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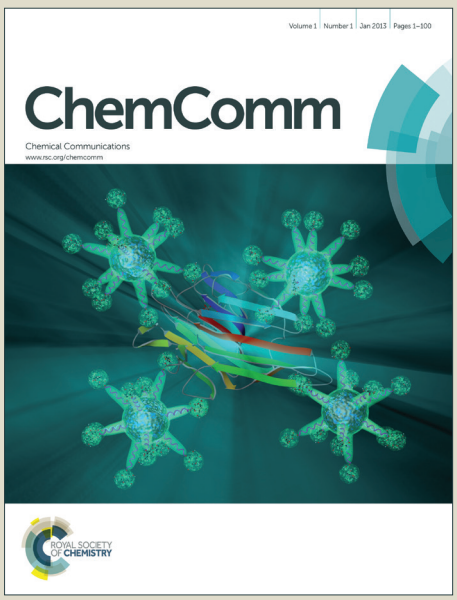

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Tryptoline-3-hydroxypyridinaldoxime conjugates as efficient reactivators of phosphorylated human acetyl and butyrylcholinesterases

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Two promising uncharged reactivators for inhibited human BChE and AChE have been described. These compounds show an ability to reactivate VX-inhibited BChE largely superior to those of known pyridinium aldoxime. Moreover, these oximes also exhibit a good ability to reactivate VX-, tabun- and paraoxon-inhibited human AChE.

Poisoning by organophosphorus (OP) compounds remains a serious health problem worldwide (Fig. 1). OP were originally developed as pest control agents but were also used as chemical warfare agents on the battlefield in armed conflicts. They were used intentionally against the Iraqi Kurdish population in the late 1980s. They have also been used in terrorist attacks in Tokyo subway in 1995 and more recently during the civil war in Syria. Moreover, OP pesticides intoxication causes 200,000 fatalities annually worldwide.[1]

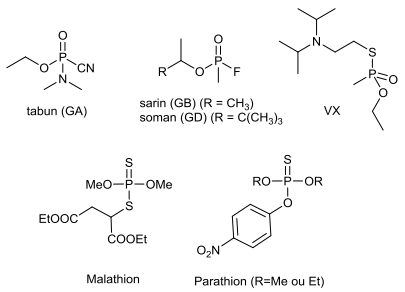


Figure 1 Examples of some warfare agents (top line) and pest control agents (lower line)

The acute toxicity of OP results from the irreversible inhibition of acetylcholinesterase (AChE), which regulates cholinergic transmission in the peripheral and central nervous system (CNS) by hydrolysing acetylcholine (ACh). The inhibition of AChE causes the accumulation of the neurotransmitter ACh in the synaptic cleft, leading to overstimulation of cholinergic receptors, seizures, respiratory arrest, and death. Depending on the class of OP and the administrated dose, death can occur within minutes Therefore, it is important to develop effective antidotes against OP poisoning. Among the therapeutic approaches currently studied to develop effective countermeasures, the reactivation of OP-inhibited AChE[2] and the detoxification of OP with bioscavengers[3] in the bloodstream are two of the most advanced approaches.

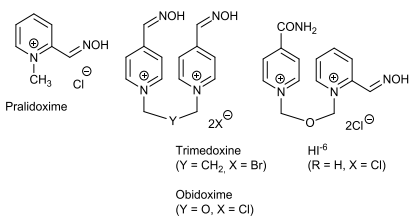
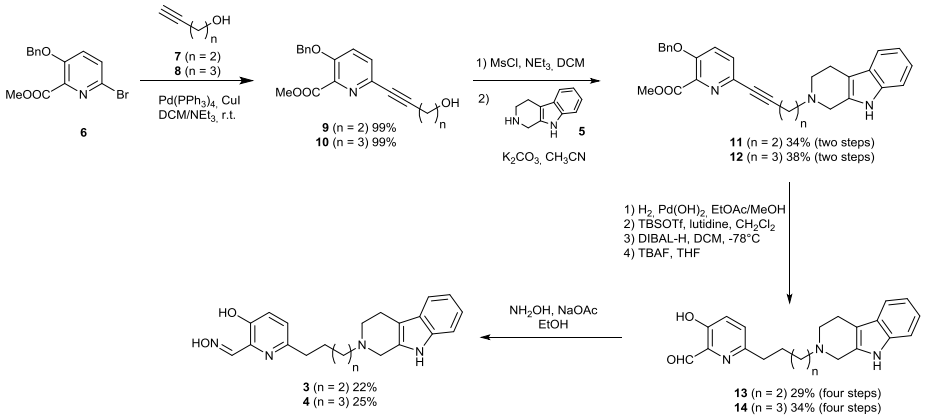


