MOLECULAR DOCKING OF CHROMONE DERIVATIVES COMPARE WITH IBUPROFEN BY THE PAR1 RECEPTORS AS AN ANITIINFLAMMATORY AGENTS

Datta Avhad*[1], Dinesh Chaple[1], Pratyush Kumar[2], Neha Dubey[3] and Nikita Naidu[4]

[1]Department of Pharmaceutical Chemistry, Priyadarshini J L College of Pharmacy, Nagpur.
[2]Priyadarshini J L College of Pharmacy, Nagpur.
[3]Manipal College of Pharmaceutical Science, Manipal.
[4]Smt. Kishoritai Bhoyar college of Pharmacy, Nagpur.

ABSTRACT

Inflammation is the widely spread symptoms of the different serious condition of disease its responsible the chemical and internal factors. In this find out the best affinity towards the crystal structure of muirne thrombin complex with the extracellular fragment of murine PAR3 (PDB ID: 2PUX) which controls the inflammation. Flavonoids category must shows the anti-inflammatroy actions here using chromone derivatives. The Default parameters of the software program have been applied similar to the protocol followed elsewhere. Briefly, Lamarckian Genetic Algorithm (LGA) with default atomic salvation parameters 126 Å (x, y, and z) grid box in ratio of (60:60:60) for scoring energy was set at co-ordinates as X = 24.320; Y = 25.104 and Z = 26.480 with 0.375 angstroms grid points spacing. Care was given during the grid box preparation to ensure that the active site of receptor was surrounded by the 3D grid box centered at its active ligand binding site location. Camparison between the standard and synthesized derivatives then found to be same amino acid present in Ibuprofen and 5-Nitro-2-phenyl-4H-1-benzopyran-4-one Leu:99; His:57; Tyr:60A; Lys:60F; Asn:98; Glu:97A; Trp:60D; Ile:174; Trp:215. Potent comp 1a studied details about their structural and predicted properties by online molinspoiration calculator.

KEYWORDS: Anti-inflammatory, Chromone, murine PAR3, Ibuprofen, molinspoiration.

1. INTRODUCTION

Based on visual observation, from long time characterised inflammation by five prefencially points, contain redness (rubor), swelling (tumour), heat (calor; only applicable to the body' extremities), pain (dolor) and loss of function (functio laesa). Types of the inflammatory agents used in the treatment of inflammatory conditions, to know the biochemical processes involved in the disease. Traditionally, the standard treatments for serious inflammatory disorder or may be disease i.e. arthritis, joint pain, skin diseases has been to use a non-steroidal anti-inflammatory drug (NSAID), such as aspirin, for pain relief and to use corticosteroids drugs in an attempt to reduce other symptoms of the disease.[1]

Protease activated receptors: PAR1, PAR3 and PAR4 are all considered thrombin receptors, whereas PAR2 is activated by trypsin and other ligands. PARs play roles in hemostasis and thrombosis, platelet signaling, and tissue injury. These receptors are involved in the process of inflammation and tissue repair. For instance, thrombin binding to PAR1 leads to activation of p115 RhoGEF, and RhoA. Activation of RhoA is responsible for stress fiber formation, and increased calcium flux triggers other signaling pathways which ultimately lead to myosin light chain-dependent contraction of endothelial cells. Thrombininduced increase in vascular endothelial permeability contributes to edema often seen in inflammatory disorders such as acute lung injury.[2]

