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# Dexibuprofen-Dextran Macromolecular Prodrugs: Synthesis, Characterization and Pharmacological Evaluation

**Arun Rasheed*, K. Aishwarya, B. Niyaz Basha, B. Sravya Reddy and A. Swetha**

*Department of Pharmaceutical Chemistry, Sree Vidyanikethan College of Pharmacy, Sree Sainath Nagar, Tirupati, Andhra Pradesh, India*

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## Abstract

Dexibuprofen (DI) is a non-narcotic analgesic and anti-inflammatory drug. A biodegradable polymer dextran has been utilized as a carrier for synthesis of dexibuprofen-dextran prodrugs to improve aqueous solubility, to increase therapeutic efficiency and to reduce its gastrointestinal side effects. The synthesis involves the condensation of acyl imidazole derivatives of dexibuprofen with dextran of different molecular weights (10000 and 20000) to obtain dexibrofen-dextran prodrugs DD10 and DD20 respectively. The structure of synthesized prodrugs was confirmed by IR and NMR spectroscopy. The molecular weight was determined by Mark-Howink Sakurada viscosity equation and the degree of substitution was obtained as 15.6 % and 16.5 % for the prodrugs. A hydrolysis study was performed in buffer solutions at different pH and in simulated colonic fluid (SCF). The hydrolysis followed first order kinetics. Much faster hydrolysis was observed at pH 9.0 than in pH 7.4 buffer solution and pH 7.4 SCF. The analgesic activity of the prodrugs was not much better compared to the parent drug. The anti-inflammatory activity was found as 60.8 % and 65.74 % inhibition when compared to the inhibition of 52.69 % of parent drug. The prodrugs showed remarkable reduction in ulcerogenicity as compared to parent dexibuprofen. The results thus proved that dextran can be employed as a promoiety for the drug delivery of dexibuprofen and showed comparable anti-inflammatory and ulcerogenic effect than the parent drug.

**Key words:** Dexibuprofen, macromolecular prodrug, anti-inflammatory, ulcer index

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## Introduction

The design and development of functional polymers as key agents in drug delivery have attracted significant attention in recent years, where the polymers have been utilized as carriers for

delivery of active pharmaceutical agents [1-4]. When taken orally, high-molecular-weight characteristics of polymers render them to be non-absorbed through the gastrointestinal (GI) tract. There have been significant developments in the area of polymeric delivery systems and polymeric prodrugs, where functional polymers have been utilized to prepare prodrugs of small molecule to improve therapeutic indices of original drugs [5-6]. The prodrugs with the polymer can temporarily mask acidic function of the non steroidal anti-inflammatory drugs like DI and decrease its GI toxicity due to the direct contact effect. The literature reveals that in most of macromolecular or polymeric prodrug approaches, the drug is either linked by physical entrapment or chemical linkage to polymeric carriers. Dextrans can be used as promoiety due to their excellent physico-chemical properties and physiological acceptance [7-10]. Dexibuprofen has a tendency of GIT disturbance, peptic ulceration and GIT bleeding properties. The concept of polymeric prodrug has been adopted for preparation of dextran prodrugs of dexibuprofen in order to improve its physico-chemical colon site specificity and reduced GIT side effects [11].

## Results and Discussion

The structure of synthesized prodrugs was confirmed by analytical and spectral data. The NMR spectra of DI prodrugs showed characteristic shifting of glucocidic ring anomeric proton signals from δ 4.91 (H-1) to δ 5.17 (H-1), H-2 proton from δ 3.42 (H-2) to δ 3.94 (H-2) which indicates the formation of an ester linkage at position C2. The disappearance of NMR signals in the range of δ 10.58 to δ 11.20 ppm for carboxylic group in the DI dextran prodrugs suggests that the free carboxylic group of drug was conjugated with hydroxyl group of dextran macromolecule and ester bond was formed. The signals of the aromatic ring of DI were found as δ 7.25 and are in agreement with the anticipated structure. The IR spectra of the DI prodrugs showed characteristic stretching at 1730 $cm^{-1}$ and confirm the formation of ester linkage. A strong O-H stretching vibration of polymeric association at 3430 $cm^{-1}$ and weak C-H stretching of allkane at 3070 $cm^{-1}$ were also found. It also showed the characteristic absorption stretching at 1570 $cm^{-1}$ for aromatic ring. The synthesized prodrugs were found to be sparingly soluble in methanol, acid and alkali. An absorption maximum in borate buffer (pH 9) was observed at 210 nm which was same as that of DI. The degree of substitution was determined by UV spectrophotometry and was found as 15.6 % for DD10 and 16.5 % for DD20.

The DI prodrug did not show any hydrolysis in acidic medium (pH 1.2) for 4 h. The hydrolysis of DI prodrugs at pH 7.4 demonstrated a slow rate of hydrolysis and relatively much faster hydrolysis was observed at pH 9 as well as SCF and also follows first order kinetics. The half lives were found to be 3.89 h and 3.25 h for DD10 and DD20 respectively.

The synthesized prodrugs were subjected for pharmacological evaluation such as anti-inflammatory, analgesic and ulcerogenic activities. The DI prodrugs showed improved anti-inflammatory activity as compared to the parent drug while the prodrugs were not showing much improved analgesic activity than the parent drug. A remarkable reduction in the ulcer index was observed for the prodrugs. The parent drug DI has a high ulcer index of 16.66 where as the DD10 and DD20 have a reduced ulcer index of 9.66 and 6.66 respectively.

A normal histological finding was observed for the samples of the control group rats. Small hemorrhagic areas and patches of inflammatory cell infiltrations were present in the lumen of the

glands and lamina propria when treated with parent drug, but normal histological findings were displayed for both DD10 and DD20 group. This reveals that the prodrugs are not producing any ulceration in the gastric region.

## Materials and Methods

The dexibuprofen was obtained as gift sample from Alkem Laboratories, India. Dextran (molecular weight - 10000 and 20000) and N,N–carbonyldiimidazole (CDI) were purchased from Sigma-Aldrich Chemicals Ltd, USA. Silica gel GF 254 for TLC was obtained from Sisco Laboratories, India. All other solvents and chemicals were of reagent grade and obtained from Qualinges fine chemicals, India. The melting points were recorded using melting point determination apparatus by Sigma Instrument, India and are uncorrected. The IR spectra were recorded on Shimadzu 8300 FT-IR spectrophotometer (Japan) using KBr pellets in the range 4000 to 400 $cm^{-1}$. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded in DMSO on NMR spectrophotometer (Bruker DRX 300, USA). Chemical shifts are expressed as $\delta$ (ppm) values. The degree of substitution and hydrolysis studies were determined by Elico UV Spectrophotometer (India). The elemental analysis was performed using Carlo-Erba Model 1108 Analyzer (Italy) and found values are almost near to that of the theoretical values.

***Synthesis of Dextran Prodrugs***

Dextran prodrugs of dexibuprofen were prepared by first activating the carboxylic group using CDI to obtain dexibuprofen acylimidazole (DDI), which were then condensed with dextran of different molecular weight (10000 and 20000) *in situ* to get DD10 and DD20 respectively (Scheme 1) [12]. The progress of the reaction was monitored by thin layer chromatography, which was performed on silica gel GF 254 as stationary phase and n-hexane: water: ethyl acetate: glacial acetic acid (30:10:10:2.5) as mobile phase. N,N–carbonyldiimidazole is moisture-sensitive and, therefore, dry solvents were used throughout and anhydrous conditions were maintained during the experiment. The physicochemical properties of prodrugs are given in Table 1. The IR and NMR spectral data of DD prodrugs are IR (KBr, max $cm^{-1}$): 1730 (C=O str.), 3070 (C-H str.), 736 (C-H aromatic bending), 3430 (-OH str.of polymeric -OH dextran), 1568 (str. of aromatic ring). $^{1}H$ NMR (DMSO d6, ppm): 7.27- 7.52 (m, 8H, aromatic ring), 3.89 (q, 2H, -CH2), 1.46 (t, 3H, -CH3), 5.30-3.63 (m, anomeric protons of glucosidic ring), 2.0- 2.49 (-OH of dextran monomer).

