Synthesis of new metoclopramide derivatives and in vitro evaluation of their human cholinesterases protection against Chlorpyrifos

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ABSTRACT

Objective: The purpose of this study was to synthesize three new derivatives of metoclopramide by diazotization and replacement reactions and then to quantify in vitro their protection effects on blood cholinesterases using chlorpyrifos as a potent inhibitor.

methods: The three new metoclopramide derivatives were synthesized via Sandmeyer reaction, the chemical structures of these derivatives were identified by physico-chemical and spectroscopic (U.V. and FTIR) techniques.

Results and conclusion: The results of in vitro evaluation of their human cholinesterases inhibition and protection against chlorpyrifos indicated that the (OH) functional group binds to cholinesterase (ChE) at the same organophosphorous (OP) binding site and shows some competition and protection ability but not to a significant degree, while the (Cl and I) functional groups bind to the ChE at site differs from OP binding site, as well as the (I) functional group has more selectivity for enzyme moiety and binds more strongly than the (Cl) group, so that shows more ChE inhibiting ability.

Keywords: metoclopramide, Sandmeyer reaction, cholinesterase, chlorpyrifos.

الخلاصة

الأهداف: إن الغرض من هذه الدراسة هو تصنيع ثلاث مشتقات جديدة من عقار الميتوكلوبرامايد باستخدام سلسلة من التفاعلات الاستعاضة الكيميائية ثم تقيم مدى قابليتهم على حماية خميرة الكولين استراز في دم الإنسان ضد المثبطات القوية باستخدام المثبط الفعال الكلوربايريفوس خارج جسم الكائن الحي.

طرق العمل: تم تصنيع ثلاثة مشتقات جديدة من الميتوكلوبرامايد باستعمال تفاعل ساندماير كما تم التأكد من أشكالها الكيميائية بواسطة التعرف على الخواص الفيزيوكميائية وأطياف الأشعة تحت الحمراء وفوق البنفسجية.

النتائج والاستنتاج: قد دلت نتائج الدراسة على أن مجموعة الهيدروكسيل الفعالة ترتبط بخميرة الكولين استيراز بنفس موقع ارتباط مركبات الفوسفات العضوية لذلك فقد أبدت بعض المنافسة وبالتالي بعض الحماية لهذه الخميرة ولكن ليس إلى الدرجة الهامة، بينما مجموعتي الكلورايد والايوديد الفعالتين ترتبطان بخميرة الكولين استيراز بموقع مختلف عن موقع ارتباط مركبات الفوسفات العضوية مع إن مجموعة الايوديد قد أبدت انتقائية أكبر تجاه الارتباط بالخميرة من مجموعة الكلورايد .

Metoclopramide (methoxychloroprocainamide, MCP) is a dopamine D_2-receptor antagonist benzamide derivative developed in the 1960s and also has an agonistic effect at serotonin receptors (5-HT4)[1]. Clinically, MCP is used owing to its prokinetic and antiemetic effects, these effects of MCP may be enhanced due to inhibition of the cholinesterase[2,3]. The drug is weakly inhibiting cholinesterase activity both in vivo and in vitro[4] and is reported to prevent organophosphorous compounds (OP) poisoning in man (using in vitro system)[5] and in animals both in vitro and in vivo[6]. The protective action of metoclopramide is thought to be a

competition for the active site of enzyme with the more potent OP, thus metoclopramide may reduce the toxicity of different OP compounds as dichlorvos and daizinon[7].

Organophosphorous compounds are serine esterase and protease inhibitors widely used in agriculture as insecticides, in industry and technology as softening agents and additives, and some of them are declared as chemical warfare agents[8]. The single most important mechanism of the toxic action of these insecticides in man and animals is the inhibition of cholinesterase (ChE) at the nerve terminals resulting in an accumulation of acetylcholine that subsequently causing a series of muscarinic, nicotinic and central nervous system effects[9,10].

Chlorpyrifos (phosphorothionic acid *O*, *O*-diethyl *O*-[3,5,6-trichloro-2-pyridyl] ester) is an organophosphorous insecticide which is considered by the U.S. Environmental Protection Agency to be moderately toxic or slightly toxic depending on the formulation [11].

