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SYNTHESIS, PHARMACOKINETIC AND PHARMACOLOGY OF MUTUAL PRODRUGS OF FENBUFEN AND PROPYPHENAZONE

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ABSTRACT: Mutual prodrugs of fenbufen and propyphenazone were synthesized with the aim of getting better therapeutic index through avoidance of gastrointestinal problems and to check the efficiency of release of parent drug in the presence of spacer. These mutual prodrugs were synthesized by direct esterification and by using glycine as a spacer. The title compounds (FP1 & FP2) were characterized by spectral methods and the release of the parent drug from the mutual pro-drug was studied in two different non-enzymatic buffer solutions at pH 1.2 and pH 7.4. The biological activity of the title compounds (FP1 & FP2) was determined by tail-flick method, carrageenan-induced paw edema method, and ulcerogenic potential. From the results obtained it was concluded that these compounds exhibit better biological activity and less gastro-intestinal side effects than parent drug fenbufen. Both mutual prodrugs (FP1 & FP2) exhibited 42 and 32% respectively hydrolysis profile in buffer solutions of pH 7.4 and showed almost negligible hydrolysis i.e., 4.6 and 5.9 % respectively at pH 1.2 over a period of 10 h.

INTRODUCTION: Non-steroidal anti-inflammatory drugs (NSAIDs) are the most usually prescribed medications for the treatment of chronic inflammatory diseases such as arthritis [1, 2]. Prolonged use of these drugs exhibit several undesired side effects; the most important are gastro-intestinal (GI) irritation and ulceration, which represent still an unresolved therapeutic problem [3]. Prostaglandins have a significant role in maintaining regular GI physiology and controlling gastric acid secretion.

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NSAIDs inhibit prostaglandin synthesis causing adverse effects on GI function that eventually leads to gastric erosion, haemorrhage, peptic ulcer formation, and inflammation in the small and large intestine [4].

Fenbufen (4-(4-Biphenylyl)-4-oxobutanoic acid) from the NSAID category is used in the treatment of rheumatic and other musculoskeletal disorders. GI complaints are the most frequently reported side effects of fenbufen [5]. Propyphenazone is a non-acidic pyrazole drug and has excellent analgesic and antipyretic activity with less anti-inflammatory activity [6]. The therapeutic dose of fenbufen is 600-1000 mg per day, which is similar to therapeutic doses (500-1000 mg per day) of propyphenazone which makes the coupling of fenbufen and propyphenazone to form a hybrid drug most efficacious.

Glyceride prodrugs of biphenyl acetic acid (a metabolite of fenbufen) have been synthesized as a safe anti-inflammatory agent with enhanced absorption pertaining to the absorption of natural glycerides by M S Y Khan and his team [7]. Husain and co-workers have also reported the synthesis of the 1, 3, 4-oxadiazole derivatives of fenbufen as safer anti-inflammatory and analgesic agents [8]. Many other amide analogs and gastro-protective chimeric derivatives of fenbufen were also reported in literature [9, 10].

Naproxen-propyphenazone and mefenamic acid-propyphenazone ester mutual prodrugs have also been synthesized with an intention to get a better therapeutic index through prevention of GI irritation and bleeding [11, 12].

The aim of this work was to unite fenbufen with propyphenazone to get many benefits related to synergistic analgesic effect with reduced GI irritation. The metabolism of propyphenazone has been reported to proceed *via* the formation of 3-hydroxymethyl-propyphenazone (HMP), which is pharmacologically active as the parent drug [13, 14]. Coupling of both compounds as a hybrid drug or through a spacer as a mutual pro-drug result in a potent analgesic, anti-inflammatory compound with the reduction of the main adverse local effects related to the activity of NSAIDs.

MATERIALS AND METHODS:

Materials: Propyphenazone was received as a gift from VANI PHARMA Labs Limited, Hyderabad, AP, India, and drug fenbufen was purchased from Sigma-Aldrich Chemicals Pvt. Ltd. New Delhi. The analytical grade reagents and solvents were used. The preliminary compound 3-bromomethyl-propyphenazone was synthesized in a pure crystalline form according to the method reported by Lucius *et al.,* [15] The melting points of finally synthesized compounds were determined in an open capillary using Decibel melting point apparatus and recorded in ℃ without correction. The reactions were monitored by TLC on precoated silica gel G plates using iodine vapors as detecting agent. The infrared spectra for the synthesized compounds were recorded by the BRUKER ALPHA Eco-ATR spectrophotometer. Proton nuclear magnetic resonance spectra (1H NMR) were recorded on NMR spectrophotometer AGILENT 500/54 AR (software Agilent Vnmr J version 3.2) using tetramethyl silane as an internal standard. 1H NMR spectra were recorded with DMSO 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-MS (AGILENT 6520, Software Mass Hunter). Elemental analysis was performed with CHNS Analyser (EURO EA3000, software callidus) at IPC, Ghaziabad. The hydrolysis data and drug content determination were performed by a UV-visible spectrophotometer Pharma Spec-1700 (SHIMADZU).

