Chemico-Biological Interactions 237 (2015) 125–132

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Synthesis and in vitro kinetic study of novel mono-pyridinium oximes as reactivators of organophosphorus (OP) inhibited human acetylcholinesterase (*h*AChE)

Aditya Kapil Valiveti [a], Uma M. Bhalerao [a], Jyotiranjan Acharya [a,*], Hitendra N. Karade [a], Raviraju Gundapu [a], Anand K. Halve [b], Mahabir Parshad Kaushik [a]

[a] Process Technology Development Division, Defence Research & Development Establishment, Jhansi road, Gwalior 474 002, India
[b] School of Studies in Chemistry, Jiwaji University, Gwalior, India

ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 26 May 2015
Accepted 3 June 2015
Available online 10 June 2015

Keywords:
Acetylcholinesterase
Nerve agents
Tabun
Reactivation
Inhibition
Oxime

ABSTRACT

A series of mono pyridinium oximes linked with arenylacetamides as side chains were synthesized and their in vitro reactivation potential was evaluated against human acetylcholinesterase (*h*AChE) inhibited by organophosphorus inhibitors (OP) such as sarin, VX and tabun. The reactivation data of the synthesized compounds were compared with those obtained with standard reactivators such as 2-PAM and obidoxime. The dissociation constant (K_D) and specific reactivity (k_r) of the oximes were also determined by performing reactivation kinetics against OP inhibited *h*AChE. Among the synthesized compounds, oximes 1-(2-(4-cyanophenylamino)-2-oxoethyl)-4-((hydroxyimino)methyl)pyridinium chloride (**12a**) and 4-((hydroxyimino)methyl)-1-(2-(4-methoxyphenylamino)-2-oxoethyl)pyridinium chloride (**2a**) were found most potent reactivators for *h*AChE inhibited by sarin. In case of VX inhibited *h*AChE majority of the oximes have shown good reactivation efficacies. Among these oximes 1-(2-(benzylamino)-2-oxoethyl)-4-((hydroxyimino)methyl)pyridinium chloride (**18a**), 4-((hydroxyimino)methyl)-1-(2-(4-(methoxycarbonyl)phenylamino)-2-oxoethyl)pyridinium-chloride (**14a**) and **12a** were found to surpass the reactivation potential of 2-PAM and obidoxime. However, the synthesized oximes showed marginal reactivation efficacies in case of tabun inhibited *h*AChE. The pKa value of the oximes were determined and correlated with their observed reactivation potential.

1. Introduction

Poisoning by organophosphorus (OP) nerve agents viz. VX, sarin and tabun (Fig. 1A) and pesticides pose serious life-threatening situations to the mankind [1]. The ready availability of the raw materials, ease in their preparation and extreme toxicities of these toxicants made them as chemical weapons of mass destruction [2]. The use of nerve agents on the civilian population was witnessed by several instances in the history viz. Iran–Iraq war (1980–1988), sarin attack in Tokyo subway (1995) and recent sarin gas attack at Damascus in Syrian civil war (August 2013) [3]. All these incidents produced severe casualties all over the world. In addition, approximately 300,000 deaths have been recorded annually because of intentional (suicidal) and unintentional (occupational) means of poisoning by OP pesticides and insecticides in the developing countries around the globe [4]. Despite serious and continued efforts to prevent synthesis, storage and use of these compounds by the Chemical Weapons Convention (CWC) [5], repeated use of chemical warfare agents during military conflicts and terrorist attacks indicate that they constitute a persistent threat for the civilization [6].

The OP compounds (Fig. 1A) exert their toxicity by inhibiting the activity of the enzyme acetylcholinesterase (AChE), an enzyme responsible for hydrolysis of neurotransmitter acetylcholine (ACh). This lead to the accumulation of endogenous ACh thereby triggering a variety of clinical manifestations in the autonomic nervous system (both central and peripheral nervous system) and finally leading to death due to respiratory failure [7].

Current medical protection against the toxicity of OP poisoning consists of a regimen of anti-cholinergic drugs, such as atropine to counteract the accumulation of acetylcholine, an anti convulsant e.g. diazepam to reduce the CNS related symptoms and an oxime reactivator to reactivate OP-inhibited AChE [8]. Quaternary pyridinium oximes such as 2-pyridinealdoxime methochloride

* Corresponding author. Tel.: +91 751 2390205, +91 751 2340245; fax: +91 751 2341148.
E-mail addresses: jracharya@rediffmail.com, j.acharya@drde.drdo.in (J. Acharya).

http://dx.doi.org/10.1016/j.cbi.2015.06.007
0009-2797/

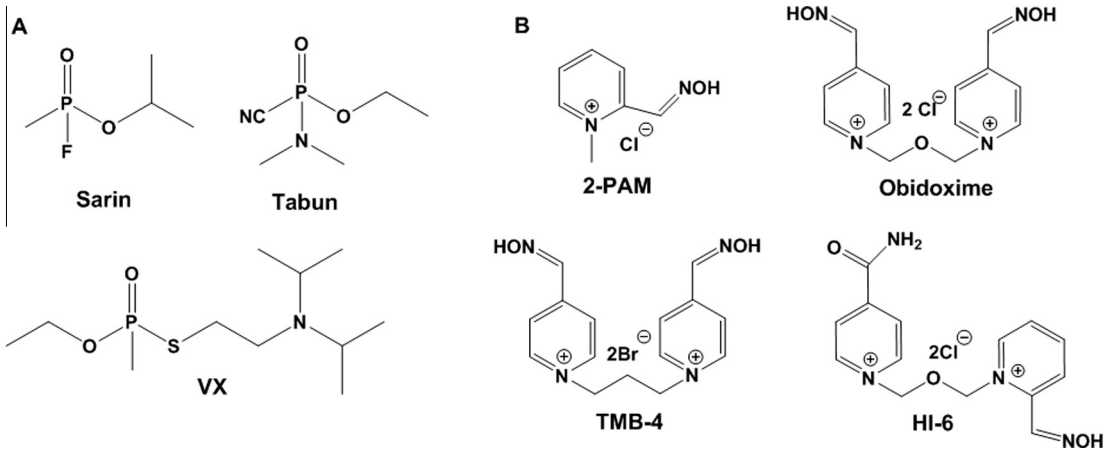

Fig. 1. (A) Structures of organophosphorus nerve agents; **(B)** structures of oxime reactivators.

