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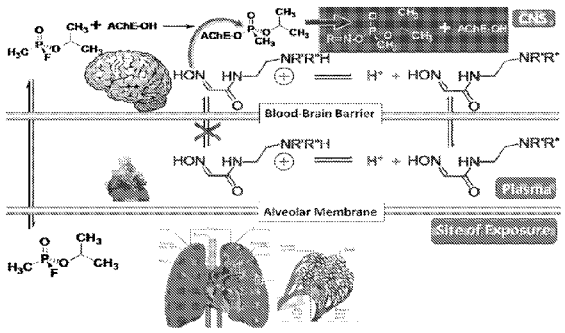
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[Continued on next page]

(54) Title: CENTRALLY ACTIVE AND ORALLY BIOAVAILABLE ANTIDOTES FOR ORGANOPHOSPHATE EXPOSURE AND METHODS FOR MAKING AND USING THEM

Figure 1



(57) Abstract: In alternative embodiments, the invention provides nucleophilic hydroxyimino- acetamido alkylamine antidotes that cross the blood-brain barrier (BBB) to catalyze the hydrolysis of organophosphate (OP)-inhibited human acetylcholinesterase (hAChE) in the central nerve system (CNS). The hydroxyimino-acetamido alkylamines of the invention are designed to fit within AChE active center gorge dimensions, bind with reasonable affinity, and react with the conjugated phosphate atom in the gorge. The hydroxyimino- acetamido alkylamines of the invention are also designed to possess ionization states that govern affinity and reactivity for the two linked hAChE re-activation steps. In alternative embodiments, the invention provides pumps, devices, subcutaneous infusion devices, continuous subcutaneous infusion devices, infusion pens, needles, reservoirs, ampoules, a vial, a syringe, a cartridge, a disposable pen or jet injector, a prefilled pen or a syringe or a cartridge, a cartridge or a disposable pen or jet injector, a two chambered or multi- chambered pump, a syringe, a cartridge or a pen or a jet injector, comprising a compound of the invention.

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CENTRALLY ACTIVE AND ORALLY BIOAVAILABLE ANTIDOTES FOR ORGANOPHOSPHATE EXPOSURE AND METHODS FOR MAKING AND USING THEM

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Patent Application Serial No. (USSN) 61/765,596, filed February 15, 2013. The aforementioned application is expressly incorporated herein by reference in its entirety and for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

This invention was made with government support under grant no. U01 NS058046, awarded by the National Institutes of Health (NIH), DHHS. The government has certain rights in the invention.

TECHNICAL FIELD

This invention relates to antidote chemistry for organophosphates and their detoxification. In alternative embodiments, the invention provides nucleophilic hydroxyimino-acetamido alkylamine antidotes that cross the blood-brain barrier (BBB) to catalyze the hydrolysis of organophosphate (OP)-inhibited human acetylcholinesterase (hAChE) in the central nerve system (CNS). The hydroxyimino-acetamido alkylamines of the invention are designed to fit within AChE active center gorge dimensions, bind with reasonable affinity, and react with the conjugated phosphate atom in the gorge. The hydroxyimino-acetamido alkylamines of the invention are also designed to possess ionization states that govern affinity and reactivity for the two linked hAChE re-activation steps.

BACKGROUND

A recent spur of interest in centrally acting reactivators of organophosphate $(OP)^{3-}$ inhibited acetylcholinesterase (AChE) reflects a compelling need for antidotal therapy capable of efficient reinstatement of CNS AChE activity in OP-intoxicated individuals. Exposure to uncharged, lipophilic OPs from both pesticide and nerve agents leads to inhibition of peripheral and CNS AChE within minutes of exposure due to rapid OP distribution through the various body compartments of exposed individuals. However,

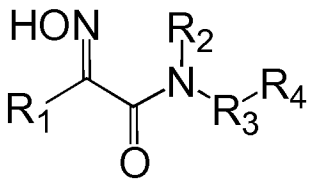
attention has been accorded primarily to the development of re-activator antidotes with an ionizable cationic site since quaternary ammonium ligands have a clear preference for association with predominantly aromatic active center gorge of AChE. When administered *in vivo*, quaternary oxime reactivators largely remain in blood and peripheral tissues incapable of crossing the blood-brain barrier and reactivating OP-inhibited brain AChE.

The organophosphate antidote 2-PAM, designed by Irwin Wilson in the 1950's, remains as a staple antidote for nerve agent and insecticide poisoning to this day. The compound is limited by three factors: its quaternary ammonium moiety prevents the compound from crossing the blood-brain barrier, so 2-PAM does not reactivate CNS acetylcholinesterase; the quaternary change precludes rapid absorption following oral administration requiring parenteral administration, and the volume occupied by the pyridinium aldoxime moiety precludes it attacking the covalently linked phosphate with an optimal attack angle.

SUMMARY

In alternative embodiments, the invention provides compounds having one of the following structures or compositions having one or more compounds of the following structures, or equivalents thereof, or a stereoisomer thereof, or an analog thereof, or a pharmaceutically acceptable salt thereof, or a bioisostere thereof; or, the invention provides a composition comprising an isolated compound consisting essentially of, or consisting of:

(a) a compound having the formula:



wherein:

R_1 is selected from the group consisting of: -H, -alkyl, and –aryl;

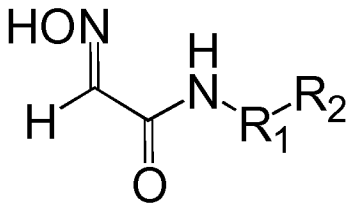
R_2 is selected from the group consisting of: -H, -alkyl, and -aryl ;

R_3 is selected from the group consisting of: $-(CH_2)_n-$, and $(CH_2)_n-CH(CH_3)-$, wherein **n** is the integer 0, 1, 2, 3, 4 or 5;

R_4 is selected from the group consisting of : -H, -alkyl (wherein optionally the alkyl is

selected from the group consisting of: -methyl, -ethyl, -propyl, -butyl, -i-propyl, and -i-butyl), -cycloalkyl (wherein optionally the cycloalkyl is selected from the group consisting of: -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclohexyl, -cycloheptyl, and -cyclooctyl), –aryl (wherein optionally the aryl is selected from the group consisting of: -phenyl, -naphthyl, -thienyl, and -indolyl), -saturated and nonsaturated heterocyclics (wherein optionally the saturated or nonsaturated heterocyclic is selected from the group consisting of: -aziridine, -oxirane- thiirane, -azirine, -oxirene, -thiirene, -azetidine, -oxetane, -thietane, -azete, -oxete, -thiete, -pyrrolidine, -oxolane, -thiolane, -pyrrole, -furan, -thiophene, -piperidine, -oxane, -thiane, -pyridine, -pyran, -thiopyran, -azepane, -oxepane, -thiepane, -azepine, -oxepine, -thiepine, -azocane, and -azocine), and bridged compounds (wherein optionally the bridged compound is selected from the group consisting of: -adamantanes, -amantadines, -biperidenes, -memantines, -methenamines, -rimantadines, -norbornanes, and -triazoles);

(b) a compound having the formula:



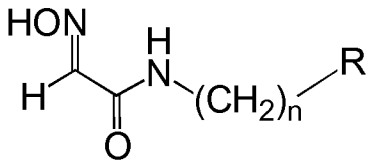
wherein

$\mathbf{R_1}$ is selected from the group consisting of: $-(CH_2)_n-$, and $(CH_2)_n-CH(CH_3)-$, wherein **n** is the integer 0, 1, 2, 3, 4 or 5;

$\mathbf{R_2}$ is selected from the group consisting of: -H, -alkyl (wherein optionally the alkyl is selected from the group consisting of: -methyl, -ethyl, -propyl, -butyl, -i-propyl, and -i-butyl), -cycloalkyl (wherein optionally the cycloalkyl is selected from the group consisting of: -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclohexyl, -cycloheptyl, and -cyclooctyl), –aryl (wherein optionally the aryl is selected from the group consisting of: -phenyl, -naphthyl, -thienyl, and -indolyl), -saturated and nonsaturated heterocyclics (wherein optionally the saturated or nonsaturated heterocyclic is selected from the group consisting of: -aziridine, -oxirane- thiirane, -azirine, -oxirene, -thiirene, -azetidine, -oxetane, -thietane, -azete, -oxete, -thiete, -pyrrolidine, -oxolane, -thiolane, -pyrrole, -furan, -thiophene, -piperidine, -oxane, -thiane, -pyridine, -pyran, -thiopyran, -azepane, -oxepane, -thiepane, -azepine, -oxepine, -thiepine, -azocane, and -azocine), and bridged

compounds (wherein optionally the bridged compound is selected from the group consisting of: -adamantanes, -amantadines, -biperidenes, -memantines, -methenamines, -rimantadines, -norbornanes, and -triazoles);

(c) a compound having the formula:

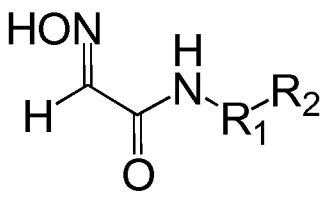


wherein

N is the integer 0, 1, 2, 3, 4 or 5 ;

R is selected from the group consisting of: -H, -alkyl (wherein optionally the alkyl is selected from the group consisting of: -methyl, -ethyl, -propyl, -butyl, -i-propyl, and -i-butyl), -cycloalkyl (wherein optionally the cycloalkyl is selected from the group consisting of: -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclohexyl, -cycloheptyl, and -cyclooctyl), –aryl (wherein optionally the aryl is selected from the group consisting of: -phenyl, -naphthyl, -thienyl, and -indolyl), -saturated and nonsaturated heterocyclics (wherein optionally the saturated or nonsaturated heterocyclic is selected from the group consisting of: -aziridine, -oxirane- thiirane, -azirine, -oxirene, -thiirene, -azetidine, -oxetane, -thietane, -azete, -oxete, -thiete, -pyrrolidine, -oxolane, -thiolane, -pyrrole, -furan, -thiophene, -piperidine, -oxane, -thiane, -pyridine, -pyran, -thiopyran, -azepane, -oxepane, -thiepane, -azepine, -oxepine, -thiepine, -azocane, and -azocine), and bridged compounds (wherein optionally the bridged compound is selected from the group consisting of: -adamantanes, -amantadines, -biperidenes, -memantines, -methenamines, -rimantadines, -norbornanes, and -triazoles);

(d) a compound having the formula:

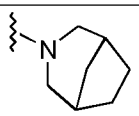
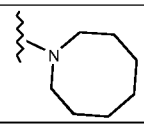
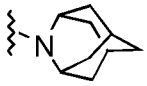
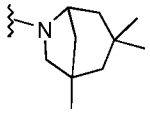
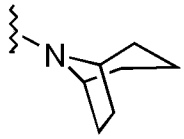
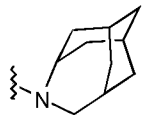
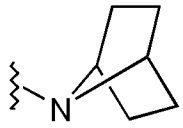
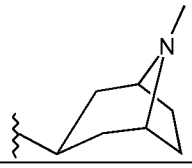
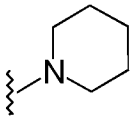
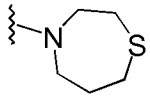
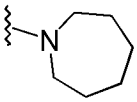
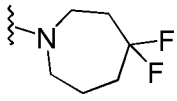


wherein

$\mathbf{R_1}$ is selected from the group consisting of: $-(CH_2)_n-$, and $(CH_2)_n-CH(CH_3)-$,

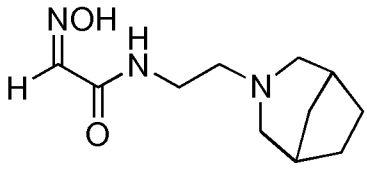
wherein **N** is the integer 0, 1, 2, 3, 4 or 5;

R_2 is selected from the group consisting of:

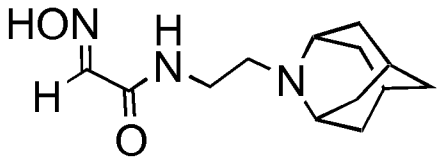
 R_2	 R_2
 R_2;	 R_2;
 R_2;	 R_2;
 R_2;	 R_2;
 R_2;	 R_2;
 R_2;	 R_2;

and any combination thereof);

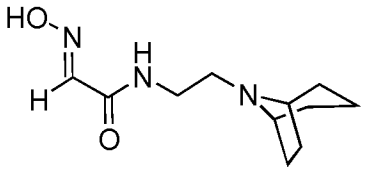
(e) a compound having the formula:



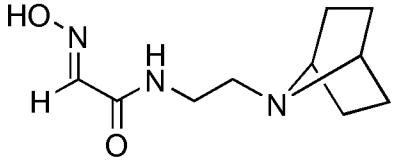
(RS3-43D);



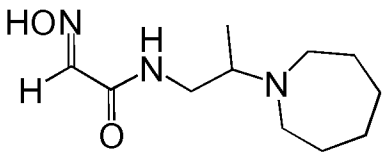
(RS3-36D);



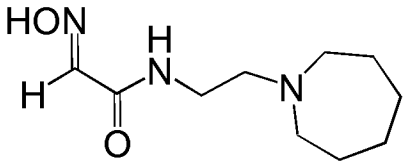
(RS2-237D)



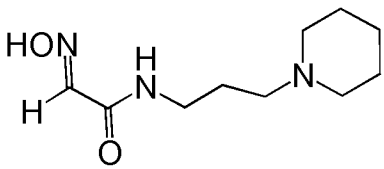
(RS2-234D);



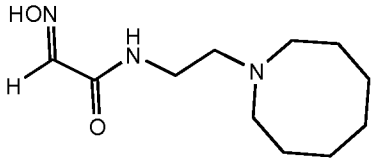
(RS2-245C);



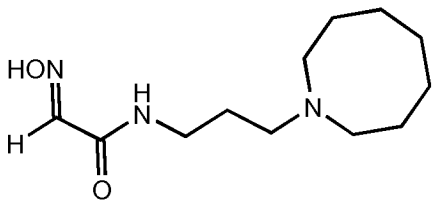
(RS194B) ;



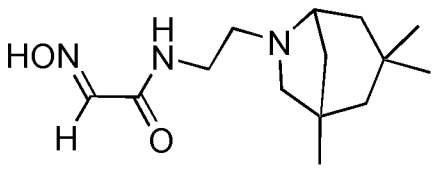
(RS191E) ;



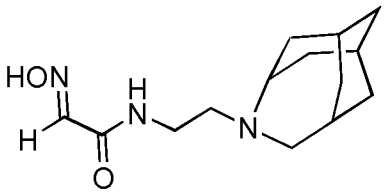
(RS251A) ;



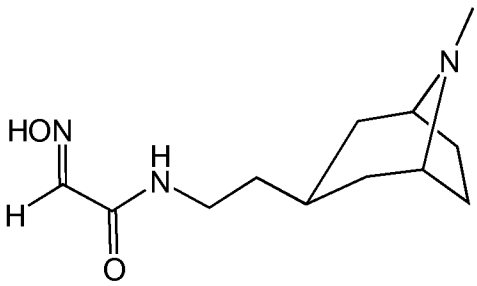
(RS251B) ;



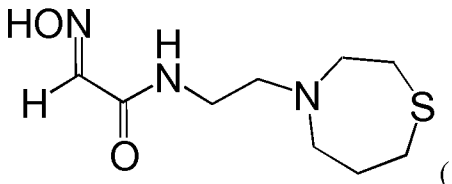
(RS218A) ;



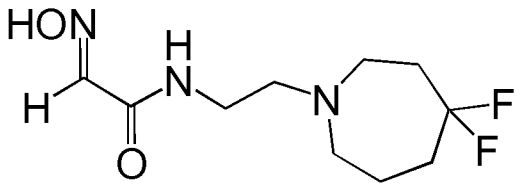
(RS2-138B) ;



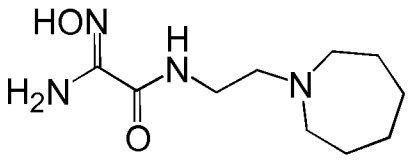
(RS2-57B) ;



(RS2-148B) ;



(RS2-140B) ;



(RS292A); and

a combination thereof.

In alternative embodiments, the invention provides compounds having one of the following structures or compositions having one or more of the following structures, or equivalents thereof, or a stereoisomer thereof, or an analog thereof, or a pharmaceutically acceptable salt thereof; or, the invention provides a composition comprising an isolated compound consisting essentially of, or consisting of:

having a structure as set forth in Table 1 (Figure 3) to Table 2 (Figure 4), or equivalents thereof, or pharmaceutically acceptable salt thereof,

wherein optionally the compound is RS2 138B, RS194B or RS191E;

wherein optionally the compound is RS251A, RS251B, RS218A, or RS2–57B; or

wherein optionally the compound is RS2-148B, RS2-140B, RS3-43D, RS3-36D, RS2-237D, RS2-234D and RS2-245C (Figure 4A).

In alternative embodiments, the invention provides formulations comprising a compound or composition (e.g., pharmaceutical composition) of the invention, wherein optionally the formulation is a liquid, aerosol, powder or emulsion formulation. In alternative embodiments the compositions and compounds of the invention are formulated as a pharmaceutical composition, or formulated for enteral or parenteral administration, wherein optionally the compound or formulation is administered orally, parenterally, by inhalation spray, nasally, topically, intrathecally, intrathecally, intracerebrally, epidurally, intracranially or rectally.

In alternative embodiments, the invention provides a pump, a device, a subcutaneous infusion device, a continuous subcutaneous infusion device, an infusion pen, a needle, a reservoir, an ampoule, a vial, a syringe, a cartridge, a disposable pen or jet injector, a prefilled pen or a syringe or a cartridge, a cartridge or a disposable pen or jet injector, a two chambered or multi-chambered pump, a syringe, a cartridge or a pen or a jet injector, comprising a compound of the invention, or a formulation of the invention.

In alternative embodiments, the invention provides methods for treating, ameliorating or protecting (preventing) an organophosphate toxicity or poisoning or toxic exposure, or for treating, ameliorating or protecting (preventing) organophosphate inhibition of an acetylcholinesterase (AChE), comprising:

administering to a patient or an individual in need thereof, a compound of the invention, or a formulation of the invention, or

administering the compound of the invention, or the formulation of the invention, using a pump, a device, a subcutaneous infusion device, a continuous subcutaneous infusion device, an infusion pen, a needles, a reservoir, an ampoules, a vial, a syringe, a cartridge, a disposable pen or jet injector, a prefilled pen or a syringe or a cartridge, a cartridge or a disposable pen or jet injector, a two chambered or multi-chambered pump, a syringe, a cartridge or a pen or a jet injector of the invention.

In alternative embodiments of the methods, the organophosphate toxicity, poisoning or toxic exposure is caused by exposure of the patient or individual to an alkyl methylphosphonate or related nerve agent, or an alkylphosphorate insecticide,

and optionally the organophosphate (OP) is or is a component of a toxin, an herbicide, an insecticide, or a nerve gas or nerve agent,

and optionally the organophosphate (OP) is or comprises a parathion, a malathion, a methyl parathion, a chlorpyrifos, a diazinon, a dichlorvos, a phosmet, a fenitrothion, a tetrachlorvinphos, an azamethiphos or an azinphos methyl, or the nerve agent is a soman (*O*-Pinacolyl methylphosphonofluoridate), a tabun (Ethyl *N*,*N*Dimethyl-phosphoramido-cyanidate) or a sarin ((*RS*)-propan-2-yl methylphosphonofluoridate).

In alternative embodiments of the methods, the acetylcholinesterase (AChE) is in the central nerve system (CNS), or the acetylcholinesterase (AChE) is a human acetylcholinesterase (hAChE).

In alternative embodiments, the invention provides for treating, preventing or ameliorating excessive acetylcholine stimulation in the CNS, including the brain, or in the periphery, including the peripheral nervous system (PNS), comprising:

administering to a patient or an individual in need thereof, a compound of the invention, or a formulation of the invention, or

administering a compound of the invention, or a formulation of the invention, using a pump, a device, a subcutaneous infusion device, a continuous subcutaneous infusion device, an infusion pen, a needles, a reservoir, an ampoules, a vial, a syringe, a

cartridge, a disposable pen or jet injector, a prefilled pen or a syringe or a cartridge, a cartridge or a disposable pen or jet injector, a two chambered or multi-chambered pump, a syringe, a cartridge or a pen or a jet injector of the invention.

In alternative embodiments of the methods, the excessive acetylcholine stimulation in the CNS, PNS or brain is caused by a drug, a drug overdose, or a poisoning or a toxic exposure to a drug, and optionally the drug overdose causing the excessive acetylcholine stimulation is caused at least in part by physostigmine, pyridostigmine, neostigmine, diisopropyl phosphorofluoridate (DFP), and/or echothiophate.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

All publications, patents, patent applications cited herein are hereby expressly incorporated by reference for all purposes.

DESCRIPTION OF DRAWINGS

The drawings set forth herein are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 schematically illustrates that the uncharged and protonated forms of acetamido oximes (highlighted with darker background) of the invention will equilibrate in blood upon intramuscular injection to OP exposed individual; the uncharged form will cross the blood-brain barrier and enter brain tissue where it will re-equilibrate; the protonated form will bind AChE-OP adduct and recover AChE catalytic activity (shown in the rectangle), as discussed in detail, below.

Figure 2 schematically illustrates Scheme 1, the preparation of exemplary oximes of the invention, including: *N*-Substituted 2-hydroxyiminoacetamides RS194B, RS194C, RS69N, RS218A, RS69L, and RS191E, which were prepared from ethyl glyoxylate (*1*) in two steps, as discussed in detail in Example 1, below.

Figure 3 illustrates Table 1, which also schematically illustrates the structures of exemplary compounds of this invention, and their activity, as discussed in detail in Example 1, below.

Figure 4 illustrates Table 2, which also schematically illustrates the structures of exemplary compounds of this invention, and exemplary activities, as discussed in detail in Example 1, below.

Figure 5 graphically illustrates the concentration dependence of oxime reactivation of sarin (***A***)-, VX (***B***)-, cyclosarin (***C***)-, paraoxon (***D***)-, and tabun (***E***)-inhibited (conjugated) hAChE; dependence for the exemplary compound, the lead oxime, RS194B (*black circle*) and the exemplary compound, the initial lead, RS41A (*open circle*) compared with reference uncharged (DAM (*gray diamond*) and MINA (*gray square*)) and cationic (2PAM (*gray square*) oximes, as discussed in detail in Example 1, below.

Figure 6 graphically illustrates the pH dependence of UV spectra of 50 μM of the exemplary compound RS41A and pH dependence of $A_{270\ nm}$ of 50 μM (***A***) of the exemplary compound RS41A (***B***), and RS194B along with corresponding pK_a values calculated by nonlinear regression using Equation 1 (***C***), as discussed in detail in Example 1, below.

Figure 7 illustrates the pH dependence of ^{1}H NMR spectra of 2.0 mM of the exemplary compound RS194B in D_2O buffers (***A***, ***B***, and ***C***) along with corresponding pK_a values calculated from the observed pH-induced difference in chemical shifts (***D***, ***E***, and ***F***) by nonlinear regression using Equation 1; NMR signals in panels *A*, *B*, and *C* were normalized relative to the maximal peak height in the given chemical shift region; spectra were aligned using a benzene external standard singlet at 7.16 ppm, as discussed in detail in Example 1, below.

Figure 8 graphically illustrates concentration dependence of oxime reactivation of VX-inhibited hAChE by the exemplary compound, the lead oxime, RS194B (***A***), initial lead oxime RS41A (***B***), and reference oxime 2PAM (***C***) and the exemplary compound oxime RS186B (***D***) measured at pH 6. 4 (○), pH 7.4 (□), and pH 8.4 (△) at 37 °C in 0.10 M phosphate buffers, as discussed in detail in Example 1, below.

Figure 9 describes the pharmacokinetics of the exemplary compound RS194B (***circles***) and the exemplary compound RS41A (***squares***) in mice; brain (***gray lines***) and plasma (***black lines***) compound concentrations were determined at discrete time points upon single, 80 mg/kg (RS194B), or 30 mg/kg (RS41A) dose administered to mice i.m.; each point represents average of determinations from three mice. S.E. of determination are indicated by *error bars*; times required for halving maximal compound concentrations

in plasma and in brain are indicated by *dashed lines* for each of two oximes, as discussed in detail in Example 1, below.

Figure 10 illustrates an image of a computational molecular modeling of VX-inhibited AChE showing the reversible Michaelis type complex and covalent pentacoordinate trigonal bipyramidal intermediate for interaction of initial lead oxime RS41A (***A***) and the exemplary compound, the lead oxime, RS194B (***B***), as discussed in detail in Example 1, below.

Figure 11 graphically illustrates the free energy relationships between nucleophilic reactivities and oxime group ionization states of selected oxime reactivators. Rate constants (k) of maximal pH-dependent ATCh oximolysis (*black line* and *circles*), oximolysis at pH 7.4 (*gray line* and *circles*), and an average overall rate constant k_r ($M^{-1}min^{-1}$) for oxime reactivation of VX, sarin, cyclosarin, and paraoxon inhibited hAChE (*white diamonds*) in relation to pK_a values were determined for reactivator oxime groups, as discussed in detail in Example 1, below.

Figure 12 illustrates Table 3, which describes a summary of p$\boldsymbol{K_a}$ values for the exemplary compound, the lead oxime, RS194B and other selected oximes determined by four different techniques from pH-dependent changes in oxime UV spectra (***cf.*** Figure 6), oxime ^{1}H NMR spectra in D_2O (***cf.*** Figure 7), oxime-induced ATCh oximolysis (***cf.*** supplemental Fig. S3), and oxime-induced VX Flu-MP oximolysis (***cf.*** supplemental Fig. S4), as discussed in detail in Example 1, below.

Figure 13 illustrates Table 4, which describes the Kinetic constants for reactivation of VX-hAChE conjugate by the exemplary compounds of the invention, the oximes RS194B, RS41A, RS186B, and reference oxime 2PAM determined in 0.1 M phosphate buffers pH 6.4, 7.4, and 8.4, as discussed in detail in Example 1, below.

Figure 14 illustrates Table 5, which describes data from the therapy of OP-exposed mice with the exemplary compounds, the lead oximes, RS194B and RS41A and standard reference oxime 2PAM; protective index is the ratio of OP LD_{50} for OP-exposed animals treated with oxime (+atropine) and for animals given OP alone (***cf.*** supplemental Tables S2–S6), as discussed in detail in Example 1, below.

Figure 15 illustrates Table 6, which describes the combination of therapy and pretreatment of OP-exposed mice with the exemplary compound oxime RS194B at i.m. administered doses equivalent to 25, 10, or 5% its LD_{50} dose of 500 mg/kg (***cf.*** supplemental Tables S2–S6), as discussed in detail in Example 1, below.

Figure 16 graphically illustrates data showing the plasma kinetics in mice following intravenous, intramuscular and oral dosing; and, PK profiles of the exemplary RS194B (structure illustrated) concentration in plasma following various routes and doses of administration to the mouse, as discussed in detail in Example 1, below.

Figure 17 illustrates data showing the bio-availability parameters of the exemplary RS194B; where bio-availability (F) is estimated from pharmacokinetic (PK) parameters determined upon intravenous and oral administration to the mouse, and toxicity of RS194B determined upon intramuscular administration to the mouse: $LD_{50} \geq 500$ mg/kg, as discussed in detail in Example 1, below.

Figure 18 graphically illustrates data showing an exemplary loading and maintenance dose scheme; the loading dose is given intramuscularly and the maintenance doses are given orally; PK profiles of the exemplary compound RS194B concentration in plasma and brain following loading oral dose of 220 mg/kg and subsequent maintenance oral doses of 110 mg/kg to the mouse, as discussed in detail in Example 1, below.

Figure 19 illustrates data showing *in vitro* reactivation data for an antidote congener of the exemplary compounds RS194B and RS138B; showing kinetic constants for reactivation of paraoxon-, sarin- , cyclosarin- and VX-hAChE conjugates by the exemplary RS2 138B, the principal uncharged lead oxime, the exemplary compound RS194B, and reference oximes 2PAM, MINA and DAM; reactivation rate constants k_2, K_{ox} and k_r were determined from the time dependence of OP-hAChE reactivation, as discussed in detail in Example 1, below.

Figure 20 illustrates an *in silico* computational molecular model of reversible binding for the exemplary RS138B to the VX-hAChE conjugate, characterized by K_{ox} ; the ethoxy methylphosphonylated conjugated active serine side chain is rendered as orange (P) red (O) and white (C) sticks; the face of tetrahedral P atom open to nucleophilic oxime attack is facing the viewer, as discussed in detail in Example 1, below.

Figure 21 schematically illustrates the structures of the exemplary RS2 138B and RS194B compounds; the *In vitro* reactivation potency of RS2 138B makes it the most promising structural analog of the lead reactivator RS194B, as discussed in detail in Example 1, below.

Figure 22, or Figure S1 supplemental Fig. S1, illustrates the ^1H NMR spectra of 50.0 mm RS194B in D_2O buffer at pH 6.0; Fig. 22A illustrates the integration-based peak

assignment, an peak integrals are indicated; Fig. 22B illustrates confirmation of peak assignment by DQF-COSY spectrum (at a 1.5 to 4.0 ppm expansion) of the same solution; spectra were taken in 20 mM D_2O phosphate-pyrophosphate buffer pH 6.0 containing 100 mM NaCl, as discussed in detail in Example 1, below.

Figure 23, or Figure S2 (supplemental Fig. S2), graphically illustrates pH dependence of ^1H NMR spectra of 2.0 mM 2PAM in D_2O buffers pH 5 – 10; Fig. 23A) ^1H NMR spectrum of 2.0 mM 2PAM at pH 5 along with peak assignment; Fig. 23B) expanded view of the spectrum in the chemical shift region 7.8 – 8.9; Fig. 23C) pH dependent change in chemical shifts for the 8.7 ppm singlet; and, Fig. 23D) 8.77 ppm doublet; the corresponding pK_a values were calculated from the observed pH induced difference in chemical shifts (Fig. 23E and Fig. 23F) by nonlinear regression using equation (1), as discussed in detail in Example 1, below.

Figure 24, or Figure S3 (supplemental Fig. S3), graphically illustrates pH dependence of rates of 1.0 mM ATCh hydrolysis by 0.1 mM oximes (oximolysis) for the exemplary compounds RS194B (red); RS41A (blue symbols and curve); RS186B (green symbols); RS150D (magenta); and, RS174C (teal), and also: MINA (black inverted triangles and curve) and 2PAM (white circles and black curve); grey and white/grey circles stand for spontaneous ATCh hydrolysis in the absence of oxime and DTNB hydrolysis in the absence of oxime or ATCh, respectively, as discussed in detail in Example 1, below.

Figure 25, or Figure S4 (supplemental Fig. S4), graphically illustrates pH dependence of rates of 0.1 mM VX Flu-MP hydrolysis by 0.5 mM oximes (OP oximolysis) for RS194B (red symbols and curve) RS41A (blue symbols and curve) and 2PAM (black and white symbols and curve); grey circles stand for spontaneous VX Flu-MP hydrolysis in the absence of oxime, as discussed in detail in Example 1, below.

Figure 26, or Figure S5 (supplemental Fig. S5), graphically illustrates free energy relationships between nucleophilic reactivities and oxime group ionization states of selected oxime reactivators; rate constants (k) of maximal pH dependent ATCh oximolysis (black line and circles), oximolysis at pH 7.4 (grey line and circles) and oxime reactivation k_r ($M^{-1}min^{-1}$) of: Fig. 26A) VX, Fig. 26B) Paraoxon, Fig. 26C) cyclosarin and Fig. 26D) sarin inhibited hAChE (yellow diamonds) in relation to pK_a values determined for reactivator oxime groups; the exemplary reactivator compound RS194B is indicated by cross-haired symbols; k_r for RS174C and RS150D were extrapolated from reactivation

rates determined at single (0.67 mM) oxime concentration, as discussed in detail in Example 1, below.

Figure 27, or Figure S6 (supplemental Fig. S6), graphically illustrates free energy relationships between nucleophilic reactivities and oxime group ionization states of selected oxime reactivators; rate constants (k) of maximal pH dependent ATCh oximolysis (black line and circles), oximolysis at pH 7.4 (grey line and circles) and maximal oxime reactivation rate k_2 (min^{-1}) (yellow diamonds and red line) of: Fig. 27A) VX, Fig. 27B) Paraoxon, Fig. 27C) cyclosarin, Fig. 27D) sarin inhibited hAChE and Fig. 27E) average k_2 (min^{-1}) in relation to pK_a values determined for reactivator oxime groups. The lead reactivator RS194B is indicated by cross-haired symbols. k_r for RS174C and RS150D were extrapolated from reactivation rates determined at single (0.67 mM) oxime concentration, as discussed in detail in Example 1, below.

Figure 28 illustrates Table S1, showing maximal oxime concentrations in brains and plasma of mice and halving time of those concentrations determined upon i.m. administration of a single oxime dose (cf. Figure 5); distribution coefficients (log D) were calculated from oxime structures using ChemAxon software package, as discussed in detail in Example 1, below.

Figure 29 illustrates Table S2, showing antidotal efficacy of oximes RS41A and RS194B given i.m. to mice after (therapy) and before and after (pretreatment + therapy) s.c. VX exposure; the oximes were administered at doses equal to 25% of their LD_{50} dose. LD_{50} (s.c) of VX = 28.3 μg/kg, LD_{50} (i.m.) of RS41A = 200 mg/kg, LD_{50} (i.m.) of RS194B = 500 mg/kg, CD-1 strain male mice; oximes in therapy were diluted in atropine (10 mg/kg) , as discussed in detail in Example 1, below.

Figure 30 illustrates Table S3, showing antidotal efficacy of oximes RS41A and RS194B given i.m. to mice after (therapy) and before and after (pretreatment + therapy) s.c. sarin exposure; the oximes were administered at doses equal to 25% of their LD_{50} dose. LD_{50} (s.c) of sarin = 238.3 μg/kg, LD_{50} (i.m.) of RS41A = 200 mg/kg, LD_{50} (i.m.) of RS194B = 500 mg/kg, CD-1 strain male mice; oximes in therapy were diluted in atropine (10 mg/kg) , as discussed in detail in Example 1, below.

Figure 31 illustrates Table S4, showing antidotal efficacy of oximes RS41A and RS194B given i.m. to mice after (therapy) and before and after (pretreatment + therapy) s.c. tabun exposure; the oximes were administered at doses equal to 25% of their LD_{50} dose. LD_{50} (s.c) of tabun = 565.8 μg/kg, LD_{50} (i.m.) of RS41A = 200 mg/kg, LD_{50} (i.m.)

of RS194B = 500 mg/kg, CD-1 strain male mice; oximes in therapy were diluted in atropine (10 mg/kg) , as discussed in detail in Example 1, below.

Figure 32 and Figure 33 illustrate Table S5, showing antidotal efficacy of oximes RS41A and RS194B given i.m. to mice after (therapy) and before and after (pretreatment + therapy) s.c. paraoxon exposure; the oximes were administered at doses equal to 25% of their LD_{50} dose. LD_{50} (s.c) of paraoxon = 712.9 μg/kg, LD_{50} (i.m.) of RS41A = 200 mg/kg, LD_{50} (i.m.) of RS194B = 500 mg/kg, CD-1 strain male mice. Oximes in therapy were diluted in atropine (10 mg/kg) , as discussed in detail in Example 1, below.

Figure 34 illustrates Table S6, showing antidotal efficacy of oximes RS41A and RS194B given i.m. to mice after (therapy) and before and after (pretreatment + therapy) s.c. soman exposure; the oximes were administered at doses equal to 25% of their LD_{50} dose. LD_{50} (s.c) of soman = 136.1 μg/kg, LD_{50} (i.m.) of RS41A = 200 mg/kg, LD_{50} (i.m.) of RS194B = 500 mg/kg, CD-1 strain male mice; oximes in therapy were diluted in atropine (10 mg/kg) , as discussed in detail in Example 1, below.

Figure 35 graphically illustrates alternative exemplary structures of the invention, as discussed in detail in Example 1, below.

Figure 36 graphically illustrates: ***Top panels,*** *In vitro* efficacy of the exemplary blood-brain-barrier penetrant oxime RS194B for reactivation of OP-hAChE conjugates obtained by paraoxon, sarin, cyclosarin and VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate) inhibition; dependences of rates of reactivation on concentrations of RS194B, our initial lead RS41A and uncharged (MINA, DAM) or charged (2PAM) reference oximes; ***Lower panels*****:** *In vitro* oxime efficacy corrected for *in vivo* toxicity of the each oxime; LD_{50} values for i.m. administration to mice are 106 mg/kg, 200 mg/kg and 500 mg/kg for 2PAM, RS41A and RS194B, respectively, as discussed in detail in Example 1, below.

Figure 37 graphically illustrates antidote indices for oxime re-activating agents; the Antidote Index is defined here by ([oxime]/LD50); reactivation rates are found in Figure 36.

Like reference symbols in the various drawings indicate like elements.

Reference will now be made in detail to various exemplary embodiments of the invention, examples of which are illustrated in the accompanying drawings. The following detailed description is provided to give the reader a better understanding of

certain details of aspects and embodiments of the invention, and should not be interpreted as a limitation on the scope of the invention.

DETAILED DESCRIPTION

The invention provides nucleophilic hydroxyimino-acetamido alkylamine antidotes that cross the blood-brain barrier (BBB) to catalyze the hydrolysis of organophosphate (OP)-inhibited human acetylcholinesterase (hAChE) in the central nerve system (CNS). In alternative embodiments, the hydroxyimino-acetamido alkylamines of the invention are designed to fit within AChE gorge dimensions and interact with the conjugated phosphate atom in the gorge. In alternative embodiments, the hydroxyimino-acetamido alkylamines of the invention are designed for optimized fit within the gorge and ionization equilibria that govern affinity and reactivity for the two linked hAChE re-activation steps. In alternative embodiments, compositions of the invention comprise uncharged but ionizable *N*-substituted 2-hydroxyimino-acetamido alkylamine reactivators of phosphorylated human acetylcholinesterase (hAChE) intended to catalyze the hydrolysis of organophosphate (OP)-inhibited hAChE in the CNS. In alternative embodiments, compositions of the invention comprise the so-called RS194B, an azepine analog, and RS2-138B structures and compounds.

In alternative embodiments, compositions of the invention are rapidly absorbed from the site of administration (e.g., oral, inhalation, or intramuscular, or i.m.), cross the blood-brain barrier as a neutral species, show enhanced nucleophicity from the oximate ionization species, selectively form a reversible complex with the active center gorge of an AChE, displace the covalently attached OPs, e.g., from a organophosphate toxicant such as a pesticide or a nerve agent, and restore AChE activity in the brain and periphery. In alternative embodiments, compositions and methods of the invention provide immediate protection from exposure, as well as prevention, of OP exposure, e.g., protection or prevention of immediate and recurring seizures that result from excessive acetylcholine stimulation in the brain.

In alternative embodiments, compositions of the invention have two ionization equilibria: a neutral oxime going to an anionic oximate, and a neutral amine going to a protonated amine. Hence, in these embodiments of compositions of the invention, there are four ionization states: (1) a cationic state with a positive charge on the amine (this may be the ionization state that binds best in the active center gorge); (2) an anionic state

with a negative charge on the oximated (this is the active species in solution that can promote the hydrolysis of organophosphate esters; however, we cannot be so sure for the enzyme since the neutral species may bind but the enzyme may facilitate a proton transfer to form the oximated, hence the attacking species could be the zwitterion, having a positive change on the amine and a negative charge on the oximate); (3) the zwitterion, as described above, that possesses a cationic (positive) and a anionic (negative) ion or moiety; and (4) a neutral species (this is the one that crosses the blood-brain barrier and is responsible for oral absorption). The ratio of species will always adjust according to the overall pH and pKa of the residues.

In alternative embodiments, compositions of the invention whose cationic species can bind within the active center gorge, so positioned that the oximate (the reactive warhead) can attack the conjugated phosphorus. In alternative embodiments, the oximate can access the phosphorus in the narrow and impacted region of the gorge. In alternative embodiments, compositions of the invention can have a small fraction of neutral species, perhaps 10% to 20%, so that the compound will cross the blood-brain barrier and can be absorbed after oral administration.

With these exemplary properties, compositions of the invention can access the CNS and peripheral nervous system and reactivate cholinesterases inhibited by pesticides and nerve agents. In alternative embodiments, compositions of the invention can be an antidote for poisoning by organophosphate (e.g., diisopropylfluorophosphates and echothiophate) and carbamylating drugs (e.g., physostigmine, neostigmine and pyridostigmine). In alternative embodiments, compositions of the invention can assist in the catalysis of the organophosphate itself before it gets to the enzyme; hence, compositions of the invention can have a scavenging capacity for the parent organophosphate; catalysis is carried out by acetylcholinesterase or butyrylcholinesterase and the oxime facilitates this.

In alternative embodiments, compounds of the invention can not only cross the blood-brain barrier to reactivate acetylcholinesterase in the CNS and peripheral nervous system, but compounds of the invention also are effective as antidotes and protective (prophylactic) agents when given orally. In alternative embodiments, for example, as a prophylactic agent, the oral dosage route does not have the most rapid onset, but is an ideal route for achieving prolonged antidote activity; this can be the most practical route for prophylactic use against massive releases of volatile gases, for example, prophylactic

or therapeutic use in accidents or in poison gas (e.g., nerve agent) warfare, e.g., paraoxon, sarin, cyclosarin and VX attacks.

In alternative embodiments, compositions of the invention have improved antidote efficacy for various organophosphates, e.g., methylphosphonate nerve agents and phosphorate insecticides, and have a lower toxicity than 2-PAM, and an increased half-life in the brain when compared with other compounds. In alternative embodiments, compositions of the invention, e.g., the compound RS194b, are designed to have an optimized fit within an AChE gorge, and ionization equilibria dictating affinity and reactivity for the two linked reactivation steps.

For example, Figure 1 schematically illustrates that the uncharged and protonated forms of acetamido oximes (shown in blue) of the invention will equilibrate in blood upon intramuscular injection to OP exposed individual. The uncharged form will cross the blood-brain barrier and enter brain tissue where it will re-equilibrate. The protonated form will bind AChE-OP adduct and recover AChE catalytic activity (shown in the red rectangle). Thus, in alternative embodiments, compositions of the invention are antidotes to various organophosphates, including methylphosphonates and other nerve agents as well as the phosphorate insecticides, and methods of the invention are used to treat, ameliorate or prevent organophosphate nerve agent poisoning or toxic effects subsequent to organophosphate insecticide exposure. Furthermore, in alternative embodiments, compositions of the invention act as a universal antidote to non-aging nerve agents, e.g., compositions of the invention can be used as countermeasures to organophosphate nerve agents, e.g., as those that could be used chemical terrorism. In alternative embodiments, compositions of the invention limit the toxicity and threat potential of organophosphate nerve agents by assisting the catalytic breakdown of the organophosphates by e.g., butyrylcholinesterase in plasma and tissues. In alternative embodiments, compositions of the invention limit the toxicity and threat potential of organophosphate nerve agents by assisting the butyrylcholinesterase catalysis of the organophosphate.

Bioisosteres of Compounds of the Invention

In alternative embodiments, the invention also provides bioisosteres of compounds of the invention. In alternative embodiments, bioisosteres of the invention are compounds of the invention comprising one or more substituent and/or group replacements with a substituent and/or group having substantially similar physical or

chemical properties which produce substantially similar biological properties to a compound of the invention, or stereoisomer, racemate or isomer thereof. In one embodiment, the purpose of exchanging one bioisostere for another is to enhance the desired biological or physical properties of a compound without making significant changes in chemical structures.

For example, in one embodiment, bioisosteres of compounds of the invention are made by replacing one or more hydrogen atom(s) with one or more fluorine atom(s), e.g., at a site of metabolic oxidation; this may prevent metabolism (catabolism) from taking place. Because the fluorine atom is only slightly larger than the hydrogen atom the overall topology of the molecule is not significantly affected, leaving the desired biological activity unaffected. However, with a blocked pathway for metabolism, the molecule may have a longer half-life or be less toxic, and the like.

Products of Manufacture, Kits

The invention also provides products of manufacture and kits for practicing the methods of this invention. In alternative embodiments, the invention provides products of manufacture and kits comprising all the components needed to practice a method of the invention.

The invention provides kits comprising compositions and/or instructions for practicing methods of the invention. In alternative embodiments, the invention provides kits comprising: a composition used to practice a method of any of the invention, optionally comprising instructions for use thereof.

In alternative embodiments, the invention provides pumps, devices, subcutaneous infusion devices, continuous subcutaneous infusion device, infusion pens, needles, reservoirs, ampoules, vials, syringes, cartridges, disposable pen or jet injectors, prefilled pens or syringes or cartridges, cartridge or disposable pen or jet injectors, two chambered or multi-chambered pumps, syringes, cartridges or pens or jet injectors comprising a composition or a formulation of the invention. In alternative embodiments, the injector is an autoinjector, e.g., a SMARTJECT® autoinjector (Janssen Research and Development LLC); or a MOLLY®, or DAI®, or DAI-RNS® autoinjector (SHL Group, Deerfield Beach, FL). In alternative embodiments, the injector is a hypodermic or a piston syringe.

Formulations and pharmaceutical compositions

In alternative embodiments, the invention provides compounds and compositions, including formulations and pharmaceutical compositions, for use in *in vivo*, *in vitro* or *ex vivo* methods for catalyzing the hydrolysis of organophosphate (OP)-inhibited human acetylcholinesterase (hAChE) in the central nerve system (CNS); or, for treating, ameliorating or protecting (preventing) an organophosphate toxicity or poisoning or toxic exposure, or for treating, ameliorating or protecting (preventing) organophosphate inhibition of an acetylcholinesterase (AChE); or, for treating, preventing or ameliorating excessive acetylcholine stimulation in the CNS, or the brain.

In alternative embodiments, the pharmaceutical compositions of the invention can be administered parenterally, topically, orally or by local administration, such as by aerosol or transdermally. In alternative embodiments, pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, capsules, suspensions, taken orally, suppositories and salves, lotions and the like. Pharmaceutical formulations of this invention may comprise one or more diluents, emulsifiers, preservatives, buffers, excipients, etc. and may be provided in such forms as liquids, powders, emulsions, lyophilized powders, sprays, creams, lotions, controlled release formulations, tablets, pills, gels, geltabs, on patches, in implants, etc. In practicing this invention, the pharmaceutical compounds can be delivered by transdermally, by a topical route, formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols. Oral carriers can be elixirs, syrups, capsules, tablets, pills, geltabs and the like.

In alternative embodiment, compositions of the invention are delivered orally, e.g., as pharmaceutical formulations for oral administration, and can be formulated using pharmaceutically acceptable carriers well known in the art in appropriate and suitable dosages. Such carriers enable the pharmaceuticals to be formulated in unit dosage forms as tablets, pills, powder, dragees, capsules, liquids, lozenges, gels, syrups, slurries, suspensions, etc., suitable for ingestion by the patient. Pharmaceutical preparations for oral use can be formulated as a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable additional compounds, if desired, to obtain tablets or dragee cores. Suitable solid excipients are carbohydrate or protein fillers include, e.g., sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose,

hydroxypropylmethyl-cellulose, or sodium carboxy-methylcellulose; and gums including arabic and tragacanth; and proteins, e.g., gelatin and collagen. Disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

In alternative embodiments, liquid carriers are used to manufacture or formulate compounds of this invention, or a composition used to practice the methods of this invention, including carriers for preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compounds. The active ingredient (e.g., a composition of this invention) can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can comprise other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators.

In alternative embodiments, solid carriers are used to manufacture or formulate compounds of this invention, or a composition used to practice the methods of this invention, including solid carriers comprising substances such as lactose, starch, glucose, methyl-cellulose, magnesium stearate, dicalcium phosphate, mannitol and the like. A solid carrier can further include one or more substances acting as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier can be a finely divided solid which is in admixture with the finely divided active compound. In tablets, the active compound is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins. A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free flowing form such as a powder or granules, optionally mixed with a binder (e.g., povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid

diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropyl methylcellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

In alternative embodiments, concentrations of therapeutically active compound in a formulation can be from between about 0.1% to about 100% by weight.

In alternative embodiments, therapeutic formulations are prepared by any method well known in the art, e.g., as described by Brunton et al., eds., Goodman and Gilman's: The Pharmacological Bases of Therapeutics , 12th ed., McGraw-Hill, 2011; Remington: The Science and Practice of Pharmacy, Mack Publishing Co., 20th ed., 2000; Avis et al., eds., Pharmaceutical Dosage Forms: Parenteral Medications, published by Marcel Dekker, Inc., N.Y., 1993; Lieberman et al., eds., Pharmaceutical Dosage Forms: Tablets, published by Marcel Dekker, Inc., N.Y., 1990; and Lieberman et al., eds., Pharmaceutical Dosage Forms: Disperse Systems, published by Marcel Dekker, Inc., N.Y., 1990.

In alternative embodiments, therapeutic formulations are delivered by any effective means appropriated for a particular treatment. For example, depending on the specific antitumor agent to be administered, the suitable means include oral, rectal, vaginal, nasal, pulmonary administration, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) infusion into the bloodstream. For parenteral administration, antitumor agents of the present invention may be formulated in a variety of ways. Aqueous solutions of the modulators can be encapsulated in polymeric beads, liposomes, nanoparticles or other injectable depot formulations known to those of skill in the art. In alternative embodiments, compounds of the invention are administered encapsulated in liposomes (see below). In alternative embodiments, depending upon solubility, compositions are present both in an aqueous layer and in a lipidic layer, e.g., a liposomic suspension. In alternative embodiments, a hydrophobic layer comprises phospholipids such as lecithin and sphingomyelin, steroids such as cholesterol, more or less ionic surfactants such a diacetylphosphate, stearylamine, or phosphatidic acid, and/or other materials of a hydrophobic nature.

The pharmaceutical compositions can be formulated in any way and can be administered in a variety of unit dosage forms depending upon the condition or disease and the degree of illness, the general medical condition of each patient, the resulting

preferred method of administration and the like. Details on techniques for formulation and administration are well described in the scientific and patent literature, see, *e.g.*, the latest edition of Remington's Pharmaceutical Sciences, Maack Publishing Co., Easton PA ("Remington's"). For example, in alternative embodiments, these compositions of the invention are formulated in a buffer, in a saline solution, in a powder, an emulsion, in a vesicle, in a liposome, in a nanoparticle, in a nanolipoparticle and the like. In alternative embodiments, the compositions can be formulated in any way and can be applied in a variety of concentrations and forms depending on the desired *in vivo*, *in vitro* or *ex vivo* conditions, a desired *in vivo*, *in vitro* or *ex vivo* method of administration and the like. Details on techniques for *in vivo*, *in vitro* or *ex vivo* formulations and administrations are well described in the scientific and patent literature. Formulations and/or carriers used to practice this invention can be in forms such as tablets, pills, powders, capsules, liquids, gels, syrups, slurries, suspensions, etc., suitable for *in vivo*, *in vitro* or *ex vivo* applications.

In practicing this invention, the compounds (e.g., formulations) of the invention can comprise a solution of compositions (e.g., apratoxin F and apratoxin G compounds, and/or apratoxin F and apratoxin G stereoisomers, derivatives and analogs) disposed in or dissolved in a pharmaceutically acceptable carrier, e.g., acceptable vehicles and solvents that can be employed include water and Ringer's solution, an isotonic sodium chloride. In addition, sterile fixed oils can be employed as a solvent or suspending medium. For this purpose any fixed oil can be employed including synthetic mono- or diglycerides, or fatty acids such as oleic acid. In one embodiment, solutions and formulations used to practice the invention are sterile and can be manufactured to be generally free of undesirable matter. In one embodiment, these solutions and formulations are sterilized by conventional, well known sterilization techniques.

The solutions and formulations used to practice the invention can comprise auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and can be selected primarily based on fluid volumes, viscosities and the like, in accordance with the particular mode of *in vivo*, *in vitro* or *ex vivo* administration selected and the desired results.

The compositions and formulations of the invention can be delivered by the use of liposomes. In alternative embodiments, by using liposomes, particularly where the liposome surface carries ligands specific for target cells or organs, or are otherwise preferentially directed to a specific tissue or organ type, one can focus the delivery of the active agent into a target cells in an *in vivo*, *in vitro* or *ex vivo* application.

The compositions and formulations of the invention can be directly administered, e.g., under sterile conditions, to an individual (e.g., a patient) to be treated. The modulators can be administered alone or as the active ingredient of a pharmaceutical composition. Compositions and formulations of this invention can be combined with or used in association with other therapeutic agents. For example, an individual may be treated concurrently with conventional therapeutic agents.

Nanoparticles, Nanolipoparticles and Liposomes

The invention also provides nanoparticles, nanolipoparticles, vesicles and liposomal membranes comprising compounds and compositions used to practice the methods of this invention, e.g., methods for catalyzing the hydrolysis of organophosphate (OP)-inhibited human acetylcholinesterase (hAChE) in the central nerve system (CNS); or, for treating, ameliorating or protecting (preventing) an organophosphate toxicity or poisoning or toxic exposure, or for treating, ameliorating or protecting (preventing) organophosphate inhibition of an acetylcholinesterase (AChE); or, for treating, preventing or ameliorating excessive acetylcholine stimulation in the CNS, or the brain

The invention provides multilayered liposomes comprising compounds used to practice this invention, e.g., as described in Park, et al., U.S. Pat. Pub. No. 20070082042. The multilayered liposomes can be prepared using a mixture of oil-phase components comprising squalane, sterols, ceramides, neutral lipids or oils, fatty acids and lecithins, to about 200 to 5000 nm in particle size, to entrap a composition used to practice this invention.

Liposomes can be made using any method, e.g., as described in Park, et al., U.S. Pat. Pub. No. 20070042031, including method of producing a liposome by encapsulating an active agent (e.g., a compound of the invention), the method comprising providing an aqueous solution in a first reservoir; providing an organic lipid solution in a second reservoir, and then mixing the aqueous solution with the organic lipid solution in a first mixing region to produce a liposome solution, where the organic lipid solution mixes with

the aqueous solution to substantially instantaneously produce a liposome encapsulating the active agent; and immediately then mixing the liposome solution with a buffer solution to produce a diluted liposome solution.

. In one embodiment, liposome compositions used to practice this invention comprise a substituted ammonium and/or polyanions, e.g., for targeting delivery of a compound (e.g., e.g., a compound of the invention) used to practice this invention to a desired cell type or organ, e.g., brain, as described e.g., in U.S. Pat. Pub. No. 20070110798.

The invention also provides nanoparticles comprising compounds (e.g., a compound of the invention) used to practice this invention in the form of active agent-containing nanoparticles (e.g., a secondary nanoparticle), as described, e.g., in U.S. Pat. Pub. No. 20070077286. In one embodiment, the invention provides nanoparticles comprising a fat-soluble active agent of this invention or a fat-solubilized water-soluble active agent to act with a bivalent or trivalent metal salt.

In one embodiment, solid lipid suspensions can be used to formulate and to deliver compositions used to practice this invention to mammalian cells *in vivo*, *in vitro* or *ex vivo*, as described, e.g., in U.S. Pat. Pub. No. 20050136121.

Delivery vehicles

In alternative embodiments, any delivery vehicle can be used to practice the methods or used to practice this invention, e.g., to deliver compositions of the invention (e.g., a compound of the invention) to mammalian cells *in vivo*, *in vitro* or *ex vivo*. For example, delivery vehicles comprising polycations, cationic polymers and/or cationic peptides, such as polyethyleneimine derivatives, can be used e.g. as described, e.g., in U.S. Pat. Pub. No. 20060083737.

In one embodiment, a dried polypeptide-surfactant complex is used to formulate a composition used to practice this invention, e.g. as described, e.g., in U.S. Pat. Pub. No. 20040151766.

In one embodiment, a composition used to practice this invention can be applied to cells using vehicles with cell membrane-permeant peptide conjugates, e.g., as described in U.S. Patent Nos. 7,306,783; 6,589,503. In one aspect, the composition to be delivered is conjugated to a cell membrane-permeant peptide. In one embodiment, the composition to be delivered and/or the delivery vehicle are conjugated to a transport-

mediating peptide, e.g., as described in U.S. Patent No. 5,846,743, describing transport-mediating peptides that are highly basic and bind to poly-phosphoinositides.

In one embodiment, electro-permeabilization is used as a primary or adjunctive means to deliver the composition to a cell, e.g., using any electroporation system as described e.g. in U.S. Patent Nos. 7,109,034; 6,261,815; 5,874,268.

Dosaging

The pharmaceutical compositions and formulations of the invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a subject already exposed to a toxin, or exposed to any agent or chemical causing or resulting in excessive acetylcholine stimulation in the brain, e.g., exposure to a drug, a drug overdose, or a poisoning or a toxic exposure to a drug, and optionally the drug overdose causing the excessive acetylcholine stimulation is caused at least in part by: physostigmine, neostigmine, pyridostigmine, diisopropylfluorophosphate, or echothiophate an amount sufficient to cure, alleviate or partially arrest the clinical manifestations of the agent and/or its complications (a “therapeutically effective amount”).

The amount of pharmaceutical composition adequate to accomplish this is defined as a "therapeutically effective dose." The dosage schedule and amounts effective for this use, i.e., the “dosing regimen,” will depend upon a variety of factors, including the stage of the disease or condition, the severity of the disease or condition, the general state of the patient's health, the patient’s physical status, age and the like. In calculating the dosage regimen for a patient, the mode of administration also is taken into consideration.

The dosage regimen also takes into consideration pharmacokinetics parameters well known in the art, i.e., the active agents’ rate of absorption, bioavailability, metabolism, clearance, and the like (see, e.g., Hidalgo-Aragones (1996) J. Steroid Biochem. Mol. Biol. 58:611-617; Groning (1996) Pharmazie 51:337-341; Fotherby (1996) Contraception 54:59-69; Johnson (1995) J. Pharm. Sci. 84:1144-1146; Rohatagi (1995) Pharmazie 50:610-613; Brophy (1983) Eur. J. Clin. Pharmacol. 24:103-108; the latest Remington’s, supra). The state of the art allows the clinician to determine the dosage regimen for each individual patient, active agent and disease or condition treated. Guidelines provided for similar compositions used as pharmaceuticals can be used as

guidance to determine the dosage regiment, i.e., dose schedule and dosage levels, administered practicing the methods of the invention are correct and appropriate.

The invention will be further described with reference to the following examples; however, it is to be understood that the invention is not limited to such examples.

EXAMPLES

EXAMPLE 1:

This example demonstrates the effectiveness of compositions of the invention as a antidotes to organophosphates and organophosphate poisoning and toxicity.

We present a systematic structural optimization of uncharged but ionizable *N*-substituted 2-hydroxyiminoacetamido alkylamine reactivators of phosphylated human acetylcholinesterase (hAChE) intended to catalyze the hydrolysis of organophosphate (OP)-inhibited hAChE in the CNS. Starting with the initial lead oxime RS41A identified in our earlier study and extending to the azepine analog RS194B, reactivation rates for OP-hAChE conjugates formed by sarin, cyclosarin, VX, paraoxon, and tabun are enhanced several-fold *in vitro*. To analyze the mechanism of intrinsic reactivation of the OP-AChE conjugate and penetration of the blood-brain barrier, the pH dependence of the oxime and amine ionizing groups of the compounds and their nucleophilic potential were examined by UV-visible spectroscopy, ^{1}H NMR, and oximolysis rates for acetylthiocholine and phosphoester hydrolysis. Oximolysis rates were compared in solution and on AChE conjugates and analyzed in terms of the ionization states for reactivation of the OP-conjugated AChE. In addition, toxicity and pharmacokinetic studies in mice show significantly improved CNS penetration and retention for RS194B when compared with RS41A. The enhanced intrinsic reactivity against the OP-AChE target combined with favorable pharmacokinetic properties resulted in great improvement of antidotal properties of RS194B compared with RS41A and the standard peripherally active oxime, 2-pyridinealdoxime methiodide. Improvement was particularly noticeable when pretreatment of mice with RS194B before OP exposure was combined with RS194B reactivation therapy after the OP insult.

The invention provides an ideal centrally acting reactivator that combines the properties of efficient reactivation and blood-brain barrier penetration. Our initial approach was to develop a library of uncharged oxime reactivators amenable to

protonation (1) and identify a lead structure. A reactivator, the so-called RS41A (Table 1), is comparable in its *in vitro* reactivating potency to standard pyridinium oxime reactivator 2PAM, was selected from a large synthetic library.

In this study we describe systematic variations of RS41A structure and a detailed evaluation of associated reactivating properties leading to several enhanced reactivators culminating in the several-fold improved azepine analog RS194B. By virtue of enhanced association with OP-hAChE conjugates, RS194B showed substantially improved *in vitro* reactivation kinetics compared with the initial lead RS41A. Moreover, the low toxicity of RS194B in the mouse animal model in combination with its enhanced intrinsic reactivity results in substantial improvement of its therapeutic properties compared with RS41A and the standard oxime, 2PAM. The improvement was particularly noticeable when pretreatment of mice with RS194B before OP exposure was combined with RS194B reactivation therapy after the OP insult. We describe here the mechanistic basis for this enhancement in terms of the intrinsic reaction constants and pharmacokinetic profiles.

Since RS194B crosses the blood-brain barrier and has a reasonable fraction of neutral, non-ionized species at physiological pH, it can also be expected to be cross gastrointestinal membranes and hence will be efficacious after oral administration.

Materials and Methods

Enzyme: Highly purified monomeric hAChE was prepared as described e.g. in Sit et al. (2011) J. Biol. Chem. 286, 19422–19430; Cochran et al. (2011) J. Biol. Chem. 286, 29718–29724.

Organophosphates: Low toxicity, nonvolatile fluorescent methylphosphonates (Flu-MPs) (see e.g., Amitai (2007) Toxicology *233, 187–198*) were used in *in vitro* experiments as analogues of nerve agents sarin, cyclosarin, and VX. The Flu-MPs differ from actual nerve agent OPs only by the structure of their respective leaving groups. Inhibition of hAChE by Flu-MPs results in OP-hAChE covalent conjugates identical to the ones formed upon inhibition with the corresponding volatile OPs. Paraoxon was purchased from Sigma. Nerve agent OPs tabun, VX, sarin, and soman used in *in vivo* experiments were purchased from NC Laboratory (Spiez, Switzerland).

Oximes: 2-Pyridinealdoxime methiodide (2PAM), monoisonitrosoacetone (MINA), and 2,3-butanedione monoxime (DAM) were purchased from Sigma.

Preparation of exemplary Oximes of the invention: *N*-Substituted 2-hydroxyiminoacetamides RS194B, RS194C, RS69N, RS218A, RS69L, and RS191E were prepared from ethyl glyoxylate (*1*) in two steps, see Scheme 1, illustrated as Figure 2. Condensation with hydroxylamine provided ethyl glyoxylate oxime (*2*) followed by subsequent amidation with the corresponding primary amine.

Scheme 1

In Vitro Oxime Reactivation Assays

hAChE activities were measured using a spectrophotometric assay (see reference 18, below) at 25 °C in 0.1 m sodium phosphate buffer, pH 7.4, containing 0.01% BSA and 1.0 mm substrate acetylthiocholine (ATCh). OP-hAChE conjugates were prepared, and oxime reactivation was performed (at 37 °C in 0.1 m sodium phosphate buffer, pH 7.4, containing 0.01% BSA) as described in references 1, 16, below). The first order reactivation rate constant (k_{obs}) for each oxime + OP conjugate combination was calculated by nonlinear regression, as described in reference 19. The dependence of reactivation rates on oxime concentrations and determination of maximal reactivation rate constant k_2, Michaelis-Menten type constant K_{ox}, and the overall second order reactivation rate constant k_r were conducted as previously described in reference 19. The pH dependence of oxime reactivation of AChE was performed in 0.1 m phosphate buffers pH 6.4, 7.4, or 8.4 containing 0.01% BSA.

Oxime pK_a Determinations

Protonation of ionizable groups in oximes was monitored either using UV-visible spectrophotometry or by NMR spectrometry. A series of 20 mM phosphate-pyrophosphate buffers pH 5.0, 6.0, 7.0, 8.0, 8.5, 9.0, 9.5, 10, 10.5, and 11 (containing 0.1 m NaCl) were prepared in either H_2O or D_2O. For D_2O buffers, pD values were determined by correcting the pH reading by +0.4 pH units (*cf.* Ref. 20, see below). UV spectra of oximes between 220- and 320-nm wavelengths were recorded on Cary 1E (Varian) UV-visible spectrophotometer at the above pH values, and absorbance at 270 nm ($A_{270\,nm}$) was plotted as a function of pH yielding pK_a values by nonlinear regression of Equation 1:

$$A_{270\,nm} = A^{max}{}_{270\,nm} / (1 + [H^+] / Ka)$$

^{1}H NMR spectra recorded on Bruker DRX-500™ (Bruker, USA) spectrometer in 20 mm phosphate-pyrophosphate D_2O buffers pH 5.0, 6.0, 7.0, 8.0, 8.5, 9.0, 9.5, and 10

(containing 0.1 m NaCl) were overlaid and aligned using benzene as an external standard placed in a separate capillary tube within the NMR sample probe. Two-dimensional $^{1}H,^{1}H$ double-quantum filtered COSY spectra were recorded on a Bruker DRX-600™ spectrometer.

Computational Molecular Modeling

Interactions of oximes RS41A and RS194B within the active center gorge of VX-inhibited hAChE were studied for the reversible complex and for the trigonal bipyramidal intermediate for the reactivation step using a simulated annealing molecular dynamics approach (see reference 21). Covalent conjugates of hAChE ethylmethylphosphonylated at the active Ser-203 were generated by pasting ethylmethylphosphonylated Ser-203 of mouse AChE structure (PDB 2JGH) into the native WT hAChE structure (PDB 3LII) and subsequent semiempirical quantum mechanical adjustment of partial charges by InsightII™ suite (Accelrys, San Diego, CA). All water molecules were removed from the PDB structures, and a dielectric constant of four was used in calculation to mimic the interior of the hAChE active center gorge. In calculations for formation of the reversible complex, distances between oximate oxygen and the P atom of the VX conjugate were flexibly constrained to a distance between 0 and 3.00 Å. In trigonal bipyramidal intermediate calculations, the oximate oxygen was covalently linked to the P atom rehybridized to pentacoordinate geometry (see reference 21). Ten calculations were made for each oxime at both the reversible complex and trigonal bipyramidal intermediate steps allowing the oxime molecule and hAChE residues 72, 124, 286, 297, 341, and VX-conjugated Ser-203 to rotate freely, whereas all other enzyme side chains were fixed.

Acute Oxime Toxicity and Oxime Treatment of OP-exposed Mice

Male CD-1 mice (25–30 g body weight) were purchased from Rudjer Bošković Institute, Zagreb, Croatia. Mice were fed on a standard diet, had free access to water, and were kept in Macrolone cages at 21°C, exchanging light and dark cycles every 12 h. For the experimental sequence, mice were divided into groups of four. The mice were treated in accord with the approval of the Ethical Committee of the Institute for Medical Research and Occupational Health in Zagreb, Croatia.

Acute intramuscular (i.m.) toxicity (LD_{50}) was based upon 24-h mortality rates calculated according to Thompson (see reference 22) and Weil (see reference 23). Each LD_{50} was evaluated from the results obtained with four to six doses of a given oxime

(dissolved in water plus a minute amount of HCl to form the corresponding hydrochloride salt).

Antidotal activity against OP poisoning was tested by giving male CD-1 mice the studied oximes i.m. (at the specified dose) together with atropine sulfate (10 mg/kg) 1 min after subcutaneous OP administration (see references 24, 25) or p.o. (by oral administration) 15 min before OP followed by i.m. (intramuscular) atropine injection 1 min after OP. Stock solutions of nerve agents were prepared in isopropyl alcohol or in propylene glycol. Further dilutions were made in saline immediately before use. Alternatively, mice were pretreated i.m. with oximes (at specified dose but without atropine) 5 or 15 min before subcutaneous OP administration. The combination of pretreatment and antidotal therapy was performed by i.m. pretreatment with oxime 15 min before subcutaneous OP administration followed by i.m. administration of oxime (dissolved in 5 mg/ml atropine sulfate) 1 min after the OP exposure.

The antidotal efficacy of oximes was expressed as protective index (PI) with 95% confidence limits and the maximal dose of OP. The PI was the ratio of LD_{50} between OP with antidote and OP given alone. The maximal dose of organophosphate was the highest multiple of the OP LD_{50}, which was fully counteracted by the oximes.

Oxime Pharmacokinetics in Mice

Female CD-1 mice 4–8 weeks old (22–34 g of body weight) were purchased from Harlan (Livermore, CA). Mice were fed Purina Certified Rodent Chow #5002. Food and purified water were provided *ad libitum*. Mice were kept in hanging polycarbonate cages at 21–23 °C, exchanging light and dark cycles every 12 h. General procedures for animal care and housing were in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (1996) and the Animal Welfare Standards incorporated in 9 CFR Part 3, 1991.

For experiments mice were divided into groups of three. In the pharmacokinetic studies, 80 mg/kg RS194B or 30 mg/kg RS41A oxime was administered i.m. as a single dose in the absence of OP. Three animals were injected for every time point analyzed. Alternatively oxime was administered orally in a single dose, or repetitively every two hours after the initial p.o. administration. Brain and plasma were collected at each time point following the oxime administration. Blood (approximately 300 µl) was collected from the retro-orbital sinus of mice under isoflurane anesthesia into tubes containing

EDTA, processed to plasma within 30 min of collection, and then stored frozen at ≤−80 °C (± 10°C).

Brains were collected and analyzed individually at each time point. Brain weight was documented for each animal before storage on dry ice. Brains were stored at ≤−80 °C (± 10 °C) until analysis. Concentration of the oxime in body compartments was determined by LC-MS using multiple reaction monitoring electrospray ionization detection in positive ion mode.

Results

Systematic Variations of RS41A Structure

Our recent characterization (see reference 1) of a library of 135 uncharged, structurally diverse oximes revealed hydroxyimino acetamido amines as efficient reactivators of OP-hAChE conjugates. In particular, the selected lead structure RS41A appeared similar in reactivation potency to the standard reference oxime 2PAM.

With the aim of better understanding structure/activity relationships of the hydroxyimino acetamido alkylamine reactivators and specifically improving RS41A reactivation properties, we synthesized new reactivators by systematically modifying structure, see Table 1, which describes the structures and initial screen of relative rate constants (k_{obs}) for OP-hAChE reactivation of selected uncharged reactivators (0.67 mm) in comparison to cationic pyridinium aldoxime 2PAM; Nonenzymic oximolysis rates of ATCh (1 mm) by 1.0 mm oximes and calculated percentages of oximate anion and neutral species at pH 7.4 are also given as well as volume and surface area of R in the general oxime formula HO=NCHC(O)NH-R. Experiments were performed in duplicate at 37 °C in 0.10 m phosphate buffer, pH 7.4.

In Table 1: Nonenzymic oximolysis rates of ATCh (1 mM) by 1.0 mM oximes and calculated percentages of oximate anion and neutral species at pH 7.4 are also given as well as volume and surface area of R in the general oxime formula HO¢NCHC(O)NH-R. Experiments were performed in duplicate at 37 °C in 0.10 M phosphate buffer, pH 7.4. * The approximate k_{obs} values determined for 0.67 mM 2PAM were: 0.087 min (for paraxon (POX)), 0.16 min^{-1} (for sarin), 0.025 min^{-1} (for cyclosarin (CS)), 0.15 min^{-1} (for VX). The experimental uncertainty of individual constant determination in the screen was about 50%. Structures and initial screen of relative rate constants (k_{obs}) for OP-

hAChE reactivation of selected uncharged reactivators (0.67 mM) in comparison to cationic pyridinium aldoxime 2PAM.

In analyzing the alkylamine substituent attached to the 2-hydroxyimino acetamide, we distinguished two elements: the heterocycle "handle" and an intervening methylene "linker." Starting with the 2-hydroxyimino acetamido amine RS157A (no linker), ethyl, *n*-propyl, and *n*-butyl linkers were introduced along with primary amine, dimethylamine, pyrrolidine, piperidine, azepane, azocane, and bridged, polycyclic handles. Small amine and dimethylamine handles did not prove helpful, resulting in largely inactive reactivators (the last four compounds in Table 1, illustrated in Figure 3) irrespective of the associated linker length. Both ethyl and propyl linkers proved effective.

Of four pairs of compounds containing an identical handle, the ethyl linker proved more efficient in two pairs (RS194B *versus* RS194C and RS41A *versus* RS69L) and the propyl linker in the other two pairs (RS251B *versus* RS251A and RS191E *versus* RS69N). Overall, the most effective reactivators were RS194B and RS191E where RS41A structure was extended either through linker or handle size, but combining both longer linker and larger handle (as in compound RS194C) was not productive. The further increase in handle size to either an eight-membered azocane ring (RS251A and RS251B), bridged azepane (RS218A), or seven membered rings (RS2–57B) or by making it bicyclic yielded a respectable but not superior reactivator.

Analysis of molecular volumes and solvent-accessible surface areas for the N substituents (Table 1, or Figure 3) suggests that the most efficient reactivators fall in the group of those having volume/surface area ratio between 0.87 and 0.92, suggesting that for this series of relatively similar compounds, a more spheroid rather than planar shape appears more productive. Intrinsic reactivities of all 16 compounds from Table 1 reflected in their measured rates of ATCh oximolysis as well as the calculated percents of respective oximate anions were similar, emphasizing the importance of the shape and charge distribution for the productive interaction with OP-AChE conjugates leading to recovery of enzyme activity.

Reactivation Kinetics of Selected Oximes in Vitro

To distinguish whether molecular recognition or intrinsic chemical reactivity of RS41A was dominant in influencing overall reactivation potency, we analyzed reactivation kinetics of seven leading congeners of RS41A and three reference oximes,

2PAM, MINA, and DAM, and determined their individual reactivation constants k_2 and K_{ox} ; see Table 2, or Figure 4; and Fig. 5).

Table 2 describes: Kinetic constants for reactivation of paraoxon-, sarin-, cyclosarin-, VX- and tabun-hAChE conjugates by principal uncharged lead oxime RS194B initial lead RS41A, several oximes leading from RS41A to RS194B, and reference oximes 2PAM, MINA, and DAM; and Maximal reactivation rate constant (k_2, min^{-1}), apparent dissociation constant of (oxime · OP-hAChE conjugate) reversible complex (K_{ox}, mm), and overall second order reactivation rate constant (k_r, M^{-1} min^{-1}) were determined from reactivation curves as presented in Fig. 6. All constants were determined from triplicate experiments. S.E. of determined kinetic constants were typically less than 30% that of the mean.

Figure 5 graphically illustrates the concentration dependence of oxime reactivation of sarin (***A***)-, VX (***B***)-, cyclosarin (***C***)-, paraoxon (***D***)-, and tabun (***E***)-inhibited (conjugated) hAChE**.** Dependence for the lead oxime RS194B (*black circle*) and the initial lead RS41A (*open circle*) compared with reference uncharged (DAM (*gray diamond*) and MINA (*gray square*)) and cationic (2PAM (*gray square*) oximes (measured at 37 °C in 0.10 m phosphate buffer pH 7.4). See also Figure 36.

In the analysis we assume that K_{ox} mainly reflects initial reversible interaction of oxime reactivator with an OP-hAChE conjugate, whereas k_2 reflects the interaction of the reactivating oxime or oximate nucleophile with the phosphate moiety of the OP-conjugated hAChE in leading to the trigonal bipyramidal intermediate for reactivation. A pairwise comparison of six reactivators containing the identical handle and different methylene linkers reveals that trigonal bipyramidal intermediate for the oximate reaction prefers a shorter linker, whereas the initial reversible binding within the gorge appears frequently better for the propyl derivatives. Indeed, k_2 constants for the pairs RS194B *versus* RS194C, RS69N *versus* RS191E, and RS41A *versus* RS69L were frequently larger in respective ethyl derivatives, whereas K_{ox} constants appeared smaller in respective propyl derivatives, indicating stronger initial reversible interaction between an oxime and OP-hAChE. On the other hand, increases in the handle size from pyrrolidine to piperidine and azepane rings resulted primarily in smaller K_{ox} values, whereas k_2 constants remained largely unchanged. One could thus conclude that enhancement of reactivation rates observed for RS194B *versus* initial lead RS41A results primarily from improved molecular recognition while preserving ability to form productive trigonal

bipyramidal intermediate adducts. In further increasing size of handle from azepane to substituted tropane (RS194B to RS218A), a similar trend of lowering K_{ox} can be observed for paraoxon and sarin conjugates. However, due to simultaneous loss of reaction efficiency (reflected in reduced k_2 constants), the overall reactivation efficiency of RS218A was not greater than that of RS194B. Hence, RS194B oxime, an azepane analog of the initial lead RS41A, appears as a several fold better reactivator and significantly superior to the reference α-ketoximes, DAM and MINA, primarily due to enhanced molecular recognition of OP-hAChE conjugates.

pK$_a$ Determinations for Lead Oximes

The initial lead RS41A and the best uncharged reactivator RS194B include at least two groups ionizable in the physiologically relevant pH range, affecting their reactivation potencies. Deprotonation of the oxime group is assumed to be essential for the oxime nucleophilic reactivity, whereas protonation of the handle ring nitrogen (azepane or pyrrolidine) should improve molecular recognition of a largely aromatic and partly anionic active center gorge of OP-hAChE adducts. Our goal was, therefore, to determine pK_a values of both ionizable groups for both RS41A and RS194B oximes. Initially UV-visible spectroscopy was used to record change in UV spectra of both oximes in the pH range 1–13, see Figure 6. The increase in absorbance of both compounds at 270 nm results from deprotonation of the oxime hydroxyl, enabling us to determine a common pK_a value of 8.8 for both compounds, see Figure 12, Table 3).

Figure 6 graphically illustrates the pH dependence of UV spectra of 50 μm RS41A and pH dependence of $\boldsymbol{A_{270\ nm}}$ of 50 μM) RS41A (***B***), and RS194B along with corresponding p$\boldsymbol{K_a}$ values calculated by nonlinear regression using Equation 1 (***C***).

Table 3 (illustrated as Figure 12) describes a summary of p$\boldsymbol{K_a}$ values for lead oxime RS194B and other selected oximes determined by four different techniques from pH-dependent changes in oxime UV spectra (***cf***. Figure 6), oxime ^{1}H NMR spectra in D_2O (***cf***. Figure 7), oxime-induced ATCh oximolysis (***cf***. supplemental Fig. S3), and oxime-induced VX Flu-MP oximolysis (***cf***. supplemental Fig. S4).

Figure 7 illustrates the pH dependence of ^{1}H NMR spectra of 2.0 m**m** RS194B in D_2O buffers (***A***, ***B***, and ***C***) along with corresponding p$\boldsymbol{K_a}$ values calculated from the observed pH-induced difference in chemical shifts (***D***, ***E***, and ***F***) by nonlinear regression using Equation 1. NMR signals in panels *A*, *B*, and *C* were normalized relative to the

maximal peak height in the given chemical shift region. Spectra were aligned using a benzene external standard singlet at 7.16 ppm.

Ionization states of the lead reactivator RS194B and the reference oxime 2PAM were studied in more detail using ^{1}H NMR spectrometry in D_2O medium (supplemental Figs. S1 (Figure 22) and S2 (Figure 23) and Fig. 7). Figure 22, or Figure S1, illustrates the ^{1}H NMR spectra of 50.0 mm RS194B in D_2O buffer at pH 6.0; Fig. 22A illustrates the integration-based peak assignment, an peak integrals are indicated; Fig. 22B illustrates confirmation of peak assignment by DQF-COSY spectrum (at a 1.5 to 4.0 ppm expansion) of the same solution; spectra were taken in 20 mM D_2O phosphate-pyrophosphate buffer pH 6.0 containing 100 mM NaCl.

We focused on position and appearance of three peaks in the RS194B spectrum (supplemental Fig. S1, or Figure 22), the amido H peak present at approximately 7.65 ppm at pH 7.0, and two triplets coming from protons surrounding the azepane nitrogen and appearing at approximately 3.37 ppm and approximately 3.73 ppm at pH 7.0. Peak assignments were confirmed using the double-quantum filtered COSY spectra (supplemental Fig. 1B, Figure 22B). Due to dissociation of the proton or deuteron, all three peaks shift to higher field at higher pH. The singlet at approximately 7.65 ppm shifts slightly to approximately 7.59 ppm at pH 10 (Fig. 7*A*). The triplet at approximately 3.38 ppm shifts slightly to approximately 3.28 ppm at pH 10 and becomes a complex multiplet (Fig. 7*B*), whereas the triplet at 3.73 ppm shifts to 3.68 ppm (Fig. 7*C*). Plotting the change of chemical shift in D_2O as a function of pH yields three pK_a values: 9.1 (equivalent to 9.1–0.5 = 8.6 in H_2O (see reference 27)) for loss of the imino hydrogen and pK_a values 9.3 (8.8 in H_2O) and 9.2 (8.7 in H_2O) for the azepane and pyrrolidine protons on the nitrogens (Fig. 7, *D*, *E*, and *F*; Table 3, Figure 12). Both ionizable groups should be largely protonated at the physiological pH of 7.4, thus rendering a cation dominant over the neutral, zwitterion, and anionic species.

In the ^{1}H NMR spectrum of 2PAM in D_2O, both the aldoxime CH peak and peaks of all aromatic protons shift simultaneously as a function of pH (supplemental Fig. S2, Figure 23). As expected, only the *N*-methyl peak at 2.8 ppm did not shift (data not shown). This indicates that the ionization state of the oxime group affects delocalization of aromatic system of 2PAM. This may influence the π-orbital interactions between the pyridinium ring and aromatic residues in the AChE gorge and consequently binding orientation of the reactivator. Analysis of pH-induced shifts of both the amido H peak

(8.70 ppm at pH 7.0; supplemental Fig. S2C) and the doublet of aromatic proton at position 6 (8.75 ppm at pH 7.0; supplemental Fig. S2D) reveal a D_2O pK_a value of 8.6 (8.1 in H_2O) for the oxime group ionization (supplemental Fig. S2, E and F; Table 3).

pH Dependence of Oxime Nucleophilicity

The rates of oxime-induced hydrolysis (oximolysis) of ATCh and Flu-MP analog of VX measured spectrophotometrically and spectrofluorometrically were considered to be a measure of nucleophilic reactivity of an oxime. The pH dependence of ATCh oximolysis by RS41A, RS194B, and 2PAM revealed respective pK_a values of 9.0, 9.0, and 8.0 (supplemental Fig. S3, Figure 24) and pK_a values of 8.9, 8.7, and 8.1 for VX Flu-MP oximolysis (supplemental Fig. S4, Figure 25), consistent with UV and ^{1}H NMR-based pK_a determinations (Table 3). In supplementary Fig. 3 (Fig. S3; or Figure 24), Reaction rates were measured spectrophotometrically at 22 ^{0}C in 20mM phosphate-pyrophosphate buffers (containing 100 mM NaCl); and resulting pKa values calculated by nonlinear fit using equation (1) are given in this figure and in Table 3. In supplementary Fig. 4 (Fig. S4; or Figure 25), reaction rates were measured spectrofluorometrically at 22 ^{0}C in 20mM phosphate-pyrophosphate buffers (containing 100 mM NaCl); resulting pK_a values calculated by nonlinear fit using equation (1) were: 8.9 ± 0.1 (for RS194B); 8.7 ± 0.1 (for RS41A) and 8.1 ± 0.2 (for 2PAM). As expected, maximal oxime nucleophilicity was higher in oximates with higher pK_a values. However, at pH 7.4, the oxime-oximate pK_a comes into play where 2-PAM would have a greater nucleophilic capacity because of the greater fraction of oximate species.

pH Dependence of Reactivation Kinetics for Lead Oximes

Reactivation of VX-hAChE conjugate by RS41A and RS194B oximes as well as by RS186B and 2PAM measured in 0.1 m phosphate buffers at pH 6.4, 7.4, and 8.4 reveals no systematic changes of the overall reaction rate (reflected in k_r constant) (Table 4), whereas the nucleophilic first order reactivation rate constant k_2 did show a small increase with pH for all oximes. Consistent with its lowest pK_a value, the increase in k_2 was largest for 2PAM. However, although molecular recognition of 2PAM, reflected in K_{ox} constant, increased at lower pH, it did not change appreciably for the other two oximes. Thus, the protonated forms of both RS194B and RS41A oximes that dominate in large ratios at pH 6.4 as well as pH 7.4 with amine pK_a values around 8.9 (Table 3) did not bind to VX-hAChE conjugate as well as cationic 2PAM. The presence of a

zwitterionic species at pH 8.4 (more than 50% of all forms for 2PAM and less than 50% for RS oximes) did not improve binding of RS oximes, whereas it slightly compromised binding of 2PAM (Table 4; Fig. 8).

Table 4 describes the Kinetic constants for reactivation of VX-hAChE conjugate by oximes RS194B, RS41A, RS186B, and reference oxime 2PAM determined in 0.1 m phosphate buffers pH 6.4, 7.4, and 8.4.

Figure 8 graphically illustrates concentration dependence of oxime reactivation of VX-inhibited hAChE by the lead oxime RS194B (***A***), initial lead oxime RS41A (***B***), and reference oxime 2PAM (***C***) and oxime RS186B (***D***) measured at pH 6. 4 (○), pH 7.4 (□), and pH 8.4 (△) at 37 °C in 0.10 m phosphate buffers.

Acute Oxime Toxicity and Oxime Treatment of OP-exposed Mice

The acute i.m. toxicity for mice of RS41A and in particular the lead reactivator RS194B was found to be relatively low (Table 5). Both oximes were less toxic than standard reference oxime 2PAM (LD_{50} = 106 mg/kg), whereas RS194B was similarly toxic as HI-6 (LD_{50} = 450 mg/kg) (data not shown), known as the least toxic standard oxime antidote.

Table 5 describes the therapy of OP-exposed mice with the exemplary compounds, the lead oximes, RS194B and RS41A and standard reference oxime 2PAM. Protective index is the ratio of OP LD_{50} for OP-exposed animals treated with oxime (+atropine) and for animals given OP alone (***cf.*** supplemental Tables S2–S6).

OP-exposed mice treated with 125 mg/kg of the exemplary compound RS194B (a dose roughly equivalent to 25% of its LD_{50}) recovered significantly better from OP exposure than mice treated with an equivalent dose of RS41A and notably better than animals treated with 2PAM, with paraoxon-exposed mice being an exception (Table 5). Treatment of VX-exposed mice with lower RS194B doses equivalent to 10% of its LD_{50} (50 mg/kg) and 5% of its LD_{50} (25 mg/kg) yielded significant animal protection comparable with or better than 25% LD_{50} dose treatment by 2PAM, emphasizing the unique therapeutic efficacy of the exemplary compound RS194B oxime (Table 6). Table 6 describes the combination of therapy and pretreatment of OP-exposed mice with the exemplary compound oxime RS194B at i.m. administered doses equivalent to 25, 10, or 5% its LD_{50} dose of 500 mg/kg (***cf.*** supplemental Tables S2–S6).

Protective index is the ratio of OP LD_{50} for OP-exposed animals treated with oxime and for animals given OP alone. Maximal dose of poison (MDP), a highest multiple of OP LD_{50} fully counteracted by the oxime, is given in parentheses. Oximes in therapy (but not in pretreatment) were administered together with atropine. ND, not determined.

Pretreatment of mice with RS194B i.m., either 5 or 15 min before VX exposure provided notable protection effects only at the highest oxime dose applied (Table 6). However, a combination of 15-min oxime pretreatment and post-VX exposure therapy with 125 mg/kg RS194B produced an exceptionally high protective index of 45 and ensured survival of all mice against VX dose of 31.8 multiples of its LD_{50} that equals 900 μg/kg of VX. Combining pretreatment with therapy enhanced RS194B protective indices, albeit to a smaller extent, also for paraoxon, soman, and tabun (Table 6).

Oral administration (p.o.) of RS194B oxime 15 min before OP, followed by an atropine i.m. injection 1 min after OP provided outsdanding protection of VX exposed mice reflected in very high protective index (PI=40; Figure 15A). Administering reference oxime 2PAM orally under equivalent conditions completely failed to protect mice exposed to VX (PI=1.1; Table 15A).

RS41A and RS194B Pharmacokinetics in Mice

Upon i.m. administration of a single dose of 80 mg/kg RS194B or 30 mg/kg RS41A, maximal concentrations determined in plasma were 10 and 27 μg/ml, respectively (equivalent to 54 and 125 μm) observed at the initial collection time point (Fig. 9; supplemental Table S1). The time needed for maximal plasma concentrations to decay by half was 11 min and 12 min for RS41A and RS194B, respectively. The decay kinetics over this interval likely reflects the distribution into tissue from the plasma as well as total body elimination. The distinctly non-first order elimination of the RS compounds from plasma is consistent with a multicompartmental analysis. Both compounds rapidly penetrated the blood-brain barrier. Maximal brain concentrations determined as 1.2 and 7.9 μg/ml (approximately 6.5 and approximately 37 μM), respectively, for RS41A and RS194B, reduced in half in 30 and 60 min. RS194B established between 15 and 40 min post-administration an apparent steady state concentration in brain, presumably due to the increased rate of brain accumulation, and plasma declines in concentration (supplemental Table S1).

Figure 9 describes the pharmacokinetics of RS194B (***circles***) and RS41A (***squares***) in mice. Brain (***gray lines***) and plasma (***black lines***) compound concentrations were determined at discrete time points upon single, 80 mg/kg (RS194B), or 30 mg/kg (RS41A) dose administered to mice i.m. Each point represents average of determinations from three mice. S.E. of determination are indicated by *error bars*. Times required for halving maximal compound concentrations in plasma and in brain are indicated by *dashed lines* for each of two oximes.

The resulting brain/plasma ratios at the t_{max} were thus significantly, 2–3-fold higher, for RS194B (0.30) than for RS41A (0.12). Brain levels of both oximes, in particular RS194B, at the final time point (180 min; Fig. 9) were higher than plasma concentrations, suggesting that elimination from this tissue lagged compared with systemic peripheral clearance.

Figure 16 graphically illustrates data showing the plasma kinetics in mice following intravenous, intramuscular and oral dosing. The important point here is the rapid oral absorption reaching peak plasma levels in 20 to 30 minutes. Decay is also rapid. PK profiles of RS194B (structure illustrated) concentration in plasma following various routes and doses of administration to the mouse.

Figure 17 illustrates data showing the bio-availability parameters. These are not very accurate; nevertheless they show oral absorption to be approximately 60% or better. Bioavailability of RS194B (F) estimated from pharmacokinetic (PK) parameters determined upon intravenous and oral administration to the mouse. The Lethal Dose in 50% of the mice. Because of amounts of material and solubility we didn't go to higher ranges. Toxicity of RS194B determined upon intramuscular administration to the mouse: $LD_{50} \geq 500$ mg/kg.

Figure 18 graphically illustrates data showing a loading and maintenance dose scheme. The loading dose is given intramuscularly and the maintenance doses are given orally (gastric lavage as is the case in the previous Figure 17). Plasma levels are measured before administration of the next maintenance dose. What this shows is that we can maintain a steady state concentration and would not see cumulative toxicity of the antidote. PK profiles of RS194B concentration in plasma and brain following loading oral dose of 220 mg/kg and subsequent (2hr.) maintenance oral doses of 110 mg/kg to the mouse. Steady-state is reached with repetitive dosing. No signs of toxicity over this time period were noted, justifying considerably higher RS194B doses.

Figure 19 illustrates data showing *in vitro* reactivation data for an antidote congener of RS194B, RS138B. Note that for the various organophosphates, it has comparable reactivation rates for both the phosphorate insecticides and the phosphonate nerve agents. A docking model of the compound within the active center gorge of acetylcholinesterase containing a conjugated organophosphate is shown. Kinetic constants for reactivation of paraoxon-, sarin- , cyclosarin- and VX-hAChE conjugates by RS2 138B, the principal uncharged lead oxime RS194B and reference oximes 2PAM, MINA and DAM. Reactivation rate constants k_2, K_{ox} and k_r were determined from the time dependence of OP-hAChE reactivation.

Figure 20 illustrates an *In silico* computational molecular model of reversible binding for RS138B to the VX-hAChE conjugate, characterized by K_{ox} . One of preferred oxime conformations obtained by the modeling. The oxime structure represented by Connolly solvent accessible surface is colored by charge distribution (red – negative and blue – positive charge). Connolly surface of AChE is cut in half to reveal interior of the active center gorge (orange). The opposing side of the surface inaccessible to solvent is colored gray. Residues of the AChE peripheral site were allowed flexibility together with oxime, during computations. The ethoxy methylphosphonylated conjugated active serine side chain is rendered as orange (P) red (O) and white (C) sticks. The face of tetrahedral P atom open to nucleophilic oxime attack is facing the viewer.

Figure 21 illustrates RS2 138B and RS194B. The *In vitro* reactivation potency of RS2 138B makes it the most promising structural analog of the lead reactivator RS194B. The *In vivo* therapeutic efficacy of RS2 138B in the VX exposed mouse is: the i.m. LD_{50} in mg/kg is 238. The protective index is 10.8 (range is 9.1 to 12.7).

Discussion

The data presented herein are based on our recent revelation that 2-hydroxyimino acetamidoalkyl amines, although devoid of a permanent cationic charge, can efficiently reactivate OP conjugated hAChE *in vitro* (see reference 1). Formation of protonation equilibria around two ionizable groups in those oxime structures, an oxime group and an additional amine group, results in coexistence of charged, zwitterionic, and uncharged reactivator species around physiological pH values. Although zwitterionic and cationic species have the best chance of productive interaction with OP-hAChE conjugates, the

uncharged species can be expected to cross the blood-brain barrier delivering reactivator into CNS.

Our starting point for optimization was the structure of initially recognized lead hydroxyiminoacetamido alkylamine reactivator, RS41A. Introduction of systematic structural modifications in both its aliphatic linker and heterocyclic handle led to oxime structures with several fold improved potency for *in vitro* reactivation of four OP-hAChE conjugates culminating with new lead oxime structure RS194B. Not only were we able to design a more efficient reactivator, but through detailed analysis of reactivation kinetics, we obtained insights into regulatory structural constraints imposed by varying geometries of OP-hAChE conjugates. Reversible binding of a reactivator leading to progressive reactivation is thus facilitated by propyl linker and a bridged azepane heterocycle, whereas optimal geometry of AChE-OP-oxime trigonal bipyramidal intermediate is achieved with ethyl linker. The greater *in vitro* reactivation rate of RS194B is, therefore, largely a reflection of its improved molecular recognition, *i.e.* better binding to OP-hAChE conjugates as indicated by smaller K_{ox} constants while maintaining similar k_2 constants, in comparison to RS41A. This effectively means that improved oxime·OP-hAChE interactions in the reversible complex were also preserved in the subsequent reaction trigonal bipyramidal intermediate. Computational molecular modeling of two reaction steps for RS41A and RS194B oximes consistently reveals more pronounced similarity in geometries of reversible complex and trigonal bipyramidal intermediate for RS194B oxime and not with RS41A oxime (Fig. 10). Additionally, modeling suggests that the main anchoring point of both RS41A and RS194B oximes in interaction with VX-hAChE is the aromatic amino acid cluster of the AChE peripheral site.

Figure 10 illustrates an image of a computational molecular modeling of VX-inhibited AChE showing the reversible Michaelis type complex (***white sticks***) and covalent pentacoordinate trigonal bipyramidal intermediate (***yellow sticks***) for interaction of initial lead oxime RS41A (***A***) and the lead oxime RS194B **(*B*).** Ten conformers of each oxime are shown in each of two interaction states. The phosphorus atom is colored *purple*. The solvent-accessible part of the hAChE Connolly surface is represented in *orange*, and the solvent-inaccessible part of the hAChE molecule is in *dark gray*. Pronounced overlapping similarity in global geometries of the reversible complex (*white sticks*) and trigonal bipyramidal intermediate (*yellow sticks*) was observed for RS194B oxime, but not with RS41A oxime.

Analysis of ionization states of RS194B by UV spectroscopy, ^{1}H NMR spectrometry, and pH dependence of oximolysis reveals that both oxime group and amino group in the reactivator handle have pK_a constants in the range between 8.6 and 9.0. At physiological pH 7.4, therefore, more than 90% of the compound exists in the protonated, cationic form. That seems to be reflected in reasonable *in vitro* reactivation kinetics where the initial reversible binding is improved for RS194B *versus* RS41A (lower K_{ox}) while maintaining respectable reactivity (a small decrease in k_2). A low fraction of oximate is thus counteracted by high nucleophilicity (supplemental Fig. S3) and preferred for CNS reactivators due to lower ionization of the oximate facilitating blood brain barrier penetration.

Despite the suggestion that reactivators with lower oxime pK_a may be intrinsically more reactive at physiological pH in the absence of enzyme (Fig. 11 and, see Ref. 26), their overall reactivation potency expressed by constant k_r was not found to correlate closely with the oximate pK_a (in the wide pK_a range 6.5–10.1) for reactivation of any of OP-hAChE conjugates analyzed individually (supplemental Fig. S5) or as an average (Fig. 11). The linear increase of the maximal rate of reactivation log k_2 with the increase in pK_a, observed for five studied reactivators (supplemental Fig. S6E), however, reveals a greater dependence of reactivator nucleophilic strength for reactivity of AChE-OP-oxime trigonal bipyramidal intermediate when compared with fractional availability of oximate anion at physiological pH determined by the oximate pK_a. This observation may indicate an effective proton extraction mechanism for oxime reactions in the hAChE active center gorge. On the other hand domination of the protonated amine at pH 7.4 influences the capacity of RS194B to enter the CNS. Nevertheless, pharmacokinetic studies in mice indicate that both RS194B and RS41A oximes penetrate CNS quickly and can be found there at up to 37 and 6.5 μm concentrations, which are ~12–30% of corresponding plasma concentrations.

Figure 11 graphically illustrates the free energy relationships between nucleophilic reactivities and oxime group ionization states of selected oxime reactivators. Rate constants (k) of maximal pH-dependent ATCh oximolysis (*black line* and *circles*), oximolysis at pH 7.4 (*gray line* and *circles*), and an average overall rate constant k_r ($m^{-1}min^{-1}$) for oxime reactivation of VX, sarin, cyclosarin, and paraoxon inhibited hAChE (*white diamonds*) in relation to pK_a values were determined for reactivator oxime groups. The lead reactivator RS194B data is indicated by cross-haired symbols. k_r for

RS174C and RS150D (not extensively studied in this series) were extrapolated from reactivation rates determined at single (0.67 mm) oxime concentration.

Very good antidotal actions of RS194B in treatment of OP exposed mice relative to RS41A or 2PAM treatments undoubtedly result from its improved intrinsic reactivation potency combined with its lower toxicity and superior pharmacokinetic profile including faster CNS penetration and longer retention. However, a favorable antidotal action of RS194B was noticeable even at doses equivalent to 5 and 10% its LD_{50} value. RS194B was most efficient in therapy of VX, sarin, and paraoxon exposed mice, where it was superior to both RS41A and to a standard reactivator 2PAM (except for paraoxon intoxication). Although RS194B, when given solely as a pretreatment modality, produced limited prophylactic protection to subsequent OP exposure, consistent with its relatively low binding affinity for AChE conjugates (large K_{ox} values), the combination of pretreatment and therapy at the highest RS194B dose resulted in outstanding antidotal efficiency reflected in the protective index of 45.

Oral therapeutic efficacy of RS194B proved to be outstandingly consistent with excellent bioavailability of this compound determined in PK experiments. This critical observation allowed for the design of entirely new, previously unreported classes of oxime reactivator antidotes of the invention, all suitable to treat very large numbers of OP exposed populations in a pronounced non-invasive and cost-effective manner.

Several classes of novel compounds have been recently suggested as promising centrally active reactivators of OP-exposed hAChE. Two non-quaternary pyridine aldoxime phenyltetrahydroisoquinoline derivatives directed to interact with the AChE peripheral site showed outstanding potency for *in vitro* reactivation of VX and tabun-conjugated hAChE (see reference 2). Thus, uncharged, extended ligands with high affinities for the peripheral site form an alternative means of directing nucleophiles to the organophosphate conjugated active center (see reference 28). The overall second order rate constant (k_r) for reactivation of VX-hAChE conjugate by phenyltetra-hydroisoquinoline derivatives was an order of magnitude larger than the corresponding RS194B constant despite nearly an order of magnitude smaller maximal reactivation rate constant (k_2) and largely due to their high apparent affinity for VX-hAChE conjugate (K_{ox} = 6–47 μm). However, these compounds lack pharmacokinetic, toxicity, and efficacy analyses.

A similar series of α-ketoaldoxime derivatives of polycyclic peripheral site-directed ligands also exhibited initial promise in reactivation of VX and sarin-conjugated hAChE, whereas tabun-hAChE conjugate reactivation was less efficient (see reference 3). Finally, several amidine-based oximes indicated positive *in vitro* and *in vivo* initial properties for reactivation of hAChE inhibited by charged or less volatile nerve agent analogues (see reference 4). Although *in vitro* reactivation approached 2PAM reactivation levels, *in vivo* efficacy for treatment of nerve agent-exposed mice is difficult to assess as low toxicity nerve agent surrogates were used as toxicants.

In summary, this study presents uniquely comprehensive characterization of a novel series of *N*-substituted 2-hydroxyimino acetamido alkylamine reactivators of nerve agent-conjugated hAChE. Through systematic steps of structural modifications, we refined the initial lead RS41A into a superior RS194B reactivator of VX-, sarin-, paraoxon-, cyclosarin-, and tabun-conjugated hAChE both *in vitro* and *in vivo*. Cyclosarin has yet to be tested *in vivo*. Outstanding intrinsic reactivation potencies of this oxime, resulting in part from its favorable interaction with the peripheral site of AChE, in combination with its low toxicity results provide the lead for a pan-reactive, orally effective antidote for the treatment of OP-exposed mice, either post-exposure or in combination of prophylactic and antidotal oxime treatments of OP-exposed animals.

In one study, VX exposed mice were treated with the exemplary RS194B, or 2PAM, with intramuscular (i.m.) or oral (p.o). oxime administration. Protective Index is the ratio of LD_{50} values of the organophosphate determined in the presence and in the absence of an oxime. Atropine was always administered i.m. 1 min after VX. These data (summarized in table, below) show true superiority for RS 194B over 2-PAM as an oral antidote or protectant agent. The additional efficacy seen here arises from the superior oral bioavailability of RS194B from the oral route. Oral dosing should also be considered as a maintenance dose to provide longer term antidote action. The volatile organophosphate agents will deposit in lipid reservoirs and slowly leach from those sites. Hence, prolonged protection is warranted after an initial long term exposure.

The following table shows the reactivation data graphically illustrated in Figure 5, divided by the lethal dose (LD50) of the respective antidote, giving *Antidote Indices* (reactivation rate/LD50). This shows the superiority of RS194B over 2-PAM, RS41A and the other potential antidotes:

oxime	Protective Index	
	oxime + atropine 1 min after VX i.m.	oxime : 15 min before VX p.o. atropine: 1 min after VX i.m.
RS194B		
125 mg/kg **i.m.**	**18**	**-**
200 mg/kg **p.o.**	**-**	**40**
2PAM		
26 mg/kg **i.m.**	**9.3**	**-**
42 mg/kg **p.o.**	**-**	**1.1**

Oxime doses were set as 25% of individual oxime LD_{50} values for i.m. administration and were increased 1.6-fold for oral administration.

The abbreviations used are: OP, organophosphate; AChE, acetylcholinesterase; hAChE, human hAChE; ATCh, acetylthiocholine; 2PAM, 2-pyridinealdoxime methiodide; MINA, monoisonitrosoacetone; DAM, 2,3-butanedione monoxime; PI, protective index; Flu-MP, fluorescent methylphosphonates; VX, *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate.

Synthetic procedures

All reactions were performed with commercially available ACS grade reagents and solvents. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer. All chemical shifts were reported in ppm relative to solvent resonances, as indicated (DMSO-d_6 δ2.49, ^{1}H; δ39.49, ^{13}C), ($CDCl_3$ δ7.26, ^{1}H; δ77.0, ^{13}C). ^{1}H NMR coupling constants (J) are given in Hz. The following compounds were synthesized by known literature methods; 2-(azepan-1-yl)ethanamine, 3-(azepan-1-yl)propan-1-amine and 2-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octan-6-yl)ethanamine (Vooturi et al., 2010), **RS41A** (Sit et al., 2011) and **RS186B** (Sato et al., 1988). All other primary amines were obtained commercially.

General method for the preparation of acetamido oximes RS194B, RS194C, RS69N, RS218A, RS69L, and RS191E

The desired amine was added to solution of ethyl glyoxylate oxime **2** in ethanol

with stirring. The solution was stirred at 50 °C overnight and cooled to room temperature. The resulting precipitate was filtered, washed with cold ethanol and dried under vacuum.

2-(Hydroxyimino)-*N*-(2-(azepan-1-yl)ethyl)acetamide (RS194B). Prepared according to the general method. Ethyl glyoxylate oxime **2** (1 g, 8.5 mmol), 2-(azepan-1-yl)ethanamine (1.3 g, 9.1 mmol), ethanol (14 mL). White solid, yield (1.1 g, 61 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.90 (s, 1H), 7.88 (s, 1H), 7.42 (s, 1H), 3.20 (q, J = 6.8 Hz, 2H), 2.61-2.58 (m, 4H), 2.54 (t, J = 6.8, 2H), 1.53 (app s, 8H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 161.6, 143.7, 56.0, 54.6, 36.7, 27.9, 26.6; LCMS (ESI) (*m/z*): $[M+H]^+$ calculated for $C_{10}H_{19}N_3O_2$, 214.3; found, 214.5.

2-(Hydroxyimino)-*N*-(3-(azepan-1-yl)propyl)acetamide (RS194C). Prepared according to the general method. Ethyl glyoxylate oxime **2** (0.15 g, 1.3 mmol), 3-(azepan-1-yl)propan-1-amine (0.23 g, 1.5 mmol), ethanol (1.3 mL). White solid, yield (0.21 g, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.86 (s, 1H), 8.17 (s, 1H), 7.41 (s, 1H), 3.16 (q, J = 8.0 Hz, 2H), 2.52-2.48 (m, 2H), 2.42 (t, J = 8.0 Hz, 2H), 1.61-1.48 (m, 12H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 161.6, 143.8, 55.4, 54.9, 37.2, 27.8, 27.0, 26.5; LCMS (ESI) (*m/z*): $[M+H]^+$ calculated for $C_{11}H_{21}N_3O_2$, 228.3; found, 228.5.

2-(Hydroxyimino)-*N*-(2-(piperidin-1-yl)ethyl)acetamide (RS69N). Prepared according to the general method. Ethyl glyoxylate oxime **2** (0.15 g, 1.3 mmol), 2-(piperidin-1-yl)ethanamine (0.19 g, 1.5 mmol), ethanol (1.3 mL). White solid, yield (0.17 g, 66%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H), 7.91 (s, 1H), 7.42 (s, 1H), 3.24 (q, J = 8, 2H), 2.37-2.29 (m, 6H), 1.50-1.41 (m, 4H), 1.36 (app d, J = 4, 2H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 161.6, 143.7, 57.3, 54.0, 35.9, 25.6, 24.0; LCMS (ESI) (*m/z*): $[M+Na]^+$ calculated for $C_9H_{17}N_3O_2$, 216.2; found, 216.3.

2-(hydroxyimino)-*N*-(2-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octan-6-yl)ethyl) acetamide (RS218A). Prepared according to the general method. Ethyl glyoxylate oxime **2** (0.20 g, 1.7 mmol), 2-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octan-6-yl)ethanamine (0.40 g, 2 mmol), ethanol (4 mL). White solid, yield (0.27 g, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.86 (s, 1H), 7.80 (s, 1H), 7.39 (s, 1H), 3.14 (app s, 2H), 3.04 (s, 1H), 2.86 (d, J = 8, 1H), 2.66 (app s, 1H), 2.57-2.51 (m, 1H), 2.08 (d, J = 8, 1H), 1.49 (app s, 2H), 1.32 (d, J = 12, 2H), 1.18 (s, 3H), 1.15-1.03 (m, 2H), 0.99 (s, 3H), 0.84 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 161.6, 143.6, 64.7, 61.8, 56.5, 51.5, 44.9, 41.2, 40.9, 40.1, 38.3,

36.5, 31.8, 29.8, 25.7; LCMS (ESI) (*m/z*): $[M+H]^+$ calculated for $C_{14}H_{25}N_3O_2$, 268.4; found, 268.5.

2-(hydroxyimino)-*N*-(3-(pyrrolidin-1-yl)propyl)acetamide (RS69L). Prepared according to the general method. Ethyl glyoxylate oxime **2** (0.17 g, 1.4 mmol), 3-(pyrrolidin-1-yl)propan-1-amine (0.22 g, 1.7 mmol), ethanol (2.5 mL). White solid, yield (0.15 g, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.88 (s, 1H), 8.20 (s, 1H), 7.41 (s, 1H), 3.17 (q, J = 6.8 Hz, 2H), 2.41-2.35 (m, 6H), 1.68-.61 (m, 4H), 1.58 (t, J = 6.8 Hz, 2H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 161.6, 143.8, 54.9, 53.6, 37.3, 28.1, 23.1; LCMS (ESI) (*m/z*): $[M+H]^+$ calculated for $C_9H_{17}N_3O_2$, 200.2; found, 200.4.

2-(hydroxyimino)-*N*-(3-(piperidin-1-yl)propyl)acetamide (RS191E). Prepared according to the general method. Ethyl glyoxylate oxime **2** (0.15 g, 1.3 mmol), 3-(piperidin-1-yl)propan-1-amine (0.22 g, 1.5 mmol), ethanol (5 mL). White solid, yield (0.15 g, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 8.22 (s, 1H), 7.40 (s, 1H), 3.16 (q, J = 8 Hz, 2H), 2.27-2.23 (m, 6H), 1.58 (pent, J = 8 Hz, 2H), 1.48 (pent, J = 8 Hz, 4H), 1.36 (app d, J = 4, 2H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 161.6, 143.7, 56.6, 54.1, 37.5, 26.0, 25.6, 24.2; LCMS (ESI) (*m/z*): $[M+H]^+$ calculated for $C_{10}H_{19}N_3O_2$, 214.3; found, 214.5.

References

(1) Vooturi, S. K.; Firestine, S. M. *J. Comb. Chem.* **2010**, *12* (1), 151–160; (2) Sit, R.K.; Radić, Z.; Gerardi, V.; Zhang L.; Garcia, E.; Katalinić, M; Kovarik, Z.; Fokin, V.V.; Sharpless, K. B.; and Taylor, P. *J. Biol. Chem.***2011**, 286, 19422- 19430; (3) Sato, N.; Saito, N. *J. Heterocycl. Chem.* **1988**, *25* (6), 1737-1740.

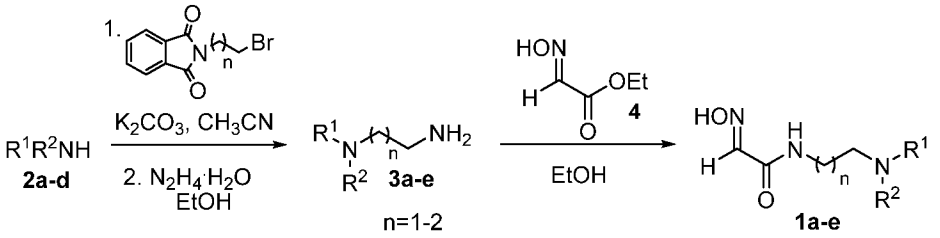
Further synthetic schemes

Our previous studies have indicated that the tertiary amine containing region of the oxime molecule is a critical modulator of activity. Herein we explored the effect of the tertiary amine size on the rate of reactivation towards organophosphate inhibited acetylcholinesterase. Uncharged reactivators demonstrate the ability to cross the blood brain barrier and are a logical step towards improving existing treatments for organophosphate poisoning. Building on our lead compound, **RS194B**, we demonstrate here 10 additional derivatives (**1a-k**), as illustrated in Figure 35.

By use of previous protocol, *N*-substituted 2-hydroxyiminoacetamides **1a-e** were synthesized, as shown in scheme 1. The synthesis begins by reacting the pthalimide

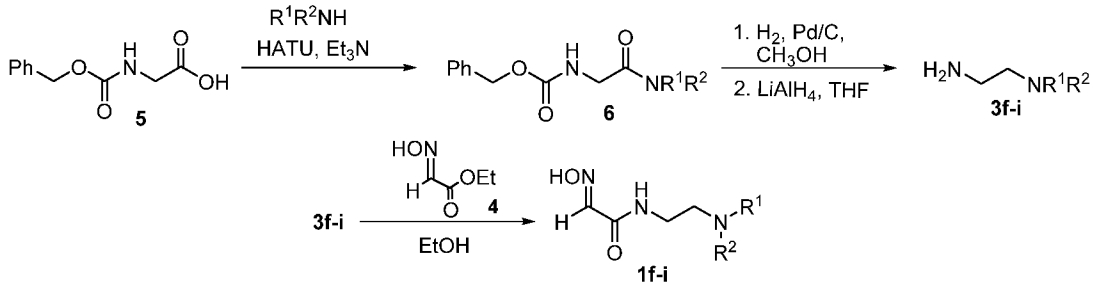
protected amino alkyl bromide with the desired secondary amine (**2a-d**) to yield the protected intermediates. In the next step, deprotection of the intermediate using hydrazine reveals the desired amine (**3a-e**) in moderate yield, and followed by subsequent amidation with ethyl glyoxylate oxime **4** delivers oxime derivatives **1a-e**.

Scheme 1



Furthermore, to obtain oxime derivatives **1f-i** the corresponding primary amines **3f-i** were first synthesized following a different route, shown in scheme 2. In the first step the acid chloride reacts with glycine under Schotten–Baumann conditions to form N-protected glycine **5**. Next, carboxamidation of **5** with desired secondary amines (**2e-h**) was done to obtain carboxamide **6**. Removal of Cbz group of **6**, and followed by reduction with $LiAlH_4$ in THF gave primary amines **3f-i**. Amidation of ethyl glyoxylate oxime **4** with corresponding primary amines provided acetamide oxime derivatives **1f-i**.

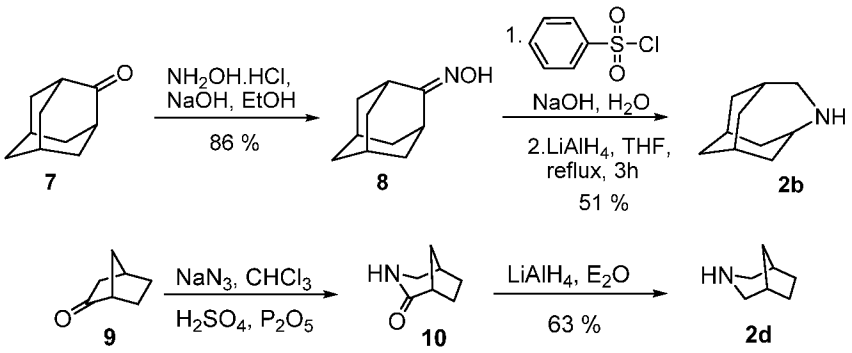
Scheme 2



The synthesis of secondary amine 4-azatricyclo[4.3.1. $1.^{3,8}$]undecan **2b** was straightforward and involved an initial condensation of 2-adamantanone **7** with hydroxylamine to afford adamantan-2-oxime, followed by Beckmann rearrangement to form the 4-azatricyclo[4.3.1. $1.^{3,8}$]-undecan-5-one (**8**) (scheme 3). Reduction of 4-azatricyclo amide derivative **8** furnished 4-azatricyclo [4.3.1. $1.^{3,8}$]undecane **2b**. Similarly, the secondary amine 1,4-thiazepane **2a** was also prepared following the same synthetic route. Further, the bicylic amine **2d** was made from starting material norcamphor **9** in two steps. 3-Azabicyclo[3.2.1]octan-2-one (**10**) was obtained by

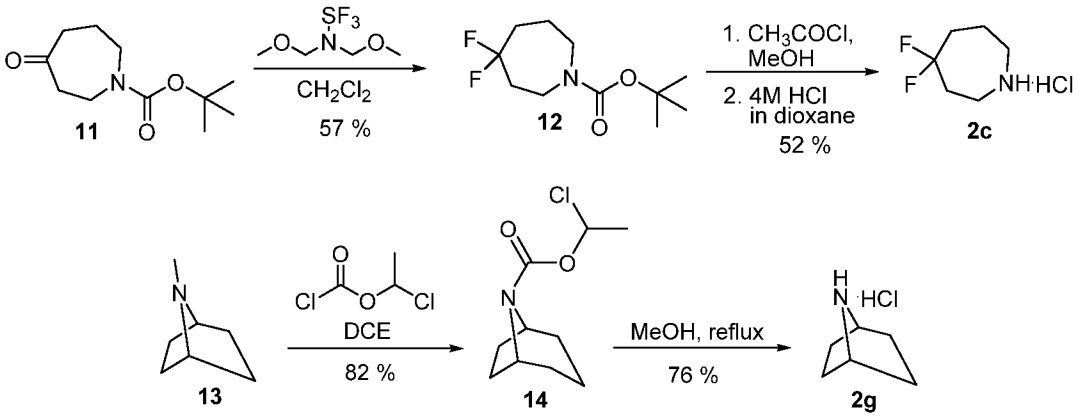
carrying out curtius rearrangement on **9**. Finally, reduction with $LiAlH_4$ in Et_2O delivered 3-azabicyclo[3.2.1]octane in moderate yield **2d**.

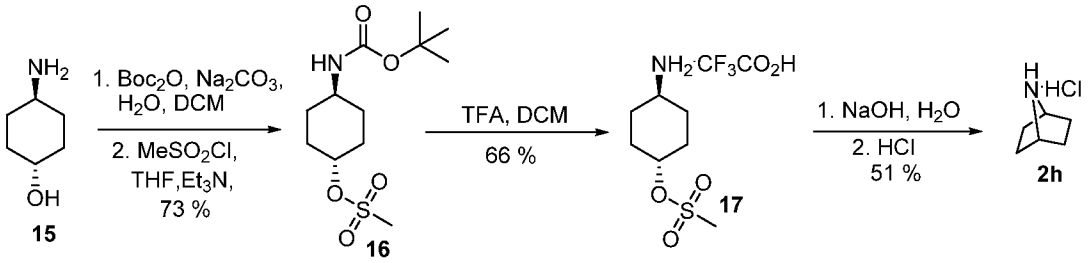
Scheme 3



It was decided to incorporate fluorine in the azepane ring at position **4** in **RS194B**, scheme 4, to achieve a modest p*K*a reduction of the cycylic amine and thus N-(2-(4,4-difluoroazepan-1-yl)ethyl)-2-(hydroxyimino)acetamide **1c** was synthesized. Difluoride **2c** was obtained from **11** following treatment with aminosulfurane and subsequent Boc deprotection. To synthesize oxime derivative **1h**, bicyclic amine 8-azabicyclo[3.2.1]octane hydrochloride **2g** was obtained via chloroethyl chloroformate mediated dealkylation of the tertiary amine **13**. Further, trans-4-aminocyclohexanol **15** was protected using Boc-anhydride and then mesylation was done to obtain Boc-protected cyclohexyl methanesulfonate **16**. In the next step, Boc group was removed under acidic condition and the TFA salt of cyclohexyl methanesulfonate was cyclized under basic condition to deliver 7-azabicyclo[2.2.1] heptanes **2h** in moderate yield.

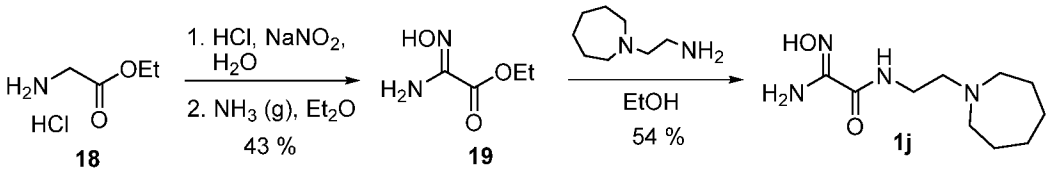
Scheme 4





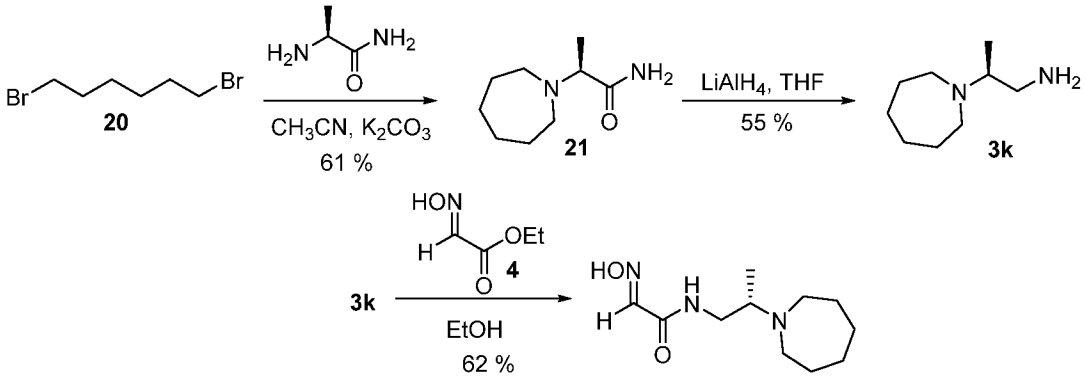
After successful synthesis of oximes **1a–i**, modification of vicinity of the nucleophilic oxime was made and the oxime derivative **1j** was prepared. Glycine ester hydrochloride **18** was converted into 2-chloro-2 (hydroxyimino)acetate, followed by treatment with NH_3 (g) afforded 2-amino-2-(hydroxyimino)acetate **19**. Amidation of **19** with 2-(azepan-1-yl)ethan-1-amine delivered acetamide oxime **1j**.

Scheme 5



To investigate the effect of introducing methyl group in the linker, the oxime derivative **1k** was prepared. The primary amine **3k** was first synthesized in two steps. The appropriate tertiary amine carboxamide **21** was prepared from the readily available L-alaninamide by reaction with 1,6-dibromohexane under basic conditions to construct the azepane ring. Reduction of carboxamide **21** with $LiAlH_4$ in THF afforded **3k** and subsequent amidation with ethyl glyoxylate oxime **4** delivered acetamide oxime **1k**.

Scheme 6

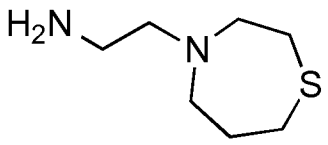


General Synthetic Protocols

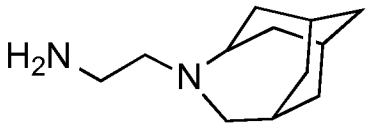
All reactions were performed with commercially available ACS grade reagents and solvents. Anhydrous *N*,*N*-dimethylfomamide (DMF), acetonitrile and chloroform, dichloromethane were used as received without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer. All chemical shifts were reported in ppm relative to solvent resonances, as indicated (DMSO-d_6 δ2.50, ^{1}H; δ39.50, ^{13}C), ($CDCl_3$ δ7.26, ^{1}H; δ77.16, ^{13}C). ^{1}H NMR coupling constants (J) are given in Hz.

A. General method for the preparation of primary amines (3a-e). A 15 mmol solution of secondary amine (**2a–e**) in 80 mL of acetonitrile was treated with 18.75 mmol of *N*-bromopthalimide and K_2CO_3 (45.0 mmol). The resulting mixture was refluxed overnight. After the reaction was completed, 90 mL of saturated $NaHCO_3$ was added followed by extraction with ethyl acetate. The combined organic layers were acidified with 2 N HCl and washed with water. The pH of the aqueous layer was adjusted to pH 12 using 4 N NaOH and then extracted with methylene chloride. The organic solution was dried over Na_2SO_4 and evaporated to yield the product, which is used in the next reaction without further purification.

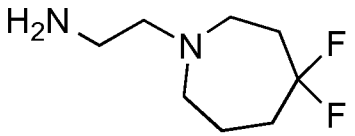
The *N*-alkylated pthalimide obtained above was dissolved in 80 mL of ethanol and hydrazine hydrate (3 equiv) was added. The reaction was refluxed for 3 h and cooled to room temperature, and the resulting precipitate was removed by filtration. The filtrate was concentrated and the residue was diluted with 30 mL of EtOAc. The resulting precipitate was removed by filtration, the filtrate was concentrated to dryness and kugelrohr distillation was done to obtain pure amine.



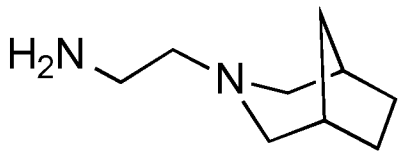
2-(1,4-thiazepan-4-yl)ethanamine (3a). Prepared according to the general method A. Clear oil, yield 1.1 g (46 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.92 (t, J = 5.6 Hz, 2H), 2.85 (t, J = 5.6 Hz, 2H), 2.75-2.64 (m, 6H), 2.60 (t, J = 5.7 Hz, 2H), 1.87 (pent, J = 5.8 Hz, 2H), 1.59 (s, 2H). ^{13}C NMR (400 MHz, DMSO-d6) δ 59.6, 58.8, 53.7, 39.9, 34.4, 32.1, 31.1.



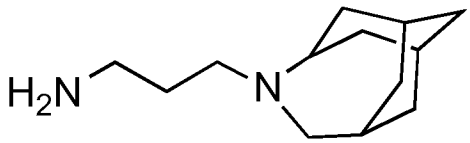
2-(4-Azatricyclo[4.3.1.1^{3,8}]undecan-4-yl)ethanamine (3b). Prepared according to the general method A. Clear oil, yield 1.7 g (58 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.92 (t, J = 5.4 Hz, 1H), 2.76 (d, J = 3.8 Hz, 2H), 2.64 (dd, J = 9.1, 4.5 Hz, 4H), 2.04 (s, 2H), 1.86-1.76 (m, 4H), 1.75-1.40 (m, 9H). ^{13}C NMR (400 MHz, DMSO-d6) δ 60.0, 55.8, 54.8, 40.9, 32.8, 31.3, 29.0, 26.4, 17.3, 16.8.



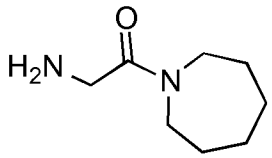
2-(4,4-Difluoroazepan-1-yl)ethanamine (3c). Prepared according to the general method A. Light yellow oil, yield 1.3 g (48 %).



2-(3-Azabicyclo[3.2.1]octan-3-yl)ethanamine (3d). Prepared according to the general method A. Clear oil, yield 1.4 g (59 %)

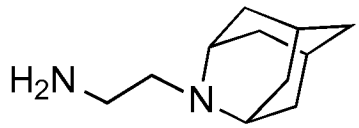


3-(4-Azatricyclo[4.3.1.13^{,8}]undecan-4-yl)propan-1-amine (3e). Prepared according to the general method A. Clear oil, yield 1.6 g (51 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.99 (s, 1H), 2.77 (d, J = 3.8 Hz, 2H), 2.72 (t, J = 6.7 Hz, 2H), 2.61 (t, J = 7.1 Hz, 2H), 2.05 (s, 1H), 1.90-1.75 (m, 6H), 1.69-1.63 (m, 3H), 1.62-1.52 (m, 5H), 1.49-1.45 (m, 2H).



2-Amino-1-(azepan-1-yl)ethanone (3f). To a stirred solution of ((benzyloxy)carbonyl)glycine (1 g, 4.78 mmol) in 35 mL of dichloromethane at rt, HATU (2 g, 5.3 mmol) and DIPEA (1.8 g, 14.3 mmol) were added. The reaction mixture was stirred for 10 min, and then azepane (1 g, 10.5 mmol) was added, and stirred for another 3 h. The resulting reaction mixture was filtered and the filtrate was concentrated. The obtained residue was purified by flash column chromatography (EtOAc/hexane, 8:2) to obtain 1.1 g (79 %) of benzyl(2-(azepan-1-yl)-2-oxoethyl)carbamate as a clear oil.

Benzyl(2-(azepan-1-yl)-2-oxoethyl)carbamate (1 g, 3.4 mmol) was dissolved in 40 mL of MeOH, degassed and placed under argon. 10% Pd/C (0.25 g) was added, and the contents were thoroughly degassed, placed under hydrogen via balloon and stirred overnight. The resulting reaction mixture was filtered through Celite, and the Celite pad was subsequently washed with DCM and MeOH. The resulting filtrate was concentrated in vacuo and kugelrohr distillation was done to afford 0.50 g (94 %) of the title compound as clear oil. ^{1}H NMR (400 MHz, CDCl3) δ 3.50 (t, J = 6.0 Hz 2H), 3.41 (s, 2H), 3.30 (t, J = 6.0 Hz, 2H), 1.78 (s, 2H), 1.72-1.63 (m, 4H), 1.51 (dd, J = 5.9, 3.0 Hz, 4H). ^{13}C NMR (400 MHz, DMSO-d6) δ 172.2, 46.4, 46.2, 43.1, 28.9, 27.6, 27.2, 26.9.

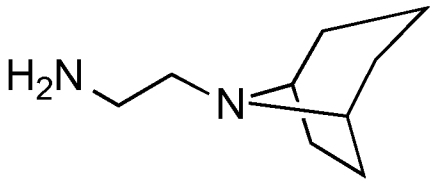


2-(Azaadamantan-2-yl)ethanamine (3g). To a stirred solution of ((benzyloxy)carbonyl)glycine (0.70, 3.3 mmol) in 25 mL of dichloromethane at rt, HATU (1.40 g, 3.6 mmol) and DIPEA (0.90 g, 6.9 mmol) were added. The reaction mixture was stirred for 10 min, and then 2-azaadamantane (0.54 g, 4 mmol) was added, and stirred for another 3 h. The resulting reaction mixture was filtered and the filtrate was concentrated. The obtained residue was purified by flash column chromatography (EtOAc/hexane, 7:3) to obtain 0.90 g (83 %) of benzyl(2-(2-azaadamantane-2-yl)-2-oxoethyl)carbamate as a clear oil.

Benzyl(2-(2-azaadamantane-2-yl)-2-oxoethyl)carbamate (0.85 g, 2.6 mmol)

was dissolved in 25 mL of MeOH, degassed and placed under argon. 10% Pd/C (0.25 g) was added, and the contents were thoroughly degassed, placed under hydrogen via balloon and stirred overnight. The resulting reaction mixture was filtered through Celite, and the Celite pad was subsequently washed with DCM and MeOH. The resulting filtrate was concentrated in vacuo to deliver 1-(2-azaadamantane-2-yl)-2-aminoethan-1-one (0.43 g, 85 %).

To a stirred suspension of LAH (0.24 g, 9.7 mmol) in THF (20 mL) at 0 °C was added dropwise to a solution of 1-(2-azaadamantane-2-yl)-2-aminoethan-1-one (0.40 g, 2.1 mmol) in 20 mL of THF. The reaction mixture was stirred overnight at rt. The resulting reaction mixture was quenched with careful addition of H_2O (0.30 mL), followed by 15% aqeous NaOH (0.60 mL), and H_2O (0.90 mL). This crude mixture was filtered through a small pad of Celite, and the filter cake was washed with ether (10 mL). The filtrate was concentrated and kugelrohr distillation was done to afford 0.29 g (76 %) of the title compound as a clear oil. ^{1}H NMR (400 MHz, cdcl$_3$) δ 2.75 (s, 2H), 2.71-2.63 (m, 4H), 2.03-1.95 (m, 6H), 1.78 (s, 2H), 1.72 (s, 2H), 1.53 (s, 2H), 1.50 (s, 2H). ^{13}C NMR (400 MHz, DMSO-d6) δ 55.9, 51.8, 40.2, 37.3, 33.5, 27.5.

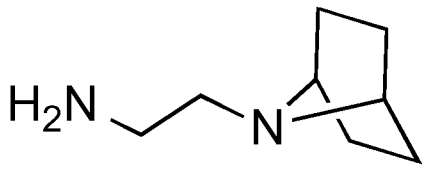


2-(8-Azabicyclo[3.2.1]octan-8-yl)ethanamine (3h). To a stirred solution of ((benzyloxy)carbonyl)glycine (0.80, 3.8 mmol) in 25 mL of dichloromethane at rt, HATU (1.60 g, 4.2 mmol) and DIPEA (1.5 g, 11.6 mmol) were added. The reaction mixture was stirred for 10 min, and then 8-azabicyclo[3.2.1]octane hydrochloride (1.1 g, 7.4 mmol) was added, and stirred for another 3 h. The resulting reaction mixture was filtered and the filtrate was concentrated. The obtained residue was purified by flash column chromatography (EtOAc/hexane, 8:2) to obtain 1.1 g (95 %) of benzyl(2-(8-azabicyclo[3.2.1]heptan-7-yl)-2-oxoethyl)carbamate as a clear oil.

Benzyl(2-(8-azabicyclo[3.2.1]heptan-7-yl)-2-oxoethyl)carbamate (1.1 g, 3.6 mmol) was dissolved in 40 mL of MeOH, degassed and placed under argon. 10% Pd/C (0.28 g) was added, and the contents were thoroughly degassed, placed under hydrogen via balloon and stirred overnight. The resulting reaction mixture was filtered through

Celite, and the Celite pad was subsequently washed with DCM and MeOH. The resulting filtrate was concentrated in vacuo to deliver 2-amino-1-(8-azabicyclo[3.2.1]octan-8-yl)ethan-1-one (0.59 g, 97 %).

To a stirred suspension of LAH (0.40 g, 10.5 mmol) in THF (20 mL) at 0 °C was added dropwise to a solution of 2-amino-1-(8-azabicyclo[3.2.1]octan-8-yl)ethan-1-one (0.59 g, 3.5 mmol) in 20 mL of THF. The reaction mixture was stirred overnight at rt. The resulting reaction mixture was quenched with careful addition of H_2O (0.40 mL), followed by 15% aqeous NaOH (0.80 mL), and H_2O (1.3 mL). This crude mixture was filtered through a small pad of Celite, and the filter cake was washed with ether (10 mL). The filtrate was concentrated and kugelrohr distillation was done to afford 0.35 g (65 %) of the title compound as a clear oil. ^{1}H NMR (400 MHz, cdcl$_3$) δ 3.09 (s, 2H), 2.69 (t, J = 6.3 Hz, 2H), 2.34 (t, J = 6.3 Hz, 2H), 1.91-1.82 (m, 2H), 1.78 (s, 2H), 1.72-1.57 (m, 2H), 1.54-1.47 (m, 2H), 1.45-1.35 (m, 2H), 1.29 (d, J = 12.9 Hz, 2H). ^{13}C NMR (400 MHz, DMSO-d6) δ 60.0, 55.8, 54.8, 40.9, 32.8, 31.3, 29.0, 26.4, 17.3, 16.8.

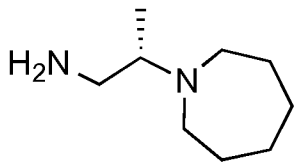


2-(7-Azabicyclo[2.2.1]heptan-7-yl)ethanamine (3i). To a stirred solution of ((benzyloxy)carbonyl)glycine (0.80, 3.8 mmol) in 25 mL of dichloromethane at rt, HATU (1.60 g, 4.2 mmol) and DIPEA (1.5 g, 11.6 mmol) were added. The reaction mixture was stirred for 10 min, and then 7-azabicyclo[2.2.1]heptane hydrochloride (0.76 g, 5.7 mmol) was added, and stirred for another 3 h. The resulting reaction mixture was filtered and the filtrate was concentrated. The obtained residue was purified by flash column chromatography (EtOAc/hexane, 8:2) to obtain 1 g (91 %) of benzyl(2-(7-azabicyclo[2.2.1]heptan-7-yl)-2-oxoethyl)carbamate as a clear oil.

Benzyl(2-(7-azabicyclo[2.2.1]heptan-7-yl)-2-oxoethyl)carbamate (1 g, 3.5 mmol) was dissolved in 40 mL of MeOH, degassed and placed under argon. 10% Pd/C (0.26 g) was added, and the contents were thoroughly degassed, placed under hydrogen via balloon and stirred overnight. The resulting reaction mixture was filtered through Celite, and the Celite pad was subsequently washed with DCM and MeOH. The resulting

filtrate was concentrated in vacuo to deliver 2-amino-1-(7-azabicyclo[2.2.1]heptan-7-yl)ethan-1-one (0.50 g, 93 %).

To a stirred suspension of LAH (0.37 g, 9.7 mmol) in THF (20 mL) at 0 °C was added dropwise to a solution of 2-amino-1-(7-azabicyclo[2.2.1]heptan-7-yl)ethan-1-one (0.50 g, 3.2 mmol) in 20 mL of THF. The reaction mixture was stirred overnight at rt. The resulting reaction mixture was quenched with careful addition of H_2O (0.40 mL), followed by 15% aqeous NaOH (0.80 mL), and H_2O (1.3 mL). This crude mixture was filtered through a small pad of Celite, and the filter cake was washed with ether (10 mL). The filtrate was concentrated and kugelrohr distillation was done to afford 0.31 g (69 %) of the title compound as clear oil. ^{13}C NMR (400 MHz, DMSO-d6) δ 61.6, 56.4, 56.1, 40.9, 37.6, 36.1, 35.7, 32.2, 31.7, 26.7.



(*S*)-2-(Azepan-1-yl)propan-1-amine (3k). 1,5-Dibromopentane (3.0 g, 12.3 mmol mmol) was added to a suspension of L-alaninamide (1.5 g, 12 mmol), potassium carbonate (5 g, 36 mmol), and potassium iodide (6 mg) in CH_3CN (36 mL). The mixture was refluxed overnight and then cooled to rt. The mixture was treated with 75 mL of 1N HCl and extracted with dichloromethane. The organic layer was discarded. The aqueous layer was basified with 4N NaOH and extracted with dichloromethane (3x30 mL). The solvent was evaporated to dryness to obtain 1.50 g (73%) of (S)-2-(azepan-1-yl)propanamide.

To a stirred suspension of LAH (1.5 g, 41.1 mmol) in THF (80 mL) at 0 °C was added dropwise a solution of (S)-2-(azepan-1-yl)propanamide (1.4 g, 8.2 mmol) in 40 mL of THF. The reaction mixture was stirred overnight at 50 °C and cooled to rt. The resulting reaction mixture was quenched with careful addition of H_2O (1.5 mL), followed by 15% aqeous NaOH (3 mL), and H_2O (5 mL). A thick gel-like precipitate was formed. The reaction mixture was filtered through a small pad of Celite, and the filter cake was washed with ether (50 mL). The filtrate was concentrated and kugelrohr distillation was done to afford 0.90 g (66 %) of the title compound as a clear oil.

^{1}H NMR (400 MHz, cdcl$_3$) δ 2.68-2.56 (m, 3H), 2.56-2.47 (m, 1H), 2.44-2.35 (m, 3H), 1.66-1.43 (m, 10H), 0.81 (d, J = 6.3 Hz, 3H).^{13}C NMR (400 MHz, DMSO-d6) δ 62.8, 50.7, 45.5, 29.9, 26.9, 11.4.

B. General method for the preparation of acetamide oximes 1a-k. The desired primary amine (3a-j) was added to a solution of ethyl glyoxylate oxime 4 in ethanol with stirring. The solution was stirred overnight at room temperature. The resulting precipitate was filtered, washed with cold ethanol and dried under vacuum.

***N*-(2-(4-Azatricyclo[4.3.1.1^{3,8}]undecan-4-yl)ethyl)-2-(hydroxyimino)acetamide (RS2-138B)** Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.75 g, 6.41 mmol), 2-(4-azatricyclo[4.3.1.1^{3,8}]undecan-4-yl)ethanamine (1.4 g, 7.0 mmol), ethanol (10 mL). White solid, yield (1 g, 60 %). ^{1}H NMR (400 MHz, dmso) δ 11.91 (s, 1H), 7.87 (s, 1H), 7.42 (s, 1H), 3.19 (dd, J = 12.8, 6.4 Hz, 2H), 2.95 (d, J = 5.1 Hz, 1H), 2.77 (d, J = 3.8 Hz, 2H), 2.66 (t, J = 6.8 Hz, 2H), 2.04 (s, 1H), 1.82 (d, J = 10.7 Hz, 4H), 1.69 (d, J = 13.6 Hz, 2H), 1.59-1.40 (m, 6H).^{13}C NMR (400 MHz, DMSO-d6) δ 161.6, 143.7, 60.5, 56.8, 55.9, 37.3, 37.0, 35.7, 35.4.

***N*-(2-(2-Azaadamantan-2-yl)ethyl)-2-(hydroxyimino)acetamide (RS3-36D)** Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.15 g, 1.3 mmol), 2-(2-azaadamantan-2-yl)ethanamine (0.25 g, 1.4 mmol), ethanol (4 mL). White solid, yield (0.26 g, 79 %). ^{1}H NMR (400 MHz, dmso) δ 11.92 (s, 1H), 7.86 (s, 1H), 7.42 (s, 1H), 3.15 (dd, J = 12.8, 6.5 Hz, 2H), 2.75 (s, 2H), 2.68 (t, J = 6.9 Hz, 2H), 1.93 (d, J = 6.9 Hz, 6H), 1.75 (s, 2H), 1.50 (d, J = 10.8 Hz, 4H).^{13}C NMR (400 MHz, DMSO-d6) δ 161.6, 143.7, 51.7, 50.9, 37.3, 36.6, 32.9, 26.8.

(*S*)-*N*-(2-(Azepan-1-yl)propyl)-2-(hydroxyimino)acetamide (RS2-245C) Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.50 g, 4.27 mmol), (*S*)-2-(azepan-1-yl)propan-1-amine (0.77 g, 4.91 mmol), ethanol (7 mL). White solid, yield (0.60 g, 62 %). ^{1}H NMR (400 MHz, dmso) δ 11.95 (s, 1H), 7.77 (s, 1H), 7.42 (s, 1H), 3.20-3.00(m, 2H), 2.83 (sext, J = 7.1 Hz, 1H), 2.67-2.61 (m, 2H), 2.52 (s, 2H), 1.52 (s, 8H), 0.88 (s, 3H). ^{13}C NMR (400 MHz, DMSO-d6) δ 161.5, 143.7, 58.7, 50.2, 41.5, 39.5, 29.2, 26.4, 12.3.

***N*-(2-(Azepan-1-yl)-2-oxoethyl)-2-(hydroxyimino)acetamide (RS2-90C)** Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.30 g, 2.56 mmol), 2-amino-1-(azepan-1-yl)ethanone (0.46 g, 2.94 mmol), ethanol (6 mL). White solid, yield (0.34 g, 58 %). ^{1}H NMR (400 MHz, DMSO-d6) ^{1}H NMR (400 MHz, dmso) δ

12.04 (s, 1H), 8.02 (s, 1H), 7.50 (s, 1H), 4.02 (d, J = 5.2 Hz, 1H), 3.41 (dd, J = 11.7, 5.7 Hz, 2H), 1.74-1.63 (m, 1H), 1.63-1.54 (m, 1H), 1.54-1.35 (m, 2H).^{13}C NMR (400 MHz, DMSO-d6) δ 167.2, 161.7, 143.3, 46.1, 45.4, 28.2, 27.1, 26.7, 26.2.

***N*-(2-(8-Azabicyclo[3.2.1]octan-8-yl)ethyl)-2-(hydroxyimino)acetamide (RS2-237D)** Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.17 g, 1.5 mmol), 2-(8-azabicyclo[3.2.1]octan-8-yl)ethanamine (0.25 g, 1.6 mmol), ethanol (5 mL). White solid, yield (0.24 g, 71 %). ^{1}H NMR (400 MHz, DMSO) δ 11.92 (s, 1H), 7.92 (s, 1H), 7.42 (s, 1H), 3.19 (q, J = 6.5 Hz, 2H), 3.11 (s, 2H), 2.36 (t, J = 6.9 Hz, 2H), 1.93 - 1.73 (m, 2H), 1.65-1.50 (m, 3H), 1.49 (d, J = 7.5 Hz, 2H), 1.39-1.19 (m, 3H). ^{13}C NMR (400 MHz, DMSO-d6) δ 161.6, 143.7, 59.2, 51.2, 37.8, 30.6, 26.0, 16.3.

***N*-(2-(7-Azabicyclo[2.2.1]heptan-7-yl)ethyl)-2-(hydroxyimino)acetamide (RS2-234D)** Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.23 g, 1.94 mmol), 2-(7-azabicyclo[2.2.1]heptan-7-yl)ethanamine (0.30 g, 2.14 mmol), ethanol (5 mL). White solid, yield (0.28 g, 68 %).

***N*-(2-(3-Azabicyclo[3.2.1]octan-3-yl)ethyl)-2-(hydroxyimino)acetamide (RS3-43D)** Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.35 g, 3.0 mmol), 2-(3-azabicyclo[3.2.1]octan-3-yl)ethanamine (0.53 g, 3.44 mmol), ethanol (7 mL). White solid, yield (0.41 g, 60 %). %). ^{1}H NMR (400 MHz, DMSO) δ 11.92 (s, 1H), 7.82 (s, 1H), 7.41 (s, 1H), 3.21 (d, J = 5.6 Hz, 2H), 2.61 (d, J = 8.5 Hz, 2H), 2.36 (d, J = 6.1 Hz, 2H), 2.07 (s, 2H), 2.00 (d, J = 9.9 Hz, 2H), 1.65-1.35 (m, 5H), 1.29 (d, J = 10.6 Hz, 1H).^{13}C NMR (400 MHz, DMSO-d6) δ 161.6, 143.7, 60.8, 59.5, 56.1, 37.2, 35.9, 34.6, 28.3.

***N*-(3-(4-Azatricyclo[4.3.1.1^{3,8}]undecan-4-yl)propyl)-2-(hydroxyimino)acetamide (RS2-150C)** Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.50 g, 4.27 mmol), 3-(4-azatricyclo[4.3.1.1^{3,8}]undecan-4-yl)propan-1-amine (1.0 g, 4.91 mmol), ethanol (9 mL). White solid, yield (0.70 g, 59 %). ^{1}H NMR (400 MHz, dmso) δ 11.84 (s, 1H), 8.18 (s, 1H), 7.41 (s, 1H), 3.17 (q, J = 6.6 Hz, 2H), 2.95 (s, 1H), 2.72 (d, J = 3.8 Hz, 2H), 2.55 (t, J = 6.8 Hz, 2H), 2.04 (s, 1H), 1.81 (s, 4H), 1.73-1.62 (m, 2H), 1.60-1.42 (m, 8H).^{13}C NMR (400 MHz, DMSO-d6) δ 161.6, 143.9, 60.8, 55.8, 55.6, 37.2, 37.1, 35.5, 30.9, 27.8, 26.1.

***N*-(2-(Azepan-1-yl)ethyl)-2-(hydroxyamino)-2-iminoacetamide (RS292A)** Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.30 g, 2.56 mmol), 2-(azepan-1-yl)ethanamine (0.42 g, 2.94 mmol), ethanol (6 mL). White solid,

yield (0.32 g, 54 %). ^{1}H NMR (400 MHz, dmso) δ 9.85 (s, 1H), 7.65 (s, 1H), 5.62 (s, 2H), 3.19 (q, J = 6.3 Hz, 2H), 2.61-2.57 (m, 2H), 2.53 (t, J = 6.6 Hz, 2H), 1.53 (s, 8H). ^{13}C NMR (400 MHz, DMSO-d6) δ 161.6, 143.7, 56.0, 54.6, 36.7, 28.0, 26.6.

***N*-(2-(4,4-Difluoroazepan-1-yl)ethyl)-2-(hydroxyimino)acetamide (RS2-140B)**

Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.30 g, 2.56 mmol), 2-(4,4-difluoroazepan-1-yl)ethanamine (0.52 g, 2.94 mmol), ethanol (6 mL). White solid, yield (0.37 g, 58 %).

***N*-(2-(1,4-Thiazepan-4-yl)ethyl)-2-(hydroxyimino)acetamide (RS2-148B)**

Prepared according to the general method B. Ethyl glyoxylate oxime 3 (0.35 g, 3 mmol), 2-(1,4-thiazepan-4-yl)ethanamine (0.55 g, 3.45 mmol), ethanol (7 mL). White solid, yield (0.44 g, 61 %). ^{1}H NMR (400 MHz, dmso) δ 11.88 (s, 1H), 7.93 (s, 1H), 7.42 (s, 1H), 3.20 (q, J = 6.3 Hz, 2H), 2.90 (t, J = 5.1 Hz, 2H), 2.85 (t, J = 5.9 Hz, 2H), 2.70 - 2.59 (m, 6H), 1.80 (pent, J = 5.9 Hz, 2H). ^{13}C NMR (400 MHz, DMSO-d6) δ 161.7, 143.7, 58.1, 54.6, 52.9, 36.8, 33.5, 31.0, 30.6.

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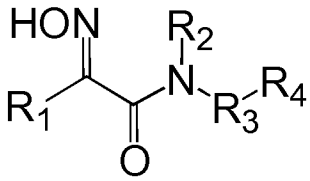
A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A compound having one of the following structures or compositions having one or more compounds of the following structures, or equivalents thereof, or a stereoisomer thereof, or an analog thereof, or a pharmaceutically acceptable salt thereof, or a bioisostere thereof; or

a composition comprising an isolated compound consisting essentially of, or consisting of:

(a) a compound having the formula:



wherein:

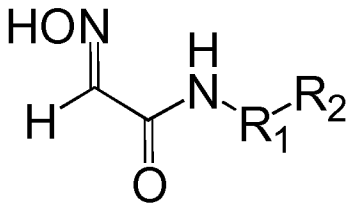
$\mathbf{R_1}$ is selected from the group consisting of: -H, -alkyl, and –aryl;

$\mathbf{R_2}$ is selected from the group consisting of: -H, -alkyl, and -aryl ;

$\mathbf{R_3}$ is selected from the group consisting of: $-(CH_2)_n-$, and $(CH_2)_n-CH(CH_3)-$, wherein **n** is the integer 0, 1, 2, 3, 4 or 5 ;

$\mathbf{R_4}$ is selected from the group consisting of : -H, -alkyl (wherein optionally the alkyl is selected from the group consisting of: -methyl, -ethyl, -propyl, -butyl, -i-propyl, and -i-butyl), -cycloalkyl (wherein optionally the cycloalkyl is selected from the group consisting of: -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclohexyl, -cycloheptyl, and -cyclooctyl), –aryl (wherein optionally the aryl is selected from the group consisting of: -phenyl, -naphthyl, -thienyl, and -indolyl), -saturated and nonsaturated heterocyclics (wherein optionally the saturated or nonsaturated heterocyclic is selected from the group consisting of: -aziridine, -oxirane- thiirane, -azirine, -oxirene, -thiirene, -azetidine, -oxetane, -thietane, -azete, -oxete, -thiete, -pyrrolidine, -oxolane, -thiolane, -pyrrole, -furan, -thiophene, -piperidine, -oxane, -thiane, -pyridine, -pyran, -thiopyran, -azepane, -oxepane, -thiepane, -azepine, -oxepine, -thiepine, -azocane, and -azocine), and bridged compounds (wherein optionally the bridged compound is selected from the group consisting of: -adamantanes, -amantadines, -biperidenes, -memantines, -methenamines, -rimantadines, -norbornanes, and -triazoles);

(b) a compound having the formula:

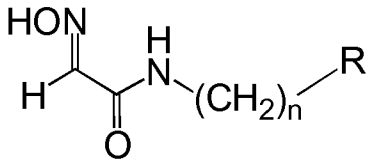


wherein

$\mathbf{R_1}$ is selected from the group consisting of: $-(CH_2)_n-$, and $(CH_2)_n-CH(CH_3)-$, wherein **n** is the integer 0, 1, 2, 3, 4 or 5;

$\mathbf{R_2}$ is selected from the group consisting of: -H, -alkyl (wherein optionally the alkyl is selected from the group consisting of: -methyl, -ethyl, -propyl, -butyl, -i-propyl, and -i-butyl), -cycloalkyl (wherein optionally the cycloalkyl is selected from the group consisting of: -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclohexyl, -cycloheptyl, and -cyclooctyl), –aryl (wherein optionally the aryl is selected from the group consisting of: -phenyl, -naphthyl, -thienyl, and -indolyl), -saturated and nonsaturated heterocyclics (wherein optionally the saturated or nonsaturated heterocyclic is selected from the group consisting of: -aziridine, -oxirane- thiirane, -azirine, -oxirene, -thiirene, -azetidine, -oxetane, -thietane, -azete, -oxete, -thiete, -pyrrolidine, -oxolane, -thiolane, -pyrrole, -furan, -thiophene, -piperidine, -oxane, -thiane, -pyridine, -pyran, -thiopyran, -azepane, -oxepane, -thiepane, -azepine, -oxepine, -thiepine, -azocane, and -azocine), and bridged compounds (wherein optionally the bridged compound is selected from the group consisting of: -adamantanes, -amantadines, -biperidenes, -memantines, -methenamines, -rimantadines, -norbornanes, and -triazoles);

(c) a compound having the formula:



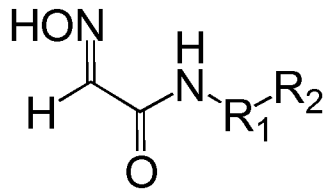
wherein

N is the integer 0, 1, 2, 3, 4 or 5 ;

R is selected from the group consisting of: -H, -alkyl (wherein optionally the alkyl is selected from the group consisting of: -methyl, -ethyl, -propyl, -butyl, -i-propyl, and -i-butyl), -cycloalkyl (wherein optionally the cycloalkyl is selected from the group consisting of: -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclohexyl, -cycloheptyl, and -

cyclooctyl), –aryl (wherein optionally the aryl is selected from the group consisting of: -phenyl, -naphthyl, -thienyl, and -indolyl), -saturated and nonsaturated heterocyclics (wherein optionally the saturated or nonsaturated heterocyclic is selected from the group consisting of: -aziridine, -oxirane- thiirane, -azirine, -oxirene, -thiirene, -azetidine, -oxetane, -thietane, -azete, -oxete, -thiete, -pyrrolidine, -oxolane, -thiolane, -pyrrole, -furan, -thiophene, -piperidine, -oxane, -thiane, -pyridine, -pyran, -thiopyran, -azepane, -oxepane, -thiepane, -azepine, -oxepine, -thiepine, -azocane, and -azocine), and bridged compounds (wherein optionally the bridged compound is selected from the group consisting of: -adamantanes, -amantadines, -biperidenes, -memantines, -methenamines, -rimantadines, -norbornanes, and -triazoles);

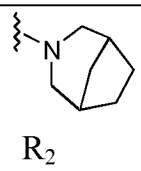
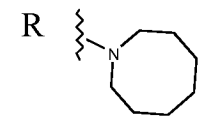
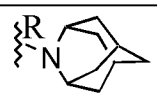
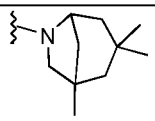
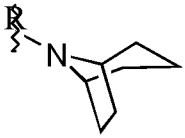
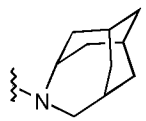
(d) a compound having the formula:

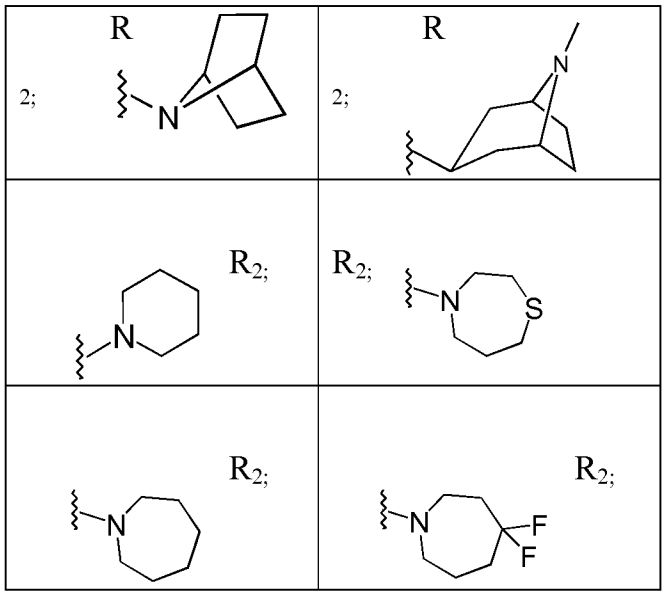


wherein

$\mathbf{R_1}$ is selected from the group consisting of: $-(CH_2)_n-$, and $(CH_2)_n-CH(CH_3)-$, wherein **N** is the integer 0, 1, 2, 3, 4 or 5;

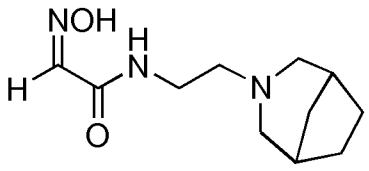
$\mathbf{R_2}$ is selected from the group consisting of:

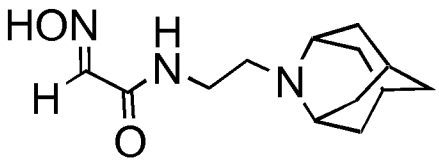


and any combination thereof);

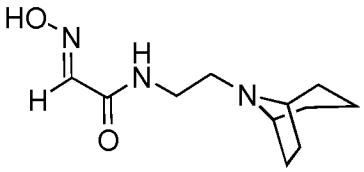
(e) a compound having the formula:



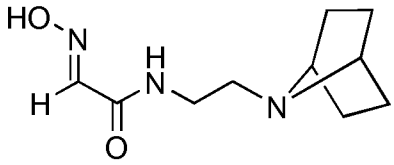
(RS3-43D);



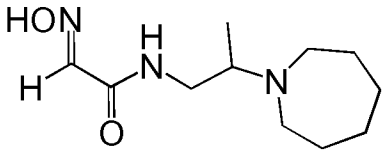
(RS3-36D);



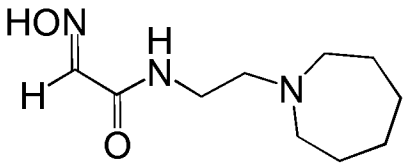
(RS2-237D)



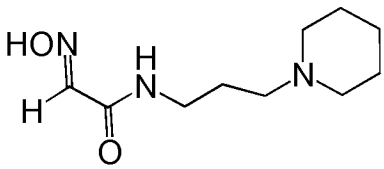
(RS2-234D);



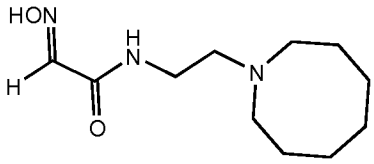
(RS2-245C);



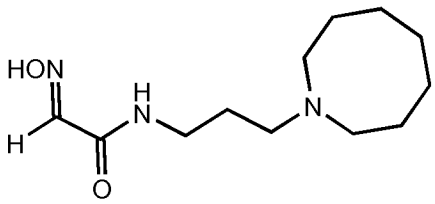
(RS194B) ;



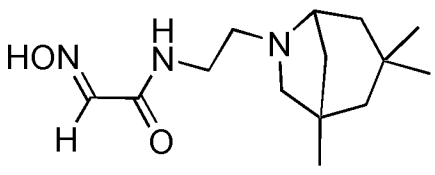
(RS191E) ;



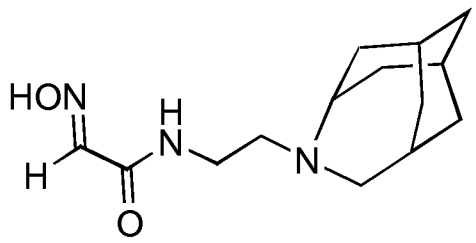
(RS251A) ;



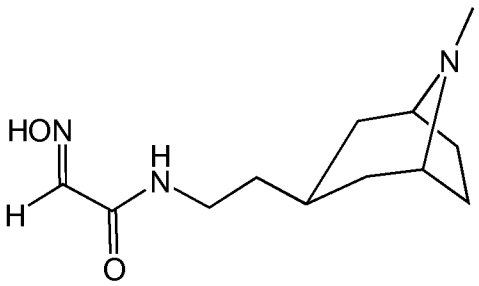
(RS251B) ;



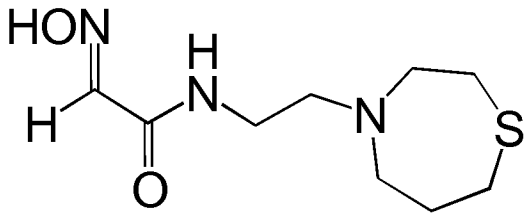
(RS218A) ;



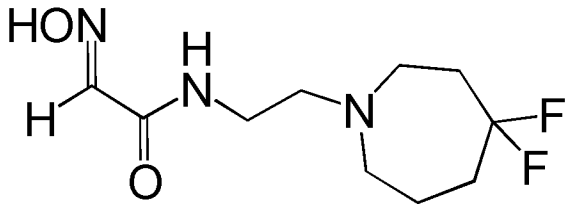
(RS2-138B) ;



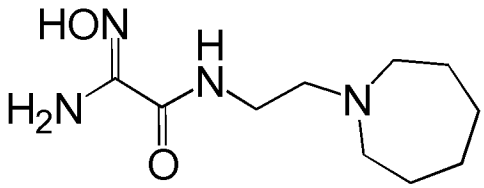
(RS2-57B) ;



(RS2-148B) ;



(RS2-140B) ;



(RS292A); and

a combination thereof;

(f) compound having a structure as set forth in Table 1 (Figure 3) to Table 2 (Figure 4), or equivalents thereof, or pharmaceutically acceptable salt thereof,

wherein optionally the compound is RS2 138B, RS194B or RS191E;

wherein optionally the compound is RS251A, RS251B, RS218A, or RS2–57B; or

wherein optionally the compound is RS2-148B, RS2-140B, RS3-43D, RS3-36D, RS2-237D, RS2-234D or RS2-245C (Figure 4A); or

(g) any combination of any of the compounds of (a) to (f).

2. A formulation comprising a compound or composition as set forth in claim 1, wherein optionally the formulation is a solid, liquid, aerosol, powder or emulsion formulation.

3. A pharmaceutical composition comprising a compound or composition as set forth in claim 1, wherein optionally the pharmaceutical composition is formulated for enteral or parenteral administration.

4. A pump, a device, a subcutaneous infusion device, a continuous subcutaneous infusion device, an infusion pen, a needles, a reservoir, an ampoules, a vial, a syringe, a cartridge, a disposable pen or jet injector, a prefilled pen or a syringe or a cartridge, a cartridge or a disposable pen or jet injector, a two chambered or multi-chambered pump, a syringe, a cartridge or a pen or a jet injector, comprising a compound as set forth in claim 1, or a formulation as set forth in claim 2, or a pharmaceutical composition as set forth in claim 3.

5. A method for treating, ameliorating or protecting (preventing) an organophosphate toxicity or poisoning or toxic exposure, or for treating, ameliorating or protecting (preventing) organophosphate inhibition of an acetylcholinesterase (AChE), comprising:

administering to a patient or an individual in need thereof, a compound as set forth in claim 1, or a formulation as set forth in claim 2, or a pharmaceutical composition as set forth in claim 3, wherein optionally the compound or formulation is administered enterally or parenterally,

wherein optionally the compound or formulation is administered orally, parenterally, by inhalation spray, nasally, topically, intrathecally, intrathecally, intracerebrally, epidurally, intracranially or rectally, or

administering the compound as set forth in claim 1, or the formulation as set forth in claim 2, or the pharmaceutical composition as set forth in claim 3, using a pump, a device, a subcutaneous infusion device, a continuous subcutaneous infusion device, an infusion pen, a needles, a reservoir, an ampoules, a vial, a syringe, a cartridge, a disposable pen or jet injector, a prefilled pen or a syringe or a cartridge, a cartridge or a disposable pen or jet injector, a two chambered or multi-chambered pump, a syringe, a cartridge or a pen or a jet injector as set forth in claim 4.

6. The method of claim 5, wherein the organophosphate toxicity, poisoning or toxic exposure is caused by exposure of the patient or individual to an alkyl methylphosphonate or related nerve agent, or an alkylphosphorate insecticide,

and optionally the organophosphate (OP) is or is a component of a toxin, an herbicide, an insecticide, or a nerve gas or nerve agent,

and optionally the organophosphate (OP) is or comprises a parathion, a malathion, a methyl parathion, a chlorpyrifos, a diazinon, a dichlorvos, a phosmet, a fenitrothion, a tetrachlorvinphos, an azamethiphos or an azinphos methyl, or the nerve agent is a soman (*O*-Pinacolyl methylphosphonofluoridate), a tabun (Ethyl *N*,*N*Dimethyl-phosphoramido-cyanidate) or a sarin ((*RS*)-propan-2-yl methylphosphonofluoridate).

7. The method of claim 5, wherein the acetylcholinesterase (AChE) is in the central nerve system (CNS), or the acetylcholinesterase (AChE) is a human acetylcholinesterase (hAChE).

8. A method for treating, preventing or ameliorating excessive acetylcholine stimulation in the brain, comprising:

administering to a patient or an individual in need thereof, a compound as set forth in claim 1, or a formulation as set forth in claim 2, or a pharmaceutical composition as set forth in claim 3,

wherein optionally the compound or formulation is administered enterally or parenterally,

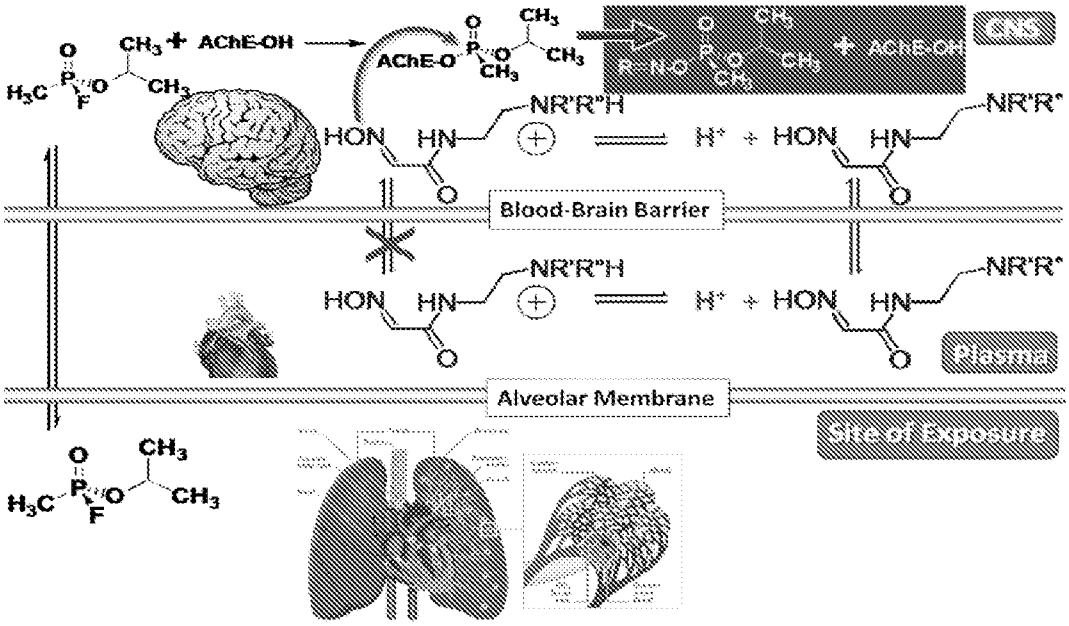
wherein optionally the compound or formulation is administered orally, parenterally, by inhalation spray, nasally, topically, intrathecally, intrathecally, intracerebrally, epidurally, intracranially or rectally, or

administering the compound as set forth in claim 1, or the formulation as set forth in claim 2, or a pharmaceutical composition as set forth in claim 3, using a pump, a device, a subcutaneous infusion device, a continuous subcutaneous infusion device, an infusion pen, a needles, a reservoir, an ampoules, a vial, a syringe, a cartridge, a disposable pen or jet injector, a prefilled pen or a syringe or a cartridge, a cartridge or a disposable pen or jet injector, a two chambered or multi-chambered pump, a syringe, a cartridge or a pen or a jet injector as set forth in claim 5.

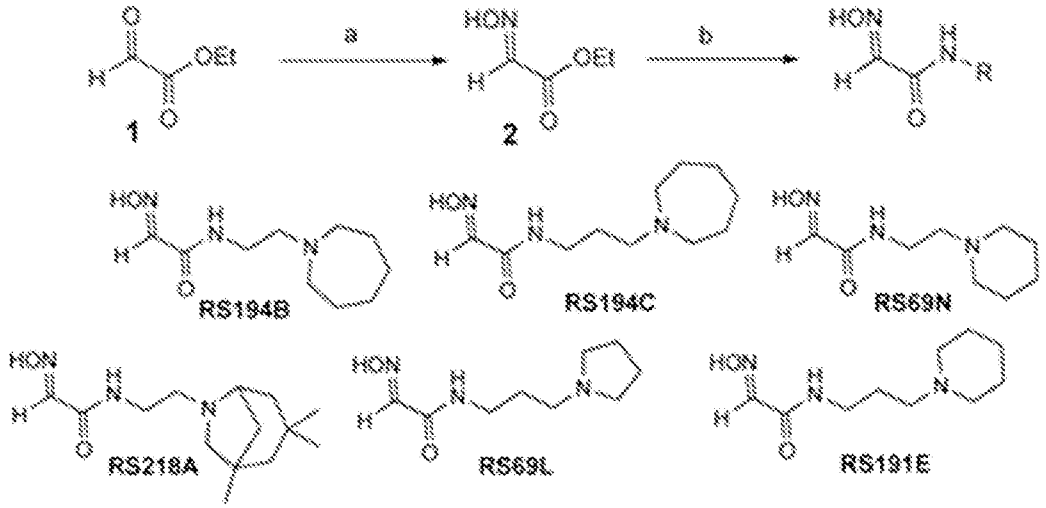
9. The method of claim 8, wherein the excessive acetylcholine stimulation in the brain, the CNS or the PNS is caused by a drug, a drug overdose, or a poisoning or a toxic exposure to a drug, and optionally the drug overdose causing the excessive acetylcholine stimulation is caused at least in part by physostigmine, neostigmine, pyridostigmine, diisopropylfluorophosphate and/or echothiophate.

Figure 1

5


AChE-OH
AChE-O
CNS
Blood-Brain Barrier
Plasma
Alveolar Membrane
Site of Exposure

Scheme 1 - Figure 2



Reagents: (a) $NH_2OH\cdot HCl$, Et_3N, CH_3CN, H_2O, *rt*; (b) RNH_2, C_2H_5OH, 50 °C

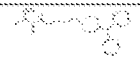
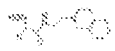
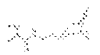
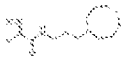
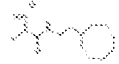
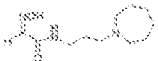
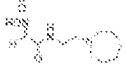
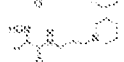
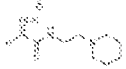
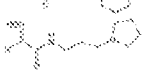
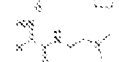
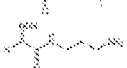
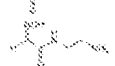
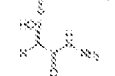
Radic Z et al. J. Biol. Chem. 2012;287:11796-11805

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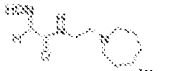
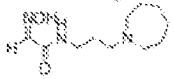
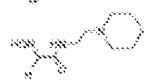
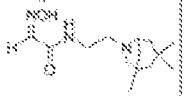
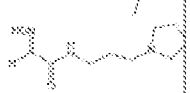
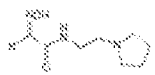
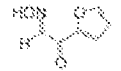
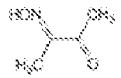
5

Table 1 - Figure 3

Oxime	Oxime structure	k_{obs} (relative to 2PAM)*					Oximolysis (d4/min)	% Oximate @ pH 7.4	% Neutral @ pH 7.4	R Volume (Å^3)	R Area (Å^2)
		Ave	POX	Sarin	CS	VX					
RS2-31B		1.1	0.24	0.85	2.4	0.95	0.099	0.25	4.4	299	324
RS189A		1.4	0.11	1.0	2.6	1.7	0.124	0.21	26	156	180
RS2-57B		0.16	0.084	0.11	0.19	0.26	0.181	0.23	0.17	161	173
RS218A		0.91	0.23	1.1	1.5	0.80	0.128	0.35	1.0	196	199
RS251B		0.82	0.34	1.0	1.2	0.74	0.074	0.13	0.31	173	188
RS251A		0.55	0.11	0.69	0.83	0.56	0.108	0.26	2.2	156	170
RS194C		0.58	0.37	0.88	0.39	0.68	0.120	0.30	0.49	157	174
RS194B		2.5	0.53	3.1	3.6	2.7	0.128	0.25	3.5	139	156
RS191E		2.0	0.39	1.6	3.5	2.7	0.126	0.30	1.4	140	161
RS69N		0.53	0.200	0.400	0.500	1.0	0.135	0.22	9.5	123	143
RS69L		0.55	0.16	0.80	0.24	1.0	0.119	0.30	1.0	124	148
RS41A		0.88	0.10	1.0	1.3	1.1	0.109	0.20	7.0	107	130
RS41B		0.26	0.073	0.21	0.20	0.56	0.127	0.21	7.0	83	107
RS160C		0.091	0.052	0.030	0.18	0.10	0.117	0.27	0.43	69	94
RS157B		0.010	0.010	0.010	0.010	0.010	0.078	0.41	1.7	52	76
RS157A		0.010	0.010	0.010	0.010	0.010	0.128	0.5	99.5	19	37

WO 2014/127315 PCT/US2014/016639

Table 2 - Figure 4

Oxime	Oxime structure	Paraoxon			Sarin			Cyclosarin			VX			Tabun		
		k_2	K_{oo}	k_p	k_2	K_{oo}	k_p	k_2	K_{oo}	k_p	k_2	K_{oo}	k_p	k_2	K_{oo}	k_p
RS194B		0.38	7.4	51	2.5	1.9	1300	0.88	3.9	230	3.1	2.1	16000	0.0018	1.8	1.0
RS194C		0.11	2.9	38	1.5	1.1	1400	0.90	4.3	210	1.9	2.4	790	n.d.	n.d.	n.d.
RS69N		> 0.7	>50	13	2.2	2.2	1000	1.4	9.4	150	2.7	3.0	900	n.d.	n.d.	n.d.
RS218A		0.16	2.2	73	0.84	0.83	1000	0.53	5.4	98	1.3	2.2	590	n.d.	n.d.	n.d.
RS69L		0.08	3.0	27	1.8	1.9	950	1.1	10	110	3.4	7.9	430	n.d.	n.d.	n.d.
RS41A		0.15	4.3	35	3.7	11	340	1.9	21	90	3.1	4.6	670	0.00082	3.6	0.22
RS191E		0.048	2.0	24	1.3	2.2	590	0.72	4.4	160	1.4	5.8	240	n.d.	n.d.	n.d.
RS186B		0.31	9.1	39	0.84	7.8	110	0.72	15	48	0.36	2.7	130	n.d.	n.d.	n.d.
MINA		> 0.20	>20	8.4	1.6	14	120	1.2	16	75	>0.7	>6.0	110	-	-	0.039
DAM		0.027	46	0.58	0.13	88	1.5	>0.30	>100	2.5	0.20	>100	1.7	-	-	0.0038
2PAM		0.27	1.8	150	1.1	0.34	3200	0.73	6.6	110	0.65	0.25	2600	0.0058	1.5	3.8

WO 2014/127315 PCT/US2014/016639

Figure 5

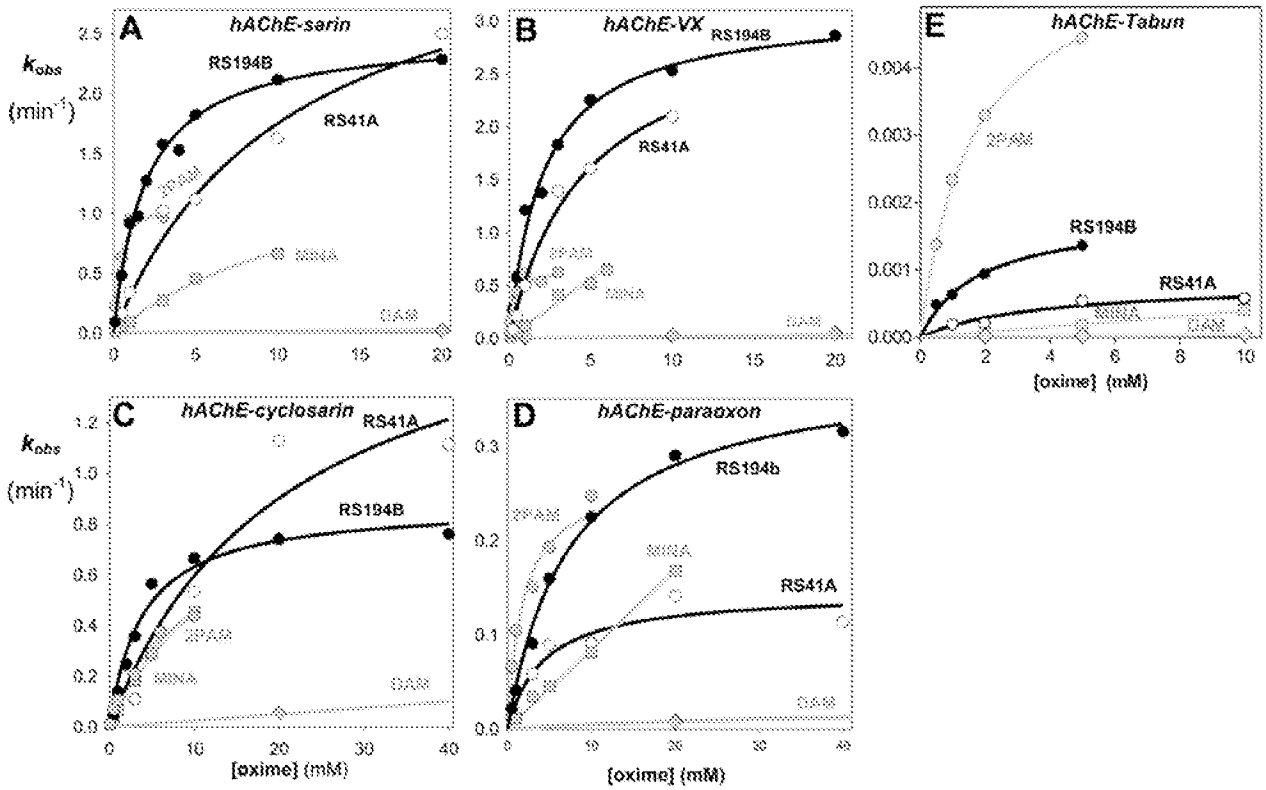

A
hAChE-sarin
RS194B
RS41A
2PAM
MMA
DAM
k_{obs} (min^{-1})
B
hAChE-VX
RS194B
RS41A
2PAM
MMA
DAM
E
hAChE-Tabun
2PAM
RS194B
RS41A
MMA
DAM
[oxime] (mM)
C
hAChE-cyclosarin
RS41A
RS194B
2PAM
MMA
DAM
k_{obs} (min^{-1})
[oxime] (mM)
D
hAChE-paraoxon
RS194b
2PAM
MMA
RS41A
DAM
[oxime] (mM)

Figure 6

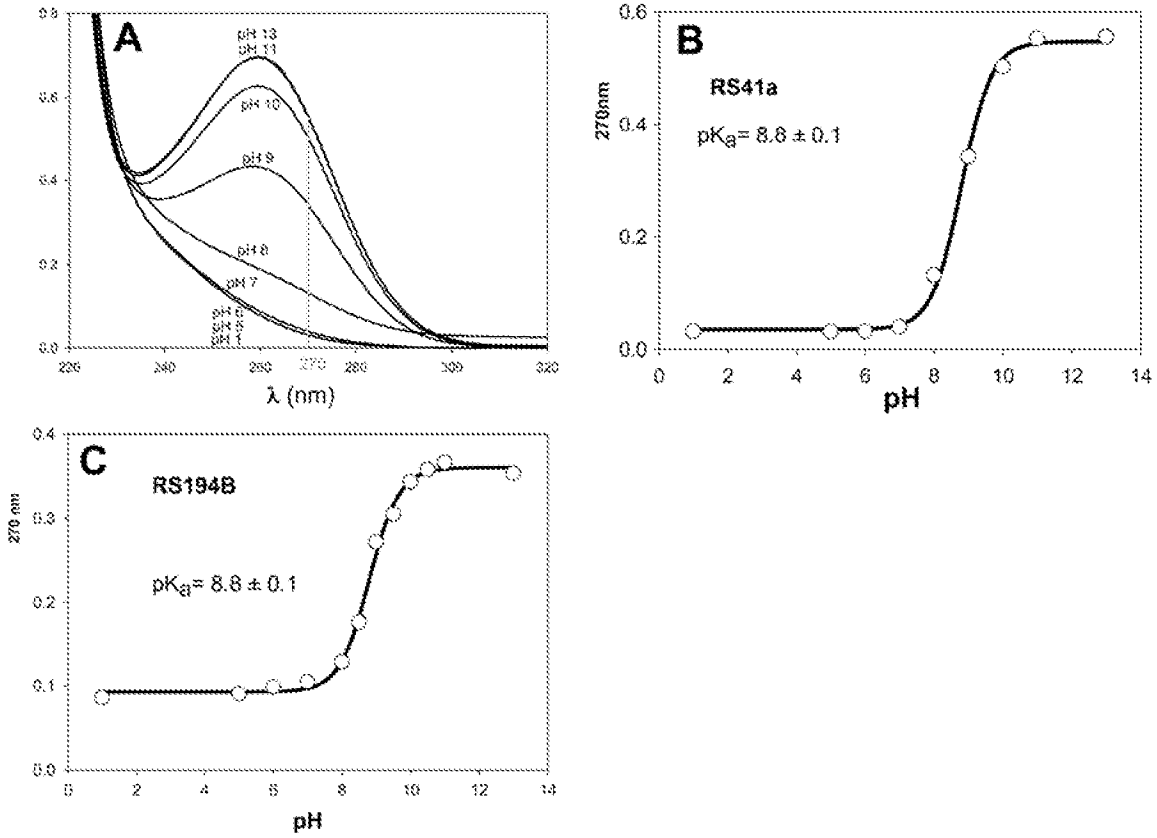

A
pH 13
pH 11
pH 10
pH 9
pH 8
pH 7
pH 6
pH 5
pH 1
λ (nm)
B
RS41a
pKa= 8.8 ± 0.1
270nm
pH
C
RS194B
pKa= 8.8 ± 0.1
270 nm
pH

Figure 7

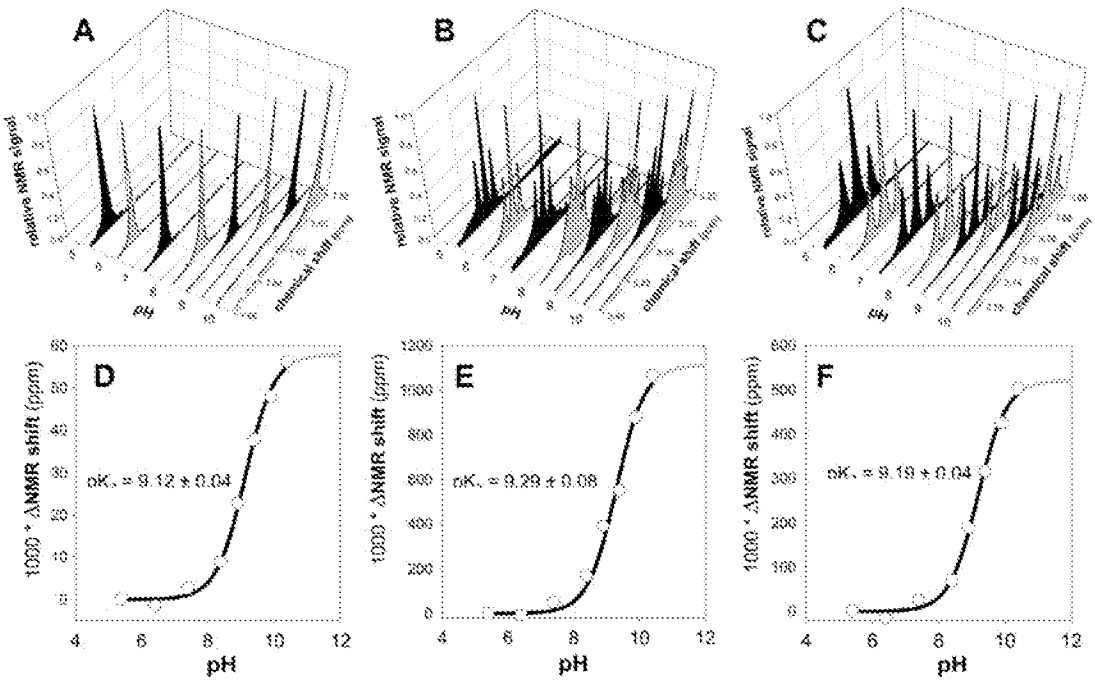

A
B
C
relative NMR signal
chemical shift (ppm)
pH
D
E
F
1000 * ΔNMR shift (ppm)
pK$_a$ = 9.12 ± 0.04
pK$_a$ = 9.29 ± 0.08
pK$_a$ = 9.19 ± 0.04
pH

Figure 8

5

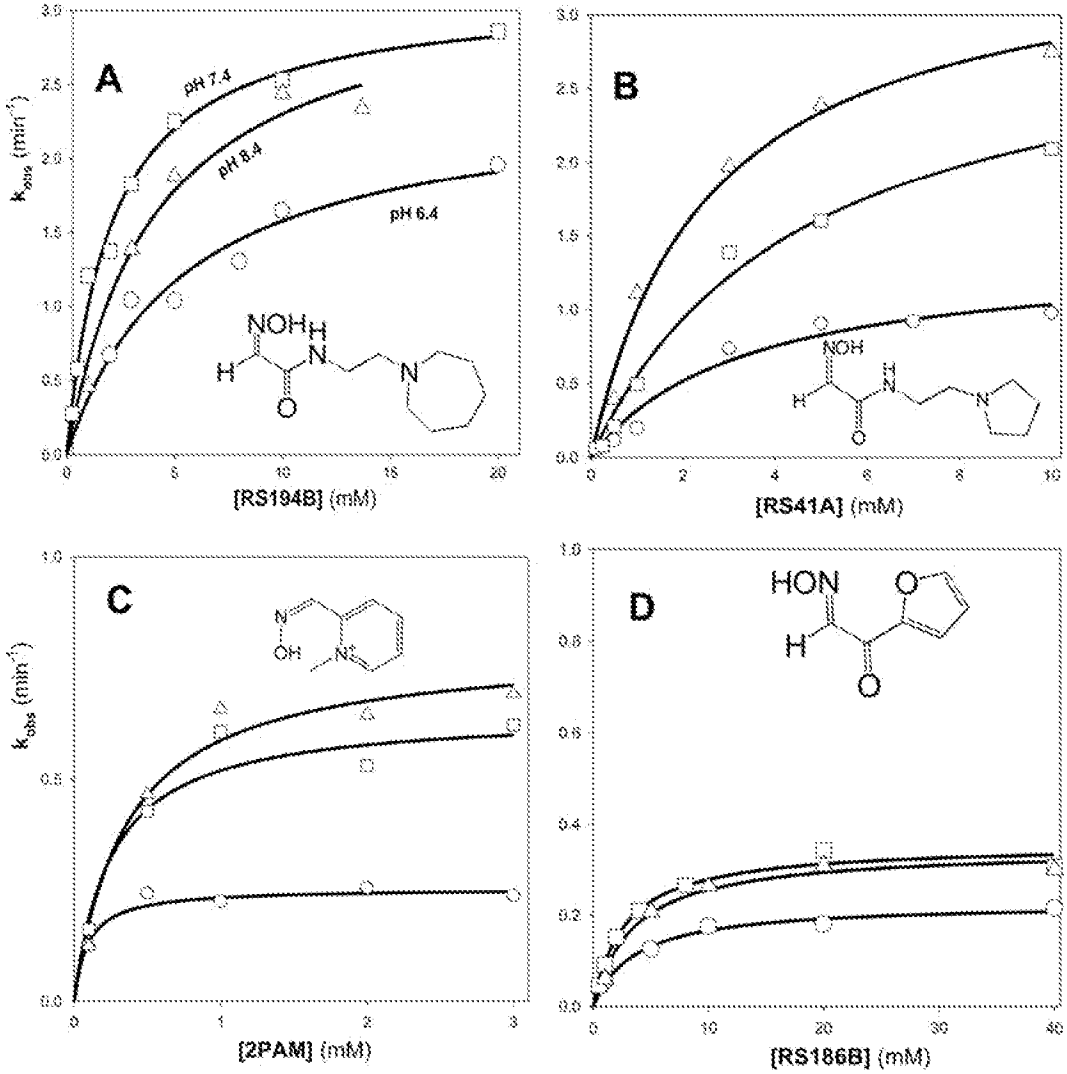

A
pH 7.4
pH 8.4
pH 6.4
k_{obs} (min^{-1})
[RS194B] (mM)
B
[RS41A] (mM)
C
[2PAM] (mM)
D
[RS186B] (mM)

Figure 9

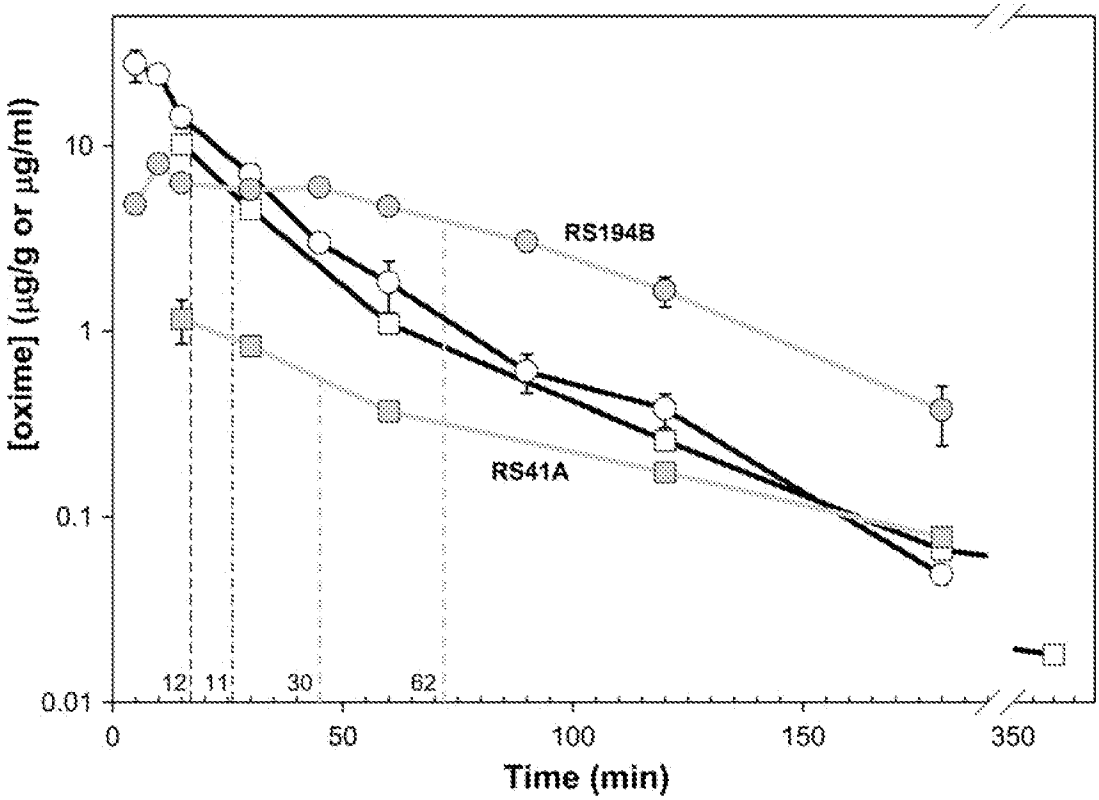

[oxime] (µg/g or µg/ml)
10
1
0.1
0.01
RS194B
RS41A
12
11
30
62
0
50
100
150
350
Time (min)

Figure 10

Fig. 10A

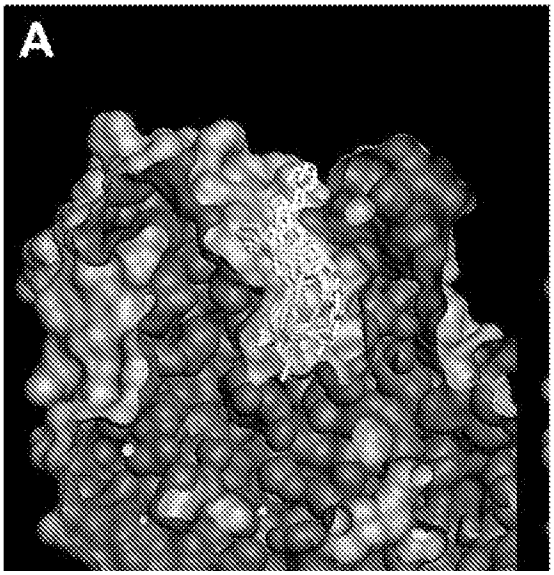


Fig. 10B

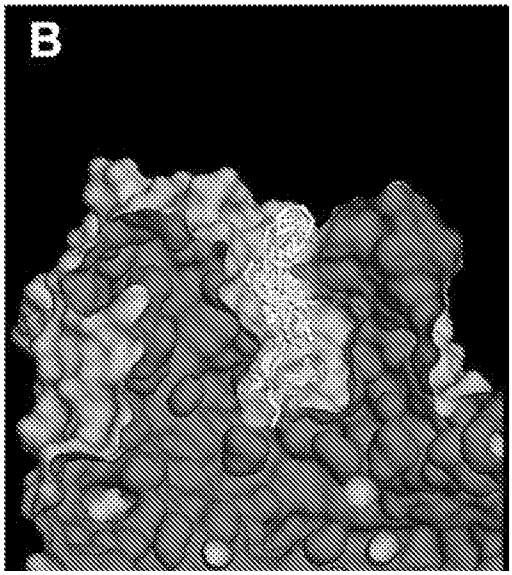


Figure 11

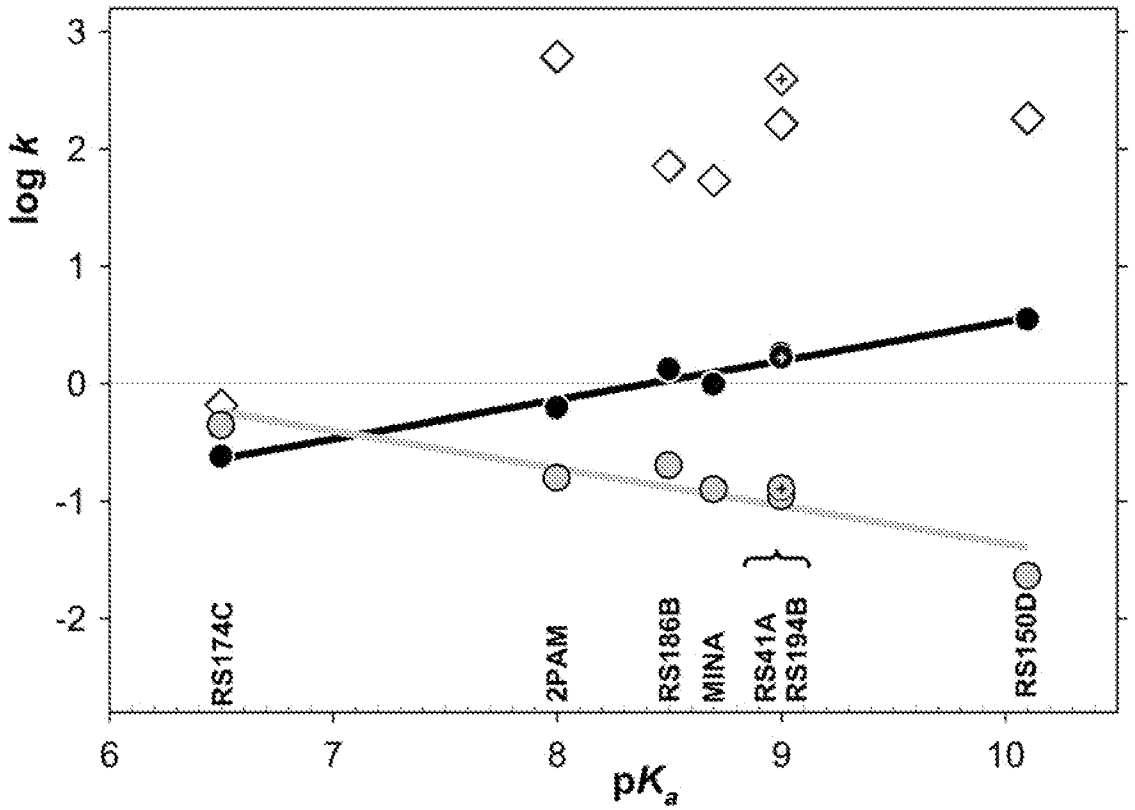

log k
3
2
1
0
-1
-2
6
7
8
9
10
pK_a
RS174C
2PAM
RS186B
MINA
RS41A
RS194B
RS150D

Figure 12 – Table 3

Constants were calculated by nonlinear regression using Equation 1. ND, not determined.

Oxime	UV spectra	Oxime pK_a	Oximolysis		
			ATCh	VX Flu-MP	Average p
RS194B	8.8 :± 0.2	9.1 :± 0.1 (-OH) 9.3 :± 0.1 (NH^+)[b] 9.2 :± 0.1 (NH^+)[c]	9.0:± 0.1	8.9 :± 0.1	8.8 (-OH) 8.8 (NH^+)
RS41A	8.8 :± 0.1	ND	9.0:± 0.1	8.7:± 0.1	8.8
RS186B	8.3 :± 0.1	ND	8.5:± 0.1	ND	8.4
RS150D	ND	ND	10.1:± 0.1	ND	10.1
RS174C	ND	ND	6.5:± 0.1	ND	6.5
MINA	8.3 :± 0.1	ND	8.7:± 0.1	ND	8.5
2PAM	ND	8.6 :± 0.1 (-OH)	8.0:± 0.1	8.1 :± 0.2	8.1

[a] pK_a values determined in D_2O, as shown, are typically higher than those determined in H_2O by ~0.5 (27) and were corrected before calculating the average pK_a.
[b] Based on pH induced shift of 3.37 ppm triplet in D_2O (Figs. 3*B* and supplemental S1).
[c] Based on pH induced shift of 3.73 ppm triplet in D_2O (Figs. 3*C* and supplemental S1).

Figure 13 – Table 4

TABLE 4

Kinetic constants for reactivation of VX-hAChE conjugate by oximes RS194B, RS41A, RS186B, and reference oxime 2PAM determined in 0.1 M phosphate buffers pH 6.4, 7.4, and 8.4

Oxime	pH	k_2	K_{ox}	k_r
		min^{-1}	mM	M^{-1}
RS194B	6.4	2.1 :± 0.2	3.2 :± 1.0	620 :± 130
	7.4	3.1 :± 0.1	2.1 :± 0.3	1600 :± 130
	8.4	3.3 :± 0.3	4.2 :± 1.0	790 :± 120
RS41A	6.4	1.4 :± 0.2	3.3 :± 1.4	410 :± 110
	7.4	3.1 :± 0.3	4.6 :± 0.9	670 :± 80
	8.4	3.5 :± 0.3	2.5 :± 0.6	1400 :± 210
2PAM	6.4	0.25 :± 0.01	0.091 :± 0.028	2800 :± 780
	7.4	0.65 :± 0.08	0.25 :± 0.12	2600 :± 910
	8.4	0.80 :± 0.04	0.36 :± 0.08	2200 :± 390
RS186B	6.4	0.23 :± 0.01	3.6 :± 0.7	63 :± 10
	7.4	0.36 :± 0.02	2.7 :± 0.4	130 :± 18
	8.4	0.35 :± 0.02	3.5 :± 0.6	99 :± 15

Figure 14 – Table 5

TABLE 5

Oxime Tabun	LD_{50}		Dose	Protective index VX	Sarin	Paraoxon	Soman
	mg/kg		*mg/kg*				
RS41A	200 (160.9–248.8)	50	4.5 (3.8–5.3)	1 (ND)	1 (ND)	1.3 (1.1–1.5)	1.2 (1.0–1.4)
RS194B	500[a]	125	18 (12.4–25.7)	10 (6.6–15.3)	9.4 (7.9–11.3)	1.8 (1.5–2.1)	1.5 (1.2–1.7)
2PAM	106 (94.0–118.4)	26.4	9.3 (7.3–13.0)	6.7 (5.9–7.5)	47 (36.7–59.5)	1.5 (1.3–1.8)	1.3 (1.1–1.5)

[a] Estimated from 50% lethality at the maximal administered dose due to solubility limitations.

WO 2014/127315 PCT/US2014/016639

Figure 15 – Table 6

TABLE 6

Combination of therapy and pretreatment of OP-exposed mice with oxime RS194B at doses equivalent to 25, 10, or 5% its LD_{50} dose of 500 mg/kg (*cf.* supplemental Tables S2–S6)

Protective index is the ratio of OP LD_{50} for OP-exposed animals treated with oxime and for animals given OP alone. Maximal dose of poison (MDP), a highest multiple of OP LD_{50} fully counteracted by the oxime, is given in parentheses. Oximes in therapy (but not in pretreatment) were administered together with atropine. ND, not determined.

Protective index (MDP) (95% confidence limits)

RS194B	Pretreatment before VX, 5 min	Pretreatment before VX, 15 min	Therapy 1 min after VX	Pretreatment 15 min and therapy 1 min after VX
25 mg/kg	2.0 (1.6) (1.8–	1.6 (ND) (1.2–	5.3 (4) (4.8–	
50 mg/kg		1.3 (1.0) (1.1–1.6)	11 (7.9) (8.5–13.7)	7.3 (5.0) (5.6–9.6)
125 mg/kg	3.7 (2.5) (2.9–4.7)	3.6 (2.5) (3.0–4.2)	18 (10.0) (12.4–25.7)	45 (31.8) (37.2–54.3)

Pretreatment 15 min and therapy 1 min after OP

	Paraoxon	Soman	Tabun	Sarin
125 mg/kg	22 (7.9) (17.0–27.5)	2.8 (2.5) (ND)	2.0 (ND) (1.3–3.1)	4.5 (3.2) (3.8–5.3)

WO 2014/127315 PCT/US2014/016639

Figure 16

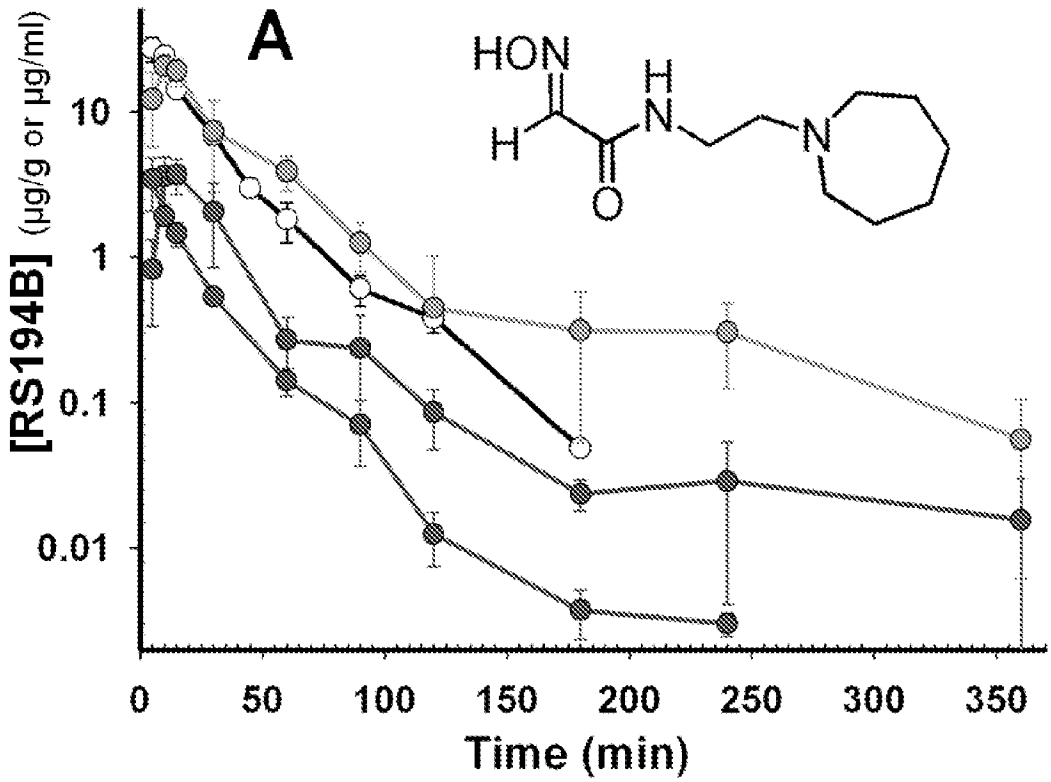

A
HON
H
N
H
O
N
[RS194B] (µg/g or µg/ml)
10
1
0.1
0.01
0
50
100
150
200
250
300
350
Time (min)

Figure 17

intravenous

Dose (mg/kg)	$t_{1/2}$ (hr)	C_{max} (ng/ml) Mean	C_0 (ng/ml)	AUC_{last} (hr·ng/ml) Mean	AUC_{inf} (hr·ng/ml)
20	0.9728	3457	6212	1270	1274

oral

Dose (mg/kg)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} Mean	AUC_{last} (hr·ng/ml) Mean	AUC_{inf} (hr·ng/ml)	F (%)
50	1.2	0.25	3693	2149	2175	68.3
200	1.2	0.167	20540	12391	12484	98.0

WO 2014/127315 PCT/US2014/016639

Figure 18

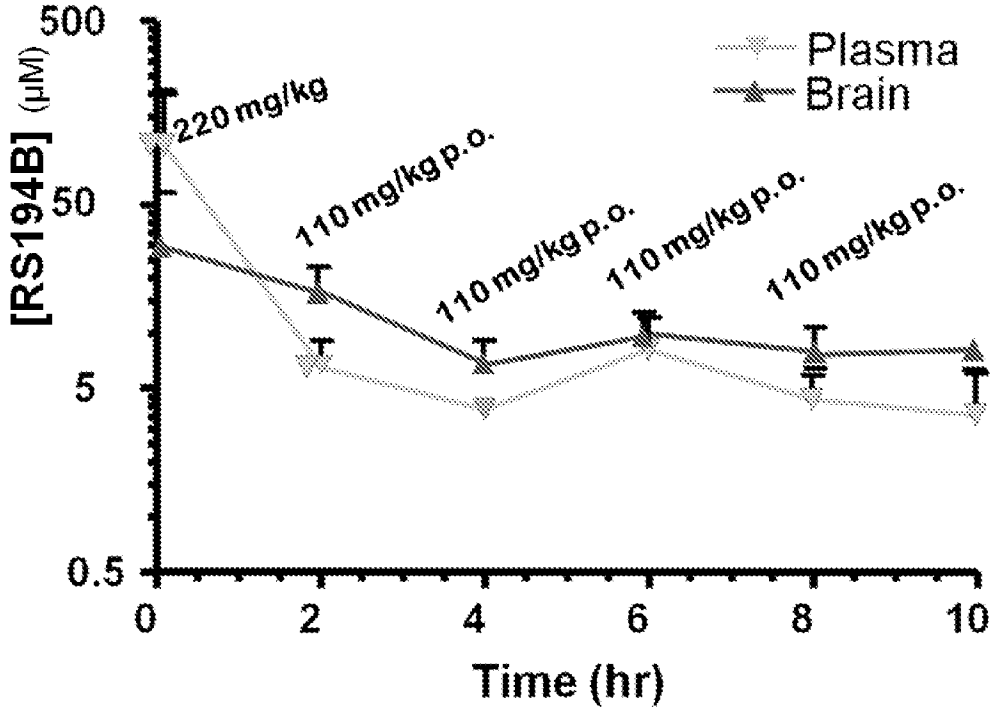

500
50
5
0.5
[RS194B] (μM)
Plasma
Brain
220 mg/kg
110 mg/kg p.o.
110 mg/kg p.o.
110 mg/kg p.o.
110 mg/kg p.o.
0
2
4
6
8
10
Time (hr)

Figure 19

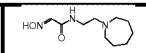
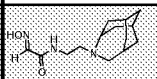
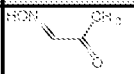
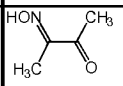
oxime		paraoxon			sarin			Cyclosarin			VX		
		k_2 (min^{-1})	K_{ox} (mM)	k_r ($M^{-1}min^{-1}$)	k_2 (min^{-1})	K_{ox} (mM)	k_r ($M^{-1}min^{-1}$)	k_2 (min^{-1})	K_{ox} (mM)	k_r ($M^{-1}min^{-1}$)	k_2 (min^{-1})	K_{ox} (mM)	k_r ($M^{-1}min^{-1}$)
RS 194B		0.38	7.4	51	2.5	1.9	1300	0.88	3.9	230	2.8	1.6	1800
RS2 1388		0.23	2.2	100	2.4	1.2	2000	0.83	6.8	120	2.6	1.6	1600
MINA		> 0.20	> 20	8.4	1.6	14	120	1.2	16	75	> 0.7	> 6.0	110
DAM		0.027	46	0.58	0.13	88	1.5	> 0.30	> 100	2.5	0.20	> 100	1.7
2PAM		0.27	1.8	150	1.1	0.34	3200	0.73	6.6	110	0.73	0.3	2400

Figure 20

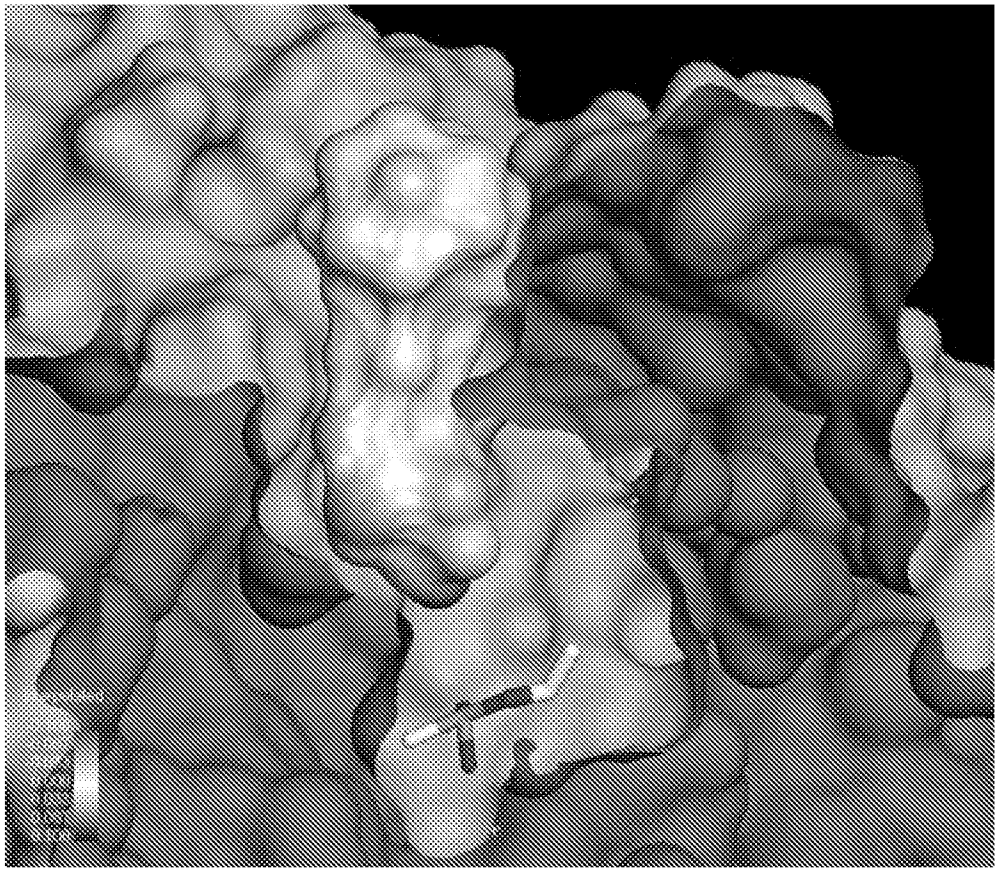
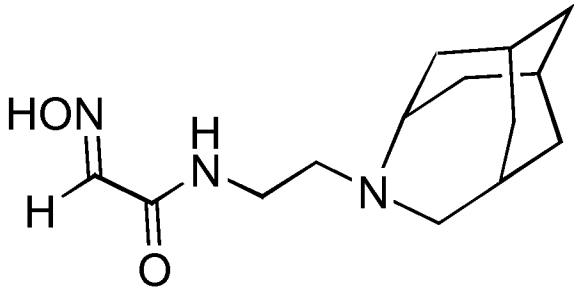


Figure 21

RS2 138B



RS 194B

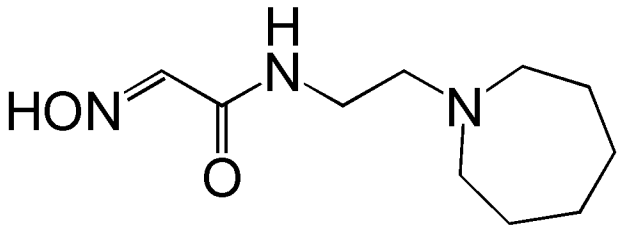
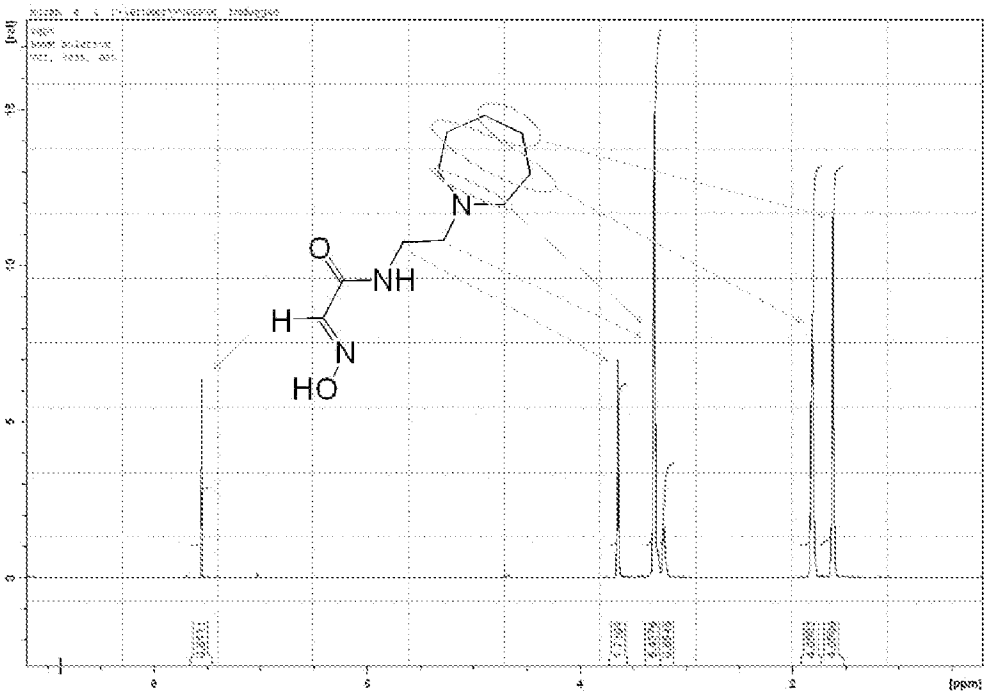


Figure 22

A



B

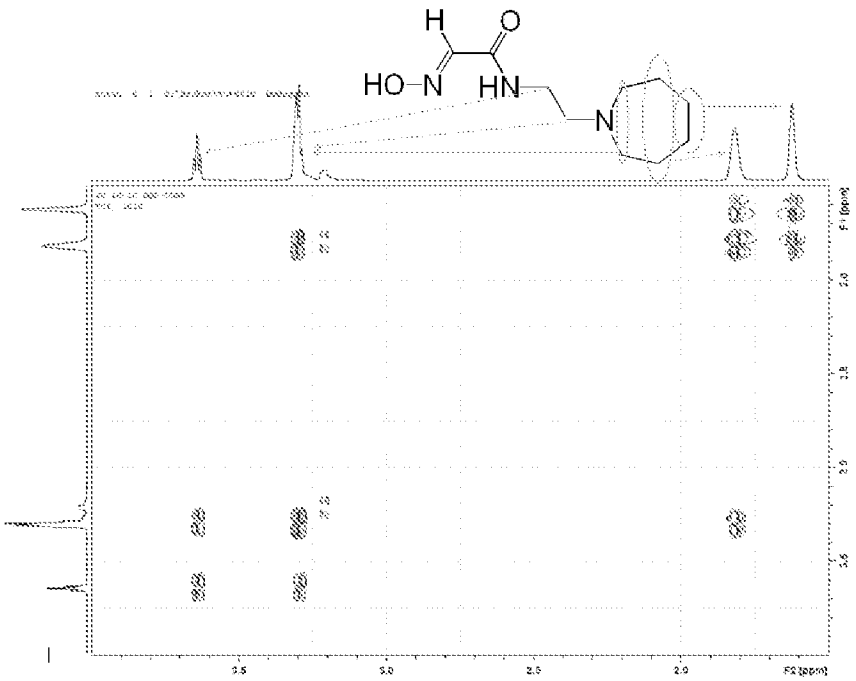


Figure 23 (supplemental Fig. S2)

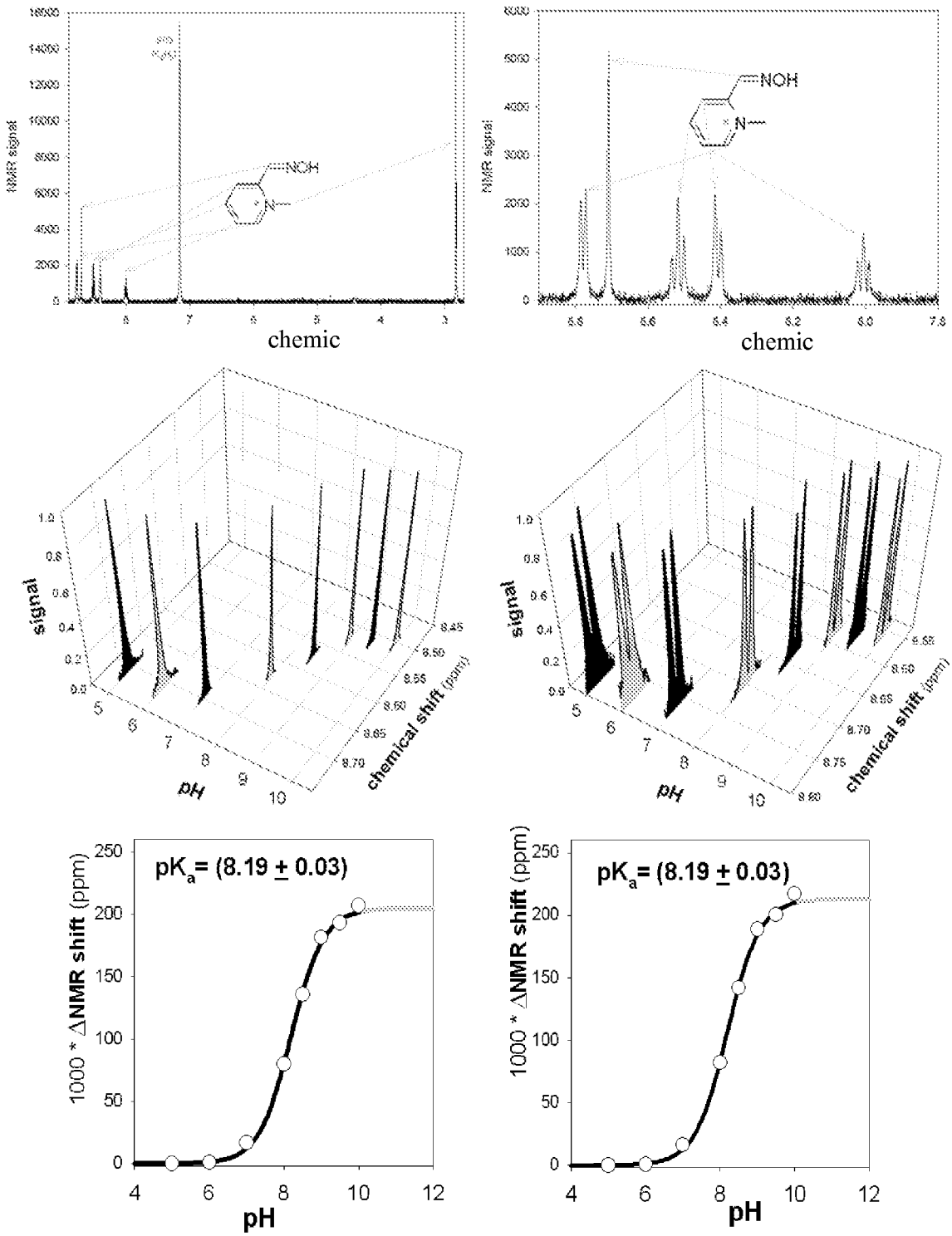


Figure 24 (supplemental Fig. S3)

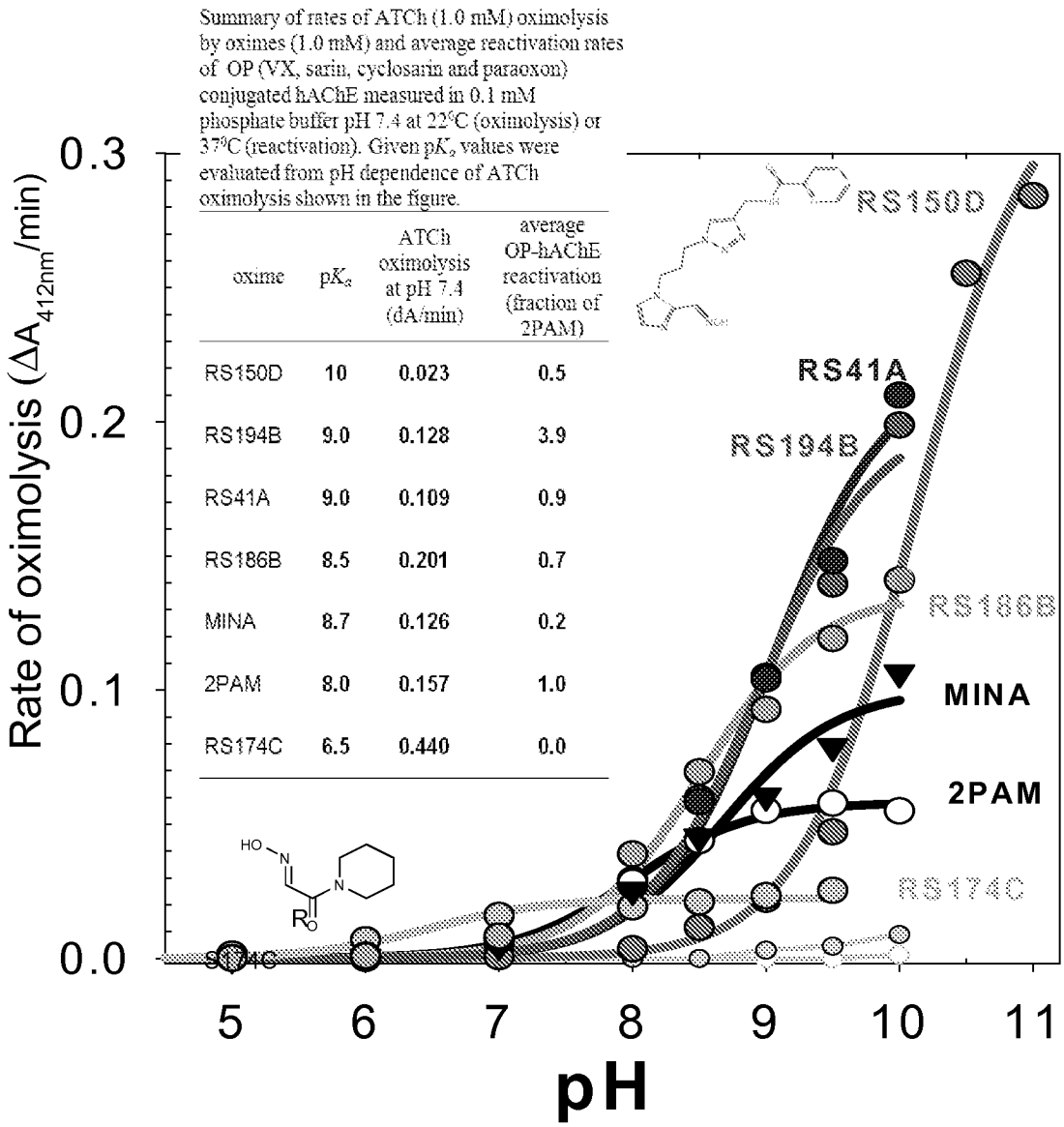


Figure 25 (supplemental Fig. S4)

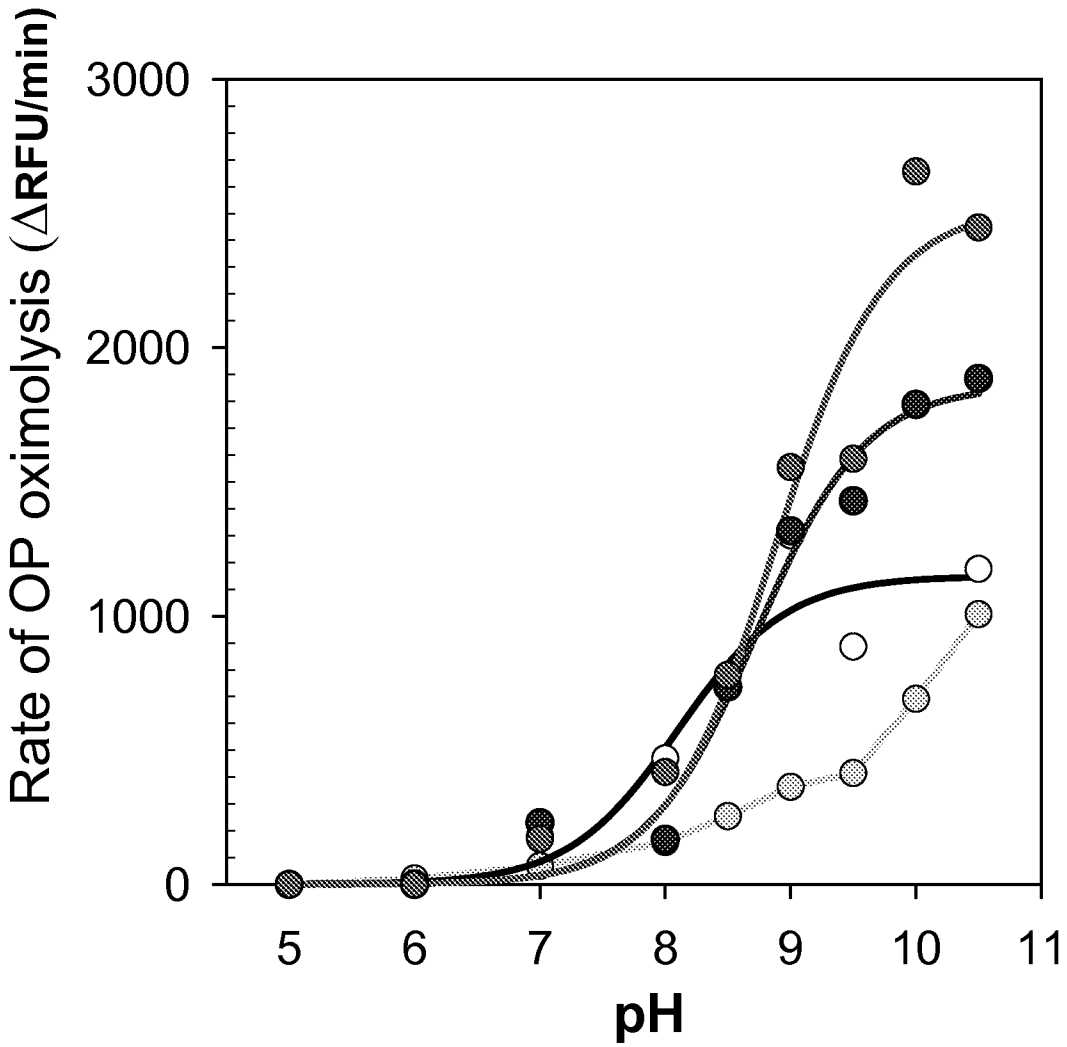


Figure 26 (supplemental Fig. S5)

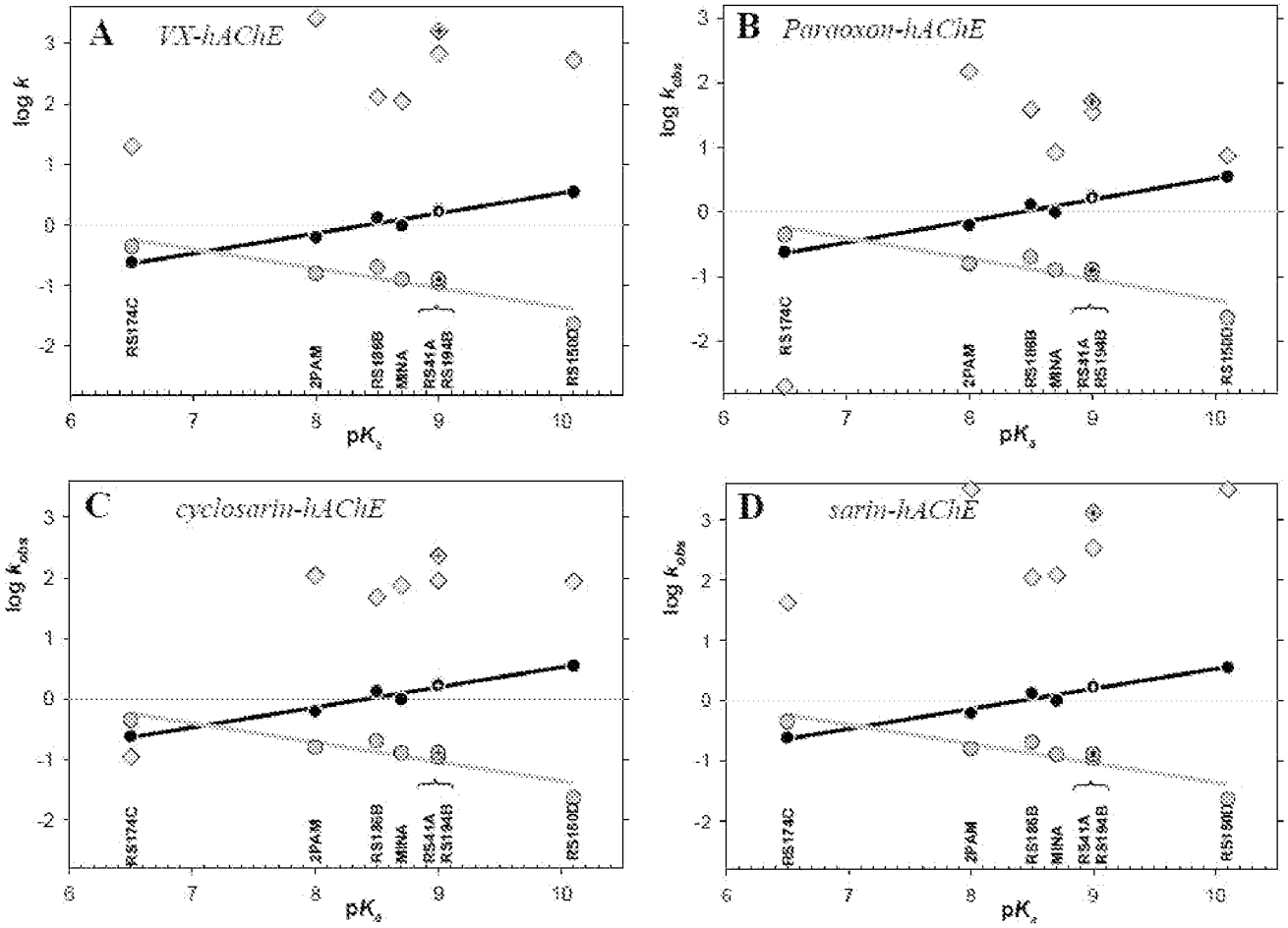


Figure 27 (supplemental Fig. S6)

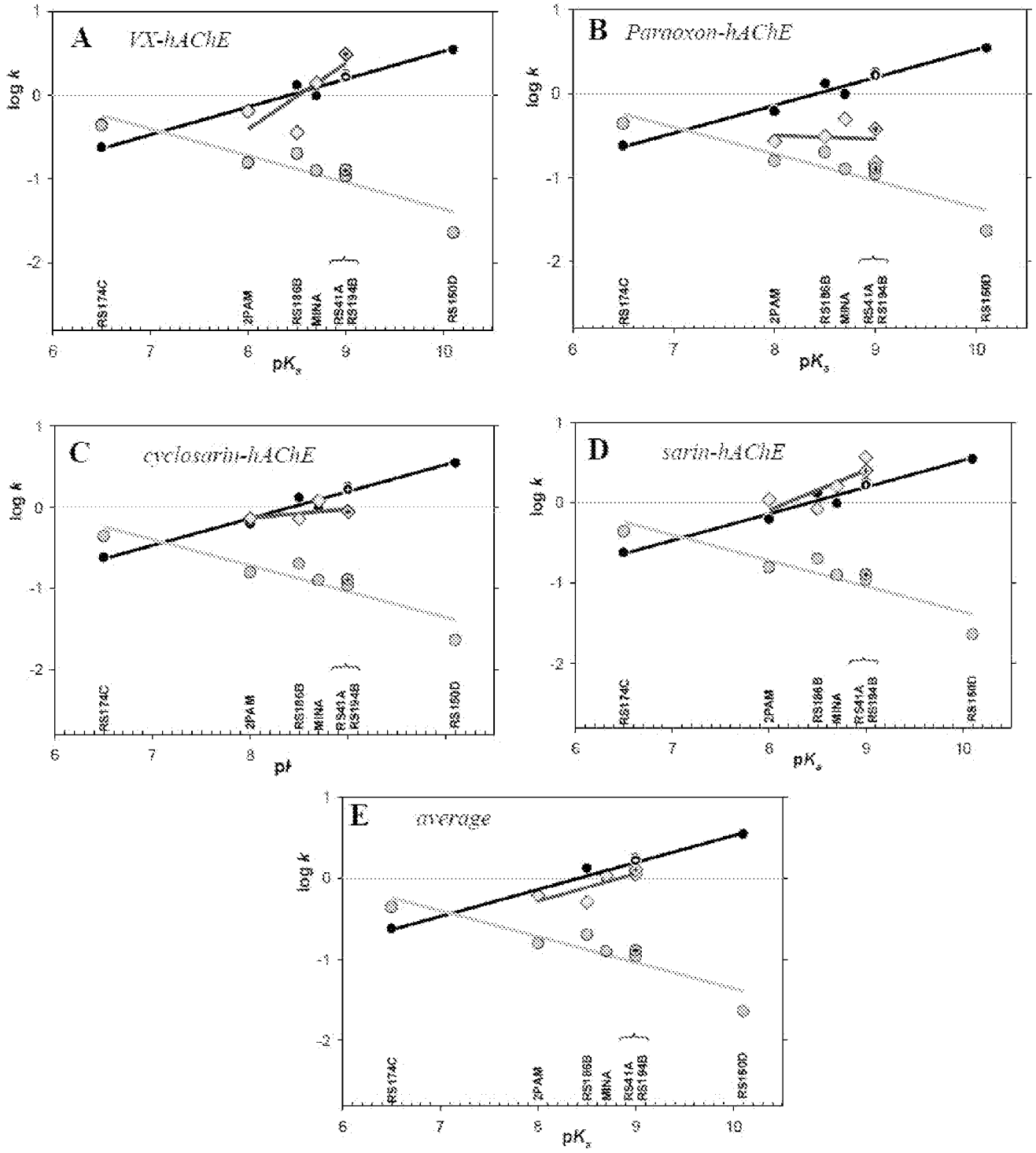


Figure 28

oxime	i.m. dose (mg/kg)	[oxime]$_{max}$					time for ([oxime]$_{max}$)/2			log D
		(µg/ml)		(µM)			(min)			
		Brain	plasma	brain	plasma	brain/plasma	brain	plasma	brain/plasma	
RS41A	30	.2	10	6.5	54	0.12	30	11	1.7	1.09
RS194B	80	.9	27	37	125	0.30	22	12	4.2	0.48

WO 2014/127315 PCT/US2014/016639

Figure 29

Table S2

VX		Therapy 1 min after exposure				Pretreatment 15 min before VX & Therapy 1 min after exposure	
		RS41A		RS194B		R194B	
LD_{50}	(µg/kg)	Survived/ treated	Symptoms	Survived /	Symptoms	Survived/ treated	Symptoms
3.18	90.0	4 / 4	Mice were hypoactive; no symptoms.	4 / 4	No visible symptoms.		
4.0	113.2	3 / 4	Strong tremor immediately upon application; respiratory disturbance; three mice recovered fully after 1 h.	4 / 4	No visible symptoms.		
5.0	141.5	1 / 4	Strong tremor immediately upon application; respiratory disturbance; light salivation; surviving mouse weak after 24h.	4 / 4	No visible symptoms.		
6.3	178.3	0 / 4	Strong tremor and salivation immediately upon application.	-	-		
7.9	223.6	1 / 4	Strong tremor immediately upon application. Surviving mouse weak after 24h.	-	-		
10.0	283.0	2 / 4	Strong tremor immediately upon application. Surviving mouse weak after 24h.	4 / 4	Light tremor 10 min upon application. All symptoms disappeared after 1 h.		
12.6	356.6	0 / 4	Strong tremor and respiratory disturbance immediately upon application.	3 / 4	Strong tremor 2 min upon application, respiratory disturbance. Surviving mice recovered fully after 24h.		
15.9	450.0	0 / 4	Strong tremor and respiratory disturbance immediately upon application	3 / 4	Strong tremor immediately upon application, respiratory disturbance, salivation. Surviving mice recovered fully after 24h.		
20.0	566.0			1 / 4	Strong tremor immediately upon application, salivation. Surviving mouse weak but active after 24h.	4 / 4	Mice hypoactive 5 min upon application. All symptoms disappeared after 30 min.
25.2	713.2			1 / 4	Strong tremor immediately upon application, salivation. Surviving mouse weak after 24h.	1 / 4	Strong tremor 2min upon application, respiratory disturbance, surviving mouse active after 24h.
31.8	899.9					4 / 4	Strong tremor 2min upon application, respiratory disturbance, surviving mice active after 24h.
40.0	1132					2 / 4	Strong tremor 2min upon application. Surviving mice weak after 24h.
50.4	1426.3					2 / 4	Strong tremor immediately upon application, respiratory disturbance, salivation, surviving mice weak after 24h.
63.0	1785.2					0 / 4	Strong tremor immediately upon application, respiratory disturbance, salivation,
LD_{50}		**127.3 µg/kg**		**504.4 µg/kg**		**1272.9 µg/kg**	
PI		**4.5 (3.8 - 5.3)**		**17.8 (12.7 - 25.7)**		**45 (37.2 – 54.3)**	
MDP		**3.18**		**10**		**31.8**	

WO 2014/127315 PCT/US2014/016639

Figure 30

Table S3						
Sarin		**Therapy 1 min after exposure**			**Pretreatment 15 min before sarin & Therapy 1 min after exposure**	
		RS41A		**RS194B**		**RS194B**
LD_{50}	(µg/kg)	Survived/ treated	Symptoms	Survived/ treated	Symptoms	Survived/ treated	Symptoms
3.18	757.8	0 / 4	Mild but persistent tremor 10 min upon application.	-		4 / 4	Strong tremor immediately upon application. All mice quickly and fully recovered after 24 h.
4.0	953.2			4 / 4	No visible symptoms.	3 / 4	Strong tremor immediately upon application. Surviving mice completely recovered after 24 h.
5.0	1191.5			2 / 4	Strong tremor immediately upon application, convulsions, surviving mice recovered after 24 h.	1 / 4	Strong tremor immediately upon application, convulsions, surviving mouse weak but active after 24 h.
6.3	1501.3			3 / 4	Strong tremor 1min upon application, convulsions, surviving mice recovered after 24 h.	0 / 4	Strong tremor immediately upon application.
7.9	1882.6			3 / 4	Strong tremor immediately upon application, convulsions, surviving mice recovered after 24 h.	2 / 4	Strong tremor immediately upon application, convulsions, surviving mice weak after 24 h.
10.0	2383.0			2 / 4	Strong tremor immediately upon application, convulsions, surviving mice recovered after 24 h.	2 / 4	Strong tremor immediately upon application, convulsions, surviving mice weak after 24 h.
12.6	3002.6			1 / 4	Strong tremor immediately upon application, convulsions, surviving mouse recovered after 24 h.	-	
LD_{50}		**-**		**2384 µg/kg**		**1072 µg/kg**	
PI		**-**		**10 (6.6 – 15.3)**		**4.5 (3.8 – 5.3)**	
MDP		**-**		**4**		**3.18**	

WO 2014/127315 PCT/US2014/016639

Figure 31

Table S4							
Tabun		Therapy 1 min after exposure				Pretreatment 15 min before tabun & Therapy 1 min after exposure	
		RS41A		RS194B		RS194B	
LD_{50}	(µg/kg)	Survived/ treated	Symptoms	Survived/ treated	Symptoms	Survived/ treated	Symptoms
0.79	449.0	4 / 4	No visible symptoms.	-			
1.0	565.8	3 / 4	Mild but persistent increasing tremor upon application. Surviving mice recovered completely after 1h.	3 / 4	Mild increasing tremor upon application. Surviving mice recovered after 24h.		
1.26	712.9	2 / 4	Strong tremor immediately upon application. Surviving mice recovered completely after 24h.	4 / 4	Slight tremor only. All mice recovered.		
1.59	899.6	0 / 4	Strong tremor immediately upon application.	1 / 4	Tremor immediately upon application, respiratory disturbance, surviving mouse weak after 24h.	3 / 4	Mild but persistent increasing tremor upon application. Surviving mice recovered completely after 24h.
2.0	1131.6			0 / 4	Strong tremor immediately upon application.	1 / 4	Strong tremor immediately upon application, convulsions, surviving mouse recovered after 24h.
2.52	1425.8					2 / 4	Strong tremor immediately upon application, convulsions, surviving mice active after 24h.
3.18	1799.2					1 / 4	Strong tremor immediately upon application, convulsions, surviving mouse weak after 24h.
LD_{50}		**672.8**		**831.8 µg/kg**		**1133.4 µg/kg**	
PI		**1.2 (1.0 – 1.4)**		**1.5 (1.2 – 1.7)**		**2.0 (1.3 – 3.1)**	
MDP		**0.79**		**1.26**		**/**	

WO 2014/127315 PCT/US2014/016639

Figure 32 (Table S5 part I)

Table S5							
Paraoxon		Therapy 1 min after exposure				Pretreatment 15 min before paraoxon & Therapy 1 min after exposure	
		RS41A		RS194B		RS194B	
LD_{50}	(µg/kg)	Surviv	Symptoms	Surviv	Symptoms	Surviv	Symptoms
1.0	712.9	0 / 4	Strong motor irritation immediately upon application.				
4.0	2851.6			4 / 4	Mild symptoms upon application. Mice recovered fully after 10 min.		
5.0	3564.5			2 / 4	Mild increasing tremor upon application. Surviving mice recovered after 40 min.		
6.3	4491.3			4 / 4	Strong tremor immediately upon application, salivation, all mice recovered after 60 min.		
7.9	5631.9			3 / 4	Strong tremor immediately upon application, salvation, respiratory disturbance, surviving mice recovered after 2 h.	4 / 4	No visible symptoms.
10.0	7129.0			2 / 4	Strong tremor immediately upon application, salvation, surviving mice recovered after 24 h.	3 / 4	Mild tremor upon application. Surviving mice recovered completely after 24h.

WO 2014/127315 PCT/US2014/016639

12.6	8982.5		0 / 4	Strong tremor immediately upon application, salvation, respiratory disturbance.	2 / 4	Increasing tremor immediately upon application. Surviving mice recovered after 24 h.
15.9	11335		1 / 4	Strong tremor immediately upon application, salivation, surviving mouse weak after 24 h.	3 / 4	Strong tremor immediately upon application, repiratory distrurance, surviving mice weak after 24 h.
20.0	14816				3 / 4	Strong or increasing tremor immediately upon application, respiratory disturbance, surviving mice weak after 24 h.
25.2	18668				1 / 4	Strong tremor immediately upon application, respiratory disturbance, surviving mouse weak after 24 h
31.8	23557				0 / 4	Strong tremor immediately upon application.
LD_{50}		**-**		**6731.3 µg/kg**		**16032.5 µg/kg**
PI		**-**		**9.4 (7.9 – 11.3)**		**21.6 (17.0 – 27.5)**
MDP		**-**		**6.3**		**7.9**

Figure 33 (Table S5 part II)

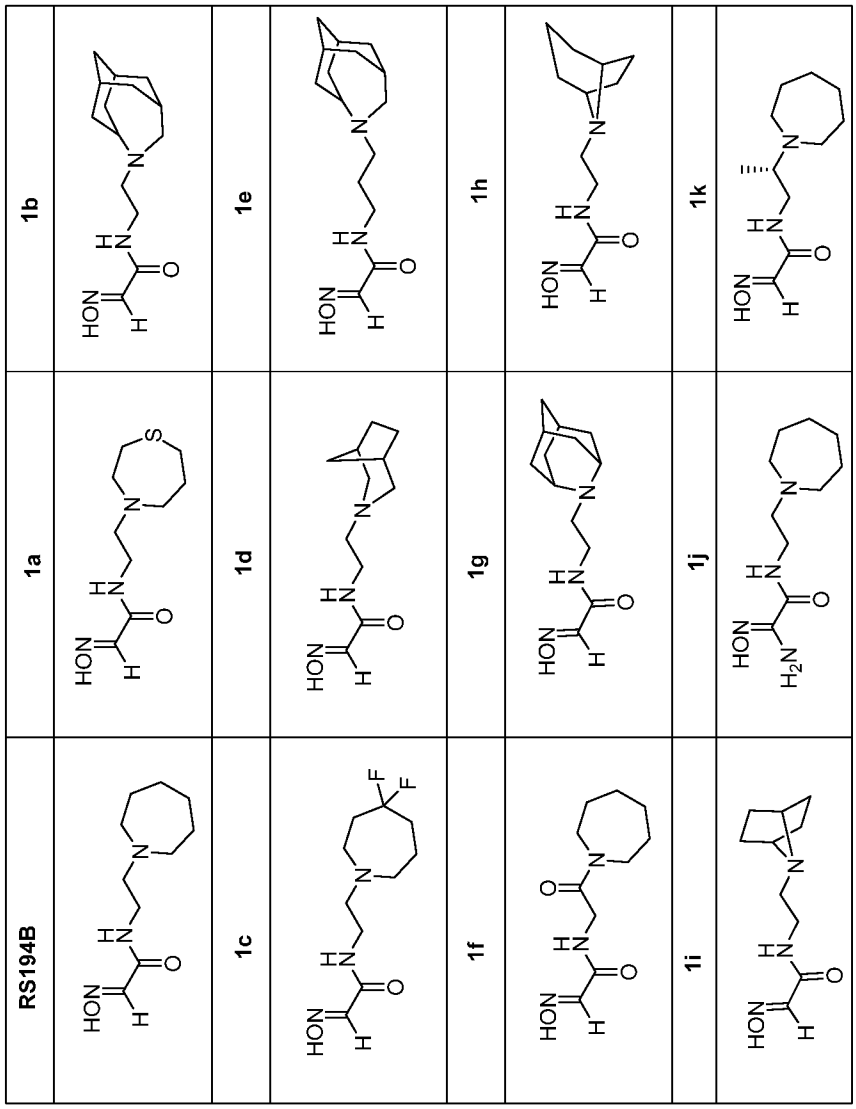
Figure 34

Table S6							
Soman		Therapy 1 min after exposure				Pretreatment 15 min before soman & Therapy 1 min after exposure	
		RS41A		RS194B		RS194B	
LD_{50}	(µg/kg)	Survived/ treated	Symptoms	Survived/ treated	Symptoms	Survived/ treated	Symptoms
1.0	136.1	4 / 4	Mice hypoactive upon application.	4 / 4	Mice hypoactive upon application. All mice recovered after 24 h.		
1.26	171.5	1 / 4	Increasing tremor and convulsions immediately upon application. Surviving mouse was weak but active after 24 h.	4 / 4	Mice hypoactive upon application, diarrhea, all mice recovered after 24 h.	3 / 4	Mice were hypoactive upon application occasionally having light tremor. Surviving mouse weak after 24 h.
1.59	216.4	1 / 4	Increasing tremor and convulsions immediately upon application. Surviving mouse was weak and hypoactive after 24 h.	3 / 4	Mice hypoactive with increasing tremor upon application, diarrhea, motor coordination disturbance, surviving mice recovered after 24 h.	3 / 4	Mice hypoactive with increasing tremor upon application. Surviving mice weak but active after 24 h.
2.0	272.2	0 / 4	Strong tremor immediately upon application.	1 / 4	Mice hypoactive with increasing tremor upon application, diarrhea, motor coordination disturbance, surviving mouse weak after 24 h.	4 / 4	Mice weak with occasional tremor upon application. All mice weak after 24 h.
2.52	342.9			0 / 4	Increasing tremor immediately upon application, diarrhea.	4 / 4	Mice weak with occasional tremor upon application. All mice weak but active after 24 h.
3.18	432.8					0 / 4	Strong tremor immediately upon application, convulsions, respiratory disturbance,
4.0	544.4					0 / 4	Strong tremor immediately upon application, convulsions.
LD_{50}		**171.5**		**242.6 µg/kg**		**385 µg/kg**	
PI		**1.26 (1.1 – 1.5)**		**1.8 (1.5 – 2.1)**		**2.8 (–)**	
MDP		**1.0**		**1.26**		**2.52**	

WO 2014/127315 PCT/US2014/016639

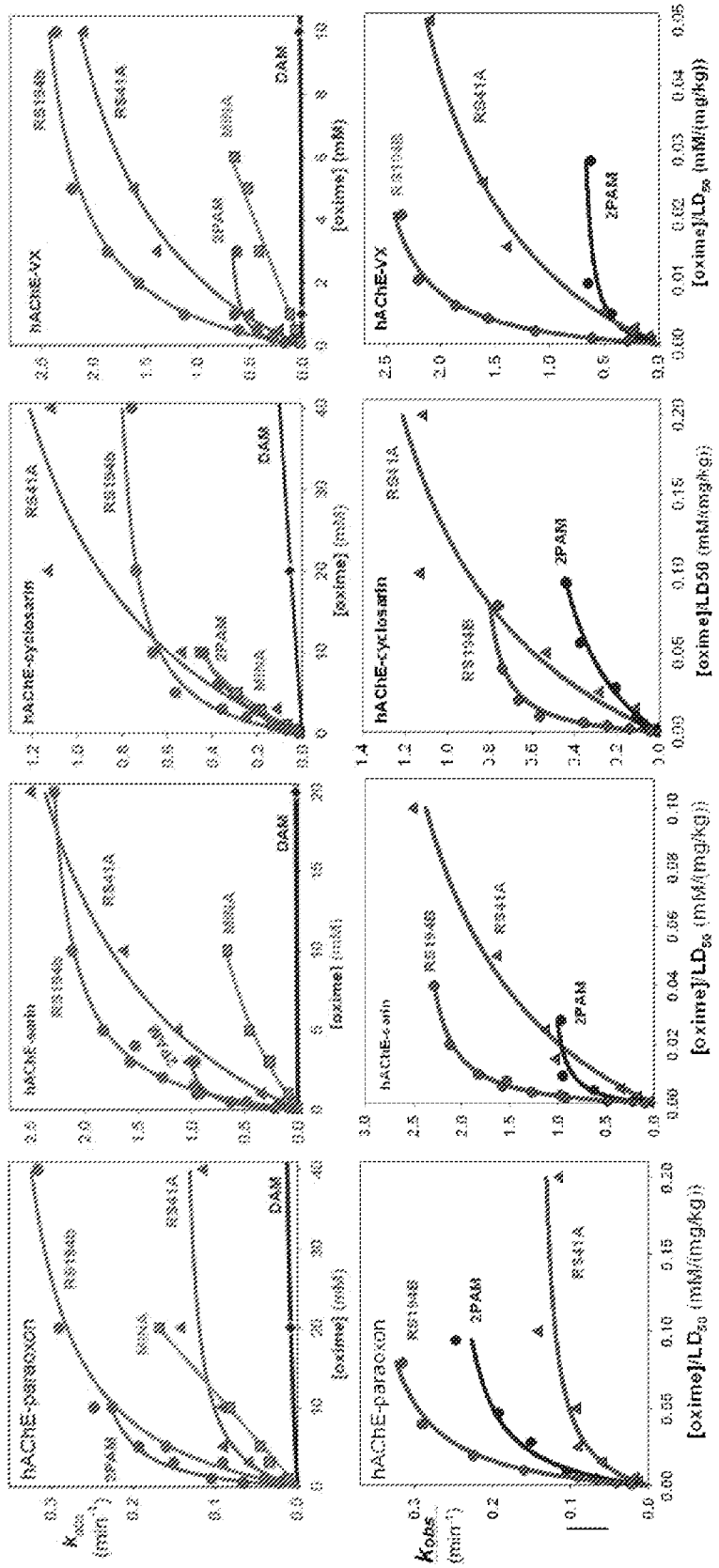
35/37

Figure 35



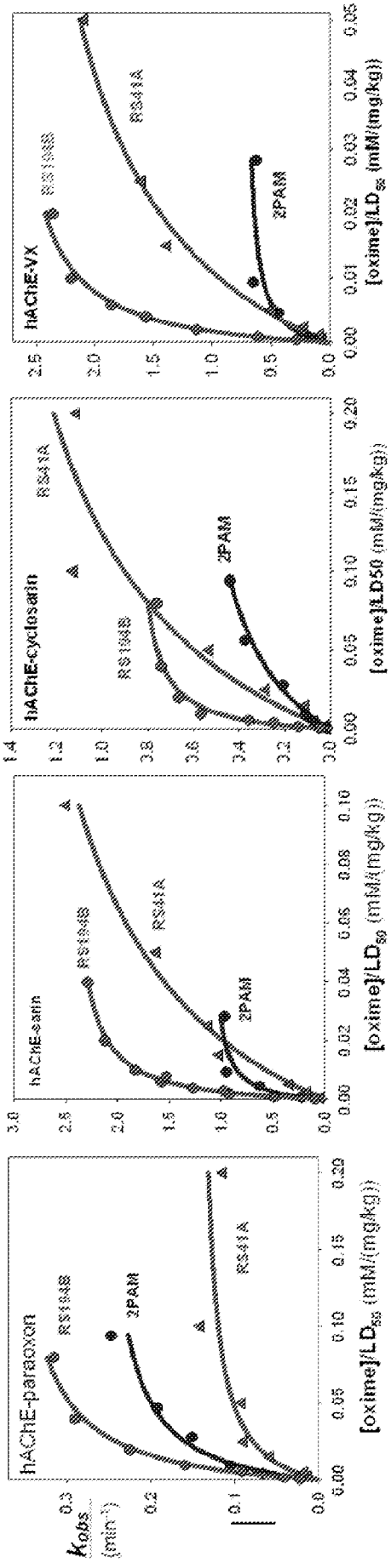
36/37

Figure 36



37/37

Figure 37



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/016639

A. CLASSIFICATION OF SUBJECT MATTER

C07D 403/02(2006.01)i, C07D 225/02(2006.01)i, A61K 31/46(2006.01)i, A61P 25/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D 403/02; A61K 31/506; A61K 31/4545; C07D 225/02; A61K 31/46; A61P 25/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords:AchE, organophosphate, acetylcholinesterase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013-0035351 A1 (STANTON F. MCHARDY et al.) 07 February 2013 See claims 1-24, examples 1-19	1-4
X	RAIDIC, ZORAN et al., `Mechanism of interaction of novel uncharged, centrall y active reactivators with OP-hAChE conjugates`, Chemico-Biological Interact ions Vol.203, 2013, pages 67-71 Available online 4 september 2012 See abstract, Tables 1,2	1-4
X	RADIC, ZORAN et al., `Refinement of Structural Leads for Centrally Acting Ox ime Reactivators of hosphylated Cholinesterases`, Journal of Biological Chem istry, 2012 Vol 287, No.15, pages11798-11809, 6 April, 2012 See abstract, Tables 1-3	1-4

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 2014 (27.06.2014)	**30 June 2014 (30.06.2014)**

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/016639

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 5-9
because they relate to subject matter not required to be searched by this Authority, namely:
The subject-matter of claims 5-9 relates to a method of treatment of the human body, and relates to a subject matter which this International Searching Authority is not required,under PCT Article 17(2)(a)(I) and Rule 39.1(IV), to search.

2. ☒ Claims Nos.: 6,7,9
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 6,7,9 are unsearchable because they are worded in reference to multiple dependent claims which are not searchable due to not being drafted in accordance with the third sentences of Rule 6.4(a).

3. ☒ Claims Nos.: 5,8
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2014/016639

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2013-0035351 A1	07/02/2013	None	