Experimental:

Mutual Prodrugs of Fenbufen were Prepared by Two Methods:

A. Without Spacer (FP1)
B. With Spacer (FP2)

Synthesis of Fenbufen–propyphenazone Mutual Prodrug without Spacer (FP1):

Step-1: Synthesis of 3-bromomethyl propyphenazone (BMP): 3-bromomethyl propyphenazone, $C_{14}H_{17}BrN_2O$, was synthesized from propyphenazone and bromine, according to Lucius *et. al.,* [15] with some alterations to increase yield and purity as follows;

Propyphenazone (10 g) was dissolved in 25 mL CH_2Cl_2 in 100 mL round bottom flask fitted with dropping funnel; it was set aside over an ice bath and bromine solution (6.95 g; 2.23 mL bromine dissolved in 5 mL CH_2Cl_2) was poured drop-wise and gradually with the help of magnetic stirring for 1 h. The brown color of the bromine faded quickly upon its addition. The temperature was maintained 10- 15 ℃ during the reaction. The progress of reaction was checked by TLC using Ethyl acetate (EA): Hexane (2:1) ratio. An aliquot of 10% cold aqueous sodium carbonate solution (30 mL) was added with strong shaking. The organic phase was separately collected and dried with Na_2SO_4, filtered, and concentrated under vacuum at 40 ℃ until the volume was reduced to about 10 mL. The reaction mixture was cooled to room temperature and about 15 mL diethyl ether was added. This solution was kept 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 ºC). The modifications were made to the reported method incorporated: (a) The use of cold sodium carbonate aqueous solution to prevent formation of any 3-hydroxymethyl- propy-phenazone; (b) The use of diethyl ether instead of dichloromethane to obtain more yield of BMP crystals; and (c) The use of cold diethyl ether for crystallization instead of dichloromethane at room temperature to get more pure crystals of BMP.

Step-2: Preparation of 3- Hydroxymethyl Propyphenazone (HMP): BMP (0.309 g, 1 mmol) was refluxed with 5.0 mL of water. The progress of reaction was checked by TLC, using EA: Hexane (2:1). As the reaction was completed, reaction mixture was cooled, extracted with dichloro-methane (DCM), and the DCM layer was collected. It was washed with water and drying was carried out over anhydrous sodium sulphate. Concentration of DCM layer resulted in white hydroxyl derivative. Recrystalization with hot water gave pure white crystals.

Step 3: Coupling of HMP to Carboxylic Acid Group Containing Fenbufen: A solution of carbonyldiimidazole (CDI) (0.443g, 2.7 mmol) in anhydrous dimethylformamide (2.5 mL) was added to a cold solution of fenbufen (2 mmol) dissolved in anhydrous DMF (3.5 mL). The mixture was left for 30 min and a solution of HMP (0.5g, 2 mmol) in dry DMF (10 mL) was poured drop wise. The reaction mixture was magnetically stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with DCM. Organic layer was separately collected, washed with water and dried over anhydrous sodium sulphate. It was concentrated to obtain the product.

Synthesis of Fenbufen-propyphenazone Mutual Pro-drug with Spacer (FP2):

Step-1: Synthesis of glycinyl-3-hydroxymethyl propyphenazone (Gly-HMP): A mixture of HMP (2.46 g, 10 mmol), dimethylaminopyridine (50 mg), and 2.27 g dicyclohexylcarbodiimide (DCC) was added in an ice- cold solution of Boc-glycine (1.75 g, 10 mmol) in 30 mL dichloromethane. The reaction mixture was magnetically stirred at 4 ºC for 1 h, and left overnight at room temperature. The precipitated dicyclohexylurea (DCU) was collected by filtration and the filtrate was washed with cold 0.05 N HCl and then followed by saturated solution of $NaHCO_3$ and finally with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure.

The product was then treated with 10 mL of 6N HCl in dioxane and stirred for 1 h followed by evaporation under vacuum at room temperature. The obtained product was triturated with 100 mL of dry ether and kept for 2 h in refrigerator. The precipitated product was filtered and vacuum dried to yield crystals of Gly-HMP.

Step-2: Preparation of Fenbufen- glycine-3-hydroxypropyphenazone (FP2): A solution of CDI (1 g, 6.16 mmol) in anhydrous DMF (5 mL) was added dropwise, at 4 ºC to a solution of fenbufen (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 dropwise. The reaction mixture was stirred for 10 min at 4 ºC, kept for 3 h at room temperature, and then evaporated under vacuum. The residue was purified by column chromatography (EA:Hexane; 2:1).

