Novel Squalene-Hopene Cyclase Inhibitors Derived from Hydroxycoumarins and Hydroxyacetophenones

Giancarlo Cravotto,* Gianni Balliano, Silvia Tagliapietra, Simonetta Oliaro-Bosso, and Gian Mario Nano

Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino; Via Giuria 9, 10125 Torino Italy.
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Squalene-hopene cyclase (SHC) is a useful model enzyme for predicting molecular interactions with oxidosqualene cyclase (OSC). Structure–activity relationships were investigated for numerous coumarin-derived inhibitors of SHC, and structural simplifications are suggested. Both umbelliferone and 2,4-dihydroxyacetophenone provide convenient starting nuclei for the design of SHC inhibitors. Derivatives bearing an ω-epoxyfarnesyl moiety or just a plain alkyl chain showed an inhibitory effect on a recombinant SHC from *Alicyclobacillus acidocaldarius* expressed in *Escherichia coli*.

Key words 7-alkyloxycoumarin; 4-alkyloxyacetophenone; squalene hopene cyclase; oxidosqualene cyclase; secoammoresinol; secoferulenol

One of the most intensively studied enzymes of sterol biosynthesis, oxidosqualene cyclase (OSC), catalyzes the conversion of an acyclic compound, 2,3-oxidosqualene, to the first cyclic intermediate along the pathway. This is lanosterol in all non-photosynthetic organisms, and cycloartenol in plants.[1] While several mammalian OSCs were being isolated, characterized, cloned and sequenced,[2—9] hundreds of OSC inhibitors have been designed and tested[10—14] as potential anti-fungal[15] or cholesterol-lowering drugs.[16] Recently, a novel series of orally active inhibitors have been tested on human liver microsomal OSC, and their effectiveness as cholesterol-lowering drugs has been evaluated in hyperlipidemic hamsters.[17] OSC inhibitors are also under investigation as potential anti-trypanosomal agents.[18,19]

The design of new OSC inhibitors received a further boost when the complete structure of a squalene hopene cyclase (SHC) was elucidated.[20] This bacterial enzyme, isolated from the thermophilic bacterium *Alicyclobacillus acidocaldarius*, proved particularly interesting because its sequence revealed a remarkable degree of homology and partial identity with eukaryotic OSCs.[21,22] SHC may therefore be used as a model to predict how newly designed inhibitors may interact with OSCs. Recently, the interaction of several new inhibitors of human OSC[17] with the active site of SHC was investigated by co-crystallization experiments.[23] Because comparative studies on the two enzyme types[24,25] have shown close correspondence of their inhibitor activities, we recently investigated a group of meroterpenoids that proved to be excellent inhibitors of SHC.[26] The basic structure of bioactive oxyprenylcoumarins is derived from umbelliprenine (7-farnesyloxycoumarin) by the insertion of either a tetrahydrofuran unit (farnesylferol C, **1**) or 1–2 oxiran rings (**2**, **3**) (Fig. 1). While farnesyferol C (IC_{50} 7.0 μM) is a naturally occurring compound, umbelliprenine-10′,11′-monoepoxyde (**2**) (IC_{50} 2.5 μM) and diepoxyde (**3**) (IC_{50} 1.5 μM) have been previously obtained by synthesis.[26]

This encouraging start prompted us to extend the study of structure–activity relationships by modifying both the heterocyclic nucleus and the side chain, hopefully to improve molecular recognition.

Results and Discussion

We proceeded towards molecular simplification and also covered a few isosters, *i.e.* thiocoumarin and quinolindione derivatives. 2- and 4-thiocoumarin nuclei were accessed by a published procedure,[27,28] while 2,4-quinolinediol was commercially available. *S*-Geranylthiocoumarins **7** and **8** were prepared *via* a Mitsunobu reaction.[29] 4-*O*-Geranyl-quinolin-2-one (**10**) was prepared according to Grundon.[30]

The isosters synthesized by us (**7**—**10**) showed lower inhibitory activities compared to either the *O*-geranylcoumarins or the ω-epoxy derivative **6**. A shorter side chain, as in aurapten[31] (7-geranyloxycoumarin, **5**) and 6′,7′-epoxyaurapten[32] (**6**) or prenyletin[33,34] (**4**) was also deleterious for the inhibitory activity.

All relevant structure modifications are shown in Fig. 2; results of the SHC inhibition test are summarized in Table 1.

