DANIEL ANGELO POLISEL

CURRENT STATUS AND TRENDS OF ACETYLCHOLINESTERASE REACTIVATORS: THE TRIMEDOXIME CONTRIBUTION

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agroquímica, área de concentração Química/Bioquímica, para obtenção do título de Doutor.

Prof. Dr. Teodorico de Castro Ramalho

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CURRENT STATUS AND TRENDS OF ACETYLCHOLINESTERASE REACTIVATORS: THE TRIMEDOXIME CONTRIBUTION

STATUS ATUAL E TENDÊNCIAS DE REATIVADORES DE ACETILCOLINESTERASE: A CONTRIBUIÇÃO DA TRIMEDOXIMA

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agroquímica, área de concentração Química/Bioquímica, para obtenção do título de Doutor.

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RESUMO GERAL

Compostos Organofosforados são compostos com alto potencial de toxicidade em diversos seres vivos, especialmente em humanos. Essa classe de compostos age no sistema nervoso, inibindo a ação da acetilcolinesterase, enzima responsável pela hidrólise do neurotransmissor acetilcolina, processo natural que controla o fim da estimulação dos receptores, controlando a transmissão de impulsos nervosos. Com a inibição da acetilcolinesterase não ocorre a interrupção da interação dos receptores nervosos com a acetilcolina, gerando uma superestimulação desses, resultando em problemas de controle e coordenação de contrações musculares, conhecida como crise colinérgica. A falta de controle das contrações musculares compromete diversos sistemas do organismo, em casos de intoxicação aguda em especial o sistema respiratório, levando o indivíduo à morte por parada cardiorrespiratória. Apesar dos conhecidos riscos à saúde por parte dos compostos organofosforados, eles estão presentes na sociedade sendo amplamente utilizados na agricultura e veterinária no controle de várias classes de pestes. Além disso, ao longo da história, compostos organofosforados foram extensamente estudados e utilizados como armas químicas, sendo constatado seu uso em conflitos militares e atentados terroristas. Tendo isso em mente, a necessidade de pesquisa e desenvolvimento de antídotos para a intoxicação por esses compostos é uma demanda atual, uma vez que as atuais drogas são em geral específicas para determinados organofosforados e não existe um antídoto de amplo espectro. Este trabalho é composto por dois artigos, sendo o primeiro uma revisão bibliográfica das principais contribuições no campo da reativação da acetilcolinesterase inibida, através de diferentes classes de reativadores nos anos recentes. O segundo artigo consiste no estudo teórico, através de ferramentas de modelagem computacional do processo de reativação da acetilcolinesterase inibida tanto por representantes de armas químicas como por pesticidas, pela Trimedoxima, sendo observado bom potencial para a reativação da enzima inibida por VX. Além disso, são discutidas as contribuições dos parâmetros termodinâmicos que governam o processo de reativação por esta oxima, tendo como objetivo avançar no entendimento do processo de reativação da acetilcolinesterase inibida por diferentes organofosforados na busca por um reativador de amplo espectro.

Palavras-chave: Organofosforados. Química Computacional. Trimedoxima. Reativação AChE.

GENERAL ABSTRACT

Organophosphorus compounds are compounds with high potential for toxicity in many living beings, especially in humans. These compounds class acts on the nervous system, inhibiting the acetylcholinesterase function, the enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine, a natural process that controls the end of receptors´ stimulation, controlling the nerve impulses transmission. With the acetylcholinesterase inhibition, there is no interruption of the nerve receptors interaction with acetylcholine, generating an overstimulation of these, resulting in control and coordination of muscles contractions trouble, known as cholinergic crisis. The lack of muscle contractions control compromises several body systems, in cases of acute intoxication, especially the respiratory system, leading the individual to death due to cardiorespiratory arrest. Despite the known health risks of organophosphorus compounds, they are present in society and are widely used in agriculture and veterinary medicine on several classes of pests controlling. In addition, throughout history, organophosphorus compounds have been extensively studied and used as chemical weapons, and their use has been confirmed in military conflicts and terrorist attacks. Bearing this in mind, the need for research and development of antidotes for intoxication by these compounds is a current demand, since current drugs are generally specific for certain organophosphorus and there is no broad-spectrum antidote. This work consists of two articles, the first being a literature review of main contributions in the inhibited acetylcholinesterase reactivation field, through different reactivators classes in recent years. The second article consists on the theoretical study, through computational modeling tools, of acetylcholinesterase inhibited reactivation process by both chemical weapons and pesticides representatives, through Trimedoxime, with results showing a good reactivation potential for the enzyme inhibited by VX. Furthermore, the thermodynamics parameters that govern the reactivation process contributions, through Trimedoxime, were discussed, aiming to advance in the understanding of the acetylcholinesterase inhibited reactivation process by different organophosphorus in the search for a broad-spectrum reactivator.

Keywords: Organophosphorus. Computational Chemistry. Trimedoxime. AChE Reactivation.

Sumário

PRIMEIRA PARTE

1 - INTRODUÇÃO

O fósforo é um elemento muito presente em várias áreas do cotidiano, como na medicina, na agricultura e na área de engenharia de materiais. É constituinte de uma série de moléculas biológicas, como parte de membranas celulares, participa do metabolismo de glicídios, presente em nucleotídeos, entre outras funções. Uma aplicação desse elemento, é em sua forma pentavalente, ligado à cadeias carbônicas formando compostos organofosforados (OP), tendo várias aplicações, como por exemplo pesticidas e fármacos (FU *et al.*, 2020; JONES; O'LEARY; O'SULLIVAN, 2020).

Com o rápido crescimento da população mundial, surge a demanda do aumento da produção de alimentos. O uso de pesticidas acaba sendo uma alternativa eficiente nesse contexto, para se obter maior produtividade, uma vez que as áreas de plantio adequadas no planeta são limitadas. Pesticidas OP vem sendo utilizados desde a década de 60, sendo que atualmente estima-se que essa classe de compostos seja responsável por 38% do mercado mundial de produtos para controle de pestes (PUNDIR; MALIK; PREETY, 2019; SARLAK *et al.*, 2021). Apesar da necessidade do uso de pesticidas a base de OP, esses compostos podem ser extremamente prejudiciais à saúde. O uso inadequado desses produtos, devido à sua persistência no ambiente, podem contaminar o solo, mananciais, acumular em alimentos e com isso, por alguma cadeia de abastecimento, causar o envenenamento por OP (LIU *et al.*, 2020).

Na década de 30, o cientista alemão Schrader, pesquisava o uso de OP para o controle de pestes, mas devido à um acidente de laboratório descobriu-se o grande efeito nocivo que essa classe tinha sobre o sistema nervoso, despertando assim o interesse do uso bélico de tais compostos. Surgiram a partir daí diversos OP para serem utilizados como armas químicas de guerra, também chamados de agentes neurotóxicos, exemplos como Sarin, Tabun, VX, entre outros. Alguns desses compostos já foram utilizados em ocasiões ao longo da história como conflitos armados e ataques terroristas (GUSAROVA; TROFIMOV, 2020).

O envenenamento por OP ocorre pela inibição da enzima Acetilcolinesterase (AChE), enzima responsável pelo processo de transmissão dos impulsos nervosos. A inibição da AChE, gera uma superestimulação do sistema nervoso, chamado de crise colinérgica, responsável por vários sintomas como excesso de lacrimação, diarreia, perda

do controle das contrações musculares, especialmente do sistema respiratório, que em casos de envenenamento agudo, leva o indivíduo à morte por parada cadiorrespiratória (MUKHERJEE; GUPTA, 2020; PANNU *et al.*, 2020).

Devido à rápida ação dos agentes neurotóxicos, o tratamento deve ser iniciado rapidamente. O atual tratamento consiste em duas linhas terapêuticas concomitantes, a aplicação de uma droga para competir com a acetilcolina (ACh) pelo sítio dos receptores nervosos, como por exemplo a atropina para interromper a superestimulação, juntamente com o uso de uma substância reativadora da função enzimática da AChE, como as oximas. As oximas são uma classe de compostos químicos, que devido à sua nucleofilicidade, são capazes de remover o OP do sítio ativo da AChE, num processo chamado de reativação (AMEND *et al.*, 2020).

Devido à bons resultados, a classe das oximas é muito estudada, contando atualmente com uma grande gama de estruturas propostas, por exemplo oximas mono-piridínicas, bipiridinícas, sem anéis piridínicos, dentre outras variações estruturais. Apesar do grande número de pesquisas a respeito do assunto e o grande número de oximas desenvolvidas, não existe um reativador universal, demandando assim novas pesquisas buscando reativadores mais eficientes com amplo espectro de ação e baixa toxicidade (AMEND *et al.*, 2020).

O objetivo deste trabalho é fazer uma revisão da literatura das principais classes de reativadores, além de testar a atividade *in vitro* da Trimedoxima frente a AChE inibida por diferentes OP e juntamente com ferramentas de modelagem computacional, avaliar teoricamente os parâmetros termodinâmicos que regem a reação de reativação desses sistemas.

2 - REFERENCIAL TEÓRICO

2.1 - Compostos Organofosforados

Os estudos dos compostos envolvendo a ligação fósforo-carbono tem registros desde a Idade Média. Estudos mais consistentes sobre as propriedades dessa classe de compostos foram ganhando destaque a partir do século XIX, alguns exemplos são em 1820 por Lassaigne, estudando a esterificação de ácido fosfórico, em 1845 Thinard e colaboradores estudaram uma série de derivados de fosfinas, o que deu base para um rápido avanço dos estudos na área. A segunda metade do século XIX teve ícones como Michaelis e Arbuzov dando contribuições principalmente no estudo de compostos de fósforo trivalente. Futuramente, o cientista alemão Schrader e colaboradores descobririam e estudariam as propriedades tóxicas de OP em animais, trabalhos precursores de atuais usos industriais, agropecuários e bélicos destes compostos (KOSTOUDI; PAMPALAKIS, 2022; SANTOS *et al.*, 2007).

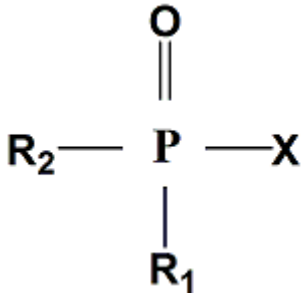
O átomo de fosforo é polarizável, com baixa a média eletronegatividade, geralmente com valência igual a três ou cinco. Quando pentavalente normalmente faz três ligações simples e uma ligação dupla com Oxigênio ou outro átomo bivalente. Os compostos fosforados são facilmente oxidados por oxigênio, ozônio, peróxidos e outros oxidantes (WANG, J. J. *et al.*, 2020). O átomo de fósforo faz ligações muito estáveis com átomos de carbono, com isso, diversos compostos de fósforo são precursores à moléculas essenciais à vida, como constituintes do protoplasma, ácidos nucléicos, coenzimas, nucleotídeos, intermediários metabólicos e fosfatídeos (SANTOS *et al.*, 2007).

Como citado anteriormente, OP vem sendo amplamente utilizados na sociedade em diversas áreas como indústria, veterinária, medicina, agricultura e usos militares (KUMAR; JOSHIBA, 2021). Porém o uso inadequado desses compostos é altamente nocivo à saúde, além de serem potenciais contaminantes ambientais, por exemplo no uso não planejado na agricultura. Segundo a Organização Mundial da Saúde, todo ano ocorrem por todo o planeta milhões de casos de intoxicação por OP, além de aproximadamente 200.000 mortes. A maioria desses casos se deve ao envenenamento ocupacional e à tentativas de suicídio (BRENET *et al.*, 2020; HULSE *et al.*, 2020). Além

do uso na agricultura, OP são a estrutura base para diversos agentes químicos de guerra. Tais agentes químicos de guerra, como Sarin, Tabun e VX, foram empregados ao longo da história em conflitos armados, por exemplo na Síria em 2013 e em alguns atentados terroristas, como no Japão em 1995 e o recente assassinato do irmão do ditador Norte-Coreano Kim Jong-nam (MATULA *et al.*, 2020; MUKHERJEE; GUPTA, 2020).

A estrutura geral dos OP está representada na Figura 1, em que R1 e R2 podem ser radicais arila, alquila, ariloxila ou alcoxila e X grupos variados, como halogênios, alifáticos, aromáticos, etc. (BORNEMANN *et al.*, 2020).

Figura 1 - Estrutura geral dos OPs.



Fonte: (ALVIM *et al.*, 2014).

2.2 - Armas Químicas

Armas químicas de guerra são definidas como compostos químicos cujas propriedades tóxicas são utilizadas com a finalidade de matar, ferir ou incapacitar seres humanos, normalmente associados à operações militares e atos terroristas (SIERRA; MARTÍNEZ-ÁLVAREZ, 2020).

Apesar de algumas descobertas consideráveis de novas substâncias para usos bélicos ao longo dos séculos XVIII e XIX, foi durante a 1ª Guerra Mundial que aconteceu um grande marco na história das armas químicas, em seu uso para destruição em massa. Em 1914, o cientista Fritz Haber do Instituto de Física de Berlim, criava o conceito de nuvem de gás tóxico. Devido à escassez de granadas, Haber pensou que uma nuvem de gás químico poderia incapacitar os combatentes inimigos sem a utilização de explosivos, além disso, a liberação do gás se dispersaria por uma área muito mais ampla do que ataques de artilharia. Devido à sua abundância na indústria alemã, o gás cloro foi selecionado para tal finalidade (SMART, 1997). Em abril de 1915, ocorreu o primeiro

ataque alemão utilizando gás cloro em Ypres, na Bélgica. Após cerca de 10 minutos 1000 soldados franceses haviam morrido e outros 4000 foram incapacitados. O uso dessa tecnologia foi considerado um grande marco nas guerras, talvez o mais importante desde a invenção da pólvora (FITZGERALD, 2008).

Devido ao sucesso da nova forma de ataque, o período entre as grandes guerras foi um momento de grande mobilização, tanto para o desenvolvimento de antídotos como para o desenvolvimento de novos agentes químicos. Na década de 1930, o alemão Schrader e colaboradores pesquisando compostos organofosforados com a finalidade de seu uso na agricultura, visando o aumento da produção de alimentos para a recuperação da Alemanha pós 1ª Guerra Mundial, acabou acidentalmente descobrindo o grande efeito que tal classe de compostos tinham sobre os seres humanos (RANCOURT *et al.*, 2020).

2.2.1 - OP como Armas Químicas

Em 1936, o químico alemão Dr. Gerhard Schrader obteve o primeiro composto organofosforado, que foi relatado para a Seção de Armas Químicas das forças armadas alemãs. Os militares alemães ficaram impressionados com os efeitos produzidos no sistema nervoso pelo novo composto e o recomendaram para novas pesquisas. Tal composto foi denominado Tabun (SZINICZ, 2005). Em meados de 1938, Schrader desenvolve um agente similar, o Sarin, cinco vezes mais tóxico que o Tabun. Nesse cenário, os militares alemães recrutaram diversos químicos para desenvolverem novos agentes neurotóxicos e começaram a construir uma planta piloto para produzir estes compostos em 1939 (SMART, 1997).

Apesar de todos esses eventos, a 2ª Guerra Mundial não tem registros de utilização significativa de armas químicas de guerra, nem mesmo pela Alemanha, mesmo possuindo grandes estoques desses compostos. Não é claro o motivo do não uso, mas especula-se que seria pelo medo da tecnologia de produção dos novos compostos neurotóxicos estar em posse dos Aliados após avanços sobre o território alemão (PITA, 2008; SMITH, 2008).

Após o término da guerra, houve a descoberta de várias instalações com grandes estoques de agentes químicos e vários cientistas alemães foram capturados, juntamente com documentos que relatavam a existência dos agentes neurotóxicos Sarin, Tabun e

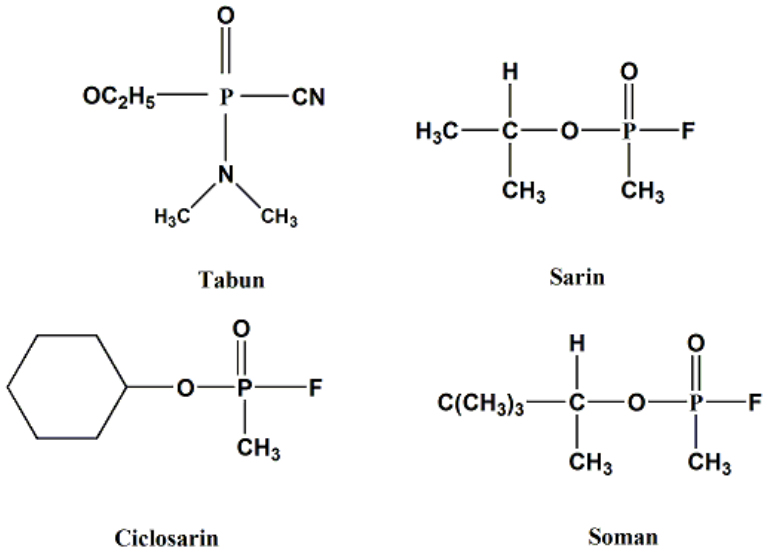
Soman, além de informações sobre a existência de uma substância que combatia os efeitos desses compostos, a atropina (FITZGERALD, 2008; PITA, 2008).

Ao longo dos anos, como muitos países possuíam a tecnologia de produção dos agentes neurotóxicos, existiram ocasiões em que houve a eminência do uso dos agentes químicos; porém, por medo de retaliações, em poucas ocasiões os compostos foram realmente postos em ação. Alguns casos que valem a pena ressaltar são os atentados terroristas nos metrôs do Japão em 1994 e 1995, de autoria de um grupo religioso conhecido como Aum Shinrikyo (Verdade Suprema), que utilizaram o VX. O ataque de 1994, ocorrido na cidade de Matsumoto, deixou 8 mortos e 200 feridos. Já o ataque de 1995, ocorrido na capital Tóquio, atingiu 5 vagões de um metrô, resultando em 11 mortes e aproximadamente 5000 hospitalizados (SAKURADA; OHTA, 2020; SUGIYAMA *et al.*, 2020).

Já no século XXI, alguns casos que se tem conhecimento do uso de OP são o uso de gás Sarin na Guerra Civil da Síria em 2013, onde estima-se a morte de 1300 pessoas, dentre elas grande número de civis, e o assassinato do irmão do ditador norte-coreano, Kim Jong-nam em 2017 na Malásia, utilizando o composto VX (AMEND *et al.*, 2020; MUKHERJEE; GUPTA, 2020).

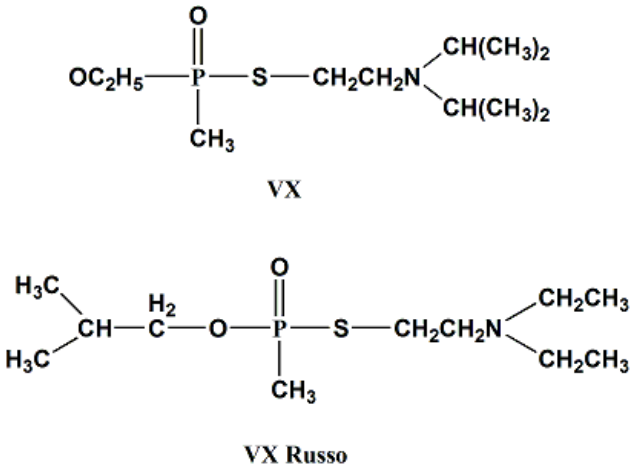
Historicamente, os agentes neurotóxicos organofosforados são classificados basicamente em dois grupos: a série G, em que a letra é devido ao país de origem, do inglês *Germany*, tendo como os representantes mais famosos o Tabun (GA), Sarin (GB), Soman (GD) e Ciclosarin (GF) (Figura 2); o segundo grupo é a série V, cuja literatura relata referir-se a palavras inglesa *Venenous,* devido à alta toxicidade desse grupo, tendo como os principais representantes o VX e o VX Russo (VR) (Figura 3) (NORRRAHIM *et al.*, 2020).

Figura 2 - Estruturas dos principais agentes neurotóxicos da série G.



Fonte: (RAMALHO *et al.*, 2016).

Figura 3 - Estruturas dos principais agentes neurotóxicos da série V.



Fonte: (RAMALHO *et al.*, 2016).

