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IN SILICO STUDIES OF ATROPINE DERIVATIVES ON TO CBPF FOLLOWED BY GENOTOXICITY STUDIES

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ABSTRACT

Computational studies were carried out with the aim of identifying a potential atropine derivative of choline binding protein F (PDB ID: 2X8O), other than atropine monohydrate sulphate. Among the various atropine derivatives, atropine-n-oxide HCL which got docked with an energy of -232.16 kcal mol^{-1}, was considered for further analysis. Since the ligand was present in its unrefined form, it was subjected to the geometrical optimization technique as implemented in the GAUSSIAN software package. There was, however, no marked difference in the dock energy between unrefined and optimised atropine-n-oxide HCL,when docked onto the CBPF protein. As a part of genotoxocity study, which is yet to be conducted at a later stage, the identified potential ligand, atropine-n-oxide HCL, was docked to onto DNA to explore its affinity. The study showed that the ligand molecule got docked with an energy of -189.76 kcal mol^{-1} .

KEY WORDS: **CBPF, DNA, docking, HEX, atropine-n-oxide HCL, atropine derivative structures.**

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INTRODUCTION

Atropine is an alkaloid present in Datura stramonium, Atropa belladona, Mandragora officinarum and other plants of the family Solanaceae. This is a secondary metabolite and it serves as a drug with a wide variety of effects [1, 18, 23]. The molecule also could be a competitive antagnist for muscarinic acetylcholine receptor (mAchR) [1]. Because of its high medicinal application, atropine is considered as a "core medicine" by the WHO and has been mentioned in essential drug list (WHO model list of essential medicines, 2005) [2]. History of Roman and Islamic Empires says about the application of Solanaceae plants containing tropine alkaloids for anaesthesia in combination with opium. Cleopatra has used atropine extracts to dialte her pupils, in the hope that she would appear more alluring [3, 16, 17]. During renaissance period, atropine was taken only by extraction method but now atropine is available in powder form also [4]. LD50 of atropine is estimated to be 453 mg per person per oral [5]. The compound can be synthesized by the reaction of tropine with tropic acid in the presence of hydrochloric acid. Biosynthesis of atropine starts with l-phenylalanine when it first forms phenylpyruvic acid which is subsequently converteded to phenyl-lactic acid. Coenzyme A then couples phenyl-lactic acid with tropine forming littorine and undergoes rearrangement with P450 forming hyoscyamine aldehyde. A dehydrogenase then reduces the aldehyde to a primary alcohol making (-)-hyoscamine, which upon racemization forms atropine [6, 19, 21, 22].Atropine is widely used to selectively antogonize muscarine acetyl choline receptors (mAchRs) 1. Even nicotinicn acetyl choline receptors (nAchRs) also have been identified as one of the target for atropine [7,24, 25, 26]. Many studies have been reported to show that atropine play a role in inhibiting several types of ligand gated ion channels [8, 9] .Atropine can reduce the frequency of transient lower oesophageal sphincter relaxation (TLOSR) [10].A survey of PDB database showed the deposition of a total of 4 crystal structures of proteins complexed with atropine. The PDB IDs of these structures include :2X8O [11], 2X8P (Martin et al., 2013)[12], 1TH6 [13] and 2ARM (Singh et al ., 2006) [14]. Study by Sing et al has revealed that phospholipase is involved in the release of arachidonic acid - precursor for the biosynthesis of pro- inflammatory eicosonoids. Therefore, specific inhibitors of these enzymes may act as potent anti-inflammatory agents. Atropine and anisic acid - inhibits PL A (2) isolated and purified from the venom of Daboia russeli pulchella (Viper). These 2 compunds - bound to the enzyme at the substrate binding cleft of PLA (2), stablized by H- bonding and hydrophobic interactions. Genotoxicity is also an another important factor to be considered in any drug designing programme. The assay can reveal the toxicity of drug molecule at genetic level [27, 28]. Atropine sulphate monohydrate, one of the derivative(CASNo#5908-99-6). Based on AMES test, atropine sulphate monohydrate showed a negative genotoxocity level [15, 29]. Other derivatives of atropine family include atropine-methyl-nitrate, atropine oxide, atropine-n-oxide, atropine-n-oxide HCL; however, there is lack of sufficient evidence pointing toward the medical application of these molecules [20] Based on these observations, few gaps have been identified in atropine research. Till date, 3D structure for many of the atropine derivatives are not available. Further, there are no information about the interaction of atropine derivatives with DNA / proteins other than atropine mono hydrate sulphate. The main aim of the present investigation therefore was to explore remaining atropine derivative (atropine-methyl-nitrate, atropine oxide, atropine-n-oxide, atropine-n-oxide HCl) using both computational approach and experimental studies as a possible alternative for atropine.

