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Structure Based Drug Design and Synthesis of Ibuprofen analogs as Cox Inhibitors

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ABSTRACT

Cyclooxygenase-2 (COX-2) is one of the important targets for treatment of inflammation related diseases. Structure based drug design was carried out for about 1000 molecules and then molecules having good interactions with 3NT1 protein were synthesized. According to the results of molecular docking, the synthesized novel ibuprofen schiff bases derivatives have selective COX-2 inhibition. In conclusion, the newly synthesized ibuprofen derivatives can be used in model *in vivo* studies.

Key words: Cyclooxygenase-2 and Ibuprofen Schiff bases, Docking.

Inflammation is a complex reaction of the body as a response to cellular injury or damage that is characterised by tissue swelling, capillary dilation, anti-histamine activity, redness, heat and pain.[1] It serves as a defence mechanism by eliminating the noxious agents and of damaged tissues and cells. Inflammation is critical for our survival against a hostile world as it is our body's natural mechanisms to defend against a diverse variety of pathogens including bacteria, viruses, fungi, tumors and a number of various other harmful agents (chemicals, radiation, burns, and various wounds). Chronic inflammation might lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma)[1].

The X-ray crystallography of 3-D structures of COX-1 and COX-2 as well as complexes with NSAIDs has thrown light on the mechanism of action [2,3,4] . COX-1 and COX-2 are almost similar enzymes consisting of a long narrow channel with a hairpin bend at the end (active site). Arachidonic acid released from damaged membranes or tissues adjacent to the opening of the enzyme channel mostly hydrophobic is sucked in, twisted around the hairpin bend and subjected to chemical reactions resulting in the formation of the cyclopenta ring of Prostaglandins. Experiments have revealed the site of catalysis at about half-way down the channel and mechanism of action of NSAIDs at that site[5]. Subtle differences existing at the active site in COX-1 and COX-2 can be expected to regulate specificity has been convincingly shown by the detailed study of complexes of the classical, nonspecific NSAIDs, flurbiprofen and indomethacin with selectivity for COX-2. It was postulated that L-valine at 523 in the active site of COX-2 as against the bulkier isoleucine in COX-1 gave better access to the inhibitor in the case of former.

2. MATERIALS AND METHODS

The chemicals and reagents (Table I) used in the present project were of AR and LR grade, procured from Hi-media, S.D Fine Chem. Ltd, Avra labs.

2.1. Molecular Docking[6,7,8]:

Molecular docking is a key tool in structural molecular biology and computer-aided drug design.

