Malaysian Journal of Analytical Sciences
(MJAS)
Published by Malaysian Analytical Sciences Society

A BRIEF REVIEW ON THE DESIGN AND SYNTHESIS OF NEW ANTIDOTES IN THE TREATMENT OF ORGANOPHOSPHORUS POISONINGS

(Ulasan Ringkas pada Reka Bentuk dan Sintesis Antidot Baru untuk Merawat Keracunan Organofosforus)

Mas Amira Idayu Abdul Razak[1,2], Mohd Nor Faiz Norrrahim[2], Ong Keat Khim[2,3], Siti Aminah Mohd Noor[3],
Norhana Abd Halim[3], Noor Aisyah Ahmad Shah[3], Victor Feizal Knight[2], Wan Md. Zin Wan Yunus[2,3],
Noor Azilah Mohd Kasim[2,3]*

[1]Faculty of Defence Science and Technology
[2]Centre for Chemical Defence
[3]Centre for Defence Foundation Studies
Universiti Pertahanan Nasional Malaysia, Kem Perdana Sungai Besi, 57000 Kuala Lumpur, Malaysia

**Corresponding author: azilah@upnm.edu.my*

Received: 13 November 2019; Accepted: 3 September 2020; Published: 12 October 2020

Abstract

Organophosphorus (OP) are highly toxic compounds commonly used as pesticides and chemical warfare nerve agents. Exposure to OP compounds can lead to death due to respiratory failure. OP compounds function to inhibit the enzyme acetylcholinesterase (AChE) which controls the transmission of nervous impulses and accumulation of acetylcholine (Ach) at cholinergic receptor sites. The inhibition of AChE by OP compounds can cause motor convulsions and epileptic seizures. There are several drugs used to treat OP poisonings, such as atropine, diazepam, and oximes. However, none of them are considered to be effective in treating OP poisonings. Recently, several new researches have been done to introduce strategies on designing oximes to treat OP poisonings. Therefore, this brief review summarizes several recent findings on the design and synthesis of new oximes that can reactivate the inhibited AChE.

Keywords: organophosphorus, nerve agents, acetylcholinesterase, acetylcholine, oximes

Abstrak

Peranti agen saraf organofosforus (OP) sering digunakan sebagai agen racun perosak dan agen saraf dalam peperangan kimia. Pendedahan kepada OP boleh menyebabkan kematian akibat kegagalan sistem pernafasan. OP menghalang enzim asetilkolinesterase (AchE) yang berfungsi untuk mengawal penghantaran impuls saraf dan pengumpulan asetilkolin (Ach) di tapak reseptor kolinergik yang boleh menyebabkan sawan motor dan sawan epilepsi. Terdapat beberapa kaedah yang digunakan untuk merawat keracunan organofosforus seperti atropin, diazepam, dan oksim. Walaubagaimanapun, tidak ada satu pun yang berkesan untuk merawat keracunan OP. Baru-baru ini, beberapa penyelidikan telah dijalankan untuk memperkenalkan strategi baru dalam rek bentuk penawar atau antidot yang boleh merawat agen saraf OP. Oleh itu, tinjauan semula ini meringkaskan beberapa strategi baru dalam reka bentuk dan sintesis penawar atau antidot baru yang dapat mengaktifkan semula AchE yang terhalang.

Kata kunci: sebatian organofosforus, agen saraf, asetilkolinesterase, asetilkolin, antidot

Introduction

Organophosphorus (OP) are highly toxic compounds which are usually used as chemical warfare nerve agents, such as tabun, soman, sarin, and VX [1,2,3,4]. Figure 1 shows several examples of chemical warfare agents.

The OP compounds show their toxic effects by phosphorylating the active site of serine in acetylcholinesterase (AChE) [5]. It hydrolyses acetylcholine (ACh) to choline, thus terminating cholinergic neurotransmissions [6]. The subsequent irreversible inhibition of AChE results from the accumulation of ACh in both central and peripheral synapses [7], which causes several side effects, such as cholinergic crisis, respiratory distress, convulsive seizures, respiratory failure, and fatality [8].

Decades ago, the action mechanism of oximes was investigated [9]. Several indications showed that the oximes have the potency to reactivate the inhibited AChE. The inhibited AChE is reactivated by transferring the phosphoryl group from the AChE due to their large affinity and strong nucleophilicity [10,11]. During reactivation, phosphorylated oximes are formed. Some of the phosphorylated oximes appeared to be potent inhibitors of AChE [12,13]. Figure 2 shows the phosphorylated oximes which are commercially used in researches of OP treatments.

