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Synthesis and structure–activity relationships of novel warfarin derivatives

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Abstract—4-Hydroxycoumarins such as warfarin **1** have been the mainstay of oral anticoagulation therapy for over 20 years. Yet little detail is known about the molecular interactions between 4-hydroxycoumarins with vitamin K epoxide reductase (VKER), inhibition of which produces a deficiency of vitamin K and consequently a deficiency of vitamin K-dependent proteins involved in thrombus formation. Using molecular probes, such as 4-sulfhydrylwarfarin **7** and 4-chlorowarfarin **10** it is shown in vitro that inhibition of VKER by warfarin is dependent on deprotonation of the 4-hydroxycoumarin moiety. In addition, the nature of the substituent on carbon 3 of the 4-hydroxycoumarin modulated inhibition. More specifically, a linear isoprenyl side chain increased inhibition of VKER when compared to cyclical substituents as present in warfarin. An example of a 4-hydroxycoumarin with an isoprenyl side chain is the natural product ferulenol **19** derived from Ferula communis. Ferulenol **19** confers ~22 times more potent inhibition than warfarin and is ~1.5 more potent than the rodenticide brodifacoum in this in vitro assay. Based on these data it is hypothesized that 4-hydroxycoumarins bind to the active site of VKER thereby mimicking the transition state of the elimination of water from substrate 2-hydroxyvitamin K.
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1. Introduction

4-Hydroxycoumarins such as warfarin **1**, acenocoumarol **2** and phenprocoumon **13** have been the mainstay of oral anticoagulation therapy for over 20 years.[1] 4-Hydroxycoumarins bind to vitamin K epoxide reductase (VKER), thereby inhibiting the reduction of vitamin K epoxide to dihydrovitamin K.[2–5] Dihydrovitamin K is a co-factor for the post-translational carboxylation of glutamic acid residues in about 20 proteins, several of which (the vitamin K-dependent clotting factors) play a pivotal role in regulating the coagulation of blood.[6–8] During the carboxylation reaction dihydrovitamin K is oxidised to vitamin K epoxide. The reduction of vitamin K epoxide by VKER provides a mechanism of recycling this co-factor. Recycling is impaired by inhibition of VKER resulting in under-carboxylated clotting factors with reduced activity manifested as a decreased tendency for fibrin clot formation, which is measured as a prolongation of the bleeding time (prothrombin time).[9]

More than 60 years ago the discovery of dicoumarol **15** as the causative agent of haemorrhagic disease in cattle fed on spoiled sweet clover hay spurned the development of 4-hydroxycoumarins.[10] This led to the development of warfarin as a rodenticide.[11] After an attempted suicide with warfarin it became apparent that a single large dose was less toxic than anticipated and the idea to use warfarin for human therapy for the treatment and prevention of thrombotic disease gained increasing acceptance.[12]

Despite the widespread use of 4-hydroxycoumarins, in particular warfarin, no systematic investigation of the in vitro structure–activity relationships of 4-hydroxycoumarins is reported. Here we report on the effect of structural modifications of warfarin on the inhibition of VKER activity in vitro. Some of the modifications involving the 4-hydroxy group of warfarin were aimed to explore the effect of acidity of this substituent. Other modifications were designed to explore the role of the side chain attached to carbon 3 of the coumarin moiety. More specifically we replaced the aromatic substituent of warfarin with linear carbon chains of repeating isoprenyl units as found in vitamin K. One of these compounds is the anticoagulant ferulenol **19** which is of particular interest since it is a toxin produced by

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Ferula communis (Giant Fennel), a plant endemic to Mediterranean countries.[13] Ingestion of the plant by livestock can cause a haemorrhagic disease (Ferulosis) similar to the haemorrhagic sweet clover disease.[14]

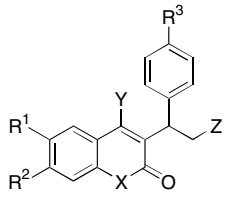
2. Results and discussion

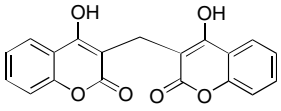
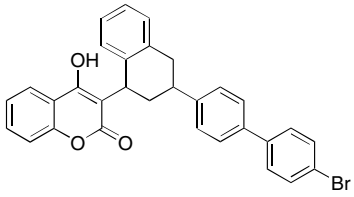
2.1. Chemistry

