Design and Synthesis of Stable N-[2-(aryl/heteroaryl substituted)ethyl] propanamide Derivatives of (S)-Ketoprofen and (S)-Ibuprofen as Non-Ulcerogenic Anti-Inflammatory and Analgesic Agents

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ABSTRACT

The carboxylic acid groups of (*S*) ketoprofen and (*S*) ibuprofen were brought into reaction with substituted ethylamine derivatives to form (*S*)-2-(4-isobutylphenyl)- and (*S*)-2-(3-benzoylphenyl)-*N*-[2-(aryl/heteroaryl substituted) ethyl]propanamide derivatives. Then, these sets were evaluated in terms of their in vivo anti-inflammatory and analgesic properties using the carrageenan-induced paw edema and *p*-benzoquinone-induced writhing models. Among the synthesized compounds, (*S*)-2-(4-isobutylphenyl)-*N*-[2-(pyrrolidin-1-yl)ethyl]propanamide (4f) showed the highest activity at the 100mg/kg dose inducing no gastric lesions when compared to the parent compound, ibuprofen. In vitro studies on chemical stability revealed that the amide derivative with the highest activity (4f) was chemically stable in simulated gastric (pH 1.2) and intestinal fluids (pH 7.4). In 80% v/v human plasma, the amide derivative was found to be stable against plasma hydrolases over the experimental period. The most active compound, (*S*)-2-(4-isobutylphenyl)-*N*-[2-(pyrrolidin-1-yl)ethyl]propanamide, was also studied in 10% rat liver homogenate (pH 7.4) to identify its release pattern as a prodrug.

Keywords: Ketoprofen, Ibuprofen, anti-inflammatory, NSAIDs

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed medication in the management of pain, inflammation, and fever. They exert their therapeutic activity by non-selectively inhibiting cyclooxygenase-derived prostaglandin synthesis[1-2]. This mechanism of action is inherently responsible for their gastrointestinal (GI) [3-7], renal [8-10] and hepatic [11-13] side effects observed in

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long-term treatments. To improve the GI safety profile of NSAIDs, the following four strategies have been identified: (a) development of selective cyclooxygenase-2 (COX-2) inhibitors; (b) co-administration of a proton pump inhibitor with the NSAID; (c) linking a nitrate-based nitric oxide (NO)-releasing moiety to classical NSAIDs (NO-NSAIDs); and (d) preparing ester or amide derivative as prodrugs. The former three strategies have different advantages and limitations. For example, despite the relatively safe profile of COX-2 inhibitors in the GI tract, their adverse cardiovascular effects reported in some patients undergoing chronic treatment have attracted considerable attention, which resulted in the withdrawal of rofecoxib from the market[14-15]. Organic nitrate-based NO-NSAIDs such as NCX-4016[16], nitronaproxen[17], NCX-2216[18-19] and NO-diclofenac (5)[20] suppress prostaglandin synthesis as effectively as the parent NSAIDs [21-23] but have been shown not to impair the GI tract both in animals and humans. However, an important drawback to this design is the fact that production of NO from nitrate esters requires a three-electron reduction, and this metabolic activation can decrease the efficiency of these drugs when they are used continuously, thus increasing nitrate tolerance[24-26].

The fourth strategy is based on the fact that GI mucosal injury is caused by two different mechanisms[27-29]. The primary mechanism involves a local action comprising a direct contact mechanism, and an indirect effect on the GI mucosa. The direct effect can be attributed to a combination of local irritation produced by the acidic group of the NSAIDs and local inhibition of prostaglandin synthesis in the GI tract. The indirect effect is associated with a combination of an ion-trapping mechanism of NSAIDs in mucosal cells and back diffusion of hydrogen ions from the lumen into the mucosa. The subsequent mechanism is based on a generalized systemic action occurring after absorption, which can be demonstrated following intravenous dosing. These direct and indirect effects can be altered by producing amide and ester derivatives of these structures as prodrugs[30-35].

Ketoprofen and ibuprofen are aryl propionic acid derivatives with known GI side effects of the prolonged use of frequently prescribed NSAIDs. In this present study, we synthesized *N*-[2-(aryl/heteroaryl substituted)ethyl]propanamide derivatives of ketoprofen and ibuprofen (compounds 3a-f and 4a-f) to investigate their anti-inflammatory properties, GI ulceration, and their potential as analgesics and prodrugs.

