European Journal of Medicinal Chemistry 157 (2018) 151–160

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Discovery of a potent non-oxime reactivator of nerve agent inhibited human acetylcholinesterase

Martijn Constantijn de Koning [a, *], Gabriele Horn [b], Franz Worek [b], Marco van Grol [a]

[a] TNO, Lange Kleiweg 137, 2288, GJ, Rijswijk, the Netherlands
[b] Bundeswehr Institute of Pharmacology and Toxicology, Neuherbergstrasse 11, 80937, Munich, Germany

ARTICLE INFO

Article history:
Received 19 May 2018
Received in revised form
29 June 2018
Accepted 4 August 2018
Available online 6 August 2018

Keywords:
Acetylcholinesterase
Nerve agents
Chemical warfare agent
Reactivator
Non-oxime

ABSTRACT

Organophosphorous (OP) compounds (such as nerve agents) inhibit the enzyme acetylcholinesterase (AChE) by covalent phosphylation of a key serine residue in the active site of the enzyme resulting in severe symptoms and ultimately death. OP intoxications are currently treated by administration of certain oxime compounds. The presently fielded oximes reactivate OP-inhibited AChE by liberating the phosphylated serine. Recent research towards new reactivators was predominantly devoted to design, synthesis and evaluation of new oxime-based compounds dedicated to overcoming some of the major limitations such as their intrinsic toxicity, their permanent charge which thwarts penetration of brain tissues and their inability to effectively reactivate all types of nerve agent inhibited AChEs. However, in over six decades of research only limited success has been achieved, indicating that there is a need for alternative classes of compounds that could reactivate OP-inhibited AChE. Recently, a number of non-oxime compounds was discovered in which the 4-amino-2-((diethylamino)methyl)phenol (ADOC) motif proved to be able to reactivate OP-inhibited AChE to some extent. In this paper several structural derivatives of ADOC were synthesized and screened for their ability to reactivate human AChE (hAChE) inhibited by the nerve agents VX, sarin, tabun, cyclosarin and paraoxon. We here disclose that one of those compounds showed a remarkable ability to reactivate OP-inhibited hAChE in vitro and that it is the most potent non-oxime reported to date.

1. Introduction

Organophosphorus (OP) compounds, such as certain pesticides (e.g., chlorpyrifos) and the nerve agents sarin (GB), soman (GD), tabun (GA), VX, Russian VX (VR) and cyclosarin (GF) are highly toxic [1]. Their toxicological target is the enzyme acetylcholinesterase (AChE) which catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) in cholinergic synapses [2,3]. OPs inhibit AChE through covalent phosphylation of a serine residue in the active site of AChE [1]. The inhibition of AChE results in accumulation of ACh levels which leads to overstimulation of cholinergic receptors, failure of the cholinergic synaptic transmission, flaccid muscle paralysis and seizures in the central nervous system. Exposure to OP pesticides is a worldwide issue in agriculture [4] resulting in over 200.000 deaths annually, and unfortunately, the use of OP chemical warfare agents (CWA) by state actors as well as by terrorists has been documented several times in the last decades [5]. These events underline the existence of a continuous threat to civilian and military personnel by these compounds and consequently, effective medical treatment strategies are needed.

The treatment of OP-poisoning comprises the administration of an anticonvulsant drug such as diazepam, atropine as an anti-muscarinic agent and an oxime. Currently fielded oximes, such as 2-PAM [6] (Fig. 1), liberate the blocked active site serine in AChE by the nucleophilic displacement of the phosphyl residue, thereby restoring the activity of the enzyme. However, the currently employed oximes have several disadvantages, such as a relatively high toxicity, a lack of broad spectrum efficiency towards all possible OP-AChE adducts, and limited brain accessibility as a result of the presence of one or multiple permanent charges. These problems have been addressed in decades of research towards more effective oximes, but unfortunately only with limited success [7–9]. To date, no broad spectrum reactivator has been discovered. Recent advances have been made in the design and synthesis of

* Corresponding author.
E-mail addresses: m.dekoning@tno.nl (M.C. de Koning), Gabriele-Horn@gmx.de (G. Horn), franzworek@bundeswehr.org (F. Worek), marco.vangrol@tno.nl (M. van Grol).

https://doi.org/10.1016/j.ejmech.2018.08.016
0223-5234/

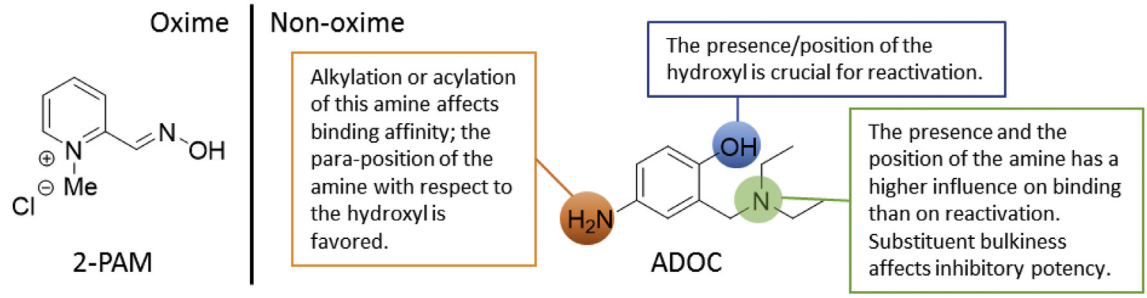

Fig. 1. Comparison of the structures of a current oxime (2-PAM) and a non-oxime reactivator, including summary of previously reported structure-activity trends.

effective, non-ionic oxime reactivators for enhanced brain permeability [10–23], but most of the reported molecules are structurally complex and sometimes difficult to synthesize in large quantities, thwarting *in vivo* evaluation. Moreover, the lack of charge often results in reduced reactivity of the oxime functionality, complicating design of effective oxime-based reactivators. Apparently, there is a need to further expand the chemical space for designing reactivators beyond that of oximes.

A couple of years ago, Bhattacharjee et al. reported a study in which in silico pharmacophore modeling and virtual screening strategies were employed to identify potential reactivators from large libraries of virtual compounds [24,25]. A number of selected compounds displayed some ability to in vitro reactivate eel AChE inhibited by diisopropylfluorophosphate (DIFP), a nerve agent simulant. In a similar effort, Katz et al. discovered a number of compounds with reactivation potency by in vitro screening of large libraries of bioactive compounds and approved drugs [26,27]. Astonishingly, none of the compounds reported by Bhattacharjee or by Katz possessed the oxime structural motive that has dominated the past 60 years of reactivator development. While the active compounds reported by Battacharjee showed little structural resemblance, the compounds reported by Katz displayed common structural features such as basic moieties (pyridine, imidazole and piperazine) [27] or a Mannich phenol [28] (*i.e.,* phenols featuring a benzylic amine in the α-position). In particular, 4-amino-2-((diethylamino)methyl)phenol (ADOC, Fig. 1) exhibited promising reactivation potential for paraoxon-ethyl (PXE) and DIFP-inhibited AChE [26]. The latter discovery triggered subsequent studies, for instance by Bierwisch et al. who investigated the kinetic interactions of amodiaquine, an ADOC based anti-malaria drug, with AChE as well as with butyrylcholinesterase (BChE) [29]. Cadieux et al. reported a study [30] in which they characterized ADOC for activity against a range of OP nerve agent-inhibited AChEs, using the standard kinetic model for reactivation to determine k_r, which is the reactivation rate constant that describes the nucleophilic displacement reaction, and the dissociation constant K_D, which reflects the affinity of the reactivator for the inhibited enzyme. The results revealed that the k_r values for ADOC mediated reactivation of AChE inhibited by GB, GF, VX and VR were higher than those of 2-PAM (GD and GA adducts could not be reactivated). However, the corresponding K_D values were less favorable for ADOC. As a consequence, the overall bimolecular reactivation rate constant ($k_{r2} = k_r/K_D$) of ADOC was only significantly better than that of 2-PAM in the case of VR-inhibited AChE. It was also found that ADOC was a strong reversible inhibitor of the native enzyme ($IC_{50} = 17.8$ μM). Cadieux et al. also synthesized a series of structural analogs of ADOC to probe the essential structural elements needed for reactivation and binding, as the mechanism by which these compounds act is currently unknown. They varied the bulk, type and position of various substituents on either the aniline, the hydroxyl or the benzylic amine (Fig. 1). The results indicated that the presence and position of the hydroxyl function was essential for reactivation, that varying the steric bulk on the benzylic amine led to drastic changes in affinity (IC_{50}), and that the nature of the benzylic amine and the aniline amine contributed to the affinity of ADOC for the inhibited enzyme. Unfortunately, none of the compounds generated in that study performed better than ADOC in vitro reactivation assays. Intriguingly, all deviations to the ADOC structure led to a (nearly) diminished reactivation potency, indicating a delicate sensitivity of biological activity to structural changes. In this contribution, the synthesis of ADOC derivatives in which relatively small adjustments were made to the structure of the benzylic amine are reported. These compounds were screened for their ability to reactivate hAChE inhibited by several nerve agents. We here present some unusual observations in the behavior of these compounds as well as the discovery of one compound that clearly out-performed ADOC in the in vitro reactivation of all nerve agent-inhibited AChE's tested (except for tabun-AChE).

2. Results and discussion

2.1. Synthesis of Mannich phenols

Fig. 2 shows the structures that were targeted for synthesis. These structures reflect the focus on making relatively small variations of the lead structure ADOC (compound **3h** in Fig. 2). The synthesis of Mannich phenols **3a-r** was accomplished following a simple, previously reported [31], 2-step general procedure. Acetamidophenol (**1**) was treated overnight in ethanol (or water) with paraformaldehyde (or formaldehyde when executed in water) and the respective amine at 90 °C. The resulting monosubstituted intermediates **2** were then separated from unreacted material and the bis-substituted product using automated flash column chromatography. The second step comprised treatment of pure **2** with 10% HCl at elevated temperature and monitoring the progress of the reaction by LC-MS. When complete, the mixture was concentrated and co-evaporated to give the HCl-salt of the target product, which did not require further purification. All of the resulting compounds were characterized by $^{1}H/^{13}C$-NMR, LC-MS and HRMS.

The envisaged variations comprised asymmetrical (**3a, 3c-g**) as well as symmetrical (**3b, 3i**) substitution of the benzylic amine with aliphatic carbon chains. Cyclic amines of varying ring-size (3–6 atoms, **3j-n**) were also targeted. The synthesis of derivatives with the smallest ring-sizes (*i.e.,* aziridine **2j** and azetidine **2k**) were unproductive, but **3l-n** (5 and 6 membered rings) were successfully isolated. Further derivatization was attempted by introducing unsaturated ring systems (**3o-r**). The synthesis of **2o** and **2p** suffered from polymerization of the amine and/or the product and could not be obtained. The imidazole (**3q**) and pyrazole (**3r**) derivatives could only be isolated in low overall yield, because of low conversion of **1** into **2q** and **2r** and the need to isolate these intermediates by HPLC purification.