Recentemente, surgiram eventos do uso de um novo grupo de agentes neurotóxicos creditado aos russos, denominado Novichok. O caso mais famoso é a tentativa de assassinato ao antigo espião russo Sergei Skripal em 2018, no reino unido. Foi relado que na ocasião, além de de Skripal e sua filha, vários civis na cidade

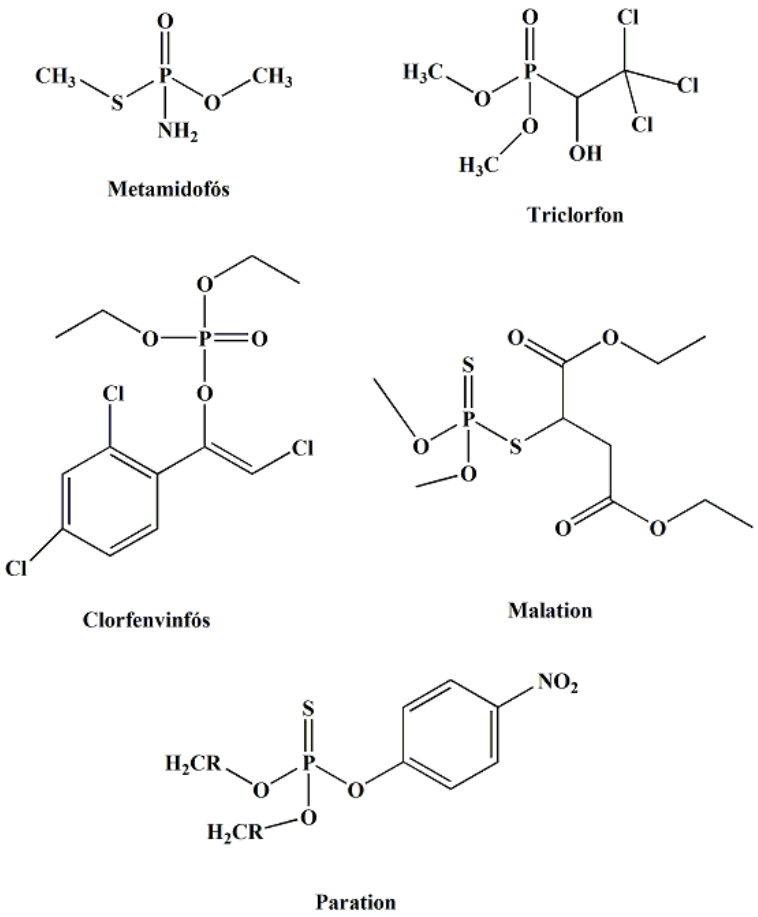
apresentaram sintomas de envenenamento pelo composto (HARVEY; MCMAHON; BERG, 2020; STEINDL *et al.*, 2021).

Apesar dos esforços da Organização para Proibição de Armas Químicas (OPCW) agindo na destruição de estoques existentes e controlando a produção de potenciais compostos e precursores de agentes neurotóxicos, sabe-se que estoques de Sarin e VX existem em 6 bases continentais dos EUA, além de outros agentes químicos de guerra. Declaradamente países como Índia, Iraque, Japão, Rússia, Síria, Israel, Coreia do Norte, entre outros possuem estoques de armas químicas (MUKHERJEE; GUPTA, 2020).

2.3 - Compostos Organofosforados na Agricultura

Como já citado anteriormente, o uso de OP como armas químicas foi originado a partir de estudos para seu uso como pesticida. Apesar do risco à saúde , OP tem sido extensivamente utilizados na agricultura nas últimas 5 décadas (PUNDIR; MALIK; PREETY, 2019). Dentre a variedade de usos como pesticidas estão a aplicação como inseticidas, anti-helmínticos, antinematóides, fungicidas e herbicidas, além de ter uso no controle de ectoparasitas em algumas espécies domésticas. Alguns exemplos de OP disponíveis no mercado são metamidofós, triclorfon, clorfenvinfós, malation e paration, representados na Figura 4. Estima-se que atualmente cerca de 38% dos pesticidas comercializados no mundo são OP (MALAKOOTIAN *et al.*, 2020).

Figura 4 - Principais representantes dos OP pesticidas.



Fonte: (JACQUET *et al.*, 2016).

Pesticidas são essenciais para o controle de pestes possibilitando o aumento da produção agrícola, visto o crescente aumento da demanda de alimentos no mundo. Com isso, pesticidas OP se tornam amplamente utilizados, devido a fatores como baixo custo, fácil síntese e alta eficiência no controle de pestes (CHU *et al.*, 2020). Porém, com a grande comercialização e utilização desses produtos surgem problemas de saúde pública, como o grande número de casos de envenenamento, seja de forma acidental devido à má manipulação ocupacional, seja por tentativas de suicídio, que são uma grande parte desses casos de envenenamento (AKHTAR *et al.*, 2020).

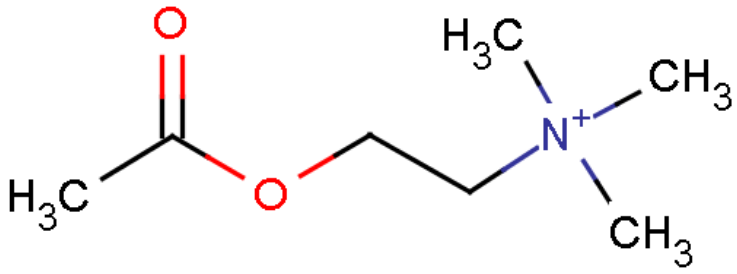
Além disso, alguns desses pesticidas e seus metabólitos são persistentes no meio ambiente, podendo contaminar solo, mananciais, acumular em alimentos e água de

sistemas de abastecimento urbanos, consequentemente, vir a contaminar os seres humanos. (MATULA *et al.*, 2020; WANG, J. Y. *et al.*, 2020).

2.4 - Acetilcolina

A ACh (Figura 5) é um neurotransmissor encontrado em vertebrados e artrópodes; é um dos principais compostos responsáveis pela sinalização do nervo para o músculo nas sinapses, designadas junções neuromusculares. No entanto, além da sua função no sistema nervoso periférico (SNP), também tem um papel importante no sistema nervoso central (SNC), no qual está envolvida a memória e aprendizagem (VENKATESAN *et al.*, 2020).

Figura 5 – Estrutura ACh.



Fonte: (FRANJESEVIC *et al.*, 2019).

A ACh é responsável pelo estímulo de dois receptores, muscarínicos e nicotínicos, pressentes no SNC e SNP. Os receptores muscarínicos estão, principalmente, associados com o SNP e com músculos lisos e cardíacos. O efeito da ligação com ACh está geralmente associado com a estimulação do sistema nervoso parassimpático (SUZUKI; MOMIYAMA, 2021). Os receptores nicotínicos encontram-se no SNC e na placa motora terminal (EPP), que são as sinapses entre os nervos e músculos esqueléticos. Com o avanço do Mal de Alzheimer, há a perda de receptores nicotínicos, constituindo evidências de um papel desses receptores na deficiência de cognição e memória (DONG *et al.*, 2021).

Quimicamente, a ACh é um éster de colina (Ch), sendo sintetizada no terminal pré-sináptico, a partir de uma molécula de Ch e uma molécula de acetil-coenzima A, reação catalisada pela enzima colina-acetiltransferase (ChAT). Estímulos nervosos

desencadeiam a liberação da ACh na fenda sináptica por exocitose. ACh liga-se aos seus receptores, desencadeando uma série de reações intracelulares, sendo em seguida hidrolisada em Ch e acetato pela AChE, interrompendo a estimulação dos receptores nervosos. Posteriormente, a Ch é reutilizada na síntese de novas moléculas de ACh e armazenada em vesículas (ROBERTS *et al.*, 2021).

2.5 - Acetilcolinesterase

A AChE é a enzima responsável pela hidrólise rápida do neurotransmissor ACh, reação que gera o impulso nervoso, que pode ser observado na seguinte reação (Equação 1):

$$CH_3COOCH_2CH_2N^+(CH_3)_3 + AChE \rightarrow CH_3CO{-}AChE + HOCH_2CH_2N^+(CH_3)_3 + H_2O \rightarrow CH_3COO^- + H^+ + AChE \quad \textbf{(1)}$$

A AChE é a principal enzima pertencente à família das colinesterases, sendo responsável pela finalização da transmissão dos impulsos nervosos nas sinapses colinérgicas. A AChE está presente no SNC e SNP (GORECKI *et al.*, 2020)

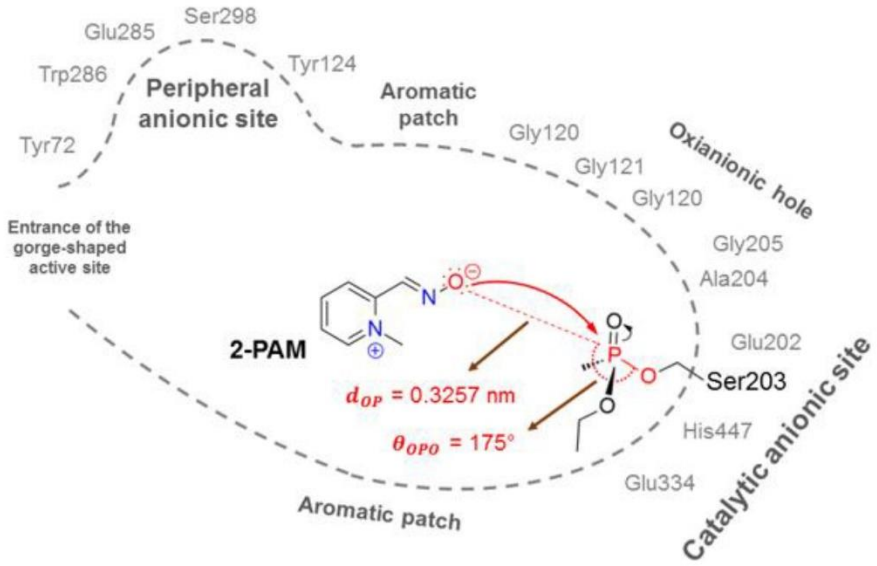
No SNP, AChE é responsável pela modulação dos impulsos nervosos que controlam os batimentos cardíacos, pela dilatação dos vasos sanguíneos e pela contração dos músculos lisos, enquanto que, no sistema nervoso central, ela está envolvida no controle motor, na cognição e na memória. O excesso de impulsos nervosos pode causar problemas, e por isso é importante que o processo de interação da ACh com o receptor seja interrompido (LENINA *et al.*, 2020).

A ACh interage com os sítios aniônicos e esterásicos da AChE, formando o complexo enzima-substrato. Então, a Ch é liberada, restando a enzima acetilada. Uma molécula de água é hidrolisada por essa enzima acetilada, formando acetato e regenerando a AChE. Os OP agem se ligando ao sítio esterásico da AChE, inibindo a capacidade catalítica da enzima, resultando em um acúmulo do neurotransmissor nos receptores e com isso, um excesso de estímulos nervosos, promovendo os sintomas da intoxicação (ARAÚJO *et al.*, 2020). Os sintomas da intoxicação dependem da dose a qual o indivíduo sofreu exposição e o tipo de organofosforado, resultando em distúrbios de numerosas

funções, tais como a paralisia das funções neuromusculares e, finalmente, parada respiratória e morte (LENINA *et al.*, 2020; WOREK *et al.*, 2004)

Além do sítio aniônico catalítico, existe um segundo sítio aniônico, conhecido como sítio aniônico periférico (Figura 6). Esse sítio, identificado com base na ligação de compostos bis-quaternários, pode estar envolvido na ação de alguns inibidores da enzima ou na inibição por excesso de substrato. Esse sítio pode ser alvo de inibidores, que impediriam a saída do produto de reação (SHAIKH *et al.*, 2020).

Figura 6 - Esquema da cavidade do sítio ativo da AChE e suas principais regiões.



Fonte: (SILVA *et al.*, 2020)

Em humanos, o sítio oxianiônico consiste em glicinas e uma alanina (Gly116, Gly117 e Ala199). Esses três resíduos peptídicos formam ligações de hidrogênio com o intermediário acil-AChE, que é formado durante o processo catalítico da ACh, estabilizando-o. O sítio aniônico de ligação ao substrato é geralmente composto por resíduos aromáticos, como triptofano e fenilalanina (Trp82 e Phe339), que possuem uma pequena carga negativa por meio da qual se ligam os amônios quaternários por interações π-cátion. O local de ligação à acila é composto por Leu286 e Val288, desempenhando um

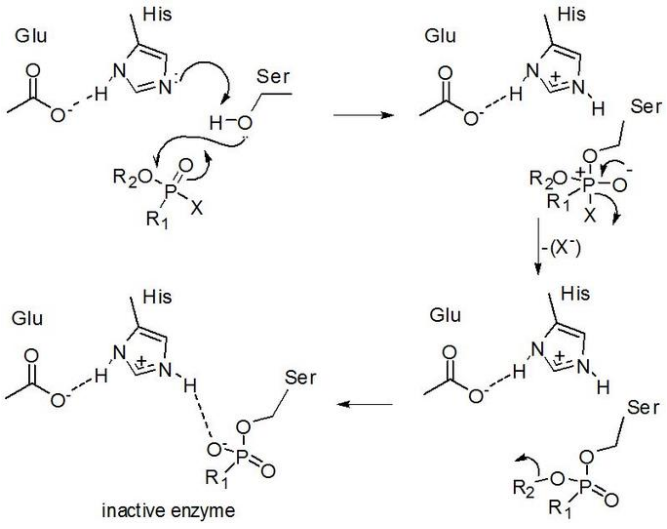
papel importante na limitação da dimensão de substratos que são capazes de entrar no sítio ativo (CAVALCANTE *et al.*, 2020; SHAIKH *et al.*, 2020).

A principal dificuldade em combater as intoxicações por OP é a baixa eficiência dos antídotos disponíveis, sendo que atualmente não existem antídotos universais ou de amplo espectro de ação. O protocolo corrente para o tratamento para envenenamento por OP inclui três estratégias: primeiro, a administração de um receptor antagonista muscarínico da ACh como por exemplo a atropina, para boquear a superestimulação dos receptores colinérgicos pela ACh; segundo, aplicação de um reativador com a função de remover o OP da enzima inibida, regenerando sua função catalítica; e terceiro, uma droga anticonvulsivante (ACHARYA *et al.*, 2011; DHANARISI *et al.*, 2020). Uma motivação para novos estudos é a descoberta de novos reativadores, com maior eficiência e com maior espectro de atividade.

2.6.1 - Envenenamento por OP

Os OP têm o poder de inibir a AChE, isso ocorre devido à formação de uma ligação covalente entre o átomo de fósforo do OP e um resíduo de serina do sítio ativo da enzima, ligação de hidrólise espontânea muito lenta, podendo considerar a reação de formação dessa ligação praticamente irreversível (Figura 7) (ALEX; MUKHERJEE, 2021).

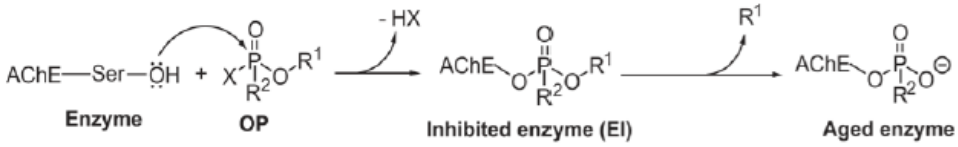
Figura 7 – Esquema geral do processo de inibição da AChE por OP.



Fonte: (SANTOS *et al.*, 2007).

Após a formação do complexo enzima-OP, uma reação secundária pode ocorrer dependendo do OP, que é a dealquilação espontânea da serina fosforilada, processo conhecido como envelhecimento. A forma envelhecida da enzima resulta na formação de um complexo estável fosfonil-AChE carregado negativamente impedindo o ataque nucleofílico por reativadores, com a liberação de um radical R, como mostrado na Figura 8 (BADAWY, 2021; FIGUEROA-VILLAR *et al.*, 2020; WARD, 2019).

Figura 8 - Esquema geral do processo de envelhecimento.



Fonte: (RAMALHO *et al.*, 2016)

2.7 - Reativação

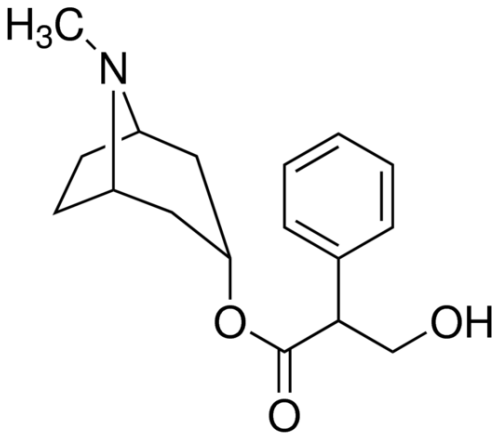
Como descrito nos tópicos anteriores, OP inibem a função catalítica da AChE pelo processo de fosforilação. Com a formação da ligação covalente entre o fósforo do OP e o oxigênio do resíduo de serina, o sítio esterásico fosforilado sofre regeneração hidrolítica com uma velocidade muito lenta. Pesquisas mostraram que grupos nucleofílicos como as oximas, hidroxilaminas e os ácidos hidroxâmicos são capazes de reativar a enzima com maior velocidade do que a hidrólise espontânea. Também foi constatado que a AChE, com sua capacidade de esterase perdida, poderia ser restaurada por compostos que deslocassem o grupo fosforil da enzima. A reativação seletiva da AChE poderia ser alcançada por um nucleófilo, contendo um nitrogênio quaternário que interagisse com o subsítio negativo do centro ativo, deixando este nucleófilo em posição oposta ao átomo de fósforo (SEMENOV *et al.*, 2020; SHIH *et al.*, 2021; SILMAN; SUSSMAN, 2005).

O principal tratamento para o envenenamento por OP é a combinação de alguns fármacos, com o intuito de diminuir os efeitos do acúmulo do neurotransmissor nas terminações nervosas e reativar a enzima fosforilada. Geralmente, indivíduos que apresentam uma sintomatologia característica de síndrome colinérgica são submetidos à combinação de fármacos, sendo uma substância antagonista à ACh, tal como o sulfato de

atropina, e um reativador da enzima AChE inibida, usualmente um composto da classe das oximas (CHAMBERS; MEEK, 2020; SAVALL *et al.*, 2020).

A atropina (Figura 9) age competindo com a ACh pelos receptores nervosos, inibindo a ação desta sobre o órgão efetor. Esta competição ocorre preferencialmente nos receptores colinérgicos muscarínicos. Desta forma, a atropina reverte apenas sintomas muscarínicos e deve ser administrada assim que se suspeitar o diagnóstico de intoxicação por compostos OP (SAMPRATHI *et al.*, 2020).

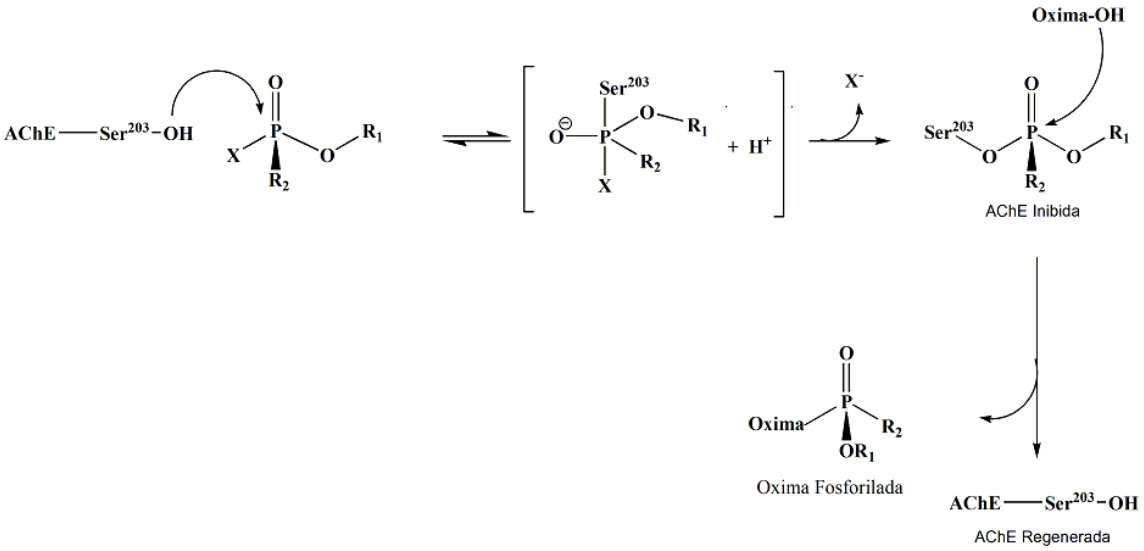
Figura 9 – Fórmula **e**strutural da Atropina.



