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SYNTHESIS, PHARMACOLOGICAL AND TOXICOLOGICAL EVALUATION OF AMIDE DERIVATIVES OF IBUPROFEN

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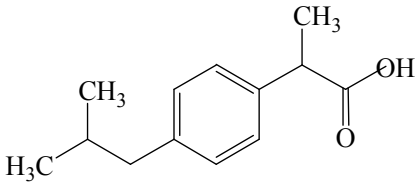
ABSTRACT: Pain, fever and inflammation have been associated with mankind since beginning. Nonsteroidal antiinflammatory drugs (NSAIDs) are the first choice drugs used for the treatment of pain, degenerative inflammatory joint diseases and rheumatic disorders. NSAIDs were found to be associated with undesirable side effects ranging from dyspepsia to symptomatic and complicated gastric and duodenal ulcers. The greatest degree of damage is generally caused by NSAIDs which contain a free carboxylic group. In our approach, Ibuprofen containing free carboxylic group has been modified into various amide derivatives using different aromatic as well as aliphatic amines, which resulted in masking of the carboxylic moiety. Evaluation of the amide derivatives of ibuprofen resulted in improved analgesic, gastroprotective as well as antiinflammatory activity.
Key words: Amide, COX, Ibuprofen, Inflammation, NSAIDs.

1. INTRODUCTION

Pain, fever and inflammation have been associated with mankind since beginning. Nonsteroidal antiinflammatory drugs (NSAIDs) are the first choice of drugs in the treatment of pain, degenerative inflammatory joint diseases and rheumatic disorders. All the NSAIDs, despite differences in their chemical structure, inhibit the enzyme COX and production of prostaglandins. It wasn't until 1990 that the possible existence of two different COX enzymes: COX-1 (constitutive) and COX-2 (inducible) were suggested. Prostaglandins play an essential homeostatic role in cytoprotection of gastric mucosa, hemostasis, renal function, gestation and parturition. There are two isoforms of the COX: COX-1 and COX-2, and inhibition of COX-1 rather than inhibition of COX-2 underlies this gastrointestinal toxicity.[1] Recently existence of a splice variant of COX-1 i.e. COX-3 has also been suggested by some research groups.[2] Though effectively addressing pain and inflammation, NSAIDs were found to be associated with undesirable side effects ranging from dyspepsia to symptomatic and complicated gastric and duodenal ulcers. Traditional NSAIDs differ in their relative inhibitory potency against two isoforms of COX: COX-1 and COX-2. The greatest degree of damage is generally caused by NSAIDs that are preferential COX-1 inhibitors and contain a free carboxylic group e.g. aspirin, indomethacin, ibuprofen etc.[3]

A common strategy in pharmaceutical research consists in the use of well-established drugs as lead compounds to design new drug candidates with improved therapeutic properties. The prodrugs show comparable anti-inflammatory activity and lesser ulcerogenicity in comparison to the parent drug.[4] We have exploited biochemical differences between the two COX enzymes to convert carboxylate-containing NSAID Ibuprofen into gastroprotective amide

prodrugs of ibuprofen, which resulted in masking the free carboxylic group and may shift its enzyme selectivity from COX-1 towards COX-2. The free carboxylic acid group found in NSAIDs such as flurbiprofen and ibuprofen forms critical interactions with residues Arg-120, Glu-524, and Tyr-355 within the cyclooxygenase active site.[5] The masking of the ibuprofen-free carboxylic group seems to be principally the basis of this reduced topical irritant action.[6] Ibuprofen **(1)** has been modified into various heterocyclic amide derivatives[7] having improved analgesic activity and lower ulcerogenic effects, as *N*-(β-hydroxyethyl)-dl-2-(4'-isobutylphenyl)propionamide **(2)** (aminoprofen), an amide derivative of ibuprofen has been used for its topical anti-inflammatory activity.