Weak and reversible inhibitor of cholinesterases, when administered before potent organophosphorous class compound, has the ability to protect a certain extent the enzyme from inhibition[12]. The putative mode of protective action of MCP is, when administered in excess, a competition for the active site of enzyme with the more potent organophosphate, this protective effect of metoclopramide is thought to be of practical usefulness for the treatment of organophosphate poisoning[13].

The purpose of this study was to synthesize three new derivatives of MCP by diazotization and replacement reactions and then to quantify in vitro their protection effects on blood cholinesterases using chlorpyrifos as a potent inhibitor.

Materials and methods

Part I - synthesis of MCP derivatives

The parent compound (metoclopramide) was supplied from Ninevah Drug Industry (Iraq). The chemical compounds used in this research were of analytical grade purity and the solvents were purified by distillation prior to use. The melting points were measured using an electro-thermal CIA 9300 apparatus, and were reported uncorrected. The IR spectra of the compounds in KBr pellets were recorded on a Buck 500 scientific FTIR spectrophotometer.

The ultraviolet-visible spectra were obtained via Carrywinn U.V. Varian U.V. - visible spectrophotometer. The chemical structures were drawn by Chemdraw Office 2001 software. Thin-layer chromatography (TLC) was carried out on TLC plastic sheets silica gel 60 F5 pre-coated , 20×20 cm, layer thickness 0.2 mm. The spots on the chromatograms were localized using U.V. light (366 nm) (Whatmann). The solvent system employed for separation composed from methanol:strong ammonia solution (98.5:1.5).

General procedure for the synthesis of diazonium salt solution[14]

Metoclopramide hydrochloride (3.54 g, 10 mmole) was dissolved in 35 ml of water in a beaker, the resulting solution was cooled by immersing in a bath of crushed ice. The cold solution of sodium nitrite (0.83 g, 12 mmole) in (12 ml) water was placed in a dropping funnel and then added dropwise into the stirred solution of metoclopramide hydrochloride in an ice bath; the reaction temperature was kept below 5°C by adding few grams of crushed ice when necessary. After the last addition, the resulting solution was stirred for 5 min in an ice bath. A drop of the solution diluted with (4 drops) of water was tested with potassium iodide-starch paper; if no immediate

blue color was obtained at the point of contact with paper, a further 1 ml of sodium nitrite solution was added, and the solution tested again after 5 min. Further adding and testing were continued until an immediate blue color was obtained. Thus, the diazonium salt solution was prepared.

Synthesis of compound I[15]

The diazonium salt solution was brought to room temperature and heated in a water bath at 100 °C for (15 min) to allow the release of nitrogen gas. Distilled water (20 ml) was added to the mixture and the pH adjusted to 4. The solution was kept for (24 h) at room temperature. The formed precipitate was collected by filtration and purified by crystallization from ethanol-water mixture (9:1), the compound purity was checked by TLC and its result showed that only a single spot was observed. The physicochemical properties of this compound are listed in Table 1.

Synthesis of compound II[16]

Cuprous chloride should be prepared freshly by dissolving 3.5 g, 14 mmol of $CuSO_4.5H_2O$ and 0.95 g, 16 mmol of NaCl in 13 ml of distilled water in a conical flask with warming on a water bath. A solution of 0.95 g, 5 mmol of $Na_2S_2O_5$ in 10 ml of distilled water was added to the previous hot solution dropwise with shaking. The mixture allowed to stand in an ice bath for 30 min. and filtered off. The filtrated particles washed with a dilute solution (0.5%) of $Na_2S_2O_5$, dissolved in 7 ml of conc HCl and kept in an ice bath for 30 min. The cuprous chloride solution was added dropwise into the cold diazonium salt solution, stirred at 20 ºC for 40 min and at 70 ºC for 40 min. The resulting mixture was stored overnight at room temperature. The product was filtered under vacuum and purified by crystallization from ethanol, the compound purity was established by TLC and its result showed that only a single spot was observed. The physicochemical properties of this compound are listed in Table 1.

Synthesis of compound III[17]

KI (1.66 g, 10 mmol) was dissolved in 10 ml water; the resulted solution was cooled to 0 ºC and added dropwise under continuous stirring into a cold diazonium salt solution. The pH was adjusted to 3.5 and after standing for 12 h at room temperature, the resulting precipitate was filtered under vacuum and purified by crystallization from an ethanol:water mixture (8:2), the compound purity was established by TLC and its result showed that only a single spot was observed. The physicochemical properties of this compound are listed in Table 1.

