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Research Article

Amide prodrugs of NSAIDs: Synthesis and biological evaluation with a hydrolysis study

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Abstract: The aim of the present study was to synthesize prodrugs of commonly used NSAIDs to overcome the gastrointestinal toxicity (irritation and bleeding) associated with their use. A total of six amide-based prodrugs (**Ia-f**) of aceclofenac, diclofenac, fenbufen, indomethacin, mefenamic acid and 4-biphenyl acetic acid were synthesized through one-pot method (single step synthesis). The structures of the synthesized prodrugs were confirmed by modern analytical techniques. The release pattern of parent drug from prodrug **Ia** was also studied by reverse phase HPLC method in acidic buffer (pH 1.2), phosphate buffer (pH 7.4), 80% plasma, 10% rat intestinal homogenate and 10% rat liver homogenate (pH 7.4). The prodrugs were also evaluated for their anti-inflammatory and ulcerogenic actions and compared to their corresponding parent drugs.
Keywords: NSAID, Prodrug, Anti-inflammatory, Hydrolysis, Ulcerogenic.

INTRODUCTION

The non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed and used drugs to reduce pain, fever and inflammation [1]. Most of these drugs elicit their therapeutic effects by inhibition of prostaglandin biosynthesis, which are important mediators of pain and edema. These NSAIDs competitively inhibit cyclooxygenase enzymes (COX) primarily responsible for conversion of arachidonic acid into prostaglandins in inflammatory processes [2]. The major drawback of long-term use of NSAIDs is their gastrointestinal (GIT) toxicity which is due to inhibition of COX-1 activity and include upper GI irritation, ulceration, dyspepsia, bleeding, and in some cases death [2,3]. GIT toxicities produced by NSAIDs involve two different mechanisms: inhibition of prostaglandin synthesis via COX-1 in the stomach responsible for inducing mucus production and a local insult exerted by direct contact of the drugs with gastric mucosa due to acidic nature of the NSAIDs [3,4]. The other isoform of cyclooxygenase enzyme, COX-2 plays an important role in producing pain and inflammation and must be selectively inhibited to minimize the GI toxic effects. For the same reason, selective COX-2 inhibitors were introduced in the market which do not significantly inhibit cyclooxygenase in the stomach and appeared to be GIT safe [5,6]. Unfortunately, these very effective COX-2 inhibitors like rofecoxib and celecoxib, were withdrawn from the market because of the severe risks of heart attack and stroke associated with them [7,8]. Therefore, research is going on to develop safe and effective NSAIDs. Recently, considerable attention has been directed towards the development of bioreversible derivatives via chemical modifications, such as prodrugs and mutual prodrugs. The chemical modification or derivatization temporarily mask the acidic group of NSAIDs and appears to be as a promising and fruitful means of reducing or abolishing the GIT toxicity due to local insult mechanism [9-11].

The concept and application of prodrug approach to NSAIDs afforded compounds with better anti-inflammatory activity, improved pharmacokinetic profile and reduced gastric ulcerogenic activity [9-12]. A prodrug is pharmacologically inactive derivative of the active drug, which undergoes chemical and/or enzymatic biotransformation, resulting in the release of active drug in the body. The parent drug then shows the desired biological response. Majority of prodrugs of NSAIDs have been synthesized by derivatization of the free carboxylic group (-COOH) of the NSAID [11, 12]. Among various type of prodrugs amide derivatives are the most common one. Many studies have reported that conversion of the carboxylic group of NSAIDs to amide functional group increases their selectivity towards COX-2 and it further helps in decreasing the GI toxicity

