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Stable ester and amide conjugates of some NSAIDs as analgesic and antiinflammatory compounds with improved biological activity

Mecit Orhan ULUDAĞ[1], Burcu ÇALIŞKAN ERGÜN[2], Duygu Aslı ALKAN[1], Nilüfer ERCAN[1], Güler Yağmur ÖZKAN[2], Erden BANOĞLU[2,*]

[1] *Department of Pharmacology, Faculty of Pharmacy, Gazi University, 06330, Ankara-TURKEY*

[2] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06330, Ankara-TURKEY*

e-mail: banoglu@gazi.edu.tr

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A set of ester and amide derivatives of some acidic NSAIDs, including ibuprofen, ketoprofen, and mefenamic acid (**1-3**), were synthesized and evaluated for their in vivo analgesic and antiinflammatory activity using the p-benzoquinone-induced writhing test and the carrageenan-induced paw edema model, respectively. Among the synthesized compounds, ester derivatives of ketoprofen showed especially potent analgesic and antiinflammatory activity as compared to the parent drug. In vitro chemical stability studies revealed that ester and amide derivatives were chemically stable in simulated gastric (pH 1.2) and intestinal fluids (pH 6.8). In 80% human plasma, the ester derivatives were found to be relatively stable against plasma esterases over periods of 24 h, indicating that the observed activity was not due to the parent NSAIDs. Most of the compounds were found to be nonulcerogenic under the tested conditions.

Key Words: Ibuprofen, ketoprofen, mefenamic acid, writhing, carrageenan

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used for the treatment of pain and chronic inflammatory ailments such as rheumatoid arthritis.[1] The majority of currently known NSAIDs mainly act peripherally

*Corresponding author

by blocking the production of prostaglandins (PG) through inhibition of cyclooxygenases (COX-1 and COX-2) to varying extents.[2] However, the prolonged utilization of these drugs limits their therapeutic use since they cause gastrointestinal and renal side effects[3] due to inhibition of constitutive COX-1 responsible for the production of PGs that are important for gastroprotection and vascular homeostasis.[3–5] In addition, a direct contact mechanism also appears to play a major role in the development of gastrointestinal lesions with acidic NSAIDs.[6] As a result, the gastric disturbance caused by NSAIDs is probably a combination of local irritations produced by the free carboxyl group in NSAIDs and by inhibition of the cytoprotective PGs on gastric mucosa. Therefore, there is still growing interest in improved NSAIDs that are biologically effective but devoid of the side effects inherent to traditional NSAIDs.

Kalgutkar et al. demonstrated that ester and amide derivatives of indomethacin and meclofenamic acid selectively inhibited COX-2 and showed that certain amide derivatives of indomethacin were potent nonulcerogenic antiinflammatory agents in an in vivo model of acute inflammation.[7–9] These common approaches to overcome the gastrointestinal side effects of known NSAIDs were also used by many other researchers over the years as has been reviewed recently.[10] For example, 2-formylphenyl esters of indomethacin, ketoprofen and ibuprofen were shown to have more potent antiinflammatory activity than the parent drugs.[11] In addition, morpholinoalkyl esters of naproxen, indomethacin and diclofenac were reported to have better bioavailability and be less irritating to the gastric mucosa than the parent compounds.[12,13] Moreover, the benzyl ester prodrug of ibuprofen showed good gastric tolerability without affecting the pharmacological activity.[14] The proline amides of ibuprofen were reported as neuroprotective agents and were shown to possess antiinflammatory activity without ulcerogenic potential.[15] The L-cysteine ethyl ester of a series of NSAIDs including ibuprofen and ketoprofen was also found to be a potent antiinflammatory and antioxidant agent while demonstrating considerably reduced gastrointestinal toxicity.[16] Based on the above findings, it is evident that derivatization of the free carboxylic acid group in NSAIDs represents a suitable strategy for obtaining novel compounds with an improved therapeutic index and even the conversion of nonselective COX inhibitor NSAIDs into selective COX-2 inhibitor drugs.

In the present study, we prepared 2 phenolic esters and 2 secondary amide derivatives of ibuprofen, ketoprofen and mefenamic acid with the aim that they would have increased pharmacological activity, low ulcerogenicity and good chemical stability.

Experimental

Chemistry

Ketoprofen, mefenamic acid, ibuprofen, EDC, and amine and phenol derivatives were purchased from Aldrich (Deisenhofen, Germany) and Merck (Darmstadt, Germany). IR (KBr) spectra were recorded on a PerkinElmer Spectrum 100N FT-NIR spectrometer. ^{1}H-NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Varian Mercury 400 MHz High Performance Digital FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of the Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm). HRMS spectra were taken on a Waters LCT Premier XE orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer using ESI (+) or ESI (-) methods (Waters Corporation, Milford, MA, USA).

Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected. Flash chromatography was performed with a Combiflash®Rf automated flash chromatography system (Teledyne Isco, Lincoln, NE, USA) using hexane-ethyl acetate or dichloromethane-methanol solvent gradients.

General synthesis of esters and amide derivatives

To a solution of appropriate carboxylic acid derivative (1 mmol) and phenol or amine derivative (1 mmol) in DCM (10 mL), DMAP (0.2 mmol) and EDC (1.1 mmol) were added and the resulting solution was stirred overnight at room temperature. The reaction mixture was quenched with 0.5 N HCl and extracted with DCM. The organic phase was washed with a 1% $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under vacuum. The residue was purified by flash column chromatography (Combiflash®Rf) using hexane-EtOAc or DCM-MeOH as eluents.