MATERIALS AND METHODS

PDB structures for Receptors and Ligands: Choline-binding protein F crystal structure from PDB ID 2X8O (http://www.rcsb.org/pdb/explore/explore.do?structureId=2X8O)[11] by taking it's cordinates out from atropine - CBPF complex with the help of Swiss-pdb viewer 4.04 (Swiss Institute of Bioinformatics)[30, 34]. DNA crystal structure from PDB ID 3PA0 (Yeh, J.I. Shivachev.B et al.)[31] by separating PNA coordinates and modification, using Swiss-pdb viewer 4.04. Atropine derivatives (atropine-m-nitrate, atropine oxide, atropine-n-oxide, atropine-n-oxide HCl, atropine monohydrate sulphate) were drawn successfully and converted to 3d formate and PDB formate with the help of Chemdraw (David A. Evans and Stewart Rubenstein., 1985)[32].

Preliminary docking studies

Derivatives of atropine docked onto CBPF protein and DNA using HEX 6.3 package[33], the best docked output was selected based on total dock energy. Docking output were done by Swiss-pdb viewer 4.04 [30, 34]. Ligand having the highest dock energy was subjected to geometry optimization using Gaussian 03W Package installed on SGI Altix UV10 for optimizing the structure [35]. For optimizing the structure, Hatreefock theory with basis set “3-21g” was considered. Standard orientation of the optimized structure generated was visualized using ARGUS lab [36] package and the structure was saved in PDB format.

Docking studies with Ligand onto DNA/Protein receptor

Geometrically optimized atropine derivative docked onto CBPF protein (from PDB ID 2X8O) and DNA (from PDB ID 3PA0) separately using HEX 6.3. Post docking analysis of protein-ligand complex was done by Swiss-pdb viewer 4.04.

RESULTS

Preliminary docking studies were attempted to identify the potential atropine derivative. Among the 5 atropine derivative ligands that were selected in the present study for docking, atropine mono hydrate sulphate got docked with highest dock energy followed by atropine-n-oxide HCl onto the crystal choline-binding protein F (table 01,

Fig 2a). Since atropine mono hydrate sulphate is already a well established drug molecule, the present study focused on the oxide derivative of atropine as potential alternative drug. Analysis of the binding site of atropine-n-oxide HCl with choline-binding protein F and DNA separately, revealed the unrefined molecule got docked onto both CBPF as well DNA molecule. A total of 8 residues (pro178,180 , ala179 , trp181 , met210 , thr212 , asn226 , lys227) of CBPF showed the interaction (Fig 2b). Since atropine-n-oxide HCl used for docking purpose is not an optimised structure, it was subjected to geometry optimization using GAUSSIAN package. The structure was optimized at the end of 6 cycles recorded energy of -9.18 x 105 kcal mol-1 (Fig 1). In addition to this, maximum force, RMS force, maximum displacement and RMS displacement computed by the package were less than the set threshold values, suggesting the molecule has converged to global minimum energy. There was only marginal difference in the dock energy when the CBPF was docked onto geometrically optimized atropine-n-oxide HCL (dock energy = -255.06 kcal mol-1) (Fig 3a) in relation to the un-optimised atropine monohydrate sulphate (- 241.07 kcal mol-1); however, the protein complexed with the refined ligand structure showed, among 15 residues, only 2 were the same (met210, thr212) as observed for unoptimized ligand, the remaining 13 being different (ala211, arg218, asp193, gly205, 208 , lys215, phe204 , ser206, 207, 209 , tyr216, 203 , val217) (Fig 3b). In case of DNA molecule docked with geometrically optimized atropine-n-oxide HCl, the dock energy value suggest that the ligand appears to more affinity towards protein than DNA. Analysis of the residues of the protein interacting with ligand strongly suggests that protein and ligand occupy two different binding sites in the case of before and after optimisation (Fig 3a and 4a)

Table 1

List of atropine derivatives and the preliminary docking score by He

SL No	Atropine and its derivatives	E total
01	Atopine	- 204.38 kcal mol^{-1}
02	atropine-m-nitrate	-136.72 kcal mol^{-1}
03	atropine oxide	-137.53 kcal mol^{-1}
04	atropine-n-oxide	-206.78 kcal mol^{-1}
05	atropine-n-oxide HCL	-232.16 kcal mol^{-1}
06	Atropine monohydrate sulphate	- 241.07 kcal mol^{-1}

Table 2

E total value of Optimised and Unoptimised Atropine-n-oxide HCL docked onto CBPF and DNA.

SL No	DNA / protein	atropine-n-oxide HCL	E total
01	DNA	Optimised	-189.76 kcal mol^{-1}
03	CBPF	Optimised	-255.06 kcal mol^{-1}
04		Unoptimised	-232.16 kcal mol^{-1}

Figure 1

3D structure of atropine n oxide HCl geometrically optimized using GAUSSIAN software package [E (RHF) = -9.18 x 10^5 kcal mol^{-1} after 6 cycles]. Hatreefock theory with 3-21g" as the basis set was used.

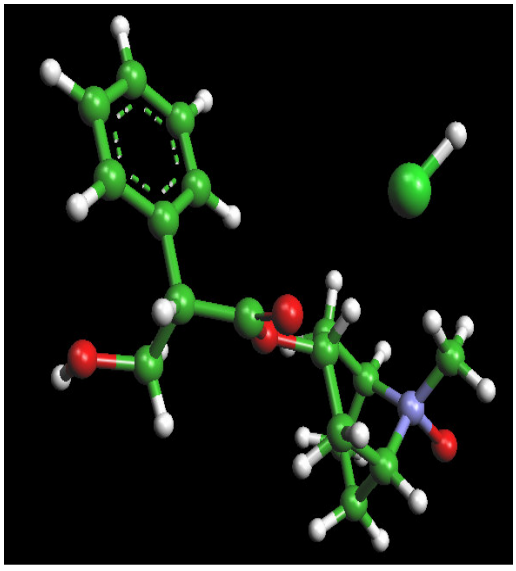


Figure 2a
CBPF docked with unoptimised Atropine N Oxide HCl using Hex Having the E Total (-232.16 kcal mol^{-1}).

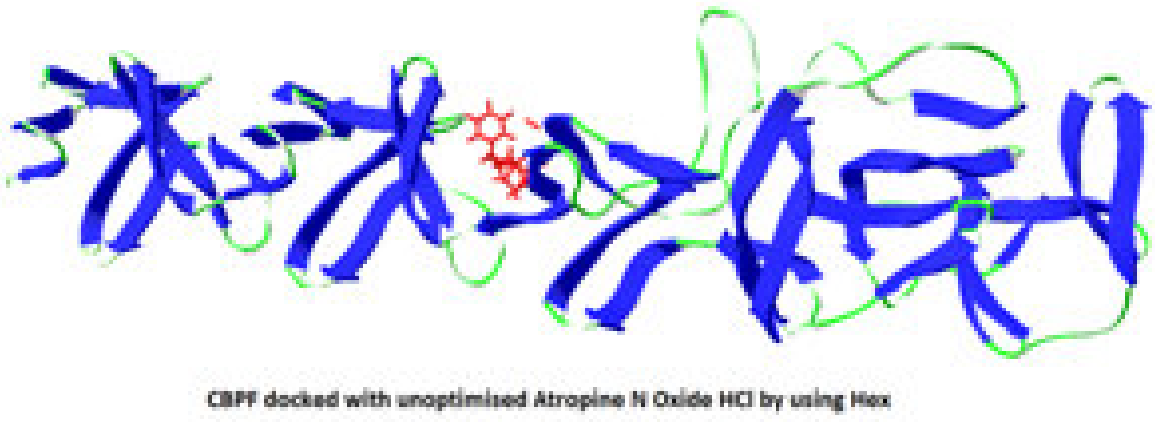


Figure 2b
Neighbour of selected residue (Atropine N Oxide HCl) with in 5.000 A^{0}.

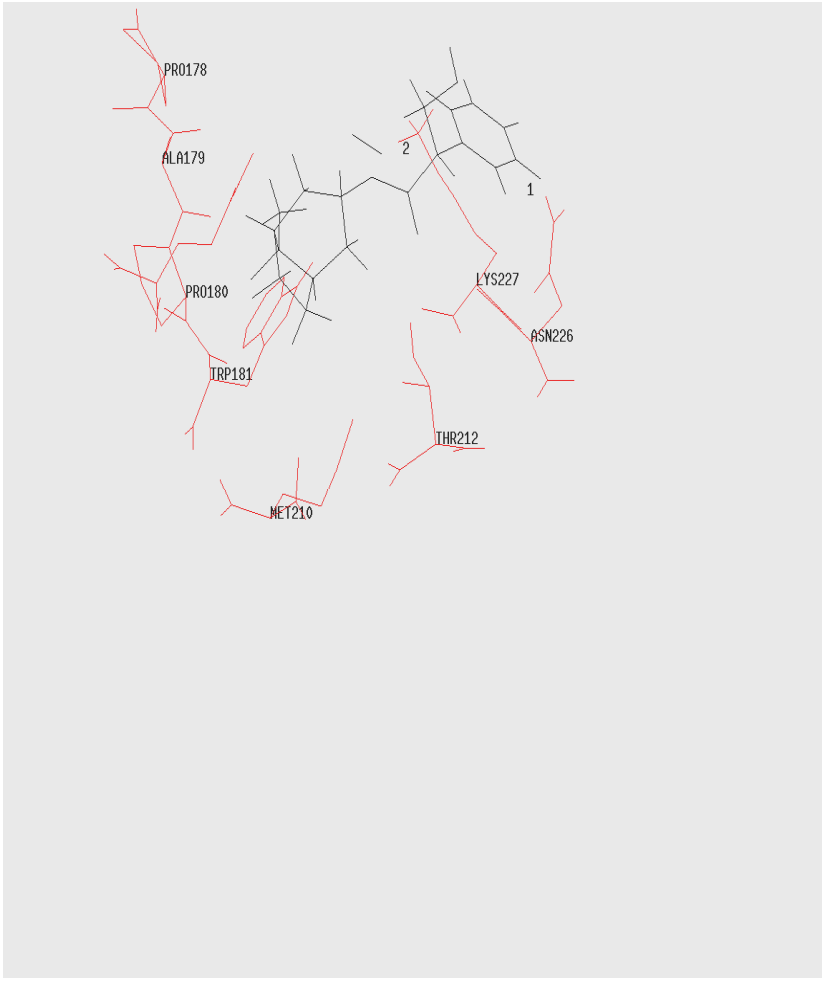


Figure 3a
CBPF docked with optimised Atropine N Oxide HCl using Hex Having the E Total (-255.06 kcal mol^{-1}).

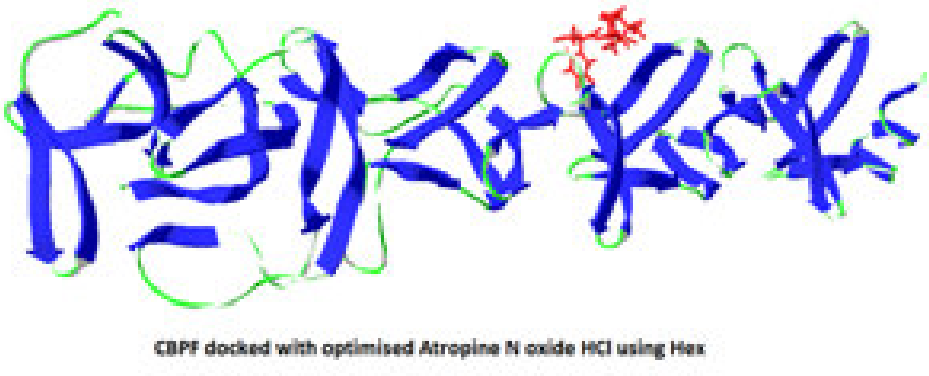


Figure 3b
Neighbour of selected residue (Atropine N Oxide HCl) with in 5.000 A^{0} .

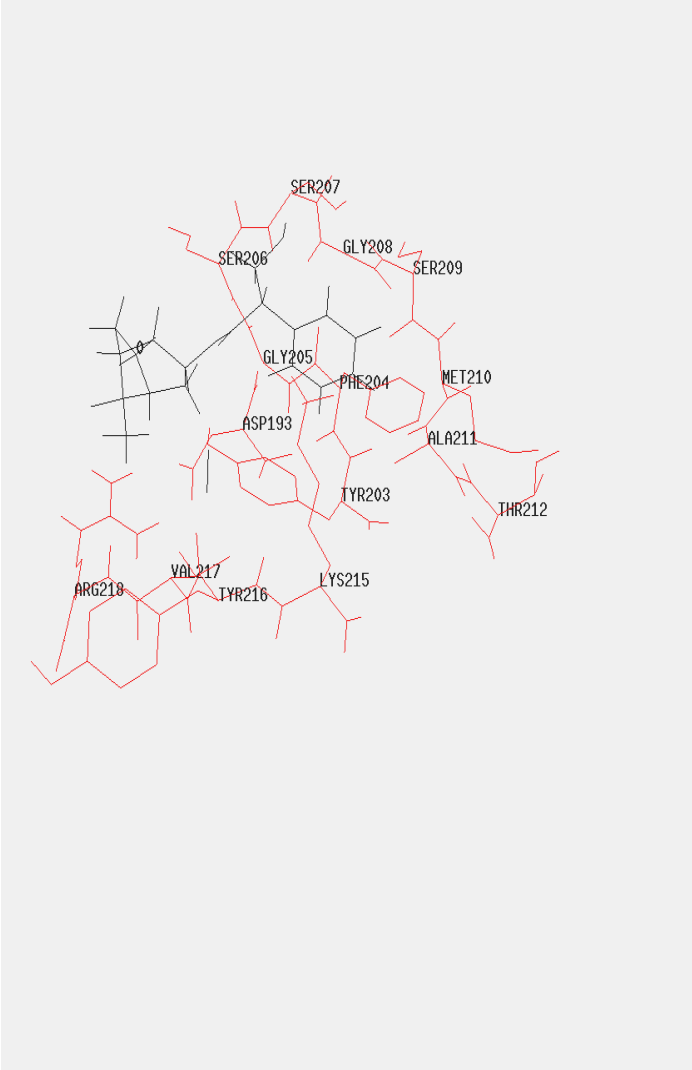


Figure 4a
DNA docked with optimised Atropine N Oxide HCl using Hex Having the E Total (-189.76 kcal mol^{-1}).

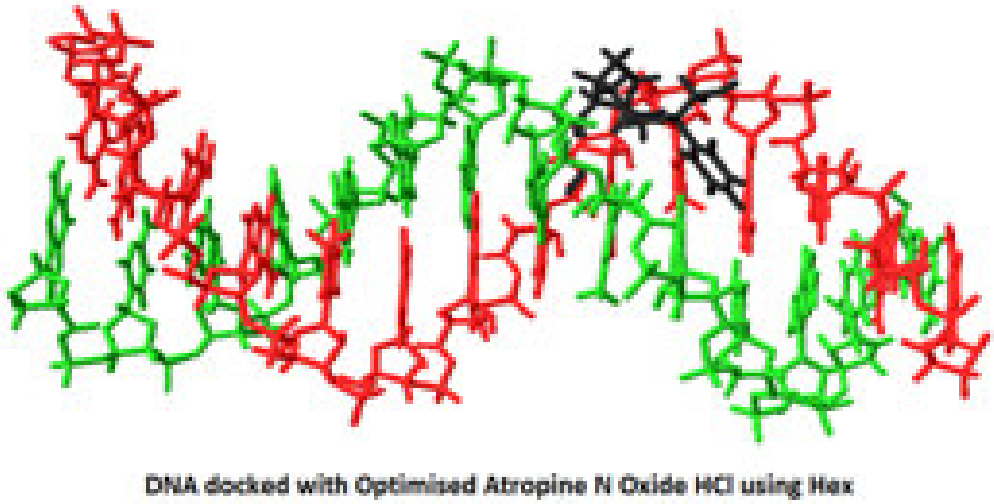
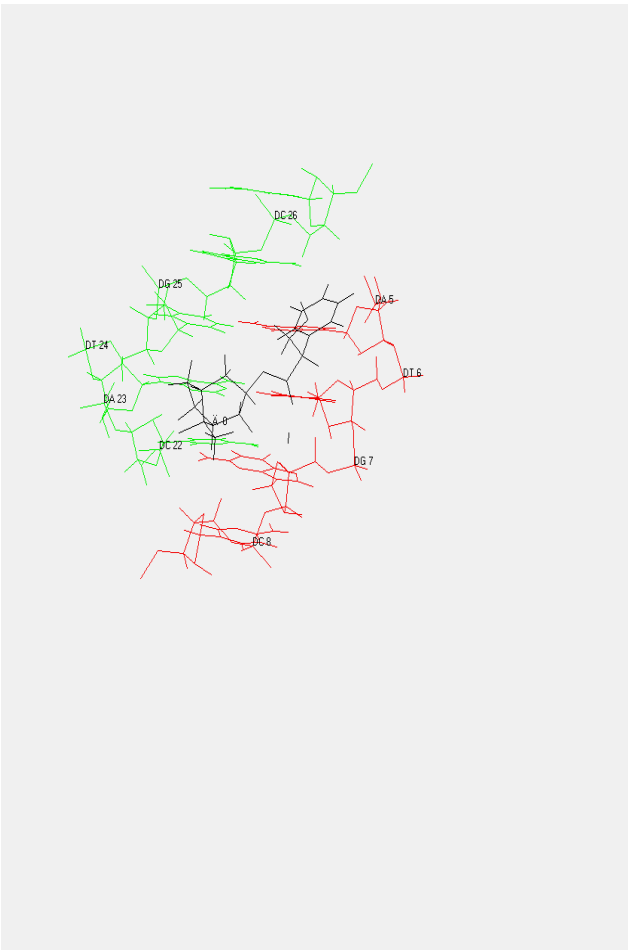


Figure 4b
Neighbour of selected residue (Atropine N Oxide HCl) with in 5.000 A^{0}.



DISCUSSION

Among the various atropine derivative that were screened for CBPF docking efficiency properties, atropine-n-oxide HCl structure got docked onto the crystal structure of choline-binding protein F with the (second) highest dock energy after the parent atropine molecule (atropine mono hydrate sulphate). However, atropine mono hydrate sulphate, being the current drug, was not considered in our study; instead, atropine derivative (atropine-n-oxide HCl) was chosen for subsequent studies, anticipating the molecule if produced with a reduced cost, could be considered as a second option for research studies oriented towards treating various neural disorders. Though there was marginal difference in the dock energy between the unoptimized and geometrically optimized atropine-n-oxide HCl, the refined ligand molecule interacted with larger number of residues compared to the unrefined ligand structure, got docked with more number of residues was present in its unoptimised form, it was optimised using geometrical optimization technique as implemented in GAUSSIAN software package. There was little difference in the dock energy between unoptimised and optimised atropine-n-oxide HCl structure when docked onto the CBPF and DNA; however, the protein complexed with unoptimised atropine-n-oxide HCl molecule shared few residues that were interacting with optimised atropine-n-oxide HCL structure, which was not seen in unoptimised one. In a study conducted by Suvannang et al 201148, aromatase inhibitors were geometrically optimized using appropriate model chemistry implemented in GAUSSIAN software, before attempting molecular docking. This explains the fact that docking program needs to have the ligands with the right molecular mechanics parameters and atom types .

CONCLUTION

Docking studies of atropine N oxide HCl with protein as well DNA shows that, ligand structure appears to function more as a protein binding drug rather than DNA binding drug. Based on this observation, it is possible to speculate that atropine N oxide HCl might have approchable medicinal property and less genotoxocity, which can be confirmed through in silico, in vitro as well as in vivo studies. To conclude, docking of refined atropine derivative molecule with CBPF showed increased number of interacting residues. Therefore, atropine-n-oxide HCL, if were to be produced with reduced cost, could be considered as a potential alternative.

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