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Hybrid organic molecules as antiinflammatory agents; a review of structural features and biological activity

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Abstract: Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used for the treatment of pain and inflammation. Some undesirable effects are linked with NSAIDs such as the gastrointestinal tract (GIT) toxicity and cardiovascular disturbances. At present the preparation of a hybrid molecular technique is being used to produce new analgesic and antiinflammatory molecules. Attachment of NSAIDs with nitric oxide and hydrogen sulfide releasing molecules produced some gastroprotective agents with improved analgesic and antiinflammatory activities. Combination of NSAIDs with different biologically active 5-membered, 6-membered, and condensed heterocyclic rings has also led to the formation of some potent molecules. Some of these hybrid molecules, e.g., ibuprofen–thiazole, exhibited less GIT toxicity, while others showed selectivity for COX-2 enzyme, e.g., quinazolinone–pyrimidine and benzothiophene–rhodanine hybrids. COX-2 selectivity was also exhibited by hybrids of NSAIDs with natural molecules such as salicylates–resveratrol, chromone–oxindole, and chrysin–indole–pyrazole. The preparation of new hybrid molecules is significant because they can serve as a lead compound for the discovery and development of safer analgesic and antiinflammatory agents.

Key words: NSAIDs, hybrids, pharmacophore, inflammation, gastrointestinal toxicity, cyclooxygenase 1 and 2, carrageenan induced paw edema

1. Introduction

Inflammation is a physiological process that results from some external or internal stimulus to the body. It is basically a part of the defense mechanism of the body.[1,2] Acute inflammation results in edema and cellular influx due to changes in vascular permeability and local hemodynamics.[3] An acute inflammatory response in the body is not very dangerous but chronic inflammatory condition produces diseases like asthma, rheumatoid arthritis, and cancer.[4] Nonsteroidal antiinflammatory drugs (NSAIDs) are the most important agents used for the treatment of inflammation. They are also used as an analgesic and antipyretic. The mechanism for the antiinflammatory activity is the inhibition of prostaglandin synthesis. Generally NSAIDs are the nonselective inhibitors of both isoforms of cyclooxygenase enzyme, i.e. COX-1 and COX-2.[5,6] Continuous use of NSAIDs even for 5 to 7 days causes some side effects such as gastric ulcers and therefore long-term use of NSAIDs is problematic.[7] Renal toxicity, hepatotoxicity, and cardiovascular side effects are some other problems associated with use of NSAIDs.[8,9] The presence of free carboxylic acid in many traditional NSAIDs is the most common cause of GIT toxicity (Figure 1).[10] The development of COXIBs as selective COX-2 inhibitors was advantageous because these compounds have less potential to cause GIT toxicity.[11] However, cardiovascular side effects are

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associated with these drugs and they resulted in the withdrawal of rofecoxib from the market, which is a selective COX-2 inhibitor.[12] Therefore, the search for new and safer NSAIDs is still very important in order to develop more active and less toxic agents.

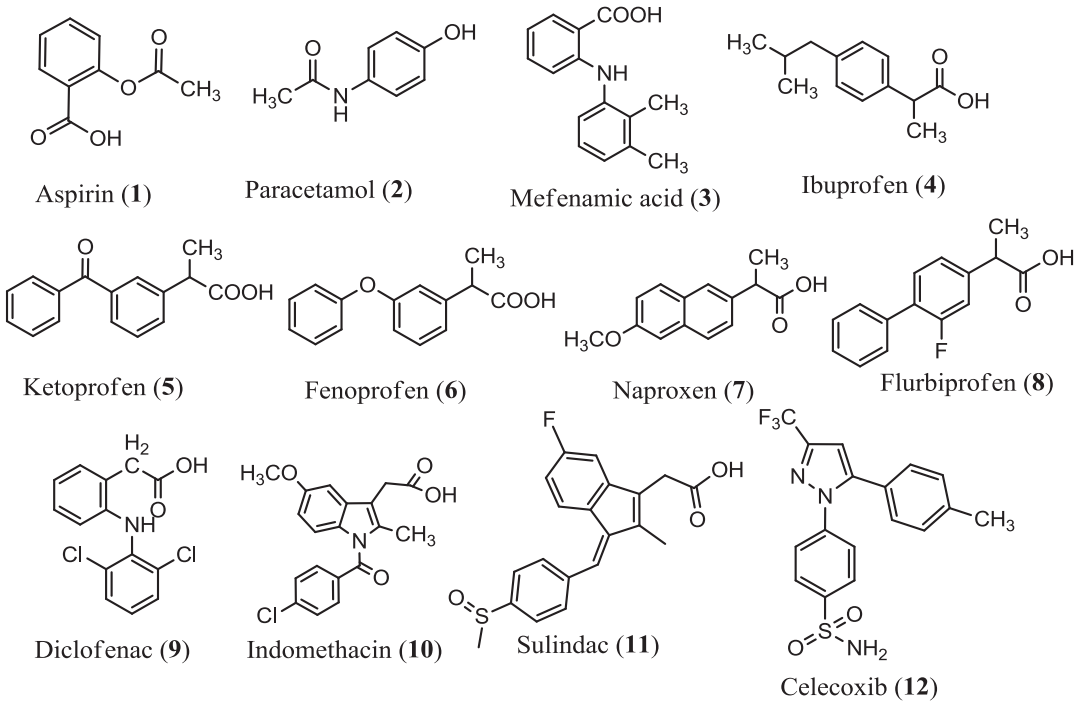


Figure 1. Structure formulas of common NSAIDs.

A hybrid molecule is a synthetic compound in which two or more compounds are combined by a chemical bond. In this technique, it is possible to combine natural or synthetic active molecules to produce a new molecule with synergistic activity and with less toxicity or side effects.[13,14] In designing new drug molecules, synthesis of the hybrid molecular technique is also being used in addition to the synthesis of new derivatives.[15] In molecular hybridization constituents are linked directly, with the help of some linker, or the active structural parts are merged into a single molecule.[16] In our work, we studied the hybrid molecules prepared by a combination of NSAIDs with other synthetic and natural molecules. Moreover, the combination of different pharmacophores other than NSAIDs having analgesic and antiinflammatory activities is also discussed. The results achieved by this strategy have been discussed with respect to their antiinflammatory and analgesic activities, GIT toxicities, selectivity for COX-1/COX-2, and inhibition of release of proinflammatory mediator's cytokines. The aim of this study was to evaluate the combination of different types of natural and synthetic molecules with NSAIDs.

2. Hybrid molecules of NSAIDs with different pharmacophores

2.1. NSAIDs hybrids with nitric oxide and hydrogen sulfide releasing molecules

NSAIDs have been attached with nitric oxide and hydrogen sulfide releasing molecules to produce new anti-inflammatory compounds (Figure 2). Naproxcinod is a nitric oxide donor having antiinflammatory, analgesic, and antipyretic properties without causing GIT and cardiovascular toxicity and is currently in the clinical trial stages of development.[17,18] In another work, hybrid molecules of aspirin were prepared in which nitric oxide and hydrogen sulfide releasing molecules were incorporated through aliphatic spacer. Hydrogen sulfide releas-

ing molecules were 4-hydroxy benzothiazide, lipoic acid, and 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione and they were attached directly to aspirin. Compound **13** showed higher antiinflammatory and analgesic activities and longer lasting effect as compared to aspirin as it was determined by carrageenan induced paw edema and acetic acid induced writhing test. It also inhibited the release of interleukin-1 during paw edema. This compound was also very effective in inhibiting the growth of different cell lines.[19,20] Hybrid molecules of aspirin with nitric oxide releasing furaxon and with nitric oxide free furazan were evaluated for their effect on the release of proinflammatory cytokines. Compound **14** significantly inhibited the release (36 ± 10% of lipopolysaccharide control) of TNF-α from human monocytes derived macrophages. These agents were also found to be cytoprotective as determined by measuring the release of lactate dehydrogenase. This effect was mediated by the release of nitric oxide because the corresponding furazan analogues, which do not contain the nitric oxide moiety, were less active. The compounds having amide group (**15**) were found to be less active as compared to nitrile group containing compounds.[21] Hybrid molecules of diclofenac with nitric oxide donating furoxan were prepared in which furoxan and diclofenac were attached through amide–ester and ester–ester linkage. Compound **16** showed excellent antiinflammatory action (85.97 ± 0.55% inhibition of inflammatory activity) followed by **17** (80.44 ± 0.62%) and **18** (79.16 ± 0.59%). Compound **16** released a higher amount of nitric oxide and was also found to be least ulcerogenic (severity index, 0.250 ± 0.11). Compound **17** (79.05 ± 1.22% inhibition) was the most

