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Synthesis, biochemical evaluation and rationalisation of the inhibitory activity of a series of 4-hydroxyphenyl ketones as potential inhibitors of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3)

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Abstract—We report the preliminary results of the synthesis and biochemical evaluation of a number of 4-hydroxyphenyl ketones as inhibitors of the isozyme of the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) responsible for the conversion of androstenedione (AD) to testosterone (T), more specifically type 3 (17β-HSD3). The results of our study suggest that we have synthesised compounds which are, in general, potent inhibitors of 17β-HSD3, in particular, we discovered that 1-(4-hydroxy-phenyl)-nonan-1-one (**8**) was the most potent (IC_{50} = 2.86 ± 0.03 μM). We have therefore provided good lead compounds in the synthesis of novel non-steroidal inhibitors of 17β-HSD3.

The biosynthesis of testosterone (T) [the precursor to dihydrotestosterone (DHT), the most potent androgen] involves the reduction of the C(17) carbonyl moiety within androstenedione (AD) and is catalysed by the type 3 isozyme of the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD3) (Fig. 1).

This enzyme has now become a potential biochemical target[1] in the fight against hormone-dependent prostate (as well as breast) cancer, the rationale being that the reduction of testosterone levels would result in a subsequent reduction in DHT, thereby leading to a loss of stimulation of the prostate cancer cells. Several isozymes of 17β-HSD are known to exist and are responsible for a number of redox reactions, including the conversion of the weak C=O containing sex steroids to the more mitogenic 17β-hydroxy containing steroids [e.g., AD to T catalysed by type 3 and conversion of estrone (E1) to estradiol (E2) catalysed by type 1 (17β-HSD1)], hence the targeting of isozymes of this enzyme in the treatment of hormone-dependent cancers.[1] In an effort to aid the design of novel inhibitors of this enzyme, we have previously undertaken the derivation of the transition-states (TS) of the reduction reaction catalysed by 17β-HSD1.[2] We concluded, from our study, that the carbonyl moiety was an important feature of any potential inhibitor of the numerous isozymes of 17β-HSD involved in the reduction (and therefore a good mimic) of the steroid C(17)=O moiety within the natural substrate. In an effort to evaluate our hypothesis, we undertook the design of a number of compounds as potential inhibitors, as such, we report here the initial results of the synthesis of a range of straight alkyl chain containing 4-hydroxyphenyl ketones and their

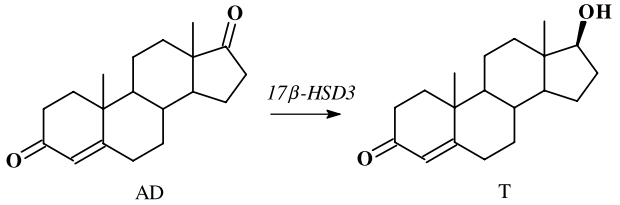

Figure 1. Conversion of AD to T catalysed by 17β-HSD type 3.

Keywords: 17β-hydroxysteroid dehydrogenase; Type 3; Inhibitors; Androstenedione; Testosterone.
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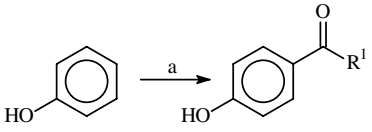
subsequent biochemical evaluation against rat testicular microsomal enzyme against 17β-HSD3 using radiolabelled AD as the substrate.

In the synthesis of the 4-hydroxyphenyl ketones considered within the current study, we utilised the reaction outlined in Scheme 1, a reaction which has been utilised extensively by us in the synthesis of the corresponding sulfamate derivatives as potent inhibitors of the enzyme estrone sulfatase.[3,4] In general, the reaction involved Friedel–Crafts acylation of phenol using the appropriate acyl chloride in the presence of anhydrous aluminium chloride ($AlCl_3$) and using anhydrous dichloromethane (DCM) as the reaction solvent. The reactions proceeded in relatively good yield (between a range of ~24% and ~56%) and without any major problems; the syntheses of 1-(4-hydroxy-phenyl)-ethanone[5] (**1**) and 1-(4-hydroxy-phenyl)-propan-1-one[6] (**2**) are given as examples.

Table 1 shows the results of the initial screening, using a modified literature-based assay,[7,8] as well as the IC_{50} values for the compounds synthesised within the current study. Due to the lack of compounds in the clinic for this biochemical target, it was not possible to compare the biological activity (and therefore relative potency) of the synthesised compounds within the current study against a single standard inhibitor. However, as the flavonoid-based compounds have been previously evaluated and found to possess good inhibitory activity against 17β-HSD3, in particular, Baicalein and 7-hydroxyflavone,[9] we have therefore used these for comparison.

In general, consideration of the biological activity obtained shows that the 4-hydroxyphenyl ketone-based compounds synthesised within the current study possess potent inhibitory activity against 17β-HSD3. Indeed of the compounds synthesised, only two are found to possess poor IC_{50} values. Detailed consideration of the inhibitory activity shows that compounds **8** (IC_{50} = 2.86 μM) and **9** (IC_{50} = 4.97 μM) are the most potent compounds within the current study. As such, **8** is approximately 65 and 23 times more potent than the two previously reported inhibitors of 17β-HSD3, namely Baicalein (IC_{50} = 185.92 μM) and 7-hydroxyflavone (IC_{50} = 66.98 μM), respectively. Compounds **6** (IC_{50} = 7.84 μM), **7** (IC_{50} = 6.52 μM) and **10** (IC_{50} = 7.55 μM) are also found to possess potent inhibitory activity in comparison to the two standard compounds (although they are all approximately 2–3 times less potent than **8** and may be considered to be equipotent to **9**).



Scheme 1. Synthesis of the 4-hydroxyphenyl ketones as potential inhibitors of 17β-HSD. Reagents: (a) acid chloride/$AlCl_3$/DCM; $R^1 = C_1$–C_{11}.

Table 1. Inhibitory data obtained for the 4-hydroxyphenyl ketones synthesised within the present study as inhibitors of 17β-HSD3

Compound	R	% inhibition [I] = 100 μM	IC_{50} value (μM)
Baicalein	—	NQ	185.92 ± 12.70
7-Hydroxyflavone	—	NQ	66.98 ± 0.95
1	CH_3	36.59 ± 0.52	1708.92 ± 170.71
2	C_2H_5	53.03 ± 2.18	150.56 ± 12.21
3	C_3H_7	60.18 ± 0.77	89.51 ± 6.73
4	C_4H_9	61.81 ± 0.89	60.52 ± 5.83
5	C_5H_{11}	76.40 ± 0.18	18.02 ± 0.96
6	C_6H_{13}	80.26 ± 0.20	7.84 ± 0.36
7	C_7H_{15}	82.58 ± 0.49	6.52 ± 0.18
8	C_8H_{17}	83.53 ± 0.48	2.86 ± 0.03
9	C_9H_{19}	81.39 ± 0.09	4.97 ± 0.25
10	$C_{11}H_{23}$	78.92 ± 0.58	7.55 ± 0.32

NQ, not quoted.

From the consideration of the biological data, we initially observe that the potency of the inhibitors appears to increase with increasing alkyl chain length and upon consideration of the physicochemical factors for the novel inhibitors, we observe a good correlation between the logarithm of the calculated partition coefficient (log *P*) (calculated using Quantum CaChe Project Leader[10]) and IC_{50} (Fig. 2), resulting in an optimum log *P* of approximately 4.1, corresponding to compound **8**. We therefore suggest that hydrophobicity may be an important physicochemical factor in determining the overall inhibitory activity in the inhibition of 17β-HSD3.

Consideration of the steroid backbone suggests that there are two modes of superimpositioning the inhibitors such that the carbonyl moiety within the inhibitor mimics the C(17)=O of the substrate: one, whereby the alkyl chain extends towards the area of space normally occupied by the rings A, B and C of the steroid substrate, placing the 4-hydroxyphenyl moiety beyond the C(15) and C(16) position of the steroid backbone. The alternative mode of superimpositioning involves the 4-hydroxyphenyl being positioned towards the steroid backbone whilst the alkyl chain is now positioned such that it extends far beyond the D-ring. In a previous study, the area of the enzyme active site corresponding to the D-ring of the natural substrate has been shown to be populated with hydrogen donor and bonding groups as well as NADPH as the reducing agent (in the conversion of E1 to E2) and is therefore

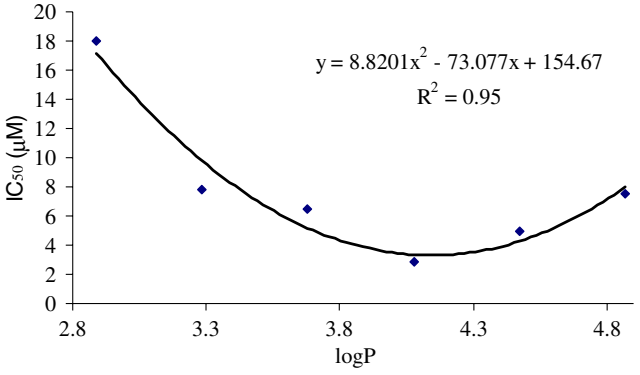


Figure 2. Plot of IC_{50} versus calculated log *P* for a small range of the compounds (from **5** to **10**) synthesised within the current study.

constrained in the volume of space available for the large acyl chains.[2] As such, binding in such a manner where the alkyl chain extends out beyond the D-ring is unlikely due to the increased unfavourable steric interactions which would therefore lead to reduced inhibitory activity—this is contrary to what is observed experimentally.

We therefore undertook a molecular modelling study involving the determination of low energy conformers and the subsequent superimpositioning of the conformers of the most potent compound (namely **8**) onto the backbone of AD (such that the alkyl chain mimicked the backbone of AD). We discovered that numerous low energy conformers of compound **8** exist, however, one low energy conformer was found which allows the alkyl chain to be positioned over the steroid backbone without a major change in energy when compared to the global minima ($\Delta E = 0.82$ kcal/mol). Occupying the same volume of space as the steroid backbone of the substrate AD (Fig. 3) allows the alkyl chain to utilise any available hydrophobic interactions. We therefore propose that the potent inhibitory activity observed within compound **8** is not only due to the increased log *P* of this compound but also due to the ability of the alkyl chain to mimic the steroid backbone and not undergo any unfavourable steric interactions.

That our hypothesis may have some validity can be observed when the larger alkyl chain containing compounds, for example **10**, are superimposed onto the steroid backbone. Detailed conformational analysis of compound **10** resulted in the discovery of numerous conformers and from the superimpositioning of the conformers onto the backbone of AD, we observed that a conformer exists which allows the C_{11} containing alkyl chain to approach the area of the active site (and therefore the appropriate hydrogen bonding group) which would normally interact with the C(3)=O moiety of the substrate (Fig. 4). We therefore suggest that an increase in alkyl chain length (beyond the C_8 or C_9 found in compounds **8** and **9**) would result in the longer alkyl chain containing inhibitors undergoing steric interaction with the hydrogen bonding group at the active site corresponding to the C(3)=O moiety, thereby reducing the inhibitory activity of the larger alkyl chain containing compounds.

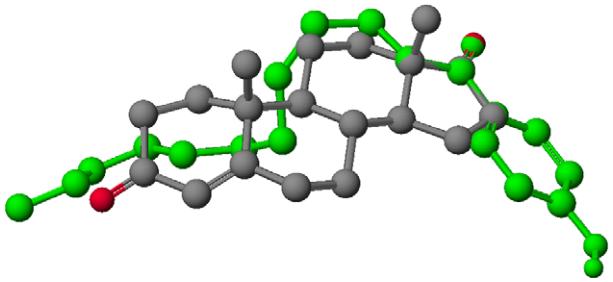


Figure 4. Superimpositioning of a low energy conformer of **10** (in green) onto the backbone of AD.

From the molecular modelling study, we also suggest that the 4-hydroxyphenyl moiety (especially within the larger chain containing compounds) is involved in hydrogen bonding interactions with the active site about the C(15) and C(16) area of the steroid backbone. Our hypothesis may be rationalised by the large differences in inhibitory activity observed with increasing alkyl chain length within the synthesised compounds (e.g., compound **1** is found to have an IC_{50} value of 1708.92 ± 170.71 μM, whereas compounds **6** and **8** are found to possess IC_{50} values of 7.84 ± 0.36 and 2.86 ± 0.03 μM, respectively). We hypothesise therefore that within the small alkyl chain containing compounds, these inhibitors may bind in either of the two modes suggested above. That is, as well as binding such that the 4-hydroxyphenyl moiety is able to hydrogen bond with groups about the C(15) and C(16), compounds **1**–**4** may also bind in an alternative manner such that the acetyl moiety is positioned about the C(15) and C(16) position of the steroid backbone resulting in decreased interaction between the inhibitor and the enzyme leading to decreased potency (Fig. 5 shows the superimpositioning of compound **2** such that the alkyl chain occupies an area about the C(15) and C(16) area of the androgen backbone). As a result of the two available modes of binding, therefore, we propose that the inhibitor is not able to bind in an effective manner (due to decreased hydrogen bonding) and as a result possesses a much greater IC_{50} value than would be expected in comparison to the other derivatives. That is, differences in log *P* cannot be utilised in rationalising the large difference in potency between the small and larger compounds, and we suggest that this difference is also due to the ineffective binding of these smaller compounds to the 17β-HSD3 active site.

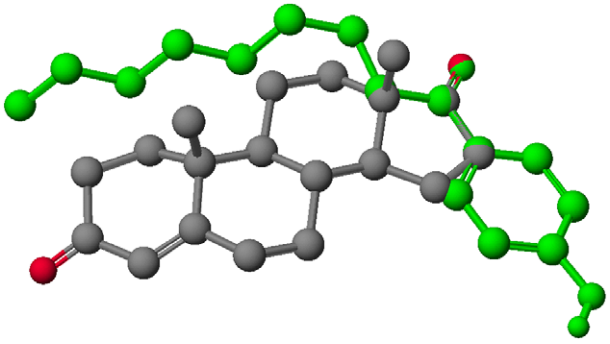


Figure 3. Superimpositioning of a low energy conformer of **8** (in green) onto the backbone of AD.

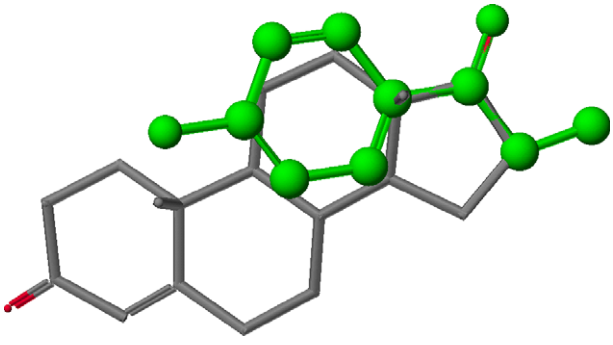


Figure 5. Superimpositioning of **2** (in green) onto the backbone of AD.

As previously mentioned, due to the extensive size of the octyl chain within compound **8** (or indeed any compound possessing an alkyl chain greater than C_4), it is only able to bind such that the alkyl chain mimics the steroid backbone, with the 4-hydroxyphenyl moiety able to undergo hydrogen bonding with the active site, leading to increased inhibitory activity. The ability of the compounds to undergo this favourable hydrogen bonding interaction is therefore another reason for the potent inhibitory activity observed within the larger inhibitors of 17β-HSD3. In an effort to validate our hypothesis, we undertook the biochemical evaluation of a range of inhibitors (not reported here) which contained various substituents (in particular groups which lacked any hydrogen bonding groups) in place of the 4-hydroxy moiety and discovered that the compounds were either weak or non-inhibitors of this enzyme.

In conclusion, from the consideration of the inhibitory activity of the 4-hydroxyphenyl ketones synthesised within the current study, we have first produced two highly potent inhibitors (compounds **8** and **9**) of 17β-HSD3. Furthermore, from the molecular modelling study, we have proposed a probable mode of action of these compounds and have therefore rationalised the structure–activity relationship observed within the current range of compounds. We have also suggested a structural modification which may allow non-steroidal inhibitors of this enzyme to possess greater inhibitory activity.

Acknowledgments

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5. 1-(4-Hydroxy-phenyl)-ethanone (**1**): $AlCl_3$ (1.50 g, 21 mmol) was added to a solution of phenol (0.50 g, 10.6 mmol) in anhydrous DCM (10 mL). The slurry was left stirring for 30 min before acetyl chloride (0.83 mL, 11.7 mmol) was added in a dropwise manner. The solution was then left to stir for 14 h. The reaction was quenched using ice-cold solution of hydrochloric acid (HCl) (1 M, 30 mL) and then extracted into diethyl ether (2× 50 mL). The combined organic layer was extracted into sodium hydroxide (NaOH) (2 M, 2× 50 mL) and then acidified to pH 2 using HCl (1 M). The product was extracted into diethyl ether (2× 50 mL) and the organic layer was washed with water (2× 50 mL) and dried over anhydrous magnesium sulfate ($MgSO_4$), filtered and the solvent removed under vacuum to give a brown solid. Flash chromatography of the crude solid gave **1** as a white solid (0.68 g, 47% yield) [mp 109.8–110.1 °C; R_f = 0.26 diethyl ether/petroleum ether 40–60 °C (30:70); lit. mp 110.2–110.4 °C]. $\nu_{(max)}$ (film) cm^{-1}: 3321.93 (OH), 1662.55 (C=O), 1603.51 (Ar C=C); δ_H ($CDCl_3$): 7.89 (2H, d, J = 8.79 Hz, Ph-*H*), 6.91 (2H, d, J = 8.97 Hz, Ph-*H*), 2.56 (3H, s, C*H*$_3$); δ_C ($CDCl_3$): 198.59 (*C*=O), 160.74, 131.19, 129.55, 115.51 (Ar *C*), 26.29 (*C*H$_3$); GC: t_R 6.06 min; LRMS (EI): 136 (M^+, 30%), 121 (M^+-CH_3, 100%); Elemental analysis, Found: C, 70.42%; H, 5.88%; $C_8H_8O_2$ requires C, 70.58%; H, 5.92%.
6. 1-(4-Hydroxy-phenyl)-propan-1-one (**2**): Compound **2** was synthesised in a similar manner to **1** except that propanoyl chloride (1.02 mL, 11.7 mmol) was used in place of acetyl chloride. The crude solid was purified via flash chromatography to give **2** as an off-white solid (0.84 g, 53% yield) [mp 158.2–158.6 °C; R_f = 0.32 diethyl ether/petroleum ether 40–60 °C (30:70); lit. mp 152–153 °C]. $\nu_{(max)}$ (film) cm^{-1}: 3222.83 (OH), 1650.16 (C=O), 1605.78 (Ar C=C); δ_H ($CDCl_3$): 7.85 (2H, d, J = 8.42 Hz, Ph-*H*), 6.81 (2H, d, J = 8.42 Hz, Ph-*H*), 2.89 (2H, q, J_{AB} = 7.32 Hz, J_{AB} = 7.14 Hz, O=C–C*H*$_2$), 1.15 (3H, t, J_{AB} = 7.14 Hz, J_{AB} = 7.32 Hz, CH$_2$C*H*$_3$); δ_C ($CDCl_3$): 198.96 (*C*=O), 152.58, 130.56, 115.26 (Ar *C*), 31.39 (*C*H$_2$CH$_3$), 8.38 (*C*H$_3$); GC: t_R 6.81 min; LRMS (EI): 150 (M^+, 9%), 121 ($M^+-C_2H_5$, 100%); Elemental analysis, Found: C, 71.84%; H, 6.71%; $C_9H_{10}O_2$ requires C, 71.98%, H, 6.71%.
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8. Preliminary screening and IC_{50} determinations of compounds: All incubations were carried out in triplicate at 37 °C in a shaking water bath. Incubation mixtures (1 mL), containing NADPH generating system (50 μL), inhibitor (varying concentration, 20 μL) and substrate (1.5 μM final concentration, 15 μL), in phosphate buffer (pH 7.4, 905 μL), were allowed to warm to 37 °C. The rat testicular microsomes were thawed and warmed to 37 °C before addition of the enzyme (0.097 mg/mL final concentration, 10 μL) to the assay mixture. The solutions were incubated for 30 min at 37 °C and the reaction was quenched by the addition of ether (2 mL). The solutions were vortexed, then left to stand over ice for 15 min. The assay mixture was extracted with further aliquots of ether (2× 2 mL) and organic layers combined into a clean tube before the solvent was evaporated. Acetone (30 μL) was added to each tube and vortexed thoroughly. Aliquots, along with steroid carriers (A and T, 5 mg/mL, approximately 10 μL), were spotted onto TLC plates and run, using a mobile phase consisting of dichloromethane (70 mL) and ethyl acetate (30 mL). After development, the separated steroids were identified, using an UV lamp, cut from the plate and placed into scintillation vials. Acetone (1 mL) was added to each vial in order to dissolve the steroid from the silica plate and then scintillation fluid (Optiscint HiSafe) (3 mL) was added. The samples were vortexed and read for tritium for 4 min per tube. In determining the IC_{50} values for the compounds studied within the current study, the inhibitory activity was determined using the method outlined above, however, for each compound, five or more inhibitor concentrations were used and the inhibitory activity determined at each concentration (in triplicate); the IC_{50} was then determined from a graph (using linear regression analysis) of the inhibitory activity versus log[I].
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10. Quantum Cache Project Leader is a trademark of Oxford Molecular Ltd (Fujitsu), Oxford Science Park, Oxford, UK.