Anti-inflammatory agents of the carbamoylmethyl ester class: synthesis, characterization, and pharmacological evaluation

Bassem Sadek¹
Amar Mansuro Hamruoni²
Abdu Adem¹

¹Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab Emirates University, ²Department of Pharmaceutical Sciences, College of Pharmacy, Al Ain University of Science and Technology, Al-Ain, United Arab Emirates

Abstract: In this study, target compounds 5–12 were synthesized via acid amine coupling of ibuprofen and naproxen with methyl ester derivatives of amino acids, namely, L-proline, sarcosine, L-tyrosine, and L-glutamic acid. When tested for anti-inflammatory activity using the acute carrageenan-induced hind paw method in rats, compounds 5–12 showed significantly greater anti-inflammatory activity, in the range of 40.64%–87.82%, compared with a placebo control group (P < 0.001). Among the newly synthesized compounds 5–12, naproxen derivatives 9–12 with anti-inflammatory activity ranging between 66.99% and 87.82% showed significantly higher (P < 0.05) potency than ibuprofen derivatives 5–8 with inhibition in the range of 22.03%–52.91% and control groups of ibuprofen (76.34%) or naproxen (75.59%, P < 0.05). Moreover, derivatives 9–12 derived from naproxen, in particular compounds 9 and 10 which achieved 83.91% and 87.82% inhibition of inflammation, respectively, showed significantly (P < 0.05) higher potency than naproxen derivatives 11 and 12. Notably, among naproxen derivatives 9–12, the gastric ulcerogenicity for 9 (ulcer index 11.73) and 10 (ulcer index 12.30) was found to be significantly lower (P < 0.05) than that of the active ibuprofen and naproxen control groups with ulcer indices of 22.87 and 24.13, respectively. On the other hand, naproxen derivatives 9–11 showed significant inhibition (P < 0.05) of prostaglandin E₂ synthesis when compared with the active control group receiving indomethacin, suggesting a correlation between the observed low ulcerogenicity and effect on prostaglandin E₂ synthesis for compounds 9 and 10. However, significant inhibition of prostaglandin E₂ observed for naproxen derivative 11 (107.51) did not correlate with its observed ulcer index (16.84). Our overall findings for carbamoylmethyl ester derivatives named 5–12 clearly suggest that the compounds showing potent antiinflammatory effect.

Keywords: carbamoylmethyl ester, anti-inflammatory, prostaglandin E₂, inhibitory properties

Introduction

Ibuprofen and naproxen are well known nonsteroidal anti-inflammatory drugs (NSAIDs) belonging to the family of propionic acid derivatives. They often cause upper gastrointestinal damage, including lesions, peptic ulcers, bleeding, and perforation which are attributed to the presence of a free carboxylic acid group responsible for local irritation and ulceration of the gastrointestinal mucosa and local blockade of prostaglandin biosynthesis.¹ NSAIDs belonging to the propionic acid class have greater selectivity for inhibition of constitutively expressed cytoprotective cyclo-oxygenase 1 than inducible cyclo-oxygenase 2, so long-term therapy with nonselective NSAIDs may cause gastrointestinal complications, ranging from stomach irritation to life-threatening
gastrointestinal ulceration and bleeding.2–5 Mounting evidence has suggested that structural modification, especially esterification, of the free carboxylic moiety of representative NSAIDs maintains anti-inflammatory activity and a reduced potential for ulcerogenicity.6–11 Prompted by these observations and applying previously described efficient chemical procedures, we report herein the synthesis of novel carbamoyl derivatives 5–12 containing various amide-linked amino acid methyl esters.12 To achieve the desired target compounds 5–12, the carboxylic acid moieties of ibuprofen and naproxen were reacted with methyl ester derivatives of amino acids, including L-proline, sarcosine, L-tyrosine, and L-glutamic acid, in the presence of thionyl chloride and benzotriazole. Because of their nontoxicity, varying degrees of lipophilicity, and lower incidence of side effects, the amino acid group was chosen especially for amidation to mask the free carboxylic acid group of ibuprofen and naproxen.13–17 The newly synthesized compounds 5–12 were screened for anti-inflammatory and ulcerogenic properties, and their ability to inhibit prostaglandin E2 synthesis.