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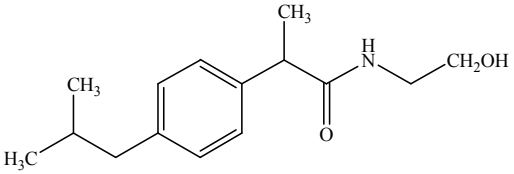

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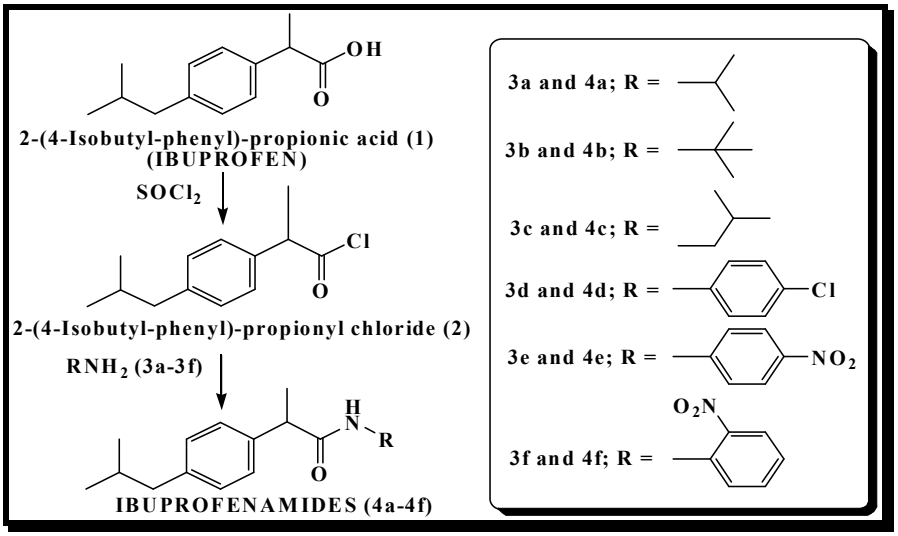
2. Experimental Work

Chemicals and Reagents

All chemicals and reagents were obtained from E. Merck (India) limited, Sigma-Aldrich, Loba Chemie,. All solvents used for chromatography and analytical work were of LR or AR grade and purchased from Merck Ltd. Reactions were monitored and the homogeneity of the products was checked by TLC. Plates for thin layer chromatography (TLC) were prepared with silica gel G and activated at 110° for 30 min. Silica gel G60 F aluminum sheets plates were used for final monitoring. Infrared (IR) spectra were obtained with Perkin Elmer 882 Spectrum and RXI, FT-IR model using a potassium bromide pellets. Elemental analyses were carried out on a Perkin–Elmer 2400 CHN elemental analyzer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with Brucker AC-300F (300 MHz) and FT NMR Avance-II Brucker AC-400F (400 MHz) spectrometers at Regional Sophisticated Instrumentation Centre, Panjab University, Chandigarh. using tetramethylsilane (TMS) used as internal standard. The spin multiplicities are indicated by the symbols: s (singlet), d (doublet), t (triplet), q (quartet), p (pentat), h (heptet), dd (double doublet), dq (double quartet), m (multiplet), bs (broad singlet) and bd (broad doublet). Melting points were determined on Veego-540 melting point apparatus and are uncorrected.

Chemistry

2-(4-Isobutyl-phenyl)-propionic acid (Ibuprofen, **1**) obtained commercially was reacted with thionyl chloride to get 2-(4-isobutyl-phenyl)-propionyl chloride **(2)**. Acid chloride was further reacted with different amines **(3a-f)** in dry pyridine to get ibuprofenamides **(4a-f)** given in scheme 1.[8] Amide prodrugs so synthesized were characterized with the help of elemental analysis and spectroscopic techniques such as: FT-IR, ^{1}H-NMR, ^{13}C-NMR.



Scheme 1

2-(4-Isobutyl-phenyl)-propionyl chloride (2)

Ibuprofen **(1)** (2.06 g, 0.01 mol) was stirred with freshly distilled thionyl chloride (5.95 mL,0.05mol) for 8 hr. Thionyl chloride was removed under reduced pressure to get 2-(4-isobutyl-phenyl)-propionyl chloride.

