Chapter-2
Review of Literature

2.1. 1,2.4-TRIAZINE AS ANTI-INFLAMMATORY AGENTS

1,2,4-Triazine derivatives gained immense research interest in medicinal chemistry for their powerful anti-inflammatory properties due to their selective inhibition of the cyclooxygenase (COX) and lipoxygenase (LOX) pathways where COX-1 regulates physiological functions like gastric mucosa protection, and COX-2 appears during inflammatory conditions. Generally, conventional NSAIDs inhibit both enzymes causing pain relief but also have gastrointestinal and renal issues, but 1,2,4-triazine derivatives selectively inhibit COX-2, sparing COX-1 and reducing adverse effects. Additionally, some derivatives also inhibit LOX, offering a dual-action mechanism that targets both prostaglandin and leukotriene pathways. As this implies, the research and development of 1,2,4-triazine-based anti-inflammatory medications remained a promising area of drug discovery, therapeutic efficacy, and safety than existing therapies (Zhang, Zhu et al. 2023).

Misra U *et al*. reported the 1,2,4-triazine derivatives, including 3-(indol-3-ylmethyl)-6-phenyl-1,2,4-triazine, compound **1** (Figure 2.1) as anti-inflammatory agents. The study focused on synthesizing indole congeners with enhanced anti-inflammatory properties, highlighting the importance of exploring novel anti-inflammatory compounds without significant adverse effects. The findings of this research provide a foundation for further study in designing safer anti-inflammatory medications, which could improve therapeutic approaches (Misra, Hitkari et al. 1996).

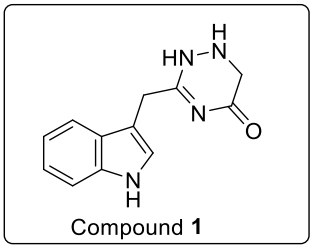


Figure 2.1. Structure of 3-(indol-3-ylmethyl)-1,2,5,6-tetrahydro-1,2,4-triazin-5-one

El-Agrody, Abd El-Latif, *et al*. reported the synthesis of compounds including 1,2,4-triazine derivative with potential anti-inflammatory activities as part of the broader study on polyfunctional heterocycles. The research focuses on chemical compounds developed from 4H-pyran, to identify molecules with intriguing and beneficial biological characteristics, including anti-inflammatory properties. Out of all synthesized derivatives, the 1,2,4-triazine containing compounds **2** and **3** (Figure 2.2) showed substantial anti-inflammatory properties along with antimicrobial and antifungal properties (El-Agrody, Abd El-Latif et al. 2001).

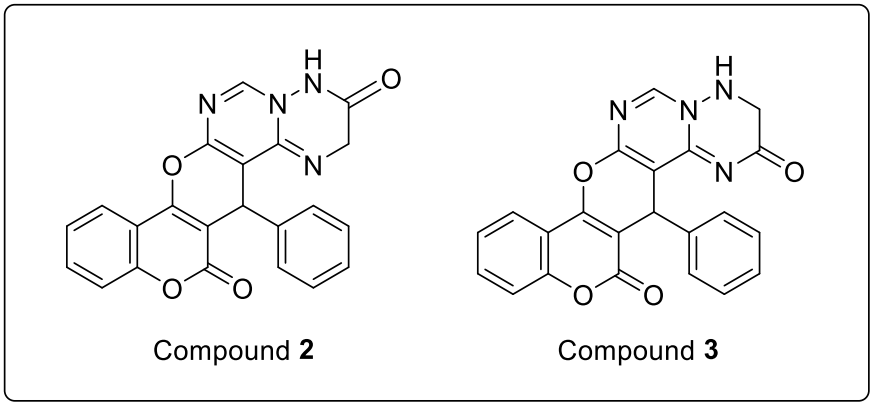


Figure 2.2. Structures of the phenyl-chromeno-pyrano-pyrimido-1,2,4-triazine dione

Amir and Shikha, synthesized numerous molecules, including those bearing the 1,2,4-triazine nucleus, and examined their biological activities. Compound 4 i.e., (2,6-dichloroanilin)benzyl]5-oxo-1,2,5,6-tetrahydro-1,2,4-triazine (Figure 2.3) demonstrated anti-inflammatory effects comparable to diclofenac, with an efficacy of 80.76% in the carrageenin-induced paw edema test. This shows that the 1,2,4-triazine structure could contribute positively to the compounds' anti-inflammatory potential. The study highlights the relevance of altering diclofenac's carboxylic acid group with heterocycles such as 1,2,4-triazine to improve the safety and efficacy of NSAIDs. This strategy attempts to maintain or improve anti-inflammatory properties while minimizing side effects such as gastrointestinal ulcers (Amir and Shikha 2004).

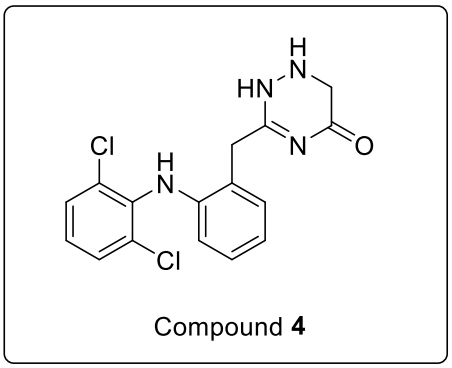


Figure 2.3. Structure of (2,6-dichloroanilino)benzyl]5-oxo-1,2,5,6-tetrahydro-1,2,4-triazine

Mullick *et al.* have synthesized a series of diaryl 1,2,4-triazine (**5**) (Figure 2.4) and evaluated them for biological potential as anti-anxiety and anti-inflammatory agents. The anti-inflammatory efficacy was investigated using the carrageenan-induced paw edema procedure, which revealed that certain derivatives achieved over 88% inhibition, outperforming the reference drug indomethacin, indicating a potential structure-activity relationship. Overall, diaryl 1,2,4-triazine derivatives constitute a substantial topic of interest in medicinal chemistry, requiring further exploration and development (Mullick, Khan et al. 2009).

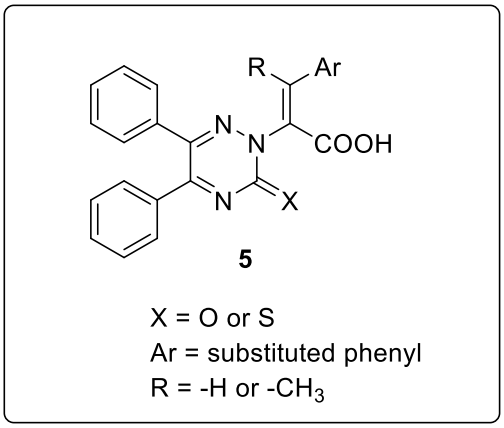


Figure 2.4. Structure of 5,6-diphenyl-1,2,4-triazine derivatives **5**

Khoshneviszadeh *et al.* presented insightful information on the synthesis, design, and biological assessment of new anti-cytokine medications which might help in the develop-

-ment of novel strategies for managing inflammation. These compounds were synthesised with eco-friendly synthetic techniques, such as substituting non-toxic substances like thiamine hydrochloride for dangerous cyanide ions. It is noteworthy that compounds **6**, **7**, **8**, and **9** (Figure 2.5) significantly inhibited cytokines revealing their anti-inflammatory potential. Furthermore, Western blotting studies demonstrated that the anti-cytokine actions of compound **6** are predominantly mediated through the suppression of the p38 MAPK signalling pathway, supporting its role as a potential candidate for future therapeutic development (Khoshneviszadeh, Ghahremani et al. 2013)

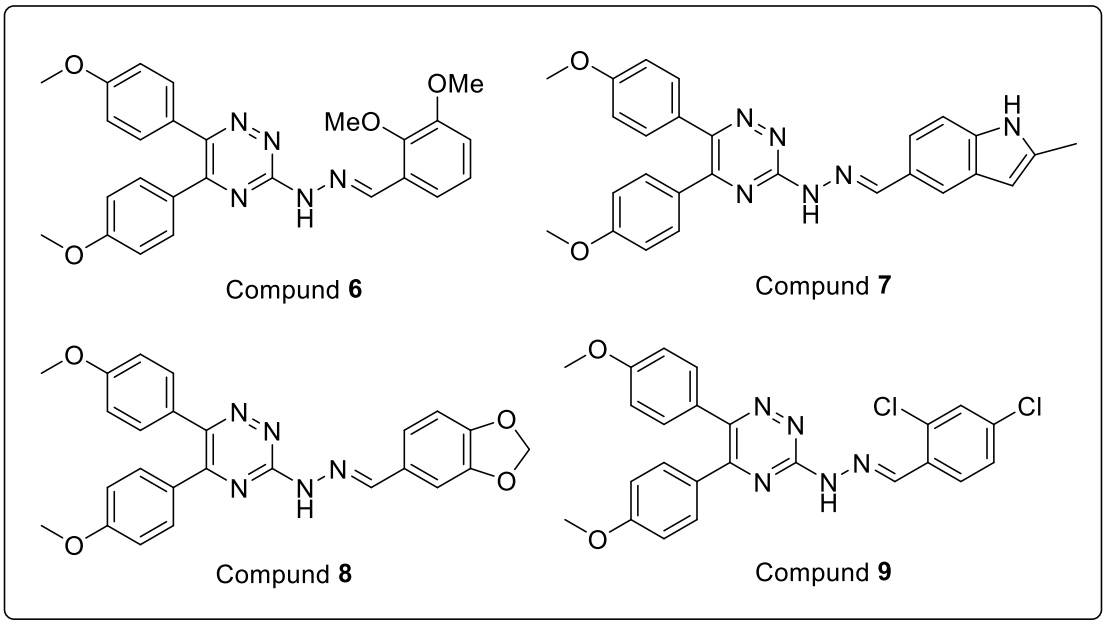


Figure 2.5. Structures of 3-Hydrazinyl-5,6-bis(4-methoxyphenyl)-1,2,4-triazine derivatives **6, 7, 8,** and **9.**

