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SYNTHESIS, *IN-VIVO* ANTI-INFLAMMATORY EVALUATION AND MOLECULAR DOCKING STUDY OF A SERIES OF SUBSTITUTED XANTHONE DERIVATIVES AS NOVEL COX-2 INHIBITORS

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Keywords:

Xanthone derivatives, Anti-inflammatory, Carrageenan induced paw edema, Discovery studio version 2.5

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ABSTRACT: A series of 3,6-bis (3′-substituted propoxy) and 3,6-bis (5′-substituted pentyloxy) xanthone derivatives was synthesized by the condensation of 3,6-bis (3′- bromopropoxy) and 3,6-bis (5′-bromopentyloxy) xanthones with primary or secondary amines. These compounds were characterized by FT-IR, ^{1}H NMR, and Mass spectral data. The anti-inflammatory study of the synthesized compounds was carried out using the carrageenan-induced rat paw edema method on Albino rats of Wistar strains. Thirty minutes after injection of 1% carrageenan solution into the sub plantar surface of the left hind paw, synthesized drugs were orally administered at a dose of 100 &200 mg/kg body weight. The compounds S3, S17, and S20 at a dose of 200 mg/kg body weight showed 63.32%,62.75%, and 60.71% paw edema reduction respectively after 6 hours as compared to standard diclofenac (10mg/kg) body weight which showed 68.27% reduction of paw edema after 6 hours. The molecular docking studies were also carried out in the active site of COX-2 enzymes (PDB ID: 1CX2) using Discovery studio version 2.5. The *in-silico* ligand binding interactions of compounds S3, S6, S14, S17, and S20 suggested approx.> 30% higher binding energy values than the standard Diclofenac (Binding energy -155.46 Kcal/mol). A significant correlation was observed between the *in-silico* and the *in-vivo* studies of the synthesized compounds. With the help of anti-inflammatory and docking, the synthesized compounds S3, S17, and S20 could be considered as possible hits as anti-inflammatory agents.

INTRODUCTION: Inflammation is essentially a protective response and the body's way of dealing with infections which involves a complex series of enzyme activation, mediator release, fluid, extra vacations, cell migration, tissue breakdown, and repair [1].

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The clinical use of classical NSAIDs for the treatment of inflammation and pain causes a spectrum of toxic effects, especially gastrointestinal effects [2].

NSAIDs perform by restricting the cyclooxygenase pathway associated with the conversion of arachidonic acid by the enzyme cyclooxygenase (COX) to produce prostaglandins (PGs). Cyclooxygenase (COX) is the major mediator of inflammation, pain, and increased body temperature (hyperpyrexia), two isoforms, namely COX-1 and COX-2. COX-1 is referred to as a "housekeeping enzyme," which maintains the

normal cellular processes such as gastric cytoprotection, vascular homeostasis, platelet aggregation, and kidney function, while COX-2 is an inducible enzyme that is undetectable in most tissues and is primarily expressed by various pro-inflammatory cytokines, lipopolysaccharides, mitogens [3-4]. On the inhibition of COX-1, inflammation is decreased. Still, the protection of the lining of the stomach is also disturbed, which can cause stomach distress as well as ulceration and bleeding from the stomach and even the intestines. There is much less gastric irritation associated with COX-2 inhibition together with the decreased risk of peptic ulceration [5].

The perfect NSAID inhibits the inducible COX-2 isoform (thereby decreasing inflammation) without having any sizeable effect on the constitutive COX-l isoform (thereby minimizing toxicity). Such an agent would increase the effectiveness without causing toxicity, particularly gastroduodenal erosions. Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Aceclofenac and Etoricoxib were the first COX-2 selective NSAID approved by Food and Drug Administration (FDA). Many researchers have recently focused on selective COX-2 inhibitors that have been found to overcome NSAID complications like gastric ulcers, transient rise in liver enzymes, hepatitis, Reye's syndrome, joint destruction (suppress bone repair and remodelling) [6], disturbed sleep pattern [7], augment insulin secretion [8], CNS side effects (headache, aseptic meningitis, drowsiness, altered mood, tinnitus, *etc.*). There is arising urge to develop a new selective COX-2 inhibitor with fewer side effects through a more effective and efficient drug discovery process.

Prostaglandin E_2 (PGE_2) levels are very low or undetectable in the brain's normal conditions, which can arise during inflammatory processes, multiple sclerosis, and AIDS-associated dementia [9]. Elevated levels of PGE_2 can affect the activities of several cell types, including neurons, and endothelial cells, and also regulate microglia/ macrophage and lymphocyte functions during inflammatory and immune processes [10]. There is considerable evidence that relates the production of prostaglandins with inflammation, pain, and fever. Therefore, the interaction between PGE_2 and other local factors, including pro and anti-inflammatory cytokines, influences inflammatory and immune responses in the central nervous system (CNS).

Xanthones are secondary metabolites from higher plants and organisms, and near about one thousand known members of naturally occurring xanthone have different types of substituents in different positions which leads to compounds possessing very diverse biological profiles, including antibacterial, antimalarial, antifungal, anti-inflammatory, anti-hypertensive, antioxidative, antithrombic, anti-cancer and anti-retroviral activity [11]. Chemically xanthones are heterocyclic compounds with dibenzo pyrone framework. So, the synthesis of new xanthone derivatives can help rationalize the relation of structural features versus activity [12]. Recent studies of hydroxy xanthones have shown that successive introduction of hydroxyl groups on the aromatic ring has increased *in-vitro* anti-malarial potency. It was found that xanthones bearing hydroxyl groups at 3 and 6 positions have the highest potency in the hydroxy xanthone series. Incorporation of amine terminated alkoxy side chains at 3 and 6 positions of the xanthone enhances COX-2 inhibitory activity. The presence of alkyl groups with nitrogen atoms may enhance the anti-inflammatory activity. So, the substitution was done only at 3 and 6 positions by propane and pentane side chains with substituted primary and secondary amines.

