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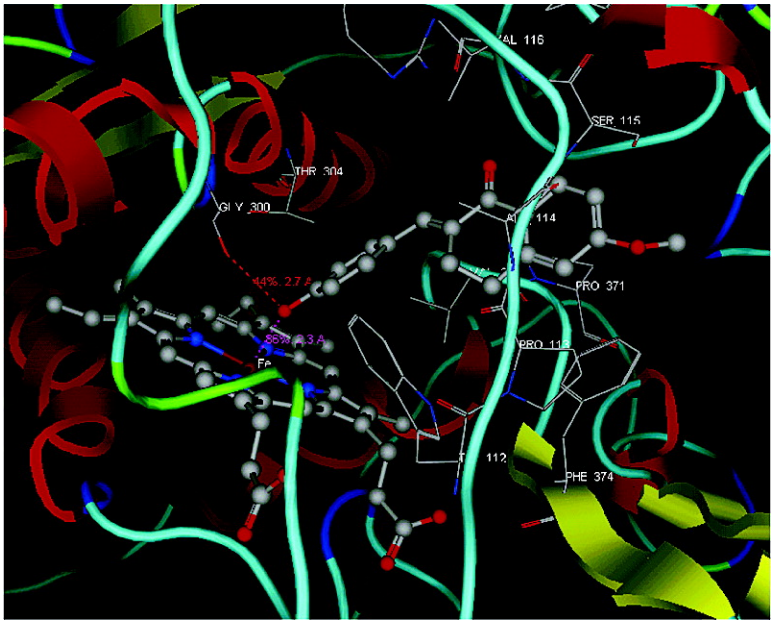

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Novel Tetralone-Derived Retinoic Acid Metabolism Blocking Agents: Synthesis and in Vitro Evaluation with Liver Microsomal and MCF-7 CYP26A1 Cell Assays

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Medicinal Chemistry and Tenovus Research Centre, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, U.K., School of Chemistry, Cardiff University, Cardiff CF10 3AT, U.K., and Department of Child Health, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, U.K.

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The potent inhibitory activity of novel 2-benzyltetralone and 2-benzylidenetetralone derivatives vs liver microsomal retinoic acid metabolizing enzymes and a MCF-7 CYP26A1 cell assay is described. In the liver microsomal assay, the 2-biphenylmethyl-6-hydroxytetralone derivatives **16a** and **16b** were found to be potent inhibitors (IC_{50} = 0.5 and 0.8 μM) compared with the broad spectrum P450 inhibitor ketoconazole and the retinoid mimetic R115866 (IC_{50} = 18.0 and 9.0 μM, respectively). In the MCF-7 CYP26A1 cell assay, the 2-(4-hydroxybenzyl)-6-methoxytetralone **5** and unsaturated benzylidene precursor **6** were found to be the most potent (IC_{50} = 7 and 5 μM, respectively), which was comparable with liarozole (7 μM) but considerably less active than R115866 (IC_{50} = 5 nM). With a CYP26A1 homology model, the tetralones were shown to be positioned in a hydrophobic tunnel with additional interactions, e.g., transition metal coordination and hydrogen-bonding interactions with GLY300, observed for the potent 4-hydroxyphenyl substituted inhibitors.

Introduction

All-*trans*-retinoic acid (atRA) is a naturally occurring retinoid responsible for growth and differentiation of mammalian epithelial tissues,[1] which exerts activity by binding to transcription-regulatory factors in the cell nucleus known as RAR (retinoic acid receptor) and RXR (retinoid X receptor), each having subtypes α, β, and δ.[2] Upon atRA binding the activated receptor transcriptionally regulates its target genes by binding to its response element retinoic acid response element (RARE) or retinoid X response element (RXRE) (Figure 1).[3,4] Retinoic acid has been used in a number of clinical situations, especially oncology and dermatology. In oncology, atRA has shown spectacular success in the treatment of acute promyelocytic leukaemia,[5,6] although the remission seen is followed by relapse within 4−6 months; this appears to be due to increased RA metabolism as a result of RA induction, leading to decreased clinical efficacy. atRA may improve the efficacy of other treatments such as radiation, cisplatin, and interferon therapies.[7,8] Retinoids have been used for some time in the treatment of psoriasis, cystic acne, and cutaneous malignancies due to hyperkeratinization as well as in the treatment of photodamaged skin.[9,10]

Vitamin A (retinol) is oxidized through retinal by dehydrogenases in the cytoplasm of target cells in low yields to all-*trans*-retinoic acid (Figure 1), which is at least 100-fold more active than retinol and accounts for its biological action. atRA has a short half-life (ca. 1 h), and its potency is reduced when it is administered systemically, due to metabolism by human liver and intestine cytochrome P450s to the inactive 4-hydroxy-RA and thence by dehydrogenases to the partially active 4-keto-RA and inactive polar metabolites (Figure 1).[11]

The specific P450s responsible for 4-hydroxylation of atRA in the human liver are CYP2C8 as a major contributor as well as 3A7, 3A5, 3A4, 2C9, and 1A1.[12] Several CYP isozymes from different rat tissues have been shown in vitro to be capable of metabolizing RA via 4-hydroxylation,[13] with RA metabolism by rat liver microsomes being mainly by the 1A1/2, 2A6, and 3A4 forms. However, in living tissues, atRA administration induces another RA-metabolizing enzyme, CYP26,[14] which recognizes only atRA as its substrate, and the expression of this isozyme can be induced by atRA both in vitro and in vivo.[15] Three members of the CYP26 family have now been identified: CYP26A1[14] and CYP26B1,[16] which metabolize atRA in the embryo and adult, and more recently,[17] CYP26C1, which may have a role in the specific metabolism of both all-trans and 9-cis isomers of RA.

An inhibitor of the metabolism of endogenous atRA would be expected to have a beneficial effect on epithelial differentiation and proliferation as an RA mimetic, with potential use as an agent for nonhormone-dependent cancers and various skin conditions. Importantly, such inhibitors should display selectivity for CYP26 because inhibition of hepatic enzymes, e.g., CYP2C8, 3A7, 3A5, 3A4, 2C9, and 1A1, may interfere with the metabolism of other endogenous compounds or drugs. A number of retinoic acid metabolism blocking agents (RAMBAs) have been described (Figure 2). The imidazoles, ketoconazole and liarozole, were reported as inhibitors of RA-metabolizing enzymes while being studied as inhibitors of 17α-hydroxylase: 17,20-lyase (P450 17α) as agents for the treatment of androgen-