Irannejad *et al.* reported the synthesis and biological evaluation of novel 3-alkylthio-4,5-diaryl-1,2,4-triazine derivatives (Figure 2.6) as selective COX-2 inhibitors. Nonsteroidal anti-inflammatory medications (NSAIDs) frequently inhibit both COX-1 and COX-2, however selective COX-2 inhibitors minimize the side effects associated with COX-1 inhibition. The triazine derivatives showed strong COX-2 selectivity and insignificant COX-1 inhibition; compound **10** had an IC_{50} of 0.10 µM for COX-2 and 39.5 µM for COX-1, which were similar to celecoxib. AutoDock Vina docking experiments verified

that **10** bound effectively, with significant associations at the COX-2 active site. According to a 3D-QSAR model using k-Nearest Neighbour Molecular Field Analysis (kNN-MFA), substituents on rings A and C impacted COX-2 activity. The compounds demonstrated significant in vivo anti-inflammatory and analgesic properties in animal models, indicating that diaryl-triazine scaffolds could be used as leads for COX-2 selective medications (Irannejad, Kebriaieezadeh et al. 2014).

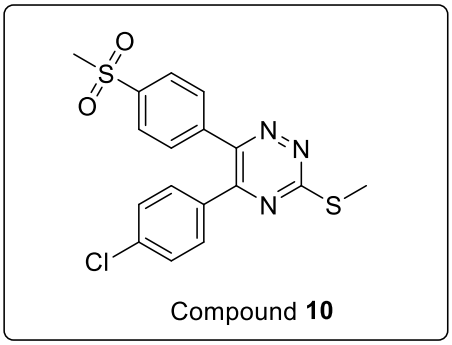


Figure 2.6. Structure of novel 3-alkylthio-4,5-diaryl-1,2,4-triazine compound **10**

Dadashpour *et al.* developed a series of ethyl 5,6-diaryl-1,2,4-triazine-3ylthioacetate (Figure 2.7) analogues to synthesise selective COX-2 inhibitors for the treatment of inflammatory conditions such as Alzheimer's disease (AD). An ethyl carboxylate side chain was introduced to improve inhibitory potential. Molecular docking experiments demonstrated that compounds had higher COX-2 binding affinity than COX-1. Compound **11** had the highest selectivity (COX-2 IC_{50} of 10.1 µM, COX-1 IC_{50} of 88.8 µM) and successful binding interactions in the COX-2 active site.

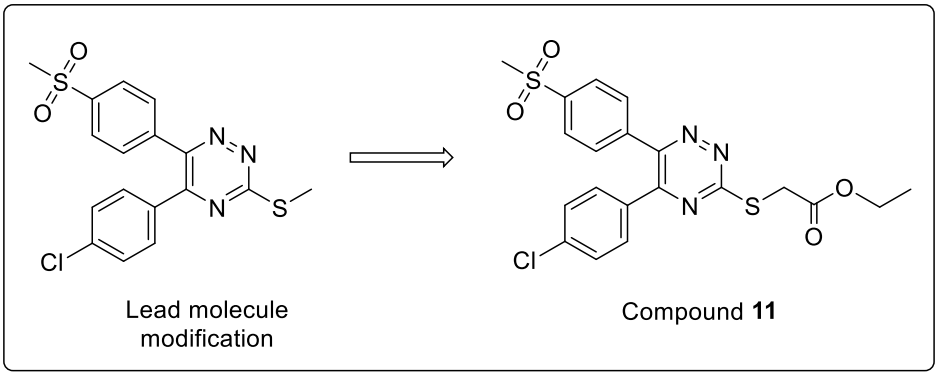


Figure 2.7. Structures of 5,6-diaryl-1,2,4-triazine-3ylthioacetate analogues

According to *in vitro* tests, compound **11** prevented 94% of the production of β-amyloid fibrils after 48 hours, which may indicate that it could be used as a treatment for AD. The research underlines the possibility of ethyl carboxylate side-chain derivatives as multitargeted COX-2 inhibitors for neuroinflammatory disorders, with fewer COX-1-related side effects (Dadashpour, Tuylu Kucukkilinc et al. 2015).

Banerjee *et al.* reported 5,6-diphenyl-1,2,4-triazine-based compounds with 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole moieties as selective COX-2 inhibitors. Amongst the synthesized derivatives, compounds **12** and **13** (Figure 2.8) showed considerable selectivity for COX-2 over COX-1, with 5,6-diphenyl-1,2,4-triazine contributing to specific binding inside the COX-2 active site, as evidenced by molecular docking experiments. Toxicity investigations exhibited negligible gastrointestinal and cardiotoxic adverse effects, as evidenced by low malondialdehyde (MDA) levels and no deleterious effects in myocardial infarction rats. Overall, these 5,6-diphenyl-1,2,4-triazine-based hybrids showed significant COX-2 selectivity and desirable safety profiles indicating potential as selective anti-inflammatory medications for further research (Banerjee, Das et al. 2016)

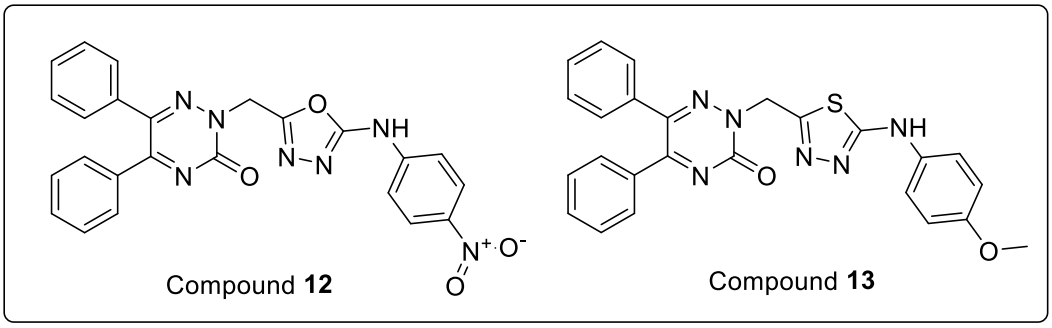


Figure 2.8. Structures of 5,6-diphenyl-1,2,4-triazine-based compounds **12** and **13**

Kaur *et al*. developed triazine-based indole-pyrazole and indolinone conjugates as selective COX-2 inhibitors to improve anti-inflammatory activity while minimising side-

-effects (Figure 2.9). Compound **14** demonstrated significant anti-inflammatory effects in *in vitro* studies and surpassed celecoxib and diclofenac in terms of COX-2 selectivity (IC_{50} = 0.02 μM). Compounds **14** and **15** significantly reduced inflammation in paw oedema *in vivo* models, while compound **15** showed very strong analgesic and anti-inflammatory effects. The research identified triazine-based conjugates, particularly compound **14**, as potential anti-inflammatory alternatives due to their strong efficacy and high COX-2 selectivity. The compound **14** not only showed higher COX-2 selectivity but also exhibited lower toxicity in preliminary assessments, rendering it a promising alternative for inflammatory diseases. The results presented suggested that similar triazine compounds might provide a safer, more tailored approach for addressing inflammatory conditions while minimising the possibility of prevalent NSAID-associated adverse effects (Kaur, Kumari et al. 2018).