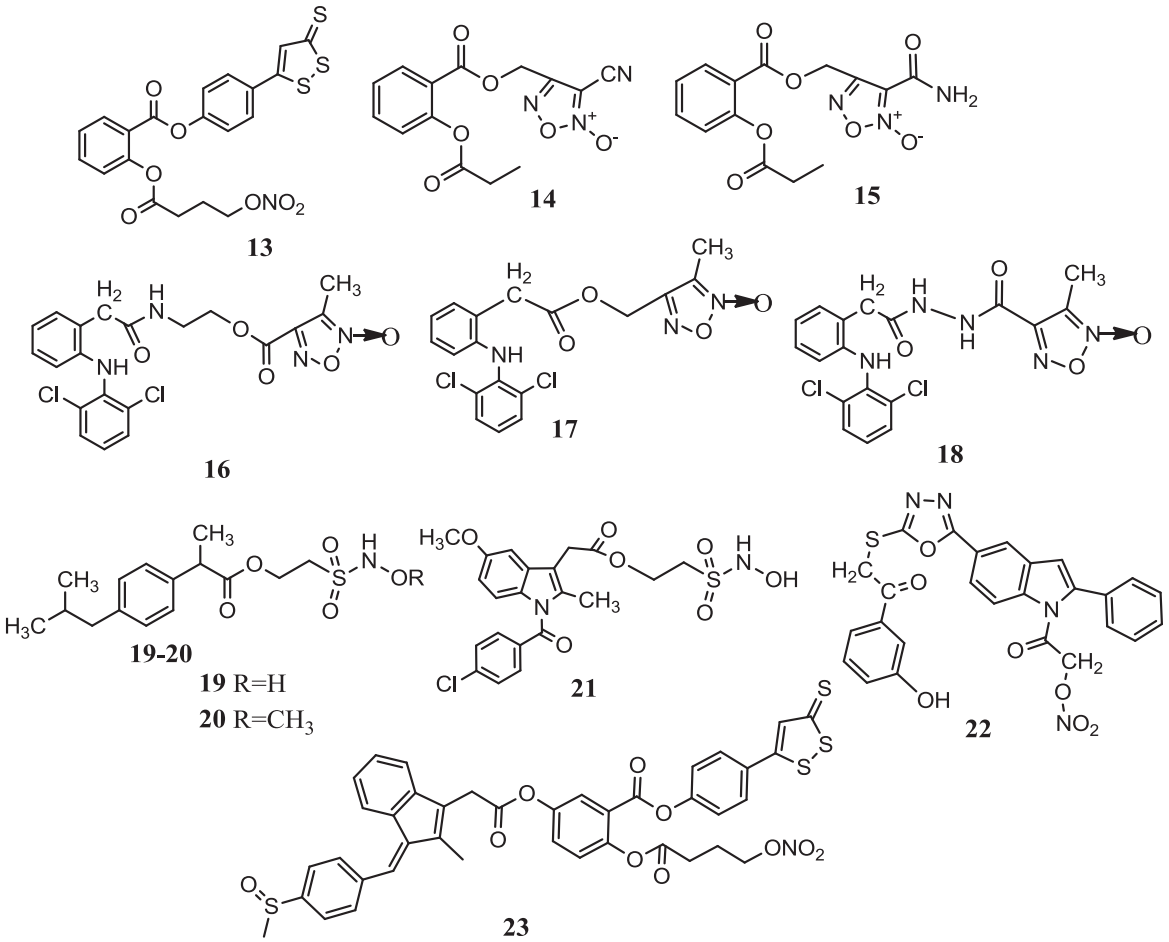


Figure 2. Hybrid molecules of NSAIDs with nitric oxide and hydrogen sulfide releasing molecules.

potent analgesic in this series.[22] Ibuprofen, (S)-naproxen, and indomethacin were attached with sulfohydroxamic acid via a two carbon ethyl bridge to produce hybrid molecules. Prominent antiinflammatory activity was exhibited by **19**, which is an ibuprofen derivative (ID_{50} 79.5%, ID_{50} = dose that inhibited edema by 50%), and **20**, which is a methyl ester (ID_{50} 78.9%) of ibuprofen. These compounds exhibited prominent release of nitric oxide ranging from 44.5% to 54.3% in phosphate buffer saline. The most potent (IC_{50} 1.1 μM) COX-1 inhibitor was a hydroxamic acid with ibuprofen and the most potent COX-2 inhibitor (IC_{50} 0.42 μM) was **21**, which is a hydroxamic acid conjugate with indomethacin. The important observation in these derivatives was that indomethacin–sulfohydroxamic acid conjugate showed no GIT toxicity. This may be due to its higher selectivity to the COX-2 enzyme.[23] Hybrid molecules of indomethacin were also synthesized with oxadiazole and organic nitrate having the general formula 2-(5-(5-(substitutedphenyl)-2-oxo-ethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1*H*-indol-1-yl)-2-oxoethyl nitrate. Compound **22** demonstrated significant analgesic (68.4% inhibition of acetic acid induced writhes) and antiinflammatory activity (70.65% inhibition of paw edema for the most potent compound). Moreover, 0.39% nitric oxide releasing activity and less gastrointestinal toxicity up to 50 mg/kg body weight were also observed for this compound.[24] A hybrid molecule of sulindac **23** with nitric oxide and hydrogen sulfide releasing molecules also exhibited prominent antiinflammatory activity (72%) and reduced GIT toxicity as evident from their ulcer index (UI). Compound **23** was found to be less ulcerogenic (UI = 10) as compared to its precursor sulindac (UI = 130). The preparation of this derivative is advantageous because long-term use of sulindac causes GIT toxicity. Compound **23** also showed potential as an analgesic, antipyretic, and anticancer agent and as an inhibitor of the release of tumor necrosis factor alpha (TNFα).[25]

4-Acetamidophenyl 2-((2-(nitrooxy)ethyl)(phenyl)amino)benzoate is a hybrid molecule prepared by combining acetaminophen and fenamate (Figure 3). A nitric oxide releasing group was attached to this hybrid molecule by ether linkage. The unsubstituted compound **24** at position # 4 of the phenyl group was the most active antiinflammatory agent (65.82% inhibition of rat paw edema after 3 h). This compound also showed the

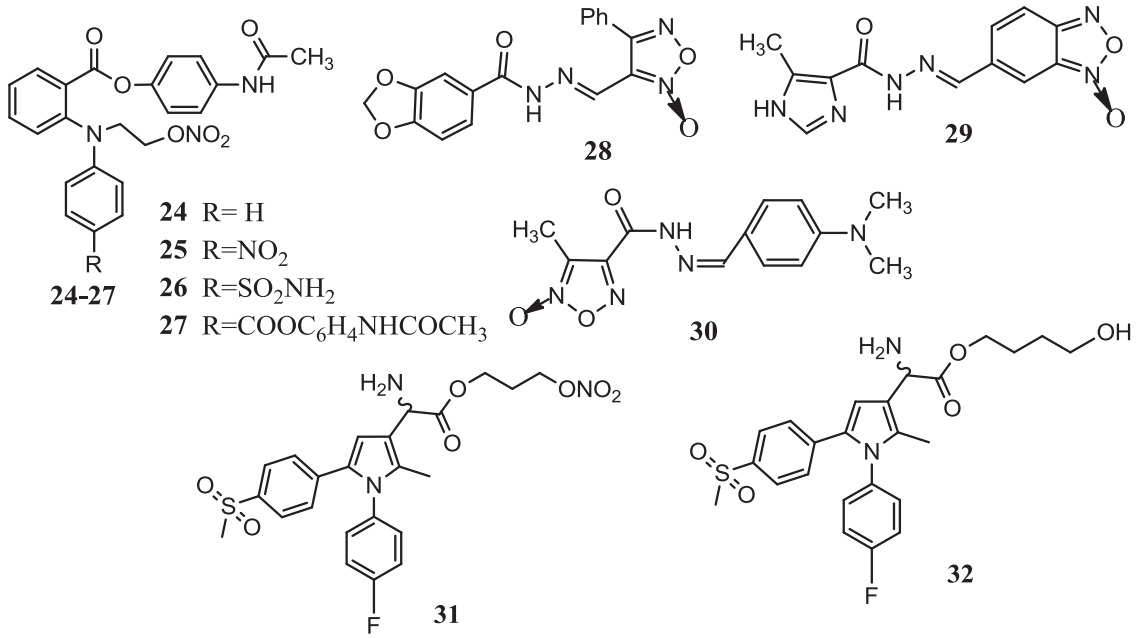


Figure 3. Hybrid molecules of NSAIDs with nitric oxide and hydrogen sulfide releasing molecules.

maximum nitric oxide (10.71%) releasing property. **25** and **26**, which contain nitro and sulfonamide at this position, were less active antiinflammatory compounds. **25**, having a nitro group (56.06% inhibition of number of acetic acid induced writhes), and **27**, having acetamidophenoxy carbonyl (61.34%) at this position, showed prominent analgesic activity.[26] Molecular hybridization was used to prepare furoxanyl-*N*-acyl hydrazones and the newly synthesized compounds were evaluated for their ability to inhibit the in vitro release of proinflammatory cytokines IL-8. The most potent IL-8 inhibitor compound **28** (percentage of IL-8 production = 4 ± 1) contains 4-phenyl furoxanoyl attached with benzodioxole through a carbohydrazide group. **29** and **30** showed excellent antiinflammatory (**29** = 31 ± 9% and **30** = 19 ± 7% edema inhibition) and analgesic activities (percentage of acetic acid induced constriction inhibition **29** = 46 ± 13 and **30** = 22 ± 5). However, **29** was found to be toxic to murine macrophage J774 cells (IC_{50} = 38 ± 1). Compound **28** also showed the release of nitric oxide (NO = 0.39 nM/min) at basic pH.[27] 1,5-Diaryl pyrrole-3-acetic acid is a new class of highly potent and selective COX-2 inhibitors.[28] Development of novel hybrid molecules having a nitric oxide group was carried out. Compound **31** exhibited COX-2 inhibition (IC_{50} 0.82 μM) and showed nitric oxide releasing activity. This was also a potent antiinflammatory compound (70% reduction of paw edema after 30 min) in this series. The corresponding alcohol derivatives also showed COX-2 inhibitory activity and the most potent compound, **32** (IC_{50} 0.22 μM), contains a hydroxyl group attached to the acetate group through a butyl chain. Introduction of the amino group in these molecules showed improved water solubility.[29]