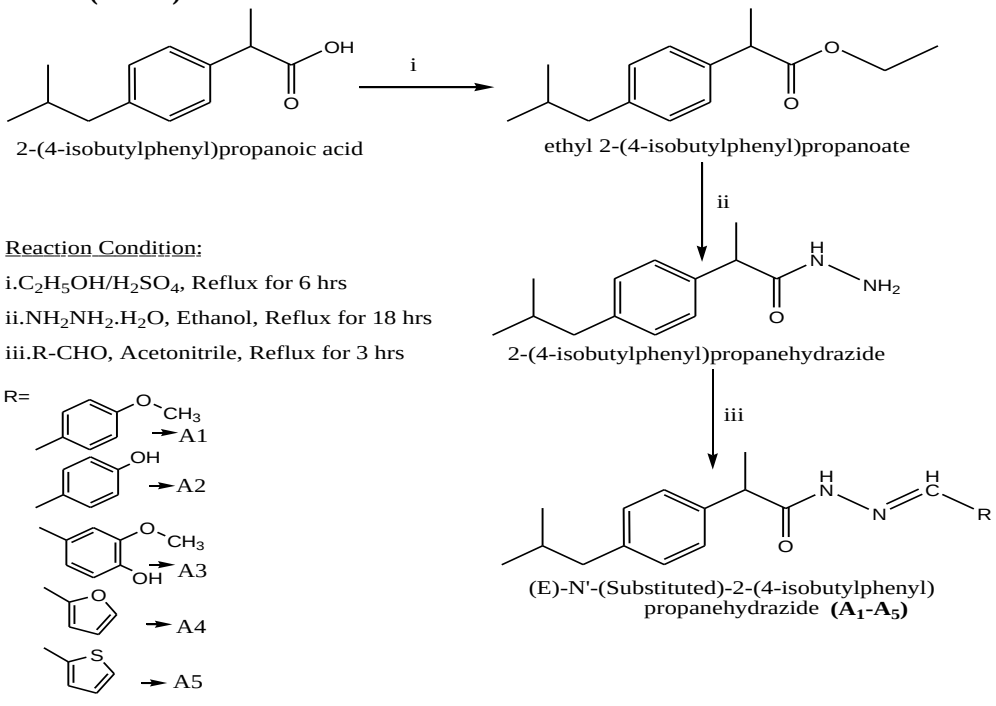
The goal of ligand-protein docking is to predict the significant binding characteristics of a ligand with a protein of known three-dimensional structure. Hence docking plays an important role in the rational design of drugs. Before doing wet lab synthesis docking studies has been carried out using Schrodinger software. About 1000 molecules have been drawn. Different modifications have been done by replacing the carboxylic group of ibuprofen with oxdiazole, thiazole, pyrazole, triazole, azetidinone, thiazolidinone, 3-methyl-1-phenyl pyrazolone and these structures have been docked against 3NT1 protein. Structures were drawn in such a way that all those molecules occupied tunnel of COX enzyme. 3NT1 protein was selected for docking as it has low resolution and also due to the crystal ligand (naproxen) present in its grid.

Intially the protein downloaded from PDB was prepared by removing chain B. Water molecules present in both the chains are removed. Energy minimisation was done. Later 1000 molecules drawn using chemdraw were converted to mol format and ligprep was created. Grid generation was done by removing crystal ligand Naproxen and the structures were docked agaist 3NT1 protein. Compounds having good interactions with the 3NT1 protein were selected for synthesis. Most of the compounds showed important interactions with amino acids Arg120 and Tyr355 of 3NT1 protein. Even validation of docked ligand naproxen and crystal ligand was done.

2.2. Chemistry:

All these compounds are synthesised in 4 steps. First step involved the esterification of ibuprofen with ethanol. Formation of ester was preliminarily confirmed by its yellow colour and its fruity odour. Second step involved the synthesis of hydrazide derivative of ibuprofen ethyl ester using hydrazine hydrate. Its formation was preliminarily confirmed by performing a test in which the formed compound was treated with Nessler's reagent, black colouration obtained on addition of reagent indicates presence of hydrazide. Third step involves the nucleophilic substitution reaction for the formation of schiff's bases from ibuprofen hydrazide and various aldehydes.

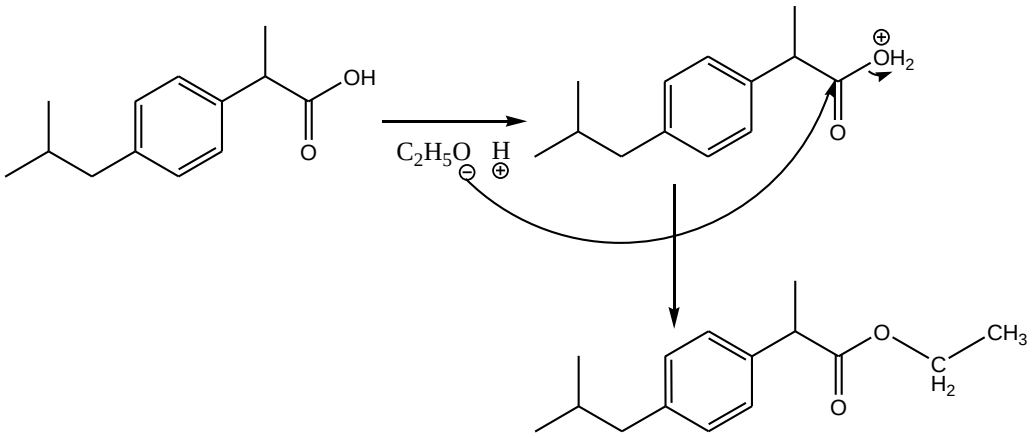
Scheme 1: Scheme involves the synthesis of (E)-N'-(Substituted)-2-(4-isobutylphenyl) propanehydrazide (A1-A5)



