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Quaternary salts of 4,3′ and 4,4′ bis-pyridinium monooximes: Synthesis and biological activity

Srinivas Rao Chennamaneni,[a] Venkateswarlu Vobalaboina[a,*] and Achaiah Garlapati[b]

[a]*Department of Pharmaceutics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506 009 AP, India*
[b]*Department of Medicinal Chemistry, University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506 009 AP, India*

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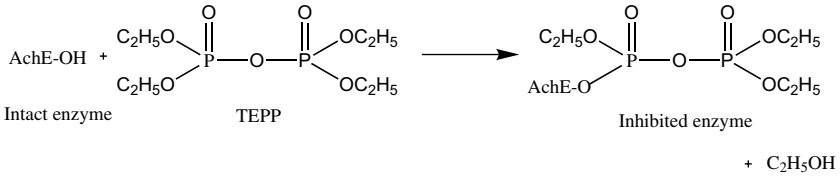
Abstract—Six unsymmetrical bis-quaternary monooximes viz. dibromides of 1-(4-hydroxyiminomethyl pyridinium)-3-(3/4-carbamoyl pyridinium)propane, 1-(4-hydroxyiminomethyl pyridinium)-4-(3/4-carbamoyl pyridinium) butane, 1-(4-hydroxyiminomethyl pyridinium)-5-(3/4-carbamoyl pyridinium)pentane were synthesized and characterized by spectral data. Their ability to reactivate tetraethyl pyrophosphate inhibited mouse total brain cholinesterase was investigated and compared with 2-pyridine aldoxime chloride (2-PAM). All the compounds were found to be more effective acetylcholinesterase reactivators when compared with the conventional oxime, 2-PAM, except the compound (**5a**) with pentylene bridge and carbamoyl group present at fourth position. The bis-pyridinium monooximes with 3-carbamoyl group were more potent reactivators than the corresponding 4-carbamoyl compounds and bis-oximes tested.

1. Introduction

Acute organophosphate (OP) pesticide poisoning is an important cause of morbidity and mortality worldwide. The mechanism of organophosphate poisoning involves phosphorylation of a serine hydroxyl group in the active site of acetylcholinesterase (AchE), leading to inactivation of this essential enzyme, which plays an important physiological role in the cholinergic nervous system (Scheme 1). The therapeutic approach to organophosphate poisoning is to reactivate AchE with a site-directed nucleophile. After reversibly binding to the active site, the nucleophile reacts with the phosphorylated hydroxyl group to release free, active enzyme (Scheme 2).[1,2] Furthermore, phosphorylated AchE can undergo a fairly rapid process of 'ageing' (loss of one alkyl or alkoxy group) so that within the course of minutes (with dimethyl OP's) or hours (with diethyl or diisopropyl OP's) it becomes completely resistant to the reactivators. Although the highly toxic nature of OP compounds has been known for many years, there still exist serious limitations in the antidotal therapy available against poisoning of these compounds. Conventional therapy against OP ester intoxication entails coadministration of atropine that antagonizes the effects of accumulated acetylcholine and an AchE 'reactivator' that restores enzyme activity.



Scheme 1. Inhibition of acetylcholinesterase by tetraethylpyrophosphate.

Keywords: Organophosphate; Pesticide; TEPP; Brain acetycholinesterase; 2-PAM; Bis-pyridinium monooximes; Reactivation.
* Corresponding author. Tel.: +91 9849 187969; fax: +91 870 2438844; e-mail: vobala@yahoo.com

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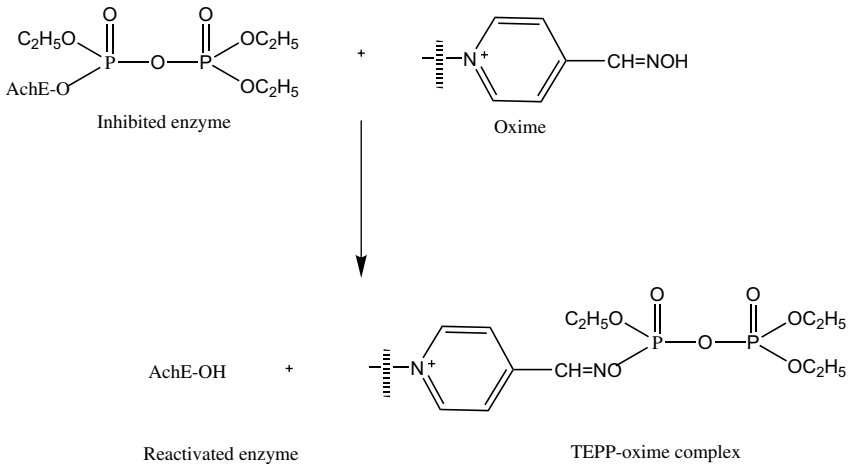


Scheme 2. Oxime induced reactivation of TEPP-inhibited acetylcholinesterase.

