between the conformational freedom of the studied molecules with K_a is observed. This study allows us to reinterpret the organophosphorus cholinesterase inhibition mechanism and consequently the 'thiono' and 'thiolo effect' based on a global 'chalcogen effect'.

Keywords Acetylcholinesterase · Conformational behaviour · Organophosphorus compounds · Structure activity relationship · Toxicity mechanism

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Introduction

It is assumed that the toxic activity of organophosphorus compounds (OPs) is related to several factors associated with toxicokinetic and toxicodynamic pathways. The potential toxicity in a given species, induced by OPs, largely depends on the inhibition of acetylcholinesterase (AChE) by the active oxygen analogue of the corresponding phosphorus triester [1, 2]. The inhibition constant or bimolecular rate constant (K_i) of the parent compound for AChE and/or its metabolic products is one of the most important determinants of the toxic character of OPs [3].

The molecular activity of anticholinesterase OPs associated with the inhibition mechanism consists in a nucleophilic attack of the serinic oxygen of cholinesterase active site to the phosphorus atom of OPs. This serinic oxygen is deeply placed within a hydrophobic pocket in the enzyme structure. Although other proposed models exist [4], the inhibition of AChE by OPs can be described by a two-step process: a binding step, characterized by the binding affinity (K_a), followed by a phosphorylation step, characterized by a phosphorylation rate constant (K_p). The K_p/K_a ratio is the bimolecular rate constant or inhibition constant (K_i).

In this model, the value of the K_a constant is associated with the event of organophosphate approximation through the hydrophobic pocket and the adequate position of the molecule in the environment of the reactive serine, whereas K_p is influenced by molecular events directly associated with the nucleophilic attack at the serinic oxygen.

It is assumed, that the weak inhibitor ability of OPs, that contain a P=S group, is related to the low charge density of the phosphorus atoms, associated with the low electronegativity value and poor electron withdrawing power of the sulfur atom, and also to the lack of hydrogen bonding between sulfur and the enzyme active centre. Both effects constitute the so-called thiono effect [5]. Therefore, an important correlation between kinetic inhibition constants and the electronic charge of the phosphorus atom can be established [6]. Nevertheless, the explanation of the thiono effect is more directly linked to the phosphorylation mechanism than to the organophosphorus compound affinity of the active site of cholinesterase.

In the same context, it is assumed that the strong inhibitor ability of OPs, which possess a P-S-R group, is related to the higher value of the P-S bond hydrolysis kinetic constants [5] as compared to the P-O bond hydrolysis kinetic constants. Thus, the so-called thiolo effect is just related to the phosphorylation mechanism.

Many algorithms to explain toxic effects have been proposed for different situations where homogeneous classes of chemicals produced different activities [7]. To find an adequate quantitative structure-activity relationship (QSAR) describing toxicity of OPs over whole organisms can be a complex task [8, 9]. However, it is possible to derive an appropriate QSAR with the anticholinesterase activity [10–12] or other molecular activities [13], for OPs connected to common molecular characteristics. Those QSARs allow to predict the kinetic parameters of AChE inhibition for hypothetical or unknown substances and to find out the molecular mechanisms of inhibition. An important role of hydrophobic, electronic, and steric interactions of OPs and AChE can be determined performing an adequate QSAR study. Consequently, descriptors associated with the leaving group properties are most important for the majority of QSARs [14]. By use of QSARs it was found, that the anticholinesterase inhibition ability of OPs is directly related to the lability of the phosphoric ester bond of the leaving group [14]. This lability can be evaluated theoretically with Hammett's constant (σ), which provides an estimation of the electron donation properties of the leaving group. Also, can be evaluated experimentally via the phosphoric ester leaving group bond vibrational frequencies or by its alkaline hydrolysis constants [15]. Although the lability of this ester bond is the most important factor of irreversible anticholinesterase ability, steric properties of OPs also affect significantly the inhibition constants. For instance, it was suggested, that the relative small dimension of the AChE active site pocket determines the resistance of insects and fish to certain OPs, which points to a steric exclusion of OPs [16] or similar features [17].