2-(4-Isobutyl-phenyl)-*N*-(amino)-propionamide (4)

To a mixture of an amine **(3a-3f)** (0.01 mol) and pyridine (2.0 mL) in acetone (25.0 ml) maintained at -10°C was added with stirring a solution of 2-(4-isobutyl-phenyl)-propionyl chloride **(2)** (2.25 g, 0.01 mol) in acetone (25.0 mL) over a period of 1.0 hr. The reaction mixture was stirred for 8 hr and poured into crushed ice. The residue obtained was filtered, dissolved in chloroform (100.0 ml), washed with 5% hydrochloric acid (3 × 50.0 mL), 5% sodium bicarbonate (3 × 50.0 mL) and finally with brine solution (2 × 25.0 mL). The organic layer was filtered, dried and crystallised from peteroleum ether: ethyl acetate (60^o-80^o) to get 2-(4-isobutyl-phenyl)-*N*-(amino)-propionamide **(4a-4f)** .

Spectral Data

Analysis Compound 4a:

IR (KBr): 3307, 3058, 2968, 2872, 1643, 1550, 1460, 1366, 1236 and 848cm^{-1}

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (d, 6H, *J* = 6.6 Hz, -$CH(CH_3)_2$), 1.04 (dd, 6H, *J* = 4.0 and 13.1 Hz -$NHCH(CH_3CH_3)$), 1.46 (d, 3H, *J* = 7.0 Hz, -$CHCH_3$), 1.85 (h, 1H, *J* = 6.7 Hz, -$CH(CH_3)_2$), 2.45 (d, 2H, *J* = 7.2 Hz, Ar-CH_2-), 3.48 (q, 1H, *J* = 7.1 Hz, -$CHCH_3$), 4.03 (m, 1H, -$NHCH(CH_3CH_3)$), 5.11 (bs, 1H, -CONH-, Exchangeable with D_2O), 7.11 (d, 2H, *J* = 8.1 Hz, Ar-H) and 7.18 (d, 2H, *J* = 8.1 Hz, Ar-H)

^{13}C NMR (30 MHz, $CDCl_3$): δ 18.58 (-$CHCH_3$), 22.39 (-$CH(CH_3)_2$), 22.39 (-$NHCH(CH_3CH_3)$), 22.39 (-$NHCH(CH_3CH_3)$), 30.18 (-$CH(CH_3)_2$), 41.41 (-$NHCH(CH_3CH_3)$), 45.05 (-$CHCH_3$), 46.79 (Ar-CH_2-), 127.32 (2 × 2° Ar-C, ibuprofen), 129.59 (2 × 2° Ar-C, ibuprofen), 138.75 (1 × 3° Ar-C, ibuprofen), 140.60 (1 × 3° Ar-C, ibuprofen) and 173.73 (-NHCO-)

Calcd for $C_{16}H_{25}NO$ (%): C, 77.68; H, 10.19; N, 5.66, and, Found: C, 65.81; H, 8.23; N, 5.31

Analysis Compound 4d:

IR (KBr): 3350, 3027, 2955, 1595, 1270 and 834 cm^{-1}

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (d, 6H, *J* = 6.8 Hz, -$CH(CH_3)_2$), 1.59 (d, 3H, *J* = 7.2 Hz, -$CHCH_3$), 1.86 (h, 1H, *J* = 6.8 Hz, -$CH(CH_3)_2$), 2.47 (d, 2H, *J* = 7.2 Hz, Ar-CH_2-), 3.67 (q, 1H, *J* = 7.1 Hz, -$CHCH_3$), 7.16 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.26 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.35 (bs, 1H, Ar-H, -CONH-, Exchangeable with D_2O), 7.53 (d, 2H, *J* = 8.7, Ar-H) and 7.89 (d, 2H, *J* = 8.9, Ar-H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 18.42 (-$CHCH_3$), 22.41 (-$CH(CH_3)_2$), 26.44 (-$CH(CH_3)_2$), 30.20 (Ar-$COCH_3$), 45.05 (-$CHCH_3$), 47.86 (Ar-CH_2-), 118.86 (2 × 2° Ar-C), 127.41 (2 × 2° Ar-C), 129.69 (2 × 2° Ar-C), 129.96 (2 × 2° Ar-C), 133.81 (1 × 3° Ar-C), 135.44 (1 × 3° Ar-C), 138.23 (1 × 3° Ar-C), 141.08 (1 × 3° Ar-C), 173.10 (-NHCO-) and 197.13 (Ar-$COCH_3$)

Calcd for $C_{21}H_{25}NO_2$ (%): C, 77.98; H, 7.79; N, 4.33, and, Found: C, 65.81; H, 8.23; N, 3.16 .

3. BIOLOGICAL STUDIES

Animals

Rats (male, Wistar, 150-200g) and mice (male, LACA, 25-35g) procured from Central Animal House, Panjab University, Chandigarh, India were used. Animals were housed under standard laboratory conditions and maintained on rat chow. Animals were allowed free access to food and water until used and fasted 24 hr prior to studies. All the experiments were carried out 'blind' in that the observer was not aware of the identity or dose of drugs administered to individual animals. All the experiments used for pharmacological and toxicological evaluation of drugs and synthesized derivatives had prior approval of the Institutional Animal Ethical Committee, Panjab University, Chandigarh.

Experimental Conditions

Unless otherwise stated, the following conditions were employed in all experiments. The test compounds were suspended in 0.5% carboxymethylcellulose (CMC) or 0.9% w/v NaCl containing 20% v/v tween-80, and administered per orally (p.o) or intra peritonially (i.p.), respectively. Control animals were given the corresponding amount of vehicle (0.5% CMC or 0.9% w/v NaCl containing 20% v/v tween-80). The test drugs were administered on molar equivalent basis of ibuprofen. A total number of 6-8 animals were taken in each group and the animals showing greatest and lowest response in a particular experiment were discarded during final calculations.

Statistical Analysis

Results are expressed as mean ± standard error of mean (SEM) of atleast 6 animals per group. Statistical significance of differences between groups was determined by one-way analysis of variance (ANOVA) followed by Dunnet's test. For the statistical determination, statistical computerized software SIGMASTAT was used. A probability (P) value of less than 0.05 was taken to indicate statistical significance.

4. Pharmacological Studies

Anti-Inflammatory Activity

Paw edema in rats is the most widely used method for for testing acute and subacute inflammation. It is based upon the ability of anti-inflammatory agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Percentage change (increase) in paw volume was calculated **(Table1**) and expressed as the amount of inflammation.[9, 10]

➢ % increase in paw volume at any time = $(V_l - V_r)/ V_r \times 100$
where, V_l = Volume of left paw
V_r = Volume of right paw (control)

Analgesic Activity

Analgesic activity was determined against acetic acid induced writhing assay.[11,12] Writhing was induced by intraperitoneal (i.p.) injection of freshly prepared acetic acid solution. The average number of writhes in each group of drug treated mice was compared with that of the control group and degree of analgesia was expressed as % inhibition (**Table 2, Figure1**) calculated from the equation:

$$\% \text{ Inhibition} = (1 - N_t/N_c) \times 100$$

where, N_c = number of writhes in control
N_t = number of writhes in drug treated mice

Anti-Ulcer Activity

Antiulcer potential of the derivatives of salicylamide, ketoprofen and ibuprofen was found. Gastric irritation properties of orally administered NSAIDs can be evaluated in fasted rats by sacrificing after predetermined time intervals. The stomachs can be removed and inspected for irritation and ulcers. The ulcers were scored on the following scale: 0 (Normal colored stomach), 0.5(Red coloration), 1.0 (Spot ulcers), 1.5 (Haemorrhagic streaks), 2.0 (Ulcers > 3 but < 5), 3.0(Ulcers > 5).[13,14] Ulcer index of all the derivatives of the Ibuprofen has been given (**Table3,Figure2).**

5. RESULTS AND DISCUSSION

Ibuprofen containing free carboxylic group has been modified into various amide derivatives using different aromatic as well as aliphatic amines, resulted in masking of the carboxylic moiety. The newly synthesized prodrugs were evaluated for their analgesic activity (acetic acid induced writhing assay), anti-inflammatory activity (carrageenan induced rat paw edema model) and antiulcer activity (ulcer index). Some of these compounds have shown better pharmacological activity than the parent drug, and had lesser gastrotoxicity. The carboxylic group of the NSAIDs can be temporarily masked and its direct effect on the gastric mucosa will be prohibited. Transformation of the aryl acetic moiety of ibuprofen to amides furnishes molecules which have better activity and lesser toxicity than the parent drug.

Table 1: Anti inflammatory activity of amide derivatives (4a- 4f) of Ibuprofen

Compound	% Increase in paw volume mean ± SEM	
	2 hrs	4 hrs
Control	42.85 ± 1.32	66.99 ± 1.88
Ibuprofen	17.81 ± 0.71	25.82 ± 0.68
4a	16.81 ± 0.83	25.96 ± 1.01
4b	22.61 ± 0.67	35.52 ± 0.71
4c	23.67 ± 0.04	35.61 ± 0.55
4d	22.61 ± 0.67	31.51 ± 0.71
4e	21.02 ± 1.23	30.81 ± 0.85
4f	27.63 ± 0.94	43.35 1.27

Table 2: Analgesics activity of amide derivatives (4a- 4f) of Ibuprofen

Compound	% Inhibition in writhings ± S.E.M.
Control	12.12±0.98
Ibuprofen	83.29±1.24
4a	54.43±2.72
4b	51.90±1.34
4c	68.10±1.04
4d	69.37±1.64
4e	58.99±1.36
4f	75.69±2.69

Table 3: Ulcer index of amide derivatives (4a- 4f) of Ibuprofen

Compound	Ulcer Index±S.E.M.
Control	0.13±0.08
Ibuprofen	4.13±0.29
4a	0.50±0.19
4b	0.63±0.12
4c	0.75±0.16
4d	1.06±0.15
4e	0.87±0.19
4f	0.94±0.18

Figure 1: Analgesic activity of amide derivatives (4a- 4f) of Ibuprofen

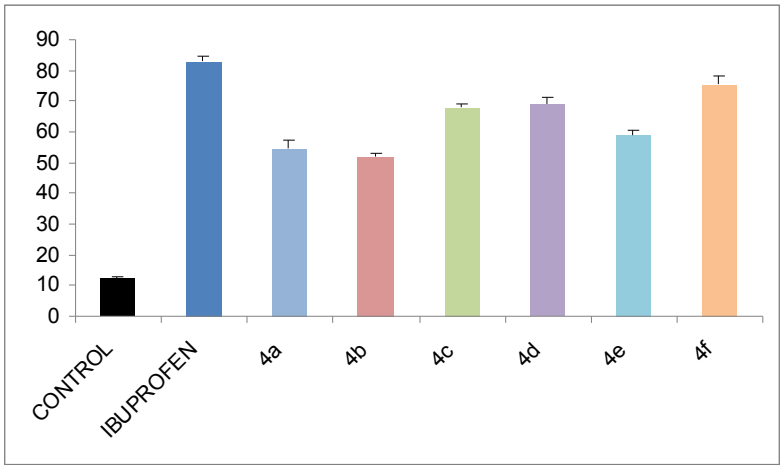
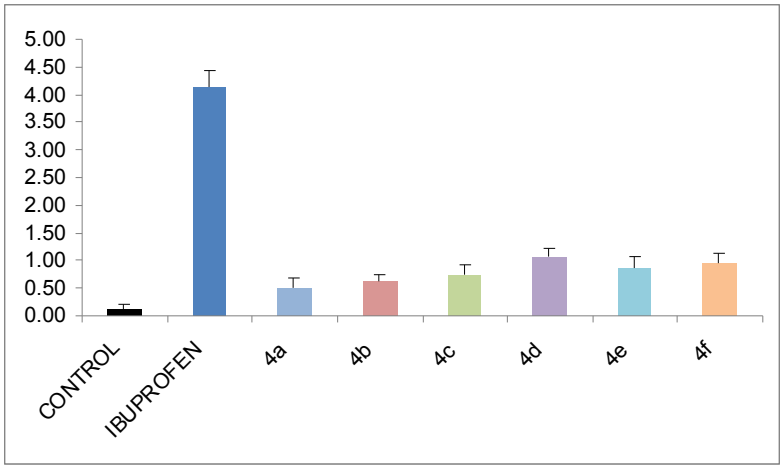


Figure 2: Ulcer index of amide derivatives (4a- 4f) of Ibuprofen



6. ACKNOWLEDGEMENT

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