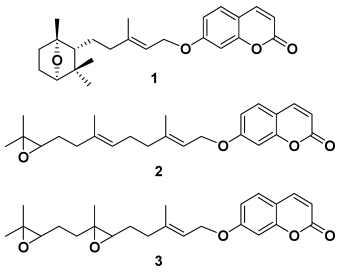


Fig. 1. Meroterpenoid Inhibitors for SHC

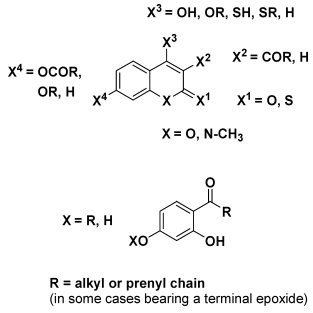
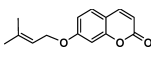
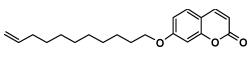
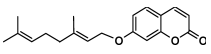
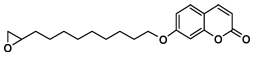
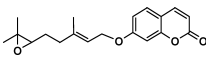
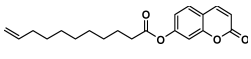
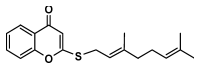
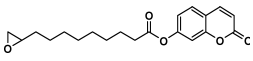
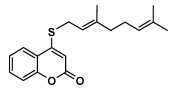
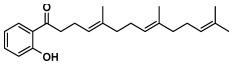
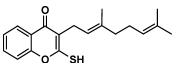
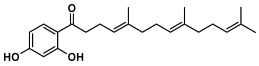
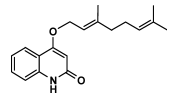
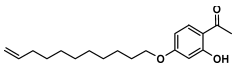
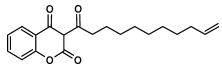
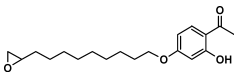


Fig. 2. Isosters and Chemical Modifications of Coumarin and Acetophenone

* To whom correspondence should be addressed. e-mail: giancarlo.cravotto@unito.it

Table 1. SHC Inhibition Values

	Compound	Inhibition[a)]		Compound	Inhibition[a)]
4		No activity[b)]	**12**		72% resid. activity[b)]
5		No activity[b)]	**13**		68% resid. activity[b)]
6		80% resid. activity[b)]	**14**		90% resid. activity[b)]
7		No activity[b)]	**15**		90% resid. activity[b)]
8		No activity[b)]	**16**		IC_{50} 60 μM
9		No activity[b)]	**17**		IC_{50} 30 μM
10		No activity[b)]	**18**		IC_{50} 48 μM
11		No activity[b)]	**19**		IC_{50} 100 μM

a) Values are means of three experiments. *b*) At 100 μM.

In compounds **11**, **12** and **14**, the farnesyl moiety carried by the corresponding natural products ferulenol (3-farnesyl-4-hydroxycoumarin) and umbelliprenine (7-farnesyloxycoumarin) is replaced with a linear alkyl chain of similar length (C_{11}). Compound **11** was obtained by acylation of 4-hydroxycoumarin with 10-undecenoyl chloride in pyridine, while ester **14** was obtained by the treatment of umbelliferone with 10-undecenoic acid in the presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP). A Mitsunobu reaction of umbelliferone and 10-undecenol afforded compound **12**. Classical epoxydation with MCPB acid afforded derivatives **13** and **15**. As shown in Table 1, the inhibitory activity fell sharply when the farnesyl moiety was replaced with a similar alkyl chain, revealing a critical detail for molecular recognition. Although an epoxide group inserted at the end of the farnesyl chain increased the inhibitory activity, it did not have the same effect when present on an aliphatic chain. Slightly increased activity was found when the side chain was bound to the heterocyclic nucleus by an ester linkage, **14**–**15**.

The coumarin skeleton itself could be usefully cut down to *o*-hydroxyacetophenone, the product of coumarin hydrolysis and decarboxylation. Our inhibition tests on these derivatives started with secoferulenol **16** and secoammoresinol **17**, two naturally occurring acetophenones,[35,36)] prepared as previously described.[37)] As shown in Table 1, both were more active than the corresponding coumarin derivatives ferulenol (IC_{50} 90 μM) and ammoresinol (7-farnesyloxy-4-hydroxycoumarin, no activity at 100 μM). Keeping in mind the structures of the original inhibitors (**1**—**3**), we also elaborated on resacetophenone (2,4-dihydroxyacetophenone), producing its 4-undecenyloxy- (**18**) and 4-(10′,11′-epoxyundecanyloxy)- (**19**) derivatives. Selective *O*-alkylation was achieved by Mitsunobu reaction; a subsequent MCPBA epoxidation gave **19**. Both compounds proved moderately active (IC_{50} 48 μM and 100 μM, respectively).