On the other hand, an interesting conformational diversity can be ascribed to OPs. The coexistence of multiple forms could be determined not only theoretically but also on the basis of experimental data [18, 19]. The existence of an important difference in the interconversional freedom of conformers for the P=O, P=S and the P=Se series has also been demonstrated [19]. For this reason, the contribution of the conformational flexibility might be a relevant factor to explain the biological behaviour of these molecules [18], and this is precisely the focus of the present work.

Experimental

A series of OPs with small size substituents was used. The series includes P=O, P=S, and P=Se groups, and methyl, amine, acetylamine, and dichlorovinyl substituents. The molecules are shown in Table 1. Substance *I* was purified [20] from trimethyl phosphate p.a. grade (Sigma Aldrich)

Table 1 Data set (*I*, *VI*, *VII*, *VIII*, *IX,* and *X*) and test set (*II*, *III*, *IV*, *V*) molecules

$X_1X_2(Y)P=Z$	Z	X_1	X_2	Y	Denomination
Trimethyl phosphate	O	$-OCH_3$	$-OCH_3$	$-OCH_3$	*I*
O,O,O-trimethyl phosphothioate	S	$-OCH_3$	$-OCH_3$	$-OCH_3$	*II*
O,O,O-trimethyl phosphoselenoate	Se	$-OCH_3$	$-OCH_3$	$-OCH_3$	*III*
O,O,S-trimethyl phosphotionate	O	$-OCH_3$	$-OCH_3$	$-SCH_3$	*IV*
O,O,Se-trimethyl phosphoselenonate	O	$-OCH_3$	$-OCH_3$	$-SeCH_3$	*V*
Dimethyl amidophosphate	O	$-OCH_3$	$-OCH_3$	$-NH_2$	*VI*
O,O-dimethyl amidophosphothioate	S	$-OCH_3$	$-OCH_3$	$-NH_2$	*VII*
O,S-dimethyl amidophosphothionate	O	$-OCH_3$	$-SCH_3$	$-NH_2$	*VIII*
2,2-dichlorovinil-dimethyl phosphate	O	$-OCH_3$	$-OCH_3$	$-O-CH=CCl_2$	*IX*
N-acethyl-O,S-dimethyl amidophosphothionate	O	$-OCH_3$	$-SCH_3$	$-NHC(O)CH_2$	*X*

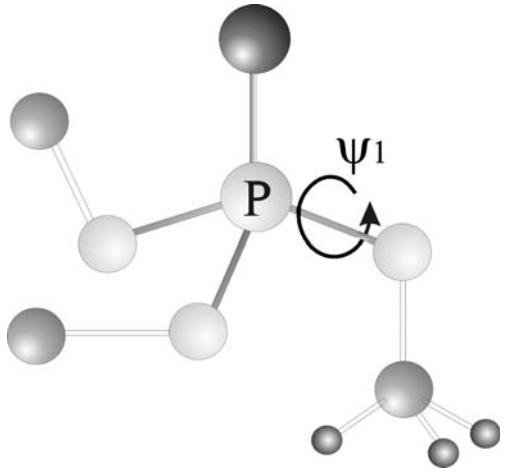

Fig. 1 Conformational freedom of organophosphates, as defined by the rotation of the dihedral angles Ψ_1

by vacuum distillation. Substances *II* [20] and *III* [18] were synthesized from trimethyl phosphite (Sigma Aldrich) and purified by vacuum distillation. Substances *VII*, [19] *VIII*, and *IX* were purified by vacuum distillation from a technical grade substance (Bayer). Substance *VI* was obtained by oxidazing substance *VII* and then purifing it by vacuum distillation [18]. Substance *X* was purified by recrystallization from a technical grade substance (Bayer).

Several molecular descriptors for this series were calculated. According to the classic scheme, electronic, steric, or hydrophobic descriptors were discriminated. Descriptors associated with the conformational behaviour were located in a fourth group.

The electronic descriptor calculated by the Mulliken method at the *ab initio* HF/STO-3G level of theory is the phosphorus partial charge ($^{+}$P). The steric descriptors are the molar refractivity (MR) and molar volume (MV), respectively, calculated by the Viswanadhan fragmentation method [21, 22] and with the minimum energy conformer by HF/STO-3G approximation. The hydrophobic descriptors are the dipole moment (μ) and the logarithm of the octanol/water partition constant (logP), respectively. They were calculated with the minimum energy conformer at the HF/STO-3G level and by the Viswanadhan fragmentation method [21]. Since there are methodological limitations [21], the logP and MR estimations for substance *III* could not be determined.