4-Isopropylphenyl 2-(4-isobutylphenyl)propanoate (1a)

Elution with hexane-EtOAc (0%-3%) afforded **1a** as a colorless oil (yield 94%); IR (KBr, cm^{-1}): 1754 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 0.90 (d, J= 6.8 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.21 (d, J= 7.2 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.59 (d, J= 6.8 Hz, 3H, $COCHC\underline{H}_3$), 1.86 (m, 1H, $C\underline{H}(CH_3)_2$), 2.47 (d, J= 6.8 Hz, 2H, $C\underline{H}_2CH$), 2.87 (m, 1H, $C\underline{H}(CH_3)_2$), 3.92 (q, J= 7.0 Hz, 1H, $COC\underline{H}CH_3$), 6.90 (m, 2H, ArH), 7.13 (d, J= 8.0 Hz, 2H, ArH), 7.17 (m, 2H, ArH), 7.29 (d, J= 8.0 Hz, 2H, ArH); HRMS calcd. for $C_{22}H_{28}O_2$ (M+1): 325.2168, found: 325.2178.

4-Tert-butylphenyl 2-(4-isobutylphenyl)propanoate (1b)

Elution with hexane-EtOAc (0%-5%) afforded **1b** as a white solid (yield 61%); mp 54 °C; IR (KBr, cm^{-1}): 1752 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 0.90 (d, J= 6.8 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.28 (s, 9H, $C(C\underline{H}_3)_3$), 1.59 (d, J= 6.8 Hz, 3H, $COCHC\underline{H}_3$), 1.86 (m, 1H, $C\underline{H}(CH_3)_2$), 2.46 (d, J= 6.8 Hz, 2H, $C\underline{H}_2CH$), 3.93 (q, J =7.2 Hz, 1H, $COC\underline{H}CH_3$), 6.91 (m, 2H, ArH), 7.13 (d, J= 8.0 Hz, 2H, ArH), 7.29 (d, J= 8.0 Hz, 2H, ArH), 7.33 (m, 2H, ArH); HRMS calcd. for $C_{23}H_{30}O_2$ (M+1): 339.2324, found: 339.2325.

1-(4-Tert-butylbenzyl)-4-[2-(4-isobutylphenyl)propanoyl]piperazine (1c)

Elution with DCM-MeOH (0%-5%) afforded **1c** as a white solid (yield 65%); mp 282 °C; IR (KBr, cm^{-1}): 1635 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 0.91 (m, 6H, $CH(C\underline{H}_3)_2$), 1.31 (s, 9H, $C(C\underline{H}_3)_3$), 1.41 (d, J= 6.8 Hz, 3H, $COCHC\underline{H}_3$), 1.86 (m, 1H, $C\underline{H}(CH_3)_2$), 2.47 (d, J = 7.6 Hz, 2H, $C\underline{H}_2CH$), 2.40-4.76 (m, 10H, piperazine-H, $NC\underline{H}_2$), 7.09-7.49 (m, 8H, ArH); HRMS calcd. for $C_{28}H_{40}N_2O$ (M+1): 421.3219, found: 421.3210.

Ethyl 1-[2-(4-isobutylphenyl)propanoyl]piperidine-4-carboxylate (1d)

Elution with DCM-MeOH (0%-4%) afforded **1d** as a colorless oil (yield 82%); IR (KBr, cm^{-1}): 1728 (C=O), 1641 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 0.87 (m, 6H, $CH(C\underline{H}_3)_2$), 1.21 (m, 3H, $OCH_2C\underline{H}_3$), 1.42 (d, J = 6.4 Hz, 3H, $COCHC\underline{H}_3$), 1.58-1.88 (m, 5H, piperidine, $C\underline{H}(CH_3)_2$), 2.37 (m, 1H, piperidine), 2.43 (d, J= 6.8 Hz, 2H, $C\underline{H}_2CH$), 2.67-3.00 (m, 2H, piperidine), 3.79 (m, 2H, piperidine, $COC\underline{H}CH_3$), 4.09 (m, 2H, $OC\underline{H}_2CH_3$),

4.29-4.57 (m, 1H, piperidine), 7.10 (m, 4H, ArH); HRMS calcd. for $C_{21}H_{31}NO_3$ (M+1): 346.2382, found: 346.2378.

4-Isopropylphenyl 2-(3-benzoylphenyl)propanoate (2a)

Elution with DCM-MeOH (0%-5%) afforded **2a** as a colorless oil (yield 32%); IR (KBr, cm^{-1}): 1754 (C=O), 1659 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 1.22 (d, J= 6.8 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.65 (d, J = 7.6 Hz, 3H, $CHC\underline{H}_3$), 2.89 (m, 1H, $C\underline{H}(CH_3)_2$), 4.03 (q, 1H, J= 7.2 Hz, $C\underline{H}CH_3$), 6.92 (m, 2H, ArH), 7.18 (m, 2H, ArH), 7.46-7.51 (m, 3H, ArH), 7.59 (m, 1H, ArH), 7.64 (m, 1H, ArH), 7.73 (m, 1H, ArH), 7.80-7.85 (m, 3H, ArH); HRMS calcd. for $C_{25}H_{24}O_3$ (M+1): 373.1804, found: 373.1812.

4-Tert-butylphenyl 2-(3-benzoylphenyl)propanoate (2b)

Elution with hexane-EtOAc (0%-20%) afforded **2b** as a colorless oil (yield 65%); IR (KBr, cm^{-1}): 1753 (C=O), 1659 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 1.29 (s, 9H, $C(C\underline{H}_3)_3$), 1.65 (d, J = 7.2 Hz, 3H, $CHC\underline{H}_3$), 4.03 (q, 1H, J= 7.2 Hz, $C\underline{H}CH_3$), 6.93 (d, J= 8.8 Hz, 2H, ArH), 7.35 (d, J= 8.8 Hz, 2H, ArH), 7.46-7.51 (m, 3H, ArH), 7.60 (m, 1H, ArH), 7.64 (m, 1H, ArH), 7.72 (m, 1H, ArH), 7.80-7.85 (m, 3H, ArH); HRMS calcd. for $C_{26}H_{26}O_3$ (M+1): 387.1960, found: 387.1941.

(3-{2-[4-(4-Tert-butylbenzyl)piperazin-1-yl]-1-methyl-2-oxoethyl}phenyl)(phenyl) methanone (2c)

Elution with DCM-MeOH (0%-2%) afforded **2c** as a yellow solid (yield 63%); mp 221 °C; IR (KBr, cm^{-1}): 1647 (C=O), 1659 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 1.29 (d, 9H, $C(C\underline{H}_3)_3$), 1.50 (m, 3H, $CHC\underline{H}_3$), 2.68-4.82 (m, 11H, piperazine-H, $NC\underline{H}_2$, $C\underline{H}CH_3$), 7.40-7.84 (m, 13H, ArH); HRMS calcd. for $C_{31}H_{36}N_2O_2$ (M+1): 469.2855, found: 469.2840.

Ethyl 1-[2-(3-benzoylphenyl)propanoyl]piperidine-4-carboxylate (2d)

Elution with DCM-MeOH (0%-5%) afforded **2d** as a colorless oil (yield 48%); IR (KBr, cm^{-1}): 1726 (C=O), 1659 (C=O), 1641 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 1.22 (m, 3H, $OCH_2C\underline{H}_3$), 1.47 (m, 3H, $COCHC\underline{H}_3$), 1.57 (m, 1H, piperidine), 1.66 (m, 2H, piperidine), 1.90 (m, 1H, piperidine), 2.44 (m, 1H, piperidine), 2.75-3.10 (m, 2H, piperidine), 3.80 (m, 1H, piperidine), 3.98 (m, 1H, $COC\underline{H}CH_3$), 4.11 (m, 2H, $OC\underline{H}_2CH_3$), 4.34-4.53 (m, 1H, piperidine), 7.43-7.79 (m, 9H, ArH); HRMS calcd. for $C_{24}H_{27}NO_4$ (M+1): 394.2018, found: 394.2008.

4-Isopropylphenyl 2-[(2,3-dimethylphenyl)amino]benzoate (3a)

Elution with hexane-EtOAc (0%-5%) afforded **3a** as a yellow solid (yield 79%); mp 102 °C; IR (KBr, cm^{-1}): 3337 (N-H), 1689 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 1.27 (d, J = 6.8 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.15 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.95 (m, 1H, $C\underline{H}(CH_3)_2$), 6.73 (m, 1H, ArH), 6.79 (d, J = 8.8 Hz, 1H, ArH), 7.02 (d, J= 6.8 Hz, 1H, ArH), 7.09 (d, J= 8.0 Hz, 1H, ArH), 7.12-7.17 (m, 3H, ArH), 7.28-7.33 (m, 3H, ArH), 8.19 (dd, J= 8.0 Hz, 1.6 Hz, 1H, ArH), 9.19 (s, 1H, NH); HRMS calcd. for $C_{24}H_{25}NO_2$ (M+1): 360.1964, found: 360.1957.

4-Tert-butylphenyl 2-[(2,3-dimethylphenyl)amino]benzoate (3b)

Elution with hexane-EtOAc (0%-5%) afforded **3b** as a pale yellow solid (yield 87%); mp 122 °C; IR (KBr, cm^{-1}): 3343 (N-H), 1690 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (s, 9H, C(C$\underline{H}_3$)$_3$), 2.15 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 6.73 (m, 1H, ArH), 6.79 (d, J= 8.8 Hz, 1H, ArH), 7.02 (d, J= 6.8 Hz, 1H, ArH), 7.09 (d, J= 8.0 Hz, 1H, ArH), 7.12-7.17 (m, 3H, ArH), 7.31 (m, 1H, ArH), 7.45 (m, 2H, ArH), 8.19 (dd, J= 8.0 Hz, 1.6 Hz, 1H, ArH), 9.19 (s, 1H, NH); HRMS calcd. for $C_{25}H_{27}NO_2$ (M+1): 374.2120, found: 374.2115.

N-(2-{[4-(4-Tert-butylbenzyl)piperazin-1-yl]carbonyl}phenyl)-2,3-dimethylaniline (3c)

Elution with DCM-MeOH (0%-5%) afforded **3c** as a white solid (yield 70%); mp 123 °C; IR (KBr, cm^{-1}): 3381 (N-H), 1623 (C=O); ^{1}H-NMR ($CDCl_3$, δ): 1.31 (s, 9H, C(C$\underline{H}_3$)$_3$), 2.14 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.45 (s, 4H, piperazine), 3.47 (s, 2H, NCH_2), 3.67 (s, 4H, piperazine), 6.76 (m, 1H, ArH), 6.92 (m, 2H, ArH), 7.00 (s, 1H, NH), 7.03-7.18 (m, 4H, ArH), 7.22 (d, J= 8.0 Hz, 2H, ArH), 7.33 (d, J= 8.4 Hz, 2H, ArH); HRMS calcd. for $C_{30}H_{37}N_3O$ (M+1): 456.3015, found: 456.2999.

Ethyl 1-{2-[(2,3-dimethylphenyl)amino]benzoyl}piperidine-4-carboxylate (3d)

Elution with DCM-MeOH (0%-5%) afforded **3d** as a white solid (yield 52%); mp 77 °C; IR (KBr, cm^{-1}): 3361 (N-H), 1725 (C=O), 1625 (C=O); ^{1}H-NMR (CD_3COCD_3, δ): 1.21 (t, J = 7.2 Hz, 3H, $CH_2C\underline{H}_3$), 1.65 (m, 2H, piperidine), 1.93 (m, 2H, piperidine), 2.11 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.64 (m, 1H, piperidine), 3.14 (m, 2H, piperidine), 4.09 (q, J = 7.2 Hz, 2H, $C\underline{H}_2CH_3$), 4.20 (m, 2H, piperidine), 6.79-6.92 (m, 3H, ArH), 7.04-7.06 (m, 2H, NH+ArH), 7.18-7.25 (m, 3H, ArH); HRMS calcd for $C_{23}H_{28}N_2O_3$ (M+1): 381.2178, found: 381.2195.

Chemical stability

The hydrolytic stability of the synthesized ester and amide derivatives was studied in near physiological conditions in hydrochloric acid (simulated gastric fluid, SGF, pH 1.3) and phosphate buffer (simulated intestinal fluid, SIF, pH 6.8) containing 1% Tween 20 at 37.4 °C. The ionic strength (μ) for each buffer was maintained at 0.5 by adding a calculated amount of potassium chloride. The reactions were initiated by adding 0.1 mL of stock solution of the compounds (10 mg/mL) to 1.9 mL of SGF or SIF in screw-capped vials that were preequilibrated at 37.4 °C. Samples were withdrawn at appropriate intervals for 24 h and analyzed by LC/MS-TOF for residual prodrug and parent drug concentrations.

Enzymatic stability

The hydrolysis rates of the ester derivatives were studied in 80% human plasma diluted with isotonic phosphate buffer (pH 7.4) at 37.4 °C. The reactions were initiated by adding 20 μL of stock solutions of the ester derivatives in DMSO (10 mg/mL) to 480 μL of diluted plasma, incubated at 37.4 °C. At appropriate time intervals, 50 μL of the plasma were taken and deproteinized by the addition of 450 μL of acetonitrile. After mixing and centrifugation for 10 min at 10,000 rpm, the supernatant was analyzed by LC/MS-TOF for intact ester and parent drug concentrations.

Pharmacological screening

Animals

Male Swiss albino mice (25-30 g) were purchased from the animal breeding laboratories of the Refik Saydam Central Institute of Health (Ankara, Turkey). They were housed in a room with controlled temperature (22 ± 1 °C), humidity (55 ± 10%), and photoperiod (12:12 h) for at least 1 week before being used. They were maintained on a standard pellet diet and water ad libitum throughout the experiment. A minimum of 6 animals was used in each group. Throughout the experiments, the animals were processed according to the suggested international ethical guidelines for the care of laboratory animals under the audit of the Gazi University Commission of Animal Ethics (Permission No: 53-3044).

Drugs

p-Benzoquinone (PBQ), λ-carrageenan (type IV), and other biochemicals used in this study were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Analgesic activity

Analgesic activity was measured using the PBQ-induced writhing (abdominal constriction) test in mice.[17] 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. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for the observation and the total number of abdominal contractions (writhing movements) was counted for the following 15 min, starting 5 min after the PBQ injection. The data represent the average of the total number of writhing movements observed. Analgesic activity was then expressed as the percentage change from the writhing controls.

Antiinflammatory activity

The carrageenan-induced hind paw edema model was used for determining antiinflammatory activity.[18] Each group contained a minimum of 7 animals, and 30 min after the subcutaneous administration of a test sample (50 mg/kg body weight) or dosing vehicle, each mouse was injected with a freshly prepared suspension of carrageenan (0.5 mg/25 μL) in physiological saline into the subplantar tissue of the right hind paw. As a control, 25 μL of saline was injected into that of the left hind paw. Paw edema was measured 90, 180, 270, and 360 min after the carrageenan injections. The difference in hind paw thicknesses was measured by caliber compasses (Ozaki Co., Tokyo, Japan). Mean values of each treated group were compared with the control group and analyzed by statistical methods.

Acute toxicity

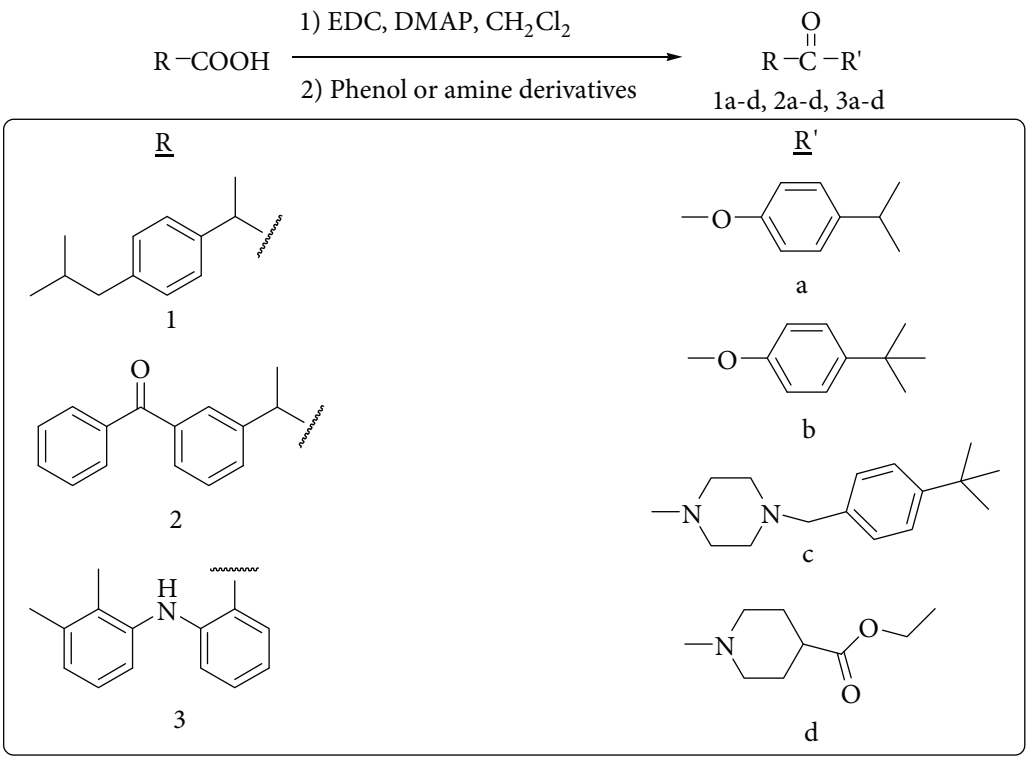
At the end of these experiments, after 48 h of observation of all animals, no morbidity or mortality was recorded.

Gastric ulcerogenic effect

The ulcerogenicity was investigated as described elsewhere.[19] The animals were sacrificed with an overdose of diethyl ether after 270 min of administration of the compounds. After abdominal dissection, the stomachs of the animals were slightly taken out. The esophagus was then tied in a knot nearest the cardia by a surgical suture. From the duodenum side, 2.5 mL of 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 (e.g. food fragments, blood clots), and examined under a dissecting microscope (20 × 6.3) to assess the formation of ulcers. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index.

Statistical analysis

The data were expressed as means ± SEM. The significance of differences between the treatment and the control group of animals was determined by 1-way ANOVA with Bartlett's test following a post hoc Student-Newman-Keuls multiple comparisons test for analgesic activity, and 2-way ANOVA following a post hoc Bonferroni test for antiinflammatory activity. Values of $P < 0.05$ were considered statistically significant.



Scheme. Synthetic pathway for the synthesis of the target ester and amide derivatives.

Results

The desired ester and amide derivatives of ibuprofen (**1**), ketoprofen (**2**), and mefenamic acid (**3**) were prepared in a one-pot synthetic procedure as shown in the Scheme. Treatment of parent NSAIDs with the appropriate phenol or amine derivatives in the presence of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCI) as the carboxyl group activator and dimethylaminopyridine (DMAP) afforded the desired products (**1a-d**, **2a-d**, and **3a-d**) in 50%-70% yields.

In the pharmacological study, we investigated the analgesic and antiinflammatory activities and the ulcerogenic potential of acute administration of both the parent NSAIDs (**1-3**) and the corresponding ester (**1a-b**, **2a-b**, and **3a-b**) and amide (**1c-d**, **2c-d**, and **3c-d**) derivatives. Animals were administered 100 mg/kg body weight doses of test compounds, and the percentage of analgesic activity by means of inhibition of writhing movements in comparison with parent NSAIDs are given in the Figure and the Table. Closer inspection of the results indicates that all compounds showed significant analgesic activity as compared with the vehicle-injected mice. The percentage inhibition of PBQ-induced writhing revealed that the ester and amide derivatives of ibuprofen displayed similar (**1b-d**) or reduced (**1a**) analgesic activities compared to the parent ibuprofen. In the ketoprofen series, ester derivatives **2a** and **2b** showed more potent and higher analgesic activity than ketoprofen, while amide derivatives **2c** and **2d** resulted in lower activity as compared to the parent drug. In addition, both ester and amide derivatives of mefenamic acid showed analgesic activity comparable to the parent NSAID.

The parent NSAIDs (**1-3**) and their corresponding ester and amide derivatives (**1a-d**, **2a-d**, and **3a-d**) were also evaluated for their in vivo systemic antiinflammatory activity using carrageenan-induced paw edema in mice at 50 mg/kg body weight doses. All compounds and parent NSAIDs showed a peak antiinflammatory activity at 270 min, and the inhibitory activity on edema formation started to diminish at that point (Table). The 4-*i*-propylphenyl ester (**1a**) showed the same pattern of inhibition, with ibuprofen also having a peak inhibition at 270 min (28%). The 4-*t*-butylphenyl ester of ibuprofen (**1b**) showed extended inhibitory activity ranging from 35% to 21%. On the other hand, the piperazine amide (**1c**) of ibuprofen did not show any notable activity, while the piperidine amide derivative (**1d**) was effective until 270 min, although with a reduced activity as compared to the parent drug. For the ketoprofen (**2**) series, the phenolic ester (**2ab**) and piperidine (**2d**) amide derivatives resulted in potent antiinflammatory activity, which was extended to all time points and was higher than or comparable to the parent drug. However, the piperazine (**2c**) amide derivative showed reduced antiinflammatory activity and the peak inhibition of edema was only evident for 180 min. With regard to mefenamic acid (**3**), it was interesting that only the 4-*i*-propylphenyl ester (**3a)** and piperidine amide (**3d**) derivatives showed inhibition of edema formation; however, this activity was not as pronounced as that of the parent drug. Moreover, the compounds that were tested for antiinflammatory activity were further monitored for their gastric toxicity on acute administration to determine the ulcerogenic potential at the time interval in which the compounds showed the highest antiinflammatory activity (270 min). Results indicated that all parent NSAIDs showed a measurable ulcerogenic index in at least 1 or 2 animals after the subcutaneous administration of 50 mg/kg doses. As seen from the Table, none of the ibuprofen or mefenamic acid prodrugs caused any sign of gastric lesions under the same conditions. However, the ketoprofen ester derivatives (**2a-b**), which exhibited the highest analgesic and antiinflammatory activity of the whole series, caused severe bleeding lesions compared to the parent drug in the tested animals.

The chemical stability of the synthesized ester and amide derivatives of selected NSAIDs were studied at 37.4 °C in aqueous buffer solutions of pH 1.2 and 6.8, which were considered as nonenzymatic simulated gastric fluid (SGF) and nonenzymatic simulated intestinal fluid (SIF), respectively. The reactions were monitored by mass spectrometry coupled with a UPLC system. The results indicated that the synthesized ester and amide derivatives were sufficiently stable in SGF (pH 1.2) and SIF (pH 6.8) for a period of 1 day (24 h) at 37.4 °C.

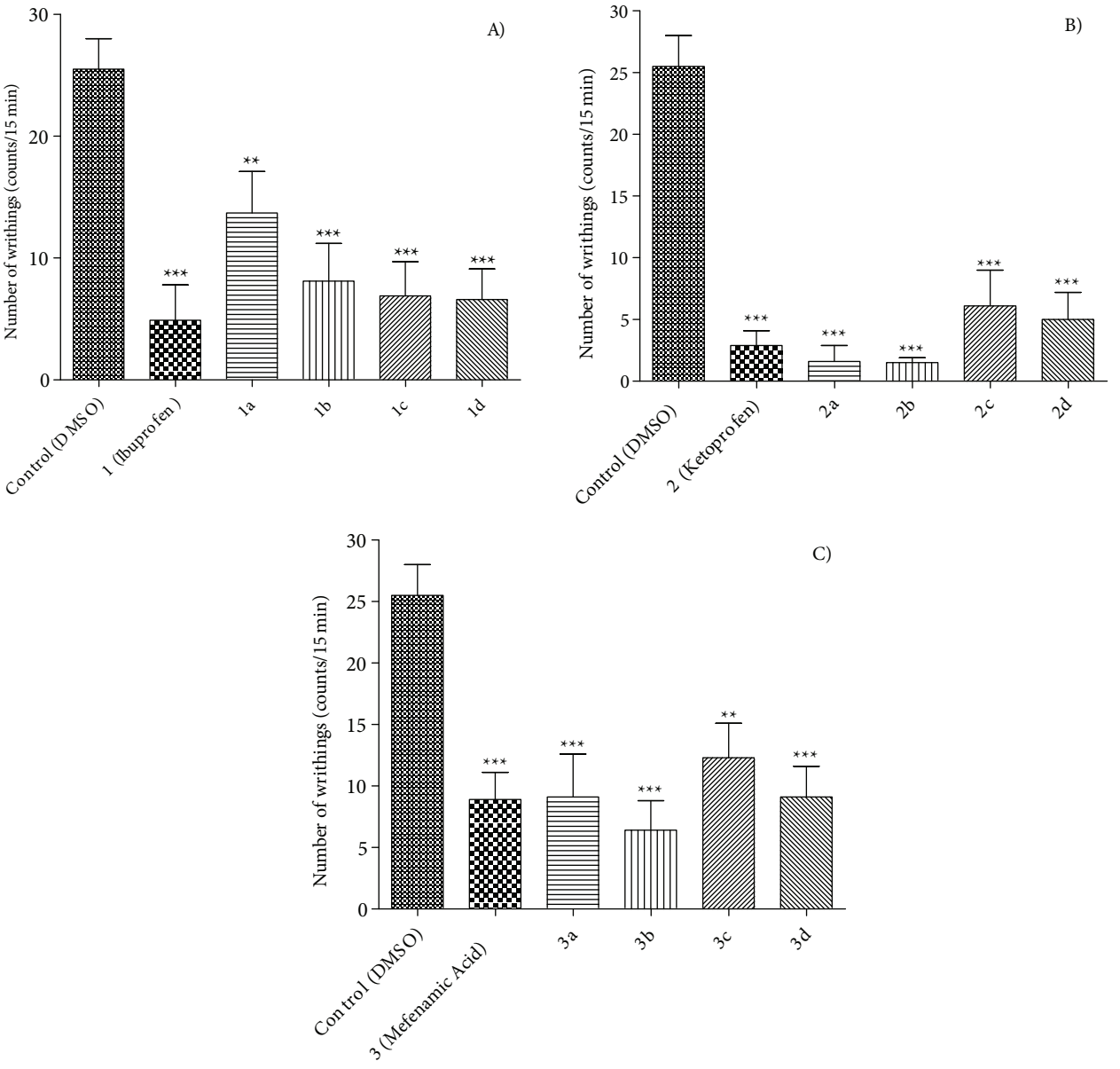


Figure. Writhing responses (means ± SEM, n = 6-9) for the A) ibuprofen, B) ketoprofen, and C) mefenamic acid derivatives compared to the control (**P < 0.01, ***P < 0.001).

Table. Analgesic and antiinflammatory effects of the test compounds on *p*-benzoquinone (PBQ)-induced abdominal constriction test and carrageenan (CG)-induced hind paw edema model in mice, respectively, and ratio of ulceration.

Test compounds	Analgesic activity, % ± SEM[a]	Swelling in thickness ($\times 10^{-2}$ mm) ± SEM (inhibition of edema, %)[b]				Ratio of ulceration[c]
		90 min	180 min	270 min	360 min	
Control	-	30.0 ± 3.5	69.3 ± 8.4	86.4 ± 7.3	79.3 ± 7.4	0:7
1	80.9 ± 11.4	31.4 ± 4.6	47.1 ± 6.8 (32.0)	57.9 ± 6.6 (33.1)*	67.9 ± 6.1 (14.4)	1:7
1a	46.4 ± 13.3	32.1 ± 3.9	47.9 ± 5.4 (30.9)	62.1 ± 5.5 (28.1)*	77.1 ± 5.5 (2.7)	0:7
1b	68.1 ± 12.0	29.3 ± 4.4 (2.4)	45.0 ± 5.1 (35.1)*	64.3 ± 6.3 (25.6)	62.1 ± 6.9 (21.6)	0:7
1c	73.1 ± 11.1	32.1 ± 3.4	63.6 ± 12.5 (8.3)	85.0 ± 5.7 (1.7)	88.6 ± 6.6	0:7
1d	74.0 ± 9.8	27.1 ± 4.7 (9.5)	48.6 ± 6.4 (29.9)	72.1 ± 7.5 (16.5)	77.9 ± 5.9 (1.8)	0:7
2	88.7 ± 4.5	26.4 ± 5.0 (11.9)	34.3 ± 3.8 (50.5)***	37.9 ± 6.6 (56.2)***	52.9 ± 5.7 (33.3)*	4:7
2a	93.6 ± 5.3	19.5 ± 4.7 (35.0)	27.5 ± 6.2 (60.3)***	40.0 ± 6.5 (53.7)***	47.5 ± 5.3 (40.1)**	2:7
2b	94.1 ± 1,5	14.6 ± 5.3 (51.4)	22.1 ± 4.2 (68.0)***	37.9 ± 7.1 (56.2)***	58.6 ± 6.4 (26.1)	5:7
2c	75.9 ± 11.3	23.9 ± 5.8 (20.5)	51.4 ± 7.1 (25.8)	75.0 ± 6.2 (13.2)	87.9 ± 7.4	0:7
2d	80.4 ± 8.5	29.6 ± 6.6 (1.4)	32.1 ± 3.9 (53.6)***	52.1 ± 5.7 (39.7)**	72.1 ± 7.7 (9.0)	0:7
3	62.1 ± 7.7	27.9 ± 7.3 (7.1)	40.7 ± 10 (41.2)*	55.0 ± 11 (36.4)**	55.7 ± 12 (29.7)	2:7
3a	64.2 ± 13.7	39.3 ± 4.1	50.7 ± 4.7 (26.8)	70.7 ± 7.5 (18.2)	90.0 ± 9.3	0:7
3b	75.0 ± 9.4	40.0 ± 3.5	70.7 ± 3.7	75.7 ± 3.2 (12.4)	80.7 ± 5.1	0:7
3c	52.0 ± 11.1	28.6 ± 3.6 (4.8)	65.7 ± 1.0 (5.2)	76.4 ± 12 (11.6)	75.0 ± 7.4 (5.4)	0:7
3d	64.3 ± 9.7	22.9 ± 5.4 (23.8)	48.6 ± 8.3 (29.9)	66.6 ± 5.3 (23.0)	78.6 ± 7.5 (0.9)	0:7

Data obtained from animal experiments were expressed as means ± standard error of mean (SEM); 1: ibuprofen, 2: ketoprofen, 3: mefenamic acid.

[a] Groups composed of 8 mice were employed for the PBQ-induced writhing test and all test drugs were subcutaneously administered to mice at doses of 100 mg/kg body weight.

[b,c] Groups composed of 7 mice were employed for the CG-induced paw edema model and all test drugs were subcutaneously administered to mice at doses of 50 mg/kg body weight.

*Significantly different from the control (*P $<$ 0.05, **P $<$ 0.01, ***P $<$ 0.001).