During the recent years, there has been intense research on fused heterocyclic compounds with pharmacological importance. Among the heterocycles the benzopyran class has drawn the attention. benzopyran refer to a fused five member pyran ring to a six member benzene ring. The benzopyran nucleus is a versatile source of biologically important molecules. It is an isomer of coumarin. The 1,4- benzopyran(1) are the most popular with pharmacological importance and have been explored most. 1,4- benzopyran posses important roles in the field of pharmacological and medicinal chemistry due to the various activities of the heterocycle core. Here shows the ketone present in the 4H-pyran-4one (2). Chromone exists as a solid, slightly soluble (in water), and an extremely weak acidic (essentially neutral) compound (based on its pKa).[3]
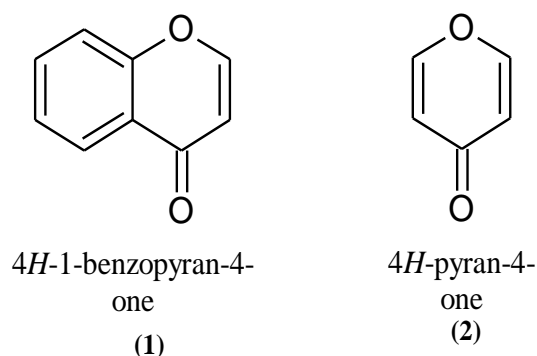
4H-1-benzopyran-4-one

(1)

4H-pyran-4-one

(2)

Figure 1: Strcture of Benzopyran.

Chromones (benzo-γ-pyrone) and related compounds are widely distributed in nature and have been found to play an important role in a number of biological processes. Chromones have versatile biological activities like anticancer shown by Agullo et al.,[4] antioxidant, anti-inflammatory, antibiotic, anti-HIV by Xu et al.,[5] and also as good vasodilators Middleton et al.[6] and were preferred due to their low mammalian toxicity as per the study by Gabor, in 1991.[7] Many natural chromones and flavones like Quercetin, Christen, Kaempferol, Myricetin, Apigenin, Luteolin etc., mainly present in apple, tomato, onion etc., found to have the property of inhibiting autooxidation reactions and scavenging of free radicals as spotted by Bors et al.; Miean and Mohamed.[8,9] This property delays the decaying of these natural fruits and vegetables. They play a key role in the prevention of cancer.[10-12] They also have "Anti-aging" properties as substantiated by Jones and Hughes.[13] The natural chromones were studied for their SAR (Structure Activity Relationships)[14] and many synthetic chromones related to the structure of natural chromones which are having similar structures, are found to possess similar activities. The automation of the study of the SAR of some natural and synthetic chromones as compiled by Kini et.al., assists to choose and design the most biologically potent chromones.[15]

2. EXPERIMENTAL
2.2 Materials and Method
2.2.1 Software's and programs
Chemsketch a chemical molecule drawing tool was used to draw the ligand compounds.[16] Avogadro software was used to convert the .mol file to .pdb format.[17] Autodock 4.0.[18] a preliminary docking program was used for the semi-flexible protein ligand docking studies. Molinspiration online property calculator was used to study the chemical properties of the compound.[19] The crystalline structure of Protease activated receptors i.e. PAR3 was downloaded from protein database and the pdb code was [PDB: 2pux]. This will act as target for computational studies. Pyrx software was used for virtual screening of library of derivatives.[20] Discovery studio 3.5,[21] and Maestro 12.7,[22] was used for molecular interaction and visualization.

2.2.2 Preparation of ligand
Library of total 35 ligands synthesized out of them 11 molecules shows the best binding affinity with the target. Structures of ligand was drawn using Chemsketch software and the structure was cleaned using the clean structure tool. The structure was saved in the working folder as .mol file. Using control all ligands were loaded into the Avogadro software and structure was optimized using optimization tool. Further, the ligands are prepared by detecting the torsion root, correcting the torsion angles, assigning charges, optimizing using UFF (Universal force field),[23] and finally converting them into the pdb format and save in the working folder.

2.2.3 Preparation of receptor
In silico analysis of crystal structure of muirne thrombin complex with the extracellular fragment of murine PAR3 (PDB ID: 2PUX). which was retrieved from protein data bank (https://www.rcsb.org)[24]. The 6LU7 protein contains two chains, B and C, which form a homodimer. The protein was visualised using PyMol (http://www.pymol.org) and unwanted chain residues, bound ligand fragment of murine, water molecules were removed.[25] Additionally to prepare protein, charges added and minimized the energy of the protein using Autodock Vina in PyRx open source software and subsequently converting it to pdbqt format.

