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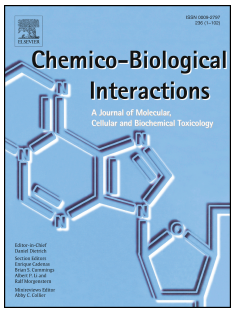

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Synthesis and *in vitro* evaluation of neutral aryloximes as reactivators of *Electrophorus eel* acetylcholinesterase inhibited by NEMP, a VX surrogate

Samir F. de A. Cavalcante[a,b,c,d,*], Daniel A. S. Kitagawa[e], Rafael B. Rodrigues[a], Leandro B. Bernardo[a], Thiago N. da Silva[c], Wellington V. dos Santos[f,g] , Ana Beatriz de A. Correa[a], Joyce S. F. D. de Almeida[e], Tanos C.C. França[d,e], Kamil Kuča[d], and Alessandro B. C. Simas[b],*

[a] Brazilian Army CBRN Defense Institute (IDQBRN), Avenida das Américas 28705, Rio de Janeiro, 23020-470, Brazil.

[b] Walter Mors Institute of Research on Natural Products (IPPN), Federal University of Rio de Janeiro (UFRJ), CCS, Bloco H, Rio de Janeiro, 21941-902, Brazil.

[c] University Castelo Branco (UCB), School of Pharmacy, Avenida Santa Cruz 1631, Rio de Janeiro, 21710-255, Brazil.

[d] Department of Chemistry, Faculty of Science, University of Hradec Králové, Rokitanskeho 62, 50003 Hradec Králové, Czech Republic.

[e] Laboratory of Molecular Modelling Applied to Chemical and Biological Defense (LMACDB), Praça General Tibúrcio 80, Rio de Janeiro, 22290-270, Brazil.

[f] Emergency and Rescue Department (DSE), Rio de Janeiro State Fire Department (CBMERJ), Praça São Salvador 4, Rio de Janeiro, 22231-170, Brazil.

[g] University Universus Veritas (UNIVERITAS), School of Biomedicine, Rua Marquês de Abrantes 55, Rio de Janeiro, 22230-060, Brazil.

Abstract

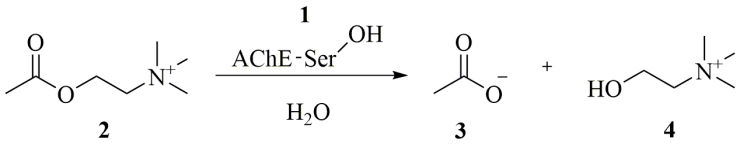
Casualties caused by nerve agents, potent acetylcholinesterase inhibitors, have attracted attention from media recently. Poisoning with these chemicals may be fatal if not correctly addressed. Therefore, research on novel antidotes is clearly warranted. Pyridinium oximes are the only clinically available compounds, but poor penetration into the blood-brain barrier hampers efficient enzyme reactivation at the central nervous system. In searching for structural factors that may be explored in SAR studies, we synthesized and evaluated neutral aryloximes as reactivators for acetylcholinesterase inhibited by NEMP, a VX surrogate. Although few tested compounds reached comparable reactivation results with clinical standards, they may be considered as leads for further optimization.

Keywords: Acetylcholinesterase reactivators; Nerve agents' surrogates; Neutral oximes; Drug screening; Chemical defence.

* Corresponding authors: Dr. Samir F. de A. Cavalcante, e-mail: samir.cavalcante@eb.mil.br; Dr. Alessandro B. C. Simas, e-mail: abcsimas@nppn.ufrj.br.

1. Introduction

Acetylcholinesterase (AChE, EC 3.1.1.7, **1**) is a serine-estearase, with pivotal role in the parasympathetic neurotransmission, ending the potential action at the post-synaptic cleft by hydrolysis of neurotransmitter acetylcholine (ACh, **2**, Scheme 1) into its two precursors, choline (**3**) and acetate (**4**). AChE is mainly located in brain, but it can also be found in erythrocytes and muscles.[1-7]



2 **3** **4**

Scheme 1. Hydrolysis of ACh by AChE.

