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Synthesis and evaluation of carrier linked Prodrug of Ketoprofen with Glucosamine

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ABSTRACT

Nonsteroidal anti-inflammatory drugs are widely used to treat the effects of inflammation through inhibition of cyclooxygenase enzyme. The major limitation to long term therapeutic use of these drugs is their gastro toxicity. The Prodrug approach can be applied to solve this problem, which is an established tool to mask the side-effects of drugs. The present work was targeted at the designing of carrier linked prodrug of Ketoprofen with glucosamine by masking the free carboxyl group. The synthesized prodrug was characterized by analytical and spectral data. The synthesized prodrug was also evaluated for its anti-inflammatory activity and ulcerogenic potential. It exhibited increase in anti-inflammatory activity and significantly less ulcerogenic ability than parent drug.

Key words: Prodrug, NSAID, Ketoprofen, glucosamine, ulcerogenicity, anti-inflammatory activity.

INTRODUCTION

Prodrug design is opening new doors in the challenging field of drug discovery, revolutioning the art of Drug development. Today drug candidates are often discontinued due to issues of poor pharmacokinetic properties or high toxicities. Prodrugs have the potential to overcome these challenges. This approach has the ability to keep promising new drug candidates alive through development and improving the safety and efficacy of existing drug products[1-3].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most useful clinical therapies for the treatment of pain, inflammation and fever. NSAIDs work by blocking the production of cyclooxygenase, which is a precursor to prostaglandins and in turn responsible for pain and swelling. Inhibition of this enzyme in micro molar concentrations of NSAIDs prevents the formation of thromboxane, prostaglandins and prostacyclin. The main side effects associated with COX-II inhibitors are due to carboxylic acid functional group. Gastrointestinal lesions produced by NSAIDs are generally attributed to either direct or indirect mechanisms. The direct contact effects result usually from local irritation produced by free acidic group of NSAIDs and local inhibition of prostaglandin synthesis in GIT. Indirect mechanism is due to generalized systemic action occurring after absorption[4-6].

This problem has been solved by derivatization of carboxylic function of NSAIDs into ester and amide mutual prodrugs using various carriers. Clinically, the majority of prodrugs are used with the aim of enhancing drug permeation by increasing lipophilicity and more recently by improving water solubility.

The general objective of the present study was to design, synthesize and evaluate novel prodrug of Ketoprofen with Glucosamine. The glucosamine is considered as promoiety because it is nontoxic, normal constituent of body, potentiates the anti-inflammatory activity and also gives additional anti-arthritic activity to parent drug.

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Ketoprofen is a member of group of compounds known as profens and is used for its antipyretic, analgesic, and anti-inflammatory properties by inhibiting cyclooxygenase-1 and -2 (COX-1 and COX-2) enzymes reversibly, which decreases production of pro-inflammatory prostaglandin precursors. Side effects include gastrointestinal ulcers, drop in red blood cell count (a result of GI bleeding), and rarely kidney damage, protein loss, and bleeding disorders. It should therefore be used with caution with liver or kidney disease, or gastrointestinal problems[7-8].

Glucosamine, an amino sugar is synthesized by the human body and is a major component of bones, cartilage, tendons and connecting tissues. Glucosamine is used extensively to treat many types of arthritis especially osteoarthritis. Glucosamine is needed to make glycosaminoglycans (GAGs), which are proteins that make up the "ground substance" of cartilage. Together, collagen and glycosaminoglycans maintain and rebuild cartilage. Glucosamine from either glucosamine hydrochloride or glucosamine sulfate has been shown to regenerate cartilage. Here glucosamine HCL is used as a source of glucosamine because it releases 85% of free glucosamine[9,10].

MATERIALS:

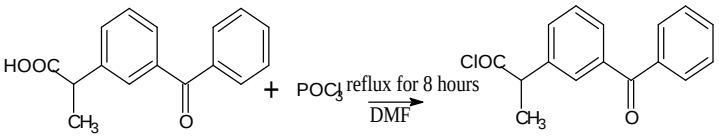
Ketoprofen was procured from Cipla Ltd. as gift sample and Glucosamine HCL was purchased from S. D. Fine chem. Ltd. All other chemicals and reagents were of analytical grade and used as received. Double distilled deionized water was used in the preparation of buffer solutions and mobile phases. The infrared spectra for the synthesized compounds were recorded using JASCO-FTIR 8400 spectrophotometer using potassium bromide pellet technique. ^{1}H NMR spectra were recorded on an EL Varian 300 MHz instrument. Chemical shifts are reported in parts per million (d) relative to tetramethylsilane (1%) as the internal standard. Agilent 6500 Q-TOF ESI-MS spectrometer was used for determination of molecular ion peak (CDRI Lucknow, India).

UV/Visible spectra were taken on JASCO V-550 UV/Visible spectrophotometer. TLC was performed on glass plate coated with silica gel 60 F254, 0.2 mm thickness. The partition coefficient of prodrug was determined by HPLC method using HPLC model JascoCo-2065 Plus with Jasco PU-2080 Plus intelligent HPLC pump, Jasco UV-2075 Plus intelligent UV/VIS detector The HPLC software used was Jasco-Borwin Chromatograph (1.5 Version). The HPLC column used was RP C-18 (Thermo Hypersil, 250 x 4.6 mm, 5 µm). Adult albino rats (180-210 g) of either sex were used in the anti-inflammatory and ulcerogenic study. Animals were housed in-group of

6 per cage under standard laboratory conditions, temperature and humidity. All experimental procedures were carried out under standard guidelines prescribed by Committee for the purpose of Control and Supervision of experiments on Animal and were approved by Institutional Animal Ethics Committee.

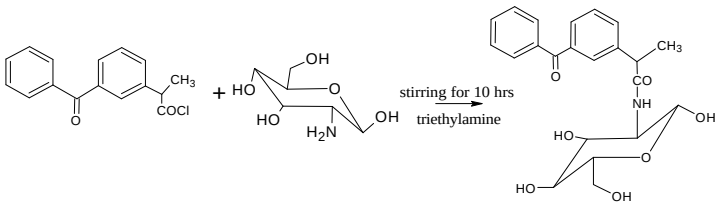
SYNTHESIS[11-13]:

Step1: Synthesis of acid chloride of Ketoprofen



Ketoprofen (0.01 mol; 2.54 g) was dissolved in 125 ml benzene. Freshly distilled Thionyl chloride (0.01 mol; 2 ml) was added to the above mixture with a drop of DMF as catalyst and was refluxed for 8 hours at 78°C. Benzene was distilled of at atmospheric pressure leaving behind crude viscous acid chloride. The viscous liquid was immediately poured on petridish and was vacuum dried to give yellowish white acid chloride. The compound was recrystallized using ethanol to get pure acid chloride derivative.

STEP 2: Prodrug synthesis by coupling of step 1 with glucosamine



Glucosamine HCL (0.01 mol; 2.17g) was suspended in 50 ml methanol. Triethylamine (0.01 mol; 1.01ml) was then added drop wise to the above mixture to liberate free D-glucosamine. The mixture was stirred for 2 hours at room temperature. The acid chloride (2.72g) was added drop wise at 10-15°C into the mixture of D-glucosamine over 1 hour. The reaction mixture was stirred for 10 hours at 10-15°C and then it was filtered. The crude product was recrystallized with methanol and dried under vacuum.

ANALYSIS: The physicochemical properties of a drug play a major role in the design, development of formulations and bioavailability. To ensure the success of a drug's development, it is essential that a drug candidate has good bioavailability and a desirable half life. Therefore, an accurate estimate of the pharmacokinetic data and a good understanding of the factors that affect the pharmacokinetics will guide drug design. Therefore in addition to characterization of proposed structure, the physicochemical parameters like solubility and partition coefficient, have to study[14-16].

Solubility studies:

Aqueous Solubility:
The aqueous solubility of synthesized prodrug was determined by stirring 200 mg accurately weighed compounds in water (10 ml) with a magnetic stirrer for 4 hrs. in a sealed flask. The solvent was filtered through 45 µm Whatman filter and quantitatively diluted with mobile phase and analysed by HPLC. The solubility was calculated in µg/ml.

Solubility in methanol, ethanol and chloroform
The solubility of the synthesized prodrug was determined by stirring 500 mg accurately weighed synthesized compounds in methanol, ethanol and chloroform, respectively (5 ml) with a magnetic stirrer for 4 hrs. in a sealed flask. The solvent was filtered through 45 µm Whatman filter and quantitatively diluted with mobile phase and analysed by HPLC.The solubility was calculated in µg/ml.

Determination of apparent partition coefficients[17,18]:
The partition coefficient is a measure of how well a substance partitions between aqueous and organic phase. It is useful parameter to predict the distribution of a drug compound in a biological system. The log P value for a compound is the logarithm (base 10) of the partition coefficient (P). The log P value determination is a useful parameter in Drug Discovery and Development and is used to predict transport properties across cell membranes, establish quantitative structure-activity relationships (QSARs), and as an indicator of protein binding characteristics.

Apparent partition coefficients (P) prodrug were determined in n-octanol/ Phosphate buffer pH 7.4 buffer at 25 ± 0.2 °C. Mutually pre-saturated phases were used. The traditional shake flask method was used, and concentrations were determined by HPLC to afford rapid evaluation and better reliability. The synthesized prodrug was dissolved in octanol (5 ml) in screw capped test tube. After addition of buffer (10 ml), the two phases were mixed on a suitable mechanical shaker such as rotary shaker for 8 h. The tubes were centrifuged at 3000 rpm for 30 min. The octanol layer was removed, diluted; 20 µl of the resulting solution was injected into the HPLC column and peak area was measured (AUCoctanol). The buffer solution was also removed, diluted, 20 µl of this solution was injected and corresponding peak area was obtained (AUCbuffer). The partition coefficient (P) was determined from the following expression.

$$P = \frac{AUCoctanol}{AUCbuffer} \times \text{dilution factor}$$

The Log P value was also determined by using following expression.

Log P = Log 10 (P)

PHARMACOLOGICAL EVALUATION

ANTI-INFLAMMATORY ACTIVITY[19.20]: The anti-inflammatory activity was evaluated using carrageenan-induced paw edema on rat method. To evaluate the anti-inflammatory activity of the conjugates 3 groups (n = 6) of Wistar rats (150–200 g) were examined. A first group of rats was used as a control without using the drug, group II received Ketoprofen 25 mg/kg group III received prodrug, where the dose was molecularly equivalent to the Ketoprofen. The synthesized compound (25 mg / kg) was suspended in 0.5% solution of carboxy methyl cellulose (CMC) in distilled water. For control 0.5% solution of CMC in distilled water was given orally. Ketoprofen 25 mg/kg was used as reference drug. Thirty minutes after the drug administered, 0.1 ml of 1% w/v carrageenan solution was injected in the plantar region of the left hind paw of the animals. The inflammation was determined using a plethysmograph at specified time interval after injecting the phlogistic agents and compared with that of the control. The data was analyzed using student's "*t*" test and the level of significance was defined at $p<0.05$. Data are expressed as mean ±SEM.

ULCEROGENIC STUDY[21,22]:
The Albino rats of either sex (100-150g) were fasted for 12 hours. Ulcerogenic activity was evaluated after oral administration of the test compounds. Control group received only 0.5% (*m*/*V*) carboxymethyl cellulose solution. After the drug treatment, the rats were fed normal diet for 4 h and were then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. Mucosal damage was assessed for each stomach according to the following scoring system: 0.0 -Normal color stomach, 0.5 -Red coloration, 1.0 -Spot ulcers, 1.5 -Hemorrhagic streaks,2.0 -Ulcers > 3mm but < 5mm, 3.0 -Ulcers > 5mm

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.
The UI was calculated as,
UI= [1× (number of lesions of grade 1) +2× (number of lesions of grade 2) + 3× (number of lesions of grade 3)]/10

For assessing the incidence of ulceration, the rats showing ulcers greater than 0.5mm in gastric mucosa were considered to have a positive ulcerogenic response.

RESULT:

Carrier linked prodrug of Ketoprofen was synthesized successfully by two methods. The sharp melting points indicate that the synthesized prodrug is crystalline compounds. The structure of synthesized prodrug by two methods was confirmed by IR and NMR spectroscopy. The physicochemical properties of prodrug and spectral data are summarized in Table 1 and 2 respectively. The method II showed better % yield and stability of prodrug at room temperature.

Table 1: Physicochemical properties of prodrug:

Molecular Formula	$C_{22}H_{25}N0_7$
Molecular Weight	415.44
% Yield	84%
Color	Yellowish solid
Odor	Odourless
Melting Point	116-118°C
R_f value	0.62{(ethyl acetate: n Hexane: Chloroform)6: 2.5:1.5}

Table 2. Spectral characterization of synthesized prodrug

UV (λmax in nm)	259 nm
IR SPECTRAL DATA (Wave number (cm-1))	3419.17(0-H stretching), 3326.61(N-H stretching), 3036.37(Aromatic C-H stretching), 2851.24, 2655.5 (AliphaticC-H stretching),1626.62(C=O stretching deformation (sec. amide)), 1436.71(Asymmetric C-H bending), (amide)), 1536.02(N-H1186.01(O-H deformation), 1270 (In plane C-H bending (aromatic)), 892,842 (Out of plane C-H bending)
1H NMR SPECTRAL DATA [δ (ppm)] in $CDCl_3$	1.368 (m){3 H (O-H)},1.944 (d){1 H CH_2},1.170 (m){1H C-H}3.480 (s){tetrahydropyran},7.26-7.758(m){4H Ar-H}, 1.170 (d){CH_3},7.791{1 H amide}, 9.023(s){1 H N-H}
Mass Spectra	415

UV Spectrum of KTP Prodrug exhibited λmax at 259 nm which was similar to respective parent drug, Ketoprofen (254 nm). The UV spectrum confirmed that the prodrug was derivative of parent compounds. The solubility of prodrug was determined in water, methanol, ethanol and chloroform.The parent drug Ketoprofen is practically insoluble in water but synthesized prodrug showed better water solubility than the parent compound Ketoprofen.

Apparent partition coefficient study in octanol /50 mM Phosphate buffer (pH 7.4) revealed increase in lipophilicity (log P) value as compared to parent drug (Refer Table. 3). This indicate that all prodrug was more lipophilic compared with parent drug. Thus, one can expect better absorption of this prodrug *in vivo*.

Table 3: Solubility data of compounds in various solvents

Solvent	Solubility(ug/ml)
Water	36.6
Methanol	269.7
Ethanol	178.4
Chloroform	134.9

Table 4. Physicochemical Properties of Prodrug

Partition Coefficient	4.3767
Log P	0.6411

Table 5. Anti-inflammatory activity of prodrug

Groups	Treatment	Paw Volume (ml) (Mean± SEM) 0 MIN	1 HR	2 HR	3 HR	4 HR
1	Control (0.5% CMC)	0.83 ± 0.009	1.75 ± 0.007	1.83 ±0.01	1.95 ± 0.007	2.205 ±0.007
2	Ketoprofen (25mg/kg)	1.115 ± 0.009	0.95 ± 0.008	0.75 ± 0.010	1.15 ± 0.03	1.30 ± 0.007
3	KTP Prodrug (25mg/kg)	1.123 ± 0.008	1.14 ± 0.009	0.68 ±0.011	0.95 ± 0.01	1.22 ± 0.008

*** p < 0.05 considered as significant when compared with the control.(One-way ANOVA followed by Dunnet's Test). All the compounds show significant p value.*

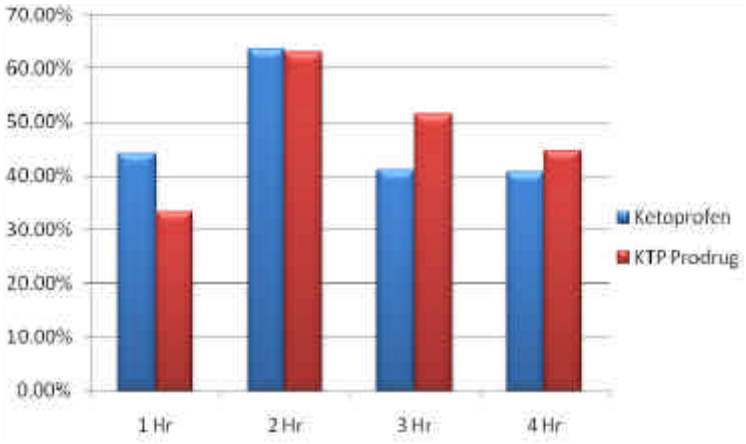


Figure 1: anti-inflammatory activity of prodrug

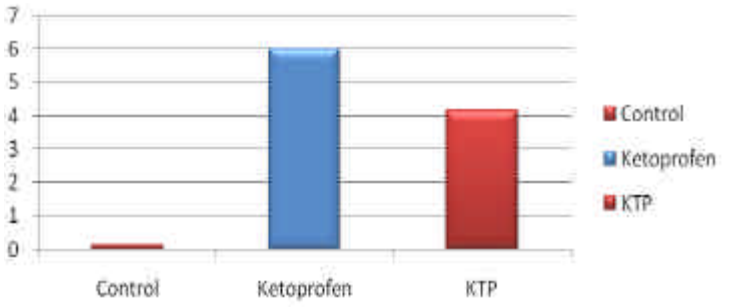


Figure 2: Ulcer Index of Prodrug

Prodrug was also evaluated for anti-inflammatory and ulcerogenicity *in vivo*. The prodrug showed comparable activity as that of parent drug. It exhibited maximum anti-inflammatory activity at 2 h and percentage inhibition (58.92%) was comparable with that of parent drug (58.38%).

The prodrug was also tested for its ulcerogenicity potential. In comparison to Ketoporfen, the prodrug was found to be considerably less ulcerogenic indicating that GI toxicity due to direct contact of the carboxylic group has been reduced.

DISCUSSION:

The synthesis of amide prodrug of Ketoprofen with glucosamine was achieved in good yield by two simple techniques. It was chemically stable to be presented in proper dosage form. The sharp melting point and unique spot on TLC indicated that prodrug was obtained in pure form. The interpretation of IR and 1 H NMR spectra confirmed the structure of prodrug. The UV spectrum of the prodrug showed the λmax nearly similar to that of Ketoprofen(λmax 254nm).The Log P value showed more lipophilicity than parent drug and indicated that prodrug can be orally administered. The prodrugs showed initially less % inhibition. After two hours, prodrug also showed comparable % inhibition at 2 hr but after that it showed maximum % inhibition than parent compound.

Although the intestinal mucosa was also examined, no detectable ulcers were noted. However, the complete elimination of ulcer formation cannot be avoided.

On the basis of the results, it was concluded that prodrug approach can be successfully applied in attaining the goal of minimized gastrointestinal toxicity without loss of desired anti-inflammatory activities of the drug.

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