SYNTHESIS, PHARMACOLOGICAL ACTIVITY AND HYDROLYTIC BEHAVIOUR OF MUTUAL PRODRUGS OF IBUPROFEN

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ABSTRACT: For reducing the gastrointestinal (GI) toxicity associated with ibuprofen (IBU), its carboxylic group was masked by synthesizing its mutual prodrugs with propyphenazone by direct coupling and by using spacer technique (amino acid was taken as a spacer). The structures of synthesized prodrugs were confirmed by 1H-NMR, 13C-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 negligible ulcerogenic tendency than IP2 and hence it could be considered as a better candidate for prodrug among the two.

INTRODUCTION: Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed drugs, and consumption is projected to increase because of the aging population and more widespread use in cardiac and cerebrovascular disease. In fact, NSAIDs are generally prescribed for pain management in musculoskeletal or osteoarticular pathologies and for rheumatic diseases, very common diseases in the general population. The therapeutic efficacy of currently available NSAIDs is significantly limited by associated GI toxicity, which causes a higher incidence of morbidity in long term NSAID users. However, both traditional NSAIDs and second-generation NSAIDs (cyclooxygenase-2 inhibitors) can lead to very expensive and serious adverse events.

Gastrointestinal, cardiovascular, and renal complications associated with NSAIDs have been shown to be dose-dependent. These GI complications are believed to be determined from the mixed effect of irritation caused by blockage of prostaglandin biosynthesis in the GI tract, as the endogenous prostaglandins are known to have cytoprotective action on the gastric mucosa and direct action of free carboxylic groups in NSAIDs. The recent failure of the selective COX-2 inhibitors...
leaves the compelling need for effective NSAIDs with improved safety NSAIDs\textsuperscript{4}. Therefore, it is necessary to consider NSAID-induced toxicity as a serious public health problem contributing significantly to the morbidity and mortality of patients receiving these drugs. For many years, several attempts have been made to develop bioreversible derivatives or prodrugs of NSAIDs containing carboxylic acid function in order to depress upper gastrointestinal (GI) irritation and bleeding\textsuperscript{5}.

In recent years, there has been an increasing interest in the design and development of mutual prodrugs, which involves combining of two different pharmacophores with similar pharmacological activities to give synergistic action. For example the mutual prodrug of etodolac and glucosamine solves not only the formulation problem of Etodolac (lower aqueous solubility, BCS class II drug) but also increased synergistic antiinflammatory and antiarthritis activity with lower toxicity and less ulcerogenic activity than the parent drug.\textsuperscript{6}

Similarly Mutual prodrug of ketoprofen and glucosamine have also been synthesized with synergistic action\textsuperscript{7}. Codrug or mutual prodrug is an approach where various effective drugs, which are associated with some drawbacks, can be modified by attaching with other drugs of same or different categories directly or via a linkage. More appropriately one can say combining two different pharmacophores with similar or different pharmacological activities elicit synergistic action or help to target the parent drug to specific site/organ/cells respectively. This approach is commonly used to improve physicochemical, biopharmaceutical and drug delivery properties of therapeutic agents\textsuperscript{8,9}.

Even though ibuprofen (IBU) is very potent and widely used among other clinically used NSAIDs, literature is abundant with its gastric and other side effects because of the presence of free carboxylic group. Frequent medication of IBU, however, is well known to cause serious GI damage, like other NSAIDs. Also, IBU has a short plasma half-life of 1-2 hours which further necessitates frequent administration to maintain therapeutic drug doses\textsuperscript{10-12}. Many IBU prodrugs i.e glyceride derivatives\textsuperscript{11}, ethylenediamine and benzathine conjugates\textsuperscript{13}, amide derivatives\textsuperscript{14}, poly (HEMP) conjugates\textsuperscript{15}, acrylic-type derivatives\textsuperscript{16}, N-hydroxymethylsuccinimide-/satin esters\textsuperscript{17}, anhydride derivatives\textsuperscript{18} etc and some mutual prodrugs such as IBU-anthraquinone\textsuperscript{19}, IBU-sulpha drugs\textsuperscript{20}, IBU-paracetamol and IBU- salicylamide\textsuperscript{21} etc were reported in literature.

Recently, IBU has been conjugated with different antioxidants (menthol, thymol and eugenol) having antiulcerogenic activity with the objective of obtaining NSAIDs- antioxidant mutual prodrugs as gastrosparing NSAIDs devoid of ulcerogenic side effects\textsuperscript{22}. Mahdi and co-workers have also reported the synthesis of mutual prodrugs of IBU by coupling with two different sulfa drugs sulfathiazole and sulfadiazine using glycolic acid spacer (-OCH2COO-) to reduce the ulcerogenic side effects of IBU by esterification of the free carboxyl group of the IBU that is responsible for the local irritation\textsuperscript{23}.

