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ORIGINAL RESEARCH

Selected AChE reactivators in different crystalline environment: salts and enzyme

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Abstract The investigation of relationships between the molecular structure of the compounds capable to reactivate acetylcholinesterase (AChE) inhibited by organophosphorus toxins, such as nerve agents and pesticides, is an important step toward synthesis of more efficient antidota. In the present article, we describe the crystal structures of two new AChE reactivators, which are bromides of (E)-1,4-bis(4-hydroxyiminomethylpyridinium)-but-2-ene (K075) and of 4,4′-bis(hydroxyiminomethyl)-1,1′-(1,4-phenylenedimethyl)-bispyridinium (K114). Their molecular geometry and intermolecular interactions in the crystalline state are compared to those in the crystal structures of the well-known AChE reactivators, obidoxime, and TMB-4. Inspection of hydrogen bonds and other short intermolecular contacts in the crystalline AChE–obidoxime complex revealed their similarity to those observed in the crystal structures of K075 and K114.

Keywords AChE reactivators · Oximes · Hydrogen bonds · Crystalline state

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Introduction

Although there are many natural and synthetic compounds that inhibit the enzyme acetylcholinesterase (AChE, EC 3.1.1.7), the organophosphorus inhibitors (OPI; e.g., nerve agents, pesticides, and flame retardants) remain one of the most dangerous and deleterious series of compounds developed by man [1]. Namely, the OPI molecule binds covalently to the hydroxyl group of the serine residue within the enzyme active site, so that the AChE is not able to cleave the neuromediator acetylcholine, which causes the permanent activation of muscarinic and nicotinic receptors. This leads to a central cholinergic crisis with symptoms of lacrimation, salivation, and miosis, with additional neuromuscular and breathing difficulties; death is caused by suffocation [2].

Various treatments are used to counteract the toxic effects of OPI produced either by pre- or by post-intoxication [2, 3]. For example, pre-treatment methods of potentially threatened persons includes weak AChE

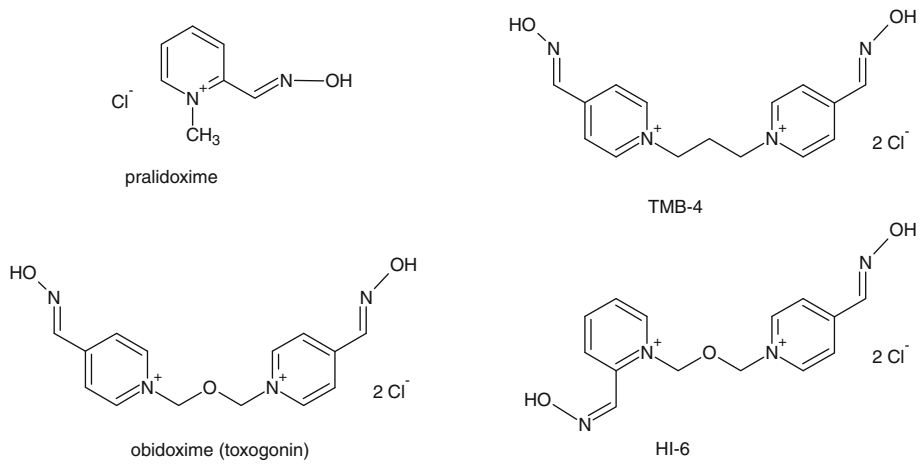

Fig. 1 Structural formulae of commercially available acetylcholinesterase reactivators

inhibitors (e.g., pyridostigmine) to sequester the enzyme, other esterases (e.g., human butyrylcholinesterase) to scavenge OPI or one of oximes (e.g., HI-6) to reactivate AChE.

Post-treatment regimens involve oxime reactivators of AChE in combination with atropine and diazepam [2–4]. Reactivators of AChE (e.g., pralidoxime, HI-6, obidoxime, and TMB−4; Fig. 1) are commonly used in the treatment of OPI intoxications [2, 3, 5–7].