Materials and methods

General

The melting points are uncorrected and were determined in open capillaries using Buechi 512 Dr Tottoli apparatus.

1H-NMR spectra were recorded on a WC 300 spectrometer (Bruker, Billerica, MA, USA) with tetramethylsilane as the internal standard. Chemical shifts are reported in ppm downfield from internal tetramethylsilane used as the reference. 

1H-NMR signals are reported in order: multiplicity (s, single; d, doublet; t, triplet; dt, doublet of triplet; q, quartet; m, multiplet), number of protons, and approximate coupling constants in Hz. 13C-NMR spectra were recorded on a DPX 400 Avance (75 mHz) instrument (Bruker). Chemical shifts are reported in ppm downfield from internal tetramethylsilane used as the reference. Elemental analyses were performed on 240B and 240C instruments (Perkin-Elmer, Boston, MA, USA). Analyses (C, H, N) indicated by the symbols of elements were within ±0.4% of the theoretical values. Chromatographic separations were done using a Chromatotron model 7924 (Harrison Research, Union, NJ, USA) with 4 mm layers of silica gel 60 PF containing gypsum (Merck, Whitehouse Station, NJ, USA). Electron ionization mass spectra were recorded using a Finnigan MAT CH5DF instrument (Thermo Fischer Scientific, MA, USA) (xenon, dimethyl sulfoxide/glycerol).

Preparation of benzotriazole-activated ibuprofen and naproxen 3 and 4

Thionyl chloride (2.64 mmol) was added to a solution of 1H-benzotriazole (8.7 mmol) in dry CH2Cl2 (60 mL) at room temperature and the reaction was stirred for 20 minutes. Ibuprofen (1) (2.2 mmol) or naproxen (2) (2.2 mmol) were added and the mixtures were stirred for 4 hours at room temperature. The white precipitate formed was filtered off and the filtrate was concentrated under reduced pressure. Each residue was diluted with 50 mL of ethyl acetate and each solution was washed with 4 N HCl (3 × 15 mL) and dried over MgSO4. Removal of the solvent under reduced pressure gave products 3 and 4, which were recrystallized from hexanes to give 3 and 4 as pure products.12

1-((1H-benzo[d][1,2,3]triazol-1-yl)-2-(4-isobutylphenyl)propan-1-one (3)

White microcrystals (95%) melting point 76.0°C–77.0°C [12]; 1H-NMR (CDCl3): 8.29 (d, J = 8.2 Hz, 1H), 8.07 (d, J = 8.1 Hz, 1H), 7.61 (t, J = 8.1 Hz, 1H), 7.4–7.5 (m, 3H), 7.11 (d, J = 7.6 Hz, 2H), 5.41 (q, J = 7.0 Hz, 1H), 2.41 (d, J = 7.2 Hz, 2H), 1.78–1.90 (m, 1H), 1.75 (d, J = 6.9 Hz, 3H), 0.86 (d, J = 6.4 Hz, 6H); 13C-NMR (CDCl3): 173.8, 146.4, 141.3, 136.6, 131.5, 130.5, 129.8, 128.0, 126.3, 120.3, 114.7, 45.2, 44.6, 30.3, 22.6,18.8; analysis calculated for C19H18N3O2: C, 74.24; H, 6.89; N, 13.67. Found: C, 74.29; H, 6.94; N, 13.83.

1-((1H-benzo[d][1,2,3]triazol-1-yl)-2-(6-methoxynaphthalen-2-yl)propan-1-one (4)

Microcrystals (92%) melting point 181.0°C–182.0°C [12]; 1H-NMR (CDCl3): 8.29 (d, J = 8.3 Hz, 1H), 8.07 (d, J = 10.1 Hz, 1H), 7.89 (d, J = 1.1 Hz, 1H), 7.70 (d, J = 8.6 Hz, 2H), 7.59–7.66 (m, 2H), 7.43–7.50 (m, 1H), 7.11 (d, J = 8.9; 2.5 Hz, 1H), 7.07 (d, J = 2.3 Hz, 1H), 5.55 (q, J = 7.0 Hz, 1H), 3.89 (s, 3H), 1.83 (d, J = 7.0 Hz, 3H); 13C-NMR (CDCl3): 173.5, 157.8, 146.2, 134.3, 133.8, 131.3, 130.3, 129.3, 128.9, 127.4, 126.9, 126.4, 126.1, 120.1, 119.1, 114.5, 105.5, 55.3, 44.7, 18.6; analysis calculated for C21H17N3O2: C, 72.49; H, 5.17; N, 12.68. Found: C, 72.14; H, 5.28; N, 12.69.

