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Bis-pyridiumaldoxime reactivators connected with $CH_2O(CH_2)_nOCH_2$ linkers between pyridinium rings and their reactivity against VX

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Abstract—New bis-pyridinium oxime reactivators connected with $CH_2O(CH_2)_nOCH_2$ linkers between two pyridinium rings were designed and synthesized, and their reactivation potency was evaluated for AChE inhibited by organophosphorus VX agent. Among the prepared compounds, 1,2-dimethoxy-ethylene-bis-*N*,*N*′-4-pyridiumaldoxime dichloride **5a** was the most potent and appeared to be the most promising compound as a potential reactivator for AChE inhibited by organophosphorus VX agent.

Exposure to organophosphorus nerve agents such as sarin, soman, cyclosarin, and VX causes acute intoxication and this toxic effect is due to the inhibition of acetylcholinesterase (AChE) followed by the increase in the amount of the neurotransmitter acetylcholine (ACh) at central and peripheral sites.[1] AChE is one of the most efficient enzymes known, and ligand binding studies and X-ray crystallography of *Topedo california* AChE (*Tc*AChE) reveal a narrow active site gorge 20 Å deep with two separate ligand binding sites.[2] The acylation site at the bottom of the gorge contains residues involved in a catalytic triad (in human AChE, E334, H447, and S203), which binds to the trimethylammonium group of acetylcholine. The peripheral site at the mouth of the gorge includes, among others, residue W286.[3] In an effort to improve drug potency and selectivity, the bivalent ligand strategy was applied to the development of AChE-targeted therapeutic agents such as treatment of Alzheimer's disease (AD).[4] In the studies of bistacrine with alkyl spaces of varying lengths confirmed that heptylene-linked tacrine dimer, bis(7)-tacrine, possessed optimum AChE inhibition potency; bis(7)-tacrine showed significantly higher potency for inhibition of rat AChE than monomeric tacrine.[5] The superior inhibitory capacity of bis(7)-tacrine relative to tacrine is attributed to dual-site binding, and studies of related ligands on rat AChE also demonstrated beneficial hydrophobic effects imparted by the alkylene tether to the peripheral site ligand.[6] The importance of these dual-site binding properties was applied to the design of bis-pyridiumaldoximes to develop potent reactivator for AChE inhibited by organophosphorus nerve agents. Recently, several bis-pyridiumaldoximes linked by a variable-length alkylene chain as AChE reactivators were designed by using a ligand docking program and the X-ray crystal structure of AChE.[7] This work was based upon the identification of two potential binding sites for bifunctional AChE inhibitors: a high-affinity tryptophan residue at position 84, deep in the catalytic gorge, and a low-affinity tryptophan residue at position 279, near the AChE surface. For example, Pang and his coworkers developed a dimeric oxime **2**, 1,7-heptylene-bis-*N*,*N*′-pyridiumaldoxime dichloride. The oxime **2b** is 100 times more potent than pralidoxime in reactivating hAChE after exposure to echothiophate or isoflurophate.[7]

Continuing our effort to develop new oxime reactivators, we newly designed and evaluated the substances differing from the currently used oximes in the length and atoms connecting chain between two pyridinium

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rings.[8] We are especially interested in oxygen atom, because oxime reactivators having the linkers of oxygen atom and methylene such as HI-6 show stronger reactivation activity compared to other commercially available reactivators (obidoxime and pralidoxime). These above-mentioned speculations prompted us to design new oximes in which a longer ether linker was introduced to connect two pyridine rings. In this report, the two pyridine rings of bis-pyridiumaldoximes were connected with $CH_2O(CH_2)_nOCH_2$ linkers between the two quaternary nitrogens.

