Journal of Academia and Industrial Research (JAIR)
Volume 3, Issue 12 May 2015

ISSN: 2278-5213

RESEARCH ARTICLE

In Silico Analysis of Inhibitors for Inflammatory Targets

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Abstract

Immunological response by the organism towards the injurious stimuli is inflammation. Healing process of the tissues is initiated by this mechanism. Absence of inflammation leads to host of diseases such as fever, atherosclerosis and rheumatoid arthritis. For this reason inflammation is closely regulated by the body. The phenothiazine nucleus is well known for its inhibitory activity towards the regulatory enzymes that contribute to inflammatory diseases. Phenothiazines biological activity against the regulatory enzymes like phosphodiesterase (PDE), prostaglandin dehydrogenase (PS) and superoxide dismutase (SOD) control the inflammation. In the present study, analogues of phenothiazines were used for molecular docking. Inhibitors, which are having good inhibitory activity against the targets, were taken from the literature. Sixteen inhibitors of phenothiazine and their IC_{50} values were taken from literature. The proteins and ligands were energy minimized using OPLS force field. High throughput Virtual Screening had been carried out for all these compounds and based upon their glide energy, glide score and their IC_{50} values, some of the ligands were selected for Induced fit docking studies. The result shows that some of the ligands maintain favourable interactions with the active site residues of the target molecule. All docking studies were performed using the molecular modelling software GLIDE of Schrodinger package.

Keywords: Phenothiazine, phosphodiesterase, prostaglandin dehydrogenase, molecular docking.

Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Ferrero-Miliani *et al.,* 2007). It is a protective attempt by the organism to remove the injurious stimuli as well as to initiate the healing process for the tissue. Infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. On the other hand, an inflammation that runs unchecked can also lead to a host of diseases such as hay fever, atherosclerosis, and rheumatoid arthritis. For this reason inflammation is strictly regulated by the body. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and it is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues (Cotran *et al.,* 1998). A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells within the injured tissue (Serhan and Savill, 2005).

Prolonged inflammation leads to chronic inflammation which causes a progressive shift in the type of cells which are present at the site of inflammation and characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Pathophysiological functions of acute and chronic inflammatory diseases such as leuktrienes, cytokines and prostaglandin released from biosynthetic cascade of arachidonic acid are catalyzed through phospholipase A2 (PLA2), lipooxygenase and cycloxygenase enzymes (Lewis and Austen, 1981; Matsuka and Hirata, 2000; Taylor and Feldmann, 2004). These regulatory enzymes are believed to play a vital role in initiating and amplifying the inflammatory disorders in the body, contributing to diseases such as asthma, autoimmune disorders etc. (Greenfider and Anthes, 2005; Crunkhorn, 2007; Schenk *et al.,* 2007). The chemical diagrams of the compounds is shown in Fig. 1 and their IUPAC names are given in Table 1 and also the IC_{50} values of the compounds is shown in Table 2. Phenothiazines are important class of heterocyclic compounds possessing a wide spectrum of diverse biological activities like antitumor, antimalarial, antipsychotic, antiinflammatory etc. (Singh *et al.,* 2003; Wagner and Wagenknecht, 2008; Blobaum and Marnett, 2007).

Table 1. IUPAC name of the docked compounds.

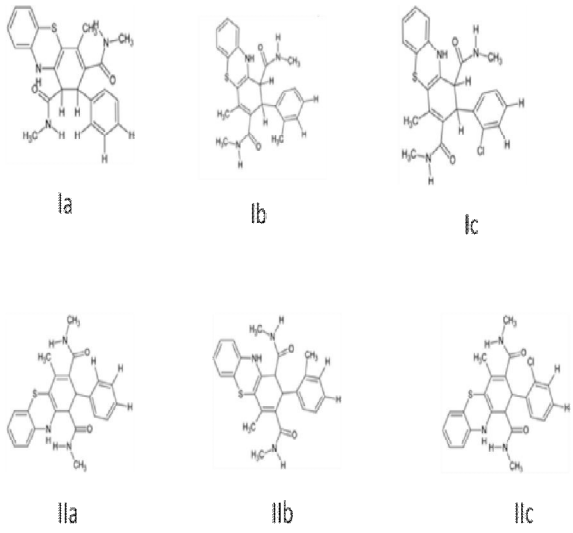
Compounds	IUPAC name
Ia	2-Phenyl-N,N0,4-trimethyl-2,10-dihydro-1H-phenothiazine-1,3-dicarboxamide.
Ib	2-(2-Methylphenyl)-N,N0,4-trimethyl-2,10-dihydro-1H-phenothiazine-1,3-dicarboxamide.
Ic	2-(2-Chlorophenyl)-N,N0,4-trimethyl-2,10-dihydro-1H-phenothiazine-1,3-dicarboxamide.
IIa	2-Phenyl-N,N0 ,4-trimethyl-10H-phenothiazine-1, 3-dicarboxamide.
IIb	2-(2-Methylphenyl)-N,N0 ,4-trimethyl-10H-phenothiazine-1,3-dicarboxamide.
IIc	2-(2-Chlorophenyl)-N,N0 ,4-trimethyl-10H- phenothiazine-1,3-dicarboxamide.