The idea of using an ethylene linker between the amide and R functional moieties is substantiated by our recent report and other studies using similar derivatives of naproxen[36]. Although compounds 3a, 4c, 4d and 4e had been previously synthesized using different methods and investigated in other subjects[37-41], they were resynthesized and included in pharmacological and kinetic studies.

METHODOLOGY

Chemistry

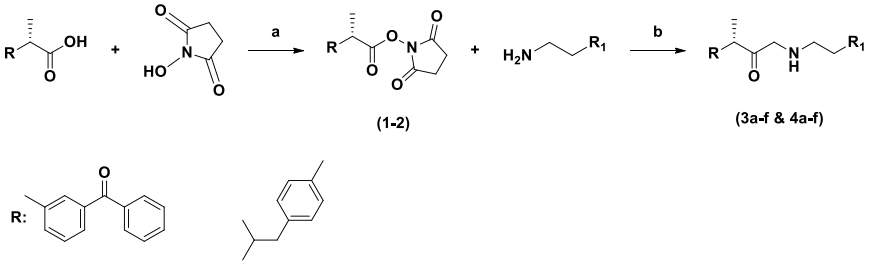
All chemicals were purchased from Aldrich Chemical Co. (Steinheim, Germany). Melting points were detected with a Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA) and uncorrected. IR spectra (KBr) were recorded on a Perkin Elmer 1720X FT-IR spectrometer (Beaconsfield, UK) and ^{1}H-NMR spectra were obtained by Bruker DPX-400, 400 MHz High Performance Digital FT-NMR using DMSO-d6 and tetramethylsilane as internal standard. All chemical shift values were recorded as δ (ppm). Mass spectra were recorded using an Agilent 1100 series LC/APCI/MS 1946 G spectrometer in the negative ionization mode. The purity of the compounds was checked by thin-layer chromatography on silica gel-coated aluminum sheets (Merck, 1.005554, silica gel HF254–361, Type 60, 0.25 mm; Darmstadt, Germany). The elemental analyses were performed with a Leco CHNS 932 analyzer (Leco Corp., MI, USA). Elemental analysis for C, H and O were within ± 0.4 % of the theoretical values. ^{1}H-NMR spectra and elemental analysis were performed at the Instrumental Analysis Laboratory of the Scientific and Technical Research Council of Turkey in Ankara.

The HPLC analyses of ibuprofen and the (*S*)-2-(4-isobutylphenyl)-*N*-[2-(pyrrolidin-1-yl)ethyl]propanamide (4f) derivative were performed on an Agilent 1100 series LC spectrometer containing a quaternary pump, multiple wave length UV detector equipped with a C-18 reverse phase column (μ-Bondapak). HPLC-grade solvents were used for HPLC analyses. The mobile phase was prepared by dissolving 500 mg of NaH_2PO4 in 150mL of water and 850 mL of methanol in a one-liter volumetric flask 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 using the detector. Naproxen was used as an internal standard.

General synthesis for amide derivatives

To the ice-cooled solution of (*S*)-ketoprofen or (*S*)-ibuprofen (22 mmol) and *N*-hydroxysuccinimide (28 mmol) in tetrahydrofuran (THF) (20 mL) were added equimolar (28 mmol) *N*,*N'*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in 20 mL dichloromethane (DCM). The mixture was stirred for 2 hours at 0°C, and refrigerated overnight for the total precipitation of dicyclohexylurea (DCU). After filtering DCU, the solution was concentrated in a vacuum. The leftover was washed with a $NaHCO_3$ solution, extracted with DCM and the separated DCM phase was evaporated. The residue was solidified with ether and crude precipitates of either (*S*)-2,5-dioxopyrrolidin-1-yl 2-(3-benzoylphenyl)propanoate **(1)** or (*S*)-2,5-dioxopyrrolidin-1-yl 2-(4-isobutylphenyl)propanoate **(2)** crystallized from ethanol-water. Then equimolar appropriate ami-

nes were added to the solution of compounds 1 or 2 (0.01 mol) dissolved in the mixture of THF and DCM (20:10 mL), and refluxed for 30 min. The precipitates of the derivatives were filtered directly or the mixture was evaporated and triturated with diethyl ether, crystallized from 1,4-dioxane-water.