Conclusion

Structural modification of our original coumarin-derived SHC inhibitors yielded significant clues for future improvements in molecular design. We proved that umbelliferone and hydroxyacetophenone are useful starting structures for the design of new SHC inhibitors. Although an epoxide group inserted at the end of the prenyl chain increased the inhibitory effect, it did not have the same effect when similarly placed on a linear aliphatic chain. On comparison of several farnesylcoumarins (ferprenine, ferulenol and ammoresinol) with the corresponding farnesylacetophenones (secoferulenol and secoammoresinol) we found the simplified aromatic moiety to be more active. The acetophenone moiety probably interacts more strongly with the aromatic residues at the active site of the enzyme.

The present study is to be extended to eukaryotic oxidosqualene cyclases.

Experimental

General IR: Shimadzu DR 8001 spectrophotometer; NMR: ^{1}H-(300 MHz) and ^{13}C-NMR (75 MHz) were recorded at room temperature with a Bruker 300 Advance spectrometer. The spectra were recorded in $CDCl_3$, and solvent signals for $CHCl_3/CDCl_3$ (7.26 ppm) were used as a reference. Reactions were monitored by TLC (Silica gel Fluka aluminium cards, 0.2 mm), spots being visualized by UV inspection and/or staining with 5% H_2SO_4 in ethanol, followed by heating. Macherey-Nagel silica gel was used for column chromatography (CC). Melting points: Büchi SMP-20 (uncorrected). Low-resolution mass spectra (LR-MS): Finnigan-MAT TSQ70 in chemical ionization with isobutane as the reactant gas. HPLC analysis: Thermo-Quest Spectra Series P200, Detector UV/VIS Jasco 875-UV or a Gilson 133 refractive index refractometer, integrator Millipore 740 Waters. Commercially available reagents and solvents were used without further purification unless otherwise noted. Analytical samples of all new compounds have been obtained by semi-preparative HPLC.

Preparation of Compounds 4—6 and 10 According to a standard procedure,[38] we refluxed 4-hydroxycoumarin in anhydrous acetone with 1 eq of alkyl bromide in the presence of excess K_2CO_3 to obtain in good yield prenyletin (**4**, yield 89%) and aurapten (**5**, yield 82%).

When treated in the same way, 2,4-quinolindiol gave 4-geranyloxy-2-quinolinone (**10**, yield 39%).[30] **5** was epoxidated at the terminal double bond by treatment with *N*-bromosuccinimide in THF/H_2O, then conversion of the resulting bromohydrin to epoxide (**6**) by treatment with a base[26,39] (yield 72%).

Preparation of *S*-Geranyl Thiocoumarins 7 and 8 A solution in anhydrous THF containing triphenylphosphine (1.5 eq), geraniol (1.5 eq) and thiocoumarin (1.0 eq) was magnetically stirred at 0 °C under N_2. Diisopropyl azodicarboxylate (DIAD) (1.5 eq) was added dropwise over a period of 5 min. The orange-red color of DIAD immediately disappeared. The weakly exothermic reaction was allowed to proceed at room temperature under stirring. Its duration ranged from 1 to 24 h, depending on the nature of the enol. After completion, as indicated by TLC monitoring (eluent hexane–ethyl acetate), the reaction mixture was evaporated. The residue was diluted with hexane–ether 3 : 1, filtered through a thin pad of Celite to remove the precipitate of triphenylphosphine oxide, and concentrated under reduced pressure. Finally, the products were purified by flash silica gel column chromatography. Isolated yields **7** (17%), **8** (29%).

2-Geranylsulfanylchromen-4-one (**7**): Yellow oil. IR (liquid film) 1643, 1587, 1356, 1130, 922, 759 cm^{-1}. LR-MS: 315 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 8.12 (1H, d, J=7.5 Hz, H-5), 7.59 (1H, m, H-7), 7.37—7.24 (2H, m, H-6, H-8), (1H, t, J=7.3 Hz,), 6.23 (1H, br s, H-3), 5.28 (1H, br t, J=6.4 Hz, H-2′), 5.10 (1H, m, H-6′), 3.67 (2H, d, J=7.6 Hz, H-1′), 1.77 (3H, s, H-10′*), 1.68 (3H, s, H-9′*), 1.61 (3H, s, H-8′). ^{13}C-NMR ($CDCl_3$) δ: 159.1, 156.7, 151.9, 143.2, 131.9, 131.4, 128.4, 124.0, 123.3, 115.4, 106.9, 38.9, 29.2, 26.1, 25.5, 17.6, 16.3. *Anal.* Calcd for $C_{19}H_{22}O_2S$: C, 72.57; H, 7.05. Found: C, 73.00; H, 7.16.