2.2. Hybrid analgesic and antiinflammatory molecules having a pyrazole ring

Synthesis of hybrid molecules of antipyrine and pyridazone was carried out and these compounds were evaluated for their in vivo analgesic and antiinflammatory activities (Figure 4). The presence of aryl piperazine attached with the pyridazine ring influences the analgesic and antiinflammatory activities. Compound **33** was the most

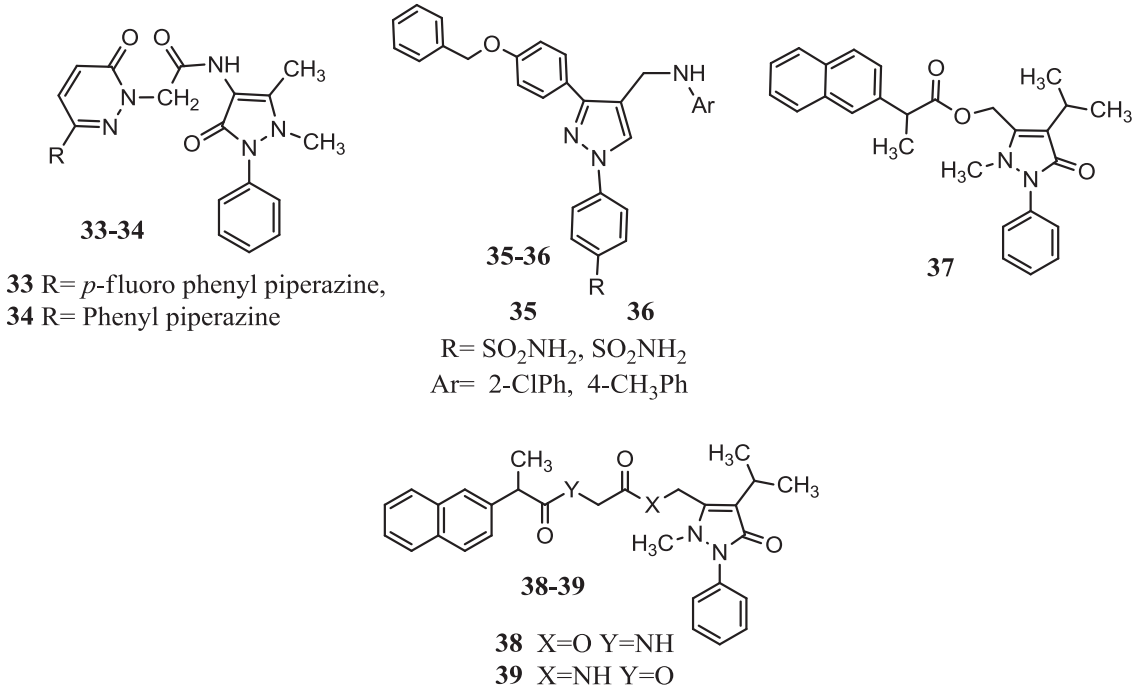


Figure 4. Pyrazole and pyrazolone containing hybrid antiinflammatory molecules.

potent antiinflammatory compound (67% inhibition of paw edema), with *p*-fluoro phenyl piperazine attached with pyridazone. The most potent analgesic compound, **34** (percentage of analgesic activity = 88.33% ± 5.87), contains phenyl piperazine attached with pyridazone. An increase in the chain length between pyridazone and antipyrine decreases the activity of compounds.[30] Synthesis of hybrid pyrazole compounds was reported in which a p-sulfonamide substituted aromatic ring was introduced at the nitrogen of position # 1. 4-Benzyloxyphenyl was attached at position # 3 and substituted phenyl group at position # 4 through a CH_2-NH linker. Two derivatives, **35** and **36**, showed potent antiinflammatory activity as it was determined by carrageenan induced paw edema. These derivatives contain 2-Cl phenyl (80.87 ± 2.67%) and 4-CH_3 phenyl (80.63 ± 0.53%) groups at position # 4 of the pyrazole ring. The presence of a sulfonamide substituted phenyl group at position # 1 is necessary for the activity of these agents. These compounds also exhibited in vitro COX-2 (IC_{50} = 2.51, 1.79 μM for **35** and **36**) inhibitory activity and selectivity (72.95 and 74.92 for **35** and **36**) over COX-1. These results were supported by docking studies where these compounds showed a strong interaction with the COX-2 enzyme.[31] Hybrid molecules of naproxen and propyphenazone were synthesized via ester or amide linkage in order to minimize gastrointestinal irritation and toxicity. Some potent compounds were produced that showed less GIT toxicity and compound **39** was found to be least toxic. The analgesic and antiinflammatory activity increases with time, which may be due to the fact that prodrugs are hydrolyzed to generate the active molecules that exhibit activity. Compound **37** produced the maximum analgesic effect (pain threshold 107.6 ± 11.95) after 4 h and **38** was the most potent as antiinflammatory agent.[32]

2.3. NSAIDs hybrids with triazole and other triazole containing antiinflammatory agents

1,2,3-Triazole possesses many different types of activities such as antibacterial, antifungal, anti-HIV, and antiinflammatory.[33] Hybrid molecules combining ibuprofen, resorcinol, and triazole were synthesized and promising antiinflammatory activity (range = 47.00% to 94.01% inhibition after 3 h) was observed. Compound **40**, in which the p-nitrobenzyl group was attached with a triazole ring, showed more interaction with COX-2 enzyme as determined by molecular docking studies (Figure 5). Some compounds in this series that contain electron withdrawing groups bearing a benzene ring exhibited good bactericidal activity, e.g., the compounds containing p-nitrobenzyl (MIC = 12 μM against *B. cereus*), p-nitrophenyl (MIC = 20.5 μM against *Bacillus subtilis*), and m-chlorophenyl (MIC 18.6 μM against *B. subtilis*) groups attached with the triazole ring.[34] Hybrid molecules of ibuprofen with heterocyclic ring thiotriazole were prepared and the triazole ring was further substituted with nitric oxide bearing diaryl rings. Compound **41** was the most potent antiinflammatory agent (62.82% inhibition of paw edema volume) and showed release of nitric oxide (NO = 0.35%). Compound **42** was the most significant analgesic agent (64.75% inhibition of acetic acid induced writhes). These agents also showed less gastrointestinal toxicity as no ulcer index was seen in rats after administration of drug. However, these compounds have high molecular weight, which may be problematic in further studies.[35] Usnic acid is a dibenzofuran isolated from *Usnea longisiima* and it has antimicrobial, antiinflammatory, and analgesic properties.[36] Usnic acid was linked with triazole substituted with various aromatic and aliphatic groups. Prominent antiinflammatory activity was observed for compounds **43** and **44**, in which triazole was substituted with methyl amino (90.94% inhibition of TNF-α release) and hydroxy methyl (89.18% inhibition of TNF-α release) group. Derivatives that contain an alkyl chain of two carbons between triazole and usnic acid were less active as compared to those that contain a propyl chain.[37] New hybrid antiinflammatory molecules (**45**–**47**) were created by combining tryptamine with phaeonol, which is an important constituent of many herbs of the

genus Phaeonia.[38] In the new hybrid molecules triazole was created by combining the azido tryptamine with propazylated phaeonol and propazylated syringic acid. Evaluation of the antiinflammatory activity was performed on BV2 cell lines on lipopolysaccharide (LPS) induced inflammation. Compounds **45** and **46** exhibited potent antiinflammatory activity with more than 90% reduction of inflammation as compared to the control. Syringic acid hybrid compound with phaeonol (**47**) was inactive as an antiinflammatory agent. Upon toxicity evaluation **45** was found to be the most cytotoxic (0% viability at 50 μM), while compound **47** was the least toxic (100% viability at 50 μM).[39] Isatin is a natural compound obtained from the genus Isatis.[40] Some novel isatin derivatives were prepared by hybridizing them with 1,2,4-triazole through alkyl linkers. Promising results were obtained as evaluation of these compounds was carried out by the inhibition of TNF-α induced expression of ICAM-1 (intracellular adhesion molecule 1). The introduction of an electron withdrawing group such as a bromine atom at position # 5 increases activity possibly due to increased lipophilicity and penetration into the cells. Compound **48** was the most active compound (89% inhibition of ICAM-1, IC_{50} 20 $\pm$ 1 μM) in this series. Compound **49** also showed prominent activity (77% inhibition of ICAM-1, IC_{50} 30 $\pm$ 2 μM).[41]

