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Topical anti-inflammatory activity of boropinic acid and its natural and semi-synthetic derivatives

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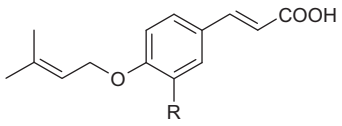
ABSTRACT

Boropinic acid is a natural isopentenyloxycinnamic acid extracted from the aerial parts of *Boronia pinnata* Sm. (Rutaceae) with soybean 5-lipoxygenase inhibitory activity. In this paper the topical anti-inflammatory activity of boropinic acid and some of its natural and semi-synthetic derivatives was evaluated using the Croton oil ear test in mice as a model of acute inflammation. Some of the tested compounds (**15**, **17**, **19**, **20**) revealed an effect comparable (ID_{50} = 0.18 ÷ 0.72 μmol/cm^2) to that of the reference drug indomethacin (ID_{50} = 0.23 μmol/cm^2), a non-steroidal anti-inflammatory drug.

3-(4′-Isopentenyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid, commonly known as boropinic acid, **1** is a secondary metabolite biosynthetically related to ferulic acid in which an isopentenyl chain is attached to the phenolic group. It has been isolated in 2000 by Ito et al. from the aerial parts of *Boronia pinnata* Sm., an Australian shrub belonging to the family of Rutaceae.[1] Since its discovery, this natural compound showed valuable pharmacological properties that have been recently reviewed.[2] In particular, boropinic acid showed valuable in vitro inhibitory effects on the growth of *Helicobacter pylori* and soybean 5-lipoxygenase (5-LOX) activity.[3]



1 R = OMe
2 R = H

Inhibition of 5-lipoxygenase suggests a possible in vivo anti-inflammatory activity of **1**. Since inflammation is a universal and physiological response involved in several diseases, compound **1** and a series of its novel natural and semi-synthetic derivatives were investigated for their anti-inflammatory activity. Continuing our research aimed to better define the pharmacological profile of **1** and its natural and semi-synthetic derivatives, we synthesized a series of isopentenyloxycinnamic acids differently substituted in position 3′ and assessed their topical anti-inflammatory activity. These compounds were drawn in such a structure in order to verify the influence of different substituents located in the aromatic ring on the biological activity.

Boropinic acid **1** and 4′-isopentenyloxycinnamic acid **2**, the latter a natural prenyloxyphenylpropanoid identified in *Esenbeckia hieronymi* Engl. (Fam. Rutaceae),[4] were synthesized as already reported.[5]

The synthesis of 3′-halo-4′-isopentenyloxycinnamic acids was accomplished starting from 3-halo-4-hydroxybenzaldehydes, that in all cases were purified by crystallization in H_2O. To this aim 3-fluoro-4-hydroxybenzaldehyde **4** was obtained in 50% yield starting from commercially available 2-fluorophenol **3** by formylation with hexamethylenetetramine and CF_3COOH at 100 °C for 24 h (Scheme 1).[6]

3-Chloro-4-hydroxybenzaldehyde **6** was synthesized in 64% yield by chlorination of commercially available 4-hydroxybenzaldehyde **5** with *N*-chlorosuccinimide (NCS) in $CHCl_3$ at 60 °C for 12 h (Scheme 2).[7]

Finally 4-hydroxy-3-iodobenzaldehyde **7** was obtained in 72% yield by iodination of 4-hydroxybenzaldehyde **5** with KI/I_2 in NH_4OH 30% (aq) as the solvent at room temperature for 6 h (Scheme 3).[8]

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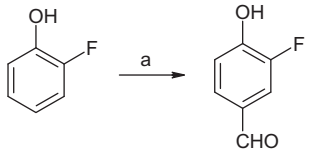
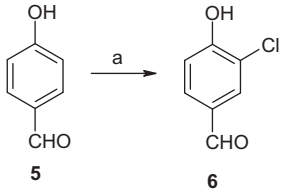


Scheme 1. Reagents and conditions: (a) hexamethylenetetramine (1 equiv), CF_3COOH (1 equiv), 100 °C, 24 h.



Scheme 2. Reagents and conditions: (a) NCS (1 equiv), $CHCl_3$, 60 °C, 12 h.