They contain a nucleophilic oxime moiety (oximate anion), which is able to cleave the OPI molecule from AChE and thereby to restore its function. However, the group of OPI called nerve agents (e.g., tabun, sarin, soman, and VX; Fig. 2) undergoes a process called "aging", where some parts of the OPI molecule are cleaved and replaced by a hydroxyl group with a negative charge [1, 8]. After this aging process, an oxime is unable to counteract the inhibited AChE [9].

While OPI nerve agents were prepared in the first half of the twentieth century, still no reactivator of AChE able to counteract the full spectrum of different OPI has been discovered [2]. Presently, the HI-6 oxime is the most versatile reactivator of AChE for intoxication by nerve agent with the exception of tabun [10]. Moreover, the changes induced by tabun into amino acids of AChE lead to a partial closing of the enzyme active site. As a result, only a few reactivators, obidoxime, and TMB−4, are able to counteract its effect [8, 9]. A drawback of these oximes is their higher toxicity than that of HI-6 [11].

In this study, we present some results concerning crystal structures of newly synthesized highly active reactivators, K075, and K114, as well as a discussion of their potential interactions with the enzyme.

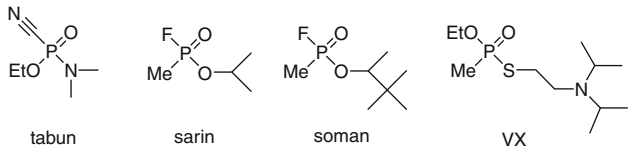

Fig. 2 OPI nerve agents—organophosphorus inhibitors with lethal effects

Experimental

Compounds K075 and K114 were synthesized according to the known procedure shown in Scheme 1. The heating of pyridinium aldoximes with dibromo linkers afforded the desired products in good yield and purity [12–14].

Single crystals of compounds K075 and K114 were obtained from water solutions by slow evaporation. The phase problem was solved by direct methods using SHELXS program [15]. The structures were refined by full-matrix least-squares method using SHELXL97 [15] with anisotropic displacement parameters for non-hydrogen atoms. All the hydrogen atoms bonded to the carbon atoms were located in the difference Fourier maps and refined isotropically using the riding model. The hydrogen atoms bonded to the oxygen atoms were located in difference Fourier maps and included in the refinement without constraints. The details of the crystal data, data collection, and refinement are listed in Table 1. The supplementary crystallographic data obtained for K075 and K114 have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition numbers: CCDC 714420 and CCDC 714421, respectively. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033; email: deposit@ccdc.cam.ac.uk].