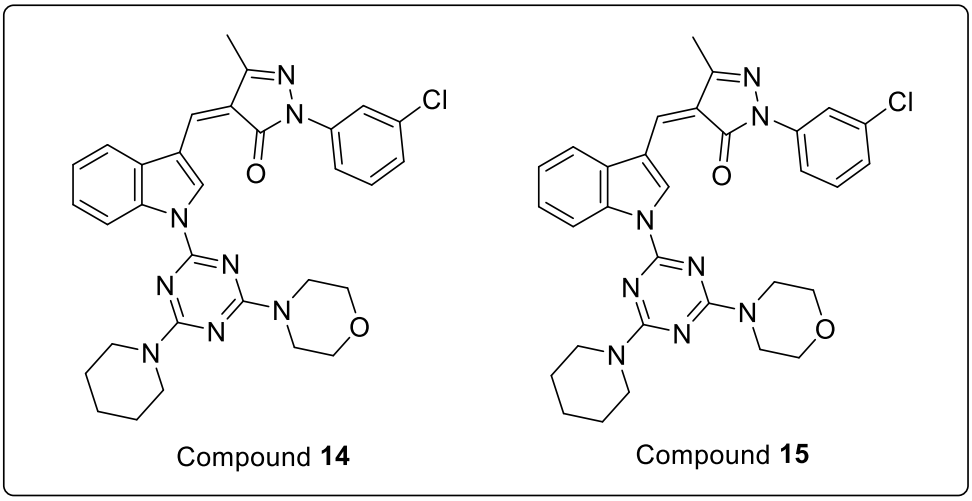


Figure 2.9. Structures of triazine-based indole-pyrazole compounds **14** and **15**

Maqbool *et al*. synthesized diphenyl 1,2,4-triazine hybrids as alpha-synulein (a-syn) fibrillogenesis inhibitors. The anti-inflammatory ability of 1,2,4-triazine molecules could provide neuroprotective effects by targeting inflammatory processes that exacerbate disorders such as Parkinson's disease. *In vitro* experiments revealed that compounds **16**, **17**, **18**, **19** and **20** (Figure 2.10) significantly inhibited a-syn fibril formation, suggesting

that the triazine moiety improves their anti-aggregation properties. Moreover, the low cytotoxicity detected in mouse embryonic fibroblast cells validates the viability of these diphenyl triazine hybrids for further exploration in the treatment of other inflammatory conditions (Maqbool, Gadhavi et al. 2020).

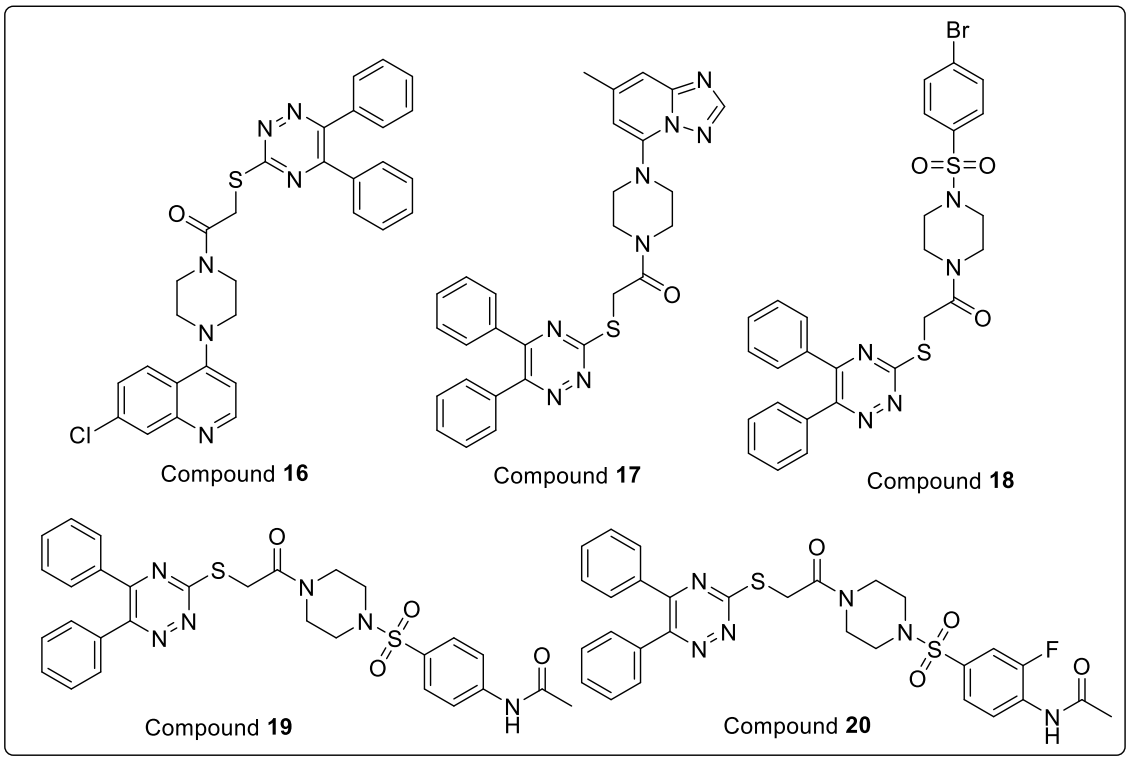
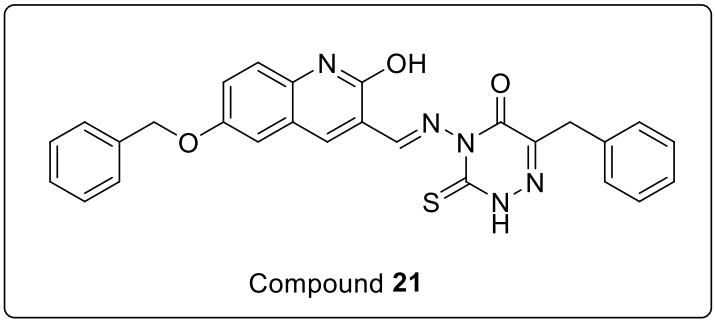


Figure 2.10. Structures of diphenyl 1,2,4-triazine compounds **16, 17, 18, 19** and **20**

Ghanim *et al.* reported the potential of 1,2,4-triazine-quinoline hybrids as promising dual COX-2/LOX inhibitors implicated in inflammatory pathways. The synthesised hybrid **21** with triazine and quinoline structure (Figure 2.11) demonstrated strong COX-2 inhibition (IC_{50}: 0.047-0.32 μM) with selectivity over COX-1 and robust LOX inhibition (IC_{50}: 1.81-3.60 μM), attaining the most potent dual inhibition, equalling conventional references like celecoxib and quercetin. The compound **21**, substantially lowered ROS levels and inhibited pro-inflammatory cytokines such as TNF-α and IL-6 in LPS-activated macrophages. Molecular docking investigations validated these findings, demonstrating favourable contacts within COX-2 and 15-LOX active sites, with compound 8e having

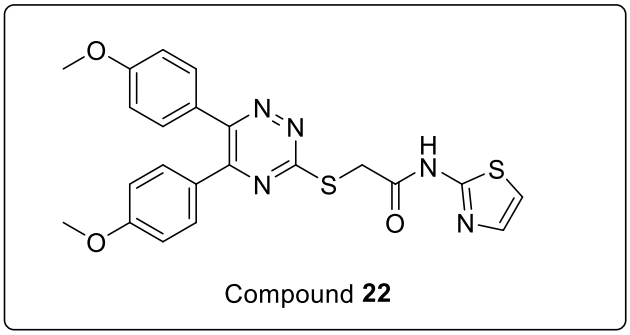
optimum docking scores that corresponded to its *in vitro* efficacy. Overall, this study highlights 1,2,4-triazine hybrids as potential prospects for anti-inflammatory drug discovery, enabling a dual inhibitory strategy with better selectivity and lower toxicity risks (Ghanim, Rezq et al. 2021).



Compound **21**

Figure 2.11. Structure of 1,2,4-triazine-quinoline compound

Ertaş *et al*. reported synthesis of a series of 2-[(5,6-diaryl-1,2,4-triazin-3-yl)thio]-N-(benzo/thiazol-2-yl)acetamide derivatives and evaluated for their inhibitory potential against COX-2 enzyme. Amongst all synthesized derivatives, compound **22** (Figure 2.12) demonstrated significant selectivity for COX-2, with an IC_{50} of 3.06 µM, suggesting its potential significance in addressing inflammatory reactions with minimal COX-1-associated adverse effects. Molecular docking experiments also validated compound **22** COX-2 selectivity, uncovering favourable interactions with COX-2 active site residues which are responsible for its higher binding affinity, thereby highlighting the compound's therapeutic potential as a selective COX-2 inhibitor (Ertaş, Biltekin et al. 2022).



Compound **22**

Figure 2.12. Structure of [(5,6-diaryl-1,2,4-triazin-3-yl)thio] derivative **22**

2.2. 1,3,4-OXADIAZOLE AS ANTI-INFLAMMATORY AGENTS

Omar *et al.* synthesized and examined 1,3,4-oxadiazole derivatives for their potential anti-inflammatory properties by cyclodesulfurizing thiosemicarbazide using dicyclohexylcarbodiimide (DCC) or sodium hydroxide. The anti-inflammatory activity of compounds such as **23**, **24**, **25**, and **26** (Figure 2.13) was evaluated in histamine-induced inflammation models using Golikov's method. These compounds demonstrated superior activity in comparison to ibuprofen. The most active compounds exhibited higher LD_{50} values in acute toxicity studies, which indicated a lower toxicity. The safety of these compounds was further enhanced by including basic substituents on the oxadiazole nucleus, which reduced gastrointestinal adverse effects. These findings underline the potential of 1,3,4-oxadiazole derivatives as better, more secure substitutes for traditional NSAIDs (Omar, Mahfouz et al. 1996).

