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Article type : Research Article

Sinapic acid phenethyl ester as a potent selective 5-lipoxygenase inhibitor: synthesis and structure activity relationship

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Abstract

Given the hepatotoxicity and an unfavourable pharmacokinetic profile of Zileuton (Zyflo®), currently the only approved and clinically used 5-Lipoxygenase (5-LO) inhibitor, the search for potent and safe 5-LO inhibitors is highly demanded. The action of several phenolic acid phenethyl esters as potential 5-Lipoxygenase (5-LO) inhibitors has been investigated. For this purpose, a series of fourteen phenethyl esters was synthesized and their impact on 5-LO inhibition was evaluated. The effects of position and number of hydroxyl and methoxy groups on the phenolic acid were investigated. The shortening of the linker between the carbonyl and the catechol moiety as well as the presence of the α,β-unsaturated carbonyl group was also explored. The sinapic acid phenethyl ester (**10**), which can be named SAPE (**10**) by analogy to caffeic acid phenethyl ester (CAPE), inhibited 5-LO in a concentration-dependent manner and outperformed both zileuton (**1**) and CAPE (**2**). With an IC_{50} of 0.3 µM, SAPE (**10**) was 3-fold more potent than CAPE (**2**) and 10-fold more potent than zileuton (**1**), the only 5-LO inhibitor approved for clinical use. Unlike CAPE (**2**), SAPE (**10**) had no effect on 12-lipoxygenase (12-LO) and less effect on cyclooxygenase COX-1 which makes it a more selective 5-LO inhibitor.

Keywords: Sinapic acid, Caffeic acid, 5-LO inhibitors, anti-leukotriene therapy.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cbdd.13360

1. INTRODUCTION

5-Lipoxygenase (5-LO), a key enzyme for arachidonic acid (AA) metabolism, catalyzes the biosynthesis of leukotriene A_4 (LTA_4).[1] The hydrolysis of LTA_4 by leukotriene A_4 hydrolase produces the proinflammatory leukotriene B_4. On the other hand, hydrolysis of LTA_4 by leukotriene C_4 synthase produces the cysteinyl leukotrienes C_4, D_4 and E_4[2]. Leukotrienes (LTs) are a class of eicosanoid inflammatory mediators which have been shown to play a crucial role in several inflammatory disorders including asthma, atherosclerosis and arthritis[3]. Additionally, malignant cells highly express 5-LO and related LT biosynthesis enzymes, consequently anti-LT therapy can not only be a promising anti-inflammatory but an anticancer strategy as well[3–9].

5-LO is a validated target for inflammation and related disorders.[10] Several 5-LO inhibitors have been reported, including iron ligand, redox, and nonredox inhibitors[11]. Given the weak potency, hepatotoxicity and an unfavourable pharmacokinetic profile of zileuton (Zyflo®) (**1**) (Figure 1), currently the only approved and clinically used 5-LO inhibitor[3,12–15], the search for potent and safe 5-LO inhibitors is highly demanded.

Recent studies from our group have shown that CAPE (**2**) is significantly more potent than Zileuton (**1**) for the inhibition of LTs biosynthesis in human polymorphonuclear leukocytes (PMNL, IC_{50} = 0.52 μM)[16]. Our research has shown that the amide as well as the cinnamic acid analogs are less active, which highlights the importance of the polyphenol and ester moieties for efficient inhibition of LTs biosynthesis. On the other hand, the corresponding ketone and ether, which may be less sensitive to hydrolysis *in vivo*, were more active than CAPE (**2**)[11,17–19].

CAPE (**2**), like other polyphenols, undergoes both sulfatation and glucuronidation[20–26] during metabolism, which generates several molecular entities which are rapidly eliminated and contribute to the poor pharmacokinetic profile and bioavailability of the molecule. By having an α,β-unsaturated carbonyl moiety, CAPE (**2**) and its analogues react as Michael's acceptor with nucleophiles such as SH cation of cysteines leading to a covalent bond with the β carbon.[27]. Therefore, derivatives of CAPE that reach their targets may be structurally quite different from the originally administered molecule, thus complicating the discovery of new molecules as well as the understanding of their mode of action. However, the α,β-unsaturated carbonyl moiety can act as radical scavenger and antioxidant[28].

In the present work, we investigated the influence of the presence of the two hydroxyls as well as the α, β-unsaturated carbonyl group present in CAPE (**2**). Phenethyl esters of various phenolic acids were synthesized and tested for 5-LO inhibition.

The development of dual or even multiple inhibitors of oxygenases is sought after, however, the discovery of specific inhibitors to a single enzyme such as 5-LO remains relevant. Therefore, the selectivity of the synthesized compounds was also explored. The best 5-LO inhibitors were also tested for the inhibition of 12-lipoxygenase (12-LO) and cyclooxygenase (COX-1). 12-LO catalyzes the stereo-specific peroxidation of polyunsaturated fatty acids generating the bioactive lipid mediator (12S)-HPETE which regulates several biological processes including platelet activation and angiogenesis.[29,30] COX-1 catalyzes the biosynthesis of prostaglandins which have several important physiological roles including the control of gastric secretions and nociception[31].

2. MATERIALS AND METHODS

2.1. Chemistry

All chemicals used were purchased from Aldrich (CA) and used without further purification. Purification of compounds was carried out by flash chromatography (Isco, Inc. CombiFlash™ Sg100c). TLC was run on silica gel coated aluminium sheets (SiliaPlate TLC, Silicycle®) with detection by UV light (254 nm, UVS-11, Mineralight® shortwave UV lamp). Melting points were obtained using a MELTEMP® (model 1001D) melting point apparatus. FTIR spectra were recorded on a Nicolet® Impact 400 spectrometer. NMR spectra were recorded on a Bruker® Avance III 400 MHz spectrometer using TMS as an internal standard. High-resolution mass measurements were performed on a Bruker® Doltonics' micrOTOF instrument in positive or negative electrospray.

2.1.1. General procedure for the esterification of phenolic acids

To a vigorously stirred solution of Na_2CO_3 (1.2 eq) in 6 mL of HMPA, 500 mg of the appropriate phenolic acid (1 eq) is added after which the reaction vessel is put under argon atmosphere. After 30 minutes of stirring, the appropriate bromide (1.1 eq) is dissolved in 1 mL of HMPA and added dropwise to the reaction mixture over a period of 30 minutes. A small amount of KI is added and the reaction vessel is thoroughly flushed with argon gas and sealed under balloon pressure. The reaction mixture is stirred in an ice bath for 72 hours under argon atmosphere. The resulting solution is quenched with 30 mL of ice water and

stirred for 30 minutes. The aqueous phase is extracted three times with AcOEt (30mL). The organic fractions are then combined, washed with brine, dried over $MgSO_4$ and concentrated. The resulting oil is purified by flash chromatography.

2-Phenylethyl (2E)-3-(4-hydroxyphenyl)prop-2-enoate (**4**)

Following our general procedure for the esterification of phenolic acids with *para*-coumaric acid (**3a**) (500 mg, 3.05 mmol, 1 eq), Na_2CO_3 (384 mg, 3.62 mmol, 1.2 eq) and (2-bromoethyl)benzene (0.47 mL, 3.45 mmol, 1.1 eq), compound **4** was obtained as a white solid after flash chromatography (0 - 20 % AcOEt/Hex), yield = 24 %, Mp = 96 - 97 °C, Rf = 0.43 (30 % AcOEt/Hex). ^{1}H-NMR (DMSO-d6, 25 °C); δ (ppm): 10.09 (s large, 1H, OH), 7.55 - 7.51 (d, *J = 15.00 Hz*, 1H, C_{Ar}-CH=CH), 7.55 - 7.51 (m, 2H, H_{Ar}), 7.33 - 7.27 (m, 4H, H_{Ar}), 7.25 - 7.20 (m, 1H, H_{Ar}), 6.79 (d, *J = 8.56 Hz*, 2H, H_{Ar}), 6.35 (d, *J = 15.97 Hz*, 1H, CH=CH-C(=O)), 4.33 (t, *J = 6.84 Hz*, 2H, CH_2O), 2.95 (t, *J = 6.84 Hz*, 2H, CH_2Ph); ^{13}C-NMR (DMSO-d6, 25 °C); δ (ppm): 167.03, 160.32, 145.29, 138.55, 130.82, 129.33, 128.84, 126.84, 125.47, 116.25, 114.48, 64.80, 34.92; HRMS *m/z* calc. for $C_{17}H_{16}O_3$ + (H^+): 269.1172; found: 269.1183.