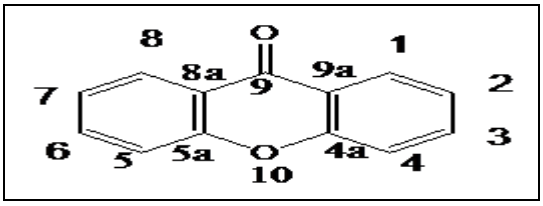


FIG. 1: STRUCTURE OF XANTHONE

A method to investigate a more effective and efficient new drug is using molecular docking. This is a part of molecular modelling that envisions the interaction orientations (conformations) between COX-2 proteins and synthetic derivatives of xanthone precisely, as shown by the formation of a stable complex using the Lib Dock program from Discovery Studio, version 2.5 (Accelrys, San Diego, CA, USA). The most preferred orientation (the best conformer) shows the lowest binding energy and is associated with the strongest interaction. This method allows us to explore and investigate many drugs for the same receptor at the

same time. According to structure-based researching methodology, the feasible prediction of binding sites was calculated, and every molecular docking result was analyzed through the scoring functions, interaction energy, and structural conformation. The drug with a better interaction between a ligand and a receptor will be chosen for use in laboratory experiments, and it saves resources and is less time-consuming [13]. This drug virtual-screening method is universally applied in structure-based drug design and can be performed for interaction modelling of ligands and proteins at an atomic level [14]. In the current study, the energy minimization of the ligands is achieved through Merck Molecular Force Field which has immense importance in reducing the phase time, and Interactions between ligands and receptors, as indicated by their scoring function, are ranked; ligands with the lowest score are investigated further. This step will make molecular docking an important part of more effective and efficient drug discovery, as it saves time and money [15].

Literature survey reveals that xanthone derivatives are compounds known for analgesic and anti-inflammatory activity. Natural xanthones can be isolated from a variety of plants, including *Garcinia mangostan* [16]. *Hypericum perforatum* [17]. *Calophylum inophylum* [18], *Gamboge hanburyi* [19.] and *Artocarpus optusus* [20]. Xanthones have an anti-inflammatory effect by inhibiting COX-2 and prostaglandin synthesis at glioma cell in rats [21]. without affecting the constitutive COX-1 [22]. There are confirmed reports that α-Mangos tin, (Garcinia mangos tana L., "the queen of fruits") firstly isolated in 1855, is a competitive antagonist of the histamine H1 receptor, which possesses biological effects as antibacterial and anti-inflammatory activity [23-24]. The help of computational studies on xanthone and its protein targets will help us in designing compounds that have potential anti-inflammatory activity. Moreover, the replacement of the carboxylic groups by amide groups in NSAID drugs such as indomethacin, meclofenamic acid, and ketoprofen confers the compound with greater selectivity for COX-2 over the COX-1enzyme.

MATERIALS AND METHODS:

Docking Molecular Process: The 3D structure of the protein was downloaded from RCSB (Research Collaboratory for Structural Bioinformatics), Protein Databank (PDB, http://www.pdb.org).The PDB ID of the selected protein complexed with SC-558 was found to be 1CX2 which were used for the docking study. The errors of the proteins were corrected by the structure preparation process in Discovery studio version 2.5 software.

Synthesis: The chemicals used were of analytical grade and obtained from Hi media Lab and Spectroc hem Pvt. Ltd. The completion of the reaction and characterization (Rf value) of the synthesized compounds were checked by thin layer chromatography. The TLC plates were prepared by using silica gel G and visualization of the spots was accomplished by using Iodine vapour lamp and UV light 25. On the basis of Polarity of the products, Mobile phases were selected. Melting point range of the synthesised compounds were determined by Melting point apparatus (Veego Model No.MP-I). The temperature range at which the compound melted was measured by a thermometer as a melting point range. Various solvents of different polarity were taken for dissolving the intermediates and final products.0.5 mg of each compound was weighed and added to 5 ml of solvent and determined the solubility of the intermediates and final products. The wavelengths of maximum absorption of the compounds were recorded on Shimadzu UV-1700 and UV -Vis's spectro-photometer instrument. The FT-IR spectra of the synthesised compounds were recorded on OPUS, Bruker -Alpha FT-IR spectrometer using KBr pellet technique. The H1-NMR spectra of the synthesized compounds were recorded in Me OD at 400.40 MHz by Bruker Avance - II 400 NMR spectrometer and 13C-NMR also recorded in Me OD at 100 MHz by Bruker Avance -II 100 NMR spectrometer. Chemical shifts are expressed as δ values (ppm), downfield from tetramethylsilane (TMS) used as internal standard. The mass spectra of the synthesized compounds were recorded in methanol on Water ZQ-4000 equipped with an Electrospray Ionization technique as an ionization method.

Chemistry: A new series of 3,6 bis (3'-substitutedpropoxy) xanthone & 3,6 bis (5'-substituted pentyloxy) xanthone derivatives has been synthesized. The series starts with the synthesis of 3, 6 dihydroxy xanthone. The two

hydroxyl groups at 3, 6 positions of 3, 6 dihydroxyxanthone were substituted with propane and pentane side chains along with selected amines to obtain the targeted compounds.

Synthesis of 3,6 dihydroxy xanthone: Eaton's reagent was prepared by dissolving phosphorus pentoxide in methane sulphonic acid in 1:10 ratio.100 ml of Eaton's reagent was added slowly to a mixture of Resorcinol (60 mmol) and 2, 4 dihydroxybenzoic acid (60 mmol). The mixture was warmed up to 70 °C for 35 min with stirring. Then the mixture was cooled to room temperature and poured the reaction mixture into ice and stirred for 2 h 30 min [Int-1]. The resulting solid collected by filtration, washed with water until pH 6 and dried at 600C [26].

Synthesis of 3,6-bis (3/-bromopropoxy) xanthone: Potassium carbonate (0.4 mmol) was added to a solution of 3,6-dihydroxyxanthone (0.2 mmol) [Int-2] and 1,3-dibromopropane 0.4(mmol) in acetone. For 24 h the mixture was stirred at room temperature. The reaction mixture was poured in ice water and filtered and extracted with ethyl acetate to obtain a yellow pale solid [27].

Synthesis of 3,6-bis (5/-bromopentyloxy) xanthone: Potassium carbonate (0.4 mmol) was added to a solution of 3,6-dihydroxyxanthone (0.2 mmol) [Int-3] and 1,5-dibromopentane (0.4mmol) in acetone. For 24 h the mixture was stirred at room temperature. The reaction mixture was poured in ice water and filtered and extracted with ethyl acetate to obtain a yellow pale solid [27].

Synthesis of 3, 6-bis(3/-substitutedpropoxy) xanthone: 3,6-bis(3/-bromopropoxy) xanthone (10mmol) and selected amine (20mmol) were dissolved in 20ml of acetone containing (20mmol) potassium carbonate and stirred at room temperature for 48 hours. The end of the reaction was confirmed by TLC with the formation of only one spot using acetone: hexane (2:1). The mixture was filtered and collected as a yellow solid [28].

Synthesis of 3, 6-bis(5/-Substitutedpentyloxy) Xanthone: 3,6-bis(5/-bromopentyloxy) xanthone (10mmol) and selected amine (20mmol) were dissolved in 20ml of acetone containing (20mmol) potassium carbonate and stirred at room temperature for 48 h. The end of the reaction was confirmed by TLC with the formation of only one spot using acetone: hexane (2:1). The mixture was filtered and collected a yellow solid.