(2-PAM), trimedoxime (TMB-4), obidoxime and HI-6 (Fig. 1B) are currently used as reactivators in the treatment of OP poisoning [9]. Though, these oximes have proven their efficacies against OP nerve agent poisoning, however there are certain limitations which constraint their scope in shaping them as universal antidote. 2-PAM is being used as an effective drug in many countries against OP poisoning, however its efficacy is limited to sarin and VX inhibited AChE and has marginal efficacy in case of other nerve agents. In view of the above, research is being continued for a far more effective reactivator that can be used as an antidote against a broad spectrum of nerve agents [10,11].

Recently several studies on bis- and mono-quaternary oximes connected with various bridging chains (prop-1,3-diyl, xylene linkers, aliphatic and heteroaromatic linkers) and side chain (benzyl, heterocyclic and functionalized aliphatic moieties) were reported for their efficacies against OP inhibited AChE [12–17]. Few of these reactivators have shown promising reactivation efficacies against specific OP inhibited AChE. Therefore further modifications in the structural features of AChE reactivator are highly essential in order to establish broad spectrum antidote in OP poisoning.

Bis-pyridinium oximes and their analogs have been widely studied against OP poisoning, however 2-PAM has been used worldwide [18]. Moreover, various studies have proved that the diffusion rate of monoquaternary oximes into the brain is higher in comparison to their bis-analogs [19]. Therefore, in continuation to our work on antidotes against nerve agents, herein we report the synthesis and in vitro evaluation of a series of mono-pyridinium oximes (connected to aromatic and aliphatic acetamide side chains) as reactivators of three different OP nerve agents (sarin, VX and tabun) inhibited *h*AChE.

2. Materials and methods

2.1. Materials

Substituted aromatic amines, benzylamine, cyclohexylamine, γ-aminobutyric acid, 6-aminocaproic acid, propargylamine, 2-, 3- and 4-pyridinealdoxime, acetylthiocholineiodide (ATChI), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), isopropanol (spectroscopic grade), potassium dihydrogenphosphate, dipotassium hydrogen phosphate, trizma-base and trizma-HCl were purchased from Sigma–Aldrich, USA and used without further purification. Glycine was obtained from E. Merck (India) and used without further purification. Chloroacetylchloride, anhydrous sodium sulfate, anhydrous sodium bicarbonate and anhydrous potassium carbonate were purchased from Qualigens, India. Solvents (dichloromethane, acetonitrile, acetone, and methanol) were purchased from S.D. Fine Chemicals (India) and dried and distilled before use. Sarin, tabun and VX were prepared in house with >98% purity (GC and ^{31}P NMR). 2-PAM was prepared according to the method of Wilson and Ginsburg [20]. Obidoxime was synthesized using reported methods [21]. The synthesized compounds as well as standards were characterized by their IR, ^{1}H NMR, ^{13}C NMR spectral and elemental analysis data (Supplementary information). The progress of reaction and purity of the compounds were checked by thin layer chromatography (TLC) using commercially available pre-coated silica on aluminum sheets purchased from E. Merck, India.

2.2. Synthesis of oximes

A two step simple (Scheme 1) protocol was used to synthesize the mono-pyridinium oximes (**1a**–**22a**).

2.2.1. Step 1: synthesis of 2-chloro-N-(4-nitrophenyl)acetamide

A suspension of 4-nitroaniline (7 g, 0.051 mol) and anhydrous potassium carbonate (7 g, 0.051 mol) in dichloromethane (30 mL) was stirred for 30 min at room temperature. The reaction mixture was cooled on an ice bath. To this was added an ice-cooled solution of chloroacetylchloride (5.7 g, 0.051 mol) in dichloromethane (20 mL) drop wise over a period of 30 min. The reaction mixture was stirred overnight at room temperature followed by reflux for additional 30 min. Excess solvent was removed and the residue was neutralized with aqueous sodium bicarbonate solution (5% w/v). The product obtained was filtered off and washed thoroughly with cold water. The crude product obtained was dried under vacuum (10 g, yield: 92%). TLC (chloroform, R_f = 0.5). The product was sufficiently pure and used in the next step directly.

2.2.2. Step 2: synthesis of 4-((hydroxyimino)methyl)-1-(2-(4-nitrophenylamino)-2-oxoethyl)pyridinium chloride

4-Pyridinealdoxime (1.0 g, 0.0082 mol) dissolved in dry acetonitrile (30 mL) taken in a two neck round bottom flask equipped with a magnetic stirrer, condenser and calcium chloride guard tube was stirred at room temperature. 2-chloro-*N*-(4-nitrophenyl) acetamide (1.76 g, 0.0082 mol) dissolved in dry acetonitrile (20 mL) was added to the reaction mixture slowly over a period of 10 min. The reaction mixture was then stirred for 3 h at room temperature followed by reflux for another 3 h. The product obtained was filtered off, washed with dry hot acetonitrile (2 × 15 mL) followed by dry hot acetone. Finally, the crude product was dried (0.52 g, yield 19%) and recrystallized from