2-Phenylethyl (2*E*)-3-(3-hydroxyphenyl)prop-2-enoate (**5**)

Following our general procedure for the esterification of phenolic acids with *meta*-coumaric acid (**3b**) (500 mg, 3.04 mmol, 1 eq), Na_2CO_3 (384 mg, 3.62 mmol, 1.2 eq) and (2-bromoethyl)benzene (0.47 mL, 3.45 mmol, 1.1 eq), compound **5** was obtained as a white solid after flash chromatography (5 - 12 % AcOEt/Hex), yield = 13 %, Mp = 98 - 99 °C, Rf = 0.75 (50 % AcOEt/Hex). ^{1}H-NMR (DMSO-d6, 25 °C); δ (ppm): 9.62 (s, 1H, OH), 7.53 (d, *J = 16.01 Hz*, 1H, C_{Ar}-CH=CH), 7.34 - 7.29 (m, 4H, H_{Ar}), 7.25 - 7.20 (m, 2H, H_{Ar}), 7.05 - 7.01 (m, *J = 1.72 Hz*, 1H, H_{Ar}), 6.85 (dd, *J = 8.0 0Hz*, *2.24 Hz*, 1H, H_{Ar}), 6.48 (d, *J = 16.01 Hz*, 1H, CH=CH-C(=O)), 4.55 (t, *J = 6.84 Hz*, 2H, CH_2O), 2.95 (t, *J = 6.84 Hz*, 2H, CH_2Ph); ^{13}C-NMR (DMSO-d6, 25 °C); δ (ppm): 166.57, 158.17, 145.28, 138.48, 135.65, 130.41, 129.36, 128.84, 126.86, 119.73, 118.16, 115.18, 65.04, 34.89; HRMS *m/z* calc. for $C_{17}H_{16}O_3$ + (H^+): 269.1172; found: 269.1170.

2-Phenylethyl (2*E*)-3-(2-hydroxyphenyl)prop-2-enoate (**6**)

Following our general procedure for the esterification of phenolic acids with *ortho*-coumaric acid (**3c**) (500 mg, 3.05 mmol, 1 eq), Na_2CO_3 (384 mg, 3.62 mmol, 1.2 eq) and (2-bromoethyl)benzene (0.47 mL, 3.45 mmol, 1.1 eq), compound **6** was obtained as white

crystals after flash chromatography (0 - 10 % AcOEt/Hex), yield = 36 %, Mp = 98 - 99 °C, Rf = 0.52 (30 % AcOEt/Hex). ^{1}H-NMR (DMSO-d6, 25 °C); δ (ppm): 10.25 (s, 1H, OH), 7.86 (d, *J = 16.17*, 1H, C_{Ar}-CH=CH), 7.60 (d, *J = 7.76 Hz*, 1H, H_{Ar}), 7.32 - 7.22 (m, 6H, H_{Ar}), 6.91 (d, *J = 8.12 Hz*, 1H, H_{Ar}), 6.82 (t, *J = 7.52 Hz*, 1H, H_{Ar}), 6.58 (d, *J = 16.13 Hz*, 1H, CH=CH-C(=O)), 4.35 (t, *J = 6.92 Hz*, 2H, CH_2O), 2.97 (t, *J = 6.84 Hz*, 2H, CH_2Ph); ^{13}C-NMR (DMSO-d6, 25 °C); δ (ppm): 157.25, 140.68, 138.54, 132.22, 129.35, 128.84, 126.83, 121.11, 119.87, 117.43, 116.62, 64.86, 34.94; HRMS *m/z* calc. for $C_{17}H_{16}O_3$ + (H^+): 269.1172; found: 269.1175.

2-Phenylethyl (2*E*)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**7**)

Following our general procedure for the esterification of phenolic acids with 3,4-dimethoxycinnamic acid (**3d**) (500 mg, 2.40 mmol, 1 eq), Na_2CO_3 (320 mg, 3.02 mmol, 1.3 eq) and (2-bromoethyl)benzene (0.37 mL, 2.72 mmol, 1.1 eq), compound **7** was obtained as a beige powder after flash chromatography (0 - 10 % AcOEt/Hex), yield = 72 %, Mp = 97 - 98°C, Rf = 0.40 (30 % AcOEt/Hex). ^{1}H-NMR ($CDCl_3$, 25 °C); δ (ppm): 7.64 (d, *J = 15.93 Hz*, 1H, C_{Ar}-CH=CH), 7.37 - 7.30 (m, 2H, H_{Ar}), 7.29 - 7.25 (m, 3H, H_{Ar}), 7.12 (dd, *J = 8.28 Hz*, *1.56 Hz*, 1H, H_{Ar}), 7.07 (d, *J = 1.44 Hz*, 1H, H_{Ar}), 6.89 (d, *J = 8.28 Hz*, 1H, H_{Ar}), 6.32 (d, *J = 15.89 Hz*, 1H, CH=CH-C(=O)), 4.45 (t, *J = 7.08 Hz*, 2H, CH_2O), 3.94 (s, 6H, OCH_3), 3.05 (t, *J = 7.04 Hz*, 2H, CH_2Ph); ^{13}C-NMR ($CDCl_3$, 25 °C); δ (ppm): 167.14, 151.15, 149.23, 144.78, 137.95, 128.93, 128.52, 127.41, 126.56, 122.67, 115.74, 111.03, 109.61, 64.88, 55.98, 55.91, 35.25; HRMS *m/z* calc. for $C_{19}H_{20}O_4$ + (H^+): 313.1434; found: 313.1437.

2-Phenylethyl (2*E*)-3-(3,4-dichlorophenyl)prop-2-enoate (**8**)

Following our general procedure for the esterification of phenolic acids with 3,4-dichlorocinnamic acid (**3e**) (500 mg, 2.30 mmol, 1 eq), Na_2CO_3 (320 mg, 3.02 mmol, 1.3 eq) and (2-bromoethyl)benzene (0.36 mL, 2.60 mmol, 1.1 eq), compound **8** was obtained as a white powder after flash chromatography (0 - 10 % AcOEt/Hex), yield = 69 %, Mp = 68 - 69 °C, Rf = 0.28 (5 % AcOEt/Hex). ^{1}H-NMR ($CDCl_3$, 25 °C); δ (ppm): 7.62 (d, *J = 1.89 Hz*, 1H, H_{Ar}), 7.57 (d, *J = 16.05 Hz*, 1H, C_{Ar}-CH=CH), 7.48 (d, *J = 8.32 Hz*, 1H, H_{Ar}), 7.37 - 7.33 (m, 3H, H_{Ar}), 7.29 - 7.26 (m, 3H, H_{Ar}), 6.42 (d, *J = 16.01 Hz*, 1H, CH=CH-C(=O)), 4.46 (t, *J = 7.04 Hz*, 2H, CH_2O), 3.04 (t, *J = 7.00 Hz*, 2H, CH_2Ph); ^{13}C-NMR ($CDCl_3$, 25 °C); δ (ppm): 166.27, 142.08, 137.74, 134.44, 134.22, 133.26, 130.90, 129.62, 128.92, 128.56, 127.04, 126.65, 119.95, 65.29, 35.15; HRMS *m/z* calc. for $C_{17}H_{14}Cl_2O_2$ + (H^+): 321.0444; found: 321.0441.

2-Phenylethyl (2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate (**9**)

Following our general procedure for the esterification of phenolic acids with ferulic acid (**3f**) (501 mg, 2.57 mmol, 1 eq), Na_2CO_3 (325 mg, 3.06 mmol, 1.2 eq) and (2-bromoethyl)benzene (0.40 mL, 2.94 mmol, 1.1 eq), compound **9** was obtained as a pale yellow oil after flash chromatography (0 - 10 % AcOEt/Hex), yield = 24 %, Rf = 0.43 (30 % AcOEt/Hex). ^{1}H-NMR (DMSO-d6, 25 °C); δ (ppm): 9.61 (s large, 1H, OH), 7.53 (d, *J = 15.89 Hz*, 1H, C_{Ar}-CH=CH), 7.34 - 7.29 (m, 5H, H_{Ar}), 7.25 - 7.21 (m, 1H, H_{Ar}), 7.10 (dd, *J = 8.20 Hz*, *1.84 Hz*, 1H, H_{Ar}), 6.79 (d, *J = 8.08 Hz*, 1H, H_{Ar}), 6.45 (d, *J = 15.93 Hz*, 1H, CH=CH-C(=O)), 4.35 (t, *J = 6.84 Hz*, 2H, CH_2O), 3.82 (s, 3H, OCH_3), 2.97 (t, *J = 6.84 Hz*, 2H, CH_2Ph); ^{13}C-NMR (DMSO-d6, 25 °C); δ (ppm): 167.06, 149.86, 148.64, 145.64, 138.56, 129.32, 128.85, 126.83, 125.99, 123.73, 115.94, 114.77, 111.63, 64.74, 56.17, 34.95; HRMS *m/z* calc. for $C_{18}H_{18}O_4$ + (H^+): 299.1278; found: 299.1280.

2-**Phenylethyl (2*E*)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoate** (**SAPE**, **10**)

Following our general procedure for the esterification of phenolic acids with sinapic acid (**3g**) (500 mg, 2.23 mmol, 1 eq), Na_2CO_3 (385 mg, 3.63 mmol, 1.6 eq) and (2-bromoethyl)benzene (0.35 mL, 2.57 mmol, 1.2 eq), compound SAPE (**10**) was obtained as a pale yellow solid after flash chromatography (0 - 20 % AcOEt/Hex), yield = 48 %, Mp = 95 - 96 °C, Rf = 0.26 (30 % AcOEt/Hex). ^{1}H-NMR (DMSO-d6, 25 °C); δ (ppm): 8.98 (s large, 1H, OH), 7.54 (d, *J = 15.85 Hz*, 1H, C_{Ar}-CH=CH), 7.34 - 7.29 (m, 4H, H_{Ar}), 7.25-7.21 (m, 1H, H_{Ar}), 7.02 (s, 2H, H_{Ar}), 6.50 (d, *J = 15.85 Hz*, 1H, CH=CH-C(=O)), 4.35 (t, *J = 6.84 Hz*, 2H, CH_2O), 3.80 (s, 6H, OCH_3), 2.97 (t, *J = 6.84 Hz*, 2H, CH_2Ph); ^{13}C-NMR (DMSO-d6, 25 °C); δ (ppm): 167.06, 148.49, 145.99, 138.85, 138.55, 129.30, 128.86, 126.84, 124.78, 115.18, 106.75, 64.75, 56.56, 34.94; HRMS *m/z* calc. for $C_{19}H_{20}O_5$ + (H^+): 329.1384; found: 329.1381.

