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Design, molecular docking, synthesis and evaluation of some novel heterocyclic analogues of diclofenac as potent analgesic and anti-inflammatory agents with less ulcerogenicity

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ABSTRACT

Diclofenac sodium is being used for the treatment of pain and inflammation. But as all the NSAIDs are suffering from severe GI toxicities, Diclofenac sodium is also not an exception to these toxocities. The GI toxicity associated with all traditional NSAIDs is mainly due to the presence of free carboxylic group. In the present research work, the main motto was to develop some new heterocyclic analogues of diclofenac as potential analgesic and anti-inflammatory agents with negligible ulcerogenicity. In this paper various heterocyclic analogues of diclofenac were designed by replacing the free –COOH with some less acidic heterocycles and evaluated for COX-1, COX-2 and LTA_4 hydrolase binding ability by molecular docking. The most potent ligands were selected as the lead and structural modifications were done to the selected lead compounds in order to increase the potency. Finally these potent ligands were synthesized and characterized by FTIR and ^{1}HNMR. Furthermore the synthesized compounds were also tested for their analgesic, anti-inflammatory and ulcerogenicity activities. Out of all the synthesized compounds, 6 new compounds were found to have significant analgesic and anti-inflammatory activities with negligible ulcerogenicity.

Keywords: Molecular Docking • Antiinflammatory activity • Analgesic activity • Acute Ulcerogenicity

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of inflammation, especially arthritis.[1] Among the most popular NSAIDs worth mentioning is diclofenac sodium, which is approved in more than 120 countries across the globe since its introduction, 28 years ago, and is ranked 30th among the top 200 drugs with respect to new prescriptions.[2]In a simplified term the inflammation process can be considered as an event of the immune response through which tissue damage occurs. The latter is accompanied by the release of several biochemical mediators such as histamine, bradykinin, platelet-activating factor, and a group of lipid material known as leukotrienes (LTs) and prostaglandins (PGs). [3] The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme prostaglandin endoperoxidase, popularly known as cyclo-oxygenase (COX).It was discovered that COX exists in two isoforms, COX-1 and COX-2, which are regulated and expressed differently. COX-1 provides cytoprotection in the gastrointestinal tract (GIT), whereas inducibleCOX-2 selectively mediates inflammatory signals. Since most of the currently available NSAIDs in the market show greater selectivity for COX-1 than COX-2, chronic use of NSAIDs, including diclofenac, may elicit appreciable GI irritation, bleeding and ulceration. The

incidences of clinically significant GI side effects due to long term use of NSAIDs are very high (30%) and cause some patients to abandon NSAID therapy.GI damage from NSAID is generally attributed to two factors. Local irritation by the direct contact of carboxylic acid (–COOH) moiety of NSAID with GI mucosal cells (topical effect) and decreased tissue prostaglandin production in tissues which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homoeostasis.Synthetic approaches based upon chemical modification of NSAIDs have been taken with the aim of improving safety profile and in turn therapeutic window of this NSAID. Several studies have described the derivatization of the carboxylate function of representative NSAID with less acidic heterocycles, viz. 1,3,4-oxadiazole,Triazole, etc. which resulted in an increased anti-inflammatory activity with reduced ulcerogenicity. Furthermore, it has been reported in the literature that certain compounds bearing 1, 3, 4-oxadiazole nucleus possess significant anti-inflammatory activity.[2] In our attempt to discover new, safer and potent agents for treatment of inflammatory diseases, we have designed some new heterocyclic analogues of diclofenac by replacing thecarboxylic acid group of diclofenac acid with less acidic heterocycle, Moreover the designed compounds were evaluated for COX-1, COX-2, LTA_4 hydrolase (since LTs also act as an important mediators for inflammation) binding ability to identify the new lead compounds by molecular docking. The most potent compounds selected as lead on which I carried out structural modification inorder to obtain new ligands with excellent binding ability. These ligands were synthesized and tested for their analgesic, anti-inflammatory and ulcerogenicity activities. Out of all the synthesized compounds, 6 new compounds showed significant analgesic and anti-inflammatory activity with negligible ulcerogenicity.

MATERIALS AND METHODS

Experimental protocols

4.1 Molecular docking

Arguslab.exe was used to perform molecular docking studies. The protein sequences for Cyclooxygenase-1, Cyclooxygenase-2, Leukotriene-A4-hydrolase (PDB code: 1Q4G [4], 6COX [5], 2R59 [6] respectively) were taken from Protein Data Bank (www.rcsb.org/pdb) and edited by removing the hetero atoms. CAST P (Computed Atlas of surface Topography of Protein) server was used to cross check the active pockets on target protein molecules. All the ligand molecules were designed and the structure was analyzed using Chemoffice2004 software. Pymol software was used to view the structure.

4.2 Synthesis

Melting points of the compound were determined by open tube capillary method with the help of VEEGO-VMPT instrument using silicon oil bath. FT-IR spectra of the powdered compound were recorded using Perkin elmer instrument. 1HNMR spectra of the synthesized compound were recorded using Bruker –Ultra shield (300 MHz).

Hydrolysis of diclofenac sodium to 2-[(2, 6-dichloroanilino) phenyl] acetic acid (1)

Diclofenac sodium (0.101 mol) was dissolved in ethanol (2.5 mol). To this solution conc.H2SO4 was added dropwise to hydrolyse the salt to acid. The acid obtained was filtered, dried. Yield: 96.34%, mp: 150-154°C. IR (KBr), *v*, cm-1: 3100-3000 (CH), 1693.38 (C=O), 3456.20 (O-H), 1417.58 (C-O-H), 3250 (N-H), 1321.15 (C-N), 2921.96(CH_2), 441.67 (C-Cl). 1H NMR (400 MHz, CDCl3, TMS): δ 3.852(s, 2H, H-16), 6.974-7.010(m, 4H, H-11, 12, 13, 14), 7.122-7.171(t, 3H, H-4, 5, 15), 7.260(s, 1H, H-2).

