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Synthesis and *in vitro* evaluation of donepezil-based reactivators and analogues for nerve agent-inhibited human acetylcholinesterase

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Abstract

Poisoning by organophosphorus nerve agents and pesticides is a serious public and military health issues with over 200 000 fatalities annually worldwide. Conventional emergency treatment consists of rapid administration of atropine and pyridinium oxime as antidote. The reactivation of acetylcholinesterase (AChE) in the central nervous system (CNS) by the oxime is inefficient due to the fact that positively charged pyridiniums do not cross readily the blood brain barrier (BBB). Herein, we described the synthesis and *in vitro* evaluation of four donepezil-based non quaternary reactivators. The compounds **1**-**4** have been prepared in 7-8 linear steps in 1-9% overall yields and oximes **1**-**3** show a better ability (8 fold higher) than pralidoxime to reactivate VX-inhibited human AChE (VX-hAChE). Besides, oxime **2** is 5 to 11 fold more efficient than pralidoxime and HI-6 respectively for the reactivation of VX-inhibited human butyrylcholinesterase (VX-hBChE).

Introduction

Organophosphorus compounds in the form of pesticides or nerve agents are a serious health problem worldwide. Pesticides exposure is one of the most common causes of poisoning throughout the world (over 200 000 deadly intoxications yearly) and organophosphorus nerve agents (OPNA) present a persistent threat to the population as a consequence of armed conflicts (e.g. Gulf War) and terrorist attacks (e.g., subway attacks in Japan in 1995 and more recently during the civil war in Syria) despite an international convention prohibiting the use of chemical weapons (Figure 1).[1]

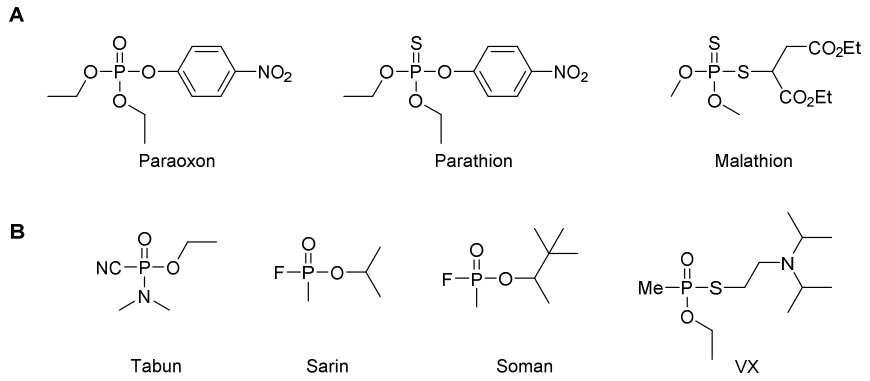


Figure 1 Structures of pesticides (A) and OPNA (B)

The acute toxicity of OPNA results from irreversible inhibition of AChE (EC 3.1.1.7), a key enzyme in neurotransmission, *via* the formation of a covalent P-O bond at the catalytic serine. Inhibition of AChE leads to the accumulation of acetylcholine neurotransmitter (ACh) in the synaptic cleft causing among other symptoms, seizures and respiratory arrest leading to death.[2, 3] The current urgency treatment of OPNA poisoning is based on the administration of a cocktail of three components: an antimuscarinic agent (e.g. atropine), an anticonvulsant drug (e.g. diazepam) and mono or bispyridinium AChE reactivator (e.g. pralidoxime, obidoxime, trimedoxime) (Figure 2). The high nucleophilicity of these alpha-nucleophiles allows the displacement of the phosphyl group from the catalytic serine, yielding to the restoration of AChE activity.

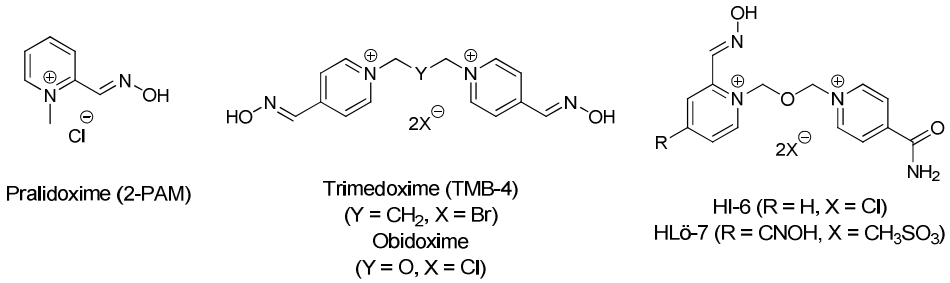


Figure 2 Structures of pyridinium AChE reactivators

Since the discovery of mono- and bis-pyridinium oximes as reactivators of inhibited AChE in 1950's, many structural modifications have been realized on these structures leading to a large library of potential reactivators.[4-7] However, despite hundreds of pyridinium oximes synthesized and evaluated during the last 70 years, this family of reactivators has serious drawbacks. Firstly, these molecules poorly cross the BBB, due to their permanent charge, limiting their efficiency to reactivate AChE in the CNS.[8] Moreover, no mono- or bis-pyridinium oximes are broad-spectrum reactivators, they have highly variable efficiency depending on the nature of the agent. During the last decade, several groups have focused their efforts on the development of new non-pyridinium reactivators able to cross the BBB for reactivating central AChE.[9-14] For example, our group described the synthesis and evaluation of new reactivator families based on 3-hydroxy pyridinaldoxime attached to AChE peripheral site ligands (e.g. phenyltetrahydroisoquinoline, tetrahydroacridine, tryptoline,…) through carbon chains with various lengths.[15-21]

Encouraged by these promising results, we envisaged to develop a new series of reactivators based on Donepezil (Aricept®). Donepezil is a well-known inhibitor of AChE and used for the treatment of Alzheimer's disease with an inhibitory activity in the nanomolar range.[22] X-ray structures of the complex of donepezil with human AChE has been solved.[23, 24] (Figure 3). The indanone moiety stacks against W286 at the peripheral site, while benzyl ring stacks against W86 at the catalytic site. The nitrogen atom of the piperidine ring makes water-mediated hydrogen bond to Y341 and Y337.

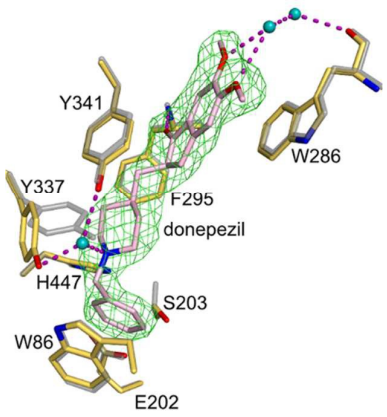


Figure 3 Donepezil-*h*AChE crystal structure.[24]

Based on these structural data, we developed a series of donepezil-based reactivators and analogues by replacing the benzyl group with 3-hydroxypyridinaldoxime. Four compounds have been synthesized in order to study the structural modifications influence on the affinity toward inhibited AChE (K_D) and the reactivation efficiency (k_{r2}) (Figure 4). The role of the ketone and/or the piperidine ring (compounds **2-4**) has also been studied.

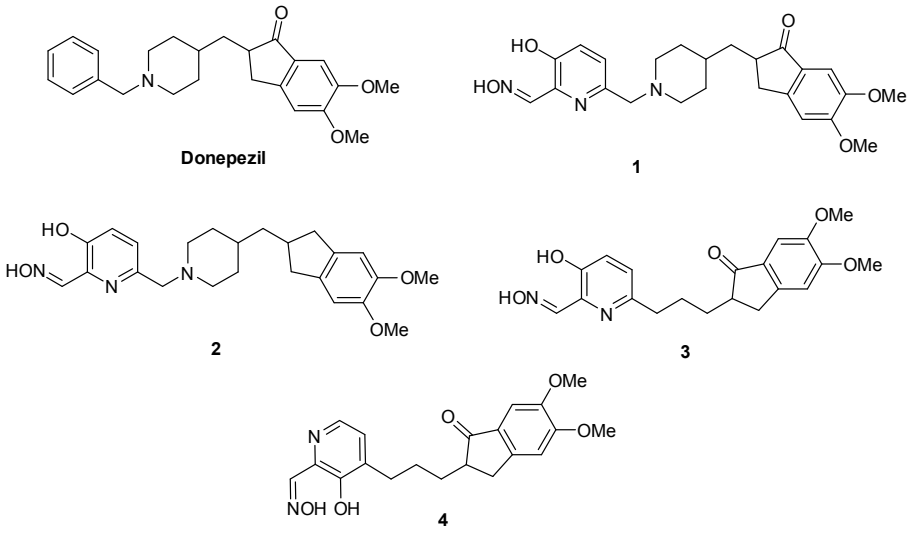
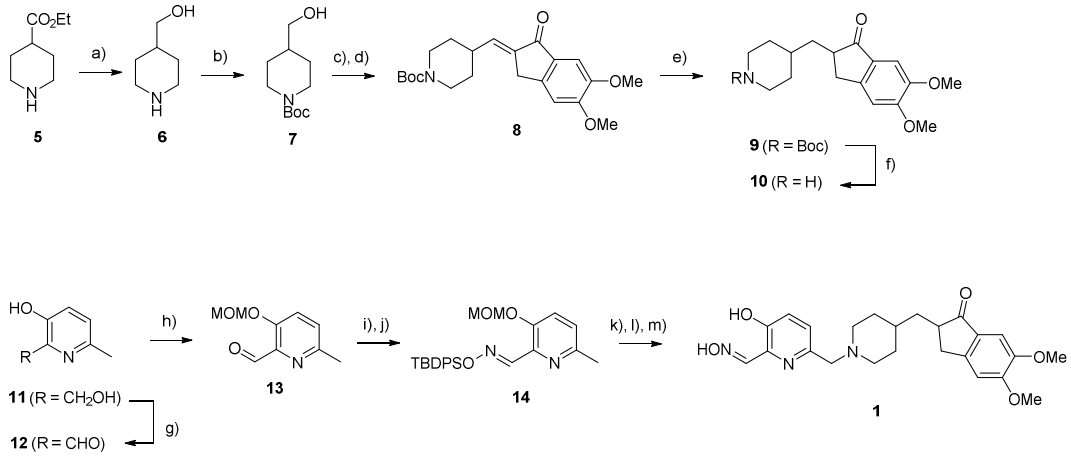


