

A Prodrug Design: Synthesis and Biological Screening of Cox II Inhibitor.

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of chronic inflammatory diseases, such as arthritis. NSAIDs are the most popularly used OTC drugs worldwide. Despite the intensive research that has been aimed at the development of NSAIDs, their clinical usefulness is still restricted by their GI side effects like gastric irritation, ulceration, bleeding, and perforation and in some cases may develop into life threatening conditions. The side effects produced by NSAIDs are generally attributed due to presence of free carboxylic acid functionality. 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 Acetyl Salicylic acid with Glucosamine. Acetyl Salicylic acid produces its anti-inflammatory effects via suppressing the activity of cyclooxygenase (COX), an enzyme which is responsible for the production of pro-inflammatory mediators such as the prostaglandins. The synthesized prodrug was characterized by analytical and spectral data. The synthesized prodrug was also evaluated for its anti-inflammatory activity and ulcerogenicity. It exhibited increase in anti-inflammatory activity and significantly less ulcerogenic ability than parent drug.

Key Words

NSAIDs, Prodrug, Parent drug, carrier, aspirin, glucosamine.

Introduction

The drug development process start from the target identification to the final product is a time and money consuming process. The total research and development (R&D) costs reported to incurred up to 1.7 billion dollars on an average of 10 years. Almost all drugs possess some undesirable physicochemical and biological properties. Prodrug approach has the ability to keep

promising new drug candidates alive through development process and improving the safety and efficacy of existing drug products. NSAIDs are the most popularly used OTC drugs worldwide. A drug molecule with optimal structural configuration and physicochemical properties for eliciting the desired therapeutic response may not necessarily possess the best molecular framework and properties for its delivery at the target site. Usually a small fraction of

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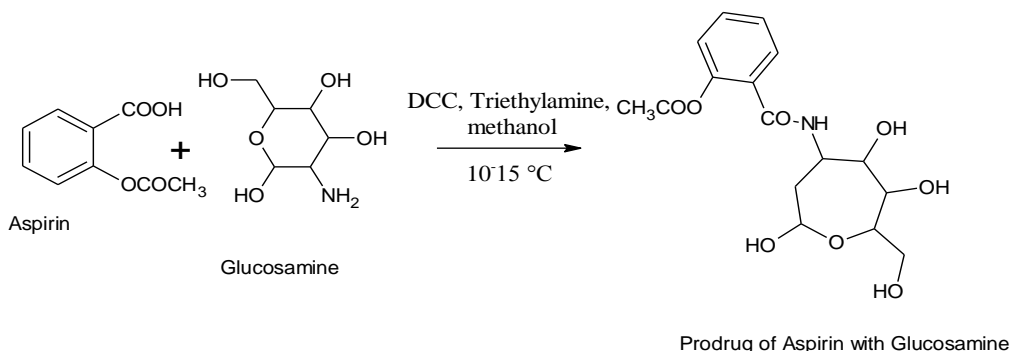
administered drug reaches the target area and the remaining fraction also interacts with non-targeted sites, resulting in an inefficient delivery and undesirable side effects. A therapeutically significant drug may have limited utilization in clinical practice because of various shortcomings sometimes, an adequate pharmaceutical formulation can overcome these drawbacks, but often the galenic formulation is in operant and a chemical modification of active molecule is necessary to correct its pharmacokinetic insufficiencies. This chemical formulation process, whose objective is to convert an interesting active molecule into a clinically acceptable drug, often involves the so-called 'prodrug design.' The prodrugs classified into two broad categories: the carrier-linked prodrugs and bioprecursors.

Initially, the term prodrug was introduced by Albert to describe any compound that undergoes biotransformation prior to exhibiting its pharmacological effect Harper referred to this process as drug latention, that is, chemical modification of a biologically active compound to form a new compound that, upon *in vivo* enzymatic attack, will liberate the parent compound.

Experimental Methods

Material used

Aspirin Was Obtained As A Gift Sample From Overseas Pharma (P) Ltd, Bangalore, Glucosamine was obtained as gift sample from Cipla India. The chemicals used in the present work were AR grade and LR grade, purchased from Ranbaxy, Merck and SD fine chemicals.



Synthetic Reaction

General Procedure

A solution of dicyclohexyl carbodiimide (2.1mmol 0.443gm) in methanol was added drop wise to cold solution of aspirin dissolved in methanol (2mmol 0.360gm). The mixture was kept cold for 30 min. 1-2

drops of anhydrous DMF (dimethylformamide) was added to it. Meanwhile a solution of glucosamine was suspended in methanol for half an hour to release free glucosamine. Then solution was cold to 10- 15⁰c (2mmol 0.346) after that solution was

added drop wise to above solution. Then mixture was kept stirred at Room Temperature overnight.

