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Derivatives of diisopropyl phenoxyphosphate with controlled reactivity for enhancement of Acetylcholine (ACh) neurotransmitter

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Abstract: Here, new phenoxide derivatives of diisopropyl flourophosphate for reaction with Lewis basic sites on acetyl cholinesterase (AChE) were designed. Such binding interaction or reaction inhibits the hydrolysis of the acetylcholine (ACh) neurotransmitter thus enhancing its concentration. This increased neurotransmitter concentration can enhance memory and cognition thus improving symptoms of neurodegenerative diseases such as Alzheimer disease and down syndrome. For docking analysis, we particularly targeted those reception sites on AChE that interacts with the ACh. This led to structural design of derivatives of diisopropyl phenoxyphosphate with controlled reactivity stemming from para substituted phenoxide leaving group. Impact of electron donating (CH_3, OCH_3) and withdrawing substituents ($COCH_3$) on para position of phenol group on rate of acyl addition elimination reaction was modeled using QM DFT technique. Difference in activation energy between electron donating and withdrawing substituents on phenoxide was noted hence making the derivatives of diisopropyl phenoxyphosphate less reactive and more selective. Docking also confirmed binding of designed derivatives with AChE. Hence novel derivatives with high binding energy and controlled reactivity were designed for retrosynthesis.

Keywords: Acetylcholinesterase; Neurotransmitter; Density Functional Theory; Molecular Docking Simulation; Diisopropyl Phosphate Derivatives

INTRODUCTION

Acetylcholine (ACh) is a neurotransmitter that is concentrated between the nerve synapses. Lower levels of ACh results in neurodegenerative diseases such as Alzheimer disease (AD) (Pandey and Singh, 2020). The decrease in the level of ACh could be due to increased activity of acetylcholinesterase enzyme (AChE) which hydrolyzes ACh into acetic acid and choline (McHardy *et al*.,2017). Thus, inhibiting AChE can enhance memory, cognition and learning capacity which is done by using AChE inhibitor drugs molecules (Pascoini *et al*., 2019). Diisopropyl fluorophosphate reacts with Lewis basic sites $–CH_2$-OH sites of AChE which undergo acyl addition elimination reaction with phosphonyl P=O group of the diisopropyl fluorophosphate, thus deactivating themselves to interact with ACh (González *et al.*, 2020). Diisopropyl fluorophosphate is highly reactive which can result in higher concentration of ACh causing prolonged muscle contraction (Millard *et al.*, 1999). While there have been many examples in recent times of designing and synthesis of AChE inhibitors (Sudhapriya *et al.*, 2019), the chief intention behind this research is to manipulate the structure of diisopropyl fluorophosphate itself to achieve stability and less reactivity. Hence, in this research, derivatives of diisopropyl phenoxyphosphate were designed to have controlled reactivity towards AChE, as shown in (A) in fig. 1 and to find the most highly selective derivative with less reactivity. Controlled reactivity was achieved by identifying the impact of substitution on para position of phenoxy leaving group on reactivity of the diisopropyl phenoxyphosphate molecule towards $–CH_2$-OH of the AChE site. QM calculation was performed on three derivatives of the diisopropyl phenoxyphosphate molecules with three different electron donating and withdrawing substituents at para position of the phenol leaving group (leaving groups in fig. 1A). Elimination of the tetrahedral intermediate was modeled by density functional theory (DFT) B3LYP method to understand the relationship between structure of leaving group and activation energy of elimination step. Activation energies of the elimination for all three derivatives was compared to see change caused by structural manipulation of leaving group. Docking was also performed to check binding energy values of the derivatives with reactive reception sites on AChE.

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MATERIALS AND METHODS

Binding energy analysis

Docking simulations were performed using molecular operating environment (MOE version 2015.10) software. Structures of all the designed molecules (1-3) were drawn on MOE. For preparation of drug target receptor sites, the X-ray co-crystallized structure of human AChE with resolution 2.35 Å (PDB ID - 4EY7) and donepezil was retrieved from RCSB-PDB (Cheung *et al*., 2012) in pdb formats.

QM Gaussian

The Gaussian 09 was used to perform QM calculations (Young, 2001) The geometries for all the tetrahedral intermediate of designed molecules (1-3) and their transition states of elimination step were optimized along with vibrational frequency analysis by using DFT with B3LYP method and basis set 6-311G+(d,p). CH_3OH was used to simulate -CH_2-OH Lewis basic sites on AChE. Activation energy was obtained by difference between energies of tetrahedral intermediate and transition states optimized molecular structures.

