In silico pharmacophore modeling on known pyridinium oxime reactivators of cyclosarin(GF) inhibited AChE to aid discovery of potential more efficacious novel non-oxime reactivators

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Summary

Cyclohexyl methylphosphonofluoridate (cyclosarin, cyclosin, GF) is a highly toxic organo-phosphorus (OP) nerve agent considered as potential warfare threats and known to be resistant to conventional oxime antidotal therapy. To gain further structural insights on the mechanism of reactivation that may aid to the discovery of novel antidotes for GF toxicity, we present here the first three-dimensional *in silico* pharmacophore model for reactivation against GF intoxication. The model was generated from an earlier published experimental data on percentage reactivations as changes of AChE/BuChE activities in whole blood after cyclosarin intoxication and administration of oximes. The pharmacophore model was found to contain a hydrogen bond donor site and two ring aromatic sites on the oximes as necessary optimal features for reactivation of GF intoxication. Our earlier reported stereoelectronic features relating to binding affinity of the oximes for tabun-inhibited (GA) AChE provided guidance to develop this model. The model was consistent with the structure activity data of the oximes. Furthermore, from a map fitting of the model, two new non-oxime compounds were identified through virtual screening of two commercial databases, Maybridge and ChemNavigator, which showed reactivation efficacy within 10-fold range of 2-PAM for DFP-inhibited AChE. Since GF is a G-simulator like DFP (diisopropylfluorophosphate), the model has the potential for discovery of novel reactivators against GF intoxication.

Keywords: 3D pharmacophore model; acetylcholinesterase (AChE); butyrylcholinesterase (BuChE); oximes; reactivation; cyclosarin (GF); *in vitro*

INTRODUCTION

Organophosphorus (OP) nerve agents, such as tabun (GA), sarin (GB), soman (GD), and cyclosarin (GF) represent an extremely toxic group of compounds. These agents are known as the G-series of nerve agents because the German scientists first synthesized them, starting with tabun (GA) in 1936, sarin (GB) in 1938, soman (GD) in 1944, and the lesser known cyclosarin (GF) in 1949 (Taylor 2006, Kuca et al. 2006, 2004). These nerve agents are considered potential warfare threats due to their high intrinsic toxicity (Bakshi et al. 2000). The potential for exposure of these compounds exists in battlefields (e.g., VX used in Iran-Iraq war), in civilian sectors by terrorists (e.g. the use of sarin in Tokyo subway), or sudden accidents due to potentially demilitarization efforts of an army unit (Bakshi et al. 2000). The acute toxicity of OP compounds in mammals is due to rapid inhibition of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7), subsequent accumulation of the neurotransmitter acetylcholine (ACh) in synapses of the central and peripheral nervous systems, and resulting overstimulation of postsynaptic cholinergic receptors (Taylor 2006). AChE is a serine hydrolase responsible for hydrolyzing (removing) ACh in humans and animals. The enzyme has a catalytic triad consisting of Ser203, His447, and Glu334 at the active site at the end of a deep gorge (~ 20 Å), the lining of which contains mostly aromatic residues that forms a narrow entrance to the catalytic Ser203 (Sussman et al. 1991). A peripheral anionic/aromatic site (PAS), comprising another set of aromatic residues Tyr72, Tyr124, Trp286 or Tyr341 and acidic Asp74 (Barak et al. 1994) is located at the rim of this gorge and provides a binding site for allosteric modulators and inhibitors. AChE is inhibited by a phosphorous group originating from the OP that is conjugated to the catalytic serine residue at the active site (Sussman et al. 1991).

The conjugate of the inhibited AChE may either undergo a process known as “aging” via an elimination reaction involving dealkylation, or prior to the “aging”, reactivation by nucleophiles, such as oximes (Ekstrom et al. 2006). Aging results in AChE that is refractory to reactivation. Oxime reactivators contain a quaternary nitrogen atom that promotes binding in the catalytic site of the AChE. Oximes function as nucleophiles to displace the phosphate moiety of the OP’s (prior to aging), which reacted with the enzyme’s active site serine hydroxyl group, thereby reactivating AChE (Bajgar 2004). From a mechanistic point of view, it is generally believed that the oxime will proximally orient to exert a nucleophilic attack on the phosphorus atom of the AChE-inhibitor complex and the AChE-inhibitor-complex will split leaving the regenerated AChE. Despite oximes being investigated for many years as treatments for OP poisoning, only five pyridinium oximes, pralidoxime (2-PAM, used by the U.S. Army), methoxime (MMC-4), trimedoxime (TMB-4), obidoxime (LüH-6, Toxogonin), and asoxime (HI-6) have been clinically considered to date for standard treatment (Taylor 2006, Kuca et al. 2007, 2006, 2004).