After going through vast literature survey and earlier reports, a possible way to solve GI side effects of IBU was found to derivatize the carboxylic functional group of the IBU to produce mutual prodrug with adequate stability at the acidic pH of the stomach and releasing the drug in a sustained manner predominantly at the intestinal pH in order to avoid direct contact of free carboxyl group of the drug to the gastric mucosa. Propyphenazone is a non-acidic pyrazole drug and has a good analgesic and antipyretic activity with no anti-inflammatory activity.

The current work is targeted at the concept of designing drug through conjuction of two different pharmacophores having similar pharmacological activities.

In the view of this background, the present study was conducted to design, synthesis, and preliminary kinetics study of mutual prodrugs of IBU with propyphenazone to get NSAIDs with lesser ulcerogenic side effects while retaining the anti-inflammatory and analgesic activity.
MATERIALS AND METHODS:
Propyphenazone was obtained as a gift from Vani Pharma Labs Limited, Hyderabad, AP, India and drug IBU was obtained as gift sample from Jarkar Pharmaceuticals, HSIDC Industrial Complex Rai, Sonepat, Haryana. The other reagents and solvents used were of analytical grade. The starting compound 3-bromomethyl-propyphenazone was prepared in a pure crystalline form according to the reported method\textsuperscript{24}.

The melting points of synthesized compounds were determined in open capillary using Decibel melting point apparatus and recorded in °C without correction. The reactions were monitored by TLC on precoated silica gel G plates using iodine vapours as detecting agent. The infrared spectra were recorded by PERKIN ELMER spectrophotometer using potassium bromide pellet technique and sodium chloride cells for liquid samples. Proton nuclear magnetic resonance spectra (\textsuperscript{1}H NMR) were recorded on Bruker Avance II 400 NMR Spectrophotometer using tetramethyl silane as an internal standard.

\textsuperscript{1}H NMR spectra were recorded with CDCl\textsubscript{3} as a solvent and the chemical shift data were expressed as values relative to TMS (Chemical shift δ in ppm). Mass spectra of the compounds were obtained using LC-MS (SHIMADZU-2010 AT, Software class VP). Elemental analyses were performed at the Analysis centre, Chemistry department, Faculty of Science, Delhi University. The hydrolysis data and drug content determination were performed by a UV-Visible Spectrophotometer Pharma Spec-1700 (SHIMADZU).

General procedure for synthesis of mutual prodrugs of Ibuprofen (IBU)
Mutual prodrugs of IBU were prepared by two methods:

a. Without spacer (IP1)

b. With spacer (IP2)

Synthesis of IBU–propyphenazone (Ibu-Propy) mutual prodrug without spacer (IP1)

Synthesis of 3-bromomethyl propyphenazone (BMP)

3-bromomethyl propyphenazone, C\textsubscript{14}H\textsubscript{17}BrN\textsubscript{2}O, was synthesized from propyphenazone and bromine, according to Lucius (1907) with some modifications to increase yield and purity as follows; About 10 g propyphenazone was dissolved in 25 mL dichloromethane (DCM) in 100 mL round bottom flask fitted with dropping funnel; it was kept over an ice bath and bromine solution (6.95 g; 2.23 mL bromine dissolved in 5 mL CH\textsubscript{2}Cl\textsubscript{2}) was added drop-wise and very slowly with the aid of stirring for 1 h.

The brown color of the bromine faded rapidly upon its addition. Temperature was maintained 10-15 °C throughout the reaction. The reaction progress was monitored by TLC using ethyl acetate (EA): hexane (2:1) ratio. An aliquot of 30 mL 10% cold aqueous sodium carbonate solution was added with vigorous shaking.

The organic phase was separated and dried with Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated under vacuum at 40 °C until the volume was about 10 mL. The reaction mixture was cooled to room temperature and about 15 mL diethyl ether was added. This solution was left to stand in stoppered flask at room temperature in the dark. The colorless crystals which formed were separated and washed with cold diethyl ether (about 5 °C).

The obtained crystals were dried overnight in vacuum desiccators over anhydrous calcium chloride, in the dark to yield 11.8 g (87.86%) of pure dried crystals of BMP. The modifications made to the reported method included, (a) The use of cold sodium carbonate aqueous solution to avoid formation of any 3-hydroxymethyl-propyphenazone; (b) Using diethyl ether instead of dichloromethane to get more yield of BMP crystals; and (c) Using cold diethyl ether for crystallization instead of dichloromethane at room temperature to get more pure crystals of BMP.