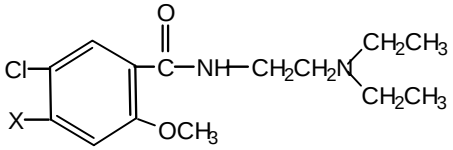

Table 1. Physicochemical properties of the synthesized compounds.

Compound no.	X	m.p.(ºC)	% yield	R_f	λ_{max} chloroform (nm)
Compound I	OH	216-218	78	0.56	472
Compound II	Cl	154-156	55	0.34	312
Compound III	I	106-108	62	0.42	398

For Metoclopramide: X= NH_2; m.p.= 182-184ºC; R_f = 0.47; λ_{max} (chloroform) = 254 nm[18]

Part II – In vitro assay of the ChE protection ability

The subjects included in this study (male and females, age 30± 10 years) were apparently healthy with no history of exposure to OP insecticides. Blood samples were collected in the 5 ml EDTA-treated test tubes and then centrifuged by (Centurion, UK) at 3000 rpm for 15 min. The erythrocytes and plasma were pooled and kept on ice for the ChE assay.

Electrometric assay of ChE activity[19-21]

The reaction mixture in a 10 ml beaker contained 3 ml of distilled water, 0.2 ml of plasma or erythrocytes and 3 ml of (pH 8.1) barbital-phosphate buffer was prepared and its pH (pH1) was measured with glass electrode using pH meter (Hanna Instruments, Romania). Then 0.1ml of aqueous acetylcholine solution (7.5%) was added and the reaction mixture was incubated at 37 °C in a water bath (Shaker bath 5BS30, UK) for 20 min. After that, the pH of reaction mixture (pH2) was measured and the enzyme activity was calculated as follows:

ChE activity (ΔpH/20 min.) = (pH1-pH2)-ΔpH of blank

The blank was without the blood aliquot. The barbital-phosphate buffer solution was prepared from 1.24 g sodium barbital, 0.163 g potassium dihydrogen phosphate and 35.07 g sodium chloride in a one liter of distilled water, the pH of buffer solution was adjusted to 8.1 with 0.5 N HCl.

In vitro ChE inhibition by the MCP derivatives and Chlorpyrifos[22]

Plasma and erythrocyte samples were collected from healthy volunteers and different MCP derivatives concentrations were prepared and then added individually in a volume of 0.1 ml to the reaction mixture to obtain the final concentrations as follows:

Chlorpyriphos: 4 μM
Compound I: 200, 100, 75, 50 and 25 μM
Compound II: 200, 100, 75, 50 and 25 μM
Compound III: 200, 100, 75, 50 and 25 μM

The concentrations of chlorpyriphos and the MCP derivatives used in the present study were obtained from preliminary experiments to validate the experimental concentrations. The chlorpyriphos or MCP derivatives were prepared in distilled water and individually added in 0.1ml to the reaction mixture of the plasma and erythrocytes. The reaction mixture containing chlorpyriphos or MCP derivatives was incubated at 37ºC for 10 min. Thereafter, the residual ChE activity was measured and the % of enzyme inhibition was calculated as follows:

% ChE inhibition = $[ChE_1-ChE_2]/ChE_1 \times 100$

Where ChE_1 is the enzyme activity without chlorpyriphos or MCP derivatives (control) while ChE_2 is the enzyme activity with chlorpyriphos or MCP derivatives (inhibitor).

In vitro ChE inhibition by the MCP derivatives with Chlorpyriphos in the same sample[23]

The same plasma and erythrocyte samples used in the previous experiment were used here and the same concentrations of MCP derivatives were also applied. 0.1 ml of each derivative concentration mentioned above was added to the reaction mixture which incubated at 37 °C in a water bath for 10 min , then chlorpyriphos diluted with distilled water (0.1 ml, 4 μM) was added after (10 min) from the addition of the MCP derivatives, then the reaction mixture was again incubated at 37°C in a water

bath for 10 min before the ChE activity and percentage of enzyme inhibition were calculated as mentioned above.

Data are presented as mean± SE and were subjected to analysis of variance followed by the least of significant difference test[24]. Paired Student's t-test was used for the means of two groups[25]. The level of significance was at $P<0.05$.