Fonte: (CANAES; FATIBELLO-FILHO, 2006).

Já as oximas têm a propriedade de reativar a AChE inibida através de um ataque nucleofílico ao átomo de fósforo do OP, através do mecanismo apresentado na Figura 10. Algumas oximas possuem um nitrogênio quaternário que se liga diretamente ao sítio aniônico da AChE, deslocando a ligação dos OP junto ao sítio esterásico por serem doadores de próton H^+, estabelecendo ligação oxima-OP e reativando a enzima (CHAMBERS; DAIL; MEEK, 2020; MACHAMER *et al.*, 2020).

Figura 10 - Esquema geral de reativação por oximas.



Fonte: (POLISEL *et al.*, 2019).

2.7.1 - Oximas

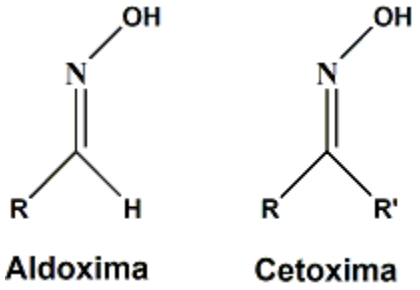
Na década de 1950, a atropina era o único antidoto disponível para tratamento por envenenamento por OP. Descobriu-se um tempo depois que a hidroxilamina serviria como remediação para OP em experimentos *in vitro*, conseguindo a primeira reação de reativação conhecida da AChE inibida, sendo que até então esse processo era considerado irreversível. Em 1955, as oximas foram identificadas como antídotos específicos mais eficientes que as hidroxilaminas (EYER; WOREK, 2007; STOJILJKOVIĆ; JOKANOVIĆ, 2006).

Ácidos hidroxâmicos e oximas são descritos como potenciais reativadores da AChE inibida. Observa-se um uso crescente de compostos contendo piridina quaternizada como reativadores da AChE. Ainda que a literatura reporte eficiência de reativação maior que 60% para alguns destes compostos e derivados, a ação contra OP específicos e a sua toxicidade têm sido fatores limitantes no processo (CHAMBERS; DAIL; MEEK, 2020; MACHAMER *et al.*, 2020).

Oximas geralmente são obtidas por uma reação de condensação entre uma substância carbonilada e uma hidroxilamina. O termo oxima foi primeiramente definido no século XIX e deriva da contração das palavras oxigênio e imina (**ox**igênio + **im**in**a**) = oxima (CISTRONE *et al.*, 2020).

As oximas são compostos químicos que obedecem à fórmula molecular geral R'RCNOH, conforme a Figura 11. Dependendo dos radicais R e R', as oximas podem ser classificadas como aldoximas ou cetoximas. Elas se diferenciam pelos radicais, quando um dos radicais é substituído por um hidrogênio a molécula é classificada como aldoxima, quando ambos os radicais são cadeias carbônicas a molécula é classificada como cetoxima. As oximas geralmente são sólidos cristalinos, pouco solúveis em água e derivados da condensação de uma porção hidroxilamida com um aldeído ou uma cetona por catálise ácida (GRITZAPIS *et al.*, 2020).

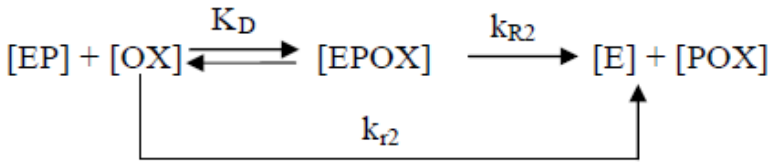
Figura 11 – Estrutura geral das oximas.



Fonte: (DEBNATH, 2018).

O processo de reativação ocorre através de duas etapas. A primeira é a formação de um complexo reversível AChE-fosfil-oxima (quando inibido por OP), com estado de transição penta-coordenado, do tipo Michaelis, seguido do deslocamento do fosfil do estado de transição. O K_D aproxima-se da constante de dissociação, a qual é inversamente proporcional à afinidade da oxima pela AChE fosforilada, e então a reatividade pode ser expressa em função da constante k_{r2}. A constante k_{r2} da taxa de reação de segunda ordem pode ser obtida pela razão $kr_2 = k_{R2}/ K_D$, como mostra a Figura 12 (EYER; WOREK, 2007).

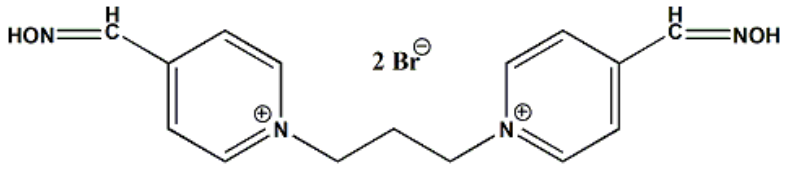
Figura 12 - Esquema da reação de reativação da AChE por uma oxima.



Fonte: (MATOS *et al.*, 2011).

As principais oximas piridínicas que são disponibilizadas atualmente para uso clínico no tratamento de intoxicação por OP são: Pralidoxima, Trimedoxima, Abidoxima, Asoxima e HLo-7. No Brasil, a Pralidoxima é a única oxima autorizada pela Agência Nacional de Vigilância Sanitária (ANVISA) para ser usada em tratamentos de envenenamento por OP (ARAÚJO; GONSALVES, 2015). Diversos estudos mostram o bom potencial de oximas bis-quaternárias, com bons resultados na reativação da AChE inibida por alguns OP, como por exemplo a Trimedoxima (Figura 13), objeto de estudo deste trabalho.

Figura 13 – Estrutura química da Trimedoxima.



Fonte: (KUCA *et al.*, 2006).

A Trimedoxima, também conhecida na literatura como TMB-4, é uma oxima bis-quaternária, com duas extremidades contendo o grupo oximato. Vem sendo alvo de estudos, mostrando bons resultados para determinados OP, como no trabalho de Kassa e colaboradores em que compararam algumas oximas, dentre elas a Trimedoxima, quanto a capacidade de reativação, proteção contra o envenenamento agudo e combater os sintomas da neurotoxicidade do envenenamento por Tabun em ratos. Os resultados mostraram que apesar de não eliminar completamente os sintomas da neurotoxicidade, a Trimedoxima era uma das melhores opções para combater o envenenamento agudo e a proteção contra esses sintomas a longo prazo (KASSA *et al.*, 2017).

Como já descrito, a Trimedoxima possui duas extremidades com grupo oximato, sendo que ambos estão na posição “para” nos respectivos anéis piridínicos. Esse fator estrutural acaba favorecendo uma melhor eficiência na reativação da AChE inibida por determinados OP como o Tabun, que segundo a literatura é melhor reativado por oximas quaternárias com o grupo oximato na posição “para”. Por outro lado, tem baixa eficiência para a reativação pela enzima inibida por Ciclosarin, que como encontrado na literatura tem melhores resultados de reativação por reativadores com o grupo oximato na posição

“orto”, sendo verificado por diversos estudos teóricos e experimentais (KASSA et al., 2020; LORKE; PETROIANU, 2019; ZORBAZ et al., 2018).

Ainda não existe uma oxima com espectro de ação satisfatório para todos os agentes neurotóxicos, sendo esta, a principal motivação para novos estudos. Dentre as abordagens dos estudos mais recentes para o desenvolvimento de novos antídotos e fármacos, está a modelagem molecular, que relaciona métodos de estrutura eletrônica e simulações computacionais, sendo muito útil para o entendimento do comportamento das moléculas e sistemas moleculares (ALENCAR FILHO; SANTOS; OLIVEIRA, 2017; LYAGIN; EFREMENKO, 2018).

2.8 - Química Computacional

Grandes avanços têm sido obtidos no desenvolvimento científico em nível elementar, chegando a um maior entendimento de processos cada vez mais complexos. Grande parte desses avanços deve-se aos trabalhos de cientistas teóricos, que caracterizam quantitativamente as forças responsáveis pelos processos químicos e seus mecanismos. Os avanços da informática nos últimos tempos possibilitaram o processamento mais rápido e eficaz de grandes quantidades de informação, juntamente com novas ferramentas e metodologias computacionais, com isso, é possível obter resultados cada vez mais precisos, que são usados para simular diversos parâmetros químicos e correlações (CARVALHO *et al.*, 2003).

Uma das principais vantagens da química computacional é o relativo baixo custo em relação a métodos experimentais, devido a altos custos de determinados reagentes e equipamentos analíticos, além do fato que o preço de equipamentos tecnológicos tende estar diminuindo pela constante renovação tecnológica. A não geração de resíduos com repetições de análises é uma importante motivação econômica e ambiental. A aplicação de métodos computacionais para solucionar problemas químicos estende-se às áreas onde a química desempenha um importante papel, direto ou indireto, como áreas farmacológicas, médicas e ciência de materiais (MCARDLE *et al.*, 2020).

Pode-se definir a química computacional como o domínio dos métodos computacionais aplicados à Química, para prever e estudar propriedades e interações dos sistemas estudados. Ela apresenta-se como um domínio interdisciplinar, que se ramifica pelas áreas clássicas da química, como Química Orgânica, Físico-Química, Bioquímica,

Química Medicinal, etc., juntamente com a ciência dos computadores (GRIMME; SCHREINER, 2018; MCARDLE *et al.*, 2020).

A química computacional é dividida em várias áreas, sendo que as principais são: a Mecânica Molecular e a Quântica. A primeira área se utiliza das leis da mecânica clássica enquanto a quântica se utiliza de leis da mecânica quântica e clássica, além de constantes físicas. Os químicos computacionais utilizam-se de softwares e metodologias específicos para o entendimento de determinados problemas. Com isso, é possível entender melhor os processos desde o nível macro até o microscópico. Uma das principais aplicações dessa metodologia é na área de química medicinal no desenvolvimento de fármacos (GOH; HODAS; VISHNU, 2017).

A química medicinal tem um papel fundamental no planejamento de novos fármacos. Ela é fundamentada no conhecimento prévio dos processos envolvidos e na seleção de alvos terapêuticos. Através de diversas estratégias, que englobam seleção, identificação e otimização de estruturas de moléculas capazes de interagir com o alvo selecionado, a Química Medicinal computacional tem potencial para desenvolver pesquisas tanto na área de desenvolvimento de novos fármacos como na obtenção de antídotos contra armas químicas de guerra, provando ser extremamente versátil e de grande importância e interesse, tanto científico como econômico (KORSHUNOVA *et al.*, 2021; LIN; LI; LIN, 2020).

A oferta de softwares para modelagem molecular, juntamente com bancos de dados, disponíveis nas redes, são fundamentais para o desenvolvimento de pesquisas visando a obtenção de novos compostos farmacológicos. Essas informações permitem uma análise rápida da atividade biológica e propriedades físico-químicas de uma série de moléculas de interesse (GOH; HODAS; VISHNU, 2017).

2.8.1 - Modelagem Molecular

Modelagem molecular, segundo a IUPAC, é a investigação das estruturas e das propriedades moleculares, pelo uso da química computacional e técnicas de visualização gráfica, visando a fornecer uma representação tridimensional sob um dado conjunto de dados. O estudo das relações entre a estrutura de um composto e sua atividade e seus análogos pode ser a chave para entender quais partes da estrutura são responsáveis por determinada atividade biológica. Tendo-se essa ciência, é possível utilizá-la como base

para a obtenção de novas estruturas com melhores propriedades e atividade (CARVALHO *et al.*, 2003; GOH; HODAS; VISHNU, 2017).

A modelagem molecular consiste em um conjunto de ferramentas que possibilita a compreensão da interação, a nível molecular, de uma substância com seu receptor, por meio de simulação computacional. Isso ocorre através da geração de estruturas realistas. Os métodos teóricos relacionados a essa técnica permitem calcular propriedades de moléculas, tais como conformações estáveis, cargas e interações atômicas, propriedades e energias de moléculas associadas; exibir, sobrepor e comparar modelos moleculares. A modelagem e suas representações gráficas são, portanto, ferramentas utilizadas para a construção, edição, visualização, análise e armazenamento de sistemas moleculares complexos (ACUNA; HOPPER; YODER, 2020; HOFFMANN; MALRIEU, 2020; MIRZAEI *et al.*, 2020).

Os softwares de modelagem são capazes de desenhar estruturas moleculares, realizando cálculos de otimização geométrica e estudos de análise conformacional. Os arquivos de saída desses cálculos podem ser utilizados como arquivos de entrada para outros programas. Sendo assim, o primeiro passo da modelagem molecular é desenhar a estrutura da molécula. Seguido da otimização da molécula, objetivando encontrar parâmetros estruturais, como comprimentos e ângulos de ligação, para posterior comparação com valores próximos aos valores determinados experimentalmente (BARREIRO *et al.*, 1997; BELLO-RIOS *et al.*, 2021).

É importante destacar que as estruturas desenhadas tridimensionalmente, não necessariamente estão na conformação mais estável. Durante o processo de obtenção de uma estrutura podem ocorrer distorções na molécula, que envolvem a formação desfavorável de parâmetros estruturais, tais como comprimentos e ângulos de ligações, além de fatores como impedimento estérico e repulsões eletrostáticas também podem ser provocados por interação entre átomos não ligados. Portanto, a minimização de energia e a análise conformacional são utilizadas interativamente, para otimizar a geometria de moléculas e corrigir possíveis distorções (CARVALHO *et al.*, 2003). Além disso, é possível avaliar interações entre estruturas, como por exemplo, em sistemas enzimáticos, modelar as interações de uma enzima e um substrato ou um possível inibidor. Essas simulações são conhecidas como Ancoragem Molecular.

2.8.2 - Ancoragem Molecular (*Molecular Docking*)

Em estudos com grandes sistemas biológicos, como o estudo de proteínas, uma das principais ferramentas de modelagem é a Ancoragem Molecular (*docking*). A simulação computacional do *docking* é uma das mais importantes técnicas de investigação das interações moleculares entre a proteína e um ligante nos casos em que a estrutura 3D da proteína já foi elucidada, por exemplo por técnicas como cristalografia por raio-x, RMN e difração de nêutrons. Esse tipo de simulação encontra a estrutura mais estável do complexo proteína-ligante e calcula essa estabilidade relativa. Para encontrar a estrutura de menor energia, sem qualquer suposição prévia, é necessário analisar todos os modos de interação, considerando a flexibilidade conformacional do ligante a ser introduzido no sítio ativo da proteína. Como esses dois problemas estão interligados, eles podem ser resolvidos ao mesmo tempo, contudo o número de combinações envolvidas é muito grande (ROCHA; RAUSCH; HEIN, 2012; SANTOS FILHO; ALENCASTRO, 2003).

Avanços foram feitos ao se propor que o método de docking em primeiro momento deveria determinar a posição e a orientação dos fragmentos rígidos do ligante e então pesquisar as conformações da região flexível do ligante de modo sistemático. A energia de interação intermolecular é calculada através da soma das contribuições de energia entre todos os átomos das duas moléculas, desconsiderando as interações entre os átomos da mesma molécula. Assim, a metodologia utiliza a energia envolvida no processo para identificar a conformação do ligante energeticamente mais favorável quando ligado ao alvo. Valores mais baixos de energia representam melhor as interações entre proteína e ligante, quando comparados com valores mais elevados (THOMSEN; CHRISTENSEN, 2006).

De maneira geral, os “softwares” de “docking” são formados por uma combinação de dois componentes: um algoritmo de busca e uma função de energia. O algoritmo é utilizado na busca de possíveis modos de ligação, e permite explorar os graus de liberdade translacional, rotacional e conformacional do ligante, bem como o de ligações rotacionáveis na proteína. A função de escore é aplicada para tentar distinguir os modos de ligação teoricamente mais próximos dos obtidos experimentalmente entre os demais modos de ligação, explorados pelo algoritmo de busca e, dessa forma, ordenar os diferentes modos de ligação apresentados. As funções de escore podem ser estabelecidas

de acordo com campos de força de mecânica molecular, parâmetros empíricos de cálculos de energia livre ou até de acordo com parâmetros denominados “knowledge-based” score (KONG *et al.*, 2020; TAO; ZHANG; HUANG, 2020)

Uma das características mais valiosas dos métodos de “docking” é a sua capacidade de reproduzir modos de ligação observados experimentalmente, funcionando até como uma forma de validação dos mesmos. Para realizar um teste desse nível, um ligante é extraído de seu complexo cristalográfico e submetido a simulações com o sítio ligante da proteína. Dessa forma, os modos de ligação obtidos nas simulações são comparados com os respectivos modos de ligação obtidos experimentalmente. Outra possibilidade inerente ao método é a capacidade de sua função de escore de ordenar ligantes de acordo com valores experimentais de atividade (TAO *et al.*, 2020). Os valores da função de desempenho do ancoramento (*Docking Scoring Function*), E_{score}, são definidos pela Equação 2:

$$E_{score} = E_{inter} + E_{intra} \quad \textbf{(2)}$$

Em que:

$$E_{inter} = \sum_{i=ligant} \sum_{j=protein} \left[E_{PLP}(r_{ij}) + 332.0 \frac{q_i q_j}{4 r_{ij}^2} \right] \quad \textbf{(3)}$$

O termo de E_{PLP} é um “potencial linear por partes”, que usa dois conjuntos diferentes de parâmetros: um para a aproximação do termo estérico (van der Waals) entre átomos, e outro potencial para a ligação de hidrogênio. O segundo termo descreve as interações eletrostáticas entre átomos carregados. É um potencial de Coulomb com uma constante dielétrica dependente da distância (D(r) = 4r). O valor numérico de 332,0 converte as unidades de energia eletrostática para quilocalorias por mol (THOMSEN; CHRISTENSEN, 2006).

E_{intra} é a energia interna do ligante:

$$E_{intra} = \sum_{i=ligant} \sum_{j=protein} E_{PLP}(r_{ij}) + \sum_{flexiblebonds} A[1 - \cos(m\theta - \theta_0)] + E_{clash} \quad \textbf{(4)}$$

As duas primeiras somas referem-se a todos os pares de átomos do ligante, excluindo os pares de átomos conectados por duas ligações. O segundo termo refere-se à energia torsional, em que θ é o ângulo de torção da ligação. A média da contribuição da energia torsional é utilizada se diversas torsões são determinadas. O último termo, E_{clash}, atribui uma penalidade de 1000 se a distância entre dois átomos pesados (mais de duas ligações distantes) for menor que 2.0 Å, punindo conformações inexistentes do ligante (Molegro ApS). Em resumo, essas funções são usadas para sobrepor automaticamente uma molécula flexível em uma molécula molde parcialmente rígida (proteína) (THOMSEN; CHRISTENSEN, 2006).

2.8.3 - Métodos Quânticos

No início do século XX, Max Planck postulou que a troca de energia entre a radiação emitida por um corpo aquecido e os átomos da parede ocorria de forma quantizada, ou seja, a energia se apresentava como pacotes indivisíveis de energia. O físico francês Louis de Broglie formulou a hipótese, futuramente comprovada experimentalmente, de que o elétron teria comportamento ondulatório. Schrödinger, em 1926, desenvolveu a equação de onda da mecânica ondulatória. Essa equação, quando aplicada ao modelo atômico desenvolvido por Bohr, demonstrou que os valores de energias quantizados estavam de acordo com os resultados experimentais obtidos por Heisenberg e Dirac, sendo que ambas abordagens foram desenvolvidas separadamente. Essa equação é conhecida como equação de Schrödinger, base para os cálculos de energia para átomos (PATEL; SHIZGAL, 2021).

O ponto de partida para descrever quanticamente o comportamento da matéria a nível atômico, no caso não relativístico, é através da resolução da equação diferencial linear de Schrödinger independente do tempo (Equação 5), que pode ser escrita em função das três dimensões espaciais da seguinte forma:

$$\left[-\frac{h^2}{2m}\left(\frac{\partial^2}{\partial x^2}+\frac{\partial^2}{\partial y^2}+\frac{\partial^2}{\partial z^2}\right)+V(x,y,z)\right]\Psi(x,y,z)=E\Psi(x,y,z) \qquad \textbf{(5)}$$

Os termos dentro do colchete são um operador matemático chamado hamiltoniano, representado comumente pela letra H. Podemos reescrever a equação simplificada de Schrödinger em função de r (Equação 5), onde r representa as três coordenadas espaciais:

$$\hat{\mathrm{H}}\Psi(r) = E\Psi(r) \quad \textbf{(6)}$$

Em química, usa-se essa equação, geralmente, para a obtenção do valor energético de sistemas (ZHANG; SHIZGAL, 2020).