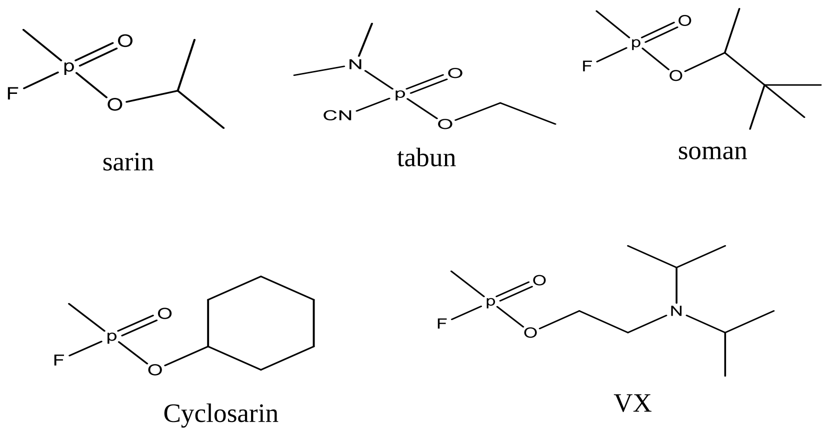
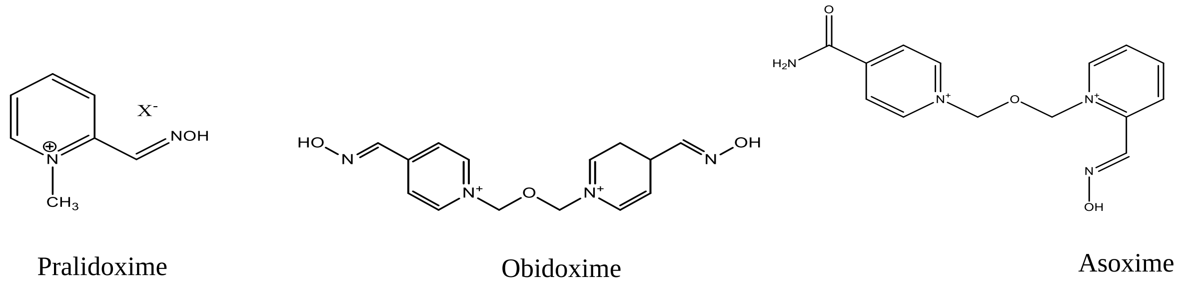
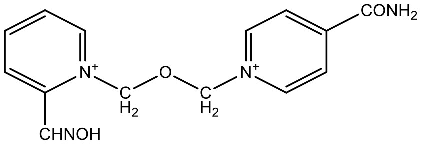
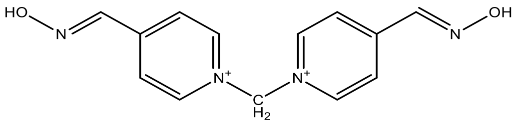


Figure 1. OP nerve agents

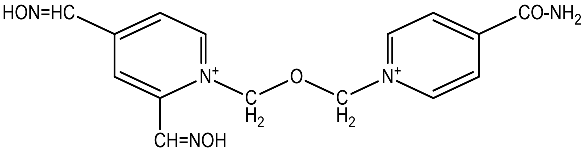




HI-6



Methoxime (MMB-4)



HLö-7

Figure 2. Structures of oximes used in the treatment of OP poisoning

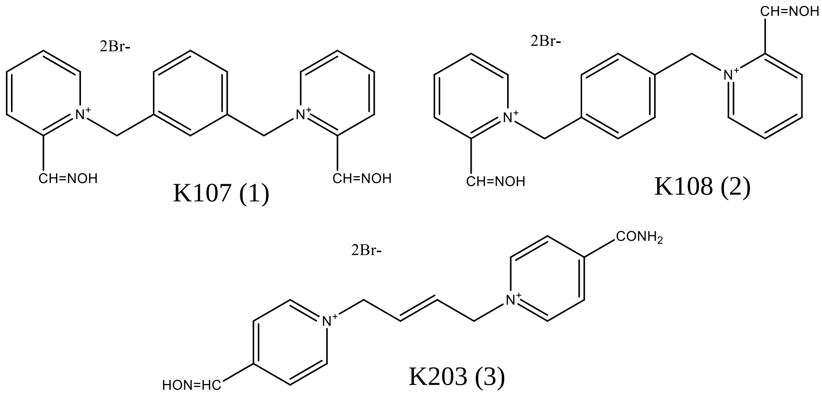
Design and Synthesis of New Antidotes (Reactivators)

In this paper, several recent strategies related to the development of new oximes are discussed.

Musilek et al. [14] has prepared a new series of 26 monooxime-monocarbamoyl bispyridinium xylene-linked compounds. The compounds were evaluated on tabun-inhibited and paraoxon-inhibited AChE. The result showed that these oximes were able to reactivate the paraoxon-inhibited AChE, but not the tabun-inhibited AChE. The molecular features of K107(1), K108(2), and K203(3) shown in Figure 3 were combined to design the monooxime-monocarbamoyl xylene-linked compounds 4-29, as shown in Table 1. Firstly, based on the in-vitro study, the symmetrical xylene-linked bispyridinium bisoximes K107 and K108 have showed promising reactivation of chlorpyrifos-inhibited and tabun-inhibited AChE [15]. Though they were selected by in-vitro screening, they showed an increase in-vivo toxicity (lower LD_{50} values) compared to all commercial oximes. However, the xylene linker was still considered as a valuable molecular tool in the design of new oximes.

