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The Design of Novel 17β-Hydroxysteroid Dehydrogenase Type 3 Inhibitors

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Abstract

17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) is expressed at high levels in the testes and seminal vesicles but has also been shown to be present in prostate tissue, suggesting its potential involvement in both gonadal and non-gonadal testosterone biosynthesis. The role of 17β-HSD3 in testosterone biosynthesis makes this enzyme an attractive molecular target for inhibition by small molecule inhibitors for the treatment of prostate cancer.

Here we report the design of selective inhibitors of 17β-HSD3 as potential anti-cancer agents. Due to 17β-HSD3 being a membrane-bound protein a crystal structure is not yet available. A homology model of 17β-HSD3 has been built to aid structure-based drug design. This model has been used with docking studies to identify a series of lead compounds that may give an insight as to how inhibitors interact with the active site. Compound **1** was identified as a potent selective inhibitor of 17β-HSD3 with an IC_{50} = 700 nM resulting in the discovery of a novel lead series for further optimization. Using our homology model as a tool for inhibitor design compound **5** was discovered as a novel potent and selective inhibitor of 17β-HSD3 with an IC_{50} ~ 200 nM.

Keywords: Hydroxysteroid dehydrogenase; 17β-HSD3; Prostate; Cancer

1. Introduction

The 17β-hydroxysteroid dehydrogenases (17β-HSDs) are a family of enzymes that catalyse the oxido-reduction of 17β-alcohol or 17-keto groups on steroids using NAD(P)H or NAD(P) as cofactor (Poirier, 2003). These enzymes catalyse the final step in male and female sex hormone biosynthesis. In humans 15 17β-HSD isozymes have been characterised, 17β-HSD types 1-4 and 6-15 belong to the short-chain dehydrogenase/reductase (SDR) family (Lukacik et al., 2006; Moeller & Adamski, 2006; Luu-The et al., 2008). The 17β-HSD5 isozyme, however, belongs to the aldo-keto reductase (AKR) family (Penning et al., 2000).

Prostate cancer is the second most common cancer in males in western countries. As 679,000 new cases are diagnosed each year it represents a therapeutic area with a great unmet medical need. The androgens testosterone (T) and dihydrotestosterone (DHT) are hormones that play an important role in the development of prostate cancer, benign hyperplasia, acne, baldness and hirsutism. Regulation of androgen biosynthesis or its action on the androgen receptor (AR) is central to the management of prostate cancer (van Bokhoven et al., 2003). 17β-HSD3 is expressed at high levels in the testes and seminal vesicles but has also been shown to be present in prostate tissue, suggesting its potential involvement in both gonadal and non-gonadal testosterone biosyntheses. The role of 17β-HSD3 in testosterone biosynthesis makes this enzyme an attractive molecular target for small molecule inhibitors for the treatment of prostate cancer.

17β-HSD3 catalyses the formation of testosterone from androstenedione (Laplante & Poirier, 2008). 17β-HSD2 catalyses the reverse reaction and oxidises testosterone to the weaker androgen androstenedione. It has been indicated that expression of 17β-HSD3 mRNA increases over 30-fold in cancerous prostate biopsies (Koh et al., 2002). Inhibition of 17β-HSD3 should therefore reduce prostate tumour growth. 17β-HSD3 inhibitors may also have potential as male anti-fertility agents.

The 17β-HSD3 isozyme uses NADPH as a co-factor in the reductive direction. The cDNA encodes a microsomal protein of 310 amino acids with an apparent molecular mass of ~ 35kDa (Moghrabi & Andersson, 1998). 17β-HSD3 is a microsomal enzyme which is bound through the *N*-terminal transmembrane domain to the endoplasmic reticulum (Lukacik *et al*., 2006).

Recently some potent selective inhibitors have been reported by Schering-Plough and Bristol-Myers Squibb (BMS) (Fig.1) (Guzi *et al*., 2004; Fink *et al*., 2006). Efficacy studies, using SCH-451659, in cynomologus monkeys showed promising results, leading to a 50% reduction in serum testosterone levels, an 85% decrease in testicular testosterone levels and a 20% decrease in prostate weight after 4 weeks dosing at 15 mg/kg, BID, p.o. (Pachter *et al.*, 2005). These results

illustrate proof of concept in higher mammals, showing that inhibition of 17β-HSD3 is a valid target for the treatment of prostate cancer.