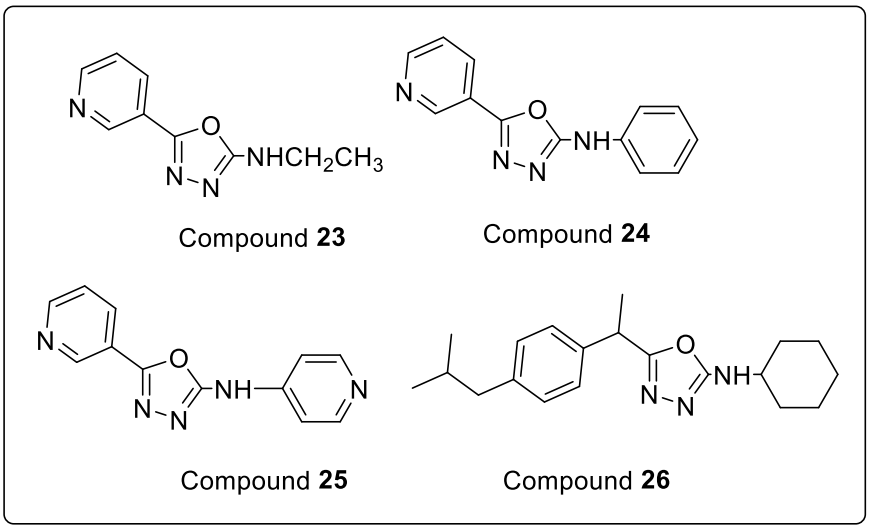


Figure 2. 13. Structures of 1,3,4-oxadiazole derivatives **23**, **24**, **25**, and **26**

Nicolaides *et al.*, and colleagues reported synthesis and evaluation of 1,3,4-oxadiazole compounds for their ability to reduce inflammation. They observed that adding a 1,3,4-oxadiazole ring considerably increased the biological activity of coumarin derivatives. Amongst the synthesized derivatives, compound **27** (Figure 2.14) demonstrated strong anti-inflammatory properties using the carrageenin-induced rat paw edema model, with

edema reduction ranging from 59.1% to 87.6%. Although certain substances reduced the activity of lipoxygenase (LOX), the fact that there was no evident link between the anti-inflammatory effects observed *in vivo* and the inhibition of LOX *in vitro* implies an alternate mechanism of action. These results demonstrate the potential of 1,3,4-oxadiazole derivatives in the development of anti-inflammatory medications (Nicolaides, Fylaktakidou et al. 1998).

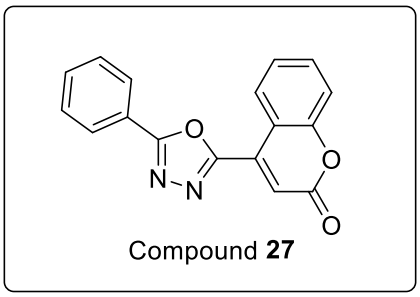


Figure 2.14. Structure of Coumarin oxadiazole compound **27**

Sharma *et al.,* reported the synthesis and evaluation of 1,3,4-oxadiazole compounds resulting from NSAID hydrazide analogues such as naproxen, probenecid, and diclofenac, that reacted with biphenyl acetic acid or p-chloro benzoic acid to yield novel derivatives. The derivative with the most anti-inflammatory efficacy, compound **28** (Figure 2.15), was made from naproxen hydrazide and biphenyl acetic acid which surpassed the other compounds. The increased activity was linked to modifications to structures, particularly higher molecular weight, which might enhance interactions at inflammation targets. Compound **28** offers the potential as a safer, more effective anti-inflammatory lead (Sharma and Khan 2003)**.**

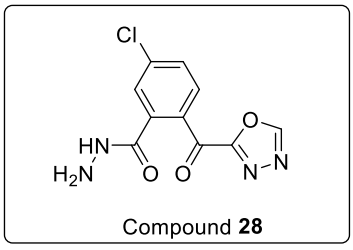


Figure 2.15. Structure of 1,3,4-oxadiazole compound **28**

Narayana, *et al.*, and colleagues reported the synthesis of 2-(6-methoxy-2-naphthyl)-5-aryl-1,3,4-oxadiazoles as potential non-steroidal anti-inflammatory and analgesic agents by reacting 6-methoxy-2-naphthoic acid hydrazide with several aromatic carboxylic acids. The compounds were characterized using spectrum methods, and pharmacological testing demonstrated potent anti-inflammatory and analgesic properties, particularly for compound **29** (Figure 2.16). These compounds' structural resemblance to diclofenac sodium and their decreased gastrointestinal adverse effects highlight their potential as safer alternatives for the treatment of pain and inflammation (Narayana, Vijaya Raj et al. 2005).

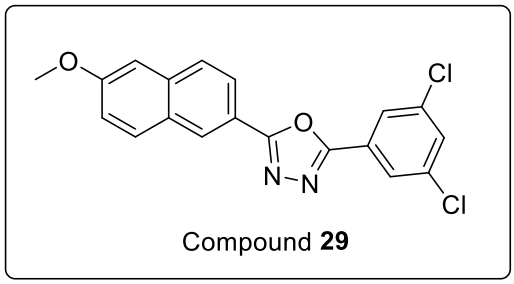


Figure 2.16. Structure of 3,5-Dichloro-phenyl-naphthyl-oxadiazole compound **29**

Amir *et al.*, highlighted the significance of the 1,3,4-oxadiazole moiety in the development of strong anti-inflammatory and analgesic medicines, with compounds **30** and **31** (Figure 2.17), demonstrating superior performance. The anti-inflammatory activity of compound **30** was 81.81%, which is comparable to naproxen, and the analgesic activity was 86.6%, which was higher than naproxen's 73%.

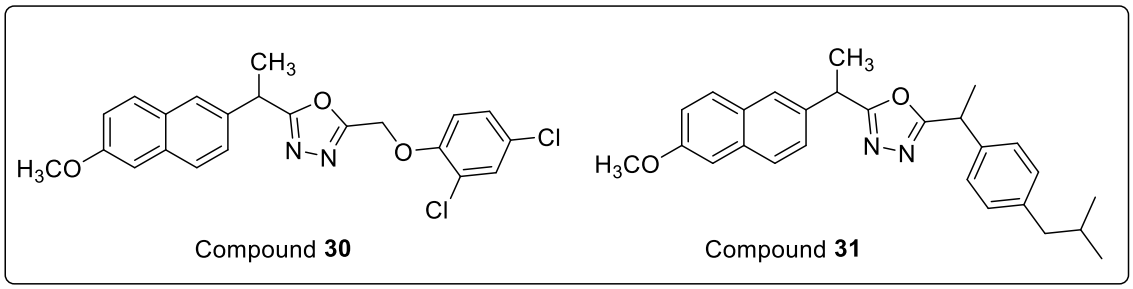


Figure 2.17. Structure of naproxen derivatives containing 1,3,4-oxadiazole, **30** and **31.**

Additionally, compound **30** had less ulcerogenic action, suggesting its potential for safe anti-inflammatory medication research. The safety profiles and efficacy were dependent on the oxadiazole core (Amir, Kumar et al. 2007).

Rai *et al.* reported the synthesis and biological evaluation of novel ether-linked bis(heterocycles) with isoxazoline and 1,3,4-oxadiazole structures for therapeutic properties. Hydrazides were generated from ester analogues and combined with aromatic aldehydes to yield hydrazones, which were then oxidized with chloramine-T to obtain 1,3,4-oxadiazoles. Pharmacological examinations revealed that the compounds had lesser anti-inflammatory activity than ibuprofen, but four compounds (**32**, **33**, **34**, and **35**) (Figure 2.18) showed substantial analgesic benefits, with compound **33** being the most effective (Jayashankar, Rai et al. 2009).

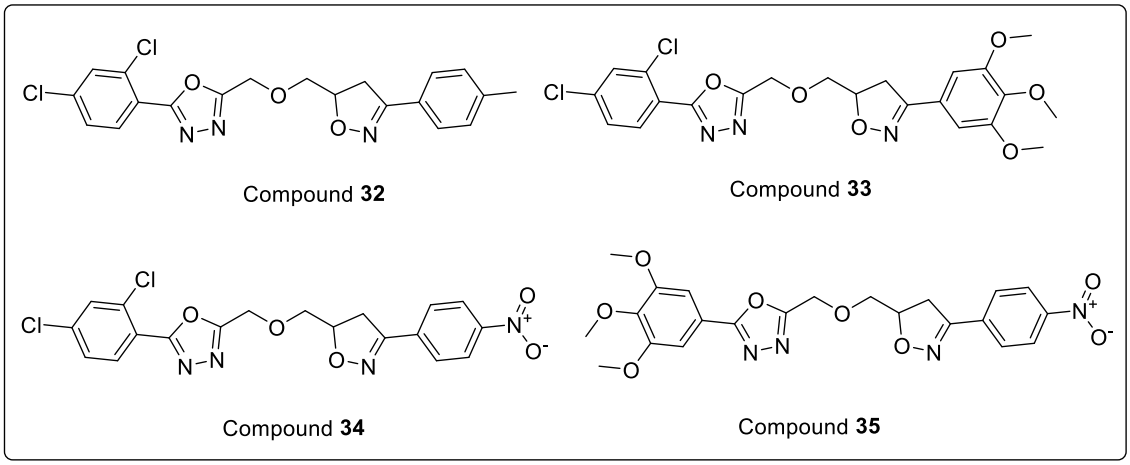


Figure 2.18. Structures of ether-linked bis(heterocycles) with isoxazoline and 1,3,4-oxadiazole

Akhter *et al*. highlighted the potential of 2,5-disubstituted 1,3,4-oxadiazole derivatives (Figure 2.19) as promising candidates for dual inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, both of which play important roles in the inflammatory reactions. Among the synthesized derivatives, **36** identified as the most promising derivative, demonstrating a remarkable 89% reduction of edema induced by carrageenan.