In 2011, Musilek et al. developed the mono-oxime bisquaternary, as shown in Figure 4a. These developed oximes consist of prop-1,3-diyl as a linker and have different moieties on a non-oxime aromatic ring. The compounds were evaluated on tabun-inhibited and paraoxon-inhibited AChE. Results revealed that these developed oximes showed promising in-vitro results, which are as good or better than existing oximes [16]. In this study, some developed oximes were found to possess better reactivation ability at a lower concentration (10 µM) than at a higher concentration (100 µM). The authors determined the inhibition data (IC_{50}) for the main compounds of interest based on suggestion by Pohanka et al. [17]. The developed oxime, as shown in Figure 4b (IC_{50}, 105 µM) was the best inhibitor. Poor inhibition on human acetylcholinesterase (hAChE) was observed at concentration of 100 µM (11%) and 10 µM (31%.). It produced 50% hAChE inhibition at the 100 µM concentration (screening concentration, 100 µM ~ IC_{50}, 105 µM). At 10 µM, the inhibition ability of this developed oxime was decreased 10-fold, had a better reactivation rate of (31%).

In the same year, Koning et al. [18] presented a concept linkage of peripheral site ligand (PSL) through a spacer to a reactivation moiety. The designed dual binding site mode of AChE contained two moieties with are A-site and P-site. The P-site contained benzhydryl-piperidine derivatives which acted as the PSL. Meanwhile, the A-site contained oxime. These two sites was conjugated via N-alkylation. The biological evaluation of the non-quaternary conjugates in Figures 5a and 5b not only demonstrate higher affinity of AChE, but also show enhanced reactivation potency as compared to neat oxime. Besides, the conjugates were able to reactivate sarin-inhibited and VX-inhibited AChE. It was revealed that the linkage of PSL resulted in increased reactivation potency of the non-quaternary conjugates. Moreover, the obtained non-quaternary conjugates were still inefficient as compared to the current pyridinium oximes.



K107 (1)

K108 (2)

K203 (3)

Figure 3. The molecular features of K107(1), K108(2), and K203(3)

Table 1. Structure of newly prepared compounds

Compound	A	Oxime	Carbamoyl	Compound	A	Oxime	Carbamoyl
4	O	2’	2	17	m	3’	4
5	O	2’	3	18	m	4’	2
6	O	2’	4	19	m	4’	3
7	O	3’	3	20	m	4’	4
8	O	3’	4	21	p	2’	2
9	M	4’	2	22	p	2’	3
10	M	4’	3	23	p	2’	4
11	M	4’	4	24	p	3’	2
12	M	2’	2	25	p	3’	3
13	M	2’	3	26	p	3’	4
14	M	2’	4	27	p	4’	2
15	M	3’	2	28	p	4’	3
16	M	3’	3	29	p	4’	4

Figure 4a. Structure of the newly developed mono-oxime-bisquaternary oxime

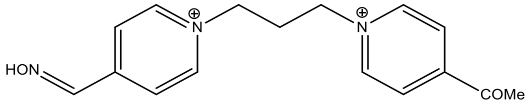
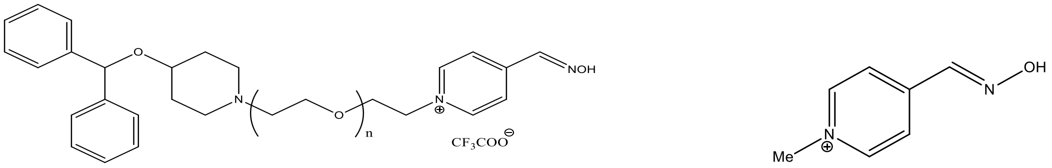


Figure 4b. Structure of the best inhibitor among the prepared mono-oxime-bisquaternary oxime



1a: n=1 (14%)
1b: n=2 (15%)
1c: n=3 (14%)

Figure 5a. Compound structure of PSL conjugates with varying spacer length and neat oxime