The idea of defining a conformational descriptor arises from the calculation of the energies of the OPs structures, that are obtained by the free rotation of each of the three P-O ester bonds (Fig. 1). Calculations of energies were performed with the molecular mechanic DREIDING force field method applying the Gaussian98 package [23]. These energy values generate an hypersurface of conformational energy. The minima represent easily accessible geometries, and the maxima correspond to geometries with restricted access due to steric or electronic reasons (Fig. 2).

Although QSAR studies have found an interesting field in the investigation of 3D descriptors, there are few publications on their application to the study of anticholinesterase activity [24–27] and even a smaller number of studies on OPs were reported in the literature [28]. Those analyses are interesting because they explain the mechanism of toxicity in the context of AChE active site structure and the inhibitor architecture, which are dynamic properties of molecules. These are Intrinsic Conformational Descriptors ('I' superscript) referred to calculations about free structures, without solvent or in another context. On the other hand, they are Restriction Conformational Descriptors ('R' descriptor) which have a positive correlation with structural energies. The concept of conformational restriction is visualized in Fig. 2. Since these functions quantify the conformational variability and the spatial energy performance of the multiple structures for a same molecule, we can define these descriptors as 4D molecular descriptors.

K_i, K_a and K_p were evaluated in the AChE activity of Wistar rat brain extracts by the Ellman's procedure. A racemic mixture was used to determine the kinetic constant of substances *VIII* and *X*. The determinations were performed in a 100 mM phosphate buffer, 1 mM magnesium chloride solution, at pH 8.0. 12.5 mM acethylthiocholine chloride (Sigma Chemical Co.) and 12.5 mM 5,5′-dithio-bis-(2-nitrobenzoic acid) (Sigma Chemical Co.) in phosphate buffer (pH 8.0) solutions stored in light protected bottles

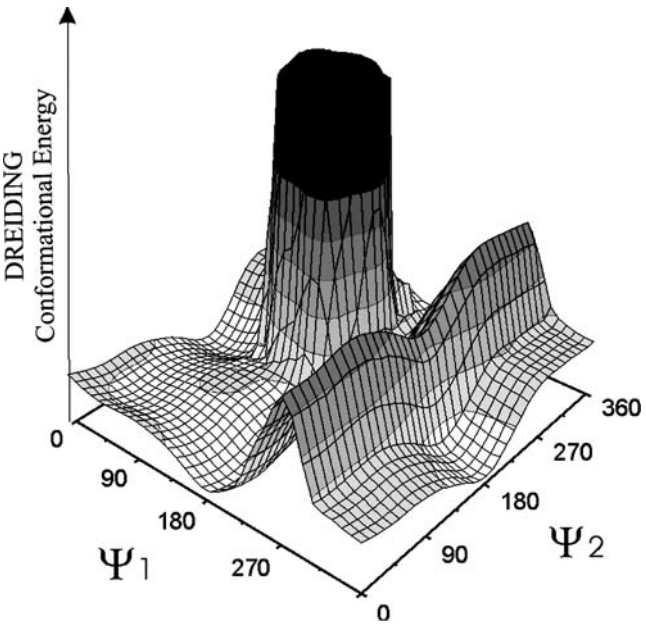

Fig. 2 Hypersurface of conformational energy for trimethylseleno phosphate (Substance III) with respect to the 2 dihedral angles Ψ. The minima (white surface regions) represent easily accessible geometries and the maxima (black surface regions) represent geometries with restricted access

Table 2 Classical molecular descriptors, definition of conformational descriptors and values calculated for molecules