Figure 4 Structure of the donepezil-based AChE reactivator and analogues

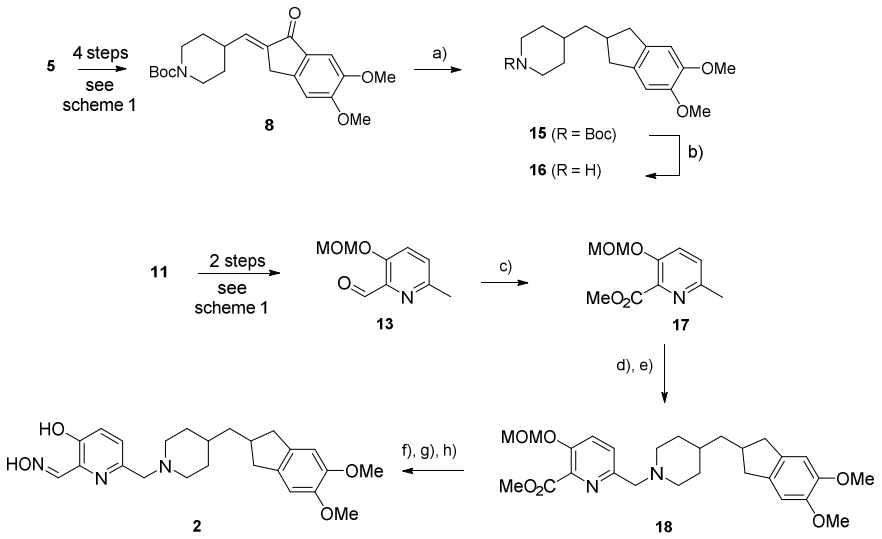
Results and discussion

The synthesis of the donepezil-based- reactivator **1** (Scheme 1) started with the preparation of the donepezil moiety **10**. The first step consisted of the reduction of ethyl isonipecotate **5** into the corresponding alcohol by using $LiAlH_4$. The protection of the secondary amine of the compound **6** as Boc-carbamate gave compound **7** in 50% yield. Then, alcohol **7** was oxidized into the corresponding aldehyde, which was used directly in the aldolisation-crotonization reaction with 5,6-dimethoxyindanone in the presence of sodium hydroxide and a phase transfer catalyst, to give the desired compound **8** in 57% yield over two steps. Different reduction conditions have been tested so as to reduce the conjugated double bond to give compound **9**. While the assays using a palladium catalyst (e.g. Pd/C, $Pd(OH)_2$) under hydrogen atmosphere gave only an over-reduction (ketone function was converted into the corresponding methylene group), we then evaluated the conditions reported by Keinan *et al.* using silicon hydride in the presence of a catalytic amount of zinc chloride and $Pd(PPh_3)_4$.[25] Under these conditions, the conjugate reduction of the α,β-unsaturated ketone **8** has been performed in 30% yield. Finally, the deprotection of the amine **9** by treatment with trifluoroacetic acid offered the donepezil moiety **10** in 80% yield. The second part of this synthesis consisted on the functionalization of the reactivator function, named 3-hydroxy pyridinaldoxime. The oxidation of alcohol **11** with MnO_2 in toluene furnished the corresponding aldehyde **12** in 76% yield. Then the protection of phenol as MOM ether has been carried out using methyl chloromethyl ether (MOMCl) in presence of potassium carbonate to afford **13** in 95% yield. The following steps consisted of the formation of oxime and its protection with TBDPSiCl. Radical bromination of the benzylic position of pyridine **14** using *N*-bromosuccinimide (NBS) followed by a nucleophilic substitution with amine **10** and treatment with trifluoroacetic acid afforded the reactivator **1** with 0.7% yield over three steps. It is noteworthy that the deprotection of oxime has been observed during the nucleophilic substitution under basic conditions. The oxime **1** has been prepared in 8 linear steps from the compounds **5** and **11** with 0.4% overall yield.



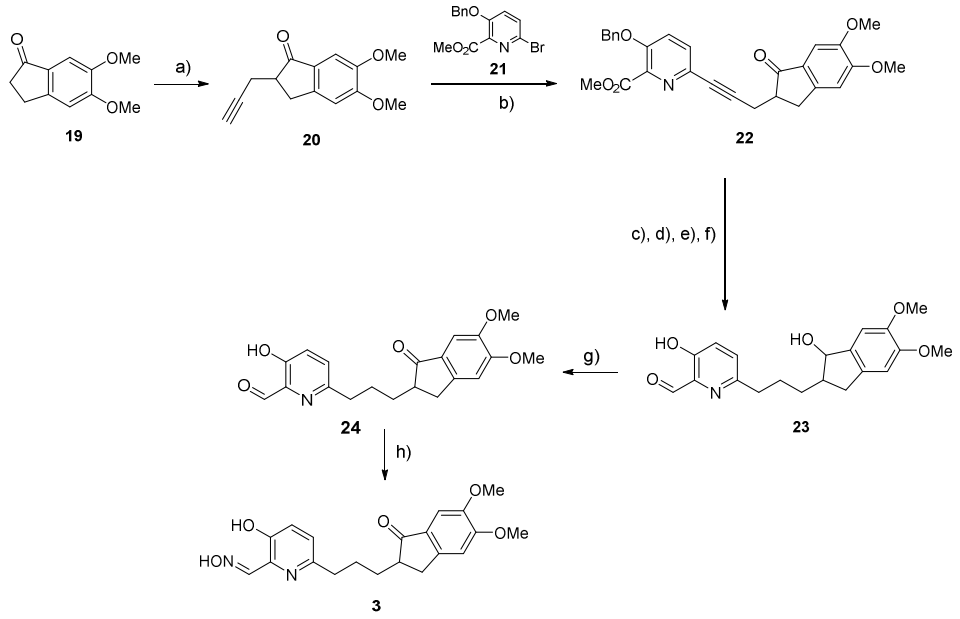
Scheme 1 *Reagents and conditions:* a) $LiAlH_4$ (1.7 equiv), THF, 92% ; b) Boc_2O (1 equiv), THF/EtOAc, 50%; c) 1) IBX (3 equiv), EtOAc; d) 5,6-dimethoxyindanone (0.9 equiv), NaOH aq. 10%, TBAB (0.1 équiv), CH_2Cl_2, 57% (two steps); e) Ph_2SiH_2 (1.8 equiv), $ZnCl_2$ (0.3 equiv), $Pd(PPh_3)_4$ 1.5 mol%, $CHCl_3$, 30%; f) TFA 20% in CH_2Cl_2, 80%; g) MnO_2, toluene, 76%; h) MOMCl (3 equiv), K_2CO_3 (4 equiv), acetone, 95%; i) $NH_2OH.HCl$ (1.1 equiv), NaOAc (1.2 equiv); j) TBDPSiCl (1.5 equiv), imidazole (1.3 equiv), CH_2Cl_2, 76% (two steps); k) *N*-bromosuccinimide (1.1 equiv), benzoyl peroxide (0.1 equiv), CCl_4, l) **10** (0.6 equiv), K_2CO_3 (1.1 equiv), DMF; m) TFA 20% (v/v) in CH_2Cl_2 then purifications (twice) by semi-preparative RP-HPLC, 0.7% (three steps)

The synthesis of the analogue **2** (Scheme 2) started with the preparation of the donepezil moiety **16**. Complete reduction of α-β unsaturated ketone **8** was performed using Pd/C under hydrogen atmosphere (1 bar) to give the over-reduced product **15** in 85% yield. Deprotection of amine by treatment with trifluoroacetic acid furnished the compound **16** in 75% yield. Direct radical bromination performed on aldehyde **13** failed, leading only to degradation products. Therefore, we decided to realize the radical bromination on the corresponding methyl ester **17**. The latter has been prepared from aldehyde **13** by treatment with iodine in basic conditions using methanol as solvent, in 78% yield.[26] Then, the photobromination of the benzylic position of the ester **17** with NBS, followed by the nucleophilic substitution with **16** furnished the compound **18** in 40% yield over two steps. The last steps consisted of the formation of oxime function and the deprotection of phenol. Reduction with DIBAL-H, followed by the deprotection of the phenol function using trifluoroacetic acid, and formation of oxime allowed to afford targeted oxime **2** in 15% yield over three steps. The oxime **2** has thus been prepared in 10 linear steps from the compounds **5** and **11** with 1% overall yield.