R_1: Phenyl, pyridin-2-yl, piperidin-1-yl, morpholin-4-yl, 1-methylpyrrolidin-2-yl, pyrrolidin-1-yl

Scheme 1: Synthetic pathway followed for the preparation of (*S*)-2-(4-isobutylphenyl) and (*S*)-2-(3-benzoylphenyl)-*N*-[2-(aryl/heteroaryl substituted)ethyl]propanamide derivatives (Compounds 3a-f &4 a-f)

Reagents and conditions: (a) DCC, DMAP, DCM, 0 °C; 2h, 4 °C; overnight; conc. $NaHCO_3$ (b) THF/DCM, reflux

(*S*)-2-(3-benzoylphenyl)-*N*-phenethylpropanamide (Compound 3a)

Yield 89%, m.p. 150-151 °C. IR (KBr) ν (cm^{-1}): 1655 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 1.28 (d, 3H, CH_3), 2.74 (t, 2H, CH_2-Ph.), 3.21 (t, 2H, CH_2-NH), 3.52 (q, 1H, CH-CO), 7.1-7.8 (m, 14H, Ph. H), 8.2 (s, 1H, NH). MS 357.2 (M^+). Anal. calcd for $C_{24}H_{23}NO_2$; C, 80.64; H, 6.49; N, 3.92 Found; C, 80.68; H, 6.45; N, 3.96.

(*S*)-2-(3-benzoylphenyl)-*N*-[2-(pyridin-2-yl)ethyl]propanamide (Compound 3b)

Yield 86 %, m.p. 135-136 °C. IR (KBr) ν (cm^{-1}): 1651 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 1.28 (d, 3H, CH_3), 3.14 (t, 2H, CH_2-Py.), 3.46-3.53 (m, 3H, CH-CO and CH_2-NH), 7.20-8.00 (m, 12H, Ph. H and Py. H), 8.2 (s, 1H, NH), 8.45 (d, 1H, Py. H). MS 358.2 (M^+). Anal. calcd for $C_{23}H_{22}N_2O_2$; C, 77.07; H, 6.19; N, 7.82 Found; C, 77.04; H, 6.15; N, 7.85.

(*S*)-2-(3-benzoylphenyl)-*N*-[2-(piperidin-1-yl)ethyl]propanamide (Compound 3c)

Yield 85 %, m.p. 147-148 °C. IR (KBr) ν (cm^{-1}): 1653 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 1.28 (d, 3H, CH_3), 1.53-1.59 (m, 6H, Pip. H), 2.45 (t, 4H, Pip. H), 2.62 (t, 2H, CH_2-Pip.), 3.30 (t, 2H, CH_2-NH), 3.52 (q, 1H,

CH-CO), 7.40-7.80 (m, 9H, Ph. H), 8.0 (s, 1H, NH). MS 364.2 (M^+). Anal. calcd for $C_{23}H_{28}N_2O_2$; C, 75.79; H, 7.74; N, 7.69 Found; C, 75.76; H, 7.77; N, 7.67.

(*S*)-2-(3-benzoylphenyl)-*N*-[2-(morpholin-4-yl)ethyl]propanamide (Compound 3d)

Yield 78 %, m.p. 143-144 °C. IR (KBr) ν (cm^{-1}): 1650 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 1.28 (d, 3H, CH_3), 2.41 (t, 4H, Mor. H), 2.53 (t, 2H, CH_2-Mor.), 3.30 (t, 2H, CH_2-NH), 3.52 (q, 1H, CH-CO), 3.65 (t, 4H, Mor. H), 7.3-7.60 (m, 9H, Ph. H), 8.0 (s, 1H, NH). MS 366.2 (M^+). Anal. calcd for $C_{22}H_{26}N_2O_3$; C, 72.11; H, 7.15; N, 7.64 Found; C, 72.09; H, 7.11; N, 7.66.