Biological Activities:

Ethics Statement: The experiments were performed after approval no. (IAEC/DU/92 dated: 27/3/15) of the protocol by the Institutional Animal Ethics Committee of laboratory animals of the department of pharmaceutical sciences, Dibrugarh University, Assam, India (Regd.No.1576/GO/a/11/ CPCSEA, dated:17/2/2011). All experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals [29].

Acute Toxicity Studies: Toxicological studies of the test compounds as a suspension in 1% Tween 80 were carried out by administering a dose of 100, 200, 500, 1000, and 2000 mg/kg body weight in Male Wistar rats weighing (80-140 g). The control group received 1% tween 80 suspensions. Animals were kept in fasting condition prior to dosing. Following the period of fasting, the animals were weighed, properly marked, and the test substances were administered. After administration of test compounds, food was withheld for a period of 1-2 hours. Fixed-dose OECD guideline No. 423; (Annexure -2d) method of CPCSEA was adopted for toxicity studies. 5 animals (n=5) were used for each step. All the Animals were observed individually after dosing during the first 30 minutes, first 24 h and especially for the first 4 hours of dosing special attention should be given and then daily thereafter, for a total of 14 days. If mortality was seen in 2 out of 3 animals, then the dose administrated was allotted as a toxic dose, and if mortality was seen in one animal, then a similar dose repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 100, 200, 500, 1000, and 2000 mg/kg body weight. Any toxicity sign of gross changes in skin and fur, eyes and mucous membranes, circulatory, respiratory and autonomic, central nervous systems, and behaviour pattern was reported. No serious toxicity signs or death were observed during the whole acute toxicity studies for each compound up to dose level 2000mg/kg body weight.

Anti-Inflammatory Activity:

Carrageenan Induced Rat Paw Edema Method: For the experiment, the male wistar rats (80-140 g) were divided into twelve groups (n=5). The animals were fasted overnight prior to the start of the experiment and water *ad libitum*. The left hind paw was marked just beyond the lateral malleolus, so as to fix a constant level up to which the rat's paw must be dipped in water. Acute inflammation was produced by injecting 0.1ml of Carrageenan 1% suspension in 1% Tween 80 under the planter surface of the left hind paw. During the whole experiment, animals were acclimatized in the animal house and were kept at 22 ± 3°C and 55 ± 5% relative humidity. The control group received normal water (10 ml/kg, p.o.).

The standard group received diclofenac at a dose of 10mg/kg p.o. The test groups received synthesized compounds at doses of 100mg/kg and 200 mg/kg p.o. After 1 hour of the administration of vehicle, synthesized compounds, and standard drug, 0.1 ml of carrageenan suspension in 1% Tween 80 was injected subcutaneously (SC) into the plantar surface of the left hind paw. Hind paw edema volume was measured plethysmographically after an interval of ½, 1, 2, 3, 4 & 6 hours after administration of carrageenan injection and recorded. The significance between the groups was tested by one-way ANOVA followed by Dunnett's t test. The data obtained is expressed as mean standard error of mean (SEM).

% Inhibition = (Mean edema of control group – Mean edema of test) × 100 / Mean edema of control group

RESULTS:

***In-silico* Work:** Protein-ligand docking studies are used to check the structure, position, and orientation of a protein when it interacts with a small molecule like ligand. Binding energy, π-π interactions, hydrogen bond interaction, the orientation of the docked compounds within the active site, and root mean square deviation (RMSD) of active site residues are some parameters to analyze protein-ligand interactions. A total of 28 substituted xanthone compounds were designed and were docked into the binding pocket of the protein 1CX2. Out of 28 designed compounds, five compounds have shown satisfactory binding affinity and binding energies with the protein 1CX2 were considered for further synthesis. Solubility, drug likeliness and drug score are the parameters calculated by Osiris property explorer shown in **Table 2**. The COX-2 binding site possesses an additional 20 pocket that is absent in COX-1, which is highly relevant to the design of selective COX-2 inhibitors. The ligands and their mode of interactions with different residues on the binding sites of the protein 1CX2 during docking simulations are shown in the **Table 3**. To understand the COX-inhibiting behaviour of the designed compounds, automated docking studies were carried out using Discovery studio version 2.5 software. The scoring function and the number of hydrogen bonding formed with the neighbouring amino acids were used to predict their binding modes, binding affinities in the active sites of COX-2 enzyme. The active site of the enzyme was found to include residues within a 10.0 Å radius to any of the inhibitor atoms and the scoring functions for docked compounds were calculated from minimized ligand-protein complexes.

X = Primary or Secondary Amine, n = Number of CH2 groups

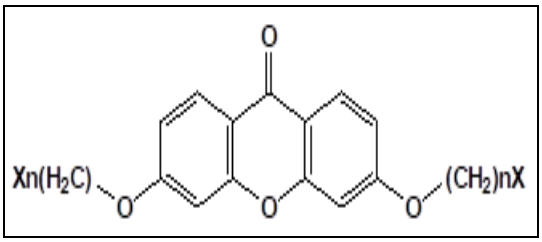


FIG. 2: GENERAL STRUCTURE OF THE PROPOSED COMPOUND

TABLE 1: LIST OF THE DESIGNED COMPOUNDS

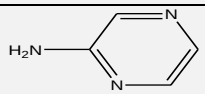
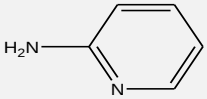
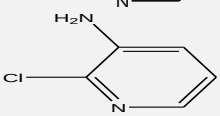
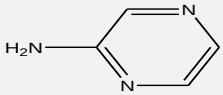
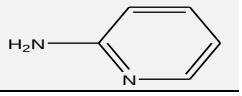
S. no.	Compound Code	No. of CH_2 groups	X ($1^0/2^0$ amine)
1	S3	3	
2	S6	3	
3	S14	3	
4	S17	5	
5	S20	5	

TABLE 2: SOLUBILITY, DRUG SCORE, AND DRUG-LIKENESS ACCOUNTED **BY OSIRIS PROPERTY OF THE SUBSTITUTED XANTHONE DERIVATIVES IS GIVEN IN THE FOLLOWING TABLE**

Molecule code	Solubility	Drug likeness	Drug score
S3	-6.06	2.7	0.41
S6	-7.92	1.87	0.31
S14	-6.86	1.3	0.29
S17	-6.41	1.26	0.35
S20	-7.95	1.53	0.33

