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DOCKING STUDIES OF NONSTEROIDAL ANTI INFLAMMATORY DRUGS AGAINST INDOLEAMINE 2, 3-DIOXYGENASE USING MOLEGRO VIRTUAL DOCKER

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ABSTRACT

Indoleamine 2, 3-dioxygenase (IDO) is the regulatory enzyme of tryptophan degrading kynurenine pathway. The over-expression of IDO, however, leads to increase concentration of kynurenine pathway metabolites, particularly the neurotoxic metabolites 3-hydroxykynurenine and quinolinic acid. Quinolinic acid recently been established as one of the key players involved in the pathogenesis of Alzheimer's disease (AD) and depression. The present result illustrates the binding of established non-steroidal anti-inflammatory drugs against IDO enzyme using Molegro Virtual Docker software (MVD). For this purpose we have selected one structure hits of protein overly expressing in disease from protein data bank (PDB) and non-steroidal anti-inflammatory (NSAIDs) (phenylbutazone, lornoxicam and allopurinol). Docking results show that all NSAIDs fit well into the active site of IDO. Energy scores for the top ranked ligand molecule phenyl butazone -122.256 K cal/mol, lornoixacam -112.28 K cal/mol and allopurinol -84.8765 K cal/mol. It is concluded that phenylbutazone, lornoxicam and allopurinol are possible lead molecules. Present results also prooved that they are competitive inhibitors of IDO. Our study also gives an idea that peripheral anti inflammation approaches might be beneficial in the therapy of inflammation-related central nervous system diseases such as Depression and Alzheimer.

Key-words: Indoleamine 2,3-dioxygenase, tryptophan, Molegro Virtual Docker software, non steroidal anti-inflammatory, quinolinic acid

INTRODUCTION

Indoleamine 2,3dioxygenase (IDO) (EC 1.13.11.52) breaks down the tryptophan (TRP) by the oxidative cleavage process. IDO is usually found in the tissues, such as neuroglia, macrophages of the central nervous system (CNS) in mammals. IDO is a haem containing monomeric enzyme (Botting, 1995). IDO utilizes superoxide and O_2 during catabolism (Taniguchi *et al*., 1979) as well as dihydroflavin mononucleotide and tetrahydrobioprotien use as cofactor (Ozaki *et al*., 1987). Modification of TRP metabolism triggered by pro inflammatory cytokines has emerged as a notion to elucidate pathophysiology of major depression. It shares TRP with the serotonin (5-HT) pathway. Pro inflammatory cytokines induce IDO in stress, stimulate the KP, and reduce serotonin synthesis; subsequently decrease serotonin synthesis may be the cause of major depression. Moreover, metabolites of the KP have both activities like neuroprotective/neurotoxic; kynurenic acid is neuroprotective whereas 3-hydroxykynurenine and quinolinic acid are neurotoxic. The over-expression of IDO, however, leads to augmented concentration of kynurenine pathway metabolites, particularly the neuroactive metabolites 3-hydroxykynurenine and quinolinic acid. Elevated levels of these metabolites have been linked to neurological conditions including cerebral malaria (Sanni *et al*., 1998), AIDS dementia complex (Sardar and Reynolds, 1995) and (Brew *et al*., 1996), AD (Winder *et al*.,1999, 2000) and Hungtintion's disease (Winder *et al*., 1999, 2000 and Okuda *et al*. ,1996). Metabolism of tryptophan through the KP, produces neurotoxic metabolites which are associated in the pathogenesis of Alzheimer disease (AD). Oxidative stress significantly damages neuronal tissue via 3-hyderoxykynurenine (3-HK) and 3-hydroxyanthranilic acid (3-HAA) which may contribute to neurodegenration through consequent amyloid-β accumulation, glial activation, and up regulation of KP. The over expression of IDO-1 and 3-HK in AD tissue sections confirms the up regulation of the kynurenine pathway in AD (Bonda *et al*., 2010). Recently Coptisine, a novel IDO inhibitor, has been reported as a potential new class of drugs for AD treatment. (Yu *et al*., 2015).

Kynurenine pathway is up-regulated in brain of AD patients, leading to increase in the excitotoxin quinolinic acid as proved by immune histochemical study. IDO activity is raised in AD (Guillmen *et al*., 2005). Quinolinic acid has a potent neurotoxic effect. Studies showed that the involvement of quinolinic acid in many psychiatric disorders, neurodegenerative processes in the brain, as well as other disorders. Though the detailed mechanisms connecting inflammation and depression still not clear, IDO plays an important role in inflammation and depression. IDO is activated by Pro-inflammatory cytokines, such as interferon-γ and tumor necrosis factor-α, (Wang *et al*., 2010).