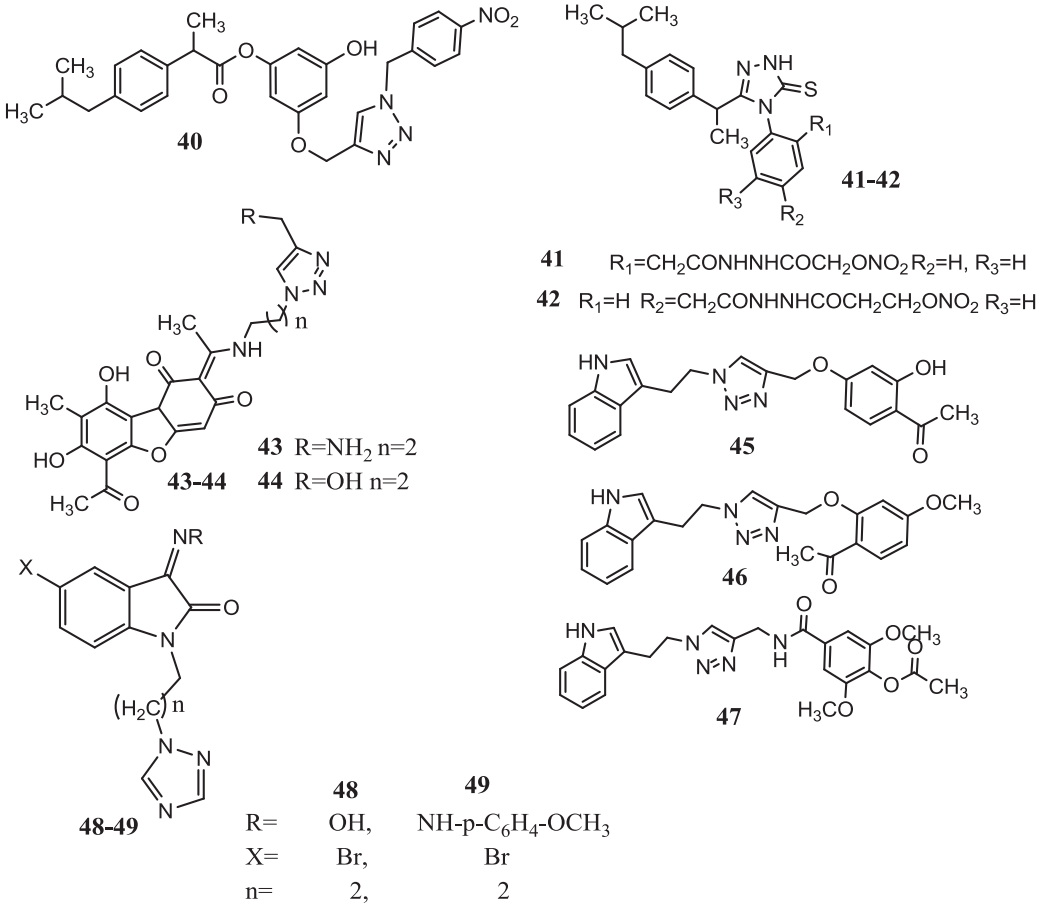


Figure 5. Triazole containing hybrids antiinflammatory molecules.

2.4. NSAIDs hybrid with acyl hydrazones

Hybrid molecules of naproxen and acyl hydrazone were synthesized using the conventional method and by microwave radiation. Higher yields of compounds were obtained using the microwave method. Compounds

were evaluated for their interaction with COX-2 by using molecular modeling studies. The effect of different substituents on the aromatic ring was calculated by dipole moment and electrophilicity index. Meta substituted compounds showed higher interaction with COX-2 as compared to ortho and para substituted compounds. The most potent inhibitor of COX-2 was **50**, which has a chloride group at position # 3 of the aromatic ring because it showed the best docking results (Figure 6). However, this compound presented a different binding mechanism to its target enzyme as compared to COX-2 inhibitors.[42] Pharmacophores of NSAIDs, acetyl salicylic acid, and n-acyl hydrazone were combined to produce new hybrid molecules. **51** was found to be a more potent (52.8 ± 0.07% edema inhibition after 4 h) antiinflammatory agent as compared to its precursor, while other derived compounds showed less activity. Higher analgesic activities for these conjugates were observed as compared to their precursor and **52**, the derivative having diclofenac (42 ± 1.1% protection of total acetic acid induced writhing), was most potent. Less gastrointestinal toxicity (gastric lesion < 1 mm diameter) was presented by these compounds as compared to the starting NSAIDs. Docking studies showed that hybrid compounds have higher selectivity for COX-2 as compared to COX-1.[43]

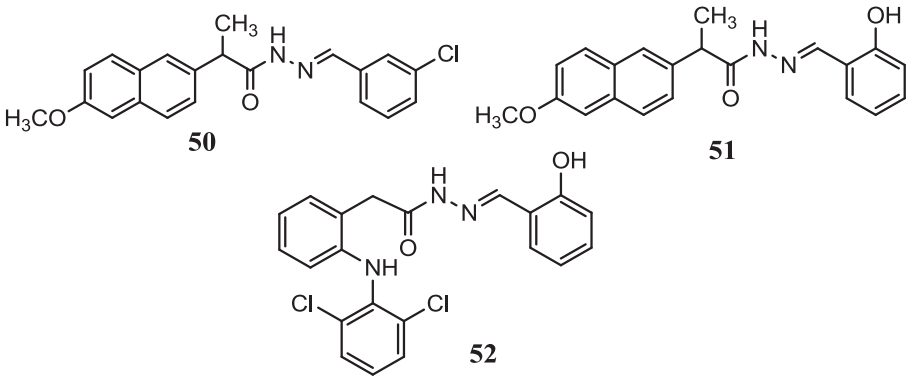


Figure 6. NSAIDs hybrids with acyl hydrazones.

2.5. Pyrimidine, dihydropyrimidines, tetrahydropyrimidine, and tetrahydropyran containing hybrids

Hybrid molecules of quinazolinone were prepared with substituted pyrimidines and dihydropyrimidines in order to increase the antiinflammatory activity. Dihydropyrimidne compounds were found to be less active than the pyrimidine derivatives. Quinazolinone and pyrimidine compounds, which contain aniline moiety, showed more antiinflammatory activity (compound **53**, **54** = 90.91% inhibition of edema after 1 h) and a lower ulcer index (UI = 14.43, 11.38) than the standard drug diclofenac (inhibition of edema = 83.64%, UI = 17.02) (Figure 7). These two compounds also showed more COX-2 inhibition as compared to COX-1 inhibition.[15] Tetrahydropyrimidine and adamantane hybrids were synthesized and evaluated for their antiinflammatory activity. It was found that compounds that contain a methyl group or benzyl group at position # 1 of the tetrahydropyrimidine ring showed excellent antiinflammatory activity, while those containing a phenyl group were found to be less active. **55** was the most active compound (–19.67% decrease in paw edema volume after 3 h); it contains a methyl group at position # 1 and the p-chlorobenzoyl group at position # 5.[44] 1,3,4-Oxadiazole derivatives having a chloroquine nucleus and substituted dihydropyrimidinones were synthesized. Antiinflammatory activity of these compounds ranges from 47.1% to 76.9%. Prominent activity was exhibited by **56** and **57** (72.1%, 76.9%), which contain chloro and nitro substituted benzene rings attached with dihydropyrimidinone. Some agents in

this series also presented antibacterial activity (zone of inhibition > 15 mm). Therefore, these compounds have the potential to be used as antibacterial and antiinflammatory agents.[45]

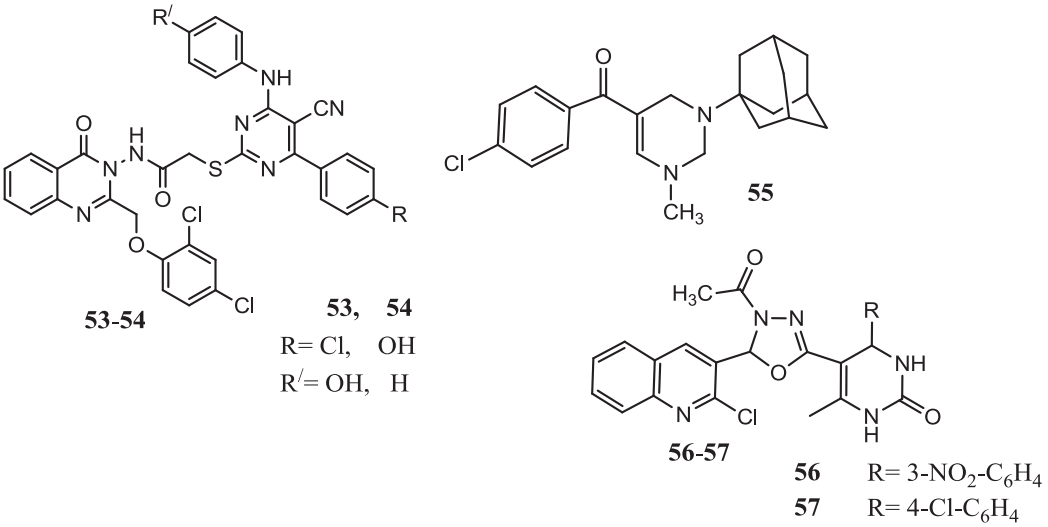
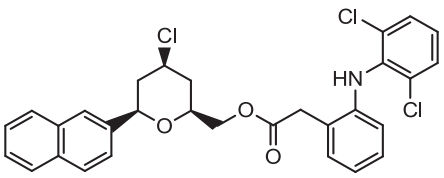


Figure 7. Pyrimidine containing antiinflammatory molecules.