2-Phenylethyl (2*E*)-3-(3,5-dimethoxyphenyl)prop-2-enoate (**11**)

Following our general procedure for the esterification of phenolic acids with 3,5-dimethoxycinnamic acid (**3h**) (500 mg, 2.40 mmol, 1 eq), Na_2CO_3 (320 mg, 3.02 mmol, 1.3 eq) and (2-bromoethyl)benzene (0.37 mL, 2.72 mmol, 1.1 eq), compound **11** was obtained as a white solid after flash chromatography (0 - 10 % AcOEt/Hex), yield = 72 %, Mp = 55 - 57°C, Rf = 0.25 (30 % AcOEt/Hex). ^{1}H NMR ($CDCl_3$, 25°C), δ (ppm)= 7.63-7.59 (d, 1H, J=15.97 Hz, ArCH=CH-), 7.37-7.27 (m, 5H, H_{ar}), 6.69-6.68 (sd, 2H, J= 2.20 Hz, H_{ar}), 6.53-6.52 (st, 1H, J= 2.20 Hz, H_{ar}), 6.44-6.40 (d, 1H, J= 15.97 Hz, ArCH=CH-), 4.47-4.44 (t, 2H, J= 7.04 Hz, -$COOCH_2CH_2Ar$), 3.84 (s, 6H, 2 $ArOCH_3$), 3.07-3.03 (t, 2H, J= 7.08 Hz, -

$COOCH_2CH_2Ar$); ^{13}C NMR ($CDCl_3$, 25°C), δ (ppm)= 166.79161.03, 144.86, 137.87, 136.29, 128.93, 128.54, 126.59, 118.57, 105.99, 102.58, 65.04, 55.44, 35.21; HRMS *m/z* calc. for $C_{19}H_{20}O_4$ + (H^+): 313.1434; found: 313.1427.

2-Phenylethyl (2*E*)-3-(3,4,5-trimethoxyphenyl)prop-2-enoate (**12**)

Following our general procedure for the esterification of phenolic acids with 3,4,5-trimethoxycinnamic acid (**3i**) (500 mg, 2.40 mmol, 1 eq), Na_2CO_3 (320 mg, 3.02 mmol, 1.3 eq) and (2-bromoethyl)benzene (0.37 mL, 2.72 mmol, 1.1 eq), compound **12** was obtained as a white solid after flash chromatography (0 - 10 % AcOEt/Hex), yield = 59 %, Mp = 98 - 100°C, Rf = 0.20 (30 % AcOEt/Hex). ^{1}H-NMR ($CDCl_3$, 25°C), δ (ppm)= 7.63-7.59 (d, 1H, J= 15.93 Hz, ArCH=CH-), 7.37-7.25 (m, 5H, H_{ar}), 6.77 (s, 2H, H_{ar}), 6.38-6.34 (d, 1H, J= 15.89 Hz, ArCH=CH-), 4.48-4.44 (t, 2H, J= 7.08 Hz, -$COOCH_2CH_2Ar$), 3.91 (s, 6H, $ArOCH_3$ meta), 3.90 (s, 3H, $ArOCH_3$ para), 3.07-3.03 (t, 2h, J= 7.04 Hz, -$COOCH_2CH_2Ar$); ^{13}C-NMR ($CDCl_3$, 25°C), δ (ppm)= 166.87, 153.45, 144.85, 140.16, 137.87, 129.90, 128.91, 128.54, 126.59, 117.28, 105.26, 64.98, 60.98, 56.18, 35.21. HRMS *m/z* calc. for $C_{20}H_{22}O_5$ + (H^+): 343.1540; found: 343.1540.

2-Phenylethyl (2*E*)-3-(1,3-benzodioxol-5-yl)prop-2-enoate (**14**)

Following our general procedure for the esterification of phenolic acids with 3,4-(methylenedioxy)cinnamic acid (**13a**) (500 mg, 2.60 mmol, 1 eq), Na_2CO_3 (338 mg, 3.19 mmol, 1.2 eq) and (2-bromoethyl)benzene (0.40 mL, 2.94 mmol, 1.1 eq), compound **14** was obtained as a white powder after flash chromatography (0 - 10 % AcOEt/Hex), yield = 46 %, Mp = 63 - 64 °C, Rf = 0.43 (30 % AcOEt/Hex). ^{1}H-NMR ($CDCl_3$, 25 °C); δ (ppm): 7.60 (d, *J = 15.93 Hz*, 1H, C_{Ar}-CH=CH), 7.36 - 7.32 (m, 2H, H_{Ar}), 7.29 - 7.25 (m, 3H, H_{Ar}), 7.05 (d, *J = 1.6 Hz*, 1H, H_{Ar}), 7.02 (dd, *J = 8.00 Hz, 1.60 Hz*, 1H, H_{Ar}), 6.83 (d, *J = 8.00Hz*, 1H, H_{Ar}), 6.27 (d, *J = 15.89 Hz*, 1H, CH=CH-C(=O)), 6.03 (s, 2H, OCH_2O), 4.44 (t, *J = 7.04 Hz*, 2H, OCH_2CH_2), 3.04 (t, *J = 7.08 Hz*, 2H, CH_2Ph); ^{13}C-NMR ($CDCl_3$, 25 °C); δ (ppm): 167.09, 149.62, 148.35, 144.56, 137.94, 128.95, 128.85, 128.52, 126.57, 124.46, 115.99, 108.55, 106.25, 101.57, 64.94, 35.24; HRMS *m/z* calc. for $C_{18}H_{16}O_4$ + (H^+): 297.1121; found: 297.1118.

2-Phenylethyl (2*E*)-3-(naphthalen-2-yl)prop-2-enoate (**15**)

Following our general procedure for the esterification of phenolic acids with 3-naphthalen-2-yl-acrylic acid (**13b**) (250 mg, 1.26 mmol, 1 eq), Na_2CO_3 (164 mg, 1.55 mmol, 1.2 eq), 6 mL

of HMPA and (2-bromoethyl)benzene (0.19 mL, 1.42 mmol, 1.1 eq), compound **15** was obtained as a white powder after flash chromatography (0 - 5 % AcOEt/Hex), yield = 70 %, Mp = 77 - 78 °C, Rf = 0.38 (30 % AcOEt/Hex). ^{1}H-NMR ($CDCl_3$, 25 °C); δ (ppm): 7.95 - 7.85 (m, 5H, H_{Ar}, C_{Ar}-CH=CH), 7.69 (dd, *J = 8.64 Hz, 1.08 Hz*, 1H, H_{Ar}), 7.57 - 7.52 (m, 2H, H_{Ar}), 7.39 - 7.27 (m, 5H, H_{Ar}), 6.57 (d, *J = 16.01 Hz*, 1H, CH=CH-C(=O)), 4.92 (t, *J = 7.08 Hz*, 2H, CH_2O), 3.08 (t, *J = 7.08 Hz*, 2H, CH_2Ph); ^{13}C-NMR ($CDCl_3$, 25 °C); δ (ppm): 166.98, 144.92, 137.94, 134.25, 133.3, 131.93, 129.96, 128.97, 128.70, 128.59, 128.56, 127.80, 127.26, 126.74, 126.61, 123.54, 118.22, 65.07, 35.26; HRMS *m/z* calc. for $C_{21}H_{18}O_2$ + (H^+): 303.138; found: 303.1387.

2-Phenylethyl 3-(3,4-dihydroxyphenyl)propanoate (**17**)

Following our general procedure for the esterification of phenolic acids with hydrocaffeic acid (**16a**) (300 mg, 1.65 mmol, 1 eq), Na_2CO_3 (211 mg, 1.99 mmol, 1.2 eq) and (2-bromoethyl)benzene (0.25 mL, 1.84 mmol, 1.1 eq), compound **17** was obtained as a pale brown powder after flash chromatography (0 - 15 % AcOEt/Hex), yield = 44 %, Mp = 70 - 71 °C, Rf = 0.32 (25 % AcOEt/Hex). ^{1}H-NMR ($CDCl_3$, 25 °C); δ (ppm): 7.34 - 7.21 (m, 5H, H_{Ar}), 6.77 (d, *J = 8.00 Hz*, 1H, H_{Ar}), 6.66 (s, 1H, H_{Ar}), 6.59 (d, *J = 8.00 Hz*, 1H, H_{Ar}), 4.31 (t, *J = 7.00 Hz*, 2H, CH_2O), 2.94 (t, *J = 6.96 Hz*, 2H, CH_2Ph), 2.82 (t, *J = 7.52 Hz*, 2H, $CH_2CH_2C(=O)$), 2.59 (t, *J = 7.68 Hz*, $CH_2CH_2C(=O)$); ^{13}C-NMR ($CDCl_3$, 25 °C); δ (ppm): 173.49, 143.59, 142.08, 137.78, 133.25, 128.93, 128.52, 126.60, 120.57, 115.37, 65.16, 36.15, 35.04, 30.24; HRMS *m/z* calc. for $C_{17}H_{18}O_4$ + (Na^+): 309.1097; found: 309.1083.

