1. OBJECTIVE
Synthesis and biological evaluation of prodrugs of some nonsteroidal anti-inflammatory drugs.

2. PLAN OF PROPOSED WORK AS PER SYNOPSIS
The study was planned as follows:

1. Synthesis of prodrugs of some NSAIDs by direct coupling and by using spacer technique (amino acid was taken as a spacer). Synthesis of Prodrugs was done in two parts with two different aims. **Part A**: Synthesis of Mutual Prodrugs of NSAIDs with synergistic effects. NSAIDs-Propyphenazone esters were synthesized as prodrugs with the aim of improving therapeutic index through prevention of GI irritation and bleeding. **Part B**: Synthesis of Mutual Prodrugs of NSAIDs with additional effects. NSAIDs-Allopurinol mutual prodrugs were synthesized with antigout activity additional to anti-inflammatory and analgesic activity of NSAIDs.

2. Characterization of synthesized compounds by determining the physicochemical properties (melting point, solubility, T.L.C).

3. Spectral (IR, $^1$H NMR, Mass spectroscopy) and elemental analysis of synthesized compounds.

4. Hydrolysis studies (In vitro release studies) in aqueous buffers (SGF of pH 1.2 & SIF of pH 7.4)

5. Pharmacological evaluation:
   - **Biological evaluation of NSAIDs- propyphenazone esters (Part A)**:
     (i) Evaluation of analgesic activity by acetic acid induced writhing method.
     (ii) Evaluation of anti-inflammatory activity by carrageenan induced paw edema method.
     (iii) Study of ulcerogenic potential of synthesized compounds.
   - **Biological evaluation of NSAIDs- allopurinol esters (Part B)**:
     (i) Screening of hypouricaemic / anti-gout activity by serum urate level determination using standard uric acid kit.
     (ii) Evaluation of anti-inflammatory activity by TNF-α Elisa kit.
     (iii) Study of ulcerogenic potential of synthesized compounds and their histopathological studies.
3. Summary of work done during the Ph.D.

1. Literature review on mutual prodrugs

Mutual prodrug is a form of prodrug in which two pharmacologically active agents are attached to each other in such a way that each drug acts as a promoiety/carrier for each other and vice versa. The association may be “synergistic” if the carrier shows the same biological action as that of parent drug or may provide “additional” benefit if it shows new pharmacological action which is lacking in parent drug. The mutual prodrug concept has shown its marked therapeutic gain in case of well-accepted and useful drugs with minor undesirable properties and in those active compounds that suffer from severe limitations, like lack of site specificity, poor bioavailability or lack of particular activity. Nowadays, Anticancer, cardiovascular, antiviral, antipsychotic and anti-inflammatory drugs are best utilizing the concept of mutual prodrug designing for their better effect. In this literature, we have reviewed mechanism of activation, contribution of mutual prodrug approach in different therapeutic areas and the development in this field including a list of patents. Various design approaches, methods of synthesis, pharmacological evaluations for mutual prodrugs have been studied under this literature study.

Article published from the above work:


2. Synthesis, characterization, in-vitro hydrolysis and pharmacodynamic profiles of potential novel mutual prodrugs of N-(2,3-xylyl anthanillic acid)

