19. BIOCHEMICAL STUDIES OF OXIMES SYNTHESIZED IN CROATIA OVER THE PAST DECADES

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About ninety new oxime compounds were synthesized in Croatia over the past two decades. These compounds contain one or two oxime groups on pyridinium, imidazolium or quinuclidinium rings, or on their derivatives, as shown on Figures 1 and 2. Almost all compounds were prepared in the Department of Organic Chemistry, Faculty of Science of the University of Zagreb. The syntheses of the major groups of compounds are described in references 1-14.

Biochemical studies of the compounds comprised the mechanism of reversible inhibition of cholinesterases, protection of the enzymes against phosphorylation by organophosphates (OPs) and reactivation of the inhibited enzymes. The enzymes were mainly human erythrocyte acetylcholinesterase (AChE; EC 3.1.1.7) and human plasma butyrylcholinesterase (BChE; EC 3.1.1.8). The OPs were primarily Sarin, Soman, Tabun, VX and DDVP. The evaluated biochemical parameters were compared with those obtained from studies with conventional oximes used as therapeutic agents against intoxication by OPs: PAM-2, HI-6 and Toxogonin (Fig. 3). Most biochemical studies were carried out at the Department of Biochemistry of the Institute for Medical Research and Occupational Health, Zagreb (11-27).

We have shown that the majority of new compounds, just like the conventional oximes, bind to two sites on AChE: the catalytic or/and allosteric site. The enzyme-oxime dissociation constants (K_d) were in the millimolar range for most compounds with only a few compounds having higher affinity (i.e. low K_d value) (Table 1). The affinities for the two sites on the enzyme were usually within one order of magnitude apart. The affinities of the oximes for BChE were different for different BChE phenotypes (Table 1); this property might be of importance, considering the effect of oximes on the therapy of poisoning by OPs.

All compounds protected AChE and BChE against phosphorylation by OPs. As the protection of the enzyme depends on the concentration of the oxime and on its affinity for the enzyme, it can be predicted from known K_d values. Theoretical equations for the effect of reversible inhibitors upon phosphorylation were derived for compounds that bind either to the catalytic or allosteric site, or to both sites on the enzyme. The equations were experimentally verified for the effect of bipyridine on the inhibition by several organophosphates (28). In the examples shown in Table 2, the protective index (PI) was calculated assuming that the protector binds only to one site of the enzyme. The higher PI measured than the calculated PI indicates however that the protector binds to both, the catalytic and allosteric sites.

Reactivation of the phosphorylated AChE was similar to reactivation by the conventional oximes with one exception BDB-113 (diimidazolium dioxime) that proved to be a better reactivator of the Tabun-inhibited enzyme than the other oximes (Table 3).

Finally, we have shown that the oximes react with thiocholine esters, like acetylthiocholine, whereby thiocholine is one of the reaction products (21, 24). This is a non-enzymic reaction that might even exceed the rate of the enzymic hydrolysis of thiocholine substrates. When enzyme activities are determined spectrophotometrically with thiol reagents (like DTNB) in the presence of an oxime, the overall rate of substrate hydrolysis must be corrected for this non-enzymic reaction because otherwise false higher activities will be reported.

The majority of new oximes was also tested *in vivo* (on mice or rats). The best effect against intoxication by Soman was obtained with a carbamate of the quinuclidinium-imidazolium oxime (29).

The efficacy of some prepared oximes as protectors and reactivating agents shown in the biochemical studies justifies their retesting and further extensive research.

SUMMARY

This paper gives a short review of biochemical research of oximes sythesized in Croatia over the past two decades. The synthesized compounds contained one or two oxime groups at various positions on pyridinium, imidazolium, or quinuclidinium rings. Aromatic rings of some compounds were substituted with aliphatic or aromatic radicals. Biochemical studies of the oximes focused on the mechanism of reversible binding of oximes to cholinesterases, reactivation of phosphorylated cholinesterases, protection of the enzyme against inhibition by the organophosphates (Soman, Sarin, Tabun, VX or DDVP), and interaction of oximes with thiocholine substrates.

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LEGENDS TO FIGURES AND TABLES

Pyridinium and dipyridinium derivatives

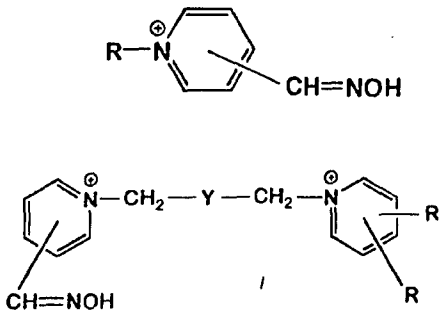
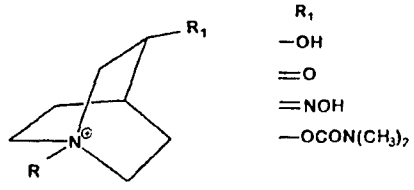


Figure 1.

General stuctures of pyrydinium and dipyridinium oximes Y stands for: - O -, $-(CH_2)_n-$ or -CO-.

Quinuclidinium derivatives



Imidazolium derivates

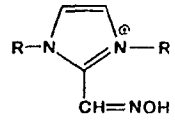
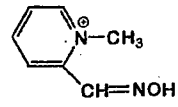


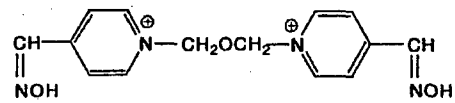
Figure 2.

General structures of quiniclidinium and imidazolium derivatives. They were prepared as oximes or these structures were combined with each other or with a pyridinium ring. Mono- or dioxime derivatives were prepared with different substituents on the rings.

PAM-2



TOXOGONIN



HI-6

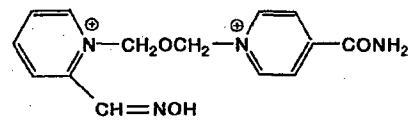


Figure 3.

Structure of the conventional oximes.

Table 1.

Dissociation constants of enzyme-oxime complexes (K_d) for the presumed binding sites evaluated from the effect of oxime upon activity of human erythrocyte acetylcholinesterase or serum butyrylcholinesterase. BDB-106 and BDB-110 are two 1-methyl-imidazolium oximes linked by $-CH_2-CH=CH-CH_2-$ (BDB-106) or linked by $(-CH_2-)_4$ (BDB-110). BDB-108 is a dipyridinium dioxime linked by $-CH_2-CH=CH-CH_2-$.

ENZYME	OXIME	K_d / mM		References
		Catalytic site	Allosteric site	
	PAM-2	0.13	0.76	21
	Toxogonin	0.16	2.0	21
	HI-6	0.031	0.16	23
	BDB-108(cis)	0.052	> 0.1	15
	BDB-108(trans)	0.045	> 0.08	15
	BDB-106	-	0.024	15
	BDB-110	-	0.011	15
Butyrylcholinesterase				
Usual	PAM-2	0.88	> 1.7	27
	HI-6	0.23	> 0.7	27
Atypical	PAM-2	1.1	> 3.4	27
	HI-6	0.47	> 2.5	27

Dash (-) means no binding observed to the catalytic site.

Table 2.

Protection of human erythrocyte AChE against phosphorylation by Soman (5nM) or VX (20 nM) (Ref. 14). PI denotes protective index. PI-measured = k_i / k_i', where k_i and k_i' are rate constants of phosphorylation in the absence and presence of the protecting compound. PI-calculated = 1 + (Concentration of the protecting compound / K_d). The PI-calculated refers to both, Soman and VX.

COMPOUND	OP	PI Measured	PI Calculated
3-oxo-1-methylquinuclidinium iodide / 1.4 mM	Soman	1.9	1.9
	VX	2.0	
3-oxo-1-methylquinuclidinium iodide / 6.0 mM	Soman	9.0	4.8
	VX	7.0	
3-oxo-1-[3-(2-hydroxyiminomethyl-3-methyl-1-imidazolio)propyl]-quinuclidinium diiodide / 0.2 mM	Soman	2.0	1.8
	VX	1.9	
3-oxo-1-[3-(2-hydroxyiminomethyl-3-methyl-1-imidazolio)-2-oxapropyl]-quinuclidinium dichloride / 0.3 mM	Soman	3.8	2.2
	VX	4.2	

Table 3.

Reactivation (%) of phosphorylated human erythrocyte acetylcholinesterase (Ref. 9). All compounds are dioximes and their aromatic rings are linked with $-CH_2-O-CH_2-$. BDB-101 and BDB-118 have pyridinium and imidazolium rings and BDB-113 has two imidazolium rings; the imidazolium rings are in all compounds substituted with benzyl.

OXIME	SARIN	VX	TABUN	SOMAN
PAM-2	66	61	0	0
Toxogonin	79	78	16	3.6
HI-6	73	85	0	2.6
BDB-101	57	4	13	1.4
BDB-113	68	22	44	0
BDB-118	82	54	12	9

KEY WORDS

Oximes, synthesis, cholinesterases, inhibition, organophosphorus compounds