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Induction of IDO leads to divert TRP metabolism, from synthesis of serotonin to the formation of neurotoxic TRP metabolites such as quinolinic acid, and leads to depression-like behaviors through both glutamate and serotonin pathways (Dantzer *et al*., 2008, Muller and Schwarz, 2007). 1-Methyltryptophan (1-MT), IDO antagonist, inhibits depression-like behaviors in LPS-challenged mice, induction of IDO paly an important role in inflammation-related depression. So, peripheral inflammation and depression are strongly related with each others it is necessary to foresee a beneficial effect of anti-inflammatory therapy on depression-like behavior. Celecoxib, a non-steroidal anti-inflammatory drug has been recommended for the treatment of such behavior (Guo *et al*., 2009). IDO is a main therapeutic target for the treatment of above mention disease. We can overcome this problem by finding the inhibitors of this enzyme. Until, the best known IDO inhibitors are 1methyl D-tryptophan (Cady and Sono, 1991), ß-Carboline (Eguchi *et al*., 1984). In 2006, effective nanomolar inhibitors were isolated from marine invertebrates extract (Brastiano *et al*., 2006; Pereria *et al*., 2006). Simultaneously new brassinin based IDO inhibitors were published (Banerjee *et al*., 2008; Gaspari *et al*., 2006). Many IDO inhibitors show either uncompetitive or noncompetitive inhibitory kinetics that are similar to those present in phenyl imidazol (PIM) and norharman ligands, which are known to directly bind with haem iron and to occupy the expected TRP binding site (Kumar *et al*., 2008). In this work we have selected one structure hits of protein overly expressing in disease from PDB. The binding stereochemistry of the NSAID with IDO has not yet been done. The main goal of this work is to discover novel IDO inhibitors by using MVD software

MATERIALS AND METHODS

In this study, docking of selected NSAID against IDO was carried out by MVD software. Storn and Price (1995) was introduced differential evolution. The scoring function of MVD depended on piecewise linear potential (PLP) originally introduced by Gehlhaar *et al*., (1998 and 1995) and modified latter by Yang and Chen , (2004) is extended with a new term, taking hydrogen bond directionality into account. Besides, docking accuracy is increased by applying a re-ranking procedure. Throughout this study 10 solutions got by 10 independent docking runs were re-ranked. NSAID like (Phenylbutazone, Lornoxicam and Allopurinol) were selected for this study. The selected ligands structures were built using ChemDraw (Fig 1) software and imported to MVD workspace in 'sdf' format. All necessary valency checks and H atom addition were thus performed using the utilities provided in MVD.

The crystal structures selected for this study is IDO complex with PIM (Fig 2) from the available crystal structure (July 2014). The crystal structures of IDO (PDB, ID, 2D0T) downloaded to MVD workspace from protein data bank (http://www.rcsb.org/pdb), under the criteria that they had a reasonable resolution (≤ 2.8 A^0) and involved the non-mutated IDO enzyme in complex with ligands. The steps involved in docking included, importing the molecules and ligands, preparing the molecules, creating template and, docking

RESULTS

Table 1 shows MolDock score, re- rank score and the hydrogen bond energy of selected 3 ligands. Table 2 shows amino acid residues present in the active site of IDO and also ligand binding amino acids. Fig. 1 shows selected ligand structures were built using ChemDraw software. Fig. 2 shows crystal structure of IDO (PDB, ID, 2D0T) complex with ligand PIM is represented in ball and stick model prepared by using MVD software. Fig 3 shows dock structure of IDO with 3 selected ligands. Ligand 1 (Phenylbutazone) bind into the active site with mole dock score -122.256 k cal/mol and binding site consist of amino acid residues like Gly 676, Ala 264, Leu 234, Ser 263. Ligand Ligand 2 (Lornoxicam) bind into the active site with mole dock score -112.28 k cal/mol and binding site consist of amino acid residues like Ser 235, Gly 236, 261. Ligand 3 (Allopurinol) bind into the active site with mole dock score -84.8765 k cal/mol and binding site consist of amino acid residues like Ser 167, Ala 264, Tyr 126.

Table 1. MolDock score; Rerank score and the hydrogen bond energy of the docked compounds.