In contrast to organophosphonates, intoxications with organophosphate pesticides result in phosphorylated AchE, which can be easily reactivated by the marketed oximes, that is, various pralidoxime salts and obidoxime. This fact prompted the synthesis of hundreds of oximes with numerous structural modifications in the last four decades. The available data suggest that obidoxime is the most effective reactivator of human AchE inhibited by pesticides, for example, paraoxon and malaoxon. The bispyridinium-dioxime, HLO-7, was slightly less effective whereas 2-PAM and HI-6 showed to be weak reactivators. According to the data available, obidoxime is considered to be the oxime of choice for the treatment of pesticide poisoning.[3–5] Reactivation by oximes of phosphorylated AchE is thought to proceed via a nucleophilic attack of the anion on the phosphorus atom. Therefore, the intrinsic reactivity of the oxime anion with regard to nucleophilic substitution at phosphoryl centres is an important factor for the reactivation reaction, in addition to affinity for the inhibited AchE.[6]

Pralidoxime is the oxime most often used worldwide and occurs in two common forms: pralidoxime chloride (2-PAM, used worldwide) and mesylate (P2S, used in the UK).[7] According to the latest research results, obidoxime seems to be the most promising AchE reactivator against pesticide poisoning. Nevertheless, the human use of this oxime is limited in Southeast Asia due to non-availability and moreover the bis-chloromethyl ether group present in its structure is carcinogenic. On the other hand, alkylene-linked bis-pyridinium oximes are efficient reactivators.[8,9] Therefore we planned to synthesize the compounds, which contain part of the chemical structure of Obidoxime and bis-pyridinium oximes such as TMB-4.

1.1. Objectives

Objectives were to synthesize new series of asymmetrically substituted 4,3′ and 4,4′ bis-pyridinium monooximes by increasing the chain length between the two-pyridine rings and by changing the substituent position in the second pyridine ring with an interest to increase the affinity of the compound for the phosphorylated AchE.

2. Materials and methods

Test compounds were mostly synthesized by conventional methods as described in the Experimental section and also by the methods established in our laboratory.

2.1. Enzymes and chemicals

Anaesthetized animals were sacrificed by decapitation and exsanguinated; the mice brains were removed and used as a source of AchE after homogenization. Tetraethyl-pyrophosphate (TEPP), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (Asch), eserine, 4-pyridinealdoxime, 1,3-dibromopropane, 1,4-dibromobutane, 1,5-dibromopentane, isonicotinamide and nicotinamide were obtained from the Sigma-Aldrich Chemicals Private Limited, Hyderabad, India. 2-PAM was provided as a gift sample by the Troika Parenterals Private Limited, Ahmedabad, India.

2.2. Chemistry

Solvents were dried or distilled before use. Melting points were obtained on a Mel-temp apparatus (Shital Scientifics), Mumbai, India, in open capillary tubes and are uncorrected. Infrared spectra (IR) were recorded with KBr pellet on a Perkin–Elmer BX Series, infrared spectrophotometer. ^{1}H NMR spectra were recorded on Avance-300 MHz spectrometer in DMSO-d_6.[14]

3. Experimental

The general procedure for the synthesis of **3**, **4** and **5** intermediate compounds were as follows.

3.1. Procedure-A

3.1.1. Step-I. 4-Pyridinealdoxime and 1,3-dibromopropane in 1:1.2 molar ratio were added to chloroform and heated at reflux with stirring for 120 h. and then cooled to room temperature. The product was collected by filtration, washed with chloroform and crystallized from acetonitrile. Intermediate compounds **4** and **5** were

synthesized by this procedure with much improved yields, 80% and 85%, respectively.

3.1.2. Step-II. Preparation of 4-carbamoyl-4-[(hydroxyimino)methyl]-1,1′-(propane) bis-pyridinium dibromide (**3a**). The intermediate **3** and isonicotinamide or nicotinamide in 1:1.2 molar ratio were added to absolute ethanol. The reaction mixture was heated at reflux for 36 h. and then cooled to room temperature. The product was collected by filtration and crystallized with absolute ethanol.

