Introduction
Chapter 1

Introduction

1.1 Background of the proposed research

Inflammation is defined as a tissue directed response to noxious and injurious external and internal stimuli. The stimuli can be classified into four categories. These are physical stimuli, infective stimuli and immunological stimuli. Upon interaction with anyone or more of stimuli, the body produces a variety of substances. These mediators are responsible for the physical symptoms such as edema, erythema and fever. The mediators can be broadly classified into four categories, namely vasoactive amines, plasma factors, arachidonic acid metabolites and lymphokines. The chronic inflammatory condition leads to the development of diseases including osteoarthritis, rheumatoid arthritis and other inflammatory diseases of the joint. Anti-inflammatory drugs often produce symptomatic relief in the inflammatory diseases, where the underlying causes of inflammation are unidentified.
Non steroidal anti-inflammatory drugs (NSAIDs) are a non-homogeneous family of pharmacologically active compounds used in the treatment of acute and chronic inflammation, pain and fever. However, nevertheless NSAIDs are the most widely used drugs; their long-term clinical use is associated with significant side effects like the onset of gastrointestinal lesions, bleeding and nephrotoxicity.

Therefore the discovery of new safer anti-inflammatory drugs represents a challenging goal for research.

Although several mediators support the inflammatory processes, the main target of NSAIDs is Cyclooxygenase (COX), the enzyme involved in the first step of the conversion of arachidonic acid to prostaglandins (PGs). These later regulate important functions in the gastric, renal, and lymphatic systems and are known to mediate all inflammatory responses. Classical NSAIDs, such as indomethacin, inhibit both isoforms of COX. COX-1 which is constitutively expressed in most tissues and organs and catalyzes the synthesis of PGs involved in the regulation of physiological cellular activities; COX-2, which is mainly induced by several stimuli such as cytokines, mitogens, and endotoxins in inflammatory sites. Thus, their therapeutic effects are mainly due to the decrease of pro-inflammatory PGs produced by COX-2, whereas their unwanted side effects result from the inhibition of constitutive COX-1 isoform.

The first compound, DUP-697 (Figure 1.1) with a clear COX-2 specificity was developed in the early 1990’s and served as template for the development of new drugs, among them Celecoxib and Rofecoxib (Figure 1.1) molecules are in clinical use as anti-inflammatory and analgesic drugs with reduced ulcerogenic
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potential. The basis of COX-2 specificity became evident once the 3D structure of COX-1 and COX-2 were resolved. It was found that change of two isoleucines (Ile 523, Ile434) in COX-1 by two valines in COX-2 enlarged the NSAIDs binding site around 25%, making accessible a hydrophobic pocket in COX-2 but not in COX-1. The aromatic group of COX-2 specific NSAID drug derived from DUP-697 occupies this pocket. Another key difference between COX-2 and COX-1 is the mutation of His513 in COX-1 by Arg in COX-2. This substitution generates a specific interaction which becomes clear when we consider that almost all COX-2 specific drugs have a methylsulfone or sulfonamide group in a position that makes the interaction with Arg possible.

There is tremendous amount of experimental and theoretical work, focused on study of COX-2 and the existence of high-resolution structural information on the binding site of NSAIDs, but still several aspects of binding mechanisms of DUP-697 related compounds to COX-2 remains unclear. Inspection of experimental data reveals that empirical rules formulated for given set of the drugs are useless when applied to a different set, even when both set of the compounds share a common background. This suggests that subtle structural changes in binding site of COX-2 might occur to adopt its structure to the inhibitor. This might be the reason for many diverse group of compounds reported to have anti-inflammatory activity. The marketed anti-inflammatory drugs contain two phenyl rings attached to heterocyclic ring systems like thiophene, oxazolidinone, pyrazoles. Studies on replacement of sulfonamide group with isosteric azido group and other groups are being reported. The survey of structures as given in Figure shows that possibly sulphonamide group and biphenyl group are essential for activity.
4-Thiazolidinone derivatives have been demonstrated to possess antibacterial, antifungal, anticonvulsant, anticancer, and anti-tubercular activities. Compounds MKT-077 and HP-236 have been reported as a registered anti-tumour and antipsychotic agents, respectively Figure 1.1. 4-Thiazolidinones have been reported as novel inhibitors of the bacterial enzyme Mur B which was a precursor for the biosynthesis of peptidoglycan. Moreover, anticonvulsant, antibacterial and antifungal properties of several N1-[4-(4-methoxybenzoylamino)benzoyl]-N2-substituted methylene hydrazines and 1-[4-(4-methoxybenzoylamino)-benzoyl]-4-alkyl-aryl thiosemicarbazides were described. 2-Aryl derivatives of thiazolidinones as lead compounds in the quest for clinically useful N-type calcium channel blockers in the treatment of pain associated with inflammation are reported [10].

A critical observation of these data indicates that, there still exists a gap to identify new compounds as anti-inflammatory agents with reduced toxicity.
Figure 1.1: Structures of new compounds with anti-inflammatory activity
4-thiazolidinones are known to have various pharmacological activities. In the present study various 4-thiazolidinone derivatives were synthesized and evaluated for their pharmacological activities.

Nimesulide a well known anti-inflammatory agent with preferential inhibition of COX-2 enzyme activity was hydrolyzed to obtain a free amino group which was later converted to the corresponding 4-thiazolidinone (A1-A8).

As derivatives of sulphonilamide are known to have antibacterial as well as diuretic activity and also many times infection is associated with inflammatory conditions, it was thought worthwhile to incorporate a sulphonamide group, and study them for analgesic and anti-inflammatory activity (B1-B8). Phenacetin and paracetamol are therapeutically used as analgesics. The metabolite para amino phenol is the main active moiety to exert the analgesic activity. Bearing this in mind para hydroxyl group (D1-D4) and para ethoxyl group (C1-C4) were attached to the phenyl ring of 4-thiazolidinone moiety to know, whether such a compound designed would be an analgesic, anti-inflammatory or both.

The 4-thiazolidinone derivatives obtained from nimesulide and sulphonilamide were further converted to the spiro derivatives (A9-A11) and (B9-B11). Coumarin derivatives are not explored so far for analgesic and anti-inflammatory activities. The coumarin was reacted with ethyl chloracetae to get the corresponding ester which in turn on reacting with hydrazine hydride, the corresponding 4-thiazolidinone derivatives (C5-C7) were synthesized, which were studied for anti-inflammatory, analgesic and antipyretic activity.
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With this background, seven series of derivatives of 4-thiazolidinones were synthesized, at the Department of Pharmaceutical Chemistry, K.L.E. University’s College of Pharmacy, Belgaum. They were evaluated for anti-inflammatory, analgesic, antipyretic and COX-2 enzyme inhibitory activity to know whether sulfonamide, 4-nitrophenoxypyphenyl, p-hydroxyphenyl, p-ethoxyphenyl and coumarinyl group substitution on 4-thiazolidinone moiety is essential for activity or not and
also the effect of introducing 5-spiro substitution at 4-thiazolidinone on the above mentioned activities and toxicity.

### 1.2 Objectives of the proposed research

1. To evaluate the novel derivatives of 4-thiazolidinones for anti-inflammatory, analgesic, antipyretic and in vitro COX-2 enzyme inhibitory activity.

2. To identify the possible mechanism of action and structure activity relationship among the compounds.

3. To evaluate the acute and subacute toxicity of active moieties.

### 1.3 Methodology adopted for study

The methodology adopted for the research work is as given below; the study tasks were completed to achieve the objectives of the study.

Phase - 1: Collect the samples of different derivatives of 4-thiazolidinones synthesized in Department of Pharmaceutical Chemistry, KLE College of Pharmacy,
Belgaum. The structure of the compounds selected, were established on the basis of physical, chemical and spectral data. In all 33 compounds belonging to seven series of 4-thiazolidinones were taken for study.

Phase - 2: The compounds selected for activity were tested for acute toxicity as per OECD guidelines to identify the therapeutic dose of the test compounds, which will be approximately between one fifth (1/5th) to one tenth (1/10th) of the toxic dose range.

Phase - 3: The 33 compounds were evaluated for anti-inflammatory activity in acute and sub acute models of inflammation.

Phase - 4: The compounds were evaluated for analgesic and antipyretic activity as many known NSAIDs also have analgesic and antipyretic activity.

Phase - 5: The test compounds were evaluated for COX-1 and COX-2 enzyme inhibitory activity, which will help us to identify possible mode of action of active compounds.

Phase - 6: Test compounds showing significant and good anti-inflammatory activity were evaluated for sub-acute toxicity studies wherein effects on hematological and histopathological parameters were studied.


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1.4 Scope of the study

As reported in literature, compounds with diverse structures have been developed as anti-inflammatory agents, indicating that there is lot of flexibility in the structure of COX-2 enzyme inhibition. So far no efforts have been made to explore the spiro compounds for anti-inflammatory activity. Hence for the first time such compounds were synthesized with an interest to see whether any optical centre introduced would play a major role in the thiazolidinones anti-inflammatory activity. The phenacetin molecule was suitably modified to a thiazolidinone derivative. Since oxygen containing compounds such as oxazolidinones, phenylbutazone are known for anti-inflammatory activity, an oxygen bearing compound coumarin was taken as lead for modification of thiazolidinone moiety. Our study will try to identify how the various modifications in the structure of 4-thiazolidinones can bring about change in activity and gives direction to develop newer agents for better anti-inflammatory activity with reduced toxicity.