Research Article

STRUCTURE BASED DESIGNING OF PYRIDAZINONE DERIVATIVES AS POTENTIAL INHIBITORS AGAINST COX-2RECEPTOR

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ARTICLE INFO

ABSTRACT

Molecular docking is a computational tool to understand the binding mode of ligands with the crystal structure of target protein, which is achieved by generatinga number of conformations (or poses) of a ligands within the active site of receptor and scoring them to identify the best binding conformation. An attempt toward designing of some pyridazinone derivatives against inflammation, as inhibitors of Cycloxygenase-2 (COX-2) molecule was carried out using *in silico* approaches of molecular docking studies. Binding conformations were compared with the co-crystallized inhibitor, complexed within the of 3D structure of target protein receptor COX-2 (PBD ID: 6 COX), used in docking simulation. The designed ligands exhibited good binding within the active site of receptor protein. But the Pyridazinone based ligands such as **3l**, **3s**, **3h** and **3p** were screened as the best potent hits, as potential inhibitors of COX-2, on the basis of molecular docking studies.

INTRODUCTION

Inflammation is the tissue reaction to infection, irritation or foreign substance. It is a part of the host defense mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin & prostaglandins. The development of non-steroids in overcoming human sufferings such as Rheumatoid arthritis has evoked much interest in the extensive search for new drugs with this property [1, 2].

There are several tissue factors or mechanisms that are known to be involved in the inflammatory reaction such as release of histamine, bradykinin and prostaglandins. In addition to local changes in an inflammatory area, there are often various responses such as rise in temperature, an increase in blood leucocytes etc. There are also increases in certain plasma proteins termed acute phase proteins [3]. Histamine has been implicated as a mediator of vasodilatation and other changes that occur during inflammation. It promotes adhesion of leukocytes to vascular endothelium by expressing adhesion molecule P-selectin on endothelial cell surface, sequestrating leukocytes at the inflammatory site [4, 5].

Cyclooxygenase (COX) is an enzyme that is responsible for the formation of important biological mediater's prostanoids i.e. prostaglandins, prostacyclins and thromboxane as shown in fig.1.

Fig. 1 Role of Cyclooxygenase (COX) and COX inhibitors during the formation of Prostaglandins (PG**)**

Pharmacological inhibition of COX can provide relief from pain, inflammation [6] Inflammation is the body's reaction to invasion by an infectious agent, antigen challenge, or even just physical, chemical or traumatic damage. Inhibition of inflammation depends on suppression of COX or PGH synthase enzyme. Prostaglandins are formed by the oxidative cyclization of arachadonic acid. Arachadonic acid is converted to PGG_2 and PGH_2. PGH_2 is converted to eicasonoids that include PGE_2, PGD_2, PGF_2, PGI_2 and thromboxane (TX) A_2.

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PGs not only play a central role in inflammation, but also regulate other critical physiological responses i.e. blood clotting, ovulation initiation of labour pain, nerve growth and development. Suppression of COX may lead to unwanted side effects such as gastric irritation and renal side effects [7]. At present three COX isoenzymes are known COX-1, COX-2, and COX-3. COX-3 is a splice variant of COX [6].

MATERIAL AND METHOD

Molecular docking is a computational tool to understand the binding mode of ligands with the crystal structure of target protein, which is achieved by generatinga number of conformations (or poses) of a ligands within the active site of receptor and scoring them to identify the best binding conformation [8].

Table-1 General structures of the designed Pyridazinone based Ligands

General structure for the designed pyridazinone based ligand

Ar

or

R

or

or

or

or

or

or

Table 2 Dock Score of series of synthesized compounds (3*a-u*) (*pdb: 6COX*)

compound	Mol Dock Score	Docking Score	H-Bond Interaction	H-Bond Distance(A°)	Interacting Residue	Interaction with compound of Structural Feature
Internal ligand S58_701	-134.092	-132.974	2	2.52	Gly 225	N-O
				2.26	Ser 143	N-O
3 *a*	-78.1184	-81.3695	2	3.03	Gly 536	N of pyridazinone with O
				2.76	Val 228	O of pyridazinone with N
	-77.5482	-80.6051	2	2.35	Asn 537	N of pyridazinone with H
				2.72	Val 228	O of pyridazinone with N
3 *b*	-109.823	-107.955	1	3.42	Asn 375	N of pyridazinone with N
	-104.374	-102.004	1	2.60	Asn 375	O of pyridazinone with N
3 *c*	-124.97	-122.784	3	3.09	Asn 537	O of NO$_2$ with N
				3.40	Val 228	O of NO$_2$ with N
				3.54	Asn 375	O of NO$_2$ with N
	-121.386	-123.873	3	2.71	Arg 376	O of NO$_2$ with N
				3.16	Arg 376	N of NO$_2$ with N
				3.51	Asn 375	O of NO$_2$ with N
3 *d*	-120.921	-118.281	0	-----	----	-----
	-119.765	-117.746	0	----	----	----
3 *e*	-114.455	-115.743	2	3.28	Arg 376	N of pyridazinone with N
				3.56	Arg 376	N of pyridazinone with N
	-113.922	-112.44	1	3.25	Arg 376	O of CF$_3$ with N
3 *f*	-115.54	----	0	----	----	----
	-112.039	----	0	----	----	----
3 *g*	-112.681	-110.033	2	3.53	Asn 375	N of pyridazinone with N
				3.21	Asn 375	N of pyridazinone with N
	-109.059	-106.744	1	3.10	Asn 375	N of pyridazinone with N
3*h*	-117.001	-117.878	1	2.64	Asp 229	N of CN with O
	-113.037	-117.399	3	3.10	Asn 375	O of pyridazinone with N
				3.08	Gly 536	N of CN with O
				2.72	Gly 533	N of CN with O
3 *i*	-112.384	-109.862	1	3.34	Asn 375	N of pyridazinone with N
	-108.316	-105.78	1	2.83	Asn 375	O of pyridazinone with N
3 *j*	-95.5091	-95.5991	3	3.08	Asn 375	N of pyridazinone with N
				3.22	Asn 375	N of pyridazinone with O
				3.07	His 226	N of pyridazinone with O
	-95.8734	-96.7929	1	3.09	Asn 375	N of pyridazinone with O
3*k*	-104.96	-104.654	1	2.72	Asn 375	O of pyridazinone with N
	-104.23	-103.828	1	2.74	Asn 375	O of pyridazinone with N
3*l*	-142.263	-143.833	4	2.40	Arg 376	O of NO$_2$ with N
				2.40	Arg 376	O of NO$_2$ with N
				3.41	Arg 376	O of NO$_2$ with N
				2.76	Arg 376	O of NO$_2$ with N
				3.47	Asn 375	N of NO$_2$ with N
	-141.262	-140.163	5	2.87	Asn 375	O of NO$_2$ with N
				3.29	Asn 537	N of NO$_2$ with N
				2.62	Asn 537	O of NO$_2$ with N
				3.02	Val 228	O of NO$_2$ with N
3 *m*	-118.708	-118.306	2	2.96	Asn 375	N of Quinoline with O
				2.86	Asn 375	O of pyridazinone with N
	-123.197	-121.49	1	2.88	Gly 225	N of quinoline with O
3 *n*	-101.445	-100.991	1	3.26	Asn 537	O of CF$_3$ with N
	-100.892	-100.965	1	3.33	Asn 537	O of CF$_3$ with N
3 *o*	-125.156	-119.837	0	----	----	----
	-123.549	-121.808	0	----	----	----
3*p*	-124.931	-118.297	3	3.12	Trp 139	N of pyridazinone with N
				3.36	Lys 333	N of pyridazinone with N
				2.59	Lys 333	O of pyridazinone with N
	-121.039	-118.764	1	3.33	Asn 375	O of pyridazinone with N

Compound				Distance	Residue	Interaction
				2.60	Glu 140	N of CN with O
	-118.335	-120.982	3	2.30	Asn 375	N of CN with O
				2.10	Trp 139	N of CN with N
3q	-121.892	-123.201	1	2.60	Gly 533	N of CN with O
	-150.059	-113.487	1	3.13	Asn 375	O of pyridazinone with N
3r	-114.946	-112.531	1	3.10	Asn 375	O of pyridazinone with N
				3.37	Asn 375	N of pyridazinone with O
	-94.6208	-95.6732	4	3.10	Asn 375	N of pyridazinone with N
				3.49	Gly 225	N of pyridazinone with O
				3.10	His 226	N of pyridazinone with O
				3.10	His 226	N of pyridazinone with O
3s	-93.9941	-95.3216	4	3.33	Asn 375	N of pyridazinone with O
				3.51	Gly 225	N of pyridazinone with O
				3.07	Asn 375	N of pyridazinone with N
3t	-135.815	-133.783	1	3.33	Asn 375	O of pyridazinone with N
	-132.032	-130.333	1	3.35	Asn 375	O of pyridazinone with N
				3.23	**Asn 375**	**O of Biphenyl with N**
				3.18	**Asn 375**	**N of NO$_2$ with N**
	-132.964	**-138.101**	**5**	**3.10**	**Arg 376**	**O of NO$_2$ with N**
				3.12	**Arg 376**	**O of NO$_2$ with N**
3u				**3.55**	**Arg 376**	**N of NO$_2$ with N**