Insert Figure 1

The design of inhibitors of 17β-HSD3 as anti-cancer agents is an active area of our research (Vicker et al., 2007). Due to 17β-HSD3 being a membrane-bound protein a crystal structure is not available. Crystallisation is difficult as the protein has to be removed from the membrane prior to crystallisation, which can alter the tertiary structure. Stabilisation of the protein tertiary structure by adding detergents is a method sometimes used to crystallise such proteins, but to date this has not been successful for 17β-HSD3. The sequence of 17β-HSD3 is known; therefore it is possible to build a homology model using a protein from the SDR super-family with high sequence identity. Homology models will obviously not give as accurate a depiction of the active site as a protein crystal structure, but a model can give a broad idea as to how inhibitor compounds, when docked, may fit in the active site. A homology model of 17β-HSD3 has thus been built to aid structure-based drug design and competitor inhibitors from Schering-Plough and BMS have been docked into it. This model has been used to identify a series of lead compounds, for which docking studies give an insight as to how these novel compounds may interact with the active site. Compound **1** was identified as a potent and selective inhibitor of 17β-HSD3 with an IC_{50} = 700 nM, resulting in the discovery of a novel lead series for further optimisation. Using our homology model as a tool for inhibitor design compound **5** was identified as a novel potent inhibitor of 17β-HSD3 with an IC_{50} ~ 200 nM. Compound **5** shows an encouraging profile *in vitro* and initial *in vivo* studies indicate inhibition of androstenedione-stimulated tumour growth in a mouse model being developed in house (Day et al., 2008).

2. Methods

Compounds **1-5** were synthesised from substituted anilino-diphenylethers via a reductive amination reaction as shown in Schemes 1 and 2 (Vicker et al., 2007). Compounds **1** and **2** were prepared using some novel in-house methodology by a reductive amination reaction with anilines and ketones using microwave chemistry as indicated below (Bailey et al., 2006). The general method for the synthesis of **1** and **2** is outlined below.

To a solution of aniline (1.00 mmol) and ketone (2.00 mmol) in dichloroethane (2.00 mL) in a microwave vial, was added $NaBH(OAc)_3$ (2.50 mmol) and AcOH (3.00 mmol). The vial was capped and the resulting solution was heated in a CEM Discover® microwave for 10 minutes at 140 °C. The power was set at 300 Watts and the pressure in the vial was variable during the reaction. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL) and the mixture then extracted with DCM (3 x 15 mL). The combined organics were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography (eluting with DCM) gave the desired products **1** and **2**.

Insert Scheme 1

1-(4-[2-(4-Chloro-phenoxy)-phenylamino]-piperidin-1-yl)-ethanone (1).

Mp. 129-130 °C, LCMS: tr= 5.05 min (50 % MeOH in water at 0.5 mL/min), m/z M+H 345.40, HPLC: tr= 2.45 min (90 % acetonitrile in water at 1.0 mL/min), >99 %, ^{1}H NMR (CDCl3, 270 MHz): δ 1.23-1.37 (2H, m, CH2), 2.01-2.14 (5H, m, CH2, CH3), 2.80-2.90 (1H, m, CH2), 3.13-3.23 (1H, m, CH2), 3.48-3.52 (1H, m, CH), 3.72-3.78 (1H, m, CH2), 4.00 (1H, s, NH), 4.37-4.42 (1H, m, CH2), 6.64 (1H, td, J = 6.4, 1.2 Hz, ArH), 6.74 (1H, dd, J = 8.2, 1.5 Hz, ArH), 6.80 (1H, dd, J = 6.4, 1.5 Hz, ArH), 6.84-6.88 (2H, m, ArH), 7.03 (1H, td, J = 7.4, 1.5 Hz, ArH), 7.21-7.27 (2H, m, ArH). ^{13}C NMR (CDCl3, 101 MHz): δ 21.4 (CH3), 31.9, 32.7, 40.2, 45.0 (CH2), 49.6 (CH), 112.2, 117.2, 118.6, 119.5, 125.2 (ArCH), 127.8 (ArC), 251, 129.7 (ArCH), 138.7, 142.9, 156.0 (ArC), 168.8 (CO). Elemental analysis: Calcd for C_{19} $H_{21}ClN_2O_2$: C 66.18, H 6.14, N 8.12%. Found C 66.0, H 6.06, N 7.90%.

1-(4-[2-(2, 4-Chloro-phenoxy)-phenylamino]-piperidin-1-yl)-ethanone (2).