The hydrolysis of ACh takes place at the estearic site of AChE, which has a catalytic triad composed by residues of serine, histidine and glutamate, which are highly conserved throughout species. In case of organophosphorus poisoning, phosphorylation of the serine hydroxyl group in the active site leads to AChE inhibition, bringing about ACh accumulation, which may be fatal depending on the dose, due to overstimulation of cholinergic innervations.[8-12] This could be avoided by the action of an AChE reactivator, *i.e.* a molecule capable of reverting the serine phosphorylation and restoring the enzyme functions. Such compounds can be discovered through kinetic studies on the isolated and purified enzyme.[13-15] In spite of interspecies differences that may occur related to affinity and reactivity towards inhibitors,[16] and the use of different isoforms, AChE from electric eel (*Electrophorus electricus*) *Ee*AChE, is largely reported in studies on novel reactivators against inhibitors or active compounds to address neurodegenerative diseases, being considered a trustable and affordable model.[17,18]

Nerve agents are strictly regulated by the Chemical Weapons Convention (CWC) and its international watchdog, the Organization for the Prohibition of Chemical Weapons (OPCW), which oversees the CWC implementation and compliance.[13] Figure 1 depicts examples of such toxic chemicals, the nerve agents sarin (**5**), soman (**6**), tabun (**7**), VX (**8**), the recently confirmed Novichok agents, e.g., A-234 (**9**), and the pesticide paraoxon (**10**).

Novichok agents, a class of compounds developed to circumvent CWC and North Atlantic Treaty Organization (NATO) standard protective gear and detection systems, have been involved in poisonings that took place in the United Kingdom. According to different sources, diverse structures have been proposed for these compounds. These structures have been considered to enter in the official documents, as CWC, and databases, as the OPCW Central Analytical Database (OCAD).[20-26]

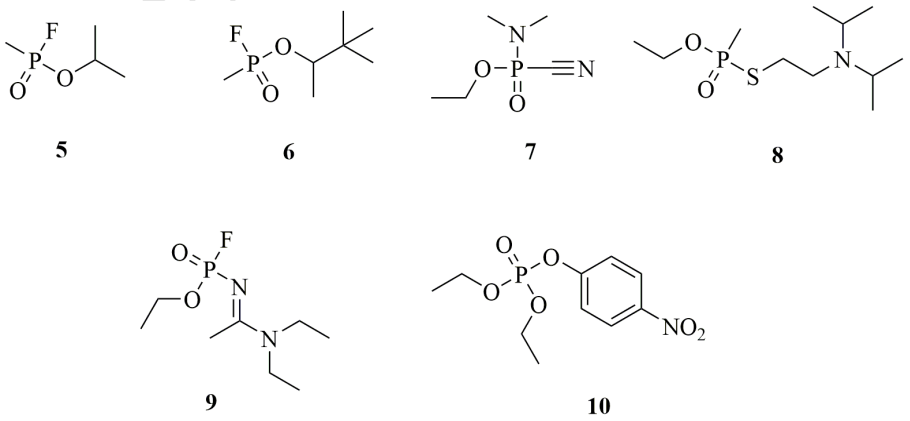


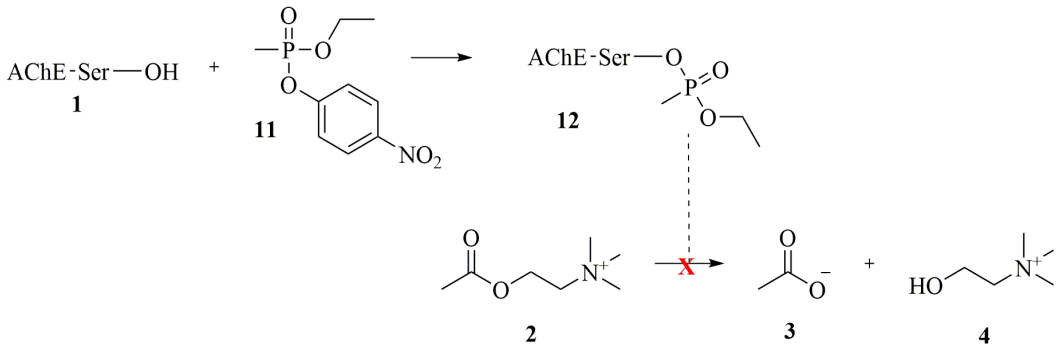
Figure 1. Examples of toxic organophosphorus compounds.

Development of antidotes towards nerve agents is essential, as there is no "universal antidote" available. The production, transfer and use of the so-called "live agents" for must be done in accordance to CWC.[19] Therefore, they are under strict control. To overcome these limitations, surrogates may be employed. They possess lower volatility and toxicity in comparison to actual agents, being easier to manipulate. Nonetheless, they afford the same enzymatic adduct, allowing the search for novel antidotes, among other studies.[23,24] An example of such compounds is *O*-(4-nitrophenyl) *O*-ethyl methylphosphonate (NEMP, **11**), a VX surrogate, which yields the same *O*-ethyl methylphosphonyl adduct.[23-25] Figure 2 depicts VX and NEMP structures, with similarities shown in bold red.

