# Medicinal Chemistry and Anti-Inflammatory Activity of Nitric Oxide-Releasing NSAI Drugs

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**Abstract:** Nitric Oxide, which acts as a non-specific cytotoxic mediator and a biological messenger on immunological competence, has been gaining significantly increasing importance. As an alternative to conventional NSAIDs having significant side effects, pharmacologically improved and therapeutically enhanced NO releasing non-steroidal anti-inflammatory drugs with less side effects are being planned to produce.

**Key Words:** Nitric oxide, NO-NSAID, anti-inflammatory.

## INTRODUCTION

As a result of the combination of one single nitrogen and oxygen atom, an inorganic gas, nitric oxide (NO) occurs which is also synthesized from L-arginine by the mediation of NO synthase (NOS) [1]. Nitric oxide exerts various physiological roles and pathological impacts in the human body. These impacts and roles influence cardiovascular system as vascular smooth muscle relaxant; cardiac muscle inhibitor and oxygen consumption attenuator of the heart; and also influence thrombocytes by inhibiting thrombocyte aggregation. NO's main effect on gastrointestinal (GI) system is the modulation of tonus and motility along the GI canal (oesophagus, lower oesophagal sphincter, stomach, pyloric sphincter, duodenum, gall bladder, oddi sphincter, ileum, colon, rectum and anal sphincter). Its impact on urinary system appears to occur on kidneys as being the most important paracrine modulator and mediator in governing the renal functions such as renal blood flow, renal autoregulation, glomerular filtration, renin secretion and salt excretion, also on bladder and ureter as being an inhibitor neuromediator secreted from nitrergic neurons both in bladder and ureter. It mainly affects learning and memory processes in the central nervous system. In addition, NO has a crucial role in the process of transpassing from fetal life into neonatal life and it also affects cellular respiration and mitochondrial electron transport chain. Nitric oxide affects both the respiratory system by regulating resistance of airways and genital system as being the main mediator in the erection process. Clinical trials revealed that NO intervenes various pathological conditions such as ischemia and reperfusion injuries, stroke, sepsis, neurotoxicity, macrophage-induced injury, vascular diseases, diabetes, autoimmune diseases, inflammation, pain, cancer, respiratory diseases and pulmonary hypertension [2].

The existing study presents the detailed description of the pain and its mechanism focuses on inflammation and non-steroidal anti-inflammatory drugs (NSAIDs) used in the treatment of inflammation. Classical NSAIDs are currently used in the treatment of inflammatory disorders. New synthetic approaches including chemical modification of NSAIDs might help improving their safety profile. There are several reports on the derivatization of the carboxylate and phenolate functionalities of the representive NSAIDs (Fig. (**1**)) which led to new agents with increased anti-inflammatory activity and reduced ulcerogenic effects [3-14].

Morever, the most crucial feature of nonsteroidal anti-inflammatory drugs releasing NO (NO-NSAIDs), recommended as an alternative to classical drugs having serious adverse effects and limited therapeutical benefits in the gastrointestinal region, are lowering the gastrointestinal toxicity of NSAIDs by releasing NO. In addition, NO has a protective effect on the stomach, which includes the vasodilatation of the local mucosal blood vessels. Within this context, a number of new molecules which are attached to the core molecule without any pharmacological and chemical connections with NSAIDs and releasing NO, have been recently synthesized. Experimental trials on both acute and chronic pain treatments using animal models were initiated to develop a new molecule with an improved pharmacological profile, a strong therapeutical effect and less adverse events. The present project aims to provide a detailed information on NO-NSAIDs research.

## POTENTIAL THERAPEUTICAL INDICATIONS AND PHARMACOLOGY OF NITRIC OXIDE – NSAIDS RELEASING NITRIC OXIDE

Traditional NSAIDs are currently used for the treatment of inflammatory disorders. However, these drugs generate serious adverse effects limiting their therapeutical benefits in the gastrointestinal tract. NO-NSAIDs are the last development in the list of advances including enteric-coated tablets, prodrugs and selective COX-2 inhibitors which are created to prevent the gastric side effects of NSAIDs. The property of NO-NSAIDs is to reduce the gastrointestinal toxicity of NSAIDs by releasing NO.Various mechanisms highlight the

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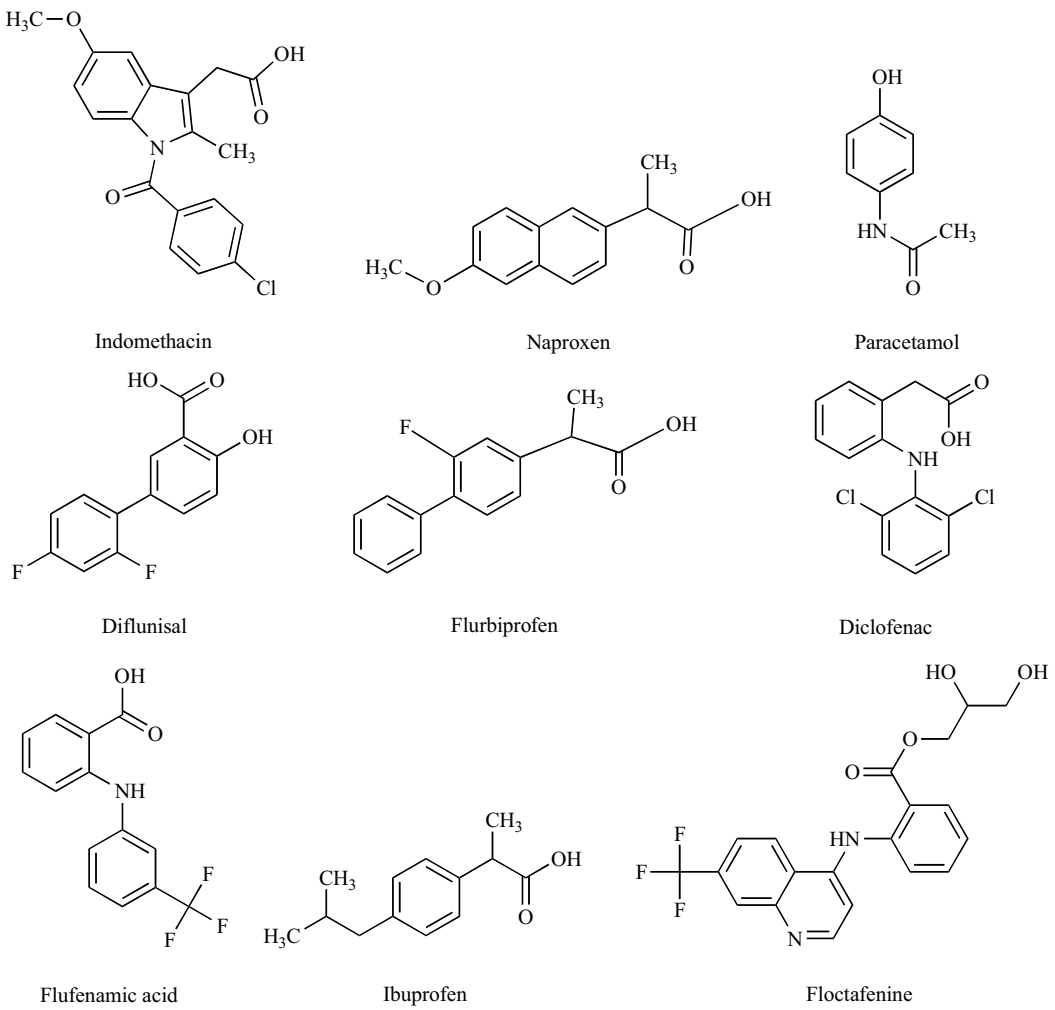


**Fig. (1).** Chemical structures of the representative NSAIDs.

gastric protective effect of NO including vasodilatation of local mucasal blood vessels [2].

A number of new NO secreting molecules attached to the core molecule without any pharmacological and chemical relations with NSAIDs have been recently synthesized. The objective is to define a new molecule with an improved pharmacological profile, stronger therapeutical effect and less toxic effect**.** Within this context, experimental trials using animal models were executed for both acute and chronic pain treatment [15].

Various new strategies were developed for the production of LOX/COX inhibitors with this dual effect such as selective or non-selective COX inhibitors and hybrid molecules releasing nitric oxide to decrease the side effects of NSAIDs [16]. Recent data indicate serious cardiovascular side effects of selective COX-2 inhibitors and in addition, these drugs have no impact on established gastric ulcer though preventing the occurrence of new ones.

In these trials, formation of hybrid molecules with non-selective COX inhibitors and the NO donor is one of the the most promising strategies, because nitric oxide supports the endogenous GIT defence mechanism *via* its protective effects such as increasing mucus, secreting bicarbonate, increasing mucosal blood flow and decreasing proinflamatory cellular activation. In addition, NO provides a protection against the adverse effects of COX-2 *via* its cardiovascular activity. Among NO-NSAIDs thus formed, nitrodiclofenac, nitroaspirin, nitroflurbiprofen and nitroketoprofen were tested in clinical trials (Fig. (**2**)). Furoxan, oxime, hydrazide and organic nitrates are nitric oxide donors used in the validation of this concept. However, long-term safety profile of these components are currently investigated.

Based on these data, hybrides and prodrugs releasing nitric oxide with anti-inflammatory, analgesic and ulcerogenic properties, are combined with nitric oxide donors as organic nitrates and oximes to gain new molecules.

Abadi A.H. *et al.* [17] synthesized six derivatives with the general formula of 2/4-(7-trifluoromethylquinolin-4-yl-amino) benzoic acid N′-(nitrooxyacetyl/propionyl) hydrazide and oxime derivatives with the formula of 1-[4-(7-trifluoromethylquinolin-4-yl-amino) phenyl)ethanol (Fig. (**3**)) and tested their capacity of nitric oxide release *in vivo* and anti-inflammatory, analgesic and ulcerogenic properties. 2-(7-Trifluoromethylquinolin-4-yl-amino)benzoic acid-N′-(2-nitrooxypropionyl)hydrazide was more potent than indo-

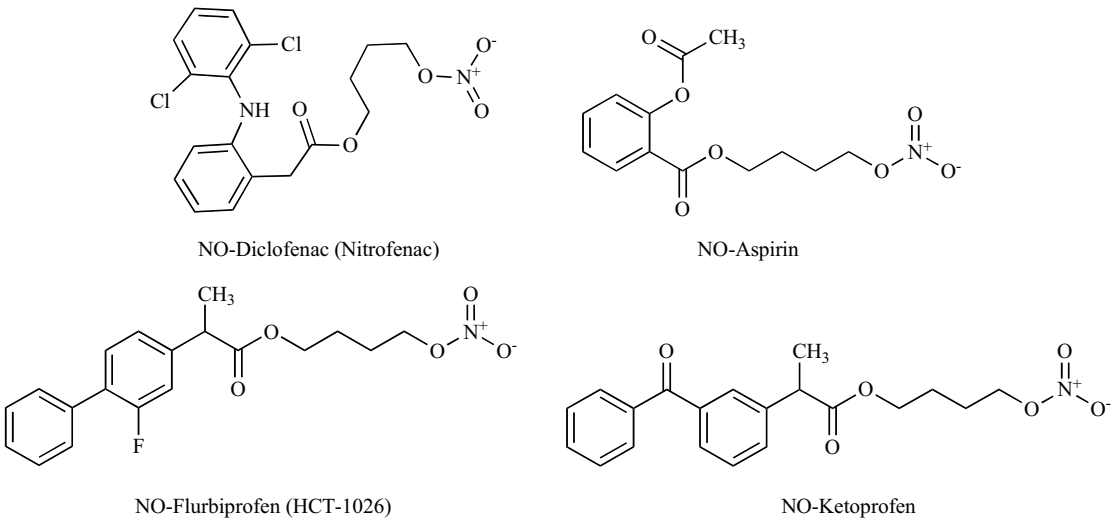


**Fig. (2).** Conservative NSAIDs examples combined with NO.

methacin which is used as standard anti-inflammatory drug. Some compounds were reported devoid of gastric ulcer tendency. In addition, nitric oxide contributed to the excellent safety profiles of these compounds.

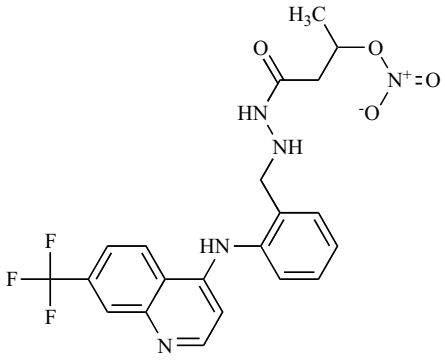

**Fig. (3)**. 2-(7-Trifluoromethylquinolin-4-yl-amino)benzoic acid-N′-(2-nitrooxypropionyl) hydrazide.

Ranatunge *et al*. [18], with a similar approach, developed an anti-inflammatory product with gastric protective effect decreasing GI injury by the COX inhibition and granting NO inhibiting COX (CINODs) as an alternative to selective COX-2 inhibitors. Within this context, glycolamide prodrugs of naproxen possessing a number of NO donor component were synthesized. Naproxen sodium and prototype AZD3582 (Fig. (**4**)) synthesized in the study where carregeenan paw oedema model was applied, exhibited similar analgesic and anti-inflammatory effects but they caused significantly less gastric injury.

In Ranatunge's study, some of the synthesized compounds showed comparable anti-inflammatory effect to naproxen sodium from classical NSAID group. Among the target compounds tested, N-methyl glycoamide appears to be the best prodrug. Some of the compounds whose activities match that of naproxen sodium at equivalent doses caused gastric injury in rats. *N*-Methyl-*N*-{{{2-(nitroxy)ethyl)oxycarbonyl}methyl}carbamoyl}methyl-(*2S*)-2-{(6-metoxy-(2-naphthyl)} propanoate was chosen to show that glycolamide nitrate could be bioactivated to release NO. Based on these data, naproxen glycolamide nitrate prodrugs appear to be the safest alternative to naproxen sodium in the treatment of inflammatory diseases and pain.

Hawkey *et al*. [19] gastrointestinal safety of naproxen and [4-(Nitrooxy]butyl-(2S)-2-(6-metoxy-2-naphthyl)propanoate] (AZD3582) were conducted. These studies are currently at the terminal period of phase II and global marketing activities have already started. Also, developed by French Pharmaceutical company NicOx SA, Naproxcinod (HCT 3012) [(*S*)-6-methoxy-α-methyl-2-naphthaleneacetic acid 4-(nitrooxy)butyl ester is currently at the investigation stage [20]. This compound is the company's lead product and in phase III.

Ziakas *et al*. [21] synthesized the nitric oxide derivatives of tolphenamic acid (TA) (Fig. (**5**)). In this trial, several TA derivatives esterified with nitrooxyalcohol were synthesized and their anti-inflammatory activities, NO releasing property and anti-oxidant effects were evaluated and finally their gastrointestinal toxicities were compared.

In this study, two different methods (Fig. (**6**) and Fig. (**7**)) were applied in the production process and it appears that the second method was preferred as the most appropriate due to low cost and feasibility.

The synthesized compounds were found to exhibit carregeenan edema lowering effect of TA. All items had significant anti-inflammatory activity. In the comparison of activity, it has been found that 2-nitrooxy-1-nitrooxymethylethyl 2-(3-chloro-2-methylphenylamino) benzoate having two nitrooxyetoxy groups demonstrated more anti-inflammatory activity than 2-nitrooxyethyl 2-(3-chloro-2-methylphenylamino)benzoate which has a single group. The results of *in vivo* and *in vitro* studies indicate that 2-nitrooxy-1-nitrooxymethylethyl 2-(3-chloro-2-methylphenylamino)benzoate (Fig. (**8**)) having two nitric acide groups is the most active item with the structure composed of two chains and two carbons having carboxylic oxygen. This product decreased the

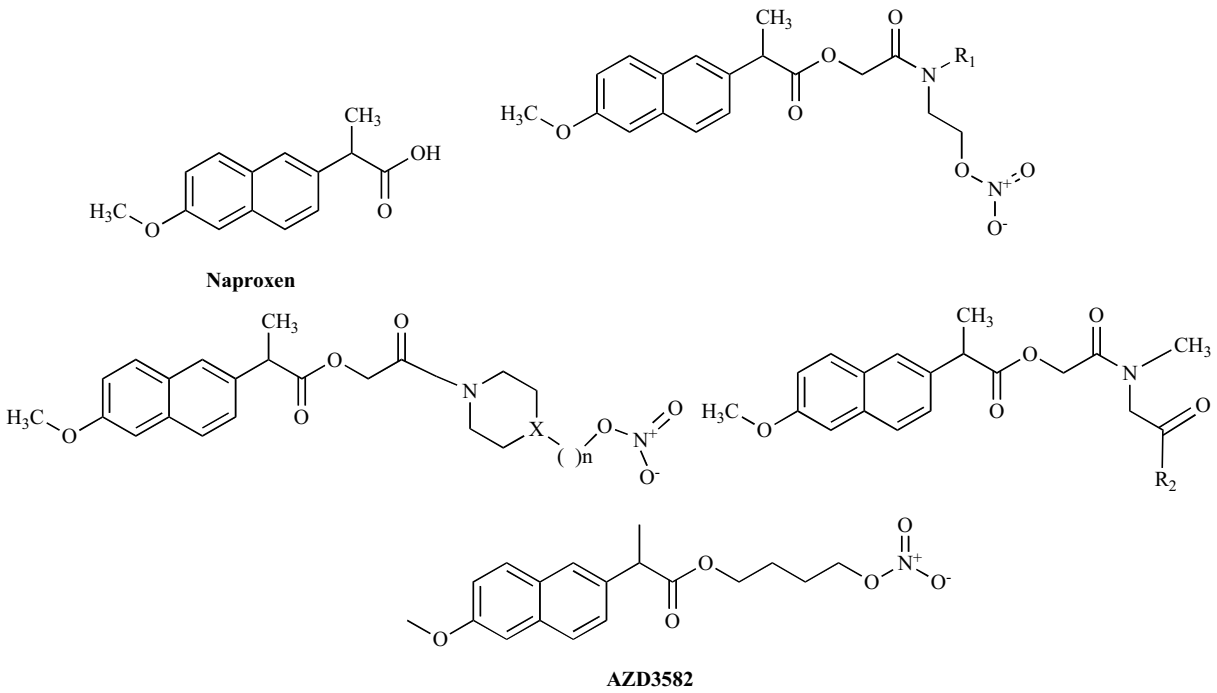


**Fig. (4).** Glycolamide prodrugs of naproxen.

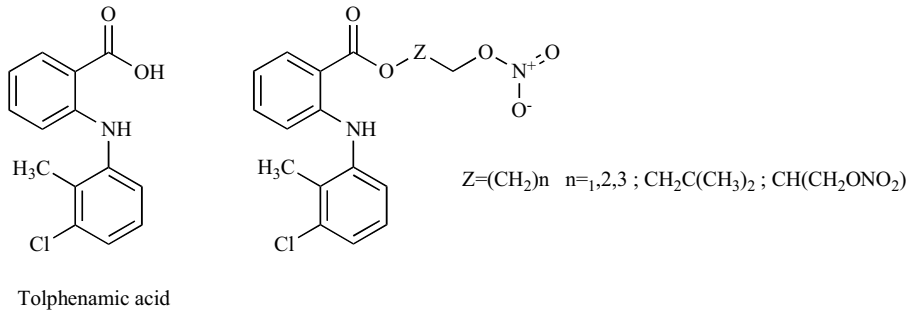


**Fig. (5).** The structure of Tolphenamic acid and its esthers together with their NO releasing components.

carrageenan rat paw oedema more than TA and other derivatives, caused less general and gastrointestinal toxicity.

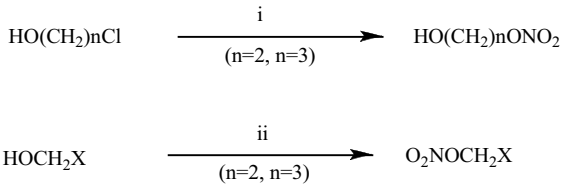


**Fig. (6).** Two methods for the production of nitrooxyalcohols.

Consequently, among carboxylic and nitric ester groups with two-three carbon chains, NO donors inhibit cyclooxygenase at optimum levels.

In several studies, nitric oxide was shown to modulate the activities of cyclooxygenase (COX) and lipooxygenase (LOX) [22]. Considering the pro-inflammatory properties of leukotrienes (LTs) and prostanoids, the capacity of drugs to inhibit the synthesis of eicosanoids (double effect inhibitors) entitle them for not only demonstrating a strong anti-inflammatory activity but also less side effects than COX-2 inhibitors and NSAIDs [23] (Fig. (**9**)).

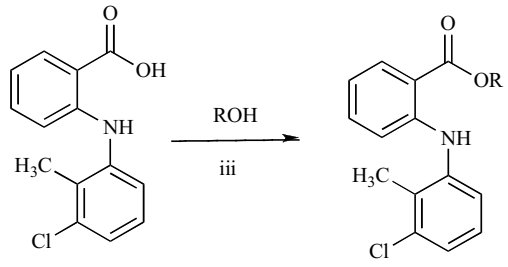


**Fig. (7).** Synthesis of final TA esthers.

Indomethacin is a nonselective cyclooxygenase (COX) inhibitor. In a study performed by Wey *et al*. [24], it was modified in three distinct regions. As a result cyclooxygenase-2 (COX-2) selectivity is increased and covalent link of an organic nitrate moiety as a nitric oxide donor was formed so that drug safety is significantly enhanced. The gastric toxicity and the increased cardiovascular risk of COX-2 selective inhibitors can be improved by supplemen-

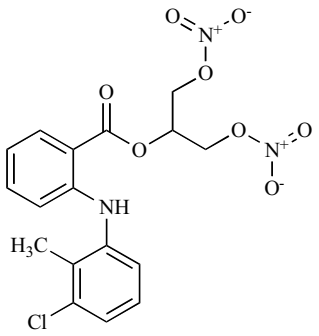

**Fig. (8).** 2-nitrooxy-1-nitrooxymethylethyl 2-(3-chloro-2-methylphenylamino)benzoate.

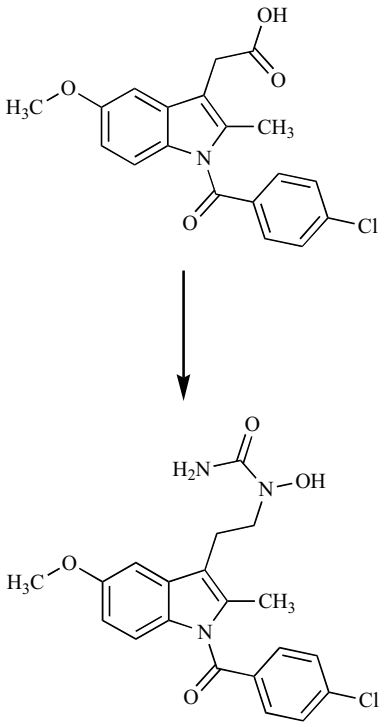

**Fig. (9).** Indomethacin derivative of double-effect COX-2/5-LOX inhibitor.

tary NO with the help of aspirin intake. The two pairs of propoxy-amide derivatives numbered 2-{1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methylindol-3-yl}-*N*-(3-hydroxypropyl)acetamide and 2-{1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methylindol-3-yl}-*N*-(3-nitrooxy)propyl)acetamide (Fig. (**10**)) yielded the best results in this study.

These compounds were further examined using *in vivo* experiments for antiinflammatory activity, gastric tolerability, and NO releasing activity. The results of GI tolerance examinations demonstrated a 98% reduction in gastric lesion score when compared to equimolar indomethacin. The concomitant use of aspirin and a COX-2 inhibitor is a popular treatment for cardiovascular prophylaxis is shown to increase gastric damage significantly when compared to using either aspirin or the COX-2 inhibitor alone. The examinations also showed that intake of aspirin with the compound 2-{1-[(4-chloro phenyl)carbonyl]-5-methoxy-2-methylindol-3-yl}-*N*-(3-nitrooxy)propyl)acetamide yields a 85% decrease in gastric-sparing effect compared to coadministration of valdecoxib and aspirin.

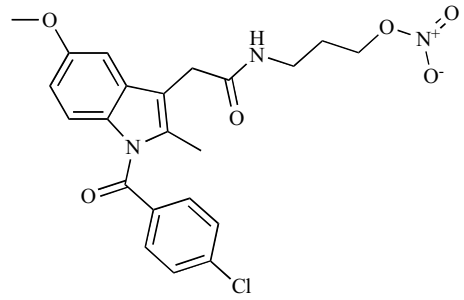

**Fig. (10).** 2-{1-[(4-Chlorophenyl)carbonyl]-5-methoxy-2-methylindol-3-yl}-*N*-(3-nitrooxy)propyl)acetamide.

The study has proved that a NO-enhanced COX-2 selective inhibitor, 2-{1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methylindol-3-yl}-*N*-(3-nitrooxy)propyl)acetamide can be synthesized from indomethacin, with a notably anti-inflammatory effect *in vivo* and almost non-observable GI liability by itself and when coadministered with aspirin.

Tepoxaline, a double-effect COX/5-LOX inhibitor, plays a role in the tricylic path of selective COX-2 inhibitors. In addition, Tepoxaline which is a hydroxamic acid (acid hydroxamide) of pyrazole content may joint 5-LOX with non-heme iron to form a "chelate". It is currently evaluated in psoriasis and romathoid arthritis [23] (Fig. (**11**)).

Among NO-NSAIDs combinations, NO and aspirin combination is an attractive new molecule. Aspirin is the most widely used NSAID in the world. It has been reported

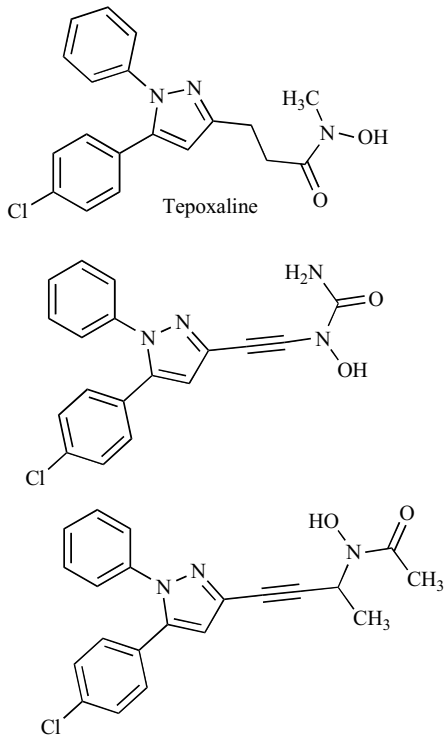


**Fig. (11).** The structure of tepoxaline and its derivatives.

that ester connections are broken down by esterases in the body and salicylates and nitrates are separately released and experimental trials on the protective effect of aspirin on GIS (GI tract) revealed that there was no relationship between acute mucosal injury and plasma salicylate concentrations [25].

NO-aspirin and NO-donors (e.g. sodium nitroprusside) prevent mucosal apoptosis and caspase activation. Caspase inhibitors maintain protection against mucosal damage. This protection shows that one of the main actions of NO-aspirin is to prevent apoptosis.

Following the approval of phase I clinical trials of NO-aspirin by American Food and Drug Administration, FDA in the year 2000, a 7-day study was performed in order to observe the positive effects of benzoic acid 2-(acetyloxy)-3-[(nitrooxy)methyl ) phenyl ester (NCX 4016) (Fig. (**12**)) in the endoscopic injury. As a result of this study, NCX 4016 was found to have the same benefits which were measured by thrombocyte aggregation, thromboxane B2 (TxB2) and thrombocyte TxB2 levels, accompanied by much less gastrointestinal side effect compared to aspirin at equivalent doses. [26]. It was proven by the measurements resulting in high levels of plasma salicylate and nitrate/nitrite that the drug is absorbed in GI tract and NO is released into circulation. However clinical trials are continued to gather new supporting results [20].

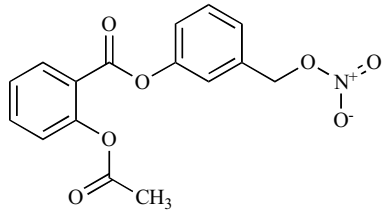

**Fig. (12).** NCX 4016.

Metabolic pathway of Nitroaspirin which is a pioneering product of NO-NSAIDs releasing NO was investigated at the single dose of 1600 mg in eight healthy white persons. NCX 4015 metabolite (Fig. (**13**)) was detected *via* LC-MS/MS analysis in plasma samples used in NCX 4016 metabolism study [27].

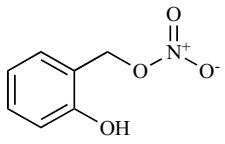

**Fig. (13).** NCX 4015.

Carini *et al*. [28] studied on NCX 4016 which is the lead of a new class of anti-inflammatory agents as a safer alternative to aspirin for the use in hypertensive patients for primary prevention of cardiovascular diseases [29, 30] intend to see the gastro protective effect of releasing NO upon NO-NSAIDs. The study has been done in the rat following p.o. and i.p. administration of 100 mg/kg, by monitoring in plasma the bioactive storage forms of NO (S-nitrosothiols, RS-NO) and its oxidation products (nitrites/nitrates, NOx) by a chemiluminescent assay. Plasma was analyzed in parallel for unchanged drug and metabolites by reverse-phase HPLC (Fig. (**14**)). At the end of the study, it is discovered that detection of unchanged drug is up to 8 h post-dosing and the only metabolite to be detected was the *O*-deacetylated derivative (NCX 4023), which was present in low concentrations up to 4 h post-dosing. It refers to NCX 4016 that does not undergo biotransformation in the upper part of gastrointestinal tract and that the stomach acts as a reservoir for the drug.

Consequently, the results of this study point out that NCX 4016 demonstrates lower gastrointestinal toxicity in comparison with aspirin in addition to the maintaining the anti-inflammatory action.

Another study of NO releasing drugs is designed by Wallace *et al*. [31] and the purpose of the study was to determine the contribution of nitric oxide (NO) and ferulic acid (antioxidant moiety) to the pharmacological properties of [3-[4-(2-fluoro-α-methyl-[1,1′-biphenyl]-4-acetyloxy)-3-methoxy-phenyl]-2-propenoic acid-4-nitrooxy butyl ester (NCX 2216) in comparison with flurbiprofen. With this aim, gastric tolerability and suppression of prostaglandin synthesis is compared. Oral flurbiprofen produced extensive gastric damage and suppressed gastric prostaglandin synthesis. In contrast, while suppressing prostaglandin production, equimolar doses of NCX 2216 did not cause detectable gastric injury. The NO-releasing moiety of NCX 2216 is shown crucial effect for the gastric safety of this compound. NCX 2216 inhibited prostanoid synthesis, however it could not be detected in plasma and even it produced only low amounts of flurbiprofen in plasma and in the brain. Inhibition of brain prostaglandin synthesis by NCX-2216 lasted for a much longer period of time than was seen with flurbiprofen. According to these results, we ascertain that a single administration of NCX 2216 can produce prolonged suppression of brain prostaglandin synthesis without causing gastric injury. Namely, an active metabolite of NCX 2216 contributes to the suppression of cyclooxygenase activity. NCX 2216 can act as an important alternative to conventional NSAIDs for long-term treatment of the inflammatory disorders (Fig. (**15**)).

The experiments with the various compounds with structural similarities to NCX 2216 support the hypothesis that it is the NO-releasing moiety of this compound that accounts for its gastric safety profile. Through NO donors, the severity of experimental gastric injury and gastrointestinal bleeding are reduced. This study demonstrated that NCX-2216 does not produce damage to the stomach, despite being able to suppress gastric PG synthesis as effectively as the parent drug. Even it shows a long-lasting supression of PG synthesis in the brain. It is additionally demonstrated that the NO-releasing moiety of NCX 2216 is inducing gastric damages, although the ferulic acid moiety does not present the same feature.

A different study exploring NO's effects was applied by Jiao *et al*. [32]. In this paper, a high-performance liquid chromatography method for simultaneous determination of ZLR-8 and its active metabolite diclofenac in the plasma of beagle dogs had been implemented. It was tested through six

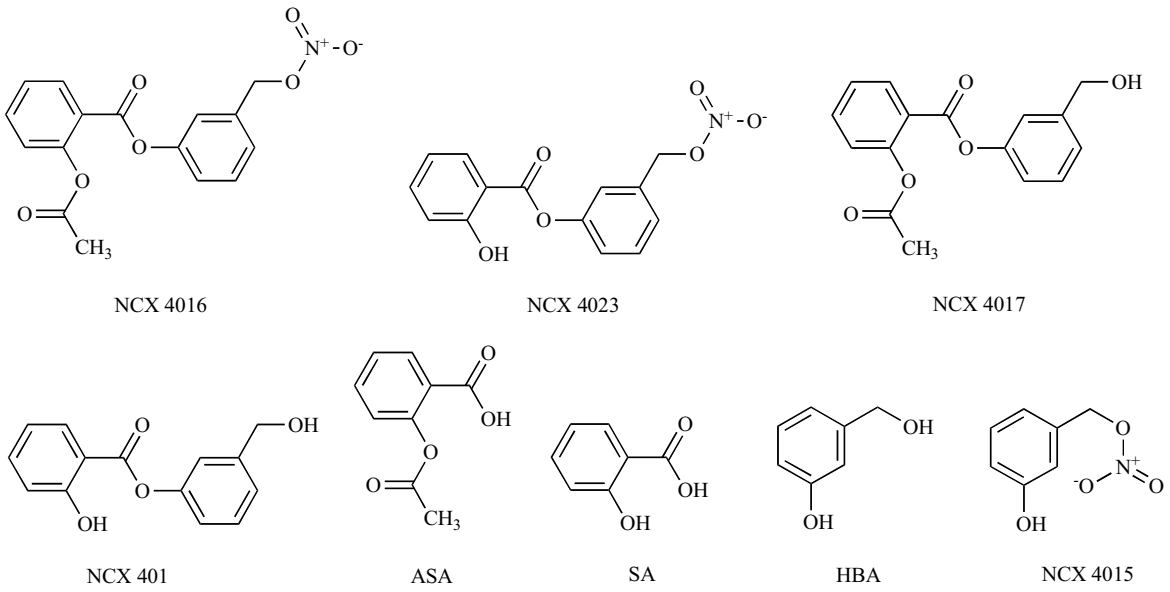


**Fig. (14).** Structures of NCX 4016 and of some postulated metabolic products.

different plasma batches. This method had been developed in preclinical pharmacokinetics studies of ZLR-8 and its active metabolite diclofenac (Fig. (**16**)).

The HPLC-UV method had been built up for the analysis of ZLR-8 and its active metabolite diclofenac in plasma. As an NO-releasing compound, ZLR-8 was synthesized with the aim of representing lower gastrointestinal toxicity in comparison to diclofenac besides maintaing the antiinflammatory action.

In conclusion, supported by this research, it was proven that NO appears to play an important role in GI homeostasis as if it is the guardian of the cell, together with prostaglandins. Formerly, in Li *et al*. [33] studies, ZLR-8 has been proved to have greater anti-inflammatory and analgesic activity and lower GI side effects than diclofenac and other NSAIDs.

Engelhardt *et al*. [34] synthesized an NO-releasing prodrug of rofecoxib (Fig. (**17**)) due to the fact that rofecoxib being a COX-2 inhibitor significantly increases the gastrointestinal injury caused by aspirin. As a result, producing the

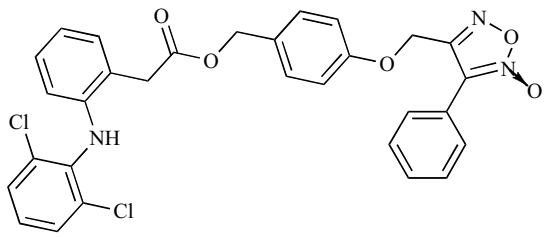

**Fig. (16).** Chemical structure of ZLR-8.

COX inhibiting nitric oxide donors (CINODs) is researched in this study intending to minimize gastrointestinal toxicity, by the mediation of nitric oxide release, compared with standard nonsteroidal anti-inflammatory drugs (NSAIDs). The process consists of five chemical steps and produces prodrug

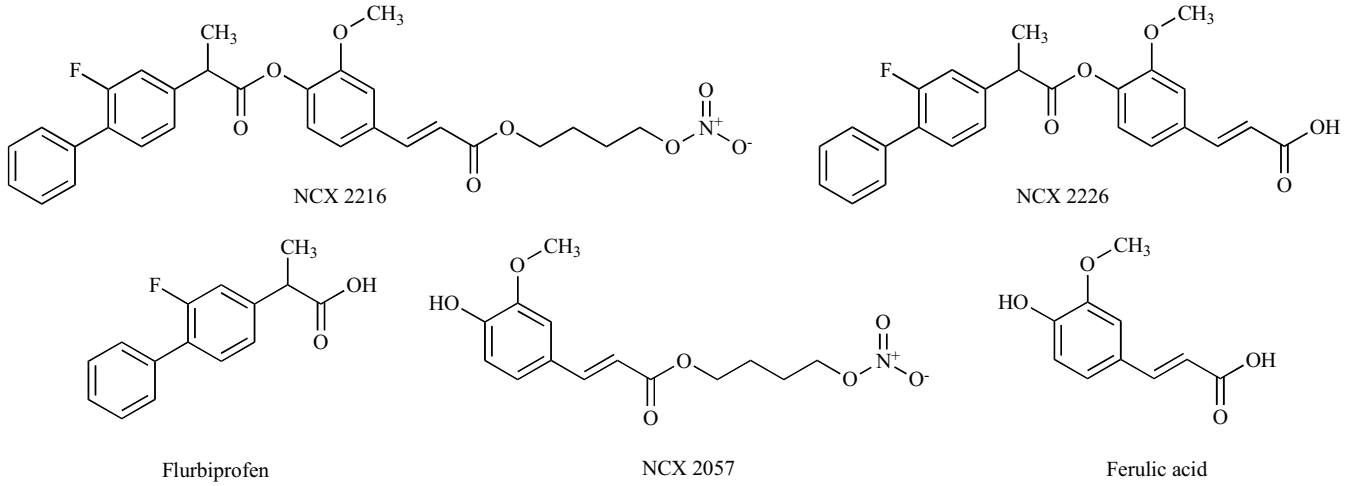


**Fig. (15).** Chemical structures of NCX-2216 and other related compounds.

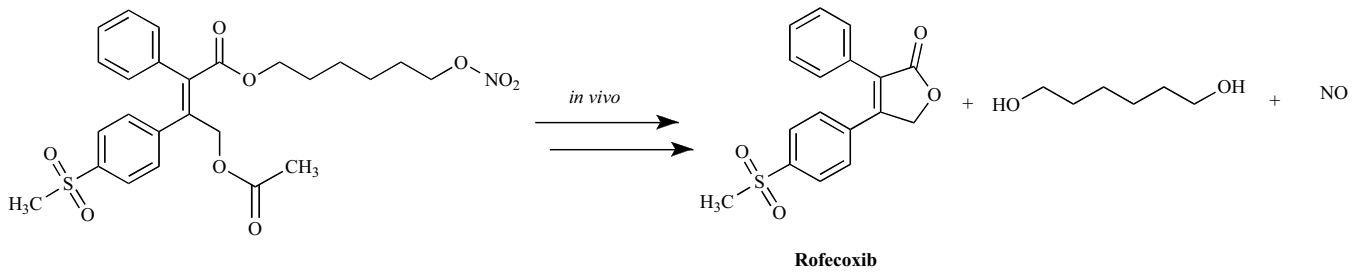


**Fig. (17).** Breakdown of pro-drug NO-Rofecoxib *in vivo*.

NO-Rofecoxib in a 64% yield from 3-phenyl-2-propan-1-ol which is commercially available.

The last recent study researching NO effects upon NO-releasing NSAIDs was developed by Laleman *et al*. [35]. They studied whether administration of nitroflurbiprofen (HCT 1026) (Fig. (**2**)), with nitric oxide NO-donating properties, regulates the increased intrahepatic vascular tone in portal hypertensive cirrhotic rats. This researcher tried to find out impaired intrahepatic NO production, which stimulated the clinical use of nitrovasodilators for the treatment of portal hypertension. The aim is to enhance therapeutic efficacy or reduce adverse effects.

The article mentioned above, aims to find out whether nitroflurbiprofen (HCT 1026), an NO releasing NSAID, can decrease the portal pressure in a rat model of thioacetamide-induced cirrhotic portal hypertension. Morever, the present paper demonstrated that nitroflurbiprofen, as well as its parent NSAID flurbiprofen, equally decreased portal hypertension in cirrhotic rats, along with preventing the hyperdynamic circulation and splanchnic hyperemia. The study is implemented in the *in situ* perfused cirrhotic rat liver and using nitroflurbiprofen and flurbiprofen equally provided lowering in the increased total intrahepatic resistance.

Consequently, regarding the results of the present study nitroflurbiprofen (HCT 1026), an NO-releasing NSAID, leads to a decreased portal pressure without systemic adverse effects in cirrhotic rats. The related positive effects are provided thanks to abrogation of TxA2 production and the replacement of the decreased intrahepatic NO availability as proven *in vivo*, in the *in situ* perfused liver, and *in vitro*. The portal hypertension is cured without any adverse effects by nitroflurbiprofen, an NO-releasing cyclooxygenase inhibitor, as a result of attenuating intrahepatic vascular resistance, endothelial dysfunction, and hepatic hyperreactivity to vasoconstrictors.

## CONCLUSION

Because conservative NSAIDs generate serious adverse effects limiting their therapeutical benefits in the gastrointestinal region, new trials are being done to find out a new molecule with an improved pharmacological profile, a stronger therapeutical effect or a less toxic effect. The trials collected in this paper prove that NO releasing NSAIDs demonstrate these features because of NO molecule's extraordinary structure.

## ABBREVIATIONS

| | | |
|---|---|---|
| COX | = | Cyclooxygenase |
| EDRF | = | Endothelium derived relaxing factor |
| FDA | = | U.S. Food and Drug Administration Agency |
| GI | = | Gastrointestinal |
| GIS | = | Gastrointestinal system |
| GIT | = | Gastrointestinal tract |
| LOX | = | Lipooxgenase |
| NO | = | Nitric oxide |
| NOS | = | Nitric oxide synthase |
| NSAID | = | Nonsteroidal anti-inflammatory drug |
| PG | = | Prostaglandin |
| CNS | = | Central nervous system |
| TA | = | Tolphenamic acid |
| TxA2 | = | Thromboxane A2 |
| TxB2 | = | Thromboxane B2 |

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