Synthesis of ethyl-[2-(2, 6-dichloroanilino) phenyl] acetate (2)

0.05 mol of 2-[(2, 6-dichloroanilino) phenyl] acetic acid (1) was dissolved in absolute ethanol (10ml), conc. H2SO4 (1ml) was added and the reaction mixture was refluxed for 22 hrs. Reaction mixture gave on processing ethyl ester (2). The solid obtained was washed with 50 ml of sodium bicarbonate solution (10%) and recrystallized from methanol. Yield: 95.14%, mp: 126-130°C. IR (KBr), *v*, cm-1: 3085.89-3029.96 (CH), 1731.96 (C=O), 1238.21(-O-C=O), 3448.49 (N-H), 1309.58 (C-N), 2947.03(–CH_2-), 453.24 (C-Cl). 1H NMR (400 MHz, CDCl3, TMS): δ 3.85 (s, 2H, H-16), 7.186-7.271(m, 4H, H-11, 12, 13, 14), 7.077-7.127(m, 3H, H-4, 5, 15), 7.501(s, 1H, H-2), 3.50(s, 2H, CH-16), 1.25 (s, 3H, H-20).

Synthesis of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (3)

Compound 2 (0.01 mol) and hydrazine hydrate (0.02 mol) were refluxed in absolute ethanol (50ml) for 20 hrs. The mixture was concentrated, cooled and poured in ice cold water. The solid thus precipitated out was filtered, dried and recrystallized from ethanol. Yield: 86.48%, mp: 136-140°C. IR (KBr), *v*, cm-1: 3050 (CH), 1454.23 (C=C1), 730.03(C=O), 3450.41, 3325.05 (N-H (Primary)), 1292.22(C-N), 1454.23(-CH_2-), 451.31 (C-Cl). 1H NMR (400 MHz, CDCl3, TMS): δ 3.64(s, 2H, H-16), 6.936-6.998(m, 4H, H-11, 12, 13, 14), 7.100-7.149 (m, 3H, H-4, 5, 6), 7.497(s, 1H, H-8), 3.806(d, 2H,H-20), 7.524(s, 1H, H-19).

Synthesis of 1-({2-[(2, 6-dichlorophenyl) amino] phenyl} acetyl)tetrahydro pyridazine-3,6-dione(4)

A mixture of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (3) (0.001 mol), succinic anhydride (0.001 mol) in 5ml absolute ethanol and glacial acetic acid (0.005 mol) was refluxed for 3 hrs. The reaction mixture was cooled and poured into crushed ice. The solid obtained was filtered, washed with 50 ml of sodium bicarbonate solution (10%) and recrystallized from ethanol. Yield: 81.22%, mp: 122-124°C. IR (KBr), *v*, cm-1: 3060 (CH), 1566.09 (C=C), 1731.96 (C=O), 3446.56 (N-H), 1298.00 (C-N), 2920.03(–CH_2-), 451.31 (C-Cl). 1H NMR (400 MHz, CDCl3, TMS): δ 3.786 (s, 2H, H-11), 7.188-7.268 (m, 4H, H-14, 15, 16, 17), 7.062-7.131(m, 3H, H-21, 22, 23), 7.499 (s, 1H, H-18), 7.507 (s, 1H, H-1).

Synthesis of 1-({2-[(2, 6-dichlorophenyl) amino] phenyl} acetyl) 1, 2-dihydro pyridazine-3, 6-dione (5)

A mixture of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (3) (0.001 mol), maleic anhydride (0.001 mol) in 5ml absolute ethanol and glacial acetic acid (0.005 mol) was refluxed for 3 hrs. The reaction mixture was cooled and poured into crushed ice. The solid obtained was filtered, washed with 50 ml of sodium bicarbonate solution (10%) and recrystallized from ethanol. Yield: 70.53 %, mp: 116-120°C. IR (KBr), *v*, cm-1: 3050 (CH), 1490.87 (C=C), 1731.96 (C=O), 3450.41 (N-H), 1298.00 (C-N), 2918.10(–CH_2-), 451.31 (C-Cl). 1H NMR (400 MHz, CDCl3, TMS): δ 3.783 (s, 2H, H-4), 7.181-7.262 (m, 4H, H-14, 15, 16, 17), 7.073-7.122 (m, 3H, H-21, 22, 23), 7.498 (s, 1H, H-18), 7.519 (s, 1H, H-1), 4.794(s, 2H, H-11).

Synthesis of 2-({2-[(2,6-dichlorophenyl)amino]phenyl}acetyl)-2,3-dihydrophthalazine-1,4-dione(6)

A mixture of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (3) (0.001 mol), phthalic anhydride (0.001 mol) in 5ml absolute ethanol and glacial acetic acid (0.005 mol) was refluxed for 3 hrs. The reaction mixture was cooled and poured into crushed ice. The solid obtained was filtered, washed with 50 ml of sodium bicarbonate solution (10%) and recrystallized from ethanol. Yield: 87.44%, mp: 110-112°C. IR (KBr), *v*, cm-1: 3062.75 (CH), 1568.02 (C=C), 1728.1 (C=O), 3450.41 (N-H), 1294.15 (C-N), 2920.03(–CH_2-), 449.38 (C-Cl). 1H NMR (400 MHz, CDCl3, TMS): δ 3.783 (s, 2H, H-15), 7.17-7.259 (m, 4H, H-18, 19, 20, 21), 7.071-7.120 (m, 3H, H-25, 26, 27), 7.495 (s, 1H, H-22), 7.521(s, 1H, H-1), 7.328-7.398 (m, 4H,H-7, 8, 9, 10).

