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# Synthesis and pharmacological evaluation of amide conjugates of NSAIDs with L-cysteine ethyl ester, combining potent antiinflammatory and antioxidant properties with significantly reduced gastrointestinal toxicity

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**Abstract**—The synthesis and pharmacological evaluation of a series of amide derivatives of NSAIDs with L-cysteine ethyl ester is described. The novel derivatives are potent antiinflammatory, antioxidant and hypocholesterolemic–hypolipidemic agents, while they demonstrate considerably reduced gastrointestinal toxicity. This molecular modification may offer a general route to safer antiinflammatory agents, potentially suitable for chronic use in conditions such as neurodegenerative disorders.


The great majority of nonsteroidal antiinflammatory drugs (NSAIDs) act via inhibition of cyclooxygenase, thus preventing prostaglandin biosynthesis. However, this mechanism of action is also responsible for their main undesirable effects, gastrointestinal (GI) ulceration and, less frequently, renal injury;[1] 15–35% of all peptic ulcer complications are caused by NSAIDs. The increase in hospitalization and deaths due to GI-related disorders parallels the increased use of NSAIDs.[2] Furthermore, it is well established that reactive oxygen species (ROS) play a decisive role in inflammatory conditions.[3] It has also been noted that some antioxidant compounds exhibit antiinflammatory activity[4,5] while oxidative stress is an important component of GI ulceration. The latter is further supported by the finding that indomethacin administration results in increased ROS production in the gastric mucosa, followed by gastric ulceration.[6,7] Thus, the discovery of molecules, which combine antiinflammatory and antioxidant activities may lead to the development of drugs with an improved therapeutic index. In this respect, the chemical derivatization of known NSAID molecules to incorporate antioxidant properties may be a useful approach, provided that the molecular modifications do not abolish the antiinflammatory activity. We have previously reported that cysteamine derivatives of NSAIDs are potent antiinflammatory and antioxidant agents.[8] Subsequently, it was shown that ester and amide derivatives of NSAIDs are potent cyclooxygenase-2 inhibitors and retain the antiinflammatory activity of the parent NSAIDs.[9–11] A large variety of substituents was tolerated in the ester or amide group of the NSAID derivatives. Thus, it appears that the carboxylic acid group of the NSAIDs, not being a requisite for antiinflammatory activity, comprises a convenient functionality for derivatization.

In this communication we report preliminary results on the design, synthesis and pharmacological evaluation of

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a series of amide derivatives of well established NSAIDs with L-cysteine ethyl ester. Due to the presence of the SH functional group, the latter moiety is likely to confer antioxidant properties to the novel compounds. The use of the natural amino acid L-cysteine as the antioxidant part of the molecule was considered advantageous in terms of potentially low toxicity of the novel derivatives.

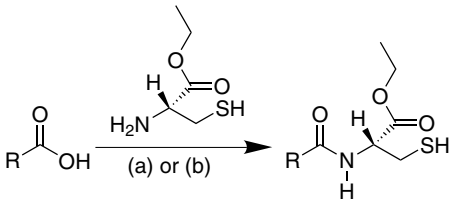
The structures and the synthesis of the novel derivatives **1**–**7** are presented in Table 1. The compounds were prepared by direct amidation of the carboxylic group of the respective NSAIDs using either N,N′-dicyclohexylcarbodiimide (DCC) or 1,1′-carbonyl-diimidazole (CDI) under the conditions indicated in Table 1. This group of compounds comprises representatives from 3 classes of NSAIDs: arylacetic acids (**1**, **2**), N-arylanthranilic acids (**3**, **4**) and arylpropionic acids (**5**–**7**).