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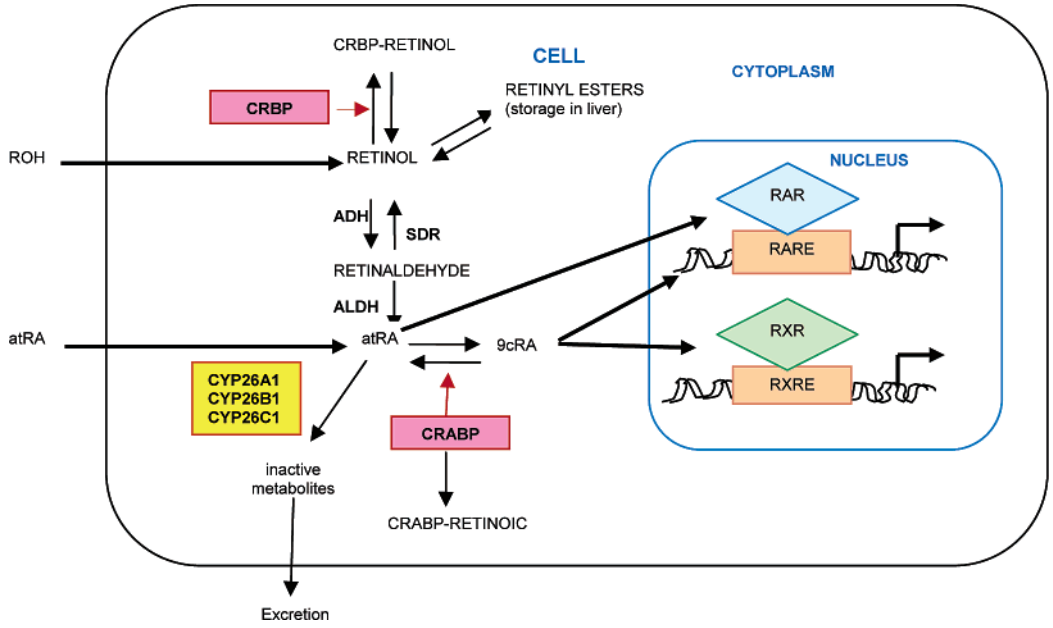


Figure 1. Alcohol dehydrogenases (ADH) and short-chain dehydrogenase/reductase catalyze the oxidation of retinol to retinaldehyde, which is subsequently oxidized by aldehyde dehydrogenases (ALDH) to retinoic acid. Isomers of retinoic acid, all-trans (atRA) and 9-cis (9cRA), are further metabolized by cytochrome P450 enzymes (including CYP26A1, B1, and C1) to inactive polar metabolites resulting in retinoid excretion. Intracelluar retinol can bind with cellular retinol-binding protein (CRBP) and retinoic acid with cellular retinoic acid-binding protein (CRABP). Retinoic acid binds to transcription-regulatory factors in the cell nucleus known as RAR (retinoic acid receptor) and RXR (retinoid X receptor).

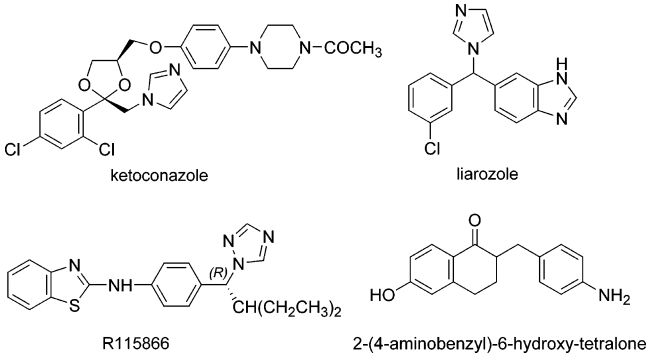


Figure 2. Retinoic acid metabolism blocking agents (RAMBAs).

dependent prostatic cancer by lowering testosterone levels.[11] Ketoconazole is not a suitable oral agent as an RA mimetic for sex-hormone-independent cancers because it inhibits several other P450 enzymes on the steroidogenic pathway of androgen synthesis and, furthermore, has a poor pharmacokinetics profile. Liarozole (Liazal) inhibits testicular (but not adrenal) P450 17 and is a potent inhibitor of aromatase ($P450_{AROM}$). Both these targets negate its potential as an oral RA mimetic for sex-hormone-independent cancers despite its effectiveness in clinical trials (oral administration) in psoriasis,[18] as well as icthyosis and hormone-resistant prostate cancer.[19,20] The triazole R115866 has been described as a novel inhibitor of CYP26 which, in vivo in rats after a single oral dose, increases endogenous tissue RA levels and mimics RA in several other of its biological actions.[15] R115866 has also shown beneficial effects when administered topically to skin.[15] A more recent derivative, R116010, has shown potent and selective inhibitory activity of retinoic acid metabolism in human T47D breast cancer cells.[21]

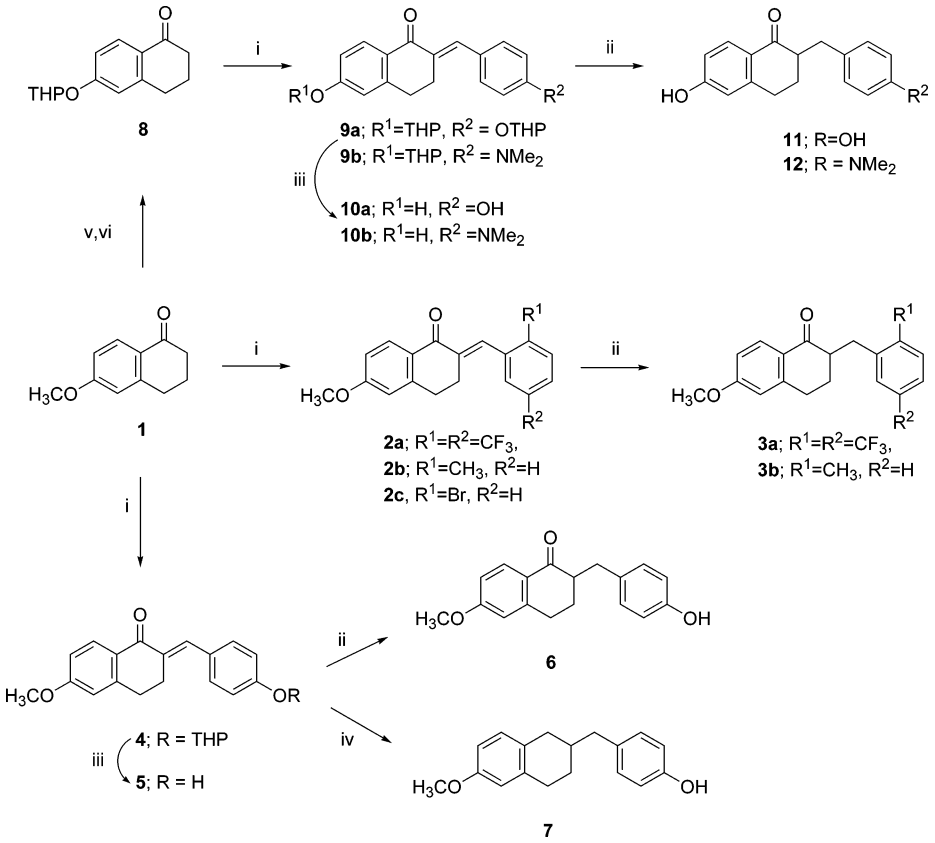
We have recently described several classes of RAMBAs in rat liver microsomal assays, other systems (human placenta and human liver microsomes, human skin homogenates), and RA-induced cell cultures (human male genital fibroblasts and HaCat cells).[22−24] The most potent inhibitor of retinoic acid metabolism was the 2-(4-aminobenzyl)-6-hydroxytetralone (Figure 2).[24,25] With this compound as a lead, a range of tetralone derivatives were prepared to probe requirements for optimal retinoic acid metabolism inhibitory activity using two in vitro assay systems: rat liver microsomes and human breast cancer MCF-7 cells expressing CYP26A1 activity.

Results

Chemistry. The method employed for the preparation of the 6-methoxy-2-(phenylmethylidene)-3,4-dihydro-2*H*-naphthalen-1-one precursors **2** involved direct condensation of the commercially available tetralone **1** with the appropriate benzaldehyde in ethanolic KOH solution.[26] This method was successfully used in the absence of a hydroxyl group on the tetralone or benzaldehyde (Scheme 1). The synthesis of hydroxyl derivatives (**5**, **10**, and **14**) required initial protection of one or both benzaldehyde and tetralone hydroxyl groups (Schemes 1 and 2) with the tetrahydropyran (THP) protecting group, which was stable under the basic ethanolic KOH condensation conditions.