Synthesis of Potassium salt of IBU:
Potassium tertiarybutoxide (0.224 g, 2 mmol) was taken in two- necked round bottom flask, fitted to a guard tube. To this 3.0 mL of toluene was added and stirred well until it completely dissolves. To the above reaction mixture 0.412 g (2 mmol) IBU was added, little by little with continuous stirring.
As soon as the addition is complete round bottom flask was stoppered, and stirred for some more time until a clear solution is obtained. Solvent was evaporated on a rotary evaporator to get 0.34g (69.67%) hygroscopic white powder of potassium salt of IBU.

Synthesis of IBU-propyphenazone ester by Coupling of BMP and potassium salt of IBU (IP1):
A solution of BMP (0.309 g, 1 mmol) and potassium salt of IBU (0.488 g, 2 mmol) in 5.8 mL of dimethylformamide (DMF) was refluxed on an oil bath at 110 °C. The reaction mixture was continuously stirred throughout the process. The progress of the reaction was monitored by TLC, using EA: Hexane (2:1). The reaction mixture was diluted with 100 mL of water and extracted with ethyl acetate. Ethyl acetate layer was collected washed with water and dried over anhydrous sodium sulphate. Finally solvent was evaporated using rotary evaporator to yield 0.56g (65.02%) of pure ester prodrug. Synthesis of IBU–propyphenazone (Ibu-Gly-Propy) mutual prodrug with spacer (IP2)

Preparation of IBU – Glycine – 3 - hydroxypropyphenazone (IP2)
A solution of CDI (1 g, 6.16 mmol) in anhydrous DMF (5 mL) was added drop-wise, at 4°C to a solution of IBU (1 g) dissolved in anhydrous DMF (10 mL). To the cold mixture, an equimolar solution of Gly-HMP in dry DMF (20 mL) was added drop-wise. The reaction mixture was stirred for 10 min at 4°C, for 3 h at room temperature and then evaporated under vacuum. The residue was purified by column chromatography (hexane / ethyl acetate; 2: 1) to yield 1.52 g (64.04%) of pure compound IP2.

3 - (bromomethyl) - 1, 2 – dihydro - 4 - isopropyl-2-methyl-1-phenylpyrazol-5-one (BMP):
Yield – 87.86%; Mp (°C) 98-100 °C; Rf value – 0.67; IR (cm⁻¹): 3023.15 (aromatic C-H str.), 2969.26 (aliphatic C-H str.), 1593.71, 1489.41 (C=C, phenyl nucleus); ¹H-NMR (δ (ppm)): 7.247-7.480 (m, 5H, Ar-H), 2.818-2.888 (m, 1H, -CH of –CH(CH₃)₂), 3.075 (s, 3H, CH₃N), 4.329 (s, 2H, CH₂Br), 1.334 (d, 6H, CH₃ of –CH(CH₃)₂, J= 6.9 Hz); ¹³C-NMR (δ (ppm)): 21.78 (CH₃ of –CH(CH₃)₂), 38.84 (CH of –CH(CH₃)₂), 39.91 (N-CH₃), 209.14 (CO), 125.61-136.56 (Ar-carbons), 42.35 (CH₂ of CH₂Br); Mass (m/z): 309 (M⁺); Elemental analysis: Calculated: C, 54.38%; H, 5.54%; N, 9.06% Found: C, 54.41%; H, 5.50%; N, 9.10%.

1, 2 - dihydro-3-(hydroxymethyl)-4-isopropyl -2-methyl-1-phenylpyrazol-5-one (HMP):
Yield – 85.36%; Mp (°C) 102-104 °C; Rf value – 0.28; IR (cm⁻¹): 2927.94 (aliphatic C-H str.), 3298.44 (O-H str.), 1344.23 (O-H in plane bending), 659.90 (O-H out of plane bending), 1492.04 (C=O, phenyl nucleus); ¹H-NMR (δ
(2, 3 - dihydro – 4 - isopropyl – 1 - methyl-3-oxo-2-phenyl-1H-pyrazol-5-yl) methyl 1 - 2 -(4-isobutylphenyl) propanoate (IP1):

Yield – 65.02%; Mp (°C) 164-166 °C; Rf value – 0.58; IR (cm⁻¹): 1734.60 (C=O str. of ester), 2959.01 (C=O band of amide), 1714.51 (C=O str., ester), 1152.12 (C–O–C str.), 1099.65; 13C-NMR (δ (ppm)): 20.13 (CH₃ of –CH(CH₃)₂), 37.56 (CH of –CH(CH₃)₂), 39.73 (N–CH₃), 122.22 (CO), 128.6-131.26 (Ar-carbons), 58.05 (CH₂ of CH₂OH); Mass (m/z): 246 (M⁺); Elemental analysis: Calculated: C, 68.27%; H, 7.37%; N, 11.37%; Found: C, 68.31%; H, 7.33%; N, 11.39%.