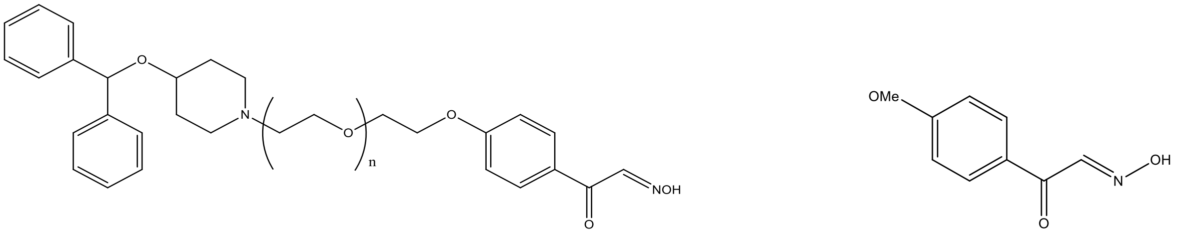
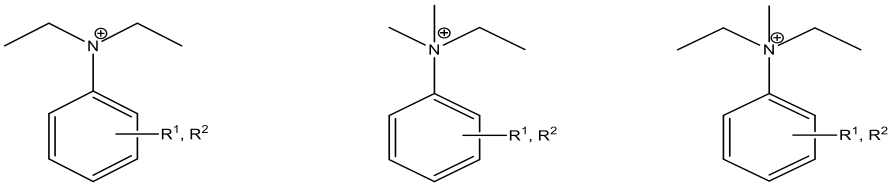


Figure 5b. Compound structure of PSL conjugates with varying spacer length and non-quaternary and neat oxime

Sit et al. [19] prepared pyridinium and non-pyridinium oximes derived from pralidoxime and edrophonium, respectively, as shown in Figure 6. The developed oximes were tested on cyclosarin-inhibited, VX-inhibited, and paraoxon-inhibited AChE. The reactivation rate was compared with pralidoxime and asoxime. These developed oximes were found to showed better reactivation potency than pralidoxime. However, none of them had better reactivation than asoxime.

A series of nine non-quaternary phenyltetrahydroisoquinoline-pyridinaldoxime conjugates were prepared by Gillon and Renard [20]. The highest reactivation efficiency was obtained with a linker length of four or five carbon atoms attached on position 6 of the pyridine ring, as shown in Figure 7. These compounds showed difficulty to reactivate tabun-inhibited and VX-inhibited AChE, which is in contrast to that of 2-PAM. Some of them were reported to have similar or more efficiency than TMB-4. Moreover, these compounds were efficient as obidoxime and TMB-4 towards paraoxon-inhibited AChE [21]. Therefore, the compounds were evaluated in-vivo because of their general efficiency and broad-spectrum. Ideally, oximes must not be strong inhibitors of hAChE at their practical reactivation concentration. The measured IC_{50} of the eight developed oximes for hAChE are as shown in Table 2. All of these developed oximes had $IC_{50} > 100$ µM, indicating that their affinity towards hAChE is lower than their affinity towards OP-inhibited AChE. Moreover, these developed oximes did not significantly inhibit the hAChE once reactivated.

McHardy et al. [22] recognized two new chemical series of non-quaternary pyridinium oximes of cyclosarin-inhibited AChE. The developed compounds shown in Figure 8 retained substantial reactivation activity, low affinity, and favorable physicochemical properties. These developed compounds also improved blood-brain barrier permeability and showed a good in-vivo reactivation of inhibited AChE.

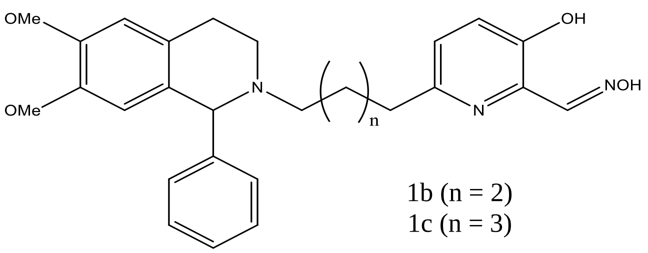


R^1 = H, OH
R^2 = CH=NOH

R^1 = H, OH, CH_2OH
R^2 = CH=NOH

R^1 = H, OH, CH_2OH
R^2 = CH=NOH

Figure 6. Structures derived from pralidoxime and endrophonium

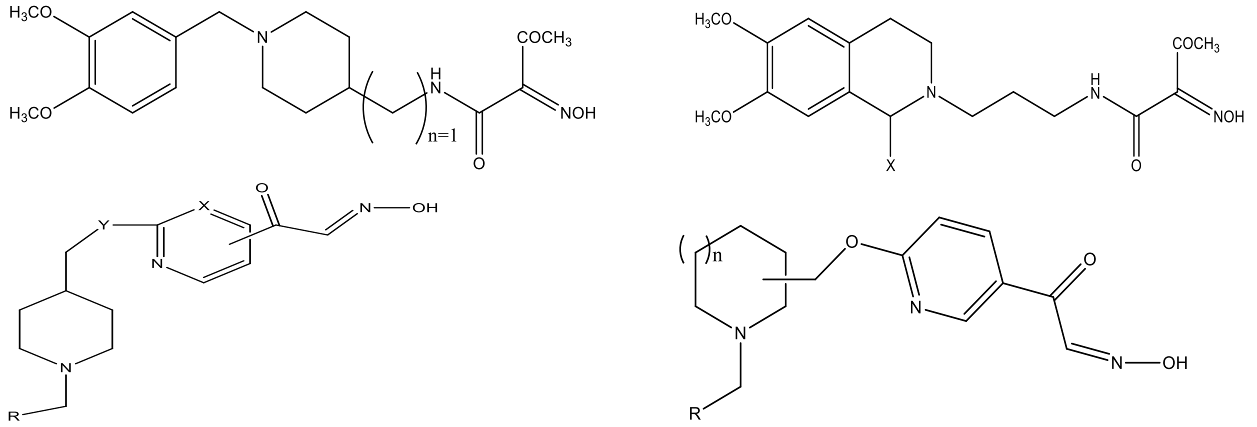


1b (n = 2)
1c (n = 3)