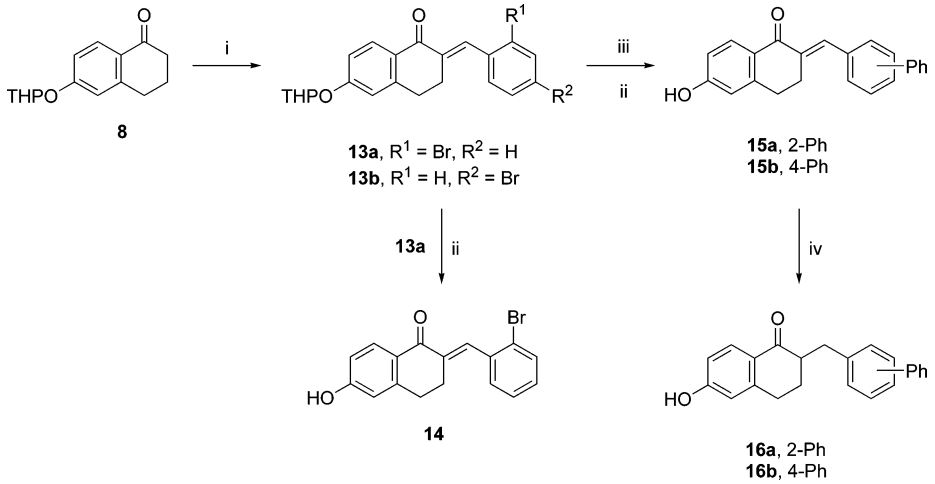
The 2-biphenyl substituted derivatives **15a**,**b** were prepared by Suzuki coupling with phenylboronic acid in the presence of $Pd(PPh_3)_4$ catalyst[27] (Scheme 2). The reduced 2-(benzyl)-3,4-dihydro-2*H*-naphthalen-1-one derivatives (**3a**, **3b**, **6**, **7**, **11**, **12**, **16a**, and **16b**) were readily obtained by hydrogenation with 10% Pd/C catalyst for 1 h at approximately 30 psi (Parr hydrogenator). When

Scheme 1[a]



[a] Reagents and conditions: (i) R−C_6H_4CHO or THPO−C_6H_4CHO, 4% KOH/EtOH, room temp, 1−72 h; (ii) 10% Pd/C, H_2, MeOH, room temp, 1 h; (iii) 2 M HCl(aq), EtOAc/2-butanone (1:1 v/v), reflux, 1 h; (iv) 10% Pd/C, H_2, MeOH, room temp, 2 h; (v) 48% HBr, reflux, 5 h; (vi) 3,4-dihydropyran, AcOH, *p*-TsOH, Et_2O, room temp, 3 h.

Scheme 2[a]



[a] Reagents and conditions: (i) Br−C_6H_4CHO, 4% KOH/EtOH, room temp, 1−4 h; (ii) 2 M HCl(aq), EtOAc/2-butanone (1:1 v/v), reflux, 1 h; (iii) phenylboronic acid, $Pd(PPh_3)_4$, toluene, 100 °C, 5 h; (iv) 10% Pd/C, H_2, MeOH, room temp, 1 h.

the hydrogenation reaction was allowed to proceed for 2 h, deoxygenation at C1 was found to occur (**7**, Scheme 1).

The 2-bromo derivatives **2c**, **13a**, and **14** gave an unexpected result in the ^1H NMR (300 MHz) spectra, with a singlet at δ 2.2−3 corresponding to the four CH_2 protons of 3,4-dihydronaphthalene. In all other compounds the four CH_2 protons were observed as two distinct multiplets at δ 2.5 and 3.0. To confirm that the 2-bromobenzaldehyde had reacted at the α-carbon rather than the (unlikely) β-carbon of the tetralone, ^1H HSQC and HMBC spectra (400 MHz) were obtained for the **2c** derivative. The HSQC spectrum confirmed that the broad singlet did correspond to the two CH_2 peaks at δ 27 and 29 in the ^{13}C NMR spectrum. The HMBC spectrum (used to determine the multiple-bond couplings over two or three bonds) indicated that (i) the CH_2 at δ 27 correlated with H-9, (ii) the CH_2 at δ 29 correlated to H-5, and (iii) the alkene proton (H-9) correlated to the C=O signal (Figure 3a). These finding all indicated that the 2-bromobenzaldehyde had reacted at the α-carbon of the tetralone. A crystal structure of

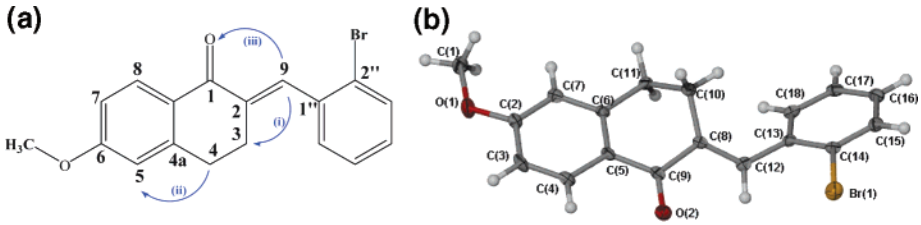


Figure 3. (a) NMR confirmed that (i) the CH_2 at δ 27 correlated with H-9, (ii) the CH_2 at δ 29 correlated to H-5, and (iii) the alkene proton (H-9) correlated to the C=O signal. (b) X-ray crystallographic structure of compound **2c**.

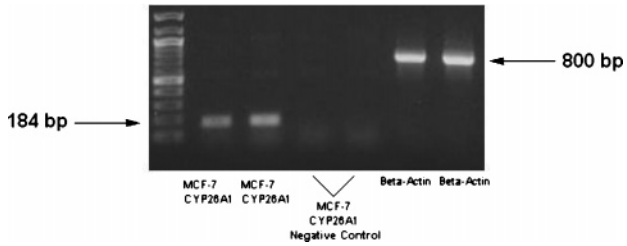


Figure 4. RT-PCR analysis of CYP26A1 mRNA expression after treatment with all-*trans*-retinoic acid (atRA). MCF-7 human breast cancer cells were treated with 10^{-7} M atRA for 9 h. Total RNA was isolated and subjected to RT-PCR. Amplified products were separated on an agarose gel containing ethidium bromide by electrophoresis.

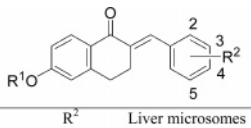
2-[1-(2-bromophenyl)methylidene]-6-methoxy-3,4-dihydronaphthalen-1-one (**2c**) confirmed the structure (Figure 3b). Therefore, we could conclude that the four protons from the two CH_2 atoms at carbons 3 and 4 appear as one broad singlet owing to accidental degeneracy.

Enzyme Inhibition. The 2-benzylidenetetralone (**2a**–**c**, **5**, **10a**,**b**, **14**, and **15a**,**b**) and 2-benzyltetralone (**3a**,**b**, **6**, **7**, **11**, **12**, and **16a**,**b**) derivatives were evaluated for their retinoic acid metabolism inhibitory activity using rat liver microsomes and a MCF-7 cell assay,[28,29] using radiolabeled [11,12-^{3}H]-all-*trans*-retinoic acid as the substrate and using ketoconazole, liarozole, and R115866 as standards for comparison.