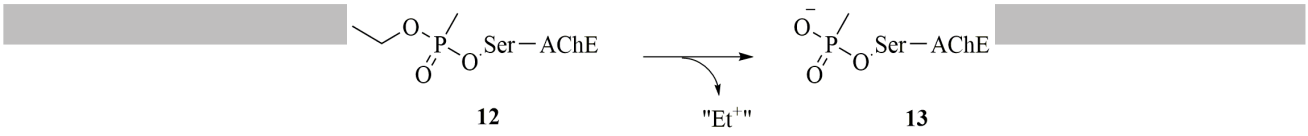
8 **11**

Figure 2. Structures of VX and NEMP.

As depicted in the Scheme 2, phosphonylation of the AChE catalytic serine residue (**12**) caused by NEMP leads to accumulation of ACh (**2**), triggering the *SLUDGEM* syndrome (salivation, lacrimation, urination, defecation, gastrointestinal disturbance, emesis, miosis and muscle spasms).[9,20] Similarly to VX, NEMP does not cause significant AChE aging, i.e., disproportion of the *O*-ethyl moiety that generates a phosphonate anion (**13**, Scheme 3).[24] Aged AChE cannot be reactivated by any of the clinically available antidotes, pyridinium oximes, due to ionic interactions with the imidazolium ring of the histidine moiety in the catalytic triad.[25,26]



Scheme 2. AChE inhibition by NEMP.



Scheme 3. Aging of *O*-ethyl methylphosphonyl-AChE adduct.

Pralidoxime (2-PAM, **14**), obidoxime (OBD, **15**), trimedoxime (TMB, **16**), HI-6 (**17**) and HLö-7 (**18**) are examples of therapeutic compounds (Figure 3).[27-30] Under physiological conditions, they yield oximates that reactivate AChE by displacing the organophosphorus moiety from the serine residue via nucleophilic attack. Although they reactivate AChE well at neuromuscular junctions, their positive charge reduces permeability through the blood-brain barrier, thereby decreasing effectiveness at the central nervous system (CNS). These limitations warrant the development of novel AChE reactivators with enhanced pharmacokinetics.[9,31-33]

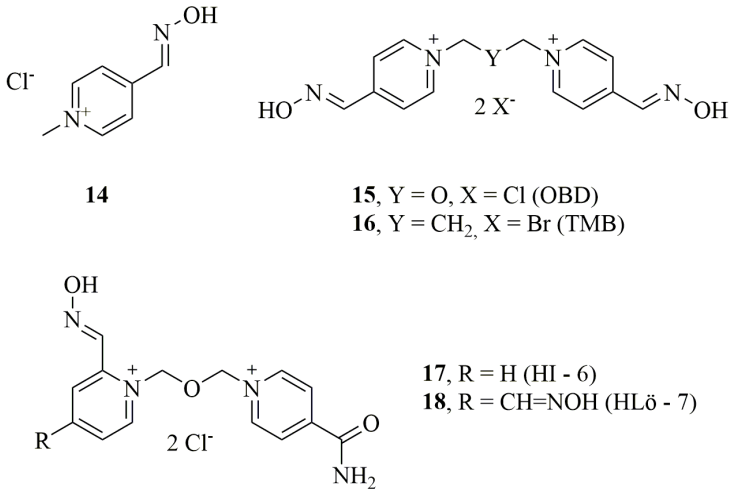


Figure 3. Clinically available antidotes.

Along with other research groups working on tackling these issues involving pyridinium oximes,[32-39] we set out to investigate the action of neutral aryloximes as reactivators for NEMP-inhibited AChE. For that, we employed the Ellman's spectrophotometric assay and commercial *Electrophorus eel* as source of AChE. Although aryloximes lacking a cationic nitrogen atom are matter-of-factly expected to perform worse than the pyridinium analogues, the *in vitro* assays with selected neutral oximes might disclose further structural features to be exploited in the design of novel AChE reactivators.