Benzaldehydes substituted in position 3 with a CH_3– and a NO_2– group were commercially available.

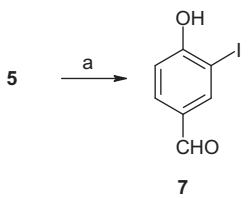
Synthesized and commercially available 3-substituted-4-hydroxybenzaldehyde were then submitted to a Wittig–Horner olefination[9] by reaction with methyl trimethylphosphonacetate and Na in MeOH at 70 °C for 24 h to obtain the corresponding cinnamic acid methyl esters **8** (X = –F, 86% yield), **9** (X = –Cl, 99% yield), **10** (X = –Br, 99% yield), **11** (X = –I, 81% yield), **12** (X = –Me, 89% yield), and **13** (X = $–NO_2$, 99% yield).[10] All these derivatives were in turn alkylated in position 4′ by reaction with isopentenyl bromide promoted by K_2CO_3 as the base in acetone at 80 °C for 2 h, followed by basic hydrolysis in the same vessel and acid/base workup to afford pure isopentenyloxycinnamic acids **14** (X = –F, 76% yield), **15** (X = –Cl, 99% yield), **16** (X = –Br, 80% yield), **17** (X = –I, 71% yield), **18** (X = –Me, 87% yield), and **19** (X = $–NO_2$, 97% yield) (Scheme 4).

Finally the last product, 3-amino-4-isopentenyloxycinnamic acid **20** was obtained in 59% yield from the nitro-derivative **19** by reduction with $SnCl_2$ in EtOH at 70 °C for 30 min. (Scheme 5).

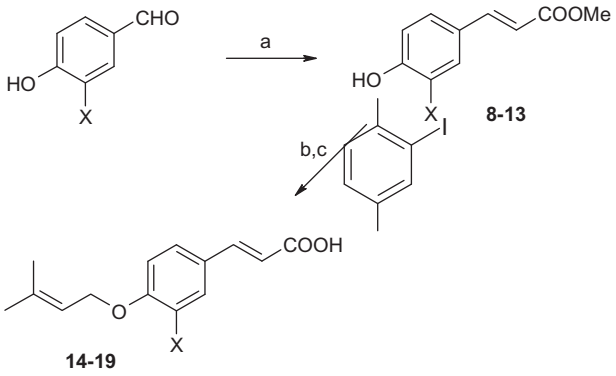
All the semi-synthetic isopentenyloxycinnamic acids are reported herein for the first time.

The topical anti-inflammatory activity of compound **1** and its derivatives **2**, and **14–20** was evaluated as inhibition of the Croton oil-induced ear dermatitis in mice.[11]

Male CD-1 mice (28–32 g, Harlan Laboratories, Udine, Italy) were anaesthetised with ketamine hydrochloride (145 mg/kg, intraperitoneally; Virbac, Milan, Italy). Inflammation was induced on the right ear (surface: about 1 cm^2) by application of 80 μg of Croton oil (Sigma Chemical Co., St. Louis, USA) dissolved in acetone. The left ear remained untreated as preliminary experiments showed that the vehicle did not affect the inflammatory response or induce irritation. Control mice received only the irritant solution, whereas the others received both the irritant and the compounds under test dissolved in acetone. Six hours later, mice were sacrificed and a plug (6 mm Ø) was excised from both the ears to quantify oedema as weight difference between the two plugs. The anti-inflammatory activity was expressed as percent of oedema reduction in mice treated with the compounds under test with regard to control mice. Oedema values, expressed as means ± standard error of the mean, were analysed by one way analysis of variance followed by Dunnett's test for multiple comparison of unpaired data. A probability level lower than 0.05 was considered as significant. Experiments complied with the Italian D.L. n. 116 of January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609 ECC) concerning animal welfare and appendix A of the European Convention ETS 123.



Scheme 3. Reagents and conditions: (a) KI/I_2 (1 equiv), NH_4OH 30%, rt, 6 h.