Essa equação é usada para descrever as funções de onda das partículas em estados estacionários. Através dela é possível obter, por exemplo, o espectro de energia do átomo de Hidrogênio, o que é base de diversos cálculos quânticos, abrangendo diversos métodos computacionais, dentre eles a Teoria do Funcional de Densidade, que corresponde à sigla do termo na língua inglesa DFT.

2.8.4 - Teoria do Funcional de Densidade (DFT)

Prever propriedades moleculares, tendências qualitativas dessas propriedades ou explicar a natureza de uma ligação química são as propostas da química computacional. Existem vários níveis ou métodos para encarar esses problemas, metodologias tradicionais como os *ab initio,* empíricos e semiempíricos e também outros mais sofisticados como a Teoria do Funcional de Densidade (DFT). Os métodos semiempíricos são baseados no formalismo de Hartree-Fock (campo autoconsistente), contudo, utilizam várias aproximações e dados retirados do experimental (PFAU *et al.*, 2020). Já o DFT contempla o sistema eletrônico por meio de funcionais, que são funções da função de onda de Schrödinger (Ψ) que espacialmente dependem da densidade eletrônica. Esse método é particularmente importante no estudo de sistemas moleculares, pois os descreve relativisticamente, aumentando a acurácia das informações. Os conceitos modernos desta teoria foram inicialmente formulados para estados não degenerados e encontram-se nos trabalhos de Hohenberg (HOHENBERG; KOHN, 1964) e Kohn (KOHN; SHAM, 1965).

Devido ao grande número de átomos em sistemas biológicos e à complexidade das interações, quebra e formação de ligações, estudar a catálise enzimática é um grande desafio para a química computacional. Métodos quanto-mecânicos de alto nível teórico são limitados a aplicações em sistemas com número relativamente pequeno de átomos. A

combinação dos métodos quanto-mecânicos e de mecânica molecular (QM/MM) excede o domínio dos cálculos QM a macromoléculas. Na região QM, os átomos são representados por núcleos e elétrons e a superfície potencial é construída dentro da aproximação de Born-Oppenheimer (BORMAN, 2004).

Um método para se obter os resultados de QM é o de funcional de densidade (Density Functional Theory, DFT), desenvolvido por Hohenberg, Kohn e Sham (KOHN; HOLTHAUSEN, 2001). Segundo Tom Ziegler (1991): "A noção básica em DFT, de que a energia de determinado sistema eletrônico possa ser expressa em função de sua densidade, é quase tão antiga quanto a própria mecânica quântica [...]". Hohenberg e Kohn mostraram também que a energia, função de onda e outras propriedades moleculares são univocamente determinadas por essa densidade de probabilidade eletrônica ρ [x,y,z] (segundo teorema de Hohenberg-Kohn), ou seja, a densidade eletrônica e o Hamiltoniano têm uma relação funcional que permite a computação de todas as propriedades moleculares no estado fundamental sem uma função de onda. Um problema desses teoremas é não mencionar como encontrar a energia do estado fundamental a partir de ρ ou a partir de ψ. Isso foi contornado por Kohn e Sham, na década de 60, quando eles propuseram que a energia puramente eletrônica de uma molécula com muitos elétrons no estado fundamental seria (Equação 7):

$$E_0 = -\frac{1}{2}\sum_{i=1}^{n}\langle\Psi_i(1)|\nabla_1^2|\Psi_i(1)\rangle - \sum_{\alpha}\int\frac{z_{\alpha}\rho(1)}{r_{1\alpha}}dv_1 + \frac{1}{2}\iint\frac{\rho(1)\rho(2)}{r_{12}}dv_1dv_2 + E_{xc}[\rho] \quad \textbf{(7)}$$

Em que Ψi (1), i=1, 2,..., n são os orbitais Kohn-Sham, e E_{xc} [ρ] é a energia de troca e correlação. Kohn e Sham também mostraram que "ρ" exato para o estado fundamental pode ser determinado pelos orbitais Ψi (Equação 8):

$$\rho = \sum_{i=1}^{n}|\Psi|^2 \quad \textbf{(8)}$$

O terceiro teorema de Hohenberg-Kohn diz que $E_0[\rho] < E_0[\rho']$, onde ρ é a densidade exata e ρ' a densidade aproximada pela expansão (Equação 8) para "n" finito. Esse teorema é equivalente ao teorema variacional de Hartree-Fock. Os orbitais Kohn-Sham podem ser determinados pela expressão (Equação 9):

$$\hat{F}_{KS}(1)\Psi_i(1) = \varepsilon_{i,KS\Psi_i}(1) \quad \textbf{(9)}$$

onde F_{KS} é o operador de Kohn-Sham apresentado pela Equação 10:

$$\hat{F}_{KS} \equiv -\frac{1}{2}\nabla_1^2 - \sum_\alpha \frac{z_\alpha}{r_{1\alpha}} + \sum_{j=1}^{n} \hat{J}_j(1) + V_{xc}(1) \quad \textbf{(10)}$$

O potencial V_{xc} é a principal diferença entre os métodos Hartee-Fock e DFT (MORGON, 1995).

Dessa forma, pode-se minimizar a energia em relação à densidade através das condições de contorno (Equação 11):

$$\frac{\partial E_0}{\partial \tilde{\rho}} = 0 \quad \textbf{(11)}$$

Sendo a integral ρ'dr = N, onde N é o número de elétrons do sistema. O principal problema do método é a falta de um processo sistemático para determinar $E_{xc}[\rho]$, assim vários tipos de funcionais já foram propostos. Este funcional é na realidade, dividido em duas partes: um de troca e outro de correlação.

Um dos funcionais de troca mais utilizados é o B3, proposto por Becke, em 1993 (BECKE, 1993), que foi utilizado neste trabalho (Equação 12):

$$E_x = (1-\alpha_0)E_x^{LSDA} + \alpha_0 E_x^{HF} + \alpha_x \Delta E_x^{B88} \quad \textbf{(12)}$$

Para correlação, foi utilizado o funcional proposto por Lee, Yang e Parr (LEE; CHUNG, 2009). A partir daí, conceitos importantes na descrição teórica das reações químicas, tais como potencial químico e conceito de dureza e moleza, são incorporados na DFT, uma vez que ρ(r) e o número de elétrons N pode ser relacionado mais facilmente do que funções de onda de muitos elétrons.

2.8.5 - Métodos Híbridos QM/MM

Como já discutido, é alta a complexidade para realizar cálculos de Mecânica Quântica (QM) para sistemas macromoleculares devido ao alto custo computacional.

Mesmo utilizando a DFT como uma alternativa viável, se nós almejarmos uma convergência de um experimento virtual para uma proposta de sistema real haverá o encarecimento do tempo de cálculo, inviabilizando ainda mais sua realização quando a função de base escolhida for muito extensa. Logo, uma boa escolha para tratar sistemas mais reais, é contornar as limitações de um cálculo utilizando-se de métodos híbridos, contendo considerações de Mecânica Quântica e Mecânica Molecular (MM), chamados métodos híbridos quanto-mecânicos (QM/MM) (CHUNG *et al.*, 2015).

As bases do método QM/MM são: uma pequena porção é selecionada para o tratamento QM, por exemplo, a um nível *ab initio*, semiempírico, ou DFT (VAN DER KAMP; MULHOLLAND, 2013). O tratamento QM, como trata de formalismo quântico, permite quebra e formação de ligações químicas. No estudo teórico de catálise enzimática, a região delimitada pela teoria QM corresponde ao sítio ativo da enzima, região que abrange resíduos de aminoácidos da enzima, substratos e cofatores. O resto do sistema, que não está diretamente ligado à reação química, é tratado através da teoria de mecânica newtoniana, pela escolha de um campo de forças (CHUNG *et al.*, 2015).

Um fator importante do método híbrido QM/MM é o acoplamento dessas duas regiões, ou seja, como as interações entre os sistemas QM e MM são tratados. A estratégia mais simples de acoplamento envolve a utilização de cargas pontuais na região QM que interagem com o resto da região de MM. No desempenho desse tipo de cálculo, a energia QM/MM de todo o sistema, $E_{TOTAL}^{QM/MM}$, é calculada como mostrado na Equação 13:

$$E_{TOTAL}^{QM/MM} = E_{TOTAL}^{MM} + E_{Região\ QM}^{QM} - E_{Região\ QM}^{MM} \quad \textbf{(13)}$$

Em que a E_{TOTAL}^{MM} é a energia MM de todo o sistema, $E_{região\ QM}^{QM}$ é a energia QM da região QM e $E_{região\ QM}^{MM}$ é a energia MM da região QM isolada. Vários métodos QM/MM ainda incluem o efeito de polarização na região QM pelo ambiente MM. Isso é importante para várias enzimas, dado o mecanismo de reação. Métodos desse tipo estão relacionados com as interações eletrostáticas entre as regiões QM e MM no cálculo (CHUNG *et al.*, 2015; LONSDALE; RANAGHAN; MULHOLLAND, 2010; VAN DER KAMP; MULHOLLAND, 2013).

Métodos QM de alto nível, combinados com métodos de MM, podem ser utilizados para estudar as reações enzimáticas, permitindo o cálculo das barreiras de

energia para o processo da reação (BORMAN, 2004). Anteriormente, devido a restrições por demanda computacional, cálculos QM eram restritos a pequenos sistemas possuindo poucas dezenas de átomos. Os efeitos de grandes sistemas em uma região QM podem agora ser estimados e corroborados a investigações experimentais com um maior grau de precisão (VAN DER KAMP; MULHOLLAND, 2013). Warshel e Levitt aplicaram o primeiro QM/MM à lisozima, da clara de ovo, em 1976 (WARSHEL; LEVITT, 1976). Eles apresentaram os conceitos de QM/MM, detalhando situações essenciais, e os aplicaram a reações enzimáticas.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1

Trends in the Recent Patent Literature on Cholinesterase Reactivators (2016–2019)

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Review

Trends in the Recent Patent Literature on Cholinesterase Reactivators (2016–2019)

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Abstract: Acetylcholinesterase (AChE) is the key enzyme responsible for deactivating the ACh neurotransmitter. Irreversible or prolonged inhibition of AChE, therefore, elevates synaptic ACh leading to serious central and peripheral adverse effects which fall under the cholinergic syndrome spectra. To combat the toxic effects of some AChEI, such as organophosphorus (OP) nerve agents, many compounds with reactivator effects have been developed. Within the most outstanding reactivators, the substances denominated oximes stand out, showing good performance for reactivating AChE and restoring the normal synaptic acetylcholine (ACh) levels. This review was developed with the purpose of covering the new advances in AChE reactivation. Over the past years, researchers worldwide have made efforts to identify and develop novel active molecules. These researches have been moving farther into the search for novel agents that possess better effectiveness of reactivation and broad-spectrum reactivation against diverse OP agents. In addition, the discovery of ways to restore AChE in the aged form is also of great importance. This review will allow us to evaluate the major advances made in the discovery of new acetylcholinesterase reactivators by reviewing all patents published between 2016 and 2019. This is an important step in continuing this remarkable research so that new studies can begin.

Keywords: organophosphorus compounds; acetylcholinesterase; therapeutic potential; reactivation process; new trends in reactivators

1. Introduction

The organophosphorus (OP) compounds are part of a very important organic class of phosphorus-based molecules. A range of these toxic substances possesses significant domestic and industrial applications [1]. The OP agents were intensively employed as warfare nerve agents in the past wars, for instance, World War II. In this regard, the most dangerous and toxic to humans are well-known nerve agents. Furthermore, these substances have remarkable importance for agricultural purposes. For instance, pesticides play an important role in combating the pests that cause damages to agricultural crops so that it is possible to enhance productivity [2]. Despite the important application of these compounds, their toxic effects are extremely harmful to humans, animals, and the environment, with high contamination rates.

Poisoning by OP may take place through skin contact, oral, and the respiratory tract. These molecules act by inhibiting the acetylcholinesterase (AChE) enzyme. This inhibition process is

irreversible in the case of no immediate treatment, resulting in a prolonged inhibition. AChE is responsible for the hydrolysis of the acetylcholine (ACh) neurotransmitter, thus balancing the synaptic activity [3]. Due to AChE inhibition, the neurotransmitter accumulates into central and peripheral cholinergic sites, leading to the over-stimulation of cholinergic receptors. The major intoxication-related symptoms are excessive salivation, lacrimation, sweating, broncho-constriction, and neuromuscular block. The latter especially affects the muscles responsible for breathing, and consequently leads to death [3–5].

There are some indications that about 3 million cases of OP poisoning occur in the world each year [6,7]. Occasionally, there is the occurrence of terrorist attacks, such as that in Syria, in 2013, where Sarin terribly affected the civilians [8]. The huge number of OP poisoning cases and the big stocks of still available nerve agents in diverse countries make necessary the discovery of large OP broad-spectrum antidotes [9–12]. Currently, the treatment procedure for OP poisoning consists of the use of two classes of drugs: competitive muscarinic receptor antagonist, such as atropine, and the use of a reactivating substance, usually one agent from the oxime class [13–15]. Unfortunately, there is no universal antidote to date, and a broad-spectrum oxime capable of reactivating all types of OP-inhibited AChE/butyrylcholinesterase (BChE) is highly desired [15–17]. In this review, the recent advances in the development of novel antidotes and therapies were analyzed according to the patents produced in the past few years.

2. Cholinesterase Enzymes–AChE and BChE

Found in various parts of the body, such as neuromuscular junctions in the peripheral nervous system (PNS), parasympathetic nervous system (PSNS), central nervous system (CNS) synapses, and linked to erythrocyte membranes in the blood, AChE plays a fundamental role in the neurosynaptic communication process (Figure 1) [18].

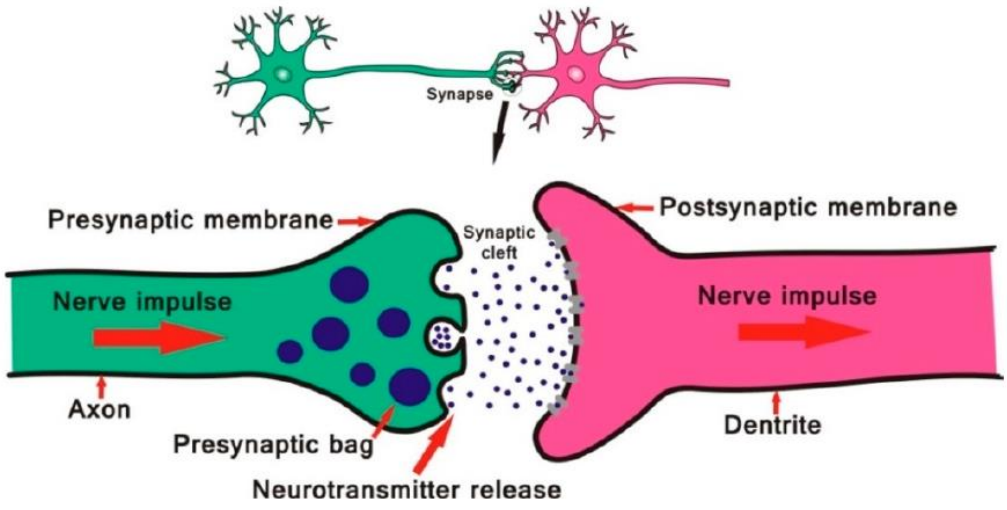


Figure 1. Neuron structure and nerve impulse transmission process.

With a determining action in the finalization of the nerve impulse propagation, AChE is responsible for maintaining the appropriate levels of ACh (Figure 2) [19]. AChE inactivates the action of ACh by hydrolyzing it into choline and acetate [20].

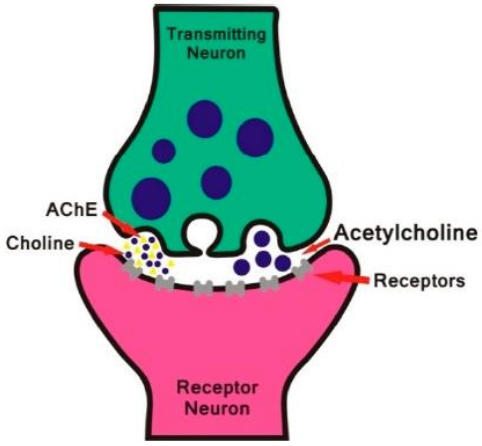


Figure 2. Representation of the acetylcholine (ACh) hydrolysis scheme.

The interaction of AChE with the neurotransmitter takes place through two essential spots. The first one is the anionic site, where there is an interaction between the nitrogen positive charge of ACh and the negative charge produced by the aspartate residue. The second one is the stearic site, where the ACh ester group performs hydrogen bonding with the tyrosine residue [21–23]. The anionic region serves to guide the substrate to the position necessary to undergo the hydrolysis process [24]. The peripheral anionic site (PAS), located at a distance equal to or greater than 4.7 Å from the hydroxyl group of Ser203, guides and accommodates the quaternary nitrogen of the ACh molecule that enters the active site, electrostatically interacting with Glu334 [25].

In addition, the butyrylcholinesterase (BChE) enzyme, such as AChE, is another cholinesterase enzyme capable of hydrolyzing choline-based esters [26]. At the molecular level, the main difference between these cholinesterases is the fact that BChE lacks six aromatic amino acids out of the fourteen that line the AChE catalytic gorge [27,28]. Thus, the BChE gorge almost doubles the width, making the BChE active site domain more accessible to a wide variety of substrates and inhibitors [29,30]. While AChE acts preferentially by hydrolyzing ACh, BChE hydrolyzes both ACh and butyrylcholine (BCh) in similar amounts [31]. However, AChE is the enzyme with the highest catalytic efficiency known today, with an enzymatic hydrolysis rate of approximately 5.000 ACh molecules/s^4 [32].

3. AChE Inhibition Processes

There are several drugs that target cholinergic synapses by inhibiting or reactivating AChE [26]. Anticholinesterase is the term used to name AChE inhibitor (AChEI) drugs. The mechanism of action of these inhibitors involves the competitive blockade of the AChE enzyme, prolonging the duration and intensity of ACh at the synaptic terminals. Regarding their binding to AChE, anticholinesterases can be classified as reversible, irreversible, and pseudo-irreversible.

Irreversible anticholinesterase agents are pentavalent phosphorus compounds that contain a labile group, such as fluoride, or an organic group [33]. Their general chemical structure can be observed in Figure 3. These agents spontaneously phosphorylate AChE, making OP poisoning very dangerous. The cholinesterase-inhibiting pesticides are well absorbed by all digestive, respiratory, and dermal routes. This property is due to their high fat solubility. They are biotransformed by oxidases, hydrolases, and transferases enzymes, whose process occurs mainly by hydrolysis, oxidation, and conjugation with glutathione. After absorption, they are rapidly and widely distributed to various tissues and organs, reaching higher concentrations in the liver and kidneys. Some highly lipophilic OPs deposit in adipose tissues and are gradually released over several days after exposure. Some works have been performed to employ the enzymatic biodegradation of these compounds by using different degrading enzymes [34–40].

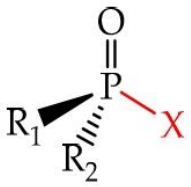
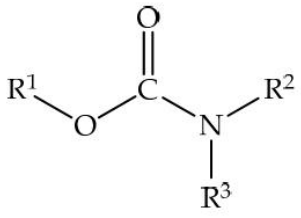


Figure 3. General structure of organophosphorus compounds.

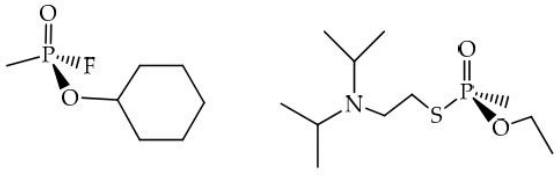
Along with OP, the carbamates (Figure 4) stand for the major class of insecticides involved in poisoning. These compounds inactivate AChE and BChE enzymes, resulting in elevated ACh levels and leading to an acute cholinergic syndrome, with the emergence of muscarinic, nicotinic, and CNS signs and symptoms. These manifestations are dependent on the dose and route of exposure involved. OP agents easily cross the blood–brain barrier (BBB), while carbamates do not effectively penetrate the CNS. The employment of carbamates results in less neurological toxicity, and these substances do not accumulate in the body [41].



