

PYRIDINIUM, IMIDAZOLIUM, AND QUINUCLIDIUM OXIMES: SYNTHESIS, INTERACTION WITH NATIVE AND PHOSPHYLATED CHOLINESTERASES, AND ANTIDOTES AGAINST ORGANOPHOSPHORUS COMPOUNDS

Ines Primožič,^a Renata Odžak,^a Srđanka Tomić,^a Vera Simeon-Rudolf,^b and Elsa Reiner^{b*}

^a Department of Chemistry, Faculty of Science, University of Zagreb, Strossmayerov trg 14, HR-10000 Zagreb, Croatia

^b Institute for Medical Research and Occupational Health, Ksaverska c. 2, P.O. Box 291, HR-10001 Zagreb, Croatia

* Telephone: ++ 385-1-4673188

Fax: ++ 385-1-4673303

Email address: ereiner@imi.hr

ABSTRACT

This report summarizes studies on pyridinium, imidazolium, and quinuclidinium oximes, which were prepared in laboratories in Croatia since the middle of the 1970s. The prepared compounds, 177 in all, include 158 new compounds. The majority of prepared compounds are mono- and bis-pyridinium oximes. *In vitro* studies comprised reversible inhibition of cholinesterases, protection of cholinesterases from phosphorylation by organophosphorus compounds (OP), and reactivation of the phosphorylated enzyme. *In vivo* studies of the oximes as antidotes in experimental poisoning by OP compounds are also reviewed. The OP compounds were predominantly chemical warfare (CW) nerve agents but also included some related organophosphates.

INTRODUCTION

Organophosphorus (OP) compounds are potent poisons. They phosphorylate the catalytic site of acetylcholinesterase (AChE), and that reaction inhibits the enzyme. Oximes are antidotes against OP poisoning because they reactivate the inhibited enzyme due to their nucleophilic properties. Since OP compounds have been introduced as pesticides, and also produced as warfare nerve agents, there has been a continuing search for an effective, and possibly universal, antidote.

In this report, we summarize and discuss data regarding oximes and related compounds synthesized and studied over several decades in laboratories in Croatia. The structures of the compounds are listed in tables 1–8. Included in the tables are references describing the synthesis of these compounds. The list comprises 177 compounds, out of which 158 compounds were declared by the authors to be new compounds. Other oximes listed in these tables are included because they are structurally related, and they were prepared and tested alongside the new compounds.

There are at present four known nucleophilic agents, which have proven very effective both as reactivators of the phosphorylated AChE, and as antidotes in OP poisoning. These are two pyridinium monooximes, PAM-2 and HI-6, and two pyridinium dioximes, Toxogonin and TMB-4. Their structures are shown in table 9. For the sake of comparison, some data on their *in vitro*

and *in vivo* properties are also reviewed.

The *in vitro* studies summarized in this paper include the interaction of oximes with native cholinesterases, protection of the cholinesterases from phosphorylation by OP compounds, reactivation of the phosphorylated cholinesterases, and the reaction of oximes with thiocholine esters. The *in vivo* effect of oximes as antidotes in experimental poisoning by OP compounds is also reviewed. In all studies, the OP compounds included primarily Sarin, Soman, Tabun, and VX. The reactions discussed in this paper are schematically presented in the Appendix, and the relevant constants defining these reactions are listed and explained.

The aim in preparing the new compounds was to search for better antidotes against OP warfare agents, in particular against Tabun and Soman. The aim of this review is to give an insight into research performed in Croatia in the field of oxime antidotes against OP compounds. So far, results on individual compounds, or on a smaller group of compounds, have been published in individual papers, often in national journals. This review summarizes all results, thus enabling a more comprehensive evaluation of the properties of the prepared compounds, and their comparison with conventional oximes. This might stimulate more detailed research on compounds that have so far proved promising, and hopefully help in creating more successful antidotes.

SYNTHESIZED COMPOUNDS

Numerous attempts had been made to improve the antidotal properties of the conventional mono- and bis-pyridinium oximes, listed in table 9, by modifying their structure. Initially, a number of mono-pyridinium oximes (**Py**; table 1) and bis-pyridinium oximes (**Py2**; table 2) were prepared to be tested as antidotes in OP poisoning. Later, attention was turned to other heterocyclic systems such as mono-imidazolium oximes (**Im**; table 3) and bis-imidazolium oximes (**Im2**; table 4), the reason being their isosteric similarity with pyridine oximes as well as the possibility of easy quaternization. In addition, mono-quaternary and bis-quaternary compounds with a heterocyclic system of quinuclidinium (**Q** and **Q2**), which embodies the functional groups of choline (i.e., acetylcholine, in its semirigid structure) were prepared and tested as well (tables 6 and 7). Finally, pyridinium-imidazolium (**PyIm**; table 5), pyridinium-quinuclidinium (**PyQ**; table 7), and quinuclidinium-imidazolium (**ImQ**; table 8) compounds were synthesized in order to determine whether conjugates, which contain two different moieties in the same molecule, will show better antidotal properties as compared to compounds with only one active moiety.

All compounds were synthesized using standard methods of organic synthesis and characterized by standard chemical and physical methods (melting point, elemental analysis, IR, NMR, MS, UV). References to original articles describing these syntheses are included in tables 1–8. In all tables, the Chemical Abstract Service Registry Numbers (CAS RN) are listed in square brackets.

INTERACTION OF OXIMES WITH NATIVE CHOLINESTERASES

Oximes are reversible ligands and their binding to cholinesterases causes inhibition of the enzyme activity. However, binding of oximes to the enzyme also protects cholinesterases from phosphorylation by OP compounds. Cholinesterases have two binding sites for reversible ligands: the

catalytic site and the peripheral allosteric site (Appendix, A and B.). The allosteric site is catalytically inactive.

Reversible ligands, such as the oximes, bind to cholinesterases either at the catalytic site, or at the allosteric site, or at both sites of the enzymes. Substrates react at the catalytic site. However, substrates such as acetylcholine can also bind to the allosteric site in cholinesterases (Appendix, A.), which causes either inhibition or activation of the enzyme.

Binding sites of reversible ligands and their affinities for cholinesterases can be evaluated by different approaches. For details concerning the evaluation of binding sites and the mechanism of action of cholinesterase inhibitors, the reader is referred to extensive publications (e.g., Quinn et al., 1995; Radić et al., 1991; Reiner et al., 2000).

Inhibition of Cholinesterases

Many compounds listed in tables 1–8 have at least been screened for their ability to inhibit AChE (86 compounds in all), but only some have been studied in more detail. Even fewer studies have been conducted on the inhibition of butyrylcholinesterase (BChE).

Screening of cholinesterase inhibition was done by determining the I_{50} value, which is the oxime (ligand) concentration causing 50 percent inhibition of the cholinesterase activity measured with a given substrate at a specified substrate concentration. Most I_{50} values were determined for inhibition of human erythrocyte AChE measured with 1.0 mM acetylthiocholine (ATCh) as substrate, at 37 °C and pH 7.4. The I_{50} is not the enzyme/inhibitor dissociation constant. Its numerical value depends on the substrate used for measuring the inhibition, and on the substrate concentration. No information about the binding site on the enzyme can be obtained from the I_{50} value, but low I_{50} values indicate a high affinity of the ligand for the enzyme.

The majority of tested compounds had I_{50} values between 0.1 and about 3 mM, while values up to 10 mM were an exception. The lowest I_{50} values in the pyridinium series (below 0.1 mM) are listed in table 10. Several imidazolium oximes stand out because their I_{50} values were in the micromolar range indicating a very high affinity for the enzyme (table 10). Other oximes had I_{50} values within the range reported for the pyridinium series. Adamantane linked to pyridinium (Py-19 to Py-21) or to imidazolium (Im-7) did not inhibit AChE (Bregovec et al., 1992). The reported I_{50} values for HI-6, PAM-2, Toxogonin, and TMB-4 were between 3 and 8 mM (Bevandić et al., 1985; Galoši et al., 1988), indicating that the standard oximes used for nerve agent treatment have lower affinities for AChE than many of the new compounds.