We reckoned that the electronic effects and other properties bestowed by electron-withdrawing or donating groups in different positions, might provide useful insights in that work. Besides the expected gain in the mentioned pharmacokinetics properties due to better permeation into the brain, additional interactions by the neutral aryl moiety could compensate the absence of the cationic nitrogen (present in clinically available compounds) in the estearic site.[9,38,42]

2. Experimental

2.1. General information

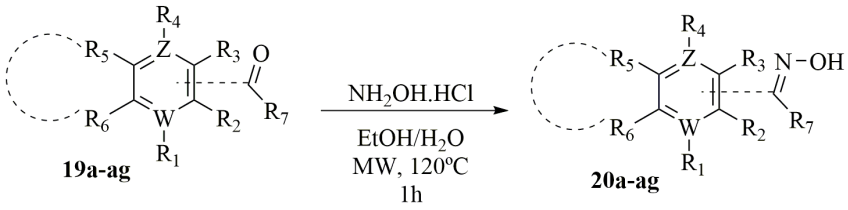
Acetylthiocholine iodide (ATCI), 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB), lyophilized acetylcholinesterase from *Electrophorus eel* (*Ee*AChE, 1000U per mg protein, type V-S, C2888), *O*,*O*-diethyl methylphosphonate, pralidoxime iodide (2-PAM), N,N-dimethylformamide (DMF, dry, oxygen-free sealed bottle), dimethylsulfoxide (DMSO, biological grade, dry, oxygen-free sealed bottle), triethylamine (TEA, dry, oxygen-free sealed bottle), absolute 2-propanol, paraformaldehyde, dichloromethane (dried over 4 Å molecular sieves before use), toluene (dried over 4 Å molecular sieves before use), sodium hydroxide (pellets), anhydrous sodium sulphate, anhydrous sodium chloride, sodium phosphate monobasic hydrate and sodium phosphate dibasic dihydrate were purchased from Sigma-Aldrich (São Paulo, Brazil). Absolute ethanol was purchased from Tedia (Rio de Janeiro, Brazil). Hydrochloric acid (37% p/p, 1.19 g/mL), chlorosulphonic acid, sodium hypochlorite, ethyl acetate, hexane and 4-nitrophenol (PNP) were purchased from Vetec (Rio de Janeiro, Brazil). Oxalyl chloride was purchased from TCI Chemicals (J1L1 Eireli, Rio de Janeiro, Brazil). Deuterated solvents ($CDCl_3$ and DMSO-d_6) containing tetramethylsilane as internal standard were purchased from Cambridge Isotopes Laboratories (Tewksbury, Massachusetts, USA). Purified water was obtained from Millipore Milli-Q system (18.2 MΩ cm at 25°C, Millipore Brazil, São Paulo, Brazil). Biotage Initiator+ Eight and Isolera ACI Chromatography System (Charlotte, North Carolina, USA) were used for synthesis of all oximes and flash chromatography, respectively. Sealed tubes (Q-Tube) were purchased from Q-Labtech (East Lyme, Connecticut, USA). TLC (Thin Layer Chromatography) aluminium plates coated with silica gel F_{254} were purchased from Merck Brazil (São Paulo, Brazil). Camag TLC-MS (Thin Layer Chromatography – Mass Spectrometry) interface was used to follow up reactions (CAMAG, Muttenz, Switzerland). NMR spectra were obtained from Varian Unity 400MHz (Santa Clara, California, USA) and Bruker Avance 400MHz (Palo Alto, California, USA) and referred to tetramethylsilane for ^{1}H and ^{13}C-NMR spectra. GC-MS (Gas Chromatography-Mass

Spectrometry) data were obtained from Agilent 6890 GC system equipped with 5975C mass spectrometer detector (Billerica, Massachusetts, USA). LCMS (Liquid Chromatography – Mass Spectrometry) data were obtained from Agilent 1210 LC system equipped with 6410B triple quadrupole mass spectrometer detector (Billerica, Massachusetts, USA). Melting points were obtained from SRS OptiMelt 100 (Stanford, CA, USA) using open capillary method and were uncorrected. SpectraMax Plus 384 microplate reader (Molecular Devices, San Jose, California, USA) was used in all assays. 96-wells microplates were purchased from Kasvi Brasil (São José dos Pinhais, Paraná, Brazil). Gilson single channel pipettes were purchased from Gilson Inc. (Middleton, Wisconsin, USA) and Eppendorf 8-channel pipettes were acquired from Eppendorf Brasil (São Paulo, Brazil). Ellman´s tests were performed in triplicate, in three different assays, by at least three different operators, measured at 24 ± 2 °C. Microsoft Excel® 2010 was used for all calculations. All disposable materials and glassware in contact with organophosphorus compounds were decontaminated with aqueous solution containing 10% w/v NaOH and 10% w/v NaClO for 48 h at room temperature before correct destination. Estimations of pKa and logP for clinical antidotes and test compounds were obtained from ChemAxon Online Suite. Agilent Chemstation E.02.02.1431 was used for area integration of GC data for purity calculations. *O*-(4-nitrophenyl) *O*-ethyl methylphosphonate (NEMP) was synthesized in accordance to literature.[23]