Scheme 1 Synthesis of investigated AChE reactivators

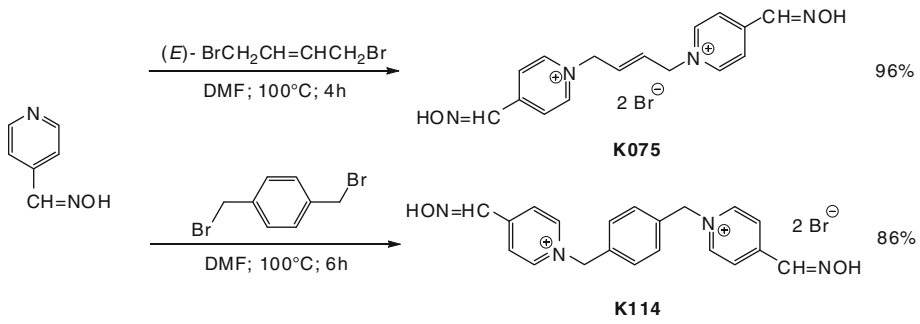

Table 1 Crystal data, the measurement and calculation details

Identification code	K075	K114
Empirical formula	$C_{16}H_{22}Br_2N_4O_4$	$C_{20}H_{20}Br_2N_4O_2$
Formula weight (g/mol)	494.20	508.22
Diffractometer	Nonius KappaCCD [16]	Nonius KappaCCD [16]
Temperature (K)	293(2) K	293(2) K
Crystal system, space group	Monoclinic, $P2_1/c$	Orthorhombic, Pcab
Unit cell dimensions	a = 9.1318 (2),	a = 7.9405 (2),
a, b, c (Å)	b = 9.0531 (2),	b = 15.0311 (3),
α, β, γ (°)	c = 13.0659 (3),	c = 17.5202 (3),
	$\alpha = \gamma = 90$, $\beta = 108.979$ (1)	$\alpha = \beta = \gamma = 90$
Volume (Å^3)	1021.45 (4)	2091.11 (8)
Z, calculated density (g/cm^3)	2, 1.607	4, 1.614
Absorption coefficient (mm^{-1})	3.996	3.900
$F(000)$	496	1016
Crystal size (mm)	0.60 × 0.35 × 0.25	0.25 × 0.20 × 0.15
θ range for data collection (°)	3.26–30.03	3.57–27.47
Limiting indices	$12 \le h \le 12$,	$-9 \le h \le 10$,
	$-12 \le k \le 11$,	$-18 \le k \le 19$,
	$-18 \le l \le 18$	$-22 \le l \le 22$
Reflections collected/unique	9115/2975 [$R_{(int)} = 0.0347$]	15124/2385 [$R_{(int)} = 0.0373$]
Completeness (%)	99.4	99.6
Absorption correction	Multi-scan [17]	Multi-scan [17]
Max. and min. transmission	0.4349 and 0.1978	0.5923 and 0.4423
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	2975/3/127	2385/0/130
Goodness-of-fit on F^2	1.080	1.047
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0350$,	$R_1 = 0.0331$,
	$wR_2 = 0.0769$	$wR_2 = 0.0750$
R indices (all data)	$R_1 = 0.0544$,	$R_1 = 0.0528$,
	$wR_2 = 0.0846$	$wR_2 = 0.0856$
Largest diff. peak and hole (e.A^{-3})	0.272 and −0.508	0.267 and −0.594

Results and discussion

The unit cell of K075 contains two (E)-1,4-bis(4-hydroxyiminomethylpyridinium)-but-2-ene cations, four bromide anions, and four water molecules. The unit cell of K114 consists of four 4,4′-bis(hydroxyiminomethyl)-1,1′-(1,4-phenylenedimethyl)-bispyridinium cations and eight bromide anions. The ORTEP [18] projections of the structures of both molecules together with water and bromide ions are shown in Figs. 3 and 4, respectively.

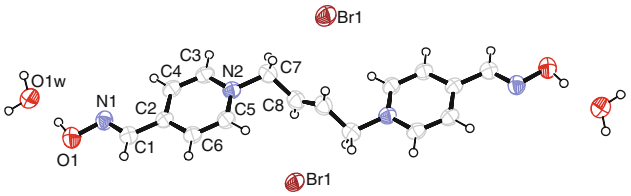

Fig. 3 The ORTEP drawing of the K075 salt and two co-crystallising water molecules

