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Esters of some non-steroidal anti-inflammatory drugs with cinnamyl alcohol are potent lipoxygenase inhibitors with enhanced anti-inflammatory activity

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ABSTRACT

Novel esters of non steroidal anti-inflammatory drugs, α-lipoic acid and indol-3-acetic acid with cinnamyl alcohol were synthesised by a straightforward method and at high yields (60–98%). They reduced acute inflammation more than the parent acids and are potent inhibitors of soybean lipoxygenase. Selected structures decreased plasma lipidemic indices in Triton-induced hyperlipidemia to rats. Therefore, the synthesised compounds may add to the current knowledge about agents acting against various inflammatory disorders.

Nonsteroidal anti-inflammatory drugs (NSAIDs) modulate the arachidonic acid cascade and are widely used for the treatment of pathophysiological inflammatory conditions. Eicosanoids are synthesised from arachidonic acid via three pathways. Cycloxygenase (COX) enzymes catalyse the formation of prostaglandins and thromboxanes; the lipoxygenase (LOX) pathway leads to leukotrienes and lipoxins, while epoxyeicosatrienoic acids are products of the cytochrome P450 enzymes. These lipid mediators generate both inflammatory and anti-inflammatory responses, through a complex communication between the arachidonic acid pathways.[1] Classical NSAIDs are mainly non-selective COX inhibitors and constitute the most commonly used approach for the treatment of inflammatory conditions. This class of drugs, as well as the second generation selective COX-2 inhibitors (coxibs) present gastrointestinal and cardiovascular undesired effects, evolved from an imbalance in homeostatic eicosanoid production. In case of COX-1/2 inhibition, a shift towards the LOX pathway is observed and leukotrienes are also important causes of gastrointestinal irritation. Concerning LOX inhibition, up to now, zileuton is the only 5-LOX inhibitor used in the clinic for the treatment of asthma, although there are some restrictions concerning its use.[2,3]

Consequently, since the selective inhibition of one pathway in the arachidonic acid cascade appears to cause undesired effects, and a combination therapy with the co-administration of two or more drugs seems to raise efficacy and safety issues, as well as compliance problems, the rational design of multi-target agents has gained interest lately.[4,5] Tenidap, a dual COX/LOX inhibitor and cytokine modulator, found to be more potent than diclofenac in the treatment of rheumatoid arthritis,[6] has been withdrawn from the clinic because of liver and kidney toxicity. However, this toxicity was attributed to reactive metabolites due to the thiophene moiety,[7,8] and replacement of the thiophene ring gave analogues with stronger activity and better gastric tolerance than tenidap.[9]

In this communication, we used eight classical NSAIDs (Fig. 1) known to be non-selective COX inhibitors, with negligible activity on LOX.[10] In addition, indole-3-acetic acid, a part of the indomethacin structure, as well as α-lipoic acid, known for its antioxidant and anti-inflammatory activity,[11,12] were included. All the above carboxylic acids were esterified with cinnamyl alcohol, giving the final, novel compounds. Cinnamic acid and its analogues have been studied for anti-inflammatory activity in a number of reports. Especially, cinnamaldehyde has been found to decrease carrageenan-induced inflammation, suppress iNOS, COX-2 and NF-κB expression,[13] as well as TNF-α.[14]

The effects of these compounds on carrageenan induced rat paw oedema and on the in vitro activity of soybean lipoxygenase were examined. Selected structures were tested for hypolipidemic activity in vivo.

