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Brief Article

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Phenyltetrahydroisoquinoline–Pyridinaldoxime Conjugates as Efficient Uncharged Reactivators for the Dephosphylation of Inhibited Human Acetylcholinesterase

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Supporting Information

ABSTRACT: Pyridinium and bis-pyridinium aldoximes are used as antidotes to reactivate acetylcholinesterase (AChE) inhibited by organophosphorus nerve agents. Herein, we described a series of nine nonquaternary phenyltetrahydroisoquinoline–pyridinaldoxime conjugates more efficient than or as efficient as pyridinium oximes to reactivate VX-, tabun- and ethyl paraoxon-inhibited human AChE. This study explores the structure–activity relationships of this new family of reactivators and shows that **1b–d** are uncharged hAChE reactivators with a broad spectrum.

INTRODUCTION

Organophosphorus nerve agents (OPNAs) are used as insecticides (pest control agents) but also for highly toxic agents as warfare agents (such as sarin, cyclosarin, tabun, soman, and VX)[1,2] in armed conflicts (e.g., Gulf Wars[3]) and terrorist attacks (e.g., subway attack in Tokyo[4]). The acute toxic effect of OPNAs is based on the irreversible phosphylation of the catalytic serine hydroxyl group at the active site of acetylcholinesterase (AChE, EC 3.1.1.7).[5] Irreversible inhibition of the enzyme leads to accumulation of the neurotransmitter (acetylcholine) in the synaptic cleft, then to overstimulation of cholinergic receptors, causing seizures, respiratory arrest, and death. Organophosphorus pesticide poisoning is also a major health problem worldwide with over 200 000 deadly intoxications yearly.[6] It is of primary importance to find an efficient treatment for acute and chronic intoxications by OPNA.

The current treatment of poisoning by OPNA is a combination of an antimuscarinic agent (e.g., atropine), AChE reactivator such as one of the standard pyridinium oximes (pralidoxime or 2-PAM, trimedoxime or TMB-4, obidoxime, HI-6, and HLö-7)[7] (Figure 1), and anticonvulsant drug (e.g., diazepam).[2] 2-PAM is the oxime currently used in the military forces of most countries. The high nucleophilicity of oximes allows the displacement of the phosphoryl group from the catalytic serine and thus induces the recovery of the enzyme catalytic activity.

Since the discovery of monopyridinium and bispyridinium oximes[7] as reactivators for inhibited AChE and despite hundreds of oximes synthesized and evaluated during the past 2 decades,[7,8] these reactivators still present serious drawbacks. First, these reactivators are permanently charged cationic compounds that poorly cross the blood–brain barrier (BBB) and cannot readily reactivate cholinesterases in the central nervous system (CNS).[9] Moreover, there is no single broad-spectrum oxime suitable for the antidotal treatment of any OPNA. For instance, HI-6 rapidly reactivates VX-AChE in vitro but is inefficient onto tabun-AChE.[10]

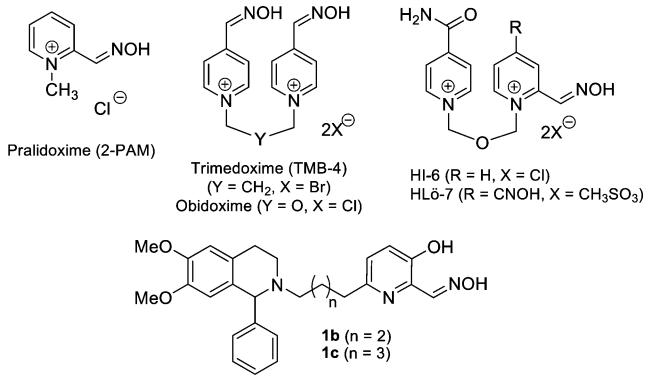

Figure 1. Structure of standard pyridinium oximes and uncharged AChE reactivators **1b,c**.

During the past couple of years, several research groups have reported new efforts toward the development of original reactivators aimed at tackling the above cited drawbacks.[11] de

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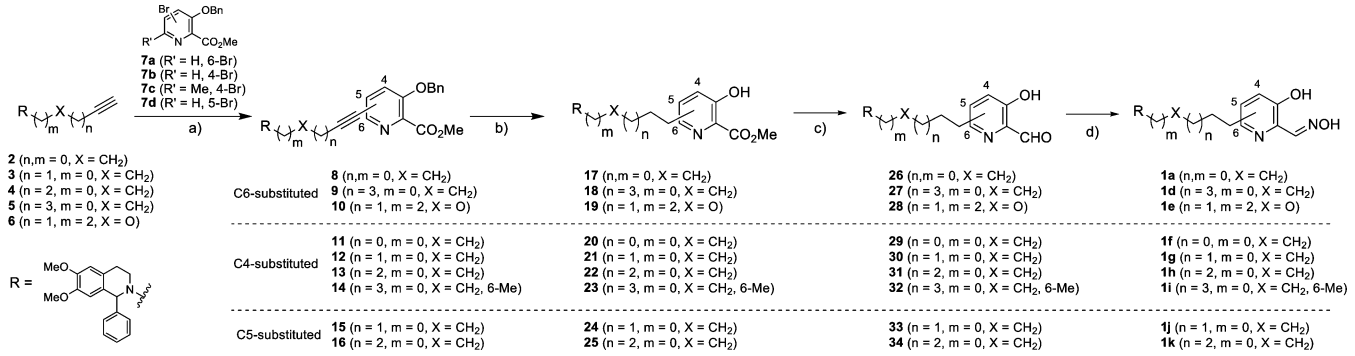
Scheme 1. Preparation of Phenyltetrahydroisoquinoline−Pyridinaldoxime Reactivators 1a,d−k[a]



[a]Conditions and reagents: (a) **7a−d**, $Pd(PPh_3)_4$, CuI, NEt_3, THF, rt, 15 h; (b) H_2 (1 atm), $Pd(OH)_2$/C, EtOAc, 15 h, rt; (c) (1) TBSCl, imidazole, DMF, rt, 2 h; (2) DIBAL-H, CH_2Cl_2, −78 °C, 10 min; (3) TBAF, THF, 0 °C, 30 min; (d) NH_2OH·HCl, NaOAc, EtOH, rt, 1 h.

Table 1. Reactivation Rate Constant (k_r, min^{-1}), Dissociation Constant (K_D, μM), and Bimolecular Reactivation Rate Constant (k_{r2}, $mM^{-1}·min^{-1}$) for Reactivation of VX-, Tabun-, and Ethyl Paraoxon-Inhibited Human AChE