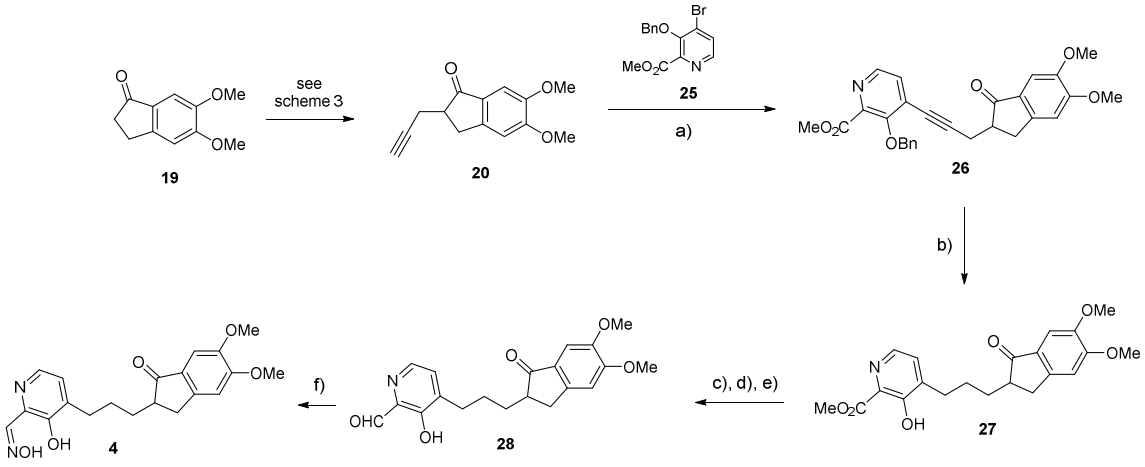
Scheme 2 *Reagents and conditions:* a) H_2 (1 atm), Pd/C, MeOH, 85%; b) TFA 20% (v/v) in CH_2Cl_2, 75%; c) I_2 (1.3 equiv), KOH (2.6 equiv), MeOH, 78%; d) *N*-bromosuccinimide (1.05 equiv), hν, CCl_4; e) **16** (0.85 equiv), K_2CO_3 (3.5 equiv), CH_3CN, 40% (two steps); f) DIBAL-H (2 equiv), CH_2Cl_2, g) TFA 20% (v/v) in CH_2Cl_2; h) NH_2OH,HCl, NaOAc, MeOH, 15% (three steps).

The synthesis of the Donepezil analogue based reactivator **3** (Scheme 3) started with the alkylation of 5,6-dimethoxyindanone **19** with propargyl bromide using lithium diisopropylamide at -78 °C to give the desired mono-alkylated product **20** in 48% yield. It is noteworthy that the starting material **19** was recovered (23%) and the di-alkylated product was also isolated by flash chromatography on silica gel (15%). The second step consisted on the Sonogashira coupling reaction between **20** and **21**, which has been prepared by following a reported procedure.[17] This cross-coupling reaction afforded **22** in 73% yield. The subsequent reduction of the alkyne and deprotection of the phenol function was then followed by a sequence of three reactions comprising (i) the protection of the phenol group with *t*-butyldimethylsilyl chloride (TBSCl), (ii) the reduction of the methyl ester to aldehyde upon DIBAL-H treatment, and finally (iii) the deprotection of the TBS ether using tetrabutylammonium fluoride (TBAF) as fluoride source. These transformations led to aldehyde **23** in 50% yield over four steps. During the reduction step with DIBAL-H, the carbonyl function on the indanone has been also reduced into the corresponding alcohol. Therefore, an additional treatment with Dess-Martin periodinane was required to furnish aldehyde **24** in 72% yield. Then, the last step consisted in the formation of the oxime function with the condensation of hydroxylamine onto the aldehyde **24**. The desired oxime **3** has thus been prepared in 8 steps from **19** with 9.5% overall yield.



Scheme 3 *Reagents and conditions:* a) propargyl bromide (1.5 equiv), LDA (1.2 equiv), THF, 48%; b) **21** (1 equiv), $Pd(PPh_3)_4$ 5 mol%, CuI (10 mol%), THF/NEt_3 (2:1 v/v), 73%; c) H_2 (1 atm), Pearlman's catalyst (20 mol%), EtOAc; d) TBDMSiCl (1.1 equiv), imidazole (3 equiv), DMF; e) DIBAL-H (4 equiv), CH_2Cl_2; f) TBAF (1.1 equiv), THF, 50% (four steps); g) Dess Martin periodinane (1 equiv), pyridine (5 equiv), CH_2Cl_2, 72%; h) $NH_2OH.HCl$ (1 equiv), NaOAc (1 equiv), EtOH, 76%

Based on the similar synthetic scheme, the synthesis of the analogue **4** (Scheme 4) started with the Sonogashira reaction between the alkyne **20** and bromo-pyridine **25**, which has been prepared by following a reported procedure.[26] The coupling product **26** has been obtained in 79% yield. Then, the reduction of the alkyne has been performed in a presence of Pearlman's catalyst under hydrogen atmosphere to afford compound **27** in quantitative yield. The sequence of three reactions comprising the protection of the phenol group as TBS ether, the reduction of the methyl ester to aldehyde, and the deprotection of the TBS ether furnished the aldehyde **28** in 3% yield over three steps. During the reduction step, the carbonyl group of the indanone has been partially reduced into the corresponding alcohol. Yet, the two products have been separated by a flash chromatography on silica gel, and to increase the global yields, the alcohol could have been oxidized in ketone as for compound **24**. The last step consisted in the formation of the oxime by treatment with hydroxylamine under basic conditions. The compound **4** has been synthesised in 7 steps from **19** with 1.1% overall yield.



Scheme 4 *Reagents and conditions:* a) **25** (1 equiv), $Pd(PPh_3)_4$ 10 mol%, CuI (20 mol%), THF/NEt_3 (2:1 v/v), 79%; b) H_2 (1 atm), Pearlman's catalyst (20 mol%), EtOAc, 99%; c) TBDMSOTf (1.1 equiv), 2,6-lutidine (3 equiv), CH_2Cl_2; d) DIBAL-H (2 equiv), CH_2Cl_2; e) TBAF (1.1 equiv), THF, 3% (three steps); f) $NH_2OH.HCl$ (1 equiv), NaOAc (1 equiv), EtOH, 99%

Reactivation tests

Since Donepezil is a very potent reversible inhibitor of hAChE (K_i = 1nM), we examined if the oxime derivatives are also inhibitors. If compounds **2**, **3** and **4** are weak reversible inhibitors of hAChE with respectively 74, 36 and 93% of residual activity at 100 µM, compound **1** is revealed to be a mild inhibitor with IC_{50} = 1.0±0.1 µM (See Supporting Information). We cannot anticipate that it is a drawback for a reactivator until toxicity tests are performed. The reactivation potencies of compounds **1**-**4** have been determined at pH 7.4 and 37 °C, close to physiological conditions (Table 1 and Supporting Information). The bimolecular reactivation rate constant (k_{r2}), which reflects the potency, has been determined. When relevant, the reactivation rate constant (k_r) and apparent dissociation constant of the reactivator/phosphyl-AChE complex (K_D) have also been determined. The reactivation parameters were compared to the reactivation constant of the marketed pyridinium aldoximes, 2-PAM and obidoxime, and to HI-6 which is about to be fielded in the French Army.

Compounds **1**-**3** are one order of magnitude more efficient than 2-PAM for the reactivation of VX-hAChE but still inferior to obidoxime and HI-6. Oxime **1** shows a similar binding affinity toward inhibited AChE as HI-6 and obidoxime ($K_D \approx 50$ µM), however *k*r is about 5-fold lower than obidoxime and HI-6. Comparison of oximes **1** and **2** show that the carbonyl function on

the indanone moiety improves the binding affinity toward VX-hAChE,[22] but possibly constraint the molecule in an orientation that results in lower k_r. Decrease in K_D balances the decrease in k_r resulting in similar k_{r2}. Comparison of oximes **3** and **4** both devoid of the piperidine ring, shows that position 6 onto the pyridine ring is the best position for anchoring. Indeed, oxime **3** is 7 fold less efficient than oxime **4**. Similarly to what is observed for oxime **2**, there is no loss of efficiency despite the loss of affinity related to the lack of piperidine ring, because k_r increases.

Compound **1**, was also tested for *in vitro* reactivation of sarin-inhibited human AChE (sarin-hAChE) (Table 1). It is less efficient than HI-6, due to a weaker affinity toward sarin-hAChE (2 fold lower than HI-6) and also lower reactivation rate constant (3.4 fold lower than HI-6).

