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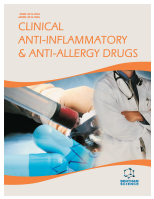
Insight into Drug Design and Development of Anti-inflammatory Agents



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Abstract: Nonsteroidal anti-inflammatory drugs available in the market suffer from side-effects like gastric ulceration, hepatotoxicity, renal toxicity, etc. Selective COX-2 inhibitors have been developed to reduce the side-effects, but unfortunately suffer from severe cardiovascular toxicity. As a result, several selective COX-2 inhibitors have been withdrawn from the market. Thus to improve the efficacy with reduced side-effects many strategies were developed. The free carboxylic group of the NSAIDs was modified into various acid derivatives to reduce gastric ulceration. Modifications of the existing anti-inflammatory drugs were carried out by various research groups so that the efficacy of the drugs improves along with selectivity. This review covers changes and structural modifications of various anti-inflammatory agents. Synthesis of various derivatives of NSAIDs was reported in the literature review. The article also summarizes the synthesis of the molecules using microwave assisted synthesis. Computational studies (*i.e.* QSAR and Docking analysis) along with information about various novel moieties acting against reported inflammation is also discussed in the article.

Keywords: Anti-inflammatory agents, docking, microwave assisted synthesis, NSAIDs, QSAR, synthesis of COX-2 inhibitors.

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INTRODUCTION

Inflammation conventionally can be defined as the normal response of our body to any injury, infection and other stimuli [1]. It can also be defined as allergic reaction towards toxic stimuli. Inflammation is a protective process of our body, but its overstated and lengthened action is harmful to our body [2]. Inflammation is an expression or outcome of a tissue damage that alters or moves the metabolic balance in the direction of catabolism. Evidence of enhanced catabolism during the inflammatory process includes proteolysis, reduction of cellular space volume or weak oxidative metabolism [3]. Presently inflammation can be identified by the incidence of five major signs or changes occurring in the body, four of them are very old and familiar, such as a tumor (tissue swelling) calor (elevated temperature of the tissue), rubor (redness at the affected part), dolor [4] (severe feeling of a harmful stimulus) and functio laesa [5], *i.e.* an inappropriate function of the affected organ. American pathologist Menkin found the sixth fundamental stimulus the only vital biochemical indication of inflammation, *i.e.* proteolysis [6] during 1940.The primary reason of inflammation is solely mechanical pressure, which includes blunt trauma [7], unknown bodies [8], vibrations and constant pressure of low intensity [9]. The original mechanism of causing inflammation by pressure is probably by tissue hypoxia. Hypoxia displays inflammatory changes through hypoxia-inducible factor (HIF). The primary evidence for inflammation is an extreme or persistent harmful stimulus. The second prime factor is cold. Some chemical causative factors affecting inflammation are bases and reducing substances [10].

ANTI-INFLAMMATORY DRUGS

Nonsteroidal anti-inflammatory drugs are primarily used against inflammation. NSAIDS have anti-pyretic, analgesic and anti-inflammatory activity [10]. Nonsteroidal anti-inflammatory drugs are supposed to produce anti-inflammatory action by inhibiting the Cyclooxygenase enzyme, an enzyme which is necessary for prostaglandin synthesis [11]. Prostaglandin is one of the mediators responsible for inflammation. These drugs are mainly classified according to the duration of action and also selectivity [12] as shown in Fig. (**1**).

STRUCTURE OF CYCLOOXYGENASE ENZYMES

Structurally COX-1 and COX-2 are very similar, except some very minute differences which are shown in (Table **1**) [13]. Both are identical in length and with molecular weight 71K. Also, both consist of about 600 amino acids among those 63% are in matching sequence. After superimposing the X-Ray crystal structure of Cyclooxygenase enzyme it was observed that all the residues are similar except two small variations. In COX 1, isoleucine is present while valine is present in COX-2 at positions 434 and 523, respectively. Secondly, on comparison the major difference found was that of the terminal endings where sequence of 17 amino acids from the N terminus is absent in COX-2, while a sequence of 18 amino acids at the C terminus of COX-2 is present. COX-2 is present on both nuclear membrane as well as endoplasmic reticulum, while COX-1 is found entirely on the surfaces of ER [14]. The major differences are found in the

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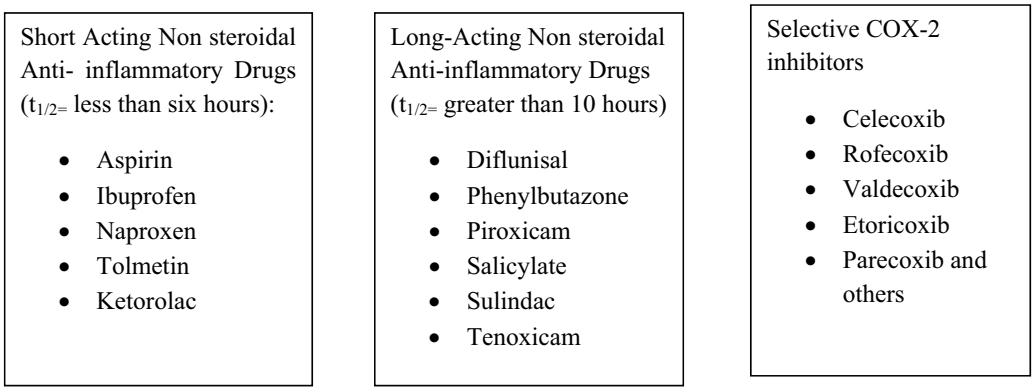


Fig. (1). Classification of anti-inflammatory drugs.

Table 1. Structural difference between COX-1 and COX-2 enzymes.

Features	COX-1	COX-2
Chromosome	9	1
Homology mRNA	Approx 60%	Approx 60%
Messenger RNA size	2.7kb	4.5kb
Protein Size	Approx 65kDA	Approx 70kDA
Intracellular location	Only Endoplasmic Reticulum	Both Endoplasmic Reticulum and nucleus
Regulation	Constitutive	Inducible
Range of Expression	2 to 4 fold	10 to 80 fold
Tissue Expression	Most tissues	Only in specific tissues
Proposed Role	House keeping	Inflammatory response

pathophysiological functions of the enzymes as shown in (Table **2**) [15-17].

SCOPE OF THE REVIEW

As we know COX-1 and COX-2 both are having severe side-effects [18, 19] which cannot be surpassed. To overcome the severe gastro-irritation problems of COX-1 inhibitors, COX-2 was discovered and launched. But later it was realized that COX-2 inhibitors were successful in reducing only the lower GI tract problems and not the upper GI tract problems. COX-1 is responsible for converting PGH_2 to thromboxane $A_{2;}$ Selective COX-2 inhibitors do not inhibit COX-1. Thromboxanes A2 is responsible for platelet aggregation and thus clot formation which results in cardiac stroke [20]. So, among the selective COX-2 inhibitors only celecoxib is available rest are banned. NSAIDs are drugs which are administered on a regular basis [21]. Thus, considering the increasing use of NSAIDs and also the side effects of the existing drugs, many researchers have tried to modify the existing NSAIDs. This was done so as to increase the selectivity of the drugs and ultimately reducing the side-effects of the same. This review consists of the details of the modification made by several scientists and also some novel moieties acting as selective COX-2 inhibitors.

