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Design, Synthesis, and Biological Evaluation of Some New Benzostyrene Incorporated Phenyl Styryl Ketone Derivatives as COX-2 Inhibitors with Anti-Inflammatory Activity

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ABSTRACT

Cyclooxygenases-II (COX-II) or Prostaglandin Endoperoxide Synthases (PGHS), a protein essential for prostaglandin biosynthesis from arachidonic acid is an important target for anti-inflammatory drugs. Despite the development of COX-II inhibitor drugs as anti-inflammatory action through an inhibitory effect on cyclooxygenase enzyme, there is still significant potential for designing new chemical entity with affordable, safe and efficacious anti-inflammatory. A strategy that has attracted considerable attention in current medicinal chemistry is based on the conjugation of two biologically active molecules into one hybrid compound. In present study 3-(2,3-dihydro-1,4-benzodioxane-6-yl)-1-substituted-phenylprop-2-en-1-one analogs were designed and these were investigated by docking studies in the binding site of 4ZOL and 4M10 enzymes using Glide v 5.6. Among the series of designed compounds were synthesized with good potential. Structural confirmation of these compounds was done by FT-IR, 1H-NMR and Ultra Performance Mass Spectroscopy (UPMS). The pharmacological evaluation of all the synthesized compounds was performed by using Carrageenan induced rat paw edema model. In silico ADME predictions for drug likeliness properties of all the designed analogs were calculated by Qik Prop v3.3 which shows that analogues are safer and can be used as a drug of choice as anti-inflammatory agents. The above study could be very useful for further design and development of new anti-inflammatory analogs.

1. INTRODUCTION

Inflammation is a biological response of vascular tissues to harmful stimuli, such as pathogen damaged cells, irritants etc. Inflammation is a protective response that involves immune cells, blood vessels, and molecular mediators. Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutically important in the treatment of osteoarthritis, multiple sclerosis, inflammatory bowl diseases and diabetic nephropathy, tumor initiation, and malignant progression. Thus, there is an urgent need for new targets that are required for the design and development of novel anti-inflammatory agents as an alternative to NSAIDs. The symptoms of inflammation are characterized by pain, heat, redness, swelling and loss of function. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair. The nonsteroidal anti-inflammatory drugs (NSAIDs) are used worldwide for therapeutic intervention of pain and inflammation. These achieve their anti-inflammatory action through an inhibitory effect on cyclooxygenase (COX) enzyme, a protein essential for prostaglandin biosynthesis from arachidonic acid.

Analgesic and anti-inflammatory relieves mild to moderate pain and reduces inflammation and fever. These agents are effective for somatic pain (e.g. musculoskeletal pain in joints, muscle and headache). They are not effective in reducing discomfort from visceral organ (Heart, liver and lung). All these analgesic and anti-inflammatory agent produce their therapeutic effects by inhibiting various prostaglandins substances involved in development of pain and inflammation as well as regulation of body temperature.

COX or PGHS are the key enzymes in the synthesis of prostaglandins from arachidonic acid (AA), the main mediators of inflammation, pain and increased body temperature (hyperpyrexia). AA is cleaved from cell membrane phospholipids by phospholipase A2. COX Convert AA into unstable endoperoxides PGG2 and PGH2. After that PGG2 and PGH2 are metabolized by synthases to primary prostaglandins PGD2, PGE2, PGF2α, TXA2 (thromboxane A2) and PGI2 (prostacyclin). Prostaglandins (PGs) are the lipid mediators made by most cells in the body except by red blood cells and released upon almost any type of chemical or mechanical stimulus.

COX exhibit in two isoforms named COX-I and COX-II, which show distinct expression patterns and distinct biological activities. COX-I expressed constitutively, is synthesized

continuously and is present in all tissues and cell types, most notably in platelets, endothelial cells, the GI tract, renal microvasculature, glomerulus, and collecting ducts. Thus COX-I is important for the production of prostaglandins of homeostatic maintenance, such as platelet aggregation, the regulation of blood flow in the kidney and stomach, and the regulation of gastric acid secretion. Inhibition of COX-I activity is considered a major contributor to NSAID GI toxicity. Thus in the present study, we report the synthesis and anti-inflammatory activity of a new series of benzostyrene substituted phenyl styryl ketone derivatives. Also, we studied the interaction of these derivatives in the binding site of 4ZOL and 4M10 using Glide docking studies.

2. MATERIALS AND METHODS

2.1 Computational studies

2.1.1 Docking validation

The most eloquent method to check the accuracy of docking method is to determine the closeness between the lowest energy conformer and scoring function. Glide score simulate an experimental binding mode as deciphered by X-ray crystallography. Assurance of docking process was done by analyzing the RMSD value, it is used to indicate whether correct docking pose was obtained by Glide or not. Normally RMSD of 2Å and higher precision analysis is not meaning full. Docking was done by using protein PDB ID 4ZOL and 4M10. The RMSD values among docked pose and its bound conformation are in range of 0.002 to 0.048, which indicates that docking was performed well for COX-II inhibitors. After this validation, all of the twenty COX-II inhibitors were docked in the binding pocket of X-ray crystallographic structure of 4ZOL and 4M10.