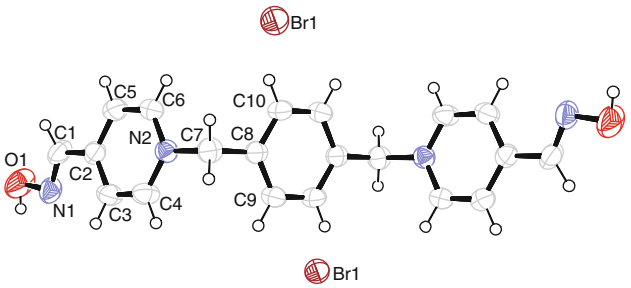

Fig. 4 The ORTEP drawing of the K114 salt

Structure of molecules in comparison with obidoxime and TMB-4

The oxime groups in both compounds have E−configuration. Both molecules are of a zigzag shape. The molecule of K075 has an inversion center, which coincides with the midpoint of the double bond between aliphatic carbon atoms, while in K114—with the middle of benzene ring. In this respect, K075 and K114 differ from the molecules of the known AChE reactivators, obidoxime [19], and TMB-4 [20]. The symmetry element of obidoxime in its chloride crystal is the crystallographic twofold axis, which passes through the central oxygen atom of the linker between two pyridine rings. The molecule of TMB-4 has an approximate (non crystallographic) twofold symmetry. Because of the molecular symmetry of K075 and K114, the values of the torsion angles which determine the mutual orientation of the pyridine ring and the linker are the same on both sides of the molecule center, namely: N2-C7-C8-C8′ = 121.6° in K075 and N2-C7-C8-C9 = 83.7° and N2-C7-C8-C10 = 95.6° in K114, respectively. The corresponding torsion angle value in obidoxime is 76.5°, while in TMB-4 two values of the torsion angles are observed: 69.9° and 66.02°. The absolute values of pseudo-torsion angles, N–C…C–N, where C denotes the first and the last atom of the linker between two pyridine rings, are 180° for the centrosymmetric K075 and K114 molecules, while for obidoxime and TMB-4 they are 135.7° and 111.6°, respectively. The differences in the lengths of the linkers and in the symmetry of the molecules lead to the differences in the overall lengths, i.e., the distances between oxygen atoms of the oxime groups. These lengths vary in the order: K075 > K114 > obidoxime > TMB-4 and are: 18.07, 17.69, 14.71, and 14.92 Å, respectively.

Intermolecular interactions

Hydrogen bonds are the most important intermolecular interactions in the crystal of K075. Their parameters are listed in Table 2. For visual interpretation of intermolecular interactions, the Mercury program was used [21]. The chains of water molecules hydrogen-bonded with bromide ions form a sort of channels along *y* axis (Fig. 5). Each water molecule is an acceptor of a proton belonging to an oxime group of one cation and a proton donor to two bromide anions. A chain with repeating fragment …H1W-O1W-H2W…Br1…(H1W), formed in this way, can be described using Etter notation as $C_1^1(4)$ [22]. The same hydrogen bonds also form a ring $R_8^{10}(58)$. Both patterns of hydrogen bonding are shown in Fig. 5.

Other short contacts, which may be qualified as O…π and N…π intermolecular interactions, are shown in Table 3. These interactions lead to intercalation of each

Table 2 Intermolecular hydrogen-bonding geometry in the crystal structures of K075, K114, obidoxime and TBM-4

D-H…A	d(D-H) (Å)	D(H…A) (Å)	d(D…A) (Å)	<(DHA) (°)
K075				
O1W-H1W···Br1#2	0.92 (2)	2.33 (2)	3.238 (2)	169 (2)
O1W-H2W···Br1#3	0.91 (2)	2.36 (2)	3.267 (2)	176 (3)
O1-H1O···O1W	0.91 (3)	1.77 (3)	2.669 (2)	171 (3)
#2 [$x + 1, y + 1, z$]; #3 [$-x + 2, y + 1/2, -z + 1/2$]				
K114				
O1-H1O···Br1#2	0.81 (4)	2.35 (4)	3.139 (2)	168 (4)
#2 [$x + 1/2, -y + 1/2, z$];				
Obidoxime [19]				
O1-H1O···Cl1	0.98	2.07	2.973	153
TMB-4 [20]				
O1-H1O···Br1	0.83	2.35	3.183	176.8