Oximes **2-4** were tested for *in vitro* reactivation of tabun- and paraoxon-inhibited human AChE (tabun-hAChE and paraoxon-hAChE) (Table 1). They displayed only marginal activity on tabun-hAChE. However, they appeared to be significantly active on paraoxon-hAChE, being one order of magnitude more efficient than HI-6, although the latter is known to be a poor reactivator for pesticides. Surprisingly, oximes **2** and **3** have very close reactivation parameters despite their structural difference.

A complementary approach to OPNA poisoning is a pre-treatment based on bioscavengers. Bioscavengers are enzymes able to neutralize organophosphorus molecules in the bloodstream before they can reach acetylcholinesterase.[27] Human butyrylcholinesterase (hBChE) is the leading candidate for stoichiometric OPNA bioscavenging. Combining hBChE to a specific reactivator can improve its scavenging efficiency.[28] In this context, *in vitro* efficiency of oxime **2** and **4** has been evaluated for the reactivation of VX-inhibited BChE (Table 1). Oxime **2** is 5 to 11 fold more efficient than pralidoxime and HI-6 respectively due to a better binding affinity to the inhibited enzyme.

Table 1 Reactivation rate constant (k_r) and dissociation constant (K_D) for the reactivation of OP-inhibited *h*AChE and OP-inhibited *h*BChE.

Oxime	k_{obs} (min^{-1}) (oxime concentration µM)	K_D (µM)	k_r (min^{-1})	k_{r2} ($mM^{-1}.min^{-1}$)
		VX-hAChE		
Obidoxime	0.39±0.04 (100)	54±12	0.6±0.06	11.1
HI-6	0.45±0.06 (100)	70±8	0.66±0.03	9.3
2-PAM	0.015±0.001 (100)	215±75	0.06±0.01	0.28
1	0.10 ± 0,006 (100)	51.4±19.1	0.12±0.02	2.3

2	0.19±0.01 (100)	>200[a]	>0.35[a]	1.8±0.05
3	0.14±0.01 (50)	>50[a]	>0.14[a]	2.7±0.1
4	0.038±0.002 (100)	>150[a]	>0.06[a]	0.039±0.001
		sarin-hAChE		
HI-6	0.69±0.06 (100)	90±34	1.17±0.19	13
1	0.11±0.01 (100)	175±47	0.34±0.05	2
		paraoxon-hAChE		
HI-6	0.005±0.001 (100)	800±140	0.09	0.1
2	0.093±0.005 (100)	160±45	0.24±0.05	1.5
3	0.070±0.003 (50)	170±70	0.3±0.1	1.8
4	0.040±0.001 (100)	-	-	-
		tabun-hAChE		
HI-6	0.055±0.004 (100)	57±22	0.074±0.010	1.3
2	0.001±0.0001 (100)	-	-	-
3	0.004±0.0002 (50)	-	-	-
4	0.001±0.0002 (50)	-	-	-
		VX-hBChE		
2-PAM	0.062±0.005 (100)	2400±400	1.4±0.2	0.58
HI-6	0.028±0.001 (100)	>400[a]	>0.11[a]	0.28±0.01
2	0.16±0.005 (100)	96±5	0.29±0.01	3.0
4	0.041±0.003 (100)	-	-	-

[a] Not determined when [Reactivator]<<K_D leading to a linear dependence between k_{obs} and [Reactivator] : $k_{obs}=k_r/K_D$*[Reactivator]. In this case, $k_{r2}=k_r/K_D$, the slope of the line, is directly obtained by fitting.

Conclusions

A series of four donepezil-based reactivators and analogues has been synthesized and evaluated for their *in vitro* reactivation phosphylated acetylcholinesterase. These compounds have been prepared in 7-8 linear steps in 0.4-9% overall yields. Oximes **1**-**3** show an ability to reactivate VX-hAChE 8-fold greater than pralidoxime. The presence of the carbonyl function of the indanone moiety confers a good affinity toward inhibited AChE, but this is balanced by a reduced reactivation rate. Oximes **2-4** do not readily reactivate tabun-hAChE, but they proved to be relevant at reactivating paraoxon-hAChE. Oxime **2** is 5 to 11 fold more efficient than pralidoxime and HI-6 respectively for the reactivation of VX-hBChE. As with the

others developed families of non quaternary reactivators (phenyltetrahydroisoquinoline[18] and tetrahydroacridine[19]), the most favourable position of the linker is the position 6 onto the pyridine ring.

Experimental section

Chemistry

General. Column chromatography purifications were performed on Merck silica gel (40-63 µm). Thin-layer chromatography (TLC) was carried out on Merck DC Kieselgel 60 F-254 aluminium sheets. Compounds were visualized by illumination with a short wavelength UV lamp (λ = 254 nm). All solvents were dried following standard procedures (CH_2Cl_2, CH_3CN: distillation over P_2O_5, DMF: distillation over BaO under reduced pressure, THF: distillation over Na/benzophenone). ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 spectrometer (Bruker, Wissembourg, France). Chemical shifts are expressed in parts per million (ppm) from $CDCl_3$ (δ_H= 7.26, δ_C = 77.16) and CD_3OD (δ_H= 3.31, δ_C = 49.00). ^1J values are expressed in Hz. Residual solvents and grease contained in NMR sample were indicated on ^1H and ^{13}C spectra (see supporting information).[29] Mass spectra were obtained with a Finnigan LCQ Advantage MAX (ion trap) apparatus equipped with an electrospray source. All analyses were performed in the positive mode.

All final oximes were confirmed to be of ≥ 95% purity based on HPLC analysis. Analytical HPLC was performed on a Thermo Electron Surveyor instrument equipped with a PDA detector under the following conditions: Thermo Hypersil GOLD C18 column (5 µm, 4.6 x 100 mm) with MeOH and 0.1% aq. trifluoroacetic acid (TFA) as eluents [0.1% aq. TFA/MeOH (90/10) (5 min), followed by linear gradient from 10% to 100% of MeOH (45 min)] at a flow rate of 1.0 mL/min and *UV detection Max Plot* 220-360 nm.

Semi-preparative RP-HPLC was performed under the following conditions: Thermo Hypersil GOLD C18 column (5 µm, 21.2 x 250 mm) with CH_3CN and tetraethylammonium bromide (TEAB) (50 mM (aq), pH 7.5) as eluents (0% CH_3CN (5 min), followed by a gradient of 0-10% CH_3CN (5 min), then 10-90% CH_3CN (105 min)) at a flow rate of 15 mL.min-1. Double UV detection was achieved at λ = 230 and 315 nm.

4-Piperidinemethanol (6)

To a suspension of $LiAlH_4$ (2.1 g, 54.4 mmol, 1.7 equiv.) in dry THF (100 mL) at 0 °C was added dropwise a solution of ethyl isonipecotate **5** (5 g, 32 mmol) in dry THF (50 mL). The mixture was stirred overnight at rt and treated carefully with water (2.1 mL), an aqueous

solution of NaOH 15% (2.1 mL) and water (6.3 mL). The resulting mixture is stirred for 1 h at rt. The solid was filtered and the filtrate was dried over $MgSO_4$. The solvent was removed under reduced pressure to afford 4-piperidinemethanol 6 (3.40 g, 92%) as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90-1.03 (m, 2H), 1.42-1.64 (m, 1H), 1.66-1.70 (m, 2H), 2.50-2.59 (m, 2H), 3.01-3.07 (m, 2H), 3.38 (d, *J* = 6.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.0, 39.1, 46.3, 67.6. MS (ESI+): *m/z* (%): 116 (100) $[M+H]^+$.

[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]methyl alcohol (7)

4-piperidinemethanol **6** (3.40 g, 29.5 mmol) was stirred in EtOAc (100 mL) and THF (44 mL) and di-*tert*-butylcarbamate (6.44 g, 29.5 mmol) was slowly added and the resulting mixture was stirred for 6 h at rt. The mixture was successively washed with an aqueous saturated solution of $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. Chromatography on silica gel (cyclohexane/EtOAc 5:5, v/v) afforded the desired compound **7** (3.2 g, 50%) as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05-1.18 (m, 2H), 1.43 (s, 9H), 1.57-1.71 (m, 3H), 1.84 (m, 1H), 2.64-2.72 (m, 2H), 3.45-3.49 (m, 2H), 4.08-4.11 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.5, 28.7, 38.8, 43.8, 67.3, 79.4, 155.0. Spectral data are in accordance with those reported in the literature.[30]

***tert*-Butyl-4-((5,6-dimethoxy-1-oxo-1*H*-inden-2(3*H*)-ylidene)methyl)piperidine-1-carboxylate (8)[31]**

[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]methyl alcohol **7** (3.2 g, 14.7 mmol) and 2-iodobenzoic acid (IBX) (12.4 g, 44 mmol, 3 equiv.) were added into EtOAc (140 mL) under inert atmosphere. The resulting mixture was refluxed for 4 h and allowed to cool at rt. The solid was filtered and the solvent was evaporated. The crude aldehyde was used in the next step without further purification.