Molecule	^{+}P	μ	logP	MV	MR	$^{I}R_A$ $\frac{\sum_{i=\Psi1,\Psi2,\Psi3}^{n} E_i^L}{n}$	$^{I}R_C$ $\frac{\sum_{i=\Psi1,\Psi2,\Psi3}^{n} \left[\ln\left(E_i^L\right)\right]}{n}$
I	1.242	0.115	0.55	80.70	28.1	126.92	2.120
II	0.998	0.857	1.29	101.49	36.09	121.68	2.451
III	1.021	2.396	ND	84.64	ND	9542.53	2.707
IV	1.005	0.785	0.90	99.09	34.54	2531.67	2.001
V	1.039	1.399	0.75	116.76	37.74	2526.83	1.923
VI	1.232	1.399	0.02	75.05	26.34	768.30	1.912
VII	0.993	2.077	0.76	85.50	34.33	26327.01	2.028
VIII	0.996	1.868	0.37	91.69	32.78	5.11	1.632
IX	1.260	4.153	1.84	125.81	42.28	6.26	1.816
X	1.030	2.932	0.00	117.38	41.16	5.69	1.698

Phosphorus partial charge ($^{+}$**P**) obtained by the Mulliken model. Dipole moment (μ) in Debye. Molar Volume (**MV**) in cm^3 /mol. ND: parameters not determined.

were used. The kinetic curves were fitted with the solution of Main's second order differential equation [29] and K_a, K_p parameters were obtained. The K_i values were obtained from the K_a/K_p ratio. This formalism allows to work with very small inhibition constants.

Substances *I*, *VI*, *VII*, *VIII*, *IX*, and *X* are the data set molecules. The structure-inhibition ability relationships of these substances were studied to obtain their kinetic and descriptor data by multiple linear regression (MLR) analysis. A good fit was defined as the one for which $r^2 > 0.800$ for one degree of freedom, $r^2 > 0.940$ for two degrees of freedom, and $r^2 > 0.995$ for three degrees of freedom. From each conformational descriptor series, correlations with a good_fit index for all the kinetic constants, K_a, K_p, and K_i, were found. Substances *II*, *III*, *IV*, and *V* are the test set molecules. Their unknown or poorly measured kinetic constants were estimated from the correlations found using the data set molecules.

Table 3 Proposed conformational quantitative structure activity correlations (^{C}QSARs) between kinetic constants and conformational descriptors

Kinetic constant	Independent factor	Descriptors							r^2
		Conformational		Steric		Hydrophobic		Electronic	
		$^{I}R_C$	$^{I}R_A$	MR	MV	μ	logP	^{+}P	
pK_a	(a) 23.70±0.05	−11.14± 0.01							0.81142
	(b) 24.01±3.07	−11.71± 1.65					1.28± 0.46		0.94748
K_p	(c) 0.549± 0.057	−0.366± 0.025			$3.79\pm 0.64\cdot10^{-3}$	$-5.79\pm 0.64\cdot10^{-2}$			0.99658
	(d) 0.487± 0.027		$-6.70\pm 0.26\cdot10^{-6}$		$1.35\pm 0.12\cdot10^{-3}$			$-4.22\pm 0.22\cdot10^{-1}$	0.99882
pK_p	(e) −0.189± 0.165		$5.39\pm 0.16\cdot10^{-5}$		$-5.96\pm 0.74\cdot10^{-3}$			1.46±0.13	0.99934
pK_i	(f) −29.84± 4.70	14.87±2.51							0.89789
	(g) −21.15± 5.40	12.38±2.20			$-4.20\pm 2.00\cdot10^{-2}$				0.95854
	(h) −19.86± 0.89	15.23±0.36		$-9.19\pm 0.97\cdot10^{-2}$				−6.67±0.46	0.99931
	(i) −20.31± 0.62	14.56±0.29			$-3.14\pm 0.24\cdot10^{-2}$			−5.28±0.35	0.99964

The analytic error of the fit constants is shown. r^2 is suggested as a good correlation index. In the data table, for example, the ^{C}QSAR(a) are defined by equation $pKa = 23.70 - {}^{I}\mathbf{R_C} \cdot 11.14$. The set molecules of ^{C}QSARs for the K_p kinetic constant were reduced to *I*, *VI*, *VII*, *VIII*, and *IX* only, because the *X* molecule incorporation leads to a bad fit.