(2*S*)-2-(3-benzoylphenyl)-*N*-[2-(1-methylpyrrolidin-2-yl)ethyl] propanamide (Compound 3e)

Yield 89 %, m.p. 159-160 °C. IR (KBr) ν (cm^{-1}): 1653 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 1.28 (d, 3H, CH_3), 1.40-1.60 (m, 6H, CH_2-Pyr and Pyr. H), 2.20-2.30 (m, 3H, Pyr. H), 2.32 (s, 3H, N-CH_3), 3.20 (t, 2H, CH_2-NH), 3.52 (q, 1H, CH-CO), 7.30-7.60 (m, 9H, Ph. H), 8.0 (s, 1H, NH). MS 364.2 (M^+). Anal. calcd for $C_{23}H_{28}N_2O_2$ Calc; C, 75.79; H, 7.74; N, 7.69 Found; C, 75.77; H, 7.78; N, 7.71.

(*S*)-2-(3-benzoylphenyl)-*N*-[2-(pyrrolidin-1-yl)ethyl]propanamide (Compound 3f)

Yield 80 %, m.p. 158-159 °C. IR (KBr) ν (cm^{-1}): 1650 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 1.28 (d, 3H, CH_3), 1.68 (t, 4H, Pyr. H), 2.25 (t, 4H, Pyr. H), 2.62 (t, 2H, CH_2-Pyr.), 3.30 (t, 2H, CH_2-NH), 3.52 (q, 1H, CH-CO), 7.30-7.60 (m, 9H, Ph. H), 8.2 (s, 1H, NH). MS 350.2 (M^+). Anal. calcd for $C_{22}H_{26}N_2O_2$; C, 75.40; H, 7.48; N, 7.99 Found; C, 75.37; H, 7.46; N, 8.02.

(*S*)-2-(4-isobutylphenyl)-*N*-phenethylpropanamide (Compound 4a)

Yield 91 %, m.p. 148-149 °C. IR (KBr) ν (cm^{-1}): 1651 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 0.84 (d, 6H, CH_3), 1.28 (d, 3H, CH_3), 1.82 (m, 1H, $(CH_3)_2$-CH), 2.43 (d, 2H, CH-CH_2), 2.74 (t, 2H, CH_2-Ph.), 3.21 (t, 2H, CH_2-NH), 3.52 (q, 1H, CH-CO), 7.0–7.3 (m, 9H , Ph. H), 7.9 (s, 1H, NH). MS 309.2 (M^+). Anal. calcd for $C_{21}H_{27}NO$; C, 81.51; H, 8.79; N, 4.53. Found; C, 81.48; H, 8.77; N, 4.56.

(*S*)-2-(4-isobutylphenyl)-*N*-[2-(pyridin-2-yl)ethyl]propanamide (Compound 4b)

Yield 88 %, m.p. 133-134 °C. IR (KBr) ν (cm-1): 1652 (C=O, amide). ^{1}H-NMR (DMSO-d6, 400 MHz), δ (ppm): 0.87 (d, 6H, CH_3), 1.28 (d, 3H, CH_3), 1.82 (m, 1H, $(CH_3)_2$-CH), 2.43 (d, 2H, CH-CH_2.), 3.14 (t, 2H, CH_2-Py.), 3.21 (t, 2H, CH_2-

NH), 3.52 (q, 1H, CH-CO), 7.1–7.24 (m, 4H , Ph. H), 7.26-7.30 (m, 2H, Py. H), 7.58 (t, 1H, Py. H), 8.0 (s, 1H, NH), 8.4 (d, 1H, Py. H). MS 310.2 (M^+). Anal. calcd for $C_{20}H_{26}N_2O$; C, 77.38; H, 8.44; N, 9.02 Found; C, 77.36; H, 8.41; N, 9.04.

(*S*)-2-(4-isobutylphenyl)-*N*-[2-(piperidin-1-yl)ethyl]propanamide (Compound 4c)

Yield 84 %, m.p. 145-146 °C. IR (KBr) ν (cm^{-1}): 1654 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 0.87 (d, 6H, CH_3), 1.28 (d, 3H, CH_3), 1.52-1.55 and 2.24 (m and t, 6H and 4H, Pip. H), 1.82 (m, 1H, $(CH_3)_2$-CH), 2.43 (d, 2H, CH-CH_2), 2.62 (t, 2H, CH_2-Pip.), 3.30 (t, 2H, CH_2-NH, 3.52 (q, 1H, CH-CO), 7.1–7.3 (m, 4H, Ph. H), 8.0 (s, 1H, NH). MS 316.3 (M^+). Anal. calcd for $C_{20}H_{32}N_2O$; C, 75.90; H, 10.19; N, 8.85 Found; C, 75.92; H, 10.17; N, 8.83.

(*S*)-2-(4-isobutylphenyl)-*N*-[2-(morpholin-4-yl)ethyl]propanamide (Compound 4d)

Yield 75 %, m.p. 141-142 °C. IR (KBr) ν (cm^{-1}): 1653 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 0.87 (d, 6H, CH_3), 1.28 (d, 3H, CH_3), 1.82 (m, 1H, $(CH_3)_2$-CH), 2.41 (t, 4H, Mor. H), 2.43 (d, 2H, CH-CH_2), 2.62 (t, 2H, CH_2-Mor.), 3.30 (t, 2H, CH_2-NH), 3.52 (q, 1H, CH-CO), 3.65(t, 4H Mor. H), 7.1–7.3 (m, 4H, Ph. H), 8.0 (s, 1H, NH). MS 318.2 (M^+). Anal. calcd for $C_{19}H_{30}N_2O_2$; C, 71.66; H, 9.50; N, 8.80 Found; C, 71.65; H, 9.53; N, 8.81.

(2*S*)-2-(4-isobutylphenyl)-*N*-[2-(1-methylpyrrolidin-2-yl)ethyl] propanamide (Compound 4e)

Yield 86 %, m.p. 154-155 °C. IR (KBr) ν (cm^{-1}): 1654 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 0.87 (d, 6H, CH_3), 1.28 (d, 3H, CH_3), 1.40-1.60 (m, 6H, CH_2-Pyr. and Pyr. H), 1.82 (m, 1H, $(CH_3)_2$-CH), 2.20-2.30 (m, 6H, N-CH_3 and Pyr. H), 2.43 (d, 2H, CH-CH_2.), 3.20 (t, 2H, CH_2-NH), 3.52 (q, 1H, CH-CO), 7.1–7.3 (m, 4H, Ph. H), 8.0 (s, 1H, NH). MS 316.3 (M^+). Anal. calcd for $C_{20}H_{32}N_2O$; C, 75.90; H, 10.19; N, 8.85 Found; C, 75.87; H, 10.14; N, 8.88.

(2*S*)-2-(4-isobutylphenyl)-*N*-[2-(pyrrolidin-1-yl)ethyl]propanamide (Compound 4f)

Yield 82 %, m.p. 156-157 °C. IR (KBr) ν (cm^{-1}): 1650 (C=O, amide). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 0.87 (d, 6H, CH_3), 1.28 (d, 3H, CH_3), 1.68 (t, 4H, Pyr. H), 1.82 (m, 1H, $(CH_3)_2$-CH), 2.43 (d, 2H, CH-CH_2), 2.50 (t, 4H, Pyr. H), 2.62 (t, 2H, CH_2-Pyr.), 3.30 (t, 2H, CH_2-NH, 3.52 (q, 1H, CH-CO), 7.1–7.3 (m, 4H, Ph. H), 8.0 (s, 1H, NH). MS 302.3 (M^+). Anal. calcd for $C_{19}H_{30}N_2O$; C, 75.45; H, 10.00; N, 9.26 Found; C, 75.41; H, 9.97; N, 9.30.

Pharmacological Screening

Animals

Male Swiss albino mice (25–30 g) and male Wistar rats weighing 160-200 g. were purchased from the animal breeding laboratories of Refik Saydam Hıfzısıhha Institute in Ankara, Turkey. For acclimatization, the animals were housed in a room with controlled temperature (22± 1 °C), humidity (55 ± 10%) and photoperiod (12:12 h) for one week. They were maintained on a standard pellet diet and water ad libitum throughout the experiment. The food was withheld on the day before the experiments only allowing free access to water. A minimum of six animals was used in each group for the examination of anti-inflammatory, analgesic and gastric ulcerogenic effects and only two animals were sacrificed for liver extracts. All the animal manipulations and experiments were carried out according to the rules and approval of the Ethical Committee for the use and care of laboratory animals of Gazi University, Ankara, Turkey.

Anti-inflammatory activity

The carrageenan-induced hind paw edema model was used to determine the anti-inflammatory activity[42]. Each group contained a minimum of six animals. Sixty min after the subcutaneous administration of a test sample (100 mg/kg body weight suspended in 0.5 % sodium carboxymethyl cellulose (CMC)) or dosing vehicle, each mouse was injected with a freshly prepared suspension of carrageenan (0.5 mg/25 µL) in physiological saline into the sub-plantar tissue of the right hind paw. As a control, 25 µL of saline was injected into the same tissue on the left side. Paw edema was measured 90, 180, 270, and 360 min after the induction of inflammation. The difference between the thicknesses of the right and left hind paws were measured using a caliber compass (Ozaki Co., Tokyo, Japan). The mean values obtained for each study group were compared with the control group and analyzed using statistical methods.

Analgesic activity

Analgesic activity was measured using phenyl-*p*-benzoquinone (PBQ)-induced writhing (abdominal constriction) test in mice[43]. According to the protocol, 30 min after the subcutaneous administration of a test sample (100 mg/kg body weight), the mice were intraperitoneally injected with 0.1 mL/10 g body weight of 2.5% (w/v) PBQ solution in distilled water. The control animals received an appropriate volume of the dosing vehicle. The mice were then kept individually for observation, and the total number of abdominal contractions (writhing movements) was counted for the next 15 min starting on the 5th minute after the PBQ injection. The data represent the average of the total number of writhing

movements observed. Analgesic activity was then expressed as the change in percentage compared to the writhing controls.

Gastric ulcerogenic effect

The ulcerogenic effect was investigated as described in a previous publication[44]. The animals were sacrificed with an overdose of diethyl ether 270 min after the administration of the compounds. Following abdominal dissection, the stomachs of the animals were taken out. Then, the esophagus was tied in a knot near to the cardia by a surgical suture. From the duodenum side, 2.5 mL of a 10% formalin solution was injected into the stomach. The distended stomach was immediately tied to the pyloric sphincter using another surgical suture to avoid leakage of the formalin solution. Finally, the stomachs were removed from the abdominal cavity and immersed in the same solution to fix the outer layer of the stomach. Each stomach was then dissected along the greater curvature, rinsed with tap water to remove the gastric contents and examined under a dissecting microscope to assess the formation of ulcers. Lesions and bleeding points were counted and documented.

Statistical analysis

The data were expressed as means ± SEM. The significance of differences between the treatment and control groups were determined using one-way ANOVA with Bartlett's test following a post hoc Student-Newman-Keuls multiple comparison test for analgesic activity, and two-way ANOVA following a post hoc Bonferroni test for anti-inflammatory activity. Values of $p<0.05$ were considered statistically significant.

Hydrolysis Studies

The most active compound, 4f, was analyzed for its hydrolysis behavior in an acidic buffer (simulated gastric fluid, pH 1.2), basic buffer (simulated intestine fluid, pH 7.4), 80% human plasma and 10% rat liver homogenate.

Acidic and basic buffers (pH 1.2 and 7.4)

In a 10 mL capacity volumetric flask, accurately weighed amount of compound 4f (10 mg) was dissolved in 5 mL methanol and kept in a bath at a constant temperature of 37°C for 10 min. The contents were then transferred to a vessel of dissolution apparatus containing 995 mL of 0.1N hydrochloric acid buffer (pH 1.2) or phosphate buffer (pH 7.4). The vessels were stirred continuously at 100 rpm 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, immediately followed by the addition of an equal aliquot of fresh 0.1N HCl (pH 1.2) or phosphate

buffer (pH 7.4). The aliquots withdrawn were extracted thrice with 5 mL 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 and diluted with the mobile phase. 20 μL of this solution was directly injected into HPLC for analysis.

80% v/v human plasma (pH 7.4)

In a 10 mL capacity volumetric flask, accurately weighed amount of compound 4f (10 mg) was dissolved in 5 mL methanol and kept in a bath at a constant temperature of 37 °C for 10 min. The content was transferred to a 250 mL beaker containing 95 mL of 80% v/v human plasma (pH 7.4) and stirred continuously. Aliquots of 2 mL were withdrawn at various time intervals, immediately followed by the addition of equal aliquots of 80% v/v human plasma (pH 7.4). The samples were shaken and centrifuged for 10 min. The amount of compound in the supernatant liquid was determined by HPLC.

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

The Wistar rats were sacrificed by cervical dislocation, and the liver was removed, washed and chopped. A 10% w/v suspension of the liver was prepared in a phosphate buffer (pH 7.4). The liver was homogenized using a tissue homogenizer to be used for hydrolysis. Compound 4f (10 mg) was dissolved in 5mL methanol in a 10 mL volumetric flask and kept in a bath at a constant temperature of 37°C for 10 min. Then, the content of the flask was 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, immediately followed by the addition of equal aliquots of 10% w/v rat liver homogenate. The samples were shaken and centrifuged for 10 min. The amount of compound in the supernatant liquid was determined by HPLC.

RESULTS AND DISCUSSION

The proposed *N*-(2-substitutedethyl)propanamide derivatives of (*S*) ketoprofen and (*S*) ibuprofen were successfully synthesized using the conventional DCC/DMAP method giving yields between 75-91%. In the IR spectra, all the compounds had a strong C=O stretching band at 1650–1655 cm^{-1}, which was accepted as an evidence for the formation of amide bond. The ^{1}H-NMR spectra of compounds showed that the phenyl protons belonging to ketoprofen and ibuprofen have been exhibited at δ 7.10–7.70 ppm as a multiplet and sometimes in aromatic region together with other aromatic groups attached to the other side of the molecules. The protons of the third carbon forming the propanamide moiety for

ketoprofen and ibuprofen are at δ 1.28 and 1.52 ppm, respectively as doublets. All the other protons were observed according to the expected chemical shift and integral values.

The mass spectroscopic fragmentation of the compounds was studied under electron ionization. Molecular ion peaks (M^+) confirmed the molecular weights of the compounds examined. The fragmentation pattern was essentially identical. In the mass spectra of the compounds, the following were detected; basic fragmentation peaks for parent ketoprofen such as m/z 209, 105, 77 with the loss of functional groups such as amide, phenethyl, carbonyl moieties and for parent ibuprofen, m/z 189, 175, 148, 133, 134 with the loss of functional groups such as amide moieties, terminal methyl remaining in the propionyl, terminal methyls in isobutyl, isobutyl with the ring system, and the isobutyl itself remained.

The parent NSAIDs and their corresponding ester amide derivatives (3a-f and 4a-f) were also evaluated for their in vivo systemic anti-inflammatory activity using carrageenan-induced paw edema in mice at a dose of 100 mg per kg body weight. During this evaluation, the compounds with similar functional groups such as 3b, 3d-f and 4b, 4d-f were found to be equal in terms of potency or more potent than parent compounds in the same time-dependent manner. These compounds were further evaluated to investigate their analgesic activity and gastric ulcerogenic effect (Table 1).

Animals were administered the selected test compounds at a dose of 100 mg per kg body weight. Table 2 presents the percentage of analgesic activity by means of inhibition of writhing movements in comparison with parent NSAIDs. Compound 3f had similar analgesic activity to ketoprofen whereas compounds 4e-f demonstrated significantly better activities than their parent compound. These results indicate that both parent NSAIDs showed a measurable ulcerogenic index in at least one animal after the subcutaneous administration of 100 mg per kg doses. None of the amide prodrugs caused any gastric mucosal lesions nor bleeding points in the gastric mucosa.

Compound 4f with the highest anti-inflammatory and analgesic activity was further evaluated for its chemical hydrolysis behaviors in simulated gastric fluid (acidic buffer, pH 1.2), simulated intestine fluid (basic buffer, pH 7.4). In addition, to explore its potential as a prodrug in various biological systems, the enzymatic hydrolysis behaviors of this compound were observed in 80% v/v human plasma and 10% w/v rat liver homogenate. The expected experimental response was the release of its parent compound as evident by the HPLC analysis. Negligible hydrolysis was observed in acidic (pH 1.2) and basic buffers, and in 80% v/v human plasma resembling a linear decrease similar to the first-order kinetic

model. The rate of conversion to the parent drug was between 8 to 15 %, which indicated that the synthesized ester and amide derivatives were sufficiently stable throughout the period of experiments.

A similar but sharper linear decrease was observed during the enzymatic hydrolysis experiment performed with 10% w/v rat liver homogenate. The rate of conversion to the parent drug ranged from 52 to 86 % after 120 and 600 min, respectively. The results of the experiments clearly show that the hydrolyzing enzymes of the liver play a much more significant role than plasma enzymes in changing these amide derivatives to their parent compounds.

Another significant result was that all the active compounds were able to inhibit the change in paw volume after carrageenan injection, which demonstrates their anti-inflammatory action. In addition, most compounds were found to be more active than the parent drugs, indicating that amidification with these NSAIDs maintains or even improves the analgesic activity.

Compared to the chemical stability studies, the synthesized amide derivative was sufficiently stable at different pH levels of the stomach and intestine. Furthermore, it was stable against enzymatic hydrolysis of plasma constituents, releasing the parent drug with degradation in the liver.

It is well evidenced that direct contact or indirect mechanisms play a major role in the production of gastrointestinal lesions following the administration of NSAIDs, and designing amide and ester derivatives can be a solution for these lesions. Furthermore, these derivatives may lead to the development of new and potent non-ulcerogenic anti-inflammatory and analgesic agents with potential clinical applications.

Table 1: Effect of compounds 3a-f and 4a-f at a dose of 100 mg per kg dose against carrageenan-induced hind paw edema in mice.

Compound	Swelling in thickness [$\times 10^{-2}$ mm] (Inhibitory percentage)			
	90 min	180 min	270 min	360 min
Control	47.6±5.4	55.0±5.4	58.6±4.1	64.8±4.0
3a	35.0±4.0 (26.5)	42.8±4.1 (22.2)	43.5±2.6 (25.8)	51.8±2.4 (20.1)*
3b	33.3±6.9 (30.0)	39.8±7.0 (27.6)	42.3±7.4 (27.8)	44.5±5.6 (31.3)*
3c	33.8±3.5 (28.9)	39.8±3.7 (27.6)	42.5±3.9 (27.5)	46.5±2.7 (28.2)
3d	31.3±4.1 (34.2)	35.5±4.2 (35.5)	37.8±4.8 (35.5)*	42.5±5.6 (34.4)**
3e	32.0±5.0 (32.8)	36.3±4.8 (34.0)	40.5±5.6 (30.9)	46.5±5.2 (28.2)*
3f	29.0±5.3 (38.4)	33.0±3.8 (40.0)*	37.0±3.5 (36.9)*	41.3±4.9 (36.3)**
4a	35.3±3.9 (25.8)	40.0±3.8 (27.3)	45.5±3.5 (22.4)	51.0±3.0 (21.3)*
4b	33.8±3.5 (28.9)	38.3±3.6 (30.4)	40.5±5.1 (30.9)**	45.0±5.6 (30.6)*
4c	34.3±4.8 (27.9)	38.3±4.8 (30.4)	42.0±4.4 (28.3)	46.3±4.0 (28.5)*
4d	26.8±5.5 (43.7)	33.0±4.8 (40.0)*	35.5±3.9 (39.4)*	38.5±3.7 (40.6)**
4e	32.5±4.0 (31.7)	37.0±3.9 (32.7)	40.0±4.1 (31.7)	44.8±4.5 (30.9)*
4f	25.3±3.4 (46.8)	30.0±3.3 (45.5)**	34.3±3.6 (41.5)	38.8±3.9 (40.1)**
Ketoprofen	34.1±4.0 (28.3)	38.4±4.3 (29.8)	38.6±3.8 (34.1)	40.4±3.1 (37.5)*
Ibuprofen	29.5±2.9 (38.2)	33.6±3.3 (38.9)	35.4±2.9 (39.5)*	37.4±2.8 (42.2)**

Table 2: Analgesic effects of compounds 3a-f and 4a-f against PBQ-induced writhings in mice and ulcer scores.

Compound	Number of writhing ± SEM	% Inhibition	Ulcer score
Control	44.2±1.8		
3b	22.3±3.0**	49.5	0/6
3d	22.8±2.1**	48,1	0/6
3e	21.1±2.3**	52.2	0/6
3f	18,9±1.3***	57.2	0/6
4b	22.3±1.3***	49.7	0/6
4d	23.3±3.0**	47.3	0/6
4e	14.0±1.6***	68.3	0/6
4f	12.3±1.9***	72.2	0/6
Ketoprofen	17.5±2.6***	60.4	1/6
Ibuprofen	16.0±1.5***	63.8	1/6

**p<0.01, ***p<0.001 significant from the control

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