Figure 2 Structures of AChE reactivators

The emergency treatment of OP poisoning consists of the rapid administration of a mixture composed of an antimuscarinic agent (e.g. atropine), an anticonvulsant drug (e.g. diazepam) and mono or bispyridinium aldoxime as AChE reactivators (Fig. 2). Nevertheless, after 50 years of research to develop new pyridinium oxime reactivators,[2] the latter have still major drawbacks. First, they poorly cross the blood brain barrier (BBB) and do not readily reactivate AChE in the CNS. Second, no single oxime is efficient against a wide variety of OP. Third, oximes cannot reactivate "aged" AChE. Therefore, this treatment is presently imperfect. Several research teams, including ours, focus their efforts on developing new generation of AChE reactivators.[4-11]



Scheme 1 Synthesis of reactivators **3** and **4**

The detoxification of OP by bioscavengers consists of the administration of exogenous enzyme able to react with OP before they reach AChE and other physiological targets.[3] The most advanced bioscavenger is presently human butyrylcholinesterase (BChE),[12, 13] which is being developed by the US Department of Defense as a preventive treatment against OP poisoning. This enzyme acts as a stoichiometric bioscavenger and a large dose of enzyme (200 mg) is estimated to protect a human against 2 x LD_{50} of soman. The development of a pseudo-catalytic bioscavenger, which consists of a combination with a reactivator, can improve OP scavenging. In this case, the reactivator would recycle the phosphorylated BChE, which can then hydrolyse another molecule of OP. Unfortunately, known mono and bispyridinium aldoximes, developed for the reactivation of OP-inhibited AChE, are inefficient to reactivate OP-inhibited BChE,[14-17] specially due to the different topology of both enzymes.[18] Therefore, the development of efficient reactivators for both enzymes represents nowadays a challenging task for the discovery of novel treatments against OP poisoning. Radić *et al.* have reported a molecule **TAB2OH** showing an ability to reactivate VX-BChE 2.6 fold higher than those of pralidoxime, but it remains less efficient than pralidoxime for OP-inhibited AChE (23 fold less efficient for VX-inhibited AChE, 9.5 fold less efficient for tabun-AChE, and 2.7 fold less efficient for paraoxon-inhibited AChE).[19]

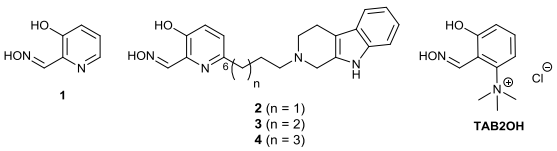


Figure 3 Structures of 3-hydroxy-pyridinaldoxime **1**, tryptoline-3-hydroxy-pyridinaldoxime conjugates **2-4** and **TAB2OH**.

Recently, we reported new uncharged reactivators aimed at crossing the BBB to reactivate OP-inhibited AChE in the CNS. Phenyltetrahydroisoquinoline-3-hydroxy-pyridinaldoxime conjugates exhibited abilities to reactivate OP-inhibited human AChE superior to those of mono and bispyridinium reactivators in *in vitro* assays.[10, 11] These results encouraged us to design and synthesize new uncharged reactivators of OP-inhibited human BChE by associating the reactivator function, 3-hydroxy-pyridinaldoxime **1**,[20, 21] with different ligands of the peripheral anionic site (PAS) of the enzyme. In order to have more active reactivators aimed at BChE reactivation, and to limit the inhibition of the dephosphylated enzymes, we have chosen tryptoline as PAS ligand, exhibiting a moderate affinity toward both enzymes (Fig. 3).[22] Molecular docking studies suggested that in order for the oxime function to be conveniently positioned towards the phosphorylated serine, the best tryptoline-based reactivators are the compounds with a linker of 3-5 methylenes groups attached onto the position 6 of the pyridine ring.

The synthesis of the novel compounds of interest **3**, **4** (Scheme 1) follows the strategy developed for the preparation of the hybrid **2** with a shorter linker, recently reported.[9] The first step consisted of the Sonogashira reaction of alkynes **7** or **8** with bromo-pyridine **6**. The desired alcohols **9** and **10** were obtained in excellent yield. Formation of the corresponding mesylates followed by a nucleophilic substitution with the tryptoline **5** furnished the compounds **11** and **12** in 34% and 38% yield respectively. This was followed by the reduction of the alkyne and deprotection of the phenol function, followed by a sequence comprising the protection of the phenol group as a TBS ether and subsequently, the reduction of methyl ester into corresponding aldehyde by using DIBAL-H, and a subsequent deprotection with TBAF furnished the aldehydes **13** and **14** in 29% and 34% yields over four steps. The last step consisted of the formation of the oxime function with the condensation of hydroxylamine onto the aldehyde. Altogether, the desired oximes **3** and **4** have been obtained in eight steps with 2% and 3% overall yield respectively.

Table 1 Reactivation rate constant (k_r), dissociation constant (K_D) and specific reactivation rate constant (k_{r2}) for the reactivation of VX-hBChE.

	k_r (min^{-1})	K_D (μM)	k_{r2} ($mM^{-1}.min^{-1}$)
Pralidoxime	1.6±0.6	2800±1200	0.57
Obidoxime	0.21[a]	409[a]	0.5[a]
HI-6	>0.11	>400	0.28±0.01
2	>0.8	>100	8±0.3[b]
3	0.77±0.04	51.8±3.9	15
4	>0.5	>100	4.5±0.3[b]

[a] from reference [14], [b] linear relationship between [Ox] and reactivation rate

The determination of apparent dissociation constant (K_D), the maximal reactivation rate (k_r), and the bimolecular rate constant ($k_{r2}= k_r/K_D$) have been measured, in order to evaluate the potential of oximes **2-4** for *in vitro* reactivation of VX-hBChE (Table 1). The results show that the compounds **2-4** are extremely promising reactivators for VX-hBChE. In particular, oxime **3** is 30-fold more efficient that obidoxime, the best VX-BChE reactivator known to date, with a higher reactivation rate (2.8-fold superior) and a higher affinity (14-fold superior) toward phosphorylated enzyme. Moreover, the compounds **2** and **3** are respectively 16-fold and 9-fold more efficient than obidoxime, respectively. The good affinity of these oximes

toward VX-BChE could be explained through a π-π interaction between tryptoline moiety and Tyr332 at the gorge entrance of hBChE.[23] Affinity of **2**, **3** and **4** toward native hBChE are 12 µM, 2.2 µM and 1.6 µM respectively. These oximes have been then evaluated for the reactivation of OP-inhibited-hAChE (Table 2). The compounds **3** and **4** shows also good abilities to reactivate AChE. For details, **4** is as efficient as HI-6 for VX-hAChE, while **3** equals trimedoxime for tabun-hAChE. In comparison with pralidoxime, **3** and **4** are up to 30 fold more efficient for VX-AChE, up to 60 fold more efficient for tabun-AChE and up to 10 fold more efficient for paraoxon-AChE. Affinity of these oximes **3** and **4** toward native hAChE are 60 µM and 40 µM respectively.

Table 2 Reactivation rate constant (k_r), dissociation constant (K_D) and specific reactivation rate constant (k_{r2}) for the reactivation of OP-hAChE.

	k_r (min^{-1})	K_D (µM)	k_{r2} ($mM^{-1}.min^{-1}$)
VX-hAChE			
pralidoxime	0.06±0.01	215±75	0.3
Obidoxime	0.60±0.05	54±12	11
HI-6	0.44±0.15	50±26	9
3	5.6±0.8	885±140	6
4	1.1±0.1	125±25	9
Tabun-hAChE			
pralidoxime	0.01±0.0005[a]	706±76[a]	0.01[a]
Trimedoxime	0.085±0.005	145±25	0.6
3	0.06±0.003	100±8	0.6
4	0.06±0.01	180±40	0.3
Ethyl paraoxon-hAChE			
pralidoxime	0.17±0.007[a]	187±19[a]	0.91[a]
Obidoxime	1.22±0.01[b]	65±17[b]	19[b]
3	1.7±0.2	190±40	9
4	0.51±0.03	83±11	6

[a] from reference [24]. [b] from reference [25]

In summary, we described a promising family of AChE and BChE reactivators. These results are very encouraging for the development of new treatment against OP poisoning and especially for the development of efficient pseudo-catalytic bioscavengers.

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Notes and references

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