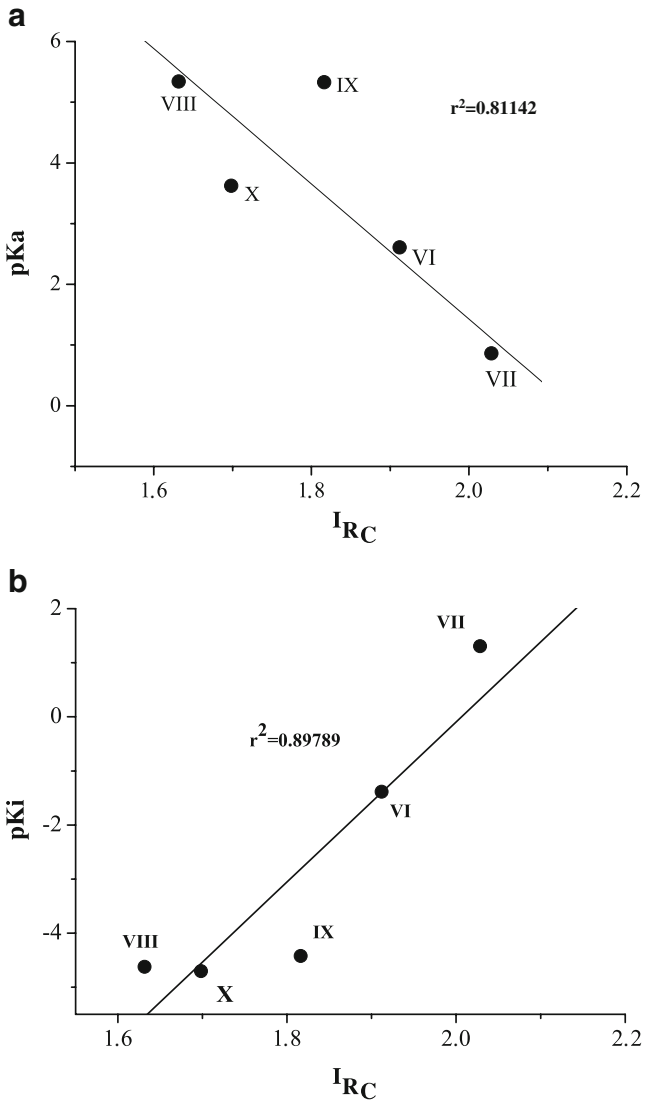

Fig. 3 $^{I}R_C$ individual correlation with pK_i and pK_a

Results

Classical descriptor values are shown in Table 2. In general, plausible results have been observed by the application of these formalisms. However, the value of the ^{+}P parameter in the frame of the application of the Mülliken's method could be underestimated for selenium compounds. On the other hand, the result of the calculations for MV for II and III would seem to be not realistic, for similar reasons already mentioned in the Viswanadhan fragmentation method [21].

Conformational descriptors are calculated by the application of functions to the set of energy values for each molecule. Two functions with good correlation behaviour were selected from a set of functions (Table 2).

In our study, good function adjustments for one, two, and three descriptors can be observed in the linear correlations (Table 3). The predominance of the conformational descriptors in all correlations is remarkable. Moreover, $^{I}R_C$ has a high degree of individual correlation with pK_i and pK_a (Fig. 3), but this result is not applicable to the other descriptors. Therefore, all proposed correlations are between the conformational structure and activity and are defined as ^{C}QSAR. Measured and predicted K_i, K_a, and K_p values are collected in Tables 4, 5, and 6.

For all substances, there is an agreement of the values found for each constant. It is possible to make certain reliable interpretations. The pK_i constant was estimated for Substance *III* according to ^{C}QSAR, and the values obtained were between 10.4 and 14.4. For Substance *II*, the pK_i values obtained were between 6.6 and 7.5. It has been reported that oxono/thiono ratios of K_a values vary from 14 to 1240, the K_p constant values vary in a narrow range from 8- to 14-fold greater than their thiono counterparts, while oxono/thiono ratios for K_i constants vary widely from 1 to 82 [30].