RESULTS

Enhanced understanding of how para substitution of electron donating and withdrawing groups on phenoxy leaving group influences reactivity is achieved by DFT calculation of activation energy (ΔG) of elimination step in which leaving group is eliminated from tetrahedral intermediate. table 1 lists the obtained activation energies. Electron donating substituents CH_3 and OCH_3 both increase the activation energy by decreasing the Lewis acidic character of P=O and destabilizing the leaving group. Electron withdrawing substituent $COCH_3$ decrease the activation energy thus making the molecule more reactive and less selective.

Binding energies listed in table 1 showed that molecule with CH_3 as para substitution has least binding energy (-7.115 kcal/mol) value with no specific interaction with 4EY7 reception sites. Molecule with OCH_3 at para position show π interaction with Trp86 site of 4EY7 and binding energy value was -7.465 kcal/mol. In case of acetophenone, $COCH_3$ reported with high binding energy value -7.670 kcal/mol. In compound (3), the hydrogen of isopropyl forms hydrogen bonds with the aryl ring of Tyr 341 site.

DISCUSSION

Neurotransmitters are needed to continue the passage of nerve impulses from one neuron to another across the synapse. Once the impulse has been transmitted AChE hydrolyzes the ACh. If the enzyme is inhibited by drug molecules such as diisopropyl fluorophosphate, then ACh accumulates and nerve impulses are enhanced, but if this process continues that prolonged muscle contraction and paralysis occurs (Sivam *et al.,* 1983). Hence, control over reactivity of the drug molecule is required. Reactivity of diisopropyl fluorophosphate can be decreased by structural modification of the leaving group to make the molecule more selective in reaction with -CH_2-OH of AChE. In this research work, reactivity of designed diisopropyl phenoxyphosphate derivatives (1-3) was fine-tuned by following two ways:

First, the control over Lewis acidic character of P atom comes from variation of the Lewis acidic character or the +δ charge on the Lewis acidic P atom of the P=O (fig. 1). The substituents at para position of the phenyl ring exert control over Lewis acidic character of the P=O. There will be higher partial +δ charge on P atom in case of presence of electron withdrawing substituent at para position ($COCH_3$) of phenoxide leaving group making the molecule more reactive with less activation energy for addition elimination reaction. Lower +δ charge makes the P atom less electrophilic resulting into less reactivity towards rate of acyl addition elimination reaction hence more selectivity can be attained. Having electron donating group at para position (CH_3, OCH_3), the aryl ring becomes more electron rich by resonance and which slows the rate of reaction and makes molecule more selective. The electron donation or withdrawing effect can be inductive, hyperconjugative, field effect or resonance. Para position is chosen to ensure coplanarity of substituent with the aryl ring, in case of resonance. Ortho substituent can become non-coplanar because of steric hindrance with sulfonyl group. Substituted leaving groups in fig. 1 shows aryl ring resonance with substituents at para position.

Second control over reactivity is exerted by stability of -δ charge on the O atom during transition state of elimination reaction (B in fig. 1). The phenoxide group acts as the leaving group with considerable -δ charge as shown in fig. 1. Less stability of this -δ charge can decrease the rate of reaction or increasing the activation energy, thus making the molecule less reactive. Electron donating substituents at para position (CH_3, OCH_3) are supposed to destabilize the phenol leaving group as in case of compound (1 and 2). Electron withdrawing group ($COCH_3$) on para position of the ring does reverse in compound (3). Hence, less reactive molecule becomes less reactive and more selective. Thus, by changing the stability of the para substituted phenoxide leaving group, control over reactivity of the molecule can be established.

CONCLUSION

Enhanced ACh neurotransmitter concentration can help in Alzheimer disease and down syndrome. The present diisopropyl flourophosphate drugs can increase ACh