Preparation of ibuprofen and naproxen containing derivatives 5–12

The appropriate benzotriazole 3 and 4 (0.65 mmol) was added to a 0.65 mmol solution of amino acid methyl ester in acetonitrile in the presence of triethylamine (1.8 mmol).
Each reaction mixture was stirred at room temperature for 6–24 hours, and completion of the reaction was checked by thin layer chromatography using ethyl acetate-hexane (1:1) as an eluting solvent. Finally, the reaction was quenched by addition of water (10 mL), and the organic compound was extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine (3 × 20 mL), dried over anhydrous Na₂SO₄, and concentrated in a vacuum. Purification was done by rotary chromatography using silica gel 60 PF containing gypsum and ethyl acetate-hexane as an eluting solvent to yield racemic products 5–12.

Methyl-1-(2-[4-isobutylphenyl]propanoyl) pyrrolidine-2-carboxylate (5)

Yield 86%, melting point 101°C–102°C; IR (KBr): ν(cm⁻¹) 1743 (ester), 1668 (amide); ¹H-NMR (CDCl₃): δ ppm = 7.19 (d, J = 7.9 Hz, 2H, Ibu-2,6H), 7.11 (d, J = 7.9 Hz, 2H, Ibu-3,5H), 4.39 (q, J = 6.7 Hz, 1H, ArCH), 3.75 (s, 3H, COOCH₃), 3.68–3.61 (m, 1H, pro-CH), 2.41 (d, J = 7.4 Hz, 2H, (CH₂)₂CHCH₂), 2.35 (2H, pro-CH₂), 2.03 (m, 2H, pro-CH₂), 1.98 (m, 2H, pro-CH₂), 1.61 (heptet, J = 6.7 Hz, 1H, (CH₂)₂CHCH₂), 1.50 (d, J = 6.7 Hz, 3H, CHCH₂); 13C-NMR (CDCl₃) δ ppm = 173.10, 170.51, 129.69, 127.41, 59.71, 53.90, 47.86, 46.40, 45.05, 28.41, 26.44, 23.40, 22.41, 18.42; MS: m/z (%) 318 (M⁺, 90); analysis calculated for C₁₉H₂₅NO₃C; C, 71.89; H, 8.57; N, 4.41. Found: C, 71.68; H, 8.43; N, 4.51.

Methyl-2-[2-[4-isobutylphenyl]-N-methylpropanamido] pentanedioate (8)

Yield 86%, melting point 89°C–90°C; IR (KBr): ν(cm⁻¹) 1738 (ester), 1662 (amide); ¹H-NMR (CDCl₃): δ ppm = 7.19 (d, J = 7.9 Hz, 2H, Ph-2H,6H), 7.11 (d, J = 7.9 Hz, 2H, Ph-3,5H), 6.67 (bs, 1H, CONH), 4.58 (dd, J = 2.1 Hz, 5.9 Hz, 1H, COOCH₂), 2.41 (d, J = 7.4 Hz, 2H, (CH₂)₂CHCH₂), 3.68–3.60 (m, 1H, CHCH₂), 3.65 (s, 3H, COOCH₃), 3.60 (s, 3H, COOCH₃), 2.17 (dt, J = 7.5 Hz, 14.1 Hz, 2H, COOCH₂CH₂), 1.92 (dt, J = 7.9 Hz, 14.2 Hz, 2H, COOCH₂CH₂), 1.61 (heptet, J = 6.7 Hz, 1H, (CH₂)₂CHCH₂), 1.50 (d, J = 6.7 Hz, 3H, CHCH₂), 0.80 (d, J = 6.7 Hz, 6H, (CH₂)₂CHCH₂); ¹C-NMR (CDCl₃) δ ppm = 173.10, 170.41, 129.69, 127.41, 53.70, 52.90, 47.86, 45.05, 39.20, 29.40, 26.44, 26.20, 21.48, 18.42; MS: m/z (%) 364 (M⁺, 100); analysis calculated for C₂₇H₄₅NO₅; C, 66.09; H, 8.04; N, 3.85. Found: C, 65.98; H, 8.24; N, 4.03.