Although these values are considered to be overestimated, they are coherent and differ in five and seven orders of magnitude, respectively, for the K_i of substances *II* and *III*. All the values considered are incompatible with the K_i* value measured for these substances in the kinetic tests. Some in vitro event of molecular modification must exist rendering it inaccessible to direct kinetic determination under these conditions. Investigating the values considered for substances *IV* and *V*, it is obvious, that there is some analogy in the difference of the order of magnitudes between the values of K_p and the measured values of K_p* for substances *II* and *III*. Also, the measured values of K_a are comparable to the K_a*. Nevertheless, the values of K_i are higher than the measured ones for substance *II* and much higher than the experimental ones for substance *III*.

Table 4 Affinity constant (K_a) values estimated and predicted by ^{C}QSARs, and measured and expressed in molar units

Molecule	Measured	(a)		(b)	
		Δ pK_a	Predicted	Δ pK_a	Predicted
I	2.35	−0.45		−0.37	
II	$*4.74\times10^{-2}$		3.97×10^{3}		1.10×10^{-3}
III	$*9.45\times10^{-2}$		2.89×10^{6}		
IV			3.90×10^{-2}		1.85×10^{-2}
V			5.28×10^{-3}		3.51×10^{-3}
VI	2.46×10^{-3}	0.21		0.96	
VII	1.37×10^{-1}	−0.24		−0.37	
VIII	4.56×10^{-6}	−0.18		−0.04	
IX	4.68×10^{-6}	1.86		0.23	
X	2.38×10^{-4}	−2.16		−0.51	

ΔpK_a is defined as (pK_a measured − pK_a estimated).

Table 5 Phosphorylation constant (K_p) values estimated and predicted by ^{C}QSARs, and measured and expressed in min^{-1}

	Measured	(c)		(d)		(e)	
		Δ pK_p	Predicted	Δ pK_p	Predicted	Δ pK_p	Predicted
I	7.38×10^{-2}	−0.01		−0.02		0.02	
II	$*5.26\times10^{-1}$		NV		2.02×10^{-1}		6.40×10^{-1}
III	$*7.57\times10^{-1}$		NV		1.06×10^{-1}		1.28×10^{-1}
IV			1.47×10^{-1}		1.79×10^{-1}		7.94×10^{-1}
V			2.07×10^{-1}		1.89×10^{-1}		7.37×10^{-1}
VI	5.94×10^{-2}	−0.05		0.02		0.06	
VII	6.72×10^{-3}	0.19		−0.01		0.03	
VIII	1.90×10^{-1}	0.00		−0.00		0.03	
IX	1.24×10^{-1}	−0.01		0.00		0.04	
X	14.3	−1.85	0.203	−1.83	0.210	−1.74	5.85×10^{-1}

Δ pK_p is defined as (pK_p measured − pK_p estimated). NV is a negatively predicted value.

However, the values of K_i for substance *V* would be compatible with the alkaline hydrolysis constant of the P-Se bond.

From the unexpected values of the kinetic constants it can be concluded, that substances *II* and *III* show a high inhibition ability, which does not correspond with their structures. Thus, these kinetic evidences suggest interactions with P-X-CH_3 isomers (X=S, Se).

Discussion

Many previous theoretical 3D studies about the required molecular structural properties for inhibitor ability are based only on the most stable conformations as obtained from vacuum calculations. Nevertheless, theoretical and spectroscopic studies have shown, that the suggested properties of stable conformations in a water environment [31] or in the active site [32, 33] are not the same as the stable conformations in vacuum, the most frequently reported calculations. Moreover, the dynamic properties of the inhibitor-biomolecule complexes have been poorly studied, both from a theoretical and an experimental point of view.

If the theoretical model were able to relate the molecular phenomenon to the measured parameters, and to phenomena, that determine the biological effect, we would have an interpretative tool derived from the correlations.

Both phenomena were used, the first one in the test set molecules and the second one in the correlations obtained. Although the number of substances in the data set molecules is low, there is a strong trend in the results, that may allow to use the method for the interpretation of the interaction mechanism. Besides, the prediction tool was used, although the interpolation of kinetic values for hypothetical or unmeasured substances is uncertain.

