Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 40 (2005) 371–376
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Synthesis, pharmacological activity and hydrolytic behavior of glyceride prodrugs of ibuprofen

M.S.Y. Khan *, Mymoona Akhter

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110 062, India

Received 14 June 2004; received in revised form 10 November 2004; accepted 17 November 2004

Available online 29 January 2005

Abstract

For reducing the gastrointestinal toxicity associated with ibuprofen its carboxylic group was condensed with the hydroxyl group of 1,2,3-trihydroxy propane 1,3-dipalmitate/stearate to give the ester prodrugs **3a** and **3b**. The release of ibuprofen from these prodrugs has been studied at pH 3, 4, 5 and 7.4 by HPLC using methanol and 0.05% phosphoric acid (80%) (70:30) as mobile phase. The prodrugs showed insignificant hydrolysis at pH 5 compared to pH 7.4 indicating that the prodrugs do not break in stomach but release ibuprofen at pH 7.4 in adequate amounts. In vivo hydrolysis studies in rats, the peak plasma concentration of ibuprofen was attained in 1.5 h in case of ibuprofen and in 2 h in prodrugs treated animals. The plasma concentration was found to be less at all times in animals treated with ibuprofen compared to the prodrugs treated animals. The maximum anti-inflammatory activity of ibuprofen was observed at 2 h whereas prodrugs showed maximum activity at 3 h and remained practically constant upto 8 h whereas a decrease in activity was observed with free ibuprofen. Further the prodrugs showed less gastric ulcers compared to ibuprofen. An average score of 0.16, 0.45, 0.97 and 0.20, 0.76, 1.02 of ulcers was observed with **3a** and **3b** compared to an average score of 0.75, 1.10, and 2.97 with ibuprofen. These prodrugs also showed significant protection against acetic acid induced writhings in rats. These finding suggested that both the prodrugs are better in action as compared to the parent drug and are advantageous in having less gastrointestinal side effects.

Keywords: NSAIDs; Glyceride prodrugs; Ibuprofen; Pharmacokinetics

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are associated with gastrointestinal side effects particularly stomach ulceration, bleeding and perforation. The side effects produced by NSAIDs are believed to involve two different mechanisms: a local action [1] exerted by direct contact of drug with gastric mucosa and a generalized systemic action following absorption, now believed to be by inhibition of the COX 1, a constitutive form of COX [2].

Acetylsalicylic acid when applied directly to the gastric mucosa produced more lesions as compared to the i.v. route; the same type of action has been found with ibuprofen [3]. These findings are further supported by Cioli et al. and Guslandi [4,5] who reported that there is no difference between the anti-inflammatory activities exerted by the two different routes.

Prodrug has been the concept of retro metabolic drug design that incorporates targeting, metabolism and the duration of action consideration into the design process. The carboxylic group of NSAIDs can be temporarily masked and its direct effect on gastric mucosa can be minimized. Ester prodrugs of naproxen have been synthesized using *N*-hydroxy methyl succinimide and *N*-hydroxy methyl isatin as promoieties to reduce their GIT irritation and improve bioavailability [6]. Polymeric prodrugs of naproxen have also been synthesized to improve upon the potency and duration of action [7]. A putative prodrug of ibuprofen and glyceryl-3-nitrate, glyceryl-1,2-diibuprofenate-3-nitrate showed less gastric irritation [8]. An increase in permeability of niflumic acid to brain by developing its triglyceride prodrugs has been observed [9]. Prodrugs of several NSAIDs, such as diclofenac, ibuprofen, ketoprofen, etc. have been synthesized using 1,4-dihydro-1-

* Corresponding author. Tel.: +91 11 260 59688x5890; fax: +91 11 260 59663.
E-mail address: msykhan@hotmail.com (M.S.Y. Khan).

0223-5234/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2004.11.009

methylpyridine-3-carboxylate as a carrier to brain to treat Alzheimer's disease [10].