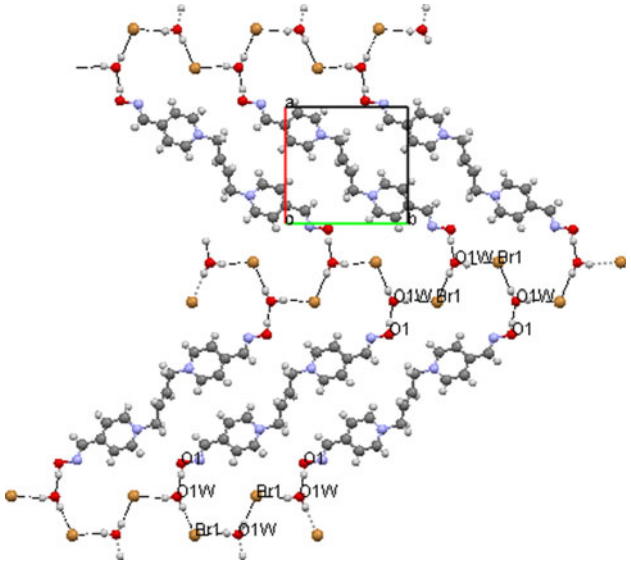

Fig. 5 Hydrogen-bonding pattern in K075

Table 3 Selected interactions in AChE complex with obidoxime and in crystal structures of K075 and K114

Shortest contacts (Å)	Crystal structures of		
	AChE-obidoxime	K075	K114
C(pyridine) …aromatic ring	3.27, 3.14	Not observed	3.69, 3.72
N(oxime) …aromatic ring	3.14, 3.47	3.23, 3.50	3.48, 3.59
O(oxime) …aromatic ring	3.44	3.39	3.14

pyridinium ring between the oxime group on one side and the water molecules on the other.

The crystal structure of K114 is stabilized by the hydrogen bonds between the hydroxyls of oxime groups and bromide anions (Table 2). "Quasi"-stacking of molecules along *x* axis becomes a very pronounced feature of the structure most probably due to the lack of water molecules in this structure (Fig. 6). The distance between the centers of pyridinium rings of the neighboring molecules, related by the glide plane "a" perpendicular to *y* axis, is 4.006 Å. The angle between the ring planes is 15.27°.

Comparison of structural motifs in K075, K114, TMB-4 and obidoxime

The type of motifs occurring in the packing of molecules in the crystal structures of AChE reactivators depends on the co-crystallising species, i.e., anions and/or water molecules (Table 2).

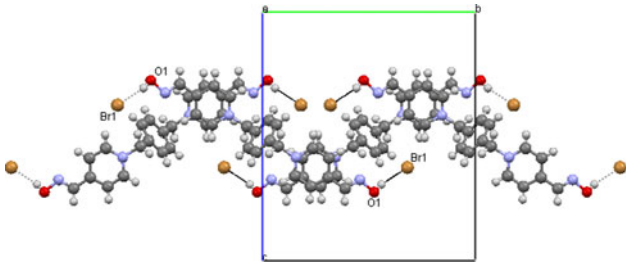

Fig. 6 Hydrogen-bonding pattern in K114

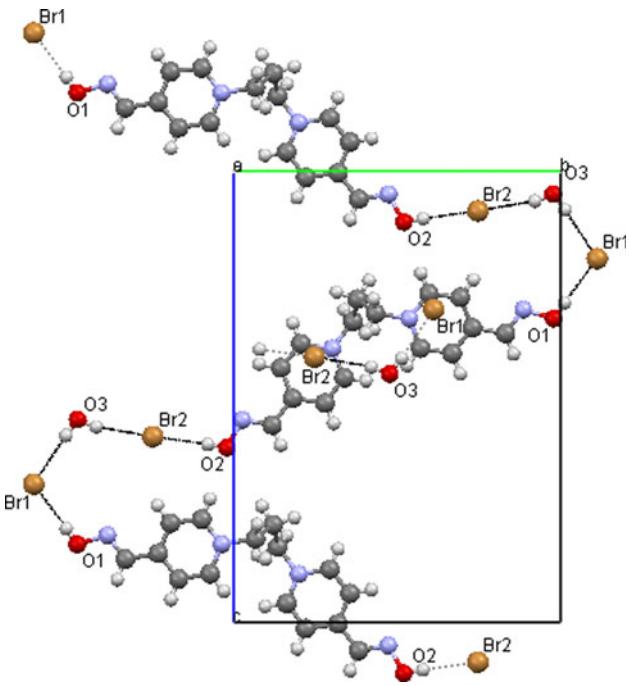

Fig. 7 Hydrogen-bonding pattern in TMB-4 [20]