In present research work we report synthesis, in-vitro hydrolysis study and pharmacological evaluation of new mutual prodrugs of mafenamic acid (MA) and 1,2-dihydro-1,5-dimethyl-4-(1-methylethyl)-2-phenylpyrazol-3-one with the aim of improving the therapeutic potency and retard the adverse effects of gastrointestinal origin. The structure of the synthesized mutual ester prodrugs (MP1 and MP2) were confirmed by IR, 1H NMR, 13C NMR, mass spectroscopy (MS) and their formation was confirmed by TLC. The purity of synthesized compounds was established by elemental analysis. The title compounds were tested for analgesic activity by acetic acid-induced writhing method; for anti-inflammatory activity by
carrageenan-induced rat paw edema method and ulcerogenicity. The study of analgesic activity revealed that both prodrugs (MP1 and MP2) have shown significant reduction (81.67%, 63.90%) in writhing response produced by acetic acid as compared to parent drug MA (61.10%). Both mutual prodrugs showed better maximum anti-inflammatory effects (71.43%, 85.71%) and for longer time as compared to parent drug MA (53.14%). The synthesized prodrugs were also found to be very less irritating to gastric mucosal membrane than parent drugs. The kinetics of ester hydrolysis was studied in simulated gastric fluid (SGF) at pH 1.2 and simulated intestinal fluid (SIF) at pH 7.4. The release of free MA from prodrugs showed negligible hydrolysis in SGF as compared to SIF. This indicated that prodrugs were sufficiently stable at pH 1.2 and do not break in the stomach, but release MA in SIF.

Scheme 1: General steps for the synthesis of MP1 Prodrug

(a) Synthesis of 3-bromomethyl propyphenazone (BMP)

(b) Synthesis of Potassium salt of Mefenamic acid

(c) Synthesis of MP1 Ester Prodrug
Scheme 2: General steps for the synthesis of MP2 Prodrug

(a) Synthesis of 3-hydroxymethyl propyphenazone (HMP)

(b) Synthesis of glycinyl-3-hydroxymethyl propyphenazone (Gly-HMP)

(c) Synthesis of MP2 Mutual prodrug

Paper published from the above work:
3. Synthesis, *in vitro* and *in vivo* evaluation of mutual prodrugs of 2-(2,3-dimethylphenyl)aminobenzoic acid

In the present investigation, 2-(2,3-dimethylphenyl)aminobenzoic acid (Indomethacin) has been modified using mutual prodrug approach. Indomethacin-propyphenazone ester/amide derivatives were synthesized as potential prodrugs by direct coupling and by using spacer technique (amino acid was taken as a spacer). The structures of synthesized prodrugs were confirmed by UV, $^1$H-NMR, Mass and FT-IR spectral methods and their purity was established by elemental analysis. The mutual prodrugs were evaluated for their drug release behaviour in enzyme-free simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4). The release of free indomethacin from prodrugs showed negligible hydrolysis in SGF as compared to SIF. Study of analgesic and anti-inflammatory properties in comparison with the reference compounds has shown that both activities were present at the same doses of investigated compounds and prodrugs were advantageous in having less gastrointestinal (GI) side effects.

Scheme 1: General steps for the synthesis of Indo-Propy Ester prodrug

(a) Synthesis of 3-bromomethyl propyphenazone (BMP)

(b) Synthesis of Potassium salt of Indomethacin
(c) **Synthesis of Indo-Propy Ester Prodrug**

\[
\begin{align*}
\text{BMP} & \quad \text{Potassium salt of Indomethacin} \\
\text{Indo-Propy Ester Prodrug} & \quad \text{(DMF + 110°C)}
\end{align*}
\]

**Scheme 2: General steps for the synthesis of Indo-Gly-Propy Mutual prodrug**

(a) **Synthesis of 3-hydroxymethyl propyphenazone (HMP)**

\[
\begin{align*}
\text{3-bromomethyl propyphenazone} & \quad \text{Water, Reflux} \\
\text{3-hydroxymethyl propyphenazone} & \quad \text{Boc-Glycine}
\end{align*}
\]

(b) **Synthesis of glycinyl-3-hydroxymethyl propyphenazone (Gly-HMP)**

\[
\begin{align*}
\text{glycinyl-3-hydroxymethylpropyphenazone} & \quad \text{Coupling reagent used: DCC / DMAP} \\
\text{Deprotection : 6N - HCl / dioxane}
\end{align*}
\]
(c) **Synthesis of Indo-Gly-Propy Mutual prodrug**

![Chemical structure of Indo-Gly-Propy Mutual Prodrug](image)