MECLOFENAMIC ACID AND MEFENAMIC ACID ANALOGUES

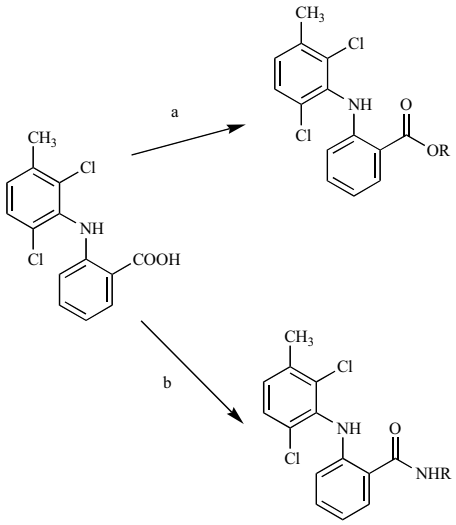
Meclofenamic acid and mefenamic acid drugs are included in the class of anthranilic acid derivatives. Both are different in their mechanism of action from other NSAIDs. This is because it blocks PGs as well as the tissue response to PGs. It cannot be administered for long duration as mefenamic acid is associated with pancytopenia, while on the other hand, meclofenamtes is associated with severe GI toxicity. Considering these side effects some derivatives of these drugs were synthesized in the recent years, which are mentioned in (Scheme **1**) [22].

S. Kalgutkar *et al.* synthesized various amide derivatives of meclofenamic acid. These derivatives were tested for selectivity and potency by *in vitro* biochemical assay using purified human COX-2 or ovine COX-1 as well as site directed mutagenesis studies. From the IC_{50} values, it has been found that the amide derivatives are more selective and potent towards COX-2 enzyme than the parent compound meclofenamic acid. Different amine derivatives were used in the synthesis as shown in the following (Scheme **1**). It was also reported that 4-nitro phenyl substituted derivative displayed maximum potency (IC_{50}=300μm) and selectivity for COX-2 inhibition. From the site directed mutagenesis studies

Table 2. Pathophysiological difference between COX-1 and COX-2 enzymes.

	COX-1	COX-2
GI Tract	Abundantly present and thus produce PGs which are cyto-protective.	Presence is less
Kidney	Production of PGs is solely mediated by COX-1 and also helps in renal homeostasis.	Enzyme observed is less.
Colorectal Adenomas and Adenocarcinomas	COX-1 is expressed in normal intestine but its levels do not change in intestinal tumors.	COX-2 expressed in trace amounts in normal intestinal tissues.
Platelet	COX-1 is the single isoform present in platelet.	Its expression in platelets is still not found.
Gestation and Parturition	Abundantly present.	Abundantly present. PGs originating from COX-2 play vital role in birth process
CNS	Mostly present in forebrain.	Present in parts like cortex, hippocampus, hypothalamus and spinal cord.

it is reported that COX-2 selectivity increases with the interaction of molecule with Arg120 and Tyr355 in the active site of COX-2 enzyme. Meclofenamic acid inhibits COX-2 enzyme by interaction with Arg120Ala but not the Tyr355Phe [23].



(Scheme 1). BOP-Cl; (**a**) ROH; (**b**) RNH_2.

N.A Razzak *et al.* synthesized two compounds which were derived from mefenamic acid. Both the compounds were examined *in vivo* as well as *in vitro* for their COX-2 binding ability. It was found that both possess extremely good COX-2 inhibitory activity. Thus, these results help in continuing the research for further development of COX-2 inhibitors so that the selectivity of the compound increases and the toxicity decreases. Synthetic route for the compound is shown in (Scheme **2**) [24]. Pharmacological screening revealed that the 2-aminopyridine substituted mefenamic acid was found to be more potent.

INDOMETHACIN AS A CORE MOIETY

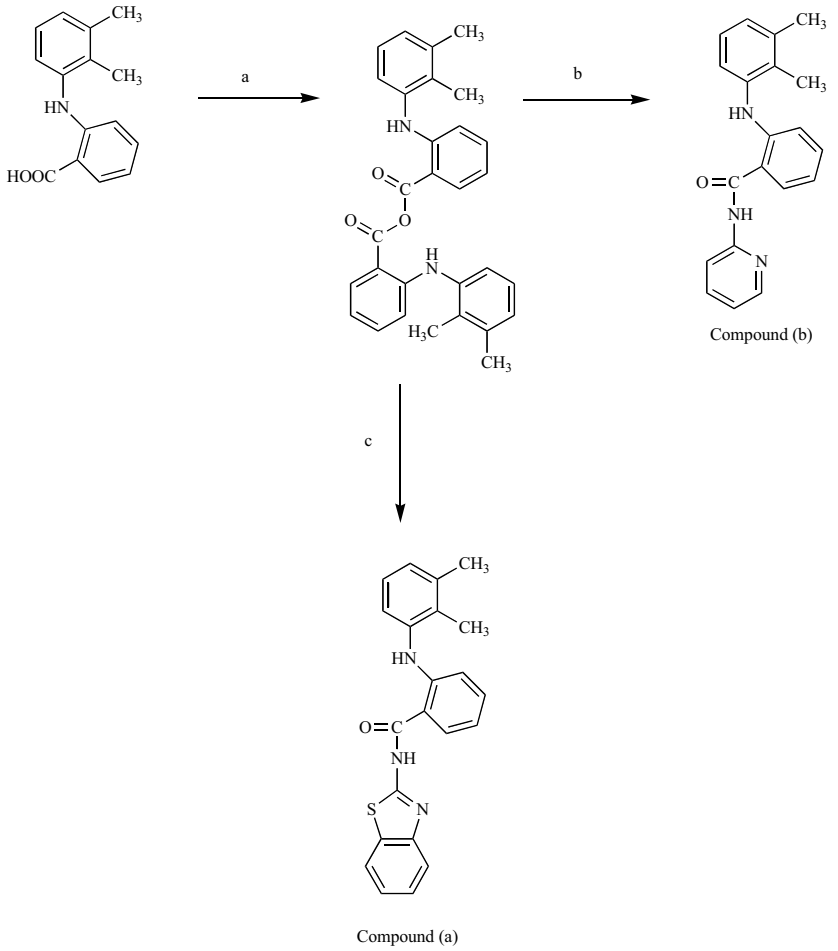
Acetic acid derivatives include indomethacin, sulindac, etc. which act as NSAIDs. Indomethacin is extensively used for acute gouty arthritis and osteoarthritis. It is highly bound to albumin and also exhibits good oral as well as rectal absorption. But the use of this drug has been limited because of gastritis, renal dysfunction and other side effects. Few studies are available for the synthesis of derivatives of indomethacin, which increases selectivity and reduces toxicity [25].

K.W. Woods *et al.* synthesized various thiazole analogues of indomethacin as shown in (Scheme **3**). The carboxylic acid group of indomethacin was replaced by thiazole moiety. The molecules were tested with human recombinant prostaglandin endoperoxidase H synthase-1 and -2 (COX-1 and COX-2). SAR studies of these molecules revealed that the thiazole analogues were found to be extremely potent as well as selective for COX-2 inhibition. Along with this extra substitution in the 1^{st} and 4^{th} position of the indole in the indomethacin were done which increased the anti-inflammatory activity. Thiazole substituted with aromatic ring at 4^{th} position is found to be more promising as compared to 2^{nd} position as reported in the article. N-(p-bromobenzyl) derivative of indomethacin increases selectivity for COX-2 enzyme as compared to N-(p-chlorobenzoyl) substitution but decreases potency [26].

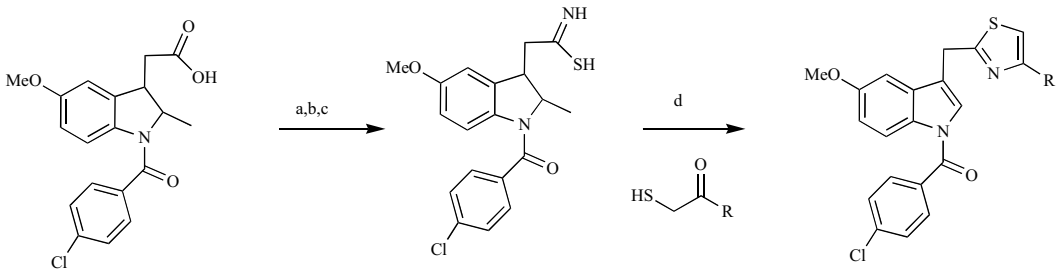
IBUPROFEN DERIVATIVES

Ibuprofen, ketoprofen and naproxen are the drugs classified as propionic acid derivatives of NSAIDs. These drugs are mainly used in arthritis. No prominent side effects were observed in over the counter doses, but high doses increase risk of GI side effects. Nausea and dyspepsia are predominant among them. Renal side effects are dependent on dose. CVS side effects were also reported [25].

Manoj Kumar *et al.* synthesized variety of amide derivatives of ibuprofen using various aliphatic as well as aromatic amines. All the NSAIDs are having major side effects of gastric-ulceration which is mainly considered to be because of the free carboxylic acid group. Thus, the free carboxylic acid in ibuprofen has been derivatized by substituted amines to amides so that improved activity with less toxicity was observed. All the derivatives were tested using *in-vitro* as



(Scheme 2). (**a**) THF, DCC (**b**) 2-aminopyridine (**c**) 2-aminobenthiazole.

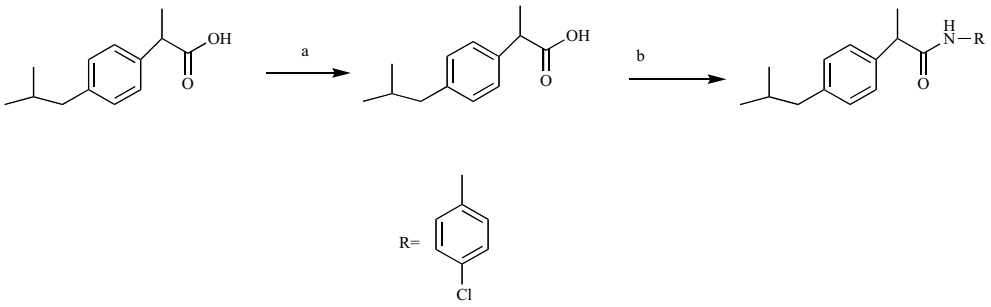


(Scheme 3). (**a**) isobutylchloroformate; (**b**) NH_3 (c) P_4S_{10}, THF/dioxane (**d**) THF, rt, 24h.

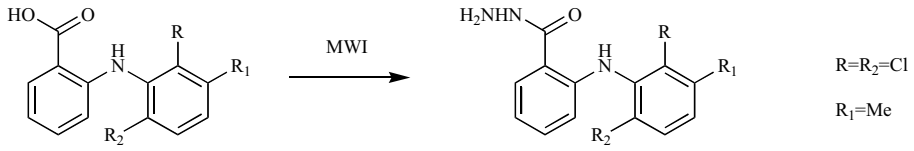
well as *in vivo* studies and were found to be more convincing as compared to ibuprofen. The synthesis of these analogues is shown in (Scheme **4**) [27].

T.A. Fadl *et al.* reported new and environmental friendly method for synthesis of fenamic acid derivatives (such as anthranilic acid derivatives). Conventional method for the synthesis of derivatives involves more than one step and is also time consuming. While the microwave assisted synthesis involves only one step and also the product obtained is in very good practical yield. Reaction between fenamic acid derivatives with hydrazine hydrate under solvent free conditions resulted in corresponding fenamic acid hydrazides in excellent yields. The synthetic pathway using microwave irradiation technique is shown in (Scheme **5**) [28].

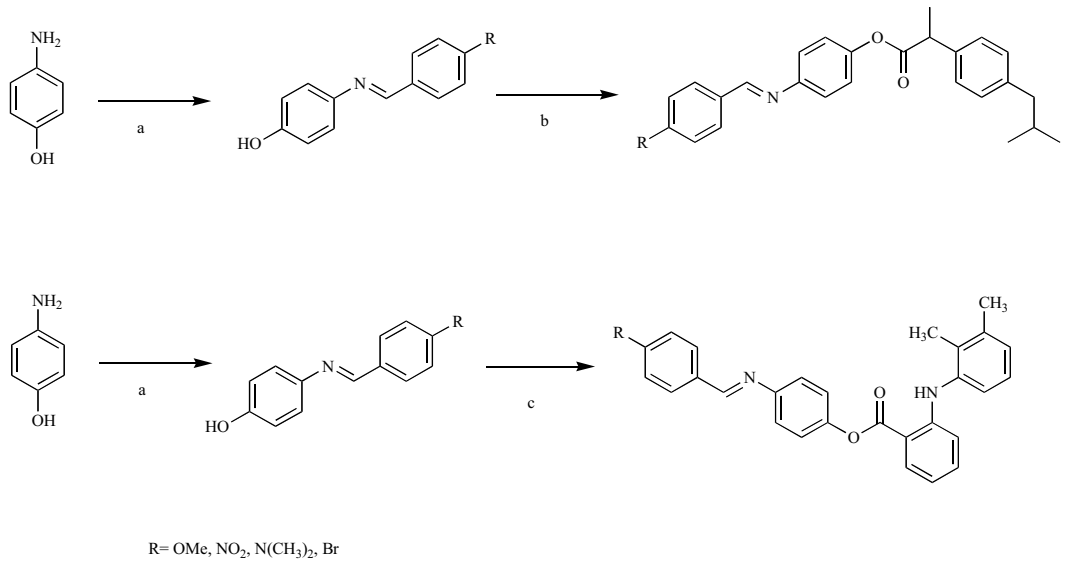
G. H. Hegazy and H. I. Ali synthesized different ester derivatives of ibuprofen and mefenamic acid to mask the free acidic group. The carboxylic acid group underwent condensation with corresponding Schiff bases using DCC as a reagent. Excellent results were obtained when these compounds were evaluated by *in vivo* studies (carrageenan rat paw edema and hot plate method). Also, these were non-ulcerogenic and found to be more selective for COX-2 enzyme. Para substituted benzylidenamino phenyl ester of ibuprofen was found to exhibit the maximum anti-edematous effect and also showed no ulcerogenic effect. The synthesis of these derivatives is shown in (Scheme **6**). Molecular docking study of eight molecules using Autodock software is also reported by the research group. The docking studies revealed



(Scheme 4). (**a**) $SOCl_2$ (**b**) RNH_2.