	VX-hAChE			tabun-hAChE			ethyl paraoxon-hAChE		
reactivator	k_r	K_D	k_{r2}	k_r	K_D	k_{r2}	k_r	K_D	k_{r2}
2-PAM	0.06 ± 0.01	215 ± 75	0.28	0.01 ± 0.0005[a]	706 ± 76[a]	0.01[a]	0.17 ± 0.007[a]	187.3 ± 19[a]	0.9[a]
obidoxime	0.60 ± 0.05	54 ± 12	11	0.040 ± 0.006	250 ± 110	0.16	1.22 ± 0.01[e]	65 ± 17[e]	19[e]
HLö-7	0.49[a]	7.8[a]	63[a]	0.020 ± 0.0007	106 ± 15	0.19	0.63 ± 0.04	210 ± 31	3
HI-6	0.44 ± 0.15	50 ± 26	9	ø[b]	ø[b]	ø[b]	0.16 ± 0.004[e]	478 ± 11[e]	0.3[e]
TMB-4				0.085 ± 0.005	145 ± 25	0.6	0.97 ± 0.01[e]	62 ± 19[e]	16[e]
1a	0.45 ± 0.11	117 ± 30	3.8	0.0034 ± 0.0002	7 ± 2	0.5	0.6 ± 0.07	208 ± 26	3
1b	0.68 ± 0.06	33 ± 4	20	0.045 ± 0.002	27 ± 4	1.7	1.17 ± 0.09	60 ± 6	19
1c	0.35 ± 0.05	6 ± 2	58	0.015 ± 0.001	5.6 ± 1.4	2.7	0.4 ± 0.041	31 ± 9	13
1d	0.29 ± 0.03	38 ± 3	7.6	0.021 ± 0.0015	14 ± 2	1.5	0.168 ± 0.004	14.5 ± 0.1	11
1e	nd[c]	nd[c]	3[c]	0.006 ± 0.0003	4 ± 1	1.5			
1f	nd[c]	nd[c]	0.8[c]	nd[d] (k_{obs} at 100 μM = 0.004 min^{-1})			nd[d] (k_{obs} at 100 μM = 0.056 min^{-1})		
1g	0.31 ± 0.09	350 ± 140	0.9	nd[d] (k_{obs} at 100 μM = 0.003 min^{-1})			0.095 ± 0.005	38.7 ± 3.5	2.5
1h	0.28 ± 0.03	113 ± 7	2.4	0.08 ± 0.006	75 ± 9	1	0.27 ± 0.01	39.9 ± 3.5	6.8
1i	0.037 ± 0.001	79 ± 1	0.5	0.028 ± 0.0008	51 ± 3	0.5	nd[d] (k_{obs} at 100 μM = 0.08 min^{-1})		
1j	0.10 ± 0.02	77 ± 26	1.3	nd[d] (k_{obs} at 100 μM = 0.002 min^{-1})			nd[d] (k_{obs} at 100 μM = 0.05 min^{-1})		
1k	nd[c]	nd[c]	0.2[c]	nd[d] (k_{obs} at 100 μM = 0.002 min^{-1})			nd[d] (k_{obs} at 100 μM = 0.018 min^{-1})		

[a]Data from ref 20. [b]No reactivation up to 5 mM HI-6. [c]If [reactivator] ≪ K_D, then there is a linear dependence between k_{obs} and [reactivator]: $k_{obs} = (k_r/K_D)$[reactivator]. In this case, k_r and K_D cannot be determined, but $k_{r2} = k_r/K_D$ is the slope of the line. [d]Not determined if k_{obs} at 100 μM is <0.005 min^{-1} for tabun-hAChE or <0.1 min^{-1} for paraoxon-hAChE. [e]Data from ref 21.

Koning et al. described uncharged reactivators, though their ability to reactivate inhibited human AChE remains modest with no efficiency for tabun-AChE.[12] Kalisiak et al. described amidine oximes with enhanced ability to cross the BBB, whose second order reactivation rates constants (efficiency rates) are greater than that of monoisonitrosoacetone (MINA).[13] Yet they remain less efficient than pralidoxime. Radić et al. reported a large library of uncharged oximes, whose promising lead is a 2-hydroxyiminoacetamide with an 8-fold improved efficiency for VX-AChE compared to pralidoxime but with 3- and 4-fold lower efficiency for paraoxon and tabun, respectively.[14] In the same period, our group described the synthesis and evaluation of two promising nonquaternary reactivators **1b,c** (Figure 1).[15] They display an in vitro ability to reactivate VX- and tabun-AChE at least equivalent to that of the best bispyridinium oximes, i.e., 1−2 order of magnitudes superior to 2-PAM. These reactivators are composed of 3-hydroxy-2-pyridinealdoxime[16] linked to a peripheral site ligand of AChE, a phenyltetrahydroisoquinoline moiety.[17] Phenyl tetrahydroisoquinoline provides a balanced affinity sufficiently high for binding to phosphylated AChE but sufficiently low for binding to the uninhibited enzyme.

Our preliminaries results encouraged us (1) to synthesize and evaluate a series of nine analogues of **1b,c** to explore the effect of structural modifications on the reactivation efficiency, namely, the linker length, attachment position of the linker onto the pyridyl ring, insertion of a heteroatom into the linker, and (2) to complete the biological evaluation with a representative pesticide, ethyl paraoxon.