4-Geranylsulfanylchromen-2-one (**8**): Yellow oil. IR (liquid film) 1721, 1595, 1192, 929, 756, 745 cm^{-1}. LR-MS: 315 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 7.83 (1H, d, J=7.9 Hz, H-5), 7.55 (1H, m, H-7), 7.32 (1H, d, J=8.1 Hz, H-8), 7.24 (1H, t, J=7.3 Hz, H-6), 6.15 (1H, s, H-3), 5.34 (1H, t, J=7.4 Hz, H-2′), 5.07 (1H, m, H-6′), 3.67 (2H, d, J=8.4 Hz, H-1′), 1.77 (3H, s, H-10′*), 1.68 (3H, s, H-9′*), 1.61 (3H, s, H-8′). ^{13}C-NMR ($CDCl_3$) δ: 159.1, 156.7, 151.9, 143.2, 131.9, 131.4, 128.4, 124.0, 123.3, 115.4, 106.9, 38.9, 29.2, 26.1, 25.5, 17.6, 16.3. *Anal.* Calcd for $C_{19}H_{22}O_2S$: C, 72.57; H, 7.05. Found: C, 72.80; H, 7.21.

3-Geranyl-2-mercaptochromen-4-one (9) In a 50 ml two-necked round-bottomed flask equipped with a magnetic stirrer and a nitrogen inlet, excess *t*-BuOK (0.600 g) anhydrous toluene (5 ml), deprenylsecoferulenol[37] (1-(2-hydroxyphenyl)-5,9-dimethyldeca-4,8-dien-1-one) (0.500 g, 1.8 mmol) and CS_2 (130 μl) were added. Progressively, the yellow solution turned to red. After 14 h the reaction was complete, as shown by TLC (eluent hexane/EtOAc 4 : 1. *Rf* **9**=0.35). Work-up: the reacted mixture was diluted with EtOAc, washed with sat. NH_4Cl and brine, dried over Na_2SO_4, filtered, and evaporated to dryness. CC (eluent hexane/EtOAc 9 : 1) yielded 115 mg of **9** (0.36 mmol, 20%). Yellow oil. IR (liquid film) 1742, 1608, 1550, 1460, 1375, 1120, 1025 cm^{-1}. LR-MS: 315 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 8.21 (1H, d, J=7.9 Hz, H-5), 7.63 (1H, m, H-7), 7.40 (2H, m, H-6), 7.31 (1H, d, J=7.9 Hz, H-8), 5.17 (1H, t, J=7.3 Hz, H-2′), 5.06 (1H, t, J=7.1 Hz, H-6′), 3.48 (1H, d, J=7.1 Hz, H-1′), 2.07 (2H, m, H-5′), 2.00 (2H, m, H-4′), 1.82 (3H, s, CH_3), 1.66 (3H, s, CH_3), 1.58 (3H, s, CH_3). ^{13}C-NMR ($CDCl_3$) δ: 184.2, 157.2, 151.9, 143.2, 131.9, 131.4, 128.4, 124.0, 123.3, 115.4, 106.9, 38.9, 29.2, 26.1, 25.5, 17.6, 16.3. *Anal.* Calcd for $C_{19}H_{22}O_2S$: C, 72.57; H, 7.05. Found: C, 72.40; H, 9.92.

3-(10-Undecenoyl)chroman-2,4-dione (11) In a 50 ml two-necked round-bottomed flask equipped with a magnetic stirrer and a nitrogen inlet, 4-hydroxycoumarin (5.0 g, 30.8 mmol) and a catalytic amount of piperidine were dissolved in anhydrous pyridine (35 ml). The solution was placed under stirring, cooled down to 0 °C, and 10-undecenoyl chloride was added dropwise. The mixture was stirred for 8 h under nitrogen at 40 °C, and the reaction monitored by TLC (eluent hexane/EtOAc 4 : 1. *Rf* **11**=0.4). Work-up: the reacted mixture was diluted with EtOAc and washed with 5% HCl and with $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated to dryness. CC (eluent hexane/EtOAc 19 : 1) yielded 7.0 g of **11** (21.3 mmol, 70%). White powder, mp 98 °C. IR (KBr) 1717, 1607, 1547, 1225, 980, 901 cm^{-1}. LR-MS: 329 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 8.08 (1H, d, J=7.90 Hz, H-5), 7.73 (1H, td, J=7.14 Hz, J'=1.39 Hz, H-7), 7.38—7.28 (2H, m, H-6, H-8), 5.90—5.76 (1H, m, H-10′), 5.03—4.93 (2H, m, H-11′a,b), 3.21 (2H, t, J=7.45 Hz, H-2′a,b). ^{13}C-NMR ($CDCl_3$) δ: 209.3, 207.5, 179.2, 160.3, 136.5, 126.0, 124.8, 117.4, 115.8, 101.5, 85.2, 68.5, 42.1, 31.4, 29.6, 29.4, 29.1, 24.5, 21.5, 19.0. *Anal.* Calcd for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37. Found: C, 73.29; H, 7.50.

7-(10-Undecenyloxy)chromen-2-one (12) Following the Mitsunobu protocol, umbelliferone (1.93 g, 11,9 mmol), 10-undecenol (2.03 g, 11.9 mmol), TPP (3.22 g, 11.9 mmol), diisopropylazodicarboxylate (DIAD) (2.47 ml, 11.9 mmol) and anhydrous THF (20 ml) were added to a 50 ml round-bottomed flask. The mixture was stirred under nitrogen at rt for 2 h, and the reaction monitored by TLC (eluent hexane/EtOAc 8 : 2. *Rf* **12**=0.45). After evaporation under vacuum the raw material was purified by CC (eluent gradient: hexane/EtOAc 9 : 1→8 : 2), yielding 2.98 g of **12** (9.5 mmol, yield 80%). White powder, mp 85 °C. IR (KBr) 1726, 1624, 1134, 837 cm^{-1}. LR-MS: 315 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 7.60 (1H, d, J=9.5 Hz, H-4), 7.35 (1H, d, J=8.4 Hz, H-5), 6.80 (2H, m, H-6, H-8), 6.25 (1H, d, J=9.5 Hz, H-3), 5.75 (1H, m, H-10′), 4.95 (2H, m, H-11′), 3.95 (1H, t, J=6.5 Hz, H-1′), 2.00 (2H, m, H-9′), 1.80 (2H, m, H-2′), 1.50—1.30 (12H, m, H-3′—8′). ^{13}C-NMR ($CDCl_3$) δ: 163.3, 161.2, 155.8, 143.3, 139.1, 127.7, 112.9, 112.8, 112.2, 101.2, 33.7, 33.3, 29.7, 29.6, 29.5, 29.3, 29.2, 29.0, 23.9, 22.6. *Anal.* Calcd for $C_{20}H_{26}O_3$: C, 76.40; H, 8.33. Found: C, 76.40; H, 8.46.

7-(9-Oxiranylnonyloxy)chromen-2-one (13) In a 100 ml round-bottomed flask, **12** (1.30 g, 4.1 mmol) was dissolved in anhydrous CH_2Cl_2 (10 ml). The solution was stirred, then cooled to 0 °C. Anhydrous sodium acetate (0.712 g, 4.1 mmol) was added all at once, and 2.76 g of MCPBA 70% (12.3 mmol) was added portionwise under stirring. After 4 h of stirring, the reaction was monitored by TLC (eluent hexane/EtOAc 8 : 2. *Rf* **13**=0.21). Work-up: the reacted mixture was washed with 5% $Na_2S_2O_3$ solution and 5% NaOH and brine. Silica gel CC eluent gradient: hexane/EtOAc 9 : 1→7 : 3 obtaining 0.954 g of **13** (2.88 mmol, yield 70%). White powder, mp 85 °C. IR (KBr) 1726, 1624, 1134, 915, 837 cm^{-1}. LR-MS: 331 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 7.60 (1H, d, J=9.5 Hz, H-4), 7.35 (1H, d, J=8.4 Hz, H-5), 6.80 (2H, m, H-6, H-8), 6.25 (1H, d, J=9.5 Hz, H-3), 3.95 (2H, t, J=6.5 Hz, H-1′), 2.75 (2H, t, J=4.9 Hz, H-11′), 2.45 (1H, m, H-10′), 1.80 (2H, m, H-2′), 1.65 (2H, m, H-9′), 1.50—1.20 (12H, m, H-3′—8′). ^{13}C-NMR ($CDCl_3$) δ: 159.1, 156.7, 151.9, 143.2, 131.9, 131.4, 128.4, 124.0, 123.3, 115.4, 106.9, 38.9, 29.2, 26.1, 25.5, 17.6, 16.3. *Anal.* Calcd for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.59; H, 7.86.

10-Undecenoic Acid 2-Oxo-2*H*-chromen-7-yl Ester (14) In a 100 ml round-bottomed flask, umbelliferone (4.0 g, 24.7 mmol), 10-undecenoic acid (5.0 ml, 24.7 mmol), DCC (5.1 g, 24.7 mmol) and DMAP (0.3 g, 2.4 mmol) were dissolved in anhydrous toluene (40 ml). After 2 h magnetic stirring at rt, the reaction was monitored by TLC (eluent hexane/EtOAc 7 : 3 *Rf* **14**=0.45). CC (eluent gradient: hexane/EtOAc 95 : 5→9 : 1→7 : 3) yielded 5.51 g of ester **14** (16.8 mmol, yield 68%). White powder, mp 57 °C. IR (KBr) 1732, 1626, 1468, 1269, 1165, 991, 927 cm^{-1}. LR-MS: 329 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 7.70 (1H, d, J=9.5 Hz, H-4), 7.48 (1H, d, J=8.4 Hz, H-5), 7.05 (2H, m, H-6, H-8), 6.40 (1H, d, J=9.5 Hz, H-3), 5.85 (1H, m, H-10′), 4.95 (2H, d, J=13.3 Hz, H-11′), 2.60 (1H, t, J=7.4 Hz, H-2′), 2.05 (2H, m, H-9′), 1.75 (2H, m, H-3′), 1.50—1.30 (10H, m, H-4′—8′). ^{13}C-NMR ($CDCl_3$) δ: 171.5, 160.3, 154.5, 153.2, 142.8, 139.0, 128.9, 118.3, 116.4, 115.9, 114.1, 110.3, 34.2, 33.6, 29.6, 29.1, 29.0, 28.9, 28.7, 24.6. *Anal.* Calcd for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37. Found: C, 73.22; H, 7.31.

9-Oxyranyl-nonanoic Acid 2-Oxo-2*H*-chromen-7-yl Ester (15) In a 100 ml round-bottomed flask, 7-(10-undecenoyloxy)coumarin (700 mg, 2.13 mmol) was dissolved in anhydrous CH_2Cl_2 (10 ml). To the ice-cold magnetically stirred solution, anhydrous sodium acetate (700 mg, 8.52 mmol) was added all at once, and MCPBA 70% (1.435 g, 6.39 mmol) was added portionwise. After 4 h of stirring, the reaction was monitored by TLC (eluent hexane/EtOAc 7 : 3. *Rf* **15**=0.27). Work-up: The reacted mixture was washed with 5% $Na_2S_2O_3$, 5% NaOH and brine. CC (eluent gradient: hexane/EtOAc 9 : 1→6 : 4) yielded 436 mg of **15** (1.27 mmol, yield 60%). White powder, mp 75 °C. IR (KBr) 1763, 1618, 1228, 1120, 916, 756 cm^{-1}. LR-MS: 345 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 7.70 (1H, d, J=9.5 Hz, H-4), 7.48 (1H, d, J=8.4 Hz, H-5), 7.05 (2H, m, H-6, H-8), 6.40 (1H, d, J=9.5 Hz, H-3), 2.70 (1H, m, H-10′), 2.60 (2H, t, J=7.5 Hz, H-11′), 2.30 (2H, t, J=7.4 Hz, H-2′), 1.75 (2H, m, H-3′), 1.50 (2H, m, H-9′), 1.40—1.20 (10H, m, H-4′—10′). ^{13}C-NMR ($CDCl_3$) δ: 170.4, 160.9, 155.1, 153.6, 139.5, 129.1, 118.3, 116.6, 116.0, 110.8, 52.3, 47.1, 34.2, 33.8, 29.6, 29.4, 29.2, 28.9, 28.7, 24.7. *Anal.* Calcd for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.63; H, 7.20.

1-(2-Hydroxy-4-undec-10-enyloxy-phenyl)ethanone (18) In a 50 ml two-necked round-bottomed flask equipped with a magnetic stirrer and a nitrogen inlet, resacetophenone (1.0 g, 6.57 mmol), 10-undecenol (1.31 ml, 6.57 mmol), TPP (2.07 g, 6.57 mmol), diisopropylazodicarboxylate (DIAD) (1.56 ml, 6.57 mmol) and anhydrous THF (20 ml) were added. The mixture

was stirred for 1 h under nitrogen at rt, and the reaction monitored by TLC (eluent hexane/EtOAc 19 : 1. *Rf* **18**=0.42). After evaporation under vacuum, the raw material was purified by CC (eluent hexane), yielding 1.28 g of **18** (4.2 mmol, yield 64%). Yellow powder, mp 41 °C. IR (KBr) 3350, 1634, 1372, 1256, 1069 cm^{-1}. LR-MS: 305 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 12.7 (1H, s, OH), 7.54 (1H, d, J=8.7 Hz, H-6), 6.37 (1H, d, overlap., H-5), 6.33 (1H, s, H-3), 5.79 (1H, m, H-10′), 4.92 (2H, m, H-11′a, 11′b), 3.91 (2H, t, J=6.4 Hz, H-1′), 2.48 (3H, s, CH_3), 2.00 (2H, m, H-9′), 1.73 (2H, m, H-2′), 1.28 (12H, m, H-3′—8′). ^{13}C-NMR ($CDCl_3$) δ: 202.1, 165.4, 165.0, 138.8, 131.9, 113.9, 113.4, 107.6, 101.0, 68.1, 33.5, 29.5, 29.2, 29.1, 28.8, 28.7, 28.6, 25.8. *Anal.* Calcd for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 75.00; H, 9.27.