Coumarins **6**–**11** (Table 1) are novel derivatives of warfarin **1** where the 4-hydroxy group is replaced by a variety of substituents. A common precursor triflate **6** was prepared in 62% yield by treating warfarin with triflic anhydride. Warfarin triflate **6** is a stable compound that reacts readily with sulfur nucleophiles to yield 4-sulfhydrylwarfarin **7** (55%), 1-*S*-*N*-acetylcysteinylwarfarin **8** (27%) and 1-*S*-glutathienylwarfarin **9** (15%). 2D NMR experiments confirmed that the *S*-glutathienyl substituent of **9** is attached to carbon 4 of the coumarin moiety. Chlorowarfarin **10** was synthesized in 9% yield from triflate **6** by reaction with tetrabutylammonium chloride. This indirect method was employed as treatment of warfarin with thionyl chloride resulted in cyclisation of the side chain onto the coumarin moiety to yield dehydrowarfarin.[15] Hydrowarfarin **11** was obtained in 24% yield by hydrogenation of triflate **6** in the presence of palladium on carbon. Other compounds listed in Table 1 were known and either purchased or synthesized using reported procedures.

Compounds with an isoprenyl side chain similar to ferulenol **19** are listed in Table 2. A novel synthetic method was developed for the preparation of known (**17**–**19**) and new (**20**–**23**) coumarin derivatives.[16] The appropriate heterocyclic precursor was employed as the sodium salt and reacted with allyl halide in the absence of a solvent. For example, reaction of the sodium salt of 4-hydroxycoumarin with farnesyl chloride afforded ferulenol **19** in 10% yield. Yields were similar as with the solvent-based method however alkylation of the 4-hydroxy group was avoided in the absence of solvent aiding the purification. Selectivity for substitution at carbon three is coherent with a single electron transfer mechanism as previously suggested.[17] A single electron is transferred from the heterocyclic anion, for example, the sodium salt of 4-hydroxycoumarin, to the allyl halide generating a coumarin radical and an allyl radical (after loss of halide ion from the allyl halide radical

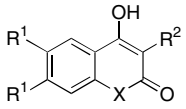
Table 1. Warfarin derivatives

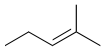
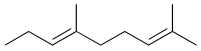
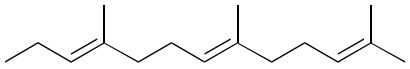
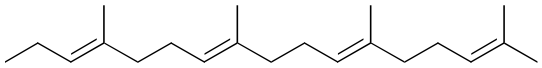
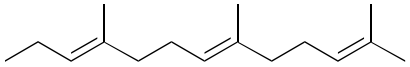
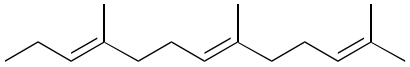
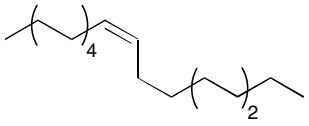


Compound[a]	R^1	R^2	R^3	X	Y	Z	IC_{50}[b] (μM)
Warfarin **1**	H	H	H	O	OH	$CO(CH_3)$	2.2
Acenocoumarol **2**	H	H	NO_2	O	OH	$CO(CH_3)$	0.77
6-Hydroxywarfarin **3**	OH	H	H	O	OH	$CO(CH_3)$	12.8
7-Hydroxywarfarin **4**	H	OH	H	O	OH	$CO(CH_3)$	250
Warfarin alcohol **5**	H	H	H	O	OH	$CHOH(CH_3)$	12.5
Warfarin triflate **6**	H	H	H	O	TfO	$CO(CH_3)$	>500
4-Sulfhydrylwarfarin **7**	H	H	H	O	SH	$CO(CH_3)$	18.3
S-(*N*-Acetyl-L-cysteinyl)warfarin **8**	H	H	H	O	*S*-(*N*-AcCys)	$CO(CH_3)$	>500
1-*S*-Glutathienylwarfarin **9**	H	H	H	O	*S*-Glut	$CO(CH_3)$	>500
4-Chlorowarfarin **10**	H	H	H	O	Cl	$CO(CH_3)$	>500
4-Hydrowarfarin **11**	H	H	H	O	H	$CO(CH_3)$	>500
4-Methoxywarfarin **12**	H	H	H	O	OCH_3	$CO(CH_3)$	>500
Phenprocoumon **13**	H	H	H	O	OH	CH_3	1.8
1-Deoxywarfarin **14**	H	H	H	Nil	OH	$CO(CH_3)$	3.0
Dicoumarol **15**							3.1
Brodifacoum **16**							0.15

[a] Compounds **1**–**14** are racemates. Compound **5** is a racemic mixture of diastereomers.
[b] SEM ± 9%.