Mp. 144-145 °C, LCMS tr= 5.40 min (50 % MeOH in water at 0.5 mL/min), m/z M+H 379.41, HPLC tr= 2.65 min (90% acetonitrile in water at 1.0 mL/min), 99 %, ^{1}H NMR (CDCl3, 270 MHz): δ 1.27-1.43 (2H, m, CH2), 2.4-2.11 (5H, m, CH3, CH2), 2.84-2.94 (1H, m, CH2), 3.15-3.25 (1H, m, CH2), 3.42-3.55 (1H, m, CH), 3.74-3.78 (1H, m, CH2), 4.06-4.08 (1H, m, NH), 4.35-4.43 (1H, m, CH2), 6.60-6.66 (1H, m, ArH), 6.72-6.77 (2H, m, ArH), 6.80 (1H, d, J = 2.5 Hz, ArH), 7.01-7.09 (1H, m, ArH), 7.13 (1H, dd, J = 8.9, 2.5 Hz, ArH), 7.44 (1H, d, J = 2.5 Hz, ArH). ^{13}C NMR (CDCl3, 101 MHz): δ 21.4 (CH3), 31.8, 32.6, 40.1, 44.9 (CH2), 49.5 (CH), 112.3, 117.0, 118.6, 119.5, 125.3, 127.9 (ArCH), 128.5 (ArC), 130.3 (ArCH), 138.2, 142.9, 151.4 (ArC), 168.8 (CO). Elemental analysis: Calcd for C_{19} $H_{20}Cl_2N_2O_2$: C 60.17, H 5.32, N 7.39 %, Found C 60.2, H 5.57, N 7.22%.

Seven membered ring ketones are less reactive than six membered ring ketones and also have added flexibility. Transformations that work on six membered rings do not always translate to their seven ring analogues. The microwave chemistry was unsuccessful for the synthesis of the seven membered ring expanded analogues and an alternative method was designed to access this novel series. For the synthesis of **3** and **4** traditional reductive amination chemistry was employed; however, reaction times were prohibitively long and a different method was used in the synthesis of **5**. The methods for the synthesis of **3-5** and their characterisation data are outlined below.

Insert Scheme 2

1-(4-[2-(4-chlorophenoxy)phenylamino]azepan-1-yl)ethanone (3)

To a solution of 2-(4-chlorophenoxy)phenylamine (113 mg, 0.51 mmol), 1-acetylazepan-4-one (159 mg, 1.02 mmol) and acetic acid (153 mg, 2.55 mmol) in DCE (4 ml), was added sodium triacetoxyborohydride (270 mg, 1.28 mmol). The reaction mixture was allowed to stir at room

temperature for 10 days. The reaction was quenched with saturated aqueous sodium sodium bicarbonate (15 mL) and extracted with ethyl acetate (2 x 15 mL). The combined organics were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography using gradient elution with 9:1 hexane:ethyl acetate to ethyl acetate gave **3** (67.1 mg, 37%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.49-2.27 (9H, m, 3 x CH_2, CH_3), 3.30-3.72 (5H, m, 5 x CH), 4.10 (1H, br s, NH), 6.59-6.67 (2H, m, Ar-H), 6.78-6.89 (3H, m, Ar-H), 7.00-7.08 (1H, m, Ar-H), 7.21-7.24 ppm (2H, m, Ar-H). LCMS: M^+H: 359.45; HRMS: $C_{20}H_{24}ClN_2O_2$, requires 381.1340, found 381.1344.

The same methodology was applied to the synthesis of **1-(4-[2-(4-chlorophenoxy)phenylamino]azepan-1-yl)ethanone** (**4)** to give a light brown oil in 40% yield. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.42-2.27 (9H, m, 3 x CH_2, CH_3), 3.30-3.72 (5H, m, 5 x CH), 4.10 (1H, br s, NH), 6.57-6.70 (2H, m, Ar-H), 6.72-6.85 (2H, m, Ar-H), 7.00-7.17 (2H, m, Ar-H), 7.43 ppm (1H, t, J = 2.5 Hz, Ar-H). LCMS: M^+H: 393.45; HRMS: $C_{20}H_{23}Cl_2N_2O_2$, requires 393.1131, found 393.1131.

1-Acetyl-5-[2-(4-Chloro-phenoxy)-phenylamino]-2,3,4,5-tetrahydro-benzo[b]azepine (5).