Compound **37** trailed close with 86% inhibition. Compounds **36**, **37**, and **38** of the 2,5-disubstituted 1,3,4-oxadiazole analogues showed strong analgesic activity, with 51-74% protection against sodium chloride-induced writhings, whereas compounds containing electronegative groups on the phenyl ring demonstrated more favourable results. Their dual COX/LOX inhibition mechanism improves anti-inflammatory efficiency while lowering gastrointestinal adverse effects commonly associated with classic NSAIDs, making them intriguing candidates for facilitating the development of more effective and safer anti-inflammatory medicines (Akhter, Akhter et al. 2011).

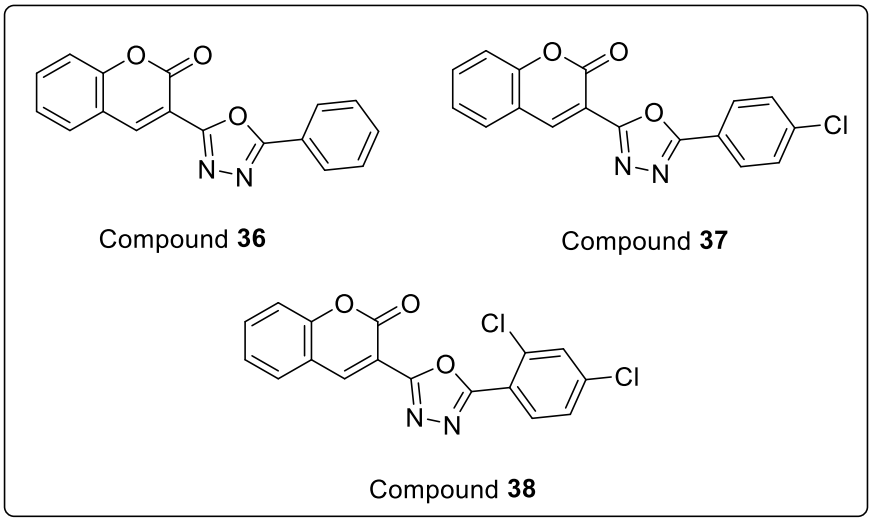


Figure 2.19. Structures of 2,5-disubstituted 1,3,4-oxadiazoles **36**, **37**, and **38**

Bala *et al*. reported N-phenyl anthranilic acid-based 1,3,4-oxadiazole compounds and most notably compounds **39** and **40** (Figure 2.20), have demonstrated significant anti-inflammatory and analgesic properties equivalent to diclofenac sodium. The inclusion of chloro groups at the para and ortho positions increases lipophilicity and membrane penetration, which improves active site binding. Molecular docking experiments showed that these drugs have superior binding interactions with the COX-2 enzyme, with docking scores much greater than those of diclofenac sodium. Compounds **39** and **40** form strong hydrogen bonds to crucial COX-2 residues, Glu 87 and Lys 492, indicating their efficacy as COX-2 inhibitors. Overall, the findings imply that these 1,3,4-oxadiazole

derivatives might serve as significant breakthroughs in the development of novel anti-inflammatory drugs with enhanced efficacy and minimal adverse effects (Bala, Kamboj et al. 2013).

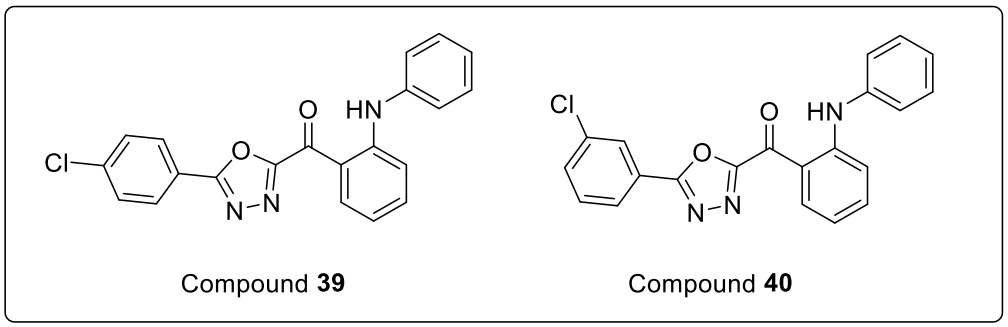


Figure 2.20. Structures of N-phenyl anthranilic acid-based 1,3,4-oxadiazole **39** and **40**

Siddiqui *et al.* synthesized and evaluated a series of N-substituted 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2-yl-2-sulfanyl acetamides generated from 4-chlorophenoxyacetic acid. The synthesis consisted of multiple steps, including the preparation of ethyl 2-(4-chlorophenoxy)acetate and its reaction to 2-(4-chlorophenoxy)acetohydrazide, and finally, ring closure to yield the oxadiazole derivative. According to biological investigations, many compounds exhibited greater antibacterial activity than ciprofloxacin against both gram-positive and gram-negative bacteria. Among all synthesized derivatives, compound **41** (Figure 2.21) exhibited the most promising antibacterial activity. The oxadiazole moiety also provides anti-inflammatory activity, making them useful in medicinal chemistry for addressing inflammatory diseases. Furthermore, the compounds demonstrated minimal cytotoxicity and moderate inhibition of the α-chymotrypsin enzyme, suggesting their potential as therapeutic agents with superior safety profiles (Siddiqui, Abbasi et al. 2014).

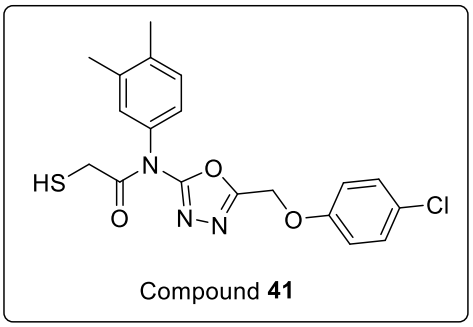


Figure 2.21. Structure of N-substituted 4-chlorophenoxy oxadiazole sulfanyl acetamide **41**

Grover *et al*. have reported a novel series of 2,5-diaryl-1,3,4-oxadiazole compounds that have been synthesized and examined for their application as selective COX-2 inhibitors and anti-inflammatory drugs. Among the investigated compounds, **42**, **43**, and **44** (Figure 2.22) appeared as the most effective COX-2 inhibitors, with IC_{50} values varying from 0.48 to 0.89 mM and selectivity indexes indicating strong selectivity against COX-2, notably compound **42** with a selectivity index of 132.83. The study suggests that these oxadiazoles may act as an innovative framework for the development of future COX-2 inhibitors, potentially providing alternative therapies for diseases like osteoarthritis and rheumatoid arthritis (Grover, Bhatt et al. 2015).

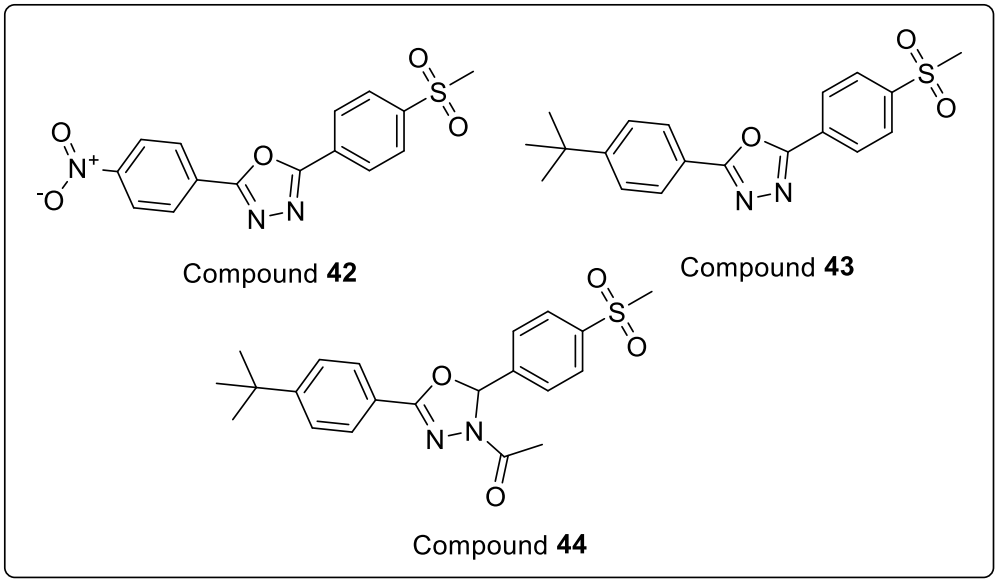


Figure 2.22. Structures of 2,5-diaryl-1,3,4-oxadiazole compounds **42**, **43**, and **44**

Abd-Ellah *et al*. have reported a novel series of 1,3,4-oxadiazole/oxime derivatives with noteworthy anti-inflammatory activities. Compound **45** (Figure 2.23) edged out indomethacin in terms of anti-inflammatory behaviour, decreasing paw edema by 96.67%; however, its analgesic effectiveness and BBB penetration were hampered by its greater molecular size. These results indicate the potential of oxadiazole/oxime hybrids as safer and more potent anti-inflammatory drugs (Abd-Ellah, Abdel-Aziz et al. 2016).

