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Studying the relationship between the structure and inhibitory power of some rodenticides

Zahra Javani[1*]

[1]*Department of Chemistry, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran*

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ABSTRACT: Considering the specific properties and applications of organic phosphorus compounds, it is important to design molecules with favorable biological potential. In this article, four compounds from the family of phosphoramides with Potential to have antiacetylcholinesterase properties were selected and after synthesis, purification and identification, they were evaluated quantitatively in terms of hydrophobicity and inhibitory power of enzyme acetylcholinesterase. Also, by using PASS software, the biological activities of each compound were investigated and in particular, the percentage of their antiacetylcholinesterase activity was extracted from the computer evaluation results. The obtained results confirm the PASS software in QSAR studies and it is possible to obtain acceptable and useful results that match what is expected in the experiment without spending time and money. The relationship between toxicity and hydrophobicity is such that with increasing logP, IC_{50} also increases. This relationship means that increasing the hydrophobic character leads to less toxicity of the phosphorus compound.

Keywords: *Inhibitory power, PASS software, Phosphorus rodenticide, QSAR*

INTRODUCTION

Extensive research on the anti-tumor and anti-AIDS activity of organophosphorus is being done. These large groups of compounds are widely used as pesticides, and most of these compounds prevent the normal function of the enzyme through reaction with the serine hydroxyl group of the acetylcholinesterase enzyme and are therefore toxic. With the restriction of the enzyme's function, the hydrolysis of acetylcholine, which plays an essential role in the transmission of nerve messages, is disturbed, and this causes the accumulation of acetylcholine in nerve membranes and eventually death. For this reason, the biological activity of antiacetylcholinesterase of organophosphorus compounds and communication It is highly regarded with important structural features such as hydrophobicity [1].

Quantitative structure-activity relationships (QSAR) have been applied for decades in the development of relationships between physicochemical properties of chemical substances and their biological activities to obtain a reliable statistical model for prediction of the activities of new chemical entities. QSAR modeling is one of the major computational tools employed in medicinal chemistry. In this method, the structural changes that affect the biological activities of a group of compounds of the same family are separated into three

(*) Corresponding Author - e-mail: Z_javani@yahoo.com

main types: electronic, spatial and hydrophobic. Other factors such as hydrogen bonding and dipole moment seem to play a lesser role. The electronic parameter is evaluated using the Hammett equation, the spatial parameter is evaluated using the Taft method, and the changes in hydrophobicity are expressed by the partition coefficients of matter in the octanol-water system. Among the three discussed evaluations, the hydrophobicity parameter can be investigated completely experimentally, and in laboratory studies, its relationship with the toxicity of the chemical compound can be indicative of the structure-activity relationship [2]. What has been proven is that the hydrophobicity parameter is one of the most important influencing parameters in biological activities, for example, the effect of the hydrophobicity of substances in binding with proteins, the effect of the hydrophobicity of substances on the interaction of substances with enzymes, the effect of hydrophobicity Materials in their inhibitory power, the effect of hydrophobicity of materials on the effectiveness of antimalarial compounds, antitumor, pesticides and anesthetics, the effect of hydrophobicity of pharmaceuticals on the double stability of drugs, the power of permeability and the formation of host-guest complexes, etc. Hydrophobicity, which is equivalent to lipophilicity, can be the affinity of a molecule or a part of a molecule to a lipid environment, which is usually investigated by measuring the distribution behavior of the compound in a two-phase system. The distribution ratio is called the division coefficient. Water is usually used as the polar phase and ethanol as the organic phase. The partition coefficient refers to the concentration ratio in the octanol phase to the water phase, which is displayed as Pow, and the hydrophobicity parameter is the logarithm of this ratio [3].

One of the methods of evaluating the biological activity of chemical compounds is the use of computer predictions, which can predict hundreds of biological activities in a short period of time and at a low cost, but with high accuracy [4]. Biological evaluations in this project were done by Prediction Activity Substance Spectra software (PASS). The basis of this software is to compare the structure of a chemical compound with substances whose biological activity is well known, and the result of this comparison is to predict the percentage of each biological activity for the studied compound.