(Scheme 5). $NH_2NH_2H_2O$, Microwave irradiation, 300W (250°C), 4-12 min.



(Scheme 6). (**a**) Ar-CHO, AcOH, EtOH, reflux, 6–8h;(**b**) ibuprofen, Schiff's base, THF/dichloromethan, dicyclohexyl carbodiimmide (DCC), rt, overnight; (**c**) mefenamic acid, Schiff's base, THF/dichloromethan, dicyclohexyl carbodiimmide (DCC), rt, overnight.

the lowest binding energy of the synthesized compounds with small RMSD (Root Mean Square Deviation) value [29].

PARACETAMOL DERIVATIVES

Paracetamol is widely used anti-pyretic and analgesic drug. It does not possess severe cardiac toxicity or GI side effects. The main problem associated with paracetamol is in its metabolism. The metabolism of this drug leads to severe hepatic and renal toxicity. This toxicity is due to the formation of N-acetylquinone imine metabolite, which depletes glutathione, and ultimately leads to cell death. To overcome this side-effect, prodrug approach was selected by santos *et al.* Esterification of paracetamol with various amino acids were carried out using o-(benzotirazol-1-yl)-N,N,N_0,N_0-tetramethyluroniumtetrafluoroborate (TBTU) as a coupling reagent. Dipeptide carriers were utilized for intramolecular cyclization of paracetamol. The hepatic levels of glutathione was measured for synthesized prodrugs and compared with paracetamol to check hepatotoxicity. The hepatotoxicity of molecules was significantly reduced by using appropriate dipeptide for synthesizing prodrug of paracetamol [30].

M. R. Yadav, *et al.* synthesized esters using paracetamol as hepato toxicity is a major issue with the drug. Ibuprofen, and mefenamic acid or indomethacin were first reacted with thionyl chloride, then the acid chloride derivative was reacted with paracetamol to give esters. All the synthesized derivatives displayed enhanced antipyretic activity than paracetamol with anti-inflammatory activity as compared to parent NSAIDs. These compounds showed no ulcerogenic toxicity as reported in the article [31].

NOVEL MOIETIES AS SELECTIVE COX-2 INHIBITORS

Several classes of heterocyclic moieties came into the market as selective COX-2 inhibitors with enhanced anti-

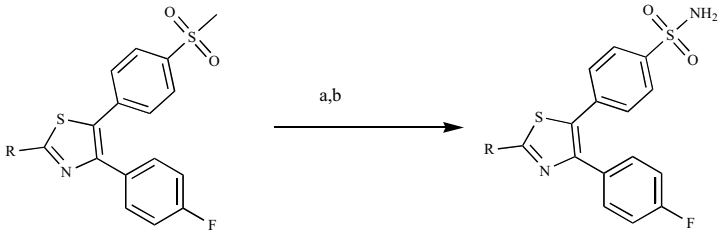
inflammatory activity and with reduction in gastric ulceration. Some examples are celecoxib, rofecoxib, etc. J.S. Carter *et al.*, reported variety of sulfonamide-substituted 4,5-diarylthiazoles which were prepared using three synthetic routes as selective COX-2 inhibitors. The sulfonamide moieties in these compounds were reported to increase the bioavailability of the compounds along with increased selectivity towards COX-2 enzymes. It is also reported that sulfonamide group is an essential pharmacophoric group to increase anti-inflammatory activity. Synthetic route is shown by (Scheme **7a**, **7b**, **7c**). *In vitro* (enzyme assay using hCOX-2) and *in vivo* (air pouch method) studies revealed maximum potency with substitutions like SO_2NH_2 and CH_3 [32].

N. Naik *et al.* synthesized novel pyrazole containing indole moieties which were proved to be good candidates for increase in anti-inflammatory activity as well as antioxidant property. The novel synthesized molecules were tested for anti-inflammatory activity and also assayed for anti-oxidant activity and the results were found to be promising. The synthetic scheme for the molecule is shown in (Scheme **8**) [33].

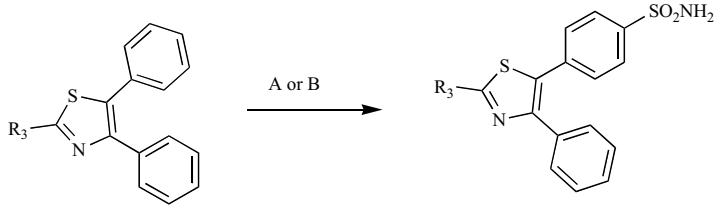
S.G. Alegaon *et al.* synthesized twenty-two 1,3,4-trisubstituted pyrazole derivatives. These analogues were further examined by the *in-vivo* studies using carrageenan-induced rat paw edema method. All the compounds investigated by rat paw edema method showed excellent activity when compared with diclofenac, which was taken as the standard. Also, they exhibited anti-cytotoxic activity against cell line MCF-7 (*in vitro* assays). Maximum activity was found in compounds substituted with OH, NO_2 (86% inhibition). The synthetic scheme of the derivatives is shown in (Scheme **9**) [34].

M.A.A. El-Sayed *et al.,* synthesized new pyrazole and pyrazoline derivatives, which were tested to inhibit ovine COX-1/COX-2 isozymes using *in vitro* cyclooxygenase (COX) inhibition assay. Most of the analogues showed enhanced COX-2 selectivity against the standard celecoxib. Those compounds which were found to be more potent during the *in vitro* study were subjected to *in vivo* screening by carrageenan induced rat paw edema method. Compounds with substitution like Br or CH_3 showed maximum inhibition against COX-2 enzyme. The synthesis of these derivatives is shown in (Scheme **10**). Molecular docking studies were performed using MOE 2008.10 software installed on 2.0G Core 2 Duo processor, to get the insight into the important functional group to increase the selectivity towards COX-2 enzyme. The results of computational studies revealed that trifluoromethyl substitution is an important pharmacophoric group for COX-2 inhibition [35].

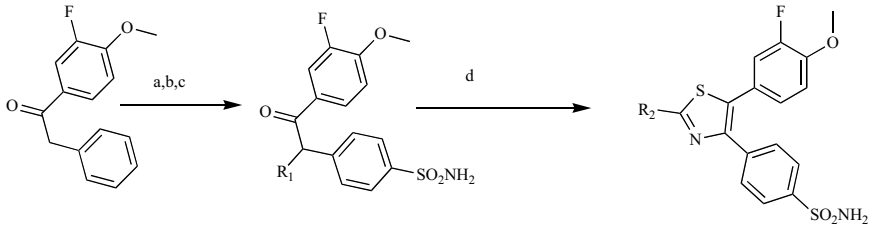
N. Kumar *et al.* synthesized a new series of 4, 6-substituted di-(phenyl) pyrimidin-2-amine by reacting chalcone derivatives with guanidine hydrochloride in the presence of dimethyl formamide. All the analogues were tested for anti-inflammatory activity using carrageenan rat paw edema method. Two compounds 4-(4-nitrophenyl)-6-phenylpyrimidine-2-amine and 4-(4-methoxyphenyl)-6-phenylpyrimidine-2-amine showed highly significant decline



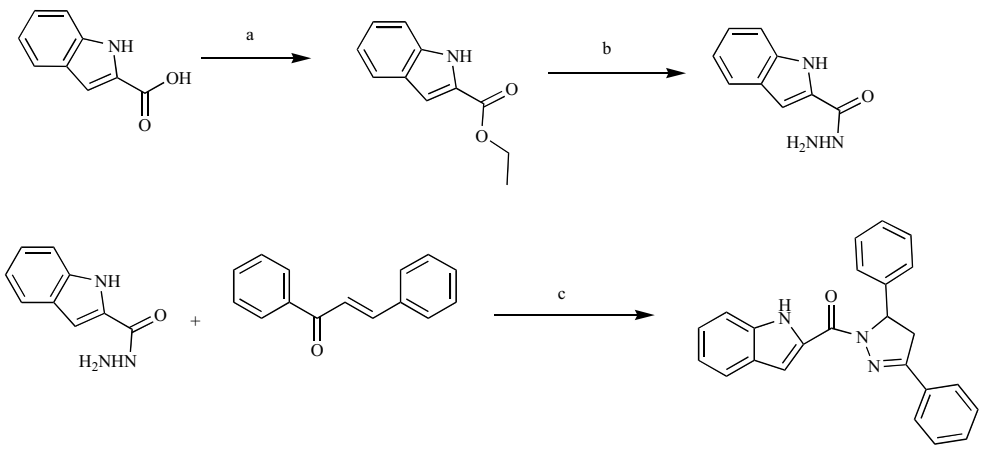
(Scheme 7a). (**a**) n-BuMgBr, THF, 0° C (**b**) 0° C, $B(Et)_3$, NH_2OSO_3H.



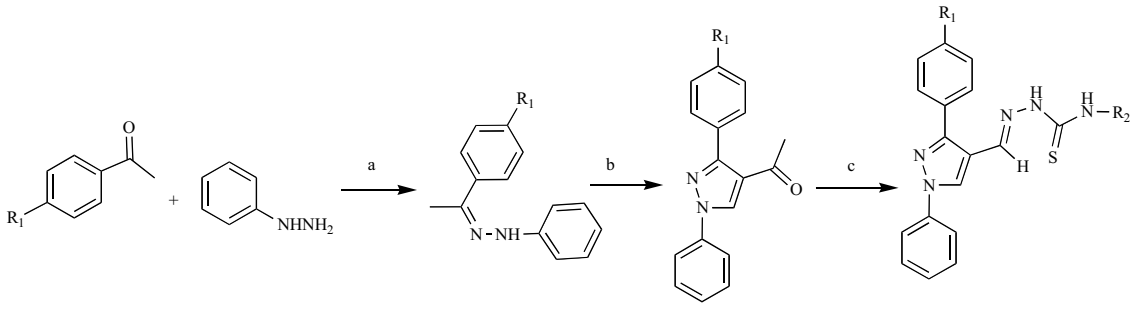
(Scheme 7b). Route A: (**a**) $CISO_3H$; (**b**) NH_4OH. Route B: (**a**) $CISO_3H$; (**b**) NH_4OH; (**c**) H_2, Pd/C.



(Scheme 7c). a) $CISO_3H$; (**b**) NH_4OH, (**c**) Br_2, HBr, HOAc; (**d**) R_3-CS-NH_2. R=H, R_2=SO_2NH_2, R_3= CH_3 substituted derivatives were found to be most potent both by *in-vitro* and *in-vivo* method.

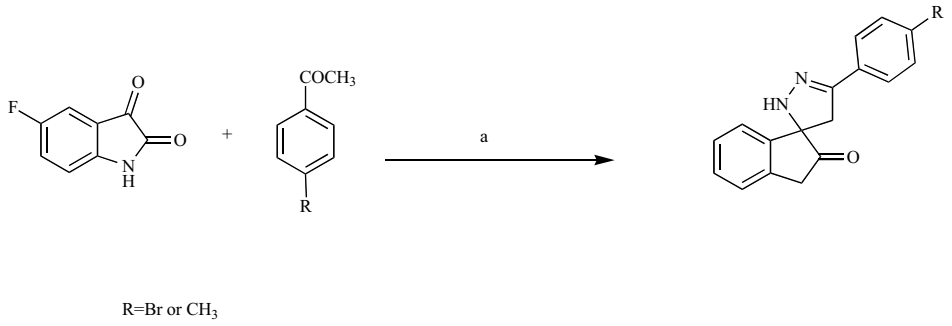


(Scheme 8). (**a**) EtOH/H_2SO_4, reflux 3h (**b**) $NH_2NH_2.H_2O$, reflux 4h (**c**) Gla AcOH, EtOH, reflux 4h.



The compounds show maximum activity with substitutions R_1=OH and R_2=H or R_1=NO_2 and R_2=H (86% inhibition).

(Scheme 9). (**a**) dry ethanol, glacial acetic acid (1 mL) reflux 5 h; (**b**) DMF/$POCl_3$,80℃; (**c**) thiosemicarbazide or 4-phenylthiosemicarbazide, ethanol: chloroform (7:3), reflux 10 h. Compounds with substitutions R_1=OH and R_2=H or R_1=NO_2 and R_2=H showed maximum activity (86% inhibition).



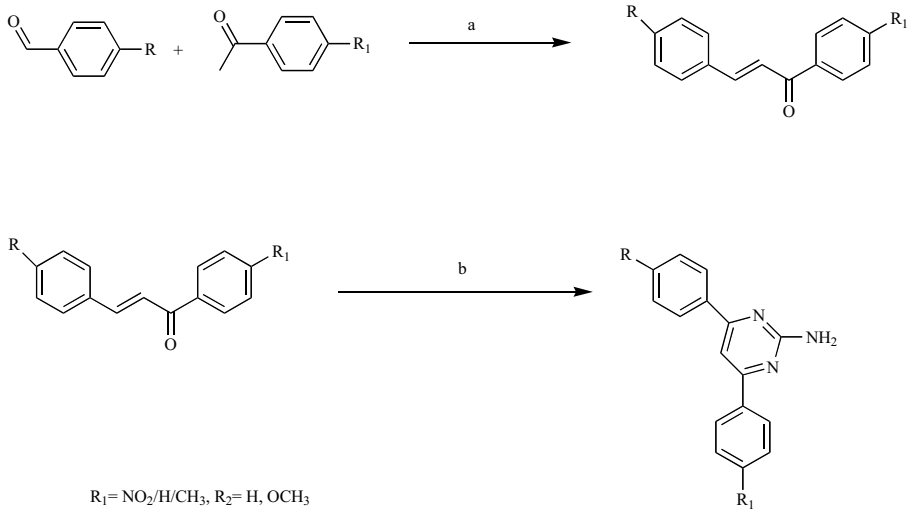
R=Br or CH_3

(Scheme 10). (**a**) $NH(CH_3)_2$/CH_3COOH/HCl, $NH_2NH_2.H_2O$/C_2H_5OH If R=Br or CH_3 then maximum inhibition of COX-2 is reported.

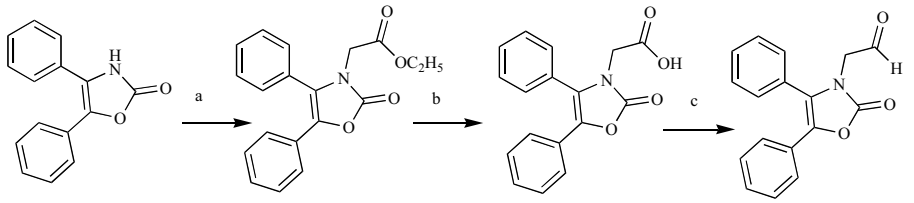
in edema volume. The result of the *in vivo* study revealed that electron withdrawing group substituted analogues showed increase in anti-inflammatory activity considering celecoxib as a standard. The synthetic scheme for the synthesized derivatives is shown in (Scheme **11**) [36].

Y. Dundar *et al.* synthesized series of 4, 5-diphenyloxazolone derivatives by various synthetic procedures as selective COX-2 inhibitors. All the derivatives synthesized were tested using *in vitro* enzyme assay and result reported as the percentage of inhibition of the purified enzymes at 10mM. It is reported in the article that presence of p-sulfone/sulfomoyl group on phenyl ring is important to increase COX-2 inhibitory activity. The result of the *in vitro* enzyme assay revealed that compound 4-(4-phenyl-3-methyl-2-oxo-3H-1,3-oxazol-5-yl)benzenesulfonamide was found to be more selective COX-2 inhibitor. The synthesis of this moiety is illustrated in (Scheme **12**) [37].

S. Hayashi *et al.* designed a series of novel [2-{[(4-substituted or 4, 5-disubstituted)-pyridin-2-yl] carbonyl}-(5- or 6-substituted or 5,6-disubstituted)-1H-indol-3-yl]acetic acid derivatives, which were synthesized and examined to yield more potent molecules as selective COX-2 inhibitors.



(Scheme 11). (**a**) EtOH, 40%NaOH, stirring 3-4 hrs, 0-2°C (**b**) Guanidine HCl, DMF, reflux, 50-60°C. R1= NO_2/H/CH_3, R_2= H, OCH_3.



(Scheme 12). (**a**) K_2CO_3, ethyl bromoacetate, acetone, reflux; (**b**) KOH, water, reflux, 1 N HCl; (**c**) ethyl chloroformate, Et_3N, amine, CH_2Cl_2. R=H.

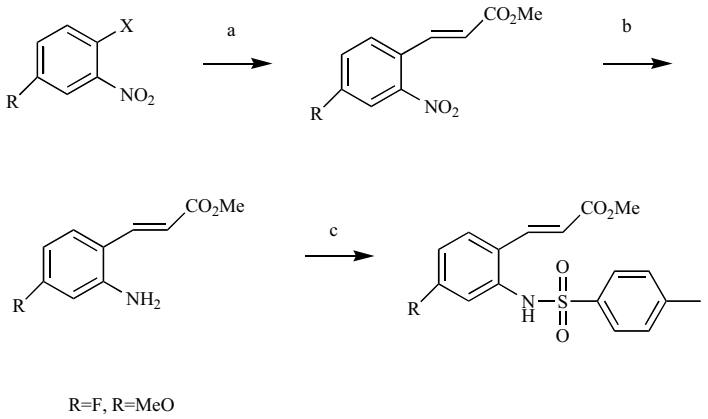
The *in vitro* COX-2 inhibitory activities in human cellular and HWB (Human Whole Blood) assays performed on synthesized molecules reported that substitution on 5th and/or 6th position on the indole ring and 4th and/or 6th position of the pyridine portion greatly enhanced the COX-2 selectivity. In this article, 2-{6-fluoro-2-[4-methyl-2-pyridinyl) carbonyl]-1*H*-indol-3-yl} acetic acid was identified as a potent and selective COX-2 inhibitor by both *in-vitro* as well as *in vivo* studies. The synthesis of the above molecule and its derivatives is shown in (Scheme **13**) [38].

M.R Yadav *et al.* designed and synthesized a series of 3, 4-diaryl-1,2,5-oxadiazoles and 3,4-diaryl-1,2,5-oxadiazole N-oxides. All the synthesized compounds were subjected to *in vitro* screening for COX-2 and COX-1 binding affinity and *in vivo* screening by the rat paw edema method. Amongst all other analogues p-methoxy (p-OMe) substituted compounds showed maximum COX-2 inhibition. Docking studies of these compounds were also done in which p-methoxy substituted compound was found to bind in the pocket of the COX-2 enzyme and also showed various hydrophobic interactions. The synthetic route for the above derivative is shown in (Scheme **14**) [39].

D. Zhao *et al.* synthesized 2-(3',5'-Difluorophenyl)-3-(4'-methylsulfonylphenyl)cyclopent-2-enone against COX-2 enzyme. This compound exhibited high selectivity and good inhibitory activity for COX-2 enzyme. Three schemes for the synthesis of this diarylcyclopentenone are described by the research group. The first reaction involves a Suzuki coupling as the vital step, while the second route includes an intramolecular friedel- crafts acylation. The reaction involving malonate alkylation and acylation followed by ring closure and decarboxylation was found to be more efficient for the synthesis as described in (Scheme **15**). During the reaction, bis alkylated product was formed as a by product which in terms of atom efficiency was not practical for large scale production. The reaction was optimized by the research group considering the amount of reagents and reaction condition to increase the yield of desired product. The best result was obtained with the use of 8 equiv. malonate, K_2CO_3, acetone, 45°C, and slow addition of bromosulfone [40].

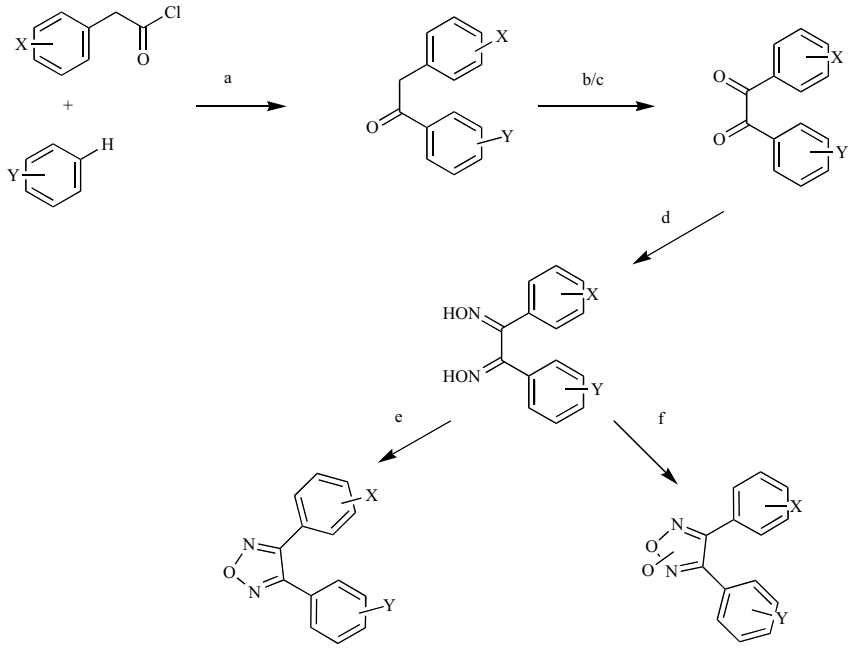
S.K Singh *et al.* designed and synthesized several 2, 3 - diaryl pyrazines and quinoxalines with 4-sulfamoyl (SO_2NH_2)/methylsulfonyl (SO_2Me)-phenyl pharmacophores and evaluated for the cyclooxygenase (COX-1/COX-2) inhibitory activity. *In vitro* screening was performed on synthesized compounds using recombinant human COX-2 enzyme. Structure activity relationships of all these compounds revealed phenyl ring substituted at 4th position with groups such as methoxy, methyl and fluoro, having immense effect on the selective COX-2 inhibitory activity. The compounds displayed higher IC_{50} ratio (COX-1/COX-2) were selected for *in vivo* screening by carrageenan induced rat paw edema method. 4-fluoro phenyl substituted pyrazine class was found to have exceptional *in vivo* potential, and signifies a new class as selective COX-2 inhibitors. The synthesis of these derivatives is shown in (Scheme **16**) [41].

W. Hu *et al.* synthesized a new series of substituted 2-sulfonyphenyl-3-phenyl-indole derivatives. The synthesized



R=F, R=MeO

(Scheme 13). (**a**) Methyl acrylate, $Pd(OAc)_2$, PPh_3,Et_3N, DMF, 130° C, 4-9 h; (**b**) Fe, NH_4Cl, $EtOH/H_2O$, reflux, 2 h; (**c**) p-toluenesulfonyl chloride, pyridine, CH_2Cl_2,10-24 h. R=F, R=MeO.



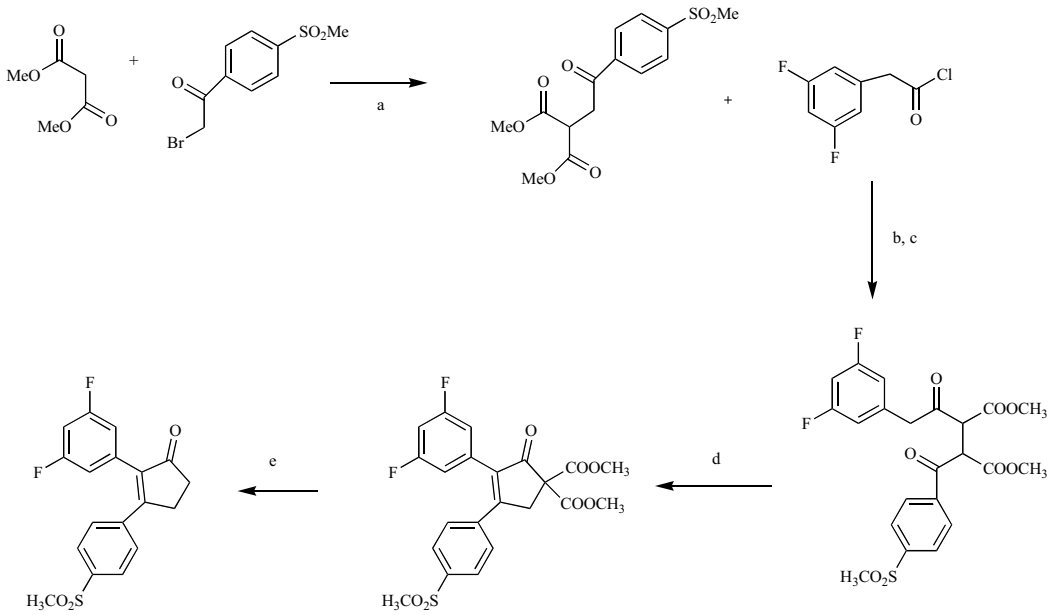
(Scheme 14). (**a**) $AlCl_3$,CH_2Cl_2, 0-60° C, 3-4h; (**b**) SeO_2, Ac_2O, reflux, 1-8h; (**c**) SeO_2, DMSO, microwave irradiation, 30-90s; (**d**) $NH_2OH.HCl$, C_6H_5N, reflux, 7-8h; (**e**) $(CH_3CO)_2O$, 180-5°C, 10 min; (**f**) aq.NaOCl(20%), 5-20° C, 14-16h.

derivatives were tested for their efficacy to inhibit COX-2 and COX-1enzymes by cellular assay using freshly harvested mouse peritoneal macrophages. The majority of the derivatives synthesized was found to be exceedingly potent and selective inhibitors of COX-2. The molecule i.e 2-aminosulfonylphenyl-3-phenyl-indole, was considered to possess higher activity and selectivity for COX-2 than celecoxib both *in vitro* and *in vivo* (using rat carrageenan-induced foot-pad edema model) screening. The route for synthesis of these derivatives is shown in (Scheme **17**) [42].

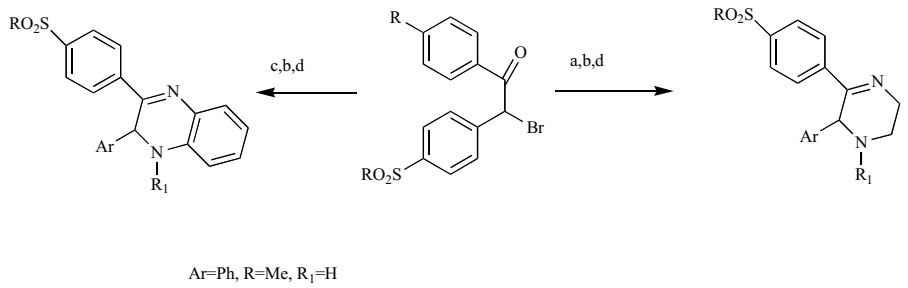
B.J. Al-Hourani *et al.,* synthesized a series of novel 5-substituted 1*H*-tetrazoles as cyclooxygenase-2 (COX-2) inhibitors using various diaryl amides with tetrachlorosilane/sodium azide. These analogues were tested using cyclooxygenase (COX) assays *in vitro* for determining their ability to inhibit COX-1 and COX-2 and also to check their potency and selectivity. Tetrazoles containing a methylsulfonyl or sulfonamide group as a COX-2 inhibitor exhibited only low inhibitory activity towards COX-2. The synthesis of these tetrazole moieties is shown in (Scheme **18**). The central ring containing tetrazole heterocycle was found to be detrimental for the COX-2 inhibitory activity as reported by the research group [43].

COMPUTATIONAL STUDIES

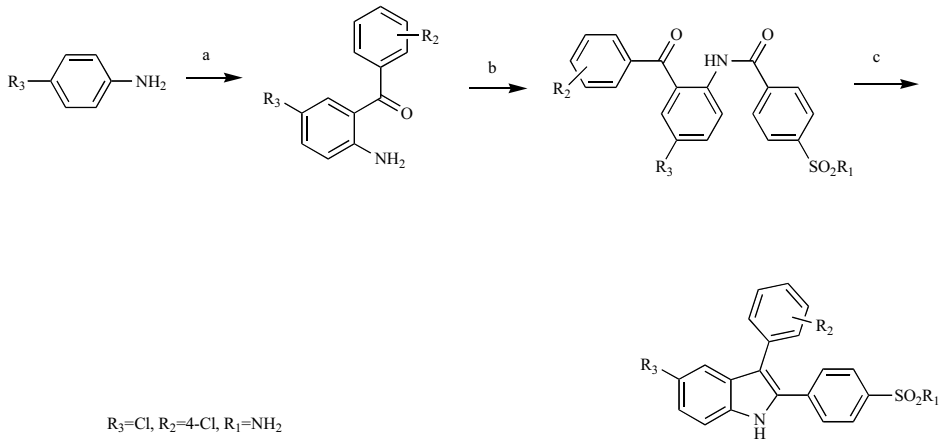
Hye-Jung Kim *et al.* carried out QSAR studies for 88 selective COX-2 derivatives specifically relating to three chemical classes such as triaryl rings, diaryl cycloalkanopyrazoles, and diphenyl hydrazides. QSAR studies were performed using comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) using sybyl 6.7. q^2 values 0.84 and 0.79 for CoMFA and CoMSIA are reported respectively. Also Comparative binding energy (COMBINE) analyses were



(Scheme 15). (**a**) K_2CO_3, Acetone, 45° C; (**b**) $MgBr_2$, Pyridine, MeCN/THF, 0° C; (**c**) -35 to -25° C, 1h; (**d**) 0.2eq. Et_3N, rt, 2h; (**e**) 3M H_2SO_4, HOAc, 90° C, 10h.



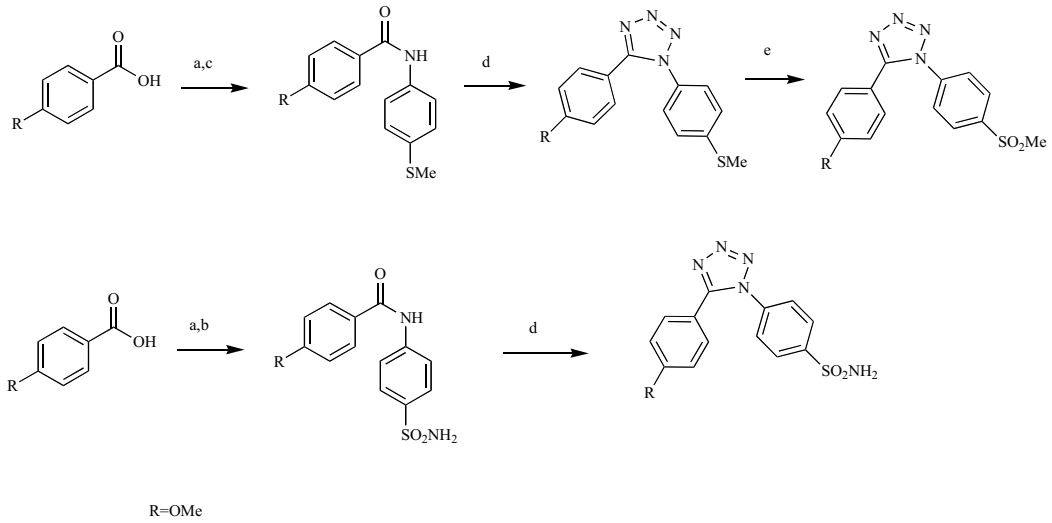
(Scheme 16). (**a**) 1,2-Diaminoethane, MeOH, 0–30 ℃, 4–6 h. (**b**) $(R_1CO)_2O$, TEA, CH_2Cl_2, reflux, 6–7 h. (**c**) O-Phenylenediamine, MeOH, 0–35 ℃, 4–6 h. (**d**) H_2O_2/AcOH, 50–60 ℃. Ar=Ph, R=Me, R_1=H.



(Scheme 17). (**a**) (i) $R_2C_6H_4COCl$, $ZnCl_2$, 205 ℃ (ii) H_2SO_4, 120 ℃; (**b**) 4-$R_1SO_2C_6H_4COCl$, THF, Et_3N, rt ; (**c**) Zn, $TiCl_4$, THF, reflux. R_3=Cl, R_2=4-Cl, R_1=NH_2.

performed using CHARMM program to identify important interacting residue in the protein. Arg513 was identified as important residue to get difference in the binding energy of ligand with protein. q^2 values for the same was obtained 0.64, 0.63, and 0.50 for triaryl rings, diaryl cycloalkanopyrazoles, and diphenyl hydrazides, respectively [44].

T. Narsinghani *et al.* carried out the QSAR studies for around 21 derivatives of meclofenamic acid analogues using



(Scheme 18). (**a**) CDI, THF; (**b**) 4-(aminosulfonyl)aniline; (**c**) 4-(methylthio)benzenamine; (**d**) $SiCl_4/NaN_3$, CH_3CN, 90 ℃, 1 d; (**e**) oxone/acetone/MeOH/H2O, 3 h, r.t. R=OMe (100% inhibition).

MOE 2002.03 software installed on P-III workstation. Several statistical significant regression equations were obtained for both COX-1 and COX-2 inhibition using sequential multiple linear regression analysis method. Statistical significant parameters generated for COX-1 inhibition *i.e.* q^2 and r^2 values are 0.612 and 0.817 while for COX-2 inhibition 0.605 and 0.794 respectively. The study revealed that the number of hydrogen bond donor atoms present in the molecule lead to decrease in selectivity [45].

S.K. Singh *et al.* designed and synthesized almost 34 compounds as selective COX-2 inhibitors with a modified pharmacophore on 1,5-diarylpyrazole. 3D-QSAR CoMFA studies of the diarylpyrazole analogues were carried out. This study consisting comparative molecular field analysis (3D-QSAR CoMFA) gave models with high predictivity. Among the 34 compounds, 24 were included in the training set and the rest 10 in the test set. q^2 value is reported around 0.571 while r^2 is reported 0.986. The results of the study suggested different substitution at C-3, C-5 and N1 of diarylpyrazole derivatives found to be more potent [46].

CONCLUSION

NSAIDs- a class of drugs widely used against various inflammatory diseases. These drugs since their discovery are associated with various unwanted and severe side-effects. The major among them are gastrointestinal bleeding and ulceration. The discovery of selective COX-2 inhibitors side effects was an effort to surpass these side-effects. Various strategies developed to overcome these side-effects are summarized in this review. Structural modifications in existing NSAIDs were done and were proven to improve efficacy, selectivity and reduce toxicity. It was found from the literature review that presence of sulfonamide group leads to increase in potency as well as selectivity for COX-2 inhibition. It is also mentioned in the literature that presence of tetrazole heterocycle as a central ring is detrimental for selective COX-2 inhibitory activity. Prodrug approach for paracetamol was also developed by scientists to reduce the hepatotoxicity of the parent drug, as it is a widely used drug. Ample computational studies were also reported for the identification of important residue of COX-2 enzyme for the development of good pharmacophore. Additional advances or structural modifications can be done in the near future based upon the literature search to develop efficient anti-inflammatory agents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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