The $^{+}$P descriptor only appears in the correlations of the K_p and K_i estimations, but not in a K_a estimation. This can be due to the fact, that the nucleophilic attack occurs on the

Table 6 Inhibition constant (K_i) values estimated and predicted by ^{C}QSARs, and measured and expressed in $M^{-1}min^{-1}$

Molecule	Measured	(f)		(g)		(h)		(i)	
		Predicted	Δ pK_i	Predicted eed	Δ pK_i	Predicted	Δ pK_i	Predicted	Δ pK_i
I	3.14×10^{-2}		−0.19		−0.20		−0.06		0.03
II	*11.12	2.51×10^{-7}		1.21×10^{-5}		3.29×10^{-8}		1.20×10^{-7}	
III	*8.01	3.80×10^{-11}		1.57×10^{-9}		3.40×10^{-15}		8.48×10^{-12}	
IV		1.22		3.52		0.185		0.386	
V		17.52		178.50		9.44		28.51	
VI	24.18		0.02		−0.75		−0.00		−0.06
VII	4.91×10^{-2}		0.99		0.95		0.06		0.01
VIII	4.16×10^{4}		0.96		0.19		0.05		0.07
IX	2.64×10^{4}		−1.59		−0.47		0.07		0.04
X	6.00×10^{4}		−0.19		0.28		−0.12		−0.08

ΔpK_i is defined as (pK_i measured − pK_i estimated).

phosphorus atom from the AChE active site, in one of the phenomena involved in the process that is described by the kinetic constants, and then the phosphorus charge value determines the magnitude of the process. Nevertheless, this charge varies with the Ψ angles [34], it is a variable and dynamic property and, therefore, the $^{+}$P Descriptor does not appear in the correlations with a good fitting. Phosphorus partial charge and enzymatic activity have been shown to be significantly related in other enzymes [35].

On the other hand, the dipole moment of the organophosphorus compounds also varies with the conformation. This can be the reason, why it appears only in one of the correlations. For steric descriptors, MV seems to be an optimal descriptor for the data set molecules. QSAR studies by MLR and artificial neural network demonstrate, that MR is of prime importance for the OPs toxicity [36].

Nevertheless, by the characteristics of the data set molecules - the small size molecules, and the small number of molecules - a successful extrapolation to molecules with high lateral groups seems to be doubtful. If we observe the individual correlation of affinity constant versus $^{I}R_{C}$ (Fig. 3), the data dispersion increases with the pK_a value, and the fitting is better in the weak inhibitor substance zone. This can be an indication that the correlations will not be valid for strong inhibitors; they will only be applicable for organophosphorus molecules with weak inhibiting power and/or small sizes.

With respect to the mechanistic interpretation, all the ^{C}QSAR of high correlations found include some of the intrinsic conformational descriptors. This suggests an important role of the conformational behaviour of individual molecules in the inhibition ability, which is represented by the intrinsic conformational descriptors. It is possible to argue that the freedom of conformational behaviour of individual molecules is of high importance during the entrance of a molecule into the hydrophobic gorge, towards the reactive serinic oxygen of the active site and in the phosphorus attack on the active site serinic oxygen. An increment of conformational restriction implies a K_a increment, a K_p decrease, and K_i decrease. This is derived for the sign of each one of the ^{C}QSARs found. This would imply that the decreased inhibiting ability of OPs with P=S and P=Se groups can be explained by a K_a increase based on an increase of the intrinsic conformational restrictions of these molecules. It has been shown that in S-aryl phosphamido thiolate analogues, an increment in the distortion energy of the X=P-O-Y dihedral angle implies an increment of the K_i values [37].

Our results focus on the affinity constant and are applied over a wide range of inhibitor abilities. This could be an alternative explanation for the argument, that states, that the smaller electronegativity and the higher size of the sulfur and selenium atoms, as compared to the oxygen atom, are not adequate for the nucleophilic attack on the phosphorus atom. For this reason, K_p diminishes drastically in thiono and seleno organophosphates. Along the same line, the explanation of the thiolo effect can be related to the different rotational freedom of the P-S and P-O bonds in oxo and thiolo OPs. Nevertheless, the intrinsic molecular conformational behaviour would play some important role in the determination of the events previous to phosphorylation and can simultaneously explain the thiono and thiolo effect. In the same sense, its was indicated broad scale rearrangement in AChE during ligand admission to the george [37]. Both facts, conformational changes of OP and protein, suggested a complex dynamic process during OP-AChE complex formation.