Quantification of the compound's biological activity in the laboratory is done by measuring the IC_{50} parameter. This parameter indicates how much of an inhibitor is required to reduce the activity of the biological component by half. The biological component can be an enzyme, cell, cell receptor, or microorganism. IC_{50} values are usually expressed as molar concentrations [5].

EXPERIMENTAL

Four compounds from the family of phosphoramides with Potential to have antiacetylcholinesterase properties were selected [6] and after synthesis, purification and identification, they were evaluated quantitatively in terms of hydrophobicity and inhibitory power of enzyme acetylcholinesterase. Also, by using PASS software (version 1.917), the biological activities of each compound were investigated (pa: Probability of activity, pi: Probability of inactivity), and in particular, the percentage of their antiacetylcholinesterase activity was extracted from the computer evaluation results [7].

First, synthesis of the precursor (N, N-dimethylamine dichlorophosphate) was done. 0.37 moles of N,N-dimethylamine hydrochloride was added to 0.37 moles of phosphoryl chloride and the reaction mixture was refluxed for 12 hours. To purify the product, the obtained liquid was distilled in vacuum. Then we reacted the prepared precursor with the molar ratio of 1 to 2 respectively with additions of sodium or potassium salt of phenol, paracrosol, parachlorophenol, paranitrophenol and 4-hydroxybenzonitrile. Each time, the reaction mixture is stirred at 4°C for 6 hours. The product is separated from the aqueous phase in the form of an oil phase and is separated by a separatory funnel. For the final purification, a chromatography column and hexane and ethyl acetate solvents were used with a ratio of 7:1.

In order to quantify the hydrophobicity parameter, we used the vibrating flask experimental method. In this method, absorption-concentration calibration curve is first drawn for the desired sample in one of two phases (aqueous or organic) in different concen-

trations by UV-Vis spectroscopy. Then the solute in one phase is extracted from the other phase by a specific volume. The concentration extracted from the substance by UV-Vis spectroscopy is measured again after separating the two phases, and the ratio of the concentration in the organic to aqueous phase is calculated, and the logarithm of this ratio is reported as the hydrophobicity parameter. The acetylcholinesterase inhibitory power of these compounds was evaluated and predicted using PASS software. This software first identifies the substructures for the studied compound and then predicts the type and probability of biological activity based on the relationship between its substructures and the substructures of the compounds in the software memory [4]. We also used the Ellman method to quantify the strength of biological ability. Based on this method, the inhibitory power of the acetylcholinesterase enzyme is measured with the IC_{50} parameter, and the smaller this number is, the greater the inhibitory power (toxicity) of the chemical compound [8].

Compound 1: $Me_2NP(O)(p\text{-}OC_6H_5)_2$

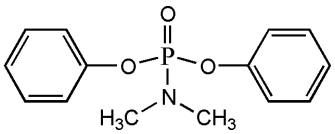

Fig. 1. N,N-dimethyl Phosphoramidic Acid diphenyl Ester.

This compound is prepared from the reaction N, N-dimethylamine dichlorophosphate and potassium salt of phenol with the ratio of 1 to 2. After purification, it is identified with NMR (Bruker DPX 250 NMR Spectrometer). Based on the shaking flask method and measurement of the distribution coefficient of this compound between the two phases of water-octanol, the hydrophobicity parameter (logP) of this compound is 1.027. Also, based on Elman's method to measure the inhibitory power of acetylcholinesterase (IC_{50}), the amount evaluated for this compound is 31.3 mM. [9]. Part of the software evaluation results using the PASS software for this compound is compiled in the Table 1. According to the evaluation, the possibility of antiacetylcholinesterase activity for this compound is 81.6%.

Compound 2: $Me_2NP(O)(p\text{-}OC_6H_4\text{-}CH_3)_2$

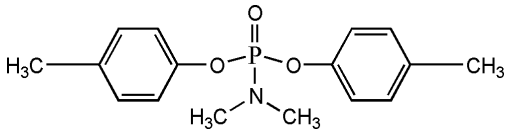

Fig. 2. N,N-dimethyl Phosphoramidic Acid Bis-(4-methylphenyl) Ester.

This compound is prepared from the reaction N, N-dimethylamine dichlorophosphate and potassium salt of paracrosol with the ratio of 1 to 2. After purification, it is identified with NMR (Bruker DPX 250 NMR

Table 1. Results of PASS software predictions for composition 1.

Pa	Pi	Biological activity
0.824	0.001	CYP2A6 human substrate
0.816	0.002	Acetylcholinesterase inhibitor
0.777	0.011	CYP2 substrate
0.796	0.012	Dynein ATPase inhibitor
0.786	0.008	GTP diphosphokinase inhibitor
0.689	0.014	CYP2C11 substrate
0.731	0.006	Granzyme B inhibitor
0.709	0.003	Carboxylesterase inhibitor
0.725	0.001	Cholinergic
0.695	0.006	Dynamin GTPase inhibitor
0.628	0.010	Thermolysin inhibitor
0.548	0.011	RNA directed DNA polymerase inhibitor
0.544	0.009	Glycerophosphocholine cholinephosphodiesterase inhibitor
0.532	0.005	Plus-end-directed kinesin ATPase inhibitor
0.523	0.006	Tropinesterase inhibitor
0.537	0.034	CYP3A substrate

Table 2. Results of PASS software predictions for composition 2.

Pa	Pi	Biological activity
0.765	0.002	CYP2A6 human substrate
0.722	0.003	Acetylcholinesterase inhibitor
0.706	0.014	CYP2 substrate
0.694	0.011	Dynein ATPase inhibitor
0.691	0.009	GTP diphosphokinase inhibitor
0.656	0.009	CYP2C11 substrate
0.648	0.016	Granzyme B inhibitor
0.630	0.007	Carboxylesterase inhibitor
0.607	0.006	Cholinergic
0.590	0.008	Dynamin GTPase inhibitor
0.581	0.006	Thermolysin inhibitor
0.548	0.011	RNA directed DNA polymerase inhibitor
0.544	0.009	Glycerophosphocholine cholinephosphodiesterase inhibitor
0.532	0.005	Plus-end-directed kinesin ATPase inhibitor
0.523	0.006	Tropinesterase inhibitor
0.537	0.034	CYP3A substrate

Spectrometer). Based on the shaking flask method and measurement of the distribution coefficient of this compound between the two phases of water-octanol, the hydrophobicity parameter (logP) of this compound is 1.63. Also, based on Elman's method to measure the inhibitory power of acetylcholinesterase (IC_{50}), the amount evaluated for this compound is 35.4 mM. [9]. Part of the software evaluation results using the PASS software for this compound is compiled in the Table 2. According to the evaluation, the possibility of antiacetylcholinesterase activity for this compound is 72.2%.

Compound 3: $Me_2NP(O)(p\text{-}OC_6H_4\text{-}Cl)_2$

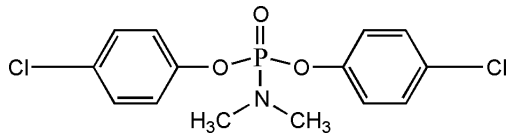

Fig. 3. N,N-dimethyl Phosphoramidic Acid Bis-(4-chlorophenyl) Ester.

Table 3. Results of PASS software predictions for composition 3.

Pa	Pi	Biological activity
0.769	0.002	CYP2A6 human substrate
0.718	0.003	Acetylcholinesterase inhibitor
0.709	0.010	Dynein ATPase inhibitor
0.690	0.009	GTP diphosphokinase inhibitor
0.621	0.006	Cholinergic
0.631	0.023	CYP3A substrate
0.623	0.020	Granzyme B inhibitor
0.599	0.008	Dynamin GTPase inhibitor
0.601	0.013	CYP2C11 substrate
0.561	0.006	Thermolysin inhibitor
0.563	0.008	Carboxylesterase inhibitor
0.562	0.010	RNA directed DNA polymerase inhibitor
0.554	0.008	Glycerophosphocholine cholinephosphodiesterase inhibitor
0.540	0.005	Plus-end-directed kinesin ATPase inhibitor
0.519	0.006	Tropinesterase inhibitor
0.521	0.025	Adenylate cyclase inhibitor

This compound is prepared from the reaction N, N-dimethylamine dichlorophosphate and potassium salt of parachlorophenol with the ratio of 1 to 2. After purification, it is identified with NMR (Bruker DPX 250 NMR Spectrometer). Based on the shaking flask method and measurement of the distribution coefficient of this compound between the two phases of water-octanol, the hydrophobicity parameter (logP) of this compound is 2.75. Also, based on Elman's method to measure the inhibitory power of acetylcholinesterase (IC_{50}), the amount evaluated for this compound is 40.9 mM. [9]. Part of the software evaluation results using the PASS software for this compound is compiled in the Table 3. According to the evaluation, the possibility of antiacetylcholinesterase activity for this compound is 71.8%.