2.2.4 Receptor-Ligand
Docking Autodock v4.0 was used to identify binding poses with associated binding energies. As per the inverse relation of energy and stability, the conformation with greater binding energy is less stable. The Default parameters of the software program have been applied similar to the protocol followed elsewhere. Briefly, Lamarckian Genetic Algorithm (LGA) with default atomic salvation parameters 126 Å (x, y, and z) grid box in ratio of (60:60:60) for scoring energy was set at co-ordinates as X = 24.320; Y = 25.104 and Z = 26.480 with 0.375 angstroms grid points spacing. Care was given during the grid box preparation to ensure that the active site of receptor was surrounded by the 3D grid box centered at its active ligand binding site location.

2.2.5 Online chemical property calculator
Molinspiration online property calculator was used for calculating the properties of the ligand. The structure of the ligand was drawn using inbuilt tool and several properties were calculated. The properties were classified broadly into two types as structural property and bioactivity. Acute oral toxicity was predicted using Protox II web server(26-27).

3. RESULTS AND DISCUSSION

The structure of ligand and receptor was critically studied and drawn through insilico tools. The grid box of X:Y:Z (50:26:40) was made using Autodock v4.0 and is represented as in fig.2.
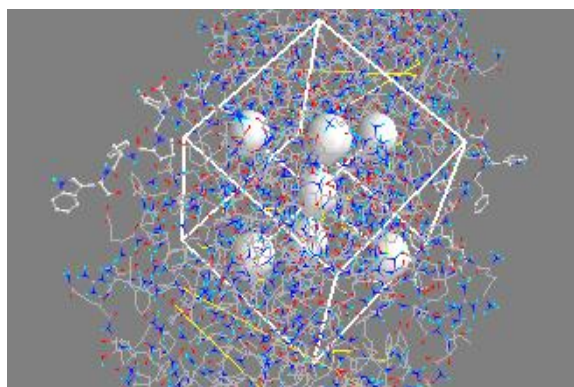
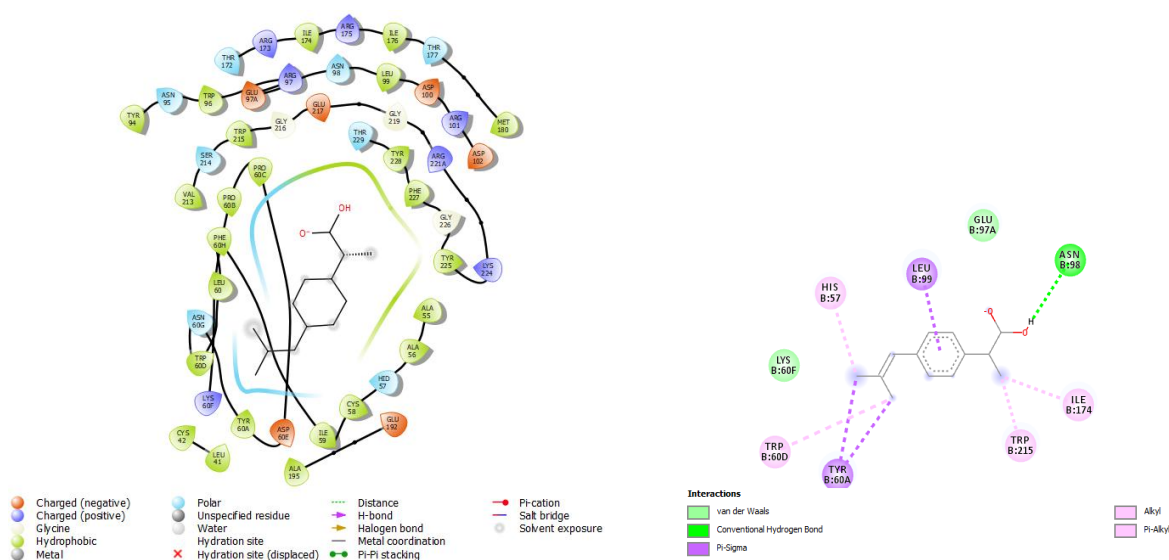**Figure 2: Grid box covers the area of Ligand active sites.**