Amongst all the OP nerve agents, countering tabun (GA) and cyclosarin (GF) inhibited AChE with oxime antidotes are most challenging. Furthermore, between tabun and cyclosarin, cyclosarin is the least examined, probably because it did not receive the necessary attention until it was reported to have been stockpiled in Iraq in the early 1990s (Karasova et al. 2009). Commonly used bisquaternary reactivators for OP-inhibited AChE, such as obidoxime and 2-PAM were reported to be ineffective and many other conventional oximes were shown to be resistant to counter the toxic effect of cyclosarin (Kuca et al. 2007, 2006, Karasova et al. 2009). Although GF, chemically known as the cyclohexyl-methylphosphonofluoridate (Chart 1) is a chemical warfare agent and perhaps was used in the first Gulf War operations (Kuca et al. 2007,

2006, Karasova et al. 2009), its mechanism for antidotal efficacy still remain unclear. In recent years, many new structural analogues of currently available oximes were synthesized to improve the efficacy to counter the toxicity of GF (Karasova et al. 2009), but none were reported to be truly satisfactory. No monopyridinium oximes, including 2-PAM (pralidoxime), were found to have satisfactory reactivation efficacy against GF-inhibited AChE (Kuca et al. 2007, 2006, Karasova et al. 2009). Amongst the bispyridinium oximes, HI-6, BI-6, HS-6 and methoxime were reported to have some reactivation efficacy against GF inhibited AChE (Taylor 2006). Thus, developing new compounds to counter GF toxicity is an important research goal. From structural perspectives, the position of the oxime group in the pyridinium ring was observed to play an important role in the reactivation efficacy of oximes and in particular, the oxime group at 4-position in the pyridinium ring was found to lower the potency of reactivators to reactivate GF-inhibited AChE, whereas reactivators with the oxime group at 2-position were found to have little better efficacy (Kuca et al. 2004, 2006).

However, despite many efforts for discovery of improved reactivation therapeutics, little success has been made so far to find out truly effective broad-spectrum AChE reactivators based on oxime chemistry and structure theory. For past several decades, efforts have only led to the development of derivatives of preexisting oxime chemical structures. Compounds from new chemical classes with good blood brain barrier (BBB) penetrability have barely been explored (Bedford et al. 1986). To date, only a few BBB-penetrable oximes have been reported in the literature, however, none of them could be further developed because of low functional activity and/or significant toxicity (Bedford et al. 1986, Okuno et al. 2008, Shih et al. 2009, Skovira et al. 2010). Therefore, new strategies are needed for development of neurologic therapeutics to counter post-exposure due to nerve agent cholinesterase poisoning.

Discovery and development of new therapeutics are expensive and complex processes with ever changing technologies. On an average, it takes about 10 years and approximately five to six million U.S. dollars to bring a new effective chemical entity from the bench of discovery to the market (Kapetanovic et al. 2008). Therefore, any technology that can improve the efficiency of the process is considered highly valuable to the pharmaceutical industry.

With the advent of high speed modern computers, astronomical memory and graphic tools, accomplishing computations and visualization of structures ranging from small to large bio-molecules including proteins have become more efficient with greater precision. The graphic tools in modern computers have not only made possible visualization of three-dimensional structures of large protein molecules, but allowed interactive virtual docking experiments between potential drug molecules and the binding sites of proteins. Applications involving *in silico* techniques have now become integral part of scientific research for obtaining molecular level information about environmental, biochemical**,** and biological processes. The current advances in these methodologies have direct applications ranging from accurate *ab initio* quantum chemical calculations of stereo-electronic properties, generation of three-dimensional pharmacophores, and performance of database searches to identify bioactive agents (Kapetanovic et al. 2008).

Increasing costs for pharmaceutical development have resulted in the emergence of many assorted approaches in recent years. Among them, virtual screening of databases to identify potential new bio-active compounds has been very successful (Lyne et al. 2002). Virtual screening is a process of intelligent use of computing to analyze large databases of chemical compounds to identify potential drug candidates. The process can serve as a complimentary tool to high-throughput screening (HTS) for rapid and effective experimental assay of large pool of

compounds. Screening compounds by this method is essentially a knowledge-based approach and thus implicitly requires certain information about the nature of the receptor binding site or the nature of ligand that is expected to bind effectively at the active site. However, the type of procedure followed in virtual screening for compound databases depends upon availability of information as input and requirement for the output. Thus, if three-dimensional structure of the target enzyme or the protein is available, small molecule docking procedures can be adopted to perform structure-based virtual screening for identification of an ideal ligand. If the three-dimensional structure of the target protein is unknown, feature based pharmacophore models can be constructed from activity data of known compounds and the developed model template can be used for virtual screening to identify potential new hits. Pharmacophores may also be developed from other molecular properties, such as the ADME properties, toxicity data, lipophilicity, and drug-related properties. Identification of new bio-active compounds using *in silico* pharmacophores has shown remarkable success in recent years (Leach et al. 2010).

In pursuit of the goal for discovery of reactivators against GF-inhibited AChE, we adopted the *in silico* strategy to develop pharmacophore models (Bhattacharjee et al. 2010, 2007, 2004) and use the generated models for virtual screening of compound databases to identify potential novel active compounds. *In silico* "three dimensional pharmacophore may be perceived as an ensemble of steric and electronic features those are necessary for optimal interaction with a specific receptor to trigger or inhibit its biological response" (Leach et al. 2010). That means, the moment a receptor recognizes the optimal features of the ligand, it spontaneously triggers complementary interactions. It is usually represented by a geometric distribution of chemical features such as hydrogen bond acceptors & donors, aliphatic & aromatic hydrophobic sites, ring aromaticity, and ionizable sites in 3D space of a molecule (Gund, 2000). The advantage of the pharmacophore is that it transcends the structural class and captures features those are responsible for the intrinsic activity of potential therapeutics as new chemical class or chemo-types from searches of compound databases (Bhattacharjee et al. 2004, 2007).