Methyl-1-(2-[6-methoxynaphthalen-2-yl]propanoyl) pyrrolidine-2-carboxylate (9)

Yield 80%, melting point 109°C–110°C; IR (KBr): ν(cm⁻¹) 1742 (ester), 1668 (amide); ¹H-NMR (CDCl₃): δ ppm = 7.75–7.77 (m, 6 H, Naphth-H), 4.02 (q, J = 7.2 Hz, 1H, CHCH₂), 3.96 (s, 3H, OCH₃), 1.69 (d, J = 7.2 Hz, 3H, CHCH₂), 2.35 (m, 2H, pro-CH₂), 2.03 (m, 2H, pro-CH₂), 1.98 (m, 2H, pro-CH₂), 2.37 (7, 2H, pro-CH₂), 2.37 (1, 2H, pro-CH₂); ¹³C-NMR (CDCl₃) δ ppm = 175.30, 171.50, 156.40, 129.30, 128.90, 127.40, 126.40, 126.10, 117.60, 105.50, 66.10, 55.30, 51.70, 50.20, 24.10, 23.90; MS: m/z (%) 341 (M⁺, 100); analysis calculated for C₂₉H₂₃NO₄; C, 70.36; H, 6.79; N, 4.10. Found: C, 70.68; H, 6.83; N, 4.07.
Methyl-3-(4-hydroxyphenyl)-2-(2-[6-methoxynaphthalen-2-yl]propanamido)propanoate (11)

Yield 59%, melting point 138°C–139°C; IR (KBr): ν(cm⁻¹): 1734 (ester), 1668 (amide); ¹H-NMR δ/ppm (CDCl₃): 6.75–7.77 (m, 8H, Naphth-H), 6.67 (bs, 1H, CONH), 4.58 (dd, J = 2.1 Hz, 5.9 Hz, 1H, COOCH), 4.02 (q, J = 7.2 Hz, 1H, CHCH₂), 3.60 (s, 3H, COOCH₃), 1.69 (d, J = 7.2 Hz, 3H, CHCH₃), 0.93 (s, 3H, OCH₳), 3.60 (s, 3H, COOCH₂), 3.60 (d, J = 7.2 Hz, 3H, CHCH₃), 1.69 (d, J = 7.2 Hz, 3H, CHCH₳), 2.17 (dt, J = 7.5 Hz, 14.1 Hz, 2H, COOCH₂CH₳), 1.92 (dt, J = 7.9 Hz, 14.2 Hz, 2H, COOCH₂CH₳); ¹³C-NMR (CDCl₃) δ/ppm = 182.6, 150.6, 134.5, 128.7, 128.4, 127.4, 117.6, 105.5, 66.5, 55.3, 51.7, 50.2, 24.1, 23.9; MS: m/z (%) 407 (M⁺, 100); analysis calculated for C₂₂H₂₃NO₆: C, 65.82; H, 6.78; N, 3.49. Found: C, 65.98; H, 6.34; N, 3.05.

Dimethyl-2-(2-[6-methoxynaphthalen-2-yl]propanamido)pentanedioate (12)

Yield 83%, melting point 107°C–108°C; IR (KBr): ν(cm⁻¹): 1738 (ester), 1662 (amide); ¹H-NMR δ/ppm (CDCl₃): 6.75–7.77 (m, 6H, Naphth-H), 6.67 (bs, 1H, CONH), 4.58 (dd, J = 2.1 Hz, 5.9 Hz, 1H, COOCH), 4.02 (q, J = 7.2 Hz, 1H, CHCH₂), 3.96 (s, 3H, OCH₳), 3.60 (s, 3H, COOCH₂), 3.60 (s, 3H, COOCH₂), 1.69 (d, J = 7.2 Hz, 3H, CHCH₳), 1.92 (dt, J = 7.5 Hz, 14.1 Hz, 2H, COOCH₂CH₳), 1.92 (dt, J = 7.9 Hz, 14.2 Hz, 2H, COOCH₂CH₳); ¹³C-NMR (CDCl₃) δ/ppm = 182.6, 150.6, 134.5, 128.7, 128.4, 127.4, 117.6, 105.5, 66.5, 55.3, 51.7, 50.2, 47.8, 45.1, 39.2, 29.3, 26.4, 26.5, 22.4, 18.5; MS: m/z (%) 407 (M⁺, 100); analysis calculated for C₂₂H₂₃NO₆: C, 65.82; H, 6.78; N, 3.49. Found: C, 65.98; H, 6.34; N, 3.05.
the individual lesions in the mucosa using Vernier calipers, and the sum of lengths of all lesions in each stomach was regarded as the ulcer index.