Table 2. IC_{50} values of screened compounds.

Compounds	IC_{50} value (µM)		
	PDE	PS	SOD
Compound-Ia	16	18	15
Compound-Ib	18	21	17
Compound-Ic	13	16	18
Compound-IIa	20	18	14
Compound-IIb	18	21	18
Compound-IIc	15	15	20

*PDE-Phospodiesatrse, *PE-Prostaglandin-D-synthase,
*SOD-Super oxide dismutase.

Fig.1. Structures of the docked compounds.



Phenothiazines exhibit promising target specific enzyme inhibition against Phosphodiestrase (PDE) (Sadanandam *et al.,* 2009). PDEs are enzymes which control cellular concentrations of the second messenger cAMP and cGMP. The PDEs are involved in many biological and metabolic processes and are proven for development of successful drugs for the treatment of a wide range of diseases. PDE9 is highly specific for cGMP (John *et al.,* 1993, 2002; Juan *et al.,* 2003; Liu *et al.,* 2005). The cGMP is involved in the biochemical mechanisms of Nonsteroidal antiinflammatory drugs (NASID) induced gastric injury (Frank *et al.,* 2005). Phosphodiestrase-9(PDE9) is a novel PDE family which has been identified. The residues in the active sites of PDE9 are found to be His-252, Met-365, ASP-402, Ile403, Ala-452, GLN-453, Phe-456-Leu-420 and Tyr-424. IBMX is a non-specific phosphodiestrase inhibitor.

Phenothiazines inhibitors were taken from the literature (Hube *et al.,* 1999) which is highly potent than IBMX. Prostaglandin D synthase (PS) or Human hematopoietic prostaglandin (PG) D synthase (H-PGDS) is the quaternary complex with glutathione (GSH), Mg^{2+} and an inhibitor, HQL-79, having antiinflammatory activities. H-PGDS inhibitors are found to suppress the progression of inflammation. It was confirmed that HQL-79 administration suppressed the muscular necrosis of *mdx* mice, an animal model of Duchenne's muscular dystrophy and the astrogliosis found in the twitcher brain (Aritake *et al.,* 2006) or after stab-wounding brain injury. The H-PGDS is a good target for designing antiinflammatory and antiallergic drugs (Inoue *et al.,* 2003). Therefore, 4-(benzhydryloxy)-1-[3-(1h-tetraazol-5-yl) propyl]piperidine (HQL-79) is an excellent lead compound for the development of novel H-PGDS inhibitors that promise to be new concept drugs against a variety of allergic and non-allergic diseases. It was found that 10H-Phenothiazines inhibitors taken from the literature are better than HQL-79. The residues in the active sites of protein are found to be Met99, Trp104, Phe102, Leu199, Tyr8, Arg14, Phe163, Gly13, Tyr152, GSH2001 and Met11. Human Superoxide dismutase (SOD1) performs an important role as an antioxidant enzyme in almost all cells of the human body, its principal catalytic action being the dismutation of superoxide anion, normal product of cell metabolism, to hydrogen peroxide and dioxygen. Each subunit of the homodimeric SOD1 contains one Cu atom, which is the site of catalytic activity and one Zn atom (Mohri *et al.,* 2006). Mutations in the SOD1 gene have been implicated as a cause of the neurodegenerative disease, Familial Amyotrophic Lateral Sclerosis (FALS), also known as motor neuron disease or Lou Gehrig's disease (Rosen *et al.,* 1993; Richard *et al.,* 2006). SOD can also play a vital role in an inflammation (Neychev *et al.,* 1994).