In the present study, we report the first *in silico* pharmacophore model for reactivation efficacy of the oximes against GF- inhibited AChE/BuChE from published literature data (Karasova et al. 2009). Structure of ten oximes were used for developing the model that includes asoxime, obidoxime, trimedoxime, and seven K-oximes (Chart 1) from their experimental percentage reactivation efficacy data in blood plasma for cyclosarin-inhibited plasma butyrylcholinesterase (E.C. 3.1.1.8; Karasova et al. 2009). Although the model was developed solely from data on GF-inhibited BuChE plasma, it was also evaluated for fitting of the oximes in whole blood after GF intoxication of AChE (Karasova et al. 2009).

MATERIALS AND METHODS

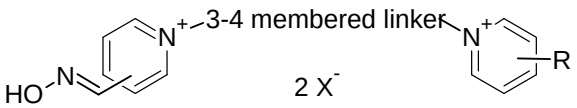
Procedure for generation of the pharmacophore model

The three-dimensional pharmacophore model for reactivation efficacy of GF inhibited AChE/BuChE in blood plasma was developed using the CATALYST 4.10 methodology (CATALYST, Accelrys Inc.). It enables the use of structure and activity data for a set of lead compounds to create a hypothesis, thus characterizing the activity of the lead set. Conformational models of molecules were generated by creating a training set of the oximes that emphasize representative coverage within a range of the permissible Boltzman population with significant abundance (within 10.0 kcal/mol) of the calculated global minimum. We selected this conformational model for pharmacophore generation within CATALYST, which aims to identify the best three-dimensional arrangement of chemical features, such as hydrophobic regions, hydrogen bond donor, hydrogen bond acceptor, and positively and/or negatively ionizable sites distributed over a three dimensional space explaining the activity variations among the training set. The hydrogen bonding features are vectors, whereas all other functions are points.

Pharmacophore generation for the ten oximes based on experimental data of GF inhibited AChE/BuChE was carried out by setting the default parameters in the automatic generation procedure in CATALYST including: function weight 0.302; mapping coefficient, 0; resolution, 287 pm, and activity uncertainty 3. Pharmacophores approximating the pharmacophore of the oximes were described as a set of aromatic hydrophobic, hydrogen bond acceptor, hydrogen bond acceptor lipid type, hydrogen bond donor, positively and negatively ionizable sites distributed over a 3D space. The statistical relevance of various generated pharmacophores was assessed on the basis of the cost relative to the null pharmacophore and the correlation coefficients. The pharmacophores were then used to estimate the activities of each of the ten oximes. These activities are derived from the best conformation generation mode of the conformers displaying the smallest root-mean square deviations (RMSD), when projected onto the hypothesis. In order to avoid chance correlation, statistical relevance of the obtained pharmacophore was assessed on the basis of cost relative to the null hypothesis and the correlation coefficient with CatScrambled confidence level of the pharmacophore (CATALYST, Accelrys Inc.). The procedure gave 85 % confidence level to the pharmacophore.

Procedure for general preparation for these compounds

The oximes used for the pharmacophore generation for reactivation efficacy of cyclosarin-inhibited AChE generally require at least one oxime group. In addition, more potent reactivators were found to require at least one oxime group at the 2-position of the pyridinium ring and a 3-4 membered linker between the two pyridinium rings (with exception for methoxime). The general basic structure for synthesis of a known cyclosarin-inhibited AChE reactivator is shown in the sketch below (oxime group may be in position 2 or 4):



RESULTS

We developed the first 3D pharmacophore model for reactivation against GF intoxication from published experimental data on GF-inhibited plasma AChE/BuChE of the oximes (Chart 1) (Karasova et al. 2009) that covered a broad range of activity, ranging from 40% to 1% reactivation potency (Table 1). The model was developed using the CATALYST pharmacophore generation methodology (CATALYST, Accelrys Inc.). The generated model contained one hydrogen bond donor function and two aromatic hydrophobic (aromatic ring) functions located in specific geometric regions surrounding the molecular space (Figure1). Although the model was developed from the experimental data for the GF-inhibited BuChE plasma, it was found to fit well onto the oxime-efficacy data for GF- inhibited AChE (Karasova et al. 2009).