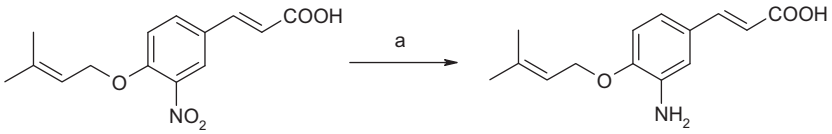
Scheme 4. Reagents and conditions: (a) $(MeO)_3POCH_2COOMe$ (3 equiv), Na (3 equiv) MeOH, 70 °C, 24 h; (b) isopentenyl bromide (1 equiv), K_2CO_3 (1 equiv), acetone , 80 °C, 2 h; (c) NaOH 2 N (aq), reflux, 30 min, acid/base workup

The results on the anti-inflammatory activity screening of compounds **1**, **2**, and **14–20**, administered at the dose of 0.3 μmol/cm^2, are reported in the Table 1, in comparison to those of the same dose of the non-steroidal anti-inflammatory drug (NSAID) indomethacin (a cycloxygenases [COXs] inhibitor), and nordihydroguaiaretic acid (a [5-LOX] inhibitor), used as references. Each compound provoked a significant oedema inhibition, which ranged from 30% (**2**) to 78% (**17** and **19**). The natural compounds, boropinic acid **1** and 4′-isopentenyloxycinnamic acid **2** induced 38% and 30% oedema reduction, respectively. While the semi-synthetic derivatives **16** and **18** provoked less than 20% oedema reduction, the other semi-synthetic compounds induced oedema reductions ranging from 46% (**20**) to 78% (**17** and **19**). The reference compounds indomethacin and nordihydroguaiaretic acid induced 62% and 36% oedema inhibition, respectively.

Thus, the natural compounds **1** and **2** as well as the most active semi-synthetic ones (**15**, **17**, **19**, **20**) were further investigated for their dose–activity relationship, in comparison to indomethacin and nordihydroguaiaretic acid.

As reported in the Table 2, boropinic acid **1** and its derivatives **15**, **17**, **19** and **20** (0.1–1 μmol/cm^2) exerted a dose-dependent anti-inflammatory effect. The relevant ID_{50} values (dose inducing 50% oedema inhibition), as index of anti-inflammatory potency, showed that the anti-inflammatory activity of compound **1** (ID_{50} = 0.72 μmol/cm^2) was slightly increased by its demethoxylation to **2** (ID_{50} = 0.50 μmol/cm^2). In addition, the semi-synthetic derivatives of **2** substituted in position 3′ with a chlorine (**15**) or iodine (**17**) atoms, but also with a nitro (**19**) or amino (**20**) functional groups, were about twice more active than **2** (ID_{50} ranging from 0.18 to 0.33 μmol/cm^2). Moreover, the anti-inflammatory potency of these compounds was comparable to that of indomethacin (ID_{50} = 0.23 μmol/cm^2) and about twice higher than that of nordihydroguaiaretic acid (ID_{50} = 0.54 μmol/cm^2).

Depicting preliminary structure–activity relationships (SAR), we have already revealed by docking studies that an unfuctionalized isopentenyloxy side chain in position 4 of the aromatic ring



Scheme 5. Reagents and conditions: (a) $SnCl_2$ (5 equiv), EtOH, 70 °C, 0.5 h.

Table 1
Anti-inflammatory activity of boropinic acid **1** and its derivatives

Substance	Nr. animals	Dose (μmol/cm^2)	Oedema (mg) [Mean ± S.D.]	% Reduction
Controls	10	—	6.9 ± 0.3	—
1	10	0.3	4.3 ± 0.2*	38
2	10	0.3	4.8 ± 0.4*	30
14	10	0.3	4.2 ± 0.3*	39
15	10	0.3	3.5 ± 0.3*	49
16	10	0.3	5.6 ± 0.3*	19
17	10	0.3	1.5 ± 0.3*	78
18	10	0.3	5.3 ± 0.3*	23
19	9	0.3	1.5 ± 0.4*	78
20	9	0.3	3.7 ± 0.3*	46
Indomethacin	10	0.3	2.6 ± 0.4*	62
Nordihydroguaiaretic acid	10	0.3	4.4 ± 0.4*	36

* $p < 0.05$ at the analysis of variance, as compared to controls.