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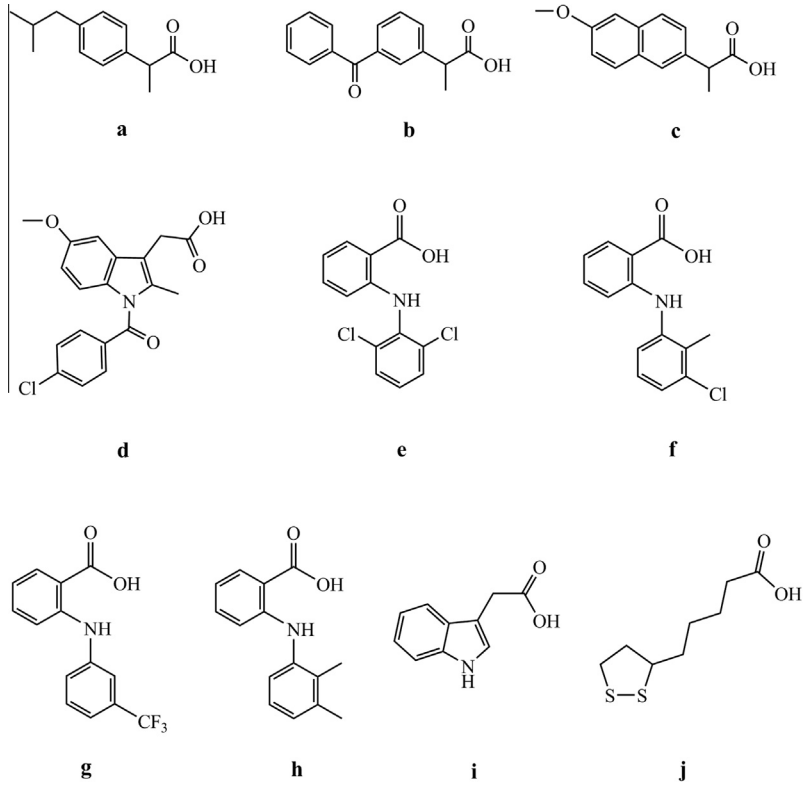
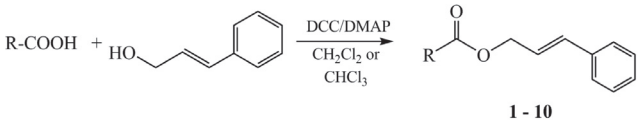


Figure 1. Structures of the carboxylic acids used: (a) Ibuprofen; (b) Ketoprofen; (c) Naproxen; (d) Indomethacin; (e) Diclofenac; (f) Tolfenamic acid; (g) Flufenamic acid; (h) Mefenamic acid; (i) 2-(1*H*-indol-3-yl)acetic acid; (j) 5-(1,2-Dithiolan-3-yl)pentanoic acid (α-lipoic acid).



Comp.	RCOOH	clogP*	Comp.	RCOOH	clogP
1	Ibuprofen	6.45	**6**	Tolfenamic acid	7.67
2	Ketoprofen	5.54	**7**	Flufenamic acid	7.34
3	Naproxen	5.59	**8**	Mefenamic acid	7.41
4	Indomethacin	6.96	**9**	2-(1*H*-indol-3-yl)acetic acid	4.18
5	Diclofenac	7.10	**10**	-Lipoic acid	5.13

Figure 2. Synthesis of compounds and their lipophilicity (C log *P*). DCC: *N*,*N*′-dicyclohexylcarbodiimide; DMAP: 4-dimethylaminopyridine. Cinnamyl alcohol: (2*E*)-3-phenylprop-2-en-1-ol. *C log *P* for windows v. 4.0, BioByte Corp.

Compounds **1**–**10** were synthesised by a straightforward method from the related acids and cinnamyl alcohol ((2*E*)-3-phenylprop-2-en-1-ol), using DDC and a catalytic amount of DMAP, at room temperature and with excellent yields (60–98%) (Fig. 2).

Their effect on acute inflammation, applying the carrageenan paw oedema model,[15] as well as the anti-inflammatory activity of the parent NSAIDs, under the same experimental conditions, are shown in Table 1. The carrageenan-induced paw oedema is a well-known and widely used model of acute inflammation. By this model, a biphasic inflammation is induced. The early phase is related to the release of histamine, serotonin, bradykinin and, to a lesser extent, of COX activation, whereas, in the delayed phase, more than one hours after administration, neutrophil infiltration, further prostaglandin production and release of pro-inflammatory cytokines are involved.[16]

All compounds could reduce paw oedema significantly, at 150 μmol/kg, ip, 3.5 h post injection. This effect seems to be dose-dependent, as indicated by the increase of activity of **1** and **10** at 300 μmol/kg. Compounds **1**–**8** were found to be more potent than their individual parent NSAIDs, indicating a further enhancement of the anti-inflammatory activity by the performed molecular modification. Compound **10** reduced oedema by 48%, more than

Table 1
Effect of compounds **1**–**10** and parent NSAIDs on carrageenan-induced rat paw oedema[a]

Compound	% Oedema reduction
1	46*
1 (0.30 mmol/kg)	68**
Ibuprofen	36*
2	55**
Ketoprofen	47*
3	60**
Naproxen	11*
4	56**
Indomethacin	42**
5	53**
Diclofenac	37**
6	42**
Tolfenamic acid	24**
7	48**
Flufenamic acid	19*
8	62**
Mefenamic acid	44*
9	33**
10	48**
10 (0.30 mmol/kg)	57**
Cinnamyl alcohol	13^{NS}

Significant difference from control: ^{NS}(not significant) $P > 0.3$, $^{*}P < 0.005$, $^{**}P < 0.001$ (Student's t test).

[a] The effect on oedema is expressed as percent of inhibition of oedema in comparison to controls. All compounds were administered ip, at a dose of 0.15 mmol/kg of body weight. Each value represents the mean obtained from 6 to 8 animals.

Table 2
Effect of compounds **1**–**10**, cinnamyl alcohol and NDGA on lipoxygenase[a]

Compound	IC_{50} (μM)
1	6.7
2	22.5
3	40.5
4	9.0
5	78
6	9.6
7	9.0
8	38
9	116
10	11.3
Cinnamyl alcohol	250
NDGA	1.3

[a] After 7 min of incubation; NDGA: nordihydroguaiaretic acid.

most of the NSAIDs included in this experiment. Lipoic acid itself has been reported[17] to possess a weak activity, about 5% oedema reduction, at similar doses (50 and 100 mg/kg, corresponding to 250 and 500 μmol/kg), under an analogous experimental setting. Compound **9** has a moderate effect on acute inflammation, while indol-3-acetic acid has no reported anti-inflammatory action. Cinnamyl alcohol, tested under the same experimental conditions, was found to have no significant effect on acute inflammation.

Soybean lipoxygenase (linoleate 13*S*-lipoxygenase) is often used as a reliable screen for LOX inhibition.[18] It has been reported that arachidonic acid binding sites in soybean lipoxygenases share almost the same similarity with animal 5-LOX.[19]

The ability of compounds to inhibit lipoxygenase, presented as IC_{50} values towards soybean lipoxygenase after 7 min of incubation, is demonstrated in Table 2. The IC_{50} of nordihydroguaiaretic acid (NDGA), an antioxidant compound acting as a nonspecific inhibitor of lipoxygenase, is also included as a reference.

The time course of LOX inhibition, as affected by the most active compounds **1** and **7** is shown in Figure 3.

All compounds inhibited lipoxygenase very significantly. Given that the NSAIDs used here have insignificant or no effect on LOX, as verified in this work and elsewhere,[10,18,20] it is apparent that the observed inhibition is a result of the esterification with cinnamyl alcohol. This is further supported by the important inhibition caused by the lipoic acid ester **10**, and even of compound **9**, considering that the parent carboxylic acids are inactive. However, cinnamyl alcohol itself has been found to offer a weak inhibition of LOX, under the same experimental conditions.

When linoleic acid was used at a concentration of 1 mM, which is higher than the saturating substrate concentration, no inhibition was observed, under the same experimental conditions. These results indicate that the examined compounds act as competitive inhibitors of lipoxygenase, since inhibition can be overcome by increasing substrate concentration.

Considering the results of the described in vivo and in vitro experiments, it could be concluded that the enhanced activity of **1**–**8**, compared with the parent NSAIDs may, at least in part, be attributed to the offered LOX inhibition. Meclofenamic acid, a NSAID, has been found to inhibit 5-LOX[10,21] and it has been suggested that this effect may add to the increased anti-inflammatory activity of this drug.[22] The contribution of LOX inhibition may be further supported by the significant anti-inflammatory activity of cinnamyl lipoate **10**, in conjunction with its strong LOX inhibition.

Atherosclerosis has been characterised as a chronic inflammatory disease of the arteries and leukotrienes derived from the 5-LOX pathway mediate various inflammatory processes during atherogenesis, leading to foam cell formation. Furthermore, it has been suggested that atorvastatin, a hypolipidemic drug, significantly alleviates atherosclerotic lesions by inhibiting the 5-LOX pathway.[23,24] Elevated plasma levels of cholesterol and

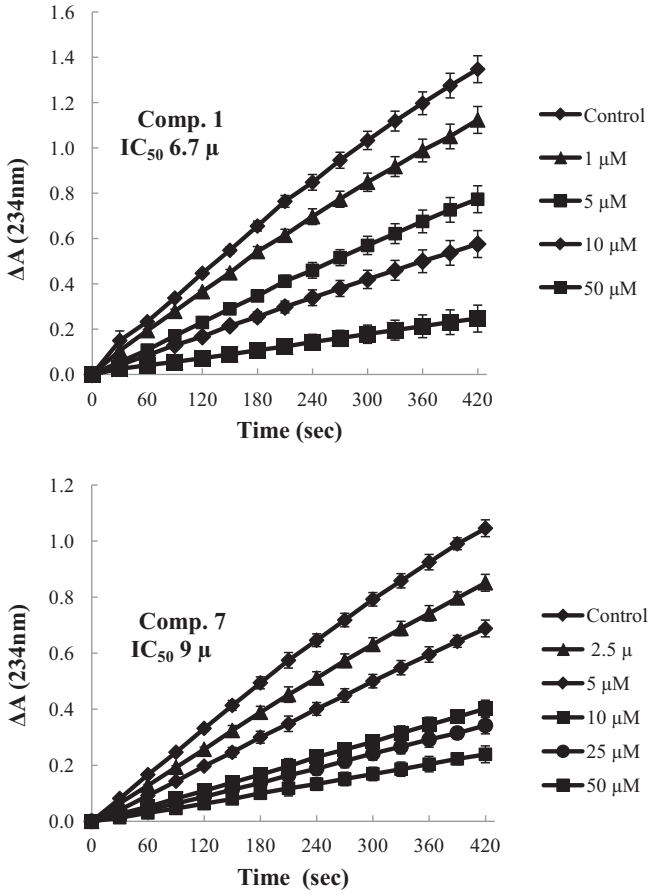


Figure 3. Time course of lipoxygenase inhibition, as affected by various concentrations of compounds **1** and **7**.

Table 3
Effect of compounds **1**, **5** and **7** on Triton WR1339 (tyloxapol) induced hyperlipidemia

Compound	% Reduction		
	TC[a]	TG	LDL-C
1	53.3**	77.5**	75.0*
5	78.4**	71.6**	76.8**
7	48.4**	50.1**	65.1**

Tyloxapol: 200 mg/kg, ip; compounds: 150 μmol/kg, ip. Significant difference from hyperlipidemic control: $^{*}P < 0.01$, $^{**}P < 0.001$ (Student's t test).
Each group was composed of six to eight rats.
[a] TC: total cholesterol; TG: triglycerides; LDL-C: LDL-cholesterol.

triglycerides constitute the most important risk factor for atherosclerosis. Moreover, a direct involvement of hypercholesterolemia in 5-LOX activity has been reported.[25] Thus, three potent anti-inflammatory compounds, **1** and **7** found to be most active LOX inhibitors, as well as **5**, a derivative of diclofenac, a NSAID used widely for myoskeletal and dental pain, migrains, moderate post-traumatic and menstrual pain, were tested for anti-dyslipidemic activity in hyperlipidemic rats. The Triton-WR1339 induced hyperlipidemia is characterised by a dramatic increase of serum cholesterol and especially triglyceride levels, the latter due to surfactant-mediated inhibition of lipoprotein lipase activity. Triton-induced hyperlipidemia occurs twenty four hours after the administration and causes significant increase in the concentrations of atherogenic C-LDL, C-VLDL and IDL in mice and rats.[26]

Results are shown in Table 3. All tested compounds reduced plasma lipidemic indices profoundly. The highest inhibition, more

than 70%, was offered by **5**, the diclofenac derivative, a potent anti-inflammatory compound, yet with moderate ability to inhibit lipoxygenase. The ibuprofen analogue **1**, the strongest LOX inhibitor, presented a very significant hypolipidemic potential. We have reported[27] that both ibuprofen and diclofenac offered a moderate to strong reduction of hyperlipidemia, however, this effect was expressed at much higher doses (300 and 500 μmol/kg, respectively). It could be concluded that esterification of NSAIDs with cinnamyl alcohol induces hypolipidemic properties.

Considering that free radicals are implicated in inflammatory processes, the synthesized compounds were tested for antioxidant activity, expressed as inhibition of rat microsomal membrane lipid peroxidation induced by ferrous ascorbate, as well as interaction with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). They were found to have no antioxidant activity in either test.

In conclusion, esterification of known NSAIDs, lipoic acid and even indol-3-acetic acid with cinnamyl alcohol enhances or confers anti-inflammatory properties and offers significant lipoxygenase inhibitory activity. Furthermore, there is strong evidence that they may also possess anti-dyslipidemic properties, thus addressing the major risk factors for atherogenesis. Concerning their effect on LOX, it could be hypothesised that, since the used cinnamyl alcohol is the *trans*-isomer, the lipophilic cinnamyl esters (Fig. 2) may occupy the hydrophobic U-shaped channel of the lipoxygenase active site that can accommodate the polyunsaturated fatty acid substrate of this enzyme.[28] Therefore, the synthesised compounds may add to the current knowledge about agents acting against various inflammatory disorders.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.10.036. These data include MOL files and InChiKeys of the most important compounds described in this article.

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