**Article communicated from the above work:**

4. **Aspirin - Propyphenazone Mutual Prodrug - Synthesis, Chemical Hydrolysis and Biological Evaluation**

Aspirin has been conjugated with propyphenazone by direct coupling and by using spacer technique (amino acid was taken as a spacer) with the objective of obtaining Aspirin-propyphenazone mutual prodrugs as safer nonsteroidal anti-inflammatory drugs (NSAIDs) devoid of ulcerogenic side effects. The structures of synthesized prodrugs were confirmed by IR, $^1$H NMR, mass spectroscopy (MS) and their purity was ascertained by TLC and elemental analysis. In vitro hydrolysis was carried out in non-enzymatic simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4). The results showed that the drug release from prodrugs was comparatively higher at pH 7.4 indicating that drug release should take place predominantly at the alkaline condition rather than at acidic one. The synthesized derivatives were screened for analgesic, anti-inflammatory and ulcerogenic activity. The percentage anti-inflammatory activity of aspirin was found as 42.86% where as an improved value of 60.57% and 52.57% were obtained for Asp-Propy and Asp-Gly-Propy mutual prodrugs respectively at the end of 6th h. Both prodrugs showed improved analgesia and reduced ulcerogenicity than aspirin, thereby proving to be better in action than parent drug.
Scheme 1: General steps for the synthesis of Asp-Propy Ester prodrug

(a) **Synthesis of 3-bromomethyl propyphenazone (BMP)**

\[
\text{Propyphenazone} \quad \text{Br}_2/\text{CHCl}_3 \quad 10-15 \, ^\circ\text{C} \quad \rightarrow \quad \text{3-bromomethyl propyphenazone} + \text{HBr}
\]

(b) **Synthesis of Potassium salt of Aspirin**

\[
\text{Aspirin} + \text{Potassium t-butoxide} \quad \rightarrow \quad \text{Potassium salt of Aspirin}
\]

(c) **Synthesis of Asp-Propy Ester Prodrug**

\[
\text{BMP} + \text{Potassium salt of Aspirin} \quad \underset{\text{Coupling}}{\overset{\text{DMF}}{\rightarrow}} \quad \text{Asp-Propy Ester Prodrug}
\]
Scheme 2: General steps for the synthesis of Asp-Gly-Propy Ester prodrug

(a) Synthesis of 3-hydroxymethyl propyphenazone (HMP)

(b) Synthesis of glycinyl-3-hydroxymethyl propyphenazone (Gly-HMP)

(c) Synthesis of Asp-Gly-Propy Mutual prodrug

Article communicated from the above work:
5. Synthesis, pharmacological activity and hydrolytic behaviour of mutual prodrugs of Ibuprofen

For reducing the gastrointestinal (GI) toxicity associated with ibuprofen (IBU), its carboxylic group was masked by synthesizing its mutual prodrugs with propyphenazone prodrugs by direct coupling and by using spacer technique (amino acid was taken as a spacer). The structures of synthesized prodrugs were confirmed by UV, $^1$H-NMR, Mass and FT-IR spectral methods and their purity was established by elemental analysis. The mutual prodrugs were evaluated for their drug release behavior in enzyme-free simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4). The release of free ibuprofen from prodrugs showed negligible hydrolysis at gastric pH in SGF as compared to SIF where they undergo significant hydrolysis and thus release IBU in adequate amounts following first order kinetics. Both IBU prodrugs were retaining anti-inflammatory activity intact and exhibited better analgesic activity along with much reduced ulcerogenicity. Prodrug IP1 however showed better analgesic activity and negligiable ulcerogenic tendency than IP2 and hence it could be considered as a better candidate for prodrug among the two.

