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Synthesis and pharmacochemical study of novel polyfunctional molecules combining anti-inflammatory, antioxidant, and hypocholesterolemic properties

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Abstract—We have designed and synthesized a series of novel molecules having a residue of a classical NSAID and an antioxidant moiety, both attached through amide bonds to a known nootropic structure, an L-proline, *trans*-4-hydroxy-L-proline or DL-pipecolinic acid residue. The compounds were found to retain anti-inflammatory and antioxidant activities, to acquire hypocholesterolemic action, and to possess a greatly reduced gastrointestinal toxicity. The novel molecules could find useful applications, among others, in slowing the progression or delaying the onset of neurodegenerative diseases.

Alzheimer's disease and related dementias (SDAT) are the greatest unmet medical challenges in neurology, with over 12 million sufferers in the world. This condition accounts for the majority of dementias diagnosed over the age of 60. It is characterized by a global, progressive decline of cognitive functions and leaves end-stage patients bedridden, dependent on custodial care, with death occurring in about 10 years after diagnosis.[1] Pharmacotherapy of SDAT has been based on the cholinergic hypothesis, that is, the dysfunction of the acetylcholine system contributes to cognitive derangement in SDAT. Therefore, standard care includes treatment with acetylcholinesterase inhibitors.[2] These agents have been proven of limited benefit, while this symptomatic treatment fails to inhibit the progress of the disease itself.

It is well documented that inflammation[3] as well as oxidative stress[4,5] are profoundly implicated in a number of pathobiochemical processes related to neurodegenerative diseases. Furthermore, increased plasma cholesterol is related to neurodegeneration.[6] Thus, the prevention of these biochemical aberrations occurring in the demented brain may be a more rational approach toward agents against this pathological condition. In this investigation, we have designed a series of novel polyfunctional molecules combining anti-inflammatory, antioxidant, and hypocholesterolemic properties, which may have the potential to slow or interrupt the progress of the disease and thus provide a more complete and effective treatment approach.

We have recently shown that the chemical derivatization of the carboxylic acid group of well-established nonsteroidal anti-inflammatory drugs (NSAIDs) may offer a viable route to anti-inflammatory agents possessing an increased safety profile.[7] Hence, amidation of the NSAID molecules with cysteamine[8] or cysteine ethyl ester[7] resulted in compounds with increased anti-inflam-

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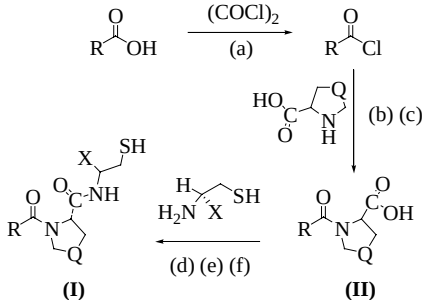
matory and antioxidant properties but with a significantly lower GI toxicity. Furthermore, it has been reported that a proline moiety is part of the pharmacophore of molecules showing nootropic or antiamnesic activity.[9,10] Thus, we have designed molecules of the general structure **I** (Table 1) in which a NSAID and cysteamine or cysteine ethyl ester have been chemically attached to a proline, 4-hydroxy-proline or pipecolinic acid moiety.

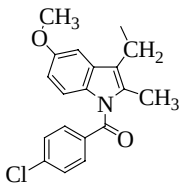
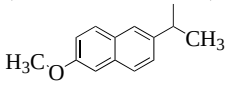
The structures and the synthesis of the novel compounds **1**–**7** are presented in Table 1. They were prepared by two successive amidations, initially of the amine group of the sodium salts of L-proline, *trans*-4-hydroxy-L-proline or DL-pipecolinic acid by the acid chloride of the NSAIDs, and then of the free carboxylic group of the intermediate **II**, using 1,1-carbonyldiimidazol (CDI) under the conditions indicated in Table 1. This group of compounds includes two representative NSAIDs (indomethacin and naproxen) and two known radical scavengers (cysteamine and cysteine ethyl ester). The structures were confirmed spectroscopically (IR, ^{1}H NMR) and by elemental analysis.