The motifs characteristic for K075 and TMB-4 [20] crystals are the loop-shaped chains linking the molecules in pairs. They consist of three water molecules and two bromide anions in K075 crystal (Fig. 5). In TMB-4, the loops are shorter (one water molecule and two bromide ions) resulting in a more "open" structure (Fig. 7).

The motifs in K114 and obidoxime [19] crystals, shown in Figs. 6 and 8, respectively, are mainly the result of the "organizing" role of bromide and chloride anions which form N–O–H…Br and N–O–H…Cl hydrogen bonds.

Inspection of enzyme–reactivator interactions

Regarding the activity of the investigated compounds as AChE reactivators, it is worthwhile to compare the intermolecular interactions observed in their crystal structures

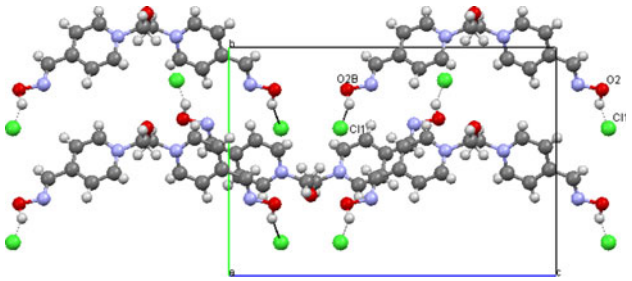

Fig. 8 Hydrogen-bonding pattern in obidoxime [19]

with those in the oxime complexes that are able to reactivate AChE-tabun conjugates.

The crystal structures of such complexes, formed by the known reactivators: HI-6, Ortho-7, and obidoxime with non-phosphorylated AChE, were determined by Ekstrom et al. [23].

These authors described also the modes of reactivator-enzyme binding. According to Ekstrom, the most important interactions with AChE amino acids are formed by such reactivator fragments as the pyridine and benzene rings, nitrogen, and oxygen atoms of oxime groups as well as by water molecules. Thus, in Table 3 we compare the parameters of selected hydrogen bonds and pyridine contacts, which occur in the crystal structures of K075 and K114, with those observed in the AChE–obidoxime complex.

In Figs. 9a and b, the surroundings of each of the obidoxime pyridinium rings are separately depicted. One of them, together with the oxime group, is located in a "sandwich-like" way between aromatic rings of Trp-286 and Tyr-72 side chains of AChE in its peripheral anionic site (PAS), while the oxygen atom, O1, forms a hydrogen bond with the carboxylic group of Val-282 (Fig. 9a). The other pyridine moiety of obidoxime interacts with the ring of Tyr-337 and its oxime group—with Phe-338 as well as with water molecule through hydrogen bonding; both these amino acid residues belong to the AChE catalytic site (Fig. 9b).

Inspection of the crystal structure fragments of K075 and K114 (Figs. 10a and b) shows that molecules of the new AChE reactivators are able to form interactions similar to those observed in the crystal of AChE complex with obidoxime. Particularly in K075, interesting contacts C4…C1 and N1…O1 resemble to those between C(pyridine) of obidoxime and aromatic ring of Trp-286 of AChE at peripheral anionic site (PAS). In the crystal structure of K114, the mutual arrangement of molecules is more complicated than in the case of K075, but there are several shorter contacts. In particular, distances: N1…C9 and O1…O6 are similar to those which occur in the AChE–obidoxime complex.