2.2.1: Synthesis of ethyl 2-(4-isobutylphenyl) propanoate: [9]

Ibuprofen (0.0048) was taken in a flat bottom flask containing 20 ml of Ethanol. To it few drops of concentrated sulphuric acid was added. The mixture was refluxed for 6hours. The reaction was monitored by TLC (30% Ethyl actetate and Petroleum ether 40/60). After completion of reaction, solvent was removed by distillation and to which cold water was added. The organic layer was washed with water, then with 5% Sodium carbonate solution and with water till it was free from acid.

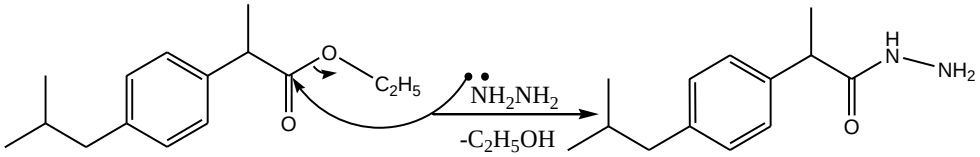
Possible Mechanism:



2.2.2: Synthesis of 2-(4-isobutyl phenyl) propanehydrazide: [10]

Hydrazine hydrate (2.5ml, 0.0085 moles) was teken in a 50 ml flat bottom flask. To it ethyl 2-(4-isobutylphenyl)propanoate (2ml, 0.0085 mole) was added and stirred for half and hour. Then 20 ml of ethanol was added to it and the reaction mixture was then refluxed for 18 hours. The reaction was monitored by TLC (30% Ethyl actetate and Pet.ether). After the reaction was over, water was added which resulted in the formation of solid that was filtered and dried. It was then recrystallized from methanol to obtain the pure product.

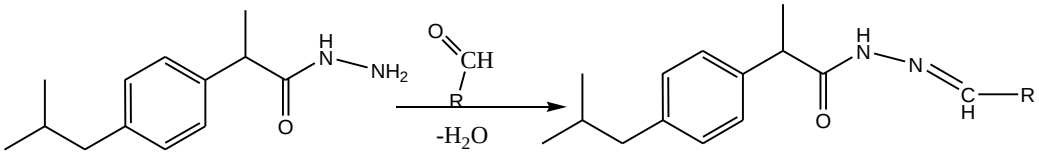
Possible Mechanism:



2.2.3: Synthesis of (E)-N'-(Substituted)-2-(4-isobutylphenyl) propanehydrazide: [11]

2-(4-isobutyl phenyl) propane hydrazide (2 g, 0.0091 moles) was transferred to 50 ml flat bottom flask containing 20 ml of acetonitrile. To it various aldehyde derivatives (0.0091 moles) was added. The reaction mixture was then refluxed for 3 hours. TLC was monitored until the completion of the reaction (50% Ethyl actetate and Pet.ether). After the reaction was over, water was added which resulted in the formation of solid that was filtered and dried. It was then recrystallized from methanol to obtain the pure product.

Possible mechanism:



3. RESULTS AND DISCUSSION

3.1. Schrodinger XP-docking results:

XP docking indicates that some of our compounds have good binding ability with COX protein (PDB ID: 3NT1) .All our synthesized compounds were found to occupy the same binding site and have similar kind of interaction as that of reported compounds as COX inhibitors.

Compound A3 (total score -11.76) demonstrated four hydrogen bond contacts. The carbonyl oxygen forms hydrogen bond with Arg120 (2.285 Å) and

Tyr355 (2.080 Å) and the Imine nitrogen forms hydrogen bond with Arg 120(2.59 Å) and Arg120 (2.03 Å).It was found to have electrostatic rewards (-0.5) and XP rotational penalty (0.3) . It showed hydrophobic interactions with Leu384, Trp387, Leu352, Val523, Val349, Ala527, Leu359, Val116, Ile112 and Phe357.