***Degree of substitution***

The degree of substitution of dexibuprofen was determined [13] by dissolving 20 mg of the dextran prodrug in 20 ml solution of phosphate buffer (pH 9.0). The reaction mixture was maintained at 70 °C for 1h and left for 24 h for complete hydrolysis. It was then neutralized with 1N HCl. The amount of dexibuprofen released during hydrolysis was extracted with chloroform and determined by UV spectrophotometer at the absorption maxima of 210 nm.

**Scheme 1: Synthesis of Dexibuprofen-Dextran Prodrug**

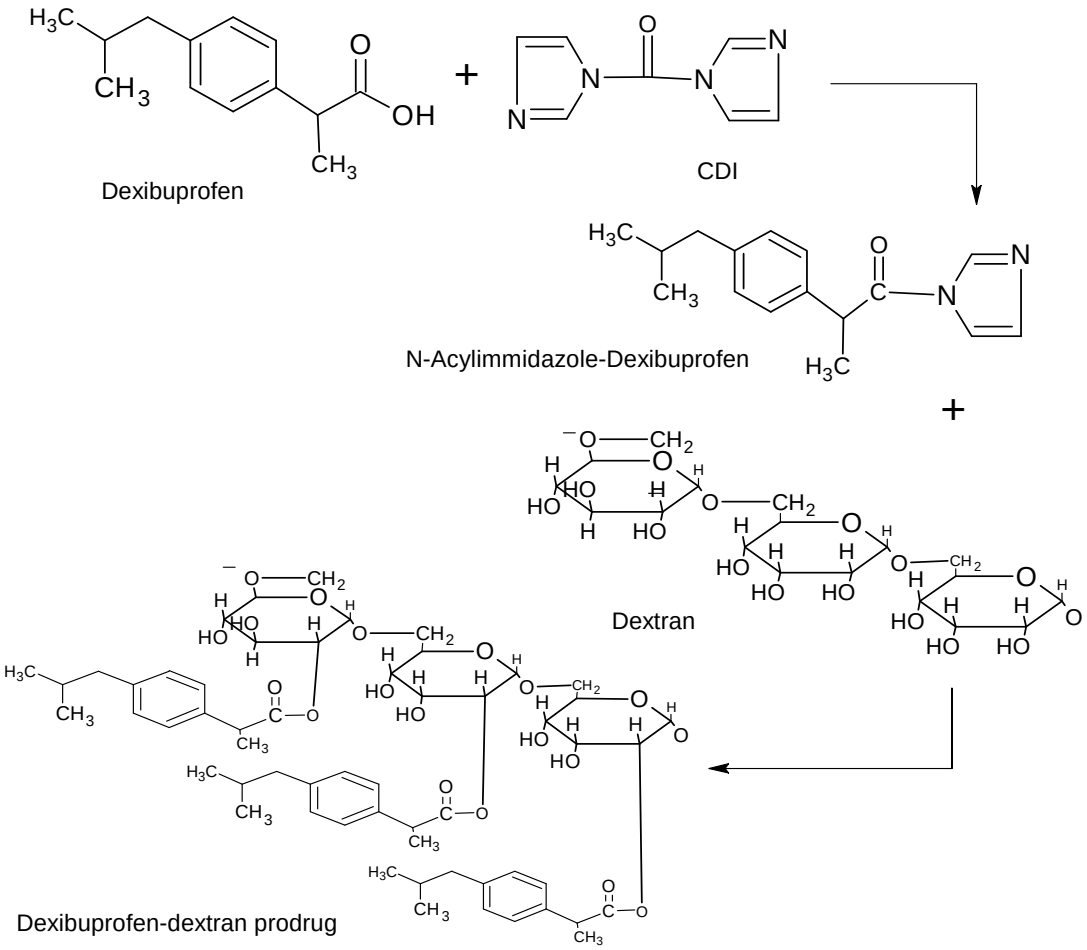


**Table (1) Physicochemical properties of prodrugs**

| Prodrug code | Colour | m.p. ($^{o}$C)* | Yield (%) | $R_f$ # value | Degree of substitution[a] | Intrinsic viscosity | Molecular Weight | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Calculated (%) | Found (%) |
| DD10 | Cream | 58-60 | 95.31 | 0.58 | 15.6 | 0.024 | 12250 | 16400 |
| DD20 | Cream | 60-62 | 96.15 | 0.56 | 16.5 | 0.028 | 22350 | 25000 |

* Uncorrected # n-hexane: water: ethyl acetate: glacial acetic acid = 30:10:10:2.5, a = amount of parent drug in mg per 100 mg of prodrug

***Molecular weight***

Intrinsic viscosities were estimated using Eq. 1. The average molecular weights were then calculated by Mark-Howink Sakurada equation (Eq 2).

$$[\eta] = [\eta \text{ rel-1}] / [c + 0.28\ c\ (\eta \text{ rel-1})] \quad [1]$$
$$\log [\eta] = \log K + a \log M \quad [2]$$

where [η] represents intrinsic viscosity, η rel is the relative viscosity at concentration c (%, w/v), M is the molecular weight and K and a are the constants.

***In vitro hydrolysis***

*In-vitro* hydrolysis of the dextran prodrugs was studied in different borate buffer solutions (pH 7.4 and pH 9.0) and SCF (pH 7.4). The rate of hydrolysis of the dextran prodrugs was computed as the percentage drug hydrolysed based on the cumulative amount of drug hydrolysed divided by the total amount of drug contained in the prodrug. The rate of hydrolysis and half-life of the prepared prodrug were calculated using

$$r = (2.303/t) \log (b/b-x)$$

where r represents hydrolysis constant, t is the time in h, b is the initial concentration of prodrug, x is the amount of prodrug hydrolyzed and (b-x) is the amount of prodrug remaining. The results are summarized in Fig. 1.

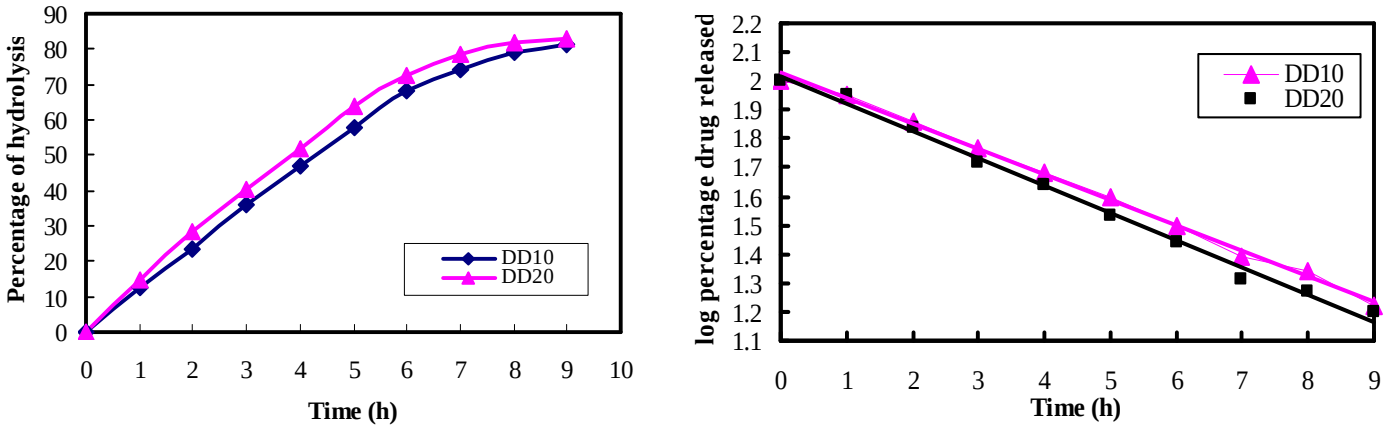


**Fig. 1: Comparative pattern of hydrolysis and first order kinetics plot of DD10 and DD20 in SCF (pH 7.4)**

***Pharmacological evaluations***

DI as well as the synthesized prodrugs were evaluated for analgesic, anti-inflammatory, ulcerogenic activity, histopathology and a comparative study was performed. Test compounds and standard drugs were administered in the form of a suspension (1 % carboxymethylcellulose as a vehicle) by oral route of administration for analgesic and anti-inflammatory studies, but for ulcerogenicity studies intraperitoneally as suspension in 2 % (m/v) acacia. Wistar albino rats of four groups, including a control and a standard group, each with six animals were selected. The selected animals were housed in acrylic cages at standard environmental conditions at 25 ± 2 °C, relative humidity of 45–55 %, in a well ventilated room maintained at 12: 12 h light: dark cycle, fed with standard rodent 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, Sree Vidyanikethan College of Pharmacy, Tirupati, India was obtained.

***Analgesic activity***

The analgesic activity of synthesized prodrugs was determined by thermal stimulus using tail flick method [14]. Analgesiometer was used for the determination of pain threshold of albino rats. The rat (100-200 g) was placed in a holder through which the tail of the rat was protruded out. The reaction time was recorded at 1, 2, 3 and 4 h after the treatment and cut-off time was 9 s. The normal reaction time, i.e. the time taken to flick the tail was noted. Animals showing delayed response were rejected. The prodrug was administered orally in 1% suspension of sodium CMC and compared with dexibuprofen as reference. The percent analgesic activity was calculated by the formula given as

$$\% \text{ Analgesic activity} = [(T_2-T_1)/ (Tc-T_1)] \times 100 \quad [3]$$

where $T_1$ - the reaction time (s) before administration of prodrug and $T_2$ -the reaction time (s) after administration of prodrug and Tc - cutoff time in sec. The analgesic activities of DD10 and DD20 are shown in Table (2).

**Table 2 Pharmacological activities of DD10 and DD20**

| **Prodrug** | **Anti-inflammatory activity** | | **Analgesic activity** | | **Mean Ulcer Index** |
|---|---|---|---|---|---|
| | **Paw volume (mL)[a]** | **Inhibition of oedema (%)** | **Basal reaction time (s)[a]** | **Pain reduction (%)** | |
| Control | 0.090 ± 0.05 | - | 3.22 ± 0.23 | - | - |
| DI | 0.233 ± 0.23 | 52.69 | 9.0 ± 0. 2 | 52 | 16.66 |
| DD10 | 0.145 ± 0.25 | 60.08 | 8.1 ± 0.23 | 45 | 9.66 |
| DD20 | 0.152 ± 0.26 | 65.75 | 8.3 ± 0.21 | 48 | 6.66 |

[a]Each value represents the mean ± SD (n =6). Significance levels $p < 0.05$ as compared with the respective control.

***Anti-inflammatory activity***

The anti-inflammatory activity was evaluated using carrageenan-induced oedema of rat paw [15, 16]. Albino rats (100-200g) were divided into four groups of six animals each. Group 1 served as control group, group II received dexibuprofen 2 mg/kg, group III and IV received prodrug DD10 and DD20 respectively, where the dose was molecularly equivalent to the free drug. The initial volume of right hind paw of albino rat was measured by plethysmometer without administration of drug. The drug was administered orally in 1% suspension of sodium CMC. After 30 min of drug administration of prodrug, carrageenen (0.1 ml, 1%) w/v solution in normal saline was injected into the planter surface of right hind paw of each animal as phlogistic agent. The volume of right hind paw of albino rats was measured after 1, 2 and 3 h. The mean difference in the volume of the right hind paw of rats was compared with control and standard. The percent inhibition of paw oedema was calculated as

Percent inhibition = (1-Vt/Vc) x100 [4]

where Vc – mean relative change in paw edema volume in control group and Vt - mean relative change in paw edema volume in test group. The % reduction in oedema at 3 h and percentage anti-inflammatory activity in comparison to standard drug DI are presented in Table (2).

***Ulcerogenic activity***

Gastrointestinal toxicity of the synthesized prodrugs was measured and compared with the parent drug by measuring ulcer index [17]. The prodrug was suspended in 10 ml of 2% w/v suspension of acacia. Measured volume of the suspension containing DI was administered orally to the test group daily for 5 days. The albino rats (100-200 g) were fasted after the administration of last dose, thereafter they were sacrificed by decapitation and the stomach was removed, opened and washed with distilled water. The lesions on the gastric mucosa were counted by visual examination using a binocular magnifier. Ulcers greater than 0.5 mm were recorded. The ulcer index (UI) was calculated by severity of gastric mucosal lesions which are graded as grade1 - less than1mm erosions, grade 2 - 1-2 mm erosions and grade 3 - more than 2 mm erosions. 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 [5]

***Histopathological studies***

The histopathological studies of stomach of rats [18] were carried out using haemotoxylin and eosin stain at Pathology Department, Sri Venkateswara Veterinary University, Tirupati, India. The stomach tissues were removed from the rats and fixed in 10% normal saline for at least 48 h.

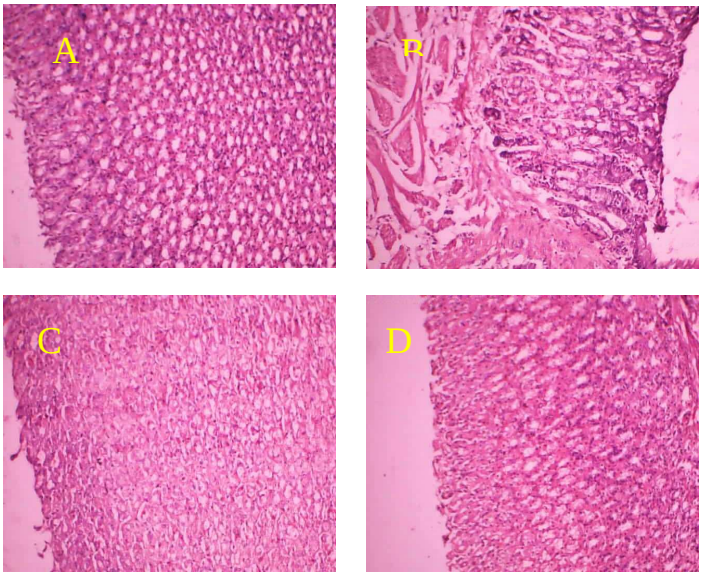


**Fig. 2: Histopathological studies of prodrugs**

A) Healthy control B) Ulcer control showing mucosal injury characterized by DI and massive mucosal infiltration of inflammatory cell C) Treated with DD10 D) Treated with DD20

These were then processed routinely and the tissues were embedded in paraffin wax. Histological sections were cut at 5-6 μm and stained with routine haematoxylin and eosin. These were then examined by a consultant histopathologist. The lesions observed were assessed for the following mucosal atrophy, the presence of inflammatory cells in the wall, oesinophils, lymphocytes and plasma cells. Photomicrographs of representative lesions at various magnifications were taken on Zeiss optical microscope (Germany), Stemi 2000-C, with a resolution of 10x45X, attached with trinocular camera and shown in Fig. 2.

## Conclusion

The DD10 and DD20 were successfully synthesized and the structures were confirmed by the spectral analysis. Both prodrugs showed excellent pharmacological response and encouraging hydrolysis rate in SIF + 80 % of human plasma. The less protein binding of the prodrugs increased its availability for hydrolysis in plasma and thus results in less dose requirement. Increased anti-inflammatory as well as reduction in ulcer index of the prodrugs were observed when compared to the parent drug. The histopathological findings reveled that there is limited ulcer formation in stomach by the prodrugs. In conclusion, the present investigations suggest that dextran can successfully be employed as promoiety for compound containing a carboxylic function. The study also proposes dextran as a polymeric carrier to achieve colon site specificity due to the presence of enzymes and alkaline pH in the colon, improved physiochemical properties and reduced gastrointestinal side effects.

***Statistical analysis***

Statistical analysis of the pharmacological activity of the synthesized prodrugs on animals was evaluated using a one-way analysis of variance (ANOVA). Student's t-test was applied for expressing the significance and the experimental data are expressed as mean ± SD (standard deviation).

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