It is the best curing agent for inflammatory diseases. The residues in the active site of SOD1 are found to be Gly141, Lys136, His63, Asp124, Gly85, Gly72, and Arg143. In the present study, 10H-Phenothiazines were used for molecular docking study. Inhibitors selected from the literature showed good inhibitory activity against these targets and these analogues contain an interesting heterocyclic ring skeleton with two carboxylic/aromatic rings, which are connected to each other *via* a sulphide and an imino bridge (Bodea *et al.,* 1968; Leuo *et al.,* 1992; Valentine *et al.,* 2005). Due to the increased importance of these heterocyclic compounds, attempts were made during the past few years in the synthesis of new generation of 10H-Phenothiazines to exert their biological activity through modulation (Motohashi *et al.,* 2000; Thomas *et al.,* 2003; Darvesh *et al.,* 2007). As mentioned in the literature different targets involved in inflammation were taken from Protein Data Bank (PDB) viz., 3DYN (PDE), 2CVD (PS) and 2C9V (SOD). The structure of the targets and the ligands were minimized using OPLS force field and the FINAL conformation was taken for docking studies. These minimized structures were subjected to High Throughput Virtual Screening (HTVS) against each of the targets. 2, 10-dihydro-1H-phenothiazines (4a-c) and 2-aryl-10H- phenothiazines (5a-c) inhibitors were taken from literature. The 10H-phenothiazines with best compounds were identified based on GLIDE energy and Docking score and were selected for Induced Fit Docking (IFD). Interaction between the target and ligands were observed for different poses. Ligand with best score and energy was selected. Molecular docking software Schrödinger GLIDE in LINUX platform was used.

Materials and methods

All computational work was performed using the GLIDE (Grid Based Ligand Docking with Energetics) software developed by Schrodinger, which runs on Red-hat Linux system. Sixteen compounds were selected from the literature for the docking studies. *In vitro* studies on enzyme inhibitory analogues 2,10-dihydro-1-H-phenothiazine (Ia-c) and 2-aryl-10-H-phenothiazine (IIa-c) exhibited promising *in vitro* enzyme inhibitory activity and further structural variation of these compounds could result in designing potent lead molecules in the therapy of many inflammatory diseases. CASTp finder was used to find the active site residues of the protein. The general procedure followed for modeling studies is as follows: All the compounds were built using MAESTRO. The energies of these compounds were minimized in GLIDE software using OPLS (Optimized Potentials for Liquid Simulations) force field. Steepest descent algorithm was used for minimization, followed by conjugate gradient method, until it reached an RMS (root mean square) gradient of 0.001 kcal/mol. Using similar procedure, the target protein molecules (PE, PS and SOD) were energy minimized. The grid was generated so that it includes the active sites of inflammatory targets. HTVS had been carried out for all compounds. HTVS to SP to XP enriched the data at every level such that only an order of magnitude fewer compounds need to be studied at the next higher accuracy level. HTVS is a rigid docking method. Here only four are selected compounds based on their Glide energy, Glide score and their IC_{50} values. Then we performed Induced Fit Docking. IFD is a Docking Method in which both protein and ligand are flexible to dock, whereas in HTVS only the ligand is flexible. Based on these IFD values, the best ligand was selected.

Results and discussion

The present work was evaluated using the interaction of 1H-phenothiazine derivatives with Phospodiestarse (PDB ID: 3DYN) using the docking program GLIDE. Most of the compounds that were docked have interaction with the active site residues like His-252, Met-365, ASP-402, Ile403, Ala-452, GLN-453, Phe-456-Leu-420, Tyr-424 and the cofactor PCG-900 is shown the Figs. 2-8 and Table 3-4 show the Glide energy and Glide score.

Fig. 2. Interaction of co-crystalized ligand with PDE.

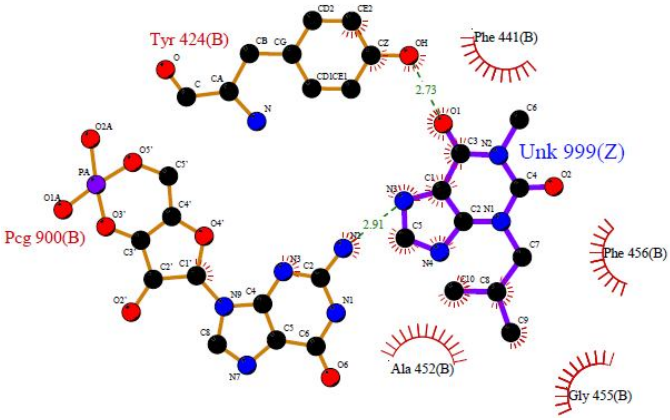


Fig. 3. Interaction of 1H-phenothiazine derivative-Ia with PDE.

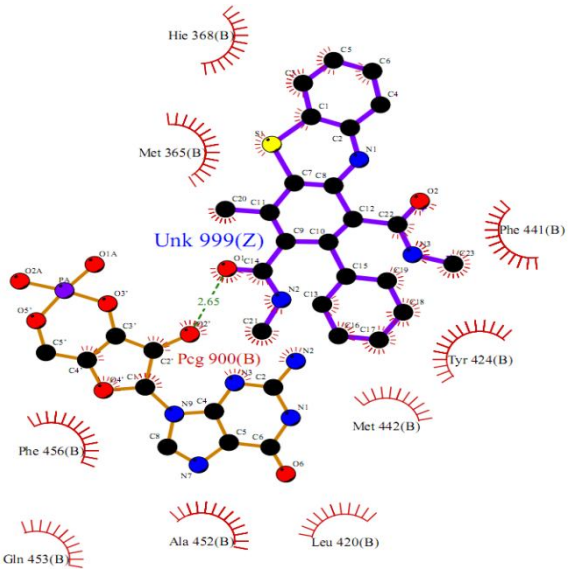


Table 3. Induced Fit Docking results (IFD) of co-crystallized ligand with PDE.