2.1.2 Ligand preparation

ChemDraw Ultra 12.0 was used to draw 2D molecular structures of designed hybrids. All these 2D structures were converted into 3D with help of Chem 3D ultra-version 8.0.3. These 3D structures were introduced into Maestro implemented in Schrödinger; energy minimization of 3D structures was done by using Ligprep v 2.4 program. Different ionization states were generated at user defined pH. ConfGen was used to generate various conformers for each ligand and minimization was done by using Impref module of Schrödinger suit by using OPLS-2005 force field to correct its bond length and bond order.

2.1.3 Protein preparation

The three-dimensional crystal structure of COX-II enzyme (PDB ID 4ZOL and 4M10) was downloaded from RCSB Protein Data Bank and prepared by protein preparation wizard. Preparation and refinement are two components of protein preparation wizards. After confirmation of chemical accuracy, addition of hydrogen's and side chain neutralization was done by using force field OPLS-2005. Only those side chains were neutralized that neither participate in salt bridge formation nor were present in contact with binding cavity. Minimization was performed until the average root mean square deviation of the non-hydrogen atoms reached 0.3 Å. In final step flip, no flip model of prepared protein was obtained and this was used for grid generation.

2.1.4 Receptor Grid Generation

Receptor grid generation starts by picking and selecting co-crystallized ligand from the active site of prepared protein. Finally, the grid was generated to define the active site of protein which was visualized in form of box at the point of workspace. The complete process was run by default settings. This grid file was further used to perform docking.

2.1.5 Docking studies

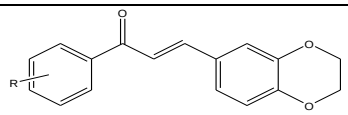
Ligand docking was done by using Glide, v 5.6. The prepared ligands and the file obtained from receptor grid generation panel were selected and all the designed analogs of 4-aminoquinoline and acridine derivatives were docked within the binding site of 3-(2,3-dihydro-1,4-benzodioxane-6-yl)-1-substituted-phenylprop-2-en-1-one (PDB ID 4ZOL and 4M10). Flexible docking was done by employing Extra Precision (XP) mode of Glide. Glide score of compounds was obtained and various interaction of ligand with protein was studied. The final energy evaluation was done with the Glide score and a single best pose was generated as output for a particular ligand with the help of following equation.

$$GScore = a*vdW + b*Coul + Lipo + H\ bond + Metal + Bury\ P + RotB + Site$$

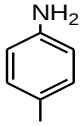
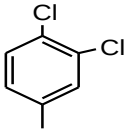
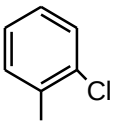
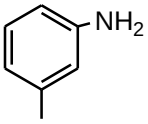
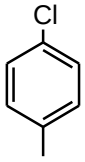
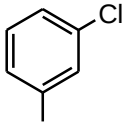
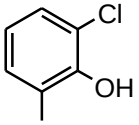
Where vdW = Vander Waal energy, Coul = Coulomb energy, Lipo = Lipophilic contact term, HBond = Hydrogen-bonding term, Metal = Metal-binding term, BuryP = Penalty for buried polar group, RotB = Penalty for freezing rotable bonds, Site = Polar interaction at active site, and the coefficient of vdW and Coul are a = 0.065, b = 0.0130. The best pose for a given

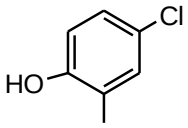
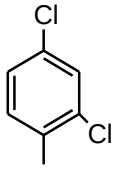
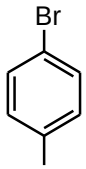
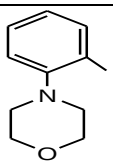
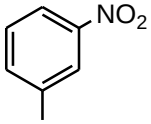
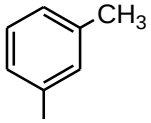
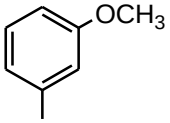
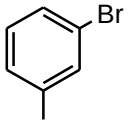
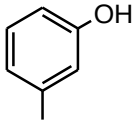
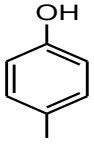
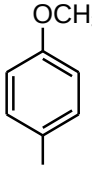
ligand was determined by the Emodel score, while different compounds were ranked using Glide score.