(2, 3 – dihydro – 4 - isopropyl – 1 – methyl - 3-oxo-2-phenyl-1H-pyrazol-5-yl)methyl 2 - (2-(4-isobutylphenyl) propanamido) acetate (IP2):

Yield – 64.04%; Mp (°C)- oily liquid; Rf value – 0.78; IR (cm⁻¹): 1723.29 (C=O str. of ester), 2959.01 (aliphatic C-H str.), 1099.65, 1176.23 (C-O-C str., ester), 1059.83 (O-C-C, bond of ester from 1° alcohol), 1457.14 (C=C, phenyl nucleus), 1397.69 (COO-CH₃), 128.12 (N=CH), 123.03 (C=O of amide), 1502.33 (N-H bend, 2° amide), 858.92 (C-N str.), 664.35 (Aromatic C-C out of plane bending), 759.63 (Aromatic C-H out of plane bending); 1H-NMR (δ (ppm)): 7.211-7.473 (m, 5H, Ar-H), 6.887-7.080 (m, 4H, Ar-H), 7.973 (s, 1H, NH), 2.850 (d, 2H, CH₂ of –CH₂CH₂CH₃, J=9.9 Hz), 4.519 (s, 2H, CH₂ of -COOCH₂-), 2.850 (s, 3H, CH₃N), 0.894 (d, 6H, -CH₃ of –CH₂CH₂CH₃, J=6.6 Hz), 2.144-2.437 (m, 1H, -CH of –CH₂CH₂CH₃, J=9.9 Hz), 1.258 (d, 6H, -CH₃ of –CH(CH₃)₂, J=7.2 Hz), 2.154-2.293 (m, 1H, CH of –CH₂CH₂CH₃, J=9.9 Hz), 13C-NMR (δ (ppm)): 21.32 (CH₃ of –CH(CH₃)₂), 29.12 (CH of –CH(CH₃)₂), 39.64 (N–CH₃), 166.21 (CO of pyrazole), 171.51 (CONH), 41.11 (NH-CH₂-COO), 169.27 (COO), 117.87-138.58 (Ar-carbons), 69.03 (CH₂ of -CH₂OCO), 18.06 (CH₃ of -CH₂CH₂CONH), 46.20 (CH of -CH₂CH₂CONH), 45.71 (CH₂ of CH₂CH₂CH₃); Mass (m/z): 491 (M⁺); Elemental analysis: Calculated: C, 70.85%; H, 7.59%; N, 8.55%; Found: C, 70.91%; H, 7.51%; N, 8.49%.

**In-vitro hydrolysis studies of Ibu prodrugs**

The hydrolysis study was carried out using Veego dissolution apparatus (VDA-BDR, USP standard). The test was conducted at 37±0.5 °C using apparatus II and pedal speed rotation of 100 rpm. Two hydrolys media were used: 900 mL of non-enzymatic simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4).

An accurate amount of 100 mg of the prodrug was used for the study. From the matrix, aliquots of 1 mL were withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 hours and were immediately replaced with 1.0 mL of fresh hydrolysis media equilibrated at 37±0.5 °C.

Free Ibu which was supposed to be released after the hydrolysis of prodrugs extracted with 5 mL methanol. The methanol layer was estimated on UV spectrophotometer for the amount of free Ibu released after hydrolysis of prodrugs in SGF and SIF. The kinetics of hydrolysis was monitored by increase of free drug concentration with time and order of reaction and half life (t½) were also calculated.

The rate of hydrolysis was calculated using equation, k= (2.303/t) log (a/a-x) where k represents hydrolysis constant, t is the time in min, ‘a’ is the initial concentration of prodrug, x is the amount of prodrug hydrolyzed and (a-x) is the amount of prodrug remaining.
**In-vivo evaluation**

The prodrugs prepared were screened for analgesic and anti-inflammatory activity as compared with that exerted by IBU and HMP, separately. Male Swiss mice (25-30 g) and male Wistar rats of Albino strain (150-200 g) were used. All the animals were obtained from Disease Free Small Animal House CCSHAU, Hisar.

The selected animals were housed in polypropylene cages at standard environmental conditions at 22 ± 2 °C, relative humidity of 45–55 %, in a well ventilated room maintained at 12: 12 h light: dark cycle, fed with standard pellet diet and water *ad libitum*. All the animals were acclimatized for a week before experiment. All animal experiments were carried out according to the guidelines of the Committee for the Purpose of Control of Experiments on Animals and approval of the Institutional Animal Ethics Committee, Maharshi Dayanand University, Rohtak, Haryana (Establishment Reg. No. 134/99/CPCSEA) was obtained. The animals were fasted with free access to water for 12 h prior to the tests. The tested compounds were prepared for oral administration in aqueous 0.5% carboxymethylcellulose (CMC) solution.