2-Phenylethyl (3,4-dihydroxyphenyl)acetate (**18**)

Following our general procedure for the esterification of phenolic acids with (3,4-dihydroxyphenyl)acetic acid (**16b**) (500 mg, 2.97 mmol, 1 eq), Na_2CO_3 (400 mg, 3.77 mmol, 1.3 eq) and (2-bromoethyl)benzene (0.46 mL, 3.36 mmol, 1.1 eq), compound **18** was obtained as a brown oil after flash chromatography (0 - 20 % AcOEt/Hex), yield = 58 %, Rf = 0.20 (30 % AcOEt/Hex). ^{1}H-NMR (MeOD, 25 °C); δ (ppm): 7.27 - 7.14 (m, 5H, H_{Ar}), 6.72 - 6.70 (m, 2H, H_{Ar}), 6.54 (dd, *J = 8.04 Hz, 1.76 Hz*, 1H, H_{Ar}), 4.27 (t, *J = 6.80 Hz*, CH_2O), 3.44 (s, 2H, $C_{Ar}CH_2C(=O)$), 2.89 (t, *J = 6.76 Hz*, 2H, CH_2Ph); ^{13}C-NMR (MeOD, 25 °C); δ (ppm): 172.56, 144.91, 144.06, 137.91, 128.59, 128.04, 126.05, 125.51, 120.28, 116.04, 114.92, 65.13, 40.14, 34.60; HRMS *m/z* calc. for $C_{16}H_{16}O_4$ + (Na^+): 295.0941; found: 295.0931.

2-Phenylethyl 3,4-dihydroxybenzoate (**19**)

Following our general procedure for the esterification of phenolic acids with 3,4-dihydroxybenzoic acid (**16c**) (500 mg, 3.24 mmol, 1 eq), Na_2CO_3 (430 mg, 4.06 mmol, 1.3 eq) and (2-bromoethyl)benzene (0.50 mL, 3.67 mmol, 1.1 eq), compound **19** was obtained as an off-white powder after flash chromatography (0 - 15 % AcOEt/Hex), yield = 39 %, Mp = 129 - 130 °C, Rf = 0.22 (30 % AcOEt/Hex). ^{1}H-NMR (MeOD, 25 °C); δ (ppm): 7.42 - 7.39 (m, 2H, H_{Ar}), 7.31 - 7.30 (m, 4H, H_{Ar}), 7.25 - 7.19 (m, 1H, H_{Ar}), 6.80 (d, *J = 8.12 Hz*, 1H, H_{Ar}), 4.47 - 4.43 (m, 2H, CH_2O), 3.07 - 3.02 (m, 2H, CH_2Ph); ^{13}C-NMR (MeOD, 25 °C); δ (ppm): 166.85, 150.32, 144.76, 138.14, 128.60, 128.11, 126.12, 122.22, 121.30, 116.00, 114.42, 65.01, 34.83; HRMS *m/z* calc. for $C_{15}H_{14}O_4$ + (Na^+): 281.0784; found: 281.0774.

2.2. 5-LO product biosynthesis assays in HEK293 cells

HEK293 cells were stably co-transfected with a pcDNA3.1 vector expressing 5-LO and a pBUDCE4.1 vector expressing 5-LO activating protein (FLAP) as previously reported[16,32]. The resulting stable double transfectants were propagated in culture and aliquots were frozen. Once thawed for a series of experiments, each aliquot of cells is cultured for a maximum of 6 weeks before being discarded. For cell stimulation of 5-LO products, transfected HEK293 cells were collected following trypsinization, washed and the cell pellet was resuspended in Hank's balanced salt solution (HBSS) (Lonza, Walkerville, MD) containing 1.6 mM $CaCl_2$ at a concentration of 5 x 10^5 cells mL^{-1}. Cells were pre-incubated with each compound or their diluent at the indicated concentration for 5 min at 37 ^oC. Cells were then stimulated for 15 minutes at 37 ^oC with the addition of 10 µM calcium ionophore A23187 (Sigma–Aldrich, Oakville, ON, Canada) and 10 µM arachidonic acid (Cayman Chemical, Ann Arbor, MI). Stimulations were stopped and processed for RP-HPLC analysis as described previously [16,33]. Data are expressed as means ± SEM of 3 independent experiments, each performed in duplicate (Fig 2 and 3).

2.3. 5-LO products biosynthesis assays in human PMNL cells

Human PMNL prepared from peripheral blood of healthy consenting volunteers as described[16] were suspended in HBSS containing 1.6 mM $CaCl_2$ (10^7 cells/ml) and pre-incubated with compounds or their diluent for 5 min at 37 ^oC in the presence of 0.4 U/ml of adenosine deaminase (Sigma-Aldrich, Oakville, On, Canada). Cells were then stimulated for 15 min at 37^oC with 1 µM thapsigargin (Sigma-Aldrich)[16]. Reactions were stopped by the addition of 0.5 volume of cold MeOH:CH_3CN (1:1) and 50 ng of PGB_2 as internal standard

and samples were stored at -20°C until processing for RP-HPLC analysis as indicated above. Data are expressed as means ± SEM of 3 independent experiments, each performed in duplicate (Fig. 4).

2.4. 12-LO and COX-1 products biosynthesis assays

Platelets isolation was performed as previously described[34]. Isolated platelets obtained from healthy consenting volunteers were pre-incubated (10^8 cells/ml) with indicated compounds or their diluent at 37˚C for 5 min in the presence of 5 mM $CaCl_2$. Stimulation was initiated with the addition of 0.5 U/ml of thrombin (Sigma-Aldrich) followed by a 15 min incubation period at 37˚C. Reactions were stopped by the addition of 3 volumes of cold MeOH:MeCN (1:1) containing prostaglandin B_2 (100ng/ml) as internal standard. Following overnight storage at -20˚C, the samples were centrifuged, the supernatant was evaporated under gas nitrogen, and resuspended in 30% methanol and analyzed by reverse-phased high-performance liquid chromatography as described above.

2.5. Free radical scavenging activity assay

The free radical scavenging activity of test compounds was measured as previously described using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a stable radical[12] with slight modifications. Particular care was taken in the preparation of the control (DPPH reagent + ethanol as a diluent without test compounds). Controls with O.D. of 0.350-0.360 at 520nm were deemed as acceptable to avoid variations in IC_{50} calculations. 1 ml of DPPH in ethanol (60 mM) was mixed with 1 mL of the test compounds at the indicated concentrations or their diluent (ethanol). Each mixture was then shaken vigorously and held in the dark for 30 min at room temperature. The absorbance of DPPH at 520 nm was then measured. The radical scavenging activity was expressed in terms of % inhibition of DPPH absorbance:

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ test})/A \text{ control})] \times 100$$

Where A control is the absorbance of the control (DPPH solution without test compound) and A test is the absorbance of the test sample (DPPH solution plus compound). Ascorbic acid was used as a positive control. Data are expressed as means ± SD of 2 independent experiments, each performed in triplicate (table 2). IC_{50} values were calculated from a sigmoidal concentration-response curve-fitting model with a variable slope on GraphPad Prism 5 software (GraphPad Software, San Diego, California).

2.6. Molecular Docking

Molecular docking was performed with AutoDock Vina 1.1.2 on 5-LO (PDB code: 3O8Y[35]) following the procedure[36] outlined in "Design, synthesis and evaluation of semi-synthetic triazole-containing caffeic acid analogues as 5-lipoxygenase inhibitors." by Daniela De Lucia et al. For optimization, the MMFF94 force field was used. Results were analyzed with Maestro 10.7 [37] and with LigPlot+[38]. Readers who want to reproduce our docking results can obtain the pdb file with all ligand and target atom coordinates. This file can be sent by email on request to the corresponding author.

2.7. Statistical analysis

Statistical analyses and graph design were performed using GraphPad Prism 5 software (GraphPad Software, San Diego, California).

3. RESULTS AND DISCUSSION

3.1. Chemistry

To prepare compounds **4**-**12**, coumaric acids (*ortho*, *meta* and *para*), 3,4-dimethoxycinnamic acid, 3,4-dichlorocinnamic acid, ferulic acid, sinapic acid, 3,5-dimethoxycinnamic acid and 3,4,5-trimethoxycinnamic acid were respectively esterified by reacting with phenethyl bromide in the presence of sodium carbonate (Na_2CO_3) and potassium iodide (KI) as a catalyst. Target compounds were thus obtained in a single step with moderate to high yields (Scheme 1).

As outlined in Scheme 2, phenethyl esters **14** and **15** having respectively a methylenedioxy 1,3-benzodioxolyl and a naphthyl moiety in place of the catechol of CAPE (**2**) were prepared by the same esterification method described in Scheme 1. The starting acids, namely 3,4-(methylenedioxy)cinnamic acid (**13a**) and 3-naphthalen-2-yl-acrylic acid (**13b**), were obtained by Knoevenagel condensation of malonic acid with piperonal or β-naphthaldehyde[39].

As shown in Scheme 3, catechol-bearing phenethyl esters **17, 18, 19** in which one, two or three carbons separate the carbonyl group from the catechol moiety were also synthesized. With the same esterification method described in Scheme 1, commercially-available 3-(3,4-dihydroxyphenyl)propanoic acid (dihydrocaffeic acid (**16a**)), 2-(3,4-dihydroxyphenyl)acetic acid (**16b**) and 3,4-dihydroxybenzoic acid (**16c**) were converted to the corresponding phenethyl esters **18, 19, 20** (Scheme 3).

3.2. Biological activity

3.2.1. 5-LO products biosynthesis assays

HEK293 cells stably transfected with 5-LO and 5-LO activating protein (FLAP) were used to screen compounds for 5-LO inhibition as previously described[33]. As shown in Figure 2, *para*-, *meta*- and *ortho*-coumaric acid phenethylesters (**4**, **5, 6**) had no inhibitory activity as opposed to Zileuton (**1**) and CAPE (**2**), which highlights the importance of multiple oxygenated positions on this aromatic ring (Figure 2).

Comparison of compound **17**, a phenethyl ester of hydrocaffeic acid, with CAPE (**2**) shows that the absence of the α,β-unsaturation in **17** does not seem to reduce the inhibitory activity. Analog **17** was equivalent with CAPE (**2**) (Figure 2). The shortening of the linker between the carbonyl and the catechol moiety does not appear to be crucial for 5-LO inhibition. Analogs **18** and **19**, having only one carbon or no carbon between the carbonyl and the catechol, were almost equipotent with Zileuton (**1**) but less active than CAPE (**2**) (Figure 2).

Replacing the 3,4-hydroxyls of CAPE (**2**) by 3,4-methoxy (**7**), 3,4-dichloro (**8**), 3,4-methylenedioxy (**14**), or phenyl (**15**) group was not favorable to a good inhibition of 5-LO since these analogs have no inhibitory activity against the biosynthesis of 5-LO products. Compound **9**, bearing 3-methoxy-4-hydroxy moiety, obtained from the ferulic acid (**3f**), also had no effect on 5-LO (Figure 3).

On the other hand, the sinapic acid phenethyl ester (**10**), which can be named SAPE by analogy to CAPE (**2**), appears to be a good inhibitor of 5-LO with potency comparable to CAPE (**2**). The OH in position 4 of SAPE (**10**) is crucial for the inhibition of 5-LO. As shown in Figure 3, analogs **11** and **12** in which the hydroxyl at position 4 is replaced by hydrogen or by a methoxy respectively, had no 5-LO inhibitory activity. The methoxy groups in position 3 and 5 are also crucial for inhibition of 5-LO since the analogs **9** and **4,** which conserve the 4-OH group but have either a single methoxy in position 3 or no methoxy (neither 3 nor 5) are not active against 5-LO (Figure 2 and 3). Compounds that approached or surpassed the inhibitory activity of CAPE (**2**) were selected for further screening in human PMNL for dose-response assays to obtain IC_{50} values.

To further probe the inhibitory activity of lead compounds selected from preliminary HEK293 cell screenings (Figures 2 and 3), 5-LO inhibition assays were undertaken in stimulated human immune cells. 5-LO is highly expressed in polymorphonuclear leukocytes (PMNL) and these cells are important physiological producers of LTB_4[16]. The

phenethylester of hydrocaffeic acid (**17)** was equipotent to slightly more active than Zileuton (**1**) and equipotent with CAPE (**2**) (Figure 4). Analogs **18** and **19** did not significantly inhibit the biosynthesis of 5-LO products in these cells (Figure 4). The number of carbons between the carbonyl and the catechol appears to be important for the inhibition of 5-LO in PMNL cells. As shown in Figure 4, whenever the carbon number decreases activity decreases. Compounds **18** and **19** having one and no carbon, respectively, were less active than **17**, which had two carbons (Figure 4).

Among the selected molecules tested for 5-LO inhibition in PMNL, SAPE (**10**) was found to be the best inhibitor exceeding the inhibitory capacity of both zileuton (**1**) and CAPE (**2**) in this model. The presence of the two methoxy groups in position 3 and 5 and the OH in position 4 seems to be an ideal combination for inhibition of 5-LO in PMNL cells. These structural changes may influence the availability, cell permeation or even the stability of inhibitors in PMNL versus HEK293 cells. The steric hindrance created by the presence of both methoxy may have an effect on the stability of the SAPE (**10**) by sterically shielding the phenolic hydroxyl group, possibly rendering it less sensitive to sulfatation and glucuronidation than a catechol such as CAPE (**2**).

The superiority of the inhibitory activity of SAPE (**10**) was confirmed in dose response experiments. Selected compounds with inhibition of at least 50% at 1 µM in PMNL were screened further through concentration-response assays in PMNL (Table 1).

Table 1. 5-LO inhibition in human PMNL cells.

Compounds	**IC_{50} (µM)** [SEM]
Zileuton (**1**)	3.1 [0.2]
CAPE (**2**)	1.01 [0.03]
17	1.01 [0.05]
SAPE (**10**)	0.3 [0.01]

SAPE (**10**) inhibited 5-LO in a dose-dependent manner and outperformed both Zileuton (**1**) and CAPE (**2**). With an IC_{50} of 0.3 µM, this phenethylester of sinnapic acid was 3-fold more potent than CAPE (**2**) and 10-fold more potent than zileuton, the only approved 5-LO inhibitor. Compound **17**, hydrocaffeic acid phenethylester, was equipotent with CAPE (**2**)

and was three times more active than Zileuton (**1**) (IC_{50} = 3.1 μM) (Table 1). SAPE (**10**), which was previously synthesized along with a few other analogues by a different esterification strategy by Shi *et al*., has almost no cytoprotective and neuritogenic activities[40] but was never tested for its inhibition of 5-LO, 12-LO or COX-1.

3.2.2. 12-LO and COX-1 products biosynthesis assays

Human platelets were used for the evaluation of products synthesized by the 12-LO and COX-1 pathways. As shown in Figure 5, CAPE (**2**) inhibits the biosynthesis of both 12-LO and COX-1 products. Including 5-LO, these results indicate that CAPE (**2**) is an inhibitor of the biosynthesis of products from all three pathways. In contrast, SAPE (**10**) had no effect on 12-LO and less effect on COX-1 indicating that this compound is a more selective 5-LO inhibitor. Therefore, the presence of the 3,5-dimethoxy and the 4-hydroxy moieties makes the phenethyl ester of sinapic acid completely inactive against 12-LO inhibition at this concentration. It is interesting to note that CAPE (**2**) surpasses the inhibitory capacity of Baicalein which is the standard commonly used as a 12-LO inhibitor. Concentration-response assays in human platelets demonstrated that CAPE (**2**) (IC_{50} = 0.07 μM) is 20-fold more active for 12-LO inhibition than Baicalein (IC_{50} = 1.52 μM).

3.3. Free radical scavenging activity assay

The free radical scavenging activity of our compounds was compared against a known antioxidant, the hydrosoluble vitamin C or ascorbic acid. The results from the DPPH assay show that all the tested compounds in this study have a higher free scavenging radical activity than vitamin C (Table 2). This potentially make these compounds good candidates as lead anti-inflammatory molecules solely from their increased free radical scavenging activity.

We previously demonstrated that the anti-radical effect of the esters of certain phenolic acid esters is not the only factor that explains their inhibitory activity against 5-LO[16,18,33]. Similarly, the free radical scavenger activity of the best 5-LO inhibitors in HEK293 cells (Figure 2 and 3) exhibit similar radical scavenging activity to CAPE (**2**) (Table 2). Analogs **17**, **18** and **19** bear the important catechol group and only differ from CAPE (**2**) by the extent of the carboxylate linker and the absence of the ethylene moiety, but only **17** shows equivalent activity against 5-LO to that of CAPE (**2**). This indicates that the linker and not differences in radical scavenging capacity is what distinguished their 5-LO inhibitory activities.

Table 2. Free radical scavenging activity

Compounds	**IC_{50} (μM)** [SEM]
CAPE (**2**)	16.5 [4.0]
17	24.6 [4.8]
18	18.0 [3.6]
19	20.4 [2.9]
SAPE (**10**)	25.4 [0.7]
Sinapic acid (**3g**)	21.9 [2.0]
Ascorbic acid	68.7 [1.6]

SAPE (**10**), which was the best inhibitor of 5-LO in HEK293 and PMNL cells, also had antiradical activity that was similar to that of CAPE (**2**). Therefore, esterification of sinapic acid (**3g**) does not seem to induce any dramatic change in radical scavenging activity. SAPE (**10**) has essentially the same anti-radical activity as its corresponding acid, which is completely inactive against 5-LO.

3.4. Molecular Docking

Molecular docking of CAPE (**2**) and the ether substituted compounds SAPE (**10**), **11** and **12** was performed using the modified 5-LO protein (PDB ID: 3O8Y). CAPE (**2**) and SAPE (**10**) are almost superimposed, root-mean-square deviation (RMSD) = 0.4158 for heavy and similarly placed atoms, with the substituted benzene at the end of the hydrophobic cavity near Ala424 (Figure 6). The length of the molecules follows the cavity until the unsubstituted benzene approaches the iron atom. For both CAPE (**2**) and SAPE (**10**), the closest atom to Fe is at a distance of 6.6 Å. The two ether substituted compounds with no measurable 5-LO inhibition, **11** and **12**, are partially superimposed, RMSD = 1.1928. Both benzene rings have the same "center" while the connecting chains take differing paths resulting in the ester oxygens pointing in opposite directions. Both molecules are the closest to the iron atom with **11**, 4.11 Å, being slightly further away than **12**, 3.88 Å.

The ether substitutions of SAPE (**10**), **11** and **12** occupy different orientations usually depending on their placement in the *meta* or *para* positions. For the strong 5-LO inhibitor SAPE (**10**), both ethers that are in a *meta* position point in the same direction (towards Tyr181 and Trp599 for the first *meta* ether and towards Val604 for the second *meta* ether). The same is true for **12**, both ethers in the *meta* position point in the same direction as their counterparts in SAPE (**10**)**,** however the additional ether in *para* points in the opposite direction (towards Ala424). For **11** with only 2 substitutions which are the ethers in the *meta* position, it positions itself so that both point in opposite directions, one is pointing towards Tyr181 and Trp599 while the second one is pointing towards Ala424. For **11** to have both ethers in opposite direction, it must rotate the substituted benzene differently compared to the other molecules.

In the tested molecules, the only detected hydrogen bond using LigPlot^{+} is between CAPE (**2**) and Leu420 (OH···O, d = 3.09 Å) (Table 3). Leu420 helps define the active site cavity[35]. Maestro detects several π-π interactions (Table 3). For the tested molecules, a minimum of one π-π interaction is present. His372 seems to be the most important, being present in four out of the five tested molecules (including Zileuton) and in the two most stable molecules. The residue His372 is an iron coordinating residue[35].

CAPE (**2**) and SAPE (**10**) are the most stable molecules tested, with their respective affinity of - 8.8 kcal/mol and - 8.7 kcal/mol, while **12** is the least stable molecule, - 8.1 kcal/mol. It appears that the molecules that have a hydroxyl substitution have more affinity for the active site than the molecules with solely ether substitutions. Overall, these molecular docking studies indicate that the molecules that are strong inhibitors of 5-LO show different positioning and affinities for 5-LO than the poorly active ether-substituted compounds that lack a hydroxyl substituent.

Table 3. Molecular modeling results for modeling affinity, π-π interactions and hydrogen bonds.

Compounds	Affinity (kcal/mol)	Maestro π-π Interactions	LigPlot$^+$ H-Bond
CAPE (**2**)	-8.8	His372	Leu420
SAPE (**10**)	-8.7	His372	-
11	-8.5	Phe177, His367	-
12	-8.1	Phe177, His367, His372, Phe421	-
(*R*)-Zileuton	-6.6	Phe421	Leu420 x 2, Asn425
(*S*)-Zileuton	-6.5	-	Leu420 x 2, Ala424, Phe421

4. CONCLUSIONS

Fourteen phenolic acid phenethyl esters were synthesized and evaluated for their 5-LO inhibition activities. Compared to zileuton (**1**) and CAPE (**2**), sinapic acid phenethyl ester (SAPE, **10**), showed high potency in HEK293 cell and in PMNL (IC_{50} = 0.3 µM) assays. Even with a lower antiradical potency than CAPE (**2**), SAPE (**10**) was found to be the best 5-LO inhibitor. SAPE (**10**) is more selective to 5-LO since, unlike CAPE (**2**), it is inactive against 12-LO and less active against COX-1. The presence of the two methoxy in position 3 and 5 and the OH in position 4 in SAPE (**10**) seems to be an ideal configuration for inhibition of only 5-LO. Although a large number of investigations have been carried out on the study of the biological effects of CAPE (**2**), one of the main constituents of honeybee propolis, reports about the activity of SAPE (**10**) are scarce. Sinapic acid (**3g**) is found in many edible plants and fruits [41] such as rapeseed where it is the predominant phenolic compound [42]. In addition, anti-inflammatory activity of sinapic acid was reported and attributed the suppression of inducible nitric oxide synthase, COX-2, and proinflammatory cytokines expressions via nuclear factor-κB inactivation [43]. Overall, this first study on the anti-inflammatory properties of SAPE (**10**) suggests that additional studies are warranted to better understand the biological effects of this compound in comparison to those of CAPE (**2**).

ACKNOWLEDGMENTS

M.T. would like to acknowledge the contribution of the Natural Sciences and Engineering Research Council of Canada (NSERC), the New Brunswick Innovation Foundation (NBIF), the Canadian Foundation for Innovation (CFI), and Université de Moncton. M.E.S. would like to thank the NBIF Innovation Research Chairs program. L.H.B. would like to acknowledge the Canadian Institutes of Health Research (CIHR), the NBIF and the Université de Moncton.

ETHICAL APPROVAL

Blood was obtained from healthy consenting volunteers. This research project was approved by the *Comité d'éthique de la recherche avec les êtres humains* at the Université de Moncton.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Figures Legends

Figure 1. Structure of Zileuton (**1**) and CAPE (**2**)

Scheme 1. Synthesis of compounds **4-12**. Reagents and conditions: i) Na_2CO_3, $PhCH_2CH_2Br$, KI, HMPA, 0^oC to RT, 12h.

Scheme 2. Synthesis of compounds **14** and **15**. Reagents and conditions: i) Na_2CO_3, $PhCH_2CH_2Br$, KI, HMPA, 0^oC to RT, 12h.

Scheme 3. Synthesis of compounds **17-19**. Reagents and conditions: i) Na_2CO_3, $PhCH_2CH_2Br$, KI, HMPA, 0^oC to RT, 12h.

Figure 2. Inhibition of 5-LO product biosynthesis by synthetic CAPE (**2**) analogs (1 μM) in stimulated HEK293 cells. Data are expressed as means ± SEM of at least 3 independent experiments. Values without one common superscript are significantly different determined by one-way ANOVA with Tukey's multiple comparison test ($p < 0.05$).

Figure 3. Inhibition of 5-LO products synthesis by synthetic CAPE (**2**) analogs (1 μM) in stimulated HEK293 cells. Data are expressed as means ± SEM of at least 3 independent experiments. Values without one common superscript are significantly different determined by one-way ANOVA with Tukey's multiple comparison test ($p < 0.05$).

Figure 4. Inhibition of 5-LO products synthesis by synthetic CAPE (**2**) analogs (1 μM) in human PMNL cells.
Data are expressed as means ± SEM of 3 independent experiments. Values without one common superscript are significantly different determined by one-way ANOVA with Tukey's multiple comparison test ($p < 0.05$).

Figure 5. Inhibition of 12-LO and COX-1 product synthesis by indicated compounds (1 μM) in human platelets. Data are expressed as means ± SEM of at least 3 independent experiments. Values without one common superscript are significantly different determined by one-way ANOVA with Tukey's multiple comparison test ($p < 0.05$).

Figure 6. Visual representation of SAPE (**10**) docked with 5-LO in 3D (left, Maestro) and 2D (right, LigPlot^{+}).

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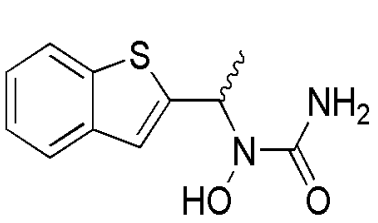
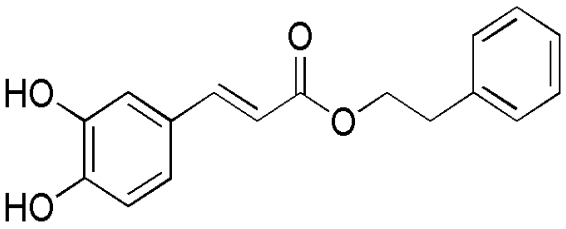
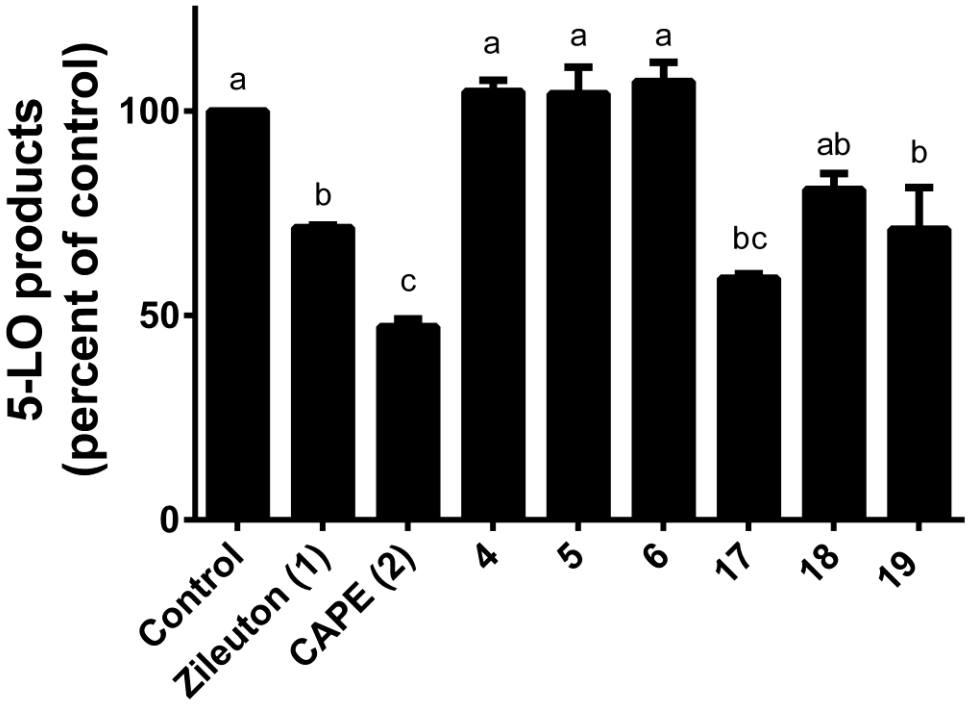
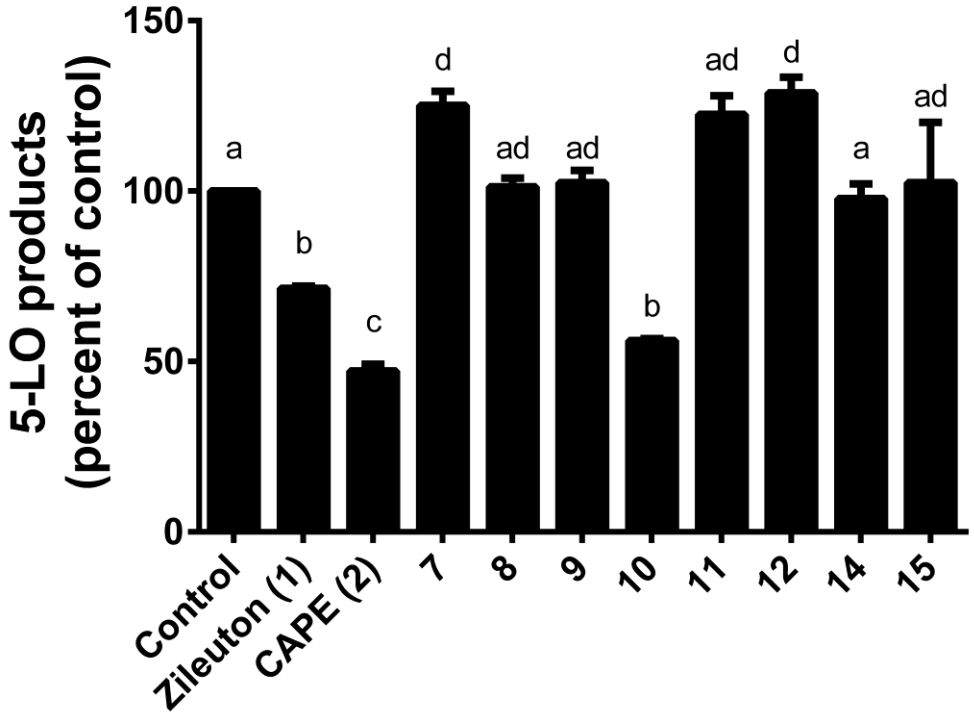


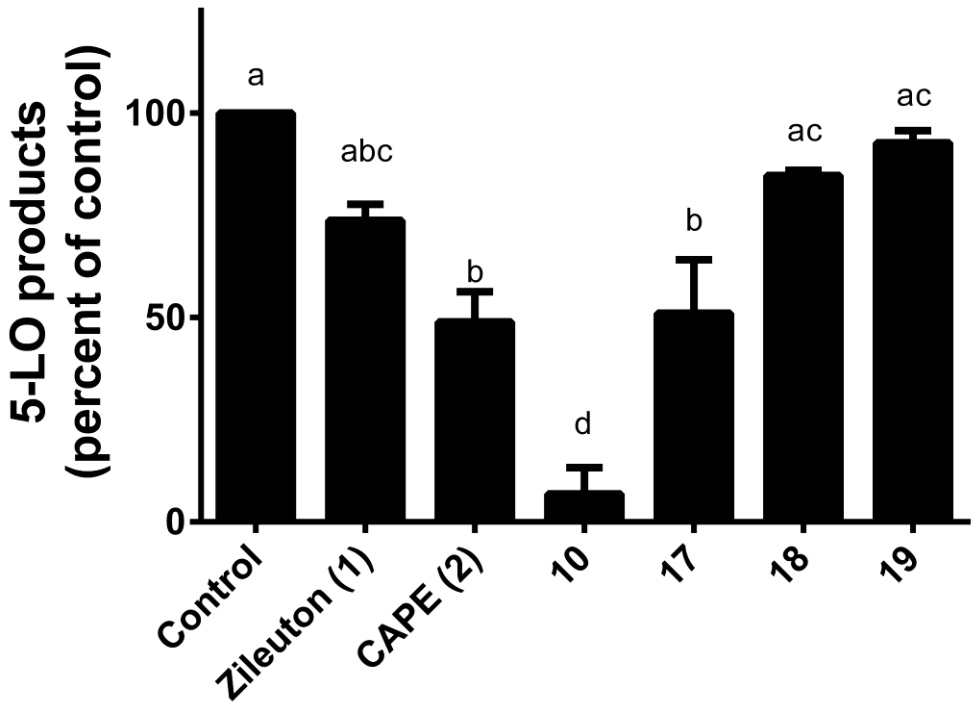
Zlleuton (**1**)

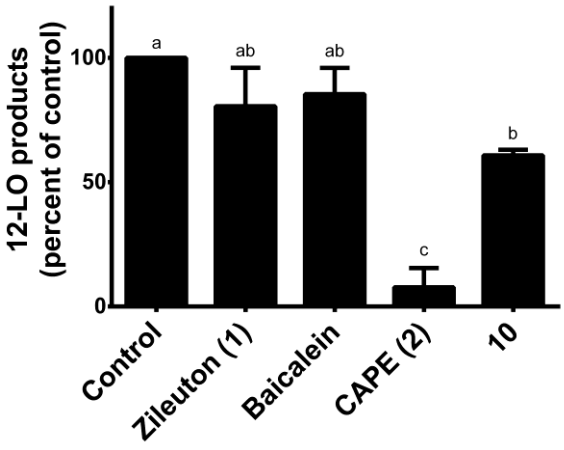


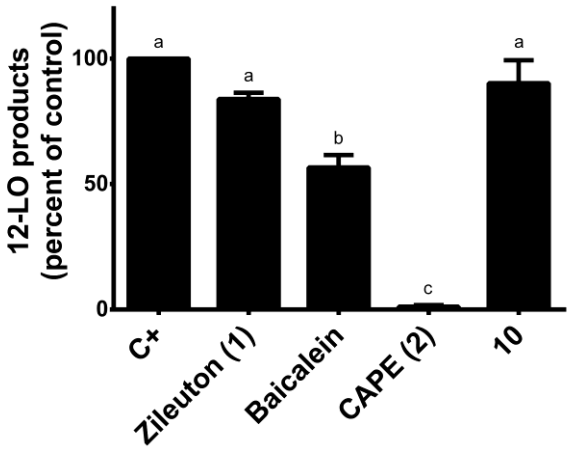
CAPE (**2**)