Results

The infrared spectra of the synthesized compounds

For compound I, the disappearance of medium absorbance band at 3386 cm^{-1} of the primary amine of metoclopramide and the appearance of medium absorbance band at 3518 cm^{-1} of free O-H group confirmed the formation of compound I. For compound II, the disappearance of medium absorbance band at 3386 cm^{-1} of the primary amine of metoclopramide and the appearance of strong absorbance bands at 812, 1092 cm^{-1} of C-Cl group confirmed the formation of compound II. For compound III, the disappearance of medium absorbance band at 3386 cm^{-1} of the primary amine of metoclopramide and the appearance of strong absorbance band at 1211 cm^{-1} of C-I group confirmed the formation of compound III.

In vitro assay studies

In vitro inhibition of plasma and erythrocyte ChE activities by the ChE inhibitor (Chlorpyriphos 4 μM) and MCP derivatives in different concentrations, and then each one of the MCP derivatives with chlorpyriphos were monitored; the results of these studies are shown in the Tables 2, 3 and 4.

Table 2. In vitro inhibition of human plasma and erythrocyte cholinesterase activities by compound I and its effect on ChEs inhibition by chlorpyriphos

Compound I (μM)	Plasma ChE		Erythrocyte ChE	
	Δ pH/20 min	% Inhibition	Δ pH/20 min	% Inhibition
Control 0 μM	1.14 ± 0.006	0	1.001 ± 0.001	0
chlorpyriphos 4 μM	0.06±0.012*	94.70	0.95±0.021*	21.40
25 μM+	0.69 ± 0.006* a	39.47	0.85 ± 0.012*	15.42
25 μM before ‡ chlorpyriphos 4 μM	0.09 ± 0.001*	92.11	0.67 ± 0.002*	33.33
50 μM+	0.52 ± 0.04* a	54.38	0.97 ± 0.013	3.48
50 μM before ‡ chlorpyriphos 4 μM	0.075 ± 0.015*	93.42	1.64 ± 0.002*	35.82
75 μM+	0.52 ± 0.022* a	54.39	0.97 ± 0.023	3.98
75 μM before ‡ chlorpyriphos 4 μM	0.095 ± 0.01*	91.67	0.47 ± 0.002* a	53.23
100 μM+	0.39 ± 0.001* a	66.23	0.96 ± 0.011	4.98
100 μM before ‡ chlorpyriphos 4 μM	0.13 ± 0.052*	89.04	0.73 ± 0.001*	27.36
200 μM+	0.24 ± 0.00*	79.39	0.87 ± 0.002*	13.43
200 μM before ‡ chlorpyriphos 4 μM	0.15 ± 0.052*	87.28	0.68 ± 0.032*	32.34

N=2-3 / concentration groups.

+ Cholinesterase inhibition was detected after (10 min) incubation of the sample with compound (I).

‡ compound (I)was added to the reaction mixture (10 min) before the chlorpyriphos addition.

* Significantly different from the respective control (0) group, $P<0.05$ [a] Significantly different from the chlorpyriphos (4 μM) , $P<0.05$

Table 2. In vitro inhibition of human plasma and erythrocyte cholinesterase activities by compound I and its effect on ChEs inhibition by chlorpyriphos

Compound I (μM)	Plasma ChE		Erythrocyte ChE	
	Δ pH/20 min	% Inhibition	Δ pH/20 min	% Inhibition
Control 0 μM	1.14 ± 0.006	0	1.001 ± 0.001	0
chlorpyriphos 4 μM	0.06±0.012*	94.70	0.95±0.021*	21.40
25 μM+	0.69 ± 0.006*a	39.47	0.85 ± 0.012*	15.42
25 μM before ‡ chlorpyriphos 4 μM	0.09 ± 0.001*	92.11	0.67 ± 0.002*	33.33
50 μM+	0.52 ± 0.04*a	54.38	0.97 ± 0.013	3.48
50 μM before ‡ chlorpyriphos 4 μM	0.075 ± 0.015*	93.42	1.64 ± 0.002*	35.82
75 μM+	0.52 ± 0.022*a	54.39	0.97 ± 0.023	3.98
75 μM before ‡ chlorpyriphos 4 μM	0.095 ± 0.01*	91.67	0.47 ± 0.002*a	53.23
100 μM+	0.39 ± 0.001*a	66.23	0.96 ± 0.011	4.98
100 μM before ‡ chlorpyriphos 4 μM	0.13 ± 0.052*	89.04	0.73 ± 0.001*	27.36
200 μM+	0.24 ± 0.00*	79.39	0.87 ± 0.002*	13.43
200 μM before ‡ chlorpyriphos 4 μM	0.15 ± 0.052*	87.28	0.68 ± 0.032*	32.34

N=2-3 / concentration groups.