Earlier reported stereoelectronic properties of some of these oximes provided guidance for generating these physico-chemical features of the pharmacophore (Bhattacharjee et al. 2010). Out of the stereoelectronic features, particularly the molecular electrostatic potential (MEP) profiles on the van der Waals surface and beyond (approximately 1.4 Å away) provided the crucial estimation of electron densities in the surrounding space of the oximes. MEP profiles provide a wealth of information about the intrinsic molecular reactivity. It is through these "interaction pharmacophore" profiles a ligand molecule interacts with the receptor. The receptor spontaneously recognizes the complementary features to promote interactions (Naray-Szabo et al. 1993, Balogh et al. 1993). These quantum chemical estimations in turn guided the selection of features for performing the pharmacophore generation in CATALYST. For example, a large extended electron density region in the surrounding space of a molecule would indicate the H-bond acceptor characteristic of a particular atom or atoms in the region, similarly the most positive potential region in a molecule would indicate the H-bond donor atom, and very weak electrostatic potential regions would indicate hydrophobic regions. Although the earlier reported stereoelectronic properties on the RHF/6-31G** optimized geometry (Bhattacharjee et al. 2010) provided guidelines for generation of the pharmacophore, it is important to note that energy minimization of a small molecule alone does not automatically stop at a local minimum of the potential energy surface if the minimum is shallow, thus leading to folding of the molecule and consequently hampering the generation of the bound conformation of a guest in the absence of its host (Wang et al. 2007). To address this possibility, molecules were mapped to the features with their pre-determined conformations generated using the "fast fit" algorithm in the CATALYST that is technically similar to the reported normal-model-analysis-monitored energy minimization procedure for generating bound conformation (Wang et al. 2007). The conformational energy for developing the set of three-dimensional conformers ranges between 0 to 20 kcal/mol. However, since one of the oximes used in our study, asoxime is reported to be highly mobile in the active site of AChE (Ekstrom et al. 2009), its reported calculated optimized RHF/6-31G** geometry was observed to be a folded conformation with intramolecular hydrogen bonding, which may not reflect the derived pharmacophore model ((Bhattacharjee et al. 2010). We performed conformation generation using the asoxime bound conformation taken from crystal structure (Ekstrom et al. 2006) to CATALYST and used the "fast" mode to develop the model. The procedure resulted in the generation of 10 alternative pharmacophores for reactivation efficacy of the inhibited enzyme for this training set. The correlation coefficients ranged from 0.91 to 0.87 for six of the ten models, while the rest four models were found to have below 0.8 correlation coefficients. The total costs of the pharmacophores varied over a narrow range (45 to 51.5 bits), and the difference between the fixed cost and the null cost is 71 bits, satisfying the acceptable range as recommended in the cost analysis. Notably, the best

pharmacophore characterized by one hydrogen bond donor function and two aromatic hydrophobic (aromatic ring) functions (Figure 1) is also statistically the most relevant pharmacophore. The predicted efficacy for the inhibited enzyme values along with the experimentally determined percentage reactivation efficacy values (Karasova et al. 2009) of the oximes are presented in Table 1. A plot of experimentally determined percentage reactivation values in the training set and their predicted values demonstrated an excellent correlation (R=0.91) within the range of uncertainty 3, indicating the predictive power of the pharmacophore (Figure 2). The statistical significance of the pharmacophores is observed to be well within the recommended range of values of the CATALYST procedure.

The observed difference of 70 bits between the fixed and the null cost clearly indicates the robustness of the correlation. Moreover, as the cost difference between the first to the tenth hypothesis and the null hypothesis was found to be between 60 and 66 bits, it could be expected that for all these hypotheses, there is about a 90% chance of representing a true correlation (R=0.91) in the data. However, to further reduce chance correlation of the best model, we performed a Fischer randomization in addition to the above statistical analysis using the CatScramble module of the CATALYST. The procedure allowed generation of 19 random spreadsheets from the training set. The statistical significance of the models was calculated. Sixteen of the randomized generated models required a total cost value lower than the model under investigation, indicating more than 85% confidence level of our pharmacophore model.

Next, the generated pharmacophore was used to estimate the activities of the individual oximes in the training set (Table 1). The potent analogs of the series map all the functional features of the pharmacophore with high scores, while the less potent compounds map fewer of the features (Figure 3). Since BI-6 and HS-6 were reported to have high reactivation efficacy for GF-inhibited AChE (Karasova et al. 2009), whereas MMC (32 % reactivation efficacy), K-33 (showed 20 %), and 2-PAM showed only 2 % efficacy toward GF-inhibited AChE/BuChE (Kuca et al. 2006, 2004; Kassa et al. 2007), we mapped the pharmacophore onto all these structures (Figure 4). The mappings indicate good fit for BI-6 and HS-6 indicating the predictive power of the model. However, the mappings onto methoxime (MMC), obidoxime, 2-PAM, K-33 and K-156 (Figure 3-4) could not well discriminate the reactivation efficacies despite the reported 32 % reactivation efficacy of MMC (Kuca et al. 2006, 2004) and varying efficacies of 5 % for K-156 (Karasova et al. 2009), 20 % for K-33 (Kassa et al. 2007), 2 % for 2-PAM (Kuca et al. 2006, 2004), and obidoxime, the well known poor reactivator of GF-inhibited AChE/BuChE (Kuca et al. 2004).

In order to further cross-validate the model, we mapped the pharmacophore onto a few down selected non-oxime compounds obtained from a sample virtual screening of Maybridge (Thermo Fisher Scientific, U.K.) and ChemNaviagtor (ChemNavigator, Inc.) databases. Five of these down selected compounds were found to have experimental reactivation efficacy within 10-fold of 2-PAM in an *in vitro* DFP-inhibited AChE assay. Surprisingly, structures of two of these non-oxime compounds (Chart 2) map well onto the present experimental reactivation efficacy model for GF-inhibited BuChE/AChE. The mappings of the model onto these two compounds, AW00554 from the Maybridge database and CNC182537715 from the ChemNavigator database are shown in Figure 5 and their predicted percentage reactivation (%) values presented in Table 1. Although the two non-oximes were not evaluated for experimental GF-inhibited BuChE/AChE so far, perfect mappings of the pharmacophore onto these non-oximes (Figure 5) indicate the potential of the model for identification, design and synthesis of novel reactivators for GF- inhibited AChE/BuChE as GF is also a G-simulator like DFP.