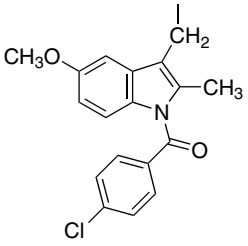
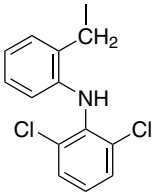
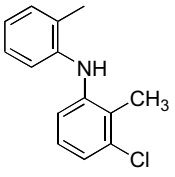
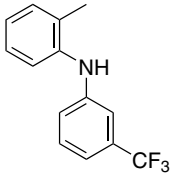
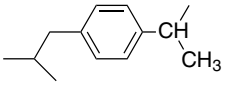
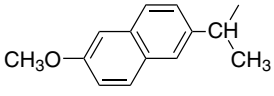
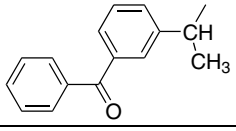
Compounds **1**–**7** were assayed in vitro for their ability to inhibit the peroxidation of rat hepatic microsomal lipids.[8,12] All compounds inhibited lipid peroxidation by 100% at 1 mM, while their $IC_{50}$ values (incubation for 45 min) were (compound, $IC_{50}$ μM): **1**, 51; **2**, 27; **3**, 180; **4**, 74; **5**, 38; **6**, 28; **7**, 650. This action may be attributed to the free SH group in the molecules, which can readily donate the sulfhydryl H atom, acting as a chain-breaking antioxidant.[13] The parent NSAIDs demonstrate limited effect on lipid peroxidation with the exception of tolfenamic acid, which has been found to possess moderate antioxidant activity.[14]

The free radical scavenging ability of the molecules was determined from the extent of their interaction with the stable free radical DPPH.[15] At equimolar concentrations, the interaction of the compounds with DPPH ranged from 70% to 90% (Table 2). The interaction of the parent NSAIDs at the same concentration was negligible, with the exception of diclofenac. Significant interaction with DPPH is observed even at concentrations of the analogues, which are 4-fold lower than that of DPPH.

The antiinflammatory activity of derivatives **1**–**7** was assessed from their ability to inhibit the paw edema induced by carrageenan in Balb/C mice[16] (Table 3). The compounds were administered ip at a dose of 150 μmol/kg and demonstrated a significant inhibition of the edema, being, in most cases, more potent than the parent NSAIDs. The increased activity of these compounds compared to the parent NSAIDs may be due to the antioxidant properties of the molecules. Furthermore, coadministration of equimolar mixtures of the parent NSAIDs and L-cysteine ethyl ester resulted in most cases in lower antiinflammatory activity than that of the test compounds. This constitutes evidence that they may act in vivo without prior hydrolysis of the amide bond. In line with this hypothesis is the finding that glycine amide derivatives of ibuprofen and naproxen are hydrolyzed very slowly in human serum ($t_{1/2} > 5$ h).[17] Furthermore, amide derivatives of indomethacin are not converted metabolically to indomethacin neither in human plasma nor by rat or human liver microsomes.[18] It should be added that the antiinflammatory activity of the compounds in the present study was estimated 3 h after their administration.

**Table 1.** Structures and syntheses for the compounds




| Compound | R | Parent RCOOH |
|---|---|---|
| **1** |  | Indomethacin |
| **2** |  | Diclofenac |
| **3** |  | Tolfenamic acid |
| **4** |  | Flufenamic acid |
| **5** |  | Ibuprofen |
| **6** |  | Naproxen |
| **7** |  | Ketoprofen |

Reagents and conditions: (a) Compounds **2**, **4** and **7**: CDI in DMF or $CH_2Cl_2$, 0 °C, 20–60 min; (b) compounds **1**, **3**, **5** and **6**: DCC/$Et_3N$ in $CH_2Cl_2$, rt, 2 h.

The gastrointestinal toxicity profile of compounds **1**–**7** and of the parent NSAIDs were evaluated in vivo following a 4-day dosage scheme in rats.[19] Thus, equimolar doses of the parent NSAIDs (producing approximately

**Table 2.** Percent interaction of compounds **1**–**7** at various concentrations with DPPH (0.2 mM) after 30 min of incubation

| Compound | 0.2 mM | 0.1 mM | 0.05 mM |
|---|---|---|---|
| **1** | 83.4 | 65.4 | 30.3 |
| **2** | 77.2 | 44.5 | 22.8 |
| **3** | 74.4 | 48.8 | 30.8 |
| **4** | 90.6 | 50.3 | 27.5 |
| **5** | 70.5 | 54.7 | 20.7 |
| **6** | 85.0 | 51.2 | 40.4 |
| **7** | 81.1 | 73.2 | 50.0 |