The experimental protocol was approved by Institutional Animal Ethics Committee (Endst. No. CBT/2010/604-1609, dated 29-10-2010). All the results were expressed as Mean ± Standard error (SEM). Data was analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. p-values < 0.05 were considered as statistically significant.

**Analgesic activity**

The analgesic activity was assessed by acetic acid induced writhing 25. Mice were divided into five groups (n=6 in each group). Group I served as control (received 0.5% w/v CMC (10 mL/kg) only). Group II and III received standard drugs i.e IBU (20 mg/Kg body weight, 96.90 μmol/kg) and HMP (23 mg/Kg body weight, 96.90 μmol/kg), Group IV and V received prodrug IP1 (42.07 mg/Kg body weight, 96.90 μmol/kg) and IP2 (47.60 mg/Kg body weight, 96.90 μmol/kg), where the dose was molecular equivalent to the free drug. The control, standard and test drugs were given by oral route while 1% v/v acetic acid solution was injected intraperitoneal to mice. Mice were pretreated with drug vehicle or standard or test drugs 1 h before acetic acid injection.

The writhing response was induced by an intraperitoneal injection of 1% v/v acetic acid solution. The mice were placed in separate boxes under observation immediately after acetic acid injection and total number of writhes, which was a parameter of chemically induced pain (i.e. constriction of abdomen, turning of trunk and extension of hind limbs), was counted for 10 min. The analgesic effect was expressed as percent reduction of writhes in comparison with the control. The percentage protection was calculated by following formula:

\[
% \text{ Protection} = 100 - \left( \frac{\text{No. of writhes in test}}{\text{No. of writhes in control}} \right) \times 100
\]

**Anti-inflammatory activity**

The carrageenan induced rat hind paw edema method 26 was used to evaluate the acute anti-inflammatory activity of the prodrugs. Rats were divided into control, standard and test groups of six animals each. Pretreatment initial paw volumes of all animals were measured using a mercury plethysmometer. The control group was given only an appropriate volume of 0.5% CMC. Standard group received IBU and HMP (equivalent dose, 96.90 μmol/kg) respectively.

To the test group, prodrugs (IP1 and IP2) were administered orally using the similar doses as employed in the analgesic activity. One hour after treatment, edema in the left hind paw of the rat was induced by injection of 0.1 mL of 1% (w/v) carrageenan solution in normal saline solution (0.9% w/v). The paw was marked with ink at the level of lateral malleolous and immersed in mercury up to this mark.

The relative change in paw volume was determined by measuring the paw volume immediately after injection and at 1, 2, 3, 4, 6 and 8 h intervals following the carrageenan administration. The percent inhibition of edema, as an indication of anti-inflammatory activity was compared with the
controls. The percentage inhibition of swelling was calculated using the following formula:

\[
\text{Inhibition (\%) = } \frac{(V_t - V_o)_{\text{control}} - (V_t - V_o)_{\text{treated}}}{(V_t - V_o)_{\text{control}}} \times 100
\]

\( V_o \) and \( V_t \) relates the average volume in the hind paw of rats (n=6) before any treatment and after anti-inflammatory agent treatment, respectively.

**Ulcerogenic Activity**

Ulcerogenesis test was performed according to the method of Kunchandy et al. \(^{27} \). Wistar rats were divided into five groups consisting of six animals in each group. All animals were fasted for 12 h prior to the administration of drug.

The first group served as control and received p.o. administration of the vehicle (0.5% CMC) only. Group II and III received IBU and HMP (in equivalent doses, 96.90 µmol/kg) respectively. Group IV and V received equivalent doses of prodrugs IP1 (42.07 mg/kg, 96.90 µmol/kg) and IP2 (47.60 mg/kg, 96.90 µmol/kg) separately. All the test drugs or standard of vehicle were administered orally to rats over a period of seven successive days.

All the rats were fasted for 24 h on the 8\(^{th} \) day. The animal was sacrificed with excessive anesthesia. The stomach was removed, opened along the greater curvature and washed gently in running tap water. The gastric mucosa of the rat was examined by means of magnifying lens and compared with that after ibuprofen administration. For each stomach, the mucosal damage was assessed according to the following scoring system: 0.0 - normal colored stomach; 0.5 - pink to red coloration of stomach; 1.0 - haemorrhagic streak; 1.5 - number of ulcers < 5; 3.0 - number of ulcers > 5; 4.0 - ulcers with bleeding.

The mean score of each treated group minus the mean score of the control group was regarded as the severity index of gastric mucosal damage.