2.2. Synthesis of neutral aryloximes

Scheme 4 illustrates the synthetic procedure for the oximes employed in this work. Their preparation was performed as follows: a microwave vial equipped with a magnetic stirrer was charged with 1 mmol of carbonyl compound, 2 mmol of hydroxylamine hydrochloride and 1.5 mL of 50% v/v water/ethanol. The system was sealed and stirred for 60 s at room temperature. Then the vial was irradiated at 120ºC with automatic power control for 1h. After completion of reaction, the solid products were filtered off and washed with cold water. If desired, the solids may be recrystallized from ethanol-water mixtures, but they usually are very pure compounds, as checked by TLC and GC. If no solid was formed, extraction with ethyl acetate (15 mL) and washing of organic layer with water (2 x 15 mL) were performed. The organic layer was collected, dried over anhydrous sodium sulphate, filtered and concentrated in the rotary evaporator to give oily products in high purity, in accordance to TLC and GC methods. If desired, oily products may be subjected to flash chromatography, using 10 to 40% ethyl acetate/hexane mixture.

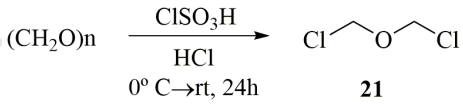
Table 1 depicts all synthesized compounds, yields and relevant estimated physicochemical properties. Spectroscopic data for all synthesised compounds are provided in Supplementary Material.



Scheme 4. Synthetic procedure for oximes.

2.3. Synthesis of bis-chloromethyl ether

Bis-chloromethyl ether (**21**) was synthesized using a slightly modified procedure from literature (Scheme 5).[43] In a 50 mL, 3-neck-round-bottomed flask equipped with a magnetic stirrer, addition funnel and thermometer, 5.25g (0.175 mmol) of paraformaldehyde was added and cooled to 0ºC. Then, 5 mL of HCl 37% p/p (pre-cooled to 0ºC) were added in one portion. The slurry was vigorously stirred and cooled to 0ºC. 7.5 mL of chlorosulphonic acid were added in such a rate that internal temperature was kept at 0-5ºC (full addition took 20 min). The suspension was then left to reach room temperature and stirred for additional 24 h. After that time, stirring was interrupted and the reaction medium separated in two layers. A yellow bottom layer was collected and stirred by 15 min with anhydrous sodium chloride to remove moisture and carefully filtered with mild vacuum. The product was pure enough for the intended purposes (9 g, 90%) and must be handled with CAUTION, as it is a known carcinogenic.

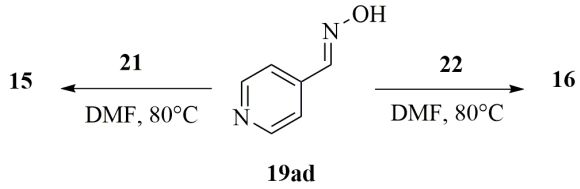


Scheme 5. Synthetic procedure for bis-chloromethyl ether.

2.4. Synthesis of standard antidotes

Obidoxime (OBD) dichloride (**15**) and trimedoxime (TMB) dibromide (**16**) were synthesized using a slightly modified procedure (Scheme 6).[44,45] In a sealed tube, pyridine-4-aldoxime (1 mmol, **19ad**) was dissolved in dry DMF (0.8 mol/L) and bis-chloromethyl ether (0.45 mmol, **21**) or 1,3-dibromopropane (**22**) was added in one portion.

Reaction was heated at 80 ºC for 6 h. Then, the reaction mixture was cooled to room temperature and cold acetone was added (5 mL per mmol of aldoxime) to full precipitation of white solids. These were filtered and washed with cold acetone, yielding 50% of OBD dichloride (77 mg) and 40% of TMB dibromide (65mg).



Scheme 6. Synthetic routes for standard antidotes used in this work.