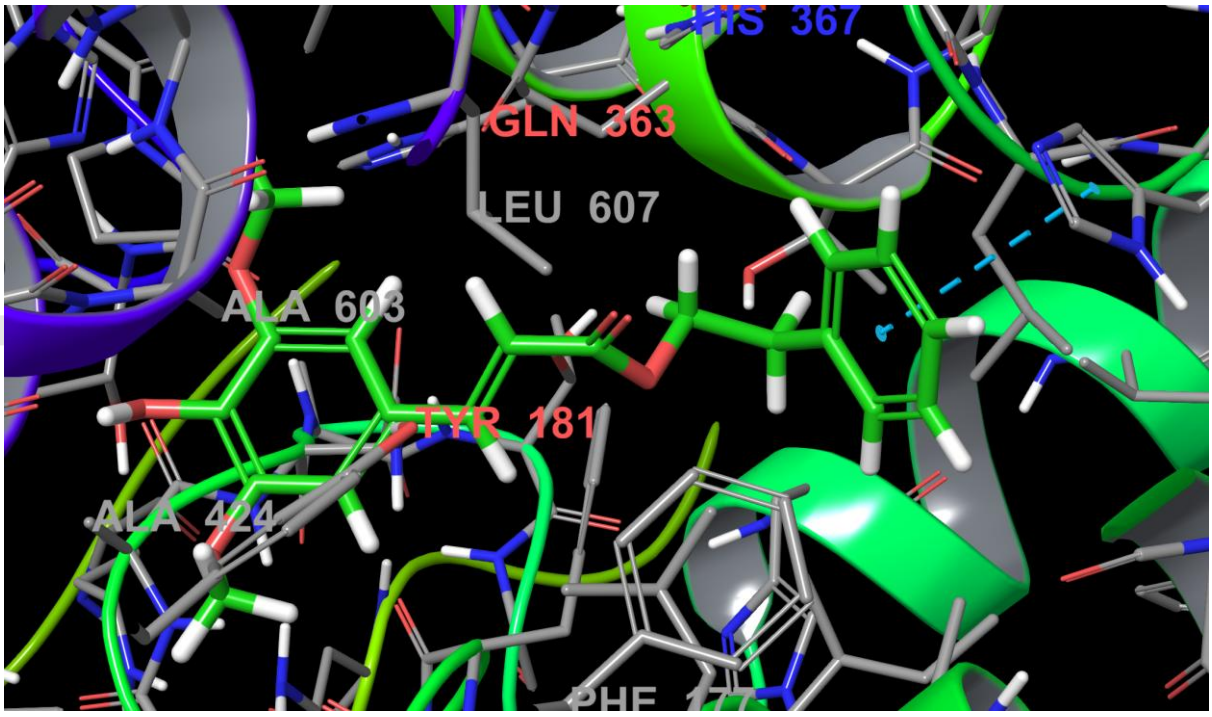


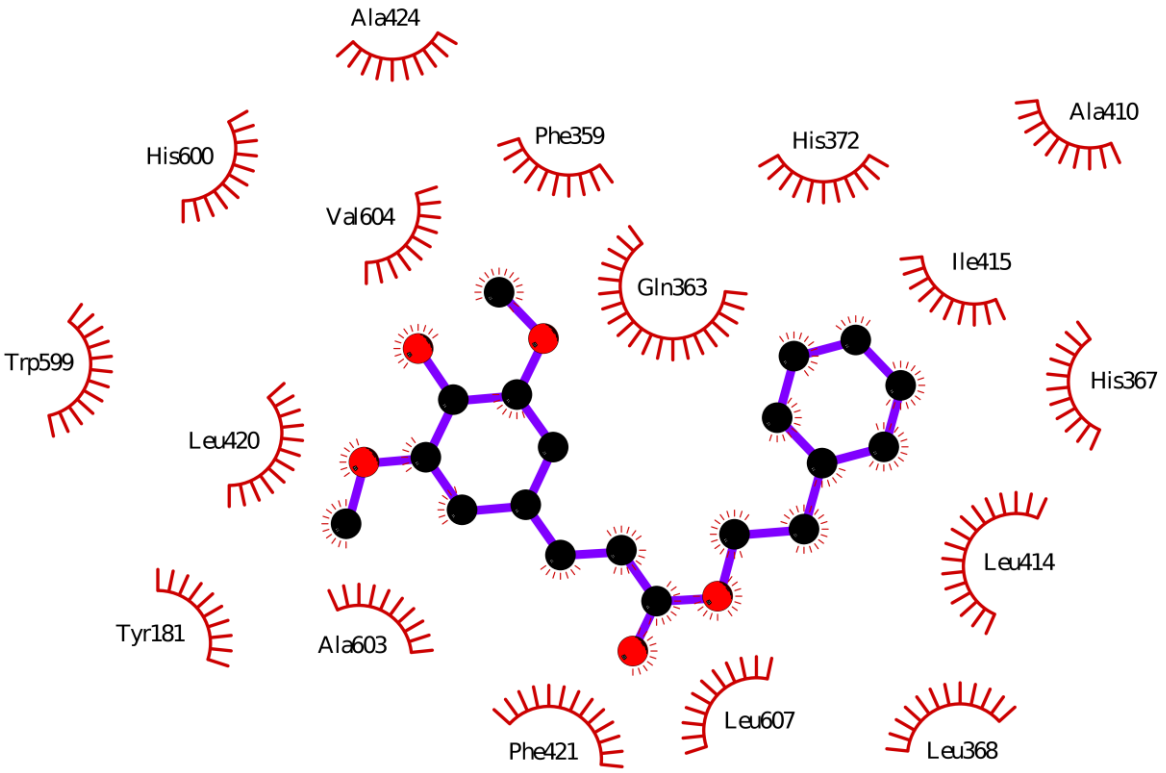


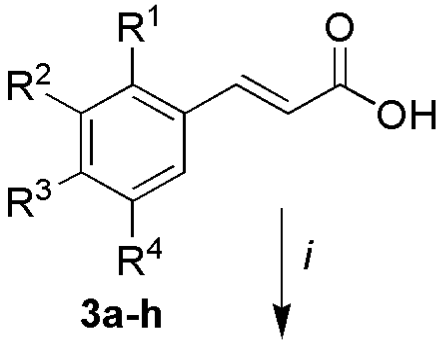




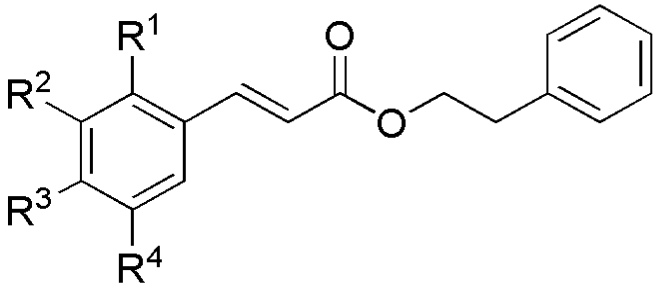








3a-h



4: $R^1 = R^2 = H$, $R^3 = OH$, $R^4 = H$
5: $R^1 = H$, $R^2 = OH$ $R^3 = R^4 = H$
6: $R^1 = OH$, $R^2 = R^3 = R^4 = H$
7: $R^1 = H$, $R^2 = R^3 = OCH_3$, $R^4 = H$
8: $R^1 = H$, $R^2 = Cl$, $R^3 = Cl$, $R^4 = H$
9: $R^1 = H$, $R^2 = OCH_3$, $R^3 = OH$, $R^4 = H$
10: $R^1 = H$, $R^2 = OCH_3$, $R^3 = OH$, $R^4 = OCH_3$
11: $R^1 = H$, $R^2 = OCH_3$, $R^3 = H$, $R^4 = OCH_3$
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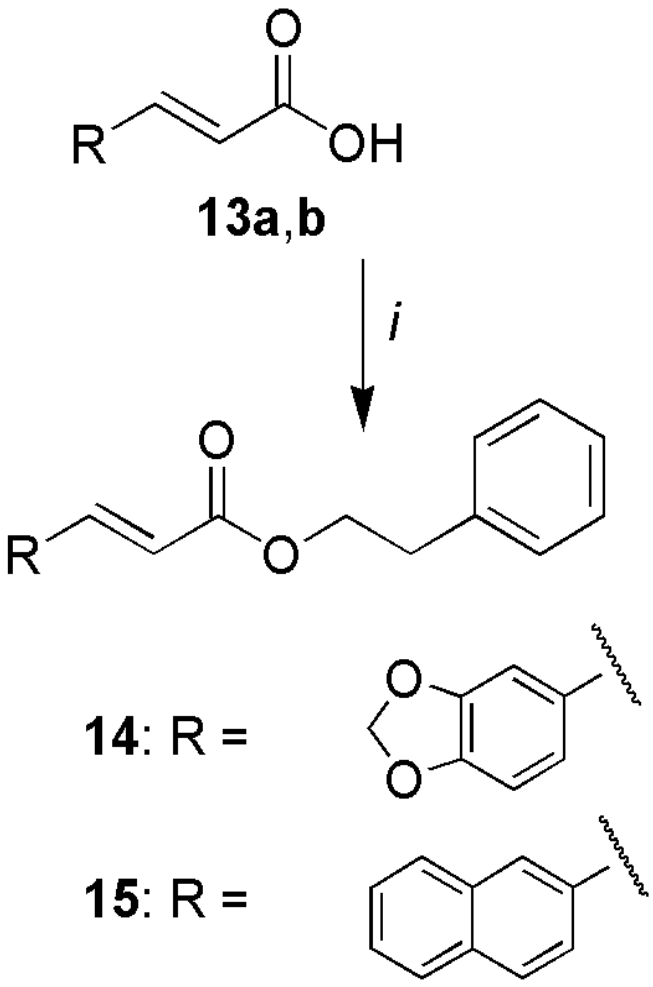
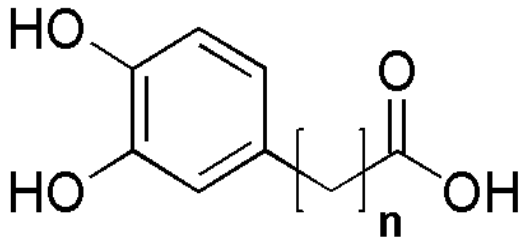
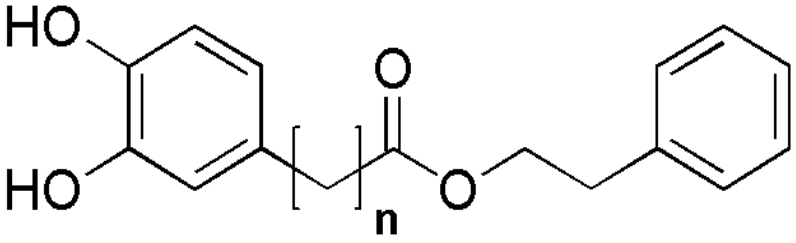




16a-c

i



17: n = 2
18: n = 1
19: n = 0