The title compounds were also prepared by an alternative method as follows.

3.2. Procedure-B

3.2.1. Step-I. Isonicotinamide or nicotinamide and 1,3-dibromopropane or 1,4-dibromobutane or 1,5-dibromopentane in 1:1.1 molar ratio were added to acetonitrile and heated at 70 °C for 20 h. The product was collected by filtration and crystallized using acetonitrile.

3.2.2. Step-II. Preparation of bis-pyridinium monooximes. The isonicotinamide or nicotinamide intermediate compounds (**6a**, **6b**, **7a**, **7b**, **8a**, **8b**) and 4-pyridinealdoxime in 1:1.6 molar ratio were added to DMF. The reaction mixture was heated at 68–70 °C for 18 h. The product was collected by filtration and washed with acetone. All the compounds were purified by recrystallization with absolute ethanol and then dried under vacuum.

Compounds **3c**, **4c** and **5c** were synthesized according to the method of Wilson and Ginsburg[1] and also by Pang et al.[9] Compounds **3c**, **4c** and **5c** were known in the literature.[8,9] **3a** and **4a** were reported while our work was in progress, however, these compounds were synthesized in this study for comparison of biological activity.

3.3. In vitro experiments

The mouse brain was removed, rinsed with cold saline, blotted dry and weighed. It was homogenized in cold condition in a sufficient amount of 0.9% NaCl to give a final solution of 100 mg. brain tissue/mL of saline. The homogenate is then centrifuged at 3000 rpm for 10 min. The reactivating efficacy of oximes was evaluated by adding TEPP to the homogenate for 10 min, and tested oximes were added for 10 min and then incubated at 30 °C for 10 min. Brain AchE activity was determined immediately by the colorimetric procedure as described by Voss and Sachsse.[10] All assays were carried out in triplicate. Percentage reactivation was calculated with the following equation:[11]

$$\frac{E_r - E_i}{E_0 - E_i} \times 100$$

In the above equation, E_0 is the control enzyme activity at 0 min, E_i is the inhibited enzyme activity and E_r is the activity of inhibited enzyme after incubation with the oxime test compounds.

4. Results

Physical data, TLC, IR and ^{1}H NMR spectra confirmed the structures and purity of the synthesized compounds. All bis-pyridinium monooxime products decomposed (>180 °C) before melting. All the synthesized compounds were evaluated for their in vitro reactivation capabilities of inhibited enzyme by colorimetry. Compounds **3a**, **4a**, **3b**, **4b** and **5b** have good reactivating capacity (antidotal property) of TEPP inhibited AchE in comparison with 2-PAM. Among the tested compounds, the compounds with butylene (**4b**) and pentylene bridge (**5b**) with carbamoyl group at third position of the second pyridine ring showed highest reactivation of AchE that is 69 ± 2.51% (**4b** and **5b**) when compared to the corresponding compounds with carbamoyl group

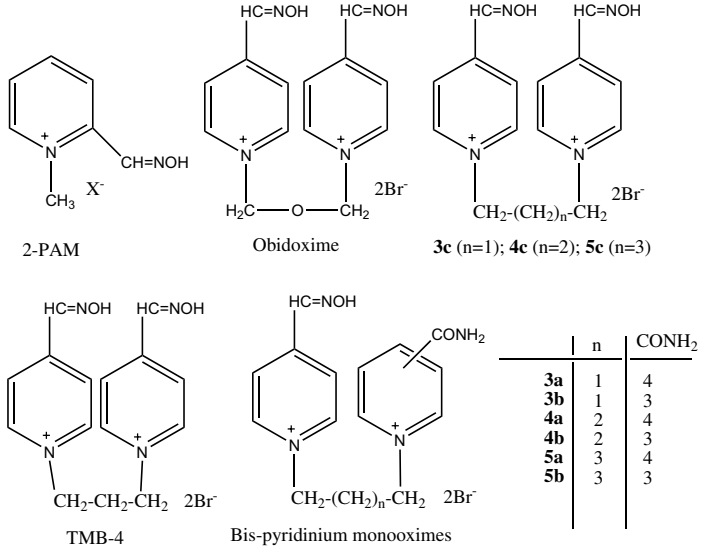


Figure 1. Structures of the standard oximes used for the treatment of OP pesticide poisoning and newly synthesized bis-pyridinium monooximes.