**Measurement of prostaglandin E\(_2\) levels**

Measurement of prostaglandin E\(_2\) levels was done using the previously described standard 6-day air pouch method for rats.\(^{22,24}\) Male Wistar rats weighing 170–200 g were divided into six groups of five animals each. The air pouch was induced as follows: on the first day of the experiment, 20 mL of air was injected subcutaneously in the back of each rat. Two days later, another 10 mL of air was injected at the same site. On the fifth day after the first injection, a further 10 mL of air was injected into the pouch. One day later and one hour before injecting the pouch with carrageenan (2 mL of 1% solution in saline), four groups of animals were treated orally with the test compounds 9–12 at a dose of 0.271 mmol/kg body weight, one group with the reference drug indomethacin (active control group, 10 mg/kg body weight, 0.028 mmol/kg) suspended in saline solution with the aid of a few drops of Tween 80, and the last group with sterile saline (placebo control group). All injections were conducted under light ether anesthesia. Six hours after injection of carrageenan, the animals were lightly anesthetized with ether and the contents of the pouch were aspirated using a Pasteur pipette and transferred into graduated plastic tubes kept in ice. The bulk of the exudates was frozen and stored at \(-20^\circ\)C until required for prostaglandin E\(_2\) assay.

Prostaglandin E\(_2\) was measured using an enzyme-linked autoimmune assay (Beckman Biomek™ 1000 Automated Laboratory Workstation apparatus, Fullerton, CA, USA) technique using a prostaglandin E\(_2\) assay kit and the reference drug (indomethacin) according to the manufacturer’s specifications (Figure 2).

**Acute toxicity**

At the end of the experiments, all animals used in the anti-inflammatory study were observed closely for 48 hours. No morbidity or mortality was documented.

**Data analysis**

The results are expressed as the mean ± SEM per group. The statistical significance of differences between groups was determined by one-way analysis of variance followed by Dunnett’s test. SigmaStat software was used to determine statistical significance. A probability (\(P\)) value of less than 0.05 was considered to be statistically significant.
Results

Chemistry
The carboxylic acid moiety of the parent compounds ibuprofen (1) and naproxen (2) was activated by reaction with thionyl chloride and benzotriazole at room temperature to yield the intermediate benzotriazoles 3 and 4, respectively (Figure 3). The reaction of 3 and 4 with the corresponding amino acid methyl esters in acetonitrile in the presence of triethylamine successfully afforded good yields (79%–89%) of the ibuprofen derivatives 5–8 and naproxen derivatives 9–12, respectively (Figures 4 and 5). Chemical structures of all the newly developed compounds (5–12) were established through spectroscopic (infrared, $^1$H-NMR, $^{13}$C-NMR, mass spectrometry) as well as elemental analysis data.

Pharmacology
When subjected to anti-inflammatory activity, compounds 5–12 showed significantly higher anti-inflammatory activity in the range of 40.64%–87.82% when compared with the placebo control group. Among all the newly synthesized compounds 5–12, the naproxen derivatives 9–12 with anti-inflammatory activity ranging between 66.99% and 87.82% were found to be significantly more potent than their corresponding ibuprofen derivatives 5–8 with inhibition in the range of 22.03%–52.91%. Moreover, the anti-inflammatory activity measured for derivatives derived from naproxen (compounds 9–12) were significantly more potent than the control groups of ibuprofen (76.34%) or naproxen (75.59%). Noticeably, within the current series of derivatives derived from naproxen (9–12), especially 9 and 10, with respective 83.91% and 87.82% inhibition of inflammation, exhibited significantly higher potency than those of 11 and 12 ($P < 0.05$).