Figure 7. The most efficient compound of non-quaternary phenyltetrahydroisoquinoline-pyridinaldoxime conjugates

Table 2. Inhibitory activity (IC_{50}) of uncharged reactivators towards native human AChE

Reactivator	**IC_{50} (μM)**
1a	260
1b	>100
1c	>100
1d	>400
1f	>200
1h	320
1j	300
1k	>500



R = phenyl, Y = O, X = CH, 2,3-pyridine/2,5-pyridine

n = 1, R = 3,4-dimethoxyphenyl, (+/1)2-piperidine

Figure 8. New chemical series of non-quaternary pyridinium oximes of cyclosarin-inhibited AChE

In 2016, the non-quaternary oximes that are efficiently used to treat soman poisoning were designed. Dual site inhibitors strategy was used to enhance the properties of non-quaternary oximes [23]. Ortho-hydroxyl-benzaldoximes were preferred as reactivation ligands of AChE to inhibit the secondary poisoning of AChE. This developed compound was similarly linked to the oximes found in HI-6. The *in-vitro* study demonstrated that some of the resulting conjugates shown in Figure 9 have potent activity against soman-inhibited AChE. The reactivation potency of these compounds was not as good as HI-6, but showed potential for the development of more efficient centrally acting reactivators for soman poisoning due to their novel non-quaternary structures, which were forecasted to be able to cross the blood-brain barrier. IC_{50} was determined, as shown in Figure 9. It was found that these newly developed oximes were moderate or weak inhibitors of hAChE with IC_{50} greater than 100 µM, thus allowed a proper affinity to hAChE for the reactivation of inhibited AChE.

In 2017, a new family of non-quaternary oximes for inhibited hAChE was developed, as shown in Figure 10. It introduced a different PSL of AChE to achieve extra affinity. The in-vitro reactivation experiments revealed that some of the compliant conjugates presented the same or even higher ability to reactivate sarin-, VX-, or tabun-inhibited AChE, in comparison with the mono- and bis-pyridinium aldoximes. It was found that oxime (**d**) was the most efficient reactivator [24]. Besides that, these newly synthesized oximes were either moderate or weak inhibitors of hAChE with IC_{50} greater than 100 µM. This might allow a proper affinity to hAChE for the reactivation of inhibited AChE. Based on previous studies by [25-27], it was confirmed that the oxime concentration of 100 µM suitable for in-vitro reactivation experiments.

Recently, Wei et al. found that the substitution of salicylaldehyde conjugate with aminobenzamide piperidine as PSL, such as **L6M1R3**, **L6M1R5** to **L6M1R7**, **L4M1R5** to **L4M1R7** shown in Figure 11 can efficiently reactivate the sarin, VX-, and tabun-inhibited AChE. The in-vitro reactivation study of these newly developed oximes was conducted [28]. Even though they were less efficient than obidoxime, some of them showed an equal or a higher reactivation efficiency as compared to 2-PAM. In most cases, it can be concluded that the introduction of PSL could increase the oximes binding affinity towards the inhibited AChE, which results in greater reactivation potency. Most of these newly developed oximes are weak inhibitors of hAChE with IC_{50} greater than 200 µM, which might allow a proper affinity to hAChE for the reactivation of inhibited hAChE [29, 30]. Oximes **L4M1R2** displayed adequate inhibition potency against hAChE. A similar phenomenon was observed in a previous study [24], where the phenol para-linked conjugates presented higher inhibition ability of hAChE than the corresponding ortho-linked conjugates.

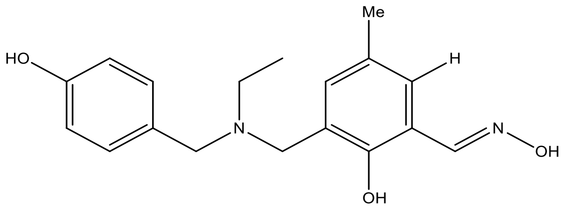
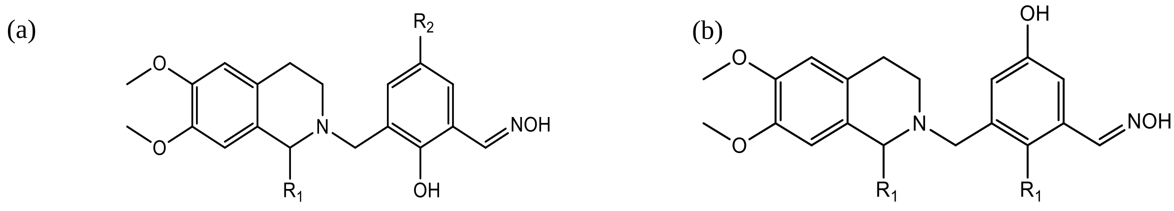
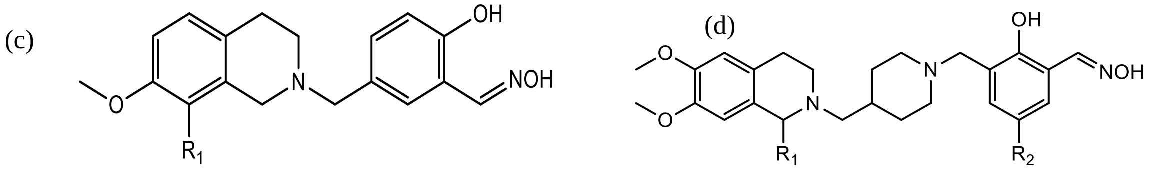


Figure 9. The most efficient structure of non-quaternary oximes to treat soman poisoning



1a: R_1 = H, R_2 = H, 1b: R_1 = Ph, R_2 = H;
3a: R_1 = H, R_2 = Me, 3b: R_1 = Ph, R_2 = Me;
4a: R_1 = H, R_2 = Cl, 4b: R_1 = Ph, R_2 = Cl;
5a: R_1 = H, R_2 = Br, 5b: R_1 = Ph, R_2 = Br;
6a: R_1 = H, R_2 = F, 6b: R_1 = Ph, R_2 = F;
7a: R_1 = H, R_2 = OMe, 7b: R_1 = Ph, R_2 = OMe;

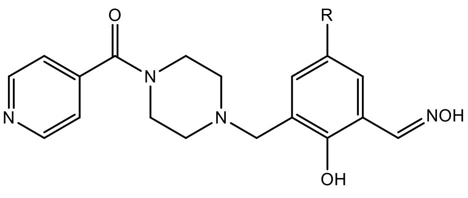
1c: R_1 = H, R_2 = H, 1d: R_1 = Ph, R_2 = H;
3c: R_1 = H, R_2 = Me, 3d: R_1 = Ph, R_2 = Me;
4c: R_1 = H, R_2 = Cl, 4d: R_1 = Ph, R_2 = Cl;
5c: R_1 = H, R_2 = Br, 5d: R_1 = Ph, R_2 = Br;
6c: R_1 = H, R_2 = F, 6d: R_1 = Ph, R_2 = F;
7c: R_1 = H, R_2 = OMe, 7d: R_1 = Ph, R_2 = OMe;



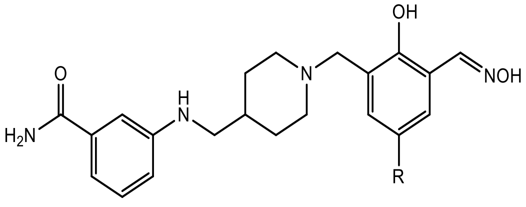
2a: R_1 = H;
2b: R_1 = Ph

2c: R_1 = H;
2d: R_1 = Ph

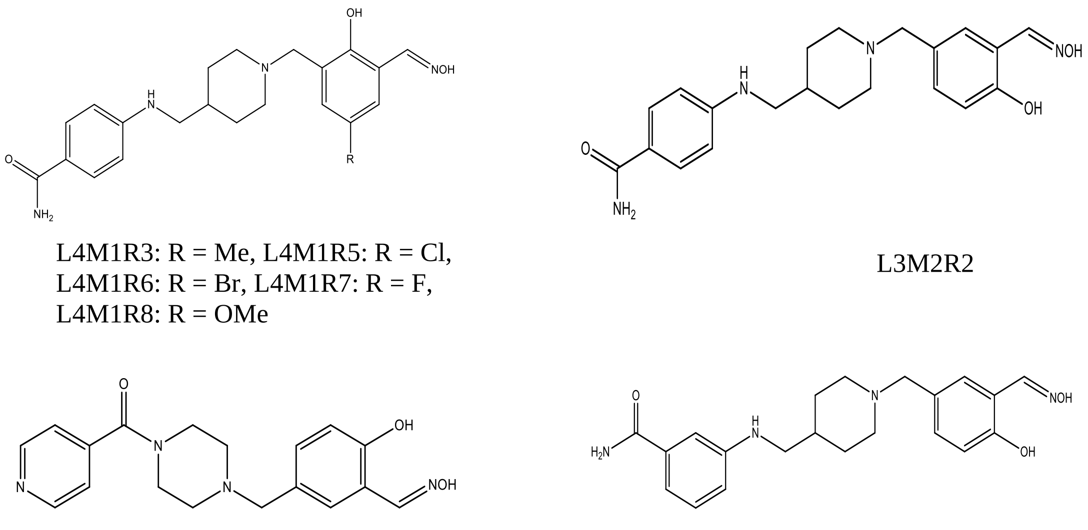
Figure 10. Family of non-quaternary developed oximes for inhibited hAChE [24]



L3M2R3: R = Me, L3M2R5: R = Cl,
L2M2R6: R = Br



L6M1R1: R = H, L6M1R3: R = Me,
L6M1R5: R = Cl, L6M1R6: R = Br,
L6M1R7: R = F, L6M1R8: R = OMe



L4M1R3: R = Me, L4M1R5: R = Cl,
L4M1R6: R = Br, L4M1R7: R = F,
L4M1R8: R = OMe

L3M2R2

L6M1R2

L4M1R2

Figure 11. The designed structures of novel non-quaternary oximes

Conclusion

Regardless of the number of efforts done for the development of new oximes to treat OP poisonings, there is still no specific oxime that can be an efficient reactivator of inhibited AChE. However, several researches had reported that the introduction of PSL not only increased the affinity of oxime towards AChE, but also enhanced the reactivation potency of inhibited AChE. Throughout the year, newly developed oximes have showed different efficiencies in treating OP poisonings. Until now, scientists are continuously working to design new oximes for the treatment of OP poisonings.

Acknowledgement

The research was supported by CHEMDEF research grant (grant code: UPNM/2018/CHEMDEF/ST4).

References

1. Sharma, R., Gupta, B., Singh, N., Acharya, J. R. and Musilek, K. (2014). Development and structural modifications of cholinesterase reactivators against chemical warfare agents in last decade: A review. *Mini Reviews in Medicinal Chemistry*, 15(1): 58-72.
2. Jokanović, M. and Stojiljković, M. P. (2006). Current understanding of the application of pyridinium oximes as cholinesterase reactivators in treatment of organophosphate poisoning. *European Journal of Pharmacology*, 553 (1–3): 10-17.
3. Jokanović, M. (2012). Structure-activity relationship and efficacy of pyridinium oximes in the treatment of poisoning with organophosphorus compounds: A review of recent data. *Current Topics in Medicinal Chemistry*, 12(16): 1775-1789.
4. Faiz Norrrahim, M. N., Idayu Abdul Razak, M. A., Ahmad Shah, N. A., Kasim, H., Wan Yusoff, W. Y., Halim, N. A., and Mohd Kasim, N. A. (2020). Recent developments on oximes to improve the blood brain barrier penetration for the treatment of organophosphorus poisoning: A review. *RSC Advances*, 10(8): 4465–4489.
5. Digiovanni, C. (2017). The spectrum of human reactions to terrorist attacks with weapons of mass destruction: Early management considerations. *Prehospital Disaster Medicine*, 18(13): 253-257.
6. Luo, C., Tong, M., Maxwell, D. M. and Saxena, A. (2008). Comparison of oxime reactivation and aging of nerve agent-inhibited monkey and human acetylcholinesterases. *Chemico-Biological Interactions*, 175 (1–3): 261-266.