Synthesis of 3-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 6-dihydro-1, 2, 4-triazin-5(2H)-one (7)

To compound (3) (0.001 mol), 2-chloroacetamide (0.001 mol) and dimethylformamide(80 ml) were added and the reaction mixture was refluxed for 30 hrs. It was then concentrated and cooled, whereupon the solid precipitated was filtered, washed with ethanol and recrystallized from DMF. Yield: 79.54%, mp: 96-100°C. IR (KBr), *v*, cm-1: 3072.39 (CH), 1581.52 (C=C), 1731.96 (C=O), 3448.49 (N-H), 1296.08 (C-N), 2916.17 (–CH_2-), 451.31 (C-Cl), 1H NMR (400 MHz, CDCl3, TMS): δ 3.805 (s, 2H, H-6), 7.073-7.150 (m, 4H, H-10, 11, 12, 13), 6.953-7.003 (m, 3H, H-18, 19, 20), 7.496 (s, 1H, H-15), 3.784 (d, 2H,H-7).

Synthesis of 5-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 3, 4-oxadiazol-2-amine (8)

To an ethanolic solution of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (3) (0.001 mol), cyanogen bromide (0.001 mol) was added. The reaction mixture was stirred with heating at 55-56º C for 2hrs. The resulting solution was cooled and it was neutralized with sodium bicarbonate (10%) solution. The solid thus precipitated was filtered, washed with water, dried and recrystallized from methanol. Yield: 89.42%, mp: 120-124°C. IR (KBr), *v*, cm-1: 3029.96 (CH), 1568.02 (C=C), 1731.96(C=O), 3350.12 (N-H), 1298.00 (C-N), 2948.96 (–CH_2-), 453.24 (C-Cl), 1095.49 (C-O-C). 1H NMR (400 MHz, CDCl3, TMS): δ 7.334-7.405 (m, 4H, H-10, 11, 12, 13), 7.076-7.136 (m, 3H, H-17,18, 19), 7.527 (s, 1H, H-14), 1.661 (s, 2H,H-7), 3.482 (s, 2H,H-6).

Synthesis of 5-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 3, 4-oxadiazole-2-thiol (9)

A mixture of 0.001 mol of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (3), KOH (0.001 mol) and carbon disulphide (5 ml) in ethanol (50 ml) was refluxed on a steam bath for 12 hrs. The solution was then concentrated, cooled and acidified with dilute HCl. The solid thus precipitated was filtered, washed with ethanol, dried and recrystallized from ethanol. Yield: 64.18%, mp: 126-130°C. IR (KBr), *v*, cm-1: 3060.82 (CH), 1479.3 (C=C), 2362.64 (S-H), 3348.19 (N-H), 1191.93 (C-N), 2920.03 (–CH_2-), 439.74 (C-Cl). 1H NMR (400 MHz, CDCl3, TMS): δ 7.320-7.390 (m, 4H, H-10, 11, 12, 13), 6.553-6.604 (m, 3H, H-17, 18, 19), 7.644 (s, 1H, H-14), 1.592 (s, 2H, H-7).

Synthesis of N-(2-{[5-(substituted aryl)-1, 3, 4-oxadiazol-2-yl] methyl} phenyl)-2, 6-dichloroaniline (10-13)

0.001 mol of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (3), and appropriate aromatic acid (0.001 mol) was dissolved in phosphorus oxychloride and refluxed for 8 hrs. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus precipitated was filtered, washed with water, dried and recrystallized from ethanol.

2, 6-dichloro-N-(2- {[5- (4-nitrophenyl) -1, 3, 4-oxadiazol-2-yl] methyl} phenyl) aniline (10)
Yield: 76.28%, mp: 107-111°C. IR (KBr), *v*, cm-1: 1473.51 (C=C), 3434.98 (N-H), 1305.72 (C-N), 783.05 (–CH_2-), 509.17 (C-Cl), 1523.66 (N=O), 1008.70 (C-O-C). 1H NMR (400 MHz, CDCl3, TMS): δ 7.499-7.568 (m, 4H, H-9, 10, 11, 12), 7.400-7.454 (m, 3H, H-22, 23, 24), 6.681-6.724 (m, 4H, H-14, 15, 18, 17), 3.788 (s, 1H, H-9), 1.256 (s, 2H, H-6).

N-(2-{[5-(4-aminophenyl)-1, 3, 4-oxadiazol-2-yl] methyl} phenyl)-2, 6-dichloroaniline (11)
Yield: 71.55%, mp: 168-172°C. IR (KBr), *v*, cm-1: 3000 (CH), 1467.73 (C=C), 3429.2 (N-H), 1311.50 (C-N), 786.90 (–CH_2-), 497.60 (C-Cl), 1012.56 (C-O-C). 1H NMR(400 MHz, CDCl3, TMS): δ 7.420-7.604 (m, 4H, H-9, 10, 11, 12), 7.215-7.337 (m, 3H, H-22, 23, 24), 6.682-6.755 (m, 4H, H-14, 15, 18, 17), 3.706 (s, 1H, H-19), 1.255 (s, 2H, H-6), 3.429 (s, 2H, H-26).