Furthermore, the gastric ulcerogenicity for naproxen derivatives 9 (ulcer index 11.73) and 10 (ulcer index 12.30) was found to be significantly lower than that of the ibuprofen and naproxen control groups which had ulcer indices of 22.87 and 24.13, respectively. Conversely, both naproxen derivatives (9 and 10) failed to show significant inhibition of

![Figure 2 Prostaglandin E$_2$ inhibition of test compounds 9–12.](https://www.dovepress.com/)

**Notes:** Doses administered: indomethacin 10 mg/kg; naproxen-containing derivatives 9–12 (0.217 mmol). Results are expressed as the mean ± standard error of six replicates. *Significantly different from control value of indomethacin ($P < 0.05$); # significantly different from 11 and 12 ($P < 0.05$). The significant difference between groups was tested by using one-way analysis of variance followed by Dunnett’s test at $P < 0.05$.

![Figure 3 Synthesis of ibuprofen and naproxen benzotriazoles 3 and 4.](https://www.dovepress.com/)

**Note:** (i) Benzotriazole, SOCl$_2$, CH$_2$Cl$_2$, room temperature, 20 minutes.
prostaglandin E₂ synthesis when compared with compounds 11 and 12 or the control group of indomethacin.

**Discussion**

**Chemistry**

Having free carboxylic acid moieties, ibuprofen and naproxen were modified into various carbamoylmethyl esters (5–12) using different aliphatic as well as aromatic amino acid methyl esters, which resulted in masking of the carboxylic acid moiety. The infrared spectra of compounds 5–12 revealed the presence of a strong band at \( \nu = 1668 \) assignable to their carbonyl amide function, in addition to an amide NH stretching vibration band at \( \nu = 3332–3340 \). The \(^1\)H-NMR spectra of compounds 7–8 and 11–12 revealed the presence of an amidic -NH group as a sharp singlet signal at \( \delta = 6.67 \), whereas the \(^1\)H-NMR spectra of 5 and 9 revealed

![Figure 4 Synthetic pathway of ibuprofen containing derivatives 5–8.](image1)

**Figure 4** Synthetic pathway of ibuprofen containing derivatives 5–8.

**Note:** (i) Methyl ester of corresponding amino acid, acetonitrile, triethylamine, 20°C, 6–24 hours.

![Figure 5 Synthetic pathway of naproxen containing derivatives 9–12.](image2)

**Figure 5** Synthetic pathway of naproxen containing derivatives 9–12.

**Note:** (i) Methyl ester of corresponding amino acid, acetonitrile, triethylamine, 20°C, 6–24 hours.
the presence of a methyl group on the amide nitrogen of sarcosine as a sharp singlet signal at δ = 3.26. The mass spectra of compounds 5–12 exhibiting the parent molecular ion peaks confirmed the assumed structures.

**Pharmacology**

From the results obtained, it appears that animals pretreated with ibuprofen derivatives 5–8 were significantly protected against inflammation (23.3%–52.9% inhibition of edema) as compared with the placebo control group, but the anti-inflammatory effects observed for compounds 5–8 were significantly lower in comparison with those of the control groups ibuprofen-treated (P < 0.05), naproxen-treated (P < 0.05) control group, as well as naproxen derivatives (P < 0.05) (Figure 4).

Among the derivatives (compounds 5–12) investigated in the current study, our findings clearly suggest that structural modification via amidation of the carboxylic acid moiety of the parent compound naproxen (9–12) showed more potent antiinflammatory effect than derivatives derived from ibuprofen. Moreover, our results show that chemical modification of naproxen, especially with small-sized amino acid esters, as achieved in derivatives 9 (83.91%) and 10 (87.82%) potently and significantly elevate their anti-inflammatory activity within the series of naproxen derivatives 9–12 as compared with the control of the naproxen group (76.34%, P < 0.05). In contrast, naproxen when modified through bulkier aromatic substitution, eg, the l-tyrosine methyl ester moiety present in compound 11, or even an aliphatic elongation of the amino acid ester side chain as found in compound 12, negatively affected the anti-inflammatory activity measured for compounds 11 and 12 (Figure 1).

Furthermore, the ulcerogenic indices for the naproxen derivatives 9–12 with more promising anti-inflammatory potencies were studied in rats (Table 1). From the data observed, it is concluded that rats pretreated with test compounds 9 and 10 had significantly less ulcerogenic potential (ulcer indices of 11.73 and 12.30, respectively) than that of the control groups pretreated with ibuprofen or naproxen (ulcer indices of 22.87 and 24.13, respectively, P < 0.05).

On the other hand, inhibition of prostaglandin E₂ synthesis determined for naproxen derivatives 9–12 showed that compounds 9 and 10 had significantly reduced (P < 0.05) prostaglandin E₂ levels compared with the control groups pretreated with the reference drug indomethacin (97.97 pg/mL, Figure 2). In contrast, naproxen derivatives 11 and 12 were found to show a significant decrease in prostaglandin E₂ levels (P < 0.05) when compared with the placebo control group, but failed to exert significantly higher inhibition when compared with the control groups pretreated with the reference compound, indomethacin, or their structurally related naproxen derivatives 9 and 10 (Figure 2).

**Conclusion**

Our results show that naproxen derivatives 9–12 had better anti-inflammatory activity compared with their corresponding ibuprofen derivatives 5–8 when ibuprofen and naproxen were used as the reference drugs. The concept of a relationship between type of chemical substitution and anti-inflammatory activity has been clarified in the present study. It can be seen clearly that amidation of the carboxylic acid moiety of naproxen with small-sized amino acid methyl esters led to a significant increase in the anti-inflammatory activity observed in the newly developed compounds, 9 and 10. However, bulkier aromatic substitution, eg, l-tyrosine methyl ester moiety as in naproxen derivative 11, or even an aliphatic elongation of the amino acid ester side chain found in 12, negatively influenced the anti-inflammatory activity measured in the current study. On the other hand, the anti-inflammatory results revealed remarkable activity for naproxen derivatives 9 and 10, which correlate largely with the results observed for inhibition of prostaglandin E₂ synthesis.

These distinct characteristics, especially for compounds 9 and 10, with high anti-inflammatory activity, low ulcerogenicity, and potent inhibitory action on prostaglandin E₂ synthesis make the newly developed derivatives, especially those derived from naproxen, potentially interesting for future structural optimization and pharmacological investigation of their ability to inhibit cyclo-oxygenase enzymes 1 and 2, given that gastric toxicity of NSAIDs is linked closely to the ability of these drugs to inhibit prostaglandin synthesis, with both cyclo-oxygenase 1 and 2 contributing significantly to mucosal defense and repair. It would also be worth performing

**Table 1** Effect of study compounds 9–12 on ulcer indices

| Compound | Animals with ulcers (n) | Incidence (%) | Ulcer index mean ± SEM (mm) |
|----------|------------------------|---------------|----------------------------|
| Control  | 0/5                    | 0             | 0                          |
| Ibuprofen| 5/5                    | 100           | 22.87 ± 6.73               |
| Naproxen | 5/5                    | 100           | 24.13 ± 6.34               |
| 9        | 4/5                    | 80            | 11.73 ± 3.96 ±6            |
| 10       | 4/5                    | 80            | 12.30 ± 4.28 ±6            |
| 11       | 5/5                    | 100           | 16.84 ± 8.14               |
| 12       | 5/5                    | 100           | 15.90 ± 7.36               |

Notes: *Significantly different from control values of ibuprofen and naproxen, respectively (P < 0.05).

Abbreviation: SEM, standard error of the mean.
further assays in appropriate cell lines to confirm that these new compounds do not have intrinsic cytotoxicity.

**Acknowledgment**

The financial support of this work by the College of Medicine and Health Sciences/United Arab Emirates University is gratefully acknowledged.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**

1. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol.* 1971;231:232–235.

2. Marnett LJ, Kaligutkar AS. Cyclooxygenase 2 inhibitors: discovery, selectivity and the future. *Trends Pharmacol Sci.* 1999;20:465–469.

3. Dannhardt G, Kiefer W. Cyclooxygenase inhibitors – current status and future prospects. *Eur J Med Chem.* 2001;36:109–126.

4. Almansa C, Alfon J, De Arriba AF, et al. Synthesis and structure-activity relationship of a new series of COX-2 selective inhibitors: 1,5-diarylimidazoles. *J Med Chem.* 2003;46:3463–3475.

5. Jackson LM, Hawkey CJ. Gastrointestinal effects of COX-2 inhibitors. *Expert Opin Investig Drugs.* 1999;8:963–971.

6. Anikina LV, Levit GL, Demin AM, et al. Synthesis and anti-inflammatory and analgesic activity of amino acids acylated with ibuprofen. *Pharm Chem J.* 2002;36:237–239.