To a mixture of 1-butyloxycarbonyl-2,3-dihydro-1H-quinolin-4-one (0.050 g, 0.25 mmol) and 2-(4-chlorophenoxy)-aniline (0.060 g, 0.27 mmol, 2.2 eq.) in toluene (5 mL) was added chlorotriisopropoxytitanium(IV) (0.3 mL, 2 eq.) and the resulting deep orange solution stirred at room temperature overnight. Saturated $NaHCO_3$ solution (10 mL) was added and the phases separated. The organic layer was separated dried over anhydrous magnesium sulphate then filtered and evaporated. The residue was re-dissolved in THF (25 mL) and cooled to 0 ^oC under nitrogen. A solution of succinic acid (0.189 g, 1.6 mmol) in THF (5 mL) was added followed by 1M borane tetrahydrofuran complex (1.6 mL, 2 eq.). The reaction was allowed to warm to room temperature before the addition of saturated $NaHCO_3$ solution (100 mL). The volatile solvent was

removed under reduced pressure then ethyl acetate (100 mL) was added and the layers separated. The organic layer was dried, evaporated and the residue purified by column chromatography (Flashmaster II, 50 g column) using 0-30% ethyl acetate/hexanes with gradient elution to give **5** (42 mg, 42%) as a white solid. Mp. 55-58^{o}C, LRMS (EI^{+}) *m/z* 429.47 (M^{+} + Na, 100%); HRMS (EI) calcd. for $C_{24}H_{23}ClN_2O_2$ (M^{+}+H) 407.1521, found 407.1523; HRMS (FAB^{+}) calcd for $C_{24}H_{23}ClN_2O_2$ (M^{+}+H) 406.14 found 406.30. ^{1}H NMR (270MHz, $CDCl_3$) δ 1.56-1.70 (2H, Broad m, CH_2), 2.01-2.18 (4H, Broad m, CH_3 and 1H from CH_2), 2.76 (1H, t, J = 7.91Hz, CH_2), 4.37-4.85 (3H, Broad m, CH and CH_2), 6.16 (1H, d, J = 7.2Hz, NH), 6.55-6.65 (1H, Broad m, CH), 6.77-6.89 (2H, Broad m, 2CH), 6.94-7.00 (2H, Broad m, 2CH), 7.73-7.94 (7H, Broad m, 7CH). ^{13}C NMR (270MHz, $CDCl_3$) δ 23.01 (CH_3), 25.87, 34.28, 45.93 (CH_2), 54.43 (CH), 122.35, 117.33, 118.64, 118.85, 119.28, 125.23, 125.96, 128.02 (CH), 128.10 (C), 128.56, 129.86 (CH), 138.89, 139.72, 139.96, 142.69, 156.02, 169.71 (C). Elemental analysis: Calcd for $C_{24}H_{23}ClN_2O_2$; C 70.84, H 5.70, N 6.88% Found C 70.83, H 5.68, N 5.71%.

The inhibition of 17β-HSD3 by compounds **1-5** was measured *in vitro* using a 293-EBNA-based cell line with stable expression of transfected human 17β-HSD3, 293-EBNA[HSD3], (Vicker et al., 2007; Day et al., 2008a). The results are shown in Table 1. The selectivity of compounds **1-5** over 17β-HSD1 and 17β-HSD2 was measured using established methods (Day et al., 2006) and compounds **1-5** were inactive on these isozymes. The observed selectivity is not unexpected as there is relatively low sequence identity between the 17β-HSDs and their active site residues vary considerably.

Insert Table 1

3. Homology Model Construction and Molecular Modelling

A phylogenetic tree (Breitling et.al., 2001) was used as an evolutionary guide to the most closely related proteins to 17β-HSD3 to identify potentially useful protein templates from which to build a homology model. It was observed that the 17β-HSD family is split into two distinct branches; the first branch contains types 1, 2, 6, 7 and 9; and the second branch contains types 3, 4, 8 and 10 and so this is the branch of interest.

The crystal structure of 7α-hydroxysteroid dehydrogenase (Tanaka et al., 1996), PDB code 1FMC (Berman *et al*., 2000), was selected as the template from which to build the homology model, as sequence alignment indicated this SDR had the highest sequence identity with 17β-HSD3 and was in the same branch of the phylogenetic tree as 17β-HSD3. This was confirmed by a BLAST analysis (Altschul et al., 1997) comparing the sequence of 17β-HSD3 with 1FMC and 1FDT (17β-HSD1) with scores of 51.2 and 43.5 respectively. From the binary complex the monomeric A unit from the protein was used in construction of the model. The sequence alignment was generated using ClustalX (Thompson et al., 1997) gap only columns having been removed. From this alignment the WhatIf homology modelling package (Vriend, 1990) was used to produce the initial model.