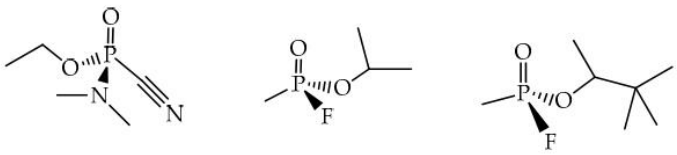
(1)

Figure 4. General structure of carbamates.

Nerve or neurotoxic agents are the most lethal group among the phosphorus-based compounds [42]. The most well-known are shown in Figure 5. They have a chiral phosphorus atom that generates a pair of optical isomers in equal proportions. Soman, also having an optically active carbon in the pinacolyl group, gives rise to another pair of enantiomers. Although this agent has two stereocenters [43], it is known in the literature that the dominant chiral center is the phosphorus atom as it determines the potential toxicity of this compound. Therefore, this agent is broadly employed for theoretical and experimental investigations [44].



(2) Cyclosarin (3) VX



(4) Tabun (5) Sarin (6) Soman

Figure 5. Chemical structures of the main warfare nerve agents.

Besides insecticidal agents, chemical warfare agents stand for an even more toxic form of OP. The first large-scale application of these OP chemical weapons occurred during World War II, and from then on, a range of compounds has been developed, presenting high toxicity and hazardousness [41]. These extremely toxic molecules are considered a serious threat to national security due to their potential use in terrorist actions. Although these chemical warfare agents were banned by the International Chemical Weapons Convention (CWC), numerous synthetic pathways for various nerve agents have been reported, and huge stocks are still available [41]. Due to their chemical reactivity, these OP agents, over the long term of human exposure, have the potential to toxically compromise the nervous system, causing several life-threatening serious diseases [45,46]. The inhibition and aging mechanisms are shown in Figure 6.

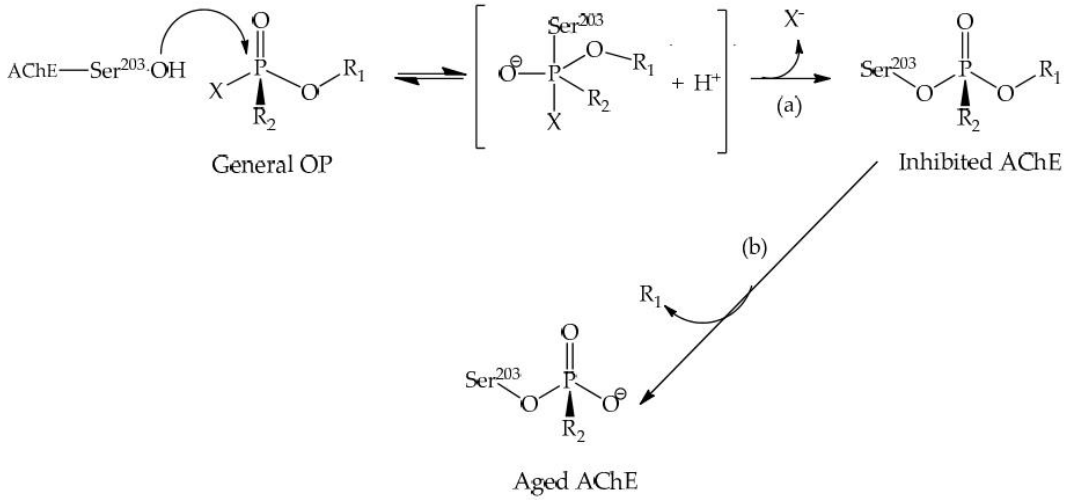


Figure 6. General representation of the (**a**) inhibition and (**b**) aging mechanisms [47].

According to Figure 6, this reaction is governed by the phosphorylation of the hydroxyl functional group [48,49]. The AChE inhibition process takes place within the active site through a biomolecular nucleophilic substitution (S_N2) reaction, with the formation of a covalent bond between the oxygen atom of serine and the central phosphorus atom of the OP [50]. After a period that varies according to the neurotoxic agent, the enzyme may be inhibited in an irreversible process known as aging (Figure 6), which is characterized by a dealkylation process [47]. Spontaneous reactivation of the enzyme may also occur, but at a practically insignificant rate [51].

Neurotoxic OP agents are substances that have several chemical and biological properties, such as high toxicity, lipophilicity, volatility. These properties favor the rapid insertion of these compounds through the airway and topical by allowing for the absorption of a reasonable concentration, as well as making them able to penetrate the BBB. In addition, the easy manufacture and low cost favor the use of some compounds of this class as agrochemicals [52]. Among the agrochemicals marketed, the OP agents were found to be the most preferred ones due to their wide spectrum bioactivity and easy availability. Some examples are ethyl paraoxon, methyl paraoxon, diazinon, and chloropyrifos (Figure 7) [41].

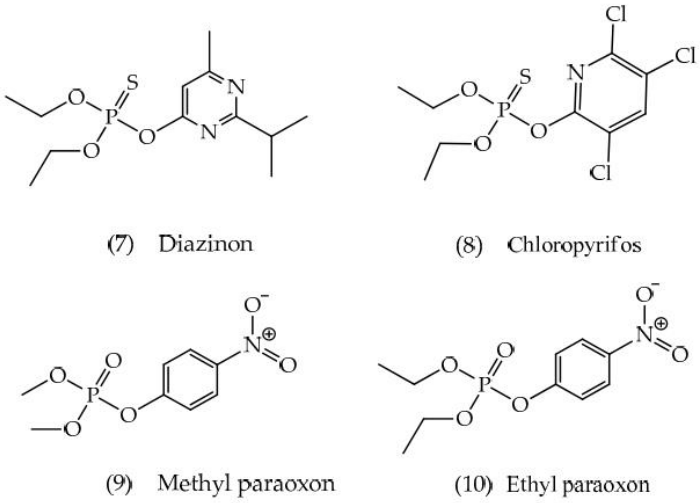


Figure 7. Chemical structures of some important pesticides.

In general, the resource adopted against neurotoxic poisoning proposes the use of atropine, diazepam (Figure 8), and an oxime [53,54]. This process will be better discussed in the next topic.

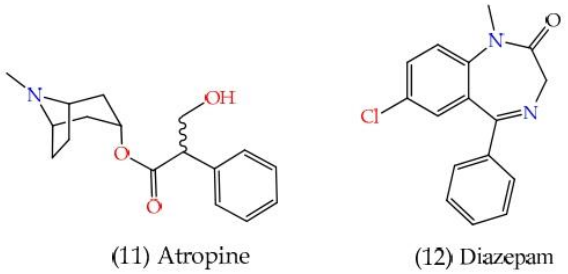


Figure 8. Representation of the chemical structures of atropine and diazepam.

Given the great utility of AChEI in medicine and the limited therapeutic arsenal for the treatment of neurodegenerative diseases, such as Alzheimer's disease (AD), as well as the problems related to this therapy [55], the evaluation of the potential of suitable compounds in inhibiting or reactivating AChE has great relevance for the development of new drugs [26].

4. AChE Reactivation Processes

Reactivators are nucleophilic substances able to cleave the covalent bond formed in the inhibition process between OP and AChE, thus restoring the enzyme catalytic activity. Some challenges still remain for future studies. Pralidoxime, discovered in 1955, was the first effective reactivator to be used as a drug in the treatment of OP intoxication, being one of the most widely used reactivators to date [56–58]. Note that each OP residue formed after inhibition differently modifies the enzyme active site, creating a selectivity of reactive molecules with the potential to bind to the enzymatic cavity [59,60]. The reactivation mechanism takes place basically in two stages: The first one is the approximation to the phosphorus atom of the AChE-OP adduct by the reactivator, forming a pentacoordinate transition state. The second step is the release of the OP-reactivator conjugate, restoring the enzyme function (Figure 9) [61,62].

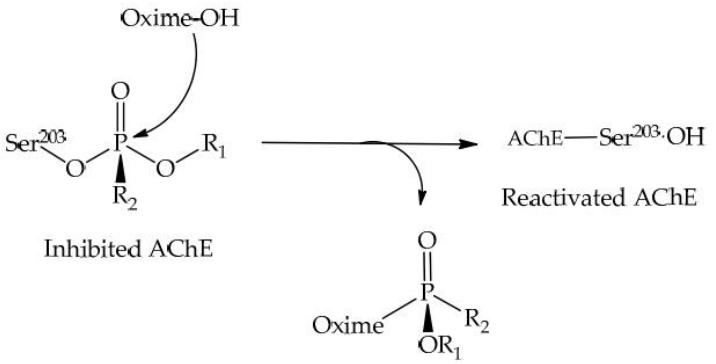


Figure 9. General representation of the reactivation process of the inhibited acetylcholinesterase (AChE).

Reactivators generally may have one, two, three, or no pyridine ring. In general, quaternary pyridine rings interact with AChE peripheral anionic site (PAS), by stabilizing the reactivator in the cavity and favoring the nucleophilic attack. Some factors listed in the literature that may influence the reactivator efficiency are the presence of quaternary nitrogen in the reactivator structure; in case of bis-pyridinium oxime, the length and rigidity of the connection chain between them; the presence of the oxime functional group; the position of the oxime group on the pyridinium ring; and the number of nucleophilic groups in the reactivator structure [63]. The first reactivators to be studied were from the oxime class. Oximes are compounds that have at one end the functional group -C=N-OH, and in biological environments, they are usually found in the conjugate base form, the oximate ion. Most of the time, oxime molecules may contain one or two oxime functional groups, such as 2-PAM (also known as pralidoxime) and HI-6 (also known as asoxime), both widely marketed (Figure 10) [56,64].

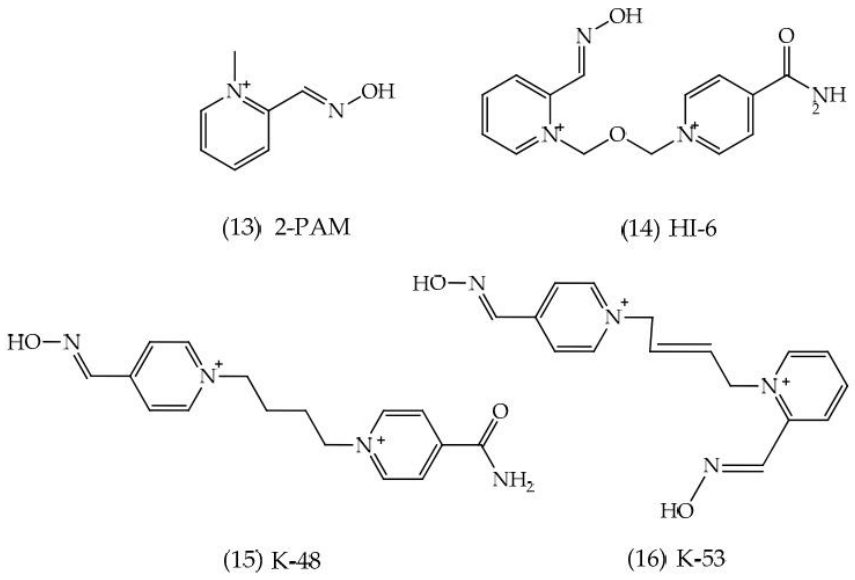


Figure 10. Representation of the chemical structures of oximes.

Over the past 50 years, the class of oximes has been exhaustively studied. Although some molecules have good efficiency with some specific OP, there are no broad-spectrum antidotes yet. It is important to mention that, generally, quaternary oximes have a permanent positive charge, which interacts with PAS, thus increasing the related efficiency. However, this charge makes it difficult for the

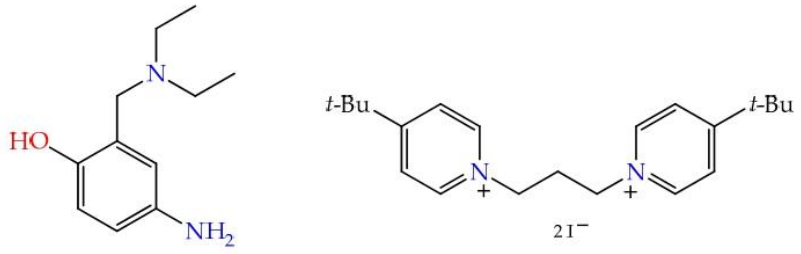
passage of the molecule through the BBB. This implies a divergent observation between good in vitro test results and not satisfactory in vivo test results [60].

Several recent works aimed at improving current antidotes by studying factors that may boost their efficiency and increase their crossing through BBB. Kuca and co-workers, for example, in in vitro tests with aryloximes and some neutral derivatives, have shown promising data on the effect of substituents, either electron-donating or electron-withdrawing with potential for existing oximes improvements, such the reactivator's potentiality as pharmacokinetics [65]. In the same line of reasoning, in a study with bisoxime, a three positive charge oxime, it was shown that even with its hydrophilicity, the reactivator was able to be stabilized in the Sarin and VX-inhibited AChE cavity through interactions with some amino acid residues, such as Tyr124 and Tyr337. Despite this finding, due to the three positive charges, this reactivator would not penetrate the BBB. However, it is an option for BChE reactivation. This enzyme is a bioscavenger for the detoxification of free nerve agents in the body [66].

Another study showed the influence of the non-nucleophilic end position on isomeric oximes, showing a large difference in the reactivation rate of each isomer in relation to AChE inhibited by different nerve agents [67]. The change in position of the carbamoyl group resulted in different reactivation percentages for different enzyme–nerve agent complexes. With the aid of theoretical studies, it was evidenced that the structural changes between the isomeric oximes resulted in different interactions with the cavity residues. In addition, one of the isomers' transition states was able to be better stabilized, especially in the cavity of the AChE-tabun cavity [67,68]. Diverse oximes-related aspects, such as interaction within AChE and reactivity, have been theoretically investigated [69]. Another approach by the same authors is the use of oximes as a pretreatment prior to acute paraoxon poisoning. The work concluded that the tested oximes, such as K-48 and K-53 (Figure 10), are able to significantly reduce the risk of death of the tested individuals when applied pre-emptively before pesticide poisoning. The tested hypothesis of using these molecules as pretreatment may be an alternative to complement the current treatment protocol [70].

Even with the benefit of oximes for the treatment of OP poisoning, as exposed previously, there is a concern about the balance between applied concentrations and the reactivator´s efficiency without causing tissue damage to the treated organisms. In their work, Kuca and coworkers studied such effects of varying concentrations of reactivators and the damage done to certain living tissues. As found in the literature, it was confirmed that all reactivators tested at high dosages caused damage to both visceral tissues and the overall health of the individual. The data obtained may be an important support for dose choices regarding different reactivators structures for newly developed drugs [71,72].

Although oximes are the main class of reactivators, several research groups are investing efforts in other non-oxime molecules. Katz et al. (2015) [73] screened a series of compounds with the potential to reactivate OP-inhibited AChE. In this regard, it highlights 4-amino-2-(diethylaminomethyl)phenol (ADOC), which showed some interesting results. This molecule piqued the interest of other groups that studied structural variations and substituent groups of its structure, such as from Konig et al. (2018) [74] and Cadieux et al. (2016) [75]. Another interesting group of molecules under study comes from tert-butyrylpyridinium, with emphasis on 1,1'-(propane-1,3-diyl)bis(4-tert-butylpyridinium)di(iodide) (MB327). Niessen et al. (2018) [76] studied the efficiency of MB327 and some of its regioisomers, finding some important results in soman-inhibited AChE reactivation. The chemical structures of ADOC and MB327 are displayed in Figure 11. Zhuang et al. (2018) [77] also screened a series of compounds derived from quinone methide precursors, that in vitro tests were able to reactivate OP-inhibited AChE, in addition to resurrecting aged-AChE. Regarding the AChE reactivation, the review from Gorecki et al. (2016) [78], which covers remarkable developments since the discovery of 2-PAM, in 1955, should be mentioned.



(17) ADOC (18) MB327

Figure 11. Chemical structures of 4-amino-2-(diethylaminomethyl)phenol (ADOC) and 1,1′-(propane-1,3-diyl)bis(4-tert-butylpyridinium)di(iodide) (MB327).

5. Update of the Recent Patents in Literature from 2016 to 2019

Faced with all exposed so far, there is an attempt to develop effective ways of reactivating AChE through specific reactivators [60]. In this context, this becomes a wide field of study, with some recent patents already produced. Some of them are described ahead in this review.

Currently, the protocol for an effective treatment of the intoxication by neurotoxic OP consists of the previous knowledge about the kind of OP from which the exposure has occurred. This situation is not easy to address in a mass-exposure event because of the short time for the patients to develop the intoxication symptoms. Therefore, quick treatment is strongly required [60]. This fact justifies the efforts made to develop novel efficient oximes and reactivators capable of treating multiple OP neurotoxic agents. However, the high complexity levels of the enzyme active site, after inhibition by diverse OP inhibitors, make necessary different therapeutic approaches owing to the size and orientation of the attached O- or N-alkyl groups [59,79,80].

The patent from Quinn and Topczewski, published in 2016 under the Pub. No.: US 2016/0151342 A1 [47], provides compounds and methods that can be used for applications in reactivation processes of the aged-AChE adduct. It is useful to counteract the intoxication caused by OP nerve agents. Regarding the employment of oximes, if administered shortly after exposure, the oxime is capable of displacing the bound OP, thus releasing the serine residue. However, as discussed previously, if the administration is delayed, a process called aging takes place by the occurrence of a solvolytic loss of an alkyl group from the AChE-OP adduct. The aged adduct is stabilized by diverse interactions with the enzyme active site, turning out to be ineffective to oxime reactivation [81]. To date, there is a lack of antidotes against aged AChE-OP adducts, but scientists worldwide are striving to discover new potent antidotes for this end [82]. Based on the exposed so far, this patent brings about the discovery of a class of molecules that can reactivate an aged AChE-OP adduct, whose process is denominated by resurrection. The method from Quinn and Topczewski can lead to the adduct reactivation with the use of alkylating agents. The invention consists of a method for treating a mammal suffering from OP intoxication, by administrating an alkylating agent, which can be performed along with a possible joint administration of ACh receptor antagonist and/or anti-seizure agent. The respective compounds discovered possess the general formula shown in Figure 12.

According to that formula, a range of molecules is possible to be synthesized. Realize that X (Figure 12) can be any suitable counter ion. R_1 and R_2 stand for a range of chemical groups, thus allowing for the development of different oxime molecules. The formula I-based molecules can be formulated as pharmaceutical compositions, with a posterior administration to a mammalian host, such as human beings. The administration can be adapted according to the chosen route, such as orally or parenterally, by intramuscular, intravenous, topical, or subcutaneous routes. Appropriate dosages of the compounds from Formula I can be determined by making a comparison regarding their in vitro and in vivo activity in animal models. The patent describes the synthesis route for some molecules, such as the molecules highlighted in Figure 13. As a matter of fact, these molecules

have suggested good potential for applications in the resurrection process of the aged-AChE. The synthesis of N-methyl-methoxypyridinium can occur through the exposure of starting pyridines to trialkoxonium tetrafluoroborate (or another alkylating agent, such as methyl triflate (MeOTf)) in an appropriate solvent before or after the oxime formation. For instance, this patent brings about promising experimental results by employing Compound **19**, demonstrating its good performance for reactivation.

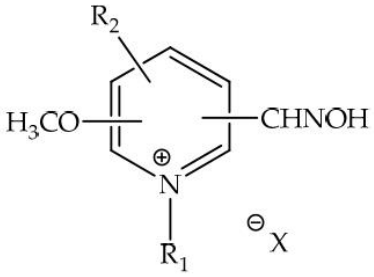
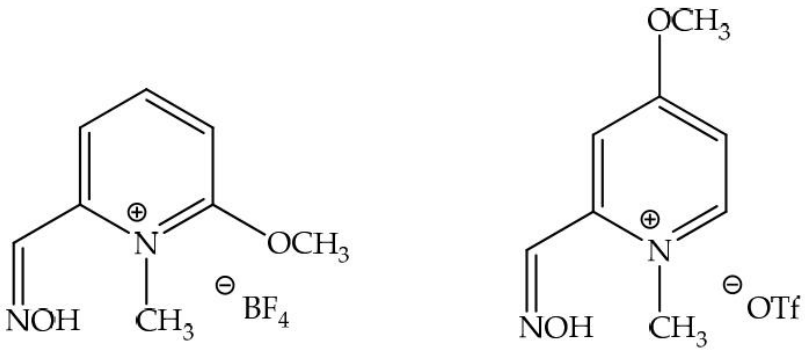


Figure 12. Formula I of the compounds developed in this patent.