■ CHEMISTRY

The syntheses of the different reactivators are shown in Scheme 1. First, Sonogashira coupling reaction between alkynes **2−6** with bromopyridines **7a−d**[15,18] afforded the desired **8−16**. Then reduction of alkyne and deprotection of phenol function furnished **17−25**. For the introduction of the required aldehyde function, the best yields were obtained through the sequence comprising the temporary protection of the phenol group as a TBS ether, the reduction of methyl ester into the corresponding aldehyde by using DIBAL-H, and a subsequent deprotection, which gave the desired products **26−34**. Finally, a

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treatment of the latter with hydroxylamine afforded the analogues **1a,d−k**, whose linker is attached to position C6, C5, or C4 of the pyridine ring. Oxime **1i** bearing an additional methyl on position 6 of the pyridine should allow us to study the interaction with residues in the AChE catalytic site and the subsequent effect on reactivation rates. Besides, the insertion of one oxygen into the linker has been evaluated (**1e** in Scheme 1) for potential H-bonds between the linker and tyrosines lining AChE active site gorge.[19]

RESULTS AND DISCUSSION

The apparent dissociation constant of the reactivator/phosphyl-AChE complex (K_D), the maximal reactivation rate (k_r), and the bimolecular rate constant ($k_{r2} = k_r/K_D$) have been measured to evaluate the potential of oximes **1a−k** for in vitro reactivation of VX-, tabun-, and ethyl paraoxon-hAChE, (Table 1 and SI for details). **1b** and **1c** are the most efficient compound for every OPNA tested and show that the general optimal linker length is 4/5 methylenes with an attachment point at position 6 onto the pyridine, i.e., in position meta relative to the oxime function. Substitution at position 5 is the most detrimental. Note that the relative linker/oxime meta position, the most favorable for the reactivation reaction, is harmful to the nucleophilicity of bispyridinium aldoxime. Indeed, the conjugation of the oxime with the pyridium nitrogen is lost, resulting in a higher oxime pK_a.

For VX-hAChE, most of the other derivatives are significantly more efficient than 2-PAM, **1d** being only marginally less efficient than HI-6 or obidoxime. For tabun-hAChE, 5 out of 9 analogues are at least as efficient as TMB-4, one of the best tabun-hAChE reactivators to date:[20] **1d** and **1e** are 2.5-fold more efficient, **1h** is 2-fold more efficient, while **1a** and **1i** are about as efficient as TMB-4.

OP pesticide poisoning is also a major health problem worldwide; therefore, these encouraging results for the reactivation of VX- and tabun-hAChE prompted us to evaluate this series of reactivators for the reactivation of human AChE inhibited by the widespread diethylphosphoryl pesticides, e.g., ethyl paraoxon. As shown in Table 1, the efficiency toward ethyl paraoxon-hAChE is correlated to that toward VX-hAChE. Oxime **1b** is as efficient as obidoxime for in vitro reactivation of paraoxon-hAChE, while **1c,d** are slightly less efficient (1.5 and 1.7 less efficient, respectively). Yet they remain among the best reactivators described to date for this family of pesticides, which further enlightens the broad spectrum of this new family of reactivators.

Ideally, reactivators must not be strong inhibitors of hAChE at their practical reactivation concentration. We measured the IC_{50} of eight reactivators for hAChE (Table 2). All of these oximes have IC_{50} > 100 μM, indicating that the affinity toward hAChE is lower than toward OPNA-inhibited AChE and that these reactivators will not significantly inhibit hAChE once it is reactivated.

Table 2. Inhibitory Activity (IC_{50}) of Uncharged Reactivators toward Native Human AChE

reactivator	IC_{50} (μM)
1a	260
1b	>100
1c	>100
1d	>400
1f	>200
1h	320
1j	300
1k	>500

CONCLUSION

We have described a series of nine analogues **1a,d−k** as hAChE reactivators to study the influence of some structural parameters on the reactivation of VX-, tabun-, and ethyl paraoxon-inhibited hAChE. The best global reactivation efficiency has been obtained with a linker length of four or five carbon atoms attached on position 6 of the pyridine ring, i.e., for **1b** and **1c**. Their derivatives show a lower ability to reactivate VX-inhibited hAChE, yet superior to that of 2-PAM. Regarding the reactivation of tabun-inhibited hAChE, i.e., the conjugate that is the most difficult to reactivate, three new compounds reported in this study, **1d**, **1e**, and **1h**, are more efficient than TMB-4, while **1a** and **1i** are as efficient as TMB-4. Moreover, this study shows that **1b−d** are as efficient as obidoxime and TMB-4 toward paraoxon-inhibited hAChE.[21] Given their general efficiency and broad spectrum, **1b−d** will be evaluated in vivo in priority as nonquaternary hAChE reactivators.

EXPERIMENTAL SECTION

The synthesis of **1d** is described below. The general chemistry, experimental information, and syntheses of all other compounds are supplied in the Supporting Information. Purity of all final compounds as determined by HPLC analysis is ≥95%.

Methyl 3-(Benzyloxy)-6-(6-(6,7-dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1*H*)-yl)hex-1-ynyl)picolinate (9). To a Schlenk tube were added **5** (500 mg, 1.4 mmol), **7a** (460 mg, 1 equiv), CuI (30 mg, 0.1 equiv), $Pd(PPh_3)_4$ (90 mg, 0.05 equiv), and degassed THF (5 mL) and NEt_3 (2.5 mL). The resulting mixture was stirred at room temperature for 15 h under argon atmosphere. After concentration under reduced pressure, the residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc 3:2) to give **9** (772 mg, 93%) as a light brown solid. R_f = 0.3 (cyclohexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.44−1.67 (m, 4H), 2.25−2.36 (m, 3H), 2.47−2.60 (m, 2H), 2.76 (dt, J = 4.4, 15.4 Hz, 1H), 2.91−3.04 (m, 1H), 3.15 (dt, J = 5.2, 11.5 Hz, 1H), 3.59 (s, 3H), 3.85 (s, 3H), 3.95 (s, 3H), 4.47 (s, 1H), 5.20 (s, 2H), 6.17 (s, 1H), 6.60 (s, 1H), 7.21−7.29 (m, 6H), 7.30−7.45 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 19.1, 25.9, 26.0, 26.9, 28.2, 46.8, 52.7, 53.4, 55.7, 55.8, 68.1, 70.8, 79.3, 90.5, 110.7, 111.6, 121.8, 126.9, 127.1, 128.1, 128.2, 128.7, 129.6, 130.0, 130.2, 135.5, 135.6, 140.0, 144.2, 147.0, 147.3, 152.8, 164.9.

Methyl 6-(6-(6,7-Dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1*H*)-yl)hexyl)-3-hydroxypicolinate (18). To a solution of **9** (245 mg, 0.5 mmol) in degassed EtOAc (10 mL) was added Pearlman's catalyst (143 mg, 0.2 equiv, 20% Pd, moisture 50%). The solution was bubbled with H_2, and the reaction was stirred at room temperature under H_2 atmosphere (1 atm) for 15 h. The mixture was filtered through Celite and concentrated under reduced pressure to furnish **18** (213 mg, 85%) as a light brown solid. The product was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.12−1.29 (m, 4H), 1.36−1.51 (m, 2H), 1.52−1.68 (m, 2H), 2.18−2.34 (m, 1H), 2.39−2.59 (m, 2H), 2.64−2.79 (m, 3H), 2.85−3.00 (m, 1H), 3.04−3.16 (m, 1H), 3.58 (s, 3H), 3.83 (s, 3H), 4.02 (s, 3H), 4.46 (s, 1H), 6.15 (s, 1H), 6.58 (s, 1H), 7.14−7.30 (m, 7H), 10.55 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.6, 27.1, 28.1, 29.2, 30.1, 37.7, 46.7, 53.2, 54.1, 55.7, 55.8, 67.8, 110.7, 111.6, 126.6, 126.9, 127.0, 128.0, 128.4, 128.7, 129.2, 129.6, 130.1, 144.3, 147.0, 154.3, 157.2, 170.2.

6-(6-(6,7-Dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1*H*)-yl)hexyl)-3-hydroxypicolinaldehyde (27). To a solution of **18** (195 mg, 0.39 mmol) in dry DMF (10 mL) were successively

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added imidazole (80 mg, 3 equiv) and TBDMSCl (128 mg, 2.2 equiv). The mixture was stirred at room temperature for 2 h under argon atmosphere. EtOAc (30 mL) was added, and the organic layer was washed with water (thrice), 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 (780 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 (780 μL), and the mixture was allowed to warm at room temperature. The organic layer was washed with an aqueous solution of NaOH (1M), dried over $MgSO_4$, and concentrated under reduced pressure. Then TBAF (430 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, a purification by flash chromatography on silica gel (cyclohexane/EtOAc 1:1) afforded the desired compound **27** (59 mg, 32%) as a pale yellow oil. R_f = 0.3 (cyclohexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.20−1.28 (m, 4H), 1.42−1.51 (m, 2H), 1.63−1.67 (m, 2H), 2.25−2.31 (m, 1H), 2.47−2.56 (m, 2H), 2.69−2.79 (m, 3H), 2.90−2.96 (m, 1H), 3.13 (dt, *J* = 5.0, 11.4 Hz, 1H), 3.59 (s, 3H), 3.85 (s, 3H), 4.47 (s, 1H), 6.16 (s, 1H), 6.60 (s, 1H), 7.20−7.28 (m, 7H), 10.02 (s, 1H), 10.63 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.7, 27.1, 28.2, 29.1, 29.8, 37.3, 46.8, 54.0, 55.7, 55.8, 67.9, 110.7, 111.6, 126.3, 126.9, 127.0, 128.1, 129.6, 129.8, 130.2, 135.7, 144.3, 147.0, 147.3, 155.2, 157.0, 198.8.

6-(6-(6,7-Dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1*H*)-yl)hexyl)-3-hydroxypicolinaldehyde Oxime (1d). To a solution of **27** (45 mg, 95 μmol) in absolute EtOH (5 mL) were added successively $HONH_2$·HCl (9.9 mg, 1.5 equiv) and NaOAc (11.7 mg, 1.5 equiv). The mixture was stirred at room temperature 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) to give **1d** (40 mg, 86%) as a white solid. R_f = 0.3 (cyclohexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.20−1.30 (m, 4H), 1.49−1.60 (m, 4H), 2.29−2.38 (m, 1H), 2.46−2.55 (m, 1H), 2.60−2.69 (m, 3H), 2.82 (dt, *J* = 4.8, 16.2 Hz, 1H), 2.95−3.05 (m, 1H), 3.17 (dt, *J* = 4.8, 11.1 Hz, 1H), 3.59 (s, 3H), 3.84 (s, 3H), 4.59 (s, 1H), 6.15 (s, 1H), 6.61 (s, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 7.19−7.28 (m, 5H), 8.37 (s, 1H), 10.00 (s, 1H), 10.48 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.1, 27.2, 27.5, 29.0, 30.0, 37.0, 46.5, 54.3, 55.7, 55.8, 67.7, 110.8, 111.5, 123.7, 124.7, 126.4, 127.4, 128.2, 129.4, 129.9, 135.0, 142.6, 147.1, 147.6, 152.5, 153.0, 153.4. MS (ESI+) *m*/*z* (%): 490 $[M + H]^+$. HRMS (ESI+): *m*/*z* calcd for $C_{29}H_{36}N_3O_4$ 490.2700; found 490.2706. HPLC: t_R = 23.2 min (purity, 95%).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Syntheses and characterization of **1a**,**d**−**k**, biological assays, and ^{1}H and ^{13}C NMR spectra of intermediate and final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

OPNA, organophosphorus nerve agent; OP, organophosphorus; hAChE, human acetylcholinesterase; BBB, blood−brain barrier; MINA, monoisonitrosoacetone; CNS, central nervous system

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