Bis-pyridiumaldoxime **5** connected with $CH_2O(CH_2)_n$-OCH_2 linkers between the two quaternary nitrogens were obtained from the alkylation of the corresponding aldoxime **4** with 1,2-bis-chloromethoxyethane **3a** and 1,4-bis-chloromethoxybutane **3b**. A mixture of **3a** and 2.3 equiv of pyridine aldoxime (**4a** or **4b**) in MeCN was heated at 45 °C for 20 h to give a bis-pyridiumaldoxime **5a** or **5b**. In the same method, bis-pyridiumaldoxime **5c** or **5d** was obtained from **3b** and pyridiumaldoxime **4a** or **4c** in DMF. The newly synthesized bis-pyridiumaldoxime **5** involving $CH_2O(CH_2)_n$-OCH_2 linkers were identified by their ^1H NMR spectra and mass spectra.[9] Preparation of 1,2-bis-chloromethoxyethane **3a** and 1,4-bis-chloromethoxybutane **3b**, and detail synthetic method of the bis-pyridiumaldoxime **5** will be reported soon in due course.

Oxime HI-6 and **2a**[7] were prepared earlier at the Korea Research Institute of Chemical Technology (Korea) and pralidoxime was purchased from Sigma–Aldrich. Nerve agent VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate) was obtained from the Military Facility Brno (Czech Republic) in 97% purity. In vitro testing of synthesized reactivators involved a standard collection of experimental procedures. The whole method is in detail described in the work of Kuca and Cabal.[10] The reactivation efficacy of tested reactivators was evaluated in 10% rat brain homogenate that was incubated with agent VX for 30 min and then, the tested oxime of appropriate concentration (10^{-5} and 10^{-3} M) was added. After 10 min of reactivation, the activity of brain AChE was measured using potentiostatic method with the usage of automatic titrator RTS 822 (Radiometer, Denmark). The data about initial rate of enzyme reaction with substrate made possible the calculation of the percentage of increase in the activity of reactivated enzyme in the reaction mixture (Figure 1).

Reactivation potency of newly synthesized reactivator **5** was evaluated using VX as an organophosphorus agent, and the results are shown in Table 1 and Figure 2. The

Table 1. Reactivation potency of newly synthesized reactivators against VX

Oxime reactivators	Reactivation potency[a] (%)	Reactivation potency[b] (%)
5a	44	15
5b	26	12
5c	13	7
5d	5	0
2a	2	10
Pralidoxime	34	0
HI-6	28	13

[a] Reactivation potency was tested at 10^{-3} M concentration.
[b] Reactivation potency was tested at 10^{-5} M concentration.

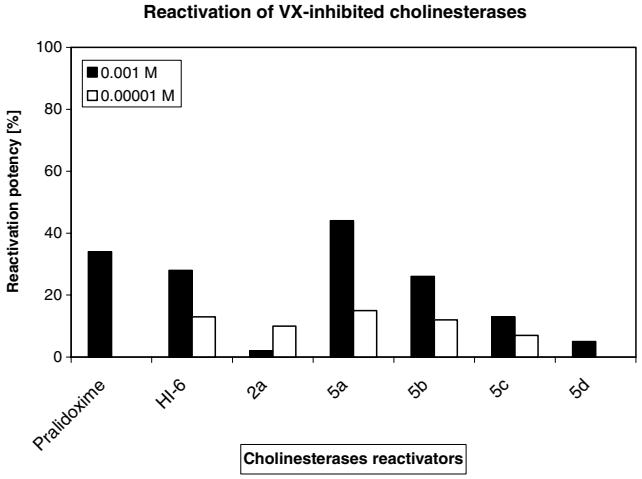


Figure 2. Reactivation potency of newly synthesized reactivators compared with pralidoxime and HI-6 (source of enzyme—rat brain homogenate; inhibitor—agent VX; time of inhibition—30 min; time of reactivation—10 min; pH 7.6; 25 °C).

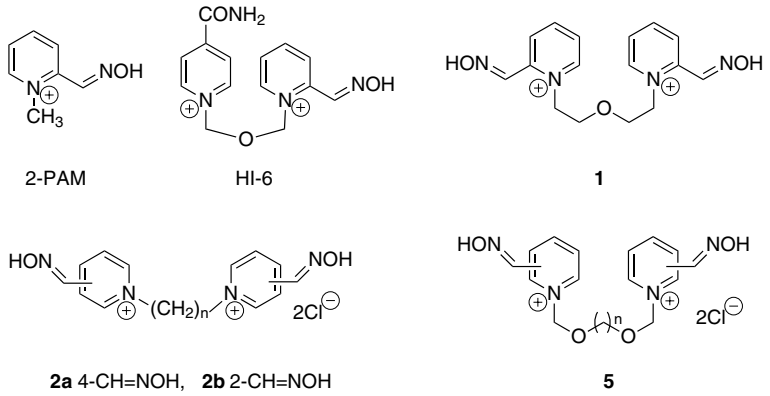
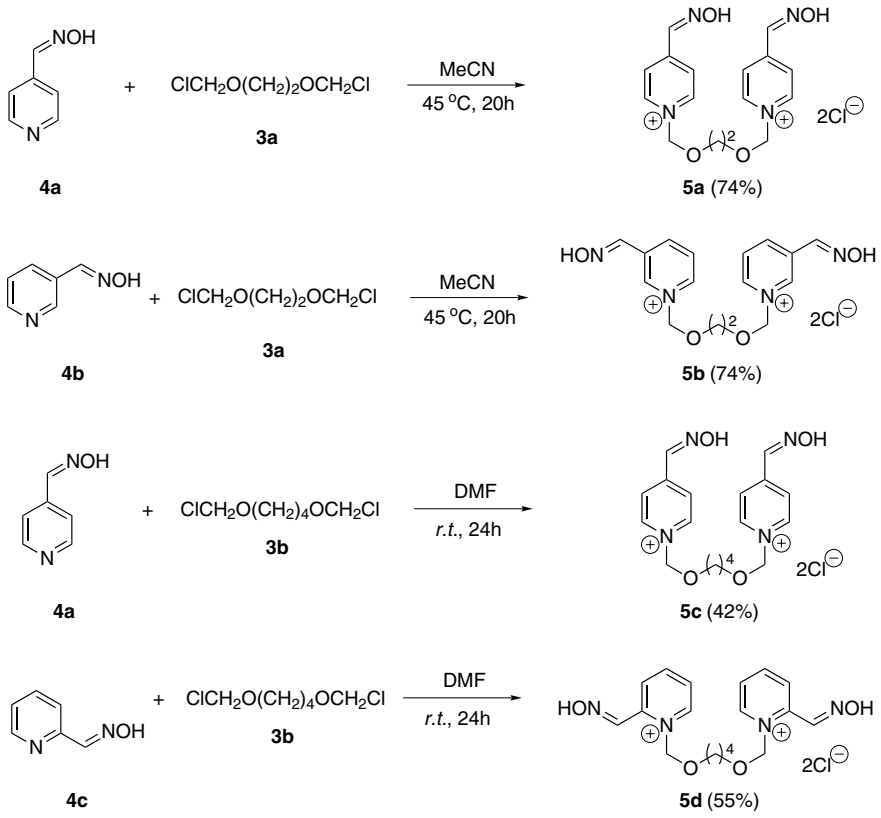


Figure 1. Structures of AChE reactivators.



Scheme 1. Synthesis of the bis-pyridiumaldoxime **5**.

compounds (**5a**–**c**) showed higher reactivation potency at 10^{-3} M concentration compared to the concentration of 10^{-5} M. Their reactivation potency, especially for **5a** and **5b**, was comparable with the potency of pralidoxime or HI-6. On the contrary, the oxime **5d** was the weakest reactivator in this study tested. At the lower 10^{-5} M concentration of the tested compounds, the compounds **5c**, **5d** and pralidoxime appeared to be poor reactivators, and **5a**, **5b**, **2a**, and HI-6 were found to exhibit moderate potency. This concentration (10^{-5} M) is considered to be attainable in human.[11] Among these compounds, **5a** was the most potent and appeared to be the most promising compound as a potential reactivator. Furthermore, at these concentrations, oxime **5a** surpassed also reactivation potency of currently the most promising oxime HI-6.[12]

The newly synthesized compounds (**5a**–**c**), especially **5a**, also showed much higher potency than **2a**. The main chemical difference in the structures between **5a** and **2a** is the incorporation with two oxygen atoms in the linker of **5a**, and this chemical difference highly influences the reactivation potency. Thus, the lone electron pairs on the oxygen atoms might cause additional interaction between the oxime **5a** and the inhibited AChE, however the oxime **2a** cannot have this type of interaction because of $(CH_2)_7$ linker. This hypothesis might be solved in future at molecular level using molecular modeling approach. It is generally known that reactivation potency of AChE reactivators depends on their chemical structure. There are many structural factors influencing their potency, for example, presence and position of quaternary nitrogen, presence, position, and number of oxime groups, and length and shape of the connection chain.[13]

In conclusion, we have synthesized the bis-pyridiumaldoxime reactivators connected with $CH_2O(CH_2)_nOCH_2$ linkers between two pyridinium rings, and evaluated their reactivation potency to reactivate AChE inhibited by organophosphorus VX agent. We examined the ability of the both linkers used in this study ($CH_2O(CH_2)_2OCH_2$ and $CH_2O(CH_2)_4OCH_2$) as a linker between two pyridinium rings to reactivate inhibited AChE by organophosphorus agents. Based upon this study, **5a** may provide a useful therapeutic potential for the reactivation of AChE inhibited by organophosphorus VX agent (Scheme 1).

Acknowledgments

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9. Compound **5a**: mp 162–165 °C; ^{1}H NMR (200 MHz, D_2O) δ 3.94 (s, 4H, OCH_2), 5.91 (s, 4H, NCH_2), 8.27 (d, J = 6.6 Hz, 4H, ArH), 8.40 (s, 2H, N=CH), 8.92 (d, J = 6.8 Hz, 4H, ArH); ^{13}C (50 MHz, D_2O) δ 69.4, 88.1, 124.3, 142.6, 145.8, 150.0; API-ES: *m*/*z* 332.1 [M-2Cl].
Compound **5b**: mp 155–157 °C; ^{1}H NMR (200 MHz, D_2O) δ 3.98 (s, 4H, OCH_2), 5.99 (s, 4H, NCH_2), 8.16–8.23 (m, 2H, ArH), 8.40 (s, 2H, N=CH), 8.83 (d, J = 8.2 Hz, 2H, ArH), 8.96 (d, J = 5.8 Hz, 2H, ArH), 9.21 (s, 2H, ArH); ^{13}C (50 MHz, D_2O) δ 69.5, 88.8, 127.9, 133.2, 140.1, 142.1, 143.8, 144.3; API-ES: *m*/*z* 332.1 [M-2Cl].
Compound **5c**: mp 128–130 °C; ^{1}H NMR (200 MHz, D_2O) δ 1.95–2.01 (m, 4H, CH_2CH_2), 3.75–3.81 (m, 4H, OCH_2), 5.88 (s, 4H, NCH_2O), 8.26–8.29 (m, 4H, ArH), 8.41 (s, 2H, N=CH), 8.90–8.94 (m, 4H, ArH); ^{13}C (50 MHz, D_2O) δ 27.7, 67.1, 88.1, 124.3, 142.5, 145.8, 149.9; API-ES: *m*/*z* 387.2 [M-CNOH].
Compound **5d**: mp 118–120 °C; ^{1}H NMR (200 MHz, D_2O) δ 1.54–1.68 (m, 4H, CH_2CH_2), 3.57–3.74 (m, 4H, OCH_2), 6.07 (s, 4H, NCH_2O), 8.06–8.14 (m, 2H, ArH), 8.49–8.53 (m, 2H, ArH), 8.60–8.64 (m, 2H, ArH), 8.71 (s, 2H, N=CH), 8.94–8.97 (m, 2H, ArH); ^{13}C (50 MHz, D_2O) δ 27.6, 73.0, 90.5, 129.5, 130.2, 144.6, 146.9, 149.1, 149.8; API-ES: *m*/*z* 359.0 [M-2Cl].
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