Compound	Residues	Hydrogen bond (D-H…A)	Distance (Å)	Glide score	Glide energy (kcal/mol)
1	PCG900 TYR424	N-H…O O-H…O	2.908 2.792	-6.443	-29.776

Table 4. Induced Fit Docking results (IFD) of PDE with compounds (Ia-Ic&IIa-II Ic).

Compound	Residues	Hydrogen Bond (D-H…A)	Distance (Å)	Glide score	Glide energy (kcal/mol)
Ia	PCG900	O-H…O	2.645	-7.643	-50.847
Ib	TYR424 PCG900	O-H…O O-H…O	2.910 2.87	-6.051	-39.808
Ic	ASN300 PCG900	O-H…O O-H…O	2.77	-5.785	-39.599
IIa	TYR424 PCG900	O-H…O O-H…O	2.96 2.88	-6.118	-42.521
IIb	TYR424 PCG900	N-H…O N-H…O	2.96 2.88	-6.449	-39.417
IIc	TYR424 PCG900	O-H…O N-H…O	2.774 3.310	-7.013	-47.081

Fig. 4. Interaction of 1H-phenothiazine derivative-Ib with PDE.

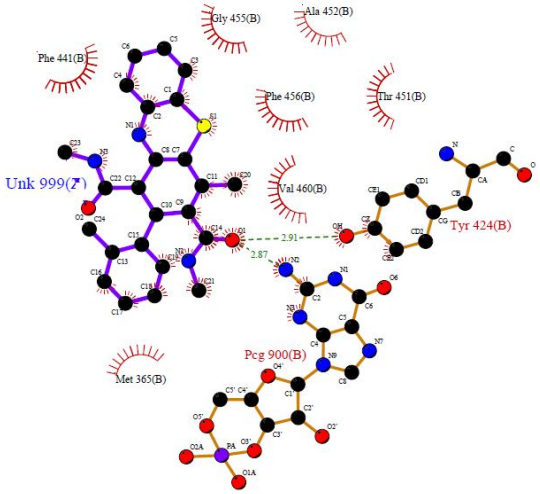


Fig. 5. Interaction of 1H-phenothiazine derivative-Ic with PDE.

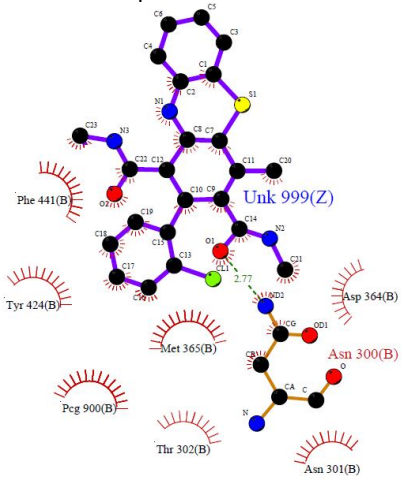


Fig. 6. Interaction of 1H-phenothiazine derivative-IIa with PDE.

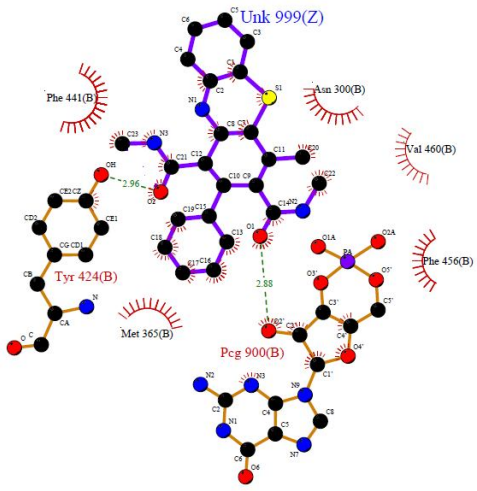


Fig. 7. Interaction of 1H-phenothiazine derivative-IIb with PDE.