Under inert atmosphere, 5,6-dimethoxy-indanone (2.35 g, 12.3 mmol), TBAB (394 mg, 1.25 mmol, 0.01 equiv.) and 10% aqueous NaOH (9 mL) were stirred vigorously for 30 min at rt into CH_2Cl_2 (17 mL). The crude aldehyde was added and the resulting mixture was heated at 50 °C for 5 h. After cooling at rt, the layers were separated. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were washed with brine, water and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the crude product was recrystallized from isopropyl ether to afford the desired product **8** as white solid (3.3 g, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41-151 (m, 2H), 1.47 (s, 9H), 1.67-1.71 (m, 2H), 2.41-2.52 (m, 1H), 2.77-2.93 (m, 2H), 3.60 (d, *J* = 1.7 Hz, 2H), 3.92 (s, 3H), 3.97 (s, 3H), 4.11-4.16 (m, 2H), 6.62 (dt, *J* = 1.8, 7.7 Hz, 1H), 6.91 (s, 1H), 7.29 (s, 1H). ^{13}C NMR (75

MHz, $CDCl_3$): δ 28.4, 29.5, 30.8, 37.2, 43.2, 56.1, 56.3, 79.6, 105.0, 107.2, 131.7, 135.9, 138.5, 144.5, 149.5, 154.8, 155.4, 192.5. MS (ESI+): *m/z* (%): 388 (100) $[M+H]^+$.

tert-Butyl-4-((2,3-dihydro-5,6-dimethoxy-1-oxo-1*H*-inden-2-yl)methyl)piperidine-1-carboxylate (9)

To a degassed mixture of *tert*-butyl-4-((5,6-dimethoxy-1-oxo-1H-inden-2(3*H*)-ylidene)methyl)piperidine-1-carboxylate **8** (3.26 g, 8.4 mmol), dihydrodiphenylsilane (2.74 g, 15 mmol, 1.8 equiv.) and $ZnCl_2$ (350 mg, 2.6 mmol, 0.3 equiv.) in $CHCl_3$ (85 mL) was added $Pd(PPh_3)_4$ (150 mg, 0.13 mmol, 0.015 equiv.). The solution was stirred at rt for 24 h. Concentration under reduced pressure and purification on silica gel (cyclohexane/EtOAc 6:4 to 5:5, v/v) afforded the desired compound **9** (950 mg, 30%) as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14-1.26 (m, 2H), 1.29-1.41 (m, 1H), 1.45 (s, 9H), 1.61-1.76 (m, 3H), 1.86-1.95 (m, 1H), 2.67-2.74 (m, 4H), 3.31-3.30 (m, 1H), 3.90 (s, 3H), 3.97 (s, 3H), 4.08-4.15 (m, 2H), 6.86 (s, 1H), 7.17 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.4, 31.6, 32.8, 33.3, 34.6, 38.7, 45.1, 56.1, 56.2, 79.3, 104.3, 107.3, 129.2, 129.2, 148.7, 149.4, 154.9, 155.5, 207.6. MS (ESI+): *m/z* (%): 390 (100) $[M+H]^+$ and 796 (38) $[2M+H_2O]^+$.

5,6-Dimethoxy-2-(piperidin-4-ylmethyl)-2,3-dihydro-1*H*-inden-1-one (10)

Tert-butyl-4-((2,3-dihydro-5,6-dimethoxy-1-oxo-1H-inden-2-yl)methyl)piperidine-1-carboxylate **9** (950 mg, 2.4 mmol) was added in a 20% (v/v) solution of TFA in CH_2Cl_2 (24 mL) at 0 °C. The mixture was stirred for 4 h and was then allowed to warm at rt. TFA and CH_2Cl_2 were removed by evaporation using a water pump and the residue was dissolved into an aqueous saturated solution of $NaHCO_3$. The aqueous layer was then extracted three times with CH_2Cl_2 and the organic layers were combined and dried over $MgSO_4$. Concentration under reduced pressure afforded the desired product **10** as white solid (560 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14-1.33 (m, 3H), 1.56-1.77 (m, 3H), 1.82-1.91 (m, 1H), 2.56-2.70 (m, 4H), 3.04-3.10 (m, 2H), 3.17-3.26 (m, 1H), 3.87 (s, 3H) 3.93 (s, 3H), 6.83 (s, 1H), 7.13 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.7, 33.3, 34.0, 34.7, 39.2, 45.1, 46.5, 56.1, 56.2, 104.3, 107.3, 129.3, 148.8, 149.4, 155.4, 207.8. MS (ESI+): *m/z* (%): 290 (100) $[M+H]^+$.

3-Hydroxy-6-methylpicolinaldehyde (12)

To a solution of 2-(hydroxymethyl)-6-methylpyridin-3-ol **11** (2 g, 14.3 mmol) in toluene (150 mL) was added MnO_2 (10 g), and the suspension was stirred at 80 °C until all starting material was consumed (reaction followed by TLC). After cooling, MnO_2 was removed by filtration through celite and the solvent was evaporated under reduced pressure to afford the

desired product **12** (1.49 g, 76%) as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.55 (s, 3H), 7.25-7.29 (m, 2H), 10.02 (s, 1H), 10.65 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.6, 126.6, 130.5, 135.7, 151.1, 157.0, 198.8. MS (ESI+): *m/z* (%): 138 (100) $[M+H]^+$.

3-(Methoxymethoxy)-6-methylpicolinaldehyde (13)

To a cooled (0 °C) solution of 3-hydroxy-6-methylpicolinaldehyde **12** (1.5 g, 10.9 mmol) and K_2CO_3 (6 g, 43.4 mmol, 4 equiv.) in acetone (110 mL) at 0 °C was added dropwise MOMCl (16 mL, 32.6 mmol, 3 equiv.).The resulting mixture was stirred for 16 h at rt. Filtration of K_2CO_3 salt and concentration under reduced pressure afforded the good product **13** as yellow liquid (1.91 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.56 (s, 3H), 3.49 (s, 3H), 5.28 (s, 2H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.55 (d, *J* = 8.7 Hz, 1H), 10.33 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.6, 56.6, 94.9, 124.7, 128.7, 140.5, 152.3, 154.1, 190.4. MS (ESI+): *m/z* (%): 182 (100) $[M+H]^+$.

3-(Methoxymethoxy)-6-methylpicolinaldehyde *O-tert*-butyldiphenylsilyl oxime (14)

To a solution of 3-(methoxymethoxy)-6-methylpicolinaldehyde **13** (1.91 g, 10.5 mmol), in dry EtOH (100 mL) were successively added $NH_2OH.HCl$ (806 mg, 11.6 mmol, 1.1 equiv.) and NaOAc (1.03 g, 12.6 mmol, 1.2 equiv.). The resulting mixture was stirred for 1 h at rt. Salts were removed by filtration and the solution was concentrated under reduced pressure. To a solution of crude product in dry CH_2Cl_2 (160 mL) were successively added imidazole (1.3 g, 19.1 mmol, 1.9 equiv.) and TBDPSCl (4 mL, 15.3 mmol, 1.5 equiv.). The mixture was stirred at rt for 18 h under argon atmosphere. Concentration under reduced pressure and chromatography on silica gel (cyclohexane/EtOAc 9:1, v/v) afforded access to the desired compound **14** as yellow oil (2.7 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (s, 9H), 2.50 (s, 3H), 3.27 (s, 2H), 4.96 (s, 2H), 7.26-7.40 (m, 8H), 7.71-7.80 (m, 4H), 8.64 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.7, 27.2, 27.3, 56.2, 94.9, 127.6, 127.9, 129.7, 133.9, 134.9, 135.8, 140.7, 150.6, 151.7. MS (ESI+): *m/z* (%): 435 (100) $[M+H]^+$.

6-((4-((5,6-Dimethoxy-1-oxo-2,3-dihydro-1*H*-inden-2-yl)methyl)piperidin-1-yl)methyl)-3-hydroxypicolinaldehyde oxime (1)

To a solution of compound **14** (1.2 g, 2.8 mmol) and NBS (541 mg, 3 mmol, 1.1 equiv.) in CCl_4 (30 mL) was added benzoyl peroxide (68 mg, 0.28 mmol, 0.1 equiv.). The mixture was stirred 2 days at 75 °C. CH_2Cl_2 was added and the organic layer was washed twice with water, dried over $MgSO_4$ and concentrated under reduced pressure. To a solution of crude product in DMF (20 mL) were added **10** (491 mg, 1.7 mmol) and K_2CO_3 (708 mg, 5.13 mmol,