The presence of CYP26A1 has not been confirmed in the MCF-7 cell line; therefore, prior to commencement of the MCF-7 cell assays, it was necessary to perform reverse transcriptase polymerase chain reaction (RT-PCR) analysis of CYP26A1 mRNA expression. This was performed after treatment of MCF-7 cells with 10^{-7} M atRA for 9 h (mirroring the assay induction conditions). After isolation of RNA and RT-PCR the amplified products were separated on a 1.5% (w/v) agarose gel containing ethidium bromide and separated at 100 V. UV illumination confirmed the presence of the 184 base pair fragment of CYP26A1 cDNA (Figure 4).

The standards were evaluated using the assay systems to determine comparison IC_{50} values. Ketoconazole had a similar profile in both assays (IC_{50}: liver microsomes, 18 μM; MCF-7 cells, 12 μM). Liarozole has previously been shown to induce atRA metabolism in rat liver microsomes,[15] a finding we also observed; however, in the MCF-7 cell assay inhibition of atRA metabolism does occur with IC_{50} activity determined as 7 μM. R115866 was not particularly active as an inhibitor of atRA metabolism in the liver microsomal assay (IC_{50} = 9 μM); however, in the MCF-7 assay it was a very potent RAMBA (IC_{50} = 5 nM). The data obtained for liarozole and R115866 were comparable

Table 1. IC_{50} Data for the Novel Benzylidene Tetralone Derivatives Using Rat Liver Microsomal and MCF-7 Assays



Compound	R^1	R^2	Liver microsomes IC_{50} (μM)[a]	MCF-7 cells IC_{50} (μM)[a]
2a	CH_3	2,5-diCF_3	> 20	50-100
2b	CH_3	2-CH_3	> 20	20-40
2c	CH_3	2-Br	> 20	9
5	CH_3	4-OH	> 20	7
10a	H	4-OH	> 20	9
10b	H	4-N(CH_3)$_2$	> 20	50-100
14	H	2-Br	2.4	20-40
15a	H	2-Ph	> 20	20-40
15b	H	4-Ph	> 20	50-100
Ketoconazole	-	-	18	12
Liarozole	-	-	Induction	7
R115866	-	-	9	0.005

[a] IC_{50} values are the average (±5%) of two experiments.

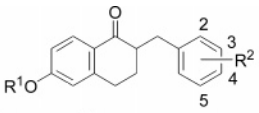
with the results obtained by Stoppie et al.[15] With the exception of the 2-bromo derivative, 2-[1-(2-bromophenyl)methylidene]-6-hydroxy-3,4-dihydro-*2H*-naphthalen-1-one (**14**, IC_{50} = 2.4 μM), the benzylidene derivatives were all poor inhibitors of retinoic acid metabolism (IC_{50} > 20 μM), using the liver microsomal assay (Table 1). Compound **14** did not retain activity in the MCF-7 assay; however, the corresponding 6-methoxybenzylidene derivative **2c** was comparable with liarozole (IC_{50}: **2c**, 9 μM; liarozole, 7 μM) in its inhibition of atRA metabolism. The 4-hydroxybenzylidene derivatives **5** and **10a** also displayed inhibitory activity (IC_{50}: **5**, 7 μM; **10a**, 9 μM) comparable with liarozole; however, **2c**, **5**, and **10a** were considerably less active than R115866 (IC_{50} = 5 nM).

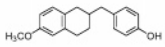
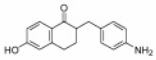
The 2-benzyl derivatives, in contrast to the 2-benzylidene derivatives, displayed potent inhibition of atRA metabolism in the liver microsomal assay (Table 2). The inhibitory activity observed using the liver microsomal assay was not observed with the MCF-7 CYP26A1 assay with the exception of the 4-hyroxy derivative, 2-(4-hydroxybenzyl)-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**6**) (IC_{50}: liver microsomes, 0.4 μM; MCF-7 cells, 5 μM). The previously[24] resolved enantiomers of the lead tetralone 2-(4-aminobenzyl)-6-hydroxytetralone were also evaluated and shown to display poor inhibitory activity in the MCF-7 assay (39 and 43 μM).

Discussion

The 2-benzyl derivatives displayed potent inhibitory activity of atRA metabolism using the rat liver microsomal assay. Inhibition of atRA metabolism in the MCF-7 assay was observed for only one of the 2-benzyl deriva-

Table 2. IC_{50} Data for the Novel Benzyltetralone Derivatives Using Rat Liver Microsomal and MCF-7 Assays



Compound	R^1	R^2	Liver microsomes IC_{50} (μM)[a]	MCF-7 cells IC_{50} (μM)[a]
3a	CH_3	2,5-diCF_3	18	50-100
3b	CH_3	2-CH_3	1.8	20-40
6	CH_3	4-OH	0.4	5
11	H	4-OH	1.5	20-40
12	H	4-N(CH_3)$_2$	1.2	25-50
16a	H	2-Ph	0.5	10-20
16b	H	4-Ph	0.8	10-20
7			2.2	25-50
		Enantiomer (-)	10.2[b]	39
		Enantiomer (+)	17.6[b]	43
Ketoconazole	-	-	18	12
Liarozole	-	-	Induction	7
R115866	-	-	9	0.005

[a] IC_{50} values are the average (±5%) of two experiments. [b] Data from ref 24.

tives, 2-(4-hydroxybenzyl)-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**6**), and three of the 2-benzylidene derivatives, 2-[1-(2-bromophenyl)methylidene]-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**2c**), 2-[1-(4-hydroxyphenyl)methylidene]-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**5**), and 6-hydroxy-2-[1-(4-hydroxyphenyl)methylidene]-3,4-dihydro-*2H*-naphthalen-1-one (**10a**); that is, inhibitory activity with the two assay systems did not generally correlate. The lack of activity of the 2-benzylidine derivatives in the rat microsomal assay may be related to a lack of flexibility or electronic effects resulting from the presence of an α,β-unsaturated ketone. Inhibition of atRA metabolism in the rat liver microsomal assay requires inhibition of the 1A1/2, 2A6, and 3A4 CYP isoforms. However, in the MCF-7 cell assay, expression of the RA-metabolizing enzyme CYP26A1 is induced by atRA (10^{-7} M); therefore, activity in the MCF-7 cell assay is more relevant, being a measure of human atRA metabolism by the specific CYP26A1 enzyme. The results obtained using both assays, particularly with reference to the potent CYP26 inhibitor R115866, would indicate that the liver microsomal assay is not a good model for human atRA metabolism. However, a comparison of results of the two assays can be used to consider selectivity. The inhibitory activity of the 4-hyroxy derivative (**6**) was retained (relative to standards) (IC_{50}: liver microsomes, 0.4 μM; MCF-7 cells, 5 μM) between the two assay systems, suggesting a broad spectrum of inhibitory activity for the CYP forms involved, including 1A1/2, 2A6, 3A4, and CYP26A1 isoforms. The benzylidene derivatives **2c**, **5**, and **10a** (IC_{50}: liver microsomes, >20 μM; MCF-7 cells, 7−9 μM) would appear to be more specific for CYP26A1 with minimal inhibition of the 1A1/2, 2A6, and 3A4 forms.

To rationalize the results obtained, FlexX[30] docking studies of all the tetralone derivatives were performed using a human CYP26A1 model,[31] built using the recently crystallized human CYP3A4[32] as a template. Docking interactions at the enzyme active site were shown to be comparable with atRA with the tetralones positioned in a hydrophobic tunnel. Additional interactions were noted for the benzylidene derivatives **5** and **10a** and the benzyl derivative **6** with coordinate binding with the heme and hydrogen bonding to GLY300 through the 4-hydroxyphenyl substituent. The 2-bromobenzylidene derivative **2c** interacted at the active site solely by hydrophobic interactions (Figure 5).

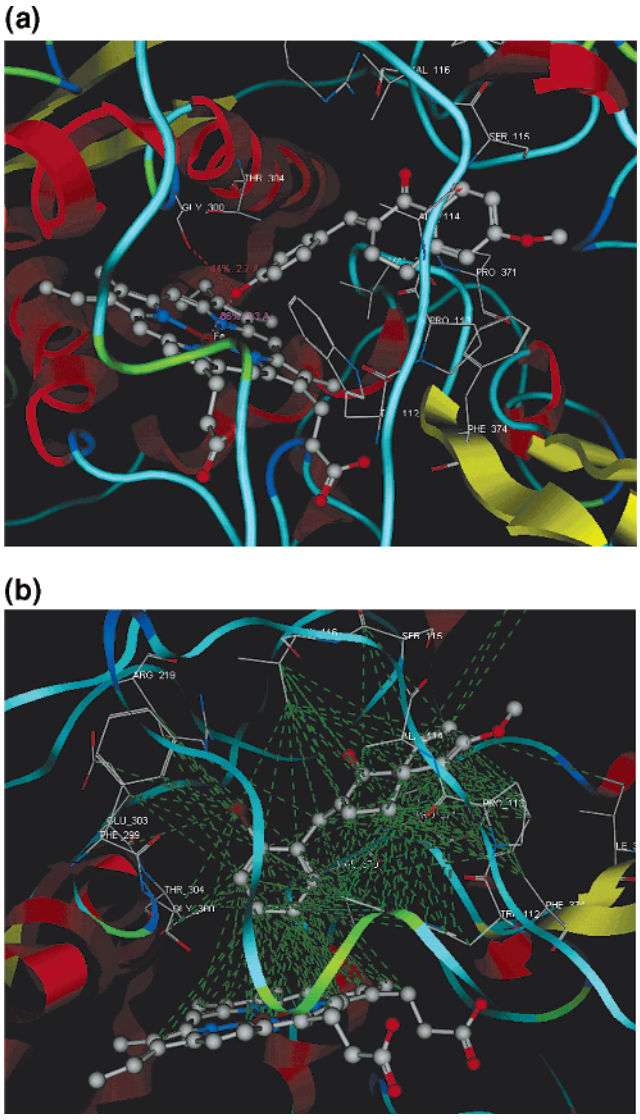


Figure 5. (a) Hydrogen (red) and transition metal (purple) interactions between the 4-hydroxyphenyl substituent of compound **5** and the active site of CYP26A1. (b) Hydrophobic (green lines) interactions between the 2-bromotetralone derivative **2c** and the active site of CYP26A1.

The enhanced activity of tetralones **5**, **6**, and **10a** may be due to the interaction of the 4-hydroxyphenyl substituent with GLY300 and the heme transition metal, resulting in enhanced binding. Interaction through the 4-hydroxy substituent may mimic the interaction of the 4-hydroxylated product of the natural atRA substrate. The activity of the 2-bromo derivative **2c** probably results from tighter binding through hydrophobic interactions and the increased size of **2c** due to the bulk of the bromo substituent, inducing a tighter fit at the active site.

Experimental Section

General Procedures. Phenol red-free RPMI-1640 medium, fetal calf serum (FCS), penicillin−streptomycin, and fungizone were purchased from Gibco Europe Ltd. (Paisley, U.K.). Tri Reagent was purchased from Sigma-Aldrich (MO).

[11,12-^{3}H]-All *trans*-retinoic acid (37 MBq/mL) was purchased from Perkin-Elmer Life Science (U.K.). All-*trans*-retinoic acid, NADPH, butylated hydroxyanisole, and ketoconazole were obtained from Sigma Chemical Company (U.K.). Acetic acid, ammonium acetate. and Optisafe 3 scintillation fluid were obtained from Fisher Scientific (U.K.). All solvents used for chromatography were HPLC grade from Fisher Scientific (U.K.).

^{1}H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX300 spectrometer operating at 300 and 75 MHz, with Me_4Si as internal standard. Mass spectra were determined by the EPSRC mass spectrometry center (Swansea, U.K.). Microanalyses were determined by Medac Ltd. (Surrey, U.K.). Flash column chromatography was performed with silica gel 60 (230−400 mesh) (Merck), and TLC was carried out on precoated silica plates (kiesel gel 60 F_{254}, BDH). Compounds were visualized by illumination under UV light (254 nm) or by the use of vanillin stain followed by charring on a hot plate. Melting points were determined on an electrothermal instrument and are uncorrected. All solvents were dried prior to use as described by the handbook Purification of Laboratory Chemicals[33] and stored over 4 Å molecular sieves under nitrogen.

The numbering of compounds for ^{1}H and ^{13}C NMR characterization and assignment of ^{1}H and ^{13}C NMR for all compounds is provided in the Supporting Information.

Enzyme Assays. Microsomal Assay for Metabolism of All-Trans Retinoic Acid. The assay performed was a modification of the general procedure previously described.[28] Tubes in duplicate, with a total volume of 500 μL, containing [11,-12-^{3}H]-all-*trans*-retinoic acid (10 μL, obtained from a stock mixture containing 15 μL of [11,12-^{3}H]-all *trans*-retinoic acid and 1 mL of 120 μM unlabeled all-*trans*-retinoic acid), inhibitor (10 μL, dissolved in acetonitrile), NADPH (4 mM, 50 μL), phosphate buffer (50 mM, pH 7.4, 410 μL), and rat liver microsomes (20 μL) were incubated in a shaking water bath for 30 min at 37 °C. The reaction was terminated by the addition of acetic acid (1% v/v, 100 μL). Then ethyl acetate (2 mL) containing 0.02% butylated hydroxylanisole was added, and the tubes were vortexed for 10 s. The tubes were left for 5 min at room temperature before removal of the organic layer (1.5 mL) from each tube and transfer to another set of tubes. The organic layer was then evaporated using a Christ centrifuge connected to a vacuum pump and a multitrap at −80 °C.

MCF-7 Cell Culture. Human MCF-7 breast cancer cells were cultured in phenol red-free RPMI 1640 medium supplemented with 5% (v/v) charcoal free fetal calf serum, antibiotics (penicillin and streptomycin), and fungizone at the same concentration of 10 iU/mL. Cells were grown in a humidified incubator (5% CO_2, 95% air) at 37 °C.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). MCF-7 cells were seeded in a 60 mm culture dish at a concentration of 1×10^6 cells in 3 mL of medium. Cells were allowed to adhere to plastic for 24 h. The cells were further incubated with 10^{-7} M all-*trans*-retinoic acid for 9 h before total cellular RNA was isolated by using Tri Reagent following the manufacturer's instructions. Total RNA amounts were quantified by measuring absorbance at 260 nm. The A_{260}/A_{280} nm absorption ratio was greater than 1.7. Denaturing agarose gel electrophoresis was performed to verify the integrity of RNA. The intensity of the 28S rRNA band was twice that of the 18S rRNA band stained by eithidium bromide. Total RNA was used to synthesize cDNA using the manufacturer's protocol provided with an AMV reverse transcriptase (Promega, U.K.) and performed in a thermocycler (Perkin-Elmer). Total RNA (2 μg) in the presence of 8 μL of $MgCl_2$ (25 mM), 2 μL of oligo(dT)$_{15}$ primer (500 ng/μL), 4 μL of reverse transcription 10× buffer, 4 μL of dNTP mix (10 mM), 1 μL of recombinant RNasin ribonuclease inhibitor, 1.6 μL of AMV reverse transcriptase, and nuclease-free water (to give a total reaction volume of 40 μL) was incubated at 42 °C for 60 min in a thermocycler. The newly synthesized cDNA was then used for PCR analysis.

To amplify a 184 base pair segment of CYP26A1 cDNA, primers were 5′-GCTGAAGAGTAAGGGTTTAC-3′ (sense) and 5′-CTTGGGAATCTGGTATCCAT-3′ (antisense).[21] β-Actin was used as the endogenous control. Amplification was performed using the PCR amplification kit by Promega (Promega, U.K.). The PCR reaction mixture contained 2.5 μL of cDNA, 2.5 μL of 10× reverse transcription 10× buffer, 0.25 μL of sense primer (100 μM), 0.25 μL of antisense primer (100 μM), 0.5 μL of dNTP mix (10 mM), 0.2 μL of Taq DNA polymerase (5 U/μL), and nuclease-free water (to give a total reaction volume of 25 μL). Taq DNA polymerase was activated at 94 °C for 5 min. The mixtures were then subjected to 30 cycles of amplification. Each cycle conditions were as follows: 94 °C for 1 min (denaturation), 55 °C for 1 min (annealing), and 72 °C for 2 min (elongation). An extra extension step was included (72 °C for 10 min) at the end of the incubation. Negative control of cDNA synthesis was carried out under the same experimental conditions but in the absence of AMV reverse transcriptase.

An amount of 20 μL of the PCR products was separated on a 1.5% (w/v) agarose gel containing ethidium bromide and separated at 100 V. The bands were visualized under UV light illumination.

MCF-7 (CYP26A1) Assay for Inhibition of Metabolism of atRA. MCF-7 cells were seeded in 12-well cell culture plates (Cornings Inc., New York) at 2.5×10^5 cells per well in a total volume of 1.5 mL. Cells were allowed to adhere to the well for 24 h. After 24 h, the medium from each well was removed, washed once with phosphate buffered saline (PBS), and replaced by fresh medium plus 10 μL of inhibitor/solvent (acetonitrile) and 10 μL of atRA (to give a final concentration of 1×10^{-7} M atRA and 0.1 μCi [11,12-^{3}H]-all-*trans* retinoic acid). The plates were foil-wrapped and incubated at 37 °C for 9 h. Each treatment was performed in duplicate. The incubation was stopped by addition of 1% acetic acid (100 μL/well), the medium was removed into separate glass tubes, and 200 μL of distilled water was added to each well. The cells were scraped off, and the contents were added to the appropriate glass tube. This procedure was repeated with a further 400 μL of water but without scraping. Ethyl acetate containing 0.05% (w/v) butylated hydroxyanisole (2×2 mL) was added to each tube. After vortexing for 15 s, the tubes were spun down at 3000 rpm for 15 min. The organic layer was then evaporated using a Christ centrifuge connected to a vacuum pump and a multitrap at −80 °C.

High-Performance Liquid Chromatography (HPLC). The HPLC system was equipped with a high-pressure pump (Milton-Roy pump) and an injector with a 50 μL loop connected to a β-RAM radioactivity detector connected to a Compaq computer running Laura data acquisition and analysis software. This enabled on-line detection and quantification of radioactive peaks. The HPLC column (10 μm C_{18} μBondapak, 3.9 mm × 300 mm, HPLC column from Waters, U.K.) operating at ambient temperature was used to separate the metabolites that were eluted with acetonitrile/1% ammonium acetate in water/acetic acid (75:25:0.1 v/v/v) at a flow rate of 1.9 mL/min. The Ecoscint was used as the flow scintillation fluid.

Chemistry. General Procedure for the Condensation of Tetralone (1 or 8) with Benzaldehyde (R−C_6H_4CHO or THPO−C_6H_4CHO) for Preparation of the Benzylidene Derivatives 2a, 2b, 2c, 9a, 9b, 13a, 13b. A mixture of the tetralone (**1** or **8**, 4 mmol) and benzaldehyde (R−C_6H_4CHO or THPO−C_6H_4CHO, 4 mmol) in 4% ethanolic KOH (10 mL) was stirred at room temperature for 1−4 h. The purification is described for each benzylidene product.

2-{1-[2,5-Di(trifluoromethyl)phenyl]methylidene}-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (2a). A mixture of 6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**1**) and 2,5-di(trifluoromethyl)benzaldehyde was stirred at room temperature for 1 h. The resulting white precipitate was collected,

washed with water, and finally recrystallized with acetone to give 2-{1-[2,5-di(trifluoromethyl)phenyl]methylidene}-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**2a**) as a white solid. Yield, 0.40 g (24%); mp 98−100 °C; R_f = 0.69 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{20}H_{14}F_6O_2$) C, H.

6-Methoxy-2-[1-(2-methylphenyl)methylidene]-3,4-dihydro-2*H*-naphthlalen-1-one (2b). A mixture of 6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**1**) and *O*-tolualdehyde was stirred at room temperature for 72 h. The resulting brown solution was evaporated in vacuo to yield a brown syrup. The crude product was extracted with CH_2Cl_2 (100 mL) and water (2 × 100 mL). The organic layer was dried with $MgSO_4$, filtered, and reduced in vacuo to give a brown residue. The brown residue was then purified by column chromatography (petroleum ether/ethyl acetate, 100:0 increasing to 87.5:12.5) to give 6-methoxy-2-[1-(2-methylphenyl)methylidene]-3,4-dihydro-*2H*-naphthlalen-1-one (**2b**) as a yellow solid. Yield, 1.57 g (71%); mp 88−90 °C; R_f = 0.65 (system petroleum ether/ethyl acetate 3:1). Anal. ($C_{19}H_{18}O_2$) C, H.

2-[1-(2-Bromophenyl)methylidene]-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (2c). A mixture of 6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**1**) and 2-bromobenzaldehyde was stirred at room temperature for 2 h. The resulting precipitate was collected, washed with water, and finally recrystallized from acetone to give 2-[1-(2-bromophenyl)methylidene]-6-methoxy-3,4-dihydro-*2H*-naphthalen-1-one (**2c**) as white crystals. Yield, 3.22 g (68%); mp 78−80 °C; R_f = 0.62 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{18}H_{15}BrO_2$) C, H.

6-(Tetrahydro-2*H*-2-pyranyloxy-2-{1-[4-(tetrahydro-2*H*-2-pyranyloxy)phenyl]methylidene}-3,4-dihydro-*2H*-naphthalen-1-one (9a). A mixture of 6-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-naphthalen-1-one (**8**) and 4-(tetrahydropyran-2-yloxy)benzaldehyde (see preparation of compound **4**) was stirred at room temperature for 1 h. The resulting precipitate was collected, washed with water, and finally purified by column chromatography (petroleum ether/ethyl acetate, 100:0 increasing to 87.5:12.5) to give the product (**9a**) as a colorless syrup. Yield, 1.00 g (41%); R_f = 0.51 (petroleum ether/ethyl acetate 4:1). HRMS (ES^+) Calcd for $C_{27}H_{30}O_5$ [M + H]$^+$ 435.2166. Found 435.2165.

2-{1-[4-(Dimethylamino)phenyl]methylidene}-6-(tetrahydro-2*H*-2-pyranyloxy)-3,4-dihydro-2*H*-naphthalen-1-one (9b). A mixture of 6-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-naphthalen-1-one (**8**) and 4-(dimethylamino)-benzaldehyde was stirred at room temperature for 72 h. The resulting brown solution was evaporated in vacuo to yield a brown syrup. The crude product was extracted with CH_2Cl_2 (100 mL) and water (2 × 100 mL). The organic layer was dried with $MgSO_4$, filtered, and reduced in vacuo to give a brown residue. The brown residue was then purified by column chromatography (dichloromethane/methanol, 100:0 increasing to 99:1) to give the product (**9b**) as an orange solid. Yield, 1.72 g (35%); mp 103−105 °C; R_f = 0.59 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{24}H_{27}NO_3$) C, H, N.

2-[1-(2-Bromophenyl)methylidene]-6-(tetrahydro-2*H*-2-pyranyloxy)-3,4-dihydro-2*H*-naphthalen-1-one (13a). A mixture of 6-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-naphthalen-1-one (**8**) and 2-bromobenzaldehyde was stirred at room temperature for 4 h. The resulting precipitate was collected, washed with water, and finally recrystallized from acetone to give 2-[1-(2-bromophenyl)methylidene]-6-(tetrahydro-2*H*-2-pyranyloxy)-3,4-dihydro-2*H*-naphthalene-1-one (**13a**) as a white solid. Yield, 7.60 g (87%); mp 76−78 °C; R_f = 0.66 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{22}H_{21}BrO_3 \cdot 0.5H_2O$) C, H.

2-(4-Bromobenzylidene)-6-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-naphthalen-1-one (13b). A mixture of 6-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-naphthalen-1-one (**8**) and 4-bromobenzaldehyde was stirred at room temperature for 1 h. The resulting precipitate was collected, washed with water, and finally recrystallized from ethanol to give 2-(4-bromobenzylidene)-6-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-naphthalen-1-one (**13b**) as a white solid. Yield, 4.55 g (51%); mp 165−167 °C [lit. mp[24] 166−168 °C]. Analytical data, including assignment of ^1H and ^{13}C NMR, are provided in Supporting Information.

6-Methoxy-2-{1-[4-(tetrahydro-2*H*-2-pyranyloxy)phenyl]-methylidene}-3,4-dihydro-2*H*-naphthalen-1-one (4). A mixture of 4-hydroxybenzaldehyde (5.0 g, 40.94 mmol), 3,4-dihydro-2*H*-pyran (8.27 g, 98.26 mmol), acetic anhydride (1 mL), and *p*-toluenesulfonic acid (50 mg) in diethyl ether (150 mL) was stirred at room temperature for 2 h. The resulting light-brown suspension turned into a clear-brown solution on stirring at room temperature. The organic layer was then washed with 2% KOH (2 × 100 mL) and water (2 × 100 mL), dried with $MgSO_4$, filtered, and reduced in vacuo to give a yellow residue. Purification by column chromatography (petroleum ether/ethyl acetate, 100:0 increasing to 87.5:12.5) gave 4-(tetrahydropyran-2-yloxy)benzaldehyde as a colorless syrup. Yield, 6.37 g (76%).

A mixture of the 6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**1**) (3.46 g, 19.64 mmol) and 4-(tetrahydropyran-2-yloxy)-benzaldehyde (4.05 g, 19.64 mmol) in 4% ethanolic KOH (80 mL) was stirred at room temperature for 12 h. The resulting precipitate was collected, washed with water, and finally recrystallized with acetone to give the product (**4**) as a light-brown solid. Yield, 3.00 g (42%); mp 100−102 °C; R_f = 0.47 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{23}H_{24}O_4$) C, H.

2-[1-(4-Hydroxyphenyl)methylidene]-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (5). Aqueous hydrochloric acid (2 M, 15 mL) was added to a solution of 6-methoxy-2-{1-[4-(tetrahydro-2*H*-2-pyranyloxy)phenyl]methylidene}-3,4-dihydro-2*H*-naphthalen-1-one (**4**) (2 g, 5.49 mmol), in 2-butanone/ethyl acetate (1:1 v/v, 40 mL), and the mixture was stirred at 100 °C for 1 h. The yellow solution was evaporated in vacuo, and the resulting yellow solid was washed with water/methanol (2:1 v/v, 40 mL) to give 2-[1-(4-hydroxyphenyl)-methylidene]-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**5**) as a yellow powder. Yield, 1.39 g (90%); mp 172−174 °C; R_f = 0.22 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{18}H_{16}O_3 \cdot 0.5H_2O$) C, H.

General Procedure for the Suzuki Coupling Preparation of the Benzylidene Derivatives 15a and 15b. A 2 M aqueous Na_2CO_3 (13.0 mL) sample was added to a solution of **13a** or **13b** (3.63 mmol) in toluene (40 mL). The mixture was bubbled with nitrogen for 1 min, and then $Pd(PPh_3)_4$ (0.18 mmol) was added to the mixture. Phenylboronic acid (7.26 mmol) in ethanol (10 mL) was added to the above mixture, and the mixture was refluxed at 100 °C for 5 h. After the reaction was complete, the residual borane was oxidized by the addition of H_2O_2 (30%, 3.5 mL) at room temperature for 1 h. The crude product was extracted with CH_2Cl_2 (100 mL) and water (3 × 100 mL). The organic layer was dried with $MgSO_4$, filtered, and reduced in vacuo to give an oily residue. Purification by flash column chromatography (petroleum ether/ethyl acetate 87.5:12.5; dichloromethane/methanol 99:1) gave 2-biphenyl-2-yl- and 2-biphenyl-4-ylmethylene-6-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-naphthalen-1-ones in 87% and 50% yield, respectively. Full analytical data is provided in Supporting Information.

Aqueous hydrochloric acid (2 M, 15 mL) was added to a solution of the biphenyl-2-ylmethylene-6-(tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-naphthalen-1-one (2.92 mmol) in 2-butanone/ethyl acetate (1:1 v/v, 40 mL), and the mixture was stirred at 100 °C for 1 h. The yellow solution was evaporated in vacuo, and the resulting yellow solid was washed with water/methanol (2:1 v/v, 40 mL) to give 2-biphenyl-2-yl- and 2-biphenyl-4-yl-methylene-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (**15a** and **15b**) as a yellow solid in yields of 91% and 95%, respectively.

2-Biphenyl-2-ylmethylene-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (15a). Mp 221−223 °C; R_f = 0.05 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{23}H_{18}O_2$) C, H.

2-Biphenyl-4-ylmethylene-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (15b). Yield, 0.38 g (95%); mp 258−260 °C; R_f = 0.05 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{23}H_{18}O_2$) C, H.

General Procedure for the Reductive Hydrogenation of Benzylidene Derivatives for the Preparation of Benzyl Derivatives 3a, 3b, 6, 11, 12, 16a, 16b. A mixture of benzylidene (2 mmol) and 10% palladium on charcoal (56 mg) in methanol (200 mL) was shaken in an atmosphere of hydrogen at room temperature for 1 h. Palladium was removed by filtration through a bed of Celite, and the filtrate was concentrated in vacuo. The purification is described for each benzyl derivative.

2-[2,5-Di(trifluoromethyl)benzyl]-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (3a). Purification by column chromatography (petroleum ether/ethyl acetate, 100:0 increasing to 87.5:12.5) gave a white residue with two close spots by TLC. The impure compound was further purified using preparative TLC to give 2-[2,5-di(trifluoromethyl)benzyl]-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**3a**) as a white solid. Yield, 19 mg (6%); mp 48−50 °C; R_f = 0.72 (petroleum ether/ ethyl acetate 1:1). HRMS (ES^+) Calcd for $C_{20}H_{16}F_6O_2$ $[M + H]^+$ 403.1127. Found 403.1121.

6-Methoxy-2-(2-methylbenzyl)-3,4-dihydro-2*H*-naphthalen-1-one (3b). Purification by column chromatography (petroleum ether/ethyl acetate, 100:0 increasing to 80:20) gave the product (**3b**) as a yellow syrup. Yield, 0.43 g (77%); R_f = 0.71 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{19}H_{20}O_2 \cdot 0.1H_2O$) C, H.

2-(4-Hydroxybenzyl)-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (6). Purification by trituration with acetone gave 2-(4-hydroxybenzyl)-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**6**) as a hygroscopic solid. Yield, 0.34 g (57%); R_f = 0.30 (petroleum ether/ethyl acetate 1:1). Anal. ($C_{18}H_{18}O_3 \cdot 0.3H_2O$) C, H.

6-(Hydroxy-2-(4-hydroxybenzyl)-3,4-dihydro-2*H*-naphthalen-1-one (11). Purification by column chromatography (dichloromethane/methanol, 100:0 increasing to 97:3) gave an off-white fluffy solid that was triturated with acetone to give 6-(hydroxy-2-(4-hydroxybenzyl)-3,4-dihydro-2*H*-naphthalen-1-one (**11**) as a light-brown solid. Yield, 30 mg (6%); mp 189−191 °C; R_f = 0.05 (petroleum ether/ethyl acetate 1:1). Anal. ($C_{17}H_{16}O_3$) C, H.

2-[4-(Dimethylamino)benzyl]-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (12). Purification by column chromatography (petroleum ether/ethyl acetate, 100:0 increasing to 50:50) gave a brown residue that was triturated with acetone to give 2-[4-(dimethylamino)benzyl]-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (**12**) as a brown solid. Yield, 42 mg (6%); mp 200−202 °C; R_f = 0.10 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{19}H_{21}NO_2 \cdot 0.1H_2O$) C, H, N.

2-Biphenyl-2-ylmethyl-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (16a). Purification by column chromatography (petroleum ether/ethyl acetate, 90:10 increasing to 65:35) gave the product 2-biphenyl-2-ylmethyl-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (**16a**) as a light-brown solid. Yield, 0.50 g (83%); mp 38−40 °C; R_f = 0.05 (petroleum ether/ethyl acetate 1:1). Anal. ($C_{23}H_{20}O_2 \cdot 0.3H_2O$) C, H.

2-Biphenyl-4-ylmethyl-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (16b). Purification by trituration with methanol gave 2-biphenyl-4-ylmethyl-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (**16b**) as a white solid. Yield, 0.20 g (63%); mp 220−222 °C; R_f = 0.32 (petroleum ether/ethyl acetate 1:1). Anal. ($C_{23}H_{20}O_2$) C, H.

4-[(6-Methoxy-3,4-dihydro-2*H*-naphthalenyl)methyl]-phenol (7). A mixture of 2-[1-(4-hydroxyphenyl)methylidene]-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**5**) (1.0 g, 3.56 mmol) and 10% palladium on charcoal (75 mg) in methanol (200 mL) was shaken in an atmosphere of hydrogen at room temperature for 2 h. Palladium was removed by filtration through a bed of Celite, and the filtrate was concentrated in vacuo to give a yellow residue that was purified by column chromatography (petroleum ether/ethyl acetate, 100:0 increasing to 70:30) to give 4-[(6-methoxy-3,4-dihydro-2*H*-naphthalenyl)methyl]phenol (**7**) as a white solid. Yield, 0.60 g (63%); mp 84−86 °C; R_f = 0.61 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{18}H_{20}O_2$) C, H.

6-(Tetrahydropyran-2-yloxy)-3,4-dihydro-2*H*-naphthalen-1-one (8). 8 was prepared as previously described from 6-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (**1**).[24]

6-Hydroxy-2-[1-(4-hydroxyphenyl)methylidene]-3,4-dihydro-2*H*-naphthalen-1-one (10a). Aqueous hydrochloric acid (2 M, 20 mL) was added to a solution of 6-(tetrahydro-2*H*-2-pyranyloxy-2-{1-[4-(tetrahydro-2*H*-2-pyranyloxy)phenyl]-methylidene}-3,4-dihydro-2*H*-naphthalen-1-one (**9a**) (2 g, 4.60 mmol) in 2-butanone/ethyl acetate (1/1 v/v, 40 mL), and the mixture was stirred at 100 °C for 1 h. The yellow solution was evaporated in vacuo, and the resulting yellow solid was washed with water/methanol (2:1 v/v, 40 mL) to give the product **10a** as a yellow powder. Yield, 0.67 g (55%); mp 260−262 °C; R_f = 0.05 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{17}H_{14}O_3.0.1H_2O$) C, H.

2-{1-[4-(Dimethylamino)phenyl]methylidene}-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (10b). Aqueous hydrochloric acid (2 M, 15 mL) was added to a solution of 2-{1-[4-(dimethylamino)phenyl]methylidene}-6-(tetrahydro-2*H*-2-pyranyloxy)-3,4-dihydro-2*H*-naphthalen-1-one (**9b**) (1.5 g, 3.97 mmol) in 2-butanone/ethyl acetate (1/1 v/v, 40 mL), and the mixture was stirred at 100 °C for 1 h. The yellow solution was evaporated in vacuo, and the resulting yellow solid was washed with water/methanol (2:1, 40 mL) to give 2-{1-[4-(dimethylamino)phenyl]methylidene}-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (**10b**) as a yellow solid. Yield, 0.82 g (70%); mp 210−212 °C; R_f = 0.05 (petroleum ether/ethyl acetate 3:1).

2-[1-(2-Bromophenyl)methylidene]-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (14). Aqueous hydrochloric acid (2 M, 15 mL) was added to a solution of 2-[1-(2-bromophenyl)-methylidene]-6-(tetrahydro-2*H*-2-pyranyloxy)-3,4-dihydro-2*H*-naphthalen-1-one (**13a**) (0.96 g, 2.42 mmol) in 2-butanone/ethyl acetate (1:1 v/v, 30 mL), and the mixture was stirred at 100 °C for 1 h. The yellow solution was evaporated in vacuo and the resulting yellow solid was washed with water/methanol (2:1 v/v, 40 mL) to give 2-[1-(2-bromophenyl)methylidene]-6-hydroxy-3,4-dihydro-2*H*-naphthalen-1-one (**14**) as a white solid. Yield, 0.60 g (63%); mp 178−180 °C; R_f = 0.20 (petroleum ether/ethyl acetate 3:1). Anal. ($C_{17}H_{13}BrO_2$) C, H.

Molecular Docking. Substrates were docked within the active site of the homology model (built using the CYP3A4 template) using the FlexX docking program of SYBYL.[30] Subsequent manipulation and interaction evaluation was performed with MOE (Molecular Operating Environment) software.[35]

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Supporting Information Available: X-ray crystallographic data for compound **2c**, the numbering of compounds for ^1H and ^{13}C NMR characterization, and assignment of ^1H and ^{13}C NMR for all compounds described. This material is available free of charge via the Internet at http://pubs.acs.org.

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