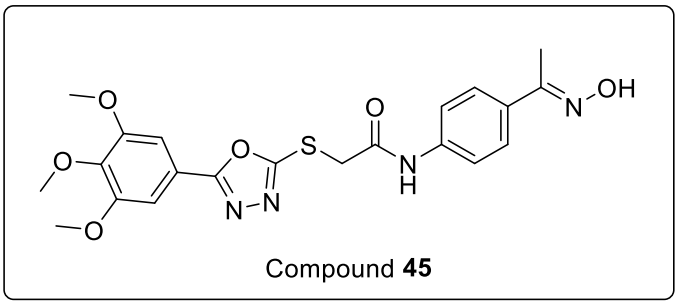


Figure 2.23. Structure of 1,3,4-oxadiazole/oxime compound **45**

Yadav *et al.* synthesized a series of novel 1,3,4-oxadiazole-2(3H)-thione analogues and investigated for their anticancer efficacy against four malignant cell lines: HeLa (cervical), U-87 (glioblastoma), Panc (pancreatic), and MCF-7 (breast). Among all compounds, **46** and **47** (Figure 2.24) displayed the most significant cytotoxicity, particularly against the HeLa cell line, with considerable apoptotic responses. The compounds **46** and **47** were further highlighted by their lack of cytotoxicity against Human Embryonic Kidney (HEK) cells, showing their promise as lead compounds in subsequent anticancer medicine discovery (Yadav, Kumar et al. 2017).

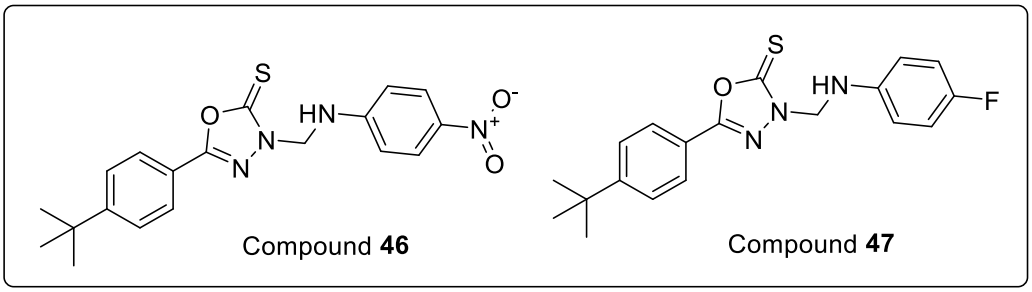


Figure 2.24. Structures of 1,3,4-oxadiazole-2(3H)-thione compounds **46** and **47**

Padejjar *et al.* have reported a novel series of 2-(5-[2-methyl-6-arylpyridin-3-yl]-1,3,4-oxadiazol-2-ylthio)-1-arylethanones and investigated them for antibacterial and anti-inflammatory activity. Amongst all synthesized compounds, compound **48** (Figure 2.25) displayed the most effective anti-inflammatory effects, attaining 106.2 % inhibitions, outperforming the conventional drug Indomethacin's relative efficacy of 70.1 %. Structural exploration demonstrated the importance of structural changes in maximizing activity by showing that halogen substitution, specifically the chlorophenyl group on the pyridine ring, improved anti-inflammatory efficiency. Overall, the arylpyridine-based 1,3,4-oxadiazoles, particularly **48** have potential antibacterial and anti-inflammatory properties (Padejjar Vasantha, Poojary et al. 2019).

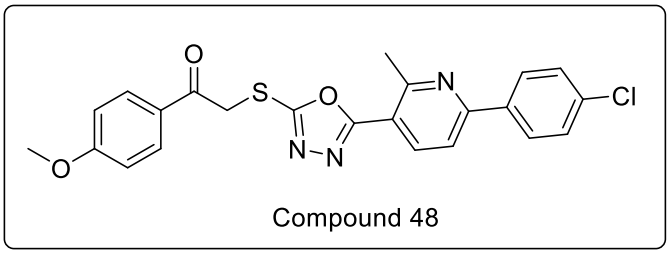


Figure 2.25. Structure of arylpyridine-based 1,3,4-oxadiazole **48**

Szczukowski *et al*. investigations have highlighted the development of pyrrolo[3,4-d]pyridazinone compounds with 1,3,4-oxadiazole-2-thione and arylpiperazine moieties, which show potential as selective COX-2 inhibitors. These compounds have considerable anti-inflammatory and antioxidant properties. They efficiently scavenge reactive species and show potential as more secure alternatives to NSAIDs such as diclofenac and ibuprofen, which induce gastrointestinal injury. Compounds **49** and **50** (Figure 2.26) have significant binding affinity to the COX-2 active site, indicating the potential for a novel class of selective COX-2 inhibitors with enhanced safety profiles and potency (Szczukowski, Krzyżak et al. 2020).

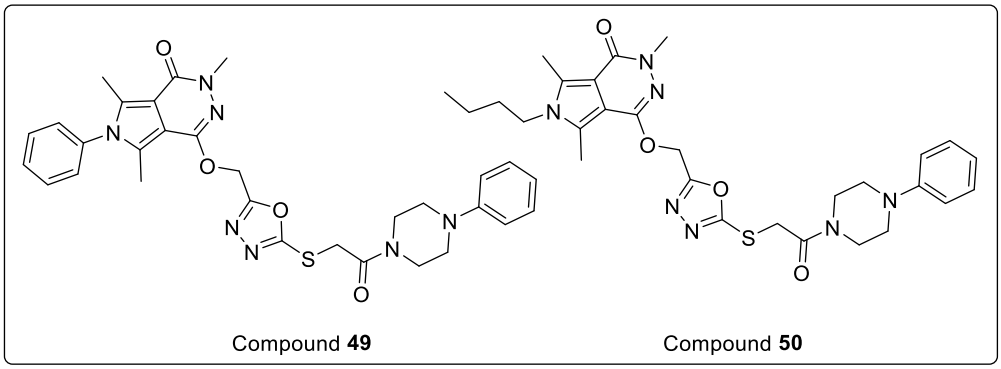


Figure 2.26. Structure of Pyrrolo[3,4-*d*]pyridazinone-Based 1,3,4-Oxadiazole

Szandruk *et al*. have reported the anti-inflammatory activities of novel 1,3,4-oxadiazole analogues of pyrrolo[3,4-d]pyridazinone, particularly compounds **51** and **52** (Figure 2.27) were investigated in a carrageenan-induced rat paw edema model. The findings suggest that both compounds considerably decreased paw oedema and compensate the increased levels of proinflammatory mediators, including prostaglandin E2 (PGE2), tumour necrosis factor-α (TNF-α), and myeloperoxidase (MPO), which are markers of inflammation and neutrophil infiltration. The study also shows that neither substance significantly changed the integrity of the stomach mucosa or liver and kidney function, indicating a good safety profile compared to conventional NSAIDs (Szandruk-Bender, Merwid-Ląd et al. 2021).

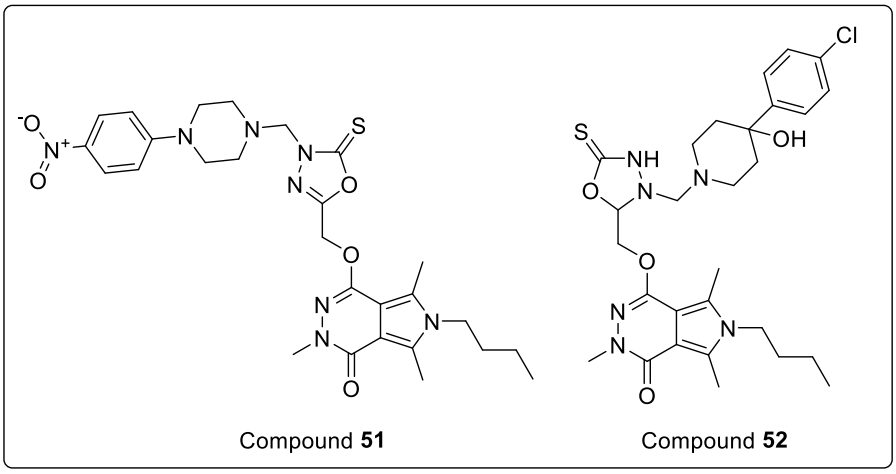


Figure 2.27. Structures of pyrrolo[3,4-d]pyridazinone-based 1,3,4-oxadiazoles derivatives **51** and **52**

Hamoud *et al*. highlighted the significance of 1,3,4-oxadiazole compounds as specific COX-2 inhibitors with noteworthy anti-inflammatory and antioxidant potential, showing minimal gastrointestinal side effects than NSAIDs. The investigation concluded that compound **53** (Figure 2.28) is the most potent and selective COX-2 inhibitor (IC_{50}: 0.04 μM, selectivity index: 337.5) which is comparable to celecoxib. Furthermore, these compounds suppress pro-inflammatory cytokines (TNF-α, IL-6), reactive oxygen species (ROS), and nitric oxide (NO), highlighting their potential in regulating inflammation and oxidative stress (Hamoud, Osman et al. 2022).