Ligand	MolDockScore	Rerank Score	Log P	MW	Torsion	Hba	HBd	HB
Phenyl butazone	-122.256	-77.7182	4.14	308	5	2	0	0
Lornoxicam	-112.28	-82.946	1.99	370	1	6	2	-4.56
Allopurinol	-84.8765	-63.9483	-1.76	137	0	5	2	-0.667

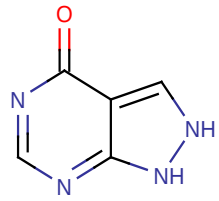
HBa= hydrogen bond acceptor, HBd= hydrogen bond donor, MW= molecular weight

Table 2. Amino acids residue around active site docked against IDO.

S.NO	Ligand name	Amino acid residues	Ligand binding amino acid
1	Phenyl butazone	Ala 264, Leu 234, Ser 263, Ser 235	Gly 676
2	Lornoxicam	Ser 235, Gly 236, Gly 261, Gly 262, Ser 263	Ser 235
3	Allopurinol	Ala 264, Ser 263, Gly 262, Tyr 126, Leu 234	Ser 167, Ala 264, Tyr 126

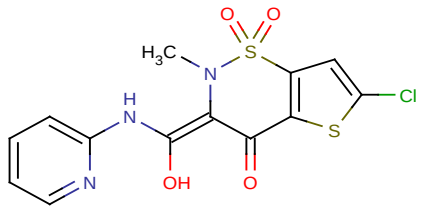
Arg=arginine; Gly= glycine; Glu= glutamate; His= histidine; Leu= leucine; Phe= phenylalanine Ser=serine; Thr= threonine; Tyr=tyrosine;Val= valine

Allopurinol



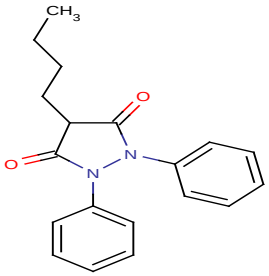
1*H*-pyrazolo[3,4-d]pyrimidin-4(2H)-one

Lornoxicam



(3*E*)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4*H*-thieno[2,3-*e*][1,2]thiazin-4-one 1,1-dioxide

Phenylbutazone



4-butyl-1,2-diphenyl-pyrazolidine-3,5-dione

Fig 1. Structure of NSAID selected for docking against IDO.

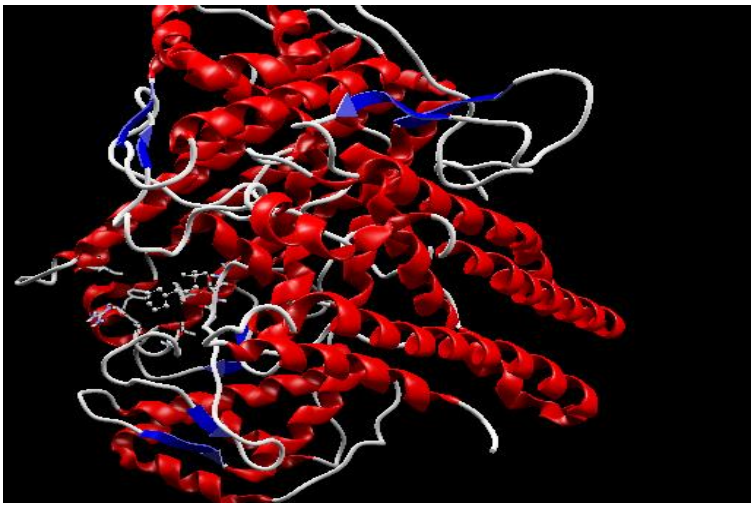
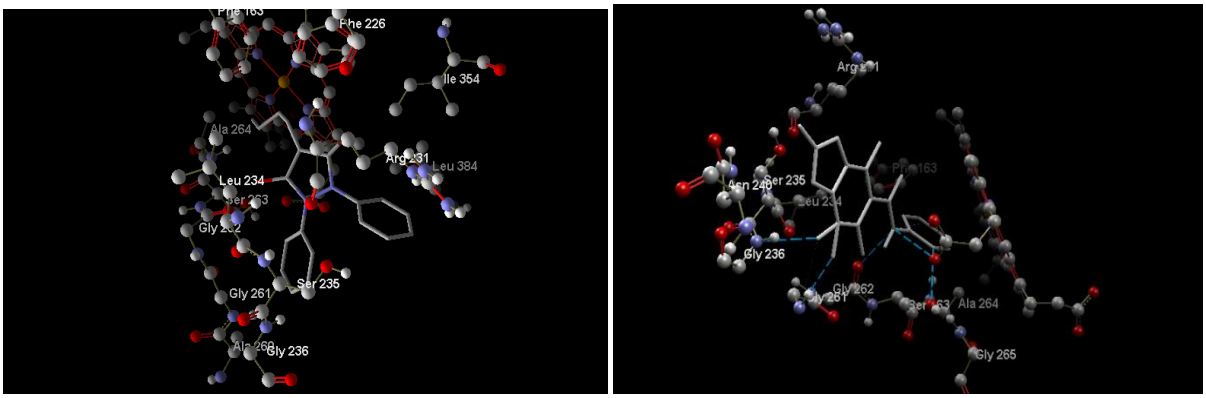


Fig 2. Structural cartoon of Indole amine 2,3-dioxygenase (PDB code 2D0T). The α helices and β strands are represented as coils (red) and arrows (white), respectively. PIM is represented in ball and stick. Model prepared using MVD.

PHENYLBUTAZONE

LORNOXICAM



ALLOPURINOL

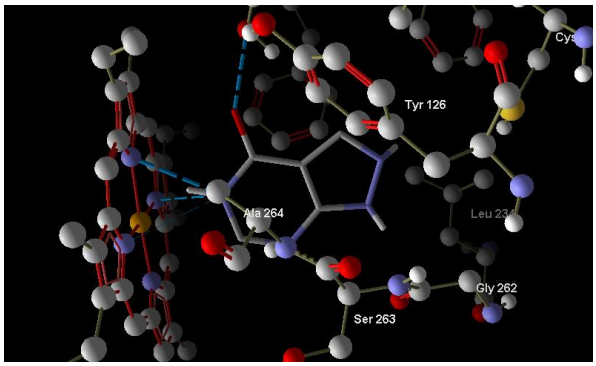


Fig 3. The best scored docking solution of IDO with the nine selected ligands (1-3). The cofactor haem is presented in lines with red color. Amino acids in the active site are presented in ball and stick with element color and ligand is presented in thick lines with element color (where carbon is grey, oxygen is red, nitrogen is blue and sulpher is yellow and hydrogen in white). Blue lines represented the hydrogen bonds in between the ligand and the active site of IDO.

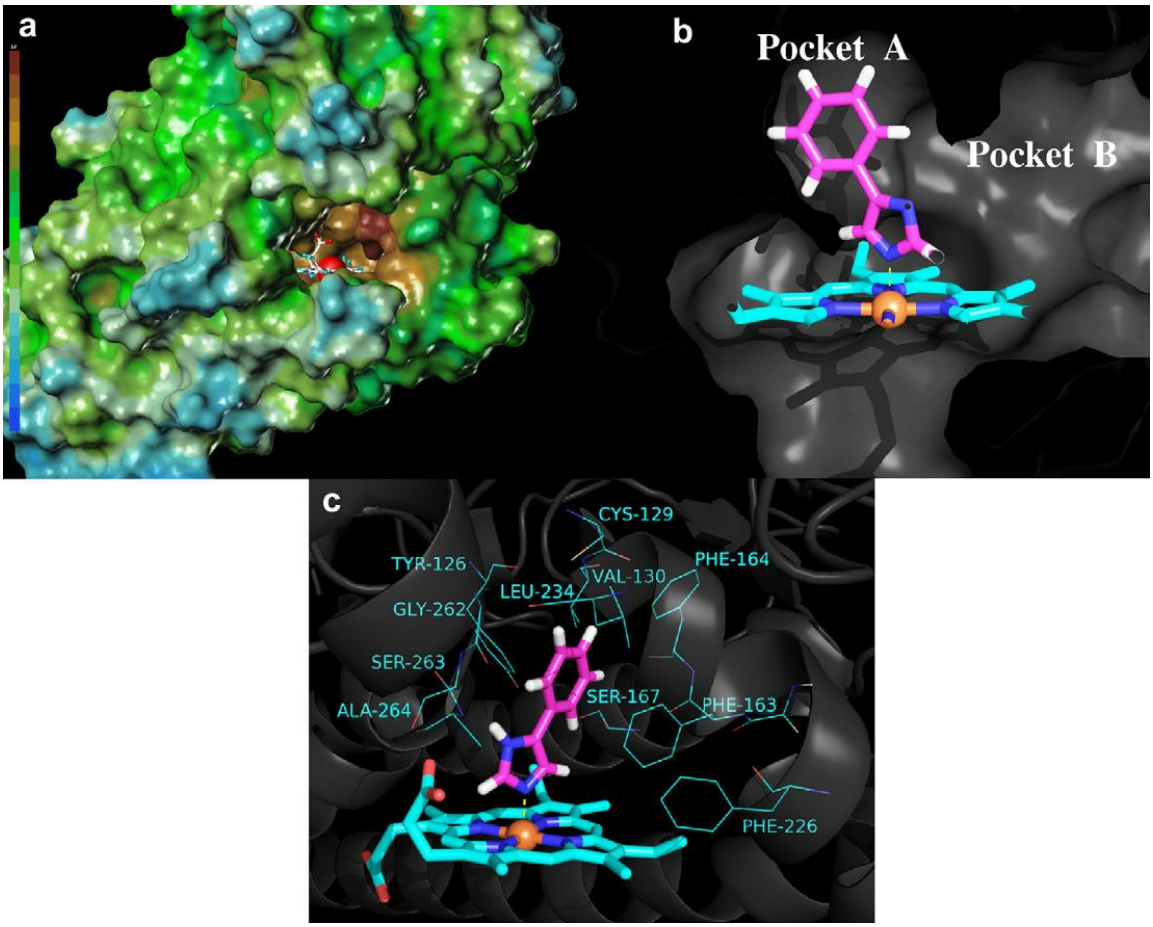


Fig 4. Lipophilicity mapped of IDO (color code: brown = high lipophilicity, green=medium lipophilicity and blue=high hydrophilicity; picture made with MOLCAD (30) and (b and c) active site of IDO with PIM as absorbed in the X-ray crystal structure (PDB ID 2D0T). (Adapted from Dolusic *et al*., 2011).

DISCUSSION

The enzyme IDO plays a physiologically important role in the human body as it can control the levels of essential amino acid, L-TRP and a number of its neurotoxic metabolites. Pro inflammatory cytokines such as interlukin -2, interferon-gamma, or tumor necrosis factor-alpha activate IDO (Weiss *et al*., 1999) leads to depression. Depression is linked with pro-inflammatory cytokines and increased use of tryptophan by activation of IDO. Prostaglandin (PGE2) is a molecule of the pro inflammatory cascade. It stimulates the Interlukin-6, the expression of cycloxegenase-2 and as cofactor the expression of IDO. Increased secretion of PGE2 has been observed in cerebral spinal fluid in serum and in saliva of depressed patient (Linnoila and Whorton, 1983) (Ohishi *et al*., 1988). It is likely that anti-inflammatory treatment is helpful in the subgroup of psychiatric patients who show signs of inflammation (Muller 2013).To identify IDO inhibitors for therapeutic purpose we have selected IDO proteins for virtual screening. For this purpose IDO was docked with the selected 3 drugs. In each docking run, the best poses were selected on the basis of their MVD score.

As previously described, each compound was automated dock within the defined grid representation after docking a score was assigned according to the quality of fit and top ten candidate were selected on the basis of exhibiting lowest predicted binding energy. These compounds were further optimized according to their energy minimization active side chain residue. More negative the energy score (K cal/mol) more is the binding affinity. Top 3 ligands with their energy scores ranging from -84.8765 K cal/mol to -122.256 K cal/mol (see Table1) was yielded through virtual screening by using the Molegro software program. Phenyl butazone -122.256 K cal/mol, lornoxicam -112.28 K cal/mol, allopurinol -84.8765 K cal/mol, respectively.

No doubt the above 3 ligand has greater binding affinity. This data also indicates that by additionally optimization these possible ligands can make a strong therapeutic inhibitor of IDO enzyme. After visualization IDO in complex with top three ligands, it was noticed that above ligand were best fitted in the cavity of receptor.

In the reported crystal structure of IDO, ligand PIM interacts with the haem iron, PIM binding site consist of amino acid residues Tyr126, Cys 129, Val 130, Phe 163, Phe 164, Ser 167, Leu234, Gly 262, Ser 263, Ala 264 and the haem ring (Fig 4, C). Additional hydrogen bond is possible due to side chain of Arg 231. Haem ring and Phe 163, Phe 226, Leu 234, Ile 354 formed hydrophobic pocket (pocket B, Fig. 4) (Sogimoto *et al*., 2006).

All of the selected 3 ligand when docked with IDO, they were oriented in the active site such as that it forms hydrogen bond with at least 3 to 4 key residues that is Ser 263, Ala 264, Gly 262, Leu 234. Allopurinol, lornoxicam directly bound with haem which showed the competitive inhibitors. Further optimization process can produce an effective therapeutic inhibitor for the IDO enzyme that can be best fitted in the cavity of receptors including the above mention top three ligands. It is suggested that IDO can be an important therapeutic target for the treatment of inflammation. Thus a better understanding of IDO biology and identification of IDO inhibitors could provide a rational for their therapeutic application in inflammation-related CNS diseases such as Depression and Alzheimer.

CONCLUSION

It is concluded that phenylbutazone, lornoxicam and allopurinol are possible lead molecules showing their highest docking score. Present results also prove that they are competitive inhibitors of IDO.

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