No systematic difference was found between I_{50} values for AChE from human and bovine erythrocytes (Deljac et al., 1982a; 1982b; and 1982c). Only one paper was published with I_{50} values for horse serum BChE (table 10).

Dissociation Constants of the Cholinesterase/Oxime Complexes

In order to compare quantitatively the affinity of an enzyme for different reversible ligands, one has to evaluate the respective enzyme/ligand dissociation constants. Schemes depicting the

reactions of reversible ligands and substrates with cholinesterases are given in the Appendix, A. and B.

Enzyme/oxime dissociation constants for binding to the catalytic site (K_a) and allosteric site (K_i) of the cholinesterases were obtained from kinetic studies of competition between substrates (usually ATCh) and the oxime. Competition between substrate and oxime at low substrate concentrations (close to the value of the Michaelis constant, K_m) reveals binding to the catalytic site, and competition at high substrate concentrations (close to the value of the substrate inhibition constant, K_{ss}) reveals binding to the allosteric site (Reiner et al., 2000).

The K_a and K_i constants were determined for both PAM-2 and Toxogonin, indicating these oximes bind to both sites in AChE (these values are presented in table 11). There is kinetic evidence that the same holds for binding of HI-6 and PAM-2 to human BChE (Reiner et al., 1995). The K_a constants for binding of HI-6 and PAM-2 to the human BChE phenotypes UU, FS, and AA ranged from 0.23 to 1.5 mM. The K_i constants could not be evaluated due to the fast oximolysis at high substrate concentrations required for the evaluation of K_i .

Only 17 compounds listed in tables 1–8 have been studied for their binding sites on human erythrocyte AChE. Their K_a and/or K_i constants are listed in table 11. One cannot generalize from these results because they represent a small percentage of the synthesized compounds, but these results do indicate that pyridinium, imidazolium, and quinuclidinium compounds have affinities for both the catalytic and allosteric site in AChE.

Protection of Cholinesterases from Inhibition by OP Compounds

Substrates and reversible ligands protect cholinesterases from phosphorylation by OP compounds (i.e., they slow down the rate of phosphorylation). Even when reversible ligands bind only to the peripheral allosteric site of the enzyme, the catalytic site can be protected from phosphorylation (Reiner, 1986).

Many oximes listed in tables 1–8 have been screened for their *in vitro* protection potency. In most studies, the extent of protection was measured by determining the oxime concentration that decreased by 50 percent the rate of enzyme phosphorylation with 20 nM Soman. Oximes with low I_{50} values (high affinity for the enzyme) protected the enzyme at lower concentrations than oximes with high I_{50} values.

A better insight into protection is obtained by determining the protective index (PI), which is the ratio of the rate constant of phosphorylation of the enzyme by a given OP compound in the absence (k_a) and in the presence (k'_a) of a given concentration of the protector ligand (oxime): $PI = k_a/k'_a$ (Appendix, C.). The k'_a is a function of the oxime concentration, and of the enzymes/oxime dissociation constants K_a and/or K_i . Measuring k'_a at different oxime concentrations, one can calculate the enzyme/oxime dissociation constants from the relationship between PI and oxime concentration. Or *vice versa*: knowing the K_a and/or K_i constants, one can predict the degree of protection (i.e., the PI value).

A theoretical model was experimentally tested for the protection of AChE from inhibition by Sarin, Soman, Tabun, and VX with the reversible ligand 4,4'-bipyridine (BP) (Reiner, 1986). The

K_a and K_i calculated from the PI values agreed with the K_a and K_i constants evaluated from the reversible inhibition of AChE by BP measured with ATCh as substrate. The theoretical model further showed that the best protection is achieved when a ligand can bind simultaneously to both sites on the enzyme, forming a ternary complex with the enzyme (Appendix, B.). The above theoretical model was tested with most compounds listed in table 11. With one exception (Py-23), the measured and calculated PIs either agreed or the measured PI was higher than the calculated PI.

From these studies, it appears that binding of a reversible ligand (oxime) to both sites in cholinesterases enhances the protective effect against phosphorylation by OP compounds. Theoretical models describing the mechanism of protection should be further developed in order to evaluate kinetic parameters that can be used in screening and comparing different ligands for their protective effects.

REACTIVATION OF PHOSPHYLATED CHOLINESTERASES BY OXIMES

The antidotal potency of oximes is primarily attributed to their abilities to reactivate the inhibited, phosphorylated, cholinesterases. Oximes reactivate phosphorylated cholinesterases by displacing the phosphoryl moiety from the enzyme by virtue of their high affinity for the enzyme and their powerful nucleophilicity. In the reactivation process, de-phosphylated enzyme and phosphylated oximes are formed, and the enzyme activity is restored (Appendix Scheme D). Reactivation is possible only if the ester substituents on the phosphorus are not hydrolyzed (i.e., if aging of the phosphylated enzyme did not occur). The rate of reactivation depends on the structure of the phosphoryl moiety bound to the enzyme, the source of the enzyme and the oxime structure. Phosphylated oximes formed during the reactivation process might be potent inhibitors of cholinesterases, which could cause re-inhibition of the reactivated enzyme. To our knowledge, no oxime described in this review has been studied in this respect.

Many oximes listed in tables 1–8 were screened for their reactivating potency using native human erythrocyte AChE inhibited by Sarin, Soman, Tabun, or VX. Several oximes also were tested on the AChE inhibited by paraoxon, dichlorvos (DDVP), medemo (*O*-ethyl-2-dimethylaminothioethyl-methylphosphonate) or armin (*O*-ethyl-*p*-nitrophenyl-ethylphosphonate) (Bregovec et al., 1986; Deljac et al., 1982a; 1982c; and 1984; Maksimović et al., 1984; Simeon et al., 1973; and 1979). Only in a few studies were the oximes tested on bovine erythrocyte AChE or horse serum BChE (Deljac et al., 1979a; and 1979b).

The reactivating potency of the oximes towards phosphylated human erythrocyte AChE was usually determined using the following procedure: washed erythrocytes were incubated with the OP for 15 min at 4 °C. Reactivation was started by dilution of the reaction mixture with the oxime solution at 37 °C. The final oxime concentration was usually 0.020 mM. After 30 min the activity of the reactivated enzyme was measured, and the result expressed as percent reactivation. A summary of the results is presented in table 12. Most of the oximes reactivated Sarin or VX inhibited AChE. Only 3 oximes (out of 72 tested) reactivated Soman inhibited AChE and 8 oximes (out of 84 tested) reactivated Tabun-inhibited AChE. Im2-13 was the only oxime to reactivate both Soman- and Tabun-inhibited AChE.

Although the data listed in table 12 are not fully comparable, some conclusions are still possible. AChE inhibited by Sarin or VX can be reactivated by the four standard oximes, as well as by many oximes reported in this paper. If AChE is inhibited by Soman or Tabun, the four standard oximes are not very effective reactivators. However, several oximes discussed in this paper reactivated AChE inhibited by Tabun, and three oximes even reactivated AChE inhibited by Soman.

In order to compare quantitatively the reactivating potency of different oximes, one has to determine the second order rate constant of reactivation (k_r). Only three papers have been published comprising k_r constants for reactivation of the AChE-OP conjugates with seven new oximes. These rate constants are presented in table 13. The highest k_r , which means the best reactivating potency, was obtained with Py2-86 (trans); which was a better reactivator than PAM-2 and Toxogonin (Milatović et al., 1989).

The degree of ionization of the oxime group is also important when judging the reactivation potency of a compound. Oximes reactivate when the oxime group is ionized, and thereby becomes negatively charged. The dissociation constants of the oxime group were determined for only 20 oximes; all but one of these were bis-pyridinium oximes (Bevandić et al., 1985; Bregovec et al., 1986; Deljac et al., 1979a; Simeon et al., 1979).