Table 2. Ferulenol derivatives



Compound	R^1	X	R^2	IC_{50}[a] (μM)
Isoprenylcoumarin **17**	H	O		79
Geranylcoumarin **18**	H	O		0.17
Ferulenol **19**	H	O		0.098
Geranylgeranylcoumarin **20**	H	O		0.081
Dimethylferulenol **21**	CH_3	O		0.34
1-Carboxyferulenol **22**	H	CO		0.13
Oleylcoumarin **23**	H	O		0.9

[a] SEM ± 10%.

anion). The coumarin and allylic radicals recombine forming 3-allyl-4-hydroxycoumarin. Consistent with this mechanistic rationale allyl chlorides were as reactive as allyl bromides, whereas alkyl halides, for example, oleyl chloride, did not produce any C-alkylated coumarin in the absence of solvent. In order to synthesize 3-oleyl-4-hydroxycoumarin **23**, a solvent-based method was employed, where a SN2-type mechanism can occur.

2.2. Inhibition of vitamin K epoxide reductase activity

Coumarin derivatives listed in Tables 1 and 2 were tested as inhibitors of rat microsomal VKER.[33] Warfarin **1**, acenocoumarol **2**, phenprocoumon **13** and dicoumarol **15** have comparable inhibitory potency with an IC_{50} in the range 0.77–3.1 μM. Brodifacoum **16**, developed as a 'superwarfarin' to overcome resistance, was a very potent inhibitor (IC_{50} = 0.15 μM).[18]

Compounds **3**–**5** are of interest as metabolites of warfarin found in human plasma and urine during warfarin therapy.[19] *S*-7-Hydroxywarfarin is the main human metabolite derived from the more active *S*-enantiomer of warfarin.[20,21] Without in vitro evidence it is frequently stated that conversion of *S*-warfarin to *S*-7-hydroxywarfarin abolishes anticoagulant activity.[22] Here racemic 7-hydroxywarfarin **4** had negligible potency as an inhibitor of VKER activity. It is therefore expected that the naturally occurring *S*-7-hydroxywarfarin does not contribute to anticoagulation, and that the metabolic conversion of *S*-warfarin to *S*-7-hydroxywarfarin does indeed terminate anticoagulant activity of *S*-warfarin.

Replacing the 4-hydroxy group of warfarin **1** (pK_a 4.85) with a sulfhydryl group resulted in compound **7** with decreased acidity (pK_a 6.60) and an 8-fold reduction of inhibition. By comparison to warfarin **1** and its sulfhydryl derivative **7**, compounds **10**–**12** do not form anions at physiological pH. Neither do compounds **10**–**12** inhibit VKER activity. These results are consistent with a mechanism of inhibition postulated by Fasco on the basis of kinetic inhibition data (Fig. 1).[23–26]

According to this mechanism deprotonated warfarin binds to the oxidised disulfide form of VKER and mimics the transition state of water elimination from 2-hydroxyvitamin K (Step 2 of Fig. 1). This implies that the 4-hydroxycoumarin heterocycle of warfarin binds to

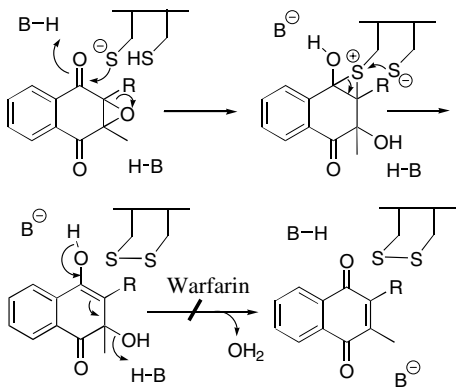


Figure 1. Mechanistic model for the reduction of vitamin K epoxide and inhibition by warfarin (adapted from Fasco).[23]

the active site of VKER in a similar orientation as the 1,4-naphthaquinone heterocycle of vitamin K. Similarly the substituent on carbon 3 of warfarin mimics the isoprenyl side chain of 2-hydroxyvitamin K substrate. In order to achieve a better fit within the active site, the 3-substituent of warfarin was replaced with an isoprenyl side chain as it occurs in vitamin K.