**Table 3.** Antiinflammatory action of compounds **1**–**7**, L-cysteine ethyl ester (CEE), the original NSAID and an equimolar mixture of NSAID and CEE, administered at 150 μmol/kg

| Compound | % Paw weight increase | % Edema inhibition |
|---|---|---|
| CEE | 29.2 ± 5.2 | 24.9 |
| Control | 38.9 ± 5.0 | — |
| **1** | 14.1 ± 6.4 | 63.3 |
| Indomethacin | 22.5 ± 4.1 | 41.6 |
| Indomethacin + CEE | 25.8 ± 7.1 | 32.8 |
| Control | 38.5 ± 6.0 | — |
| **2** | 11.8 ± 1.1 | 59.3 |
| Diclofenac | 24.5 ± 5.1 | 37.1 |
| Diclofenac + CEE | 28.2 ± 2.7 | 27.7 |
| Control | 39.0 ± 3.1 | — |
| **3** | 14.3 ± 3.7 | 51.0 |
| Tolfenamic acid | 27.6 ± 5.5 | 24.0 |
| Tolfenamic acid + CEE | 25.5 ± 3.2 | 25.9 |
| Control | 36.5 ± 4.8 | — |
| **4** | 22.5 ± 2.5 | 40.3 |
| Flufenamic acid | 55.4 ± 5.4 | 19 |
| Flufenamic acid + CEE | 61.9 ± 5.7 | 9 |
| Control | 37.8 ± 1.8 | — |
| **5** | 19.1 ± 1.8 | 61.2 |
| Ibuprofen | 31.7 ± 2.9 | 35.6 |
| Ibuprofen + CEE | 22.1 ± 3.2 | 48.4 |
| Control | 49.2 ± 3.5 | — |
| **6** | 35.6 ± 7.3 | 37.1 |
| Naproxen | 50.4 ± 6.4 | 11 |
| Naproxen + CEE | 30.1 ± 5.0 | 38.1 |
| Control | 56.6 ± 2.5 | — |
| **7** | 48.0 ± 7.4 | 29 |
| Ketoprofen | 35.9 ± 6.5 | 47 |
| Ketoprofen + CEE | 41.6 ± 4.9 | 39 |
| Control | 68.1 ± 7.8 | — |

Each value is the mean (± SD) from 4–9 mice in two independent experiments. Statistical significance compared with controls $P < 0.001$ (Student's *t*-test).

50% mortality in 4 days) and of the compounds under investigation were administered once daily for 4 days to female rats. The incidence of GI ulcers, the body weight change, the mortality and the appearance of melena defecation were recorded 24 h after the last treatment (Table 4). Evidently, the amide analogues **1**–**7** are practically devoid of GI toxicity at the doses detailed in Table 4, in marked contrast to the parent NSAIDs. The latter cause 50% mortality and 50–100% GI perforating ulcers. Although it is known that the formation of gastric lesions by NSAIDs is mainly due to inhibition of prostaglandin biosynthesis, the involvement of oxygen free radicals and lipid peroxidation in the development of the NSAID-induced mucosal damage has been proposed as an important event. Thus, indomethacin administration to rats results in a marked increase of thiobarbituric acid reactive substance formation in the liver and in the induced gastric lesions,[20] while liver glutathione levels are significantly lowered.[21] These findings indicate that NSAIDs increase the susceptibility of tissues to lipid peroxidation[22] and further suggest the potentially important contribution of lipid peroxidation in NSAID-induced ulceration. It is likely that the significantly reduced GI toxicity of the molecules **1**–**7** is due to the combination of the antioxidant properties of the compounds and the masking of the free carboxylic group of the parent NSAIDs. It is noteworthy that SH containing compounds have been shown to possess healing properties[23,24] and cysteamine and other SH-containing agents have been reported to exert a protective effect against ethanol and prostaglandin-induced gastric mucosal damage.[18] The absence of ulcerogenicity during the 4-day administration of compounds **1**–**7** constitutes further evidence that these molecules are not metabolically converted to the parent, ulcerogenic, NSAIDs.

It is well documented that inflammation[25] as well as oxidative stress[3,26] are profoundly implicated in a number of pathobiochemical processes related to neurodegenerative diseases. Thus, a number of epidemiologic studies show a lower incidence of Alzheimer's disease (AD) if NSAIDs were taken on a regular basis.[27] In a 15-year longitudinal analysis, the use of NSAIDs was associated with a lower incidence of AD.[28] However, the chronic use of NSAIDs in such conditions is seriously limited by their GI toxicity. Hence, in a double-blind AD treatment trial in which indomethacin was studied with positive results, approximately one third of patients had to discontinue because of gastrointestinal side effects.[29] Molecules **1**–**7**, having significantly reduced GI toxicity are good candidates for potential use in neurodegenerative diseases.