(19) (20)

Figure 13. Representation of the chemical structure of some molecules synthesized.

In this experiment, the human AChE (*Hss*AChE) was inhibited by exposure to an OP agent analog of Sarin, 7-(isopropyl methylphosphonyl)-4-methylumbelliferone, being incubated with Compound **19** and 2-PAM. The experimental results are displayed in Table 1. Note that the best results were achieved in larger periods of incubation.

Table 1. Resurrection essays data obtained in the experiment with inhibited *Hss*AChE (Pub. No.: US 2016/0151342 A1).

Time, Hours	% $AChE_{React}$	% $AChE_{aged.}$	% $AChE_{Res.}$
1.00	2.45 (± 0.7)	1.16 (± 0.06)	1.28 (± 0.8)
4.00	5.67 (± 0.6)	1.16 (± 0.06)	4.50 (± 0.6)
12.00	6.46 (± 1.1)	1.16 (± 0.06)	5.30 (± 1.1)
24.00	8.70 (± 1.3)	0.00 (± 0.06)	8.70 (± 1.3)
48.00	7.03 (± 0.8)	0.00 (± 0.06)	7.03 (± 0.8)

The aging process was considered irreversible in the past decades, but many attempts have been made to convert the phosphorylated oxyanion aged form to a re-alkylated form of AChE. According

to Franjesevic et al. (2019) [60], the reactivation or re-alkylation processes of aged AChE are quite challenging. At first, a substrate must bind selectively and efficiently in the enzyme active site, in an active conformation, so that it can facilitate the critical transition state for the desired re-alkylation, thus allowing for the re-alkylated phosphorylated serine to be reactivated by an efficient nucleophile. It is important to notice that all of these steps should occur inside an active site that is relatively compact and constricted. The aged state of AChE is a thermodynamically stable form of the enzyme, and the resurrection process may show some difficulties in overcoming the conformational changes and hydrogen-bonding networks within the active site of aged adduct [60].

Quinn et al. (2017) [83] demonstrated that the aged state of the enzyme makes four hydrogen bonds. One of these interactions is with the aged serine residue, between the phosphorylated oxyanion and the histidine residue, and the others with the oxyanion hole. A good stabilization of the tetrahedral intermediate in the deacylation step is observed. The action of a successful resurrector of aged AChE is crucial. This active species has to bind to disrupt the hydrogen bonds formed in the aged state and cause relevant conformational changes within the active site. These steps are important for reducing the strength of the intermolecular interactions, to allow for reactivity of the phosphorylated oxyanion. This makes possible the desired transition state for electrophilic re-alkylation. Figure 14 stands for a reaction scheme of the resurrection process of the aged AChE, proposed by Quinn and co-workers [83].

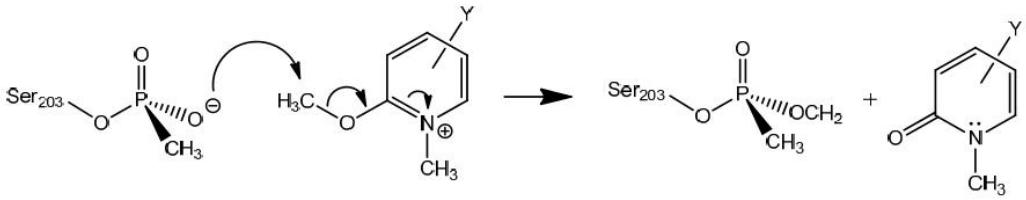


Figure 14. Proposed resurrection process for aged AChE.

Regarding the reactivation processes of OP-inhibited cholinesterase enzymes, the BChE enzyme also stands out. The patent from Chambers at al. (2017) [84], published under the Pub. No.: US 2017/0258774 A1, brings about novel oximes for reactivating the OP-inhibited BChE. The authors state that the oxime molecules can allow for a dual reactivation in the treatment process, which can take place by reactivating both serum BChE and inactivated CNS AChE. This invention lies in the field of nerve agent antidotes' development and protection against the toxic effects caused by these toxic OP inhibitors, nerve agents, and/or pesticides. For these antidotes, the administration can be performed intravenously, intraperitoneal injection, orally, nasally, topically, among many others. The researchers synthesized a range of phenoxyalkyl pyridinium oximes, according to previous work from Chambers et al. (2016) [85]. By following the line of investigation adopted, many of these oximes were employed to determine their ability to reactivate inhibited serum BChE by nerve agent surrogates and insecticidal oxons. In this line, the inhibitors investigated were PIMP (phthalimidyl isopropyl methylphosphonate, sarin surrogate), NEMP (nitrophenyl ethyl methylphosphonate, VX surrogate), NCMP (nitrophenyl cyclohexyl methyl phosphonate, cyclosarin surrogate), and DFP (diisopropyl fluorophosphate), along with two insecticidal OP: paraoxon and phorate oxon, which are metabolites of the insecticides parathion and phorate, respectively (Figure 15).

The authors demonstrated that the oximes evaluated were sufficiently capable of crossing the BBB, thus being effective as soon as these species enter the brain. The PNS is part of the nervous system that consists of nerves and ganglia on the external environment, outside of the brain and spinal cord, thus not being protected by the BBB. On the other hand, the CNS consists of the brain and spinal cord, being protected by this barrier. The present oximes showed to be more effective antidotes for OP intoxication. One feature of these oximes is the fact that they present increased lipophilicity, with the aim of enhancing the ability of these molecules to cross into the brain. By targeting BChE, crossing through the BBB does not become a great issue. On the other hand, the fact of these oximes present dual

reactivating properties makes them capable of reactivating AChE in CNS and BChE in the circulatory system. With all exposed so far, the most interesting is that these molecules can scavenge and destroy circulating OP that potentially could inhibit AChE and lead to intoxication.

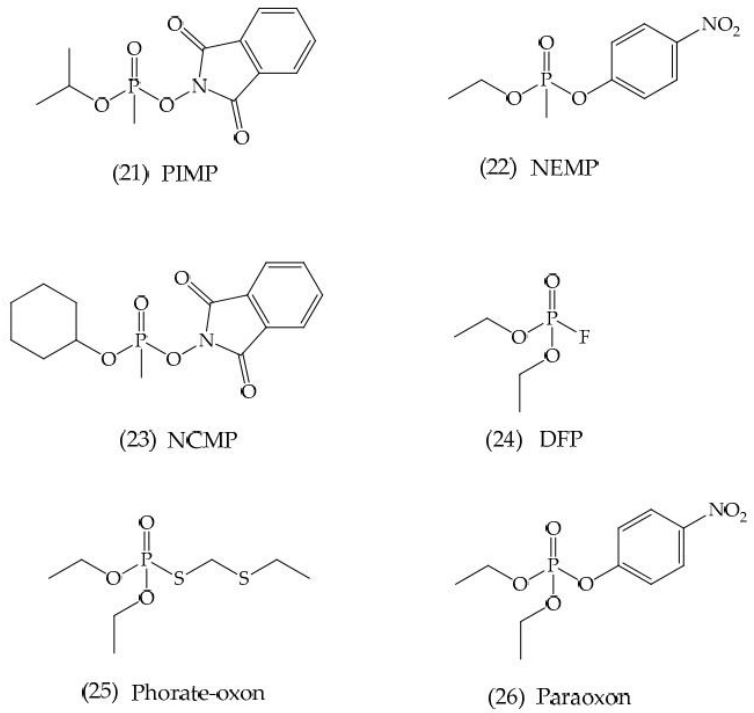


Figure 15. Representation of the chemical structures of some organophosphorus (OP) used in the patent.

Based on a range of substituted phenoxyalkyl pyridinium oximes, their goal was to identify some of these molecules that also have the potential to reactivate BChE. However, very interestingly, this enzyme can provide some protection by being inhibited by the OP neurotoxic agents. Note that this inhibition is stoichiometric, and one OP molecule is destroyed for every BChE inhibited. However, the related protection is limited by the amount of BChE present in the serum. In this patent, the authors investigated whether the BChE can be reactivated and the active site restored so that the BChE could be inhibited again, thus destroying another OP molecule. In this context, the ability of BChE to protect from intoxication could be enhanced. It is worth mentioning that the best oxime molecules from this work exhibit activity toward both AChE and BChE, thus presenting protective effects by displaying two therapeutic mechanisms, providing a substantial improvement for currently-approved antidotes.

The oximes developed, according to Chambers et al. (2016) [85], display the ability to counteract the harmful effects of OP poisoning through the restoration of the inhibited AChE in the peripheral and central nervous systems. Some of these oximes are shown to reactivate BChE as well. The identified reactivating molecules then restore the BChE activity, and multiple OP molecules could be destroyed. For that study, the serum BChE from human, guinea pig, and rat was used. The oxime molecules investigated share the common formula shown in Figure 16.

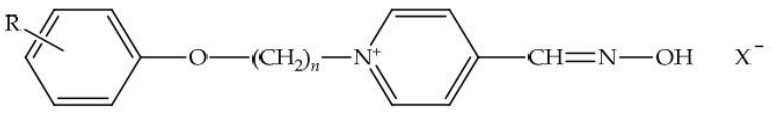


Figure 16. Representation of the general chemical formula for the oxime molecules investigated.

Wherein R = hydrogen, alkyl, alkenyl, aryl, acyl, nitro, or halo; n is an integer selected from 3, 4, or 5; and X is a pharmaceutically acceptable anion.

The phenoxyalkyl pyridinium molecules and methods of this investigation provided the military with a more efficient antidote against the intoxication caused by neurotoxic OP agents. Civilians can directly benefit from protection against terrorist attacks and OP pesticides-based poisoning. The authors showed significant broad-spectrum capability with the novel oximes to reactivate both AChE and BChE after exposure to these nerve agents. See Table 2 for more details about the chemical structures of the molecules investigated. The tested oxime molecules differ in the alkyl linker chain length (n) and/or the phenoxy ring substitution moiety (R).

Table 2. List of oxime molecules used in the patent (Pub. No.: US 2017/0258774 A1) [84].

BChE Reactivation Tested Oxime Molecules		
Tested Phenoxyalkyl Pyridinium Oxime Molecule	**Alkyl Linker Chain Length (n)**	**Phenoxy Substitute Moiety (R)**
Oxime 12 (OX12)	5	4-CH_3 –O–
Oxime 14 (OX14)	4	4-Cl–
Oxime 28 (OX28)	4	4-CH_3CH_2C(:O)–
Oxime 31 (OX31)	3	3-CH=CHCH=CH-4
Oxime 32 (OX32)	4	3-CH=CHCH=CH-4
Oxime 59 (OX59)	4	4-Ph–CH_2–O–
Oxime 98 (OX98)	4	4-$(CH_3)_3CCH_2C(CH_3)_2$ –
Oxime 99 (OX99)	5	4-$(CH_3)_3CCH_2C(CH_3)_2$ –

According to the results of the investigations from Chambers et al. (2017) [84], diverse experimental essays were performed through BChE with different OP agents and oximes. The experimental essays with paraoxon-inhibited BChE show that the novel oximes exhibited a reactivation range of 33–72%, while 2-PAM averaged 32%. In this case, all oximes presented better results in relation to 2-PAM, except OX99, whose reactivation percentage was similar to that of 2-PAM. The only difference regarding OX98 is the alkyl linker chain length (from 4 to 5), and this small modification seems to be enough to significantly decrease the efficiency of OX98. On the other hand, this difference in efficiency between OX98 and OX99 was smaller for the reactivation of the PIMP-inhibited BChE. This suggests that the enzyme active site can give rise to different conformations dependent on the type of bound OP agent, thus interfering with the interaction modes of these oximes. It is important to notice that for better performance on these oximes in the reactivation process, they should adopt an appropriate and/or correct conformation in the site. For PIMP-inhibited AChE, the new oximes presented a range of 45–73%, and an average of 46% from 2-PAM. In the case of employing NEMP as the inhibitor, the reactivation range was 18–62% (novel oximes), and an average of 8% (2-PAM). According to this experimental essay with NEMP as an inhibitor, OX98 and OX99 showed similar efficiencies, however both were inferior in relation to those of the other oximes. The oximes (OX14, OX12, OX28, OX31, and OX59) showed the best results, which were remarkably superior to that of 2-PAM. For phorate–oxon-inhibited BChE, the new oximes did not bring about good results of reactivation, with the reactivation percentage from 2-PAM being noticeably superior. In this line, the accomplishment of further studies, such as theoretical ones, to better comprehend the interaction modes and reactivity of these oximes through BChE inhibited by different OP agents is necessary. According to the reactivation experimental essays of the BChE inhibited by NCMP and DFP, all new oximes and 2-PAM did not show a good performance for reactivation. These examples show a better performance of many of the developed oximes against OP inhibitors, in comparison with the traditional 2-PAM. However, the performance of each oxime may deeply shift dependent on the type of bound OP agent.

Kovarik et al. (2006) [86] observed that the reactivation of phosphorylated BChE is not as successful with two well-known oximes, 2-PAM and HI-6, in comparison with the phosphorylated AChE. In addition, Musilova and co-workers investigated more than 20 oximes against AChE and BChE enzymes, both inhibited by the pesticide paraoxon [87]. The researchers demonstrated that none of the oximes were capable of reactivating BChE more efficiently than AChE, and this trend was also reported for diverse other OP agents bound to AChE and BChE [88,89]. This fact shows the importance of this patent, which provides novel therapeutic agents that bring about good results in reactivating both enzymes.

The current standard of care for exposure to OP-based AChEIs has changed very little over the past half-century. Accordingly, effective reactivation of OP-inhibited AChE and inactivation of OP-based AChEIs are still highly desirable and challenging. In this context, Valdez et al. (2019) [90] described a series of oxime-derived compounds capable of inactivating OP-based AChEIs (Pub. No.: US 2019/152920 A1). Figure 17 shows the general formula of oxime described by Valdez and coworkers.

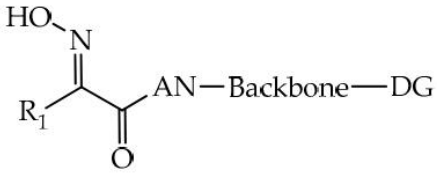


Figure 17. Representation of the general formula of oxime [90].

Wherein R_1 = H or CH_3; AN = amide nitrogen; Backbone = chemical moiety of at least two carbon atoms linking together AN and DG; DG = distal group containing a bicyclic moiety represented by Formula (II) (Figure 18).

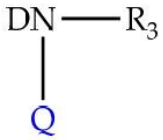


Figure 18. Representation of Formula II developed in the patent [90].

Wherein DN is nitrogen on a bicyclic core; R_3 is a hydrogen, heteroatom, functional group, or a substituted or unsubstituted linear or branched alkyl chain, aromatic or aliphatic cyclic group; Q is a heteroatom or carbon atom on the bicyclic core.

Throughout the studies from Valdez and coworkers [90], a number of related methods, systems, and compositions for inactivating one or more OP-based AChEIs, as well as the therapeutic and/or prophylactic treatment of an individual and/or decomposition of OP-based AChEIs for decontamination [90], is listed.

In detail, the authors described Formula III (Figure 19), which is one of the oximes developed in the patent. Through this formula, diverse tests have been performed to reactivate the AChE enzyme and inhibit the OP agents. The authors also described a composition consisting of an effective amount of oxime described in the patent and a saline solution to reactivate the OP-inhibited AChE, along with the description of a method for treating, preventing, and decontaminating an individual's exposure to neurotoxic OP [90].

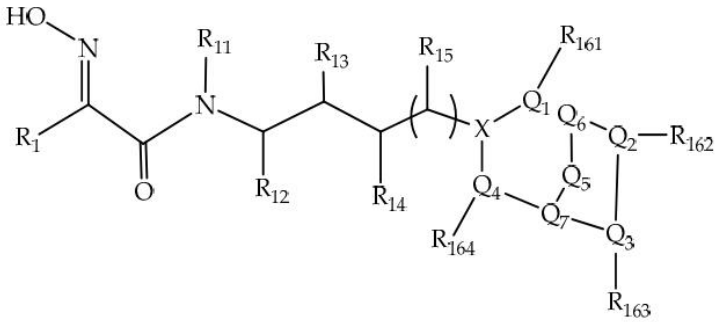


Figure 19. Formula III of an oxime developed in the patent [90].

Wherein X = N or C; R_{10}, R_1 = H, or CH_3; R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{161}, R_{162}, R_{163}, R_{164}, and R_{165} = alkyl, alkenyl, alkynyl, aryl, arylalkyl, or alkylaryl groups; Q_1, Q_2, Q_3, Q_4, Q_5, Q_6, and Q_7 = N, O, or S or saturated, unsaturated or an aromatic ring.

According to the patent, the synthesized compounds are able to penetrate the BBB, and this is a very important feature, keeping in mind that the oxime compounds thus have a more specific and efficient action. Investigations in this area arouse the interest of research groups for the potentiation of pharmacological action, physicochemical properties of these new compounds and to make them more effective in the reactivation process of AChE. However, in this patent, the oxime derivatives were the main reactivating agents used. Although these compounds are very ordinary in this area, it is necessary to search for a synthesis of new classes of compounds for this same purpose, the search for new strategies.

The reactivators presented by Valdez and coworkers [90] in the present patent are demonstrated to reactivate both AChE and BChE enzymes. To reinforce its importance, note that BChE is involved in nervous system development, detoxification of natural and synthetic toxins, hydrolysis of drugs, such as cocaine, heroin, and aspirin, fat metabolism, as well as the interaction and functional modification of other proteins, such as polyprolines and trypsin [91,92].

In 2017, Khavrutskii et al. (2017) [93] invented compounds, compositions, and methods for activating, reactivating, reversing, or preventing the deactivation of AChE and BChE, with compounds derived from Formula IV (Pub. No.: WO 2017/218886 A1, Figure 20):

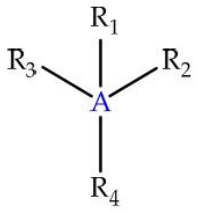


Figure 20. Formula IV for the novel molecules developed in the patent.

Wherein A is a 5- or 6-membered substituted or unsubstituted aromatic ring, cycloalkyl or heterocyclic ring; R_2 and R_3 is H,CN,OR_{10}, $S(O)_{0\text{-}2}R_{10}$, $NR_{11}R_{12}$, $C(O)NR_{13}R_{14}$, $C_{1\text{-}6}$ alkyl, and $C_{1\text{-}6}$ alkene; R_4 is H, CN, OR_{10}, $S(O)_{0\text{-}2}R_{10}$, $C_{1\text{-}6}$ alkyl, and $C_{1\text{-}6}$ alkene, $NR_{11}R_{12}$ and $C(O)NR_{13}R_{14}$; R_5 is $C_{1\text{-}4}$ alkyl, and $C_{1\text{-}4}$ alkene; R_6 is H and $C_{1\text{-}3}$ alkyl; R_7 is substituted or unsubstituted $C_{1\text{-}3}$ alkyl and substituted or unsubstituted $C(O)OR_{15}$; R_8 is $C(O)NHR_{15}$ and $C(O)OR_{15;}$ R_9 is H, CN, $C(R_6)OH$, OR_8, NR_9R_{10}, $C(O)NR_{11}R_{12}$, $C_{1\text{-}6}$ alkyl, and $C_{1\text{-}6}$ alkene; R_{10} is H, $C_{1\text{-}3}$ alkyl, and $C_{6\text{-}12}$ aryl; $R_{11,}$ $R_{12,}$ $R_{13,}$ and R_{14} is H, $C_{1\text{-}4}$ alkyl, and $C_{1\text{-}4}$ alkene; R_{15} is H and $C_{1\text{-}3}$ alkyl.

The methods disclosed by the authors of this invention relate to the administration of at least one compound of the composition comprising Formula IV. Specifically, the methods include reactivation,

deactivation, or prevention of AChE or BChE processes. The methods of the present invention include treating a subject for toxicity associated with AChE inactivation, with the methods comprising administering a therapeutically effective amount of at least one compound of Formula IV [93].

It is important to highlight that this patent focused on the synthesis of new antidotes, modifying the mechanism of action, reactivation process agents for both AChE and BChE. This is clearly a new and promising strategy; however, the patent does not mention whether the synthesized compounds penetrate the BBB and whether these new antidotes are more efficient than oxime compounds.

In general, when comparing the classes of reactivators used in the patents described here, all molecules have nucleophilic groups or a quaternary nitrogen. It is known that these pharmacophoric groups interact with the "anionic" catalytic site, by leaving the nucleophile in the opposite position to the phosphorus atom to attack and displace the phosphoryl group of the inhibited enzyme. Particularly, in the patent by Valdez et al. (2019), the binding of the quaternary N occurs within a ring. This fact is very interesting since cycles are limited to the rotation of the molecule, thus reducing the number of conformations that the molecule can adopt. Thus, the molecule is more likely to be in an active conformation as it approaches the enzyme active site. Compounds containing these pharmacophoric groups are potent reactivators of AChE [63,94].

6. Expert Commentary

The intoxication caused by neurotoxic OP agents has increasingly become a serious public health concern around the world. Many researchers have strived to find novel therapies for reversing the poisoning caused by these agents. In this context, the chemical agents denominated reactivators are the most used in the treatment protocol, along with the administration of a muscarinic receptor antagonist, such as atropine, to stop nerve receptor overstimulation. In addition, diazepam is also used as an anticonvulsant to prevent brain damage and minimize the central side effects due to nerve agent-induced incapacitation. To date, there are no broad-spectrum reactivators able to reverse the intoxication caused by different OP agents. In the current treatment protocol, previous knowledge about the kind of neurotoxic agent that the patient has been exposed to is required. Due to the intoxication level, and the speed by which the symptoms of the poisoning progress, immediate treatment is necessary. Most times, it is not possible to know the kind of OP agent the victim has been exposed to, making the administration of an antidote kit necessary. In an ideal situation, there would be a universal antidote, which would be active against a range of OP agents, and many researchers are making efforts in the search for it.

The patents analyzed employed in vitro and/or in vivo studies to investigate the performance of these molecules in reactivating cholinesterase enzymes. A more complete and reliable analysis consists of both experimental essays, given that the likely good results obtained in vitro may not be reproduced in vivo. This comes from the fact that in in vivo studies, the new compounds may find difficulties to cross the BBB, thus turning out to be inefficient. Regarding the patents, the authors also brought about a discussion concerning the administration forms of the novel antidotes, as well as their effective dosages. This analysis is important because the administration of an inappropriate dosage of these agents can result in toxicity and damages to health. In this review, we could observe different patents, which approach interesting mechanisms of action. In this context, the new antidotes may perform the reactivation of both AChE and BChE, along with the alkylating agents that allow for the reactivation of the aged adduct. These research lines are an important starting point to the discovery of promising or even universal antidotes, for a rapid and complete detoxification process.

The discovery of more effective therapies is essential to provide better and more efficient treatments for intoxicated patients. Further investigations are required to better understand the performance and mechanism of action of the novel reactivators under development. The patents analyzed show a big advance in this area, but the limited amount of registered patents shows the difficulty of getting promising and efficient reactivators. These patents present very promising reactivating compounds, and their therapeutic approaches can undoubtedly provide novel means to combat the poisoning caused

by OP neurotoxic compounds. Due to recent advances in this field, an increasing number of works, even new patents, over the next years is expected. The development of novel antidotes for OP intoxication is crucial due to the frightening numbers of intoxicated people annually in the world, majorly due to the misuse of agrochemicals. In the case of poisoning, quick detection and identification of the toxic OP agent are crucial for effective protection, considering the lack of broad-spectrum reactivators.

With regard to potential drug candidates, the reactivators investigated in these patents still suffer from the lack of biological activity data, as well as physical–chemical and mechanistic parameters. More studies become necessary to push these compounds forward, making them even more effective as reactivators. The lack of more deep in vivo studies maintains these drug candidates at the experimental level only [95].

7. Conclusions

The role of AChE for the proper functioning of the nervous system and processes involved is leading to an increase in researches about this enzyme. The current scenario marked by the use of OP agents in terrorist attacks, coupled with the increasing number of poisonings caused by the misuse of these compounds, has alerted the scientific community about the importance of studies directed to this area. For this purpose, this review has detailed the current patents related to novel cholinesterase reactivators.

The several damages caused by these toxic substances and the ease of use justify the search for the development of more efficient defense compounds against neurotoxic agents, especially OP compounds. The complexity of the inhibited AChE enzyme active site makes different approaches necessary due to the size and orientation of their specific groups. Still, as observed in this review, very important advances have been made in this field. Thus, the expectation would be to establish a treatment capable of providing coverage against a variety of OP agents.

The patents evaluated address primordial points that, in fact, contribute to the evolutionary process of the development of promising antidotes. Nowadays, the use of oximes is considered the most viable option, as they are capable of displacing the OP, thus releasing the serine residue and reactivating the enzyme. Among the described methodologies, we highlight the use of alkylating agents for aged-AChE reactivation, with joint administration of ACh receptor antagonist and/or anticonvulsant agent. The method from Quinn and Topczewski, which was detailed previously in one of the patents, points to the possibility of synthesizing a series of molecules that may contribute to the development of a range of oxime molecules.

On the other hand, regarding the development of treatments targeting aged AChE, another patent about molecules that seem to have a favorable potential for the enzyme reactivation process, which was observed from significant experimental results, was detailed. At last, this review presents essential information that serves as a starting point for researchers in medicinal chemistry, as well as clarifying the existence of coherent works developed for the same purpose. Despite the lack of a universal antidote, the range of information available allows for the discovery of new and powerful remediation techniques.

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ARTIGO 2

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Understanding the Interaction Modes and Reactivity of Trimedoxime toward *Mm*AChE Inhibited by Nerve Agents: Theoretical and Experimental Aspects

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Abstract: Organophosphorus (OP) compounds are used as both chemical weapons and pesticides. However, these agents are very dangerous and toxic to humans, animals, and the environment. Thus, investigations with reactivators have been deeply developed in order to design new antidotes with better efficiency, as well as a greater spectrum of action in the acetylcholinesterase (AChE) reactivation process. With that in mind, in this work, we investigated the behavior of trimedoxime toward the *Mus musculus* acetylcholinesterase (*Mm*AChE) inhibited by a range of nerve agents, such as chemical weapons. From experimental assays, reactivation percentages were obtained for the reactivation of different AChE–OP complexes. On the other hand, theoretical calculations were performed to assess the differences in interaction modes and the reactivity of trimedoxime within the AChE active site. Comparing theoretical and experimental data, it is possible to notice that the oxime, in most cases, showed better reactivation percentages at higher concentrations, with the best result for the reactivation of the AChE–VX adduct. From this work, it was revealed that the mechanistic process contributes most to the oxime efficiency than the interaction in the site. In this way, this study is important to better understand the reactivation process through trimedoxime, contributing to the proposal of novel antidotes.

Keywords: nerve agents; acetylcholinesterase; trimedoxime; reactivation; mechanistic studies; computational methods

1. Introduction

Chemical weapons are defined as any chemical substance whose toxic properties are used for the purpose of killing, injuring or incapacitating an enemy in war or associated military operations [1–3]. Even with the efforts from world entities to ban the use of chemical weapons, under the Chemical Weapons Convention, diverse countries still have an arsenal of these chemical substances [4–6]. Among these chemical weapons, the most toxic to humans are the well-known nerve agents, whose base structure consists of an organophosphorus compound (OP). In addition, a range of these toxic substances show potential for application in agricultural and industrial sectors [7].

From this class of OP substances, the pesticides are fundamental in agroindustrial applications [8]. Although the OP compounds are widely used for pest control, they are very dangerous and toxic to humans, animals, and the environment. These sorts of compounds act by inhibiting the

acetylcholinesterase (AChE) enzyme causing a cholinergic neurotoxic effect. In the presence of OP, the Ser203 residue from the AChE active site covalently binds to the phosphorus atom forming a phosphorylated complex [2,9]. The AChE inhibition causes an accumulation of acetylcholine (ACh), once this enzyme is responsible for the hydrolysis of this neurotransmitter. This toxic framework results in ACh accumulation, giving rise to a cholinergic syndrome, which is a set of symptoms associated with poisoning from certain toxic substances, such as OP nerve agents, caused by the overstimulation of muscarinic and nicotinic receptors [10]. Among the major symptoms of the intoxication, we can cite excessive salivation, lacrimation, urination, sweating, broncho-constriction and neuromuscular block, leading to death in severe cases of poisoning [10–12].

The current treatment protocol for OP poisoning consists mainly of the employment of a reactivating agent, commonly an oxime compound [13–16], which is capable of restoring the AChE catalytic activity through a nucleophilic attack, thus remediating the intoxication effects and reestablishing the ACh levels [17–19]. The general reactivation mechanism through oximes is represented in Figure 1.

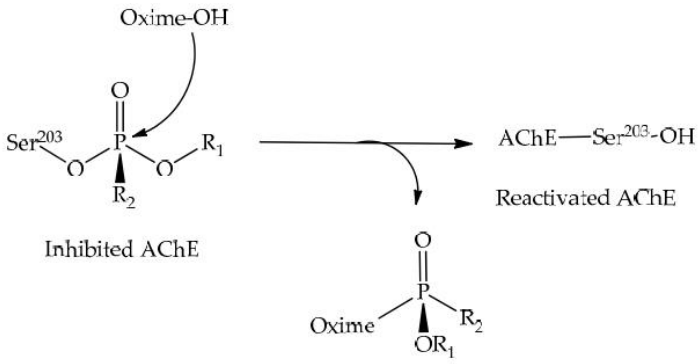

Figure 1. General representation of the reactivation process of the inhibited acetylcholinesterase (AChE).

In view of what has been exposed so far, it is important to note that there is no universal antidote to date, that is, a broad-spectrum oxime capable of reactivating all types of OP-inhibited AChE. In recent years, efforts have focused on the screening and identification of potent oximes, with sufficient permeability through the blood–brain barrier (BBB), maintaining a high reactivation rate [19–21]. In this study, we present a theoretical and experimental investigation to better understand the reactivation mechanism of the AChE inhibited by several kinds of OP agents. Based on interaction and mechanistic studies, we seek to explain the experimental data through molecular modeling in order to comprehend the reactivation process, by employing trimedoxime as the reactivating species (Figure 2). We expect to understand the interaction modes and reactivity of trimedoxime in the reactivation process of the AChE–OP adduct.

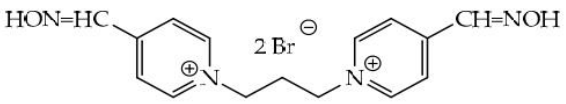

Figure 2. Chemical structure of trimedoxime.

2. Results and Discussion

2.1. In Vitro Test: Experimental Results

The results obtained through the experimental part of this work are summarized in Table 1 and Figure 3.

Table 1. Reactivation activity of trimedoxime (data obtained in triplicate experimental assays).

System	Trimedoxime	
	React. (%) Conc. 10^{-5} M	React. (%) Conc. 10^{-3} M
AChE-GA	0	30
AChE-GB	7	54
AChE-GF	0	0
AChE-GD	0	0
AChE-VX	9.8	85.3
AChE-POX	50	46
AChE-DDVP	17.3	31.5

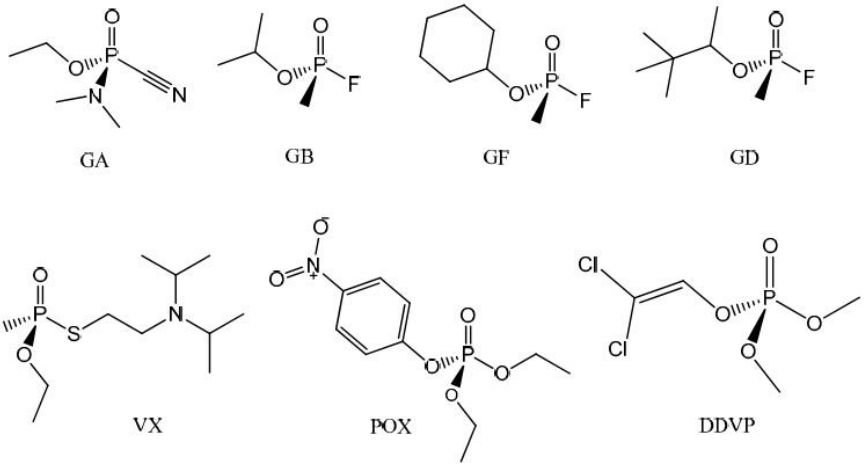

Figure 3. Chemical structures of the nerve agents used in the work.

According to the literature, the efficiency (reactivation percentage) of an oxime should be at least 10% to provide an appropriate remediation for the intoxicated patient [22,23]. In this regard, we observe different reactivation percentages through trimedoxime, taking into account its concentration as well as the type of OP–AChE complex. According to our experimental findings, note that trimedoxime demonstrated the best results at higher concentrations (10^{-3} M). At this concentration, the oxime showed a remarkable reactivation percentage of 85.3% for AChE–VX reactivation. At a concentration of 10^{-3} M, trimedoxime also exhibits a good performance in the reactivation of the AChE–sarin (GB) (54%) and AChE–paraoxon (POX) (46%) adducts. This result was more modest for AChE–Tabun (GA) (30%). An interesting outcome from this experimental investigation is the fact that trimedoxime does not reactivate the AChE–cyclosarin (GF) and AChE–soman (GD) adducts. These trends are more deeply approached in the next sections. From the experimental assays with trimedoxime at lower concentrations, we can observe that the experimental values indicate a significant reactivation percentage for AChE–POX (50%), as well as a sufficient reactivation rate for AChE–Dichlorvos (DDVP) (17.3%). Indeed, this oxime showed insufficient reactivating power for the AChE inhibited by the other OP agents investigated, such as GA, GF and GD, considering a concentration of 10^{-5} M. The chemical structures of the nerve agents used in this work are shown in Figure 3.

2.2. *Affinity and Thermodynamics: Docking Results*

According to the docking protocol, calculations were performed in order to investigate the affinity between trimedoxime and inhibited AChE. For this, a cavity prediction algorithm based on a 3D box was used to find the binding sites in the inhibited enzyme active site. The active cavity presented a volume of 113.66 $Å^3$, which was appropriate to support the reactivator.

The molecular mechanics-based calculations generated diverse poses of trimedoxime within the cavity of the inhibited complexes, and the respective intermolecular interaction energy was computed to each system. As usual in these computations, the best oxime conformation was chosen for subsequent quantum mechanics (QMs) calculations, based on the lowest interaction energies as well as the most reactive conformations. Table 2 shows the values obtained from the docking calculations for the most appropriate doses of trimedoxime with different inhibited complexes.

Table 2. Docking results for trimedoxime inside different AChE–organophosphorus compound (OP) adducts.

System	Trimedoxime	
	ΔE * (kcal mol^{-1})	**Residues**
AChE-GA	−140.9	Ser298
AChE-GB	−154.7	Tyr124, Ser298, Arg296
AChE-GF	−161.3	Tyr124, Glu285
AChE-GD	−157.7	Tyr124, Glu285
AChE-VX	−115.0	Tyr124, Phe295, Arg296
AChE-POX	−144.1	Tyr124, Glu285
AChE-DDVP	−164.8	Arg296, Ser298, Trp286

* ΔE = Intermolecular interaction energy.

According to the data reported in Table 2, note that trimedoxime showed stabilizing interactions within the inhibited enzyme complex site for all the OP agents investigated. From these results, the oxime demonstrated the lowest interaction energy in the AChE–DDVP (−164.8 kcal mol^{-1}) adduct, followed by AChE–GF (−161.3 kcal mol^{-1}) and AChE–GD (−157.7 kcal mol^{-1}). In turn, the oxime showed a less stabilizing interaction energy within the AChE–VX cavity. As shown in the experimental section, at higher concentrations, the trimedoxime demonstrated a better efficiency in the reactivation of the AChE–VX adduct. This trend leads us to believe that the interaction energy is not the only factor responsible for the performance of this antidote in the reactivation, but other factors should be involved. In this regard, the results from the mechanistic study are presented in the next section.

From Table 2, the trimedoxime was stably docked in the inhibited AChE, with intermolecular interaction energy values in the range of −115.0 to −164.8 kcal mol^{-1}. Diverse kinds of intermolecular interactions contribute to the stabilizing interaction on the site, such as hydrophobic interactions, electrostatic interactions and hydrogen bonds. It is important to mention that the AChE active site adopts distinct conformations according to the sort of OP agent. Thus, it is expected that trimedoxime interacts differently with residues from the active site. These hydrogen bond-type interactions are generally the most important in studies of biological systems.

In most of the systems investigated, trimedoxime interacted with the Tyr124 amino acid residue, and according to the literature, this interaction is described as a possible π–π stacking, which takes place between Tyr124 residue and the pyridine ring of the oxime. This interaction is indicated as having an important role in helping transition state stabilization [2,24,25]. The hydrogen bonds revealed by the interaction of trimedoxime in each inhibited system are shown in Figure 4.

From what has been discussed so far, it is important to notice that, together with the reactivation percentage, the interaction energy data do not explain the experimental trends thoroughly. The discussion of the mechanistic studies in the next section will give rise to new insights about the behavior of trimedoxime toward different AChE–OP systems.

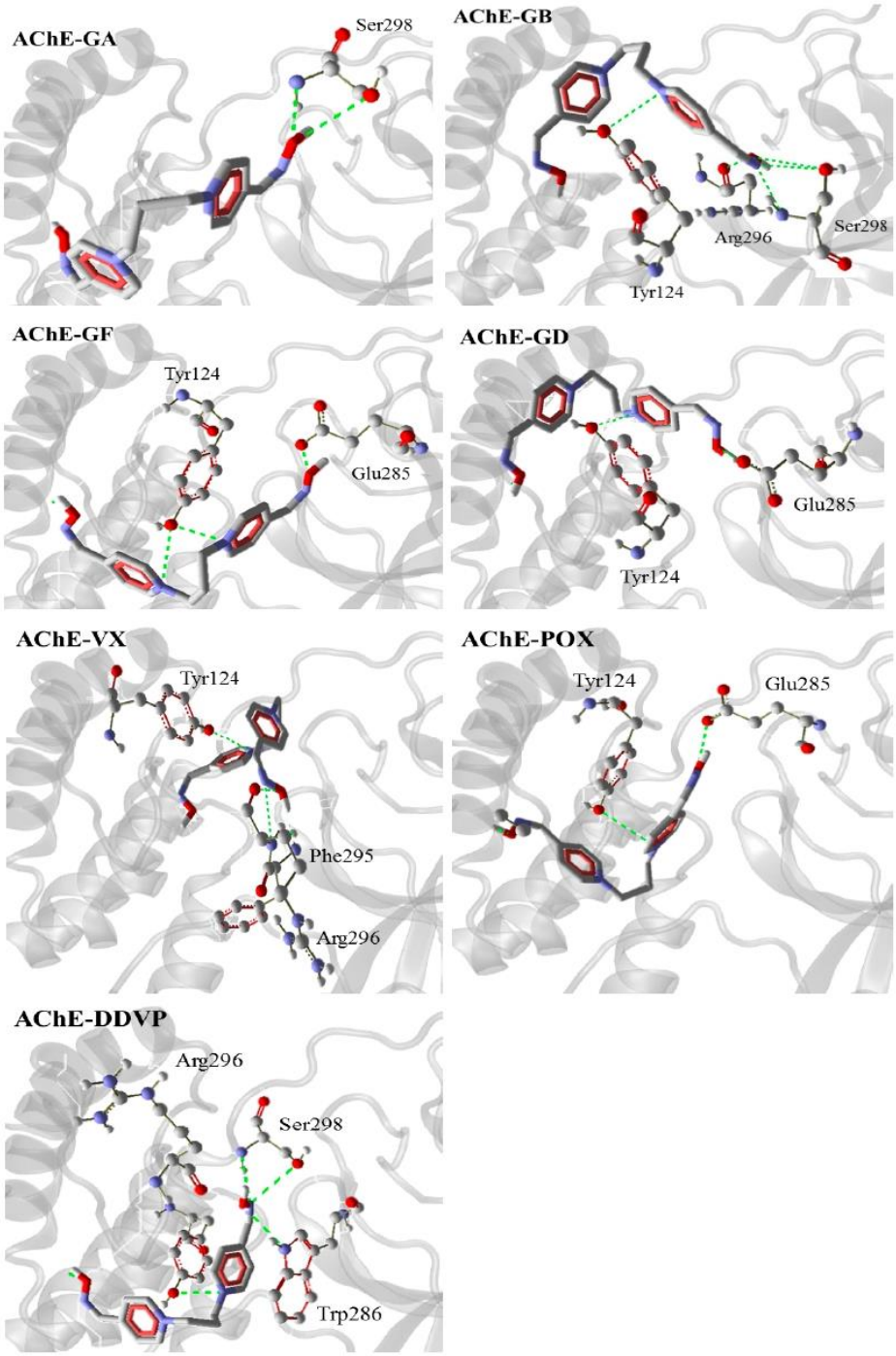

Figure 4. Representation of the hydrogen bonds performed by trimedoxime in the site.

2.3. *Investigating Kinetic Parameters for Biological Activity: Mechanistic Studies*

In the last part of this investigation, theoretical calculations were carried out to determine the relative activation energy ($\Delta\Delta E^{\#}$) through the hybrid QM/molecular mechanics (MMs) for the reactivation of each inhibited AChE system. The $\Delta E^{\#}$ values were computed based on the energy difference between the transition states and the initial system configurations from the reactants.

For the reaction mechanism simulation, the steric and electronic effects of the chemical reactions are important aspects of the reaction pathway. In addition, the strain and interaction energies are significant contributing factors that dictate the reaction course. The interaction energy is responsible for stabilizing the reaction. On the other hand, the strain energy is responsible for distorting the reactants to adopt a pentacoordinate transition state. The relation between interaction and strain energies determines the height of the reaction barrier ($\Delta E^{\#}$), the so-called activation energy. This parameter was elucidated for some of these reactions in order to better comprehend trimedoxime's behavior in the reactivation process. For this, a combined procedure of docking and DFT calculations at the QM/MM interface for the mechanism was carried out. The transition states were characterized through potential energy curves. Table 3 shows the kinetic parameters $\Delta\Delta E^{\#}$, as well as the experimental values of reactivation at the concentration of 10^{-3} M.

Table 3. Experimental reactivation percentage and relative activation energy for trimedoxime in the reactivation process.

System	Trimedoxime	
	$\Delta\Delta E^{\#}$ * (kcal mol^{-1})	**React. (%) Conc. 10^{-3} M**
AChE-GA	46.83	30
AChE-GB	33.43	54
AChE-GF	-	0
AChE-GD	-	0
AChE-VX	0	85.3
AChE-POX	41.59	46
AChE-DDVP	47.75	31.5

* $\Delta\Delta E^{\#}$ = Relative activation energy.

According to Table 3, these quantum theoretical results corroborate our experimental findings. Trimedoxime has shown itself to be very efficient in reactivating the inhibited AChE–VX at a concentration of 10^{-3} M, which is according to the reactional barrier observed in the reactivation of this inhibited complex. From Table 3, the reactivation of the AChE–VX adduct revealed the lowest barrier. This fact helps explain the higher experimental reactivation percentage of the AChE inhibited by VX, which was 85.3%. This fact suggests that its transition state is better stabilized, allowing for the oxime to interact more strongly with the nerve agent.

As we can see from Table 3, the reactivation of the AChE–GB complex showed the second most stabilizing barrier (33.43 kcal mol^{-1}), which corroborates the second best reactivation percentage found in our experimental assays (54%). In addition, a barrier of 41.59 kcal mol^{-1} was computed for AChE–POX, and for the AChE–GA and AChE–DDVP, our computations indicate very close barriers, 46.83 and 47.75 kcal mol^{-1}, respectively, which correlate very well with the close experimental reactivation percentages found for these respective systems. It is worth mentioning that our simulations for the reactivation of the AChE–GF and AChE–GD did not succeed in our study, that is, the AChE inhibited by these OP agents did not provide a feasible conformation for the nucleophilic attack by trimedoxime on the active site. This fact could be explained by the interaction modes of these toxic agents on the site, mostly due to steric hindrance effects, as well as intermolecular interactions.

From the $\Delta\Delta E^{\#}$ values in Table 3, we performed a multiple linear regression (MLR) between this parameter and the reactivation percentage, as well as the interaction energy. Our results revealed that the combination of interaction energy (ΔE) and activation energy ($\Delta\Delta E^{\#}$) is able to efficiently explain the experimental outcomes. By increasing the number of system descriptors, a better correlation between theory and experiment is expected. Based on this, the MLR between the experimental and theoretical parameters resulted in the equation below. The regression was obtained with an excellent correlation value of 0.97.

$$\% \text{ reactivation} = -0.14\Delta E - 1.22\Delta\Delta E^{\#} + 70.85 \quad (1)$$

By analyzing Equation (1), we can observe some important trends about the studied systems. Starting with the correlation value from the MLR, it shows that the docking conjugated to the QM/MM calculations result in a better representation of the systems investigated. According to the coefficients of the equation, the importance of each stage for the AChE reactivation process by trimedoxime can be evaluated. Note that the highest modulus of the coefficient of the term $\Delta\Delta E^{\#}$ (relative activation energy) indicates that the reaction step presents a greater contribution to the AChE reactivation than the interaction energy [23,25]. This means that trimedoxime can more easily fit to the transition state structure in the reactivation process. In addition, the binding mode of trimedoxime in the site is not a critical step for activity. With the information exposed in this investigation, we observe that trimedoxime stands for a significant advance in the development of more efficient reactivators for the remediation of the intoxication caused by neurotoxic nerve agents.

Previous studies have shown that there is not a direct correlation in oxime-mediated reactivation between species, and comparative studies in one species may not truly reflect the reactivation effects in humans. Due to structural differences, the active site of both enzymes from rats and humans may adopt distinct conformations in the presence of the neurotoxic agent, and the antidote might be led to specific reactional behaviors. In this context, in silico and in vitro investigations with the human AChE are equally important. These aspects will be considered in future investigations [26].

3. Materials and Methods

3.1. Experimental Details

Trimedoxime was prepared at the department of Toxicology in School of Military Health Sciences (Czech Republic), according to the synthesis route described earlier [27]. The purity of the reactivator was detected through the thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) techniques and NMR [28]. All compounds were obtained from the Brno Military Facility (95% purity and higher).

The animals employed in this experiment were handled under the supervision of the Ethics Committee of the School of Military Health Sciences in Hradec Kralove, Czech Republic. As a source of cholinesterases, a 10% rat brain homogenate (*w*/*v*) was used. The homogenate was prepared as described: ether-narcotized rats ($n = 6$) were killed by bleeding from a carotid artery. The brain was removed, washed with saline and homogenized using an Ultra-Turrax homogenizer in distilled water.

For the in vitro test, 0.5 mL of the brain homogenate was mixed with 20 µL of the isopropanol solution of the selected nerve agent and distilled water (0.5 mL). The mixture was incubated for 30 min at 25 °C to achieve a 95% inhibition of AChE. In total, 2.5 mL of sodium chloride (3 M) and distilled water were added to a volume of 23 mL. Finally, 2 mL of the substrate—ACh iodide (0.02 M)—was added. The enzyme activity (analyzed by a potentiometric titration of the decomposed ACh iodide) was measured at pH 7.6 and 25 °C on an autotitrator RTS 822 (Radiometer, Denmark). The same procedure was undertaken with the inhibited enzyme, as was a further treatment with a 10 min incubation with an aqueous solution of the reactivator (0.2 mL of 10^{-3} M), which replaced 0.2 mL of water. The activities of intact AChE (a_0), inhibited AChE (a_i) and reactivated AChE (a_r) were deduced from the consumption of the NaOH solution (0.01 M) over time; NaOH reacted with the acetate released from the decomposed ACh iodide. The reactivation percentage (%) was calculated from the measured data according to the formula (Equation (2)):

$$X = \left(1 - \frac{a_0 - a_r}{a_0 - a_i}\right) \cdot 100\ [\%] \quad (2)$$

The entire method is described in detail in the work from Kuca and Cabal [29]. This same methodology was successfully employed in the work from Polisel et al. (2019) [23].

3.2. Docking Procedure

In the docking studies, the affinity of trimedoxime with the AChE inhibited by diverse OP agents was investigated. The oxime chemical structure was constructed and optimized at the DFT level, with the B3LYP density functional method and 6-31g(d,p) basis set, as implemented in the Gaussian 09 package [30]. The oxime was then docked inside the crystallographic structure of *Mus musculus* AChE (PDB code 3ZLU; resolution = 2.60 Å) [31] inhibited by GA (Tabun), GB (Sarin), GF (Cyclosarin), GD (Soman), VX, POX (Paraoxon) and DDVP (Dichlorvos), using the Molegro Virtual Docker program (Molegro Virtual Docker (MVD®)) [32], according to similar procedures employed previously [24,33,34]. From our calculation protocol, a radius of about 20 Å was considered, where the residues of the catalytic triad were kept flexible. Due to the nature of the docking methods, the calculations carried out generated approximately 50 poses (such as conformation and orientation) for each ligand studied.

In the MVD program, the MolDock score algorithm method used as a scoring function is based on the piecewise linear potential, which is fundamentally a simplified potential whose parameters are in turn fitted to protein–ligand structures, binding data scoring functions and further extended in the Generic Evolutionary Method for molecular docking, including a new hydrogen bonding term as well as new charge schemes [32]. Along this line, the docking scoring function values, E_{score}, are usually defined by Equation (3):

$$E_{score} = E_{inter} + E_{intra} \tag{3}$$

where in:

$$E_{inter} = \sum_{i\varepsilon ligand} \sum_{j\varepsilon protein} \left[E_{PLP}\left(r_{ij}\right) + 332.0 \, \frac{qiqj}{4r_{ij}^2} \right] \tag{4}$$

Note that the E_{PLP} stands for "piecewise linear potential", which consists of the use of two different parameter sets: one for the approximation of the steric term (i.e., Van der Waals) among atoms, as well as the other to assess the potential for hydrogen bonding. As can be seen, the second term is, of course, related to the electrostatic interactions among overloaded atoms. Typically, it is a Coulomb potential with a dielectric constant dependent on the distance (which can be approximately described as $D(r) = 4r$). Hence, for this, the numerical value of 332.0 is responsible for the electrostatic energy unit to be given in kilocalories per molecule, as well [32].

E_{intra} is defined as the internal energy of each ligand. That is:

$$E_{intra} = \sum_{i\varepsilon ligand} \sum_{j\varepsilon ligand} E_{PLP}\left(r_{ij}\right) + \sum_{flexiblebonds} A[1 - \cos(m.\theta - \theta_0)] + E_{clash} \tag{5}$$

Note that the first part of the equation (double summation) is among all pairs of atoms in the ligand, taking off those connected by two bonds. Thus, in this equation, the second term denotes the torsional energy, where θ is the torsional angle of the bond. Hence, if several torsions could be determined, then each torsional energy value is considered as an average among them. The last term, E_{clash}, assigns a penalty of about 1.000 if the distance between two heavy atoms (e.g., more than two bonds apart) is smaller than 2.0 Å, but does not take into account infeasible ligand conformations [32]. Thus, the docking search algorithm that is applied in the MVD program considers an evolutionary algorithm that is based on interactive optimization techniques (inspired by Darwinian evolution theory), which implies a new hybrid search algorithm conveniently called guided differential evolution. As such, this hybrid combines the differential evolution optimization technique with a cavity prediction algorithm during the search process, allowing a fast and accurate identification of potential binding modes (poses) [32,35,36].

3.3. QM/MM Procedure

In line with the large number of atoms present in the investigated systems, a quantum mechanics (QM)-based treatment becomes infeasible due to the high computational demand. However, the covalent

bond rearrangements in the reactional process cannot be ignored and treated exclusively through molecular mechanics (MMs). In this context, hybrid quantum mechanics–molecular mechanics (QM/MM) were employed in this investigation in order to study the reaction pathway involved in the reactivation process [37]. From this protocol, the AChE active site was treated through QM methods, DFT in this case, and the rest of the system was treated with MM-based methods [38]. From these calculations, the energetic barrier of the reactivation process of each enzyme–OP complex with trimedoxime was determined. This theoretical strategy has been previously employed in other works [26,38–43]. The QM part of the calculations was performed through the Gaussian 09 package, at the DFT level and 6-31g(d,p) basis set [44,45]. The delimited QM region includes: Ser203 residue bound to the respective OP, the residues Tyr124, Phe295, Arg296, Glu285, Ser298 and Trp286, in addition to trimedoxime. In this simulation, all precursors, transition states and intermediates were calculated and characterized by identifying imaginary frequencies [25,46,47]. Each system was fully optimized at the DFT level with conjugate gradient and quasi-Newton–Raphson algorithms. The final geometries were obtained with the density functional Becke's three-parameter exchange functional and the gradient-corrected functional of Lee, Yang and Paar (B3LYP) [35,48], by using a 6-31g(d,p) basis set.

4. Conclusions

In this work, we tested the in vitro efficiency of trimedoxime and applied computational techniques to evaluate the interaction modes and reactivity of this antidote in the reactivation process of the AChE inhibited by a range of OP nerve agents. Thus, the kinetic factors and interactions that govern the AChE enzyme reactivation process were investigated. With this in mind, our theoretical outcomes show that the active site of the inhibited AChE adopts different conformations according to the kind of neurotoxic agent. Therefore, these conformational changes in the site result in different interactions and a different reactivity of trimedoxime in the active cavity.

Our findings indicate that the performance of trimedoxime enhances by increasing its concentration; the best result found was for the reactivation of the AChE–VX adduct. On the other hand, our experimental results show that trimedoxime was inefficient in the reactivation of the AChE–GF and AChE–GD complexes. Interestingly, appropriate conformations were not found when simulating the reactivation mechanisms with these complexes, which can be explained, for instance, by the steric hindrance observed in the site, thus causing a significant conformational change in the cavity.

Through the MLR analysis, we can observe that the combination of interaction energy and reaction energy is sufficient to explain the experimental data with a high correlation. However, the mechanistic part has a greater weight and contributes most to the reactivation process through trimedoxime. Therefore, this work will bring about important contributions to the field of drug design and therapies, assisting in the development of a broad spectrum and more efficient reactivators.

Author Contributions: A.A.d.C., D.A.P. and B.T.L.P. performed the theoretical calculations, data analysis, elaboration of initial versions of this manuscript and figures preparation; T.C.R., E.F.F.d.C., E.N. and K.K. contributed in the technical-scientific evaluation of the final version and adjustments of language requirements. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

AChE	Acetylcholinesterase
*Mm*AChE	*Mus musculus* Acetylcholinesterase

OP	Organophosphorus compounds
ACh	Acetylcholine
BBB	Blood Brain Barrier
GA	Tabun
GB	Sarin
GF	Cyclosarin
GD	Soman
POX	Paraoxon
DDVP	Dichlorvos
SER	Serine
Tyr	Tyrosine
ARG	Arginine
GLU	Glutamic acid
PHE	Phenylalanine
TRP	Tryptophan
QM/MM	Quantum Mechanics/Molecular Mechanics
MLR	Multiple Linear Regression
TLC	Thin Layer Chromatography
HPLC	High-performance liquid chromatography
NMR	Nuclear Magnetic Resonance
MVD	Molegro Virtual Docker

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CONCLUSÃO

Com o grande número de casos de envenenamento por agentes neurotóxicos organofosforados observados no mundo anualmente, nota-se que o estudo para o desenvolvimento de fármacos e estratégias terapêuticas para o envenenamento por esses compostos é uma demanda atual, sendo isso um problema de saúde pública mundial. Grandes esforços vêm sendo empregados afim de desenvolver tecnologias tanto para o tratamento, como prevenção para o envenenamento por esses compostos.

Atualmente, os principais antídotos, os reativadores da AChE e BChE inibidas por OP, não tem amplo espectro de ação frente a variados agentes neurotóxicos, sendo assim necessário para um tratamento efetivo conhecer o agente ao que o indivíduo foi exposto.

Com isso, vimos com a revisão da literatura que algumas patentes significativas surgiram nos últimos anos no campo do tratamento e prevenção da intoxicação por OP. Exemplos disso, são moléculas capazes de reverter o processo de envelhecimento do complexo enzima envelhecida, processo que ocorre espontaneamente conforme o tempo passa após a exposição, além disso, moléculas capazes de ultrapassar a barreira hematoencefálica, duas das principais dificuldades encontradas no desenvolvimento de fármacos efetivos, com baixa toxicidade e com ação por mais tempo após a exposição aos agentes neurotóxicos.

A efetividade de um reativador depende de vários fatores, como a capacidade de interagir satisfatoriamente com o sítio ativo da enzima inibida, uma vez que cada complexo enzima inibida por um diferente OP tem sua cavidade modificada pelas interações intermoleculares que cada inibidor tem com os resíduos de aminoácidos da enzima. Com isso, foi realizado testes teóricos e experimentais com a oxima Trimedoxima na reativação da AChE inibida por diferentes agentes neurotóxicos. Foi observado os fatores termodinâmicos e cinéticos que regem a reativação desses complexos enzima inibida por estes OP frente a Trimedoxima, colaborando para o entendimento da efetividade desta oxima principalmente com o aduto AChE-VX.

Tendo isso em mente, o estudo e desenvolvimento de candidatos à fármacos para o tratamento de envenenamento por OP é um campo promissor e vem tendo grandes avanços nos últimos anos. Apesar disso, a busca de um antídoto universal de amplo espectro de ação ainda é uma meta a ser atingida. Este trabalho teve como objetivo reunir informações sobre as principais descobertas no campo no período de 2016 a 2019 e

juntamente colaborar com informações sobre a Trimedoxima no processo de reativação da AChE inibida, visando um maior entendimento deste processo afim de somar nos atuais esforços para a meta da busca por melhores reativadores, e consequentemente, um reativador de amplo espectro.

APÊNDICE

Publicações resultantes desta tese

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Slight difference in the isomeric oximes K206 and K203 makes huge difference for the reactivation of organophosphorus-inhibited AChE: Theoretical and experimental aspects

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ABSTRACT

Studies with oximes have been extensively developed to design new reactivators with better efficiency, and greater spectrum of action. In this study, we aimed to analyze the influence of the Carbamoyl group position change in two isomeric oximes, K203 and K206, on the reactivation percentage of *Mus musculus* Acetylcholinesterase (*Mm*AChE), inhibited by different nerve agents. Theoretical calculations were performed to assess the difference for the oxime activity with inhibited AChE-complexes and the factors that govern this difference. Comparing theoretical and experimental data, it is possible to observe that this change between the oximes results in different reactivation percentage for the same nerve agent, due to the different interaction modes and activation energy for the studied systems.

1. Introduction

Chemical warfare agentes (CWA) are defined as any substance which, because of its toxic properties, whether gaseous, liquid or solid, can cause harm to humans, animals and plants. In a military context, they would be substances destined to cause death, health damage or incapacity of individuals in wars and military operations, among other scenarios [1–3]. After many armed conflicts and episodes in which CWA were utilized, with intent to stop the use of this kind of mass destruction weapons, the Chemical Weapons Convention (CWC) was created in 1993, administered by the Organization for the Prohibition of Chemical Weapons (OPCW) located in The Hague, Netherlands. It came into force in 1997, when 170 countries signed the treaty agreeing to destroy existing chemical weapons stocks within a ten-year period [4–6]. Many toxic subtances are known, but only a few are classified as CWA by the CWC, which considers for such classification several properties such as high toxicity, action quickness after dissemination, imperceptibility to the senses and persistence. Many of these toxic subtances have great agricultural and industrial importance, being difficult to replace such products with new compounds with the same action and efficiency, despite the agreement [7].

Among the CWA classes, the most dangerous and toxic to humans are the nerve agents. Nerve agents have, as their base structure, organophosphorus compounds (OP), which are also the structures of several pesticides. Pesticides based on OP are of major agroindustrial importance, and they are among the most used agricultural products, especially in third world countries [8]. Poisoning by OP may occur through skin contact, oral, and through respiratory tract as well. The OP act by inhibiting, in an irreversible form, the Acetylcholinesterase (AChE) enzyme, which accounts for the acetylcholine (ACh) neurotransmitter hydrolysis into acetate and choline. ACh is responsible for the synapse in the nerve endings of the skeletal muscle and it also plays an important role in the central nervous system [9]. The neurotransmitter accumulation at the central and peripheral cholinergic sites results in an over-stimulation of the cholinergic receptors. The main symptoms of poisoning are excessive salivation, lacrimation, urination,

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Non-conventional compounds with potential therapeutic effects against Alzheimer's disease

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