Pharmacological Evaluation of Novel Thiazolidinone Derivatives

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Doctor of Philosophy

By

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Background of the proposed research

Inflammation is defined as a tissue directed response to noxious and injurious external and internal stimuli. The mediators responsible for the physical symptoms such as edema, erythema and fever can be broadly classified into four categories, namely vasoactive amines, plasma factors, arachidonic acid metabolites and lymphokines.

In therapeutics steroidal and non steroidal anti-inflammatory drugs are used. Steroidal anti-inflammatory drugs have limitations of use because of serious adverse effects like negative calcium balance, impaired wound healing and mental depression.

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 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 molecules are in clinical use as anti-inflammatory and analgesic drugs with reduced ulcerogenic 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. 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 and pyrazoles. Studies on replacement of sulfonamide group with isosteric azido group and other groups are being reported.

4-Thiazolidinone derivatives have been demonstrated to possess antibacterial, antifungal, anticonvulsant, anticancer, and anti-tubercular activities. Derivatives of 2-Aryl thiazolidinone as lead compounds in the quest for clinically useful N-type calcium channel blockers in the treatment of pain associated with inflammation are reported.
A critical observation of these data indicates that, there still exists a gap to identify new compounds as anti-inflammatory agents with reduced toxicity.

On the basis of review of literature various 4-thiazolidinone derivatives were synthesized at the department of pharmaceutical chemistry, K.L.E. College of Pharmacy, Belgaum. The chemical structures were confirmed by physical, chemical and spectral analysis of the compounds viz, 4-nitro2-phenoxyphenyl 4-thiazolidinone (A1-A8), sulphonyl 4-thiazolidinone (B1-B8) and their spiro derivatives (A9-A11,B9-B11), Paraethoxyphenyl 4-thiazolidinone (C1-C4), Coumarinyl 4-thiazolidinones (C5-C7), Para hydroxyphenyl 4-thiazolidinone (D1-D4),
Figure 3: Chemical structures of C1-C4 and C5-C7

Figure 4: Chemical structures of D1-D4
Objectives of the proposed topic of research

1. To evaluate the novel derivatives of Thiazolidinones for analgesic, anti-inflammatory and antipyretic 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.

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 thiazolidinones synthesized in Department of Pharmaceutical chemistry, KLE College of Pharmacy, Belgaum. The selected compounds, structures were confirmed by physical, chemical and spectral analysis. In all 33 compounds belonging to seven series of thiazolidinones were taken for study.

**Phase - 2:** The compounds selected for activity are 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 inhibition activity, which will help us to identify possible mode of action of active compounds.
Phase - 6: The compounds showing highly significant anti-inflammatory activity were evaluated for sub-acute toxicity studies wherein effects on hematological and histopathological parameters were studied.
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.

Experimental Materials

Drugs:
Standard: Nimesulide at a dose of 50mg/kg b. w.
Test compounds: A1-A11, B1-B11, C1-C7, D1-D4,
Chemicals: Carrageenan, Xylol, acetic acid, Brewer’s yeast

Methodology

Toxicity Studies:

1. Acute oral toxicity studies:
   The dose of the test compounds were finalized by the acute oral toxicity studies performed in rats according to the OECD guidelines 423 (October 2000).

2. Sub acute oral toxicity studies:
   The sub acute oral toxicity studies were done as per OECD guidelines 407. The following compounds from each group were selected for sub acute toxicity study based on their showing good activity in the various tests performed: A8, B8, C3 and C7.
Pharmacological Activities

1. Anti inflammatory activity:
   
   (a) Acute models:
   
   i. Carrageenan induced paw edema in rats \(^{17}\).
   
   ii. Xylol induced ear edema in mice \(^{18}\).
   
   (b) Subacute model:
   
   i. Cotton pellet induced Granuloma in rats \(^{19}\).

2. Analgesic activity:

   (a) Acute models:
   
   i. Acetic acid induced writhing in mice \(^{20}\).
   
   ii. Caudal immersion in rats \(^{21}\).

3. Antipyretic activity:

   (a) Brewer’s yeast induced pyrexia in rats \(^{22}\).

4. COX enzyme inhibitory activity \(^{23}\).

   (a) The COX enzyme inhibitory assay was performed by using the experimental kit obtained from CAYAMAN chemicals, USA. ( Item No. 760111)

   The colorimetric COX(ovine) inhibitor screening assay utilizes the peroxidase component of cyclooxygenase. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N,N,N,N- Tetramethyl-p-phenylenediamine (TMPD) at 590nm - 610nm. The kit contained assay buffer(10X), Heme, COX-I(ovine), COX-2(ovine), Arachidonic acid, Potassium hydroxide, Colorimetric subtrate, 96 well plate.
Results

Acute Toxicity Studies

All the test compounds showed toxicity between the dose range of 300mg/kg to 1000mg/kg, except D2 showing no toxicity even at 2000mg/kg dose. Hence 50mg/kg and 100mg/kg were selected as doses for pharmacological studies.

Effect of 4-thiazolidinones on carrageenan induced paw edema:
All the 4-nitro2-phenoxyphenyl4-thiazolidinone(A1-A11) compounds showed significant (p<0.01) inhibition of edema at both doses except compound A7 showing negligible inhibition at the 1st hour. The compounds A4(56.6%), A5(49%), A6(55.5%) and A8(57.7%) showed maximum inhibition of edema and compounds with spiro group substitution at 5 position in 4-thiazolidinone(A9-A11) showed minimum inhibition of edema.

All the sulphonyl 4-thiazolidinone compounds (B1-B11) showed significant (p<0.01) inhibition of edema at both doses 50mg/kg and 100mg/kg during the 1st hour and the 3rd hour. The compounds B3(42.6%), B5(40.2%), B6(40.8%), B7(40.8%), B8(47.1%) showed maximum inhibition of edema. The 5-spiro4-thiazolidinone derivatives (B9-B11) showed less inhibition of edema at both doses tested.

The ethoxyphenyl 4-thiazolidinone compounds (C1-C4) showed significant (p<0.01) inhibition of edema. The inhibition was maximum in the 1st hour than in the 3rd hour. except for C3, at 50mg/kg dose. The coumarinyl 4-thiazolidinone compounds C5(58.7%), C6(60%), C7(60.5%) showed maximum inhibition of edema.

The hydroxyphenyl 4-thiazolidinone compounds (D1-D4) showed significant (p<0.01) inhibition of edema at both doses but compared to compounds of A, B and C series inhibition of edema was much less.

Cotton pellet induced granuloma:
All the the 4-nitro2-phenoxyphenyl4-thiazolidinone compounds (A1-A11) showed significant inhibition of granuloma at both doses tested. Compounds A1(40.7%),
A2(42.9%), A4(40%), A5(43.1%) showed maximum inhibition of granuloma. Similarly all the sulphonyl 4-thiazolidinone compounds(B1-B11) showed significant inhibition of granuloma and the inhibition was maximum with B1(39.7%), B4(42.5%), B6(41.5%), B8(39.4%).

The ethoxyphenyl 4-thiazolidinone (C1-C4) and the coumarinyl 4-thiazolidinone (C5-C7) compounds showed similar and significant inhibition of granuloma. The hydroxyphenyl 4-thiazolidinone compounds(D1-D4) showed significant inhibition of granuloma but the inhibition was much less compared to A, B and C series.

Xylol induced mouse ear edema:
The test compounds A1-A11 and B1-B11 showed significant inhibition of ear edema, however the inhibition was less with spiro substituted derivatives A9-A11 and B9-B11. Similarly all derivatives of C series showed significant inhibition of ear edema. All the compounds D1-D4 showed significant inhibition of ear edema however it was less compared to A, B and C series.

Acetic acid induced writhing in mice:
All the compounds of A, B and C series showed significant inhibition of writhing in mice.

The compounds A3, A4, A6, A7, A9, A10, B4, B7, B10, C1-C7 showed maximum inhibition of writhing, whereas compounds D1-D4 showed significant but much less inhibition.

Yeast induced pyrexia in rats:
The compounds A1-A11, showed significant reduction in pyrexia at both the doses tested. The compounds A3(36.00-0.14°C), A4(36.32-0.03°C), A5(35.84-0.15°C) and A6(35.96-0.08°C) showed maximum decrease in pyrexia at 50mg/kg. The compounds of B series showed significant reduction in pyrexia at both the doses. In C series also all the compounds showed significant decrease in pyrexia, C3 (35.80-0.18°C) at 50mg/kg showed maximum decrease in pyrexia at 30 minutes compared to other compounds. C2 (36.04-0.12°C), C3 (35.80-0.06°C), C4 (36.06-
0.07°C) at 100mg/kg showed maximum decrease in pyrexia at 30 minutes. In D series all the compounds showed significant decrease in pyrexia but the decrease was less compared to nimesulide at both the doses.

**COX inhibitory activity:**
The compounds \( A_1-A_{11} \) showed significant inhibition of COX-2 enzyme activity and very negligible inhibition of COX-1 enzyme activity. However the COX-2 enzyme inhibition by compounds \( A_2(29.59\%), \ A_9(21.30\%), \ A_{10}(24.8\%) \) and \( A_{11}(19.60\%) \) is much less.

Similarly compounds \( B_1-B_8 \) showed significant inhibition of COX-2 enzyme activity and much less COX-1 enzyme inhibition. Whereas compounds \( B_9-B_{11} \) showed much less COX-2 enzyme inhibition, the compounds \( C_1-C_7 \) showed good inhibition of COX-2 enzyme activity. Whereas compound \( C_3 \) showed significant inhibition of both COX-1 and COX-2 enzyme activity, other compounds produced much less COX-1 enzyme inhibition.

The compounds \( D_1-D_4 \) showed much less COX-1 and COX-2 enzyme inhibition.

**Subacute toxicity study:**
The 4-thiazolidinone derivatives \( A_8, \ B_8, \ C_3 \) and \( C_7 \) were evaluated for haematological parameters like RBC count, WBC differential count, haemoglobin content and histopathological parameters like stomach, liver and kidney as per the procedure of OECD guidelines. The haematological changes in rats administered with 100mg/kg dose were recorded on 7th, 14th, and 28th day of the study. Whereas the specimens’ of stomach, liver and kidney were collected on 28th day after the administration of the last dose.

In haematological parameters the results are average of 3 readings. All the test compounds did not produce any significant change in RBC, WBC differential count, haemoglobin content on 7th, 14th, and 28th day of the study.

In histopathological studies compound \( A_8 \) produced mild congestion in stom-
ach, liver and kidney. The compound B8 produced inflammatory exudates and infiltrate in stomach, mild inflammatory infiltrate in liver and inflammatory infiltrate and congestion in kidney. The compound C3 produced more inflammatory exudates and ulceration in stomach; mild necrosis and inflammatory infiltrate in liver; and tubules necrosis and inflammatory infiltrate in kidney. The compound C7 produced mild congestion and less ulceration in stomach, mild congestion and inflammatory infiltrate in liver and mild congestion and inflammatory infiltrate in kidney.
Discussion

In the present study all the thiazolidin-4-one derivatives viz., 4-nitro-2-phenoxyphenyl4-thiazolidinone, sulphonyl 4-thiazolidinone, ethoxyphenyl4-thiazolidinone, coumarinyl4-thiazolidinone, hydroxyphenyl4-thiazolidinone exhibited significant ($p<0.01$) anti-inflammatory activity in both acute and subacute models of inflammation. Our results support the earlier studies of other 4-thiazolidinone derivatives having anti-inflammatory activity \(^1\), \(^2\), \(^3\), \(^1^4\) irrespective of various substitutions to 4-thiazolidinone moiety. However the efficacy of the different derivatives was variable depending upon the various substitutions like 4-nitro phenoxyphenyl and coumarinyl moieties. The $R$, $R_1$, $R_2$, substitutions on the benzoyl moiety in all 4-thiazolidinone compounds also modified the anti-inflammatory activity. In particular derivatives of sulphonyl thiazolidinone, ethoxyphenyl4-thiazolidinone, coumarinyl 4-thiazolidinone showed significant and maximum anti-inflammatory activity compared to 5-spiro substituted derivatives of 4-thiazolidinone. The hydroxyphenyl 4-thiazolidinone derivatives showed significant but minimum inhibition of edema. In the present study, anti-inflammatory activity of all 4-thiazolidinone derivatives can be directly correlated with their COX-2 enzyme inhibitory activity, indicating their mechanism of action. All 4-thiazolidinone derivatives also showed peripheral analgesic and antipyretic activity very much similar to their anti-inflammatory activity. In sub acute toxicity study compound ethoxyphenyl 4-thiazolidinone derivative(C3) was found to produce more toxicity on stomach, liver and kidney than other compounds tested. The toxicity observed may be correlated with its significantly higher inhibition of COX-1 along with COX-2 enzyme activity. In the present study, the inhibition of COX-2 enzyme by diverse structures of 4-thiazolidinones appears to be due to the flexible nature of the COX-2 enzyme structure as indicated by earlier studies \(^3\), \(^1^0\), \(^1^1\) also.
Conclusion

Our study indicates that various 4-thiazolidinone derivatives can be further explored as anti-inflammatory agents with better efficacy and reduced toxicity. Compounds A8(4-nitrophenyl 4-thiazolidinone), B8(sulphonyl 4-thiazolidinone) and C7(coumarinyl 4-thiazolidinone) derivatives can be further selectively evaluated amongst other compounds. The study reaffirms that COX-2 enzyme structure is flexible in nature as reported by many other studies.
Bibliography