**RESULTS AND DISCUSSION:**

Bromopropyphenazone (BMP) was prepared by bromination of propyphenazone according to Meister, Lucius, Bruning in Hoechst, 1907, with some modifications to increase yield and purity. IBU-propyphenazone ester (IP1) was synthesized by coupling of BMP and potassium salt of IBU by refluxing at 110 °C as shown in scheme 1.

\[ \text{Scheme 1: General steps for the synthesis of IBU-propy prodrug (IP1)} \]

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Bromopropyphenazone (BMP) was used for preparation of hydroxymethyl-propyphenazone (HMP) by reflux with water. In order to avoid or minimize the steric hindrance effect on the hydrolysis of IBU-HMP ester prodrug (IP1), a spacer was introduced. The spacer chosen was glycine. N-Protected glycine coupled with HMP by DCC followed by N-deprotection and coupling with IBU using CDI yielded IBU amide prodrug (IP2) as illustrated in scheme 2.

The physicochemical properties were determined and shown in Table 1. Carbonyl diimidazole (CDI) is a useful coupling reagent that allows one-pot amide formation. Acyl carboxy imidazole and imidazole were initially formed but readily reacted together to yield the activated species as the acylimidazole. Practically, the acylimidazole was preformed for 1 h and then the amine was added. This reaction, which generates imidazole in situ, does not need an additional base and is even compatible with HCl salts of the amine.

### TABLE 1: PHYSICOCHEMICAL PROPERTIES OF SYNTHESIZED PRODRUGS

<table>
<thead>
<tr>
<th>Prodrug Code</th>
<th>Molecular formula</th>
<th>Mol. wt.</th>
<th>Colour</th>
<th>m.p. (°C)</th>
<th>% Yield</th>
<th>Rf value</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP1</td>
<td>C_{17}H_{23}N_{2}O_{3}</td>
<td>434</td>
<td>White</td>
<td>164-166</td>
<td>65.02</td>
<td>0.58</td>
<td>5.54</td>
</tr>
<tr>
<td>IP2</td>
<td>C_{29}H_{37}N_{2}O_{4}</td>
<td>491</td>
<td>Brown</td>
<td>Liquid</td>
<td>64.04</td>
<td>0.78</td>
<td>4.39</td>
</tr>
</tbody>
</table>

Uncorrected Ethyl Acetate: n-Hexane (2:1)

In scheme 2, the formation of Gly-HMP is an example of Steglich esterification. It is a variation of an esterification with dicyclohexylcarbodiimide (DCC) as a coupling reagent and 4-dimethylaminopyridine (DMAP) as a catalyst. The reaction mechanism involved two steps. In first...
step, the carboxylic acid reacted with DCC to an O-acyl isourea, which was more reactive than the free acid and in second step the alcohol of HMP attacks this intermediate, forming DCU and corresponding ester. The physicochemical properties were determined and shown in Table 1. The yields of prodrugs were good. The structures of prodrugs formed were confirmed by $^1$H-NMR, $^{13}$C-NMR, Mass and FT-IR spectral methods. The purity was determined by TLC. The results of elemental analysis of synthesized prodrugs were in all case within 0.4% of theoretical values and were in confirmation of desired structure.

IR stretching band ranging from 1740-1723 cm$^{-1}$ indicated the formation of an ester linkage (C=O str.). The presence of C-O str. (ester linkage) was obtained in a range of 1020-1275 cm$^{-1}$. IR stretching band at 633.83 cm$^{-1}$ in BMP indicated the presence of bromo group, whereas, IR stretching band at 3298.44 cm$^{-1}$ indicated the presence of hydroxyl group in HMP. Presence of aliphatic $^1$H alcohol in HMP was indicated by the presence of OH in plane bending at 1344.23 cm$^{-1}$ and OH out of plane bending at 659.90 cm$^{-1}$ respectively. Presence of phenyl nucleus in the synthesized compounds was indicated by the presence of skeletal stretching band of phenyl nucleus at 1590-1480 cm$^{-1}$.

The appearance of multiplet signal around $\delta$ 6.997-7.973 ppm depicted the presence of aromatic protons. The appearance of singlet signal at $\delta$ 1.991 ppm in HMP confirmed the presence of aliphatic hydroxyl group. Doublet signal observed in the range $\delta$ 1.258-1.488 ppm indicated the presence of isopropyl group -CH$_3$ of –CH(CH$_3$)$_2$ in both prodrugs.