NSAIDs such as ibuprofen, ketoprofen, naproxen, diclofenac, indomethacin, and acetyl salicylic acid were combined with 4-chloro-6-naphthyl tetrahydropyran derivatives (Figure 8). These compounds were in racemic mixture form. Better antinociceptive activity was observed for these compounds as compared to the starting molecules. The most active compound (ED_{50} = 3.17 μmol/kg) in this series was **58**, the combination of diclofenac with naphthyl tetrahydropyran derivative. This value was considerably low as compared to its precursor diclofenac (ED_{50} = 32.48 μmol/kg). These compounds showed less toxicity (LD_{50} > 2000 mg/kg) and have potential for further studies.[46]



58

Figure 8. Diclofenac hybrid with tetrahydropyran.

2.6. Thiazole, benzothiazole, and benzothiophene containing hybrids

The carboxylic group of ibuprofen was modified with 2-amino benzothiazole, which was further substituted with various groups, e.g., chloro, bromo, methyl, and nitro groups. **59** was the most prominent analgesic compound, which contains a methyl group attached with benzothiazole (Figure 9). Bromo substituted compound **60** displayed prominent antiinflammatory activity (0.18 ± 0.02% reduction of paw edema) after 150 min of formalin injection.[47] In another study benzothiophene and benzofuran were attached with rhodanine and it was further attached with various antiinflammatory pharmacophores. Benzothiophene was found to be more potent than benzofuran. Significant in vivo antiinflammatory activity (93.26% reduction of edema) as compared to standard was observed for **61** containing dimethoxy phenyl attached with benzothiophene and

rhodanine hybrid. This compound exhibited enhanced selectivity for COX-2 (IC_{50} 0.67 μM) and selectivity index (5.1) as compared to COX-1 (IC_{50} 3.4 μM). The interaction with COX-2 receptor was also supported by molecular docking studies.[48] The synthesis and analgesic and antiinflammatory activity of diphenyl thiazole and thiazolidine-4-one were reported. Thiazolidine-4-one was further attached with unsubstituted and substituted benzylidene. Compounds **62**–**65** showed moderate degrees of antiinflammatory activity. Substituted benzylidene with electron withdrawing groups produced compounds with less activity, e.g., chloro group, while electron donating substituents resulted in increased analgesic as well as antiinflammatory activity. When the benzene ring of benzylidene was replaced with furan, the resultant compound **66** showed prominent analgesic (showed lower number of acetic acid induced writhes as compared to diclofenac) and antiinflammatory activity (80% inhibition of edema as compared to standard).[49]

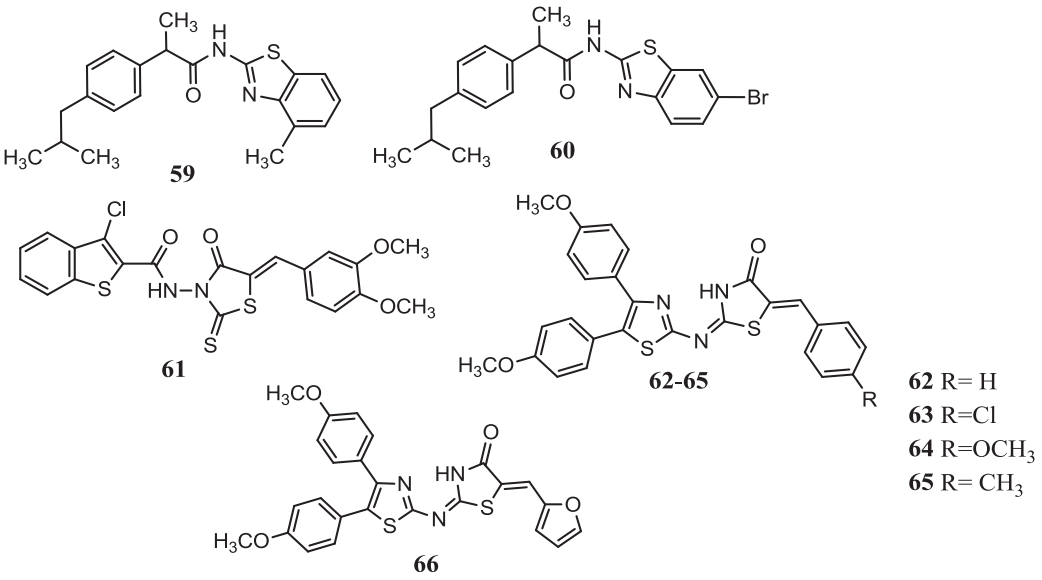


Figure 9. NSAIDs hybrids with benzothiazole and other benzothiophene, thiazole containing hybrids.

2.7. Hybrid molecules containing benzimidazole

Conjugates of benzimidazole and ibuprofen were synthesized having different aryl groups at the nitrogen at position # 1 of benzimidazole. Compound **67** was found to be an inhibitor of leukotriene formation having IC_{50} of 0.31 μM (Figure 10). Further separation of this compound into its enantiomers was carried out and both enantiomers exhibited almost equivalent activities. Compound **67** also showed in vivo antiinflammatory activities by interfering with the biosynthesis of leukotrienes as an inhibitor of 5-lipoxygenase activating protein.[50–52] Conjugates of different NSAIDs with benzimidazoles were synthesized as polyfunctional compounds. Significant antiinflammatory activity (36.8%–57.1% inhibition of paw edema volume) was observed for these compounds. The most potent agent, **68**, was the conjugate of benzimidazole with ibuprofen. These derivatives also showed reduced level of GIT toxicity (UI = 0.58 $\pm$ 0.20–1.75 $\pm$ 0.42). The least GIT toxicity causing compounds were **69** and **70**, which are flurbiprofen and mesalamine hybrids with ibuprofen. **70** also showed prominent antioxidant (EC_{50} = 0.03 $\pm$ 0.006 μM) as well as immunomodulating activities. The compounds in this series follow the Lipinski rule of five because their molecular weight and lipophilicities are less than 500 and 5, respectively.[53] Regioselective synthesis of isoxazole and mercaptobenzimidazoles hybrids was carried out. Hybrid compounds that contain electron withdrawing groups (Br and NO_2) at position # 5 of benzimidazole and with a benzene

ring (F and CN) attached at position # 3 of the isoxazole ring showed higher activity as compared to those that contain electron donating groups (OCH_3). Prominent analgesic (reaction time for potent compound = 13.87 ± 0.093, 13.92 ± 0.093 s) and antiinflammatory activity (60.76%, 58.46% edema inhibition after 180 min) was observed for **71** and **72**.[54]

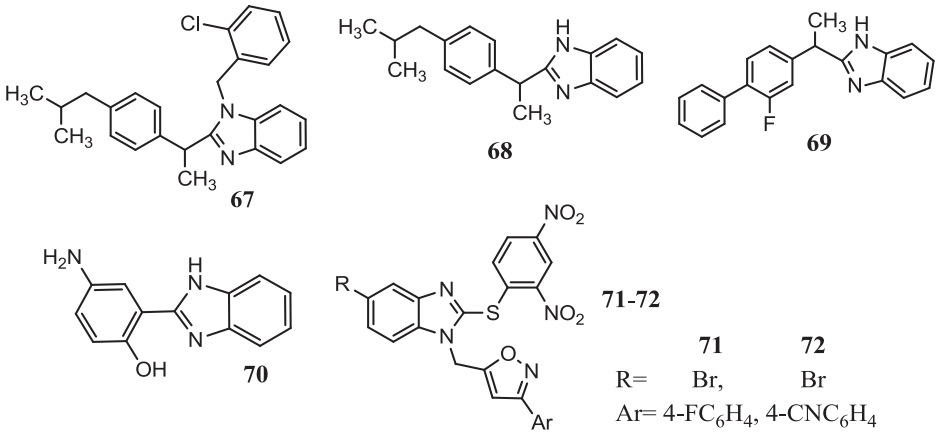


Figure 10. Benzimidazole containing hybrid molecules.

2.8. Hybrid molecules having indole and oxindole rings

Hybrid molecules of indole and oxadiazole were synthesized by connecting oxadiazole and 2-oxo-indolineylidene by a propane hydrazide chain (Figure 11). Introduction of methyl and hydroxyl groups on the aromatic ring attached with oxadiazole produced compounds with prominent analgesic and antiinflammatory activity. Derivatives **73** and **74** were the most potent analgesic (84.11% and 83.17% increase in reaction time) and antiinflammatory (42.7% and 45.5% inhibition of edema after 3 h) agents and showed less GIT (UI = 0.56 and 0.35 for **73** and **74**, respectively) toxicity. Substitution of the halogen atom in the aromatic ring resulted in the formation of less active compounds.[55] Hybrid molecules were synthesized by combining quinazoline substituted at position # 2 with an aromatic ring and isatin, which is an oxidized form of indole, based on the wide variety of biological activities of these scaffolds. In the newly synthesized compounds 2-methyl substituted derivative showed more analgesic and antiinflammatory activity as compared to 2-phenyl derivatives. The derivatives **75** and **76** showed prominent antiinflammatory activity (39% and 48% inhibition of edema after 3 h) and a lower ulcer index (UI = 0.55 ± 0.32, 0.46 ± 0.24). The introduction of aromatic and alicyclic rings at position # 1 of indole produced compounds with less antiinflammatory activity and more GIT toxicities. While in the case of analgesic activity, the alicyclic ring with one heteroatom i.e. piperidine at position # 1 of the indole ring retained the activity (percentage of analgesic activity = 44% and 40% after 3 h), the one with two heteroatoms, i.e. piperazine and morpholine, produced compounds with less activity (analgesic activity = 29% and 28%).[56] Hybrid molecules comprising imidazolidine and indole rings were evaluated for their antiinflammatory activity. Promising antiinflammatory activity was observed for compounds **77** (80.8% inhibition) and **78** (56% inhibition) in carrageenan induced air pouch inflammation. These compounds also showed analgesic activity as it was determined by inhibition (**77** = 63.1% , **78** = 52.1%) of number of writhing as compared to standard (diclofenac = 67.9% inhibition) in an acetic acid induced nociception test. Compound **77** was more effective in inhibiting the release of cytokines such as TNFα and IL-1β.[57]

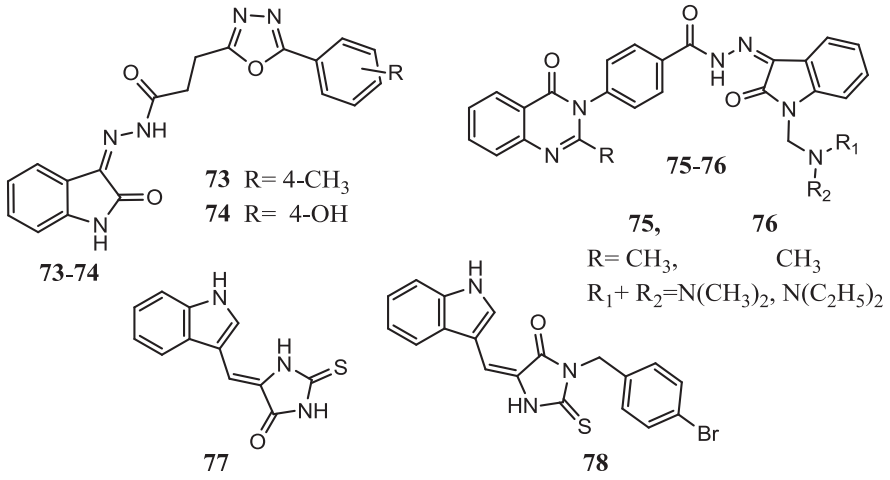


Figure 11. Indole and oxindole containing hybrid molecules.

2.9. Hybrid molecules comprising merged pharmacophores

Pharmacophores of flurbiprofen and fatty acid amide hydrolase (FAAH) inhibitors arylcarbamates were combined to produce a new hybrid molecule. Structure activity relationship studies of this hybrid showed that changing the position of carbamoyl groups to ortho or para positions decreases the COX inhibitory as well as FAAH inhibitory activity. Increasing the chain length increases the FAAH inhibitory activity and in the case of COX inhibitory activity insertion of a short chain (1–2 carbons) decreases the COX inhibitory activity while the activity increases with increasing chain length (3–7 carbons). **79** was the most potent antiinflammatory compound (IC_{50} FAAH = 0.031 ± 0.002 μM; COX-1 = 0.012 ± 0.002 μM) in this series having an n-hexyl chain attached with a carbamoyl group (Figure 12). This compound also showed COX-2 (IC_{50} = 0.43 ± 0.002 μM) inhibitory activity.[58] Naproxen is a classic nonselective COX inhibitor used as an antiinflammatory agent, while tomoxiprole is a selective COX-2 inhibitor.[59] Structural features of naproxen and tomoxiprole were merged to produce a new molecule, **80**. This compound showed analgesic and antiinflammatory activity (dose, 6.562 mg/kg) comparable to diclofenac (5 mg/kg) and celecoxib (100 mg/kg). It produced dose dependent analgesic activity and antiinflammatory activity, i.e. the highest activity was produced at higher dose, i.e. 6.562 mg/kg. Molecular docking studies showed that this compound has a strong interaction with COX-2 enzyme.[60] Paracetamol and pharmacophore of fibrates were attached to design a new hybrid molecule, **81**. This compound, ethyl 2-[4-(acetylamino)phenoxy]-2-methylpropanoate, was synthesized by the reaction of acetaminophen with ethyl-2-bromo-2-methyl-propionate. The biological activities of the compound such as antiinflammatory, hypolipidemic, antidiabetic, and antiatherosclerosis effect were predicted by using software (PASS) in which the chemical structure of a compound can be compared with that of well known biological active drugs. The compound showed a Pa (probability to be active) value greater than 0.7, which indicates that the compound will be active biologically because for a compound to exhibit biological activity the Pa value should be between 0.5 and 0.7.[61] Rutaecarpine is quinazoline alkaloid isolated from the Chinese medicinal plants and it has antiinflammatory activity.[62] Piroxicam is a member of NSAIDs that nonselectively inhibits COX-1 and COX-2. A bioisosteric analogue of rutaecarpine was synthesized by forming piroxicam in the pentacyclic ring of rutaecarpine. The resultant molecule **82** contains piroxicam moiety and is a good candidate for evaluation of antiinflammatory activity.[63] Resveratrol is a naturally occurring compound found mostly in grapes and its products. Resveratrol and salicylates hybrid molecules were synthesized by the addition of one carboxylic group

adjacent to the phenolic group in resveratrol. Compound **85** (20 mg/kg) exhibited better antiinflammatory activity (74% reduction of paw volume after 6 h) as compared to resveratrol and higher COX-2 inhibitory (IC_{50} 1.0 μM) activity as compared to **83** and **84**. It was also confirmed via molecular docking studies. Resveratrol and salicylates hybrid compounds also showed a moderate level of free radical scavenging activity.[64]

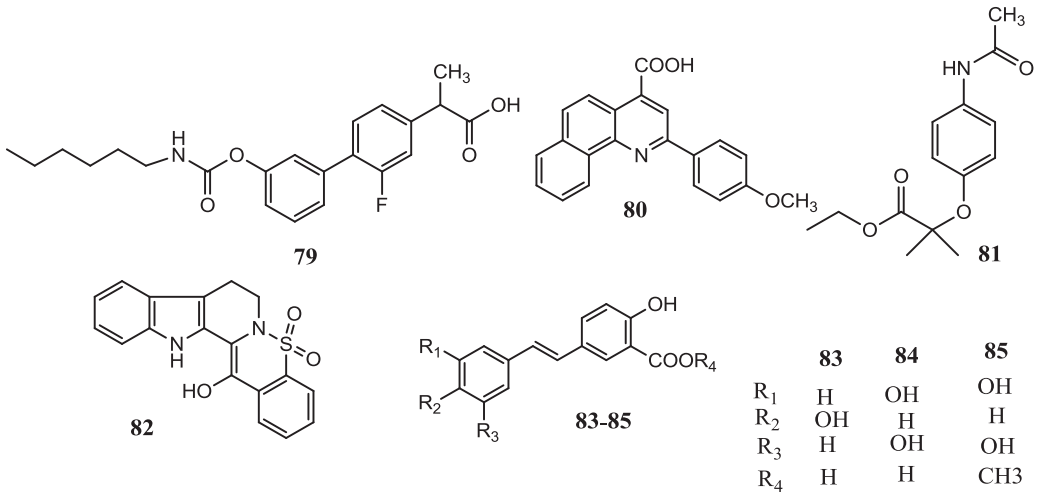


Figure 12. Hybrid molecules having merged pharmacophores.

2.10. Hybrid molecules of NSAIDs with prostaglandins, amino acids, and lipoic acid

Hybrid molecules of acetyl salicylic acid with prostaglandins compounds were prepared by the esterification of carboxylic acid group with prostaglandins. These compounds exhibited excellent analgesic activity (analgesic effect = 22.5%–37.5%) upon evaluation in mice by using a chemical stimulus test. **86** exhibited higher activity (37.5%) as compared to acetyl salicylic acid (27.5%) (Figure 13). These compounds also were less toxic for GIT as compared to acetyl salicylic acid and no sign of gastric toxicity was observed while evaluating for analgesic activity.[65] Some NSAIDs such as ibuprofen, naproxen, flurbiprofen, and acetyl salicylic acid were covalently linked to small peptides and these molecules were converted into hydrogels in water by self assembling. Dialanine and diphenyl alanine were used as conjugated amino acids. Gelation properties change by changing the small peptide from Phe-Phe to Ala-Ala. **87**, which is a hybrid of naproxen with Phe-Phe, produced the most effective hydrogel in this series. Conjugates of salicylic acid with Ala-Ala and Phe-Phe failed to form the hydrogels. These compounds showed less toxicity ($IC_{50} > 200$ μM) as they were evaluated on the HeLa cell lines.[66] Prominent antiinflammatory activity (45.5%–63% inhibition of rat paw edema) was observed for quinoline and lipoic acid hybrid compounds as compared to lipoic acid alone (29.6%). The most active compound, **88**, contains phenyl groups at position # 1 and in the side chain. **88** also exhibited lipoxygenase (100% LOX) inhibitory activity as compared to lipoic acid (29%). These were stable intact molecules as determined at different pH (7 and 9) and temperature (25 and 37 °C).[67] Kyotorphin is isolated from bovine brain sources and it has analgesic activity.[68] Combination of kyotorphin with lipophilic nitronyl nitroxide was carried out. Two compounds (**89** and **90**) were more prominent as analgesic (pain threshold variation **89** = 48.17 ± 5.77%, **90** = 60.32 ± 8.25%) and antiinflammatory compounds (**89** = 50.68% and **90** = 73.47% inhibition of ear edema). These compounds also showed free radical scavenging activity of NO, H_2O_2, and OH free radicals.[69]

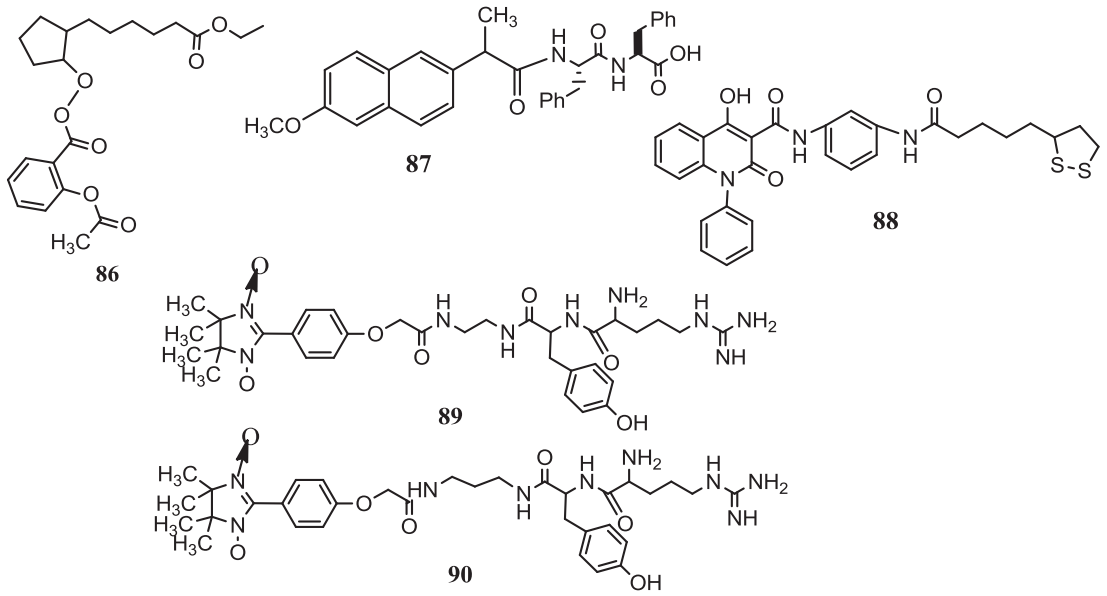


Figure 13. Prostaglandins, amino acid, lipoic acid, and kyotorphin containing analgesic and antiinflammatory agents.

2.11. Hybrid molecules of NSAIDs with terpenes

Terpenes such as oleanolic acid, imbricatolic acid, and ferruginol were connected with ibuprofen and naproxen (Figure 14). Upon evaluation for topical antiinflammatory activity, the hybrid molecule of oleanolic with ibuprofen **91** exhibited prominent antiinflammatory activity (inhibition of edema = 79.9 ± 10.6%) by using 12-O tetradecanoylphorbol 13-acetate (TPA) assay. **91** was also found to be highly cytoprotective (IC_{50} > 1000 μM).[70] Acetyl salicylic acid, naproxen, ketoprofen, and ibuprofen were attached to oleanolic acid through iminoester and ester type linkage. These compounds **92**–**95** were evaluated for activity by prediction of activity spectra for substance (PASS method). In this method different types of biological activity are predicted such as pharmacological action, adverse effects, dose, mechanism of action, etc.[71] Ester type derivatives were found to be more active because they showed a high probability (Pa = 70%) for biological activity as compared to iminoester types (Pa = 50%–70%). These compounds showed potential for antiinflammatory, chemopreventive, and hypolipemic activities. These compounds also showed stability in ethanolic solution of hydrochloric acid at room and elevated temperature.[16] Boswelic acid (BA) is the natural terpene obtained from *Boswella serrata* resin.[72] BA and keto boswelic acid (KBA) were esterified and attached with diclofenac, indomethacin, ibuprofen, and naproxen. Moderate antiinflammatory was observed for **96** and **98**, which are hybrids of BA with ibuprofen (24 h postcarrageenan injection paw edema volume = 1.26 ± 0.06) and naproxen (paw edema volume = 1.21 ± 0.07). In the case of KBA, **97** (paw edema volume = 1.26 ± 0.16) was most potent, which is a hybrid of naproxen with KBA. COX-II inhibitory activity was shown by compounds **98** and **99**, which are hybrids of ibuprofen with BA (30.23 ± 0.09% COX-2 activity) and KBA (27.66 ± 0.18% COX-2 activity) as compared to interleukin 1β treated cells (100% COX-2 activity).[73]

2.12. Hybrid molecules of NSAIDs with coumarins, chromones, chrysins, anthraquinones, and caffeic acid

Carboxylic type NSAIDs were attached with 6-substituted and 7-substituted coumarins to prepare the hybrid molecules (Figure 15). Ethanolamine was used as a linker between these two molecules. These compounds were

evaluated for inhibition of carbonic anhydrase (CA) activity on different isoforms of carbonic anhydrase. It was observed that that CA IV was significantly inhibited by these compounds (0.44–9.8 nM). These compounds showed the inhibition of isoform I, II, and VII CA at higher concentration (inhibition constant (K_I) values $>$ 100 nM). **100**, which is an ibuprofen hybrid with 7-coumarin, showed prominent pain bearing activity (weight bearing activity = 58.7 $\pm$ 1.3 g) and was also most potent in inhibiting CA IV.[74] Chrysin is a natural compound of the flavonoid class and it has antibacterial, antioxidant, antiinflammatory, anticancer, and anxiolytic properties.[75] Chrysin was combined with indole and pyrazole to produce new hybrid molecules. These compounds showed more prominent COX-2 inhibitory activity than COX-1 inhibitory activity. Compound **101** was the most potent (IC_{50} 0.7 μM) COX-2 inhibitor and it also showed a good selectivity index (SI = 168.5) over COX-1 (IC_{50} 118 μM). It contains an alkyl chain of three carbon atoms between indole and chrysin. Molecular modeling studies also showed that compound **101** has a strong interaction with the active site amino acid of COX-2.[76]

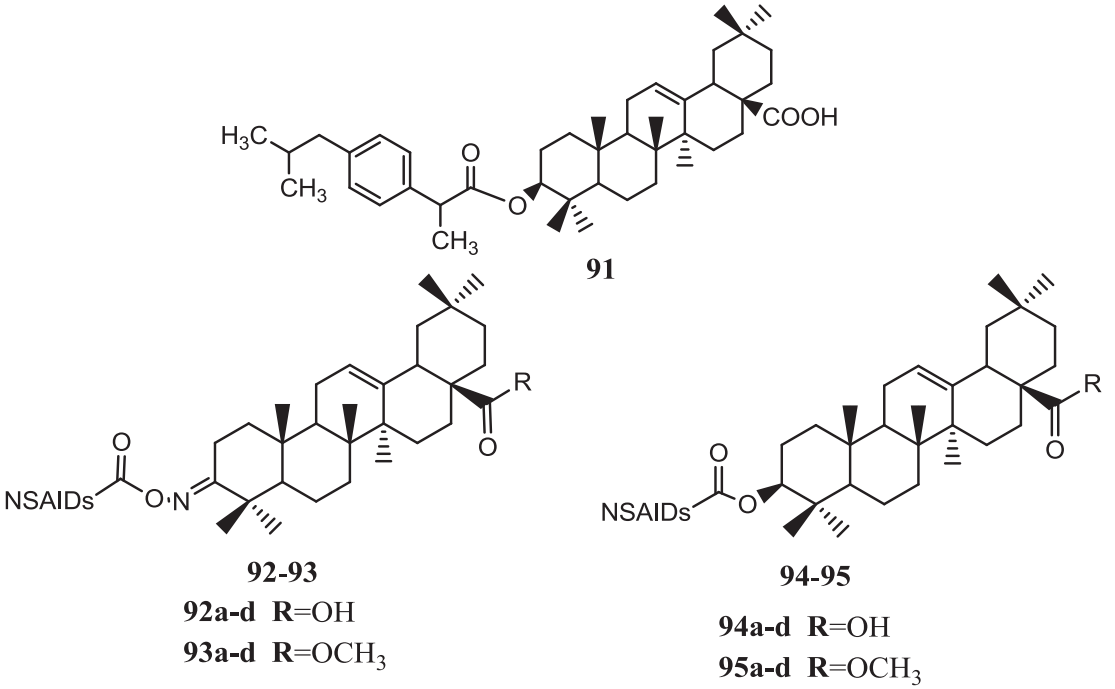


Figure 14. Hybrid molecules of NSAIDs with terpenes.