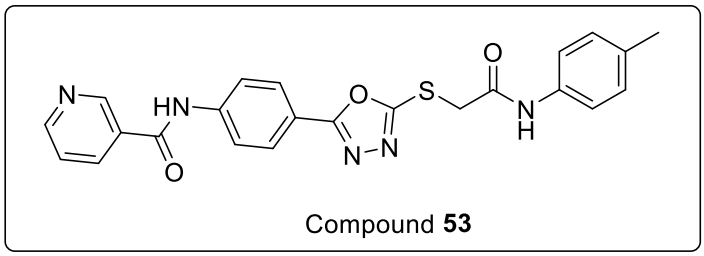


Figure 2.28. Structure of novel 1,3,4-oxadiazole-based compound **53**

2.3. BENZHYDYLPIPERAZINE AS ANTI-INFLAMMATORY AGENTS

Lewis *et al.* reported the synthesis of novel dual-function molecules that incorporate the pharmacophores of benzhydryl piperazine, an efficient H_1 receptor antagonist, and N-hydroxyurea, which is recognized for its 5-LOX inhibition activity, to target 5-lipoxygenase (5-LOX) and histamine H_1 receptors concurrently. Compound **54** (Figure 2.29) displayed considerable *in vitro* and *in vivo* efficacy, inhibiting histamine-induced bronchoconstriction by 86% in an animal model. The structural alterations made to the benzhydryl piperazine moiety were significant in increasing the compound's potency. The results presented highlight the potential of dual-targeting drugs to provide a broader treatment approach for asthma by targeting several inflammatory and bronchoconstrictive pathways, perhaps leading to better therapeutic results than typical single-target therapies (Lewis, Bayless et al. 2004).

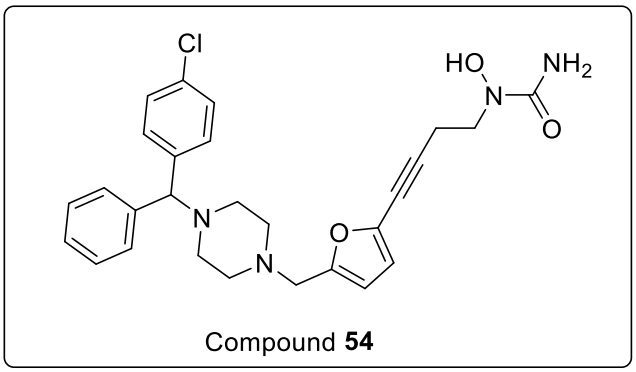


Figure 2.29. Structure of benzhydrylpiperazine and N-hydroxyurea-based compound **54**

Kumar *et al.* have synthesised a series of novel 1-benzhydryl-sulfonyl piperazine analogues via nucleophilic substitution reaction with 1-benzhydrylpiperazine and various sulfonyl chlorides, resulting in substantial yields and great purity. Amongst the synthesized derivatives, compound **55** (Figure 2.30), i.e., 1-benzhydryl-4-(4-tert-butyl-benzenesulfonyl)-piperazine, showed significant inhibition of MDA-MB-231 human breast cancer cell proliferation. This compound was listed as the most potential alternative for future preclinical investigations. The study emphasises the medicinal possibility of these analogues in breast cancer treatment, resolving the striking interest in novel chemotherapeutic drugs with improved efficiency and selectivity (Ananda Kumar, Nanjunda Swamy et al. 2007).

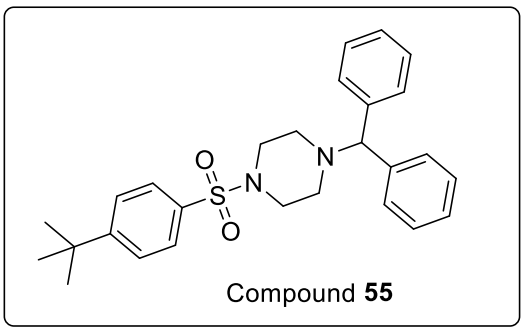
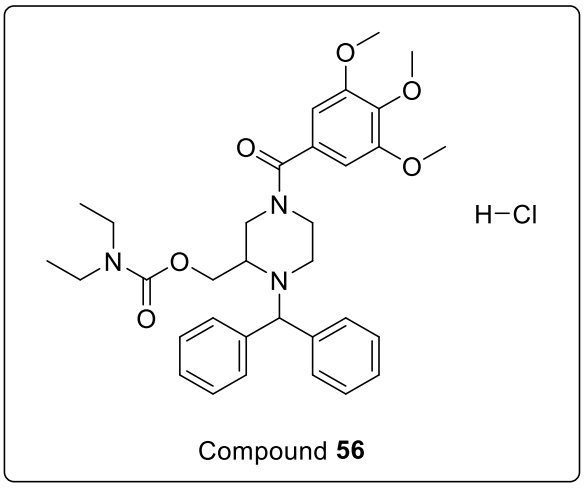


Figure 2.30. Structure of 1-benzhydryl-sulfonyl piperazine compound **55**

Wang *et al*. have reported the research that identified numerous notable compounds with strong anti-cancer capabilities. Owing to its strong anti-PAF and anti-HIV-1 properties, Compound **56** (Figure 2.31) distinguishes out among the reported derivatives and could

also be an alternative therapy for dementia linked with HIV. Furthermore, few molecules have demonstrated potential anti-HIV activity but are discussed to have lesser anti-cancer efficacy than **56**. The trimethoxybenzene compounds, notably **56**, have been identified for their ability to inhibit tubulin polymerisation, which is crucial in cancer cell proliferation. Perhaps **56** stands out as a promising candidate in the continuing hunt for potent anti-cancer medications, owing to its dual action capabilities to promote cell death in malignant cells (Wang, Xu et al. 2009).



Compound **56**

Figure 2.31. Structure of Diethylaminocarbonyloxymethyl-diphenylmethyl-trimethoxybenzoylpiperazine HCl **56**

Naveena *et al*. have focussed on a new 1-benzhydrylpiperazine derivative **57** (Figure 2.32), synthesized using nucleophilic substitution, which has strong anti-inflammatory action as well as depressive, antimalarial, and antiviral activities. The addition of the piperazine scaffold, an invaluable structure in medicinal chemistry, emphasises its capacity to interact successfully with a wide range of biochemical targets, improving its pharmacological versatility. These characteristics render the synthesised derivative a potential option for additional research in anti-inflammatory therapy, adding to the expanding study on piperazine-based molecules for medicinal purposes (Naveena, Manjunathb et al. 2010).

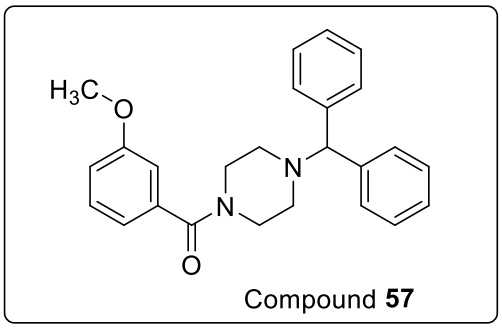


Figure 2.32. Structure 1-benzhydrylpiperazine derivative **57**

Wang *et al.* highlighted the necessity for efficient vasodilators as hypertension is a major risk factor for cardiovascular illnesses, is frequently connected to vascular damage and decreased relaxation. It has been discovered that imperatorin (IMP), a bioactive substance with significant anti-inflammatory, anticancer, and anticonvulsant qualities, causes vasodilation by blocking Ca2+ inflow and L-type calcium channels. Biological examinations in isolated artery models revealed dose-dependent relaxation, with compound **58** (Figure 2.33) demonstrating the greatest effectiveness in mesenteric arteries. The compound showed dual activity as vasodilators and anti-inflammatory agents, indicating prospective pathways for cardiovascular and inflammation-related medication development (Wang, Wang et al. 2015).

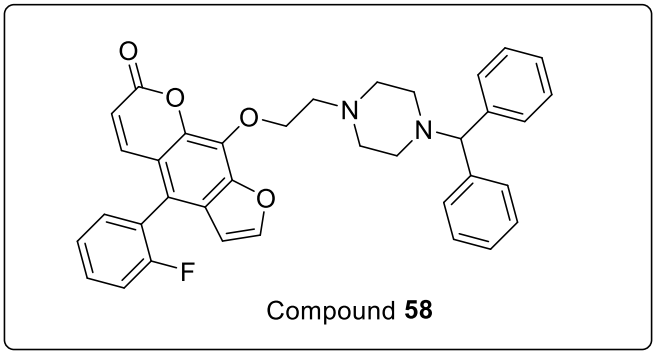


Figure 2.33. Structure of biphenyl–furocoumarin based compound **58**

Kumar *et al.* synthesized and investigated a series of 1-benzhydryl-piperazine urea derivatives for anti-inflammatory action in male Wistar rats employing the carrageenan-induced paw edema technique. The derived compounds were characterized using IR,

NMR, and LC-MS methods, and compound **59** (Figure 2.34) exhibited the most significant activity, inhibiting inflammation by 60.6%. Para-substituted and disubstituted derivatives demonstrated increased potency, showing the impact of specific structural modifications on their anti-inflammatory properties. Compound **59** is a potential candidate for further study and development as an anti-inflammatory drug with potential for enhanced oral bioavailability and therapeutic uses considering its promising activity (Kumar, Veeresh et al. 2017).

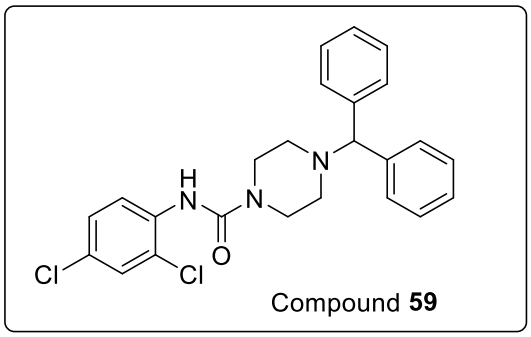


Figure 2.34. Structure of 1-benzhydryl-piperazine based compound **59**

Qiu, Pei *et al.* have reported the design and synthesis of N-heterocyclic Aloe-emodin derivatives to boost anti-inflammatory properties, particularly focuses on their capacity to inhibit RAW264.7 macrophages in producing nitric oxide (NO). Compound **60** (Figure 2.35) surpassed Aloe-emodin in terms of LPS-induced NO inhibition, having an IC50 of 5.66 ± 0.47 μM. Additionally, it hindered the NF-κB signalling pathway, a major contributor to inflammation, and decreased pro-inflammatory mediators such IL-1β, TNF-α, and PGE2. With an oral bioavailability of 55.16% and a favourable safety profile, **60** stands up as a promising option for anti-inflammatory therapy (Qiu, Pei et al. 2021).

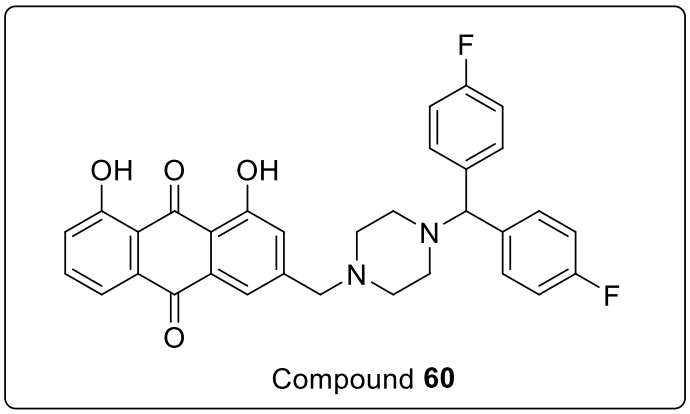


Figure 2.35. Structure of N-heterocyclic Aloe-emodin compound **60**

Marciniak *et al.* have reported the potential of phthalimide derivatives, specifically the benzhydrylpiperazine derivative **61** (Figure 2.36), as potential candidate for analgesic and anti-inflammatory medications. These derivatives have strong analgesic effects, according to behavioural tests conducted on murine analgesic models. Compound **61** has the most promising results due to the structural modifications that enhance its binding interactions with plasma proteins. The compound had high binding constants, particularly with human serum albumin (BSA), indicating significant interactions that could improve their pharmacological activity. The results indicate that the benzhydrylpiperazine derivative (F4) stands out as the best drug among the evaluated phthalimide derivatives, indicating its potential in the development of novel analgesic medicines (Marciniak, Kotynia et al. 2022).

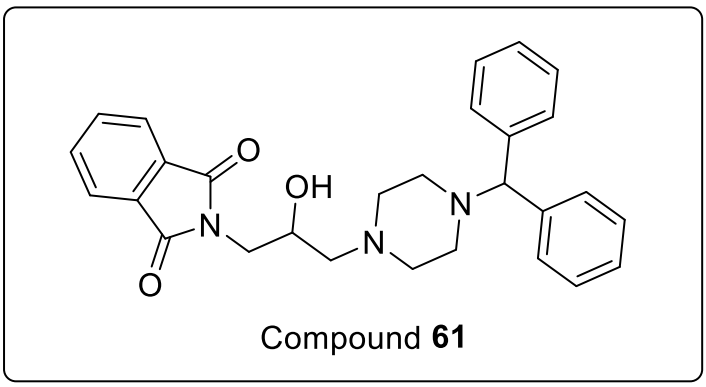


Figure 2.36. Structure of phthalimide derivative **61**

Fatima *et al*. have identified benzhydrylpiperazine analogues as intriguing alternatives for anti-inflammatory drug development owing to their varying pharmacological actions, which also include antihistaminic, antimicrobial, and anticancer activities. Amongst the synthesized derivatives, compound **62** and **63** (Figure 2.37) showed noteworthy anti-inflammatory action by successfully preventing the formation of nitric oxide (NO) in LPS-stimulated RAW 264.7 macrophage cells at non-cytotoxic doses. The addition of heterocyclic motifs, such as morpholinopyrimidine, increased its potency. This study highlights the therapeutic potential of **62** and **63** in addressing inflammation-related illnesses, opening an opportunity for future medicine development (Fatima, Zaki et al. 2023).

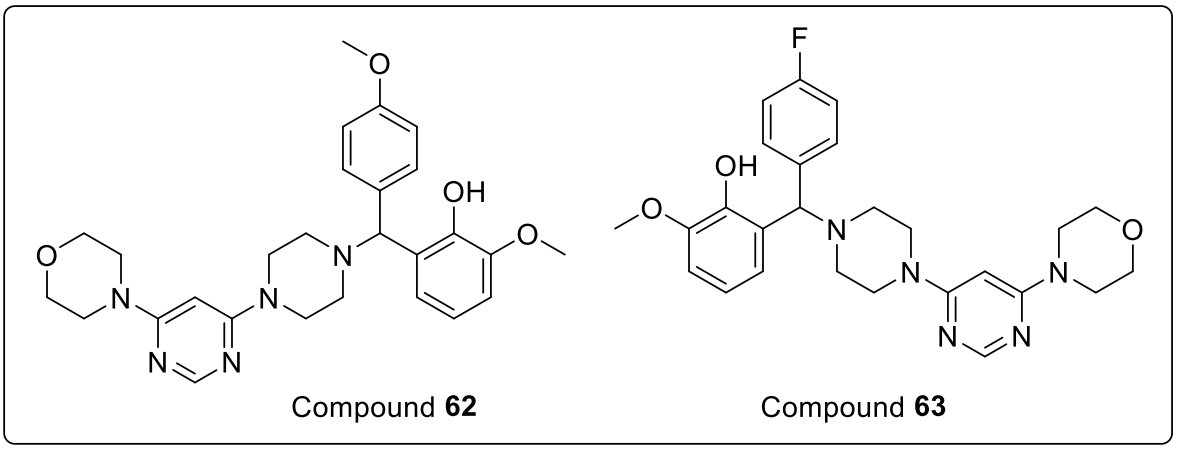


Figure 2.37. Structures of morpholino pyrimidine compounds **62** and **63**

Roy *et al*. have reported piperazine-tethered heterocyclic hybrids, including **64** (Figure 2.38), which have shown strong anti-inflammatory (IC50 = 0.68 μg/mL), strong antiproliferative (IC50 = 33.8 μg/mL), and remarkable antioxidant activity (IC50 = 0.77 μg/mL) against the MDA-MB-231 breast cancer cell line. Furthermore, **64** downregulates hypoxia-regulated CAIX genes and demonstrates significant antimicrobial action against pathogens such as Staphylococcus aureus and Pseudomonas aeruginosa, suggesting that

it might be able to target cancer pathways. **64** stands out as a potential candidate for developing oncology therapies (Roy, Remady et al. 2024).

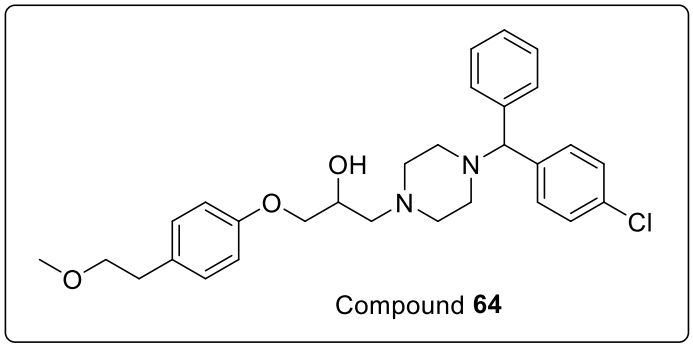


Figure 2.38. Structure of piperazine-tethered heterocyclic compound **64**