Compound A1 (total score -11.25) demonstrated two hydrogen contacts. The carbonyl oxygen forms hydrogen bond with Arg120 (2.016 Å) and Tyr355 (2.008 Å). It was found to have electrostatic rewards (-0.3) and XP rotational penalty (0.2). It showed hydrophobic interactions with Leu384, Trp387, Leu352, Val523, Val349, Ala527, Leu359, Val116, Ile112 and Phe357**.**

Compound A4 (total score -10.26) demonstrated four hydrogen bond contacts. The carbonyl oxygen forms hydrogen bond with Arg120 (2.160 Å) and Tyr355 (2.210 Å) and the Imine nitrogen forms hydrogen bond with Arg 120(2.071 Å) and Arg120 (2.325 Å) . It was found to have electrostatic rewards (-0.2) and XP rotational penalty (0.3). It showed hydrophobic interactions with Leu384, Trp387, Leu352, Val523, Val349, Ala527, Leu359, Val116, Ile112 and Phe357.

Compound A5 (total score -9.89) demonstrated four hydrogen bond contacts. The carbonyl oxygen forms hydrogen bond with Arg120 (2.118 Å) and Tyr355 (2.119 Å) and the Imine nitrogen forms hydrogen bond with Arg120 (2.570 Å) and Arg120 (1.883 Å) . It was found to have electrostatic rewards (-0.3) and XP rotational penalty (0.3). It showed hydrophobic interactions with Leu384, Trp387, Leu352, Val523, Val349, Ala527, Leu359, Val116, Ile112 and Phe357.

Compound A2 (total score -9.75) It was found to have electrostatic rewards (-0.14) and XP rotational penalty (0.11). It showed hydrophobic interactions with Leu384, Trp387, Leu352, Val523, Val349, Ala527, Leu359, Val116, Ile112 and Phe357.

Table No.1: Schrodinger XP Docking Scores

Title	XP GScore	XP HBond	XP Lipophilic EvdW	XP PhobEn	glide ecoul	glide evdw	XP Electro	XP Sitemap	XP RotPenal
A1	-11.25	-1.05	-5.34	-2.7	-4.21	-32.07	-0.31	-0.4	0.424539
A2	-9.75	0	-5.04	-2.33	-1.94	-25.76	-0.14	-0.76	0.45
A3	-11.76	-1.59	-6.34	-1.61	-7.90	-31.33	-0.59	-0.18	0.39
A4	-10.26	-1.17	-5.76	-2.34	-3.11	-32.11	-0.23	-0.19	0.44
A5	-9.80	-1.05	-5.78	-2.05	-4.31	-35.53	-0.32	-0.03	0.40
Ibuprofen	-11.4	-2.15	-4.53	-1.73	-10.95	-24.73	-0.82	0	0.33

[a] G score= glide score, [b]Lipophilic EvdW= Lipophilic term derived from hydrophobic grid potential, [c] Hbond= hydrogen bonding term, [d]Electro= Electrostatic rewards, [e]PhobEn= hydrophobic energy, [f]Rot Penal= Rotatable bond penalty.

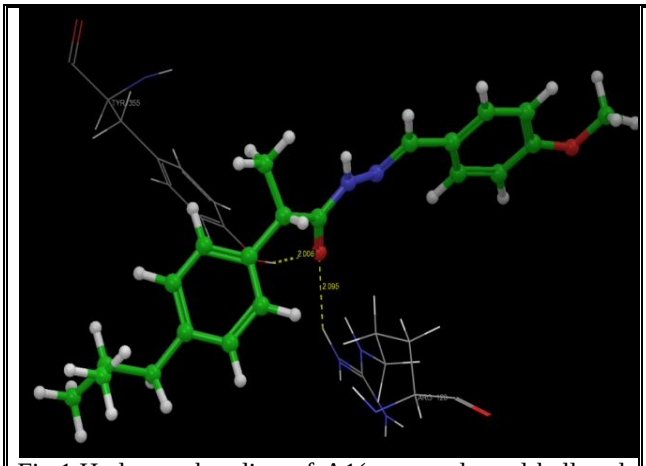


Fig.1.Hydrogen bonding of A1(green coloured ball and stick) with Arg120 and Tyr 355(yellow coloured lines) of 3NT1 protein