In the ^{C}QSAR structure referring to the nonconformational descriptors, the μ and logP parameters play a role in the K_a and K_p estimation, but not in that for K_i. It has been reported that the hydrophobic properties play a key role in the correlations of K_i for lipophylic inhibitors but in hydrophilic OPs such as phosphamido thioate analogues, the hydrophobic interactions there are not important [38]. This would imply a smaller role of the hydrophobic interactions in the formation of the enzyme-inhibitor complex for polar OPs. But according to our data, which include phosphamidate and other molecules, it is possible to affirm, that for our studied molecules with low values of K_i and small size, hydrophobic interactions are of relevance. Moreover, given the negative sign of the correlation factor that accompanies μ in the ^{C}QSAR kp estimation, it is possible to speculate, that the increase of hydrophobic interactions would imply an increase in the value of Kp and an increased capacity for the molecule to react adequately in the active site. On the other hand, the positive sign of logP in the ^{C}QSAR K_a estimation implies, that the increase of hydrophobic interactions would lead to an increase in the value of K_a and an increased difficulty to locate the molecule in the active site adequately. In this series of molecules, hydrophobic interactions play a double role in the phenomena, but they do not appear in the K_i correlations.

From these arguments arise two questions: it is a fact that the dipole moment of the conformers of OPs increases, when their conformer energy increases, then the most stable conformers in vacuum or in water solution have a smaller dipole moment. Nevertheless, the architecture of the AChE pocket would stabilize the conformers of low dipole moment, that is, the conformers of low energy in solution, but in the gorge region the conformations with a lager dipole moment are preferred. In fact, the negative sign of the correlation factor, that accompanies μ in the ^{C}QSAR K_p estimation, is an indication of the preference for low dipole moments in the phosphorylation mechanism. However, the positive sign of the correlation factor in the ^{C}QSAR pK_i

estimations indicates the tendency for a decrease of the hydrophobic interactions in the gorge region. This may mean, that a molecule will be a better inhibitor, if its bonds are easier to rotate and it can reach conformations of lower and higher dipole moment. On the other hand, the loss of the local dipole moment in the P=O, P=S, P=Se series would imply an increment in K_a and, therefore, a decrease of the K_i constant.

Both reasoning connect the hydrophobic factors and the conformational behaviour of the molecules. The first are classical objects of argumentation. It is reported that differences in hydrophobicity of oxono and thiono analogues of organophosphorus compounds may be as important as their electronic differences in determining their effectiveness as AChE inhibitors [30]. The conformational behaviour is a novel factor for the explanation of anticholinesterase ability. This work allows us to reinterpret the organophosphorus cholinesterase inhibition mechanism and, consequently, the 'thiono' and 'thiolo effect' based on a global 'chalcogen effect'.

According to our results, there is strong evidence that the conformational freedom of OPs is an important molecular property that determines the magnitude of the binding affinity of inhibitor substances. Moreover, in our system, these properties could be sufficient for the quantification of kinetic magnitudes.

This is based on the idea, that all of the inhibition events depend on static and dynamic structural characteristics of both the molecular inhibitors and the enzyme active site, that determine the organophosphorus compound toxicity and the species specificity of these compounds. We have called those dynamic molecular properties 'architectonic molecular characteristics' and the set of static and dynamic properties 'architecture' of molecules.

In that sense, the possible interpretation ability of the phosphorylation mechanisms appears to be the most interesting property of the developed intrinsic molecular descriptors. However, working with extensive training set molecules probably improves the possibility of developing intrinsic molecular descriptors with higher predictive power. We have found powerful tools for the estimation of the kinetic properties of inhibitors or substrates for the AChE and hypothetically kinetic properties in other systems with unknown architectural properties.

More complete studies of the dynamic behaviour of the OPs in water solutions and additional ^{C}QSAR could help to explain, how these structures indeed interact with the active site of AChE, and could also be useful tools to tackle other problems related to the OPs-AChE interaction.

The generalized application of conformational descriptors to other biological systems can give powerful tools to interpret molecular phenomena and to predict kinetic parameters of biological processes.

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