Compounds **1**–**7**, along with cysteamine and cysteine ethyl ester as reference compounds, were assayed in vitro for their antioxidant activity by evaluating their ability to inhibit peroxidation of rat hepatic microsomal membrane lipids.[8,11] Four compounds inhibited lipid peroxidation by 100% at 1 mM, while cysteamine and cysteine ethyl ester inhibited lipid peroxidation by 37% and 49%, respectively, at the same concentration. IC_{50} values of the new compounds (incubation for 45 min) were (compound, IC_{50} μM): **2**, 390; **4**, 320; **6**, 500; **7**, 122. This action could be attributed to a combination of proper lipophilicity with the free HS-group which can readily donate the sulfydryl H atom, acting as a chain breaking antioxidant.[12] It is noteworthy that the less lipophilic cysteamine analogues **1**, **3**, and **5** were much less active in this assay ($IC_{50} > 1$ mM) compared with the cysteine ethyl ester analogues **2**, **4**, and **6**. A similar trend was observed in a series of simpler conjugates of NSAIDs with cysteamine[8] and cysteine ethyl ester,[7] the latter being significantly more potent than the former. The parent NSAIDs and the proline intermediates **II** presented negligible antioxidant activity at 1 mM.

Table 1. Synthesis and structures of the prepared compounds



Reagents and conditions: (a) in dichloromethane at 0 °C, 30 min, 3 h rt; (b) in 2 N aq NaOH, 0 °C, 1 h; (c) acidification with 1 N hydrochloric acid, extraction with $CHCl_3$; (d) in DMF, CDI added at 0 °C, 1 h rt; (e) cysteamine HCl or cysteine ethyl ester HCl added at rt and stirred for 24 h; (f) water added, extraction with $CHCl_3$

Compound	R	Q	X
1	 (Indomethacin residue)	CH_2	H
2		CH_2	$COOC_2H_5$
3	 (Naproxen residue)	CH_2	H
4		CH_2	$COOC_2H_5$
5		CHOH	H
6		CHOH	$COOC_2H_5$
7		$(CH_2)_2$	$COOH_2H_5$

Compound, mp (°C) and yield (% final reaction): **1**, 138–140, 29; **2**, 109–111, 30; **3**, 151–152, 42; **4**, oil, 60; **5**, 140–141, 29; **6**, semi-solid, 32; **7**, oil, 39.

The radical scavenging ability of the molecules was determined from the extent of their interaction with the stable free radical DPPH.[13] At equimolar concentrations, the interaction of the compounds with DPPH ranged from 56% to 86% (Table 2). The interaction of the parent NSAIDs with DPPH at the same concentration was negligible. This interaction indicates the reducing potential of the new compounds.

The anti-inflammatory activity of derivatives **1**–**7**, along with indomethacin and naproxen as reference compounds, was assessed from their ability to inhibit paw edema induced by carrageenan in female Fischer rats[7] (Table 3). The compounds were administered ip at a dose of 300 μmol/kg and demonstrated a significant inhibition of the edema ranging from 14% to 68%. Furthermore, experimental arthritis was produced in rats by an id injection of complete Freund's adjuvant.[14] Compounds **1**–**4** were administered at a dose of 300–600 μmol/kg for 14 days, and the arthritic score was assessed on the 15th day post adjuvant injection. The tested compounds inhibited arthritis by 50–100% (Table 4). It can be seen from the results that the new compounds acquire a good antioxidant and reducing potential, while they retain considerable anti-inflammatory activity.

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	NA[a]	NA[a]	NA[a]
2	69.7	44.2	31.1
3	78.9	56.3	28.8
4	86.3	61.9	32.1
5	56.2	36.5	21.3
6	75.7	54.4	26.8
7	76.7	46.0	23.5

[a] Not active.