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Synthesis and CYP24 inhibitory activity of 2-substituted-3,4-dihydro-2*H*-naphthalen-1-one (tetralone) derivatives

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Abstract—The synthesis of novel 2-benzyl- and 2-benzylidene-3,4-dihydro-2*H*-naphthalen-1-one (tetralone) derivatives and their inhibitory activity versus kidney mitochondrial 25-hydroxyvitamin D_3 24-hydroxylase (CYP24) is described. The 2-benzylidenetetralone derivatives were found to be very weak inhibitors (IC_{50} 20 >100 μM), whereas the 2-benzyltetralone derivatives showed promising inhibitory activity (IC_{50} 0.9 μM for the most active derivative) compared with ketoconazole (IC_{50} 20 μM).

Prostate cancer, being the second leading cause of cancer death in human males, is a major disease for therapeutic intervention.[1] Androgens play an important role in the development, growth and progression of prostate cancer,[2] therefore androgen ablation therapy by gonadotropin suppression and androgen receptor blockade are current methods of treatment.[3] Although most patients respond well to this therapy, eventually many tumours recur as a result of transition of the cancer cells to androgen-independent growth.[4] Owing to the lack of effective treatments for androgen-independent metastatic prostate cancer, alternative strategies, such as 'differentiation therapy' may be useful.[5] Pro-differentiating agents of interest include retinoic acid (vitamin A) and vitamin D_3 (and their analogues).

1α,25$(OH)_2D_3$ (calcitriol) is the hormonally active metabolite of vitamin D_3, which functions as an antiproliferative and pro-differentiating agent, especially in epithelial and hematopoietic cells.[6] The use of vitamin D analogues (VDR agonists) as differentiating agents has been studied,[6] however the overall therapeutic activity of such analogues is uncertain owing to additional pharmacological effects such as transcaltachia (elevation of intracellular calcium activation of intestinal calcium uptake).[7] In addition the natural substrate calcitriol, and derivative VDR agonists, are rapidly metabolised into less active metabolites by the 24-hydroxylase (CYP24) resulting in a very limited use for either calcitriol or its derivatives as differentiating agents.[8] Therefore, compounds capable of inhibiting CYP24, the cytochrome P450 enzyme that initiates calcitriol metabolism, would have the effect of increasing endogenous levels of calcitriol so enhancing its differentiating capabilities.

Known inhibitors of CYP24 include (i) the CYP26 and CYP17 (P450 17, 17,20-lyase) inhibitor liarozole[9] and (ii) ketoconazole, the nonspecific competitive inhibitor of cytochrome P450-catalysed reactions, which inhibit both CYP24 and 1α-hydroxylase.[10] More selective CYP24 inhibitors have been described such as SDZ 89-443 and VID400.[11] All these inhibitors have a characteristic nitrogen heterocyclic moiety capable of coordinating to the Fe^{3+}-haem component of the P450 active site.

We have recently demonstrated the CYP26 inhibitory activity of a series of 2-(4-aminophenylmethyl)-tetralone derivatives,[12] therefore tetralones with varying substituents in both naphthalene and 2-aryl moieties were synthesised and evaluated for CYP24 inhibitory activity.

Keywords: 2-Substituted-3,4-dihydro-2*H*-naphthalen-1-one (tetralone) derivatives; Enzyme inhibition; 24-Hydroxylase (CYP24); Differentiating therapy.
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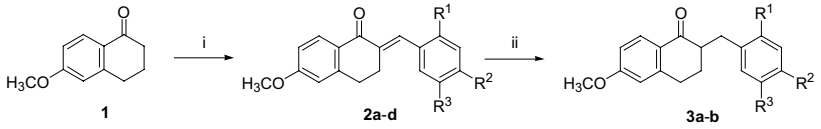
The method employed for the preparation of the 6-methoxy-2-(phenylmethylidene)-3,4-dihydro-2*H*-naphthalen-1-one derivatives (**2a**–**d**) involved direct condensation of the commercially available tetralone (**1**) with the appropriate benzaldehyde in ethanolic KOH solution.[13] This method was successfully used in the absence of a hydroxyl group on the tetralone or benzaldehyde (Scheme 1).

The synthesis of hydroxyl derivatives (**5**, **8** and **14**) required initial protection of one or both benzaldehyde and tetralone hydroxyl groups (see Schemes 2–4, respectively) with the tetrahydropyran (THP) protecting group, which was stable under the basic ethanolic KOH condensation conditions.

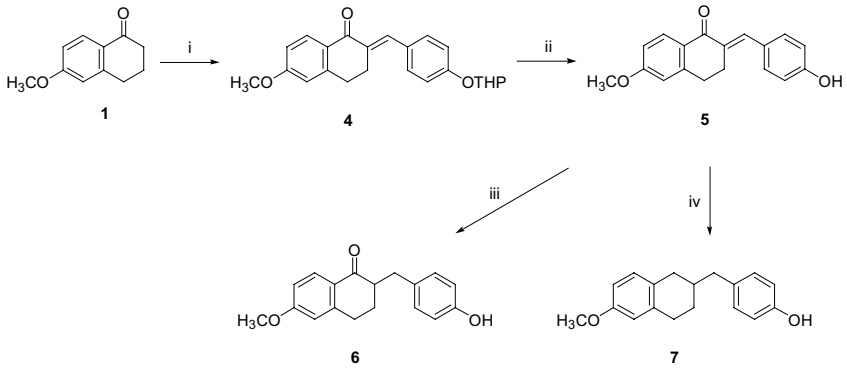
The 6-biphenyl substituted derivatives (**11a**–**b**) were prepared by Suzuki coupling with phenylboronic acid in the presence of $Pd(PPh_3)_4$ catalyst[14] (Scheme 3). The reduced 2-(benzyl)-3,4-dihydro-2*H*-naphthalen-1-one derivatives (**3a**–**b**, **6**, **9**, **12a**–**b** and **15**) were readily obtained by hydrogenation with 10% Pd/C catalyst for 1 h, at approximately 30 psi (Parr hydrogenator). When the hydrogenation reaction was allowed to proceed for 2 h, deoxygenation at C1 was found to occur (**7**, Scheme 2).

The dihydroxy derivative **15** was prepared from the corresponding THP protected tetralone and benzaldehyde (Scheme 4).

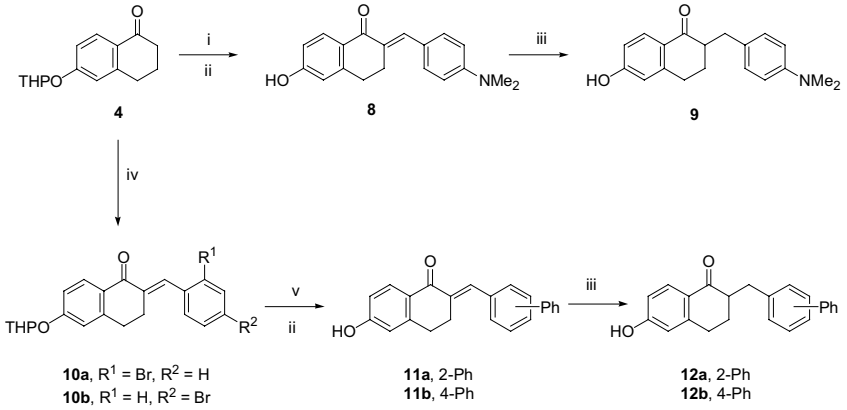
The nine 2-(benzylidene)-(**2a**–**d**, **5**, **8**, **11a**, **11b** and **14**) and eight 2-(benzyl)-tetralone (**3a**–**b**, **6**, **7**, **9**, **12a**–**b** and **15**) derivatives were evaluated for their inhibitory activity versus CYP24 using rat kidney mitochondria. The assay performed was based on a modification of the general



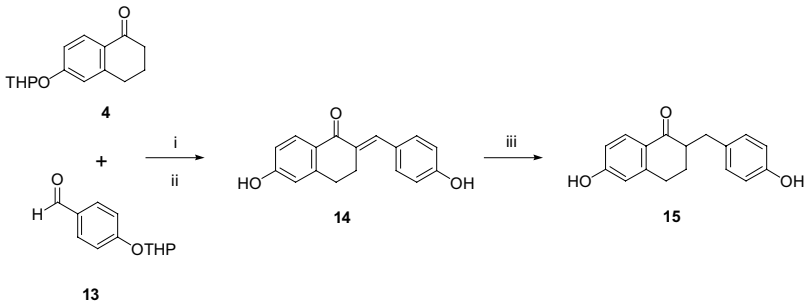
Scheme 1. Reagents and conditions: (i) R–C_6H_4CHO, 4% KOH/EtOH, rt, 1–72 h; (ii) 10% Pd/C, H_2, MeOH, rt, 1 h [**a**, $R^1 = R^3 = CF_3$, $R^2 = H$; **b**, $R^1 = CH_3$, $R^2 = R^3 = H$; **c**, $R^2 = NMe_2$, $R^1 = R^2 = H$; **d**, $R^1 = Br$, $R^2 = R^3 = H$].



Scheme 2. Reagents and conditions: (i) THPO–C_6H_4CHO, 4% KOH/EtOH, rt, 12 h; (ii) 2 M HCl (aq), EtOAc/2-butanone (1:1 v/v), reflux, 1 h; (iii) 10% Pd/C, H_2, MeOH, rt, 1 h; (iv) 10% Pd/C, H_2, MeOH, rt, 2 h.



Scheme 3. Reagents and conditions: (i) Me_2N–C_6H_4CHO, 4% KOH/EtOH, rt, 72 h; (ii) 2 M HCl (aq), EtOAc/2-butanone (1:1 v/v), reflux, 1 h; (iii) 10% Pd/C, H_2, MeOH, rt, 1 h; (iv) Br–C_6H_4CHO, 4% KOH/EtOH, rt, 1–4 h; (v) phenylboronic acid, $Pd(PPh_3)_4$, toluene, 100 °C, 5 h.



Scheme 4. Reagents and conditions: (i) 4% KOH/EtOH, rt, 1 h; (ii) 2 M HCl (aq), EtOAc/2-butanone (1:1 v/v), reflux, 1 h; (iii)10% Pd/C, H_2, MeOH, rt, 1 h.

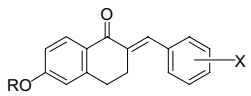
procedure previously described for CYP26,[15] using [26,27-methyl-^{3}H]-25-hydroxyvitamin D_3 (from a stock mixture containing 100 μL of [26,27-methyl-^{3}H]-25-hydroxyvitamin D_3 and 1 mL of unlabelled 25-hydroxyvitamin D_3 (25 μM), with a total of 5 μCi of radioactivity in the 1 mL of stock mixture), NADPH, inhibitor (varying concentrations using acetonitrile as solvent) and phosphate buffer (pH 7.4). After incubation in a shaking water bath for 30 min at 37 °C, the vitamin D metabolites were obtained by extraction with ethyl acetate. After evaporation of the organic solvent, the residue was analysed by a HPLC system connected to a β-RAM online scintillation detector, connected to a Compaq PC running Laura data acquisition and analysis software (Lab-Logic Ltd). The separated [^{3}H]-metabolites were quantitatively calculated from the areas under the curves. Using a control with acetonitrile instead of inhibitor, these results were expressed as 'percentage inhibition relative to control' = 100[metabolites (control)−metabolites (inhibitor)/(metabolites control)]%. Ketoconazole was used as a standard for comparison (Table 1).

The benzylidene derivatives were all poor inhibitors of CYP24, perhaps indicating the requirement for flexibility at the C2 position for optimal structure conformation with respect to interaction at the enzyme active site. In the 2-benzyltetralone series, introduction of an alkyl or aryl substituent at the 2-benzyl position, for example, 2-(2-methylbenzyl)- and 2-(2-biphenyl)-derivatives **3b** and **12a** (IC_{50} 0.9 and 2.1 μM, respectively), resulted in good activity, whereas introduction of an aryl substituent at the 4-benzyl position, for example, 2-(4-biphenyl)-derivative **12b** (IC_{50} >20 μM) substantially reduced activity. Introduction of a hydroxyl group at the 4-benzyl position was tolerated, for example **6** and **7** (IC_{50} 3.5 and 2.6 μM, respectively), however introduction of a more bulky moiety, for example, *N*,*N*-dimethyl in compound **9** (IC_{50} 18 μM) was not tolerated. These preliminary results suggest that the compounds may be orientated in the active site with a hydrophobic region or large pocket above the 2-position of the benzyl ring, and a small pocket containing an amino-acid residue capable of forming a hydrogen bond at the 4-benzyl position.

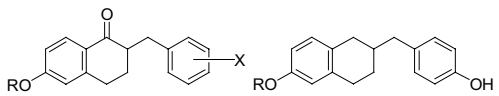
There would appear to be a slight preference for a 6-methoxy rather than a 6-hydroxy substitutent in the naphthalene ring (cf. **6** and **15**, IC_{50} 3.5 and 8.9 μM, respectively). However, no significant difference in inhibitory activity was noted for the tetralone and 1,2,3,4-tetrahydro-naphthalene structures (cf. **6** and **7**, IC_{50} 3.5 and 2.6 μM, respectively). Compared with ketoconazole, potent inhibitory activity was observed for the 2-(2-methylbenzyl)tetralone derivatives **3b**, which may

Table 1. IC_{50} data for the novel benzylidene and benzyl tetralone derivatives

Benzylidene tetralones



Benzyl tetralones



Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
2a	>100	**3a**	4.5
2b	>20	**3b**	0.9
2c	>100	**6**	3.5
2d	100	**7**	2.6
5	>20	**9**	18
8	>100	**12a**	2.1
11a	>100	**12b**	>20
11b	>100	**15**	8.9
14	>20	Ketoconazole	20

IC_{50} values are the mean of two experiments.

be a useful lead compound for further development of potent CYP24 inhibitors.

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