TABLE 3: THE SUBSTITUTED XANTHONE DERIVATIVES WITH THEIR MODE OF INTERACTIONS WITH DIFFERENT RESIDUES PRESENT ON THE BINDING SITES OF THE PROTEIN (PDB ID: 1CX2) DURING DOCKING SIMULATIONS

Molecule code	Residues involve in hydrogen bonding interactions	Residues involved in hydrophobic interactions	Binding energy (Kcal/mol)	Centre of active sites & nature of the interaction
S3	Trp387	His214,His386, His207	-270.61	π^+, π^+, π^+(3.73,5.52, 3.91), H-bonding (2.19)
S6	Arg222	His214,His207	-214.21	π^+, π^+, π^+(5.05,4.32,5.21) π-π(5.85),H-bonding (1.98)
S14	Thr206,His207, His214	His386	-205.54	π^+(4.96),H-bonding (1.88,2.38,2.39)
S17	Glu290,Arg222	His207,His214	-234.51	π^+, π^+, π^+ π^+(5.59,5.83,5.19,4.76), H- bonding (2.39,2.02)
S20	His214,His386	His207	-217.80	π^+(5.13), H-bonding(1.79,2.63)

FIG. 3: A RIBBON DIAGRAM OF THE PROTEIN 1CX2 WITH BINDING SITE

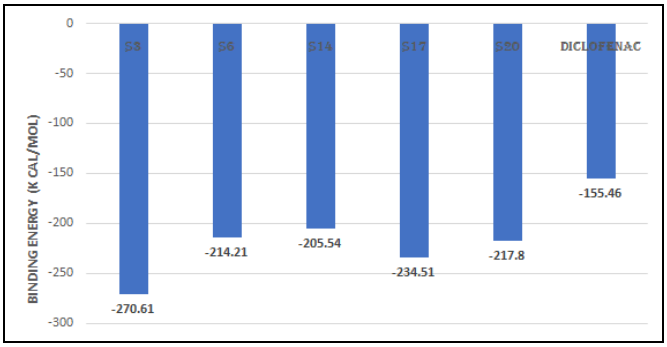


FIG. 4: BINDING ENERGY OF THE LIGANDS AND DICLOFENAC WITH THE PROTEIN 1CX2

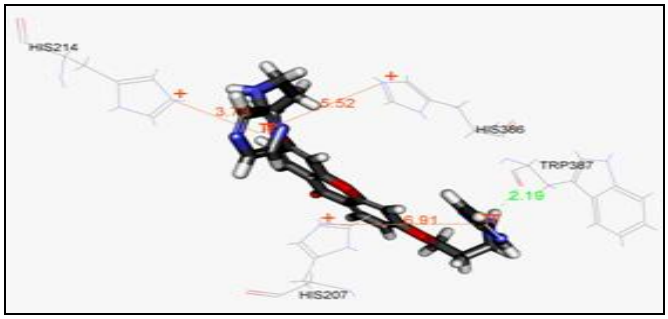


FIG. 4A: S3COMPOUND WITH 1CX2

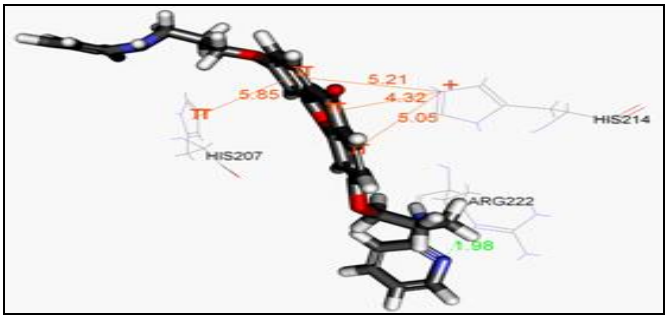


FIG. 4B: S6 COMPOUND WITH 1CX2

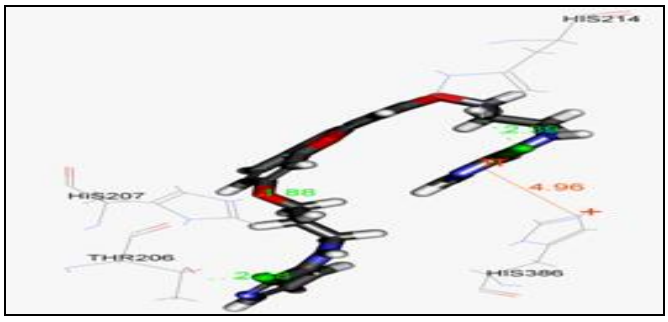


FIG. 4C: S14 COMPOUND WITH 1CX2

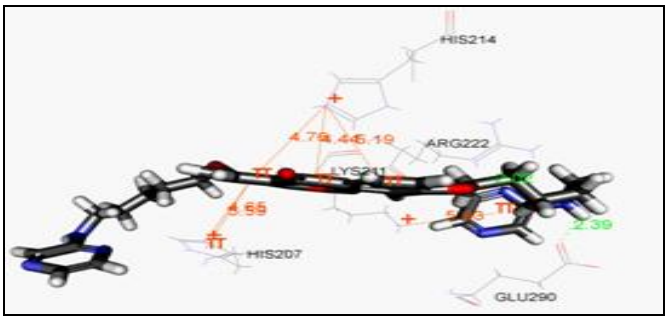


FIG. 4D: S17 COMPOUND WITH 1CX2

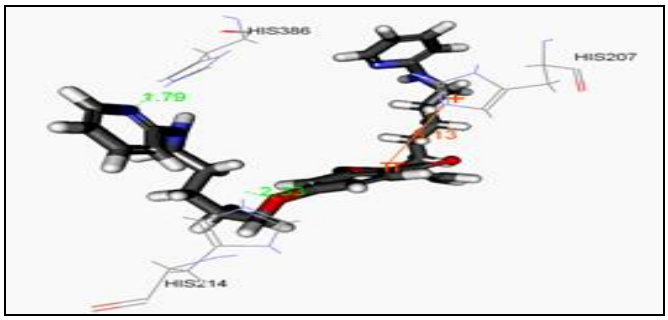


FIG. 4E: S20 COMPOUND WITH 1CX2

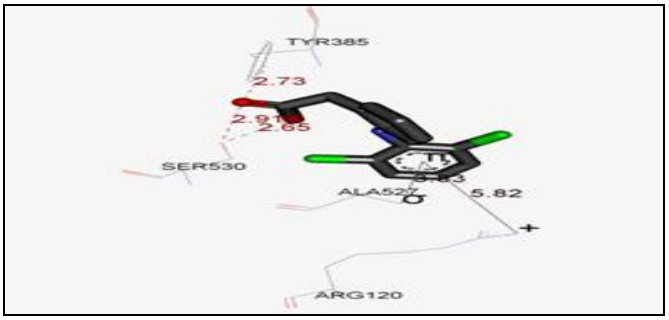


FIG. 4F: DICLOFENAC WITH 1CX2

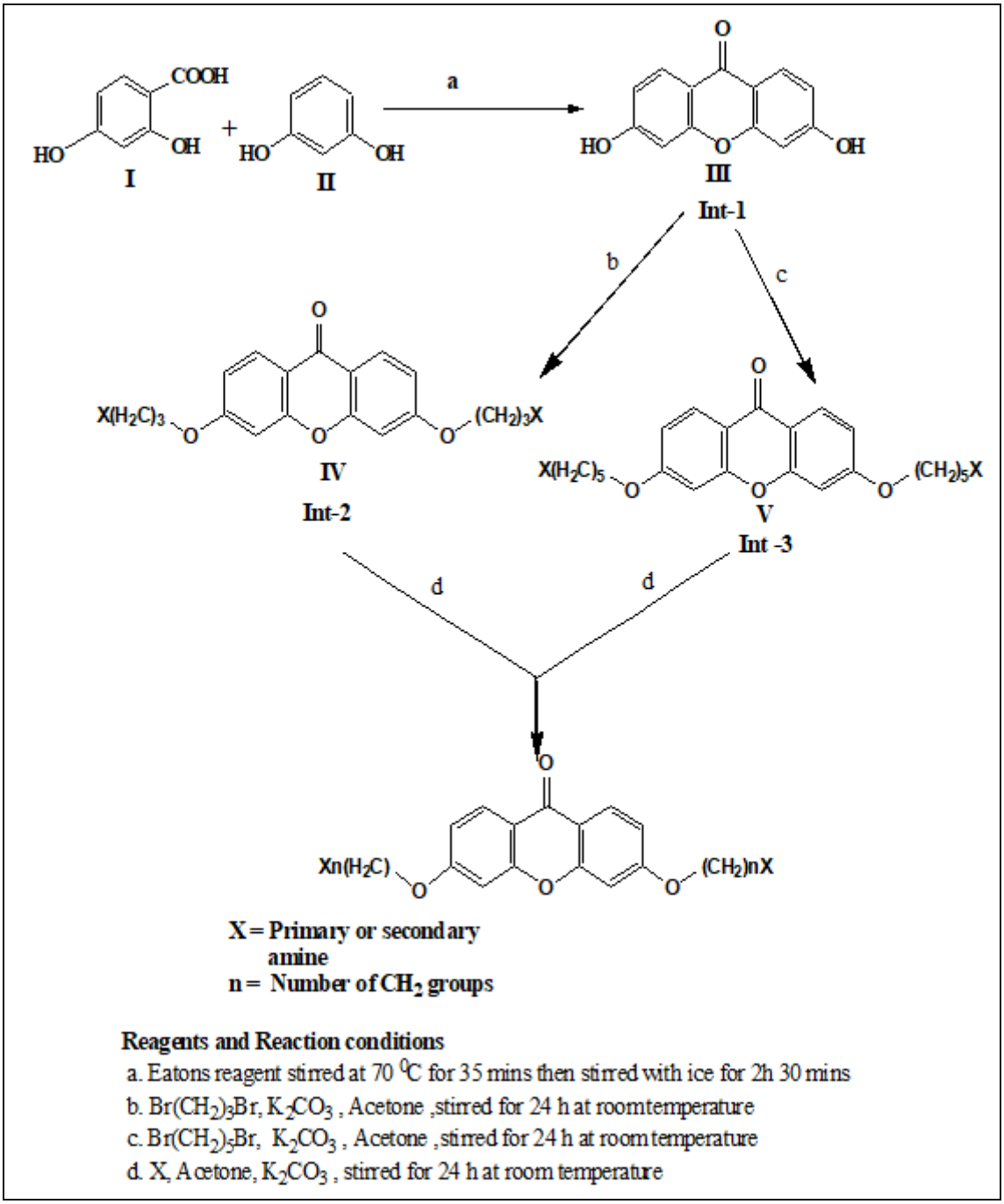
FIG. 4(A-F): INTERACTION OF THE SUBSTITUTED XANTHONE COMPOUNDS AND DICLOFENAC AND WITH THE PROTEIN 1CX2

Binding Site analysis is a rapid detection program required to identify and visualize possible binding sites and 'the distribution of surrounding residues in the active sites. A scoring function that calculates the free energy of binding of the ligand from a given pose can be used to rank the final poses of the ligands, force field evaluation, torsional and rigid body movements of the ligand are quantified for the improvement of the docking results. It has also been studied that the replacement of His513 in COX-1 by Arg222 in COX-2 plays an important role with respect to the H-bonding network in the COX-2 binding site. The selected docked compounds were synthesized in moderate yield and were recrystallized. After molecular docking study, the compounds S3, S6, S14, S17, S20 have shown binding energy greater than Diclofenac with a docking score of -155.46 Kcal/mol. The binding energies of all the active sites were observed, among which the best ligand, which shows better activity in all the active sites, was found to be S3 with a binding energy of -270.61 Kcal/mol. The other substituted xanthones showed good hydrogen-bonding interactions with the residues Trp 387, Arg 222, His214, and hydrophobic interactions with His214, His386 His207.

The results that were obtained on docking proposed that the length of the alkyl chain greatly affects the binding results. Incorporation of primary as well as secondary nitrogen in chemical structure is essential to interact more with residues *via* hydrophobic or hydrophilic or by forming both. The strong binding efficacy has been assumed due to 2-amino pyrazine substitution and incorporation of propoxy side chain in S3 as compared to S17, 3-amino-2-chloropyridine substitution in S14, 2-amino pyridine substitution in S6 and S20.

Binding energy is a versatile tool that helps us understand the ligand's affinity to the binding site present on protein. Five compounds S3, S6, S14, S17 & S20 showed the best and lowest binding energy scores among all of the ligands -270.61, -214.21, -205.54, -234.51, -217.80 kcal/mole, respectively. Furthermore, additional compounds being developed as GI-sparing anti-inflammatory drugs might be of interest. NSAIDs which show markedly reduced renal toxicity acts as dual inhibitors of both cyclooxygenase and 5-lipoxygenase, an enzyme of the arachidonic acid metabolism responsible for leukotriene biosynthesis. This compound is presently undergoing clinical testing and shows a promising pharmacological profile with low GI risks [30]. The docking study performed in our research laboratory clearly indicates the better binding efficacy of xanthone derivatives in comparison to the standard drug diclofenac.

Synthetic Work: After the docking study, five substituted xanthone compounds have been synthesized, which showed greater binding energy, as indicated in **Table 3**. The series starts with the synthesis of the starting compound 3,6 dihydroxyxanthone followed by substitution of the two hydroxyl groups at 3,6 positions of 3,6 dihydroxyxanthone with propane and pentane side chains along with selected amines to obtain the targeted compounds. The compound S17 showed 68.45% yield and melting point range of 241-245 °C which is highest compared to other compounds shown in **Table 4**. The synthesized compounds showed good solubility with the solvents such as ethanol, acetone, ethyl acetate, DMSO. All the synthesized compounds were characterized with UV-Vis's spectrophotometry, FTIR, ^{1}H NMR, Mass spectrometry.



SCHEME 1: REACTION SHOWING THE SYNTHESIS OF THE SUBSTITUTED XANTHONE DERIVATIVE

TABLE 4: PHYSICOCHEMICAL PROPERTIES OF THE SYNTHESIZED COMPOUNDS

S. no.	Compound code	Molecular Weight	Molecular formula	State	Colour	Melting point (°C)	R_f value	% Yield
1	S3	498.53	$C_{27}H_{26}N_6O_4$	Solid	Brownish yellow	230-234°C	0.67	52.45%
2	S6	496.56	$C_{29}H_{28}N_4O_4$	Solid	Light brown	236-241°C	0.72	53.62%
3	S14	565.45	$C_{29}H_{26}Cl_2N_4O_4$	Solid	Brownish yellow	232-234°C	0.68	49.35%
4	S17	554.64	$C_{31}H_{34}N_6O_4$	Solid	Greyish yellow	241-245°C	0.76	68.45%
5	S20	552.66	$C_{33}H_{36}N_4O_4$	Solid	Deep yellow	230-235°C	0.66	63.74%

Spectral Data of the Synthesised Compounds:

Compounds 3: 3, 6-bis(3-(pyrazin-2-ylamino) propoxy)-9H-xanthen-9-one UV λ_{max} (Acetone): 642nm, FTIR (cm^{-1}): 3047.74 (C-Hstr., aromatic); 1560.57 (C-aromatic) Cstr, 1684.27 (C=Ostr., ketone); 1235.87 (C-Ostr., 6-membered cyclic ether); 3421.67 (N-Hstr); 1559.39 (C-Nstr., secamine). 1HNMR (400mHz) DMSOd6, δ(ppm):

6.47,7.25 (CH, benzene), 3.58,1.93 (CH_2, methylene), 4.10(NH, aromatic C-NH), 7.93, 7.95 (CH, 2-pyrazine). MASS (m/z %): 495.31.

Compound S6: 3,6-bis(3-(pyridin-2-ylamino) propoxy)-9H-xanthen-9-one UV λ_{max} (Acetone): 644nm, FTIR (cm^{-1}): 3118.15 (C-Hstr., aromatic); 1515.79 (C-C str, aromatic); 1651.54 (C=Ostr., Ketone); 1240.85 (C-Ostr., 6-membered cyclic ether); 3502.82 (N-H str); 1157.46 (C-N str., sec amine); ^{1}H NMR (400mHz) $DMSOd_{6,}$δ (ppm): 6.587, 7.572 (CH, benzene), 3.892, 2.187 (CH_2, methylene), 4.039 (NH, aromatic C-NH), 6.629, 7.451 (CH_2, 2-pyridine) MASS (m/z %): 492.36

Compound S14: 3, 6-bis(3-(2-chloropyridin-3-ylamino) propoxy)-9H-xanthen-9-one UV λ_{max} (Acetone): 640nm, FTIR(cm^{-1}): 3185.12 (C-Hstr., aromatic); 1562.04 (C-Cstr., aromatic); 1611.92 (C=Ostr., ketone); 1240.13 (C-O.,6-membered cyclic ether); 3362.36 (N-H str); (1163.08 (C-N str., sec amine); ^{1}H NMR (400 MHz) $DMSOd_{6,}$ δ (ppm): 6.576, 6.492 (CH, benzene), 2.253, 3.828 (CH_2, methylene), 4.167 (NH, aromatic C-NH), 8.520, 7.755 (CH, 2-pyridine) MASS (m/z %): 571.45.

Compound S17: 3, 6-bis (5-(pyrazin-2-ylamino) pentyloxy)-9H-xanthen-9-one UV λ_{max} (Acetone): 642nm, FTIR (cm^{-1}): 2925.06 (C-Hstr., aromatic); 1492.67 (C-Cstr., aromatic); 1613.89 (C=Ostr., aromatic); 1242.56 (C-O., 6-membered cyclic ether); 3372.86 (N-H str); 1161.17 (CNstr., secamine); 1HNMR (400mHz) DMSOd6, δ(ppm): 6.369, 6.377 (CH, benzene), 3.527, 1.694 (CH_2, methylene), 4.015 (NH, aromatic C-NH), 7.923, 7.702 (CH, 2- pyridine); MASS (m/z %): 515.31

Compound S20: 3,6-bis (5-(pyridin-2-ylamino) pentyloxy)-9H-xanthen-9-one UV λ_{max} (Acetone): 642nm, FTIR (cm^{-1}): 3095.22 (C-Hstr., aromatic); 1572.57 (C Cstr., aromatic); 1608.84 (C=O str., aromatic); 1236.12 (C-O., 6-membered cyclic ether); 3397.41 (N-Hstr); 1162.61 (C-Nstr., secamine); ^{1}HNMR (400mHz) $DMSOd_{6,}$ δ(ppm): 6.505, 6.688 (CH benzene); 3.773, 1.924 (CH,2-methylene), 4.063 (NH, aromatic C-H); 7.56, 6.63 (CH, 2-pyridine); MASS (m/z %): 560.21.

The compound S3(3,6-bis (3-(pyrazin-2-ylamino) propoxy)-9H-xanthen-9-one) obtained as brownish yellow powder showed IR spectrum absorption band at 1684 cm-1 reminiscent of xanthone carbonyl group. C-O stretching is shown at 1235. [87] cm^{-1}, N-H stretching at 3421.67 cm^{-1}, C-Nstr., sec amine at 1559.39 cm^{-1}. ^{1}H NMR (400mHz) confirms the presence of (CH, benzene) at, δ(ppm): 6.47, 7.25. & (CH, 2-pyrazine) at δ (ppm): 7.93, 7.95. The UV λ_{max} of the compound S6 is 642 nm while m/z % value is 495.31.

The compound S6 (3,6-bis 3-pyridin-2-ylamino) propoxy)-9H-xanthen-9-one) has been obtained as light brown powder and has the IR spectrum absorption band at1651.54 cm^{-1} shows the presence of (C=O), 1240.85 cm^{-1} (C-O stretching), 3502.82 cm^{-1} (N-H stretching), 1157.46 cm^{-1}(C-N stretching sec. amine). The λ_{max} of the compound S6 is found to be 644 nm.^{1}H NMR (400mHz) confirms the presence of (CH, benzene) at δ (ppm): [6]. 587, 7.572, (CH_2, 2-pyridine) at δ (ppm): 6.629, 7.451. The m/z % of compound S6 is found to be 492.36.

The compound S14 (3,6-bis(3-(2-chloropyridin-3-ylamino) propoxy)-9H-xanthen-9-one) obtained as brownish yellow with a UV λ_{max} value of 640 nm. The IR spectrum absorption band shows the presence of C=O stretching at 1611.92 cm^{-1}, C-Hstretching, (aromatic) at 3185.12 cm^{-1}, C-O, (6-membered cyclic ether) at 1240.13 cm^{-1}, N-H stretching at 3362.36 cm^{-1}, C-N stretching (sec amine) at 1163.08 cm^{-1}. ^{1}H NMR (400 MHz) DMSOd [6] shows theδ (ppm) values: 6.576, 6.492 (CH, benzene), 2.253, 3.828 (CH_2, methylene), 4.167(NH, aromatic C-NH), 8.520, 7.755 (CH, 2-pyridine). The m/z % of the compound S14 is 571.45. All the spectral data of the compounds S3, S6 & S14 with substituents 2-amino pyrazine,2-amino pyridine, and 3-amino-2-chloro pyridine have been discussed, which brings to our knowledge that these synthesized compounds have not been encountered before as anti-inflammatory agents.

Evaluation of Anti-Inflammatory Activity by Carrageenan Induced Rat Paw Oedema Method: In this experiment, 12 groups of animals (n=5) were considered, and the anti-inflammatory activities of the synthesized compounds were compared with the standard drug diclofenac. The synthesized xanthone compounds (S3, S6, S14, S17 & S20) received doses of 100 mg/kg & 200 mg/kg and the standard drug diclofenac with a dose of 10

mg/kg. The control group received normal water (10 ml/kg p.o). The percentage inhibition of edema of the synthesized compounds together with the standard drug diclofenac is shown in **Fig. 5** and **Fig. 6.** In table 5, the hind paw edema volume was measured plethysmographically at an interval of ½, 1, 2, 3, 4 & 6 h after administration of carrageenan injection.The groups are arranged as Group 1: Control (normal water), Group 2: Diclofenac 10 mg/kg, Group 3: S3100 mg/kg, Group 4: S3200 mg/kg, Group 5: S6 100 mg/kg, Group 6: S6 200 mg/kg, Group 7: S14100 mg/kg, Group 8: S14 200 mg/kg, Group 9: S17 100 mg/kg, Group 10: S17 200 mg/kg, Group 11: S20 100 mg/kg, Group 12: S20 200 mg/kg. In wistar rats, oral administration of the synthesized xanthone compounds at a maximum dose of 2000 mg/kg did not produce any signs of toxicity, and no animals died upto three days. It showed that synthesized xanthone compounds were nontoxic in rats up to an oral dose of 2000 mg/kg body weight. Therefore, an investigation of inflammatory activity was carried out using 100 and 200 mg/kg dose levels.

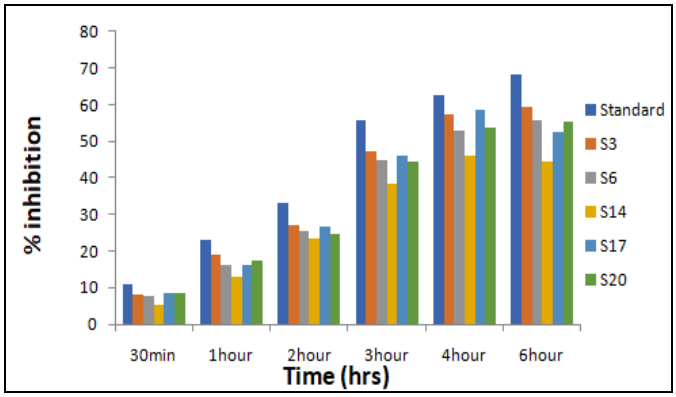


FIG. 5: ANTI-INFLAMMATORY ACTIVITY OF THE SYNTHESISED XANTHONE COMPOUNDS WITH THE STANDARD DRUG DICLOFENAC SODIUM (DOSE 100mg/kg BODY WEIGHT)

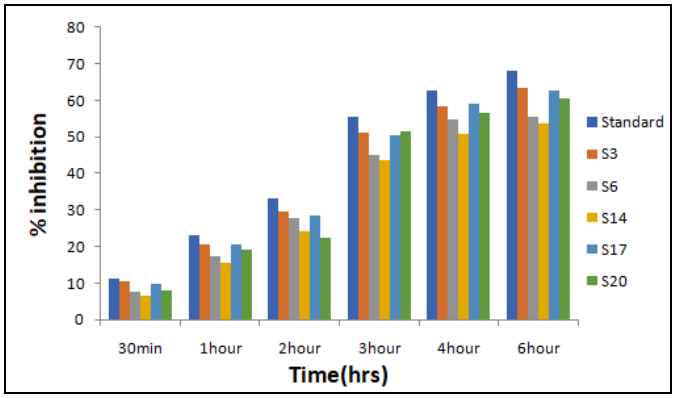


FIG. 6: ANTI-INFLAMMATORY ACTIVITY OF THE SYNTHESISED XANTHONE COMPOUNDS WITH THE STANDARD DRUG DICLOFENAC SODIUM (DOSE 200mg/kg BODY WEIGHT)

TABLE 5: ANTI-INFLAMMATORY ACTIVITY OF THE SYNTHESIZED COMPOUNDS WITH DICLOFENAC AS THE STANDARD

Group	Dose (mg/kg)	Volume displaced in ml					
		30min	1hour	2hour	3hour	4hour	6hour
Control	-	0.835 ± 0.015	0.812 ± 0.031	0.783± 0.021	0.776± 0.0.16	0.769 ± 0.014	0.722± 0.012
Diclofenac	10mg/kg	0.742 ± 0.012 (11.13%)	0.623 ± 0.15* (23.20%)	0.527 ± 0.021* (33.15%)	0.345± 0.016** (55.62%)	0.286 ± 0.022** (62.84 %)	0.245 ± 0.034** (68.27 %)
S3	100mg/kg	0.769 ± 0.013* (7.94%)	0.687 ± 0.023** (18.80%)	0.586 ± 0.012* (27.28%)	0.426 ± 0.025* (47.13%)	0.357 ± 0.016* (57.54%)	0.326 ± 0.022* (59.26%)
	200mg/kg	0.766± 0.017 (10.54%)	0.678± 0.021* (20.72%)	0.574± 0.011 (29.65%)	0.441± 0.027* (51.21%)	0.424± 0.019* (58.35%)	0.388± 0.015* (63.32%)
S6	100mg/kg	0.746± 0.031* (7.64%)	0.643± 0.032** (16.82%)	0.569 ± 0.063* (25.36%)	0.397± 0.019* (44.80%)	0.356± 0.028** (52.84%)	0.321± 0.016* (55.75%)
	200mg/kg	0.763 ± 0.022* (7.54%)	0.641± 0.028* (17.25%)	0.554± 0.018 (27.62%)	0.386± 0.021* (45.16%)	0.347± 0.031* (54.82%)	0.287± 0.015* (55.46%)
S14	100mg/kg	0.774± 0.018** (5.36%)	0.682± 0.033** (13.12%)	0.567± 0.029** (23.46%)	0.355± 0.047** (38.25%)	0.295± 0.023** (46.16%)	0.273± 0.044** (44.63%)
	200mg/kg	0.767± 0.037** (6.54%)	0.643± 0.025 (15.48%)	0.564± 0.031 (24.13%)	0.425± 0.066** (43.75%)	0.352± 0.011* (50.86%)	0.320± 0.015** (53.63%)
S17	100mg/kg	0.772±	0.683±	0.549±	0.423±	0.354±	0.318± 0.036**

		0.025* (8.45%)	0.040** (16.32%)	0.022** (26.86%)	0.039* (46.24%)	0.013* (58.50%)	(52.56%)
	200mg/kg	0.784± 0.054 (9.76%)	0.576± 0.022* (20.44%)	0.560± 0.029 (28.67%)	0.410± 0.019 (50.58%)	0.361± 0.046* (59.23%)	0.328± 0.034 (62.75%)
S20	100mg/kg	0.682± 0.019 (8.36%)	0.644± 0.037** (17.45%)	0.557± 0.038* (24.76%)	0.391± 0.30* (44.63%)	0.346± 0.018** (53.75%)	0.289± 0.027** (55.25%)
	200mg/kg	0.713± 0.015* (8.12%)	0.585± 0.021* (19.15%)	0.551± 0.016* (22.53%)	0.376± 0.026 (51.66%)	0.342± 0.013* (56.44%)	0.264± 0.025* (60.71%)

All the values are given as Mean ± SEM (n=5), Standard *vs*. all group (** p<0.01, * p< 0.05). Values given in parentheses represent the percentage of inhibition.

The anti-inflammatory activity of synthesized compounds against acute paw edema induced by Carrageenan is shown in **Table 5,** and the results are comparable to that of the standard drug diclofenac, a potent inhibitor of the prostaglandins. Carrageenan-induced paw edema remained even 6 h after its injection into the sub plantar region of the rat paw. Diclofenac inhibited the edema formation due to Carrageenan to the extent of 0.245 ±0.034 (at 6 h) at the dose of 10 mg/kg body weight. Compound S3showed dose-dependent suppression of paw edema formation at 100 and 200 mg/kg with the percentage inhibition of 59.26 % (p<0.05) and 63.32% (p<0.05) respectively. Further, the highest dose of compound S17and S20 (200 mg/kg) also significantly suppressed the edema formation with the percentage inhibition 62.75% and 60.71%, respectively shown in **Table 5**. No significant action of compounds to reduce carrageenan-induced paw edema was observed at the first two hour. Compared to the control group, known anti-inflammatory agent diclofenac at a dose of 10 mg/kg showed a significant reduction of hind paw edema at 4 h and 6 h with the percentage inhibition 62.84% and 68.27%, respectively. The results obtained clearly indicates that compound S3has appeared to be effective anti-inflammatory agents than other synthesized xanthone compounds. The inflammatory response induced by carrageenan is characterized by a biphasic response with marked edema formation resulting from the rapid production of several inflammatory mediators [31-32]. Early phase is attributed to a release of histamine, serotonin, and bradykinin, whereas late phaseis due to the over-production of prostaglandin and nitric oxide with a peak at 3h, produced by inducible isoforms of COX (COX-2) and nitric oxide synthase (iNOS) [33]. According to **Table 5,** compound S3significantly reduced paw edema at 4 h and 6 h after carrageenan injection but was less effective at 1 h and 2 h. This evidence allows us to suggest that the compounds S3, S17 & S20 acted in the second phase of the inflammatory response. The mechanism underlying the anti-inflammatory activity of the compound S3, S17 & S20 may therefore involve inhibition of the release of inflammatory mediators, such as prostaglandins and nitric oxide.

CONCLUSION: The results of the study concluded that the compound has secondary nitrogen as a substituent of the series; 3, 6-bis (3'-substituted propoxy) xanthone & 3,6-bis (5'-substituted pentyloxy) xanthone derivatives are the potential key approach to design novel anti-inflammatory agents. A molecular docking study showed that if substitution is done by substituted pyridine and pyrazine derivatives, then it exhibited better binding efficacy. Synthesis and study of acute toxicity, the anti-inflammatory activity of xanthone derivatives in rats, have been carried out successfully. Animal activity result indicates that the synthesized compounds possess significant anti-inflammatory activity at a dose of 200 mg/kg while at a dose level of 100 mg/kg shows less activity. All the synthesized compounds have shown significant activity as compared to the standard drug Diclofenac. Orally administered doses of 100 mg/kg body weight of the compounds S3, S6, S14, S17 & S20 produced 47.13%, 44.80%, 38.25%, 46.24%, 44.63% inhibition respectively after 3 h as compared to diclofenac (standard) 10 mg/kg body weight which showed 55.62% inhibition after 3 h. Moreover, orally administered doses of 200 mg/kg body weight of the compounds S3, S6, S14, S17 & S20 produced 63.32%, 55.46%, 53.63%, 62.75% & 60.71% inhibition respectively after 6 h as compared to diclofenac (standard)

10mg/kg body weight which showed 68.27% inhibition (6 h). The operational simplicity, good yield in significantly short reaction times can impose this procedure as a useful and attractive alternative to the currently available anti-inflammatory drugs. With an objective to investigate a compound for toxicity risks evaluated with the help of Osiris property explorer, we have found that most of the compounds exhibited less side effects. In light of the above observation, xanthone derivatives can lead to the development of potential COX-2 inhibitors, which shows excellent correlation between docking results, synthetic data, and *in-vivo* anti-inflammatory activity.

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