3 equiv.). The solution was stirred at 60 °C for 24 h. DMF was concentrated and the crude product was dissolved in CH_2Cl_2 (15 mL). To this solution was added TFA (3 mL) and the mixture was stirred at rt for 2 h. Solvent and the excess of TFA were removed by evaporation under reduced pressure. Purifications (twice) by semi-preparative RP-HPLC afforded the desired compound **1** (0.97 mg, 0.7%) as yellow solid. ^{1}H NMR (300 MHz, CD_3OD) δ 1.35-1.58 (m, 6H), 1.83-2.05 (m, 4H), 2.72-2.94 (m, 2H), 2.86-2.94 (m, 1H), 3.85 (s, 3H) 3.93 (s, 3H), 4.20 (s, 2H), 7.05 (s, 1H), 7.13 (s, 1H), 7.38 (s, 2H), 8.37 (s,1H). ^{13}C NMR (75 MHz, CD_3OD) δ 30.2, 30.8, 31.2, 33.4, 34.1, 39.0, 46.3, 54.9, 56.5, 56.7, 61.6, 105.3, 109.0, 116.2, 120.1, 126.0, 126.9, 129.8, 138.0, 151.1, 151.3, 151.9, 157.8, 209.9. MS (ESI+): *m/z* (%): 440 (100) $[M+H]^+$. HPLC: t_R = 26.08 min, purity = 95%. HRMS (ESI+) calcd for $C_{24}H_{30}N_3O_5^+$: 440.1938; found: 440.1942.

tert-Butyl 4-((5,6-dimethoxy-2,3-dihydro-1*H*-inden-2-yl)methyl)piperidine-1-carboxylate (15)

To a solution of **8** (1.2 g, 3.1 mmol) in degassed methanol (50 mL) was added palladium on carbon (10 wt%, 0.33 g, 0.31 mmol, 0.1 equiv.). The solution was bubbled with H_2 and stirred at rt under H_2 atmosphere (1 atm) for 2 h. The reaction mixture was then bubbled with argon for 15 min and palladium was removed by filtration over celite. Concentration under reduced pressure afforded compound **15** (1.05 g, 85%) as a white-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04-1.16 (m, 2H), 1.41-1.48 (m, 3H), 1.44 (s, 9H), 1.65-1.70 (m, 2H), 2.46-2.58 (m, 3H), 2.64-2.72 (m, 2H), 2.92-2.99 (m, 2H), 3.82 (s, 6H), 4.06-4.09 (m, 2H), 6.71 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5, 32.4, 34.8, 37.6, 39.5, 42.8, 44.1, 56.1, 79.2, 107.8, 134.8, 147.8, 154.9. MS (ESI+): *m/z* (%): 376 (100) $[M+H]^+$, 393 (50) $[M+H_2O]^+$.

4-((5,6-Dimethoxy-2,3-dihydro-1*H*-inden-2-yl)methyl)piperidine (16)

Compound **15** (1 g, 2.7 mmol) was added to a solution of TFA in CH_2Cl_2 (20% v/v, 24 mL) at 0 °C. The mixture was stirred for 1.5 h and was then allowed to warm at rt. TFA and CH_2Cl_2 were removed by evaporation using a water pump and the residue was dissolved into an aqueous saturated solution of $NaHCO_3$. The aqueous layer was then extracted three times with CH_2Cl_2 and the organic layers were combined and dried over $MgSO_4$. CH_2Cl_2 was removed under reduced pressure to afford the desired product **16** (550 mg, 75%) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08-1.15 (m, 2H), 1.41-1.44 (m, 3H), 1.68-1.72 (m, 3H), 2.45-2.63 (m, 5H), 2.91-2.98 (m, 2H), 3.03-3.07 (m, 2H), 3.82 (s, 6H), 6.72 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.9, 35.0, 37.4, 39.5, 43.6, 46.9, 56.0, 107.7, 135.0, 147.7. MS (ESI+): *m/z* (%): 276 (100) $[M+H]^+$.

Methyl 3-(methoxymethoxy)-6-methylpicolinate (17)

To a cooled solution (0 °C) of **13** (1.54 g, 8.49 mmol) in MeOH (30 mL) were added a solution of KOH (1.24 g, 22.07 mmol, 2.6 equiv.) in MeOH (30 mL) and a solution of I_2 (2.8 g, 11.03 mmol, 1.3 equiv.) in MeOH (30 mL). The resulting mixture was allowed to warm at rt and was stirred for 4 h. The reaction was quenched with a solution of $NaHSO_3$ until the brown color disappeared. MeOH was concentrated under reduced pressure and the aqueous layer was extracted twice with EtOAc. The combined organic layers were dried over $MgSO_4$. Concentration under reduced pressure and chromatography on silica gel (cyclohexane/EtOAc 7:3, v/v) afforded the desired compound **17** (1.4 g, 78%) as brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.55 (s, 3H), 3.50 (s, 3H), 3.97 (s, 3H), 5.23 (s, 2H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5, 52.6, 56.3, 95.2, 125.0, 126.5, 139.2, 150.7, 151.3, 165.5.

Methyl-6-((4-((5,6-dimethoxy-2,3-dihydro-1*H*-inden-2-yl)methyl)-3-(methoxymethoxy)picolinate (18)

To a solution of **17** (250 mg, 1.18 mmol) in CCl_4 (12 mL) was added *N*-bromosuccinimide (221 mg, 1.24 mmol, 1.05 equiv.). The resulting mixture was irradiated with a halogen lamp (300 W) for 1 h. The organic layer was washed three times with brine, dried over $MgSO_4$ and concentrated under reduced pressure. To a solution of this crude product in CH_3CN (15 mL) were added **16** (270 mg, 1 mmol) and K_2CO_3 (490 mg, 3.54 mmol, 3.5 equiv.) and the resulting mixture was refluxed overnight. After cooling, K_2CO_3 was removed by filtration through celite and the solvent was evaporated under reduced pressure. Purification by flash chromatography on silica gel (EtOAc 100%) afforded desired compound **18** (190 mg, 40%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26-1.42 (m, 5H), 1.65-1.69 (m, 2H), 2.00-2.08 (m, 2H), 2.46-2.52 (m, 3H), 2.83-2.95 (m, 4H), 3.48 (s, 3H), 3.61 (s, 2H), 3.80 (s, 6H), 3.94 (s, 3H), 5.22 (s, 2H), 6.70 (s, 2H), 7.54-7.57 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.5, 32.6, 34.4, 37.8, 39.5, 42.9, 52.7, 54.0, 54.1, 56.1, 56.5, 64.0, 95.2, 108.0, 128.8, 126.2, 135.0, 139.3, 147.8, 151.5, 152.4, 165.7. MS (ESI+): *m/z* (%): 485 (100) $[M+H]^+$.

6-((4-((5,6-Dimethoxy-2,3-dihydro-1*H*-inden-2-yl)methyl)piperidin-1-yl)methyl)-3-hydroxypicolinaldehyde oxime (2)

To a solution of **18** (190 mg, 0.39 mmol) in dry CH_2Cl_2 (10 mL) was added DIBAL-H (1 M in CH_2Cl_2, 0.78 mL, 0.78 mmol, 2 equiv.) under inert atmosphere at -78 °C. The reaction mixture was stirred at this temperature for 3 min (an extended time of reaction gave a mixture of the corresponding aldehyde and alcohol). MeOH (0.78 mL) was added and the

mixture was allowed to warm to rt. The organic layer was washed with an aqueous solution of NaOH 1 M, dried over $MgSO_4$ and concentrated under reduced pressure. To the obtained residue was added a solution of TFA (20% v/v in CH_2Cl_2, 10 mL) and the mixture was stirred for 2 h at rt. Concentration by using a water pump gave the desired intermediate. To a solution of the crude product in MeOH (5 mL) were added $NH_2OH.HCl$ (27 mg, 0.39 mmol) and NaOAc (320 mg, 3.9 mmol, 10 equiv.) and the resulting mixture was stirred overnight at rt. Concentration under reduced pressure and purification by flash chromatography (EtOAc 100%) afforded the desired compound **2** (25 mg, 15% over 3 steps) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20-1.50 (m, 5 H), 1.65-1.80 (m, 2H), 2.10-2.17 (m, 2H), 2.46-2.56 (m, 3H), 2.92-3.03 (m, 4H), 3.61 (s, 2H), 3.84 (s, 6H), 6.72 (s, 2H), 7.21 (m, 2H), 8.34 (s, 1H), 9.79 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.8, 31.7, 34.3, 37.8, 39.6, 42.6, 54.2, 56.2, 64.1, 107.9, 124.2, 125.6, 135.0, 135.8, 147.9, 153.7. MS (ESI+): *m/z* (%): 426 (100) $[M+H]^+$. HRMS (ESI+) calcd for $C_{24}H_{32}N_3O_4^+$: 426.2387; found: 426.2386. HPLC: t_R = 26.3 min, purity = 97%.