7. Soukup, O., Krusek, J. and Oz, M. (2011). Oxime reactivators and their *in vivo* and *in vitro* effects on nicotinic receptors. *Physiological Research,* 60(4): 679-86.
8. Marrs, T. C. (1993). Organophosphate poisoning. *Pharmacology and Therapeutics,* 58: 51-66.
9. Musilek, K., Dolezal, M., Gunn-moore, F. and Kuca, K. (2009). Design, evaluation and structure-activity relationship studies of the AChE reactivators against organophosphorus pesticides. *Medicinal Research Reviews*, 31 (4): 548-575.
10. Worek, F., Thiermann, H. and Wille, T. (2016). Oximes in organophosphate poisoning: 60 years of hope and despair. *Chemico-Biological Interactions*, 259: 93-98.
11. Maxwell, D. M., Koplovitz, I., Worek, F. and Sweeney, R. E. (2008). A structure-activity analysis of the variation in oxime efficacy against nerve agents. *Toxicology and Applied Pharmacology*, 231(2): 157-164.
12. Ashani, Y., Bhattacharjee, A. K., Leader, H., Saxena, A. and Doctor, B. P. (2003). Inhibition of cholinesterases with cationic phosphonyl oximes highlights distinctive properties of the charged pyridine groups of quaternary oxime reactivators. *Biochemical Pharmacology*, 66(2): 191-202.
13. Worek, F., Eyer, P., Aurbek, N., Szinicz, L. and Thiermann, H. (2007). Recent advances in evaluation of oxime efficacy in nerve agent poisoning by *in vitro* analysis. *Toxicology and Applied Pharmacology*, 219 (2–3): 226-234.
14. Musilek, K., Holas, O., Misik, J., Pohanka, M. and Novotny, L. (2010). Monooxime-monocarbamoyl bispyridinium xylene-linked reactivators of acetylcholinesterase-synthesis, *in vitro* and toxicity evaluation and docking studies. *ChemMedChem,* 5: 247-254.
15. Kuca, K., Karasova, J. and Musilek, K. (2007). Development of new reactivators of tabun inhibited acetylcholinesterase and the evaluation of their efficacy by in vitro and in vivo methods. In Defence against the Effects of Chemical Hazards: Toxicology, Diagnosis and Medical Countermeasures, 17: pp. 1-12.
16. Musilek, K., Komloova, M., Holas, O., Horova, A., Pohanka, M., Gunn-Moore, F. and Kuca, K. (2011). Mono-oxime bisquaternary acetylcholinesterase reactivators with prop-1,3-diyl linkage-Preparation, *in vitro* screening and molecular docking. *Bioorganic and Medicinal Chemistry*, 19 (2): 754-762.
17. Pohanka, M., Jun, D. and Kuca, K. (2008). Improvement of acetylcholinesterase-based assay for organophosphates in way of identification by reactivators. *Talanta*, 77 (1): 451-454.
18. Koning, M. C., Joosen, M. J. A., Noort, D., van Zuylen, A., and Tromp, M. C. (2011). Peripheral site ligand–oxime conjugates: A novel concept towards reactivation of nerve agent-inhibited human acetylcholinesterase. *Bioorganic & Medicinal Chemistry*, 19(1): 588-594.
19. Sit, R. K., Radić, Z., Gerardi, V., Garcia, E., Zhang, L., Katalinic, M., Amitai, G., Kovarik, Z., Fokin, V. V., Sharpless, K. B. and Taylor, P. (2011). New structural scaffolds for centrally acting oxime reactivators of phosphylated cholinesterases. *The Journal of Biological Chemistry.* 286(22): 19422-19430.
20. Gillon, E. and Renard, P. (2012). Phenyltetrahydroisoquinoline-pyridinaldoxime conjugates as efficient uncharged reactivators for the dephosphylation of inhibited human acetylcholinesterase. *Journal of Medicinal Chemistry*, 55: 10791-10795.
21. Worek, F., Wille, T., Koller, M. and Thiermann, H. (2012). Reactivation kinetics of a series of related bispyridinium oximes with organophosphate-inhibited human acetylcholinesterase Structure-activity relationships. *Biochemical Pharmacology*, 83(12): 1700-1706.
22. McHardy, S. F., Bohmann, J. A., Corbett, M. R., Campos, B., Tidwell, M. W., Thompson, P. M. and McDonough, J. (2014). Design, synthesis, and characterization of novel, nonquaternary reactivators of GF-inhibited human acetylcholinesterase. *Bioorganic and Medicinal Chemistry Letters*, 24(7): 1711-1714.
23. Wei, Z., Liu, Y., Wang, Y., Li, W., Zhou, X., Zhao, J. and Li, S. (2016). Novel nonquaternary reactivators showing reactivation efficiency for soman-inhibited human acetylcholinesterase. *Toxicology Letters*, 246: 1-6.

24. Wei, Z., Liu, Y. Q, Wang, S. zheng, Yao, L., Nie, H. F., Wang, Y. and Li, S. (2017). Conjugates of salicylaldoximes and peripheral site ligands: Novel efficient nonquaternary reactivators for nerve agent-inhibited acetylcholinesterase. *Bioorganic and Medicinal Chemistry*, 25(16): 4497-4505.
25. Ralph, E. C., Zhang, J. and Cashman, J. R. (2011). Amidine-oximes: Reactivators for organophosphate exposure. *Journal of Medicinal Chemistry*, 54: 3319-3330.
26. Kalisiak, J., Ralph, E. C. and Cashman, J. R. (2012). Nonquaternary reactivators for organophosphate-inhibited cholinesterases. *Journal of Medicinal Chemistry*, 55(1): 465-474.
27. Koning, M. C., Grol, M. Van. and Noort, D. (2011). Peripheral site ligand conjugation to a non-quaternary oxime enhances reactivation of nerve agent-inhibited human acetylcholinesterase. *Toxicology Letters*, 206 (1): 54-59.
28. Wei, Z., Bi, H., Liu, Y., Nie, H., Yao, L. and Wang, S. (2018). Bioorganic chemistry design, synthesis and evaluation of new classes of nonquaternary reactivators for acetylcholinesterase inhibited by organophosphates. *Bioorganic Chemistry*, 81: 681-688.
29. Mercey, G., Verdelet, T., Saint-André, G., Gillon, E., Wagner, A., Baati, R. and Renard, P. Y. (2011). First efficient uncharged reactivators for the dephosphylation of poisoned human acetylcholinesterase. *Chemical Communications*, 47(18): 5295–5297.
30. Mercey, G., Verdelet, T., Renou, J., Kliachyna, M., Baati, R., Nachon, F. and Renard, P. Y. (2012). Reactivators of acetylcholinesterase inhibited by organophosphorus nerve agents. *Accounts of Chemical Research*, 45(5): 756-766.