(2, 3-dihydro-4-isopropyl- 1-methyl-3-oxo-2-phenyl-1H-pyrazol-5-yl)methyl4-(4-biphenylyl)-4-oxobutanoate (FP1): Yield 56%; m.p. 130–132 °C; 1H NMR (400 MHz, DMSO- d6 , TMS): δ 0.95-0.94 (d, 6H, $-CH_3$ of $–CH(CH_3)$ 2, J = 4.68), 2.25-2.26 (t, 2H, CH_2 of CH_2-COO, J = 3.45), 2.59-2.62 (m, 1H, -CH of $-CH(CH_3)2$), 2.77 (s, 3H, CH_3N), 3.03-3.06 (t, 2H, CH_2 of $-CH_2$-CO-, J = 12.48), 4.22 (s, 2H, CH_2 of –CH2-O-CO-), 7.01-7.12 (m, 5H, Ar-H), 7.16-7.27 (m, 5H, Ar-H), 7.48-7.83 (m, 4H, Ar-H); ^{13}C-NMR (DMSO- d6 , 400 MHz): 21.18(CH_3 of $–CH(CH_3)2$), 23.44 (CH_3 of $–CH(CH_3)2$), 27.85 (-CHCOO), 33.10 (CH of $–CH(CH_3)2$), 36.20 ($COCH_2$), 39.62 ($N-CH_3$), 114.34-144.51 (Ar-carbons), 154.55 ($C-CH_2$- of pyrazole), 164.31 (CO of pyrazole), 173.87 (COO), 197.97 ($COCH_2$) ; IR (KBr) cm^{-1} : 2960.40 (C-H str. of methyl group), 2879.26 (C-H str, of –CH2), 1733.29 (C=O, ester), 1236.70, 1167.70 (C-O-C str.), 1099.30 (C-N str), 761.35, 660.76 (C-H def, Ar), 1252.66 (C sp2-O str), 1602.87, 1505.74 (C=C str, phenyl nucleus), 687.52, 660.76 (Mono substituted Ar ring); Anal. Calcd. C, 74.67; H,

6.27; N, 5.81; Found: C, 74.80; H, 6.42; N, 5.96. MS: m/z [M+1] 482.

(2, 3-dihydro-4-isopropyl -1-methyl-3-oxo-2-phenyl- 1H-pyrazol-5-yl)methyl 2-(4-(4-biphenylyl acetamido)benzoate (FP2): Yield 64 %; m.p. 140–142 °C; ^{1}H NMR (400 MHz, DMSO-d6, TMS): δ 1.17 (d, 6H, CH_3 of –$CH(CH_3)2$, J = 8.8), 2.50 (s, 3H, CH_3N), 2.8 (m, 1H, CH of –$CH(CH_3)2$), 4.45 (s, 2H, CH_2 of –CH_2-CO-O-), 4.53 (s, 2H, CH_2 of –CH_2-O-CO-), 7.41-7.49 (m, 5H, Ar-H), 7.71-7.74 (m, 5H, Ar-H), 7.8-7.82 (m, 4H, Ar-H); ^{13}C-NMR (DMSO- d6, 400 MHz): 21.68(CH_3 of –$CH(CH_3)2$), 23.85 (CH_3 of –$CH(CH_3)2$), 28.42 (CH of –$CH(CH_3)2$), 39.81 (N-CH_3), 40.97 (-CHCOO), 36.58 ($COCH_2$), 198.62 (COCH2), 164.84 (CO of pyrazole), 114.68-145.01 (Ar-carbons), 174.36 (CONH), 154.86 (C-N- of pyrazole) ; IR (KBr) cm^{-1} : 2960.59 (C-H str. of methyl group),1705.97 (C=O, ester), 1247.87, 1136.72 (C-O-C str.), 1435.38, 1397.91 (C-H bend, aliphatic), 1247.87 (C-O str, ester), 1494.53 (N-H str, 20 amines), 770.95 (N-H, out-of- plane bending), 701.53, 666.85 (Mono substituted Ar ring), 1582.91 (N-H in plane bending 20 amide), 3024.91 (Aromatic C-H str.); Anal. Calcd.: C, 71.22; H, 6.16; N, 7.79; Found: C, 71.38; H, 6.38; N, 7.90. MS: m/z [M+1] 539.

***In-vitro* Hydrolysis Studies of FB Pro-drugs:** Veego Paddle type dissolution apparatus (VDA-8DR USP Standard) was used for carrying out the hydrolysis study. Two hydrolysis media as per IP were used: 900 mL of non-enzymatic simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4). The test was carried out at 37 ± 0.5 ºC.

A precise amount of 100 mg of the prodrug was taken for the study. Aliquots of 1 ml were withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 h from the matrix and were immediately restored with 1.0 mL of fresh hydrolysis media equilibrated at 37 ± 0.5 ºC. Free FB was released after the hydrolysis of prodrugs extracted with 5 mL methanol. The methanol layer was estimated on UV spectrophotometer for the amount of free FB released after hydrolysis of prodrugs in SGF and SIF. The kinetics of hydrolysis was recorded by an increase of free drug concentration with time, and order of reaction and half-life ($t_{1/2}$) were also calculated. The rate of hydrolysis was calculated by first-order equation, k= (2.303/t) log (a/a-x) where k is 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 prepared prodrugs were evaluated for analgesic and anti-inflammatory activity as compared with that exerted by FB and HMP, separately. The study was carried out in Wistar rats of Albino strain (150-200 g). All the animals were obtained from Disease Free Small Animal House of Hindu College of Pharmacy, Sonepat. The animals were housed in polypropylene cages. Paddy husk provided as bedding material, which was changed every day. The cages were maintained clean. The rats were kept 3 to 5 per cage. They were fed with a standard pellet diet (Amrut rat feed, Sangli, India) and water ad libitium. They stayed in well-aerated room and a 12-hour light and dark cycle was maintained. The room temperature was kept at 22 ± 2 ºC. All the animals were acclimatized for a week before the experiment. The animals were fasted with free access to water for 12 h prior to the tests. The tested compounds were prepared in aqueous 0.5% carboxymethylcellulose (CMC) solution for oral administration. The experimental protocol was approved by the Institutional Animal Ethics Committee of Hindu College of Pharmacy, Sonepat having registration no. 585/02/c/CPCSEA, held on 27/11/2012 vide item no. I (1). All the results were expressed as mean ± standard error (SEM). Data were 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 evaluated by tail-flick method [16, 17]. The rats were separated into 5 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.*, FB (12 mg/kg body weight, 47.19 μmol/kg) and HMP (23 mg/kg body weight, 47.19 μmol/kg) respectively, Group IV and V received prodrug FP1 (22.7 mg/kg body weight, 47.19 μmol/kg) and FP2 (25.4 mg/kg body weight, 47.19 μmol/kg) separately, where the dose was molecular equivalent to the free drug. Thermal stimuli induced using hot water bath maintained at a temperature of 55 ± 0.5 ºC was pain inducing

agent. The method involved dipping an extreme 3 cm of the rat's tail in a water bath containing water at a temperature of 55 ± 0.5 ºC. Within a few minutes, the rat reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. Each animal served as its own control, and two readings were obtained for the control at 0 and 10 min intervals. The average of the two values was the initial reaction time (Ta). The reaction time (Tb) for the test groups was taken at intervals of 0.5, 1, 2, 3, and 4 h. The percentage analgesic activity was calculated using the formula:

$$\text{Percentage analgesic activity} = [1 - (T_a/T_b)] \times 100$$

T_a and T_b are latency periods of control and test group animals, respectively.

Anti-inflammatory Activity: The acute anti-inflammatory activity of the prodrugs was evaluated by using a carrageenan-induced rat hind paw edema method as described by Winter *et al.,* [18] Rats were segregated into control, standard, and test groups of six animals each. Pretreatment preliminary paw volumes of all animals were measured with the help of a mercury plethysmo-meter. An appropriate volume of 0.5% CMC was given to the control group only. The standard group received FB and HMP (equivalent dose, 47.19 µmol/kg), respectively.

To the test group, prodrugs (FP1 and FP2) were given orally using 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 point of lateral malleolous and immersed in mercury up to this point. 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 after the carrageenan administration. The percent inhibition of edema as an indication of anti-inflammatory activity was compared with the controls. The following formula was used to calculate the percentage inhibition of swelling:

$$\text{Inhibition} = (V_t - V_o)_{control} - (V_t - V_o)_{treated} / (V_t - V_o)_{control} \times 100$$

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

Ulcerogenic Activity: Ulcerogenic activity was assessed using the method as explained by Kunchandy *et al.,* [19] Five groups of six rats each were fasted for 12 h prior to the drug administration. The first group served as control and received vehicle (0.5% CMC, 10 mL/kg) only. Group II and III received FB and HMP (in equivalent doses, 47.19 µmol/kg), respectively. Group IV and V received equivalent doses of prodrugs FP1 (22.7 mg/kg, 47.19 µmol/kg) and FP2 (25.4 mg/kg, 47.19 µmol/kg) separately.

All the test drugs or standard of the vehicle were given orally to rats over a period of seven successive days. All the rats fasted for 24 h on the 8th day. The animals were sacrificed with excessive anesthesia. The stomach was removed, excised and dissected along the greater curvature, and washed gently by rinsing with isotonic saline solution. The ulcer incidence represented by the presence of hemo-rrhagic lesions and/or gastric ulcers in rats was observed by means of magnifying lens. To find out the extent of induced ulcers in each stomach, the following scoring system was employed: 0.0 - normal colored stomach; 0.5 - pink to red coloration of the stomach; 1.0 - spot ulcer; 1.5 - haemorrhagic streak; 2.0 - number of ulcers < 5; 3.0 - number of ulcers > 5; 4.0 - ulcers with bleeding.

RESULTS AND DISCUSSION:

Chemistry: By bromination of propyphenazone, according to Meister, Lucius, Bruning in Hochst, 1907 15, Bromopropyphenazone (BMP) was obtained with some alterations to enhance yield and purity. BMP was further used to synthesize hydroxymethyl-propyphenazone (HMP) by reflux with water.

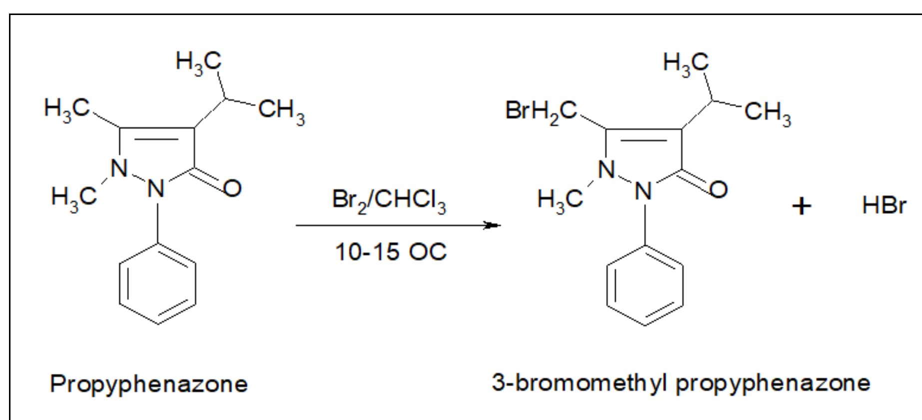
FB-propyphenazone ester (FP1) was synthesized by direct coupling of carboxyl group of fenbufen with hydroxyl group of 3-hydroxymethyl propyphenazone as shown in **Fig. 1**. In order to minimize the steric hindrance effect on the hydrolysis of FB-HMP ester prodrug (FP1), a spacer was added. Glycine was chosen as a spacer as it is a normal dietary constituent hence non-toxic in moderate doses; it possesses marked anti-inflammatory activity and is nutritional substance; hence its use as a derivatizing agent might permit more specific targeting site for enzymes involved

in the terminal phase of digestion. N-Protected glycine combined with HMP by DCC followed by N-deprotection and coupling with FB using CDI as coupling agent yielded FB amide prodrug (FP2) as shown in **Fig. 2**. The physicochemical properties were determined and presented in **Table 1**. The good yields of prodrugs were obtained.

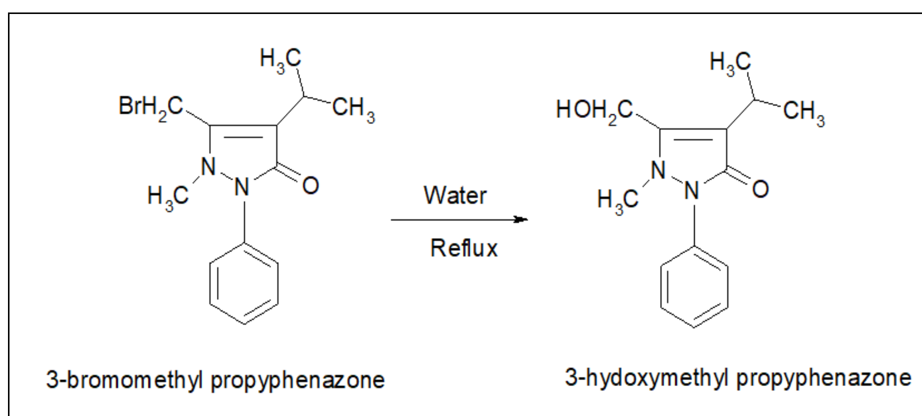
The structures of prodrugs formed were established by UV, ^{1}H-NMR, Mass and FT-IR spectral methods. The purity was checked by TLC. The results of elemental analysis of synthesized prodrugs were found to be within 0.4% of theoretical values and were observed in confirmation of desired structure.

TABLE 1: PHYSICOCHEMICAL PROPERTIES OF SYNTHESIZED PRODRUGS

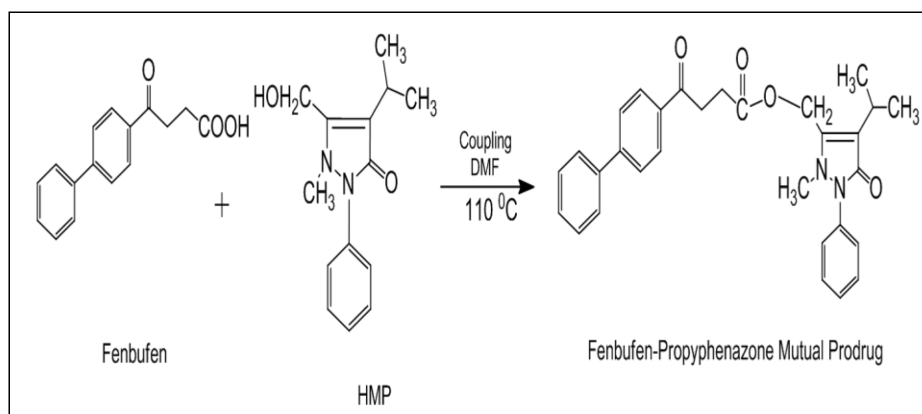
Prodrug Code	Molecular formula	Mol. wt.	Colour	Melting point (°C)	% Yield	R_f# value	Log P
FP1	$C_{30}H_{30}N_2O_4$	482	White	130-132	56	0.61	5.82
FP2	$C_{32}H_{33}N_3O_5$	539	White	145-147	64	0.35	4.67