Presence of isopropyl group (-CH of -CH(CH$_3$)$_2$) in all synthesized compounds is also indicated by multiplet signals around $\delta$ 2.414-2.449 ppm. In $^{13}$C NMR spectra, the signals for aromatic carbons had a spread from $\delta$ 117.87 to 139.37. Signals for other carbons of parent structures were observed at about $\delta$ 21.22 (CH$_3$ of –CH (CH$_3$)$_2$), $\delta$ 29.37 (CH of –CH (CH$_3$)$_2$), 172.45 (COO), 165.27 (CO of pyrazole), 73.76 (CH$_2$ of –CH$_2$OCO) and 39.37 (N-CH$_3$). Additional peaks for NH-CH$_2$-COO and CONH

were observed at $\delta$ 41.11 and $\delta$ 171.51 respectively. Further, the elemental analysis and mass spectra also supported the formation of title compounds.

**In-vitro hydrolysis of ester**

One of the crucial requirements for a prodrugs to be used, they should show a good stability in aqueous solutions and in gastrointestinal fluid, and it should be readily hydrolyzed following gastrointestinal absorption to release the parent drug. Since the carboxylic group of IBU is essential for the therapeutic action, prodrugs of prolonged action were designed in a form which the biologically active moiety can be released in its original state with time.

Therefore the release of IBU from its prodrugs was studied in -vitro in order to evaluate the possible time span in which the drug could be available from different prodrugs. The comparative patterns of hydrolysis of these prodrugs in SGF and SIF are shown in Figs. 1 and 2 respectively. The amount of IBU regenerated on hydrolysis of IP1 and IP2 in SGF (pH-1.2) was found as 26.29 and 24.02 % respectively and that in SIF (pH-7.4) was found as 94.82 and 89.52 % respectively over a period of 10 h.

![Graph of Hydrolysis in SGF](image1)

**FIG. 1. COMPARATIVE PATTERN OF HYDROLYSIS OF IP1 AND IP2 PRODRUGS IN SGF (pH-1.2)**

![Graph of Hydrolysis in SIF](image2)

**FIG. 2. COMPARATIVE PATTERN OF HYDROLYSIS OF IBU-PROPY(IP1) AND IBU-GLY-PROPY (IP2) PRODRUGS IN SIF (pH-7.4)**
The results of the hydrolytic kinetics study revealed that both followed first order kinetics. But these prodrugs showed negligible hydrolysis in acidic medium (pH 1.2) for 2 hours. From these results, this is confirmed that the release of IBU should occur predominantly at higher pH of the intestine. This may be due to the fact that ester hydrolysis is a reversible reaction in acidic pH and in alkaline pH it is irreversible and complete.

The predominant release of IBU from its prodrugs at pH 7.4 indicates potential of the prodrug to reduce the gastric complications caused by direct contact of free carboxyl group of the drug to gastric mucosa. Kinetic parameters for hydrolysis of mutual prodrugs at 37 °C are shown in Table 2. The corresponding half lives for IP1 and IP2 were found to be 14.70 and 17.14 h (in SGF, pH 1.2) and 2.28 and 3.45 h (in Phosphate buffer, pH 7.4) respectively. The half-lives and the rate constants for prodrug hydrolysis (Table 2) indicated that an esterification of carboxylic group of IBU rendered its prodrugs more stable at pH 1.2, but less stable at pH 7.4.

**In- vivo biological evaluation**

Analgesic, anti-inflammatory and ulcerogenic activities of the prodrugs were studied in comparison to equivalent doses (96.90 µmol/kg) of IBU and HMP. Results of anti-inflammatory activity by IBU and its mutual prodrugs in terms of difference in paw volume and percentage inhibition at various time intervals are presented in Tables 3 and 4 respectively.

### TABLE 3: PAW VOLUME OF TREATED GROUPS (IBUPROFEN AND ITS MUTUAL PRODRUGS) AT VARIOUS TIME INTERVALS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/Kg)</th>
<th>Change in Paw Volume (mL) (Mean± SEM)</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td></td>
<td>0.375 ± 0.0106</td>
<td>0.45±0.034</td>
<td>0.525±0.0091</td>
<td>0.475±0.011</td>
<td>0.35±0.012</td>
<td>0.125±0.0065</td>
</tr>
<tr>
<td>IBU</td>
<td>20.00</td>
<td></td>
<td>0.221±0.0085***</td>
<td>0.161±0.0057***</td>
<td>0.121±0.0122***</td>
<td>0.118±0.0073***</td>
<td>0.108±0.007***</td>
<td>0.0785±0.007***</td>
</tr>
<tr>
<td>HMP</td>
<td>23.00</td>
<td></td>
<td>0.257±0.0075***</td>
<td>0.241±0.0081***</td>
<td>0.314±0.0089***</td>
<td>0.327±0.014***</td>
<td>0.267±0.012***</td>
<td>0.11±0.009***</td>
</tr>
<tr>
<td>IP1</td>
<td>42.07</td>
<td></td>
<td>0.309±0.0000***</td>
<td>0.204±0.00063***</td>
<td>0.123±0.0010***</td>
<td>0.1±0.010***</td>
<td>0.1±0.010***</td>
<td>0.05±0.005***</td>
</tr>
<tr>
<td>IP2</td>
<td>47.60</td>
<td></td>
<td>0.325±0.00076***</td>
<td>0.2±0.012***</td>
<td>0.105±0.009***</td>
<td>0.105±0.009***</td>
<td>0.025±0.0025***</td>
<td>0.015±0.003***</td>
</tr>
</tbody>
</table>