Figure 3: a) 2D visualization of Amino acid b) Bond length between Std. Ibuprofen and Target 2pux.

Standard Ibuprofen shows the Leu:99; His:57; Tyr:60A; Lys:60F; Asn:98; Glu:97A; Trp:60D; Ile:174; Trp:215. The mean binding energy of the Ibuprofen was found to be -9.7 and the root mean square deviation (RMSD) value was found to be 13.58. The free energy was found to be -1345.90 Kcal/mol11.

VALIDATION OF RESULTS

Highest binding affinity of the compound 1a inserted with the standard ibuprofen shows similar place occupied. validation of the method and Molecular docking carried out by the PyMOL software i.e. all ligands where opened but except the 1a occupied at other site of target sites with standard ligand molecules opens into the PyMOL then inserting target 2PUX then the confirmation obtained by the grid box and the place of ligand spacified at the which amino acid shows in figure in fig 4. Violet colour indicates Target. Yellow colour: 5-Nitro-2-phenyl-4H-1-benzopyran-4-one(1a), orange colour-Ibuprofen.

In fig 5. 5-Nitro-2-phenyl-4H-1-benzopyran-4-one shows the Leu:99; His:57; Tyr:60A; Lys:60F; Asn:98;

Glu:97A; Trp:60D; Ile:174; Trp:215; Gly:215 all amino acid binding in the standard similar seen in the 5-Nitro-2-phenyl-4H-1-benzopyran-4-one so is very potent towards the anti-inflammatory activity. Hydrophobic bond, metals ions, polar bonds, Pi-Pi stocking, vander wall forces, Pi- Alkyl bond, Pi-sigma which improves the compound potentability for specific target.