One of the compounds thus generated is the natural product ferulenol **19**, which was indeed ~22 times more potent (IC_{50} = 98 nM) as an inhibitor of VKER than warfarin **1** and ~1.5 times more potent than brodifacoum **16**. The length of the isoprenyl side chain is important for VKER inhibition as at least two isoprenyl units (as in compound **18**) resulted in a ~400-fold increase in potency over the compound with only one isoprenyl unit **17**. No further significant gain was achieved by lengthening the isoprenyl side chain beyond three units.

3. Conclusion

Using chemically synthesized probes to elucidate the interaction between 4-hydroxycoumarins such as warfarin **1** and VKER, the results presented here support a non-covalent mechanism of inhibition whereby deprotonated warfarin binds to the active site of VKER. It is hypothesized that by binding to the active site of VKER warfarin mimics the transition state of the elimination of water from substrate 2-hydroxyvitamin K. An interesting finding was the superior inhibitory potency of ferulenol **19** and related compounds consistent with a potential better fit of these compounds into the active site of VKER.

4. Experimental

^{1}H NMR spectra were recorded on a Varian Unity Inova 600 spectrometer and chemical shifts are reported in parts per million relative to tetramethylsilane as the internal standard. ^{1}H NMR assignments were based on 2D NMR experiments. Compounds were purified by chromatography on silica gel (Merck silica gel 60: 230–400 mesh). Fractions were analysed by HPLC on a 4.6 × 250 mm reverse phase silica column (Zorbax SB-C8, 5 μm, 1% acetic acid/65–90% methanol/water). All test compounds were checked for purity at λ = 254, 290 and 320 nm using a Waters 996 Photodiode Array Detector, and all test compounds were at least 98% pure. Vitamin K 2,3-epoxide was synthesized and purified by chromatography on silica gel.[27]

The pK_a values of compounds **1** and **7** were determined in this work using UV-spectroscopy as described.[28]

Acenocoumarol **2** (mp 196–198 °C; lit.[29] 196–199 °C) was extracted from Sintrom® and phenprocoumon **13** (mp 174–177 °C, lit.[29] 179–180 °C) from Marcumar® tablets. An analytical standard of brodifacoum **16** (mp 235–237 °C, lit.[29] 228–230 °C) was supplied by the Forensic Science Centre, Adelaide. 4-Methoxywarfarin **12** (mp 128–129 °C; lit.[31] 124–126 °C), 6-hydroxywarfarin **3** (mp 215–217 °C; lit.[15] 219–220 °C), 7-hydroxywarfarin **4** (mp 203–205 °C; lit.[15] 208–210 °C) and warfarin alcohol **5** were synthesized according to published procedures.[30] Chemicals were purchased from Sigma–Aldrich, Australia.

4.1. [1′-*R*,*S*]-3-(1′-Phenyl-3′-oxobutyl)-2*H*-1-benzopyran-2-one-4-trifluoromethanesulfonate (6)

Warfarin **1** (18.31 g, 59.4 mmol) and triethylamine (6.31 g, 62.4 mmol) were added to $CHCl_3$ (350 mL) and stirred under nitrogen until all dissolved. The solution was cooled to −5 °C and trifluoromethanesulfonic acid anhydride (17.59 g, 62.3 mmol) in $CHCl_3$ (50 mL) added over 30 min at a rate that the temperature did not exceed 0 °C. The solution was then allowed to warm to 25 °C and the solvent removed. Ether (400 mL) was added to the residue and it was washed with 2 M HCl (2× 100 mL), $NaHCO_3$ (2× 60 mL) and brine (100 mL). The organic phase was dried over $MgSO_4$, the solvent removed and the residue chromatographed (15% ethylacetate/85% *n*-heptane) to give 16.23 g (62%) of **6** as a white solid: mp 90–91 °C; ^{1}H NMR ($CDCl_3$) δ 2.17 (s, 3H, CH_3), 3.36 (dd, 1H, CH_2), 3.75 (dd, 1H, CH_2), 4.97 (dd, 1H, CH), 7.19–7.76 (m, 9H); HRMS [EI] 440.05409 $C_{20}H_{15}O_6F_3S$ requires 440.05415.