DISCUSSIONS

Developing a perfect model for reactivation of oximes to any OP-inhibited AChE/BuChE is a complex task. The efficacy of an oxime to reactivate GF-inhibited AChE/BuChE depends on various factors including the affinity of reactivators for the inhibited enzyme and the associated physical features that have been modeled (see section Materials and Methods). These features include, electrostatic effects, hydrophobic interactions (or more specifically aromatic hydrophobic or ring aromatic interactions), hydrogen bond donors, hydrogen bond acceptors, hydrogen bond acceptors (lipid), ionizable sites, and ring aromatic sites. Affinity also takes into account the shape, size, surface area, volume, and functional groups of the oxime. This is an important parameter for overall reactivating efficacy, because reactivation is related to binding of the oxime to the inhibited enzyme followed by breakdown of the inhibited complex (rate constant of reactivation) (Kuca et al. 2005). The series of oximes evaluated here focused on the nerve agent cyclosarin (GF). An extreme example of the importance of affinity for inhibited enzyme would be the case where an oxime, such as obidoxime (Musilek et al. 2007), has high affinity (small K_R) and reactivation efficacy for tabun (GA) inhibited enzyme, but poor (large K_R) for cyclosarin (GF) inhibited enzyme (Kuca et al. 2004). Although the oxime nucleophilicity remains essentially unchanged, obidoxime is a poor reactivator of GF because it cannot bind to the inhibited enzyme, presumably because the active site with inhibitor precludes appropriate penetration and interaction. Taken together, the pharmacophore model developed here describes the general requirements and features of an oxime (or compound with appropriate features) to not only bind to cyclosarin inhibited enzyme but also dissection of the pseudo - first order rate constant of reactivation by the nucleophilic attack of the oxime. CATALYST procedure allows developing this kind of models by placing suitable constraints on the number of available chemical features, such as aromatic hydrophobic or aliphatic hydrophobic interactions, hydrogen bond donors, hydrogen bond acceptors, lipid hydrogen bond acceptors, and ring aromatic sites, to describe the affinity for the inhibited enzyme of the compounds.

In recent years, several additional studies based on quantitative derivations (Kapetanovic et al. 2008) have shown that the pharmacophore recognition process can be analyzed from three types of three-dimensional molecular field or property based similarity studies: (1) steric, (2) electrostatic, and (3) hydrophobic. It has been well documented that bioactive agents (ligands) will bind to a receptor in a similar manner by aligning their common molecular field or property characteristics. This concept is known as bioisosterism where functional groups with similar properties are used for ligand designing and has been one of the most common practices in the discovery of new leads in pharmaceutical research (Mestres et al. 1997, Naray-Szabo et al. 1993, Balogh et al. 1993). However, virtual screening of databases using the structure based drug design approach (drug-target docking protocols) with known inhibitor bound x-ray crystal structure may have bias for down selection that often result in the identification of derivatives of the bound inhibitor (Kapetanovic et al. 2008, Leach et al. 2010). Since pharmacophore transcends the structural bias, virtual screening using this procedure has the potential for discovery of novel compounds. Nonetheless, pharmacophore approach also has its shortcomings, such as (a) the biological activity is accounted only in terms of thermodynamic equilibrium, particularly by enthalpic energy considerations assuming entropy for the molecules to be similar, (b) kinetics of the processes are ignored, and (c) transport properties, diffusion and solvent effects are largely avoided. Therefore, as a note of caution for pharmacophore based approach, the following factors should be important to keep in mind: (1) very few features of a

pharmacophore may generate too many hits, (2) too many features may miss out important hits and therefore, (3) developing several pharmacophores iteratively should help.

However, our experience (Bhattacharjee et al. 2004, Bhattacharjee et al. 2007) suggests that systematic iterative refinement of the model through virtual screening and identification of novel compounds and *in vitro* evaluations should improve the quality of the model to finally down select more efficacious novel reactivators for GF-inhibited AChE. On a different note, it is interesting to observe that despite the structural difference between MMC (which has the second aromatic ring) and 2-PAM (contain one aromatic ring), the mappings of the pharmacophore resulted similar profiles as both compounds lacked mapping of one of the two aromatic features (Figure 4). Probably this deficiency of one aromatic feature contributed to lesser efficacy for the two compounds against GF-intoxication (Kuca et al. 2006, 2004, Karasova et al. 2009). Again, obidoxime, K-33 and K-156 map all the features (Figure 3-4) yet these compounds did not show good efficacy against GF-inhibited AChE. Thus, although the oxime nucleophilicity remains essentially unchanged, many oximes are poor reactivators of GF inhibited AChE/BuChE because of inability to bind to the inhibited enzyme, presumably because the active site with inhibitor precludes appropriate penetration and interaction. However, asoxime, the most potent analog of the series showed consistency to the model and observed to be similar to the bound conformation in the crystal structure of tabun inhibited AChE (Ekstrom et al. 2006). The hydrogen bond interactions and the aromatic ring interaction together with hydrophobicity were reported to be crucial for reactivation of any OP-inhibited AChE/BuChe (Ekstrom et al. 2006). Thus, the model presented here can provide an overall view for an oxime that binds well to the OP agent inhibited AChE/BuChE, and perhaps can differentiate those that do not.