Table 2
Dose-dependent anti-inflammatory activity of boropinic acid **1** and its derivatives

Substance	Nr. animals	Dose (μmol/cm^2)	Oedema (mg) [Mean ± S.D.]	% Reduction	DI_{50} (μmoli/cm^2)
Controls	19	—	6.9 ± 0.2	—	—
1	10	0.1	6.1 ± 0.3*	12	0.72
	10	0.3	4.4 ± 0.2*	36	
	10	1.0	3.1 ± 0.3*	55	
2	10	0.1	6.0 ± 0.2*	13	0.50
	10	0.3	4.4 ± 0.2*	36	
	10	1.0	2.3 ± 0.2*	67	
15	10	0.1	5.2 ± 0.3*	25	0.26
	10	0.3	3.4 ± 0.3*	51	
	10	1.0	0.6 ± 0.1*	91	
17	10	0.1	4.8 ± 0.2*	30	0.20
	10	0.3	2.3 ± 0.3*	67	
	10	1.0	0.8 ± 0.3*	88	
19	10	0.1	4.6 ± 0.3*	33	0.18
	10	0.3	2.2 ± 0.2*	68	
	10	1.0	0.6 ± 0.1*	91	
20	10	0.1	5.4 ± 0.3*	22	0.33
	10	0.3	3.9 ± 0.2*	43	
	10	1.0	1.3 ± 0.2*	81	
Indomethacin	10	0.1	5.1 ± 0.3*	26	0.23
	10	0.3	2.6 ± 0.4*	62	
	10	1.0	0.9 ± 0.2*	87	
Nordihydroguaiaretic acid	10	0.1	5.6 ± 0.3*	19	0.54
	10	0.3	4.6 ± 0.4*	33	
	10	1.0	2.4 ± 0.3*	65	

* $p < 0.05$ at the analysis of variance, as compared to controls.

is an essential feature for the inhibition of a key pro-inflammatory enzyme like 5-LOX.[12] The presence of the above cited moiety in all compounds tested could partly account also for the anti-inflammatory effect of the tested compounds. Also the cinnamic acid moiety probably plays a key role in the inhibition of 5-LOX as previously reported by Koshihara et al. in 1983.[13] Anyway, from the obtained results, the inhibition of 5-LOX seems not to be the main mechanism by which the most active compounds (**15**, **17**, **19**, **20**) reduce the oedema formation induced by Croton oil, being their activity about twice higher than that of the 5-LOX inhibitor nordihydroguaiaretic acid and comparable to that of the dual COX inhibitor indomethacin. So it could be hypothesised that, besides an activity against 5-LOX, these semi-synthetic compounds could trigger their action on other targets involved in inflammation. It seems that the presence of an isopentenyloxy side chain is still a structural requirement for the observed anti-inflammatory activity, but the introduction of bulky substituent like a iodine atom (**17**) is a feature that led to an increased activity as compared to the parent compound **2**. The same hypothesis can be made for electron withdrawing groups like the $-NO_2$ moiety, although in this case also steric hindrance has to be considered to observe efficacy. In fact the compound **14** having a fluorine atom in position 3, sharing the same electronic properties with $-NO_2$ derivative **19**, is by far less active than the latter. Also the presence of electron donat-

ing groups like the $-NH_2$ moiety (**20**), or sterically medium sized electron withdrawing groups like chlorine (**15**), led to an increased activity, even though of lower extent.

In conclusion, the findings described herein indicate that some semi-synthetic isopentenyloxycinnamic acids, structurally derived from the natural product boropinic acid **1**, could be regarded as potential novel and effective anti-inflammatory agents, the potency of which is comparable to the well known NSAID indomethacin. All tested compounds were easily synthesized starting from widely available starting materials, by good yielding and cheap synthetic routes. Four of these derivatives showed a very interesting topical anti-inflammatory activity in the Croton oil-induced ear dermatitis test in mice. For these reasons the present study could be considered as a topic for future studies aimed at better define the pharmacological profile of semi-synthetic isopentenyloxycinnamic acids. To this aim, studies to get further insights into their mechanism of action as well as to investigate the biological activity of compounds substituted in positions other than 3′ on the aromatic ring, as well as the effects of functionalized *O*-side chains, α,β-unsaturated double bonds and isosters of the carboxylic groups are now ongoing in our laboratories.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.107.

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