+ Cholinesterase inhibition was detected after (10 min) incubation of the sample with compound (I).

‡ compound (I)was added to the reaction mixture (10 min) before the chlorpyriphos addition.

* Significantly different from the respective control (0) group, $P<0.05$

a Significantly different from the chlorpyriphos (4 μM) , $P<0.05$

Table 3. In vitro inhibition of human plasma and erythrocyte cholinesterase activities by compound II and its effect on ChEs inhibition by chlorpyriphos

Compound II (μM)	Plasma ChE		Erythrocyte ChE	
	Δ pH/20 min	% Inhibition	Δ pH/20 min	% Inhibition
0 μM	1.51 ± 0.01	0	1.00 ± 0.003	0
chlorpyriphos 4 μM	0.06±0.012*	94.70	0.95±0.021*	21.40
25 μM+	1.23 ± 0.012 a	18.52	0.95 ± 0.016*	5.00
25 μM before ‡ chlorpyriphos 4 μM	0.00 ± 0.0*	100	0.29 ± 0.006* a	71.27
50 μM+	1.19 ± 0.001* a	21.45	0.91 ± 0.003*	9.50
50 μM before ‡ chlorpyriphos 4 μM	0.00 ± 0.0*	100	0.22 ± 0.002* a	77.8
75 μM+	1.21 ± 0.025* a	20.13	0.91 ± 0.003*	9.37
75 μM before ‡ chlorpyriphos 4 μM	0.00 ± 0.0*	100	0.55 ± 0.012* a	45.21
100 μM+	1.22 ± 0.001 a	19.47	0.91 ± 0.021*	9.50
100 μM before ‡ chlorpyriphos 4 μM	0.00 ± 0.00*	100	0.73 ± 0.01*	27.5
200 μM+	1.29 ± 0.012 a	14.85	0.94 ± 0.001*	6.00
200 μM before ‡ chlorpyriphos 4 μM	0.00 ± 0.00*	100	0.74 ± 0.002*	26.50

N=2-3 / concentration groups.

+ Cholinesterase inhibition was detected after (10 min) incubation of the sample with compound II.

‡ compound (II) was added to the reaction mixture (10 min) before the chlorpyriphos addition.

* Significantly different from the respective control (0) group, $P<0.05$

a Significantly different from the chlorpyriphos (4 μM) , $P<0.05$

Table 4. In vitro inhibition of human plasma and erythrocyte cholinesterase activities by compound III and its effect on ChEs inhibition by chlorpyriphos

Compound III (μM)	Plasma ChE		Erythrocyte ChE	
	Δ pH/20 min	% Inhibition	Δ pH/20 min	% Inhibition
Control 0 μM	1.52 ± 0.007	0	1.00 ± 0.002	0
chlorpyriphos 4 μM	0.06±0.015*	94.70	0.95±0.023*	21.40
25 μM+	1.06 ± 0.005* a	30.09	0.09 ± 0.012* a	86.05
25 μM before ‡ chlorpyriphos 4 μM	0.00 ± 0.0*	100	0.14 ± 0.005* a	90.71
50 μM+	0.99 ± 0.004* a	34.65	0.13 ± 0.016* a	87.0
50 μM before ‡ chlorpyriphos 4 μM	0.00 ± 0.0*	100	0.06 ± 0.006* a	94.0
75 μM+	0.88 ± 0.011* a	41.92	0.10 ± 0.008* a	89.91
75 μM before ‡ chlorpyriphos 4 μM	0.00 ± 0.0*	100	0.04 ± 0.013* a	96.21
100 μM+	0.82 ± 0.011* a	45.88	0.09 ± 0.001* a	91
100 μM before ‡ chlorpyriphos 4 μM	0.003 ± 0.002*	99.84	0.025 ± 0.007* a	97.5
200 μM+	0.80 ± 0.001* a	47.19	0.11 ± 0.012* a	89.0
200 μM before ‡ chlorpyriphos 4 μM	0.00 ± 0.0*	100	0.04 ± 0.002* a	96.0