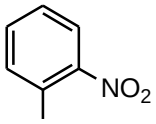
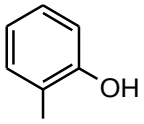
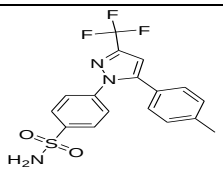
Table 1: Structures of designed compounds along with compound code, substituents and IUPAC Nomenclatures



General structure of compounds

S. No.	Compound Code	R_1	IUPAC Nomenclatures
1	HN1		(E)-3-(4-aminophenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
2	HN2		(E)-3-(3,4-dichlorophenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
3	HN3		(E)-3-(2-chlorophenyl)-1-(2,3dihydro benzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
4	HN4		(E)-3-(3-aminophenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
5	HN5		(E)-3-(4-chlorophenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
6	HN6		(E)-3-(3-chlorophenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
7	HN7		(E)-3-(3-chloro-2-hydroxyphenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one

8	HN8		(E)-3-(5-chloro-2-hydroxyphenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
9	HN9		(E)-3-(2,4-dichlorophenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
10	HN10		(E)-3-(4-bromophenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
11	HN11		(E)-1-(2-morpholinophenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
12	HN12		(E)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(3-nitrophenyl)prop-2-en-1-one
13	HN13		(E)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-m-tolylprop-2-en-1-one
14	HN14		(E)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(3-methoxyphenyl)prop-2-en-1-one
15	HN15		(E)-3-(3-bromophenyl)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)prop-2-en-1-one
16	HN16		(E)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(3-hydroxyphenyl)prop-2-en-1-one
17	HN17		(E)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one
18	HN18		(E)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(4-methoxyphenyl)prop-2-en-1-one

19	HN19		(E)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(2-nitrophenyl)prop-2-en-1-one
20	HN20		(E)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one
CEL	-		4-(5-p-tolyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide

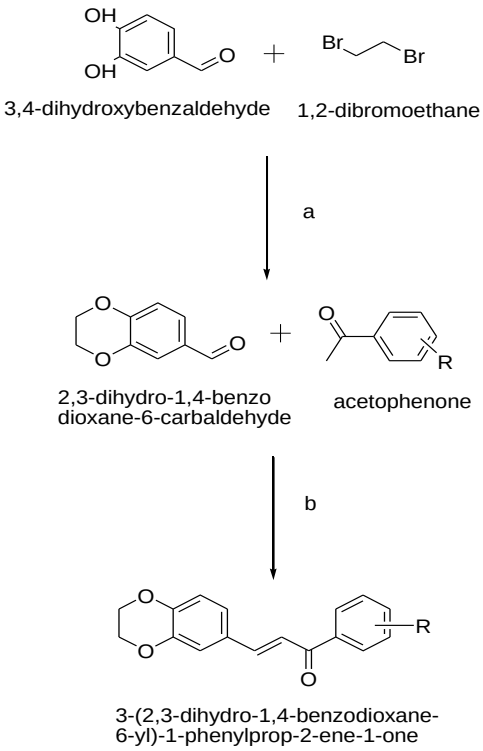
2.2 Synthesis

Chemistry

General procedure for synthesis of 3-(2,3-dihydro-1,4-benzodioxane-6-yl)-1-substituted-phenylprop-2-en-1-one (HN1-HN20) :-

The synthetic route used to prepare starting materials and the title compounds is outlined in Scheme 1. The starting material 2,3-dihydro-1,4-benzodioxane-6-carbaldehyde was prepared by reaction between 3,4-dihydroxybenzaldehyde and ethylene dibromide in dry acetone in the presence of anhydrous potassium carbonate, which on Claisen–Schmidt condensation with substituted acetophenone in absolute ethanol and 10% potassium hydroxide afforded corresponding 3-(2,3-dihydro-1,4-benzodioxane- 6-yl)-1-substituted-phenylprop-2-en-1-one derivatives (HN1-HN20).

Scheme:-



Scheme 1 :- Reagents and Conditions (a) K_2CO_3 , dried Acetone with reflux for 26 hrs (b) KOH/ Ethanol , stirred at room temperature for 11 hrs

General procedure for synthesis of Intermediate compound (1) [2,3-dihydro-1,4-benzodioxane-6-carbaldehyde] :

Anhydrous potassium carbonate (21 g) was added in portions to a stirred solution of 27.6 g of 3,4-dihydroxy benzaldehyde in 100 ml of dry acetone followed by the dropwise addition of 4.3 ml of ethylene dibromide. Another 21g of potassium carbonate and 4.3 ml of ethylene dibromide were added similarly and this was repeated twice more using a total of 84 g of potassium carbonate and 17.2 g of ethylene dibromide. Stirring and refluxing was continued for another 25 h. The reaction mixture was then filtered by sintered glass funnel and the solid residue was washed several times with acetone. The combined filtrate was concentrated to about 25 ml and the residue was poured onto crushed ice, a solid was separated which was filtered, dried and crystallized with methanol to give a low melting solid; mp 35–37ºC; 67% yield.

General procedure for synthesis of Final compound (2) [3-(2,3-dihydro-1,4-benzodioxane-6-yl)-1- substituted-phenylprop-2-en-1-one] :

25% solution of potassium hydroxide in aqueous ethanol (5 ml) was added dropwise to a mixture of substituted acetophenone (0.01 mol) and aldehyde 1 (0.01 mol) in 25 ml ethanol at 0–5 C with stirring. The stirring was further continued for 10–12 h at room temperature. After completion of the reaction, the reaction mixture was poured onto crushed ice; the resulting solid was filtered, washed with water, dried and crystallized from ethanol. mp 82–84 C; 45% yield

Thin layer chromatography was used to monitor the progress of the reactions. Infrared spectra were recorded on SHIMADZU FT/IR spectrophotometer using KBr pellets at S.G.S.I.T.S, Indore, and values were expressed in cm^{-1}.^{1}HNMR spectra were recorded using a Bruker ADVANCE II 400 NMR spectrophotometer and values were reported in ppm downfield from TMS (Tetramethylsilane) as an internal standard. The NMR spectra were obtained in DMSO. The molecular mass of synthesized hybrid was determined by mass spectroscopy. Ultra Performance Mass spectra were recorded and results were reported in terms of their m/z values. Melting points were determined by open capillary method.

The Spectral Data of the synthesized compounds is as follows:

(E)-3-(4-aminophenyl)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)prop-2-en-1-one (HN1):

Brownish black color; Yield 42%; mp: 220-222°C; IR (KBr) Ar C-H (cm^{-1}) 3043, Ar O-H (cm^{-1}) 3397 , Ar C-O (cm^{-1}) 1265, C=O(cm^{-1}) 1639 , Ar N-H (cm^{-1}) 3333, C=C(cm^{-1}) 1651 ; ^{1}H NMR (400 MHz, DMSO-δ ppm): 3.807(2H,NH-Ar), 8.624(1H,Ar-H), 2.462(1H,CH), 294(1H,CH), 4.624(1H,CH), 3.601(2O), 8.131(1H,Ar-H) ;m/z; 279[M+].

(E)-3-(4-chlorophenyl)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)prop-2-en-1-one (HN5) :

Brownish black color color; Yield 42%; mp: 221-222°C; IR (KBr) Ar C-H (cm^{-1}) 3227, Ar O-H (cm^{-1}) 3334 , Ar C-O (cm^{-1}) 1280, -Cl($cm^{-1)}$ 705, C=O($cm^{-1)}$ 1634, C=C($cm^{-1)}$ 1403; ^{1}H NMR (400 MHz, DMSO-δ ppm): 7.136(Cl), 2.626(1H,CH), 2.497(1H,CH), 2.476(1H,CH), 4.450(1H,C), 3.694(2O), 8.286(1H,Ar-H);m/z; 279[M+].

(E)-3-(2,4-dichlorophenyl)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)prop-2-en-1-one (HN9) :

Orange amorphous solid; Yield 48%; mp: 218-220°C; IR (KBr) Ar C-H (cm^{-1}) 3103, Ar O-H (cm^{-1}) 3382 , Ar C-O (cm^{-1}) 1376, C-Cl(cm^{-1}) 705, C=O(cm^{-1}) 1655, C=C(cm^{-1}) 1655; ^1H NMR (400 MHz, DMSO-δ ppm): 7.023(2Cl), 3.641(2O), 6.873(6H, Ar-H), 7.523(1H,Ar-H), 8.053(1H,Ar-H), 8.168(1H,Ar-H), 4.479(C=C) ;m/z; 279[M+].

(E)-3-(4-bromophenyl)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)prop-2-en-1-one (HN10):

Orange amorphous solid; Yield 50%; mp: 220-222°C; IR (KBr) Ar C-H (cm^{-1}) 3337, Ar O-H (cm^{-1}) 3126, Ar Br (cm^{-1}) 642, Ar C-O (cm^{-1}) 1240, C=O(cm^{-1}) 1662, C=C(cm^{-1}) 1685; ^1H NMR (400 MHz, DMSO-δ ppm): 2.382(Br), 2.607(1H,CH), 8.043(1H,Ar-H), 2.502(1H,CH), 2.36(1H,CH), 4.503(1H,CH), 3.701(2O), 8.328(1H,Ar-H) ;m/z; 279[M+].

(E)-1-(2-morpholinophenyl)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)prop-2-en-1-one (HN11) :

Black amorphous solid; Yield 40%; mp: 240-242°C; IR (KBr) Ar C-H (cm^{-1}) 3093, Ar O-H (cm^{-1}) 3075, Ar C-O (cm^{-1}) 1382, C=O(cm^{-1}) 1685 , Ar N-H (cm^{-1}) 3268, C=C(cm^{-1}) 1578; ^1H NMR (400 MHz, DMSO-δ ppm): 4.281(N-C), 3.362(1O-Ar), 2.493(1H,CH), 8.845(1H,Ar-H), 2.198(1H,CH), 2.569(1H,CH), 4.294(1H,CH), 3.701(2O), 8.328(1H,Ar-H) ;m/z; 279[M+].

(E)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3-(3-nitrophenyl)prop-2-en-1-one (HN12) :

Black amorphous solid; Yield 40%; mp: 235-238°C; IR (KBr) Ar C-H (cm^{-1}) 3083, Ar O-H (cm^{-1}) 3277 , Ar C-O (cm^{-1}) 1352, Ar NO_2(cm^{-1}) 1524, C=O(cm^{-1}) 1651, C=C(cm^{-1}) 1524; ^1H NMR (400 MHz, DMSO-δ ppm): 7.560(NO_2-Ar), 2.120(1H,CH), 8.043(1H,Ar-H), 2.502(1H,CH), 2824(1H,CH), 4.503(1H,CH), 3.701(2O), 8.512(1H,Ar-H);m/z; 279[M+].

(E)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3-(3-hydroxyphenyl)prop-2-en-1-one (HN16) : Orange amorphous solid; Yield 40%; mp: 232-234°C; IR (KBr) Ar C-H (cm^{-1}) 3361, Ar O-H (cm^{-1}) 2955 , Ar C-O (cm^{-1}) 1270, C=O(cm^{-1}) 1684, C=C(cm^{-1}) 1585; ^1H NMR awaited ;m/z; 279[M+].

2.3 *In vivo* Biological Evaluation:

All the synthesized compounds were screened for *in vivo* anti-inflammatory and their screening was carried out using Carrageenan-Induced Paw Edema Method.

Carrageenan-Induced Paw Edema Method:-

Acute inflammation is provided by injection of 0.1ml of 1% carrageenan into the sub plantar surface of rat hind paw.

Group I**:** Served as control.

Group II**:** Rats were received 0.1 ml of 1% carrageenan.

Group III**:** Rats were received indomethacin (20mg/kg/i.p)

Group IV**:** Rats were received 1.0 ml test compound (50 mg/kg/i.p) and 0.1 ml of carrageenan.

Group V**:** Rats were received 1.0ml of test compound (100mg/kg/i.p) and 0.1 ml of carrageenan.

The paw volume up to the tribiotural articulation was measured at 0, 1, 2, 3, 4, 5 and 6th hours.

At first, the animals were weighed using the digital weighing balance. Then they were marked by the picric acid solution to distinguish them according to their body weight. After that, the animals were grouped as control, test and standard respectively. The groups were fasted at a period of 24 hr prior to the study. The test compound was administered to the animals in the test group at the dose of 50 and 100 mg/kg by the feeding needle. Animals in the standard group received Indomethacin at the dose of 20 mg/kg body weight. Rats in control group received NS solution or the vehicle solution without drugs. One hour after drug administration, rats in all groups were challenged with 0.1 ml of 1% (w/v) carrageenan in sub-planter region of left hind paw. A zero hour paw volume was measured for the rats using Plethysmometer and vernier callipers before the administration of carrageenan for all groups. The volumes of edema of the carrageenan injected paws were measured at 1 hr interval for 6 hr.

The edema inhibitory activity was calculated according to the following formula-

Edema (%) inhibition = (1-D/C) ×100

Where,

D-represents the percentage difference in increased paw volume after the administration of test drugs to the rats.

C-represents the percentage difference of increased volume in the control groups.

3. RESULTS AND DISCUSSION

3.1 Docking studies:

The most significant method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy poses (binding conformation) predicted by the object scoring function, Glide score (Gscore or docking score) in this study, almost resembles an experimental binding mode as determined X-ray crystallography. It is known that RMSD value between the crystal structure and predicted conformation is widely used as an indicator of whether correct docking pose was obtained by program or not. Normally RMSD of 2 A° and higher precision analysis is not meaning full. Docking experiments were performed with crystal structures of COX-II including 4ZOL and 4M10. The RMSD values between docked posed and its bound conformation for 4ZOL and 4M10 are in the range of 0 to 0.048 for both protein, thus indicating that glide docking performed well for COX-II enzyme. The correlation of docking score with Glide Emodel energy for 4ZOL and 4M10 were found to be R^2=0.92 and R^2=0.95.After this validation, all of the 20 inhibitors of COX-II enzyme in the data set were docked into the X-ray crystallographic structure of 4ZOL and 4M10.

Table 2: Compound code, Glide score, Emodel energy, RMSD of compounds with standard.

S.No.	Compound code	Glide score		Glide EModel energy		RMSD	
		4ZOL	4M10	4ZOL	4M10	4ZOL	4M10
1	HN1	-8.196	-7.000	-62.796	-55.059	0.018	0.008
2	HN2	-7.927	-6.915	-69.078	-57.896	0.010	0.018
3	HN3	-8.349	-7.046	-69.722	-53.400	0.030	0.030
4	HN4	-7.496	-7.938	-61.826	-60.959	0.009	0.009
5	HN5	-7.557	-7.408	-64.208	52.513	0.021	0.021
6	HN6	-8.391	-8.916	-69.752	-63.671	0.046	0.046
7	HN7	-8.376	-8.437	-69.333	-64.445	0.037	0.037
8	HN8	-8.152	-7.598	-70.802	-63.802	0.043	0.048
9	HN9	-8.652	-8.443	-74.317	-67.691	0.002	0.002
10	HN10	-7.356	-8.101	-72.508	-63.550	0.048	0.048
11	HN11	-7.946	-8.542	-67.986	-61.608	0.010	0.010
12	HN12	-7.914	-8.626	-62.647	-62.415	0.032	0.032
13	HN13	-7.721	-6.449	64.652	-64.110	0.002	0.002
14	HN14	-8.277	-7.207	-62.171	-59.827	0.047	0.047
15	HN15	-7.663	-7.412	-56.429	-55.622	0.047	0.047
16	HN16	-7.285	-8.158	-58.933	-58.458	0.008	0.008
17	HN17	-8.185	-6.890	-70.933	-57.458	0.008	0.008
18	HN18	-4.676	-8.956	-73.678	-60.972	0.004	0.004
19	HN19	-7.933	-6.921	-75.742	-62.128	0.014	0.014
20	HN20	-7.547	-6.718	-59.402	-62.731	0.012	0.012
21	**CEL**	-6.703	-5.431	-54.243	-32.017	0.020	0.020

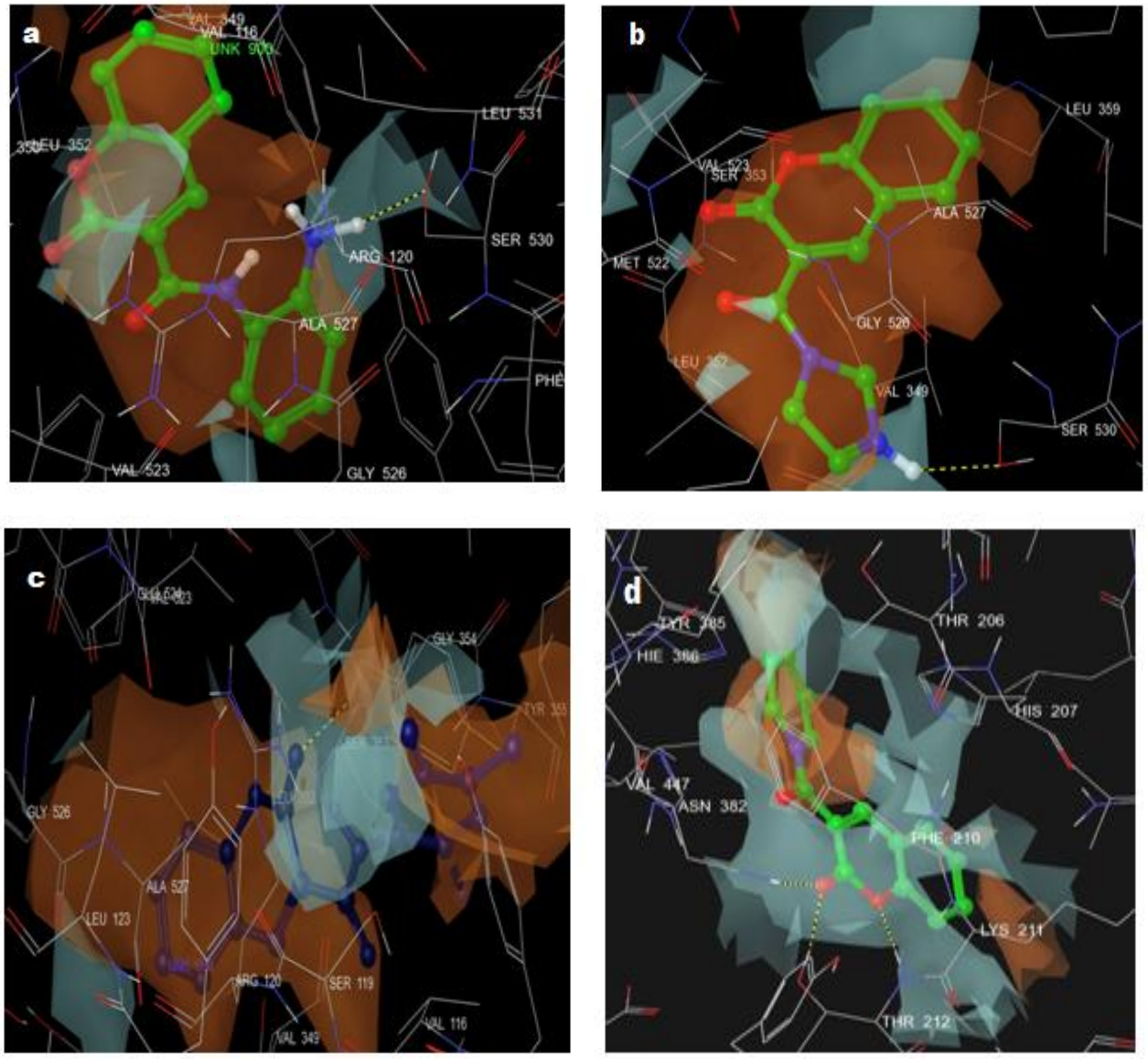


Fig.1:- Binding modes of 4ZOL with **(a)** compound HN5 **(b)** compound HN8 and 4M10 with **(c)** compound HN12 **(d)** compound HN16, showing hydrogen bond interaction with amino acid.

3.2 Biological Evaluation

3.2.1 In Vivo Biological Evaluation

Animals:-

Adult albino rats of both sex weighing 60–140g were used in evaluation of the anti-inflammatory and analgesic study of the new benzodioxane substituted styrenes **1-20**.

Biological Evaluation was done at Acropolis Institute of Pharmaceutical Education and Research Centre Indore (M.P.) (an CPCSEA approved Institute). Normalization of the animals with laboratory conditions was achieved by keeping them in laboratory one week before starting the experiments. Standard rat pellet diet was used for feeding animals and water ad libitum.

Procedures

At first, the animals were weighed using the digital weighing balance. Then they were marked by the picric acid solution to distinguish them according to their body weight. After that, the animals were grouped as control, test and standard respectively. The groups were fasted at a period of 24 hr prior to the study. The test compound was administered to the animals in the test group at the dose of 50 and 100 mg/kg by the feeding needle. Animals in the standard group received Indomethacin at the dose of 20 mg/kg body weight. Rats in control group received NS solution or the vehicle solution without drugs. One hour after drug administration, rats in all groups were challenged with 0.1 ml of 1% (w/v) carrageenan in sub-planter region of left hind paw. A zero hour paw volume was measured for the rats using Plethysmometer and vernier callipers before the administration of carrageenan for all groups. The volumes of edema of the carrageenan injected paws were measured at 1 hr interval for 6 hr.

3.2 Anti-Inflammatory Activity

In the present investigation, the *in vivo* anti-inflammatory activity was evaluated for all the newly synthesized compounds HN1-HN20 using the carrageenan-induced rat paw edema protocol. The paw edema volume was evaluated 0, 1, 2, 3 and 4 hrs after the induction of Inflammation.

Table-3 The Anti-inflammatory activity of tested compounds and reference drug (Indomethacin) in carrageenan-induced rat paw edema assay

Compounds	Change in Paw volume after drug administration				
	0 hrs	1 hrs	2 hrs	3 hrs	4hrs
Control	0.15	0.15	0.15	0.15	0.15
SD	0.17	0.16	0.14	0.12	0.11
HN1	0.19	0.18	0.17	0.14	0.12
HN2	0.16	0.16	0.15	0.15	0.14
HN3	0.15	0.15	0.15	0.14	0.13
HN4	0.20	0.19	0.19	0.18	0.15
HN5	0.22	0.20	0.19	0.17	0.15
HN6	0.15	0.14	0.13	0.11	0.10
HN7	0.24	0.22	0.20	0.19	0.18
HN8	0.19	0.18	0.17	0.16	0.15
HN9	0.20	0.18	0.15	0.14	0.12
HN10	0.18	0.17	0.16	0.15	0.12
HN11	0.17	0.15	0.14	0.14	0.12
HN12	0.15	0.14	0.13	0.12	0.11
HN13	0.14	0.13	0.12	0.11	0.10
HN14	0.14	0.12	0.11	0.10	0.09
HN15	0.23	0.22	0.21	0.20	0.20
HN16	0.22	0.21	0.20	0.19	0.18
HN17	0.19	0.18	0.15	0.14	0.13
HN18	0.11	0.10	0.10	0.09	0.09
HN19	0.15	0.14	0.13	0.12	0.11
HN20	0.15	0.15	0.14	0.12	0.11

Table -4 % Inhibition of acute inflammation (carrageenan-induced paw edema)

S.No.	Compound	Differences in Paw(4hr) (mm)	Inhibition Percentage (%)
1	HN1	0.12	88
2	HN2	0.14	86
3	HN3	0.13	87
4	HN4	0.15	85
5	HN5	0.15	90
6	HN6	0.10	82
7	HN7	0.18	85
8	HN8	0.15	88
9	HN9	0.12	88
10	HN10	0.12	88
11	HN11	0.12	89
12	HN12	0.11	90
13	HN13	0.10	91
14	HN14	0.09	80
15	HN15	0.20	82
16	HN16	0.18	87
17	HN17	0.13	91
18	HN18	0.09	89
19	HN19	0.11	89
20	HN20	0.11	89
21	SD	0.11	89

The anti-inflammatory activity of the tested compounds and reference drug (Indomethacin) were determined as the increase in paw edema volume and the results are summarized in Table 3 and as percentage inhibition (% inhibition) are summarized in Table 4.

In general, the data listed in Table 3 indicate that all of the newly synthesized compounds significantly reduce the rat paw edema volume 4 h after administration of the carrageenan compared to the reference drug, Indomethacin.

Compound **HN13** and **HN17** showed best result of 91% inhibition compared to the reference drug, Indomethacin and compound **HN5, HN12** shows 90%, compound **HN18, HN19, HN20** shows 89% inhibition compared to the reference drug, Indomethacin.

On the other hand, compounds **HN1, HN8, HN9** and **HN10** shows 88%, **HN3** and **HN16** shows 87%, **HN4, HN7** shows 85%, **HN6, HN15** shows 82% and **HN1, HN4** shows 80% inhibition, showed a slightly lower anti-inflammatory activity than Indomethacin at 4 h.

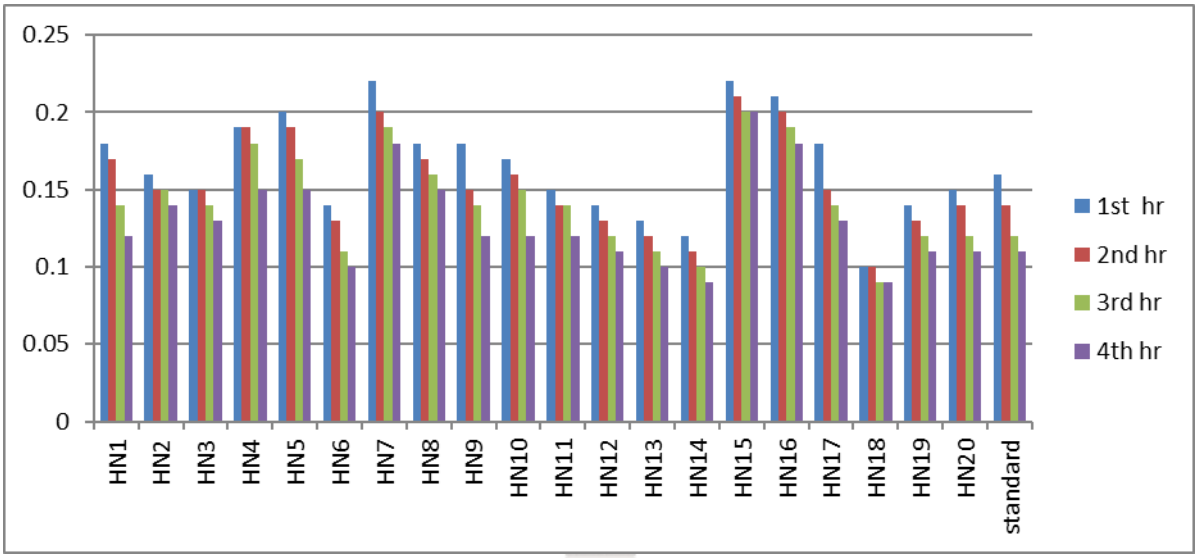


Fig. 2 Comparison of Percentage Inhibition of acute inflammation (carrageenan-induced paw edema)

4. CONCLUSION

In the present investigation, the *in vivo* anti-inflammatory activity was evaluated for all the newly synthesized compounds HN1-HN20 using the carrageenan-induced rat paw edema protocol. The paw edema volume was evaluated 0, 1, 2, 3 and 4 hrs after the induction of Inflammation.

The anti-inflammatory activity of the tested compounds and reference drug (Indomethacin) were determined as the increase in paw edema volume and the results are summarized in Table 3 and as percentage inhibition (% inhibition) are summarized in Table 4.

Furthermore, a molecular docking study of all the designed compound werw carried out to understand the binding interaction between the new compounds with COX-2 enzymes. The results of this study suggest a good binding interaction which explains the significant

biological activity. An ADME property of all the newly designed compounds was studied by QikPropv3.0. All the designed compounds were found to exhibit drug like properties from the calculated ADME properties. These studies indicate that the newly designed analogs may have a good binding affinity with Cyclooxygenase-II Inhibitor and could be used as lead for the development of anti-inflammatory agents.

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