Similarities: Ibuprofen; Leu:99; His:57; Tyr:60A; Lys:60F; Asn:98; Glu:97A; Trp:60D; Ile:174; Trp:215 **5-Nitro-2-phenyl-4H-1-benzopyran-4-one (Potent);** Leu:99; His:57; Tyr:60A; Lys:60F; Asn:98; Glu:97A; Trp:60D; Ile:174; Trp:215.
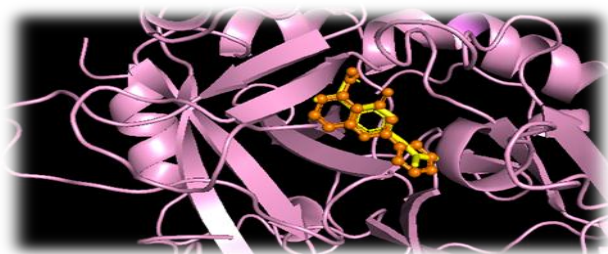
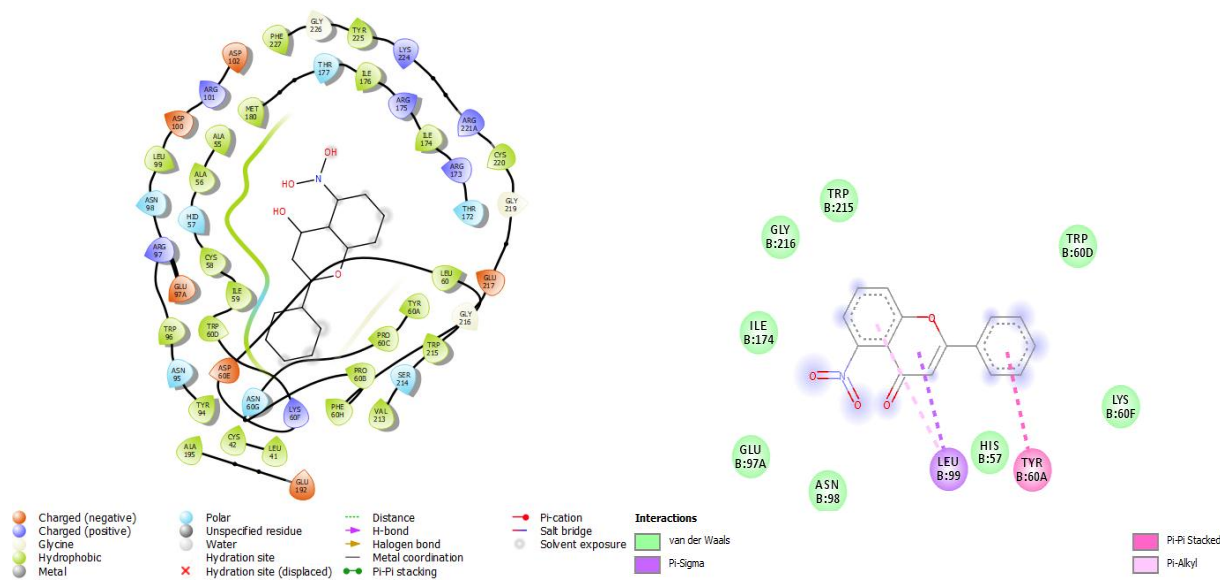**Figure 4: Comp 1a Standard Ibuprofen Binding site of Receptor**

Figure 5: a) 2D visualization of Amino acid b) Bond length between Derivatives 1a and Target 2pux

Table 1: Best Binding affinity shows the following Derivatives.

Code	Compound	IUPAC Name	Binding Affinity (kcal/mol)	Code	Compound	IUPAC Name	Binding Affinity (kcal/mol)
Std.		2-[4-(2-methylpropyl) phenyl]propanoic acid	-9.7	6f		6-Amino-5,7-dibromo-2-phenyl-4H-1-benzopyran-4-one	-8.8
1a		5-Nitro-2-phenyl-4H-1-benzopyran-4-one	-9.5	7g		6,7-Dihydroxy-5-methyl-2-phenyl-4H-1-benzopyran-4-one	9.0
2b		6,8-Dibromo-7-hydroxy-2-phenyl-4H-1-benzopyran-4-one	-8.7	8h		5,7-Dichloro-6-hydroxy-2-phenyl-4H-1-benzopyran-4-one	-8.7
3c		5,7-Dibromo-2-phenyl-4H-1-benzopyran-4-one	-8.2	9i		5,7-Dibromo-6-hydroxy-2-phenyl-4H-1-benzopyran-4-one	-7.9
4d		7-Amino-5-bromo-2-phenyl-4H-1-benzopyran-4-one	-8.6	10j		6-Methyl-2-phenyl-4H-1-benzopyran-4-one	-9.2
5e		5-Bromo-7-hydroxy-2-phenyl-4H-1-benzopyran-4-one	-9.1	11k		6-Methoxy-2-phenyl-4H-1-benzopyran-4-one	-8.5

Table 2: Results of Molinspiration online property calculaton.