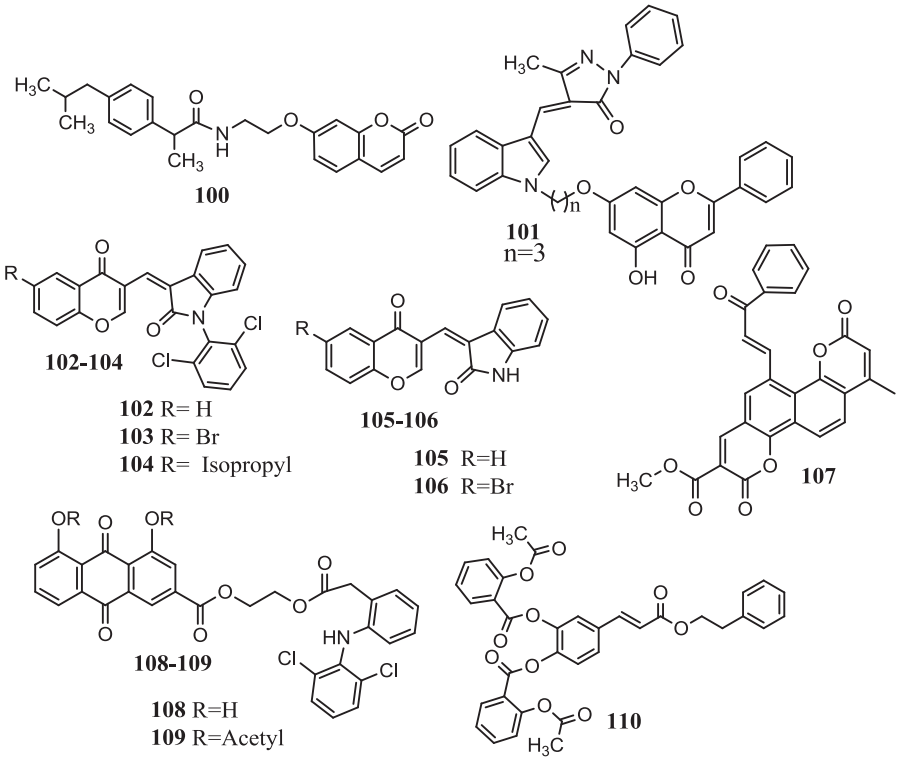


Figure 15. NSAIDs hybrid with coumarin, chromone, chrysin, anthraquinone, and caffeic acid.

Chromone–indole and chromone–pyrazole hybrid compounds were synthesized and evaluated for their activities as COX-1, COX-2, and 5-LOX inhibitors. Compound **102** and **104** exhibited prominent COX-2 inhibition activity (IC_{50} 0.0013, 0.0058 μM) but were less selective for COX-2 than COX-1. Compounds **105** and **106** produced significant inhibition (IC_{50} 0.029 and IC_{50} 0.020 μM) of COX-2 and were more selective for COX-2. Compound **103** showed a more inhibitory effect on COX-1 (IC_{50} 0.63 μM) as compared to COX-2. Compound **105** also showed significant analgesic activity. These derivatives were not active as lipoxygenase inhibitors except compound **104** (IC_{50} 0.02 μM).[77] Synthesis and antiinflammatory/antioxidant activity evaluation of pharmacophores of biscoumarin and chalcone hybrids were carried out. Antiinflammatory activities of the most active compounds in this series were 26%, 29%, and 33% as determined by inhibition of carrageenan induced paw edema. The derivatives in this series were not found to be toxic upon in vivo evaluation. The most active derivative, **107** (33% inhibition of edema), contains unsubstituted chalcone and methyl ester with the biscoumarin part. This compound also exhibited antioxidant (30% inhibition of OH free radical) and TNFα (21%) inhibition activity.[78] *Rheum palmatum* contains rhein, which possesses antiinflammatory activity.[79] Rhein was attached chemically with NSAIDs agents via a glycol ester group. Antiinflammatory activity of these compounds ranges from 13.92% to 43.98%, which is the percentage of inhibition in xylene induced mice auricle tumefaction. **108** and **109** were most potent compounds (43.89% inhibition) in this series, which are hybrids of diclofenac with rhein and with acetyl derivative of rhein. These compounds showed less ulcerogenic potential (range = 5.8–6.0) as compared to the starting NSAIDs.[80] Indomethacin and aspirin were hybridized with caffeic acid phenethyl ester (CAPE) by an ester linkage. Evaluation of the ocular antiinflammatory activity was performed on rabbits by paracentesis induced inflammation. It was observed that **110**, which is aspirin-CAPE, showed superior antiinflammatory activity at the dose of 0.01% and 0.1%

as compared to indomethacin-CAPE. It also inhibited the release of TNFα (390 $\pm$ 101 pg/mL at the dose of 0.01%) and PGE_2 (3.10 $\pm$ 1.0 pg/mL at the dose of 0.01%) in aqueous humor. Indomethacin-CAPE inhibited the production of PGE_2 at a higher dose. This difference may due to the different molecular mass, different lipophilicity, and different rates of hydrolysis, which leads to different levels in target sites.[81]

3. Discussion

Hybrid molecules of aspirin, paracetamol, fenamates, ibuprofen, indomethacin, sulindac, and diclofenac with nitric oxide and hydrogen releasing molecules demonstrated analgesic and antiinflammatory activities. These compounds showed nitric oxide releasing properties and some of them were also found to be less ulcerogenic. When aspirin was combined with a nitric oxide releasing furaxon ring, the resultant hybrid inhibited the release of proinflammatory mediator TNFα and was also found to be cytoprotective. Hybrid molecules of aspirin with caffeic acid phenethyl ester also inhibited the release of TNFα as well as the prostaglandins. Some molecules other than common NSAIDs, e.g., furaxonyl n-acyl hydrazone, inhibited the release of IL-8 in addition to analgesic and antiinflammatory activities. One of the most important combinations was indomethacin and hydroxamic acid, which exhibited COX-2 inhibitory activity without causing GIT toxicity. COX-2 inhibitory activity, as well as antiinflammatory activity, was also exhibited by pyrrole acetic acid–nitric oxide releasing molecule and pyrazole hybrids with sulfonamide substituted aromatic ring. When ibuprofen was attached with triazole through resorcinol, the resultant molecule displayed COX-2 inhibitory activity and additionally this combination inhibited the growth of bacteria. However, antibacterial activity was not observed when ibuprofen was directly linked with thio-triazole. This combination showed less GIT toxicity in addition to improved analgesic and antiinflammatory activities. Prominent inhibition of release of proinflammatory mediator TNFα was exhibited by triazole, when it was attached to natural molecules of usnic acid and isatin. Hybrid molecules of ibuprofen with benzothiazole and benzimidazole also showed analgesic and antiinflammatory activity. Mesalamine–benzimidazole hybrids exhibited antioxidant activities in addition to analgesic and antiinflammatory activities. Molecular docking studies of hybrid molecules of naproxen with acyl hydrazones showed more affinity towards COX-2 enzyme. Analgesic and antiinflammatory activities were also demonstrated by quinazolinone–pyrimidine, adamantane–tetrahydropyrimidine, oxadiazole–quinoline–dihydropyrimidine, diphenylthiazole–thiazolidine-4-one, mercaptobenzimidazole–isoxazole, benzothiophene–rhodanine, indole–oxadiazole, isatin–quinazoline, and imidazolidine–indole conjugates. Among them quinazolinone–pyrimidine, benzothiophene–rhodanine hybrids showed more selectivity for COX-2 enzyme than for COX-1. Imidazolidine–indole hybrids were effective in inhibiting the release of TNFα and interleukin-1β. Merged hybrid molecules of naproxen–tomoxiprole and resveratrol-salicylates were found to be more selective for COX-2 than for COX-1, while flurbiprofen–FAAH merged pharmacophores showed COX-1 as well as COX-2 inhibitory activity. Combination of aspirin with prostaglandin was also interesting because the resultant molecule showed analgesic activity without causing GIT toxicity. Hybrid molecules of naproxen with oleanolic acid and diclofenac with rhein exhibited prominent antiinflammatory activities. Naproxen–oleanolic acid was also found to be highly cytoprotective. Prominent analgesic activity was observed for coumarin–ibuprofen and diclofenac–tetrahydropyran combinations. Chrysin–indole–pyrazole and chromone–oxindole hybrids displayed COX-2 inhibitory activity but halogenated derivative of chromone–oxindole was more potent and also selective for COX-2. Chalcone–biscoumarin hybrids inhibited the release of proinflammatory mediator TNFα in addition to the antiinflammatory activities.

Based on these studies it was found that hybrid molecules of NSAIDs with different types of pharmacophores have been prepared. The newly synthesized compounds are screened for their interaction with COX-1

and COX-2 enzyme in addition to their analgesic and antiinflammatory activities. Interaction of new compounds with cyclooxygenase is also examined by using the molecular docking studies. There is special focus towards the development of selective COX-2 inhibitors that are free from GIT and cardiovascular side effects. Some potent hybrid molecules having selectivity for COX-2 enzymes have been synthesized. These molecules can serve as a lead compound for the design and development of new antiinflammatory molecules.

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