ANTIDOTAL EFFECT OF OXIMES AGAINST ORGANOPHOSPHATE POISONING

The antidotal effect of many oximes was tested on mice or rats by determining the therapeutic factor TF, which is the ratio of $LD_{50}(OP+oxime)$ and $LD_{50}(OP \text{ only})$. Generally, one-fourth of the LD_{50} of oxime was given together with atropine (10 mg/kg) to the animals. In some cases, 0.015, 0.03, or 0.05 mmol/kg oxime doses were given. The oxime and atropine were given intraperitoneally immediately after a subcutaneous administration of the OP. We considered an antidote effective if its TF value was at least 2.

The published TF values are summarized in table 14. Many oximes were effective antidotes against VX and Sarin, and some oximes even against Tabun and Soman. Two compounds stand out as best antidotes against Soman and Tabun respectively (Lucić et al., 1997; Mesić et al., 1991; Radić et al., 2001) One is ImQ-4: its TF values are 5.3 against Soman and 4.3 against Tabun. The other is Im2-2: its TF value is 4.9 against Tabun, but only 1.3 against Soman. Amongst good antidotes against Soman is also the compound Q-4 (TF = 3.2), which is not an oxime and can therefore not reactivate the phosphorylated AChE.

The four standard oximes are all good antidotes against VX and Sarin, with TF values between 5 and 40 for VX, and between 2 and 14 for Sarin (Maksimović et al., 1980; Bevandić et al., 1985; Mesić et al., 1991). TMB-4, Toxogonin and HI-6 were also effective against Tabun (TF values between 2 and 5; Maksimović et al., 1980; Bregovec et al., 1984b; Bevandić et al., 1985; Mesić et al., 1991; Radić et al., 2001), while against Soman only HI-6 achieved a TF value above 2 (Maksimović et al., 1980; Mesić et al., 1991; Radić et al., 2001), but lower than the TF reported for ImQ-4 (table 14).

Some correlation might be expected between TF values (table 14) and the *in vitro* reactivation of the AChE-OP conjugates (table 12). The following oximes were good reactivators and had a

high TF against VX and Sarin: Py2-62, Py2-66, Py2-67, and Py2-68 (Bregovec et al., 1984b). However, ImQ-4 did not reactivate the AChE-Soman conjugate (Radić et al., 2001) and Im2-2 was not the best reactivator of the AChE-Tabun conjugate (Mesić et al., 1991). On the other hand, Im2-13, PyIm-13 and PyQ-4, revealing good reactivation of the AChE-Soman conjugate, had a TF value only about 2 against Soman (Mesić et al., 1994a; Lucić et al., 1997; Radić et al., 2001).

It is usually considered that *in vitro* reactivation by a compound is a prerequisite for its antidotal efficacy. However, reactivators that perform well *in vitro* might fail as antidotes in OP poisoning, and *vice versa*. Quinuclidinium derivatives also may have another antidotal mechanism, in addition to reactivation of the inhibited enzyme. Sterling et al. (1991) have shown that some quinuclidinium derivatives inhibit the choline uptake into the neuron thus decreasing the acetylcholine synthesis. This might perhaps be the reason why Q-4 (which bears no oxime group) was a good antidote (table 14).

REACTION OF OXIMES WITH THIOCHOLINE ESTERS

Thiocholine esters react with oximes whereby thiocholine is released. This reaction (oximolysis) has been studied with several compounds reviewed in this paper.

The second order rate constants (measured at 37 °C and pH 7.4) for the reaction of ATCh with the bis-pyridinium oximes Py2-2, -4, -8, -12, -16, -24, -25, -34 and -86 range from 20 to 30 mol⁻¹ L min⁻¹ (Škrinjarić-Špoljar et al., 1980; 1988; and 1992). The rate constants for the reaction of ATCh with Im2-6, Im2-11, Q-1, ImQ-1, ImQ-2, ImQ-3 and ImQ-4 are within the same range (Reiner et al., 1999; Simeon-Rudolf et al., 1998; Škrinjarić-Špoljar et al., 1992). The rate constants for the reaction of ATCh with the conventional oximes PAM-2, HI-6, Toxogonin, and TMB-4 are also of the same order of magnitude (Škrinjarić-Špoljar et al., 1980; 1988; and 1992).

The reactions of several monooximes and dioximes with ATCh were studied in more detail (Simeon et al., 1981; Škrinjarić-Špoljar et al., 1992). Rate constants derived from the kinetics of the decrease in oxime concentration agreed with those derived from the kinetics of increase in the thiocholine concentration. It was further shown that in the imidazolium dioximes, Im2-6 and Im2-11, both oxime groups reacted with ATCh. However, in the reaction of the pyridinium dioximes, Toxogonin, TMB-4, and Py2-86, more thiocholine was formed than would correspond to the concentration of the oxime groups. This result was not clarified.

If cholinesterase activities are measured with thiocholine esters as substrates (Ellman et al., 1961), and if oximes are present in the samples, one has to subtract the contribution of oximolysis from the total increase of thiocholine concentration. If oximolysis is faster than the enzyme activity, this method is not suitable for cholinesterase assays. When cholinesterase activities are measured in blood samples taken from patients under oxime therapy, the oxime concentration is usually not known, and one has to be aware that due to oximolysis, the measured increase in thiocholine concentration might lead to a false conclusion concerning the enzyme activity.

CONCLUSION

The research in our Croatian laboratories to synthesize and test a variety of oxime and oxime-related compounds that are effective against nerve agents has been successful. At least on a screening level, many new compounds were found to be good antidotes against VX and Sarin, and several compounds were even effective against Tabun and Soman (table 14). More detailed studies however are needed on the *in vitro* and *in vivo* characterization of these compounds, and so far no structure-activity relationships have emerged from the published data.

We recommend that further *in vitro* studies include the determination of enzyme/oxime dissociation constants and rate constants of reactivation of the phosphorylated cholinesterases. Other parameters defining the protection of native cholinesterases from phosphorylation by OP compounds are also needed in order to validate quantitatively the new compounds and compare them with the conventional oxime antidotes.

The majority of *in vitro* studies were performed on human erythrocyte AChE, which is the target enzyme in OP toxicity. However, data on human serum BChE are also included in this review, because BChE is also inhibited by OP compounds. If inhibited BChE is effectively reactivated by oximes, BChE will act as a scavenger of OP compounds thereby decreasing the OP concentration in the organism.

Screening the antidotal property of the compounds has so far been performed on the border between prophylaxis and therapy. Both effects have probably contributed to the measured TF values, as the antidote was given immediately after the OP compound. By applying different experimental designs, the two effects (prophylaxis and therapy) could probably be studied separately.

Our conclusions regarding details of the protection and effectiveness of these compounds, and in particular our comparisons of the reactivation potency *in vitro* and effectiveness *in vivo*, should be taken with some caution. The results reported in the relevant papers were not always obtained under comparable experimental conditions with respect to inhibition and reactivation protocols, animals, application routes, and doses. However, we believe that the general conclusions concerning properties of the studied compounds are valid, and that further studies will enable selections of compounds that might broaden the choice of antidotes and treatments against OP compounds, in particular against chemical warfare agents.

REFERENCES

Bevandić, Z., Deljac, A., Maksimović, M., Bošković, B., and Binenfeld, Z. (1985) Methylthio analogues of PAM-2, TMB-4 and obidoxime as antidotes in organophosphate poisonings. *Acta Pharm Jugosl.* **35**, 213–218.

Binenfeld, Z., Deljac, V., Knežević, M., Pavlov, Lj., Maksimović, M., Markov, V., Radović, Lj., and Rakin, D. (1981) Reactivating effects of pyridinium salts on acetylcholinesterase inhibited by organophosphorus compounds. *Acta Pharm Jugosl.* **31**, 5–15.

Bregovec, I., Maksimović, M., Deljac, V., and Binenfeld, Z. (1983) Reactivators of phosphorylated and phosphonylated acetylcholinesterase. Dicarbamoyl substituted pyridinium monooximes. *Acta Pharm Jugosl.* **33**, 177–182.

Bregovec, I., Deljac, A., Maksimović, M., and Binenfeld, Z. (1984a) *In vitro* antidotal activity of phenylhydroxyiminoethyl pyridines. *Bulletin de la Societe Chimique Beograd* **49** (9), 533–536.

Bregovec, I., Binenfeld, Z., Maksimović, M., and Bošković, B. (1984b) Synthesis and therapeutic effects of bipyridyl pyridine aldoxime derivatives in poisoning by anticholinesterase agents. *Acta Pharm Jugosl.* **34**, 133–138.

Bregovec, I., Maksimović, M., Deljac, A., Deljac, V., and Binenfeld, Z. (1986) Synthesis and biological activity of methylthio substituted bis-pyridinium oximes. *Acta Pharm Jugosl.* **36**, 9–13.

Bregovec, I., Binenfeld, Z., and Maksimović, M. (1988) Synthesis and biological activity of some 3-phenylallyl substituted heterocyclic oximes and related thio compounds. *Acta Pharm Jugosl.* **38**, 119–122.

Bregovec, I., Maksimović, M., Kilibarda, V., and Binenfeld, Z. (1992) Adamantane derivatives as potential reactivators of acetylcholinesterase inhibited by organophosphorous compounds. *Acta Pharm.* **42**, 251–253.

Deljac, V., Bregovec, I., Maksimović, M., Rakin, D., Markov, V., and Binenfeld, Z. (1979a) Chemical properties and protective effect of bis-pyridinium-2-monooxime carbonyl derivatives against inhibition of cholinesterase by soman. *Acta Pharm Jugosl.* **29**, 107–110.

Deljac, V., Bregovec, I., Maksimović, M., Rakin, D., Markov, V., Radičević, R., and Binenfeld, Z. (1979b) Synthesis, chemical properties and protective effect of some asymmetrically substituted bis-quaternary pyridinium salts against inhibition of BuChE and AChE by soman. Derivatives of 4-hydroxyiminomethylpyridine. *Acta Pharm Jugosl.* **29**, 187–191.

Deljac, V., Bregovec, I., Maksimović, M., Radović, Lj., and Binenfeld, Z. (1982a) Reactivators of organophosphate inhibited acetylcholinesterase: Benzyl-substituted bis-pyridinium monooximes. *Acta Pharm Jugosl.* **32**, 113–118.

Deljac, V., Bošković, B., Maksimović, M., Bregovec, I., and Binenfeld, Z. (1982b) Synthesis and therapeutic effects of isovaleryl bis-pyridinium monooximes in acetylcholinesterase poisoning. *Acta Pharm Jugosl.* **32**, 267–274.

Deljac, V., Maksimović, M., Radović, Lj., Rakin, D., Markov, V., Bregovec, I., and Binenfeld, Z. (1982c) Reactivators of organophosphate-inhibited cholinesterase: Phenylhydroxymethyl and cyclohexylhydroxymethyl substituted bis-pyridinium monooximes. *Arch Toxicol.* **49**, 285–291.

Deljac, A., Bevandić, Z., Bregovec, I., Binenfeld, Z., and Maksimović, M. (1984) 2-Phenyl-2-hydroxyiminomethylbispyridines as antidotes in poisoning with highly toxic organophosphorus compounds. *Acta Pharm Jugosl.* **34**, 117–118.

Deljac, V., Deljac, A., Mesić, M., Kilibarda, V., Maksimović, M., and Binenfeld, Z. (1992) Reactivators of AChE inhibited by organophosphorus compounds. Butenylenic and tetramethylenic heterocyclic oximes. IV. *Acta Pharm.* **42**, 173–179.

Ellman, G.L., Courtney, K.D., Andres, V., and Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.* **7**, 88–95.

Francišković L., Škrinjarić-Špoljar, M., and Reiner E. (1993) Interactions of imidazolium and pyridinium dioximes with human erythrocyte acetylcholinesterase. *Chem-Biol Interactions.* **87**, 323–328.

Galoši, A., Deljac, A., Deljac, V., and Binenfeld, Z. (1988) Reactivators of acetylcholinesterase inhibited by organophosphorus compounds. Imidazole derivatives. *Acta Pharm Jugosl.* **38**, 23–29.

Lucić, A., Radić, B., Peraica, M., Mesić, M., Primožič, I., and Binenfeld, Z. (1997) Antidotal efficacy of quinuclidinium oximes against Soman poisoning. *Arch Toxicol.* **71**, 467–470.

Maksimović, M., Bošković, B., Radović, Lj., Tadić, V., Deljac, V., and Binenfeld, Z. (1980) Antidotal effects of bis-pyridinium-2-monooxime carbonyl derivatives in intoxication with highly toxic organophosphorus compounds. *Acta Pharm Jugosl.* **30**, 151–160.

Maksimović, M., Bregovec, I., Deljac, V., and Binenfeld, Z. (1984) Reactivators of organophosphate-inhibited cholinesterase: 4-cycloalkylcarbonyl substituted bis-pyridinium monooximes. *Arch Toxicol.* **55**, 199–202.

Mesić, M., Deljac, A., Deljac, V., and Binenfeld, Z. (1991) Reactivators of acetylcholinesterase inhibited by organophosphorus compounds. Imidazole derivatives. II. *Acta Pharm Jugosl.* **41**, 203–210.

Mesić, M., Rončević, R., Radić, B., Fajdetić, A., and Binenfeld, Z. (1994a) Reactivators of acetylcholinesterase inhibited by organophosphorus compounds. Imidazole derivatives. V. *Acta Pharm.* **44**, 145–150.

Mesić, M., Rončević, R., Radić, B., Fajdetić, A., and Binenfeld, Z. (1994b) Synthesis, acute toxicity and protective action of some new oximes in experimental soman poisoning. Imidazole derivatives. VI. *Acta Pharm.* **44**, 151–156.

Mesić, M., Deljac, A., Deljac, V., Binenfeld, Z., Maksimović, M., and Kilibarda, V. (1992) Reactivators of acetylcholinesterase inhibited by organophosphorus compounds. Imidazole derivatives. III. *Acta Pharm.* **42**, 169–172.

Milatović, D., Vorkapić-Furač, J., Škrinjarić-Špoljarić, M., and Reiner, E. (1989) Synthesis of three pyridoxal oxime derivatives and their reactivating potency against phosphorylated acetylcholinesterase. *Acta Pharm Jugosl.* **39**, 281–287.

Quinn D.M., Balasubramanian, A.S., Doctor B.P., and Taylor, P. (Eds.) (1995) *Enzymes of the Cholinesterase Family*. Plenum Press, New York. pp. 1–534.

Radić, B., Lucić, A., Peraica, M., and Bradamante, V. (1999) Treatment of carbamate and organophosphate intoxication with new oximes. *Acta Pharm.* **49**, 71–77.

Radić, B., Lucić, A., Peraica, M., Domijan, A.M., and Bradamante, V. (2001) Efficiency of imidazolium and quinuclidinium derivatives combined with HI-6 or diazepam in soman and tabun poisoning. *Acta Pharm.* **51**, 1–9.

Radić, Z., Reiner E., and Taylor P. (1991) Role of the peripheral anionic site on acetylcholinesterase: inhibition by substrates and coumarin derivatives. *Molec Pharmacol.* **39**, 98–104.

Reiner, E. (1986) Inhibition of acetylcholinesterase by 4,4'-bipyridin and its effect upon phosphorylation of the enzyme. *Croat Chem Acta* **59**, 925–931.

Reiner, E., Simeon-Rudolf, V., and Škrinjarić-Špoljar, M. (1995) Catalytic properties and distribution profiles of paraoxonase and cholinesterase phenotypes in human sera. *Toxicol Letters* **82/83**, 447–452.

Reiner, E., Škrinjarić-Špoljar, M., Dunaj, S., Simeon-Rudolf, V., Primožič, I., and Tomić, S. (1999) 3-Hydroxyquinuclidinium derivatives: Synthesis of compounds and inhibition of acetylcholinesterase. *Chem-Biol Interactions.* **120**, 173–181.

Reiner E. and Radić Z. (2000) Mechanism of action of cholinesterase inhibitors. In: *Cholinesterases and Cholinesterase Inhibitors*. (Ed. E. Giacobini), Martin Dunitz Ltd., London, pp. 103–119.

Simeon, V., Škrinjarić-Špoljar, M. and Wilhelm, K. (1973) Reactivation of phosphorylated cholinesterases in vitro and protecting effects in vivo of some pyridinium and quinolinium oximes. *Arh hig rada.* **24**, 11–18.

Simeon, V., Wilhelm, K., Granov, A., Besarović-Lazarev, S., Buntić, A., Fajdetić, A., and Binenfeld, Z. (1979) 1,3-Bispyridinium-dimethylether mono- and dioximes: Synthesis, reactivating potency and therapeutic effect in experimental poisoning by organophosphorus compounds. *Arch Toxicol.* **41**, 301–306.

Simeon, V., Radić Z., and Reiner E. (1981) Inhibition of cholinesterases by the oximes P2AM and Toxogonin. *Croat Chem Acta*. **54**, 473–480.

Simeon-Rudolf, V., Reiner, E., Škrinjarić-Špoljar, M., Radić, B., Lucić, A., Primožič, I., and Tomić, S. (1998) Quinuclidinium-imidazolium compounds: Synthesis, mode of interaction with acetylcholinesterase and effect upon Soman intoxicated mice. *Arch Toxicol*. **72**, 289–295.

Sterling, H.S., Doukas, P.H., Ricciardi Jr. F.J., and O'Neil, J.J. (1991) Quaternary and tertiary quinuclidine derivatives as inhibitors of the choline uptake. *J Pharm Sci*. **80**, 785-789.

Škrinjarić-Špoljar, M. and Kralj, M. (1980) Reactivation and protective effects of pyridinium compounds in human erythrocyte acetylcholinesterase inhibition by organophosphates in vitro. *Arch Toxicol*. **45**, 21–27.

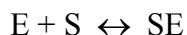
Škrinjarić-Špoljar, M., Simeon, V., Reiner, E., and Krauthacker, B. (1988) Bispyridinium compounds: Inhibition of human erythrocyte acetylcholinesterase and protection of the enzyme against phosphorylation. *Acta Pharm Jugosl*. **38**, 101–109.

Škrinjarić-Špoljar, M., Francišković, L., Radić, Z, Simeon, V., and Reiner, E. (1992) Reaction of imidazolium and pyrimidinium oximes with the cholinesterase substrate acetylthiocholine. *Acta Pharm*. **42**, 77–83.

Škrinjarić-Špoljar, M., Burger, N., and Lovrić, J. (1999) Inhibition of acetylcholinesterase by three new pyridinium compounds and their effect on phosphorylation of the enzyme. *J Enzyme Inhibition*. **4**, 331–341.

APPENDIX

Reactions presented here in a simplified schematic form are those discussed in the paper. Constants defining these reactions are explained.

A. Enzyme/substrate reactions

E = native enzyme

S = substrate (e.g., OP)

ES = Michaelis complex (S is bound to the catalytic site of E)

P = products of substrate hydrolysis

SE = reversible complex (S is bound to the allosteric site of E)

S can also bind to both sites of the enzyme, whereby a ternary complex SES is formed.

K_m = Michaelis constant for substrate hydrolysis. K_m is not the ES dissociation constant, but a function of all rate constants leading to substrate hydrolysis.

K_{ss} = dissociation constant of the reversible SE complex. K_{ss} is often referred to as the substrate inhibition constant.

B. Enzyme/oxime reactions

E = native enzyme

L = oxime (or other reversible ligand)

EL = reversible complex (L is bound to the catalytic site of E)

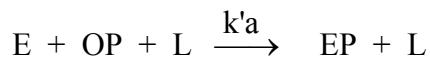
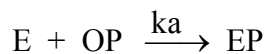
LE = reversible complex (L is bound to the allosteric site of E)

L can also bind to both sites of the enzyme, whereby a ternary complex LEL is formed.

K_a = dissociation constant of the reversible EL complex

K_i = dissociation constant of the reversible LE complex

The reciprocal value of the dissociation constant is the affinity constant. The higher the numerical value of the dissociation constant, the lower is the affinity constant (i.e., the lower is the affinity of a compound for the enzyme).

C. Phosphylation of the native enzyme in absence and presence of a reversible ligand

E = native enzyme

OP = organophosphorus compound

EP = phosphylated enzyme

L = oxime (or other reversible ligand)

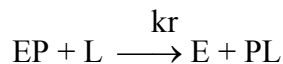
OP compounds react with cholinesterases (E) by phosphylating the catalytic site of the enzyme (i.e., a covalent bond is formed between the enzyme E and the phosphorus). Phosphylation of the enzyme is defined by a rate constant.

k_a = second order rate constant of phosphylation in the absence of an oxime

$k'a$ = second order rate constant of phosphylation in the presence of an oxime

$k_a/k'a = PI =$ protective index

The term phosphylation is used in this paper for the enzyme/OP reaction irrespective of whether the OP is a phosphate, phosphonate or phosphinate, or a thio- or dithio analogue, or an anhydrate.

D. Reactivation of the phosphylated enzyme

EP = phosphylated enzyme

L = oxime

E = native enzyme

PL = phosphylated oxime

k_r = second order rate constant of reactivation

Table 1. Mono-Pyridinium Compounds (Py)

Structure	Substituents	Abbr. [CAS RN]	Ref.
		Py-1 [7375-29-3]	
		Py-2 [2676-84-8]	Simeon et al., 1973
		Py-3 [15793-90-5]	
	3-phenyl	Py-4 [97741-89-4]	Bregovec et al., 1984a
	4-phenyl	Py-5 [97741-87-2]	
	3-cyclohexyl	Py-6 [97741-90-7]	
	4-cyclohexyl	Py-7 [97741-88-3]	
	3-NHCH ₂ CH ₂ OH	Py-8 [97741-92-9]	
	4-NHCH ₂ CH ₂ OH	Py-9 [97741-91-8]	
		Py-10 [92074-16-3]	Deljac et al., 1984
		Py-11 [92074-17-4]	
		Py-12 [69716-25-2]	Bevandić et al., 1985
		Py-13 [113038-46-3]	
	4-CHNOH	Py-14 [117819-49-5]	Bregovec et al., 1988
	2-CHNOH	Py-15 [117819-50-8]	
	4-C(SCH ₃)NOH	Py-16 [117819-51-9]	
	2-C(SCH ₃)NOH	Py-17 [117852-28-5]	
	4-COCHNOH	Py-18 [117819-52-0]	

Table 1. Mono-Pyridinium Compounds (Py) (cont.)