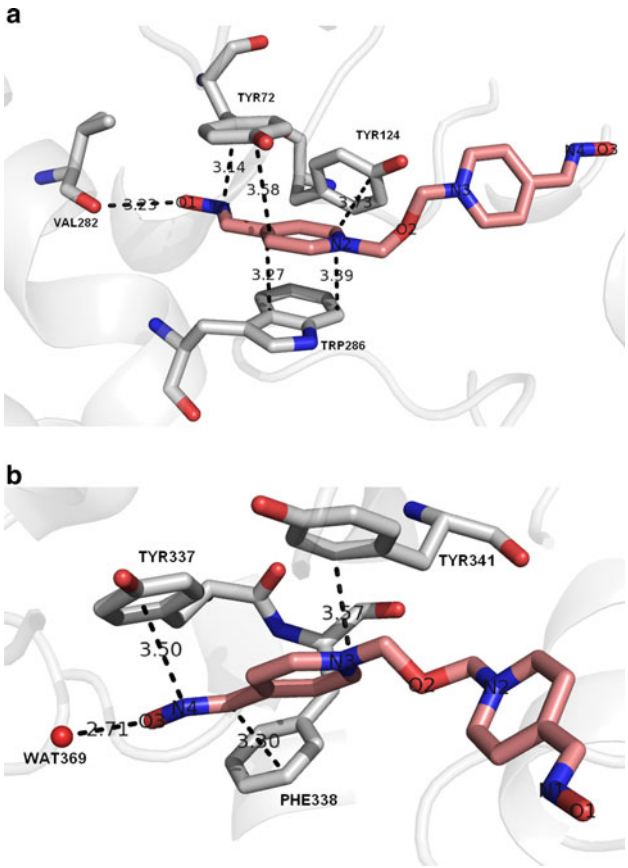

Fig. 9 Fragment of crystal structure of mAChE complex with obidoxime [23, 24]. The shortest distances (Å) between obidoxime and different residues of mAChE are marked with dashed lines. **a** Interactions with peripheral anionic site (PAS) of mAChE; **b** interactions with catalytic site of mAChE

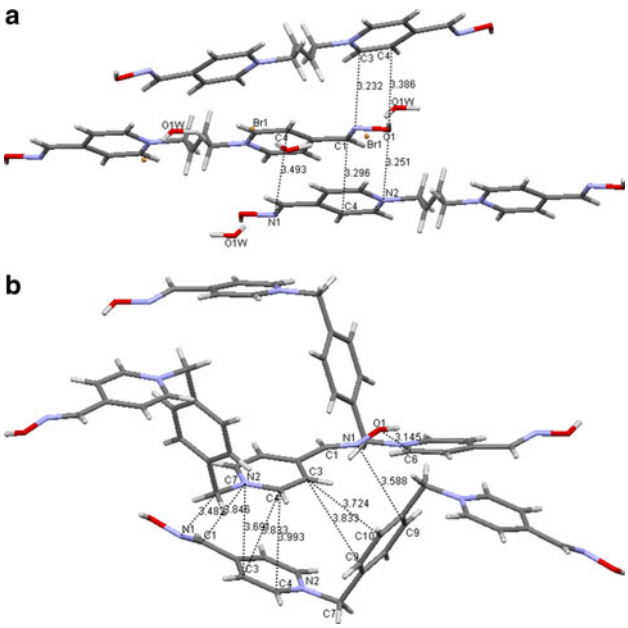

Fig. 10 Selected intermolecular interactions in the crystal structure of **a** K075 and **b** K114

Conclusions

The single crystal of (E)-1,4-bis(4-hydroxyiminomethylpyridinium)-but-2-ene (K075) and of 4,4′-bis(hydroxyiminomethyl)-1,1′-(1,4-phenylenedimethyl)-bispyridinium (K114) bromides was obtained and their structures were determined with the use of X-ray diffraction. K075 is a dihydrate in which the water molecules are hydrogen-bonded with oxygen atoms of oxime groups. In K114, the acceptors of protons of the oxime groups are bromide anions. The molecules of both compounds are centrosymmetric and in this respect they differ from the well-known obidoxime and TMB-4 whose symmetry is twofold and approximate twofold axis, respectively.