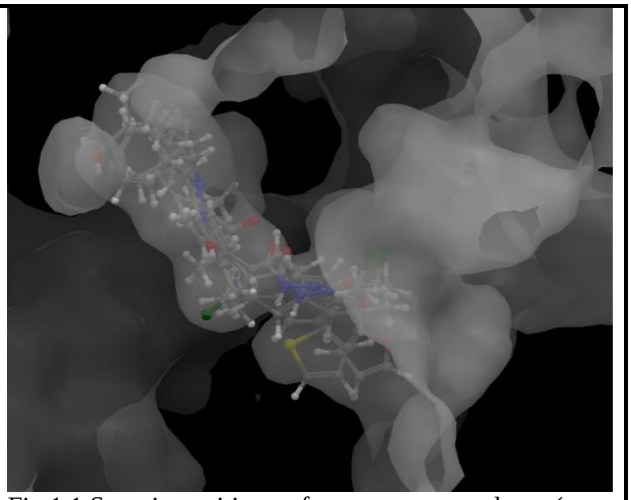


Fig.1.1.Superimposition of our compounds (gray coloured ball and stick) in the grid of 3NT1 protein

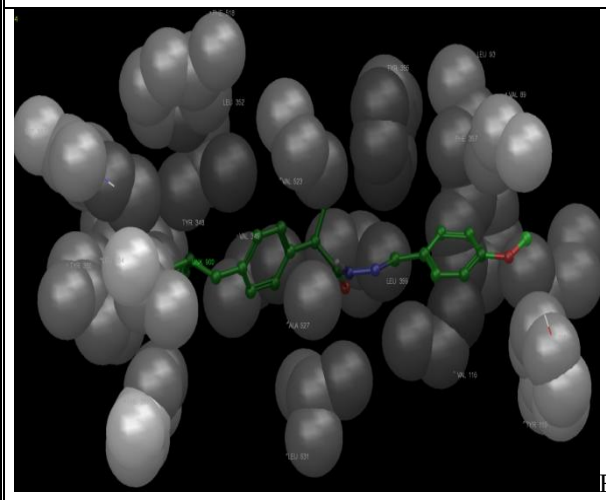


Fig.1.3.A1(green coloured ball and stick) shows hydrophobic interactions with Leu384, Ile112 and Phe357(gray coloured sphere balls) of 3NT1 protein