2-(5-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 3, 4-oxadiazol-2-yl) phenol (12)
Yield: 82.74%, mp: 140-144°C. IR (KBr), *v*, cm-1: 1195.78 (CH), 1568.02 (C=C), 1610.45 (N-H), 1309.58 (C-N), 786.90 (–CH_2-), 493.74 (C-Cl), 1010.63 (C-O-C), 3411.84 (-O-H). 1H NMR (400 MHz, CDCl3, TMS): δ 7.488 (m, 4H, H-9, 10, 11, 12), 7.245 (m, 3H, H-22, 23, 24), 6.421 (m, 4H, H- 15, 16, 17, 18), 3.704 (s, 1H, H-19), 1.249 (s, 2H, H-6), 8.492 (s,1H, H-26).

2,6-dichloro-N-(2-{[5-(3, 5-dimethoxyphenyl) -1, 3, 4-oxadiazol-2-yl] methyl} phenyl) aniline(13)
Yield: 87.42%, mp: 233-237°C. IR (KBr), *v*, cm-1: 1469.66 (C=C), 1600.81 (N-H), 1307.65 (C-N), 2933.53 (–CH_2-), 495.67 (C-Cl). 1H NMR (400 MHz, CDCl3, TMS): δ 7.58 (m, 4H, H-9, 10, 11, 12), 7.325 (m, 3H, H-22, 23, 24), 6.704 (m, 3H, H-14, 18, 16), 2.232 (s, 1H, H-9), 1.259 (s,2H, H-6), 3.890 (s, 3H, H-26, 27).

5. Pharmacology

5.1 Animals

Swiss albino mice of either sex weighing 20–25 g and Wistar rats weighing in the range 100–120 g were used for pharmacological screening. All the animals were housed under standard ambient conditions of temperature (25 ± 2 °C) and relative humidity of 50 ± 5%. A 12:12 h light: dark cycle was maintained. All the animals were allowed to have free access to water and standard palletized laboratory animal diet 24 h prior to pharmacological studies. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of College, registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

5.2 Preparation of test compounds

After suspending the test compounds in 1.0% aqueous solution of sodium carboxymethyl cellulose (CMC), test samples were administered to test animals orally. The positive and negative control group animals received the same experimental handling as those of the test groups except that the drug treatment, control group animals received only appropriate volumes of vehicle and of the reference drug, diclofenac sodium, respectively.

5.3 Anti-inflammatory activity

Anti-inflammatory activity was performed by the following procedure of Jayashankar et al. [7] The animals were divided into 12 groups each having six animals. A freshly prepared suspension of carrageenan(1% w/v , 0.1 ml) was injected to the planter region of left hind paw of each rat. One group was kept as control and the animals of the other group were pretreated with the test drugs suspended in Carboxy methyl cellulose (1%w/v) given orally 30 min before the carrageenan treatment. The paw volumes were measured immediately after injection and after 3 h with the help of plethysmometer. Mean increase in paw volume was measured and the percentage of inhibition was calculated.

5.4 Analgesic activity

Analgesic activity was tested by the acetic acid induced writhing method. [8] The mice were divided into 8 groups of six animals each. A 1 % aqueous acetic acid solution (i.p. injection, 0.1 mL) was used as a writhing inducing agent. Mice were kept individually in the test cage before acetic acid injection and habituated for 30 min. Screening of analgesic activity was performed after i.p. administration of test drugs and the reference drug (diclofenac acid) at a dose of 10 mg /kg body weight. All the compounds were injected as CMC suspension (1 %). One group was kept as control and received 1 % CMC. After 20 minutes of drug administration, 0.10 mL of 1 % acetic acid solution was given to mice intraperitoneally. Severity of the writhing response was recorded for 20 min after administration of acetic acid solution. The analgesic activity was expressed in terms of % protection.

5.5 Acute ulcerogenicity studies

Acute ulcerogenicity screening was done according to method reported by Mohammed Ajmal et al.[9] The mucosal damage was examined by means of 4x binocular magnifier. For each stomach specimen, the mucosal damage was assessed according to the following scoring system.

Ulcer score	Descriptive observation
0	Normal colored stomach
0.5	Red coloration
1	Spot ulcers
1.5	Haemorrhagic streak
2	Ulcers
3	Perforation

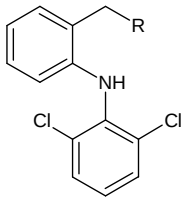
The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage. Data are expressed as mean ulcer score ±SEM, data analyzed by one way ANOVA followed by Newman keul's multiple range test to determine the significance of the difference between the standard group and rats treated with the test compounds. The differences in results were considered significant when P was found to be <0.01.

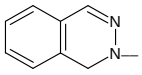
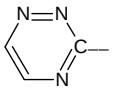
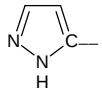
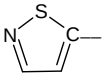
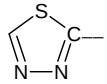
RESULTS AND DISCUSSION

2.1 Molecular docking

Various compounds were designed by replacing the carboxylic acid group present in the diclofenac with less acidic heterocycles like phthalazine, triazine, 1, 3, 4-oxadiazole, pyridazine, pyrole, pyrazole, imidazole, thiazole etc. using chemoffice2004 software and docking simulation was carried out to all the designed compounds against 6COX (COX-2 enzyme) With the help of arguslab program and the docking scores of each compound was analysed. Out of all the designed compunds, ten compounds showed very good interaction energies (tab.1.) even better than that of diclofenac acid.