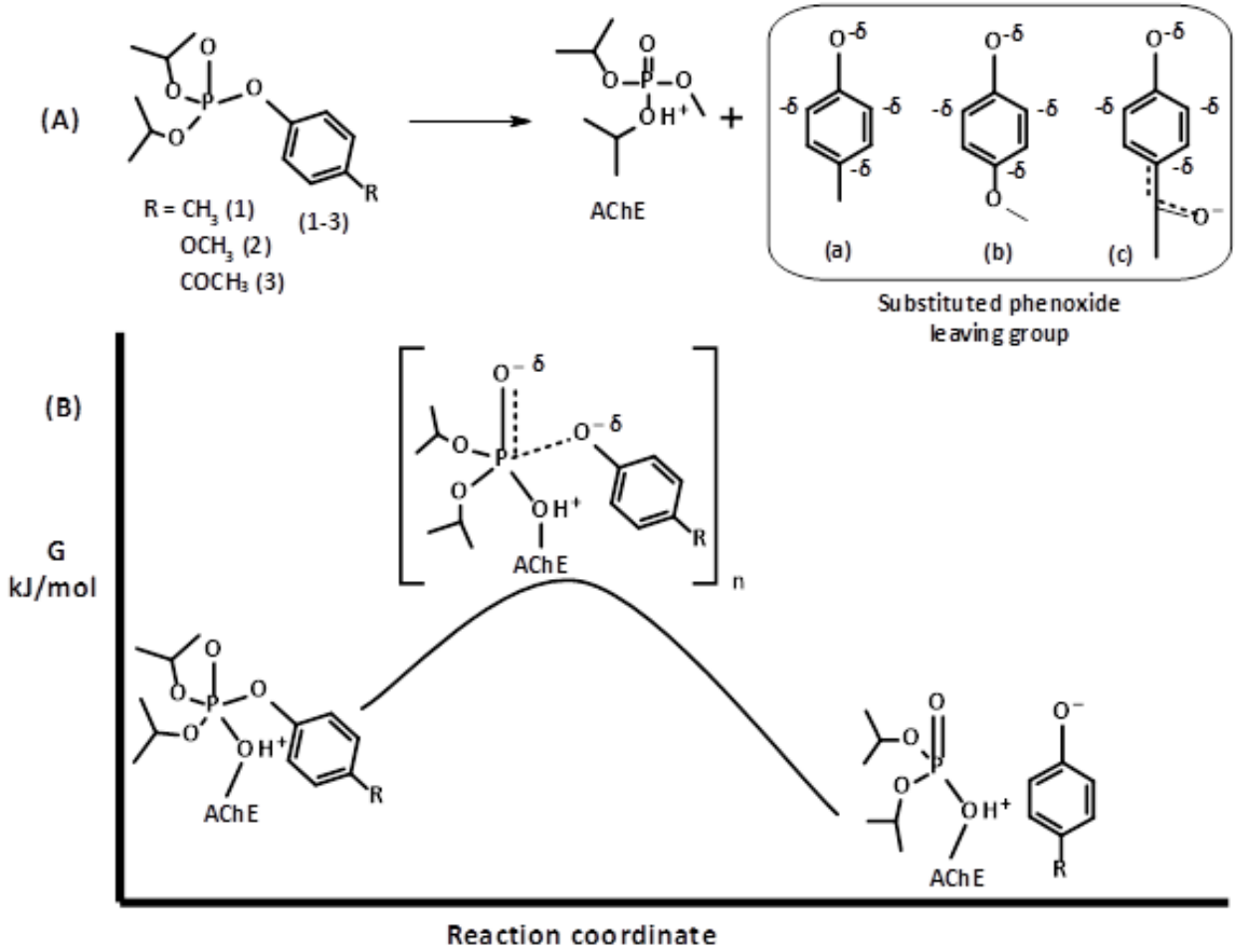

Fig. 1: (A) Reaction of diisopropyl phenoxyphosphate with OH sites of AChE. Resonance contributors of the para substituted phenoxide anion leaving group are also presented. (B) Tetrahedral intermediate THI of diisopropyl phenoxyphosphate undergoing elimination of phenoxide leaving group.

Table 1: Activation energies of compounds and their binding energy values on AChE.

S. No.	Substituent at para position	Energy of tetrahedral intermediate Hartrees (kJ/mol)	Energy of elimination step transition state (kJ/mol)	Activation energy (kJ/mol) = Energy of reactants – energy of transition state	Binding Energy (-kcal/nmol)
1	CH_3	934.8 (2454422.61)	1071.7 (2813749)	136.9 (280666)	7.115
2	OCH_3	1010.1 (2651938.9)	1151.2 (3022476)	141.1 (370458)	7.495
3	$COCH_3$	1377.8 (3617414.2)	1260.1 (3308445.3)	117.7 (309021)	7.670

concentration to very high levels because of higher reactivity. Hence, structural design of the designed derivatives was based on the controlling their reactivity. It was found that by changing electron donating and withdrawing substituents at para position phenol leaving group the rate of acyl addition elimination reaction was changed. Electron donating CH_3 and OCH_3 groups induced less Lewis acidic character on the P=O and destabilizing the leaving group thus decreasing rate of reaction thus making the molecule more selective. Reactant molecules their transition states were modeled using QM DFT technique. Docking showed high binding energy between the designed derivatives and acetylcholine esterase (AChE).

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