N=2-3 / concentration groups.
+ Cholinesterase inhibition was detected after (10 min) incubation of the sample with compound (III).
‡ compound (III) was added to the reaction mixture (10 min) before the chlorpyriphos addition.
* Significantly different from the respective control group, P<0.05
a Significantly different from the chlorpyriphos (4 μM), P<0.05

Discussion

The Sandmeyer reaction is a versatile method for replacing the amine group of a primary aromatic amine (e.g. metoclopramide) with a number of different substitutents[26]. The amine is treated with nitrous acid (HNO_2) under acidic conditions, which produces the diazonium ion. The diazonium ion can then undergo substitution reaction with various reactants, although the substitution can be simplistically viewed as a direct ionic substitution reaction (anion as nucleophile, molecular N_2 as a premier leaving group), the actual mechanism is actually more complicated[26.] A large number of studies have been devoted to determine the mechanism of Sandmeyer reaction which proceeds with liberation of the diazo nitrogen,

the most important one depends upon the polarity of the C-N^+ bond[27].

There is a good correlation between ChE inhibitions in vivo and in vitro, and usually the in vitro ChE inhibition assists in the clinical interpretation of pesticide intoxication[28].

In the present study, chlorpyriphos inhibited plasma ChE activity more than erythrocyte ChE, this effect has been reported by others[29]. The weak or reversible ChE inhibitors can reduce or prevent toxicity of OP when given before it[30], such inhibitors as diphenhydramine[31,32], alpha- 2-adrenoceptor agonists[33], metoclopramide [35] and phenothiazines[34], but when weak ChE inhibitors given after OP, may increase the toxicity of latter compound[35].

In order to get more protection ability for ChE by MCP, three new MCP derivatives were synthesized. At different concentrations, these derivatives inhibited ChE activity at different percentages, depending on their selectivity for the enzyme and the affinity power of the chemical group that modified.

According to Table 2, compound I showed more selectivity for plasma ChE (inhibit plasma ChE more than erythrocyte ChE), but still less than that of original compound at all the used concentrations[36]. When compound I used with chlorpyriphos, the selectivity for plasma ChE provided few protection effect and reduced the inhibition by chlorpyriphos but not statistically significant, but for erythrocyte ChE, compound I showed the same effect of parent compound (i.e. increased significantly the chlorpyriphos inhibition of erythrocyte ChE). These results proposed that the OH functional group of compound I may be responsible for the selectivity for plasma more than erythrocyte ChE and may bind to the enzyme moiety at the same binding site of chlorpyriphos resulting in slightly reduction in its ChE inhibiting ability.

According to Table 3, compound II showed different results when compared with compound I and the original compound. The inhibition by compound II alone for both plasma and erythrocyte ChE was very low but when it combines with chlorpyrifos, the inhibition of both plasma and erythrocyte ChE increased to higher levels and more than that of the compound I and the original compound for all the concentrations used. These results proposed that the selectivity for enzyme is low (i.e. the functional group responsible for binding to the enzyme moiety has been lost or weakened) and the Cl functional group of compound II may be responsible for the enhancement of ChE inhibition by chlorpyriphos because of the difference in the binding sites to enzyme moiety.

According to Table 4, the inhibition of erythrocyte ChE by compound III reached a maximum level and more than that of compounds I, II and the parent compound. The plasma ChE inhibition was low when compared with the compound I and the parent compound, but higher than compound II. When compound III used with chlorpyriphos, the plasma ChE inhibition was increased while the erythrocyte ChE inhibition was reached a maximum level when compared with all derivatives and the parent compound (Table 2, 3 and 4). These results make an idea of that the functional group of compound III may be responsible for increasing the selectivity of binding to the enzyme moiety and enhancing the ChE inhibition by chlorpyriphos because of the difference in the binding sites.

Conclusion

The Change in functional groups of the weak ChE inhibitor by the synthesis of

new derivatives may clear the picture about how the weak ChE binds to the enzyme moiety and how it competes, decreases or increases the ChE inhibition of the strong OP compounds. This research proposed that the OH functional group may bind to the ChE enzyme at the same OP binding site and may show some competition and protection ability but not to a significant degree. While the Cl and I functional groups may bind to the ChE enzyme at sites differ from OP binding site; the I functional group has more selectivity for enzyme moiety and binds more strongly than the Cl functional group, so that it shows more ChE inhibiting ability.

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