4.2. [1′-*R*,*S*]-4-Sulfhydryl-3-(1′-phenyl-3′- oxobutyl)-2*H*-1-benzopyran-2-one (7)

Sodium hydrosulfide hydrate (500 mg of NaHS.xH$_2$O) was dissolved in deoxygenated water (10 mL) and warmed under nitrogen to 80 °C. Warfarin triflate **6** (1.97 g, 4.46 mmol) was dissolved in pyridine (10 mL) and immediately added. The stirred mixture was cooled, ethylacetate (40 mL) added and the organic phase washed with 5% H_3PO_4 (2× 40 mL), $NaHCO_3$ (2× 20 mL), 5% H_3PO_4 (20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$ and the solvent removed. The residue was chromatographed (35% ethylacetate/65% *n*-heptane) to give 800 mg (55%) of **7** as a beige solid: mp 183–185 °C; ^{1}H NMR ($CDCl_3$) for the cyclical hemithioketals (4-SH group onto carbon 3′ of side chain) δ 1.74 (s, 3H, CH_3 minor isomer), 1.79 (s, 3H, CH_3 major isomer), 2.26 (dd, 1H, CH_2 major), 2.42 (dd, 1H, CH_2 minor), 2.57 (dd, 1H, CH_2 major), 2.77 (dd, 1H, CH_2 minor), 4.45 (dd, 1H, CHPh major), 4.81 (dd, 1H, CHPh minor), 7.19–7.76 (m); only a trace of the open-chain non-hemiketal form was detected. pK_a = 6.60. HRMS [EI] 324.08199 $C_{19}H_{16}O_3S$ requires 324.08202.

4.3. [1′-*R*,*S*]-4-*S*-(*N*-Acetyl-L-cysteinyl)-3-(1′-phenyl-3′-oxobutyl)-2*H*-1-benzopyran-2-one (8)

N-Acetyl-L-cysteine (265 mg, 1.63 mmol) was dissolved in deoxygenated pyridine (5 mL) and warfarin triflate **6** (0.858 g, 1.95 mmol) added. The resultant solution was stirred at 90 °C for 3 min, cooled, ethylacetate (40 mL) added and the organic phase washed with 2 M HCl (2× 30 mL). The organic phase was extracted with $NaHCO_3$ (3× 20 mL) and the extracted aqueous phase acidified with H_3PO_4 to pH 2 and extracted with ethylacetate (2× 30 mL) and dried over $MgSO_4$. The

solvent was removed and the residue chromatographed (15% acetic acid/85% ethylacetate) to give 200 mg (27%) of **8** as an off-white solid (from ethylacetate/acetic acid). ^{1}H NMR ($DOCD_3$) for the mixture of diastereomers A and B δ 1.82 (s, 3H, *N*-acetyl, A), 1.96 (s, 3H, *N*-acetyl, B), 2.25 (s, 3H, CH_3, A), 2.26 (s, 3H, CH_3, B), 3.37–3.87 (m, 4H, CH_2, A and B), 4.57 (m, 1H, CH, B), 4.62 (m, 1H, CH, A), 5.56 (dd, 1H, CHPh, A), 5.70 (dd, 1H, CHPh, B), 7.2–8.2 (m, 9H, A and B); HRMS [ESI] 453.12424 $C_{24}H_{23}NSO_6$ $(M-H)^+$ requires 453.12461.

4.4. [1′-*R*,*S*]-4-*S*-Glutathienyl-3-(1′-phenyl-3′-oxobutyl)-2*H*-1-benzopyran-2-one (9)