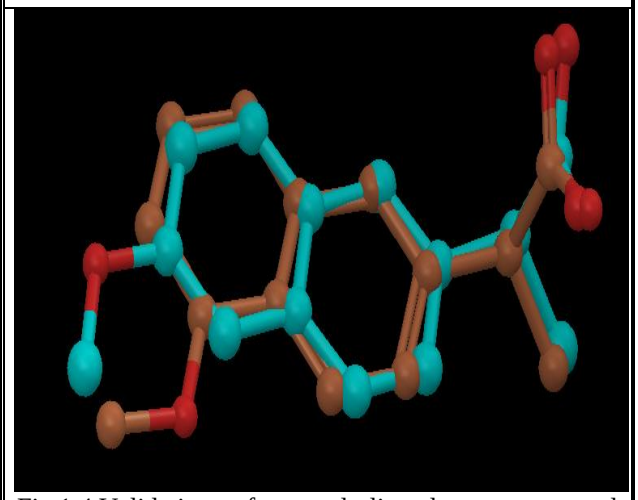


Fig.1.4.Validation of crystal ligand naproxen and redocked naproxen

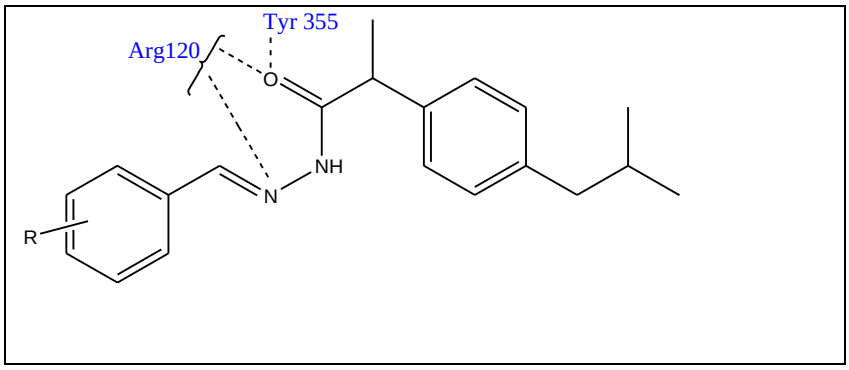
Structural Activity Relationship of Ibuprofen analogs:

It was concluded based on the docking results.

Compound A3 (E)-N'-(4-hydroxy-3-methoxybenzylidene)-2-(4-isobutylphenyl) propanehydrazide having total score of -11.76 demonstrated four hydrogen bond interactions. The carbonyl oxygen forms hydrogen bond with Arg120 (2.285 Aº) and Tyr355 (2.080 Aº) and the Imine nitrogen forms hydrogen bond with Arg 120 (2.59 Aº) and Arg120 (2.03 Aº). The hydrophobic groups present made it form hydrophobic interactions with Leu384, Trp387, Leu352, Val523, Val349, Ala527, Leu359, Val116, Ile112 and Phe357 of 3NT1 protein. XP lipophilic EvdW and sitemap are found be high than ibuprofen.

Compound A1- (E)-N'-(4-methoxybenzylidene)-2-(4-isobutylphenyl)propanehydrazide is having long chain structure which made it to fix in the tunnel of COX easily. It demonstrated two hydrogen contacts. The carbonyl oxygen forms hydrogen bond with Arg120 (2.016 Aº) and Tyr355 (2.008 A). The hydrophobic groups present in the compound showed hydrophobic interactions with surrounding amino acids like Leu384, Trp387, Leu352, Val523, Val349, Ala527, Leu359, Val116, Ile112 and Phe357**.** XP lipophilic EvdW, XP PhobEn, glide evdw and XP sitemap were found to high compared

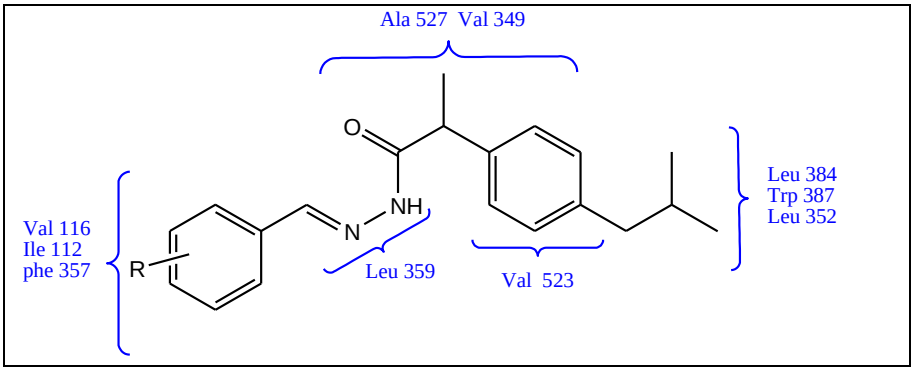
to ibuprofen. All the Compounds retained hydrogen bonding interactions with Arg120 and Tyr355 which was also seen with standard COX-2 inhibitors.Apart from those two hydrogen bonding, Imine nitrogen of 'A' series compounds is showing additional interactions with Arg120 and Tyr355.