5,6-Dimethoxy-2-(prop-2-ynyl)-2,3-dihydro-1H-inden-1-one (**20)**

To a cooled (0 °C) solution of diisopropylamine (170 µL, 1.2 mmol, 1.2 equiv.) in THF (10 mL) was added *n*-BuLi (1.6 M in hexane, 750 µL, 1.2 mmol, 1.2 equiv.). The mixture was stirred 15 min before cooled to -78 °C. A solution of 5,6-dimethoxyindanone **19** (192 mg, 1 mmol) in THF solution (5 mL) was added and the reaction mixture was stirred 15 min at -78 °C. Then, propargyl bromide (170 µL, 80 wt% in toluene, 1.5 mmol, 1.5 equiv.) was added and the solution was allowed to warm at rt. The mixture was concentrated and purified by flash chromatography on silica gel (cyclohexane/EtOAc 3:7, v/v) to give **20** (111 mg, 48%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87 (t, *J* = 2.7 Hz, 1H), 2.44-2.56 (m, 1H), 2.73 (ddd, *J* = 2.7, 4.5, 16.8 Hz, 1H), 2.78-2.87 (m, 1H), 2.97 (dd, *J* = 4.5, 16.8 Hz, 1H), 3.28 (dd, *J* = 4.5, 16.8 Hz, 1H), 3.88 (s, 3H), 3.95 (s, 3H), 6.87 (s, 1H), 7.15 (s, 1H).^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 31.7, 45.7, 56.0, 56.2, 69.5, 81.3, 104.2, 107.3, 129.0, 149.0, 149.4, 155.6, 204.8.

Methyl 3-(benzyloxy)-6-(3-(5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)prop-1-ynyl)picolinate (22)

To a Schlenk tube were added **20** (400 mg, 1.74 mmol), bromopyridine **21** (560 mg, 1.74 mmol) (prepared as reported in the literature[17]), CuI (32 mg, 0.174 mmol, 0.1 equiv.), $Pd(PPh_3)_4$ (100 mg, 0.087 mmol, 0.05 equiv.), degassed THF (5 mL) and NEt_3 (2.5 mL). The resulting mixture was stirred at rt for 15 h under argon atmosphere. After concentration under reduced pressure, the residue was purified by flash chromatography on silica gel

(cyclohexane/EtOAc 1:1, v/v) to give **22** (600 mg, 73%) as a beige solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.70 (dd, *J* = 8.4, 16.5 Hz, 1H), 2.92-3.12 (m, 3H), 3.36 (dd, *J* = 7.5, 17.1 Hz, 1H), 3.93 (s, 3H), 3.98 (s, 3H), 3.99 (s, 3H), 5.21 (s, 2H), 6.92 (s, 1H), 7.20 (s, 1H), 7.25-7.50 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 32.1, 46.0, 56.1, 56.3, 70.7, 80.2, 87.3, 104.3, 107.4, 121.6, 126.9, 128.2, 128.7, 128.9, 130.2, 135.0, 135.4, 140.0, 149.1, 149.5, 153.0, 155.7, 164.8, 204.9.

3-Hydroxy-6-(3-(1-hydroxy-5,6-dimethoxy-2,3-dihydro-1H-inden-2-yl)propyl)picolinaldehyde (23)

To a solution of **22** (300 mg, 0.64 mmol) in degassed EtOAc (20 mL) was added Pearlman's catalyst (182 mg, 0.13 mmol, 0.2 equiv., 20 wt% Pd, moisture 50%). The solution was bubbled with H_2 and the reaction was stirred at rt under H_2 atmosphere (1 atm) for 1 h. The mixture was filtrated through celite and concentrated under reduced pressure. To a solution of the residue in dry DMF (3 mL) were successively added imidazole (122 mg, 1.8 mmol, 3 equiv.) and TBDMSCl (99 mg, 0.66 mmol, 1.1 equiv.). The mixture was stirred at rt for 2 h under argon atmosphere. The organic layer was washed with brine (5 times), dried over $MgSO_4$ and concentrated under reduced pressure. To a solution of the resulting residue in dry CH_2Cl_2 (10 mL) was added dropwise DIBAL-H (2.4 mL, 1 M in CH_2Cl_2, 2.4 mmol, 4 equiv.) at -78 °C. Then, the reaction mixture was stirred at this temperature for 10 min. The reaction was quenched with MeOH (2.4 mL) and the mixture was allowed to warm at rt. The organic layer was washed with an aqueous solution of NaOH 1 M, dried over $MgSO_4$ and concentrated under reduced pressure. Then, TBAF (660 µL, 0.66 mmol, 1.1 equiv., 1 M in THF) was added at 0 °C to the residue in dry THF (20 mL) and the mixture was stirred for 30 min at this temperature. After concentration under reduced pressure, a purification by flash chromatography on silica gel (EtOAc/MeOH 1:1, v/v) afforded **23** (112 mg, 50%) as a colourless oil. The product was isolated as a mixture of 2 diastereoisomers (A-75% and B-25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59-1.65 (m, 1H-A + 1H-B), 1.75-2.06 (m, 2H-A + 2H-B), 2.11-2.14 (m, 1H-A), 2.17-2.28 (1H-B), 2.37-2.51 (m, 1H-A + 1H-B), 2.70 (dd, *J* = 8.7, 15.6 Hz, 1H-A), 2.85-2.95 (m, 2H-A + 2H-B), 3.10 (dd, *J* = 8.7, 15.3 Hz, 1H-B), 3.88 (s, 3H-A + 3H-B), 3.90 (s, 3H-B), 3.91 (s, 3H-A), 4.71 (br t, *J* = 5.1 Hz, 1H-B), 5.06 (br t, *J* = 5.1 Hz, 1H-A), 6.73 (s, 1H-B), 6.77 (s, 1H-A), 6.92 (s, 1H-B), 6.99 (s, 1H-A), 7.30-7.40 (m, 2H-A + 2H-B), 10.07 (s, 1H-B), 10.09 (s, 1H-A), 10.73 (br s, 1H-A + 1H-B).

6-(3-(5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)propyl)-3-hydroxypicolinaldehyde (24)

To a solution of **23** (50 mg, 0.14 mmol) and pyridine (57 µL, 0.7 mmol, 5 equiv.) in dichloromethane (5 mL) under argon atmosphere was added Dess Martin periodinane (15 wt% in dichloromethane, 300 µL, 0.14 mmol). The mixture was stirred for 1 h at rt and then concentrated and purified by flash chromatography on silica gel (cyclohexane/EtOAc 7/3, v/v) to give **24** (35 mg, 72%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48-1.54 (m, 1H), 1.74-1.89 (m, 2H), 1.90-2.03 (m, 1H), 2.58-2.94 (m, 4H), 3.17 (dd, *J* = 7.5, 16.8 Hz, 1H), 3.83 (s, 3H), 3.89 (s, 3H), 6.79 (s, 1H), 7.09 (s, 1H), 7.19-7.27 (m, 2H), 9.95 (s, 1H), 10.58 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.4, 31.3, 32.6, 37.2, 47.5, 56.1, 56.2, 104.3, 107.4, 126.5, 129.4, 129.9, 135.7, 149.0, 149.4, 154.4, 155.5, 157.0, 198.7, 207.4. MS (ESI+) *m/z* (%): 356 $[M+H]^+$.

6-(3-(5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)propyl)-3-hydroxypicolinaldehyde oxime (3)

To a solution of **24** (33 mg, 0.093 mmol) in absolute EtOH (5 mL) were added successively $HONH_2{\cdot}HCl$ (6.5 mg, 0.093 mmol) and NaOAc (7.6 mg, 0.093 mmol). The mixture was stirred at rt for 30 min under argon atmosphere. After concentration under reduced pressure, the residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc 1:1, v/v) to give **3** (26 mg, 76%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43-1.56 (m, 1H), 1.77-1.86 (m, 2H), 1.91-2.02 (m, 1H), 2.64-2.81 (m, 4H), 3.21 (dd, *J* = 7.5, 17.1 Hz, 1H), 3.88 (s, 3H), 3.94 (s, 3H), 6.83 (s, 1H), 7.04 (d, *J* = 8.4 Hz, 1H), 7.14 (s, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 8.43 (s, 1H), 9.83 (bs, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.8, 31.2, 32.6, 37.2, 47.6, 56.1, 56.3, 104.3, 107.4, 124.4, 125.0, 129.3, 134.6, 149.3, 149.4, 152.7, 153.1, 153.9, 155.6, 208.0. MS (ESI+) *m/z* (%): 371 $[M+H]^+$. HRMS (ESI+) calcd for $C_{20}H_{23}N_2O_5^+$: 371.1607; found: 371.1607. HPLC: t_R = 23.1 min, purity = 95%.

Methyl 3-(benzyloxy)-4-(3-(5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)prop-1-ynyl)picolinate (26)

To a Schlenk tube were added alkyne **20** (115 mg, 0.5 mmol), bromopyridine **25**[26] (161 mg, 1 equiv.), CuI (19 mg, 0.2 equiv.), $Pd(PPh_3)_4$ (58 mg, 0.1 equiv.) and degassed THF (5 mL) and NEt_3 (2.5 mL). The resulting mixture was stirred at rt for 15 h under argon atmosphere. After concentration under reduced pressure, the residue was purified by flash chromatography on silica gel (EtOAc/cyclohexane 2:3, v/v) to give **26** (187 mg, 79%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.70-2.90 (m, 3 H), 3.00-3.15 (m, 1H), 3.20-3.40 (m, 1H), 3.81 (s, 3H), 3.87 (s, 3H), 3.94 (s, 3H), 5.03 (s, 2H), 6.78 (s, 1H), 7.07 (s, 1H), 7.20-7.40 (m, 5H), 7.49 (d, *J* = 4.8 Hz, 1H), 8.39 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (75 MHz,

$CDCl_3$) δ (ppm) 21.6, 31.8, 45.7, 52.8, 56.1, 56.2, 66.4, 72.3, 76.3, 98.4, 104.2, 107.3, 128.2, 128.3, 128.8, 130.2, 136.5, 144,5, 148.9, 149.5, 155.8, 165.2, 204.6.