Although the pharmacophore model developed for this study was solely from structure-activity relationships and comprised of only three chemical functions localized in space, it proved to be quite predictive. It presents a powerful template for future discovery, design and synthesis of reactivators of GF-inhibited BuChe/AChE. It may be used for conducting a targeted assay of compound databases including commercial databases. Even though a high throughput *in vitro* reactivation assay for determining the AChE reactivation efficacy may be developed, time and resource costs prohibited an exhaustive search of these entire chemical databases. Thus, a rational strategy for development of a predictive pharmacophore tool should be useful to identify a limited number of down selected compounds for *in vitro* screening. The pharmacophore model template can be used to conduct an *in silico* screening of databases by generating multi-conformer format of the structures in the databases. This screen will evaluate the goodness of fit between the compounds in the chemical database and the pharmacophore model. The database search algorithm in CATALYST accounts for molecular flexibility of the compounds by considering each compound as an ensemble of conformers. For each compound, multiple conformations can be generated using the catDB utility algorithm of CATALYST, and conformational energies may be assigned with respect to an energy-minimized structure.

We are continuing to explore new strategies to translate the potent intrinsic activity of known compounds into new candidates though *in silico* modeling, identification, design and synthesis with vastly superior BBB penetration abilities to obtain broad spectrum *in vivo* efficacious novel agents to counter OP-inhibited intoxication including that of GF toxicity.

CONCLUSIONS

Our theoretical analysis of reactivation efficacy of known oximes against cyclosarin (GF) inhibited AChE/BuChE led to the generation of the first *in silico* pharmacophore model, which accounted for reactivation efficacy of several other reactivators for cyclosarin inhibited AChE and provided insights on the mechanism of AChE reactivation efficacy of the oximes. The study demonstrated how molecular characteristics of a set of oximes can be organized to be both statistically and mechanistically significant for potent reactivation efficacy that may have application for discovery of novel chemotypes for neurologic therapeutics. In addition, the model could be useful to unravel a possible rationale for the target-specific reactivation efficacy of non-oxime compounds. The chemically significant molecular characteristics disposed in a three dimensional space generated a pharmacophore that is found to be quite satisfactory in correlating true activity with the estimated activity of the compounds. One hydrogen bond donor and two ring aromatic hydrophobic sites appear to be the necessary functional features for potent reactivation efficacy against GF-inhibited AChE. From fitting of the model, two new non-oxime compounds were identified, which had shown *in vitro* reactivation efficacy within 10-fold range of 2-PAM for DFP-inhibited AChE. Since GF is also a G-simulator like DFP, the model has the potential to be a powerful template for discovery of novel reactivators against GF intoxication.

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Table 1. Experimental (taken from ref. Karasova et al. 2009) and predicted (by the present pharmacophore) percentage of reactivation for GF-inhibited AChE-reactivator (Correlation = 0.91).

Compound	Experimental percentage reactivation (%) *(ref kara)*	Predicted percentage reactivation (%) *(ref kara)*	Error*
asoxime	40.0	39.0	-1.0
trimedoxime	22.0	9.7	-2.3
K-203	7.0	7.5	1.1
obidoxime	6.0	6.2	1.0
K-156	5.0	1.8	-2.8
K-075	1.0	0.93	-1.1
K-074	1.0	1.4	1.4
K-027	1.0	2.9	2.9
K-206	1.0	1.2	1.2
K-269	1.0	1.2	1.2
AW00554	NA	9.0	NA
CNC182537715	NA	20.0	NA

* Values in the error column represent the ratio of predicted percentage reactivation to experimental percentage reactivation or its negative inverse if the ratio is less than one. Uncertainty = 3.0. (NA= not available)