1-[2-Hydroxy-4-(9-oxyranyl-nonyloxy)phenyl]ethanone (19) As described for the preparation of **15**, employing **18** (231 mg, 0.763 mmol), anhydrous sodium acetate (311 mg, 2.29 mmol), MCPBA 70% (512 mg, 2.29 mmol) and anhydrous CH_2Cl_2 (10 ml) we recovered 176 mg of **19** (yield 72%) after CC purification (eluent hexane/EtOAc 19 : 1). TLC (eluent hexane/EtOAc 8 : 2): *Rf* **19**=0.47. An analytical sample was obtained by HPLC (eluent hexane/EtOAc 9 : 1). Yellow oil. IR (liquid film) 1638, 1508, 1427, 1370, 1257, 1134, 802 cm^{-1}. LR-MS: 321 (MH^+, CI-MS). ^{1}H-NMR ($CDCl_3$) δ: 12.7 (1H, s, OH), 7.62 (1H, dd, J=11.3, J'=2.3 Hz, H-6), 6.43 (1H, dd overlap., J=6.5 Hz, H-5), 6.40 (1H, s, H-3), 3.99 (2H, t, J=6.5 Hz, H-1′), 2.91 (1H, br s, H-10′), 2.75 (1H, t, J'=4.2 Hz, H-11′a), 2.55 (3H, s, CH_3), 2.47 (1H, t, J'=4.2 Hz, H-11′b), 1.79 (2H, m, H-2′), 1.53 (2H, m, H-9′), 1.51—1.26 (12H, m, H-3′—8′). ^{13}C-NMR ($CDCl_3$) δ: 202.4, 183.7, 165.6, 165.1, 132.1, 113.6, 107.9, 101.1, 68.3, 52.3, 47.1, 38.6, 32.4, 29.3, 29.2, 29.1, 29.0, 28.8, 26.1. *Anal.* Calcd for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81. Found: C, 71.08; H, 8.90.

General Procedure for SHC Inhibition Tests IC_{50} values (inhibitor concentrations that reduced by 50% the rate of enzymatic conversion of squalene to hopene) were determined at 10 μM substrate concentration. [^{14}C]squalene was obtained by incubating 1 μCi of [^{14}C]mevalonolactone with the S_{10} supernatant of a pig liver homogenate (25 mg of protein), following the method of Popják,[40] in the presence of the oxidosqualene cyclase inhibitor U-18666A. Under these conditions, the bulk of radioactivity of nonsaponifiable extract was shared between squalene and oxidosqualene, which can be easily separated by TLC.[41]

