
Synthesis and Pharmacological Evaluation of Mutual Prodrugs of Some Nonsteroidal Antiinflammatory Drugs with Glucosamine

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Mutual prodrugs of ibuprofen and diclofenac with glucosamine were synthesized by reacting their acid chlorides with glucosamine with an aim to reduce gastrointestinal side effects possessed by these drugs. *In vitro* hydrolysis of these prodrugs in HCl buffer (pH 1.2) indicated that they were quite stable in gastric acid whereas in phosphate buffer (pH 7.4) undergo hydrolysis following first order kinetics, releasing free drug and glucosamine. These compounds were characterized by analytical and spectral data. The amides were evaluated for analgesic and antiinflammatory activity, Freund's adjuvant-induced arthritis assay and ulcerogenic potential. They showed better to comparable analgesic and antiinflammatory activity with excellent antiarthritic activity and negligible ulcerogenicity.

Ibuprofen and diclofenac are widely used nonsteroidal antiinflammatory drugs (NSAIDs) that are associated with major gastrointestinal (GI) side effects, which are generally attributed to a direct and/or an indirect mechanism¹⁻⁴. The direct contact effect results usually from local irritation produced by acidic group of NSAIDs and local inhibition of prostaglandin synthesis in the GIT. The indirect mechanism is due to a generalized systemic action occurring after absorption following intravenous dosing^{4,5}.

A possible way to solve this problem is to derivatize the carboxylic function to produce prodrugs with adequate stability at the acidic pH of the stomach. Thus derivatization may on one hand prevent local irritation of the stomach mucosa and on the other hand be capable of releasing the parent drug spontaneously or enzymatically in the blood following its absorption⁶⁻⁹. Various amino acid salts, esters and amino acid conjugates of ibuprofen have been reported with lower ulcerogenic tendency¹⁰⁻¹³. Until now, except for benorylate, mutual prodrug concept has not been employed to mask the free carboxylic group of NSAIDs in order to

overcome their gastric irritancy. Utility of amide in the design of prodrug of carboxylic acid has been recently reported¹⁴.

The present work reports the synthesis, physico-chemical characterization, *in vitro* hydrolysis and biological evaluation of mutual prodrugs of ibuprofen and diclofenac with glucosamine for possible use in the management of arthritis with less or no gastric side effects. The rationale behind the use of glucosamine to mask COOH group temporarily has been described below.

Glucosamine is an amino sugar, which is body's basic starting material for the production of natural joint components like critical joint lubricants and shock absorbers¹⁵. Glucosamine hydrochloride and sulphate are being used as antiarthritic agents as well as a nutritional supplement in conditions such as joint ache, stiffness, severely restricted movements, and serious pain¹⁵. It has significantly outperformed ibuprofen with respect to reducing pain in patients suffering from degenerative joint diseases of the knee¹⁷. In some of the clinical studies glucosamine was consistently shown to relieve pain in 3-4 w, restore joint flexibility, produce no known side effects and treat the cause and not just pain¹⁸. These prodrugs

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have the additional advantage of producing nontoxic, nutrient by-product, i.e. glucosamine on cleavage, which after its release is expected to be available to produce the above effects.

MATERIALS AND METHODS

All the chemicals used in the synthesis were of synthetic grade. The purity of the compounds was ascertained by TLC on precoated silica gel 60 F₂₅₄ plates (Merck, Mumbai) using UV light as detecting agent. The melting points of the final products were determined by open capillary method and are uncorrected. The absorbance maxima (λ_{max}) were determined in ethyl acetate and chloroform on a Jasco V-530, UV/Vis double beam spectrophotometer. IR spectra were recorded on a Jasco FT-IR in potassium bromide (anhydrous, IR grade) pellets. The NMR spectra were recorded in CDCl₃ using a 1H NMR Varian Mercury 300 Hz with super conducting magnet. Elemental analysis (C, H and N) was carried out using a CE Instruments EA 1110 Elemental analyzer.

Synthesis of mutual prodrug of ibuprofen with glucosamine (RP-1):

Ibuprofen (0.024 mol; 5 g) was dissolved in 125 ml benzene. Freshly distilled thionyl chloride (0.027 mol; 2.5 ml) was added to the above mixture with a drop DMF as catalyst and was refluxed for 3 h at 80°. Benzene was distilled off at atmospheric pressure leaving behind acid chloride. Glucosamine hydrochloride (0.026 mol; 5.5 g) was suspended in 50 ml of methanol. Triethylamine (0.026 mol; 3.6 ml) was then added dropwise, to the above mixture to liberate free D-glucosamine. The mixture was stirred for 2 h at room temperature. The acid chloride was slowly added dropwise at 10-15° into the mixture of D-glucosamine over 1 h. The reaction mixture was stirred for 6 h at 10-15° and then it was filtered. The crude product was recrystallised with methanol and dried under vacuum. Yield: 66.23%, mp: 225°, Rf: 0.51 in chloroform:methanol (5:1), C₁₉H₂₉O₆N requires C, 62.12; H, 7.90; N 3.31; found C, 59.7; H, 8.23; N 3.37. IR (KBr): 3470 (OH str. sec. OH), 3310 (N-H str. sec. amine), 3045 (aromatic-CH str.), 2951 (CH str. CH₃), 2926 (CH str. -CH₂), 1620 (C=O, str. amide I band), 1543 (N-H bend sec. amide II band), 1284 (OH bending, sec. alcoholic), 1111 (C-O str. alcoholic), 1022 (in plane CH bending-aromatic ring), 850 (out of plane NH wagging), 680 (out of plane ring bending-aromatic) cm⁻¹. NMR (CDCl₃): δ 1.01 (s, 6H-methyl), δ 1.52 (d, 3H-methyl), δ 2.00 (s, 4H -alcohol), δ 2.22 (s, 1H-methine), δ 2.51 (d, 2H-methylene), δ 3.86 (s, 1H-tetrahydropyran), δ 3.89 (q, 1H-methine), δ 6.95 (s, 1H-

tetrahydropyran), δ 7.07 (s, 4H-1-benzene), δ 8.00 (d, 1H-sec. amide).

Synthesis of mutual prodrug of diclofenac with glucosamine (RP-2):

For this synthesis, diclofenac (0.24 mol; 7.12 g) was used and same procedure was followed as mentioned for RP-1. Yield: 52.27%, mp: 200-202°, Rf: 0.67 in benzene:methanol (4:1), C₂₀H₂₁O₆N₂Cl₂ requires C, 52.63; H, 4.60; N 6.14; found C, 52.86; H, 4.39; N 6.42. IR (KBr): 3354 (N-H, str. sec. amine), 1628 (C=O, sec. amide, I band), 1579 (sec. amide, NH bending amide II band), 1464 (C-H bending, CH₂), 1304 (C-N vibration aromatic sec. amine), 1304 (O-H sec. alcohol bend), 1091 (C=O, str. sec. alcohol), 864 (aromatic substitution, 2 adjacent hydrogens), 765 (aromatic substitution, 3 adjacent hydrogens), cm⁻¹. H-NMR (CDCl₃): δ 1.113 (δ , 2H-CH₂), δ 1.362 (δ , 2H-CH₂), δ 1.635 (δ , 2H-CH₂), δ 1.806-1.847 (δ , 1H-CH-sat.), δ 2.096 (s, 4H-OH), δ 1.806- 1.847 (δ , 1H-CH sat.), δ 3.469 (s, 2H-CH₂), 3.785 (s, 1H-Ar₂NH), δ 6.395 and δ 6.420 (δ , 1H-O=C -NH-CH), δ 7.266-7.42 (m, 7H-Ar-H).

In vitro hydrolysis kinetics:

The kinetics of chemical hydrolysis of the synthesized compounds was studied at 37° in aqueous buffer solutions of pH 1.2 and pH 7.4. The total buffer concentration was generally 0.05 M and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The reaction was monitored by UV for the increase in concentration of free drug with time and the order of reaction and half life ($t_{1/2}$) were calculated.

Hydrolysis in 0.05 M hydrochloric acid buffer (pH 1.2):

RP-1 (10 mg) was introduced in 900 ml of HCl buffer (pH 1.2)¹⁹ taken in the basket and was kept in a constant temperature bath at 37±1°. The solution was occasionally stirred and 5 ml aliquot portions were withdrawn at various time intervals and were transferred to a separating funnel containing chloroform. Free ibuprofen which was released after hydrolysis was extracted with 5 ml portions of chloroform. The chloroform layer was estimated on UV spectrophotometer at 263 nm for free ibuprofen released after hydrolysis of the conjugate. For RP-2, free diclofenac was extracted with ethyl acetate and was estimated on UV spectrophotometer at 276 nm for free diclofenac released after hydrolysis of the prodrug. This UV assay was specially developed for determination of free drug involving extraction and was validated as per USP XXIV edition using different

parameters like accuracy, selectivity, sensitivity and reproducibility. Three readings were taken for each parameter.

Hydrolysis in 0.05 M phosphate buffer (pH 7.4)²⁰:

Same procedure as described in (a) was followed for RP-1 and RP-2, where instead of HCl buffer, phosphate buffer (pH 7.4) was used. Rate of hydrolysis was calculated using equation, $K = (2.303/t) \log (a/a-x)$, where K is hydrolysis constant, t is time in min, a represents initial concentration of prodrug, x is the amount of prodrug hydrolyzed and (a-x) is the amount of prodrug remaining. The hydrolysis kinetic studies were carried out in triplicate. The K values from the plots were calculated separately and then average K and S.D. values were calculated. The results of the same are summarized in Table 1.

Pharmacological evaluation:

Biological evaluation of the synthesized compound was carried out in the department of Pharmacology, Poona College of Pharmacy and its animal facility is approved by CPCSEA (Reg. No.100/1999/CPCSEA). The experimental protocols for the same have been approved by the Institutional Animal Ethics Committee. The biological screening of amide prodrugs was carried out with their homogenized suspension in 0.5% carbopol. Sprague-Dawley rats of either sex (150-200 g) were used for screening of all activities except antiarthritic activity where only male rats (100-150 g) were used and were distributed into control, standard and test (6 animals each) groups. The test compound and standard were administered orally to the animals at doses equimolar to the standard.

TABLE 1: HYDROLYSIS OF MUTUAL PRODRUGS OF IBUPROFEN AND DICLOFENAC WITH GLUCOSAMINE.

Compound	K*± SD Phosphate buffer	t ½ (min)	
		Hydrochloric acid buffer	Phosphate buffer
RP-1	0.002727±0.0001	-----	254.10
RP-2	0.01152±0.001	-----	70.16

Hydrolytic rates of glucosamine conjugates of ibuprofen and diclofenac were determined in hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4) and time necessary for 50% hydrolysis (t_{1/2}) and K values were calculated. *mean of three sets of experiments.

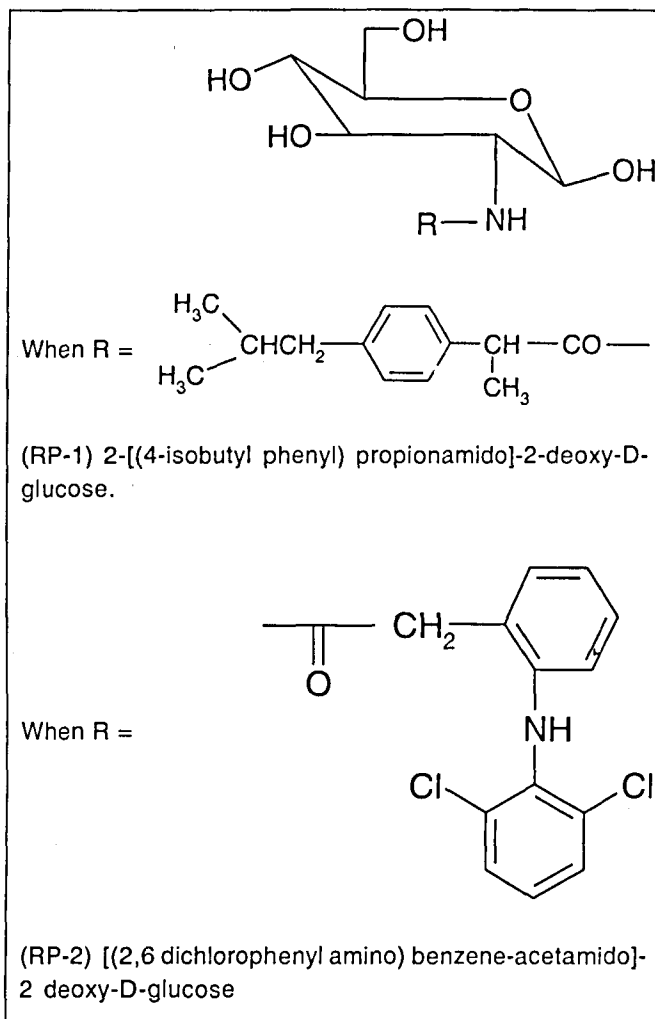


Fig. 1: Structures of mutual prodrugs of ibuprofen and diclofenac with glucosamine.

Analgesic activity:

Analgesic activity²¹ was evaluated using carrageenan induced hyperalgesia method, on a UGO Basile Analgesymeter. The suspensions of test compounds were prepared in 0.5% carbopol in water. At 0 h hyperalgesia was induced by injecting 0.1 ml of 1% carrageenan (C-3889 Type IV, Sigma Chemicals, St. Louis, MO, USA) in distilled water subcutaneously into the planter surface of the hind paw. The rats received test compounds at equimolar doses, 2 h after carrageenan injection and were evaluated for paw hyperalgesia 1 h later. The force at which a rat withdrew its hind paw, vocalized or struggled was multiplied by 10, and recorded as the withdraw force (g) or pain threshold (g). Percent increase in pain threshold was calculated by using the formula, % Increase in pain

threshold = (mean of drug - mean of control / mean of control) × 100

Antiinflammatory activity:

Antiinflammatory activity testing was carried out using carrageenan-induced rat paw edema method of Winter *et al.*²². The test compounds were administered orally at equimolar doses to standard. One hour after this treatment edema was induced by injecting 0.1 ml of 1% w/v suspension of carrageenan in subplantar region the hind paw. Paw volumes were measured before and after the administration of carrageenan using a UGO-Basile Plethysmometer. The percent inhibition was calculated using formula $(1 - V_t / V_c) \times 100$, where V_t and V_c are the mean relative changes in the paw volume in test and control, respectively.

Antiarthritic activity:

Antiarthritic activity was evaluated using Freund's adjuvant induced arthritis method²³. On day one, 0.1 ml of complete Freund's adjuvant (F-5881, Sigma, St. Louis, MO, USA) was injected into the subplantar region of the hind paw. The volume of paw was measured on 3 and 7 d using a UGO-Basile Plethysmometer. On the 13th d animals received standard and test compounds at equimolar doses. The paw volume was measured on 13th, 18th and 21st d. The average foot swelling in the group receiving drug was compared with control group and percent inhibition of edema was determined.

Ulcerogenic activity:

The ulcerogenic activity was determined by the cold stress method of Rainsford and Whitehouse²⁴. Test compounds and standards were administered orally at doses, RP-1 and RP-2 at 150 mg /kg and 17.4 mg/kg respectively whereas ibuprofen and diclofenac at 28.4 mg/kg and 11.5 mg/kg respectively. After oral administration, animals were stressed by exposure to cold (-15° for 1 h). The animals were kept in separate polypropylene cages. After 2 h of drug administrations, the animals were sacrificed using chloroform. The stomach was opened along the greater curvature and washed with distilled water. The number of lesions was examined by means of a magnifying lens. All ulcers larger than 0.5 mm were counted and recorded as average number of ulcers per animal and were scored according to the method reported by Cioli *et al.*¹ and the ulcer index was determined. The results of biological evaluation are summarized in Table 2.

RESULTS AND DISCUSSION

The synthesized compounds were subjected to physico-chemical characterization. The IR spectra showed absorption band for secondary amide which is characteristic of the anticipated structure. The NMR spectrum clearly indicated the formation of amide bond between the COOH group of parent drug and NH₂ group of glucosamine. All the above results confirm the correctness of the anticipated structure.

TABLE 2: PHARMACOLOGICAL ACTIVITIES OF MUTUAL PRODRUGS OF IBUPROFEN AND DICLOFENAC WITH GLUCOSAMINE.

Compound	Oral dose (mg/kg)	Analgesic activity (6 h). Increase in % pain threshold*	Antiinflammatory activity (6 h). % Inhibition of edema*	Antiarthritic activity (21 st d). % Inhibition of edema*	Ulcer Index ± S.D.*
Ibuprofen	16.00	94.7	54.2	15.6	6.0±1.0
RP-1	28.46	93.8	41.7	37.5	1.5±0.5
Diclofenac	4.00	134.6	76.4	11.3	22.3±15
RP-2	6.00	119.3	81.7	28.2	1.6±0.4

The analgesic, antiinflammatory, antiarthritic and ulcerogenic activities of ibuprofen and diclofenac in comparison with their glucosamine conjugates RP-1 and RP-2. *(p < 0.05), Increase in % pain threshold and % inhibition of edema was calculated by taking into consideration mean relative changes in pain threshold and paw volume in test and control groups (6 animals each) respectively ±SE. therefore the percent values are not accompanied by standard deviation values. *Dose mentioned in the text.

Hydrolysis kinetics of synthesized compounds show that they did not undergo hydrolysis in 0.05 M hydrochloric acid buffer (pH 1.2) indicating that they would not release the active drug in acidic pH of the stomach. Their hydrolysis in 0.05 M phosphate buffer (pH 7.4) indicated that RP-1 and RP-2 hydrolyzed following first order kinetics with a half lives of 254.1 min and 70.2 min, respectively.

Results of analgesic activity reveal that RP-1 showed similar increase in the pain threshold (93.8%) as that of ibuprofen (94.7%), whereas RP-2 showed better analgesic activity (40.4%) than diclofenac (23.1%). Results of antiinflammatory activity reveal that RP-1 showed comparable inhibitory activity (41.7%) to that of ibuprofen (54.2%) and RP-2 showed better activity (81.7%) than diclofenac (76.4%). RP-1 and RP-2 showed excellent antiarthritic activity (37.5% and 28.2%, respectively) as compared to 15.6% and 11.3% activity of ibuprofen and diclofenac, respectively. Both RP-1 and RP-2 showed negligible ulcerogenic tendency.

From the earlier discussion it is quite natural to presume that the most serious side effect of NSAIDs i.e. its ulcerogenicity is overcome by their conjugation with glucosamine. Moreover, ability of the synthesized compounds to produce significantly high antiarthritic activity as compared to parent drug clearly justifies the correctness of the hypothesis and rationale behind the use of glucosamine to produce true mutual prodrugs with NSAIDs.

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