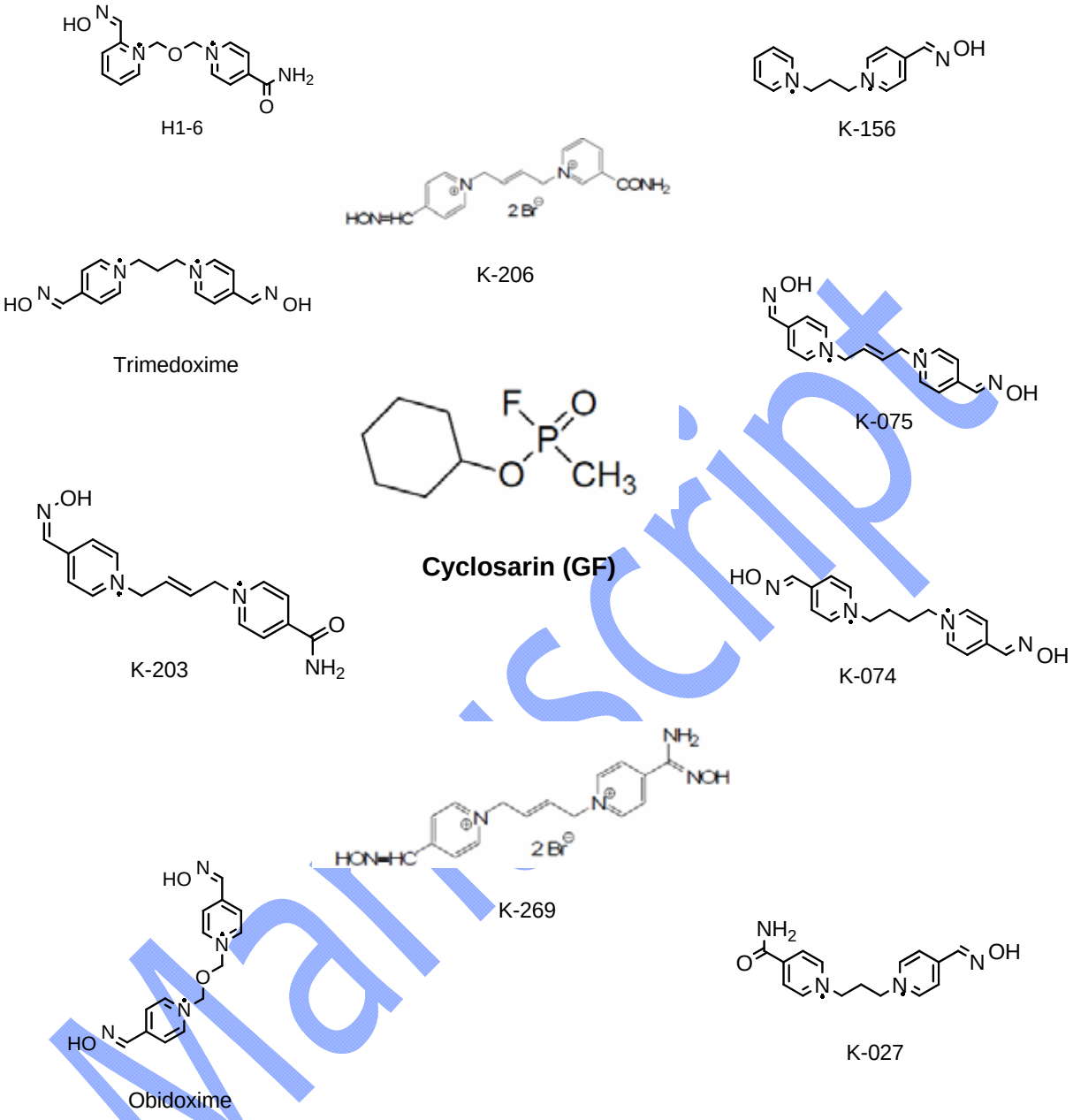
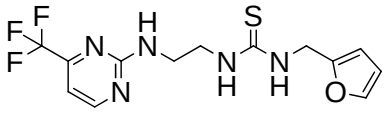


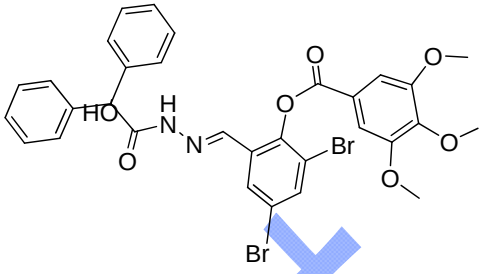
Chart 1. Structures of the oximes used for developing the pharmacophore model and structure of cyclosarin (GF).

ID# AW00554 (Maybridge)



1-(furan-2-ylmethyl)-3-(2-(4-(trifluoromethyl) pyrimidin-2-ylamino)ethyl)thiourea

CNC_ID 182537715 (ChemNavigator)



(*E*)-2,4-dibromo-6-((2-(2-hydroxy-2,2-diphenylacetyl) hydrazono)methyl)phenyl 3,4,5-trimethoxybenzoate

Chart 2. Structure of the two compounds, AW00554 (Maybridge database) and CNC182537715 (ChemNavigator database) those map well onto the model (Figure 5) and showed reactivation efficacy within 10-fold range of 2-PAM against DFP-inhibited AChE *in vitro*.

Fig. 1. Pharmacophore model for percentage reactivation efficacy of oximes against GF-inhibited AChE/BuChE based published literature data (Karasova et al. 2009).

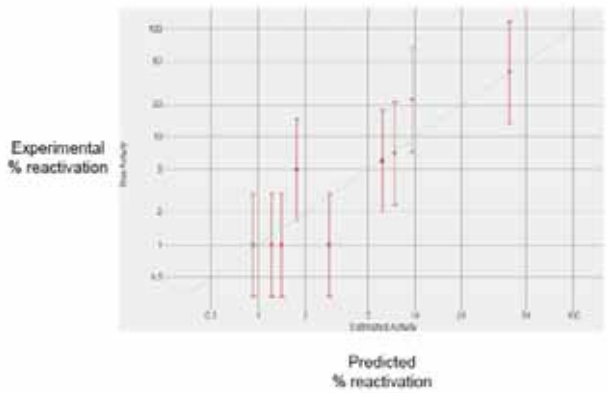


Fig. 2. Correlation (R = 0.91) between the experimental (Karasova et al. 2009) and predicted percentage reactivation efficacy of the ten oximes against GF- inhibited AChE/BuChE obtained from mapping of the pharmacophore model.