Furthermore, increased plasma cholesterol is related to neurodegeneration[30] and we have reported that several classical NSAIDs possess hypocholesterolemic and hypolipidemic action.[31] We have demonstrated that the combination in a single molecule of both hypocholesterolemic and antioxidant properties is an asset for their antiatheromatic activity.[32,33] Thus, the novel NSAID derivatives of the present study were evaluated for their hypocholesterolemic activity and preliminary results are presented in Table 5. Compounds **1**, **2**, **5** and **6** as well as the respective parent NSAIDs were administered ip to hypercholesterolemic rats and 24 h later the plasma total cholesterol (TC), LDL and triglyceride (TG) concentrations were determined in blood taken from the aorta.[32,34] The results in Table 5 demonstrate that the parent NSAIDs possess significant hypocholesterolemic activity. Amidation of the free carboxylic group of the parent NSAIDs results in either retention (**5**, **6**) or decrease (**1**, **2**) of hypocholesterolemic potency.

In conclusion, the amidation of the carboxylic group of known NSAIDs with L-cysteine ethyl ester is well tolerated and, in most cases, results in compounds with

**Table 4.** Gastrointestinal toxicity of the novel NSAID amides, parent drugs and L-cysteine ethyl ester (CEE)

| Compound | Dose (μmol/kg) | Mortality (%)[a] | Perforating ulcers (%)[b] | Body weight change[c] | Incidence of melena |
|---|---|---|---|---|---|
| **1** | 84 | 0 | 0 | 3.4 | — |
| Indomethacin | 84 | 50 | 80 | −13.0 | + |
| CEE | 84 | 0 | 0 | −1.9 | — |
| **2** | 240 | 0 | 0 | 3.1 | — |
| Diclofenac | 240 | 50 | 75 | −13.5 | + |
| CEE | 240 | 0 | 0 | −2.0 | — |
| **3** | 760 | 0 | 0 | 7.5 | — |
| Tolfenamic acid | 760 | 50 | 50 | −5.3 | + |
| CEE | 760 | 0 | 0 | −1.0 | — |
| **4** | 1200 | 0 | 0 | 3.6 | — |
| Flufenamic acid | 1200 | 50 | 50 | −9.2 | + |
| CEE | 1200 | 0 | 0 | −1.2 | — |
| **5** | 1600 | 0 | 0 | 1.0 | — |
| Ibuprofen | 1600 | 50 | 88 | −12.7 | + |
| CEE | 1600 | 0 | 0 | −1.6 | — |
| **6** | 1350 | 0 | 0 | 9.2 | — |
| Naproxen | 1350 | 50 | 100 | −15.8 | + |
| CEE | 1350 | 0 | 0 | −1.5 | — |
| **7** | 200 | 0 | 0 | 0.3 | — |
| Ketoprofen | 200 | 50 | 83 | −18.9 | + |
| CEE | 200 | 0 | 0 | −1.0 | — |

[a] Dead per total × 100.
[b] Percent of animals developing perforating intestinal ulcers.
[c] In g/100 g body weight. Standard deviation of the weight change is always within 10% of the average value.

**Table 5.** Hypolipidemic action of the test compounds, as expressed as the reduction of total cholesterol (TC), low density lipoprotein (LDL) and triglycerides (TG) in rat plasma

| Compound | Dose (μmol/kg) | % Decrease | | |
|---|---|---|---|---|
| | | TC | LDL | TG |
| **1** | 500 | 37.0 | 60.5 | 19.8 |
| Indomethacin | 500 | 74.4 | 72.2 | 78.6 |
| **2** | 500 | 54.7 | 92.6 | 31.6 |
| Diclofenac | 500 | 87.4 | 86.3 | 96.1 |
| **5** | 250 | 48.1 | 68.7 | 27.5 |
| Ibuprofen | 300 | 38.2 | 31.6 | 41.6 |
| **6** | 500 | 33.8 | 46.8 | 29.6 |
| Naproxen | 500 | 53.0 | 43.5 | 25.5 |

Each value is the mean from 4–9 rats in two independent experiments. Statistical significance compared with hyperlipidemic controls $P < 0.001$ (Student's *t*-test).

increased antiinflammatory activity. Furthermore, this molecular modification confers to the molecules antioxidant and free radical scavenging properties, while it also reduces dramatically their GI toxicity. This kind of chemical derivatization of NSAIDs may offer a viable route to safer antiinflammatory agents which, having additional beneficial properties such as antiatheromatic activity, may comprise good candidates for long term administration in neurodegenerative diseases or other conditions involving chronic inflammation.

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