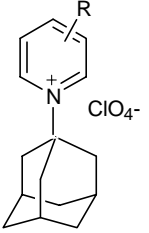
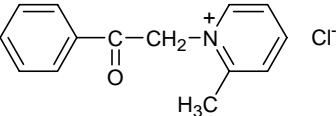
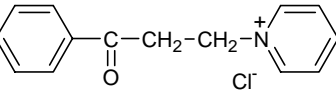
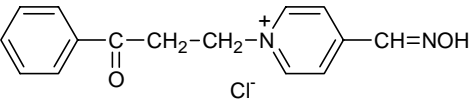
Structure	Substituents	Abbr.[CAS RN]	Ref.
	H	Py-19 [93588-29-5]	Bregovec et al., 1992
	2-CHNOH	Py-20 [145614-00-2]	
	4-CHNOH	Py-21 [145855-13-6]	
		Py-22 [115260-54-3]	Škrinjarić-Špoljar et al., 1999
		Py-23 [70013-89-7]	
		Py-24 [56795-68-7]	

Table 2. Bis-Pyridinium Compounds (Py2)

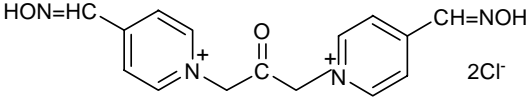
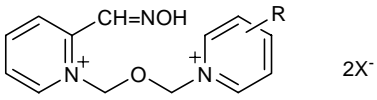
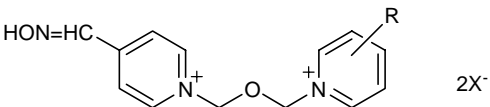
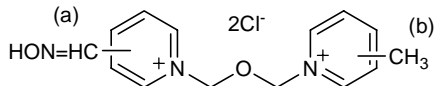
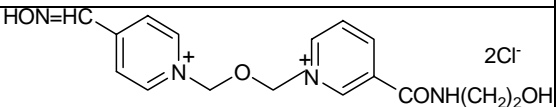
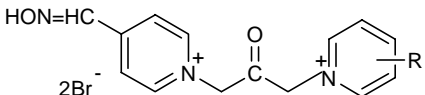
Structure	Substituents		Abbr. [CAS RN]	Ref.
			Py2-1 [35838-61-0]	Simeon et al., 1973
	R	X		Deljac et al., 1979a
	3-benzoyl	I	Py2-2 [65320-89-0]	
	3-benzoyl	Cl	Py2-3 [65321-24-6]	
	3-cyclohexylcarbonyl	I	Py2-4 [65320-92-5]	
	3-cyclohexylcarbonyl	Cl	Py2-5 [71752-85-7]	
	4-benzoyl	I	Py2-6 [65320-93-6]	
	4-benzoyl	Cl	Py2-7 [71752-86-8]	
	4-cyclohexylcarbonyl	I	Py2-8 [71752-87-9]	
	4-cyclohexylcarbonyl	Cl	Py2-9 [71752-88-0]	
	R	X		Deljac et al., 1979b
	3-benzoyl	I	Py2-10 [73973-81-6]	
	3-benzoyl	Cl	Py2-11 [73944-44-2]	
	3-cyclohexylcarbonyl	I	Py2-12 [73944-45-3]	
	3-cyclohexylcarbonyl	Cl	Py2-13 [73944-46-4]	
	4-benzoyl	I	Py2-14 [73944-47-5]	
	4-benzoyl	Cl	Py2-15 [73944-48-6]	
	4-cyclohexylcarbonyl	I	Py2-16 [73944-49-7]	
4-cyclohexylcarbonyl	Cl	Py2-17 [83972-65-0]		
	a	b		Simeon et al., 1979
	4	2	Py2-18 [70441-83-7]	
	4	4	Py2-19 [70441-82-6]	
	2	2	Py2-20 [70441-84-8]	
2	4	Py2-21 [70441-85-9]		
			Py2-22 [78368-87-3]	Binenfeld et al., 1981
	H		Py2-23 [78368-90-8]	
	2-CH ₃		Py2-24 [74634-62-1]	
	4-CH ₃		Py2-25 [74634-63-2]	

Table 2. Bis-Pyridinium Compounds (Py2) (cont.)

Structure	Substituents		Abbr. [CAS RN]	Ref.
	3-CONH ₂		Py2-26 [78368-91-9]	Binenfeld et al., 1981
	4-CONH ₂		Py2-27 [78380-06-0]	
	3-CONHCH ₂ CH ₂ OH		Py2-28 [78368-92-0]	
	4-CONHCH ₂ CH ₂ OH		Py2-29 [78368-93-1]	
	2-CH ₃		Py2-30 [78368-84-0]	
	3-CONHCH ₂ CH ₂ OH		Py2-31 [78368-85-1]	
	4-CONHCH ₂ CH ₂ OH		Py2-32 [78368-86-2]	
	Y	R		
	O		Py2-33 [78368-89-5]	
	CH ₂		Py2-34 [74634-64-3]	
	CO		Py2-35 [78368-95-3]	
	CO		Py2-36 [78368-94-2]	
	a	b		
	2	2	Py2-37	Deljac et al., 1982a
	2	3	Py2-38 [83972-82-1]	
	2	4	Py2-39 [83972-83-2]	
	4	3	Py2-40 [83972-71-8]	
	4	4	Py2-41 [83972-72-9]	
	a	b		
	2	3	Py2-42 [78851-95-3]	Deljac et al., 1982b
	2	4	Py2-43 [85126-23-4]	
	4	3	Py2-44 [85126-24-5]	
4	4	Py2-45 [85126-25-6]		
	a	b, R		
	2	2, cyclohexyl	Py2-46 [81924-40-5]	Deljac et al., 1982c
	2	3, cyclohexyl	Py2-47 [81904-51-0]	
	2	4, cyclohexyl	Py2-48 [81904-52-1]	
	2	2, phenyl	Py2-49 [81904-53-2]	
2	3, phenyl	Py2-50 [81904-54-3]		

Table 2. Bis-Pyridinium Compounds (Py2) (cont.)

Structure	Substituents		Abbr.[CAS RN]	Ref.
	2	4, phenyl	Py2-51 [81904-55-4]	Deljac et al., 1982c
	4	2, cyclohexyl	Py2-52 [81904-56-5]	
	4	3, cyclohexyl	Py2-53 [81904-57-6]	
	4	4, cyclohexyl	Py2-54 [81904-58-7]	
	4	3, phenyl	Py2-55 [81904-59-8]	
	4	4, phenyl	Py2-56 [81904-60-1]	
	a	b		Bregovec et al., 1983
	2	4	Py2-57 [89547-33-1]	
	2	5	Py2-58 [89547-34-2]	
	4	4	Py2-59 [89547-35-3]	
	4	5	Py2-60 [89547-36-4]	Bregovec et al., 1984b
	a	b, R		
	2	2,	Py2-61 [95575-09-0]	
	2	4,	Py2-62 [95575-03-4]	
	2	4,	Py2-63 [95575-04-5]	
	4	2,	Py2-64 [95575-06-7]	Bregovec et al., 1984b
	4	4,	Py2-65 [95575-07-8]	
	2	2	Py2-66 [95575-02-3]	
	4	4	Py2-67 [95575-08-9]	
	R	a		Maksimović et al., 1984
	cyclopentyl	2	Py2-69 [92953-51-0]	
	cyclopentyl	4	Py2-70 [92953-49-6]	
	cycloheptyl	2	Py2-71 [92982-27-9]	
	cycloheptyl	4	Py2-72 [92953-50-9]	Deljac et al., 1984
			Py2-73 [92074-18-5]	
			Py2-74 [92074-19-6]	

Table 2. Bis-Pyridinium Compounds (Py2) (cont.)

