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Investigation of the reactivation kinetics of a large series of bispyridinium oximes with organophosphate-inhibited human acetylcholinesterase

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HIGHLIGHTS

- We investigated the reactivation of OP-inhibited human AChE by 32 oximes in vitro.
- The reactivation kinetics were determined for tabun-, cyclosarin- and paraoxon-inhibited AChE.
- The reactivating potency of oximes was dependent on the OP and the oxime.
- No broad spectrum oxime being superior to standard oximes could be identified.
- It appears doubtful whether the bispyridinium template may ultimately lead to a superior broad spectrum oxime.

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ABSTRACT

The limited effectiveness of the established oximes obidoxime and pralidoxime resulted in ongoing research on novel oximes for the reactivation of acetylcholinesterase (AChE) inhibited by organophosphorus compounds (OP). In order to get more insight into the ability of bispyridinium oximes to reactivate human AChE inhibited by structurally different OP the reactivation kinetics of 31 compounds was determined with tabun-, cyclosarin- and paraoxon-inhibited AChE under identical experimental conditions. The determined affinity (K_D), reactivity (k_r) and hybrid reactivation rate constants (k_{r2}) enabled theoretical calculations and gave insight into distinct structural features which are important for the reactivation of AChE inhibited by different OP. Several oximes with superior reactivating potency towards selective OP–AChE conjugates were identified but none of the tested oximes can be considered as a broad spectrum reactivator. In the end, the data of this and previous studies gives rise to the question whether further modifications of the bispyridinium structure could ever result in a universal reactivator or whether future research should be directed to different templates.

1. Introduction

The repeated homicidal use of highly toxic organophosphorus compounds (OP) against military forces and civilian populations, in particular the recent attacks with the OP nerve agent sarin in Syria, emphasizes the need for effective therapies (Pita and Domingo, 2014; Dolgin, 2013). The acute toxicity of OP nerve agents and pesticides is caused by inhibition of the pivotal enzyme acetylcholinesterase, followed by accumulation of acetylcholine and overstimulation of cholinergic receptors (Holmstedt, 1959). The life threatening disturbance of numerous body functions requires effective and specific antidotes, at present the muscarinic antagonist atropine and an oxime to reactivate OP-inhibited AChE (Sidell, 1997; Cannard, 2006).

Convincing in vitro and in vivo data have demonstrated the limited effectiveness of the established oximes obidoxime and pralidoxime (2-PAM; Table 1) (Eyer and Worek, 2007) and induced research programs in different countries to identify more effective

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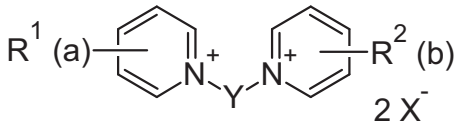
Table 1
Chemical structure and inhibitory activity of tested oximes.



Code	*a*	R^1	*Y*	*b*	R^2	*X*	IC_{50} (μM)
MMB-4	4	CHNOH	CH_2	4	CHNOH	Br	>1000
K191	4	CHNOH	$(CH_2)_2$	4	CHNOH	Br	567
TMB-4	4	CHNOH	$(CH_2)_3$	4	CHNOH	Br	215
K074	4	CHNOH	$(CH_2)_4$	4	CHNOH	Br	108
K305	4	CHNOH	$(CH_2)_5$	4	CHNOH	Br	55
K027	4	CHNOH	$(CH_2)_3$	4	$CONH_2$	Br	>1000
K048	4	CHNOH	$(CH_2)_4$	4	$CONH_2$	Br	>1000
K203	4	CHNOH	CH_2—CH=CH—CH_2	4	$CONH_2$	Br	>1000
K075	4	CHNOH	CH_2—CH=CH—CH_2	4	CHNOH	Br	195
K114	4	CHNOH	*p*-xylene	4	CHNOH	Br	31
Obidoxime	4	CHNOH	CH_2—O—CH_2	4	CHNOH	Cl	>1000
K117	4	CHNOH	$(CH_2)_2$—O—$(CH_2)_2$	4	CHNOH	Br	605
K127	4	CHNOH	$(CH_2)_2$—O—$(CH_2)_2$	4	$CONH_2$	Br	>1000
K263	4	CHNOH	CH_2—CH=CH—CH_2	4	tert-butyl	Br	395
K156	4	CHNOH	$(CH_2)_3$	–	–	Br	301
K255	4	CHNOH	CH_2—CH=CH—CH_2	–	–	Br	914
HLö 7	2.4	CHNOH	CH_2—O—CH_2	4	$CONH_2$	DMS	411
K239	2.4	CHNOH	CH_2—O—CH_2	2.4	CHNOH	Cl	>1000
K246	4	CHNOH	CH_2—CH=CH—CH_2	2	$CONH_2$	Br	41
K208	2	CHNOH	$(CH_2)_3$	4	CHNOH	Br	80
ICD585	2	CHNOH	$(CH_2)_3$	4	$CONH_2$	Br	210
K308	2	CHNOH	$(CH_2)_5$	4	CHNOH	Br	34
K053	2	CHNOH	CH_2—CH=CH—CH_2	4	CHNOH	Br	161
HS3	2	CHNOH	CH_2—O—CH_2	4	CHNOH	Cl	>1000
HI-6	2	CHNOH	CH_2—O—CH_2	4	$CONH_2$	Cl	281
K005	2	CHNOH	$(CH_2)_3$	2	CHNOH	Br	54
K033	2	CHNOH	$(CH_2)_4$	2	CHNOH	Br	14
K068	2	CHNOH	CH_2—CH=CH—CH_2	2	CHNOH	Br	10
HS4	2	CHNOH	CH_2—O—CH_2	2	CHNOH	I	371
K129	2	CHNOH	$(CH_2)_2$—O—$(CH_2)_2$	2	CHNOH	Br	119
K108	2	CHNOH	*p*-xylene	2	CHNOH	Br	>1000
2-PAM	2	CHNOH	CH_3	–	–	Cl	>1000

The inhibition of native human AChE by oximes was tested with 10 concentrations (1–1000 μM) in duplicate with SD < 5%.

compounds (Worek and Thiermann, 2013; Musilek et al., 2011a; Reiner and Simeon-Rudolf, 2006). The only mechanism of action of oximes verified as relevant for therapy is reactivation of OP-inhibited AChE by removal of the phosphyl moiety from the active site of the enzyme (Aldridge and Reiner, 1972; Eyer and Worek, 2007). This enables the in vitro testing of the reactivating potency of oximes by determination of the compound and inhibitor specific affinity and reactivity constants (Worek et al., 2004; Wang and Braid, 1967). However, published data revealed a remarkable impact of the experimental procedure, e.g. buffer, pH, temperature and AChE source, on the determined reactivation rate constants which hampers the comparison of data from different laboratories (Worek and Thiermann, 2013). In order to enable a more comprehensive evaluation of the ability of novel oximes to reactivate OP-inhibited AChE, we recently determined the reactivation kinetics of smaller sets of oximes under identical conditions that may be assumed relevant for human poisoning (Worek et al., 2012c,b,a). These data provided a limited insight into structural requirements for successful reactivation of inhibited AChE and we found it valuable to extend the database by investigating the reactivation kinetics of human AChE inhibited by tabun, cyclosarin and paraoxon-ethyl with a larger number of structurally different bispyridinium oximes. The data of the present study should enable calculations on required oxime concentrations for the reactivation of OP-inhibited AChE and should give insight into potential structure–activity relationship and into the future potential of bispyridinium compounds as broad spectrum reactivators.

2. Materials and methods

2.1. Materials

Paraoxon-ethyl was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide (ATCh) were supplied by Sigma–Aldrich (Taufkirchen, Germany). Tabun (ethyl-*N*,*N*-dimethyl phosphoroamidocyanidate) and cyclosarin (cyclohexylmethylphosphonofluoridate) were made available by the German Ministry of Defence.

All K-oximes were prepared by Assoc. Prof. Musilek and Prof. Kuca (Kim et al., 2005; Kuca et al., 2003a,b; Musilek et al., 2005, 2006, 2007, 2008, 2011b). Obidoxime was purchased from Merck (Darmstadt, Germany), TMB-4 and pralidoxime (2-PAM) from Sigma–Aldrich (Taufkirchen, Germany). HLö 7 was synthesized by J. Braxmeier (Chemisches Labor, Döpshofen, Germany). HI-6 was made available by Dr. Clement (Defence Research Establishment Suffield, Ralston, Alberta, Canada), HS3 and HS4 were provided by Prof. Eyer (University of Munich, Munich, Germany) and MMB-4 was obtained from Prof. Fusek (University of Defence, Hradec Kralove, Czech Republic).

All other chemicals were from Merck (Darmstadt, Germany).