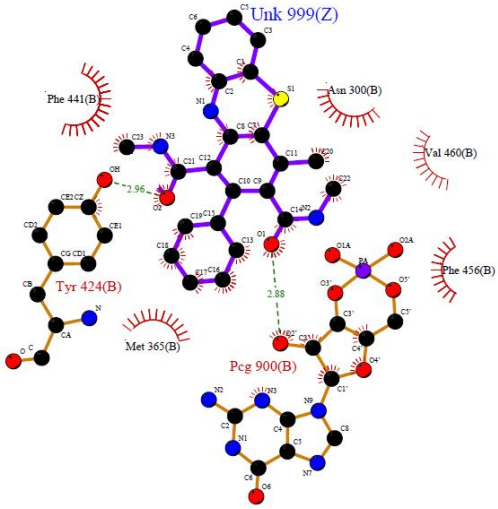


Fig. 8. Interaction of 1H-phenothiazine derivative-IIc with PDE.

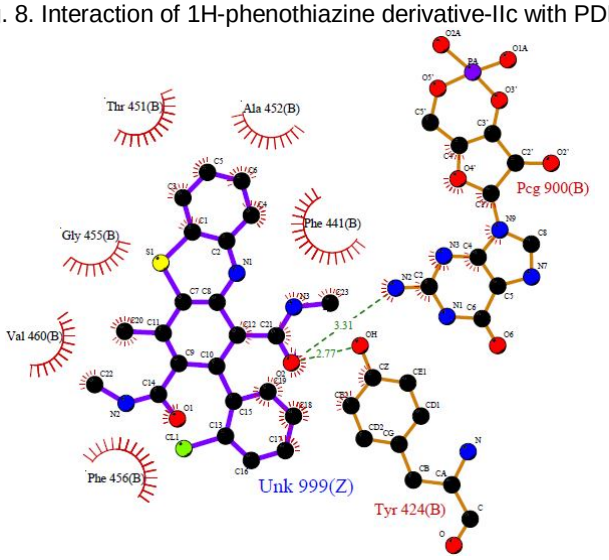


Fig. 9. Interaction of co-crystal with PE.

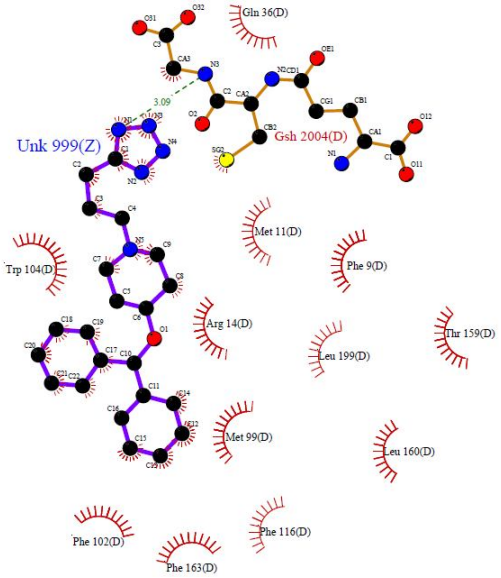


Fig.10. Interaction of 1H-phenothiazine derivative-Ia with PE.

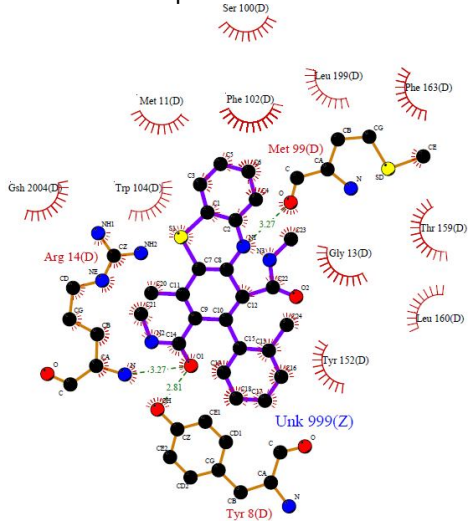


Fig. 11. Interaction of 1H-phenothiazine derivative-Ib with PE.

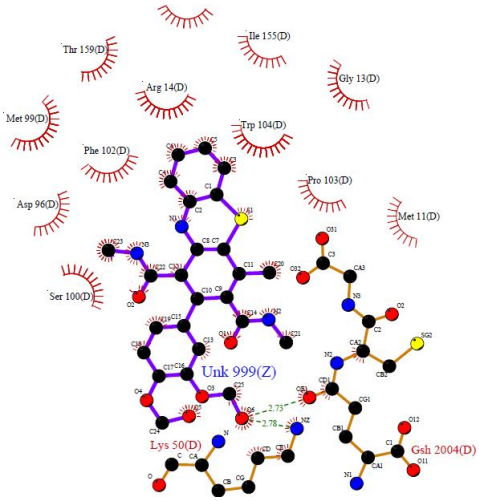


Fig. 12. Interaction of 1H-phenothiazine derivative-Ic with PE.

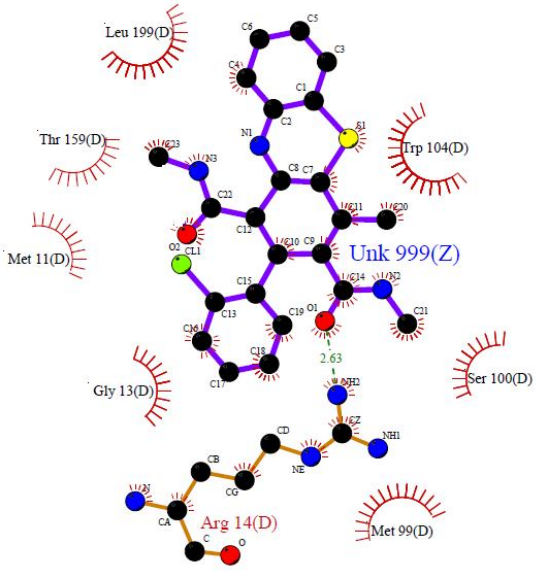


Fig. 13. Interaction of 1H-phenothiazine derivative-IIa with PE.

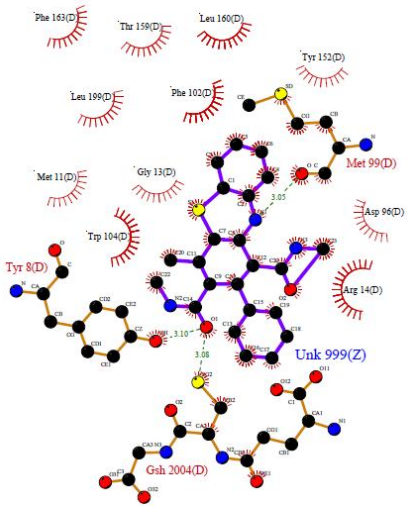


Table 5. Induced Fit Docking results of co-crystallized with PE.

Compound	Residues	Hydrogen bond (D-H…A)	Distance (Å)	Glide score	Glide energy (kcal/mol)
HQL-79	GSH2004	N-H…N	3.09	-7.494	-49.987

Table 6. Induced Fit Docking results of PE with compounds (Ia-Ic&IIa-II Ic).

Compound	Residues	Hydrogen Bond (D-H…A)	Distance (Å)	Glide score	Glide energy (kcal/mol)
Ia	TYR8 ARG14 MET99	N-H…O N-H…O N-H…O	2.81 3.27 3.27	-9.635	-59.058
Ib	GSH2004 LYS50	O-H…O N-H…O	2.73 2.78	-9.640	-61.396
Ic	ARG14	N-H…O	2.63	-7.496	-49.786
IIa	TYR8 GSH2004 MET99	O-H…O N-H…O N-H…O	3.10 3.08 3.05	-9.986	-60.023
IIb	GSH2004 ARG14	O-H…O N-H…O	3.21 2.75	-8.915	-56.803
IIc	GSH2004 ARG14	N-H…O N-H…S	2.75 3.15	-6.753	-46.899

Fig. 14. Interaction of 1H-phenothiazine derivative-IIb with PE.

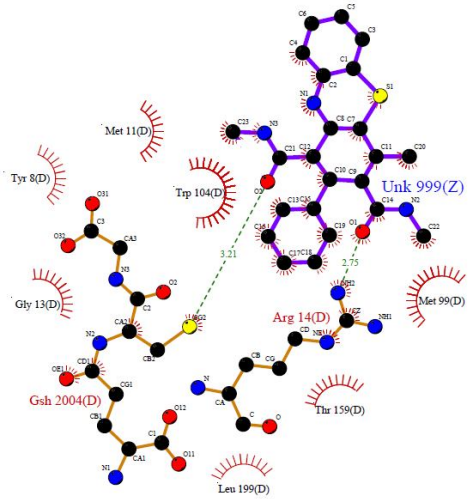
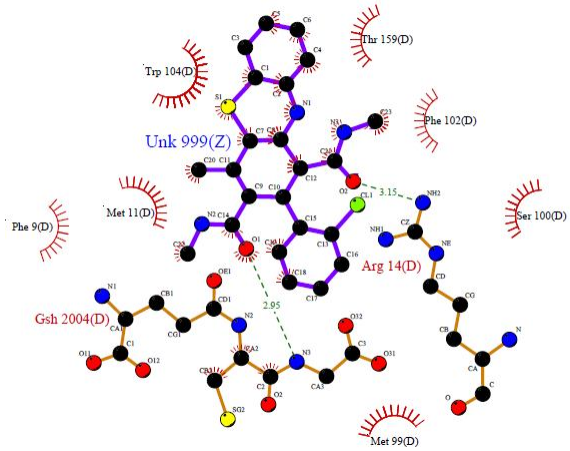


Fig. 15. Interaction of 1H-phenothiazine derivative-IIc with PE.



Similarly, the same work was carried out for Prostaglandin D synthase (PDB ID: 2CVD). Compounds showed strong hydrogen bond interaction with the active site residues Met-99, Trp-104, Phe102, Leu-199, Arg-14, Phe-163, Gly-13, Tyr-152, Met-11 and the cofactor GSH2001 is shown in the Figs. 9-15 and Table 5-6 show the Glide energy and Glide score. Docking studies had comes out for Superoxide dismutase (PDB ID: 2C9V). For this target also, the 1H-phenothiazine inhibitors showed good interaction with the active site residues Gly141, Lys136, His63, Asp124, Gly85, Gly72, and Arg143 is shown in Figs. 16-21 and Table 7 show the Glide energy and Glide score.

Fig. 16. Interaction of 1H-phenothiazine derivative-Ia with SOD.

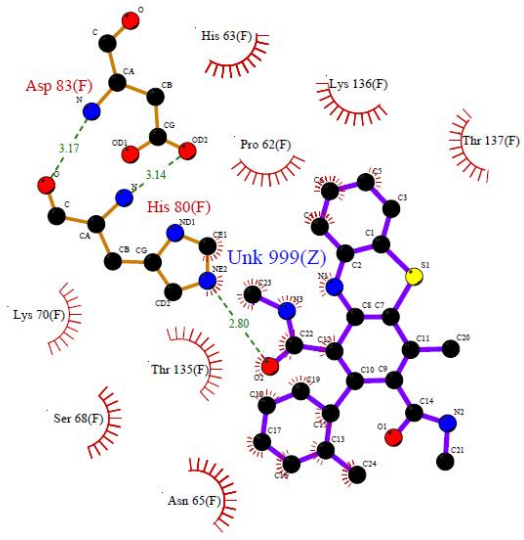


Table 7. Induced Fit Docking results (IFD) of SOD with compounds (Ia-Ic & IIa-II Ic).

Compound	Ligand conformation	Hydrogen Bond (D-H…A)	Distance (Å)	Glide score	Glide energy (kcal/mol)
Ia	ASP83 ASP83 HIS80	N-H…O O…H-N N-H…O	3.17 3.14 2.80	-5.810	-43.503
Ib	ASN65 LYS136	N-H…O N-H…O	2.76 3.04	-3.762	-41.229
Ic	ASN65 LYS136	N-H…O N-H…O	2.84 2.91	-4.063	-38.746
IIa	HIS20 ARG143 ARG143	N-H…O N-H…O N-H…O	2.88 2.80 2.86	-3.861	-38.086
IIb	LYS136 ASN65	N-H…O N-H…O	3.04 2.76	-2.521	-29.279
IIc	LYS136 ASN65	N-H…O N-H…O	2.91 2.84	-3.506	-31.276

Fig. 17. Interaction of 1H-phenothiazine derivative-Ib with SOD.

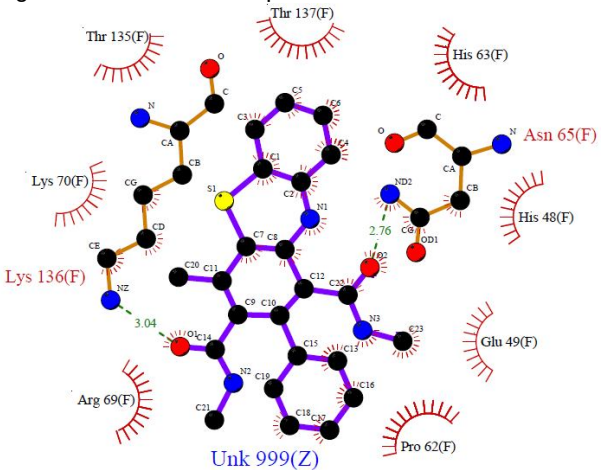


Fig. 18. Interaction of 1H-phenothiazine derivative-Ic with SOD.

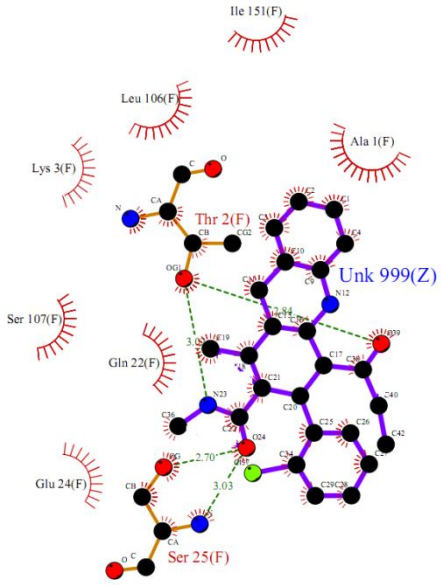


Fig. 19. Interaction of 1H-phenothiazine derivative-IIa with SOD.

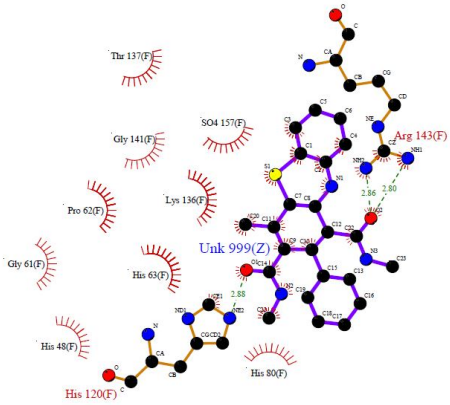


Fig. 20. Interaction of 1H-phenothiazine derivative-IIb with SOD.

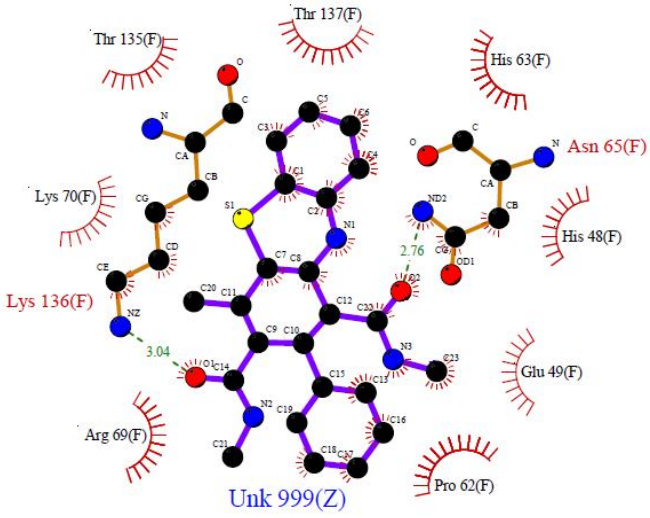
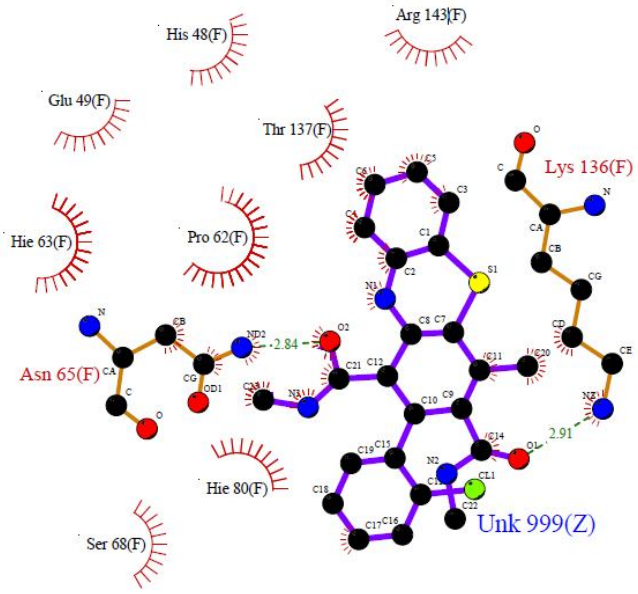


Fig. 21. Interaction of 1H-phenothiazine derivative-IIc with SOD.



Among the compounds which were docked, compounds Ia-c and IIa-c showed best docking score and glide energy compared to co-crystal ligand. The results of the docking studies suggest that the compounds 2,10-dihydro1H-phenothiazine derivatives (Ia-c) and 2-aryl-10H-phenothiazines (IIa-c) proposed show orientation close to active site and these compounds may be used as leads for designing future pharmaceuticals that may be used as potential inhibitors of the receptors. The native protein structure of SOD was taken from the PDB ID: 2C9V. The same six compounds were selected for IFD studies and docked against the target protein. Key residues of the receptor were identified using active site prediction tool based on physico-chemical descriptors (Singh *et al.,* 2011).

Conclusion

The Phosphodiesterase, Prostaglandin D synthase and Superoxide Dismutase have the potency to modulate a large range of inflammatory mediators which release through independent mechanism. The receptors have been shown to be particularly involved in this inflammatory process, indicating that these receptors subtypes could be good targets. This study focuses on a series of ligands which can be used for rational drug design and molecular docking studies. The above mentioned studies were made on potential phenothiazine derivatives which turn out to be effective inhibitors the receptors. All the compounds were docked into the active sites of the receptors. As a result, we can conclude the that 2,10-dihydro-1H-phenothiazine analogues Ia-c, and 2-aryl-10H-phenothiazine analogues (IIa-c) might be the most potent inhibitors based on Glide energy, Glide score and Hydrogen bond interactions with the residues in the active site of the receptors.

References

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