STEP 1: SYNTHESIS OF 3-BROMOMETHYL PROPYPHENAZONE (BMP)

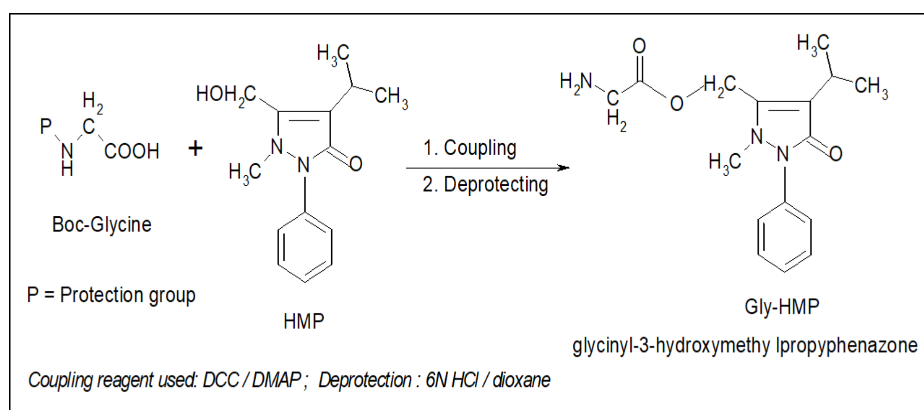


STEP 2: PREPARATION OF 3- HYDROXYMETHYL PROPYPHENAZONE (HMP)

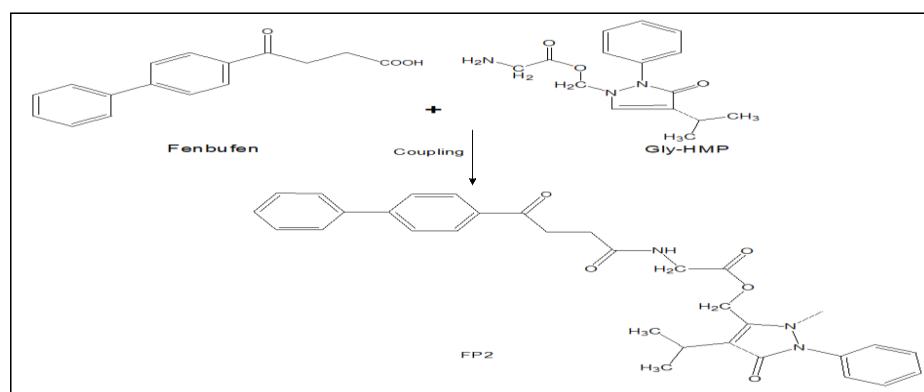


STEP 3: COUPLING OF HMP TO CARBOXYLIC ACID GROUP CONTAINING FENBUFEN

FIG. 1: SYNTHESIS OF FP1 MUTUAL PRODRUG



STEP 1: SYNTHESIS OF GLYCINYL-3-HYDROXYMETHYL PROPYPHENAZONE (GLY-HMP)



STEP 2: SYNTHESIS OF FEN-GLY-PROPY MUTUAL PRODRUG (FP2)

FIG. 2: SYNTHESIS OF FP2 MUTUAL PRODRUG

***In-vitro* Hydrolysis of Ester:** Since the carboxylic functional group of FB is necessary for the therapeutic action, prodrugs of long-lasting action were designed in such a form from which the pharmacologically active moiety can be released in its original state with time [12]. Therefore the release of FB from its prodrugs was studied *in-vitro* in order to assess the possible time span in which the drug could be accessible from different prodrugs. The comparative study of hydrolysis of these prodrugs in SIF is represented in **Fig. 3**.