2.5. Ellman's spectrophotometric assays

Ellman's assays were performed in accordance to our previously published procedure,[48] using 96-wells microplates, with maximum volume of 200 µL. Briefly, for AChE inhibition, we set the microplate reader to 412 nm and pipetted 70µL of *Ee*AChE (2.14 U/mL, prepared from commercial lyophilized), 80µL of DTNB 0.4 mg/mL, 20 µL of phosphate buffer solution (PBS, pH 7.60 ± 0.10), 10 µL of inhibitor (positive control, A_i; CAUTION as NEMP is a toxic organophosphorus compound) or 10 µL of PBS (negative control, A_0), and waited for 10 min for inhibition reaction. Then, we added 20 µL of 1 mmol/L of ATCI and read the absorbance in different times to calculate the enzyme inhibition. AChE inhibition percent was given by Equation 1. Slight modifications were put forth for aging and spontaneous hydrolysis assays. We set as inhibition reaction time 15 and 75 min for aging and 10 min and 12 h for spontaneous hydrolysis.

$$\%I = 100 \times \frac{A_0 - A_i}{A_0} \quad \textbf{(1)}$$

For AChE reactivation, we set the microplate reader to 412 nm and pipetted 70 µL of *Ee*AChE (2.14 U/mL, prepared from commercial lyophilized), 80 µL of 0.4 mg/mL DTNB, 10 µL of inhibitor (positive control) or 10 µL of PBS (negative control), and waited for 10 min for inhibition reaction. Following this time, we added 20 µL standard antidotes or test molecules in different concentrations (1000, 100 and 10 µmol/L as final concentrations) and waited for 30 min for reactivation reaction. At last, added 20 µL of 1 mmol/L of ATCI and read the absorbance (A_r) in different times to calculate the enzyme reactivation. AChE reactivation percent was given by Equation 2.

$$\%R = 100 \times \frac{A_r - A_i}{A_0 - A_i} \quad \textbf{(2)}$$

3. Results and Discussion

All oximes synthesized were evaluated as NEMP-inhibited *Ee*AChE reactivators in three different concentrations, in accordance to our procedure.[48] Table 1 shows the calculated physicochemical properties of assayed compounds and standard antidotes. NEMP solution was freshly prepared from crude compound in absolute ethanol at approximately 20 μmol/L, (0.5 mg in 100 mL, 1 μmol/L in each well after all dilutions). At this concentration, NEMP caused inhibition of 93 ± 1% of *Ee*AChE after 10 min of incubation in our Ellman's assay conditions. Major impurity, 4-nitrophenol, did not interfere in absorbance measurements.[29,30] Results were determined after 30 minutes of reactivation reaction. Results for the most active compounds are shown in Table 2 (full results on the *Ee*AChE reactivation are shown in the Supplementary Material).

We also evaluated if NEMP, in our Ellman inhibition assay's conditions, would cause *Ee*AChE aging. We performed two sets of experiments, modifying the enzyme-surrogate incubation time to 15 and 75 min, respectively. Then, we added 2-PAM 100 μmol/L and let the reactivation reaction occurs for 30 min before addition of substrate (ATCI) and read the absorbance. *Ee*AChE inhibition level by crude NEMP solution was similar for both incubation times, 95 ± 1%, and reactivation rates were virtually the same, 56% and 59%, respectively. These results (shown in Table 3) put forward the idea that NEMP indeed does not age significantly *Ee*AChE under our test conditions. As a complimentary study, we also measured spontaneous enzyme reactivation using NEMP as inhibitor. After incubating *Ee*AChE with crude NEMP solution for 10 min and 12 h at room temperature, we added ATCI and read the absorbance. Enzyme inhibition level after 10 min of incubation with NEMP was 93 ± 1%, while after 12 h inhibition was 55 ± 3% (see Table 4). This result suggests that spontaneous hydrolysis of *O*-ethyl methylphosphonyl moiety from serine residue at the estearic site might be occurred, rendering some enzyme activity. This result also supports our hypothesis that NEMP does not significantly age *Ee*AChE.[23,24]

The synthesised compounds, as expected, displayed better lipophilicity (as indicated by estimated logP values, Table 1) than standard antidotes (entries 34-36), thus, potentially enabling more effective passage through the hematoencephalic barrier. Higher concentration of reactivator in CNS is necessary for AChE reactivation in the brain.[41,42] Moreover, most calculated pKa values compare well with the standard antidotes.

All selected oximes were evaluated as NEMP-inhibited *Ee*AChE reactivators at three different concentrations via Ellman´s procedure.[48] Few compounds retrieved comparable reactivation results in comparison to standard antidotes. Nonetheless, some structural factors identified could be included in further investigations on novel cholinesterase reactivators. Considering that 100 µmol/L is the maximum attainable concentration *in vivo* for clinically available reactivators,[49,50] we decided to also assay our compounds at this condition. This would provide comparable results. We employed 2-PAM (**13**) as main antidote reference and OBD (**14**) and TMB (**15**), under the same assay conditions, to confirm the current knowledge that bispyridinium compounds are substantially more efficient in AChE reactivation.[32] Care was taken to discount the absorbance of the tested compounds, avoiding calculation errors.