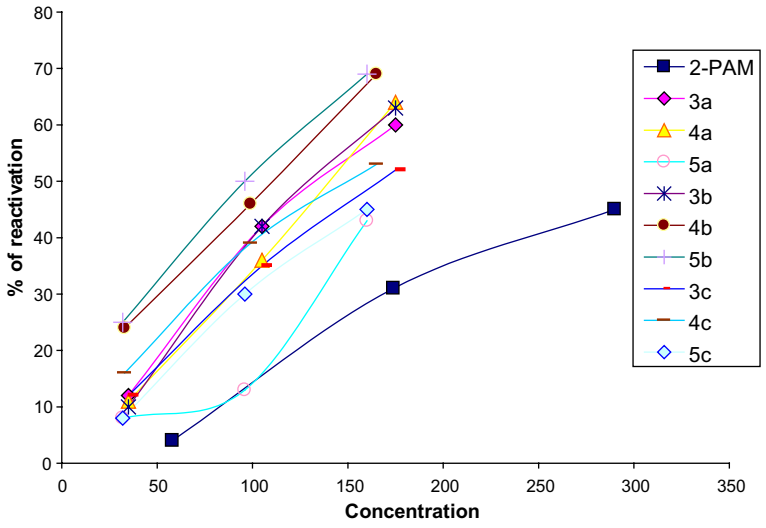
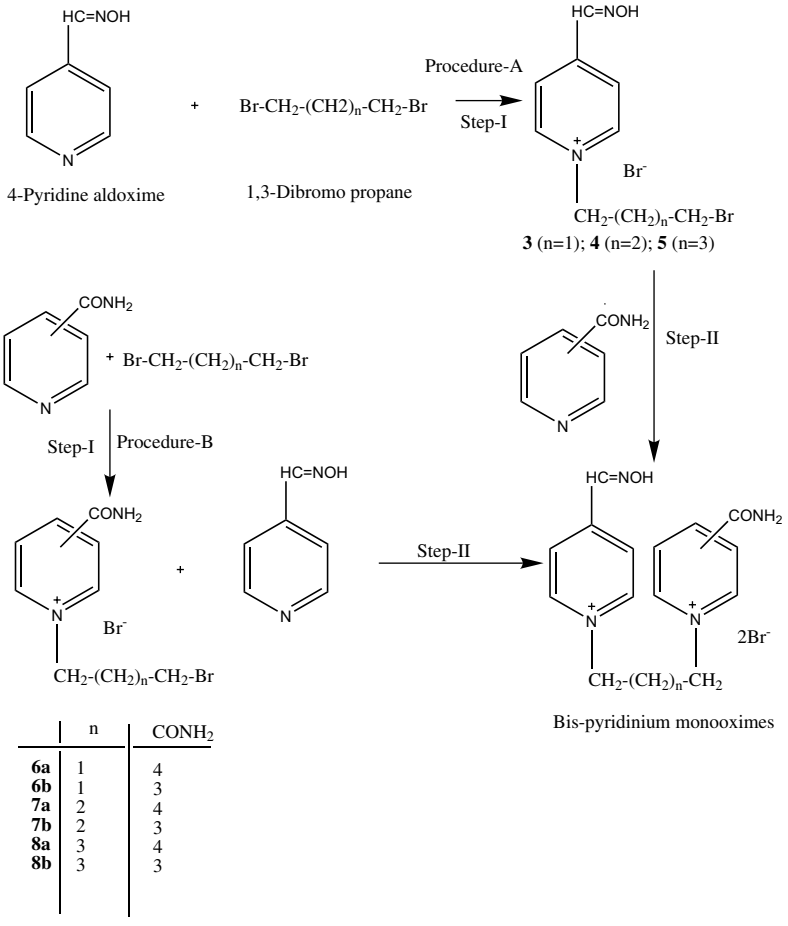


Figure 2. Reactivation of mouse brain AchE inhibited with TEPP by 2-PAM and test compounds.



	n	$CONH_2$
6a	1	4
6b	1	3
7a	2	4
7b	2	3
8a	3	4
8b	3	3

Scheme 3. Synthesis of alkylene-linked bis-pyridinium monooximes.

at fourth position with 64 ± 2.7%, 43 ± 2.6% for **4a** and **5a**, respectively. Compounds **3a** and **3b** have shown 60 ± 2.4% and 63 ± 2.8% reactivation, respectively (Fig. 2).

5. Discussion

The compounds were synthesized by the general synthetic route shown in Scheme 3. Selected

Table 1. Physical data of the compounds

Reaction	Conditions of the reaction	Molecular formula	Mp (°C)[a]	Yield (%)
3a[b]	Absolute ethanol, reflux, 36 h	$C_{15}H_{18}N_4Br_2O_2$	222–224	68
3b[b]	Absolute ethanol, reflux, 36 h	$C_{15}H_{18}N_4Br_2O_2$	220–222	64
4a[c]	DMF, 68–70 °C, 18 h	$C_{16}H_{20}N_4Br_2O_2$	260–262	62
4b[c]	DMF, 68–70 °C, 18 h	$C_{16}H_{20}N_4Br_2O_2$	258–260	58
5a[c]	DMF, 68–70 °C, 18 h	$C_{17}H_{22}N_4Br_2O_2$	218–220	64
5b[c]	DMF, 68–70 °C, 18 h	$C_{17}H_{22}N_4Br_2O_2$	220–222	60

[a] Melting points are uncorrected.
[b] Procedure-A.
[c] Procedure-B.

physicochemical data of propylene, butylene and pentylene bridge compounds are given in Table 1.

All the conventional oximes available so far differ from each other by the number of pyridinium rings present (mono-pyridnium vs bis-pyridinium oximes) and position of the oxime group on the pyridinium ring, but in the case of bis-pyridinium oximes, they differ by the chemical structure of the bridge between both pyridinium rings only (see Fig. 1). The bis-pyridinium monooximes, on the other hand, differ from bis-pyridinium oximes both in length of the side chain and position of the substituent on the second pyridine ring. The bis-pyridinium monooximes **4b** and **5b** have shown higher reactivation (69%) than the conventional oxime 2-PAM (45%). The bis-pyridinium monooximes with carbamoyl group at third position of the pyridine ring were more potent reactivators when compared with the compounds having carbamoyl group at fourth position, corresponding bisoximes (**3c**, **4c**, **5c**) and the conventional oxime 2-PAM. Further increase in chain length from 3- carbon bridge (propylene) (**3b**) to 4-carbon bridge (butylene) (**4b**) resulted in slight increase in activity. However, the compound with 5-carbon (pentylene) bridge (**5b**) showed the same activity to that of 4-carbon bridge. Two compounds have been reported from our series, while our work was in progress by Kuca et al. having excellent antidotal property, but they were tested against nerve agent poisoning.[12,13]

6. Conclusion

Three new series of asymmetrically substituted 4,3′ and 4,4′ bis-pyridinium monooximes bridged by propylene, butylene and pentylene groups were prepared. Evaluation of these compounds as antidotes for anti-AchE intoxication in the mouse brain model revealed that their effectiveness depends significantly on the position of the substituent and length of the side chain. The bis-pyridinium monooximes reactivate the inhibited enzyme faster than the mono-pyridinium and bis-pyridinium oximes.

However, in vivo pharmacological evaluation of the synthesized oximes would strengthen our findings.

7. Competing interests

Competing interests are synthesis of bis-pyridinium monooximes with hexylene, heptylene, octylene and nonylene groups and their biological evaluation in vitro and in vivo.

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14. The new compounds gave satisfactory ^{1}H NMR spectra: **3b**: 2.71 S, 2H, (CH_2–*CH_2*–CH_2); 4.80 m, 4H (*CH_2*–CH_2–*CH_2*); 8.21–8.35 m, 4H (Py); 8.61–9.00 m, 3H, (CO*NH_2*, *CH*=NOH); 9.23–9.54 m, 4H (Py).Compound **4b**: 1.83–1.98 m, 4H, (CH_2–*CH_2*–*CH_2*–CH_2); 4.69 m, 4H (*CH_2*–CH_2–CH_2–*CH_2*); 8.18–8.31 m, 4H (Py); 8.58–8.95 m, 3H, (CO*NH_2*, *CH*=NOH); 9.15–9.48 m, 4H (Py). Compound **5b**: 1.32–1.36 m, 2H, (CH_2–CH_2–*CH_2*–CH_2–CH_2); 2.01–2.04 m, 4H (CH_2–*CH_2*–CH_2–*CH_2*–CH_2); 4.65–4.70 m, 4H, (*CH_2*–CH_2–CH_2–CH_2–*CH_2*); 8.19–8.31 m, 4H (Py); 8.59–8.97 m, 3H, (CO*NH_2*, *CH*=NOH); 9.23–9.53 m, 4H (Py).