Oedema is expressed as mean change in paw volume ± SEM
N= 6 animals. *p<0.05, **p<0.01, ***p<0.001 as compared to control. ▲ p<0.05, ▲▲ p<0.01, # p<0.001 as compared to ibuprofen.

### TABLE 4: % INHIBITION OF IBUPROFEN AND ITS MUTUAL PRODRUGS AS COMPARED TO CONTROL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/Kg)</th>
<th>% Inhibition</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>IBU</td>
<td>20.00</td>
<td></td>
<td>40.96</td>
<td>64.28</td>
<td>76.95</td>
<td>75.16</td>
<td>69.14</td>
<td>37.2</td>
</tr>
<tr>
<td>HMP</td>
<td>23</td>
<td></td>
<td>31.47</td>
<td>46.44</td>
<td>40.19</td>
<td>31.16</td>
<td>23.71</td>
<td>12</td>
</tr>
<tr>
<td>IP1</td>
<td>42.07</td>
<td></td>
<td>17.63</td>
<td>54.77</td>
<td>76.66</td>
<td>78.95</td>
<td>71.43</td>
<td>61.20</td>
</tr>
<tr>
<td>IP2</td>
<td>47.60</td>
<td></td>
<td>13.33</td>
<td>55.55</td>
<td>80</td>
<td>77.89</td>
<td>92.85</td>
<td>88.00</td>
</tr>
</tbody>
</table>

IBU exhibited maximum anti-inflammatory effect (76.95 %) at 3rd h where as second parent drug HMP showed it maximum effect (46.44 %) at 3nd h. Both mutual prodrugs showed better maximum inhibition and for longer time as compared to both parent drugs. IP1 ester prodrug displayed maximum activity (78.95 %) at 4th h where as IP2 prodrug displayed its maximum activity (92.85 %) at 6th h. IP2 prodrug showed very high % inhibition i.e. 80.00, 77.89, 92.85 and 88.00% during 3rd, 4th, 6th and 8th h respectively.

Comparative pattern of paw volume of treated groups and anti-inflammatory activity (%)
Inhibition) at various time intervals are shown in Fig 3 and 4 respectively.

The results showed that the mutual prodrug synthesized with spacer technique using glycine amino acid as a spacer was found to be more effective than prodrug made by direct esterification of parent drugs. The anti-inflammatory activity of prodrugs significantly improves over time.

This means that the prodrug per se is devoid of anti-inflammatory activity and the observed latent activity results from hydrolysis to the parent drugs. Both prodrugs exhibited highly significant results (p< .001) as compared to control and also possessed significantly better activity as compared to IBU.

The analgesic activity was determined by acetic acid induced writhing method and the results are shown in Table 5. The results indicated that IP1 showed significant reduction (86.67 %, p<0.001) in writhing response produced by acetic acid as compared to parent drug IBU (54.43%) where as IP2 prodrug didn’t produce statistically significant percentage protection (56.67 %, p not less than 0.05 ) as compared to parent drug IBU. The animals which were treated with IBU, HMP and their mutual prodrugs showed significant (p< 0.001) analgesic activity when compared with control animals.

Ulcerogenic potential of synthesized prodrugs was tested in comparison to the parent drugs following oral administration for 7 days in rats and the results are shown in Table 5.

Screening for ulcerogenic activity showed that synthesized compounds had less tendencies (p< 0.001) to form ulcer when compared to that of the parent drugs. Photographs illustrated the gastric mucosal injury in Fig 5.
CONCLUSIONS: The mutual prodrugs of IBU were successfully synthesized and structures were confirmed based on spectral analysis. Both prodrugs showed encouraging hydrolysis rate in SIF and excellent pharmacological response. The in-vitro and in-vivo evaluation of synthesized prodrugs revealed improvement in the therapeutic index of parent drugs. The derivatives were characterized by prodrug profile, adequate chemical stability and reduced ulcerogenic liability. On the basis of above observations, it is concluded that both IBU prodrugs were retaining anti-inflammatory activity intact and exhibited better analgesic activity along with much reduced ulcerogenicity but prodrug IP1 however showed better analgesic activity and negligable ulcerogenic tendency than IP2 and hence it could be considered as a better candidate for prodrug among the two.

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REFERENCES:


