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Synthesis of 8-geranyloxypsoralen analogues and their evaluation as inhibitors of CYP3A4

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Abstract—Furanocoumarins have been shown to inhibit CYP3A4 in vitro with varying degrees of potency [*Pharmacogenetics* **1997**, *7*, 391–396; *Chem. Res. Toxicol.* **1998**, *11*, 252–259; *Drug Metab. Dispos.* **1997**, *25*, 1228–1233; *Br. J. Pharmacol.* **2000**, *130*, 1369–1377]. In this study, we report the effects of a series of novel furanocoumarins based on the naturally occurring derivative 8-geranylepoxypsoralen which has been shown to be a more potent inhibitor of CYP3A4 than its 5-position-substituted counterpart bergamottin [*Drug Metab. Dispos.* **2000**, *28*, 766–771; *Jpn. J. Pharmacol.* **2000**, *82*, 122–129]. Compounds were designed, synthesised and tested for their ability to inhibit CYP3A4 activity in human liver microsomes using testosterone as the marker substrate. Both the saturated and unsaturated phenolic furanocoumarin derivatives were found to be inactive. However, the 8-alkyloxy-furanocoumarin analogues were shown to inhibit CYP3A4 activity in a dose dependent manner, with IC_{50} values ranging from 0.78 ± 0.11 to 3.93 ± 0.53 μM. The reduced furan derivative dihydro-8-geranyloxypsoralen showed a 4-fold decrease in inhibitory potency, suggesting that the furan moiety plays a role in the interaction between these compounds and CYP3A4.

1. Introduction

Grapefruit juice has been found to cause a marked increase in the oral bioavailability of many therapeutic agents,[1–10] in some cases leading to toxicity. Such interactions are believed to result from the mechanism-based inhibition of cytochrome P450 3A4 (CYP3A4) activity in the intestine, by furanocoumarin compounds. 8-Geranyloxypsoralen (**1**) is a naturally occurring furanocoumarin and has been shown to be a more potent inhibitor of CYP3A4 than its 5-substituted counterpart bergamottin (**2**)[5,6] (Fig. 1). Possible hypotheses for this difference are (a) an increased affinity of the former for the enzyme or (b) decreased steric hindrance at the active site of CYP3A4, thus increasing the rate of formation of the mechanism-based enzyme–inhibitor complex.

To evaluate the potency of inhibition of CYP3A4 activity of other furanocoumarins substituted at the 8-position, we have synthesised a series of analogues (Fig. 2) comparable to ones synthesised previously and tested in the 5-substituted geranyloxy series (data submitted for publication).

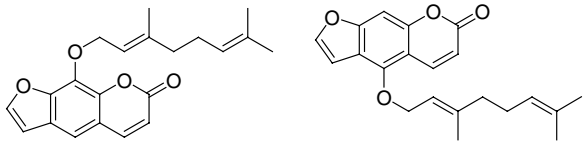

Figure 1. 8-Geranyloxypsoralen (**1**) and bergamottin (**2**).

We have investigated two possible sites of interaction with CYP3A4, namely (1) the 6′,7′-position of the geranyloxy chain and (2) the unsaturated furan ring. A series of furanocoumarins with differing functionalities along the geranyloxy side chain were synthesised (Fig. 2, compounds **1**, **5**–**7**) and tested to allow the role of hydrogen bonding in the interaction with CYP3A4 to be examined (Fig. 3(A)). The inactivation of the enzyme by bergamottin monomer and dimer analogues is postulated to occur at the unsaturated 2,3 bond (Fig. 3(B)) in the furan system.[2] It has been proposed that a 2,3-epoxide intermediate is formed, which reacts and covalently

Keywords: Furanocoumarins; Grapefruit juice; Cytochrome P450; CYP3A4.
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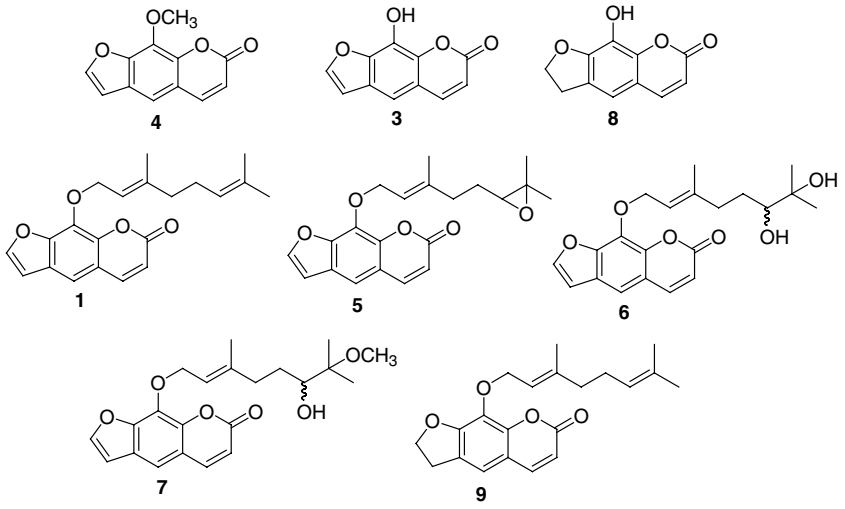


Figure 2. Furanocoumarin synthesised in the 8-geranyloxy series.

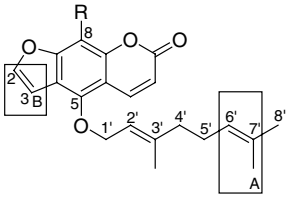


Figure 3. Proposed sites of interaction between furanocoumarins and CYP3A4, where area A highlights the 6′,7′-position along the geranyloxy chain and area B indicates the 2,3-position of the furan ring where epoxidation is believed to occur.

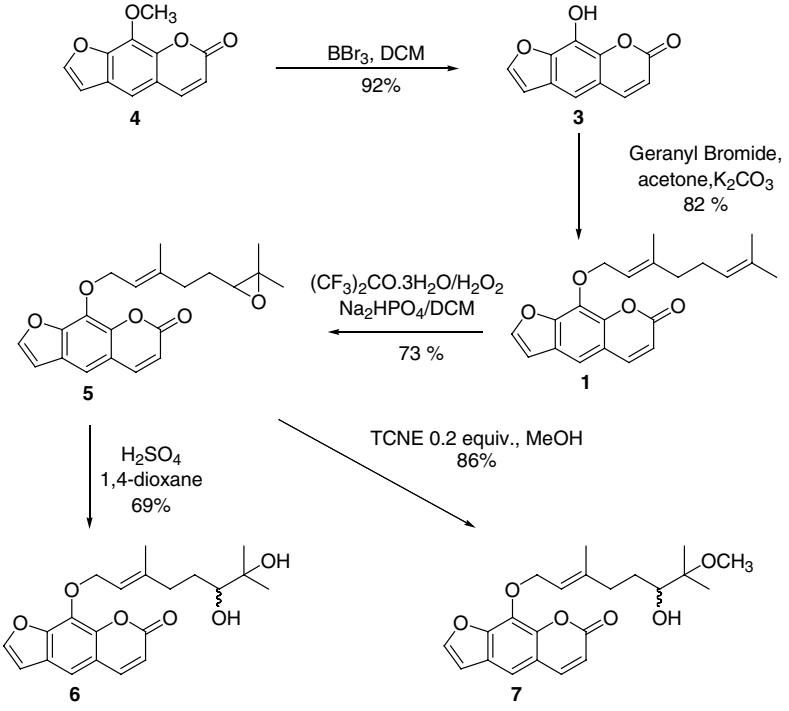
binds to the active site of the enzyme. To evaluate this hypothesis, we have synthesised and tested two reduced furan analogues (Fig. 2, compounds **8** and **9**).

2. Results

2.1. Synthetic approach

Synthesis of xanthotoxol (**3**) was achieved by the demethylation of the commercially available starting material xanthotoxin (**4**). Treatment with an excess of boron tribromide[11] afforded the desired phenol in excellent yields (Scheme 1).

The synthesis of 8-geranyloxypsoralen (**1**) was performed by alkylation of the xanthotoxol (**3**) with geranyl bromide under basic conditions, affording the desired compound in good yields (Scheme 1). 8-Geranyloxypsoralen also provided a good intermediate for the synthesis



Scheme 1. Synthetic routes employed in the synthesis of 8-geranyloxypsoralen (**1**) and analogues from xanthotoxin (**4**).

of a number of the other furanocoumarins. The synthesis of 6′,7′-epoxy-8-geranyloxypsoralen (8-EGP, **5**) was initially carried out by treatment of 8-geranyloxypsoralen (**1**) with *m*-chloroperoxybenzoic acid.[12] However, this resulted in the formation of the desired epoxide (8-EGP, **5**) in low yields, and of by-products, a bis-epoxide (**10**) (Fig. 4) and the hydrolysed phenol xanthotoxol (**3**). It was thought that the high acidity of *m*-chloroperoxybenzoic acid was causing hydrolysis of the labile ether linkage, and that a procedure that could selectively epoxidise the terminal double bond under basic conditions would be more successful. Thus, conditions previously used in the selective epoxidation of 3,20-bis-ethylenedioxy-17α-hydroxy-19-norpregna-5(10),9(11)-diene[13] were employed. Hexafluoroacetone trihydrate, hydrogen peroxide, and disodium hydrogenphosphate were stirred vigorously in dichloromethane at −10 °C for 30 min to generate the active dioxirane species. After addition of 8-geranyloxypsoralen (**1**), the reaction mixture was allowed to warm to room temperature and left stirring overnight (Scheme 1). Selective epoxidation of the geranyl terminal double bond was observed with no formation of the hydrolysis by-product xanthotoxol (**3**). Purification afforded 6′,7′-epoxy-8-geranyloxypsoralen (**5**) in good yields.

Like the epoxide (**5**), preparation of 6′,7′-dihydroxy-8-geranyloxypsoralen (8-DOHGP, **6**) was initially carried out under acidic conditions,[12] which again resulted in a low yield of the desired product and a high yield of the hydrolysed phenolic by-product (**3**). A number of dilute acids (1M) were then tested, and sulfuric acid was found to mediate the formation of the diol (**6**), producing only a small quantity of the phenolic by-product (**3**). Treatment of the epoxide (**5**) with a catalytic amount of dilute sulfuric acid was scaled up, affording 8-DOHGP (**6**) in good yields (Scheme 1). After purification by column chromatography, 8-DOHGP (**6**) was obtained as a colourless oil. In a previous study[14], this compound had been produced as a solid with a melting point of 92–94 °C. However, a number of unsuccessful attempts were made to crystallise the compound, which was identified as the desired product from other analytical data. We prepared the corresponding hydroxy-ether (**7**) by ring opening of 8-EGP (**5**) using camphor sulfonic acid in methanol, thus introducing the ether linkage at the more substituted position. When employing these conditions, problems of acid hydrolysis of the ether substituent at the 8-position were again encountered, leading to the formation of xanthotoxol (**3**). A mild catalyst for the methanolysis of epoxides that would leave acid-labile functions intact was required, and tetracyanoethylene

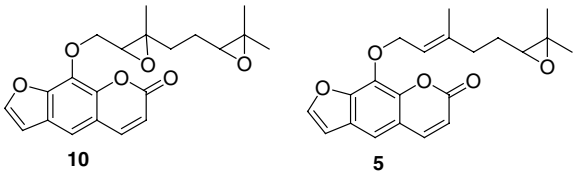
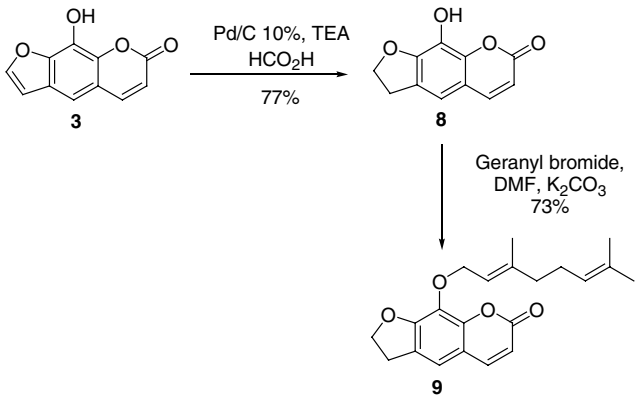


Figure 4. The desired mono epoxide (8-EGP, **5**) and the bis-epoxide by-product (**10**) obtained from the epoxidation of 8-geranyloxypsoralen (**1**) using *m*-chloroperoxybenzoic acid.



Scheme 2. Synthesis of the 2,3-dihydroderivaties **8** and **9**.

(TCNE) was used for this purpose.[15,16] Treatment of 8-EGP (**5**) with a catalytic amount of TCNE in methanol gave 6′-hydroxy-7′-methoxy-8-geranyloxypsoralen (**7**) (Scheme 1) with the highly regioselective introduction of the methoxy group at the more substituted oxirane carbon affording the secondary alcohol. ^{1}H NMR analysis with D_2O shake saw the disappearance of the peak at 2.48 and the simplification of the multiplet at 3.39–3.33 to a doublet of doublets at 3.26 (J 2.5, 9.9), and confirmed the presence of dicyanoketene dimethyl acetal ($(CN)_2C{=}C(OCH_3)_2$), as a singlet around δ 4.1. Removal of this by-product, formed during the methanolysis of TCNE, proved difficult by column chromatography due to the close running nature of the analytes. When the reaction was scaled up, the product could be purified by recrystallisation rather than chromatography, affording a 16% increase in yield. The activation of the C–O oxirane bond by alcohols is thought to be initiated by single electron transfer to the π-system of TCNE from the oxygen of the epoxide.[16]

The reduction of the unsaturated furan derivative xanthotoxol (**3**) using both transfer hydrogenation and catalytic hydrogenation[17,18] methods at low and high pressure was investigated. Initially the unreactive nature of these furans towards catalytic hydrogenation was thought to be due to the limited solubility of the starting material in a number of solvents. However, high yields of marmesins have been obtained by transfer hydrogenation with Pd/C, formic acid and triethylamine.[19] Using this procedure, dihydro-8-hydroxypsoralen (8-DHOH, **8**) was obtained in good yields (77%) (Scheme 2). The reaction time was kept as short as possible to prevent hydrogenation of the unsaturated lactone, which was detected after 15 min. Subsequent alkylation afforded the dihydro-8-geranyloxypsoralen derivative (**9**) (Scheme 2).

2.2. Inhibition of CYP3A4

With the exception of xanthotoxol (**3**) the furanocoumarin monomers showed a dose-dependent inhibition of the formation of 6β-hydroxytestosterone from testosterone (Table 1). Thus, the absence of the alkyl chain from the 8-geranyloxy structure resulted in a loss of activity with IC_{50} values >100 μM. The 8-geranyloxy series

Table 1. IC_{50} values for the inhibition of CYP3A activity in human liver (HL7) for the furanocoumarin derivatives (**1** and **3**–**9**) and bergamottin (**2**)

Inhibitor	IC_{50} (μM)
Xanthotoxol (**3**)	>100
Xanthotoxin (**4**)	NR
8-GP (**1**)	3.93 ± 0.53
8-EGP (**5**)	0.78 ± 0.11
8-DOHGP (**6**)	1.89 ± 0.31
8-OHOMeGP (**7**)	0.92 ± 0.03
8-DHOH (**8**)	>100
8-DHGP (**9**)	15.6 ± 0.5
Bergamottin (**2**)	4.48 ± 0.42

No results (NR) were obtained for xanthotoxin (**4**) because it co-eluted with the 6β-hydroxytestosterone metabolite peak. Values are means ± SD from three experiments.

showed an increase in potency with the addition of more polar substituents at the 6′,7′-position on the side chain. The epoxide (8-EGP, **5**) and the hydroxy-ether (8-OHOMeGP, **7**) derivatives were approximately twice as potent as 6′,7′-dihydroxy-8-geranyloxypsoralen (**6**) and 8-geranyloxypsoralen (**1**). Similar potencies were noted for 8-geranyloxypsoralen (8-GP, **1**) and bergamottin (**2**). Saturation of the furan ring in dihydro-8-geranyloxypsoralen (8-DHGP) (**9**) resulted in a 4-fold decrease in potency compared to its unsaturated counterpart (**1**). An absence of significant inhibitory activity was observed for both the saturated and unsaturated compounds lacking the geranyl side chain.

The pharmacophore models[20] tested provided a good qualitative fit to the furanocoumarin monomers (**1** and **2**). The furanocoumarin ring system can interact with three of the four binding regions suggested by Ekins et al.,[20] with the third hydrophobe (Fig. 4) or hydrogen bond donor region (Fig. 5) lying immediately to the right of the ring system. Both 8-geranyloxypsoralen (**1**) and bergamottin (**2**) show a good fit with the two proposed hydrophobic regions situated near the central aromatic ring, and the alkyl chain with the hydrogen bond acceptor region positioned around the carbonyl group of the lactone (Fig. 5). Incorporation of these compounds into the model suggested for quinine (Fig. 6) indicates a good fit between the two proposed hydrophilic regions on the active site and the oxygen atoms within the lactone portion of the ring system. As with the other model, the alkyl linkage lies within a hydrophobic region.

3. Discussion

Similar potencies for the inhibition of CYP3A4 activity were observed between bergamottin (**2**) (geranyl sidechain attached at the 5-position) and 8-geranyloxypsoralen (**1**). This finding may suggest that both

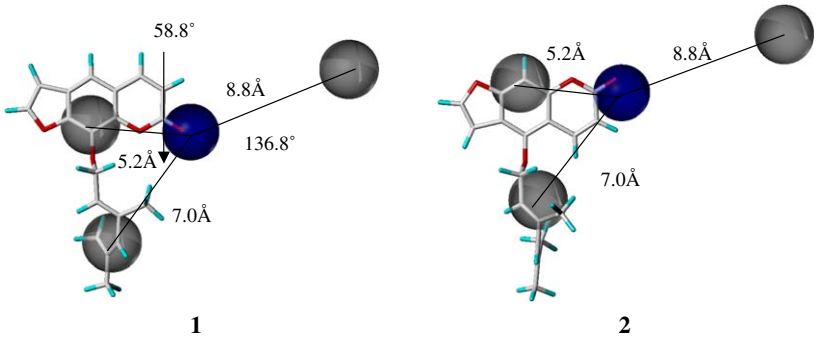


Figure 5. 8-Geranyloxypsoralen (**1**) and bergamottin (**2**) fitted by the CYP3A4 Catalyst K_i pharmacophore produced by Ekins et al.[20] from a published data set derived from the inhibition of midazolam 1-hydroxylation. The pharmacophore consists of hydrophobic regions highlighted in grey and hydrogen bond acceptor regions in blue. The inter-bond angles and distances between the pharmacophore features are inset.

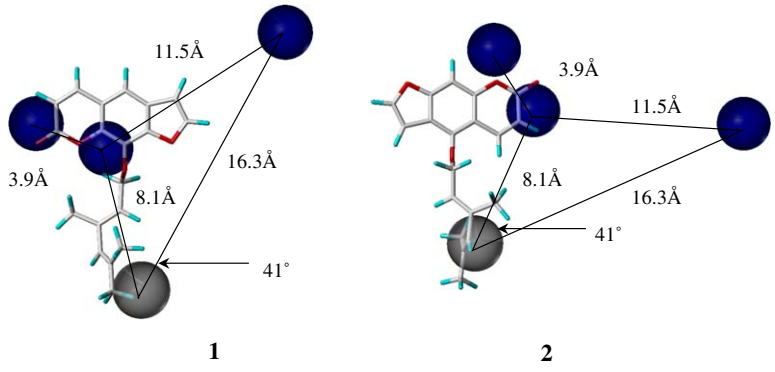


Figure 6. 8-Geranyloxypsoralen (**1**) and bergamottin (**2**) fitted by the CYP3A4 Catalyst K_i pharmacophore produced by Ekins et al.[20] from a published data set derived from the inhibition of quinine metabolism. The pharmacophore consists of hydrophobic regions highlighted in grey and hydrogen bond acceptor regions in blue. The inter-bond angles and distances between the pharmacophore features are inset.

compounds bind to the same, or a very similar, region of the enzyme active site and that the tricyclic ring system of each compound may be able to orientate differently, allowing enzymatic attack at the furan moiety to form a reactive intermediate.[2] The weak inhibition of CYP3A4 by the phenolic derivative xanthotoxol (**3**) suggests that the hydroxyl group, possibly through hydrogen bonding, might result in a change in orientation of the molecule to the extent that the furan double bond is no longer in the correct plane for enzymatic attack, and formation of the epoxide intermediate is now unfavourable. The requirement of an alkyl chain for binding to the enzyme is supported by the good fit of the two pharmacophore models to compounds 1 and 2. One common structural feature within the pharmacophore models appears to be the presence of a hydrophobic binding region along the alkyl chain. The data obtained suggest that the presence of a hydrophobic substituent on the phenolic oxygen at this region is essential for binding possibly through van der Waals forces and may affect the orientation of the ring system. The integration of these monomers into the pharmacophore models has given a tentative insight into the binding of the furanocoumarin ring system to the active site of CYP3A4.

Based on earlier work [reviewed in Ref. 2], reducing the furan ring of the furanocoumarins should prevent the formation of the epoxide and thus the inactivation of the enzyme. In support of this hypothesis, dihydro-8-geranyloxypsoralen (**9**) showed a 4-fold decrease in potency compared to 8-geranyloxypsoralen (**1**). Koenigs and Trager have shown that some dihydro-furanocoumarins are mechanism-based inhibitors of CYP2A6[21] and CYP2B1.[22] CYP2A6 was found to activate 8-dihydromethoxypsoralen to either the 2-hydroxylated or the 3-hydroxylated derivative. The latter can dehydrate to the unsaturated furanocoumarin xanthotoxin, which then undergoes epoxidation and ring opening to a 2′3-dihydroxylated derivative, resulting in CYP2A6 inactivation. Thus, both the mono- and the dihydroxylated metabolites of this compound contribute to enzyme inactivation.[21] We did not determine whether the dihydro-8-geranyloxy furanocoumarin (**9**) is a mechanism-based inhibitor of CYP3A4. However, it is clear that the mechanism of inhibition by these compounds is complex and may proceed by a mixture of competitive, non-competitive and mechanism-based inhibition pathways.

In conclusion, a series of 8-alkyloxyfuranocoumarin analogues were found to inhibit CYP3A4 activity in a dose-dependent manner and with moderate potency. Furthermore, our findings provide additional evidence that the furan moiety plays a role in the interaction between these compounds and CYP3A4.

4. Experimental

4.1. Chemistry

All chemicals were of analytical grade or of a higher purity and were purchased from Sigma–Aldrich Company Ltd (Gillingham, Dorset, UK), Fisher Scientific (Loughborough, UK), Roche Diagnostics Ltd (Lewes, UK), VWR International Ltd (Poole, UK), or were donated by SAFC Pharma (Manchester, UK). ^{1}H and ^{13}C NMR were recorded on Bruker AC 250 instrument. Chemical shifts (δ_H, δ_C) are reported in ppm and coupling constants (J) are in Hertz (Hz). Infra-red spectra were determined by direct sample analysis using a Bruker Goldengate ATR Vector 22 Spectrometer and are reported by wave numbers (cm^{-1}). Electron Impact (EI) and Chemical Ionisation (CI) mass spectrometry were carried out on a Micromass Prospec magnetic sector instrument by Sheffield University Mass Spectrometry Department or a Kratos Concept 1S instrument by Manchester University Mass Spectrometry Department. Melting points were determined on a Shardon scientific hot stage microscope apparatus and are quoted uncorrected in °C. Thin layer chromatography (TLC) was carried out on aluminium backed Merck Kiesgel plates, with detection by UV (254 nm) fluorescence. Chromatography was carried out using Merck Silica gel 60 (<63 μm) or Fisher Matrex 35–70 μm.

4.1.1. Xanthotoxol (3). Xanthotoxin (**4**) (5.98 g, 27.6 mmol) was dissolved in DCM (100 mL) and stirred under argon. BBr_3 (100 mL, 1 M in DCM 100 mmol) was added dropwise to the solution and the mixture was allowed to stir at room temperature for 4 h. The reaction mixture was poured slowly into a stirred solution of saturated aqueous sodium bicarbonate (400 mL) and allowed to stir for 1 h. The resulting colourless precipitate was recovered by filtration. The filtrate was neutralised with aq HCl (1 M) to pH 7, to yield further product, which was also recovered by filtration. Both solids were combined and stirred in water (100 mL) for 1 h. Recovery by filtration and drying under vacuum yielded the product as an off-white solid (5.13 g, 25.4 mmol, 92%). Mp 253–254 °C, lit.[23] 247 °C; υ_{max} cm^{-1} 3290, 1695, 1589; δ_H (d_6-acetone; 250 MHz) 8.03 (1H, d, $J = 9.6$ Hz), 7.93 (1H, d, $J = 2.2$ Hz), 7.44 (1H, s), 6.98 (1H, d $J = 2.2$ Hz), 6.33 (1H, d, $J = 9.6$ Hz); δ_C (d_6-acetone; 63 MHz); 160.4, 154.7, 147.9, 145.7, 140.6, 131.0, 126.5, 117.2, 114.8, 111.1, 107.7; *m/z* (EI) 202 (100%, M^+), Found (EI) 202.0265; $C_{11}H_6O_4$ requires 202.0266.

4.1.2. 8-Geranyloxypsoralen (1). Geranyl bromide (825 μL, 4.16 mmol) was added to a stirred mixture of 8-hydroxypsoralen (**3**) (840 mg, 4.16 mmol) and K_2CO_3 (574 mg, 4.16 mmol) in acetone (20 mL) under argon. The reaction mixture was stirred under reflux overnight (17 h). The reaction mixture was neutralised by addition of aqueous citric acid (10% w/v) before removal of acetone under reduced pressure. The resulting brown residue was dissolved in DCM (20 mL), washed with water (2 × 20 mL), brine (10 mL) and dried ($MgSO_4$). Removal of solvent yielded a brown oil, which was purified by column chromatography, eluting with ethyl acetate–hexane (1:4). After purification, translucent yellow oil was obtained which was crystallised by triturating with hexane and cooling. Recovery by filtration afforded colourless crystals of the title compound (**1**) (1.16 g, 3.43 mmol, 82%). Mp 53–54 °C, lit.[24]

53–54 °C; υ_{max} cm^{-1} 1706, 1585; δ_H ($CDCl_3$; 250 MHz) 7.78 (1H, d, J = 9.6, 7.71 Hz) (1H, d, J = 2.2 Hz), 7.38 (1H, s), 6.84 (1H, d, J = 2.2 Hz), 6.38 (1H, d, J = 9.6 Hz), 5.62 (1H, t, J = 7.2 Hz), 5.05 (3H, m), 2.03 (4H, d, J = 3.0 Hz), 1.71, 1.66, 1.58 (9H, 3× s); δ_C ($CDCl_3$; 63 MHz) 158.9, 147.1, 145.0, 142.7, 142.3, 141.5, 130.1, 129.9, 124.2, 122.1, 117.8, 114.8, 113.0, 111.6, 105.1, 68.4, 37.9, 24.7, 24.0, 16.0, 14.9; *m/z* (EI) 338 (4%, M^+), 202 (100%, $R_{Ar}OH$), found (EI) 338.1518; $C_{21}H_{22}O_4$ requires 338.1518.

4.1.3. 6′,7′-Epoxy-8-geranyloxypsoralen (8-EGP, 5). Hexafluoroacetone trihydrate (4.52 mL, 32.4 mmol) was added to a stirred suspension of H_2O_2 (3.31 mL, 30%, 32.4 mmol) and Na_2HPO_4 (63.22 g, 22.68 mmol) in DCM (5 mL) at room temperature. After 30 min, 8-geranyloxypsoralen (**1**) (5.47 g, 16.2 mmol) in DCM (20 mL) was added to the mixture. After 18 h, aqueous sodium sulfite (110 mL, 10% w/v) was added and stirring was continued for a further hour. The product was extracted with DCM (3 × 25 mL). The organic extracts were combined and the solvent was removed under reduced pressure. Purification by column chromatography, eluting with ethyl acetate–hexane (3:7) yielded a pale yellow oil, which afforded colourless crystals upon sonication in hexane (4.92 g, 13.9 mmol, 73%). Mp 79–81 °C; υ_{max} cm^{-1} 2959, 1713, 1584; δ_H ($CDCl_3$; 250 MHz) 7.78 (1H, d, J = 9.6 Hz), 7.71 (1H, d, J = 2.2 Hz), 7.38 (1H, s), 6.83 (1H, d, J = 2.2 Hz), 6.39 (1H, d, J = 9.6 Hz), 5.66 (1H, m), 5.05 (2H, d, J = 7.0 Hz), 2.66 (1H, t, J = 6.2 Hz), 2.34–2.10 (2H, m), 1.75 (3H, s), 1.60–1.52 (2H, m), 1.29, 1.26 (6H, 2× s); δ_C ($CDCl_3$; 63 MHz) 160.5, 148.6, 146.2, 144.4, 143.9, 142.1, 131.5, 125.8, 120.0, 116.4, 114.7, 113.3, 106.8, 69.9, 63.9, 58.4, 36.1, 27.1, 24.8, 18.7, 16.6; *m/z* (CI) 354 (10%, M^+), 203 (20%, $R_{Ar}OH+H$), found (CI) 354.1469; $C_{21}H_{22}O_5$ requires 354.1467.

4.1.4. 6′,7′-Dihydroxy-8-geranyloxypsoralen (6). Dilute sulfuric acid (5 μL, 1 M) was added to a stirred solution of **5** in 1,4-dioxane (10 mL) at room temperature. After 45 min, more acid (5 μL) was added and the reaction mixture was stirred for a further 30 min. On completion, the acid was neutralised by addition of a few drops of saturated aqueous sodium bicarbonate solution. The product was extracted with ethyl acetate (2 × 10 mL). The combined organic layers were washed with water and dried ($MgSO_4$). Removal of solvent under reduced pressure furnished a translucent oil. The product was purified by column chromatography eluting with ethyl acetate–hexane (7:3) yielding the desired product (**6**) as a translucent oil (72 mg, 0.19 mmol, 69%). υ_{max} cm^{-1} 3500, 3000, 1706, 1583; δ_H ($CDCl_3$; 250 MHz) 7.79 (1H, d, J = 9.6 Hz), 7.69 (1H, d, J = 2.2 Hz), 7.36 (1H, s), 6.81 (1H, d, J = 2.2 Hz), 6.35 (1H, d, J = 9.6 Hz), 5.65 (1H, m), 5.01 (2H, m), 3.30–3.23 (1H, m), 2.67 (1H, d, J = 4.5 Hz), 2.45 (1H, s), 2.26–2.04 (2H, m), 1.69 (3H, s), 1.56–1.31 (2H, m), 1.16, 1.12 (6H, 2 × s); δ_C ($CDCl_3$; 63 MHz) 159.1, 146.9, 145.0, 142.9, 142.1, 141.2, 129.8, 124.3, 118.3, 114.8, 112.9, 111.8, 105.1, 75.4, 71.3, 68.4, 34.8, 27.6, 24.9, 21.4, 14.7; *m/z* (EI) 372 (47%, M^+), 202 (24%, $R_{Ar}OH$), found (EI) 372.1568; $C_{21}H_{24}O_6$ requires 372.1573.

4.1.5. 6′-Hydroxy-7′-methoxy-8-geranyloxypsoralen (7). A solution of **5** (2 g, 5.64 mmol) and tetracyanoethylene (TCNE) (145 mg, 1.13 mmol, 0.2 equiv) in anhydrous methanol (200 mL) was stirred under argon at room temperature. After 1 h 30 min, the solvent was removed under reduced pressure to yield a translucent oil. The product was crystallised from ether:hexane (1:1) yielding off-white crystals (1.88 g, 4.87 mmol, 86%). Mp 110–112 °C; υ_{max} cm^{-1} 3434, 2926, 1720, 1584; δ_H ($CDCl_3$; 250 MHz) 7.79 (1H, d, J = 9.6 Hz), 7.71 (1H, d, J = 2.2 Hz), 7.38 (1H, s), 6.83 (1H, d, J = 2.2 Hz), 6.38 (1H, d, J = 9.6 Hz), 5.65 (1H, m), 5.05 (2H, d, J = 7.1 Hz), 3.39–3.33 (1H, m), 3.22 (3H, s), 2.48 (1H, m), 2.39–2.38 (1H, m), 2.14–2.01 (1H, m), 1.72 (3H, s), 1.46–1.31 (2H, m), 1.11, 1.08 (6H, 2 × s); D_2O shake saw the disappearance of the peak at 2.48 and the simplification of the multiplet at 3.39–3.33 to a doublet of doublets at 3.26 (J = 2.5, 9.9 Hz); δ_C ($CDCl_3$; 63 MHz) 160.5, 148.7, 146.6, 144.3, 143.9, 143.1, 131.5, 125.8, 119.6, 116.4, 114.6, 113.3, 106.7, 77.4, 76.1, 70.1, 49.0, 36.6, 29.3, 20.6, 18.8, 16.6; *m/z* (EI) 386 (5%, M^+), 202 (100%, $R_{Ar}OH$), found (EI) 386.1744; $C_{22}H_{26}O_6$ requires 386.1729.

4.1.6. Dihydro-8-hydroxypsoralen (8). Xanthotoxol (**3**) (600 mg, 2.97 mmol) was dissolved in acetone at 40 °C under argon. Pd/C (1.2 g, 10%), triethylamine (4.96 mL, 35.6 mmol) and formic acid (1.12 mL, 29.7 mmol) were added to the solution. After 15 min, ^1H NMR indicated that the reaction had gone to completion. The solution was filtered through Celite and removal of solvent yielded a dark oil. The residue was dissolved in DCM before washing with saturated aqueous sodium bicarbonate (15 mL), aqueous citric acid (15 mL, 10% v/v), water (15 mL) and brine (15 mL), and dried ($MgSO_4$). Removal of solvent under reduced pressure yielded a brown oil (520 mg), which was purified by column chromatography. Elution with ethyl acetate–hexane (3:7). The desired compound (**8**) was crystallised on the column and was removed by washing with ethyl acetate (100%). Removal of solvent under reduced pressure afforded cream crystals (464 mg, 2.27 mmol, 77%). Mp 201–202 °C, lit.[25] 202 °C; υ_{max} cm^{-1} 3371, 1677, 1612; δ_H (d_6-acetone; 250 MHz) 8.56 (1H, br s), 7.82 (1H, d, J = 9.5 Hz), 7.01 (1 H, d, J = 1.0 Hz), 6.14 (1H, d, J = 9.5 Hz), 4.69 (2H, t, J = 8.5 Hz), 3.29 (2H, dd, J = 1.0, 8.5 Hz); δ_C (d_6-acetone; 63 MHz); 160.7, 151.8, 145.3, 144.6, 129.8, 126.1, 115.0, 114.1, 112.3, 73.6, 29.8; *m/z* (EI) 204 (100%, M^+), 176 (67%, M-CO), found (EI) 204.0489; $C_{11}H_8O_4$ requires 204.0423.

4.1.7. Dihydro-8-geranyloxypsoralen (9). Geranyl bromide (244 μL, 1.23 mmol) was added to a stirred suspension of **8** (250 mg, 1.22 mmol) and K_2CO_3 (337 mg, 2.44 mmol) in DMF (10 mL) under argon. The mixture was heated to 80 °C for 3 h. The potassium carbonate was neutralised by addition of aqueous citric acid (10% w/v), before removal of the solvents under reduced pressure. The resulting residue was extracted into ethyl acetate (2 × 10 mL). The combined organic layers were washed with water, brine and dried ($MgSO_4$). Removal of the solvent under reduced pressure yielded a brown oil, which was purified by chromatography eluting with ethyl acetate–hexane (3:7). A translucent yellow oil was

obtained, which was crystallised from ether–hexane (1:1). Recovery by filtration afforded colourless crystals of the desired compound (303 mg, 0.89 mmol, 73%). Mp 49–50 °C; υ_{max} cm^{-1} 1714, 1614; δ_H ($CDCl_3$; 250 MHz) 7.58 (1H, d, $J = 9.5$ Hz), 6.97 (1H, s), 6.20 (1H, d, $J = 9.5$ Hz), 5.55 (1H, t, $J = 7.1$ Hz), 5.03 (1H, m), 4.80 (2H, d, $J = 7.1$ Hz), 4.74 (2H, t, $J = 8.7$ Hz), 3.3 (2H, t, $J = 8.7$ Hz), 2.04 (4H, bs), 1.69, 1.66 (9H, 3× s); δ_C ($CDCl_3$; 63 MHz) 160.9, 155.4, 148.2, 144.0, 142.6, 131.6, 130.3, 125.4, 123.9, 119.7, 117.4, 113.5, 112.2, 73.1, 69.4, 39.6, 29.2, 26.4, 25.6, 16.8, 16.5; *m/z* (EI) 340 (4%, M^+), 204 (100%, M-R_{alkyl}), found (EI) 340.1665; $C_{21}H_{24}O_4$ requires 340.1675.

4.2. Experimental procedures

4.2.1. Source and preparation of tissue samples. Human liver tissue (coded HL7) was obtained with written consent from a patient undergoing surgery for the removal of a hepatocellular tumour secondary to colon cancer. Macroscopically normal tissue close to the resection line was used. The work was approved by the South Sheffield Research Ethics Committee. Human liver microsomes were prepared as described previously.[26]

4.2.2. Incubation conditions. Human liver microsomes (0.2 mg/mL) were incubated with testosterone (37 μM) and the test compounds (0.5–100 μM) at 37 °C in the presence of KCl (1.15%), phosphate buffer (0.2 M, pH 7.4) and a NADPH generating system, for 10 min in a total volume of 1 mL. The reaction was terminated by the addition of ethyl acetate (2 mL). 16-α-Hydroxytestosterone (1.5 μg) or 11-β-hydroxytestosterone (1 μg) was added as the internal standard. Samples were gently mixed for 15 min, before centrifugation at 1500*g* for 15 min. The organic layer was evaporated to dryness under reduced pressure.

4.2.3. Determination of 6β-hydroxytestosterone by HPLC. Sample residues were reconstituted with mobile phase (150 μL) and an aliquot was injected onto the HPLC. A Hypersil C_8 BDS column (150 mm × 4.6 mm; 5 μm particle size) was used. The mobile phase was methanol–water (55:45 v/v) delivered at a flow rate of 1 mL/min. Eluants were detected by UV at 254 nm. The lower limit of determination of the assay was 30 pmol/mL, and the coefficient of variation at 328 pmol/mL was less than 5%.

4.2.4. Molecular modelling. The models were derived from the two Catalyst Hypotheses generated by Ekins et al.[20] based on the inhibition of midazolam 1-hydroxylation and of quinine metabolism. The pharmacophore models were fitted to 8-geranyloxypsoralen (**1**) and bergamottin (**2**) using Sybyl (v 7.0) software. The compounds were aligned by manual fitting of the hydrogen bond acceptor and the hydrophobic regions of the enzyme to 8-geranyloxypsoralen (**1**) and bergamottin (**2**).

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