Moreover, the enzymatic hydrolysis of ester derivatives **1a-b** and **2a-b** in human plasma also revealed that they were relatively stable against plasma esterases, since most of the ester derivatives remained intact after the 24-h incubation period. The highest conversion rate to parent drug was observed with the isopropylphenyl ester of ketoprofen (**2a**), whereby 12% and 51% of **2a** was converted to ketoprofen after 270 min and after 24 h of incubation in human plasma, respectively. The tertiarybutylphenyl ester derivative (**2b**) hydrolyzed more slowly, with a hydrolysis rate of about 6% and 23% at 270 min and 24 h, respectively. For ibuprofen derivatives **1a** and **1b**, the conversion rate to parent drug was 7%-8% at 270 min, whereas it was 16%-18% after 24 h of incubation in human plasma.

Discussion and conclusion

It is already known that a large variety of substituents can be tolerated in the ester and amide groups of NSAID derivatives, indicating that the free carboxylic acid of NSAIDs is not a significant requirement for antiinflammatory activity.[10] Therefore, it appears that the carboxyl group of the parent NSAID is a convenient functionality for derivatization. We have previously reported that piperazine and piperidine amide derivatives of certain heteroaromatic structures contribute to the analgesic and antiinflammatory activity.[20–24] Mean-

while, others have also reported that various compounds having partial piperidine carboxylic acid structures bear potent analgesic and antiinflammatory activity. In addition, during the course of our ongoing studies of antiinflammatory compounds, we also determined that preparation of *i*-propyl- and *t*-butyl-phenol esters and 4-*t*-butyl-benzylpiperazine and ethyl 4-piperidinecarboxylate amides of pyrazole-3-propanoic acids produced compounds that effectively inhibited the LTB_4 biosynthesis in human leukocytes (unpublished results). Therefore, we have implemented these results on selected contemporary NSAIDs, namely ibuprofen and ketoprofen as arylpropionic acid derivatives and mefenamic acid as an N-arylanthranilic acid derivative, and prepared targeted ester and amide derivatives **1a-d**, **2a-d**, and **3a-d**.

The resulting change of paw volume in mice indicated that all active compounds were able to inhibit the change in paw volume at 180 and 270 min after carrageenan injection, providing an implication toward their probable mechanism of action. Since prostaglandins are secreted in the third and fourth hours after carrageenan injection, it was indicated that all synthesized ester and amide derivatives still maintained the inhibition of the arachidonic acid pathway effectively. Among the chosen arylpropionic acids, derivatization of ketoprofen to its lipophilic esters (**2a-b**) produced more potent derivatives in terms of analgesic and antiinflammatory activity, while the amidification was not as effective as the esterification. However, the ulcerative potential of these ester derivatives was also comparable to the parent ketoprofen, indicating that the COX-1 inhibition may prevail with these compounds. In terms of ibuprofen esters, **1a-b** showed comparable activity to ibuprofen without producing gastric lesions in the tested animals. However, for the mefenamic acid derivatives, neither esterification nor amidification produced an increase in antiinflammatory activity. Analgesic activity results of the compounds also showed good correlation with their antiinflammatory activities in that compounds **1b-d**, **2a-b**, **3a-b**, and **3d** strongly inhibited the peripheral pain response in the mice, as the amount of writhing was found to be significantly diminished as compared to the control animals treated with vehicle (Figure). In addition, most of the compounds were found to be active in comparison with parent drugs, indicating that esterification and amidification with these NSAIDs improved the analgesic activity.

According to the chemical stability studies, the synthesized ester and amide derivatives were sufficiently stable at pH 1.2 for 24 h, so no hydrolysis was expected in the stomach. In addition, the chemical stability of the compounds at pH 6.8 for 1 day suggested that they can be absorbed from the intestines almost intact. The chemical stability of the reported ester and amide derivatives of ibuprofen, ketoprofen, and mefenamic acid at pH values simulating gastric and intestinal fluids revealed that the designed compounds might have increased absorption as compared to parent NSAIDs. Moreover, it is well-evidenced that the direct contact mechanism appears to play a major role in the production of gastrointestinal lesions upon administration of NSAIDs.[6] Therefore, the chemical stability of the reported compounds might indicate that stable ester and amide derivatives of an NSAID might also have a potentially improved therapeutic index compared to contemporary parent drugs. Furthermore, the enzymatic stability of the ester derivatives after 270 min and 24 h of incubation in human plasma establishes evidence that they may act in vivo without prior hydrolysis of the ester bond. It should be added that the antiinflammatory activity of the compounds in the present study showed a peak activity at 270 min after carrageenan injection. Therefore, the observed stability of the synthesized ester derivatives may fulfill the essential requirements for the oral delivery system of NSAIDs, in which the acid stable ester group might also prevent the direct contact effects with the stomach mucosa.

In conclusion, the prepared ester and amide derivatives of ibuprofen, ketoprofen, and mefenamic acid

were sufficiently chemically stable in SGF and SIF. Additionally, the increased lipophilicity of the resulting derivatives may attain intact absorption at a higher rate than the parent drugs. Moreover, the enzymatic stability of the ester derivatives also indicated that the activities observed with these molecules were not due to the parent NSAIDs. Therefore, we can conclude that the preparation of suitable ester and amide derivatives of contemporary NSAIDs may represent a potentially useful method for developing compounds with more potent analgesic and antiinflammatory activity than the parent compounds, in addition to reduced gastrointestinal side effects and increased chemical and enzymatic stability.

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