of parent drug [13]. Amide-based prodrugs have desirable characteristics with reasonable *in vitro* chemical stability which allows them to be formulated with adequate shelf lives. They also function as amidase substrate and are very labile to hydrolysis *in vivo*. Literature survey revealed that a number of amide-prodrugs of NSAIDs have been prepared with improved pharmacological profile [14,15]. Fernandes et al., in 2014 reported the synthesis, anti-inflammatory and antimicrobial activity of carboxamide derivatives of Naproxen prepared by condensing with substituted anilines [16]. Some of the amide prodrugs of naproxen exhibited significant antibacterial and anti-inflammatory activity. In many studies conducted elsewhere, sulfonamides have been coupled with free carboxylic acid of NSAIDs in hope of obtaining safer anti-inflammatory agents. Makhija et al., prepared the mutual prodrugs of diclofenac, and flurbiprofen, conjugated with sulphonamides[17]. They rationalized that coupling of sulphonamides with NSAIDs as mutual amide produrg can increase COX-2 selectivity, because of the presence of side pocket in the structure of COX-2 enzymes where sulfonamide group can easily fit. Thus, it will not only enhance the anti-inflammatory activity but also decrease the inhibition of COX-1 enzyme and will prevent against gastric mucosal damage [17]. It was observed that synthesized prodrugs were less ulcerogenic than their parent nonsteroidal anti-inflammatory drugs and showed better activity profile in terms of analgesic and anti-inflammatory activity as compared to their respective parent drugs. Authors proposed that possible route of hydrolysis of the synthesized prodrugs was probably by cleavage of amide bond between anti-inflammatory and sulphonamide molecule by peptidases and various other amidases present in intestine, but not in stomach, where it was hypothesized to remain as intact molecule. Thus, preventing gastric side-effects produced by NSAID's.

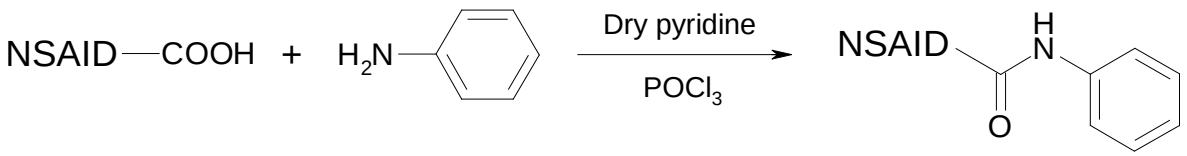
Thus, prompted by the above findings, we aimed to prepare amide-prodrugs of some NSAIDs, having improved pharmacological profile including lesser ulcerogenicity (GIT toxicity). A total of six prodrugs (**Ia-f**) were synthesized through one-pot method (single step synthesis) and tested for their anti-inflammatory and ulcerogenic actions and compared to their corresponding parent drug. Hydrolysis behavior of a prodrug (**Ia**) was also studied by reverse phase HPLC method in hydrochloric acid buffer (pH 1.2), phosphate buffer (pH 7.4), 80% human plasma, 10% rat intestinal homogenate and 10% rat liver homogenate (pH 7.4).

MATERIALS AND METHODS

Chemistry

All the reagents and solvents were obtained from S.D. Fine chemicals or E. Merck (India) Ltd. The NSAIDs were gift samples from ARBRO Pharmaceuticals, New Delhi, India. Melting points were recorded on a liquid paraffin bath in open capillary tubes and are uncorrected. Progress of the reaction mixtures was monitored using silica gel G coated TLC plates in the solvent system Toluene:Ethyl acetate:Formic acid (5:4:1, v/v/v). The spots were located by exposure to iodine vapors or under UV light. ^{1}H-NMR spectra of the prodrugs were recorded on a Bruker spectropsin DPX-300 MHz in DMSO; chemical shift (δ) values are reported in parts per million (*ppm*) using tetramethylsilane as internal reference. Mass spectra were recorded on LCMS/MS (Perkin-Elmer and LABINDIA, Applied Biosystem) Model no. API 3000. IR spectra were recorded on FT/IR (Jasco), Model no.410. Elemental analyses were performed on a Perkin-Elmer 240 analyzer and were found in the range of ± 0.4% for each element analyzed (C,H,N). The protocol for the synthesis of title compounds is presented in **Scheme I**

The HPLC analysis of aceclofenac and its prodrug (**Ia**) was done on HPLC (Shimadzu), Model no. LC-10AT *VP* (Japan) containing a Quaternary pump, UV detector and equipped with C-18 reverse phase column (μ-Bondapak). HPLC-grade solvents were used for HPLC analysis. The mobile phase was prepared by dissolving 500 mg of NaH_2PO_4 in 150mL of water and 850 mL of methanol in a liter volumetric flask. It was degassed and filtered through 0.2 µm Whatmann filter prior to use. The flow rate was 1mL/min and the eluent was monitored at 275 nm through UV detector (Shimadzu).



Prodrug	NSAID
Ia	Aceclofenac
Ib	Diclofenac
Ic	Fenbufen
Id	Indomethacin
Ie	Mefenamic acid
If	4-Biphenyl acetic acid

Scheme 1: Protocol for synthesis of title prodrugs (**Ia-f**).

General procedure for the synthesis of the prodrugs (Ia-f)

To a solution of an appropriate NSAID (aceclofenac/diclofenac/fenbufen/indomethacin/ mefenamic acid/4-biphenyl acetic acid) (4 mmol) in dry pyridine (4-6 mL), dry aniline (equimolar) was added. The reaction flask was then transferred to an ice bath and freshly distilled phosphorous oxychloride (0.5 mL) was added dropwise with continuous stirring. The reaction mixture was stirred for 4 h while maintaining the temperature below 5°C. It was left overnight and then decomposed by adding into ice cold water. A solid mass separated out was filtered, washed with water and crystallized from methanol to give TLC pure compound (**Ia-f**).

2-Oxo-2-(phenylamino) ethyl-2-(2-(2,6-dichlorophenylamino)phenyl)acetate (**Ia**). Light brown crystals; Partition coefficient: 5.72; Yield: 82%; R_f value: 0.68; m.p. 125 ˚C; IR (KBr) (cm^{-1}): 3267 (N-H, amide), 3095 (ArC-H), 1637 (C=O, amide), 1516 (N-H, aromatic amine), 1296 (C-N, aromatic amine), 1096 (C-Cl), 748 (C-N, amide), ^{1}H-NMR (DMSO) δ ppm: 3.94 (s, 2H, CH_2CO), 4.74 (s, 2H, OCH_2), 6.24 (s, 1H, NH), 7.00-7.07 (m, 5H, phenyl), 7.17-7.38 (m, 4H, disubstituted phenyl), 7.51-7.66 (m, 3H, trisubstituted phenyl), 10.11 (s, 1H, CONH). MS (*m/z*): 429/430/431 (M^+/M+1/M+2). Anal Calcd. for $C_{22}H_{18}Cl_2N_2O_3$: C, 61.55; H, 4.23; N, 6.53. Found: C, 61.48; H, 4.16; N, 6.58.

2-[2-(2,6-Dichlorophenylamino)phenyl]-N-phenylacetamide (**Ib**). Light pink crystals; Partition coefficient: 5.93; Yield: 80%; R_f value: 0.69; m.p. 140-142 ˚C; IR (KBr) (cm^{-1}): 3177 (N-H, amide), 3087 (ArC-H), 1640 (C=O, amide), 1515 (N-H, aromatic amine), 1334 (C-N, aromatic amine), 1095 (C-Cl), 723 (C-N, amide). ^{1}H-NMR (DMSO) δ ppm: 3.87 (s, 2H, CH_2CO), 6.29 (s, 1H, NH), 6.80-6.88 (m, 5H, phenyl), 7.04-7.12 (m, 4H, disubstituted phenyl), 7.32 (t, 1H, H-4, trisubstituted phenyl), 7.92 (d, 2H, H-3,5, trisubstituted phenyl), 10.46 (s, 1H, CONH). MS (*m/z*): 371/372/373 (M^+/M+1/M+2). Anal Calcd. for $C_{20}H_{16}C_{l2}N_2O$: C, 64.70; H, 4.34; N, 7.55. Found: C, 64.70; H, 4.34; N, 7.55.

4-(1,1-Biphenyl-4-yl)-4-oxo-N-phenylbutanamide (**Ic**). Cream colored crystals; Partition coefficient: 5.06; Yield: 68%; R_f value: 0.66; m.p. 138 ˚C; IR (KBr) (cm^{-1}): 3211 (N-H, amide), 3056 (ArC-H), 2872 (CH), 1640 (C=O, amide), 732 (C-N, amide). ^{1}H-NMR (DMSO) δ ppm: 2.53 & 3.45 (t, each, 2xCH_2), 7.12-7.89 (m, 14H, 3x phenyl), 9.84 (s, 1H, CONH**)**. MS (*m/z*): 329 (M^+). Anal Calcd. for $C_{22}H_{19}NO_2$: C, 80.22; H, 5.81; N, 4.25. Found: C, 80.01; H, 5.56; N, 4.41.

2-(2-Methyl-5-methoxy-1-(4-chlorobenzoyl)indolin-3-yl)-N-phenylacetamide (**Id**). Light brown crystals; Partition coefficient: 5.28; Yield: 72%; R_f value: 0.68; m.p. 83-85 ˚C; IR (KBr) (cm^{-1}): 3214 (N-H, amide), 3038 (ArC-H), 1637 (C=O, amide), 1265 (C-O-C), 1092 (C-Cl), 732 (C-N, amide). ^{1}H-NMR (DMSO) δ ppm: 2.28 (s, 3H, CH_3), 3.43 (s, 2H, CH_2CO), 3.74 (s, 3H, OCH_3), 6.70-6.92 (m, 3H, indole), 6.95-7.19 (m, 4H, *p*-disubstituted phenyl), 7.27-7.43 (m, 5H, phenyl), 8.60 (s, 1H, CONH). MS (*m/z*): 418/419//420 (M^+)/M+1/M+2). Anal Calcd. for $C_{25}H_{23}ClN_2O_2$: C, 71.68; H, 5.53; N, 6.69. Found: C, 71.37; H, 5.59; N, 6.45.

2-(2,3-Dimethylphenylamino)-N-phenylbenzamide (**Ie**). Pale yellow crystals; Partition coefficient: 5.99; Yield: 71%; R_f value: 0.65; m.p. 60-61 ˚C; IR (KBr) (cm^{-1}): 3125 (N-H, amide), 3068 (ArC-H), 1640 (C=O, amide), 1515 (N-H, aromatic amine), 1312 (C-N, aromatic amine), 750 (C-N, amide). ^{1}H-NMR (DMSO) δ ppm: 2.28 and 2.31 (s, each, 3H, 2xCH_3), 6.06 (s, 1H, NH), 6.67-6.84 (m, 3H, trisubstituted phenyl), 7.06-7.23 (m, 5H, phenyl), 7.47-7.89 (m, 4H, disubstituted phenyl), 8.58 (s, 1H, CONH). MS (*m/z*): 316 (M^+). Anal Calcd. for $C_{21}H_{20}N_2O$: C, 79.72; H, 6.37; N, 8.85. Found: C, 79.68; H, 6.34; N, 8.92.

2-(Biphenyl-4-yl)-N-phenylacetamide (**If**). Brown crystals; Partition coefficient: 4.85; Yield: 75%; R_f value: 0.63; m.p. 95 ˚C; IR (KBr) (cm^{-1}): 3203 (N-H, amide), 3092 (ArC-H), 1640 (C=O, amide), 786 (C-N, amide). ^{1}H-NMR (DMSO) δ ppm: 3.68 (s, 2H, CH_2CO), 7.04-7.12 (m, 4H, *p*-disubstituted phenyl), 7.30-7.44 (m, 5H, phenyl), 7.59-7.63 (m, 5H, phenyl), 8.04 (s, 1H, CONH). MS (*m/z*): 287 (M^+). Anal Calcd. for $C_{20}H_{17}NO$: C, 83.59; H, 5.96; N, 4.87. Found: C, 83.62; H, 6.03; N, 4.89.

Biological evaluation

The experiments were performed on Wistar Allbino rats of either sex, weighing 160-200 g. The protocol of the animal experiments was approved by the Institutional Animal Ethics Committee (IAEC). The *in-vivo* anti-inflammatory activity of the synthesized prodrugs and their parent drugs was evaluated by carrageenan-induced rat paw edema method of Winter *et al* [18]. The drugs (aceclofenac, diclofenac, fenbufen, indomethacin, mefenamic acid and 4-biphenyl acetic acid) were administered orally at a dose of 20 mg/kg of body weight, the prodrugs (**Ia-f**) were administered molecularly equivalent to their corresponding parent drug. The prodrugs and parent NSAIDs were also evaluated for their ulcerogenic potential at different dose levels (25, 50, and 100 mg/kg body weight) by the method of Cioli *et al* [19].

Anti-inflammatory activity

The rats were randomly divided into thirteen groups of six animals in each group. One group was kept as control and received only 0.5% carboxymethyl cellulose (CMC) solution. The other groups received standard drugs (aceclofenac, diclofenac, fenbufen,

indomethacin, mefenamic acid and 4-biphenyl acetic acid; 20 mg/kg, *p.o.*) and test compounds (molecular equivalent; *p.o.*). Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1mL was injected subcutaneously into the sub-plantar region of the right hind paw of each rat, 30 min after the administration of the test compounds and standard drugs. The paw volume was measured by a digital plethysmometer (Panlab; LE 7500) at 2 h and 4h after carrageenan injection. The percentage inhibition of edema was calculated using following formula:

$$\text{Inhibition (\%)} = \frac{[(V_t - V_0)_{control} - (V_t - V_0)_{treated}]}{(V_t - V_0)_{control}} \times 100$$

Where, V_0 and V_t are average volumes of hind paw of rats before any treatment and after the treatment, respectively. The data are expressed as ±SEM, statistical evaluation was performed using analysis of variance followed by t-test for sub-group comparison.

Ulcerogenic assay

Gastrointestinal toxicity (ulcerogenic activity) of the prodrugs was determined at three different doses viz. 25, 50, and 100 mg/kg body weight. The rats were divided into different groups of six in each group. One group served as control and received *p.o.* administration of vehicle (suspension of 1% CMC). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and 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 gastric mucosa of the rats was examined by means of a 4x 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 up to 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. No mucosal damage was found in control group with severity index of 0.0. The data are expressed as ±SEM, statistical evaluation was performed using analysis of variance followed by t-test for sub-group comparison.

Hydrolysis studies

Prodrug **Ia** was studied for its hydrolysis behavior in different systems. The hydrolysis kinetics of the prodrug was studied in acidic buffer (pH 1.2), basic buffer (pH 7.4), 80% human plasma, 10% rat liver homogenate and 10% rat intestinal homogenate in phosphate buffer (7.4).

Kinetic study in acidic buffer (pH 1.2) and in basic buffer (pH 7.4)

Accurately weighed prodrug **Ia** (10 mg) was dissolved in 5 mL of methanol, in a volumetric flask (10 mL capacity) and the flask was kept in a constant temperature bath at 37˚C for 10 min. The contents were then transferred to a vessel of dissolution apparatus (USP dissolution apparatus II i.e., paddle method) [20] containing 995 mL of 0.1N hydrochloric acid buffer (pH 1.2) or phosphate buffer (pH 7.4). The two buffer systems were prepared as per IP method [21]. The contents of the vessel stirred continuously at 100 rpm for 2 h and aliquots of 10 mL were withdrawn at selected time intervals of 5, 30, 60, 120, 180, 240, 300, 360, 420, 480, 560 and 600 minutes. An equal aliquot of fresh 0.1N HCl (pH 1.2) or phosphate buffer (pH 7.4) was added to the vessel immediately.

The aliquots withdrawn were extracted thrice with 5 mL of chloroform. The organic phases were mixed and washed thrice with distilled water (3 mL). The water extracts were discarded. The organic phase was evaporated to dryness. The residue was dissolved in the mobile phase and suitably diluted with it. 20 µL of this solution was injected for direct analysis by HPLC.

Kinetic study in 80% v/v human plasma (pH 7.4)

Hydrolysis kinetics of prodrug **Ia** studied at 37˚C in phosphate buffer (pH 7.4) containing 80% v/v human plasma. The prodrug (10 mg) was dissolved in 5 mL methanol in a 10 mL volumetric flask and kept in a constant temperature bath at 37˚C for 10 minutes. The contents were transferred to a 250 mL beaker containing 95 mL of 80% v/v human plasma (pH 7.4). The contents of the beaker were stirred continuously and aliquots of 2 mL were withdrawn at various time intervals and equal aliquots of 80% v/v human plasma (pH 7.4) were added to the beaker immediately. The samples so withdrawn were shaken and centrifuged for 10 min. The amount of prodrug in supernatant liquid was determined by HPLC.

Kinetic study in 10% w/v rat liver homogenate (pH 7.4)

Hydrolysis kinetics of prodrugs **Ia** studied in phosphate buffer (pH 7.4) containing 10% w/v rat liver homogenate [22, 23]. Wistar rats (150-175 g) were sacrificed by cervical dislocation and the liver was removed, washed and chopped. A 10% w/v suspension of the liver was prepared in phosphate buffer (pH 7.4). The liver was homogenized using a tissue homogenizer and the homogenate was used for hydrolysis purpose. The prodrug Ia (10 mg) was dissolved in 5mL methanol in a 10 mL volumetric flask

and kept in a constant temperature bath at 37˚C for 10 min. The contents were then transferred to a 250 mL beaker containing 95 mL of 10% w/v rat liver homogenate (pH 7.4). The beaker was kept on a rotating shaker (60 rpm) at 37˚C and aliquots of 2 mL were withdrawn at various time intervals. Equal aliquots of 10% w/v rat liver homogenate were added to the beaker immediately. The samples so withdrawn were shaken and centrifuged for 10 min. The amount of prodrug in supernatant liquid was determined by HPLC.

Kinetic study in 10% w/v rat intestinal homogenate (pH 7.4)

Hydrolysis kinetics of prodrug **Ia** studied in phosphate buffer (pH 7.4) containing 10% w/v rat intestinal homogenate [22]. Wistar rats (150-175 g) were sacrificed by cervical dislocation. The intestine was removed and washed free of food matter and chopped. The pieces of intestine were taken in a tared beaker. A 10% w/v suspension of the intestine was prepared in phosphate buffer (pH 7.4). The intestine was homogenized using a tissue homogenizer and the homogenate was used for hydrolysis studies. The prodrug Ia (10 mg) was dissolved in 5mL methanol in a 10 mL volumetric flask and kept in a constant temperature bath at 37˚C for 10 minutes. The contents were then transferred to a 250 mL beaker containing 95 mL of 10% w/v rat intestinal homogenate (pH 7.4). The beaker was kept on a rotating shaker (60 rpm) at 37˚C and aliquots of 2 mL were withdrawn at various time intervals. Equal aliquots of 10% w/v rat intestinal homogenate were added to the beaker immediately. The samples so withdrawn were shaken and centrifuged for 10 min. The amount of prodrug in supernatant liquid was determined by HPLC.

RESULTS AND DISCUSSION

Chemistry

The prodrugs (**Ia-f**) of NSAIDs were successfully synthesized by one-pot method in a single step. These type of compounds are generally synthesized in two steps; preparation of chloroacetyl derivative (-COCl) and then condensation with amino group. In this study, the title prodrugs were synthesized in dry pyridine in presence of $POCl_3$ in one step only. An amide linkage (-CONH-) was established between the free carboxylic group (-COOH) of the NSAID and amino group ($-NH_2$) of aniline. The protocol for the synthesis of the title prodrugs is presented in **Scheme 1**. The structure of the synthesized compound was established on the basis of IR, ^{1}H-NMR, Mass spectra and by elemental analyses results.

Biological evaluation

Carrageenan induced rat paw edema method showed that among all the synthesized 6 prodrugs, 4 prodrugs (**Ia, Ic, Id** & **If**) showed better anti-inflammatory activity as compared to their respective parent drugs (aceclofenac, fenbufen, indomethacin & 4-biphenyl acetic acid) at same dose level (20 mg/kg). 2 Prodrugs (**Ib** & **Ie**) showed slightly lesser activity than that of their respective parent drugs (diclofenac & mefenamic acid). The highest increase in anti-inflammatory activity was observed in the case of **If** which showed 76.14% inhibition whereas its parent drug, 4-biphenyl acetic acid, showed 72.08% inhibition (**Table 1**).

Statistical significance testing using one way analysis of variance showed that the anti-inflammatory activity of standard drug and their prodrug were effective in comparison with the control group.

Table 1: Anti-inflammatory activity of the synthesized prodrugs (Ia-f) and their parent drugs.

Treatment	Mean Paw Volume ± SEM		% Swelling		% Inhibition	
	2nd h	4th h	2nd h	4th h	2nd h	4th h
Control	0.637 ± 0.0005	0.591 ± 0.0005	88.46	74.85	-----	-----
Ia	0.308 ± 0.0008*	0.251 ± 0.0007*	60.42	30.73	51.65	57.53
Aceclofenac	0.324 ± 0.0006*	0.278 ± 0.0006*	66.15	47.10	49.13	52.96
Ib	0.239 ± 0.0009	0.188 ± 0.0011**	49.38	17.50	62.48	68.20
Diclofenac	0.228 ± 0.0006**	0.187 ± 0.0006**	45.22	19.11	64.21	68.36
Ic	0.235 ± 0.0010**	0.198 ± 0.0008**	45.96	22.98	63.11	66.50
Fenbufen	0.263 ± 0.0008*	0.215 ± 0.0006*	61.35	31.90	58.71	63.62
Id	0.222 ± 0.0005**	0.186 ± 0.0005**	40.51	17.72	65.15	68.53
Indomethacin	0.258 ± 0.0006*	0.211 ± 0.0007*	62.26	32.70	59.49	64.30
Ie	0.369 ± 0.0011*	0.321 ± 0.0010*	66.97	45.25	42.07	45.69
Mefenamic acid	0.352 ± 0.0009*	0.314 ± 0.0007*	60.00	42.73	44.74	46.87
If	0.188 ± 0.0005*	0.141 ± 0.0009**	40.30	05.22	70.50	76.14
4-Biphenyl acetic acid	0.210 ± 0.0010*	0.165 ± 0.0005**	46.85	15.38	67.03	72.08

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

In ulcerogenic test, the test results indicated that all the 6 prodrugs were significantly less ulcerogenic than their respective parent drugs at all the three dose levels. Maximum reduction in ulcerogenicity was observed in prodrug **If** which showed severity index of 1.333 whereas its parent drug, 4-biphenyl acetic acid, showed severity index of 2.667 (**Table 2**). The results clearly indicate that the synthesized prodrugs were better (safer) than their parent drugs in terms of GIT toxicity.

Table 2: Ulcerogenic activity (Severity index) of prodrugs and their respective parent drugs

Treatment group	Severity index ± SEM		
	25 mg/kg	50 mg/kg	100 mg/kg
Control	0.0	0.0	0.0
Ia	0.417 ± 0.083**	0.750 ± 0.105*	1.167 ± 0.211*
Aceclofenac	0.834 ± 0.167	1.083 ± 0.153	1.50 ± 0.224
Ib	0.667 ± 0.112*	1.417 ± 0.153*	1.917 ± 0.238*
Diclofenac	1.417 ± 0.201	2.333 ± 0.211	2.667 ± 0.211
Ic	0.750 ± 0.214*	0.916 ± 0.153*	1.50 ± 0.130*
Fenbufen	0.917 ± 0.153	1.333 ± 0.167	1.920 ± 0.238
Id	1.250 ± 0.167*	1.583 ± 0.105*	1.917 ± 0.271*
Indomethacin	2.167 ± 0.167	2.50 ± 0.224	2.833 ± 0.167
Ie	1.0 ± 0.130*	0.916 ± 0.105*	2.0 ± 0.260*
Mefenamic acid	1.333 ± 0.280	2.0 ± 0.260	2.50 ± 0.224
If	0.333 ± 0.103**	0.667 ± 0.206**	1.333 ± 0.206**
4-Biphenyl acetic Acid	1.50 ± 0.182	2.083 ± 0.201	2.667 ± 0.211

Data are represented as mean ± S.E.M., $n = 6$, $^*P < 0.01$, $^{**}P < 0.001$.

Hydrolysis studies

The hydrolysis studies were carried out in acidic buffer (pH 1.2), basic buffer (pH 7.4), 80% v/v human plasma, 10% w/v rat liver homogenate and 10% w/v rat intestinal homogenate in phosphate buffer (pH 7.4) to study the hydrolytic behavior of prodrugs in the above media and thus the fate of the prodrug in the system. Under experimental conditions, the prodrug hydrolyzed to release the parent drugs as evident by HPLC analysis (**Fig. 1**). Negligible hydrolysis was observed in acidic buffer (pH 1.2) system. In other systems, (at constant pH; pH 7.4 and temperature) the prodrug hydrolyzed and the reaction displayed 1st order kinetics as the k_{obs} was fairly constant and a straight line plot could be obtained by plotting log concentration of residual prodrug v/s time. The rate constant and the half life ($t_{1/2}$) of the prodrug were calculated from the linear regression equation by correlating the log concentration of the residual prodrug v/s time (**Fig. 1**). The results indicated that the prodrug **Ia** showed half life ($t_{1/2}$) of 9.63 h with K_{obs} 0.0012 in phosphate buffer system, $t_{1/2}$ of 3.12 h with K_{obs} 0.0037 in 80% human plasma, $t_{1/2}$ of 3.85 h with K_{obs} 0.003 in 10% rat liver homogenate and $t_{1/2}$ of 3.30 h with K_{obs} 0.0012 in 10% intestinal homogenate (**Table 3**).

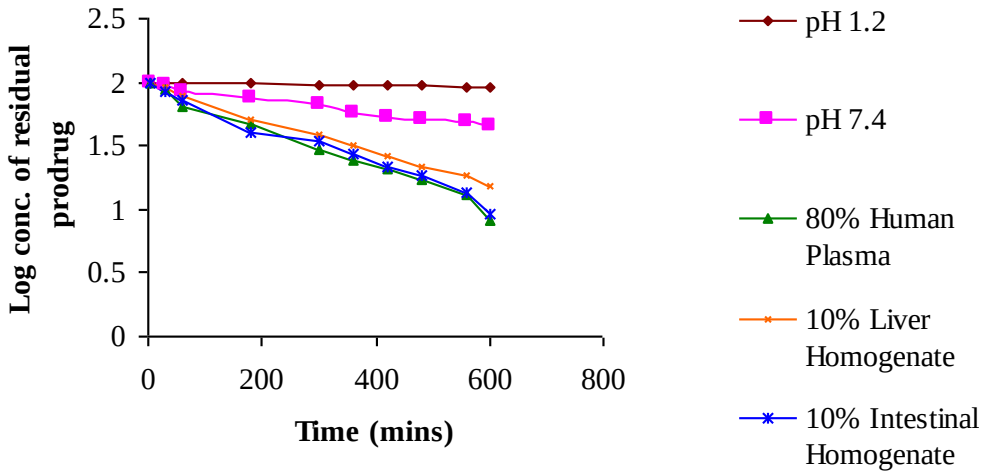


Fig-1: Hydrolysis plot of Ia at pH 1.2, pH 7.4, 80% human plasma, 10% liver homogenate and 10% intestinal homogenate in phosphate buffer (pH 7.4)

Table 3: The observed k values and half lives ($t_{1/2}$) of prodrug (Ia).

Medium	k_{obs}	Half life ($t_{1/2}$) (h)
pH 7.4	0.0012	9.63
80% Human plasma	0.0037	3.12
10% liver homogenate	0.003	3.85
10% intestinal homogenate	0.0012	3.30

CONCLUSION

Six amide-based NSAID prodrugs were successfully synthesized in a single step. Biological activity data results (anti-inflammatory activity) showed that majority of the compounds (**Ia**, **Ic**, **Id** &**If**) showed improved activity as compared to their parent counterparts, yet a slight decrease in activity was observed in few cases (**Ib** & **Ie**). Gastrointestinal toxicity potential of these prodrugs was evaluated at three different doses in rats, and as expected, all of the compounds showed about 50-70% reduction in gastric ulceration to their parent drug. Hydrolysis behavior of the prodrug **Ia** in different systems (acidic buffer, basic buffer, 80% human plasma, 10% rat liver homogenate and 10% rat intestinal homogenate) was studied by HPLC analyses and the rate constant (k_{obs}) and the half-lives ($t_{1/2}$) were calculated from the linear regression equation correlating the log concentration of the residual prodrug v/s time. The results indicated the prodrug **Ia** was resistant to hydrolysis at acidic pH. However, it hydrolyzed to different extent at basic pH in other systems. The present study reveals the importance of exploring old drugs to obtain compounds of potential pharmaceutical interest.

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