Sn. No.	Structural property		Predicted property	
	Property	Value	Site	Binding efficiency
1	miLogP	3.65	GPCR ligand	-0.34
2	TSPA	76.03	Ion channel receptor	-0.12
3	Natoms	20	Kinase inhibitor	-0.14
4	MW	267.24	Nuclear receptor ligand	-0.08
5	nON	5	Protease inhibitor	-0.47
6	nOHNH	0	Enzyme inhibitor	0.03
7	Volume	223.33		

4. CONCLUSION

The study can be extended further in designing effective molecules by the help of Docking studies. These studies can be helpful to design molecule with better specificity at receptor level and be safe. The compounds 1a showed best affinity toward anti-inflammatory receptor. The autodock 4.0 software used for molecular docking. All the derivatives synthesized and their validation by TLC method, UV spectrometers results should be under specified parameters then it uses for the anti-inflammatory activity. Here the structural activity was done by molinspiration online software. It shows the structural parameters miLogP, TSPA, natooms, MW, Non, Nohnh, Volume and predicted parameters GPCR ligand, Kinase inhibitor, Ion channel receptor, Nuclear receptor ligand, Protease inhibitor, Enzyme inhibitors.

5. REFERENCES

1. Winter CA, Risley EA, Nuss GV: Carrageenin-induced edema in hind paw of the rat as an assay for anti inflammatory drugs. Proc Soc Exp Biol Med, 1962, 111: 544-547.
2. Lei SUN, Richard D YE; Role of G protein-coupled receptors in inflammation; Acta Pharmacologica Sinica 2012; 33: 342–350.
3. https://pubchem.ncbi.nlm.nih.gov/compound/9211.
4. G Agullo; LG Payrastre; S Manenti; C Viala; C Remesy; H Chap and B Payrastre. Biochem Pharmacol. 1997;1649 -1657.
5. HX Xu; M Wan; H Dong; PP But. Biol Pharm. Bulletin, 2000; 1072-1075.
6. E Middleton; C Kandaswami; C Theoharis; Pharmacol Rev., 2000; 673-751.
7. M Gabor; Z Razga. Acta Physiol Hun, 1991; 197–207.
8. W Bors; W Heller; C Michel and M Saran. Flavonoids as antioxidants: Determination of radical-scavenging efficiencies, in Methods in Enzymology: OxygenRadicals in Biological Systems (Ed.: Packer L and Glazer AN) Academic Press, Inc., New York, 1990; 6-8.
9. Miean; S J Mohamed. J of Agric Food Chem, 2001; 3106−3112.
10. F Traganos; B Ardelt; M Halko; S Bruno and Z Darzynkiewicz. Cancer Res., 1992; 6200 – 6208.
11. RL Singhal; YA Yeh; N Praja; E Olah; GW Sledge and G Weber. Biochem Biophys Res Commun, 1995; 425– 431.
12. M. Alexandrakis, L. Singh, W. Boucher, R.Letourneau R, P. Theoflopoulos and T.C. Theoharides, IJImun pharmacol, 1999; 379 –390.
13. E.Jones, R.E. Hughes, Exp. Gerontol, 1982; 213–217.
14. S.Kumar, A.K. Pandey, Scientific World J., 2013.
15. J.H. Kini,N.K. Srinivas,V.K. Pai,Y.D. Bodke, Int. J. Sci. Res., 2013; 435-440.
16. Chemsketch, 2014.
17. Avogadro, 2010.
18. Autodock v4.0., 2014.
19. Molinspiration online property calculator, 2018.
20. Pyrex, 2010.
21. Discovery studio2011; 3.5.
22. Maestro, 2020; 12(7).
23. Bab, A., Chen, Z., et al; Crystal structure of muirne thrombin complex with the extracellular fragment of murine protease activated receptors PAR3 NAD PAR4; Proc. Natl Acad Sci USA, 2007: 11603-11608.
24. rcsb.org./structure/2PUX.
25. PyMOL 2.5.org/2.
26. Banerjee P, Eckert OA, Schrey AK and Preissner R., 2018. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Res., 2018.
27. Kumar P and Asnani A. Docking of 3,5-diphenyl-pyrazoline with monoamine oxidase A receptor and InSilico structural property calculation, Journal of Drug Delivery & Therapeutics, 2019; 9(3-s): 43-45.