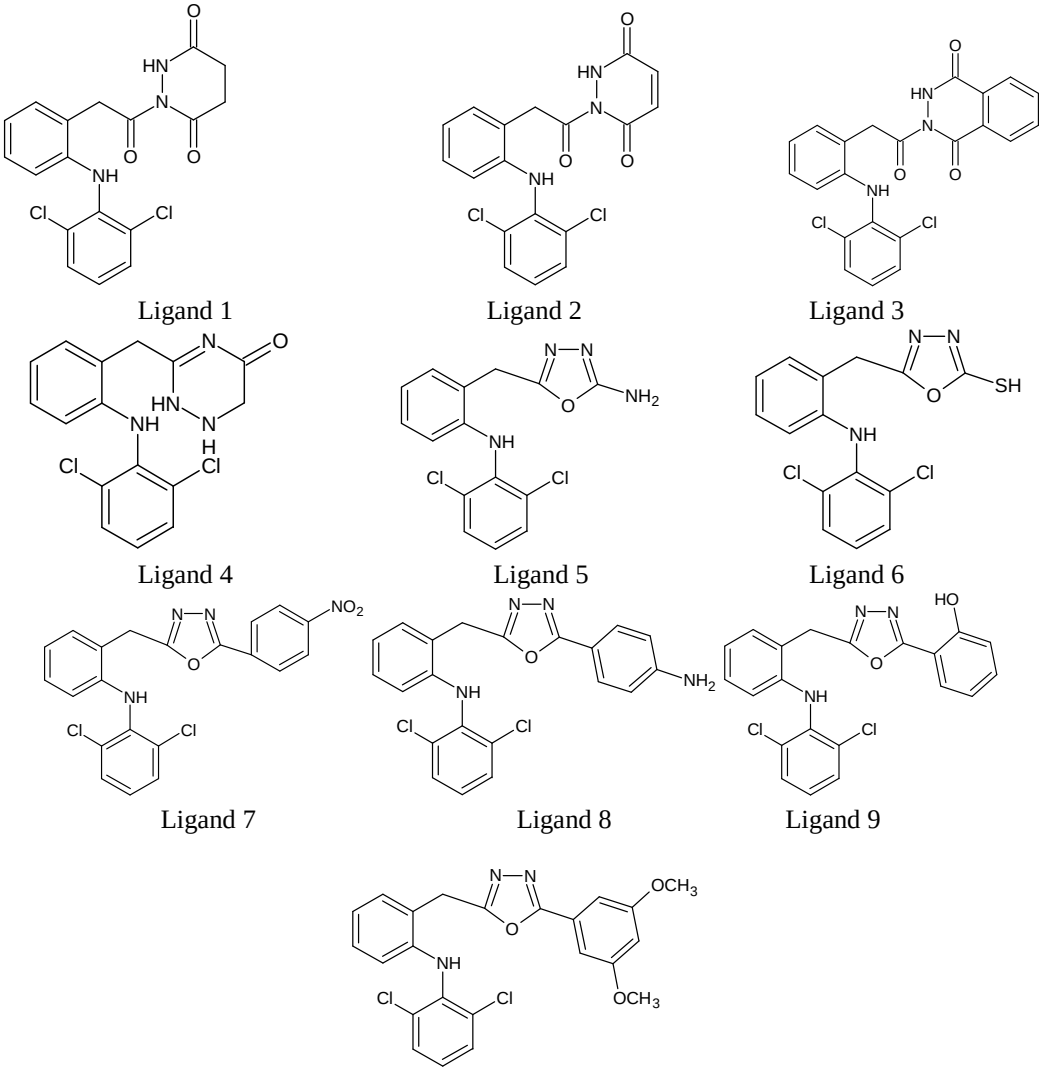
Tab. 1. Docking scores (kcal/mol) of newly designed lead compounds



S.no	*R*		*Energy level (kcal/mol)*	*S.no*	*R*	*Energy level (kcal/mol)*
1.		*	-13.1794	6.		-11.7939
2.		*	-12.8261	7.		-11.7861
3.		*	-12.4157	8.		-11.7745
4.		*	-11.8757	9.		-11.535
5.			-11.796	10.		-11.4964

Where* ⟶ more potent compounds

Fig.1.Structures of ligands designed from selected lead compounds



Ligand 1 Ligand 2 Ligand 3

Ligand 4 Ligand 5 Ligand 6

Ligand 7 Ligand 8 Ligand 9

Ligand 10

2.2 Lead optimization

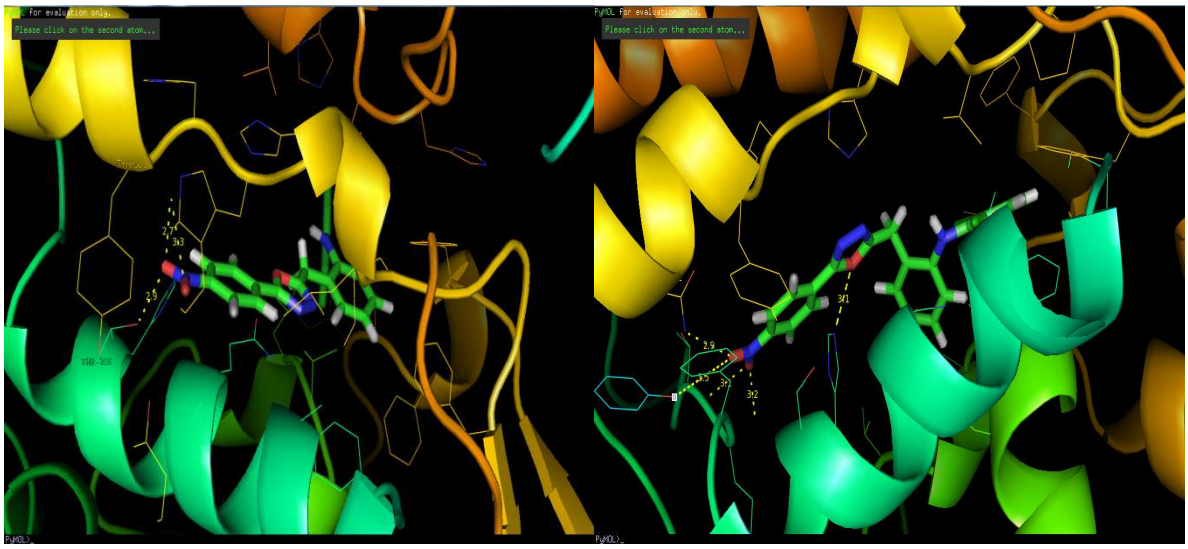
Among the above ten compounds, structures containing less acidic heterocycles like phthalazine, triazine, pyridazine and 1, 3, 4-oxadiazole nucleus showed highly negative interaction energy in molecular docking studies. The compounds which showed highly negative energy were considered as potent compound. The most potent compounds were selected as the lead on which we carried out structural modification in order to increase the binding ability. Structures of the newly designed ligands from the lead compounds were given in fig.1.

Tab. 2. Docking scores (kcal/mol) of various newly designed ligands (1-10) with COX-1(1Q4G), COX-2 (6COX), LTA_4 hydrolase(2R59)

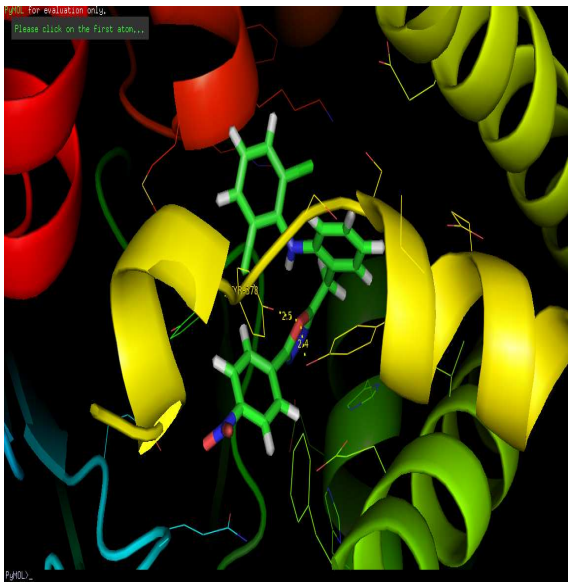
Ligand code	COX-1(1Q4G)		COX-2(6COX)		LTA_4 hydrolase (2R59)	
	Energy level(kcal/mol) or Dock score	Length of Hydrogen bonds formed	Energy level(kcal/mol) or Dock score	Length of Hydrogen bonds formed	Energy level(kcal/mol) or Dock score	Length of Hydrogen bonds formed
1	-11.4531	2.2 ,2.5 ,3.0	-12.3424	2.7	-12.1989	2.5,2.5, 2.7,3.0
2	-10.5472	2.9	-11.9594	2.5	-9.97001	1.8,2.8, 3.4,3.6
3	-12.4313	3.0	-13.3476	3.2	-11.2979	2.8,2.9

4	-11.8235	2.4	-12.5163	2.2	-11.5081	2.2,2.4,2.6 ,2.9,3.4
5	-11.6318	-	-11.3897	2.3	-9.29692	-
6	-11.9601	2.8	-11.5789	-	-9.46334	-
7	**-12.5957**	**2.7,2.9, 3.3**	**-12.1641**	**2.9,2.9, 3.1, 3.1,3.5**	**-11.0432**	**2.4,2.5**
8	-13.5629	-	-13.001	-	-11.4282	2.1,2.1
9	-13.7718	3.5	-12.6564	3.0	-11.6101	2.4,2.6
10	-12.8009	3.0	-11.4368	2.3	-10.4464	2.4,2.9

Fig. 2. Binding mode of ligand-7 with COX-1(1Q4G), COX-2 (6COX) and LTA_4 hydrolase enzymes (2R59)



COX-1(1Q4G) **COX-2 (6COX)**



LTA_4 hydrolase enzyme (2R59)

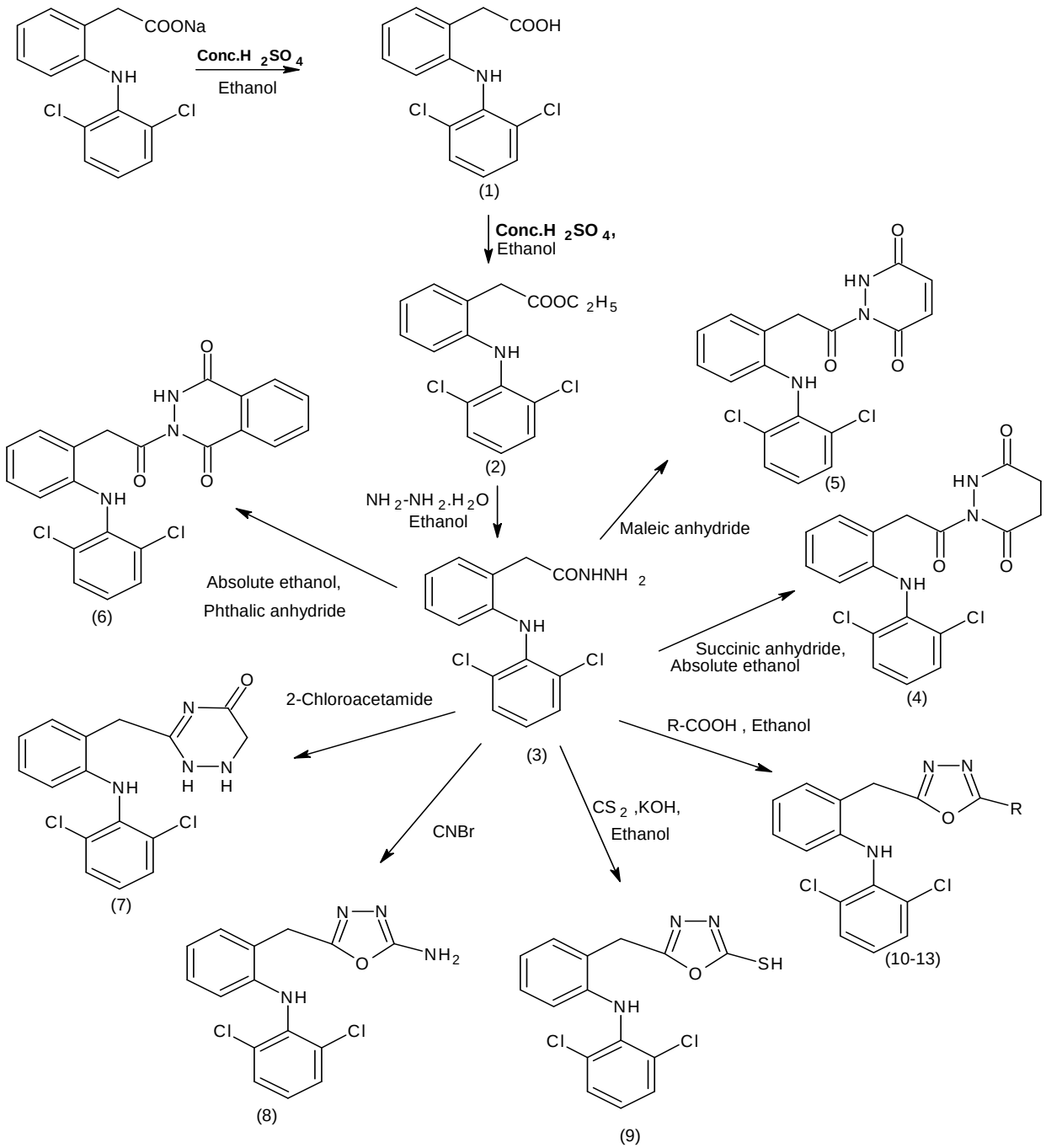
The binding ability of this newly designed ligands with COX-1, COX-2 and *LTA_4Hydrolase* enzymes were determined with the help of molecular docking studies using arguslab program. The binding scores of designed ligands 1-10 with COX-1, COX-2, *LTA_4Hydrolase* enzymes ranging from -13.7718 to -10.5472 Kcal/mol, -13.3476 to -11.3897 Kcal/mol and -12.1989 to -9.29692 Kcal/mol respectively (tab.2.) and the binding mode of ligand 7 with COX-1 ,COX-2, *LTA_4Hydrolase* enzymes was showed in Fig.2. These data clearly indicates their potency as

cyclooxygenase inhibitors and LTA_4Hydrolase inhibitors. Almost all the designed ligands showed good interaction energy than the diclofenac which showed the following interaction energies -8.4293 Kcal/mol, -8.3123 Kcal/mol and -8.0792 Kcal/mol with COX-1, COX-2, *LTA_4Hydrolase* enzymes respectively.

2.3 Chemistry

The synthetic route used to synthesize the above potent ligands is outlined in scheme 1. 2-[(2, 6-dichloroanilino) phenyl] acetic acid (1) was prepared from diclofenac sodium by hydrolysis in the pressene of conc.H_2SO_4 and ethanol. Ethyl-[2-(2, 6-dichloroanilino) phenyl] acetate (2) was prepared from 2-[(2, 6-dichloroanilino) phenyl] acetic acid (1) by esterification in the pressence of conc.H_2SO_4 and ethanol. [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (3) was prepared from ethyl-[2-(2, 6-dichloroanilino) phenyl] acetate

Scheme 1



R= p-Nitro phenyl (10), p-Amino phenyl (11), o-Hydroxy phenyl (12), 3, 5-dimethoxy phenyl (13).

(2) by treatment with hydrazine hydrate in absolute ethanol. The reaction of [2-(2, 6- dichloroanilino) phenyl] acetic acid hydrazide (3) with succinic anhydride in absolute ethanol afforded 1-({2-[(2, 6-dichlorophenyl) amino] phenyl} acetyl) tetra hydro pyridazine-3, 6-dione (4). Compound (3) was allowed to react with maleic anhydride in absolute ethanol to give1-({2-[(2, 6-dichlorophenyl) amino] phenyl} acetyl) 1, 2-dihydro pyridazine-3, 6-dione (5). Reaction of acid hydrazide(3) with phthalic anhydride in absolute ethanol afforded 2-({2-[(2,6-dichlorophenyl)amino]phenyl}acetyl)-2,3-dihydrophthalazine-1,4-dione(6). Compound (3) was refluxed with 2-chloroacetamide and dimethylformamide to yield 3-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 6-dihydro-1, 2, 4-triazin-5(2*H*)-one (7). 5-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 3, 4-oxadiazol-2-amine (8) was prepared from acid hydrazide(3) in the presence of cyanogen bromide. The reaction of [2-(2,6-dichloroanilino) phenyl] acetic acid hydrazide (3) with carbondisulfide in an alkaline medium afforded 5-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 3, 4-oxadiazole-2-thiol (9). Various 5-substituted oxadiazoles(10-13) were prepared by the treatment of acid hydrazide (3) with various substituted aromatic acid in the presence of phosphorus oxychloride.

The structures of various synthesized compounds were assigned on the basis of different spectral studies. The physical data, FTIR and ^{1}HNMR data for all the synthesized compounds were reported in experimental protocols.

2.4 Pharmacology

In the pharmacological study, we have investigated anti-inflammatory and analgesic activity as well as the acute ulcerogenicity of all the synthesized compounds. Diclofenac, the parent compound, was used as a reference standard. The animals were maintained at 25 ± 2 °C, 50 ± 5% relative humidity and 12 h light/dark cycle. The animals were fasted for 24 h prior to the experiments and water provided ad libitum.

2.5 Anti-inflammatory activity

Anti-inflammatory activity of the synthesized compounds was evaluated by carrageenan induced rat paw oedema model. Screening of anti-inflammatory activity was performed after administration of test drugs or reference drug (diclofenac acid in case of standard group) at a dose of 10 mg/kg body weight. 1% carrageenan produced increase in paw volume (oedema) of all the animals of various groups. The significant ($p < 0.01$) reduction of rat paw oedema was observed by most of the test compounds at 4 h compared to control group (Tab.3.). Compound 4, 6, 7, 9, 11, 12 possess significant anti-inflammatory activity when compared to control group at $p<0.01$.

2.6 Analgesic activity

The analgesic activities of the compounds were studied by using acetic acid induced writhing test in mice. The compounds, which exhibited significant anti-inflammatory activity comparable to that of diclofenac acid, were screened for analgesic activity. The analgesic activity was evaluated after i.p. administration of test drugs or reference drug (diclofenac acid in case of standard group) at a dose of 10 mg/kg body weight. These compounds presented an important analgesic profile measured by the classical acetic acid induced writhing model. From the results of acetic acid induced writhing test, it was noticed that all compounds possess significant analgesic activity (Tab.4.).

Tab.3. Anti-inflammatory activity of various synthetic drugs

Compound code	Paw volume(ml) as measured at 3 hour	Percentage inhibition of paw oedema
Control	5.10±0.98	-
reference	1.70±0.28	66.66%
4	2.60±0.28	49.01%*
5	3.52±0.86	30.09%
6	2.38±0.40	53.33%*
7	2.30±0.36	54.90%*
8	3.20±0.38	36.07%
9	2.58±0.48	49.41%*
10	3.12±0.73	38.82%
11	2.32±0.52	54.50%*
12	2.40±0.60	52.94%*
13	3.40±0.80	33.33%

Tab.4. Analgesic activity of various synthetic drugs

Compound code	No. of writhes in 20 min after treatment (Mean ± SEM)	% inhibition
Normal saline	36±3.0	-
reference	6±0.8	83.3%
4	14.0±2.3	61.1%*
6	12.5±1.2	65.27%*
7	8±0.7	77.7%*
9	13±1.5	63.8%*
11	11±1.0	69.4%*
12	9±0.6	75%*

Tab.5. Acute ulcerogenicity studies of various synthetic drugs

Compound code	Ulcer index(mean± SEM)
Normal saline	0.30± 0.06
reference	2.3± 0.06
4	0.75± 0.34*
6	0.90± 0.22*
7	0.75± 0.30*
9	0.50± 0.10*
11	0.58± 0.12*
12	0.60± 0.09*

Data analyzed by one way ANOVA followed by Newman's keul's multiple range test.
* *values were considered significant at P< 0.01.*

2.7 Acute ulcerogenicity studies

The compounds which showed significant Anti-inflammatory activity and analgesic activity were also screened for acute ulcerogenicity by pyloric ligation model. The mucosal damage was examined by using 4x binocular magnifier and severity index was calculated. Close inspection of the results obtained by ulcerogenecity studies indicate that ulcerogenic activity (tab.5.) of various synthesized compounds ranging from 0.58± 0.12 to 0.90± 0.22, whereas the standard diclofenac sodium showed high severity index of 2.3±0.06. Hence it can be said that gastrointestinal tolerance to this compounds are better than that of diclofenac sodium.

CONCLUSION

Various heterocyclic analogues of diclofenac were designed and the potent ligands were identified with help of molecular docking. Moreover these potent ligands were synthesized and screened for analgesic, anti-inflammatory and ulcerogenic potential. Most compounds exhibited significant analgesic and anti-inflammatory activity. Compounds 4, 6, 7, 9, 11, 12 showed strong analgesia in acetic acid induced writhing tests. Among all the synthesized compounds, compounds 4, 6, 7, 9, 11, 12 exhibited most prominent and consistent anti-inflammatory activity. From the detailed analysis of the results of acute ulcerogenicity studies, we conclude that the synthesized compounds have not only retained the anti-inflammatory profile of diclofenac acid but also have helped in enhancing the anti-inflammatory activity and are devoid of the deadlier gastrointestinal toxicities. Moreover close inspection of results of in vivo experiments; we can conclude that the cyclization of hydrazide moiety to various less acidic heterocyles yielded compounds with therapeutic efficacy.

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