The amino acids which formed important hydrophobic interactions with the ligand are Trp387, Leu352, Val523, Ala527 and Val349. This interaction was clearly exemplified and quantified for all compounds**.**

Additional hydrophobic interactions with Ile112, Tyr385, Val349, Leu359, Val116, Phe35, Val89 and Phe357 were found.

It is known that selectivity of a molecule is increased by interaction of molecule with extra binding pocket created by Ile112 and all molecules are showing hydrophobic interaction with that aminoacid (Ile112)



All the molecules were occupying the tunnel (grid) of COX enzyme. Hydrogen bonding interactions are more for compounds A1, A3, A4.The C=O group and C=N group are essential for interactions as they are showing hydrogen bonding with amino acids of protein. Compounds are having long chain structure which made them to occupy the tunnel of COX enzyme.

3.1.1. (E)-N'-(4-methoxybenzylidene)-2-(4-isobutylphenyl)propanehydrazide

Yield 64%, mp 118-120 ºC, IR 3174 (NHstr); 2833 (O-CH_3); 1666 (C=N imine str); 2951 (CH str); NMR 0.853-0.875 (d, 6H, J=6.6 Hz, Aliphatic CH_3), 1.528-1.522 (d, 3H, J=1.8 Hz, Aliphatic CH_3), 1.766-1.789 (m, 1H, J=6.9 Hz), 2.396-2.420 (d, 2H, J=7.2 Hz, Alipatic CH_2), 4.662-4.686 (m, 1H, J=7.2 Hz, CH), 7.244-7.264 (d, 4H, J=6 Hz, Aromatic CH), 3.852 (s, 3H, OCH_3), 8.078 (s, 1H, Imine CH), 8.913 (s, 1H, Imine NH)

3.1.2. (E)-N'-(4-hydroxybenzylidene)-2-(4-isobutylphenyl)propanehydrazide

Yield 67%, mp 166-170 ºC, IR 3622(OHstr); 3305(NH str);1656(C=N imine str);2960(CH str); NMR 0.762-0.774 (d, 6H, J=3.6 Hz, Aliphatic CH_3), 1.384-1.407 (d, 3H, J=6.9 Hz, Aliphatic CH_3), 1.707-1.718 (m, 1H, J=3.3 Hz), 2.296-2.319 (d, 2H, J=6.9 Hz, Alipatic CH_2), 4.584-4.607 (m, 1H, J=6.9 Hz, CH), 7.205-7.226 (d, 4H, J=7.2 Hz, Aromatic CH), 5 (s, 1H, OH), 7.972 (s, 1H, Imine CH), 9 (s, 1H, Imine NH)

3.1.3. (E)-N'-(4-hydroxy-3-methoxybenzylidene)-2-(4-isobutylphenyl)propanehydrazide

Yield 64%, mp 100-103 ºC, IR 3651(OH str); 3190(NH str);2893(O-CH_3 str);1654(C=N imine str); NMR 0.851-0.873 (d, 6H, J=6.6 Hz, Aliphatic CH_3), 1.529-1.552 (d, 3H, J=6.9 Hz, Aliphatic CH_3), 1.786-1.809 (m, 1H, J=6.9 Hz), 2.396-2.420 (d, 2H, J=7.2 Hz, Alipatic CH_2), 4.630-4.653 (m, 1H, J=6.9 Hz, CH), 7.244-7.264 (d, 4H, J=6 Hz, Aromatic CH), 5.9 (s, 1H, OH), 3.975 (s, 3H, OCH3) 8.465 (s, 1H, Imine CH), 8.936 (s, 1H, Imine NH)

3.1.4. (E)-N'-((furan-2-yl)methylene)-2-(4-isobutylphenyl)propanehydrazide

Yield 64%, mp 120-124 ºC, IR 3233(NH str);2998(CH str);1654(C=N imine str); NMR 0.814-0.849 (d, 6H, J=10.5 Hz, Aliphatic CH_3), 1.297-1.321 (d, 3H, J=7.2 Hz, Aliphatic CH_3), 1.706-1.729 (m, 1H, J=6.9 Hz), 2.358-2.383 (d, 2H, J=7.5 Hz, Alipatic CH_2), 4.558-4.582 (m, 1H, J=7.2 Hz, CH), 6.584-6.594 (d, 2H, J=3 Hz, Heteroaromatic CH), 7.045-7.072 (d, 4H, J=8.1 Hz, Aromatic CH), 7.200-7.236 (d, 1H, J=10.8 Hz, Heteroaromatic CH), 8.086 (s, 1H, Imine CH), 9.148 (s, 1H, Imine NH)

3.1.5. (E)-2-(4-isobutylphenyl)-N'-((thiophen-2-yl)methylene)propanehydrazide

Yield 64%, mp 129-135 ºC, 3188(NH str);2974(CH str);1662(C=N imine str); NMR 0.808-0.830 (d, 6H, J=6.6 Hz, Aliphatic CH_3), 1.343-1.365 (d, 3H, J=6.6 Hz, Aliphatic CH_3), 1.724-1.746 (m, 1H, J=6.6 Hz), 2.354-2.378 (d, 2H, J=7.2 Hz, Alipatic CH_2), 4.474-4.497 (m, 1H, J=6..9 Hz, CH), 7.338-7.348 (d, 2H, J=3 Hz, Heteroaromatic CH), 7.231-7.240 (d, 4H, J=2.7 Hz, Aromatic CH), 7.603-7.616 (d, 1H, J=3.9 Hz, Heteroaromatic CH), 8.078 (s, 1H, Imine CH), 8.413 (s, 1H, Imine NH)

4. CONCLUSION:

The structure based drug design (SBDD) was performed against 3NT1 protein using XP on Dell Precision T-1500 workstation. About 1000 molecules having various heterocyclic rings of synthetic and natural origin have been docked against the COX-2 protein (PDB Code: 3NT1) and the molecules having good interactions similar to those of standard were selected for synthesis.

4.1. Molecular modeling: SBDD was pursued by the preparation of protein and ligand followed by creation of a grid in the binding site of COX-2. Results revealed that our analogues (A1-A5; B1-B5) had crucial interactions with the important amino acids of cyclooxygenase enzyme. H-bonding formed by top scored molecules is similar to those of standard inhibitors. Most the molecules showed H-bonding with Arg120 and Tyr355 which is essential for interactions. Hydrophobic interaction of our molecules were found to be with amino acids Trp387, Leu352, Val523, Ala527, Val349, Ile112, Tyr385, Val349, Leu359, Val116, Phe35, Val89 and Phe357. B1-B5 having azetidinone ring makes it to occupy more in the COX-2 binding site and were found to have sitemap score of 0.7. The carbonyl group and imine group are essential for activity as they are having hydrophilic interactions with Arg120 and Tyr355. Compound A3 and A2 were found to have promising in silico cyclooxygenase inhibition activity with a total score indicating overall interactions as -11.75 and -11.25 respectively.

4.2. Chemical Synthesis & Analytical Characterization: Modeled compounds were synthesized by taking ibuprofen as the starting material. Synthetic scheme involved four steps to get final products. First step is about conversion of ibuprofen into acid ester; later the acid ester was treated with hydrazine hydrate to form ibuprofen hydrazide. In step 3, ibuprofen hydrazide was reacted with various aldehydes to form ibuprofen Schiff bases. The formation of final compounds (A1-A5) was confirmed by IR, NMR and mass analytical data. NH peak was found at 3200-3300 cm^{-1}, C=O at 1760-1720 cm^{-1}, C=N at 1660-1640 cm^{-1}, CH aromatic strectching at 2800-2900 cm^{-1}, C-Cl bending at 740-750 cm^{-1} in IR spectroscopic interpreration. Proton NMR chemical shift (δ) confirms the presence of NH proton at 9-10, OH proton at 5, OCH_3 proton at 4-4.5, and imine CH proton near 4.

4.3.Future scope: Synthesized ibuprofen analogs A1-A5 have the potential to be explored for further detailed investigation such as in vitro enzyme inhibition study against COX-2 protein, in vivo anti-inflammatory activity and ulcerogenic potential.

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