7. Mishra A, Ravichandran V, Jain PK, Dixit VK, Agrawal RK. Synthesis, characterization and pharmacological evaluation of amide prodrugs of ketorolac. *Eur J Med Chem.* 2008;43:2464–2472.

8. Shanbhag VR, Crider AM, Gokhale R, Harpalani A, Dick RM. Ester and PGE2 inhibitory properties of novel phenylcarbamoylmethyl ester derivatives. *Pharm Biochem Med.* 2009;73:618–626.

9. Roy SD, Manoukian E. Permeability of ketorolac acid and its ester analogs (prodrug) through human cadaver skin. *Eur J Med Chem.* 2002;36:232–236.

10. Levit GL, Anikina LV, Vikharev YB, et al. Synthesis and antiinflammatory and analgesic activity of naproxen amides with amino acid derivatives. *Pharm Chem J.* 2002;36:232–236.

11. Barsoum FF, Georgey H, Abdel-Gawad N. Anti-inflammatory activity and PGE2 inhibitory properties of novel phenylcarbamoylmethyl ester-containing compounds. *Molecules.* 2009;14:667-681.

12. Katrizký AR, Jishkariani D, Narindoshvili T. Convenient synthesis of ibuprofen and naproxen aminoacyl, dipeptidoyl and ester derivatives. *Chem Biol Drug Des.* 2009;73:618–626.

13. Mishra A, Veerasamy R, Kumar P, Jain PK, Dixit VK, Agrawal RK. Synthesis, characterization and pharmacological evaluation of amide prodrugs of ketorolac. *Eur J Med Chem.* 2008;43:2464–2472.

14. Rasheed A, Kumar CKA. Novel approaches on prodrug based drug design. *Pharm Chem J.* 2008;42:677–686.

15. Parmeshwari KH, Prashant RM, Rajani G, Mange RY. Prodrug designing of NSAIDs. *Mini Rev Med Chem.* 2009;9:124–139.

16. Kumar R, Jain S, Jain N, Singh M. Synthesis and biological evaluation of some novel analogue of p-hydroxyaniline. *Acta Pharm Sci.* 2008;50:183–188.

17. Rasheed A, Kumar CKA. Design, synthesis, hydrolysis kinetics and pharmacodynamic profiles of histidine and alanine conjugates of aceclofenac. *Acta Pharm.* 2010;60:99–109.

18. Winter CA, Fisley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc Soc Exp Biol Med.* 1962;111:544–547.

19. Franklin PX, Pillai AD, Rathod PD, et al. 2-amino-5-thiazolyl motif: a novel scaffold for designing anti-inflammatory agents of diverse structures. *Eur J Med Chem.* 2008;43:129–134.

20. Barsoum FF, Hosni HM, Girgis AS. Novel bis(1-acyl-2-pyrazolines) of potential antiinflammatory and molluscicidal properties. *Bioorg Med Chem.* 2006;14:3929–3937.

21. Hamza YE, Sammour OA, Abdel-Latif HA. Enhancement of dissolution of indometacin and modulation of its pharmacodynamics and ulcerogenicity via solid dispersions. *Pharm Ind.* 1994;56:286–291.

22. Khayyal MT, El-Ghazaly MA, Abdallah DM, Okpanyi SN, Kelber O, Weiser D. Mechanisms involved in the anti-inflammatory effect of a standardized willow bark extract. *Arzneimittelforschung.* 2005;55:677–687.

23. Rasheed A, Kumar CK, Mishra A. Synthesis, hydrolysis studies and pharmacodynamic profiles of amide prodrugs of dexibuprofen with amino acids. *J Enzyme Inhib Med Chem.* 2011;26:688–695.

24. Edwards JW, Sedgwick AD, Willoughby DA. The formation of a structure with the features of synovial lining by subcutaneous injection of air: an in vivo tissue culture system. *J Pathol.* 1981;134:147–156.

25. Wallace JL, McKnight W, Reuter BK, Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology.* 2000;119:706–714.

26. Wallace JL. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn’t the stomach digest itself? *Physiol Rev.* 2008;88:1547–1565.