The intermolecular interactions in the crystals of K075 and K114 are similar to those in the crystalline hydrate of TMB-4 bromide and of obidoxime chloride, respectively. This similarity, together with the comparable reactivation potencies of K075 and obidoxime [13], suggests that the interactions occurring in their crystals may play an important role in the reactivation activity. This conclusion is confirmed by the comparison of the interactions observed in the crystalline AChE–obidoxime complex and in the investigated crystals.

Determination of the exact relationship between structure and AChE reactivation activity of bis-pyridinium oxime derivatives requires further biological and crystallographic studies.

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References

1. Marrs TC (1993) Pharmacol Ther 58:51
2. Bajgar J (2004) Adv Clin Chem 38:151
3. Newmark J (2007) Neurologist 13:20
4. Saxena A, Sun W, Luo C, Myers TM, Koplovitz I, Lenz DE, Doctor BP (2006) J Mol Neurosci 30:145
5. Hagedorn I, Gündel WH, Schoene K (1969) Arzneimittelforschung 19:603
6. Lüttringhaus A, Hagedorn I (1964) Arzneimittelforschung 14:1
7. Poziomek EJ, Hackley BE, Steinberg GM (1958) J Org Chem 23:714
8. Luo C, Tong M, Maxwell DM, Saxena A (2008) Chem Biol Interact 175:261
9. Ekstrom F, Akfur C, Tunemalm AK, Lundberg S (2006) Biochemistry 45:74
10. Lundy PM, Raveh L, Amitai G (2006) Toxicol Rev 25:231
11. Bartosova L, Kuca K, Kunesova G, Jun D (2006) Neurotox Res 9:291
12. Kuca K, Cabal J, Musilek K, Jun D, Bajgar J (2005) J Appl Toxicol 25:491
13. Musilek K, Kuca K, Jun D, Dohnal V, Dolezal M (2006) Bioorg Med Chem Lett 16:622
14. Musilek K, Kuca K, Jun D, Dohnal V, Dolezal MJ (2005) J Enzym Inhib Med Chem 20:409
15. Sheldrick GM (1998) SHELX97 [includes SHELXS97, SHELXL97, CIFTAB]—programs for crystal structure analysis (Release 97-2). Institüt für Anorganische Chemie der Universität, Göttingen, Germany
16. Nonius (1997) COLLECT. Nonius BV, Delft, The Netherlands
17. Otwinowski Z, Minor W (1997) Methods in enzymology. In: Carter CW Jr, Sweet RM (eds), Macromolecular crystallography, part A, vol 276. Academic Press, New York, p 307
18. Farrugia LJ (1997) J Appl Crystallogr 30:565
19. van Havere W, Lenstra ATH, Geise HJ, van den Berg GR, Benschop HP (1982) Acta Crystallogr B 38:1635
20. Bustamante CD, Staples RJ (1999) Zeitschrift für Kristallographie 214:141
21. Macrae CF, Bruno IJ, Chisholm JA, Edgington PR, McCabe P, Pidcock E, Rodriguez-Monge L, Taylor R, van de Streek J, Wood PA (2008) J Appl Crystallogr 41:466
22. Etter MC, MacDonald JC, Bernstein J (1990) Acta Crystallogr B 46:256
23. Ekstrom F, Pang YP, Boman M, Artursson E, Akfur C, Borjegren S (2006) Biochem Pharmacol 72:597
24. Delano WL (2002) The Pymol molecular graphics system. http://www.pymol.org