Biological screening for anti-inflammatory activity

The anti inflammatory activity was evaluated by using carrageenan induced paw edema on rat.

Materials

Animal

Experiment was carried out on wistar rats of albino strains. They were obtained from drug testing laboratory, Bangalore. The animals were housed in polypropylene cages. Paddy husk provided as bedding material, which was changed every day. The place where maintained clean .The rats were kept 3 to 5 in cage. They were fed with standard pellet diet and water ad libitum. They were kept in well aerated room and 12 hr light and dark cycle was maintained. The room temperature was maintained at 22+/-20⁰c.

Species	Ratuess norvegicuss
Strain	Wistar
Body weight	150-200 g
Number /group(6)	6

Drug treatment

The drug was prepared as suspension using 0.5% w/v cmc. Drug and vehicle was administered orally one hour before the induction of inflammation.

Apparatus

Plethysmographs consist of glass tube (11 cm long, 2cm outer diameter, 1.6cm inner diameter.)With a side arm (10cm long, 0.6cm outer

diameter,0.4cm inner diameter.) Mercury is filled with 8.5 cm. The side arm was used to the measuring the changes in the level of mercury with the help of travelling microscope .When the rat paw were dipped into the tube.

Calibration of Plethysmograph

Plethysmograph was initially filled to a known weight of mercury and zero reading was taken by using travelling microscope (tm). Mercury was added upto 2ml in 0.1 ml portion and changes in the height of mercury in the side arm was measured each time with travelling microscope. Volume of mercury added was plotted against increases in the height. Slope was calculated using linear curve obtained.

Grouping

Sr. No.	Treatment	Dose
1	0.5% cmc (p.o.) +1% carrageenan (i.p)	0.1ml
2	Aspirin (p.o.)+1% carrageenan (i.p)	(25 mg/kg) 0.1%
3	ASP Prodrug	(25 mg/kg) 0.1%

Procedure

Rats were given the drug suspension orally and after one hour; the rats were challenged by a subcutaneous injection of 0.1ml of 1% v/v carrageenan solution into the planter region of left hind paw. The paw was marked with ink at level of lateral malleolous and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediate after injection and every hour for four hours. Changes mercury level was measured by travelling

microscope as change in height (in cm). This value obtained was converted into changes in volume (in ml) by interpolating them on plethysmograph calibration curve.

Result and Discussion

In the present study carrier linked prodrugs of Acetyl salicylic acid was synthesized by linking with Glucosamine as per the procedures described in schemes. Thin layer chromatography was performed on silica gel G glass plates using suitable solvents systems to ascertain the reaction completion. The percentage yield, melting point and analytical data of the synthesized compounds were calculated for IR and NMR spectra confirmed the structure of the compounds in Fig.1 and Fig 2. The parent drugs (Acetyl salicylic acid) as well as the synthesized prodrugs were evaluated for anti-inflammatory and ulcerogenic activity in Fig.3 and Fig 4. The statistical significance was tested by one way ANOVA followed by Dunnett's test. The data showed significantly higher anti-inflammatory activity as well as reduction of the ulcer index of the prodrugs when compared to the parent drug. On the basis of the above observations, it is concluded that these prodrugs can be successfully applied to attain the goal of minimized gastro-intestinal toxicity without loss of the desired anti-inflammatory and analgesic activity of the drug.

Physical Characterization

Synthesized compounds were scaled for yield and purified by recrystallisation with suitable solvent system. The purified compounds were

assigned for physical constant determination and further subjected for spectral analysis like Thin layer chromatography, Infrared spectroscopy, Nuclear magnetic resonance and melting point (Table 1).

Conclusion

Instead of synthesized new compounds which is time and money consuming. Prodrug design is now emerging as effective tool for drug design. In this research, amide prodrug of aspirin with glucosamine was successfully synthesized and characterized. Prodrug showed more anti-inflammatory activity and low ulcerogenic activity as compared to aspirin.

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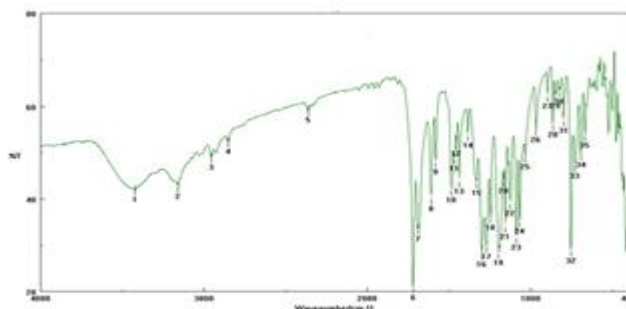


Fig 1: IR Spectroscopy.