Figure 3

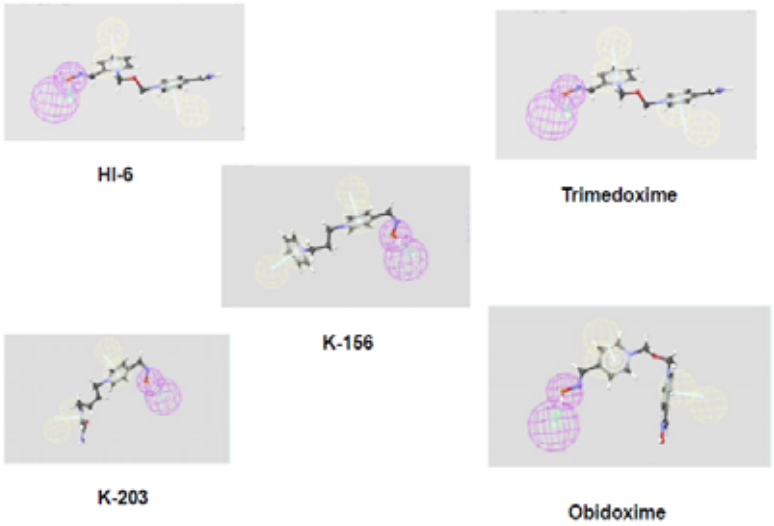


Fig. 3. Mapping of the pharmacophore onto asoxime, trimedoxime, K-203, obidoxime, and K-156 showing that all the potent analogs in Table 1 map the functional features to a certain degree.

Figure 4

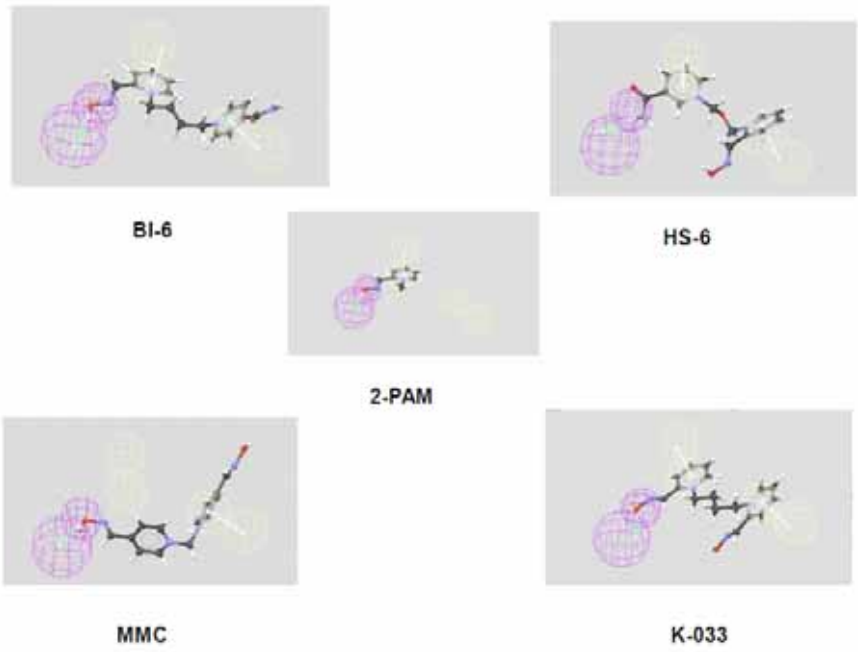


Fig. 4. Mapping of the pharmacophore onto BI-6, HS-6, 2-PAM, MMC, and K-033showing that the potent analogs map all the functional features, while the less potent compounds map fewer of the features.

Figure 5

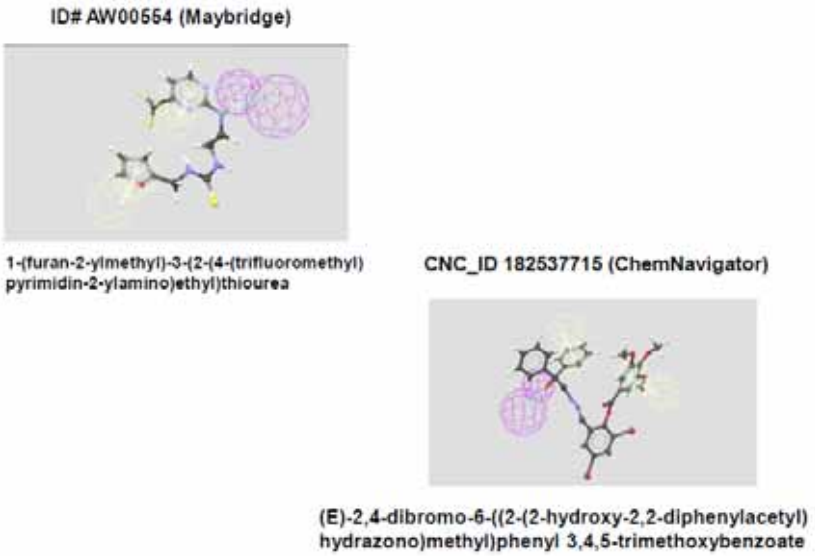


Fig. 5. Mapping of the pharmacophore onto AW00554 (Maybridge database) and CNC182537715 (ChemNavigator database) showing that the model maps well onto these structures.