**Scheme 1: General steps for the synthesis of Ibu-Propy Ester prodrug**

(a) **Synthesis of 3-bromomethyl propyphenazone (BMP)**

(b) **Synthesis of Potassium salt of Ibuprofen**

(c) **Synthesis of IP1 Ester Prodrug**
Scheme 2. General steps for the synthesis of Asp-Gly-Propy Ester prodrug

(a) **Synthesis of 3-hydroxymethyl propyphenazone (HMP)**

(b) **Synthesis of glycinyl-3-hydroxymethyl propyphenazone (Gly-HMP)**

(c) **Synthesis of IP2 Mutual prodrug**

Article communicated from the above work:
6. Synthesis, pharmacological activity and hydrolytic behaviour of mutual prodrugs of carboxylic acid containing NSAIDs:

Novel prodrugs of selected NSAIDs were synthesized and their animal activity was performed in order to obtain better and safer NSAIDs with improved pharmacological activities. Aceclofenac, diclofenac, ketoprofen were used as model drugs in this study. Prodrugs of selected NSAIDs were synthesized by direct coupling and by using spacer technique (amino acid was taken as a spacer). The results of present work indicated that the prodrugs of NSAIDs synthesized had better analgesic, anti-inflammatory and reduced or negligible ulcerogenic potential when compared to their parent NSAIDs. All prodrugs were successfully synthesized and the structures were confirmed by spectral analysis. All prodrugs showed encouraging hydrolysis rate in SIF and excellent pharmacological response. Increased anti-inflammatory as well as reduction in ulcer index of the prodrugs were observed when compared to the parent drug.

Scheme 1: General steps for the synthesis of NSAID-Propy Ester prodrug

(a) Synthesis of 3-bromomethyl propyphenazone (BMP)

(b) Synthesis of Potassium salt of Carboxylic acid containing NSAID

(c) Synthesis of NSAID-Propyphenazone Ester Prodrug
Scheme 2: General steps for the synthesis of NSAID-Gly-Propy Ester prodrug

(a) Synthesis of 3-hydroxymethyl propyphenazone (HMP)

(b) Synthesis of glycinyl-3-hydroxymethyl propyphenazone (Gly- HMP)

(c) Synthesis of NSAID- Glycine-3-hydroxypropyphenazone (NSAID-Gly-HMP)

Mutual prodrug
7. Synthesis, Hydrolysis kinetics and Pharmacological evaluation of Mutual Prodrugs of 1H-Pyrazolol [3, 4-d] – pyrimidin-4-ol and Carboxylic acid containing NSAIDs

Allopurinol (1H-Pyrazolol [3, 4-d] – pyrimidin-4-ol) is used for treating acute gout caused by excessive levels of uric acid in the blood (hyperuricemia). The uric acid forms crystals in joints (gouty arthritis) and tissues, causing inflammation and pain. Non-steroidal anti-inflammatory drugs (NSAIDs) are also prescribed in gout attack to relieve inflammation and pain. But they suffer from several undesired side effects, the most important being gastrointestinal (GI) irritation and ulceration. The mutual prodrug designing is one of the several strategies used to overcome this drawback. The rationale behind the mutual prodrug concept is to achieve temporary blockade of the free carboxylic group present in the NSAIDs till their systemic absorption and also have additional effect of allopurinol.

The aim of this research was to develop mutual prodrugs of allopurinol and NSAIDs in order to avoid the practice of clinically co-administering these two drugs for enhancing the pharmacological activity with different mechanism of action. With this concept, their mutual prodrugs were synthesized. The structures of these synthesized compounds were determined from their spectral and elemental analysis. The prodrugs showed better anti-inflammatory, analgesic activity than parent drugs. The hypouricaemic activity of prodrugs were evaluated by uricase inhibitor potassium oxonate method. The prodrugs were significantly less irritating to mucosa. The hydrolysis studies of prodrugs showed that the prodrugs were resistant to hydrolysis at pH 1.2 than pH 7.4.
Scheme 1: General steps for the synthesis of NSAID-Allopurinol mutual prodrugs

Paper presented from the above work: