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Design, synthesis, and evaluation of 4-(4′-aminobenzyl)-2-oxazolidinones as novel inhibitors of the cytochrome P-450 enzyme aromatase

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Abstract

The synthesis of a series of N-alkylated 4-(4′aminobenzyl)-2-oxazolidinones is described using a synthetically useful scheme which avoids the use of phosgene—since the derivatization is undertaken with the oxazolidin-2-one ring intact. The compounds were tested for human placental aromatase (AR) inhibition in vitro, using [1β, 2β-^{3}H]androstenedione as substrate for the AR enzyme. The compounds were found, in general, to be more potent than the standard compound, aminoglutethimide (AG), and as such proved to be good lead compounds in the search for more specific AR inhibitors.

1. Introduction

The estrogen synthetase enzyme aromatase (AR) consists of a cytochrome P-450 hemeoprotein and an NADPH-cytochrome P-450 reductase and is involved in the

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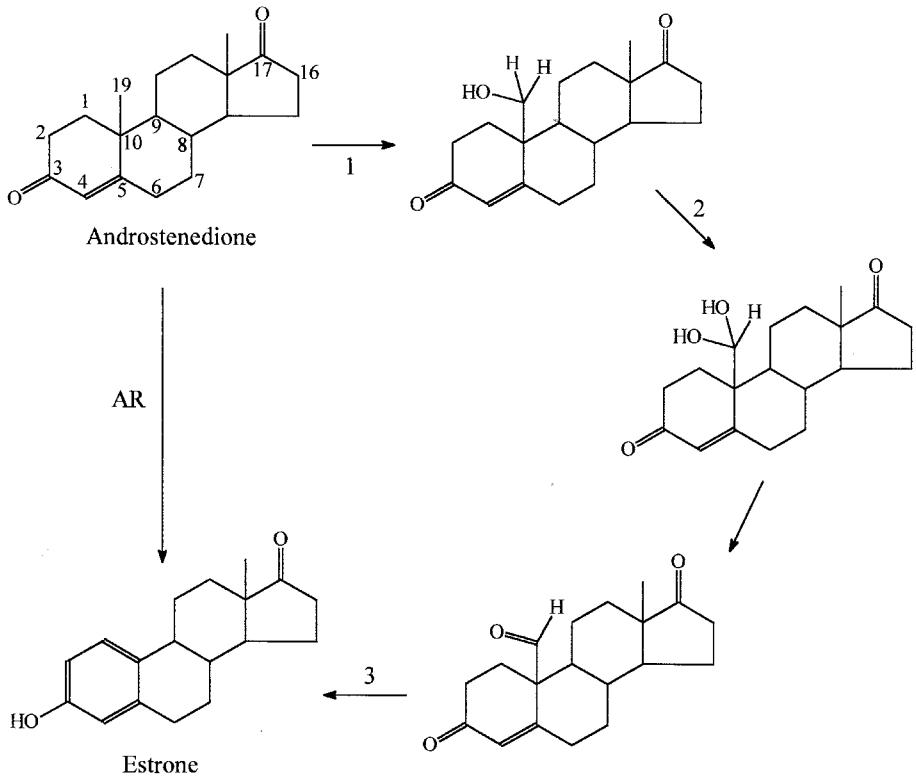
final step of the steroidal cascade. The exact nature of the mechanism of the conversion of the androgens (C_{19}) to the estrogens (C_{18}) (Scheme 1) is at present unclear, however, some aspects have been elucidated [1]. It has been shown that three sequential oxidative steps occur on the C(19) methyl group of the substrate, resulting in the loss of the methyl moiety as formic acid and the formation of the benzoid A-ring (aromatization).

Recent work on the mechanism of AR, and the general P-450 family of enzymes, has suggested that hydroxylations undertaken by the P-450 heme involve a ferroxy radical (Fe^{IV}–O·) in an overall oxygen rebound process [1]. That is, the ferroxy oxygen atom abstracts a hydrogen atom from the C(19) methyl of androstenedione, resulting in the production of the Fe^{IV}–OH and substrate $CH_2^{\cdot}$ radical, which is then neutralized by the abstraction of the hydroxyl from the Fe^{IV}–OH, resulting in the formation of Fe^{III} and the mono-hydroxylated steroid (Fig. 1). The process is repeated resulting in the production of the dihydroxy steroid which then undergoes non-enzymatic oxidation to the C(19) aldehyde. The final step involves the cleavage of the C(10)–C(19) bond due to attack by the P-450 heme, however, the nature of the attacking iron species is unclear. Although the experimental data have been utilized in proposing probable mechanisms for the aromatization reaction, the specific



Scheme 1. Aromatization of androstenedione.

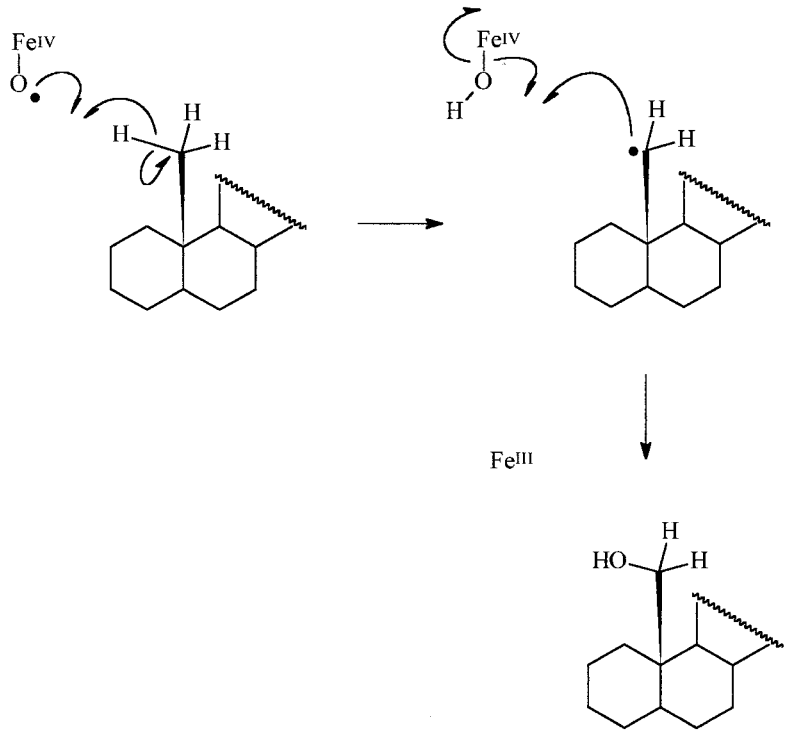


Fig. 1. Proposed mechanism for the hydroxylation steps in the aromatization of androstenedione.

mechanism has eluded us since the orientation (or position) of the different reacting species has not been considered as the exact structure of the active site is not clear.

The regulation of the enzymes involved in the biosynthesis of steroidal hormones has proved to be useful in the control of hormone-dependent cancers such as breast cancer [2]. In particular, AR inhibitors have been shown to be useful in the second line therapy of estrogen-dependent breast cancer, as such, steroidal and non-steroidal compounds have been synthesized as potential drugs. Non-steroidal AR inhibitors (Fig. 2) include liarazole (**1**), an azolyl-substituted benzimidazole, and the 1-[(benzofuran-2-yl)phenylmethyl]-imidazoles (**2**). Compounds based upon pyrrolidine-2,5-dione [such as **3**] and piperidine-2,6-dione [such as aminoglutethimide (**4**) and pyridoglutethimide (**5**)] also exist, but are less potent than **1** or **2**.

It was this latter series of compounds [**3**, **4**, and **5** (Fig. 2)] which prompted us to consider the oxazolidinone ring system within Evans' chiral auxiliary [3] (4-benzyl-oxazolidin-2-one) as a potential mimic for the steroid A ring within the natural substrate, androstenedione (AD), and therefore a possible inhibitor against P-450-dependent AR. That is, initial modelling, involving the superimpositioning of the two available enantiomers of 4-benzyl-oxazolidin-2-one onto AD [in a similar manner to Banting et al. [4] (1988)], suggested that the carbonyl group of this compound may be able to mimic the D-ring carbonyl moiety of the steroid backbone

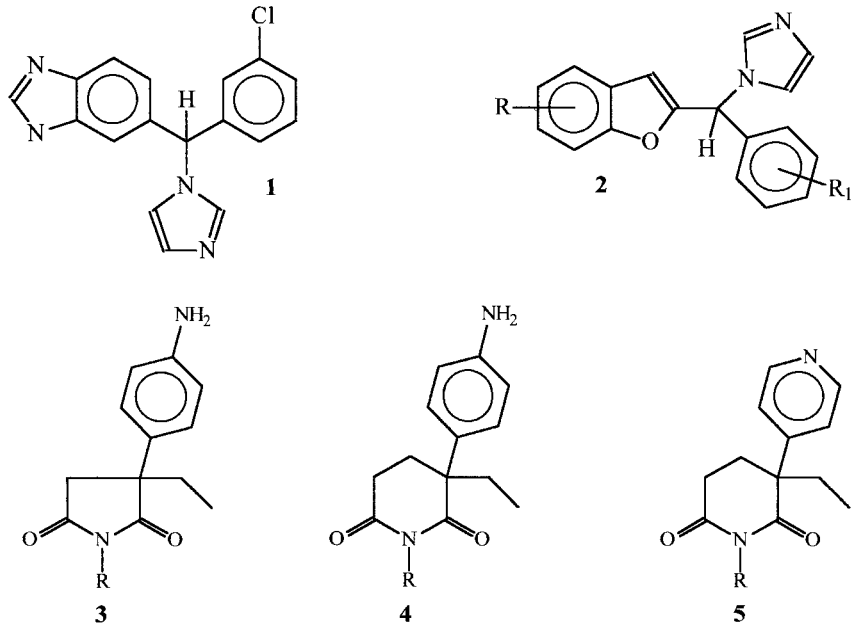


Fig. 2. A selection of non-steroidal inhibitors of AR.

whilst allowing the derivatized phenyl ring to bind in a Type II manner to the cytochrome P-450 heme (Fig. 3). That is, the derivatization of the phenyl ring with an amine group would allow the phenylamine nitrogen atom to form a dative covalent bond with the Fe atom of the P-450 heme. The oxazolidin-2-one ring was also observed to be a good mimic of the pyrrolidine-2,5-dione and piperidine-2,6-dione rings, in particular the C=O moieties (Fig. 3). Evans' chiral auxiliary has been

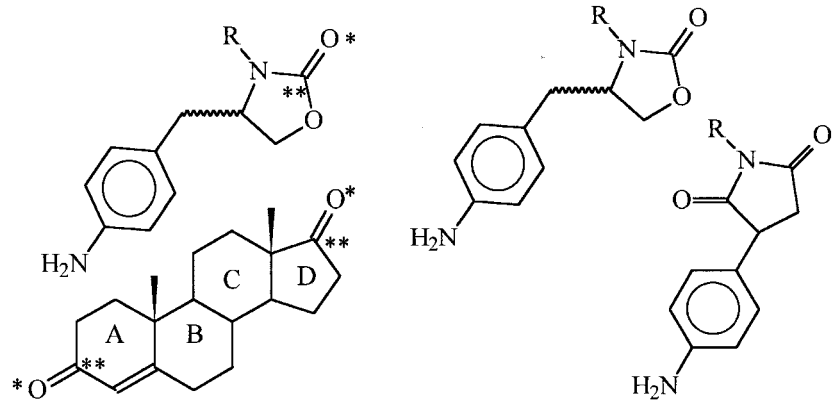


Fig. 3. Two dimensional consideration of the modes of superimpositioning of Evans' auxiliary onto AD and a derivative of **3** (the asterisks show two sets of atoms on the substrate and the atoms on the oxazolidineone ring used in the superimpositioning study).

extensively used in organic synthesis [5] and in particular stereochemical synthesis [6], however, its use as a potential enzyme inhibitor has been ignored. Indeed, the derivatization of the sterically important phenyl ring has not been considered extensively within the literature, instead work on the total synthesis of the chiral auxiliary with a 4-substituted functionality (such as an OH group) has been investigated using phosgene in the production of the oxazolidin-2-one ring [7].

In this report, we consider the modelling of 4-amino derivatives of Evans' chiral auxiliary (in designing the potential inhibitors), derivatization of the phenyl ring of this highly useful chiral auxiliary, and the biochemical evaluation of some of the synthesized compounds as potential anti-cancer compounds involving the inhibition of the cytochrome P-450 enzyme, AR. Furthermore, we provide a general and extremely useful scheme which may be modified so as to allow the synthesis of the highly desirable phenol derivative (via the diazotization of the amine), and which would therefore allow the chiral auxiliary to be attached to solid support—the synthetic scheme therefore does not require the synthesis of the oxazolidin-2-one ring system. To the authors' knowledge, this has not been previously reported, indeed, workers have utilized severe conditions (such as the use of phosgene) to produce alternative chiral auxiliaries to allow attachment to solid support for combinatorial chemistry.

2. Experimental

2.1. Molecular modelling

The construction of the substrate–heme complex (SHC) and the theoretical aspects of this novel approach have been discussed at length elsewhere [8–10] and as such, will not be considered here.

The proposed oxazolidin-2-one based inhibitors were constructed within the Alchemy III [11] molecular modelling software (using atoms/fragments/groups available within the Alchemy structure libraries). The completed molecules were then subjected to an initial minimization using the conjugate-gradient algorithm available within Alchemy until the gradient fell to below 10^{-6} (resulting, in general, in 500 or more iterations per structure). Conformational analysis was then performed on flexible parts of the inhibitors using Powersearch [11] (using the systematic search method with energy window of 5–10 kcal/mol and bond rotations of 30–60°). The low energy conformers produced were retained for further study.

The novel substrate–heme complex approach is based upon the mimicking of the inhibition process involving the binding of the inhibitor directly to the Fe atom of the P-450 heme. As such, the low energy conformers obtained were, in turn, bonded to the heme of the substrate–heme complex and the iron–phenylamine nitrogen bond rotated so as to obtain the distances between the carbonyl groups of the substrate–heme complex and the C(2)═O and C(5)═O of the inhibitor—it is presumed that the inhibitor's C═O group will mimic the substrate C═O and therefore hydrogen bond with the part of the active site which is proposed to bind the C(3)═O or C(17)═O of the substrate. The conformer displaying the smallest distance for each compound

was retained—the distance is considered to be inversely proportional to the strength of any interaction between the inhibitor and the active site.

2.2. Chemistry

The reagents used were either general purpose or analytical grade and were obtained from either Aldrich Chemical, Gillingham, Dorset; BDH, Poole, Dorset or Lancaster Synthesis, Morecombe, Lancs. Melting points were determined with an Electrothermal Instrument and infra-red spectra were determined using a Perkin–Elmer 681 FT-infrared spectrophotometer. ^{1}H (300 MHz) and ^{13}C NMR (75.5 MHz) were determined, using TMS as an internal standard, on a Brucker NMR spectrophotometer. Mass Spectra were determined by SERC Mass Spectrometry Centre, University of Wales College of Swansea. The purity of the compounds was checked through the use of a HPLC system using different mobile phases; $[\alpha]_D$ was determined at 20 °C, and at concentration of 0.25 M, using an AA-10 Automatic Polarimeter (from Optical Activity).

*4(S)-(4′-Nitrobenzyl)-oxazolidin-2-one (**6**)*: (*S*)-4-Benzyl-oxazolidin-2-one (1.0 g, 5.6 mmol) was dissolved in anhydrous dichloromethane (DCM) (15 ml). Nitric acid (3.38 ml, 5 M) was added slowly and left to stir at room temperature. After 2 h, the reaction mixture was quenched in an excess of ice-cold aqueous sodium hydrogen carbonate. The organic layer was separated and the aqueous layer further extracted with DCM (2×20 ml). The combined organic layers were dried over magnesium sulfate ($MgSO_4$). The crude product was purified by column chromatography (DCM 80: ethyl acetate 20) to give **6** as a yellow oil (yield, 65%, 0.82 g). $\nu_{(max)}$(Film)/cm^{-1}: 3279.6 (NH), 2918.1–2855.6 (CH-aliphatic), 1752 (C=O), 1519 (NO_2), 1347.4 (C=C aromatic), 1182.8–1163.2 (aromatic); δ_H (300 MHz, $CDCl_3$): 8.13 (2H, d, $J = 8$ Hz, CH-aromatic), 7.37 (2 H, d, $J = 8$ Hz, CH-aromatic), 6.26 (1 H, s, NH), 3.95 (1H, m, N–CH), 3.81 (2H, m, O–CH_2), 2.93 (1 H, d, $J = 8$ Hz, CH-benzyllic), 2.55 (1 H, d, $J = 8$ Hz, CH-benzyllic); δ_c (75.5 MHz, $CDCl_3$): 41.19 ($\underline{C}H_2$), 53.25 ($\underline{C}H$), 69.34 ($\underline{C}H_2$), 124.18 (CH-aromatic), 130.06 (CH-aromatic), 143.47 (alternative C), 147.27 (alternative C), 159.41 (C=O); LRMS m/z (EI$^+$): 222 ($M^+ + 1$); $[\alpha]_D = -63$, (*S*)-4-benzyl-oxazolidin-2-one was found to possess $[\alpha]_D = -62$ (literature value [12] $[\alpha]_D = -62$).

*4(S)-(4′-Aminobenzyl)-oxazolidin-2-one (**7**)*: 4(*S*)-(4′-Nitrobenzyl)-oxazolidin-2-one (0.2 g, 0.9 mmol) was dissolved in ethanol (7 ml). Saturated ammonium chloride solution (2.5 ml) and indium powder (0.5 g) were added. The reaction mixture was stirred under reflux for 4 h. After cooling, the reaction mixture was diluted with water (15 ml) and filtered through celite. The aqueous filtrate was adjusted to pH ~9 with sodium hydroxide (NaOH) (2 ml, 4 M) and extracted with DCM (3×10 ml). The combined organic layers were dried over $MgSO_4$. The crude product was purified by column chromatography (DCM 80: ethyl acetate 20) to give **7** as a dark yellow oil (yield 47%, 0.082 g). ν_{max}(Film)/cm^{-1}: 3348.9 (C–N), 2923.1–2852.6 (CH-aliphatic), 1745.5 (C=O), 1517.7 (C=C), 1408–1023.4 (aromatic); δ_H(300 MHz, $CDCl_3$): 6.88 (2H, d, $J = 8$ Hz, CH-aromatic), 6.56 (2H, d, $J = 8$ Hz, CH-aromatic), 5.81 (1H, m, N$\underline{H}$), 4.09 (1H, m, N–CH), 3.92 (2H, m, O–CH_2), 3.69 (2H, brs, NH_2), 3.05 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.49 (1H, d, $J = 8$ Hz, CH-benzyllic);

δ_C (75.5 MHz, $CDCl_3$): 159.45 (C=O), 145.53 (C), 129.89 (C), 125.59 (CH-aromatic), 115.55 (CH-aromatic),69.65 ($\underline{C}H_2$), 54.12 ($\underline{C}H$), 40.59 ($\underline{C}H_2$); HRMS: $M^+ =$ 192.0899 required $C_{10}H_{12}N_2O_2M^+ = 192.0899$; $[\alpha]_D = -63$.

2.3. Biochemistry

All non-radioactive steroids, NADPH (mono-sodium salt), and D-glucose-6-phosphate (mono-sodium salt) were obtained from Sigma Chemical, Poole, Dorset. D-Glucose-6-phosphate dehydrogenase (suspension in ammonium sulfate) was purchased from Boehringer–Mannheim GmbH, Mannheim, Germany. [1,2-^{3}H]Androstenedione (55.3 Ci/mmol) was purchased from Dupont (UK), Stevenage, Herts. All unlabelled laboratory reagents were of analar grade and obtained from British Drug House (BDH), Poole, Dorset. Radioactivity was determined on a KLB Wallac 1217 Rackbeta liquid scintillation counter. The scintillation fluid used was 'Cocktail T' from Parkard Instrument, Illinois, USA. An MES Mistral 3000 was used for low speed centrifugation (2000–3000*g*) and an MSB 65 M European ultracentrifuge for high speed 4000–35,000 rpm (8500–105,000*g*).

For this study, the method of Thompson and Siiteri [13] was used, where the aromatization reaction was quenched by mixing an aliquot of the incubation mixture with activated charcoal suspended in mercuric chloride solution. The tritiated water formed is thus separated from the unchanged radiolabelled steroid which is adsorbed onto the activated charcoal. The charcoal was subsequently removed by centrifugation and aliquots of the supernatant were dispersed in 'Cocktail T' and radioactivity counted by a scintillation counter.

The human placental aromatase enzyme was thawed under cold running water and allowed to warm to 37 °C before use. Incubations, in triplicate, were carried out in phosphate buffer (50 mM, pH 7.4) containing NADPH generating system (50 μl, substrate (0.84 μM, 7 μl), and inhibitor (10 μl, 100 μM final concentration), in a shaking water bath. The incubation mixtures were warmed to 37 °C and the reaction started by the addition of placental microsomal protein (50 μl, 0.19 mg/ ml). After incubation for 5 min, an aliquot (300 μl) from each assay tube was removed and added to tubes containing activated charcoal (1%, 900 μl) and mercuric chloride (1 mM, 300 μl) and thoroughly mixed. The tubes were allowed to stand on ice for 20 min and then centrifuged at 3600 rpm for 15 min. Aliquots of the supernatant (500 μl) were removed and dispersed in scintillation fluid (2 ml) and the mixture counted for 1 min.

3. Results and discussion

3.1. Molecular modelling

Using the novel SHC approach [8–10], we undertook a design process to determine whether the 4′-aminophenyl derivatives would possess inhibitory activity against AR. From the results of the molecular modelling study (Figs. 3 and 4) we observe that after ligation to the P-450 heme and the rotation of the bond connecting

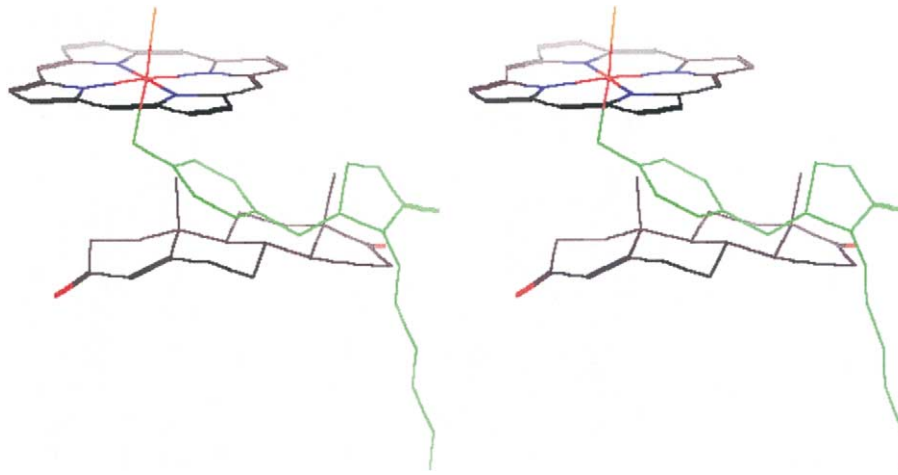


Fig. 4. Modelling of compound **13** (bound to the heme via the phenylamine nitrogen) onto the SHC for AR to show protrusion towards the C(17) and C(13) area of the steroid backbone.

the heme Fe atom to the inhibitor nitrogen atom, the molecules were able to undergo conformational change (ΔE was found to be less than l0 kcal/mol) so as to allow the inhibitor's C=O group to mimic the hydrogen bonding interaction which is presumed to occur between the C(17)=O or the C(3)=O of the steroid and the active site.

From the initial observations, we concluded that the compounds based upon 3-alkylated-(4′-aminobenzyl)-2-oxazolidinone should possess inhibitory activity since they were able to utilize either the steroid C(3) and/or C(17) carbonyl binding groups within the enzyme active site. The effect of the alkyl chain could not be determined easily from molecular modelling, however, from the modelling study we were able to propose that the different enantiomers possessed different inhibitory profiles. That is, from the modelling of the *R*- and *S*-enantiomers of 3-hexyl-(4′-aminobenzyl)-2-oxazolidinone (**13** and **23**, respectively), we observed that the hexyl moiety appeared to be positioned such that in **13**, the hexyl chain was found to be positioned below the steroidal plane (Fig. 4). In **23**, however, the hexyl chain was found to be positioned beyond the C(13) and C(17) area of the steroid backbone, an area which is proposed to be highly restricted in space (Fig. 5). The different binding profiles suggest that the enantiomers, and therefore the observed inhibitory activity would differ considerably between the *R*- and *S*-forms (since the steric interactions present in the latter form would result in a lowered inhibitory activity whereas the *R*-form undergoes no such interaction and would therefore be expected to possess greater inhibitory activity).

3.2. Chemistry

The general method employed to prepare the final compound from the starting *R*- or *S*-enantiomer of Evan's chiral auxiliary is shown in Scheme 2. Overall, the reactions proceeded without major difficulties and in good yield—as an example,

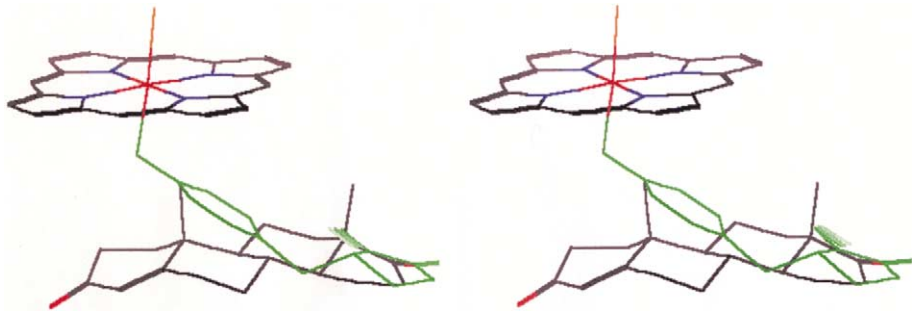
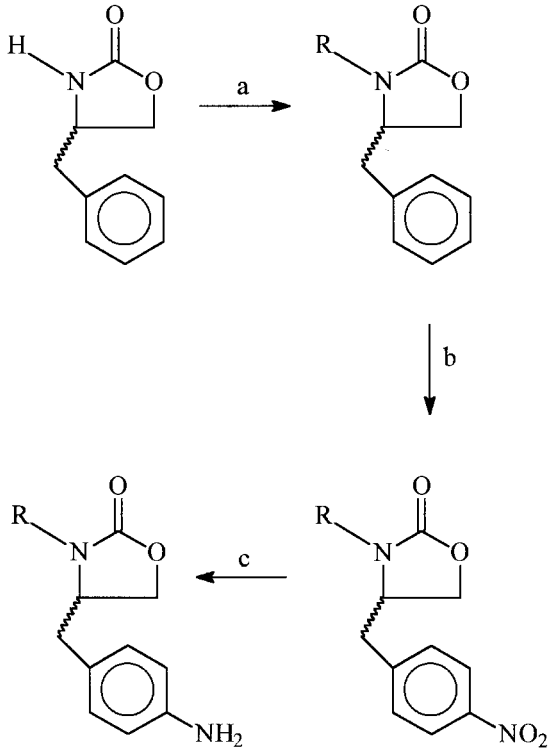


Fig. 5. Modelling of compound **23** (bound to the heme via the phenylamine nitrogen) onto the SHC for AR to show protrusion towards the C(17) and C(13) area of the steroid backbone.



Scheme 2. Synthesis of novel AR inhibitors using the *R*- and *S*-enantiomers of the Evans Chiral auxiliary (a = NaH or K_2CO_3/DMF/Δ/R-X; b = fuming HNO_3/DCM/0 °C; c = In/NH_4Cl/Δ) (R=H, Me, Et, Pr to dodecyl; X = I, Br).

the synthesis of the 4(*S*)-(4′-aminobenzyl)-oxazolidin-2-one (**7**) is given. The remaining compounds were synthesized in a similar manner and the spectral data for the N-alkylated compounds are reported in Table 1 together with the yields.

Table 1
^{1}H and ^{13}C NMR data for the 4-(*R*)-3-alkylated-(4′-amminobenzyl)-2-oxazolidinone

R =	Compound Number	^{1}H NMR	^{13}C NMR	Yield (%)
Me	8	6.89 (2H, d, $J = 8$ Hz, Ar–H), 6.60 (2H, d, $J = 8$ Hz, Ar–H), 3.95 (1H, m, N–CH), 3.81 (2H, m, O–CH_2), 3.12 (2H, brs, NH_2), 2.93 (1H, d, $J = 6$ Hz, CH-benzyllic), 2.83 (3H, s, CH_3), 2.55 (1H, d, $J = 6$ Hz,CH-benzyllic)	158.73 (C=O), 145.54, 128.88, 124.80, 115.57 (C–Ar), 66.76 (O–CH_2), 58.08 (N–CH), 37.41 (Ar–CH_2), 18.26 (CH_3)	88
Et	9	6.86 (2H, d, $J = 8$ Hz, Ar–H), 6.58 (2H, d, $J = 8$ Hz, Ar–H), 4.06 (1H, m, N–CH), 3.82 (2H, m, O–CH_2), 3.65 (2H, brs, NH_2), 3.11 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.47 (1H, d, $J = 8$ Hz CH-benzyllic), 1.11 (2H, m, $CH_2(CH_3)$), 0.82 (3H, t, $J = 7$ Hz, CH_3)	158.24 (C=O), 145.62, 128.82, 124.78, 115.56 (C–Ar), 66.85 (O–CH_2), 55.74 (N–CH), 37.48 (Ar–CH_2), 18.22 (CH_2), 12.67(CH_2)	90
Pr	10	6.89 (2H, d, $J = 8$ Hz, Ar–H), 6.61 (2H, d, $J = 8$ Hz, Ar–H), 4.13 (1H, m, N–CH), 3.97 (2H, m, O–CH_2), 3.49 (2H, brs, NH_2), 3.02 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.51 (1H, d, $J = 8$ Hz CH-benzyllic), 1.56 (2H, m, $CH_2CH_2CH_3$), 1.08 (2H, m, $CH_2CH_2CH_3$), 0.83 (3H, t, $J = 7$ Hz, CH_3)	158.35 (C=O), 148.38, 129.86, 124.86, 115.50 (C–Ar), 66.76 (O–CH_2), 56.12 (N–CH), 37.44 (Ar–CH_2), 20.70 (CH_2), 18.37 (CH_2), 11.18 (CH_3)	86
Bu	11	6.68 (2H, d, $J = 8$ Hz, Ar–H), 6.41 (2H, d, $J = 8$ Hz, Ar–H), 3.80 (1H, m, N–CH), 3.72 (2H, m, O–CH_2), 3.36 (2H, brs, NH_2), 3.26 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.76 (1H, d, $J = 8$ Hz CH-benzyllic), 1.27 (2H, m, $CH_2(CH_2)_2CH_3$), 1.08 (4H, m, $CH_2(CH_2)_2CH_3$), 0.96 (3H, t, $J = 7$ Hz, CH_3)	158.36 (C=O), 146.65, 128.81, 124.74, 115.52 (C–Ar), 66.76 (O–CH_2), 56.04 (N–CH), 37.37 (Ar–CH_2), 28.40 (CH_2), 19.17 (CH_2), 18.23 (CH_2), 13.71 (CH_3)	93
Pe	12	6.80 (2H, d, $J = 8$ Hz, Ar–H), 6.61 (2H, d, $J = 8$ Hz, Ar–H), 4.10 (1H, m, N–CH), 3.94 (2H, m, O–CH_2), 3.62 (2H, brs, NH_2), 3.03 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.49 (1H, d, $J = 8$ Hz CH-benzyllic), 1.50 (2H, m, $CH_2(CH_2)_3CH_3$), 1.28 (6H, m, $CH_2(CH_2)_3CH_3$), 0.91 (3H, t, $J = 7$ Hz, CH_3)	158.25 (C=O), 145.62, 129.85, 124.84, 115.47 (C–Ar), 66.74 (O–CH_2), 56.08 (N–CH), 37.45 (Ar–CH_2), 28.82 (CH_2), 27.20 (CH_2), 27.07 (CH_2), 22.33 (CH_2), 14.00 (CH_3)	90

Hex	13	6.82 (2H, d, $J=8$ Hz, Ar–H), 6.54 (2H, d, $J=8$ Hz, Ar–H), 4.05 (1H, m, N–CH), 3.87 (2H, m, O–CH_2), 3.51 (2H, brs, NH_2), 2.96 (1H, d, $J=8$ Hz, CH-benzyllic), 2.43 (1H, d, $J=8$ Hz CH-benzylic), 1.42 (2H, m, $CH_2(CH_2)_4CH_3$), 1.20 (8H, m, $CH_2(CH_2)_4CH_3$), 0.84 (3H, t, $J=7$ Hz, CH_3)	158.41 (C═O), 145.76, 128.73, 124.56, 115.47 (C–Ar), 66.74 (O–CH_2), 57.62 (N–CH), 37.30 (Ar–CH_2), 28.58 (CH_2), 27.04 (CH_2), 26.85 (CH_2), 22.22 (CH_2), 18.10 (CH_2), 13.89 (CH_3)	84
He-pt	14	6.73 (2H, d, $J=8$ Hz, Ar–H), 6.57 (2H, d, $J=8$ Hz, Ar–H), 4.02 (1H, m, N–CH), 3.87 (2H, m, O–CH_2), 3.52 (2H, brs, NH_2), 3.30 (1H, d, $J=8$ Hz, CH-benzyllic), 2.81 (1H, d, $J=8$ Hz CH-benzylic), 1.36 (2H, m, $CH_2(CH_2)_5CH_3$), 1.09 (10H, m, $CH_2(CH_2)_5CH_3$), 0.68 (3H, t, $J=7$ Hz, CH_3)	158.26 (C═O), 145.52, 129.87, 124.86, 115.53 (C–Ar), 66.75 (O–CH_2), 56.10 (N–CH), 37.47 (Ar–CH_2), 31.73 (CH_2), 28.84(CH_2), 27.84(CH_2), 27.42 (CH_2) 26.68 (CH_2), 22.58 (CH_2), 14.09 (CH_3)	88
Oct	15	6.66 (2H, d, $J=8$ Hz, Ar–H), 6.46 (2H, d, $J=8$ Hz, Ar–H), 4.03 (1H, m, N–CH), 3.89 (2H, m, O–CH_2), 3.53 (2H, brs, NH_2), 2.23 (1H, d, $J=8$ Hz, CH-benzyllic), 2.28 (1H, d, $J=8$ Hz CH-benzylic), 1.32 (2H, m, $CH_2(CH_2)_6CH_3$), 1.03 (12H, m, $CH_2(CH_2)_6CH_3$), 0.64 (3H, t, $J=7$ Hz, CH_3)	158.31 (C═O), 145.73, 129.81, 124.66, 115.47 (C–Ar), 66.75 (O–CH_2), 55.06 (N–CH), 37.38 (Ar–CH_2), 31.75 (CH_2), 29.66 (CH_2), 29.19 (CH_2), 27.48 (CH_2), 27.39 (CH_2), 26.70 (CH_2), 22.62 (CH_2), 14.10 (CH_3)	91
Non	16	6.91 (2H, d, $J=8$ Hz, Ar–H), 6.46 (2H, d, $J=8$ Hz, Ar–H), 4.03 (1H, m, N–CH), 3.71 (2H, m, O–CH_2), 3.50 (2H, brs, NH_2), 3.04 (1H, d, $J=8$ Hz, CH-benzyllic), 2.87 (1H, d, $J=8$ Hz CH-benzylic), 1.34 (2H, m, $CH_2(CH_2)_7CH_3$), 1.05 (14H, m, $CH_2(CH_2)_7CH_3$), 0.68 (3H, t, $J=7$ Hz, CH_3)	159.19 (C═O), 145.47, 129.88, 125.03, 115.52 (C–Ar), 66.73 (O–CH_2), 56.10 (N–CH), 42.04 (Ar–CH_2), 37.47 (CH_2), 31.88 (CH_2), 29.54 (CH_2), 29.30 (CH_2), 29.22 (CH_2), 27.43 (CH_2), 26.73 (CH_2), 22.68 (CH_2), 14.14 (CH_3)	91
Dec	17	6.70 (2H, d, $J=8$ Hz, Ar–H), 6.42 (2H, d, $J=8$ Hz, Ar–H), 4.02 (1H, m, N–CH), 3.76 (2H, m, O–CH_2), 3.56 (2H, brs, NH_2), 3.28 (1H, d, $J=8$ Hz, CH-benzyllic), 2.82 (1H, d, $J=8$ Hz CH-benzylic), 1.36 (2H, m, $CH_2(CH_2)_8CH_3$), 1.03 (16H, m, $CH_2(CH_2)_8CH_3$) 0.96 (3H, t, $J=7$ Hz, CH_3)	158.43 (C═O), 145.36, 128.84, 125.05, 115.70 (C–Ar), 66.78 (O–CH_2), 57.07 (N–CH), 37.39 (Ar–CH_2), 31.88 (CH_2), 29.55 (CH_2), 29.26(CH_2), 29.11 (CH_2), 27.38 (CH_2), 26.70 (CH_2), 26.30 (CH_2), 24.86 (CH_2), 22.66 (CH_2), 14.911 (CH_3)	91
Me	18	6.94 (2H, d, $J=8$ Hz, Ar–H), 6.65 (2H, d, $J=8$ Hz, Ar–H), 4.18 (1H, m, N–CH), 3.98 (2H, m, O–CH_2), 3.41 (2H, brs, NH_2), 3.01 (1H, d, $J=6$ Hz, CH-benzyllic), 2.88 (3H, s, CH_3), 2.58 (1H,d, $J=6$ Hz, CH-benzyllic)	158.06 (C═O), 144.52, 129.88, 123.98, 115.05 (C–Ar), 66.71 (O–CH_2), 54.38 (N–CH), 37.03 (Ar–CH_2), 12.76 (CH_3)	93

Table 1 (*continued*)

R =	Compound Number	^{1}H NMR	^{13}C NMR	Yield (%)
Et	19	6.87 (2H, d, $J = 8$ Hz, Ar–H), 6.57 (2H, d, $J = 8$ Hz, Ar–H), 4.11 (1H, m, N–CH), 3.99 (2H, m, O–CH_2), 3.33 (2H, brs, NH_2), 3.04 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.51 (1H, d, $J = 8$ Hz CH-benzylic), 1.18 (2H, m, CH_2CH_3), 1.11 (3H, t, $J = 7$ Hz, CH_3)	158.05 (C=O), 145.51, 129.88, 124.88, 115.50 (C–Ar), 66.81 (O–CH_2), 55.78 (N–CH), 37.63 (Ar–CH_2), 34.21 (CH_2), 12.74 (CH_2)	86
Pr	20	6.73 (2H, d, $J = 8$ Hz, Ar–H), 6.45 (2H, d, $J = 8$ Hz, Ar–H), 3.97 (1H, m, N–CH), 3.79 (2H, m, O–CH_2), 3.24 (2H, brs, NH_2), 2.98 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.32 (1H, d, $J = 8$ Hz CH-benzylic), 1.36 (2H, m, $CH_2CH_2CH_3$), 1.11 (3H, t, $J = 7$ Hz, CH_3)	158.45 (C=O), 145.55, 129.84, 124.88, 115.55 (C–Ar), 66.79 (O–CH_2), 56.04 (N–CH), 37.41 (Ar–CH_2), 20.16 (CH_2), 18.25(CH_2), 11.13 (CH_3)	86
Bu	21	6.94 (2H, d, $J = 8$ Hz, Ar–H), 6.65 (2H, d, $J = 8$ Hz, Ar–H), 4.15 (1H, m, N–CH), 3.79 (2H, m, O–CH_2), 3.46 (2H, brs, NH_2), 3.05 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.55 (1H, d, $J = 8$ Hz CH-benzylic), 1.60 (2H, m, $CH_2(CH_2)_2CH_3$), 1.24 (4H, m, $CH_2(CH_2)_2CH_3$), 0.83 (3H, t, $J = 7$ Hz, CH_3)	157.93 (C=O), 146.54, 128.88, 123.88, 115.51 (C–Ar), 66.74 (O–CH_2), 57.54 (N–CH), 37.51 (Ar–CH_2), 22.17 (CH_2), 20.73 (CH_2), 18.37 (CH_2), 11.20 (CH_3)	90
Pe	22	6.92 (2H, d, $J = 8$ Hz, Ar–H), 6.71 (2H, d, $J = 8$ Hz, Ar–H), 4.15 (1H, m, N–CH), 4.05 (2H, m, O–CH_2), 3.48 (2H, brs, NH_2), 3.01 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.51 (1H, d, $J = 8$ Hz CH-benzylic), 1.54 (2H, m, $CH_2(CH_2)_3CH_3$), 1.24 (6H, m, $CH_2(CH_2)_3CH_3$), 0.92 (3H, t, $J = 7$ Hz, CH_3)	158.22 (C=O), 145.51, 129.88, 125.01, 115.50 (C–Ar), 66.73 (O–CH_2), 56.11 (N–CH), 37.48 (Ar–CH_2), 28.84 (CH_2), 27.10 (CH_2), 23.35 (CH_2), 14.01 (CH_3)	93
Hex	23	6.71 (2H, d, $J = 8$ Hz, Ar–H), 6.42 (2H, d, $J = 8$ Hz, Ar–H), 4.14 (1H, m, N–CH), 3.93 (2H, m, O–CH_2), 3.35 (2H, brs, NH_2), 2.87 (1H, d, $J = 8$ Hz, CH-benzyllic), 2.33 (1H, d, $J = 8$ Hz CH-benzylic), 1.34 (2H, m, $CH_2(CH_2)_4CH_3$), 1.01 (8H, m, $CH_2(CH_2)_4CH_3$), 0.69 (3H, t, $J = 7$ Hz, CH_3)	158.24 (C=O), 145.58, 129.86, 124.90, 115.48 (C–Ar), 66.74 (O–CH_2), 56.10 (N–CH), 37.48 (Ar–CH_2), 34.08 (CH_2), 27.08(CH_2), 22.35 (CH_2), 14.21 (CH_3)	88

Hept	24	6.76 (2H, d, $J=8$ Hz, Ar–H), 6.60 (2H, d, $J=8$ Hz, Ar–H), 4.28 (1H, m, N–CH), 4.12 (2H, m, O–CH_2), 3.04 (2H, brs, NH_2), 2.84 (1H, d, $J=8$ Hz, CH-benzyllic), 2.63 (1H, d, $J=8$ Hz CH-benzylic), 1.25 (2H, m, $CH_2(CH_2)_5CH_3$), 1.10 (10H, m, $CH_2(CH_2)_5CH_3$), 0.87 (3H, t, $J=7$ Hz, CH_3)	158.31 (C=O), 145.57, 129.85, 124.86, 115.48 (C–Ar), 66.75 (O–CH_2), 56.08 (N–CH), 37.42 (Ar–CH_2), 31.70 (CH_2), 28.91 (CH_2), 27.39 (CH_2), 26.65 (CH_2), 20.43 (CH_2), 15.33 (CH_2), 14.05 (CH_3)	91
Oct	25	6.91 (2H, d, $J=8$ Hz, Ar–H), 6.61 (2H, d, $J=8$ Hz, Ar–H), 4.08 (1H, m, N–CH), 3.92 (2H, m, O–CH_2), 3.40 (2H, brs, NH_2), 2.98 (1H, d, $J=8$ Hz, CH-benzyllic), 2.58 (1H, d, $J=8$ Hz CH-benzylic), 1.52 (2H, m, $CH_2(CH_2)_6CH_3$), 1.19 (12H, m, $CH_2(CH_2)_6CH_3$), 0.84 (3H, t, $J=7$ Hz, CH_3)	158.43 (C=O), 145.35, 129.46, 125.03, 114.84 (C–Ar), 66.68 (O–CH_2), 55.98 (N–CH), 42.01 (Ar–CH_2), 37.45 (CH_2), 31.74 (CH_2), 29.18 (CH_2), 27.40 (CH_2), 26.68 (CH_2), 22.61 (CH_2), 18.23 (CH_2), 14.09 (CH_3)	88
Non	26	6.92 (2H, d, $J=8$ Hz, Ar–H), 6.73 (2H, d, $J=8$ Hz, Ar–H), 4.09 (1H, m, N–CH), 3.94 (2H, m, O–CH_2), 3.44 (2H, brs, NH_2), 3.04 (1H, d, $J=8$ Hz, CH-benzyllic), 2.53 (1H, d, $J=8$ Hz CH-benzylic), 1.52 (2H, m, $CH_2(CH_2)_7CH_3$), 1.23 (14H, m, $CH_2(CH_2)_7CH_3$), 0.84 (3H, t, $J=7$ Hz, CH_3)	158.34 (C=O), 145.07, 129.96, 126.63, 116.67 (C–Ar), 66.67 (O–CH_2), 55.95 (N–CH), 37.28 (Ar–CH_2), 33.99 (CH_2), 31.82 (CH_2), 29.48 (CH_2), 29.26 (CH_2) 29.22 (CH_2), 27.42 (CH_2), 26.70 (CH_2), 22.64 (CH_2), 14.11 (CH_3)	91
Dec	27	6.60 (2H, d, $J=8$ Hz, Ar–H), 6.34 (2H, d, $J=8$ Hz, Ar–H), 4.10 (1H, m, N–CH), 3.92 (2H, m, O–CH_2), 3.40 (2H, brs, NH_2), 3.03 (1H, d, $J=8$ Hz, CH-benzyllic), 2.54 (1H, d, $J=8$ Hz CH-benzylic), 1.22 (2H, m, $CH_2(CH_2)_8CH_3$), 1.07 (16H, m, $CH_2(CH_2)_8CH_3$), 0.87 (3H, t, $J=7$ Hz, CH_3)	158.51 (C=O), 145.53, 129.67, 12.68, 115.67 (C–Ar), 66.74 (O–CH_2), 57.40 (N–CH),37.21 (Ar–CH_2), 31.72 (CH_2), 30.69 (CH_2), 29.38 (CH_2), 29.14 (CH_2) 27.22 (CH_2), 26.55 (CH_2), 22.51 (CH_2), 21.71 (CH_2), 17.82 (CH_2), 13.90 (CH_3)	91

It should be noted, however, that with the increase in chain length (N-alkylation), the nitration step required an increase in the reaction time. That is, with the more hydrophobic chains, a greater length of time was required for the nitration reaction to proceed to completion, with the result that the N-dodecyl derivative required some 7 h (yield 80%) whereas the smaller alkyl chain containing compounds took 3 h. The reduction of the nitro to the amine (using a mixture of indium and ammonium chloride) also proceeded in good yield and without any major problems.

In an effort to ensure that the chiral centre had not undergone any kind of alteration/racemization, the $[\alpha]_D$ values of the compounds were determined and were found to be consistent with the starting enantiomer. For example, the starting compound in the synthesis of the *S*-enantiomer [namely (*S*)-4-benzyl-oxazolidin-2-one] was found to possess (under the given conditions) a $[\alpha]_D$ value of -62. Although the value of the number may be expected to change, the direction of rotation of the plane of polarized light would not. The amino compounds [containing the (*S*)-4-benzyl-oxazolidin-2-one] were all found to possess a negative $[\alpha]_D$ value (ranging from -62 to -84, where R=H to decyl respectively). The derivatives of (*R*)-4-benzyl-oxazolidin-2-one were found to possess a similar range, from $+63$ to $+85$, where R=H to decyl, respectively.

3.3. Inhibition of human placental aromatase

The novel inhibitors, in general, were found to possess a good range of inhibitory activity (Tables 2 and 3) [for example, *N*-nonyl-4(*S*)-(4′-aminobenzyl)-oxazolidin-2-one (**26**) possessed 10% inhibition whilst N-nonyl-4(*R*)-(4′-aminobenzyl)-oxazolidin-2-one (**16**) possessed 73% inhibition] compared to the standard compound aminoglutethimide (AG) which possessed 53% inhibition at $[I] = 100\,\mu M$. A detailed consideration of the inhibitory activity (initial screening and IC_{50} values) of the N-alkylated derivatives of the two enantiomers shows that, in general, the *R*-form appears to be more potent than the inhibitors based upon the *S*-enantiomer (Tables 1 and 2). Indeed, further consideration of the IC_{50} values shows that the trend in inhibitory activity between the *S*- and the *R*-forms is very different. For example, within the first series of compounds (**8**–**17**), inhibitory activity increases and is observed to reach a maximum with the pentyl (**12**) and hexyl (**13**) derivatives (Fig. 6); within the series of inhibitors of the *S*-enantiomer, however, the inhibitory activity decreases with an increase in alkyl chain length (Fig. 7). As such, the inhibitory data correlate very closely with the observations from the molecular modelling studies and therefore gives strong support for the novel SHC approach.

The biochemical evaluation of the synthesized compounds also adds further support for the existence of a large hydrophobic pocket. Consideration of the inhibitory activity of the *R*-enantiomer based compounds shows that the C_{10} (**17**) possesses greater inhibitory activity than the majority of the compounds based upon the *S*-enantiomer—only the methyl-substituted compound (**18**) possessed greater inhibitory activity than **17** (Table 2). We have previously reported an extensive overall model for the active site of AR where the existence of a protrusion has been proposed to exist below the steroidal plane, which is able to accommodate alkyl chains of up to C_{12} in length [10].

Table 2
Initial screening data for *R*- and *S*-enantiomers of Evans' chiral auxiliary and AG (values are mean of triplicate determinations)

Compound (R =)	Compound number	Percentage of inhibition ([*I*] = 100 μM)
R-enantiomer		
Me	**8**	38
Et	**9**	53
Pr	**10**	68
Bu	**11**	86
Pe	**12**	93
Hex	**13**	87
Hept	**14**	86
Oct	**15**	76
Non	**16**	73
Dec	**17**	62
S-enantiomer		
Me	**18**	65
Et	**19**	60
Pr	**20**	50
Bu	**21**	42
Pe	**22**	37
Hex	**23**	21
Hept	**24**	15
Oct	**25**	12
Non	**26**	10
Dec	**27**	10
AG	–	53

Table 3
IC_{50} of some of the synthesised inhibitors (values are mean of triplicate determinations)

Compound	IC_{50} (μM)	Relative potency
10	4.2 ± 0.2	22.67
11	1.5 ± 0.2	63.45
12	0.83 ± 0.05	114.70
13	1.2 ± 0.1	79.33
14	3.6 ± 0.06	26.44
20	12.66 ± 0.3	7.52
21	15.00 ± 0.3	6.35
22	16.82 ± 0.2	5.66
23	35.00 ± 0.5	2.72
24	71.26 ± 0.25	1.34
25	77.0 ± 0.15	1.24
26	85.8 ± 0.05	1.11
27	125.0 ± 0.4	0.76
AG	95.2 ± 0.5	1

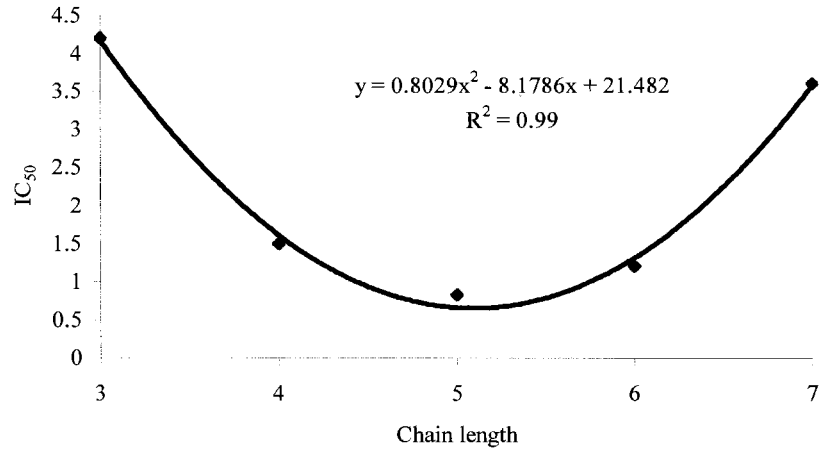


Fig. 6. Plot of chain length versus IC_{50} of the *R*-enantiomer based inhibitors.

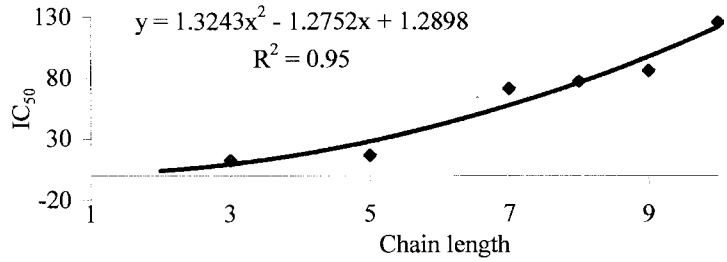


Fig. 7. Plot of IC_{50} versus chain length to show the decrease in the potency of the *S*-enantiomer based compounds with an increase in alkyl chain length.

Modelling of compounds based on the *S*-enantiomer (e.g., **23**, Fig. 5) onto the SHC showed that the alkyl chain protruded towards the C(17) and C(13) area of the steroidal backbone, an area which is proposed to be sterically constrained due to the protein backbone, resulting in greatly reduced inhibitory activity. As such, the current study therefore adds further support to the previous studies. Rotation of the benzyllic bond (so as to produce a conformer whereby the alkyl chains do not protrude towards the sterically constrained area of the protein backbone) results in an unfavourable conformational change which gives rise to a large increase in energy ($\Delta E > 20$ kcal/mol), and therefore a decrease in the inhibitory activity.

4. Conclusion

In conclusion, the results of the biochemical evaluation of the two sets of enantiomers of 3-alkylated-(4′-aminobenzyl)-2-oxazolidinone support the molecular modelling study and in particular provides further support for the SHC approach

which has previously been used to rationalize the inhibitory activity of a wide range of inhibitors of AR and other cytochrome P-450 enzymes.

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