Glutathione (1.40 g, 4.56 mmol) and warfarin triflate **6** (1.66 g, 3.78 mmol) were reacted as described for the synthesis of **8**. The mixture was stirred for 3 min and 25 mL of 0.2 M HCl added until pH 2. The aqueous layer was washed with 20 mL of ethylacetate. Product **9** was present in the aqueous layer and purified by preparative HPLC on a 22.0×250 mm reverse phase Zorbax SB-C8 column (water containing 52% methanol and 4.3% triethylamine adjusted to pH 5.5 with acetic acid). Two fractions were collected containing separated diastereomers (A and B) of **9**. The combined yield of **9** was 270 mg (15%) each as a glassy solid. ^{1}H NMR (CD_3CO_2D) for diastereomer A δ 2.14 (m, 1H, β-H of glu), 2.23 (m, 1H, β-H of glu), 2.23 (s, 3H, CH_3), 2.58 (m, 2H, γ-CH_2 of glu), 3.30 (dd, 1H, β-H of cys), 3.50 (dd, 1H, β-H of cys), 3.55 (dd, 1H, H of CH_2 of 3-side-chain), 3.79 (dd, 1H, H of CH_2 of 3-side-chain), 4.01 (t, 1H, α-H of glu), 4.04 (q, 2H, CH_2 of gln), 4.79 (dd, 1H, α-H of cys), 5.61 (t, 1H, CHPh), 7.12–8.11 (m, 9H); ^{1}H NMR (CD_3CO_2D) for diastereomer B δ 2.12 (m, 1H, β-H of glu), 2.22 (m, 1H, β-H of glu), 2.22 (s, 3H, CH_3), 2.52 (m, 1H, γ-H of glu), 2.61 (m, 1H, γ-H of glu), 3.44 (dd, 2H, β-CH_2 of cys), 3.58 (dd, 1H, H of CH_2 of 3-side-chain), 3.76 (dd, 1H, H of CH_2 of 3-side-chain), 3.99 (t, 1H, α-H of glu), 4.00 (q, 2H, CH_2 of gln), 4.89 (dd, 1H, α-H of cys), 5.58 (t, 1H, CHPh), 7.12–8.10 (m, 9H); HRMS [ESI] (diastereomer A) 598.17832 $C_{29}H_{32}N_3SO_9$ $(M+H)^+$ requires 598.17756. HRMS [ESI] (diastereomer B) 598.17849 $C_{29}H_{32}N_3SO_9$ $(M+H)^+$ requires 598.17756.

4.5. [1′-*R*,*S*]-4-Chloro-3-(1′-phenyl-3′-oxobutyl)-2*H*-1-benzopyran-2-one (10)

Tetrabutylammonium chloride (2.58 g, 9.28 mmol) was dissolved in DMSO (4 mL) at 60 °C. Warfarin triflate **6** (1.246 g, 2.83 mmol) was dissolved in pyridine (4 mL) and immediately added. The mixture was stirred at 60 °C for 1 h and poured onto 5% H_3PO_4 (50 mL). It was extracted with ethylacetate (2× 30 mL), and the organic phase washed with 5% H_3PO_4 (30 mL), $NaHCO_3$ (2× 20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent removed. The residue was chromatographed twice (20% ethylacetate/80% *n*-heptane) to give 80 mg (8.6%) of **10** as white solid: mp 134–135 °C; ^{1}H NMR (DMSO-d_6) δ 2.15 (s, 3H, CH_3), 3.53 (d, 2H, CH_2), 5.06 (t, 1H, CH), 7.21–7.96 (m, 9H); HRMS [EI] 327.07939 $C_{19}H_{16}{}^{35}ClO_3$ $(M+H^+)$ requires 327.07880. A second peak for $C_{19}H_{16}{}^{37}ClO_3$ (33% of 327 peak) was detected at 329.1.

4.6. [1′-*R*,*S*]-3-(1′-Phenyl-3′-oxobutyl)-2*H*-1-benzopyran-2-one (11)

A mixture of warfarin triflate **6** (1.103 g, 2.50 mmol) and 5% Pd on C (1.29 g) in ethylacetate (20 mL) and methanol (20 mL) was stirred under an atmosphere of hydrogen (ambient pressure) for 14 h. The atmosphere was replaced by nitrogen and the mixture filtered. The filtrate was washed with $NaHCO_3$ (2× 20 mL), 5% H_3PO_4 (20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent removed. The residue was chromatographed (35% ethylacetate/65% *n*-heptane) to give 176 mg (24%) of **11** as an off-white solid: mp 143–144 °C; ^{1}H NMR (DMSO-d_6) δ 2.12 (s, 3H, CH_3), 3.23 (dd, 1H, CH_2), 3.39 (dd, 1H, CH_2), 4.52 (t, 1H, CHPh), 7.20–7.70 (m, 9H), 8.03 (s, 1H, 4-CH); HRMS [EI] 293.11803 $C_{19}H_{17}O_3$ $(M+H^+)$ requires 293.11777.

4.7. [1′-*R*,*S*]-2-(1′-Phenyl-3′-oxobutyl)-1,3-indandione (14)

1,3-Indandione (1.85 g, 12.66 mmol), benzalacetone (2.40 g, 16.42 mmol) and trifluoroacetic acid (50 μL) were refluxed in ethanol (20 mL) for 2 days. The solvent was removed and ethylacetate (40 mL) added to the residue. The organic phase was washed with 2 M HCl (2× 30 mL) and extracted with $NaHCO_3$ (3× 20 mL). The aqueous phase was acidified with H_3PO_4 to pH 2, extracted with ethylacetate (2× 30 mL) and dried over $MgSO_4$. The solvent was removed and the residue chromatographed (33% ethylacetate/67% *n*-heptane) to give 520 mg (14%) of **14** as a yellow solid: mp 115–116 °C [lit.[32] 115–116 °C]. ^{1}H NMR (DMSO-d_6) δ 2.09 (s, 3H, CH_3), 3.10–3.42 (m, 2H, CH_2), 3.90 (m, 1H, CH), 7.04–7.85 (m, 9H); HRMS [EI] 293.11786 $C_{19}H_{17}O_3$ $(M+H^+)$ requires 293.11777.

4.8. 3-*trans*,*trans*-Farnesyl-4-hydroxy-2*H*-1-benzopyran-2-one (ferulenol) (19)

4-Hydroxycoumarin sodium salt (38.7 g) was placed in a mortar and *all-trans*-farnesylchloride (20.3 g, 0.0843 mol) added in 2 mL portions and worked into a paste using a pestle. Water (1.5 mL) was added dropwise during this process until the paste had the desired consistency. The paste was placed into a glass flask, which was inserted into a heating bath at 100 °C. The paste was regularly mixed using a spatula and as the temperature of the contents approached 100 °C (the paste tended to dry) regular addition of small amounts of water (8 mL in total) was made. After 150 min, the contents were suspended in 80 mL of toluene and the suspension filtered. The filtered solid was extracted with toluene (4× 50 mL) and the solvent evaporated from the combined filtrates. The oily residue was chromatographed (17% ethylacetate/83% *n*-heptane) and fractions containing ferulenol were combined and concentrated to 300 mL. The resultant solution was chilled at −18 °C for 2 days and filtered to yield 3.09 g (10.0%) of ferulenol **19** as a white crystalline solid: mp 55–56 °C [lit.[13,17] 61 °C]. ^{1}H NMR ($CDCl_3$) δ 1.58 (s, 3H, CH_3),

1.62 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.85 (s, 3H, CH_3), 2.00–2.09 (m, 4H, $2CH_2$), 2.18 (m, 4H, $2CH_2$), 3.45 (d, 2H, α-CH_2), 5.08 (m, 2H, C=CH), 5.47 (t, 1H, C=CH), 7.25–7.31 (m, 2H, CH), 7.52 (m, 1H, CH), 7.77 (dd, 1H, CH); HRMS [EI] 367.22715 $C_{24}H_{31}O_3$ ($M+H^+$) requires 367.22732.

4.9. 3-*all-trans*-Geranylgeranyl-4-hydroxy-2*H*-1-benzopyran-2-one (20)

To 4-hydroxycoumarin sodium salt (1.02 g, 4.82 mmol) was added *all-trans*-geranylgeranyl bromide (1.213 g, 3.44 mmol) as described for the synthesis of **19**. The crude product was chromatographed (20% ethylacetate/80% heptane) to give 180 mg (12%) of **20** as a waxy white solid: mp 44–45 °C. ^1H NMR ($CDCl_3$) δ 1.58 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.85 (s, 3H, CH_3), 1.95–2.10 (m, 8H, $4CH_2$), 2.18 (m, 4H, $2CH_2$), 3.45 (d, 2H, CH_2), 5.08–5.12 (m, 3H, C=CH), 5.47 (t, 1H, C=CH), 7.25–7.31 (m, 2H, CH), 7.52 (m, 1H, CH), 7.77 (dd, 1H, CH). HRMS [EI] 435.28959 $C_{29}H_{39}O_3$ ($M+H^+$) requires 435.28992.

4.10. 3-*trans*,*trans*-Farnesyl-6,7-dimethyl-4-hydroxy-2*H*-1-benzopyran-2-one (21)

To 6,7-dimethyl-4-hydroxycoumarin sodium salt (3.30 g, 15.6 mmol) was added farnesyl bromide (2.20 g, 7.7 mmol) as described for the synthesis of **19**. The crude product was chromatographed (ethylacetate 16%/*n*-heptane 84%) to yield 219 mg (7.2%) of **21** as a white solid: mp 92–93 °C. ^1H NMR ($CDCl_3$) δ 1.58 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.84 (s, 3H, CH_3), 2.01 (m, 2H, CH_2), 2.07 (m, 2H, CH_2), 2.18 (m, 4H, $2CH_2$), 2.30 (s, 3H, ring CH_3), 2.34 (s, 3H, ring CH_3), 3.42 (d, 2H, α-CH_2), 5.08 (tq, 1H, C=CH), 5.10 (tq, 1H, C=CH), 5.46 (tq, 1H, C=CH), 7.07 (s, 1H, OH), 7.33 (s, 1H, CH), 7.48 (s, 1H, CH); HRMS [EI] 395.25883 $C_{26}H_{35}O_3$ ($M+H^+$) requires 395.25862.

4.11. 3-*trans*,*trans*-Farnesyl-2-hydroxy-1,4-naphthoquinone (22)

To 2-hydroxy-1,4-naphthoquinone sodium salt (10.65 g, 54.3 mmol) was added farnesyl bromide (5.1 g, 17.9 mmol) as described for the synthesis of **19**. The crude product was chromatographed (ethylacetate 15%/*n*-heptane 85%) to give 61 mg (1%) of **22** as a yellow solid: mp 68–69 °C. ^1H NMR ($CDCl_3$) δ 1.55 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.79 (s, 3H, CH_3), 1.90–2.09 (m, 8H, $4CH_2$), 3.32 (d, 2H, α-CH_2), 5.03 (tq, 1H, C=CH), 5.06 (tq, 1H, C=CH), 5.21 (tq, 1H, C=CH), 7.28 (s, 1H, OH), 7.67 (t, 1H, CH), 7.74 (t, 1H, CH), 8.06 (d, 1H, CH), 8.13 (d, 1H, CH); HRMS [EI] 379.22732 $C_{25}H_{31}O_3$ ($M+H^+$) requires 379.22763.

4.12. 3-Oleyl-4-hydroxy-2*H*-1-benzopyran-2-one (23)

DMSO (200 mL) was heated to 80 °C under nitrogen and finely powdered 4-hydroxycoumarin sodium salt (22.58 g) added in portions allowing each to dissolve before adding the next portion. Oleyl bromide (12.94 g, 0.0391 mol) was added from a syringe over 10 min. Stirring was continued for 10 min at 80 °C. The hot reaction mixture was poured onto a mixture of toluene (300 mL) and 2 M HCl (200 mL), mixed and the phases separated. The organic phase was washed with water (200 mL), dried over $MgSO_4$, the solvent evaporated and the residue chromatographed of ethylacetate (16% ethylacetate/84% heptane). Two products were isolated. First eluting from the column was 4.73 g (29.4%) of 4-*O*-oleyl-coumarin: mp 32–34 °C (from *n*-heptane). Product **23** eluted second (263 mg, 1.6%) and was obtained as a white solid: mp 76–77 °C (from *n*-heptane). ^1H NMR ($CDCl_3$) δ 0.87 (d, 3H, CH_3), 1.20–1.34 (m, 22H, $11CH_2$), 1.60 (m, 2H, CH_2), 1.67 (s, 2H, CH_2), 1.98 (m, 2H, CH_2), 2.63 (t, 2H, α-CH_2), 5.30–5.36 (m, 2H, HC=CH), 7.29–7.32 (m, 2H, 2CH), 7.52 (m, 1H, CH), 7.89 (dd, 1H, CH); HRMS [EI] 412.29762 $C_{27}H_{40}O_3$ ($M+H^+$) requires 412.29775.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.01.014.

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