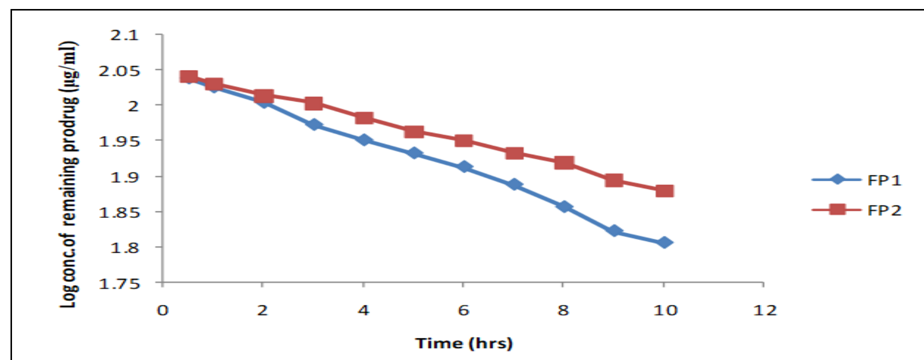


FIG. 3: COMPARATIVE PATTERN OF HYDROLYSIS OF FP1 AND FP2 IN SIF (PH 7.4) SHOWING FIRST ORDER KINETICS

The amount of FB revived on hydrolysis of FP1 and FP2 in SGF (pH 1.2) was found as 4.6 and 5.9 %, respectively, and that in SIF (pH 7.4) was found as 42 and 32% respectively over a period of 10 h. The results of the hydrolytic kinetics study showed that both followed first-order kinetics and are shown in **Fig 3**. But these prodrugs showed insignificant hydrolysis in an acidic medium (pH 1.2) for 2 h. From these results, this is established that the release of FB should occur mainly at higher pH of the intestine. This may be due to the reason that ester hydrolysis is a reversible reaction in

acidic pH, and in alkaline pH it is irreversible and complete [20]. The predominant release of fenbufen from its prodrugs at pH 7.4 shows the potential of the prodrug to decrease the gastric complications caused by direct contact of the free carboxyl group of the drug to gastric mucosa. Kinetic parameters for hydrolysis of mutual prodrugs at 37 ºC are shown in **Table 2**. The corresponding half-lives for FP1 and FP2 were found to be 26 and 36 h (in SGF, pH 1.2) and 14 and 20 h (in Phosphate buffer, pH 7.4), respectively. The half-lives and the rate constants for prodrug hydrolysis **Table 2** specified that esterification of carboxylic group of FB made prodrugs more stable at pH 1.2, but less stable at pH 7.4.

TABLE 2: KINETIC PARAMETERS FOR HYDROLYSIS OF MUTUAL PRO-DRUGS FP1 AND FP2 AT 37 ºC

Prodrug code	$K_{observed}$ $(h^{-1})^a$	$T_{1/2}$ $(h)^a$	$K_{observed}$ $(h^{-1})^b$	$T_{1/2}$ $(h)^b$
FP1	0.027	26	0.056	14
FP2	0.019	36	0.038	20

[a]In SGF (pH 1.2), [b]In SIF Phosphate buffer (pH 7.4)

***In-vivo* Biological Evaluation:** Analgesic, anti-inflammatory, and ulcerogenic activities of the prodrugs were studied in comparison to equivalent doses (47.19 µmol/kg) of FB and HMP. Findings of anti-inflammatory activity by FB and its mutual prodrugs in terms of a difference in paw volume and percentage inhibition at different time intervals are shown in **Table 3**. FB revealed maximum anti-inflammatory effect (52%) at 3rd h where as second parent drug HMP showed it maximum effect (41%) at 3rd h.

Both mutual prodrugs showed better maximum inhibition and for a longer time as compared to both parent drugs. FP1 ester prodrug exhibited maximum activity (68%) at 4th h whereas FP2 prodrug showed its maximum activity (85%) at 3rd h.

The interpretation of results showed that the mutual prodrug synthesized with spacer technique using glycine amino acid as a spacer was observed to be more effective than prodrug prepared by direct esterification of parent drugs. Both prodrugs displayed extremely significant results (p< .0001) as compared to control and also possessed significantly enhanced activity as compared to FB.

TABLE 3: PAW VOLUME AND PERCENTAGE INHIBITION OF TREATED GROUPS (FB AND ITS MUTUAL PRODRUGS) AT VARIOUS TIME INTERVALS

Treatment	Dose (mg/kg)	Change in Paw Volume (mL) (Mean± SEM) and Percentage inhibition											
		1 h		2 h		3 h		4 h		6 h		8 h	
Control	-	0.38 ± 0.005	0.0	0.44 ± 0.004	0.0	0.518 ± 0.0037	0.0	0.48 ± 0.004	0.0	0.35 ± 0.033	0.0	0.13 ± 0.006	0.0
FB	12.0	0.23 ± 0.005***	39	0.23 ± 0.004***	48	0.25 ± 0.006***	52	0.29 ± 0.006***	39	0.26 ± 0.008**	24	0.10 ± 0.005*	22
HMP	23.0	0.26 ± 0.006 ***Δ	32	0.24 ± 0.004***	46	0.30 ± 0.006 ***ΔΔΔ	41	0.32 ± 0.005***ΔΔΔ	32	0.26 ± 0.0089**	25	0.11 ± 0.006*	14
FP1	22.7	0.32 ± 0.004 ***#σ	16	0.20 ± 0.004***Δ Δσ	54	0.17 ± 0.014 *** # σ	68	0.15± 0.008***# σ	68	0.13 ± 0.007***# σ	62	0.07 ± 0.007*** Δ α	44
FP2	25.4	0.29± 0.007*** # α	24	0.11 ± 0.004 *** # σ	76	0.08 ± 0.007 *** # σ	85	0.078 ± 0.003 *** # σ	85	0.09 ± 0.006***# σ	74	0.06 ± 0.008 ***ΔΔγ	53

Oedema is shown as mean change in paw volume ± SEM. n= 6 animals. * p < 0.05, ** p <0.01, *** p <0.0001 as compared to control, Δ p < 0.05, ΔΔ p <0.01, ΔΔΔp<0.001, # p < 0.0001 as compared to fenbufen, α p <0.01, γ p<0.001, σ p < 0.0001 as compared to HMP

The analgesic activity was evaluated by tail flick method and the result was shown in **Table 4**. Results of analgesic activity revealed that Fen-Gly-HMP ester showed significantly much better inhibition of pain (61%, p<0.0001) as compared to fenbufen (39%) at 3rd h where as FP1 ester also produced better percentage protection (59%, p<0.0001) at 3rd h. The statistical analysis showed that fenbufen and its both mutual prodrugs were more significantly effective (p<0.0001) analgesic drugs than control. Ulcerogenic potential of synthesized prodrugs was tested in contrast to the parent drugs following successive oral administration for 7 days in rats, and the results are shown in **Table 5**. Screening for ulcerogenic activity showed that synthesized compounds had fewer tendencies (p< .0001) to form ulcers when compared to that of the parent drug.

TABLE 4: ANALGESIC ACTIVITY OF FENBUFEN AND ITS MUTUAL PRODRUGS

Treatment	Dose (mg/ kg)	Latentiation Period in seconds (Mean±SEM) and Percentage Analgesic activity										
		0 h	0.5 h		1 h		2 h		3 h		4 h	
Control	-	1.48 ±0.042	1.50 ±0.047	0.0	1.50 ±0.027	0.0	1.55 ±0.010	0.0	1.56 ±0.057	0.0	1.50 ±0.047	0.0
FB	12.0	1.73 ±0.033 *	1.74 ±0.043 **	14	2.00 ±0.038 α	25	2.60 ±0.05 α	40	2.59 ±0.055 α	39	2.40 ±0.066 α	38
HMP	23.0	1.86 ±0.076 ***	2.07 ±0.059 αΔΔΔ	28	2.44 ±0.046 α#	39	3.23 ±0.044 α#	52	3.01 ±0.076 αΔΔΔ	48	2.76 ±0.074 αΔΔ	46
FP1	22.7	1.85 ±0.030***	1.87 ±0.029 ασ	20	2.60 ±0.028 α#σ	42	3.54 ±0.061 α#σσσ	57	3.79 ±0.048 α#σσσσ	59	3.50 ±0.068 α#σσσσ	57
FP2	25.4	2.10 ±0.075 αΔΔΔ	2.37 ±0.045 α#σσσ	37	2.80 ±0.030 α#σσσσ	46	3.80 ±0.038 α#σσσσ	59	4.04 ±0.056 α#σσσσ	61	3.91 ±0.045 α#σσσσ	62

n= 6 animals. *p < 0.05, ** p <0.01, *** p<0.001, α p < 0.0001 as compared to control. Δ p< 0.05, ΔΔ p <0.01, ΔΔΔp<0.001, # p < 0.0001 as compared to fenbufen, σ p < 0.05, σσ p <0.01, σσσ p<0.001, σσσσ p < 0.0001 as compared to HMP

TABLE 5: ULCEROGENIC POTENTIAL OF FENBUFEN AND ITS ESTERS

Treatment	Dose (mg/kg)	Average number of ulcer score ± SEM
Control	-	0.00 ± 0.00
FB	12.0	3.50± 0.341****
HMP	23.0	1.16± 0.307*#
FP1	22.7	1.08± 0.238*#
FP2	25.4	0.66± 0.105#

Ulcerogenic potential is expressed as average number of ulcer score ± SEM. n= 6 animals, ****p < 0.0001, *p< 0.05 as compared to control, #p <0.0001 as compared to fenbufen

CONCLUSION: The mutual prodrugs of FB with propyphenazone were successfully synthesized and characterized by data based on spectral analysis. Both prodrugs showed improved hydrolysis rate in SIF and significant pharmacological response. The *in-vitro* and *in-vivo* evaluation of synthesized prodrugs revealed improvement in the therapeutic index of parent drugs. The major contribution of the ulcerogenic effect of FB may be due to its acute local contact with stomach wall. Conversion of FB into its mutual prodrug by direct esterification as well as using spacer technique render its slow diffusion into the gastric lumen as confirmed by *in-vitro* dissolution data could alleviate the problem of gastric ulceration by minimizing its direct exposure to the ulcerprone area of the stomach. It is worthy to mention that these prodrugs may be effectively applied in achieving the goal of reduced gastrointestinal toxicity without loss of required analgesic and anti-inflammatory activity of the parent drugs.

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