As neutral aryloximes are devoid of some structural features for complementary interactions inside enzymatic gorge, we decided to assay all compounds at 1000 µmol/L as well. Such experiments might be useful in disclosing other relevant molecular moieties for further development of reactivators with improved pharmacokinetics. Reactivation percent was calculated by equation 2, with absorbance read after 30 min of incubation of test compounds with NEMP-inhibited *Ee*AChE. We set as threshold 10 ± 1% of reactivation of NEMP-inhibited *Ee*AChE for selection of promising candidates, in accordance to literature.[48]

Table 1. Oximes **20a-ag** synthesized for assay in this work

Entry	Code	Oxime	pKa	logP	Yield %
1	20a	2-hydroxybenzaldoxime	6.61 (OH= 9.99)	1.39	85
2	20b	3-hydroxybenzaldoxime	7.09 (OH= 9.74)	1.39	95
3	20c	4-hydroxybenzaldoxime	7.57 (OH= 10.15)	1.39	93
4	20d	2-methoxybenzaldoxime	6.69	1.54	91
5	20e	3-methoxybenzaldoxime	7.20	1.54	92
6	20f	4-methoxybenzaldoxime	7.69	1.54	91
7	20g	2-bromobenzaldoxime	6.96	2.46	94

8	20h	3-bromobenzaldoxime	6.69	2.46	89
9	20i	4-bromobenzaldoxime	6.80	2.30	95
10	20j	2-chlorobenzaldoxime	7.26	2.30	94
11	20k	3-chlorobenzaldoxime	6.67	2.30	90
12	20l	4-chlorobenzaldoxime	6.82	1.84	96
13	20m	2-fluorobenzaldoxime	7.11	1.84	86
14	20n	3-fluorobenzaldoxime	6.80	1.84	90
15	20o	4-fluorobenzaldoxime	5.52	2.57	81
16	20p	2-trifluoromethylbenzaldoxime	6.13	2.57	88
17	20q	3-trifluoromethylbenzaldoxime	6.29	2.57	88
18	20r	4-trifluoromethylbenzaldoxime	8.08	2.21	94
Entry	Code	Oxime	pKa	logP	Yield %
19	20s	2-methylbenzaldoxime	7.97	2.21	83
20	20t	3-methylbenzaldoxime	8.14	2.21	84
21	20u	4-methylbenzaldoxime	8.21	2.94	87
22	20v	3-nitrobenzaldoxime	5.83	1.64	90
23	20w	4-nitrobenzaldoxime	5.80	1.64	93
24	20x	4-isopropylbenzaldoxime	8.71	1.80	95
25	20y	4-(N,N-dimethylamino)benzaldoxime	8.80	2.52	90
26	20z	4-(N,N-diethylamino)benzaldoxime	7.18 (OH= 10.60)	1.23	94

27	20aa	Vanillin oxime	6.69	2.46	90
28	20ab	Isovanillin oxime	6.21 (OH= 10.47)	1.23	90
29	20ac	Orthovanillin oxime	7.18 (OH= 10.14)	1.23	90
30	20ad	Pyridine-4-aldoxime	10.21	0.48	98
31	20ae	Pyridine-2-aldoxime	9.02	1.15	98
32	20af	Isatin 3-oxime	7.13 (NH= 15.51)	0.96	98
33	20ag	N-benzylisatin 3-oxime	7.31	2.55	93
34	13	Pralidoxime (2-PAM)	7.63	-3.26	-[a]
35	14	Obidoxime (OBD)	7.51, 8.11	-6.93	50
36	15	Trimedoxime (TMB)	8.63, 9.24	-7.04	40

[a] Commercial source

Table 2 shows the *Ee*AChE reactivation percent for the most active compounds. Under our Ellman's conditions, we observed that the best results for reactivation of NEMP-inhibited *Ee*AChE were at 1000 μmol/L, the highest concentration assayed (entries 1-7). Reference antidote 2-PAM and bispyridinium oxime TMB, also exhibited the same profile (entries 8 and 10, respectively), differently from the bispyridinium oxime OBD (entry 9) which showed opposite results, being more active at 100 μmol/L. These results may suggest that, for the neutral or pyridinium monooximes, reactivation potency is concentration-dependent, and corroborates with the literature findings, that oximes present different reactivation profiles for each inhibitor, being capable of even acting as AChE inhibitors.[51]

All tested neutral oximes showed essentially no reactivation at 10 μmol/L. At 100 μmol/L, four compounds (**20q**, 3-trifluoromethyl analogue; **20r**, 4-trifluoromethyl analogue; **20af**, isatin 3-oxime, and **20ag**, *N*-benzylisatin 3-oxime) reactivated NEMP-inhibited *Ee*AChE approximately at 60% of the 2-PAM reactivation, indicating that these are interesting compounds for further structural optimization.