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References

1) Nes W. D., *Recent Adv. Phytochem.*, **24**, 283—327 (1990).
2) Abe I., Sankawa U., Ebizuka Y., *Chem. Pharm. Bull.*, **40**, 1755—1760 (1992).
3) Moore W. R., Schatzman G. L. J., *Biol. Chem.*, **267**, 22003—22006 (1992).
4) Corey E. J., Matzuda S. P. T., *J. Am. Chem. Soc.*, **113**, 8172—8174 (1991).
5) Kelly R., Miller S. M., Lai M. H., Kirsch D. R., *Gene*, **87**, 177—183 (1990).
6) Roessner C. A., Min C., Hardin S. H., Harris-Haller L. W., McCollum J. C., Scott A. I., *Gene*, **127**, 149—150 (1993).
7) Lees N. D., Skaggs B., Kirsch D. R., Bard M., *Lipids*, **30**, 221—226 (1995).
8) Baker C. H., Matsuda S. P. T., Liu D. R., Corey E. J., *Biochem. Biophys. Res. Commun.*, **213**, 154—160 (1995).
9) Abe I., Prestwich G. D., *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 9274—9278 (1995).
10) Abe I., Rohmer M., Prestwich G. D., *Chem. Rev.*, **93**, 2189—2206 (1993).
11) Corey E. J., Virgil S. C., Cheng H., Hunter-Baker C., Matsuda S. P. T., Singh V., Sarshar S., *J. Am. Chem. Soc.*, **117**, 11819—11820 (1995).
12) Ceruti M., Balliano G., Viola F., Grosa G., Rocco F., Cattel L., *J. Med. Chem.*, **35**, 3050—3058 (1992).
13) Ceruti M., Rocco F., Viola F., Balliano G., Milla P., Arpicco S., Cattel L., *J. Med. Chem.*, **41**, 540—554 (1998).
14) Abe I., Liu W., Oelhschlager A. C., Prestwich G. D., *J. Am. Chem. Soc.*, **118**, 9180—9181 (1996).
15) Jolidon S., Polak-Wyss A., Hartman P. G., Guerry P. G., "Recent Advances in the Chemistry of Anti-infective Agents," Royal Soc. Chem., Cambridge, U.K., 1993, pp. 223—233.
16) Morand O. H., Aebi J. D., Dehlmlow H., Ji Y. H., Gains N., Lengsfeld H., Himber J. J., *Lipid Res.*, **38**, 373—390 (1997).
17) Dehlmlow H., Aebi J. D., Jolidon S., Ji Y. H., von der Mark E. M., Himber J., Morand O. H., *J. Med. Chem.*, **46**, 3354—3370 (2003).
18) Buckner F. S., Griffin J. H., Wilson A. J., Van Voorhis W. C., *Antimicrob. Agents Chemother.*, **45**, 1210—1215 (2001).
19) Oliaro-Bosso S., Ceruti M., Balliano G., Matsuda S. P. T., Milla P., Rocco F., Viola F., 93rd AOCS Annual Meeting & Expo, Montreal, Quebec, (Canada), on May 5—8, 2002.
20) Wendt K. U., Poralla K., Schulz G. E., *Science*, **277**, 1811—1815 (1997).
21) Wendt K. U., Lenhart A., Schulz G. E., *J. Mol. Biol.*, **286**, 175—197 (1999).
22) Wendt K. U., Schulz G. E., Corey E. J., Liu D. R., *Angew. Chem. Int. Ed.*, **39**, 2821—2833 (2000).
23) Lenhart A., Reinert D. J., Aebi J. D., Dehmlow H., Morand O. H., Schulz G. E., *J. Med. Chem.*, **46**, 2063—2092 (2003).
24) Viola F., Ceruti M., Cattel L., Milla P., Poralla, K., Balliano G., *Lipids*, **35**, 297—303 (2000).
25) Ceruti M., Balliano G., Rocco F., Milla P., Arpicco S., Cattel L., Viola F., *Lipids*, **36**, 629—636 (2001).
26) Cravotto G., Balliano G., Robaldo B., Oliaro-Bosso S., Chimichi S., Boccalini M., *Bioorg. Med. Chem. Lett.*, **14**, 1931—1934 (2004).
27) Di Braccio M., Roma G., Leoncini G., Poggi M., *Farmaco*, **50**, 703—711 (1995).
28) Majumdar K. C., Biswas A., Mukhopadhyay P. P., *Synthesis*, **15**, 2385—2389 (2003).
29) Cravotto G., Tagliapietra S., Palmisano G., Nano G. M., *Heterocycles*, **60**, 1351—1358 (2003).
30) Grundon M. F., Ramachandran V. N., Donnelly M. E., *Perkin Trans 1*, **2**, 633—635 (1981).
31) Angioni A., Cabras P., D'hallewin G., Pirisi F., M. Reniero F., Schirra M., *Phytochemistry*, **47**, 1521—1525 (1998).
32) Aziz M., Rouessac F., *Tetrahedron Lett.*, **28**, 2579—2582 (1987).
33) Pistelli L., Bertoli A., Bilia A. R., Morelli I., *Phytochemistry*, **41**, 1579—1582 (1996).
34) Ganguly N. C., Sukai A. K., Dutta S., De P., *J. Ind. Chem. Soc.*, **78**, 380—382 (2001).
35) Al-Yahya M. A., Muhammad I., Mirza H. H., El-Feraly F. S., *Phytother. Res.*, **12**, 335—339 (1998).
36) Mizukami H., Ogihara Y., *Chem. Pharm. Bull.*, **46**, 1781—1784 (1998).
37) Appendino G., Tagliapietra S., Cravotto G., Nano G. M., *Gazz. Chim. Ital.*, **119**, 385—388 (1989).
38) Gonzales A. G., Jorge D., *Anales de Quimica*, **79**, 265—269 (1983).
39) Van Tamelen E. E., Nadeau R. G., *J. Am. Chem. Soc.*, **89**, 176—177 (1967).
40) Popják, G., "Methods in Enzymology, Steroids and Terpenoids," Vol. XV, ed. by Clayton R. B., Academic Press, New York, 1969, pp. 438—443.
41) Balliano G., Viola F., Ceruti M., Cattel L., *Biochim. Biophys. Acta*, **959**, 9—19 (1988).