Methyl 4-(3-(5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)propyl)-3-hydroxypicolinate (27)

To a solution of **26** (185 mg, 0.39 mmol) in degassed EtOAc (20 mL) was added Pearlman's catalyst (112 mg, 0.2 equiv., 20% Pd, moisture 50%). The solution was bubbled with H_2 and the reaction was stirred at rt under H_2 atmosphere (1 atm) for 1 hour. The mixture was filtrated through celite and concentrated under reduced pressure to afforded **27** (185 mg, 99%) as a beige solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.39 – 1.53 (m, 1H), 1.63 – 1.76 (m, 2H), 1.84 – 1.97 (m, 1H), 2.56 – 2.71 (m, 4H), 3.16 (dd, *J* = 7.5, 17.1 Hz, 1H), 3.81 (s, 3H), 3.87 (s, 3H), 3.94 (s, 3H), 6.78 (s, 1H), 7.07 (s, 1H), 7.19 (d, *J* = 4.5 Hz, 1H), 8.07 (d, *J* = 4.5 Hz, 1H), 10.80 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.2, 29.2, 31.3, 32.5, 47.3, 53.1, 56.0, 56.2, 104.2, 107.3, 128.9, 129.3, 129.4, 132.0, 140.5, 141.0, 148.9, 149.3, 155.4, 157.5, 170.3, 207.3.

4-(3-(5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)propyl)-3-hydroxypicolinaldehyde (28)

To a solution of **27** (145 mg, 0.38 mmol) in dry CH_2Cl_2 (20 mL) were successively added 2,6-lutidine (132 µL, 3 equiv.) and TBDMSOTf (96 µL, 1.1 equiv.). The mixture was stirred at rt for 15 h under argon atmosphere. The organic layer was washed with water (once), dried over $MgSO_4$ and concentrated under reduced pressure. To a solution of the resulting residue in dry CH_2Cl_2 (10 mL) was added dropwise DIBAL-H (760 µL, 1 M in CH_2Cl_2, 2 equiv.) at -78 °C. Then, the reaction mixture was stirred at this temperature for 10 min. The reaction was quenched with MeOH (760 µL) and the mixture was allowed to warm at rt. The organic layer was washed with an aqueous solution of NaOH (1 M), dried over $MgSO_4$ and concentrated under reduced pressure. Then, TBAF (420 µL, 1 M in THF, 1.1 equiv.) was added at 0 °C to the residue in dry THF (20 mL) and the mixture was stirred for 30 min at this temperature. After concentration under reduced pressure, the crude product was purified by a flash chromatography on silica gel (cyclohexane/EtOAc 1:1 v/v) to afford the compound **28** (4.0 mg, 3%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.56 – 1.61 (m, 1H), 1.68 – 1.83 (m, 2H), 1.93 – 2.04 (m, 1H), 2.66 – 2.78 (m, 4H), 3.25 (dd, *J* = 8.4, 17.1 Hz, 1H), 3.90 (s, 3H), 3.96 (s, 3H), 6.86 (s, 1H), 7.16 (s, 1H), 7.29 (d, *J* = 4.5 Hz, 1H), 8.24 (d, *J* = 4.5 Hz, 1H), 10.06 (s, 1H), 10.98 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.2, 28.8, 31.4,

32.6, 47.3, 56.1, 56.3, 104.3, 107.4, 129.4, 129.9, 135.9, 140.5, 142.4, 148.9, 149.5, 155.6, 157.5, 199.0, 207.3.

4-(3-(5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)propyl)-3-hydroxypicolinaldehyde oxime (4)

To a solution of **28** (4 mg, 0.011 mmol) in absolute EtOH (5 mL) were added successively $HONH_2 \cdot HCl$ (0.78 mg, 1 equiv.) and NaOAc (0.92 mg, 1 equiv.). The mixture was stirred at rt for 30 min under argon atmosphere. After concentration under reduced pressure, the residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc 1:1 v/v) to give **4** (4.3 mg, 99%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.43 – 1.57 (m 1H), 1.66 – 1.80 (m, 2H), 1.92 – 2.04 (m, 1H), 2.64 – 2.74 (m, 4H), 3.21 (dd, *J* = 5.1, 17.4 Hz, 1H), 3.87 (s, 3H), 3.93 (s, 3H), 6.84 (s, 1H), 7.04 (d, *J* = 4.5 Hz, 1H), 7.14 (s, 1H), 8.06 (d, *J* = 4.5 Hz, 1H), 8.42 (s, 1H), 9.81 (br s, 2H), 10.14 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.5, 29.3, 31.5, 32.6, 47.5, 56.1, 56.3, 104.3, 107.4, 125.3, 129.3, 134.8, 138.8, 140.9, 149.2, 149.4, 153.1, 154.0, 155.6, 208.0. MS (ESI+) *m/z* (%): 371 $[M+H]^+$. HRMS (ESI+) calcd for $C_{20}H_{23}N_2O_5^+$: 371.1607; found: 371.1602. HPLC : t_R = 24.8 min (purity = 93%).

Inhibition of hAChE and hBChE by OPNAs.

Recombinant *h*AChE and *h*BChE were produced and purified as previously described. [32, 33] VX and tabun were from DGA maîtrise NRBC (Vert le Petit, France). Paraoxon-ethyl was purchased from Sigma-Aldrich. Isopropyl *p*-nitrophenyl methylphosphonate (INMP) was used as surrogate of sarin.[34] HI-6 was from Pharmacie Centrale des Armées (Orléans, France). All other chemicals including paraoxon were from Sigma. Stock solution of VX and tabun were 5 mM in isopropanol. The inhibition of 120 µM *h*AChE or 100 µM of *h*BChE is realized with a 5-fold excess of OPNAs and was performed in tris buffer (20 mM, pH 7.4, 0.1% BSA) at 25 °C. After 20 minutes incubation, inhibited *h*AChE or *h*BChE was desalted on PD-10 column (GE Healthcare).

IC_{50} measurements.

Compounds **1-4** were dissolved in MeOH to make 5 mM stock solutions. Recombinant hAChE activity was measured spectrophotometrically (absorbance at 412 nm) in the presence of various concentrations of oximes in 1 mL Ellman's buffer (sodium phosphate 0.1

M, pH 7.4, 0.1% BSA, 5% MeOH, 0.1 mg/mL DTNB, 25 °C) and 1 mM acetylthiocholine. The concentration of compound **1** producing 50% of enzyme inhibition was determined by non-linear fitting using ProFit 7.0 (Quantumsoft) using the standard IC_{50} equation: $\%Activity=100*IC_{50}/(IC_{50} + [Ox])$.

Reactivation of *h*AChE and *h*BChE inhibited by OPNAs.

OPNA-inhibited *h*AChE was incubated at 37 °C with different concentrations of oxime in 0.1 M phosphate buffer, pH 7.4, 0.1% BSA, 5% methanol. Methanol was used for complete dissolution of the oximes. 10-µl aliquots of mix was transferred to 1-mL cuvettes (100-fold dilution) at time intervals ranging from 1 to 10 minutes depending on the reactivation rate, for measurement of *h*AChE activity (1 mM acetylthiocholine) or *h*BChE activity (1 mM butyrylthiocholine), in Ellman's buffer (phosphate 0.1 M, pH 7.4, 0.1% BSA, 0.5 mM DTNB, 25 °C).[35] The increase in absorbance at 412 nm was followed on a Uvikon 943 spectrophotometer.

The enzyme activity in the control (non-inhibited enzyme) remained constant during the experiment. To take into account the significant inhibition effect of compound **1** at the tested concentration, the later was added in the control at a concentration matching that of the sample. The percentage of reactivated enzyme ($\%E_{react}$) was calculated as the ratio of the recovered enzyme activity and activity in the control. The apparent reactivation rate k_{obs} for each oxime concentration, the dissociation constant K_D of inhibited enzyme-oxime complex (E-POx) and the maximal reactivation rate constant k_r, were calculated by non-linear fit using ProFit 7.0 (Quantumsoft) and the standard oxime concentration-dependent reactivation equation derived from the following scheme:

$$\text{E-P} + \text{Ox} \underset{}{\overset{K_D}{\rightleftharpoons}} \text{E-POx} \xrightarrow{k_r} \text{E} + \text{POx}$$

$$\%E_{react} = 100 \,.\, (1\text{-}e^{k_{obs} \cdot t}) \quad \text{and} \quad k_{obs} = \frac{k_r[Ox]}{K_D + [Ox]}$$

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The synthesis and *in vitro* evaluation of four donepezil-based non quaternary reactivators are reported. Oximes **1**-**3** show a better ability (8 fold higher) than pralidoxime to reactivate VX-hAChE, and oxime **2** is 5 to 11 fold more efficient than pralidoxime and HI-6 respectively for the reactivation of VX-hBChE.