Ligand preparation

The hypothetical pyridazinone based compounds were selected on the basis of literature. The ligand molecules were prepared using Marvin 5. 11. 0 and converted to 3D structure from the 2D using build and optimization method & then clean in 3D. The resulting structure will be saved in MDL Molfile (*. mol) format. A single, low energy, 3D structure with correct chiralities for each successfully proposed input structure will be generated. Then the generated structures were imported into the workspace of docking software Molegro virtual docker 4.0.2. Molecule can be incorporated into MVD using MDL (sdf/sd/mol/mdl) file format which contains bonding information. In this step the preparation of molecules were assigned bonds, bond order and hybridization, charges, explicit hydrogens and flexible torsion in ligands.

Target preparation

The 3D structure of COX was retrieved from the Protein Data Bank (www.rcsb.org). Finally, the 3D structures of protein COX were imported into the workspace of MVD with the removal of all water molecules having more than 5 Å specific distance. The standard Molegro algorithm was employed for rendering the missing charges, protonation states, and assigning of polar hydrogen to the receptor.

Preparation of ligand molecules

The ligands were built in Marvin Sketch 5.11.4. All hydrogens in the structure were added, 2D molecules were cleaned into 3Dand the conformational energy of molecules was minimized usingMMFF94 force field. The resulting structures were saved in Marvin Sketch as MDL Molfile (*.mol). After that ligands were imported in MVD, which help in assigning the missing bond orders, charges, bonds and hybridization states of the imported ligands.

Docking studies on MVD

MVD was used for docking studies, which has been considered more accurate than other docking tools comparatively [9]. MVD was used to calculate the interaction energies between ligands and macromolecular systems from the 3D structures of the protein and ligands.

The candidates with the best conformation and energy scores were selected. The algorithm used to be the MolDock Score, which is an adaptation of Differential Evolution (DE) algorithm. Finally docking of ligands and protein was performed in docking wizard with the score function Moldock score. The parameters for docking were set as default, which includes no. of runs 10, maxi-mum iterations 1500, maximum population size 50, but maximum no. of poses was increased to 10.

Docking energy calculations

The MolDock score energy, E_{score} was calculated with the help of equation 1, where E_{inter} represent the ligand-protein interaction energy whereas E_{intra} was the internal energy of the ligand. E_{inter} was calculated by equation 2 whereas calculation of E_{intra} was made with the help of equation 3 (M.H. Christensen *et al.*, 2006).

$$E_{score} = E_{inter} + E_{intra} \tag{1}$$

$$E_{inter} = \sum_{i=ligand} \sum_{j=protein} [E_{PLP}(r_{ij}) + 332.0\, q_i q_j / 4r^2_{ij}] \tag{2}$$

$$E_{intra} = \sum_{i=ligand} \sum_{j=protein} [E_{PLP}(r_{ij})] + \sum_{flexible\ bonds} A[1-\cos(m\theta-\theta_0)] + E_{clash} \tag{3}$$

E_{PLP} represents the term "piecewise linear potential" which comprized two different parameters, one for the potential energies of hydrogen words and second for the Vander wall interactions between atoms. Second term in the equation 2 was used for the calculation of electrostatic interactions between charged atoms. The second term in the equation 3 was for the calculation of torsional energy where θ is the torsional angle. The last term E_{clash} in the equation 3 was assigned for a penalty term of 1,000 kcal mol^{-1} if the distance between two heavy atoms is smaller than 2.0 Å.