Structure	Substituents				Abbr. [CAS RN]	Ref.
	Y		X			Bevandić et al., 1985
	O		Cl		Py2-75 [99761-22-5]	
	CH ₂		Br		Py2-76 [99761-21-4]	
	a, R₁		b, R₂			Bregovec et al., 1986
	2, SCH ₃		2, H		Py2-77 [106656-52-4]	
	2, SCH ₃		2, SCH ₃		Py2-78 [106656-49-9]	
	4, SCH ₃		4, H		Py2-79 [106656-50-2]	
	2, H		4, SCH ₃		Py2-80 [106656-51-3]	
	2, SCH ₃		4, H		Py2-81 [106656-53-5]	
	R₁	R₂	R₃	R₄		Milatović et al., 1989
	CH ₃	OH	CHNOH	CH ₂ OH	Py2-82 [127525-99-9]	
	CHNOH	H	H	H	Py2-83 [127526-00-5]	
	H	H	CHNOH	H	Py2-84 [127526-01-6]	
	Y		a			Deljac et al., 1992
	CH=CH		2		Py2-85 [144310-41-8]	
	CH=CH		4		Py2-86 [4611-87-4]	
	(CH ₂) ₂		2		Py2-87 [133325-76-5]	
	(CH ₂) ₂		4		Py2-88 [2438-48-4]	

Table 3. Mono-Imidazolium Compounds (Im)

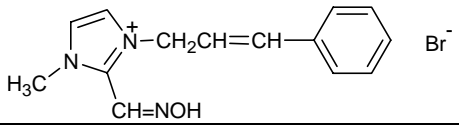
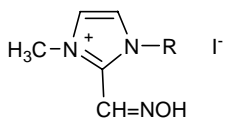
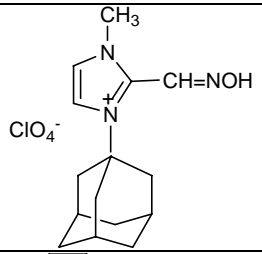
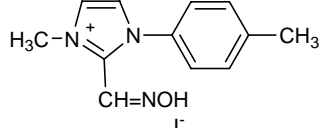
Structure	Substituents	Abbr. [CAS RN]	Ref.
		Im-1 [117819-53-1]	Bregovec et al., 1988
	methyl	Im-2 [43193-21-1]	Galoši et al., 1988
	benzyl	Im-3 [49702-55-8]	Mesić et al., 1991
	phenyl	Im-4 [61393-39-3]	
	<i>p</i> -methoxybenzen	Im-5 [61393-44-0]	
	<i>p</i> -fluorbenzen	Im-6 [144120-27-4]	Mesić et al., 1992
		Im-7 [145855-15-8]	Bregovec et al., 1992
		Im-8 [160193-37-3]	Mesić et al., 1994a

Table 4. Bis-Imidazolium Compounds (Im2)

Structure	Substituents			Abbr. [CAS RN]	Ref.	
	R	Y	X			
	methyl	O	I	Im2-1 [53035-14-6]	Galoši et al., 1988	
	benzyl	O	I	Im2-2 [138173-96-3]	Mesić et al., 1991	
	phenyl	O	I	Im2-3 [138173-97-4]		
	<i>p</i> -methoxybenzen	O	I	Im2-4 [138173-98-5]		
	<i>p</i> -fluorbenzen	O	I	Im2-5 [144120-26-3]	Mesić et al., 1992	
	methyl	CH=CH	Br	Im2-6 [144310-42-9]	Deljac et al., 1992	
	benzyl	CH=CH	Br	Im2-7 [144310-48-5]		
	phenyl	CH=CH	Br	Im2-8 [144310-44-1]		
	<i>p</i> -methoxybenzen	CH=CH	Br	Im2-9 [144329-52-2]		
	<i>p</i> -fluorbenzen	CH=CH	Br	Im2-10 [144329-53-3]		
	methyl	(CH ₂) ₂	Br	Im2-11 [144310-43-0]		
	benzyl	(CH ₂) ₂	Br	Im2-12 [144310-49-6]		
	phenyl	(CH ₂) ₂	Br	Im2-13 [144310-45-2]		
	<i>p</i> -methoxybenzen	(CH ₂) ₂	Br	Im2-14 [144310-46-3]		
	<i>p</i> -fluorbenzen	(CH ₂) ₂	Br	Im2-15 [144310-47-4]		
	<i>p</i> -methylbenzen	O	I	Im2-16 [160193-38-4]		Mesić et al., 1994a
	methyl	O-(CH ₂) ₂ -O	Cl	Im2-17 [117941-41-0]		Mesić et al., 1994b
	benzyl	O-(CH ₂) ₂ -O	Cl	Im2-18 [159980-47-9]		
	phenyl	O-(CH ₂) ₂ -O	Cl	Im2-19 [159980-46-8]		
	methyl	O-(CH ₂) ₃ -O	Cl	Im2-20 [159980-48-0]		
	benzyl	O-(CH ₂) ₃ -O	Cl	Im2-21 [160061-51-8]		
	phenyl	O-(CH ₂) ₃ -O	Cl	Im2-22 [159980-49-1]		
	methyl	CH ₂	Br	Im2-23 [43193-24-4]		
	benzyl	CH ₂	Br	Im2-24 [159980-42-4]		
	phenyl	CH ₂	Br	Im2-25 [159980-41-3]		
	methyl	(CH ₂) ₄	Br	Im2-26 [159980-43-5]		
	benzyl	(CH ₂) ₄	Br	Im2-27 [144310-49-6]		
	phenyl	(CH ₂) ₄	Br	Im2-28 [159980-44-6]		

Table 5. Pyridinium-Imidazolium Compounds (PyIm)

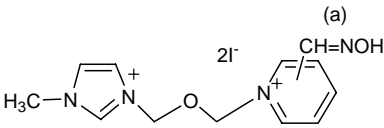
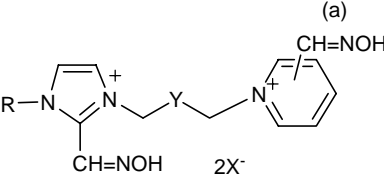
Structure	Substituents				Abbr. [CAS RN]	Ref.		
	a					Galoši et al., 1988		
	2				PyIm-1 [115820-89-8]			
	4				PyIm-2 [115820-91-2]			
	R	a	Y	X				
	methyl	2	O	I	PyIm-3 [115820-88-7]	Galoši et al., 1988 Mesić et al., 1991		
	methyl	4			PyIm-4 [115820-90-1]			
	benzyl	2			PyIm-5 [138174-02-4]	Mesić et al., 1991		
	benzyl	4			PyIm-6 [138173-99-6]			
	phenyl	2			PyIm-7 [138174-03-5]			
	phenyl	4			PyIm-8 [138174-00-2]			
	<i>p</i> -methoxybenzen	2			PyIm-9 [138174-04-6]			
	<i>p</i> -methoxybenzen	4			PyIm-10 [138174-01-3]			
	<i>p</i> -fluorbenzen	2			PyIm-11 [144120-24-1]	Mesić et al., 1992		
	<i>p</i> -fluorbenzen	4			PyIm-12 [144120-25-2]			
	<i>p</i> -methylbenzen	2			PyIm-13 [160193-39-5]	Mesić et al., 1994a		
	<i>p</i> -methylbenzen	4			PyIm-14 [160193-40-8]			
	methyl	4			CH ₂	Br	PyIm-15 [159980-50-4]	Mesić et al., 1994b
	<i>p</i> -fluorbenzen	4					PyIm-16 [159980-51-5]	

Table 6. Mono-Quinuclidinium Compounds (Q)

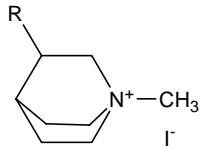
Structure	Substituents	Abbr. [CAS RN]	Ref.
	=NOH	Q-1 [192509-79-8]	Lucić et al., 1997
	=O	Q-2 [6659-51-4]	Simeon-Rudolf et al., 1998
	-OH	Q-3 [6659-52-5]	Reiner et al., 1999
	-OCON(CH ₃) ₂	Q-4 [243663-66-3]	

Table 7. Bis-Quinuclidinium (Q2) and Pyridinium-Quinuclidinium Compounds (PyQ)

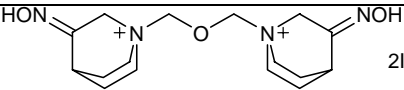
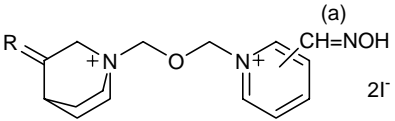
Structure	Substituents	Abbr. [CAS RN]	Ref.	
		Q2-1 [192509-82-3]	Lucić et al., 1997	
	R	a		
	O	2		PyQ-1 [192509-91-4]
	O	4		PyQ-2 [192509-92-5]
	NOH	2		PyQ-3 [192509-86-7]
NOH	4	PyQ-4 [192509-89-0]		

