Tropical Journal of Pharmaceutical Research, June 2009; 8 (3): 221-229

Available online at http://www.tjpr.org

Research Article

Flurbiprofen- and Suprofen-Dextran Conjugates: Synthesis, Characterization and Biological Evaluation

Sushant K Shrivastava[1*], DK Jain[2], Prabhat K Shrivastava[1], and Piyush Trivedi[3]

[1]Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi, U.P., [2]Department of Pharmacy, IPS Academy, Indore, M.P., [3]School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, M.P., India

Abstract

Purpose: *To synthesize and characterize the dextran conjugates of suprofen and flurbiprofen, and also evaluate their biological activities.*

Methods: *Suprofen and flurbiprofen were individually reacted with carbonyldiimidazole to form acylimidazole, which, in turn, was reacted with the dextran of varying molecular weight (40 000, 60 000, and 110 000) to form drug-dextran conjugates. The structures of the synthesized dextran conjugates were confirmed by IR and NMR spectroscopy. In vitro hydrolysis of the conjugates were studied in buffer solutions (pH 7.4 and 9.0) and 80% human plasma (pH 7.4). The analgesic and antipyretic activities, as well as the ulcerogenic index of the conjugates were also evaluated in albino rats.*

Results: *The mean degree of substitution of flurbiprofen and suprofen was between 8.0 to 9.5 % and 7.5 to 9.0 %, respectively. In vitro hydrolysis studies on the conjugates indicate faster hydrolysis at pH 9.0 than in pH 7.4 buffer solution and 80% human plasma (pH 7.4) with the process following First order kinetics. The analgesic activity of flurbiprofen-dextran conjugate (FD-110) suprofen-dextran conjugate (SD-110) was 64.23 and 41.50% which compare well with those of their parent drugs - flurbiprofen (72.60%) and suprofen (44.30%). Similar findings were made in respect of the antipyretic activity. Both flurbiprofen and suprofen showed deep ulceration, swelling and high intensity perforation in the gastric mucosa after seven days administration of flurbiprofen and suprofen with the ulcerogenic indices of 29.69 and 31.0 respectively, cpmpare with 5.88 and 6.06 for FD-110 and SD-110, respectively.*

Conclusion: *Dextran can be employed as a pro-moiety or carrier for the delivery of flurbiprofen and suprofen and showed comparable analgesic and antipyretic activities with the parent drugs but with lower ulcerogenic indices.*

Keywords: *Flurbiprofen, Suprofen, Dextran conjugates, In vitro hydrolysis, Analgesic-antipyretic activity*

Received: 9 Oct 2008 *Revised accepted: 15 Dec 2008*

**Corresponding author: Email: sushant_itbhu@rediffmail.com; Phone: +91-543-2307049, Fax+91-542-2368428*

INTRODUCTION

Not too long ago, polymeric prodrug conjugates were ushered into the era of polymeric drug delivery. The task of obtaining a versatile polymer as an ideal candidate in drug delivery can be intricate since it has to surmount several vigorous clinical barriers[1]. Dextran has excellent physicochemical properties and physiological acceptance such as the capacity to be stored in depots, unique pharmacokinetic profiles, potential body distribution and pharmacological efficacy[2,3]. The literature reveals that in most of the macromolecular or polymeric prodrug approaches, the drug is either linked by physical entrapment or by chemical linkage to polymeric carriers[4-10].

Nonsteroidal anti-inflammatory drugs are among the most frequently used groups of drugs to treat the disorders caused by inflammation[11-13]. The phenyl propionic acid derivatives such as flurbiprofen {2-(3-fluoro-4-phenyl-phenyl) propanoic acid} and suprofen {2-(4-(thiophene-2-carbonyl) phenyl) propanoic acid} possess analgesic and antipyretic actions in addition to anti-inflammatory action[14-18]. Unfortunately, however, they also possess certain undesirable gastro-intestinal (GI) side effects such as gastric ulceration and hemorrhage which are attributed in part to the presence of an acidic group, and this can be masked temporarily by conjugating with a biopolymer dextran. Previously, we synthesized and evaluated dextran conjugates of flurbiprofen and suprofen for their anti-inflammatory action[19,20]. In this study, the dextran conjugates of flurbiprofen (FD) and suprofen (SD) were prepared with the intention of achieving reductions of GI side-effects while retaining their analgesic and antipyretic activities.

MATERIALS AND METHODS

Materials and equipment

Flurbiprofen and suprofen powders were obtained as gifts from Cipla Ltd, Mumbai, India. Dextran (molecular weight - 40 000, 60 000 and 1 10 000) and N, N^1–carbonyldiimidazole (CDI) were purchased from Sigma-Aldrich Chemicals Ltd, USA. Silica gel G for TLC and solvents for HPLC analysis were purchased from Merck India. All other solvents and chemicals were of reagent grade and obtained from Qualinges fine chemicals, Mumbai.

Ultra-violet spectra of the synthesized conjugates were generated using Shimadzu 160-A, UV/Visible Spectrophotometer. I*n vitro* hydrolysis of SD and FD conjugates was performed using a water-HPLC system (Rexdale, Canada) consisting of a model 6000A pump, a 710 B WISP auto injector, and a 490 multiple-wave length UV detector operated at ambient temperature. Separation was carried out on octadecyl-bonded silica (5µm, ODS-3) stainless steel column (10 x4.6 mm, i.d.) along with a 5 cm guard column of the same material and particle size 10 µm. The mobile phase composition for separation was acetonitrile: water: phosphoric acid in the ratio 45:54:1 v/v and acetonitrile: 0.67 M KH_2PO_4: triethylamine in the ratio 35: 65: 0.02 v/v, respectively, for SD and FD conjugates. The flow rate was maintained at 1.0 ml/min.

The infrared spectra were recorded on Shimadzu 8300 FT-IR spectrophotometer using KBr pellets in the range 4000 to 400/cm. ^{1}H NMR spectra were obtained on a Bruker DRX -NMR spectrophotometer operating at a frequency of 300 MH_Z in DMSO-d_6.

Synthesis of dextran conjugates

Dextran conjugates of flubriprofen and suprofen were prepared by first activating the carboxylic group using CDI to obtain flubriprofen and suprofen acylimidazole (FAI and SAI) [21] which were then condensed with

dextran of different molecular weight (40 000, 60 000, and 110 000) *in situ* to get FD-40, FD-60, FD-110 and SD-40, SD-60, SD-110, respectively, as shown in Scheme 1. The progress of the reaction was monitored by thin layer chromatography, which was performed on silica gel (Merck No. 5554) as stationary phase and chloroform: methanol (7:3) as mobile phase. N, N – carbonyl diimidazole (CDI) is moisture-sensitive and, therefore, dry solvents were used throughout and anhydrous conditions were maintained during the experiment.

The IR and NMR spectral data of FD conjugates; IR (KBr, vmax cm^{-1}): 1728.7 (C=O str.), 2963 (aromatic str.), 736 (C-H aromatic bending), 3400-3278 (-OH str.of polymeric -OH dextran), 1568.(str. of biphenyl ring), 1010 (C-F str.) ^{1}H NMR (DMSO d_6, ppm): 7.27- 7.52 (m, 8H, aromatic ring), 3.89 (q, 2H, $-CH_2$), 1.46 (t, 3H, $-CH_3$), 5.30-3.63 (m, anomeric protons of glucosidic ring), 2.0-2.49 (-OH of dextran monomer). The IR and NMR spectral data of SD conjugates; IR (KBr, vmax cm^{-1}): 1723.6 (C=O str.), 2918 (aromatic str.), 742 (C-H aromatic bending), 3400-3243 (-OH str.of polymeric -OH dextran), 2970 (C-H str. of alkene), 1025 (thienyl str.), 1350 (C-S str.) ^{1}H NMR (DMSO d_6, ppm): 7.6- 8.0 (m, 4H, aromatic ring), 7.29-7.55 (m, 3H, thiophene ring), 3.94 (q, 2H, $-CH_2$), 1.23 (t, 3H, $-CH_3$), 5.32-3.44 (anomeric protons of glucosidic ring), 2.0-2.5 (-OH of dextran monomer)

Degree of substitution

The degree of substitution of flurbiprofen and suprofen was determined by dissolving 20 mg of the dextran conjugate in 20 ml solution of phosphate buffer (pH 9.0). The reaction mixture was maintained at 70°C for one hour and left for 24 h for complete hydrolysis. It was then neutralized with 1N HCl. The amount of flurbiprofen and suprofen drugs released due to hydrolysis was extracted with chloroform and determined by HPLC at the absorption maxima of 248.4 nm and 296 nm respectively[22,23]. *In vitro* hydrolysis of dextran conjugates was determined by HPLC using the mobile phase indicated earlier. The amount of hydrolyzed dextran conjugates was derived from the measurement of the peak area in relation to those of the standard drug response under same conditions.

Molecular weight determination

The molecular weight of the conjugates was determined by viscometery using Mark-Houwink Sakurada equation[24]., which correlates molecular weight and intrinsic viscosity.

$$[\eta] = k M^{\alpha} \quad \text{..... (1)}$$

where [η] is the intrinsic viscosity, M is the molecular weight, and k and α are constants having values 7.24×10^{-4} (dl/g) and 0.52 respectively. The values were determined by plotting η_{sp}/ C against C. Molecular weight was then calculated using the above equation.

***In vitro* hydrolysis**

In-vitro hydrolysis of the dextran conjugates was studied in different phosphate buffer solutions (pH 7.4 and 9.0 and 80% human plasma pH 7.4) and the rate of hydrolysis of the dextran conjugates was computed as the percent drug hydrolysed based on the cumulative amount of drug hydrolysed divided by the total amount of drug contained in the conjugate. The rate of hydrolysis and half-life of the prepared conjugate were calculated.

$$k = \frac{2.303}{t} \times \frac{a}{a-x} \quad \text{..... (2)}$$

$$(t_{1/2}) = \frac{0.693}{k} \quad \text{..... (3)}$$

where k is the rate constant, t is the time in hours, a is the initial concentration of conjugate, x is the amount of the conjugate hydrolyzed into the free drug, a-x is the amount of drug remaining in conjugated form and $t_{½}$ is the half life of conjugate.

Table 1: Degree of substitution (DS) and molecular weight (Mw) of flurbiprofen- and suprofen dextran conjugates

S/no.	Dextran conjugate	DS[a]	Calculated Mw	Actual Mw
1	FD-40	9.5	42350	48400
2	FD-60	9.0	62226	72000
3	FD-110	8.5	112102	129600
4	SD-40	9.0	42343	52900
5	SD-60	8.4	62187	67600
6	SD-110	8.0	112082	122500

a = amount of parent drug in mg per 100 mg of conjugate

Table 2: Hydrolysis data for dextran conjugates in phosphate buffer human plasma at 37 ± 0.5 ºC

Dextran Conjugate	Half-life ($t_{½}$)[a] of dextran conjugates (hr^{-1})		
	pH 7.4	pH 7.4 (80% human plasma)	pH 9.0
FD-40	13.81	12.77	0.98
FD-60	17.65	15.71	1.07
FD-110	21.17	21.34	1.13
SD-40	47.80	36.64	3.55
SD-60	49.14	45.23	3.60
SD-110	52.90	46.10	3.96

a = Average half-life of four trials.

Table 3: Analgesic activity of the parent drugs and their dextran conjugates

Test Compound	Oral dose (mg/kg)	% Analgesic activity at different time (min)						
		15	30	45	60	75	90	120
Flurbiprofen	2.00	18.30	30.08	41.60	61.60	70.00	72.60	65.56
FD-40	21.05	07.25	20.00	31.20	40.74	53.40	58.24	51.23
FD-60	22.20	10.50	24.13	33.60	44.80	55.30	61.40	57.14
FD-110	23.52	12.06	26.50	35.10	48.70	58.60	64.23	60.50
Suprofen	5.00	15.20	23.72	28.81	36.00	40.60	44.30	42.60
SD-40	55.55	05.76	11.53	23.07	28.84	35.30	38.46	36.20
SD-60	59.52	07.14	16.07	25.00	33.00	36.00	40.30	38.60
SD-110	62.50	08.70	19.80	27.60	34.00	38.00	41.50	40.00

FD: Flurbiprofen-dextran conjugate SD: Suprofen-dextran conjugate Number of animal in each group six
Statistical significance $p < 0.05$ in relation to control

Table 4 Antipyretic activity[a] and ulcerogenic index of the parent drugs and their dextran conjugates

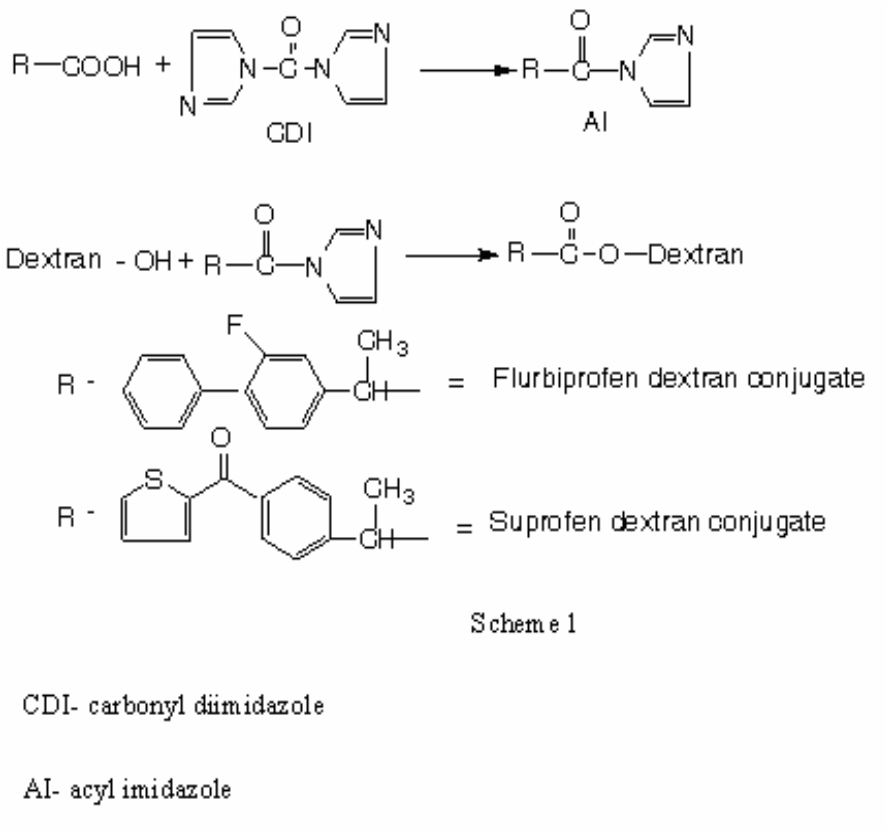
Test Compound	Oral dose (mg/kg)	Antipyretic activity (ºF) Mean ± S.E.					Ulcerogenic Index
	0 hr	1 hr	2 hr	3 hr	4 hr	(UI)	
Flubriprofen	2.00	101.6 ± 0.197	101.1 ± 0.507	99.9 ± 0.741	99.1 ± 0.90	98.5 ± 0.93	29.69
FD-40	21.05	99.2 ± 0.458	98.7 ± 0.354	98.2 ± 0.466	97.4 ± 0.50	97.1 ± 0.467	9.16
FD-60	22.2	99.5 ± 0.401	99.0 ± 0.120	98.6 ± 0.330	98.3 ± 0.466	97.8 ± 0.433	7.06
FD-110	23.52	100.1 ± 0.499	99.5 ± 0.166	98.8 ± 0.387	98.4 ± 0.578	98.0 ± 0.619	5.88
Suprofen	5.00	102.1 ± 0.456	101.2 ± 0.542	100.8 ± 0.691	99.6 ± 0.882	99.0 ± 0.936	31.0
SD-40	55.55	99.6 ± 0.636	99.1 ± 0.381	98.5 ± 0.456	96.8 ± 0.555	96.1 ± 0.562	9.83
SD-60	59.52	100.1 ± 0.199	99.8 ± 0.136	99.1 ± 0.381	98.2 ± 0.542	97.3 ± 0.477	9.90
SD-110	62.50	100.8 ± 0.552	100.2 ± 0.155	99.6 ± 0.385	99.0 ± 0.675	98.4 ± 0.610	6.06

FD: Flurbiprofen-dextran conjugates; SD: Suprofen-dextran conjugates; [a]Number of animal in each group six; $p < 0.05$ in relation to control (One-way ANOVA)

Biological evaluation

Analgesic activity

Analgesic activity was evaluated by the tail-flick method of Davies *et al* [25] using Techno-analgesiometer (Inco, Ambala, India). The suspension of drug or drug conjugate was prepared in 2% gum acacia. Ten experimental groups of *albino* rats, each having six rats weighing between 100-150 g were taken and the radiant heat from the wire was passed onto the tail, which was placed on the bridge of the analgesiometer. After recording the normal reaction time, the reaction time for tail-flick response was determined in different groups of rats by administering the test compounds orally at 15 min interval over a period of 120 min. The analgesic activity of these compounds was calculated using the following formula:



Scheme 1: Drug dextran conjugate synthesis

% analgesia = $[1-t_2/ t_1] \times 100$ …..…… (4)

where, t_1 = reaction time (sec) before drug administration and t_2 = reaction time (sec) after drug administration

Antipyretic activity

The antipyretic activity was evaluated by the method of Niemegeers *et al* and Teotino *et al* in which ten experimental groups of albino rats each having six animals were induced pyrexia by injecting a suspension of 15 % dried Brewer's yeast in 2% gum acacia in normal saline subcutaneously and the stabilized temperature was recorded after 18 hr. The test compounds were then administered orally to the rats and the rectal temperature recorded at hourly interval for a 4 h [26, 27].

Ulcerogenic index

The ulcerogenic index was determined by the method of Khan and Khan[28] and as previously reported by Shrivastava *et al*[19,20]. Wistar rats were randomly assigned to control and experimental groups, with six rats in each group. The suspension of the drug and its dextran conjugates in 2% gum acacia mucilage was administered orally to the rats for seven days. The rats were fasted for 8 h prior to dosing and 4 h post-dosing and then sacrificed. The abdomen was opened at the mid-line, and the stomach as well as the first 3 cm of the duodenum were removed. The stomach was opened along the greater

curvature and washed with saline water. The mucus was wiped off and observed for ulcer in the glandular portion of the stomach. The number of ulcer spots was noted and the severity of ulcer was scored by means of magnifying lens (10 X). The ulcerogenic index (UI) of these compounds was computed using the relationship (Eq 5) described by Robert *et al*[29].

Ulcerogenic index = number of ulcers + ulcer score + % incidence / number of animals …(5)

RESULTS

IR and NMR data

The IR spectra of flurbiprofen and suprofen dextran conjugates showed a characteristic absorption stretching at 1720-1730 cm^{-1} which confirms an ester linkage. A strong O-H stretching vibration of polymeric association at 3400-3200 cm^{-1} and a weak C-H stretching of alkene at 2970 cm^{-1} was found in both types of conjugates. FD conjugates showed characteristic absorption stretching at 1580-1510 cm^{-1} and 1010 cm^{-1} for biphenyl and C-F, respectively, whereas stretching at 1025 cm^{-1} and 1350 cm^{-1} was observed for thienyl and C-S, respectively, for SD conjugates[30,31]. ^1H NMR spectra showed a characteristic shifting of glucosidic ring anomeric proton signals from δ 4.91 (d, 1H, H-1) to δ 5.2 (s, 1H, H-1) and H-2 proton from δ 3.42 (m, 1H, H-2) to δ 3.89 (s, 1H, H-2) for FD, while the shifting of glucosidic ring anomeric proton signals from δ 4.91 (d, 1H, H-1) to δ 5.17 (s, 1H, H-1) and H-2 proton from δ 3.42 (m, 1H, H-2) to δ 3.94 (s, 1H, H-2) for SD indicates the formation of ester linkage at C-2 position of glucosidic ring. The disappearance of ^1H NMR signals in the range of 10.86-11.25 ppm for carboxylic group in all the drug-dextran conjugates suggests that the free carboxylic group of drug was conjugated with hydroxyl group of dextran macromolecule and formed the ester bond. The signals of biphenyl aromatic ring of flurbiprofen and thienyl carbonyl benzene ring of suprofen were found to be δ 7.27-7.52 (m, 8H, aromatic ring) and δ 7.29-8.0 (m, 7H, aromatic ring and thiophene ring) for FD and SD, respectively, which were in agreement with the anticipated structures.

Degree of substitution and hydrolysis

The degree of substitution of flurbiprofen and suprofen was found to be between 8.0 to 9.5 % and 7.5 to 9.0 % respectively. The molecular weights of the conjugates are summarized in Table 1. The results of *in-vitro* hydrolysis studies in solutions of different phosphate buffer medium i.e., pH 7.4, 9.0 and 80% human plasma (pH 7.4) at 37 ± 0.5 ºC are shown in Table 2 and they indicate a slow rate of hydrolysis at pH 7.4 and relatively faster hydrolysis at pH 9.0. Hydrolysis followed First order kinetics.

Biological activities

The maximum analgesic activity of flurbiprofen, suprofen and their dextran conjugate were observed after 75 and 90 min, respectively. The percent analgesic activity of FD-110 (64.23) and SD-110 (41.50) were identical to those of their parent drugs - flurbiprofen (72.60) and suprofen (44.30). The antipyretic activity of flurbiprofen and suprofen dextran conjugates were also comparable with those of their parent drugs. The results are summarized in Table 3 and 4.

It was observed that parent drugs, flurbiprofen and suprofen, showed deep ulceration, swelling and high intensity perforation in the gastric mucosa after a seven-days administration ulcerogenic index of 29.69 and 31.0, respectively. On the other hand, the conjugates, FD-40 and FD-60, showed ulcerogenic index of 9.16 and 7.06, respectively but in the case of FD-110, only oedematous gastritis with a much lower ulcerogenic index of 5.88 was observed. The suprofen conjugates were also showed similar pathological changes as flurbiprofen. SD-40 and SD-60 showed more ulcers in gastric mucosa with ulcerogenic index of 9.83 and 9.90, respectively. The ulcerogenic index

observed for SD-110 was 6.06. All the results of biological evaluation were statistically significant ($p < 0.05$) in relation to the control sample.

DISCUSSION

The dextran conjugates of flurbiprofen and suprofen were synthesized using N, N' carbonyldiimidazole which reacts with the free acidic group of the drug to form active acylimidazole. The acylimidazole active moiety condensed with the hydroxy group of dextran to form ester conjugates. The purity of synthesized conjugates was confirmed by TLC which showed different R_f values of conjugates from the drug substance. The characteristic band in IR spectra was obtained which confirmed the formation of ester bond between the free acidic groups of drug and the hydroxy group of the dextran molecule. ^{1}H NMR spectra showed disappearance of acidic proton and characteristic shifting of anomeric proton signals which indicates the formation of an ester linkage at C-2 position. It was also observed that the molecular weight of dextran increased as the degree of substitution is decreased. The hydrolysis study indicate that ester conjugates of dextran showed greater specific base catalytic hydrolysis at pH 9.0 and this may be attributed to the basic character of the carbohydrate alkoxide ion. The increased tendency of these dextran derivatives to undergo hydrolysis in the pH range 6 to 10 may be due to intermolecular catalysis by the neighboring hydroxy group. The rate data for hydrolysis of flurbiprofen and suprofen dextran conjugates at different pH and 80% human plasma was high which indicates that the hydrolysis reaction in plasma may have been affected by enzymatic interference. The hydrolytic regeneration of flurbiprofen and suprofen from the conjugates was studies in the different buffers followed first order kinetics. The half-life of the conjugates was higher in pH 7.0 and human plasma (pH 7.0) than in pH 9.0 buffer solution which suggests that the drugs would be absorbed faster at pH 9.0.

The analgesic and antipyretic activities of flurbiprofen and suprofen dextran conjugates were comparable to those of their parent drugs. While flurbiprofen and suprofen showed deep ulceration, swelling and high intensity perforation in the gastric mucosa after seven days, the dextran conjugates of the drugs manifested oedematous congestion, hemorrhagic gastric mucosa and negligible ulcers. SD-40 and SD-60 conjugates showed more ulcers in the gastric mucosa than SD-110 and this may be due to the chemical nature of carrier drug linkage.

The results shows that the molecular weight of dextran play an important role in ulcer activity, in that as the molecular weight of dextran increased, the ulcerogenic index of conjugates decreased.

CONCLUSION

Data obtained from hydrolysis, UV, HPLC, FT-IR, and ^{1}H NMR studies and molecular weight determination demonstrated that dextran can be successfully employed as pro-moiety/carrier for flurbiprofen and suprofen which have an acidic function. The resulting conjugates retained the analgesic and antipyretic activities of the parent compunds and also showed remarkable reductions in ulcerogenicity when compared with their parent compounds.

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