Compound Selection

On the basis of above literature data, we selected sixty hypothetical compounds and docking studies were performed using (PDB ID 6 COX) for anti-inflammatory & (PDB ID 2 OOX) for analgesic activity using Molegro Virtual Docker. However, all hypothetical compounds were found to possess good results for anti-inflammatory and analgesic activity. But, out of sixty compounds twenty one compounds (3*a- u*) which

we selected were found to possess best results for analgesic and anti-inflammatory activity. The docking output results of the best twenty one compounds are presented in Table 2.

Fig. 2 Binding mode of 3s (blue) into the binding site of pdb: 6COX. It has Mol dock score -94.6208 and docking score- 95.6732 and form 4 hydrogen bonds shown as blue lines, between N of pyridazinone with O, N of pyridazinone with N, N of pyridazinone with O, N of pyridazinone with O with distance of 3.37 A°, 3.10 A°, 3.49 A°, 3.10 A° respectively.

Fig. 3 Binding mode of 3h (blue) into the binding site of pdb: 6COX. It has Mol dock score -113.037 and docking score- 117.399 and form 3 hydrogen bonds shown as blue lines, between O of pyridazinone with N, N of CN with O, N of CN with O with distance of 3.10 A°, 3.08 A°, 2.72 A° respectively.

Fig. 4 Binding mode of 3l (blue) into the binding site of pdb: 6COX. It has Mol dock score -141.262 and docking score- 140.163 and form 5 hydrogen bonds shown as blue lines, between N of NO_2 with N, O of NO_2 with N, N of NO_2 with N, O of NO_2 with N, O of NO_2 with N, with distance of 3.47 A°, 2.87 A°, 3.29 A°, 2.62 A°, 3.02 A° respectively.

Fig. 5 Binding mode of 3u (blue) into the binding site of pdb: 6COX. It has Mol dock score -132.964 and docking score- 138.101 and form 5 hydrogen bonds shown as blue lines, between O of biphenyl with N, N of NO_2 with N, O of NO_2 with N, O of NO_2 with N, N of NO_2 with N with distance of 3.23 A°, 3.18 A°, 3.10 A°, 3.12 A°, 3.55 A° respectively.

RESULTS AND DISCUSSION

The crystallographic structure of receptor revealed the presence of a large hydrophobic group. The synthesized compounds were embedded in that hydrophobic pocket & forming H-bonds. Docking studies revealed following information with respect to anti-inflammatory activity given in **Table 2** and in **fig 2-5.**

(Anti-inflammatory activity, pdb=6COX)

- Compounds **3l, 3s, 3h, 3 p** having functional group 2, 4 dinitro of A.P.A(biphenyl) based pyridazinone, O of pyridazinone ring, cyano group of A.P.A.(toluene) based pyridazinone, N of pyridazinone ring respectively exhibits better binding affinity to the receptor as compared to others.

Compound **3u,** having functional group 2, 4 dinitro group of A.P.A. (biphenyl ether) based pyridazinone showed very good binding affinity towards the receptor with highest Mol dock score -132.964 and docking score- 138.101 and form 5 hydrogen bonds, between O of Biphenyl with N, N of NO_2 with N, O of NO_2 with N, O of NO_2 with N, N of NO_2 with N with distance of 3.23 A°, 3.18 A°, 3.10 A°, 3.12 A°, 3.55 A° respectively.

CONCLUSION

The present work describes the possible mechanism of pathogenesis of inflammation, which is part of the host defence mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin & prostaglandins. In addition to local changes in an inflammatory area, there are often various responses such as rise in temperature, an increase in blood leucocytes etc. Crystal structure of COX was employed to perform structural based designing of some pyridazinone based analogues as potential agents against inflammation and *in silico* analysis including molecular docking studies were performed succesfully. Theses analogues exhibited comparative to good antinflammatory properties with receptor COX, considered as main receptor involved in pathogenesis of inflamation. Reported results revealed that the analogues of the series such as Compounds **3l, 3s, 3h, 3p** having functional group 2, 4 dinitro of A.P.A(biphenyl) based pyridazinone, O of pyridazinone ring, cyano group of A.P.A.(toluene) based pyridazinone, N of pyridazinone ring respectively exhibits better binding affinity and exhibited strong hydrogen bond interactions with COX-2.

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How to cite this article:

Deepika Choudhary., Nisha Devi and Sukhbir Lal Khokra.2016, Structure Based Designing Studies of Pyridazinone Derivatives as Potential Agents Against Receptor Cox-2: A Drug Target For Antinflammatory. *Int J Recent Sci Res.* 7(6), pp. 12182-12187.