From results obtained at 1000 µmol/L, we could identify 3 additional compounds. 2-Chlorobenzaldoxime (**20j**), result in agreement with data that showed that 2-chloroaryloximes successfully reactivates AChE inhibited by different nerve agents);[52] 2-trifluoromethylbenzaldoxime (**20p**) and orthovanillin oxime (**20ac**) could all reactivate NEMP-inhibited *Ee*AChE. The activity of the trifluoromethyl derivatives may be related, at least in part, to polar hydrophobicity effects.[53,54] Isatin-derived oximes stood out in the high-concentration experiments, even surpassing 2-PAM and OBD as reactivators. Isatin derivatives, diversely available, have also been reported as cholinesterase inhibitors.[56-59] and are clearly a choice for further development. [60,61]

Table 2. Percent of *Ee*AChE reactivation by the most active oximes

Entry	Oxime	Reactivator Concentration (µmol/L)		
		1000	100	10
1	20j	36 ± 2	1 ± 0	0
2	20p	10 ± 1	1 ± 0	0
3	20q	32 ± 2	13 ± 1	3 ± 0
4	20r	32 ± 3	13 ± 1	1 ± 0
5	20ac	13 ± 1	4 ± 1	1 ± 0
6	20af	34 ± 1	13 ± 1	2 ± 0
7	20ag	45 ± 5	10 ± 1	1 ± 0
Entry	Oxime	Reactivator Concentration (µmol/L)		
		1000	100	10
8	2-PAM (13)	29 ± 2	21 ± 1	4 ± 0
9	OBD (14)	26 ± 1	46 ± 1	15 ±1
10	TMB (15)	57 ± 2	39 ± 1	17 ±1

Table 3. Percent of NEMP-inhibited *Ee*AChE aging

Inhibiton time (min)	%*Ee*AChE Inhibition[a]	%*Ee*AChE Reactivation[b]
15	95 ± 1	59 ± 3
75	95 ± 1	56 ± 5
Δ%*Ee*AChE Reactivation		3

[a] Crude NEMP 1 μmol/L, [b] 2-PAM 100 μmol/L

Table 4. Percent of NEMP-inhibited *Ee*AChE spontaneous hydrolysis

Inhibiton time	%*Ee*AChE Inhibition[a]
10 min	93 ± 1
12 h	55 ± 3

[a] Crude NEMP 1 μmol/L

4. Conclusion

In conclusion, using NEMP, a VX surrogate, as acetylcholinesterase inhibitor, we assayed a series of simple, neutral oximes, aiming at identifying scaffolds for further drug development. Based on the reactivation profile shown in our assays, we were able to identify seven active compounds which are now under structural optimization for development of novel cholinesterase reactivators. All active compounds were concentration-dependent as reactivators for NEMP-inhibited *Ee*AChE. One of the compounds identified from the study (an isatin derivative) has been modified and successfully afforded new, active cholinesterase reactivators for organophosphorus-inhibited cholinesterases. As it is also known that oximes may act as reversible inhibitors of cholinesterases, our molecules are also being investigated in order to identify scaffolds that could act as AChE inhibitors, useful against neurodegenerative diseases.

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Conflict of Interests

None.

Authors' Information

ORCID numbers for authors: Samir F. de A. Cavalcante: 0000-0002-3478-2747; Joyce S. F. D. de Almeida: 0000-0002-1426-6681; Tanos C.C. França: 0000-0002-6048-8103; Kamil Kuča: 0000-0001-9664-1109; Alessandro B. C. Simas: 0000-0002-0470-3024.

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Supplementary Material

Supplementary data (NMR and MS spectra of synthesized compounds, full results on *Ee*AChE reactivation for all synthesised compounds) can be found in the online version.

Highlights:

- A practical method for synthesis of neutral aryloximes is presented;
- A VX surrogate was used as inhibitor for *Electrophorus eel* Acetylcholinesterase;
- Neutral aryloximes retrieved promising predicted properties for use as reactivators;
- Seven compounds were identified as potential leads for further optimization.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: