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New rodenticides design based on experimental results of real rodenticides's structural study

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ABSTRACT: Organophosphorus rodenticides are used non-systemically and with contact, digestive and penetrating effects to control a wide range of rodent and sucking pests in the agricultural industry, and by inhibiting the acetylcholinesterase enzyme, they disrupt the rodent's nervous system and destroy them. The findings of the experimental studies of this research (including the hydrophobicity parameter of some organic phosphorus compounds and the level of inhibition of the corresponding enzyme by these compounds as inhibitors) show that the toxicity of the compound decreases in exchange for an increase in hydrophobicity. Therefore, the design of hydrophilic organic phosphorus compounds produces a more lethal product, and on the contrary, a less effective product is obtained with hydrophobic alternatives. For a group of chemical compounds with specific biological properties, reliable equations can be obtained by using quantitative structure-activity relationships, which are effective in the design of new substances with the desired level of effectiveness.

Keywords: *Acetylcholinesterase inhibition, Hydrophobicity, IC_{50}, logP, Organophosphorus rodenticides.*

INTRODUCTION

According to the type of use, pesticides are herbicides, insecticides, fungicides, anthelmintics, and germicides, and in terms of chemical structure, they can be classified into 4 categories: organochlorine, organophosphorus, carbamates, and pyrothyroid pesticides. Synthetic phosphorus insecticides are organic molecules containing phosphorus [1]. At the same time as the Second World War, these groups of poisons were synthesized by the Germans under the name of war gases, and then their insecticidal properties were determined [2]. The mechanism of toxicity of organophosphorus compounds is inhibition of acetylcholinesterase enzyme (AChE). When the cholinesterase enzyme is deactivated, acetylcholine (ACh) accumulates in the nervous system, resulting in continuous nerve stimulation. Acetylcholinesterase enzyme is one of the important compounds in the body of living organisms and catalyzes the hydrolysis of acetylcholine, which is a neurotransmitter in nerve interfaces. Lack of time hydrolysis of acetylcholine leads to very important disorders in the nervous system. The force that brings acetylcholine to the acetylcholinesterase enzyme is very weak van der Waals forces, so that the enzyme can do its job well, these weak forces must be created and broken alternately. Some organophosphates affect the active site of the enzyme and prevent it from activity by forming a strong and irreversible covalent bond with the enzyme.

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In inhibiting the acetylcholinesterase enzyme, first a hydrogen ion is separated from the phenyl group of the amino acid tytrosine by phosphorus compounds, and by connecting it to the inhibitory ester oxygen group, the phosphorus atom becomes partially positively charged. In the next step, a bond is formed between the oxygen of the amino acid serine and phosphorus. This relatively strong bond leads to the formation of phosphorylated enzyme. In this case, the active site of the enzyme is blocked by the amino acid part of serine and finally the catalytic action of the enzyme is stopped [3-5].

The half maximal inhibitory concentration (IC_{50}) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. IC_{50} is a quantitative measure that indicates how much of a particular inhibitory substance (e.g. drug) is needed to inhibit, in vitro, a given biological process or biological component by 50%. The biological component could be an enzyme, cell, cell receptor or microorganism. IC_{50} values are typically expressed as molar concentration. IC_{50} can be determined with functional assays or with competition binding assays. The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC_{50} values can be used to compare the potency of two antagonists [6,7]. Quantitative structure-activity relationship (QSAR) is a computational modeling method for revealing relationships between structural properties of chemical compounds and biological activities. QSAR modeling is essential for drug discovery. Hydrophobicity (lipophilicity) has been recognized for its importance in QSAR studies [8]. Lipophilicity affects drug absorption, bioavailability, hydrophobic drug-receptor interactions, metabolism of molecules, as well as their toxicity. LogP has become also a key parameter in studies of the environmental fate of chemicals. Through the review of literature, it was found that a number of QSAR. Lipophilicity refers to the ability of a chemical compound to dissolve in fats, oils, lipids, and non-polar solvents such as hexane or toluene. Such non-polar solvents are themselves lipophilic and the axiom that "like dissolves like" generally holds true. Thus lipophilic substances tend to dissolve in other lipophilic substances, but hydrophilic ("water-loving") substances tend to dissolve in water and other hydrophilic substances [9,10].

EXPERIMENTAL

In this study some rodenticides with the general formula $Me_{(2)}NP(O)(p\text{-}OC_{(6)}H_{(4)}\text{-}X)_{(2)}$, where X= H, CH_3, Cl, NO_2 and CN (Fig. 1) have been synthesized in water (without organic solvent) and characterized by $^{(31)}P$, $^{(31)}P\ \{^{(1)}H\}$, $^{(13)}C$ and $^{(1)}H$ NMR spectroscopy.

Since lipophilicity has been recognized for its importance in QSAR studies, efforts have been made to determine the logP values. The ability of these rodenticides to inhibit human acetylcholinesterase (hAChE) has been evaluated by a modified Ellman's assay and spectrophotometric measurements [11-14].

Synthesis

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

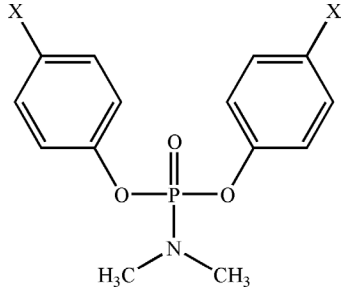

Fig. 1. General formula of some rodenticides.

used with a ratio of 7:1.

Identification of synthesized compounds

In order to confirm the structure of synthetic compounds, Nuclear magnetic resonance (NMR) has been used (*Bruker* Avance DPX 250 *NMR* Spectrometer). A single peak in phosphorus NMR indicates the purity of the material. For each compound, the chemical structure of the compound is confirmed by interpreting the ^{13}C, ^{1}H , ^{31}P $\{^{1}H\}$ spectra.

Precursor: N, N-dimethylamine dichlorophosphate

^{31}P $\{^{1}H\}$ NMR (101.25 MHz, $CDCl_3$, 25°C, H_3PO_4 external), δ (ppm): 19.68 (s). ^{31}P NMR, δ (ppm): 19.38-19.98 (m). ^{1}H NMR (250.13 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 2.88 (d, $^{3}J_{PNCH}$ = 15.7 Hz, 6 H, NMe_2). ^{13}C NMR (62.90 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 35.78 (d, $^{2}J_{P-C}$ = 3.7 Hz, 2C, NMe_2).

Compound 1: N,N-dimethyl Phosphoramidic Acid diphenyl Ester, $Me_2NP(O)(p\text{-}OC_6H_5)_2$

^{31}P $\{^{1}H\}$ NMR (101.25 MHz, $CDCl_3$, 25°C, H_3PO_4 external), δ (ppm): 1.6 (s). ^{31}P NMR, δ (ppm): 1.29-1.89 (h, $^{3}J_{P-H}$=10.1 Hz). ^{1}H NMR (250.13 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 2.72 (6H, $2CH_3$, $^{3}J_{P-H}$=10.1 Hz), 6.76-7.22 (10H, m, ArH). ^{13}C NMR (62.90 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 152.0 (d), 129.7 (s), 124.9 (s), 120.0 (s), 36.7 (s).

Compound 2: N,N-dimethyl Phosphoramidic Acid Bis-(4-methyl-phenyl) Ester, $Me_2NP(O)(p\text{-}OC_6H_4\text{-}CH_3)_2$

^{31}P $\{^{1}H\}$ NMR (101.25 MHz, $CDCl_3$, 25°C, H_3PO_4 external), δ (ppm): 2.15. ^{31}P NMR, δ (ppm): 1.95-2.35 (h, $^{3}J_{P-H}$=10.1 Hz). ^{1}H NMR (250.13 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 2.08 (6H, s, p-CH_3), 2.32 (6H, d, $^{3}J_{P-H}$=10.1 Hz, $2CH_3$), 6.66 (8H, s, ArH). ^{13}C NMR (62.90 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 145.0 (d), 134.2 (s), 130.0 (s), 119.7 (s), 36.75 (s), 20.6 (s).

Compound 3: N,N-dimethyl Phosphoramidic Acid Bis-(4-chloro-phenyl) Ester, $Me_2NP(O)(p\text{-}OC_6H_4\text{-}Cl)_2$

^{31}P $\{^{1}H\}$ NMR (101.25 MHz, $CDCl_3$, 25°C, H_3PO_4 external), δ (ppm): 1.68. ^{31}P NMR, δ (ppm): 1.38-1.99 (h, $^{3}J_{P-H}$=10.1 Hz). ^{1}H NMR (250.13 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 2.78 (6H, d, $^{3}J_{P-H}$=10.1 Hz, $2CH_3$), 6.65-7.30 (8H, m, ArH). ^{13}C NMR (62.90 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 149.2 (d), 129.8 (s), 121 (s), 116.7 (s), 36.6 (s).

Compound 4: N,N-dimethyl Phosphoramidic Acid Bis-(4-nitro-phenyl) Ester, $Me_2NP(O)(p\text{-}OC_6H_4\text{-}NO_2)_2$

^{31}P $\{^{1}H\}$ NMR (101.25 MHz, $CDCl_3$, 25°C, H_3PO_4 external), δ(ppm): 0.5 (s). ^{31}P NMR, δ (ppm): 0.26-0.68 (h, $^{3}J_{P-H}$=10.1 Hz). ^{1}H NMR (250.13 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 2.78 (6H, d, $^{3}J_{P-H}$=10.1 Hz, $2CH_3$), 6.65-7.30 (8H, m, ArH). ^{13}C NMR (62.90 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 149.2 (d), 129.8 (s), 121 (s), 116.7 (s), 36.6 (s).

Compound 5: N,N-dimethyl Phosphoramidic Acid Bis-(4-cyano-phenyl) Ester, $Me_2NP(O)(p\text{-}OC_6H_4\text{-}CN)_2$

^{31}P $\{^{1}H\}$ NMR (101.25 MHz, $CDCl_3$, 25°C, H_3PO_4 external), δ (ppm): 1.28. ^{31}P NMR, δ (ppm): 1.07-1.49 (h, $^{3}J_{P-H}$=10.1 Hz). ^{1}H NMR (250.13 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 2.78 (6H, d, $^{3}J_{P-H}$=10.1 Hz, $2CH_3$), 6.65-7.30 (8H, m, ArH). ^{13}C NMR (62.90 MHz, $CDCl_3$, 25°C, TMS), δ (ppm): 145.0 (d), 134.2 (s), 130.0 (s), 119.7 (s), 36.75 (s), 20.6 (s).

Measurement of hydrophobicity (logP)

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 concentrations by UV-Vis spectroscopy. Then the solute in one phase is extracted from the other phase by a specific volume.

Table 1. Log P value for $(Me_2N)P(O)(O\text{–}C_6H_5)_2$ by the shake-flask method.

n-octanol/buffer(v/v)	C_1	Ā(n=3)	C_2	logP
1:2	$10^{-2}\times3.48$	0.276	$10^{-3}\times2.7$	1.037
1:4	$10^{-2}\times3.48$	0.245	$10^{-3}\times2.4$	1.035
1:6	$10^{-2}\times3.48$	0.214	$10^{-3}\times2.1$	1.010
Grand mean log P value ± SD				
1.027± 0.015				

Table 2. Log P value for $Me_2NP(O)(p\text{-}OC_6H_4\text{-}CH_3)_2$ by the shake-flask method.

n-octanol/buffer (v/v)	C_1	Ā(n=3)	C_2	logP
1:4	$10^{-2}\times3.08$	0.140	$10^{-3}\times5.16$	1.74
1:6	$10^{-2}\times3.08$	0.169	$10^{-3}\times6.8$	1.62
1:8	$10^{-2}\times3.08$	0.181	$10^{-3}\times7.47$	1.53
Grand mean log P value ± SD				
1.63 ± 0.075				

Table 3. Log P value for $Me_2NP(O)(p\text{-}OC_6H_4\text{-}Cl)_2$ by the shake-flask method.

n-octanol/buffer (v/v)	C_1	Ā(n=3)	C_2	log P
1:15	$10^{-2}\times2.89$	0.103	$10^{-5}\times3.78$	2.77
1:20	$10^{-2}\times2.89$	0.105	$10^{-5}\times4.19$	2.73
1:25	$10^{-2}\times2.89$	0.106	$10^{-5}\times4.22$	2.72
Grand mean log P value ± SD				
2.75± 0.035				

Table 4. Log P value for $Me_2NP(O)(p\text{-}OC_6H_4\text{-}NO_2)_2$ by the shake-flask method.

n-octanol/buffer (v/v)	C_1	Ā(n=3)	C_2	log P
1:15	$10^{-2}\times3.45$	0.195	$10^{-5}\times3.56$	0.73
1:20	$10^{-2}\times3.45$	0.192	$10^{-5}\times3.52$	0.71
1:25	$10^{-2}\times3.45$	0.190	$10^{-5}\times3.48$	0.70
Grand mean log P value ± SD				
0.71± 0.054				

Table 5. Log P value for $Me_2NP(O)(p\text{-}OC_6H_4\text{-}CN)_2$ by the shake-flask method.

n-octanol/buffer (v/v)	C_1	Ā(n=3)	C_2	log P
1:15	$10^{-2}\times4.86$	0.215	$10^{-5}\times3.95$	0.25
1:20	$10^{-2}\times4.86$	0.212	$10^{-5}\times3.92$	0.29
1:25	$10^{-2}\times4.86$	0.209	$10^{-5}\times3.87$	0.29
Grand mean log P value ± SD				
0.28± 0.32				

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 (Table 1-5).

Reducing the inhibitory power on acetylcholinesterase enzyme (Measurement of toxicity (IC_{50} parameter))

A- Enzyme activity determination:

We measure the activity of acetylcholinesterase enzyme, which is the power of hydrolysis of a certain amount of its specific substrate in a certain time. For this purpose, we prepare a solution of enzyme and substrate along with the element reagent as follows:

We mix 50 microliters of the prepared 10 millimolar substrate solution with 847 microliters of aluminum solution and 100 microliters of phosphate buffer in a one milliliter cuvette. Add 3 microliters of enzyme to the solution and measure the absorbance at 412 nm for this solution exactly 1 minute after adding the enzyme. The absorbance value is a measure of enzyme activity. The reference solution for absorption measurement will be a solution that contains all the mentioned substances except the enzyme. The enzyme used in this

research shows an absorbance of 0.73 in 1 minute.

B- Determination of enzyme activity in the presence of inhibitor:

As above, we measure the activity of the enzyme, but this time in the presence of an inhibitor (phosphorus composition). In this step, in addition to enzyme, substrate, reactant element and phosphate buffer, we use phosphorus compound solution with different concentrations. In this way, we prepare a thick solution with a specific concentration of the synthesized compound in the phosphate buffer and add a certain amount of this solution to the cuvette each time. Each time we use 50 microliters of substrate, 847 microliters of element solution, 3 microliters of enzyme and different amounts of buffer and phosphorus solution so that the total of these two becomes 100 microliters. All materials are mixed in the cuvette, except the substrate, and absorption is measured after 1 minute immediately after adding the substrate. The absorbance-inhibitor concentration diagram is drawn. A concentration of phosphorus compound that halves the initial absorption is determined from the graph and reported as IC_{50} (Fig. 2).

RESULT AND DISCUSSION

The results related to hydrophobicity and toxicity for 5 studied compounds are collected in the table 6.

The best QSAR equation for a group of 5 from the family of N,N-dimethyl phosphoramidic acid diesters is a relationship that relates $log1/IC_{50}$ as a representative parameter of the antiacetylcholinesterase activity of the phosphoramide compound to logP, makes R^2=0.9926 (Fig. 3).

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

Table 6. logP and IC_{50} for compounds 1-5.

IC_{50}	LogP	X
31.3	1.027	H
35.4	1.63	CH_3
40.9	2.75	Cl
28.7	0.71	NO_2
26	0.28	CN

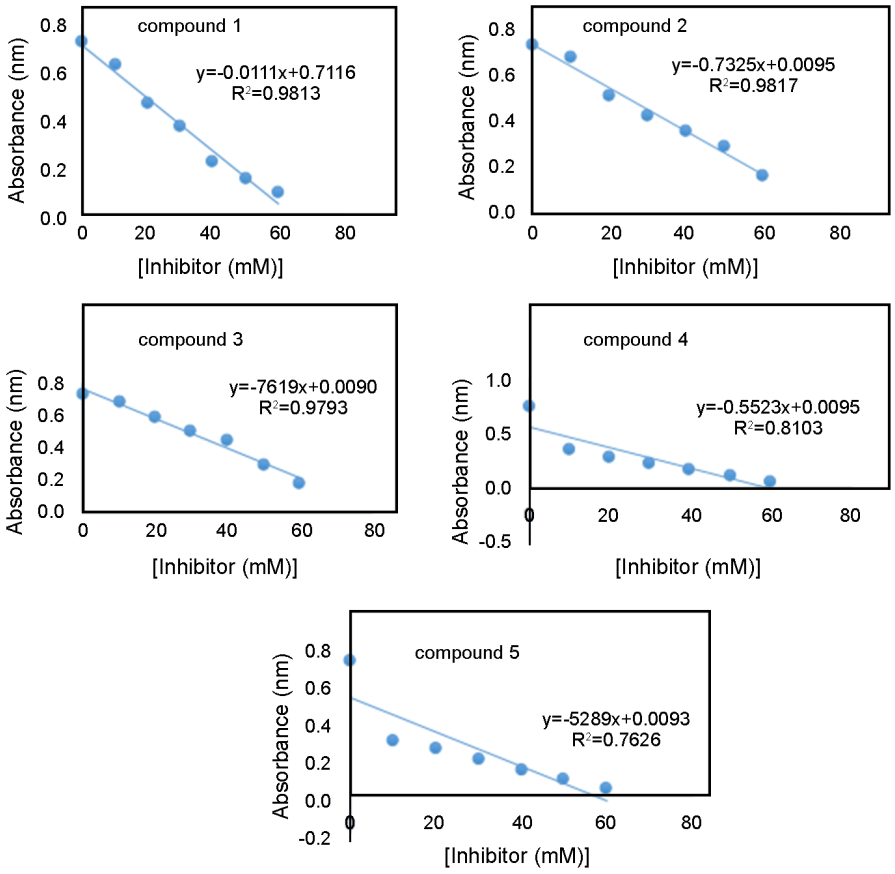

Fig. 2. Inhibition graph for compounds 1-5.

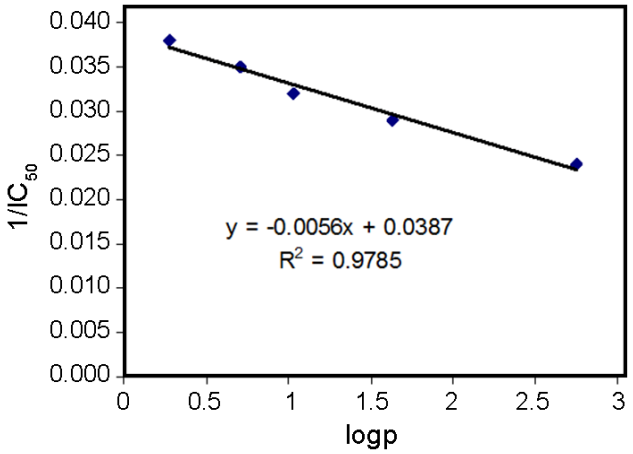

Fig. 3. The best QSAR graph for compounds 1-5.

compound. In other words, the combination of N,N-dimethyl Phosphoramidic Acid Bis-(4-chloro-phenyl) Ester (compound 3) with the most hydrophobicity, the least toxicity and the composition of N,N-dimethyl Phosphoramidic Acid Bis-(4-cyano-phenyl) Ester (compound 5) with the least hydrophobicity, have the most toxicity. Since the protein structures of the enzymes have hydrophilic and hydrophobic centers, the last result can probably be attributed to the strong interaction of the phosphorus combination with the hydrophobic centers of the protein structure of the acetylcholinesterase enzyme.

Therefore, by designing more hydrophobic compounds, we can expect compounds with less toxicity and vice versa.

For example, it can be expected that the compound whose hydrophobicity is 0.23 based on computing software (HyperChem) has more toxicity than the 5 experimentally studied compounds (Fig. 4).

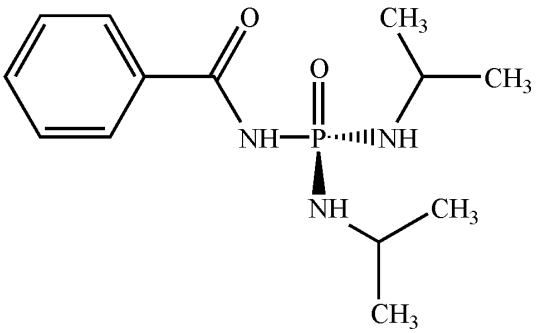

Fig. 4. The proposed compound based on the QSAR equation predicting the anti-acetylcholinesterase effect is stronger than the 5 known compounds.

CONCLUSIONS

For a group of chemical compounds with specific biological properties, reliable equations can be obtained by using quantitative structure-activity relationships, which are effective in the design of new substances with the desired level of effectiveness.

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