Table 8. Imidazolium-Quinuclidinium Compounds (ImQ)

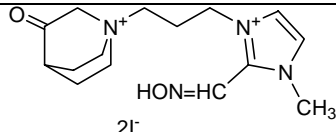
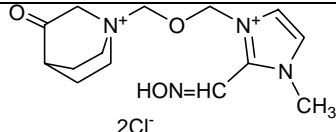
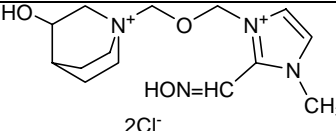
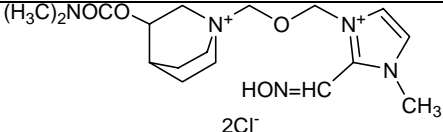
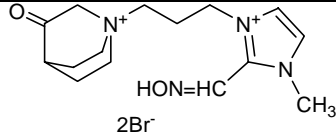
Structure	Abbr. [CAS RN]	Ref.
	ImQ-1 [208182-94-9]	Simeon-Rudolf et al., 1998
	ImQ-2 [208182-96-1]	
	ImQ-3 [243663-67-4]	Reiner et al., 1999
	ImQ-4 [243663-68-5]	
	ImQ-5 [208182-95-0]	Simeon-Rudolf et al., 1998

Table 9. Conventional oximes used as antidotes in the therapy of OP poisoning

Structure	Name [CAS RN]
	PAM-2 (Pralidoxime) [6735-59-7]
	TMB-4 (Trimedoxime) [56-97-3]
	Toxogonin (Obidoxime) [114-90-9]
	HI-6 [34433-31-3]

Table 10. Inhibition of cholinesterases by the indicated oximes
(I₅₀ values are explained in the text.)

Human erythrocyte AChE

I₅₀ = 0.020 μM

Oxime: Im2-13 (Radić et al., 1999)

I₅₀ = 0.32 – 3.0 μM

Oximes: PyIm-9, PyIm-11, Im2-12, Im2-16
(Deljac et al., 1992; Mesić et al., 1994a)

I₅₀ = 6.0 – 80 μM

Oximes: ImQ-4, Py-4, Py2-28, Py2-35, Py2-38, Py2-39, Py2-75
(Bevandić et al., 1985; Binenfeld et al., 1981;
Bregovec et al., 1984a; Deljac et al., 1982a; Lucić et al., 1997)

I₅₀ = 0.1 – 3 mM

Oximes: Majority of 86 tested compounds.

Horse serum BChE

I₅₀ = 0.04 – 0.5 mM

Oximes: Py2-10 to Py2-17 (Deljac et al., 1979b)

Table 11. Inhibition of human erythrocyte AChE by the indicated oximes
(The enzyme/oxime dissociation constants K_a and K_i are explained in the Appendix.)

Oxime	K_a /mM	K_i /mM	References
Py2-16	0.025	(a)	Škrinjarić-Špoljar et al., 1988
Py2-12	0.028	(a)	ibid.
Py2-8	0.033	(a)	ibid.
Py2-4	0.058	(a)	ibid.
Py-23	0.059	(b)	Škrinjarić-Špoljar et al., 1999
Im-2	0.073	(a)	Simeon-Rudolf et al., 1998
ImQ-4	0.20	(c)	Reiner et al., 1999
ImQ-1	0.24	(a)	Simeon-Rudolf et al., 1998
ImQ-2	0.25	(a)	ibid.
ImQ-3	0.31	(c)	Reiner et al., 1999
Q-4*	0.37	(c)	ibid.
Q-3*	0.55	(c)	ibid.
Im2-11	(d)	0.011	Francišković et al., 1993
Im2-6	(d)	0.024	ibid.
Py-24	(d)	0.33	Škrinjarić-Špoljar et al., 1999
Py-22	0.069	0.027	ibid.
Q-2*	1.6	4.6	Simeon-Rudolf et al., 1998
PAM-2	0.13	0.76	Simeon et al., 1981
Toxogonin	0.16	1.99	ibid.

(a) There is kinetic evidence that the compounds bind to the allosteric site, but K_i could not be evaluated due to fast oximolysis at high substrate concentrations

(b) No kinetic evidence that the compound binds to the allosteric site

(c) K_i not evaluated

(d) No kinetic evidence that the compounds bind to the catalytic site

* Q-2, Q-3 and Q-4 have no oxime group

Table 12. Reactivation (%) of human erythrocyte AChE-OP conjugates by the indicated oximes

AChE-VX: 109 oximes tested
At least 50 % reactivation: 43 oximes
Most other oximes also reactivate

AChE-Sarin: 88 oximes tested
At least 50 % reactivation: 43 oximes
Most other oximes also reactivate

AChE-Tabun: 84 oximes tested
About 70 % reactivation: Py2-86 (trans), Py2-88 and Im2-8
(Deljac et al., 1992)
44 % reactivation: Im2-2 (Mesić et al., 1991)
About 20 % reactivation: Im2-13, PyIm-5, PyIm-8 and PyIm-10
(Deljac et al., 1992; Mesić et al., 1991)
All other oximes do not reactivate

AChE-Soman: 72 oximes tested
71 % reactivation: Im2-13 (Radić et al., 1999)
About 35 % reactivation: PyIm-13 and PyQ-4
(Lucić et al., 1997; Mesić et al., 1994b)
All other oximes do not reactivate

Table 13. Second order rate constants of reactivation (kr) of the human erythrocyte AChE-OP conjugates by the indicated oximes

Oxime	OP			References
	VX	Sarin	Tabun	
kr *				
Py2-3	0.2	0.2	-	Milatović et al., 1989
Py2-18	6	-	-	Simeon et al., 1979
Py2-19	4	-	-	ibid.
Py2-84	0.2	1	0.3	Milatović et al., 1989
Py2-86(trans)	45	35	8	Francišković et al., 1993
Im2-6	6	8	4	ibid.
Im2-11	8	5	4	ibid.
PAM-2	1.8	1.6	0	Milatović et al., 1989
Toxogonin	2.2	3.2	0.3	ibid

* $kr = 10^3 \times \text{mol}^{-1} \text{L min}^{-1}$

The kr constant is explained in the Appendix.

Table 14. Antidotal effect of oximes (plus atropine) against intoxication of mice or rats by the indicated OPs

$$TF = LD-50 (OP+oxime) / LD-50 (OP \text{ only})$$

VX:	19 oximes tested
	TF > 10 Twelve oximes
	TF = 2.4 – 9.3 Seven oximes

Sarin:	20 oximes tested
	TF = 5.8 – 9.0
	Oximes: Py2-8, Py2-62, Py2-66, Py2-67, Py2-68, Im2-2 (Bregovec et al., 1984b; Maksimović et al., 1980; Mesić et al., 1991)
	TF = 2.4 – 4.8 Eleven oximes
	TF < 2 Three oximes

Tabun:	27 oximes tested
	TF = 4.9 Oxime: Im2-2 (Mesić et al., 1991)
	TF = 3.2 – 4.4
	Oximes: Im2-25, PyIm-5, PyIm-6, Im-3, Q-4*, ImQ-4 (Mesić et al., 1991; Radić et al., 2001)
	TF = 2.1 – 3.0 Eight oximes
	TF < 2 Twelve oximes

Soman:	43 oximes tested
	TF = 5.3 Oxime: ImQ-4 (Lucić et al., 1997)
	TF = 3.2 – 3.8
	Oximes: Py2-8, Py2-42, Q-1, Q-4*, PyQ-2 (Deljac et al., 1982b; Lucić et al., 1997; Maksimović et al., 1980; Radić et al., 2001)
	TF = 2.1 – 2.9 Seventeen oximes
	TF < 2 Eighteen oximes

* Q-4 has no oxime group