Our aim has been to reduce the gastric irritation due to direct contact of the drugs with the gastric mucosa and increase their absorption pertaining to the basic concept of glyceride absorption. Triglycerides being the major constituents of dietary fat and their absorption involves simple hydrolysis mainly by pancreatic lipases to monoglycerides and free fatty acids. These prodrugs, therefore, do not involve the risk of unwanted effects after they are hydrolyzed. In the present paper we report the synthesis of glyceryl derivatives of ibuprofen and their anti-inflammatory, analgesic activity, their in vitro hydrolysis and plasma levels in rats. The compounds synthesized will act as prodrugs of the ibuprofen which upon administration would release the parent drug as a result of hydrolysis (enzymatic and/or non-enzymatic) in the body.

2. Chemistry

The synthesis of the title compounds is illustrated in Fig. 1. 1,2,3-Trihydroxy-1,3-dipalmitate and 1,2,3-trihydroxy-1,3-distearate were synthesized by the method of Bentley and McCrae [11] and then condensed with the acid chloride of ibuprofen. The yields of the compounds were good. The structures of the synthesized compounds were established by elemental analysis, ^{1}H NMR, mass and FT-IR spectral methods. The purity was determined by TLC. The results of elemental analysis of the synthesized compounds were in all cases within ± 0.4% of the theoretical values.

3. Biological investigations

The anti-inflammatory activity of the compounds was carried out on Wistar rats by the Winter et al. [12] method, analgesic activity was carried out on Swiss albino mice by Seigmund et al. [13] method and ulcerogenic studies were carried out by Cioli et al. [4] method in Wistar rats. In vitro hydrolysis studies of prodrugs were carried out by HPLC method at pH 3, 4, 5 and 7.4 and in vivo hydrolysis were carried out in Wistar rats by reverse phase HPLC method [14].

4. Results and discussion

4.1. Hydrolysis studies

The hydrolysis studies were carried out in aqueous buffer so as to study whether the prodrugs hydrolyze in aqueous medium and to what extent or not, suggesting the fate of the prodrugs in the system. Hydrolysis kinetics of the synthesized glyceride prodrugs **3a** and **3b** were studied in aqueous buffer solution at pH 7.4. Under the experimental conditions the target compounds hydrolyzed to release the parent drug (Fig. 2) as evident by HPLC analysis. At constant pH and temperature the reaction displayed strict first order kinetics as the k_{obs} was fairly constant and a straight plot was obtained on plotting log concentration of residual prodrug v/s time. The rate constant (k_{obs}) and the corresponding half-lives ($t_{1/2}$) for the respective prodrugs were calculated from the linear regression equation correlating the log concentration of the residual prodrug v/s time. The data are given in Table 1.

To examine the degradation of glyceryl prodrugs in pH as of stomach, pH 3, 4 and 5 were selected, because the mean fasting stomach pH of adult is approximately 2 and increases to 4–5 following ingestion of food. NSAIDs are not recommended to be taken in fasting state; consequently pH 3, 4 and 5 were selected to mimic the appropriate clinical range. An assay time of 2 h was selected, after which time stomach emptying would normally be effectively complete [15].

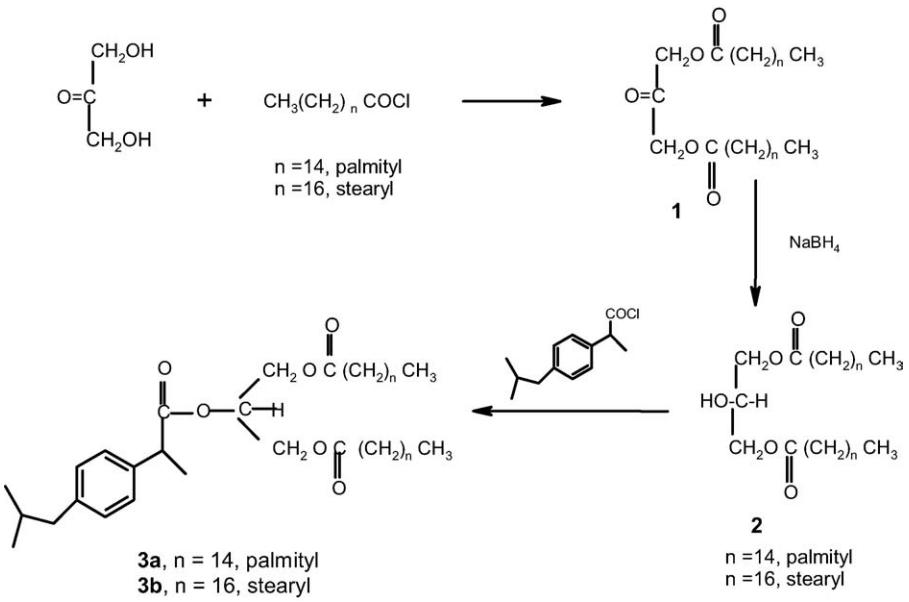

Fig. 1.

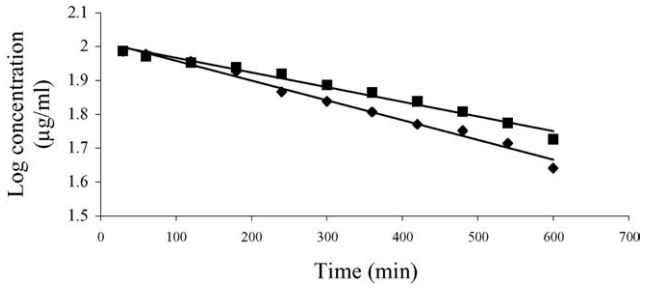

Fig. 2. First order hydrolysis plot of ibuprofen prodrugs **3a** and **3b** in phosphate buffer of pH 7.4.

Table 1
The observed k value and $t_{1/2}$ of glyceride prodrugs (**3a** and **3b**) of ibuprofen at pH 3, 4, 5 and 7.4

pH	**3a**		**3b**	
	k_{obs} (h^{-1})	$t_{1/2}$ (h)	k_{obs} (h^{-1})	$t_{1/2}$ (h)
3	2.5×10^{-4}	44.42	4.6×10^{-4}	25.10
4	2.2×10^{-4}	52.5	3.07×10^{-4}	37.62
5	1.6×10^{-4}	72.18	6.1×10^{-4}	18.93
7.4	1.1×10^{-3}	10.5	9.5×10^{-4}	12.15

Hydrolysis of the prodrugs **3a** and **3b** were found to be more at pH 3 than at pH 4 and 5. At pH 5 corresponding to fed state 32% of the prodrugs hydrolyzed to release the parent compound suggesting that very less of the prodrugs hydrolyze in the stomach as expected. At pH 7.4, 58% of the prodrugs hydrolyzed to parent compound indicating that the prodrugs will undergo hydrolysis in the system easily, which was observed in in vivo studies as well. Presence of some additional compounds was indicated by HPLC by a few additional peaks, which may be intermediate degradation products, however, we have no firm evidence of the identity of these compounds and could not quantify them.

The rate of hydrolysis of the prodrugs was also studied in rat plasma. Ibuprofen, **3a** and **3b** were given orally to the rats and at different time intervals, the concentration of ibuprofen was estimated in plasma. A comparison of the plasma concentration–time curves of ibuprofen indicated that the ester was rapidly hydrolyzed to the parent drug. Maximum plasma concentration of the liberated ibuprofen from the glyceride prodrugs **3a** and **3b** was attained after 2 h compared with the 1.5 h for the parent drug treated animal. Higher plasma concentrations of ibuprofen were observed in rats treated with prodrugs compared to the parent drug treated animals. The plasma concentration of ibuprofen was eight times higher even after 8 h of administration of **3a** and **3b** compared with free ibuprofen treated animals, indicating a sustained release. In conclusion the in vivo evaluation study indicated that the glyceride prodrugs of ibuprofen might be considered as a potential biolabile prodrug form of ibuprofen with sustained action for 8 h, Fig. 3. The C_{max} of ibuprofen was 26.8 µg ml^{-1} and was attained in 1.5 h with an $(AUC)_{0-t}$ of 69.705, whereas C_{max} of **3a** was 34.3 µg ml^{-1} and **3b** was 33.6 µg ml^{-1} attained in 2 h with an $(AUC)_{0-t}$ of 142.55 and 145.99, respectively.

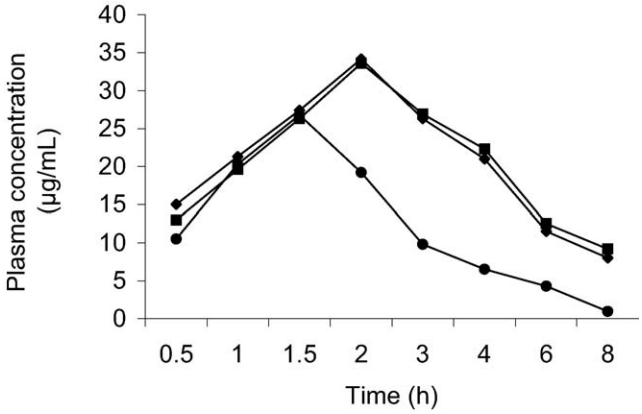

Fig. 3. Plasma level of ibuprofen in rats after oral administration of the prodrugs **3a** and **3b** and ibuprofen.

4.2. Anti-inflammatory activity

The inhibition of swelling in carrageenan-induced edema in rat paw brought about by oral administration of the drugs is shown in Table 2. The percentage of swelling inhibition were calculated using Eq. 1.

$$\text{Inhibition}\,(\%) = \{[(V_t - V_o)_{control} - (V_t - V_o)_{treated}]/(V_t - V_o)_{control}\} \times 100 \quad (1)$$

(V_t and V_o relates to the average volume in the hind paw of the rats (n = 6) before any treatment and after anti-inflammatory agent treatment, respectively. The prodrugs showed better activity compared to the free ibuprofen. The maximum anti-inflammatory activity of prodrugs (**3a** and **3b**) was observed at 3 h and remained practically constant upto 8 h. The anti-inflammatory activity of free ibuprofen, however, decreased with time. Statistical significance testing using one way analysis of variance showed that the anti-inflammatory activity of ibuprofen and prodrugs were effective in comparison with the control group. However, differences in the potency of anti-inflammatory activity of the prodrugs compared to the free ibuprofen were observed over a long period (8 h). Thus glyceride prodrugs were proved to be a suitable promoiety for ibuprofen.

Table 2
Percentage of inhibition caused by ibuprofen, **3a** and **3b** in carrageenan-induced oedema in rats

	Time (h)				
	1	2	3	4	8
Ibuprofen	40 ± 3.6*	52.38 ± 2.64*	44.00 ± 3.50*	42.71 ± 2.99**	36.1 ± 2.19**
3a	38.4 ± 4.6*	43.5 ± 3.9*	54.16 ± 3.22*	53.24 ± 2.57**	52.8 ± 2.85**
3b	39.5 ± 2.68*	42.8 ± 3.56*	55.29 ± 2.12*	53.8 ± 4.48**	53.6 ± 2.09**

Data are represented as mean ± S.E.M., n = 6, *$P < 0.01$, **$P < 0.001$ with respect to control.

Table 3
Percentage protection in acetic acid induced writhings by ibuprofen, glyceride prodrugs **3a** and **3b** in rats

Treatment	Number of writhings (average)	% Protection
Control	23.33 ± 1.45	–
Ibuprofen	11.00 ± 1.81*	52.85
3a	11.83 ± 1.87*	49.42
3b	11.66 ± 1.88*	50.02

*$P < 0.001$, compared with control.

4.3. Analgesic activity

The percent protection in mice brought about by administration of the drugs is shown in Table 3. The prodrugs showed analgesic activity comparable to the parent drug. The percent protection was calculated using Eq. 2.

$$\text{Protection (\%)} = 100 - [\text{number of writhings in test/number of writhings in control} \times 100] \quad (2)$$

4.4. Ulcerogenic study

The ulcerogenic effect of ibuprofen and prodrugs **3a** and **3b** were studied at three doses viz. 25, 50 and 100 mg kg^{-1}. It was observed that the ulcerogenic dose for prodrugs was almost double the dose of ibuprofen. Less number of ulcers were seen at all doses in animals treated with prodrugs compared with the animals treated with ibuprofen. At 100 mg kg^{-1} dose all the animals treated with ibuprofen developed ulcers compared to 35% and 42% produced by the prodrugs. These findings suggested successful masking of the carboxylic function of the ibuprofen. Results are given in Table 4.

5. Conclusions

The glyceride prodrugs containing ibuprofen were successfully synthesized. The prodrugs released ibuprofen quantitatively at pH 7.4, but were resistant to hydrolysis at pH 3, 4 and 5 indicating that the prodrugs are resistant to acidic conditions. In vivo studies showed sustained release of ibuprofen upto 8 h as also evident from the longer anti-inflammatory effect observed with prodrugs treated animals compared to ibuprofen treated animals. This is also indicated by the half-life (10 and 12.5 h for **3a** and **3b**, respectively) of the prodrugs at pH 7.4 corresponding to pH of the body. The prodrugs were found to be significantly less ulcerogenic with enhanced anti-inflammatory activity than the parent drug.

Table 4
Erosive effects observed on the gastric mucosa of rats treated with single oral dose of ibuprofen and glyceride prodrugs (**3a** and **3b**)

Compound	Dose (mg kg^{-1})	Percentage of rats with ulcers	Severity index
Ibuprofen	25	20	0.75*
	50	45	1.10*
	100	100	1.97*
3a[a]	92.35	5	0.16*
	184.5	15	0.45*
	369	35	0.97*
3b[a]	97.81	7	0.20*
	195.5	28	0.76*
	391	42	1.02*

'Severity index': mean score of each treated group minus the mean score of the control group. *$P < 0.001$.
[a] Dose is molecularly equivalent to ibuprofen.

6. Materials and methods

Except dihydroxy acetone which was purchased from E. Merck KGa A, Germany, all other reagents were obtained from E. Merck (India) Ltd. Ibuprofen and flurbiprofen were procured from Sun Pharmaceuticals, Mumbai. All the solvents used in these studies were dried and distilled before use. Wistar rats of either sex weighing between 150 and 200 g and Swiss albino mice of either sex weighing between 25 and 30 g were procured from Animal House of Jamia Hamdard.

6.1. Compounds characterization

^{1}H NMR spectra of the compounds were recorded on a Bruker Spectrospin Avance DPx200, 300 MHz in $CDCl_3$ and the mass spectra were recorded on JEOL 5 × 102/DA-6000 Mass spectrometer/Data System using Argon/Xenon (6 kV, 10 mA) as the FAB gas. IR spectra were recorded on Perkin Elmer Spectrophotometer. TLC of the synthesized compounds was carried out in petroleum ether/ethylacetate (5:1) solvent system and the spots were developed by exposure to iodine vapors.

HPLC analysis of ibuprofen and its derivatives was done on a Shimadzu Model LC-10AT*VP* (Japan) system containing a quaternary pump, UV detector and equipped with c18 reverse phase column.

Dissolution was carried out by SR8 Plus dissolution test apparatus Hanson Research USA.

Samples were filtered with 0.45 μm millipore filter and eluted with methanol and 0.05% phosphoric acid (70:30) at 2 ml per min. The eluent was monitored at 220 nm (UV detector, Shimadzu Model SCL-10AVP, Japan).

6.2. Preparation of 1,3-dipalmityl-2-(4-isobutyl phenyl propionyl) glyceride (*3a*)

Compound **3a** was prepared by dissolving 1,3-dipalmityl glyceride (5.7 g, 0.010 mol) [11] in 50 ml of freshly distilled CH_2Cl_2. Dry pyridine (0.95 g, 0.012 mol) and ibuprofen acid chloride [16] (2.46 g, 0.011 mol) were added at once, the contents were stirred for 40 h at room temperature and then diluted with 100 ml of water followed by extraction with 2 × 25 ml of CH_2Cl_2. The CH_2Cl_2 extracts were combined, washed with 1% HCl and water, dried over anhydrous sodium sulfate and evaporated to dryness. The solid mass so obtained was crystallized from petroleum ether, yield: 52%, m.p. 54–57 °C. It was TLC pure, R_f 0.69 and was characterized on the basis of ^{1}H NMR, IR and mass spectral data.

^{1}H NMR δ (ppm) $CDCl_3$- 0.85–0.91 (12H, m, 2 × CH_3 of palmitic acid unit + $\underline{CH_3}$–CH–$\underline{CH_3}$), 1.25 (m, 24 × CH_2), 1.50 (d, 3H, J = 7 Hz, $\underline{CH_3}$–CH), 1.61 (m, 4H, 2 × CH_2 β to CO), 1.84 (m, 1H, CH_3–$\underline{CH}$–CH_3), 2.34 (t, 4H, J = 7.5 Hz, 2 × CH_2 α to CO), 2.44 (d, 2H, J = 7 Hz, CH_2 benzylic), 3.7 (q, 1H, J = 7 Hz, CH_3–$\underline{CH}$), 4.14 (m, 5H, 2 × CH_2 + CH of glycerol), 7.10 (d, 2H, J = 8 Hz, A_2B_2), 7.22 (d, 2H, J = 8 Hz, A_2B_2); IR (KBr/ν_{max} cm^{-1}) 2918, 2850, 1730 (C=O), 1470, 1180, 875 (*p*-disubstituted phenyl); *m/z* 756 (M^+) (molecular ion peak was not observed), 551(16%), 501 (13%), 313 (19%), 283 (29%), 281 (24%), 265 (100%), 239 (14%), 189 (43%). Anal. $C_{48}H_{84}O_6$.

6.3. Preparation of 1,3-distearyl-2-(4-isobutyl phenyl propionyl) glyceride (**3b**)

It was prepared following the same procedure as in **3a** except that 1,3-distearyl glyceride (5.7 g, 0.010 mol) was used, yield, 48%; m.p. 46–48 °C. It was TLC pure, R_f 0.61 and was characterized on the basis of ^{1}H NMR, IR, and mass spectral data.

^{1}H NMR δ (ppm) $CDCl_3$- 0.85–0.90 (12H, m, 2 × CH_3 of stearic acid unit + $\underline{CH_3}$–CH–$\underline{CH_3}$), 1.25 (m, 28 × CH_2), 1.47 (d, 3H, J = 7 Hz, $\underline{CH_3}$–CH), 1.60 (m, 4H, 2 × CH_2 β to CO), 1.84 (m, 1H CH_3–$\underline{CH}$–CH_3), 2.35 (t, 4H, J = 7.5 Hz, 2 × CH_2 α to CO), 2.44 (d, 2H, J = 7 Hz, CH_2 benzylic), 3.7 (q, 1H, J = 7 Hz, CH_3–$\underline{CH}$), 4.14 (m, 5H, 2 × CH_2 + CH of glycerol), 7.10 (d, 2H, J = 8 Hz, A_2B_2), 7.22 (d, 2H, J = 8 Hz, A_2B_2). IR (KBr/ν_{max} cm^{-1}) 2919, 2849, 1733 (C=O), 1472, 1183, 875 (*p*-disubstituted benzene). *m/z* 812 (M^+) (molecular ion peak was not observed), 607 (18%), 529 (11%), 341 (15%), 311 (24%), 309 (19%), 267 (12%), 265 (100%), 189 (45%). Anal. $C_{52}H_{92}O_6$.

6.4. Hydrolysis study in aqueous buffers

The hydrolysis kinetics of prodrugs **3a** and **3b** was studied at pH 7.4, 3, 4 and 5 using acetate and phosphate buffers. The total buffer concentration was 0.02 M and constant ionic strength of 0.5 M for each sample was maintained by adding KCl. The total buffer volume was 900 ml. The mixture was equilibrated at 37 °C for 1 h. To this mixture 100 mg of each sample was added and the mixture agitated by an overhead stirrer. At selected time intervals of 15, 30, 45, 60, 75, 90, 105 and 120 min, 0.1 ml of the solution was removed and diluted with mobile phase upto 10 ml, and 20 μl of this solution was injected for direct analysis by HPLC. At pH 7.4 the samples were withdrawn at time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h [14].

6.5. In vivo studies

Rats weighing between 150 and 200 g from Animal House of Jamia Hamdard were divided into three groups of six animals each. The rats were used to compare the bioavailability of prodrugs **3a** and **3b** with that of ibuprofen following oral administration. In group I each animal received a dose of 20 mg kg^{-1} administered as homogenous suspension of ibuprofen in aqueous solution of sodium CMC (0.5%), groups II and III received **3a** and **3b**, respectively, a dose molecularly equivalent to ibuprofen. At appropriate time intervals 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h blood samples were withdrawn from rats into heparinized tubes and centrifuged for 15 min at 4000 rpm to separate plasma. The samples were collected in heparinized tubes and stored in deep freezer until analysis. To 0.5 ml of plasma 1.5 ml of acetonitrile was added and precipitated proteins were separated by centrifugation at 4000 rpm. The clear supernantant liquid obtained was directly used for analysis using 20 μl after filtering through 0.45 μm millipore filter and eluted with methanol and 0.05% phosphoric acid (70:30) at 2 ml per min. Flurbiprofen (1 mg ml^{-1}) was used as internal standard and 0.1 ml of this solution was added to each sample. The eluent was monitored at 220 nm. The mean concentration of ibuprofen at respective time intervals was taken [14].

6.6. Anti-inflammatory activity

The anti-inflammatory activity was evaluated using carrageenan-induced paw oedema on rat [12] method. Wistar rats (150–200 g) were divided into four groups of six animals each. Group I served control group without using the drug, group II received ibuprofen 20 mg kg^{-1}, groups III and IV received prodrugs **3a** and **3b**, 73.9 and 78.3 mg kg^{-1}, respectively, where the dose was molecularly equivalent to the ibuprofen. A stock solution of 4, 18.47 and 19.57 mg ml^{-1} was prepared as a homogeneous suspension in aqueous solution of sodium CMC (0.5% w/v) and each animal received 0.75–1.0 ml orally of the respective drugs. Thirty minutes after administration of drugs, each rat received a subplanter injection of 0.1 ml of 1% carrageenan solution in its left hind paw. The measurement of the hind paw volume was carried out using a Ugo Basile Plethysmometer before any treatment (V_o) and in any interval (V_t) after the administration of the drugs. All the results are expressed as mean ± S.E.M. Statistical evaluation was performed using analysis of variance followed by *t*-test for sub group comparison.

6.7. Analgesic activity

Analgesic activity was carried out by using acetic acid induced writhing method [13] in Swiss albino mice (25–30 g) of either sex. A 1% v/v solution of acetic acid was used as writhing inducing agent. Test compounds were administered orally 3 h prior to acetic acid injection. Number of writhings for 10 min in control and test compounds were counted and compared. Analgesic activity was measured as percent decrease in writhings in comparison to control. Mice were divided into four groups of six animals each. Group I served as a control group without using the drug, while group II received ibuprofen 20 mg kg^{-1}, groups III and IV received prodrugs **3a** and **3b** 73.9 and 78.3 mg kg^{-1}, respectively, where

the dose of prodrugs was molecularly equivalent to the ibuprofen. A stock solution of 1, 3.69 and 3.91 mg ml^{-1} was prepared as a homogeneous suspension in aqueous solution of sodium CMC (0.5% w/v) and each animal received 0.5–0.6 ml orally of the respective drugs. Acetic acid was administered intraperitoneally 1 ml/100 g body weight of the animal. All the results are expressed as mean ± S.E.M. Statistical evaluation was performed using analysis of variance followed by *t*-test for sub group comparison (level of significance is $P < 0.001$).

6.8. Ulcerogenicity

Gastrointestinal toxicity was determined by the method of Cioli et al. [4]. The studies were carried out on healthy Wistar rats (150–200 g) at three different doses viz. 25, 50 mg, and 100 mg kg^{-1}. The animals were divided into 10 groups of six each, group I served as control and received vehicle only. Groups II–IV received pure ibuprofen 25, 50 and 100 mg kg^{-1}, respectively. Groups V–X received test compounds **3a** and **3b** in dose molecularly equivalent to 25, 50 and 100 mg kg^{-1} of ibuprofen, respectively. The animals were fasted 8 h prior to a single dose of each of the control and test compounds and sacrificed 17 h later during which period food and water were available. The gastric mucosa of the rats was examined by means of a 4× binocular magnifier. The lesions were counted and divided into large (greater than 2 mm in diameter), small (1–2 mm) and punctiform (less than 1 mm).

For each stomach the severity of mucosal damage was assessed according to the following scoring system:

- 0- no lesions or upto five punctiform lesions;
- 1- more than five punctiform lesions;
- 2- one to five small ulcers;
- 3- more than five small ulcers or one large ulcer;
- 4- more than one large ulcer.

The mean score of each treated group minus the mean score of the control group was considered as the 'severity index' of gastric damage (level of significance is $P < 0.001$ with respect to control).

Acknowledgements

The authors are thankful to late Hakim Abdul Hameed Saheb (Founder Chancellor and builder of Jamia Hamdard) and Mr. A. Mueed (President, Hamdard National Foundation) for providing the facilities to carry out this research work. One of the authors (M.A.) is thankful to UGC for providing financial assistance and also to Mr. Iyaz Ahmed (Department of Pharmacology, Jamia Hamdard) and Mr. Naseem Charoo (Department of Pharmaceutics, Jamia Hamdard) for their help during the course of carrying out pharmacological and pharmacokinetic studies.

References

[1] C.A. Guyton, J.E. Hall, in: Textbook of Medical Physiology, ninth ed, Harcourt Asia Pte. Ltd, 1998, pp. 846.
[2] J.R. Vane, Y.S. Bakhle, R.M. Bolting, Annu. Rev. Pharmacol. Toxicol. 38 (1998) 97.
[3] C.J. Pfeiffer, L.G. Lewandowski, Arch. Int. Pharmacodyn. Ther. 190 (1971) 5.
[4] V. Cioli, S. Putzolu, V. Rossi, B.P. Corza, C. Corradino, Toxicol. Appl. Pharmacol. 50 (1979) 283.
[5] M. Guslandi, Drugs 53 (1997) 1.
[6] N. Mahfouz, F.A. Omar, T. Aboul-Fadl, Eur. J. Med. Chem. 34 (1999) 551–562.
[7] C.F. Wang, H.N. Chiang, W.B. Chem, J. Pharm. Pharmacol. 54 (2002) 1129.
[8] J.I. Mathew, A. Humphrey, M.W. Moynihan, C.R. Powell, J. Pharm. Pharmacol. 53 (2001) 345.
[9] L. El Kihel, J. Bourass, P. Richomme, J.Y. Petit, Y. Letourneux, Arzeneim-Forsch/Drug Res 461 (1996) 1040.
[10] L. Perioli, V. Ambrogi, C. Bernardini, G. Grandolini, M. Ricci, S. Giovagnoli, et al., Eur. J. Med. Chem. 39 (2004) 715.
[11] P.H. Bentley, W. McCrae, J. Org. Chem. 35 (1970) 2082.
[12] C. Winter, E. Risley, G. Nuss, Proc. Soc. Exp. Biol. Med. 111 (1962) 544.
[13] E. Seigmund, R. Cadmus, G. Lu, Proc. Soc. Exp. Biol. Med. 95 (1957) 729.
[14] M. Lalande, D.L. Wilson, I.J. McGilveray, J. Chromatogr. 337 (1986) 410.
[15] J.N. Hunt, W.R. Spurrel, J. Physiol. 113 (1951) 157.
[16] A.I. Vogel, Textbook of Practical Organic Chemistry, fourth ed, ELBS/Longman, London, 1987 pp. 455.