Compound 4: $Me_2NP(O)(p\text{-}OC_6H_4\text{-}NO_2)_2$

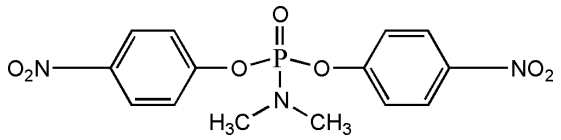

Fig. 4. N,N-dimethyl Phosphoramidic Acid Bis-(4-nitro-phenyl) Ester.

This compound is prepared from the reaction N, N-dimethylamine dichlorophosphate and potassium salt of paranitrophenol with the ratio of 1 to 2. After purification, it is identified with NMR (Bruker DPX 250 NMR Spectrometer). Based on the shaking flask method and measurement of the distribution coefficient of this compound between the two phases of water-octanol, the hydrophobicity parameter (logP) of this compound is 0.71. Also, based on Elman's method to measure the inhibitory power of acetylcholinesterase (IC_{50}), the amount evaluated for this compound is 28.7 mM. [9]. Part of the software evaluation results using the PASS software for this compound is compiled in the Table 4. According to the evaluation, the possibility of antiacetylcholinesterase activity for this compound is 82.6%.

RESULT AND DISCUSSION

The results of the evaluations (hydrophobicity parameter based on the shaking flask method, acetylcholinesterase inhibitory power based on Ellman's method and the possibility of having antiacetylcholinesterase

Table 4. Results of PASS software predictions for composition 4.

Pa	Pi	Biological activity
0.754	0.005	Carboxylesterase inhibitor
0.745	0.002	CYP2A6 human substrate
0.826	0.003	Acetylcholinesterase inhibitor
0.725	0.003	Glycerophosphocholine cholinephosphodiesterase inhibitor
0.699	0.002	Tropinesterase inhibitor
0.678	0.012	Dynein ATPase inhibitor
0.681	0.016	CYP2 substrate
0.659	0.011	GTP diphosphokinase inhibitor
0.639	0.007	Arylformamidase inhibitor
0.640	0.013	Chloramphenicol O-acetyltransferase inhibitor
0.641	0.022	CYP3A substrate
0.611	0.006	Cholinergic

Table 5. Results of the evaluations for study compounds 1-4.

P_a (%)	IC_{50} (mM)	LogP	Compound
81.6	31.3	1.027	1
72.2	35.4	1.63	2
71.8	40.9	2.75	3
82.6	28.7	0.71	4

activity based on software evaluations) are compiled in Table 5.

The first result that should be mentioned at the end of this research is the credibility and value of PASS software in the research process. As mentioned, this software has played an essential role in choosing a family of compounds for QSAR studies and has minimized the deviation from the right path in the research. It is clear that having an initial vision before starting any research project is the first step to achieve success and reach the goal. With this valuable result, it is possible to confidently design and synthesize compounds with specific effectiveness. The second result is that the relationship between toxicity and hydrophobicity is such that with increasing logP, IC_{50} also increases. This relationship means that increasing the hydrophobic character leads to less toxicity of the phosphorus compound. In a general summary, it can be stated that at the beginning of the research path, the preliminary investigations by the software provide a suitable and favorable start for the researcher.

CONCLUSIONS

In the review of QSAR studies, it is possible to rely on the results of software evaluation with a combination of and perform the desired biologically effective design with an acceptable confidence factor. With structural changes, a wide range of compounds can be evaluated by software, and biological parameters can be predicted for each compound without the need for laboratory equipment and costs and spending a lot of time.

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AUTHORS BIOSKETCHES

Zahra Javani, Assistant Professor, Department of Chemistry, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran, *E-mail: Z_javani@yahoo.com*