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Synthesis and biological evaluation of a series of substituted benzyl imidazole-based compounds as potential anti-bacterial agents

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Objective The use of azole based compounds in the treatment of fungal infections is well documented (Martin 1999). In the search for novel antifungal agents, we undertook the design and synthesis of substituted benzyl imidazole-based compounds as potential 14α-demethylase inhibitors. As expected, these compounds were found to be good anti-fungal agents and their mode of action was found to be due to the ligation of the haem moiety (and therefore inhibition) of 14α-demethylase. However, we also considered the potential use of these compounds as anti-bacterial agents. Here, we report the synthesis and evaluation of these compounds as potential anti-bacterial agents.

Method In the synthesis of the compounds, the azole functionality was reacted with a substituted benzyl bromide in the presence of a suitable base, e.g. anhydrous potassium carbonate. For example, in the synthesis of N-1-benzyl-imidazole (**1**): imidazole (2 g, 29.4 mmol) was added to anhydrous potassium carbonate (1.02 g, 7.34 mmol) and anhydrous tetrahydrofuran (THF) (50 mL). The mixture was stirred at room temperature for 10 min before the addition of benzyl bromide (2.51 g, 14.7 mmol). The mixture was then stirred under reflux for 24 h. After filtration, the THF was removed under vacuum to leave a yellow solid that was dissolved in dichloromethane (DCM) (40 mL) and washed with water (3 × 50 mL). The organic layer was then extracted using hydrochloric acid (2 M, 3 × 30 mL) followed by water (2 × 50 mL). The combined acid layer was neutralised with solid saturated sodium bicarbonate and then extracted into DCM (2 × 40 mL). The combined DCM layer was washed with water (3 × 50 mL), dried over anhydrous magnesium sulphate and filtered. Removal of DCM under vacuum gave 1 as a yellow solid (0.93 g, yield 40%) (m.p. 71.5–72.5 °C). ν(max) (Film) cm^{-1}: 3387.3 (NCN imidazole), 2938.9 (CH aliphatic); δH ($CDCl_3$): 7.46 (1H, s, NCHN imidazole), 7.18 (5H, m, ArH), 7.00 (1H, s, NCH imidazole), 6.81 (1H, s, NCH imidazole), 5.02 (2H, s, $PhCH_2$); δC ($CDCl_3$): 137.43 (NCN), 129.78, 128.97, 128.24, 127.26 (ArC), 136.18, 119.30 (ImC), 50.76 ($PhCH_2$); GCMS tR 8.24 mins. m/z 158 (M^+), 91 (base peak). The synthesised benzyl imidazole-based compounds were screened for minimum inhibitory concentration (MIC) using *in vitro* agar diffusion and broth dilution assay against a range of bacteria, including *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus mirabilis* and *Bacillus subtilis*.

Results From the results of the initial screening against the range of bacteria, we observe that the compounds are good biostatic compounds—all of the compounds considered here were found to inhibit the growth of the range of bacteria considered within this study. In general, the compounds were found to inhibit *E. coli* at or below 5 μM, whilst the same compounds inhibited the growth of *S. aureus* at or below 10 μM. As such, these compounds are good lead compounds in the design of further potent azole-based anti-bacterial agents.

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The synthesis and evaluation of a range of non-steroidal inhibitors of the type 3 isozyme of 17β-hydroxysteroid dehydrogenase (17β-HSD) through the derivatisation of the steroid backbone

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Objective The type 3 isozyme of the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD3) is responsible for the conversion of the C(17) = O group of androgens to the reduced hydroxy moiety, and is responsible for the formation of the potent androgens. 17β-HSD3 is now a potential target in the fight against hormone-dependent prostate cancer; however, no crystal structure exists for this enzyme. In the initial design of novel inhibitors of 17β-HSD3, we concluded that compounds that are able to mimic the steroid structure would possess inhibitory activity. More specifically, we concluded that groups able to mimic the steroidal C(17) = O group would be expected to act as a substrate and would therefore undergo the reduction step. Here, we report the initial results of: the derivation of the transition-state (TS) of 17β-HSD3 for the forward reduction reaction (Penning 1997), the synthesis and biochemical evaluation and the rationalisation of the observed inhibitory activity using the TS.

Method The proposed inhibitors were synthesised involving the initial oxidative ring cleavage of the steroid backbone, leading to a ‘keto-acid’, which was derivatised to the ester functionality involving the reaction with a range of alcohols so as to give the appropriate ester. The reactions proceeded in good yield and without any major problems (typically, the yield for the ring cleavage reaction was 80%, whilst for the esterification reaction it was 60%). In an effort to show that the C(17) = O was important in the inhibition process, we reduced the C(17) = O with sodium borohydride to give the C(17)-OH functionality. The synthesised compounds were then evaluated for inhibitory activity against 17β-HSD3 using modified literature procedures (Lota et al 2006).

Results Consideration of the initial screening data shows that the compounds are, in general, weak inhibitors of 17β-HSD3. For example, the most potent compound is the pentyl ester (namely, 3-(3a,6-dimethyl-3,7-dioxo-dodecahydro-cyclopenta[a]naphthalen-6-yl)-propionic acid pentyl ester) possessing an inhibitory activity of 29% at [I] = 100 μM. On evaluating the C(17) reduced form of this compound, and subsequently evaluating it against 17β-HSD3, we discovered that the compound possessed greatly reduced inhibitory activity against 17β-HSD3. As such, the initial results of this study would appear to support our previous hypothesis, in particular, that compounds which are able to mimic the C(17) = O group within the steroid substrate may act as potential inhibitors of 17β-HSD3. Furthermore, using the TS for 17β-HSD3, we propose that compounds containing large alkyl chains undergo steric hindrance with the active site corresponding to the C(3) area of the steroid backbone and which results in a decrease in inhibitory activity, as such, the design of further potent novel inhibitors of 17β-HSD3 would be required to reduce the steric interaction.

Conclusion The results of our study show that there is some validity to our initial hypothesis that in the inhibition of 17β-HSD3, mimicking the steroid C(17) = O group aids the inhibition process; as such, we have provided a range of good lead compounds.

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Synthesis, biochemical evaluation and molecular modelling of a range of 4-substituted phenyl alkyl azoles as inhibitors of 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$)

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Objectives The biosynthesis of androgens from C_{21} steroids is catalysed by a single enzyme and involves two distinct steps. The initial 17α-hydroxylation is undertaken by the 17α-hydroxylase (17α-OHase) component to give the 17α-hydroxylated intermediate; the cleavage of the C(17)-C(20) bond is carried out by the 17,20-lyase component, resulting in the formation of the appropriate androgen. No crystal structure exists for 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$); however, in our efforts we have developed a novel technique (the substrate-haem complex (SHC) approach), which we have utilised to produce a representation of the active site of the overall enzyme complex (Ahmed 1995). In an effort to add support to the SHC, here, we report the initial results into the synthesis of a range of compounds based upon 4-substituted phenyl alkyl azoles, their biochemical evaluation and molecular modelling of inhibitors both components.

Method The synthesis of the final azole compounds was achieved through the *N*-alkylation of the azole using the appropriate 4-substituted phenyl alkyl bromide

and a suitable base. In general, the reactions proceeded in good yield and no major problems were encountered. However, in the case of the larger alkyl chain containing compounds, where the alkyl chain was greater than propyl, it was necessary to synthesise the appropriate 4-substituted phenyl alkyl bromide. The biochemical evaluation of the synthesised compounds was undertaken using standard literature assay procedure (Li et al 1996).

Results The results show that the compounds were, in general, equipotent or more potent than the standard compound for $P450_{17\alpha}$, namely ketoconazole (KTZ); the most potent imidazole-based inhibitor, 4-fluorophenyl heptyl imidazole ($IC50 = 57.5 \pm 1.5$ nM against lyase and $IC50 = 173.62 \pm 0.1$ nM against 17α-OHase), was found to be ~29 and ~22 times more potent than KTZ ($IC50 = 1660 \pm 150$ nM against lyase and $IC50 = 3760 \pm 10$ nM against 17α-OHase). The 4-bromophenyl pentyl imidazole compound was found to possess an IC50 value of 58.1 ± 5.2 nM against lyase and an IC50 value of 500.0 ± 40 nM against 17α-OHase, and was therefore more potent than KTZ. Modelling the compounds onto the SHC for $P450_{17\alpha}$ shows that the synthesised compounds are able to fit within the active site without undergoing any unfavourable steric interactions and adds further support to the requirement of both a polar-polar interaction between the inhibitor and the active site, as well as the Fe-azole interaction. On consideration of the physicochemical properties of the inhibitors, we find a good correlation between the calculated log of the partition coefficient (logP) (between octanol and water) and the IC50 values for the inhibitors with an optimum logP of 3.5. Indeed, we suggest that whilst the ability of the 4-substituted phenyl moiety to interact with the active site is an important factor, the ability of the inhibitors to mimic the logP value of the substrate is of even greater importance in determining the overall inhibitory activity.

Conclusion The compounds synthesised within this study are excellent lead compounds in the design (using the SHC approach) of further novel inhibitors of $P450_{17\alpha}$.

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Synthesis of ester derivatives of pregnenolone in the mapping of the active site of 17α-hydroxylase corresponding to the C(3) area of steroidal back bone

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Objective The enzyme complex 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) is responsible for the conversion of C_{21} progestins and pregnanes to the corresponding C_{19} androgens and requires both NADPH and oxygen in the sequential oxidative steps. $P450_{17\alpha}$ catalyses both the hydroxylation of the C_{21} steroids, via the 17α-hydroxylase (17α-OHase) component, followed by the cleavage of the C(17)-C(20) bond, via the 17,20-lyase (lyase) component. No crystal structure exists for $P450_{17\alpha}$; however, it has been proposed that two binding sites exist at the active site (Ahmed 1995). In an attempt to discover more specific information regarding the active site of 17α-OHase component, in particular, the available conformational space about the area of the active site corresponding to the steroid C(3) position, we considered the synthesis of ester derivatives of pregnenolone. Here, we report the synthesis of the target compounds, their biochemical evaluation and the rationalisation of their inhibitory activity.

Method For 17α-OHase activity (in comparison with ketoconazole (KTZ)), rat testicular microsomal suspension was thawed under cold running water, and vortexed. The final incubation assay mixture (1 mL) consisted of sodium phosphate buffer (50 mM, pH 7.4, 905 μL), radiolabelled progesterone as substrate (1.5 μM, 15 μL), NADPH-generating system (50 μL) and inhibitor (10 μM or 100 μM, 20 μL). Tubes were warmed to 37°C for 5 min and the assay initiated by the addition of microsomal enzyme (final concentration 0.16 mg/mL, 10 μL) and vortexed. The assay mixture was incubated for 15 min. The reaction was quenched by the addition of ether (2 mL), vortexed and placed on ice. The organic layer was then removed and placed into a separate tube. The assay mixture was further extracted with ether (2×2 mL), and the organic layers combined. The solvent was removed under a stream of nitrogen, acetone (30 μL) was added to each tube and the solution spotted onto silica-based TLC plates along with carrier steroids (progesterone, 17α-hydroxyprogesterone, testosterone and androstenedione, 5 mg/mL). The TLC plates were developed using the mobile phase DCM:ethyl acetate (7:3). The separated spots were identified under UV light and each spot cut out and placed into scintillation vials. Acetone (1 mL) and scintillation fluid (Optiscint HiSafe) (3 mL) were added to each vial, vortexed and counted for 3 min for ^3H.

Results The results show that the esters are much weaker inhibitors than KTZ—the most potent inhibitor was found to be the acetyl derivative, which was found to possess 30% inhibition at inhibitor concentration of 10 μM in comparison to KTZ, which was found to possess 70% inhibition under similar conditions. Modelling the inhibitors onto the substrate haem complex (SHC) for 17α-OHase shows that the area about the active site corresponding to the C(3) area of the steroidal backbone is indeed limited and that groups larger than the propyl results in a large increase in steric hindrance and therefore a decrease in inhibitory activity.

Conclusion The compounds have proved to be good compounds in mapping the active site of 17α-OHase and have shown the conformational space within this area of the active site to be limited.

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