The homology model was refined initially using YASARA (Krieger et al., 2002) using the following methodology. Hydrogen atoms were added using the add hydrogen command and terminal groups were modified so that they could be recognised by the YASARA program. The system was optimised in several stages. In the first stage, the system was minimised with the atoms of the backbone atoms constrained. Minimisation was performed using iteratively the NOVA and AMBER force fields as implemented in YASARA. This minimisation step was necessary to remove any steric clashes which may have existed between the side chain atoms and the backbone whilst still protecting the secondary and tertiary structures from damage by misplaced or clashing side chain groups. After three cycles of this process the backbone loop regions were freed and a further three minimisations were performed. This allowed the loop

regions to adapt to any strain caused by the insertion or deletion of residues without disruption to the secondary and tertiary structure. The SYBYL 7.1 program was used to add the cofactor and a rapid minimisation was performed with fixed backbone atoms to allow the protein to adapt to the presence of the cofactor. A final minimisation was performed using the AMBER FF99 force field as implemented in SYBYL 7.1. No constraints were imposed on any of the atoms during this minimisation as this programme is more tolerant of highly strained and sterically clashing situations and is therefore gentler in the treatment of such systems. Once the system was relaxed out of its initial high energy environment SYBYL 7.1 could be employed to perform minimisations. After each minimisation calculation a PROCHECK (Laskowski et al., 1993) calculation was performed to assess the quality of the model and to ensure that it was improving; the Ramachandran plot obtained after the last minimisation stage is shown in Fig. 2.

The minimised protein structure was then subjected to simulated annealing calculations within SYBYL 7.1, the system was subjected to 15 cycles of simulated anneals with heating to 500° K for 1000 fs, followed by cooling to 100° K for 1500 fs. This process was performed to move the protein out of any high energy local minima to lower energy states. This process generated a series of structures from which the single homology model was developed. These structures were then examined and five representative structures were chosen based on the calculated energy of the structure and the Ramachandran plots. These five structures were minimised using SYBYL 7.1 and the AMBER FF99 force field. These optimised structures were used to dock several compounds from a Schering-Plough patent (Guzi *et al.*, 2004). The docking was performed using version 2 of the GOLD package (Jones et al., 1997). Each ligand was docked into the active site of our structures a total of 30 times. The final structure was selected by visual examination of the inhibitor-ligand complexes obtained from this procedure. These inhibitors are known to be potent against 17β-HSD Type 3. For this to be the case the compounds must firstly be able to fit into the active site as well as to bind strongly to it. These were the guidelines used to choose a single

protein model which was subjected to a further series of simulated annealing calculations, this time with SCH-451659 in the active site. The system underwent 15 cycles of simulated anneals with heating to 500° K for 1000 fs, followed by cooling to 100° K for 1500 fs. These calculations were performed in SYBYL 7.1 using the AMBER FF99 force field.

The results of the simulated annealing were inspected with reference to both the quality of the protein structure and the conformation of the cofactor and ligand. A single structure was then selected and minimised once again using SYBYL 7.1 and the AMBER FF99 force field. The Ramachandran plot, which gives an indication of the quality of the model, for the final minimised system is depicted in Fig. 2. The coordinates of the model are available on request from the corresponding author.

Insert Figure 2

It was observed that there is potentially a strong pi-stacking interaction between Phe205 and one of the aromatic rings of SCH-451659 (not shown). Also, with some movement there could additionally be a further pi-stacking interaction between the second aromatic ring and Tyr212. Also, from the docking a hydrophobic interaction with Val213 and an aromatic ring is observed. The *t*-butyl group occupies a lipophilic pocket formed by Ile148, Phe151, Trp153 and Leu252 which could explain the improved activities observed when lipophilic groups of increasing size are placed in this region.

As a final test of the model for use as an aid in hit identification a 17β-HSD3 inhibitor from BMS (Fig1. 1.) was also docked into this homology model to ensure that the active site was not overly optimised for the Schering-Plough type compound and would accept non-related inhibitors. The docking was again performed using the GOLD docking package. The ligand was docked into the active site a total of 30 times.

The homology model below (Fig. 3.) indicates that the substrate binding site in the protein is highly hydrophobic in nature. Key hydrophobic residues are indicated in grey. The aromatic rings

on the diphenyl ether group in our inhibitors may potentially form pi-pi interactions with Phe205 as well as other hydrophobic interactions with residues such as Val213 and Leu252. The homology model was then used as a tool in structure-based drug design by docking potential target molecules to discover novel 17β-HSD3 inhibitors

Insert Figure 3

Compound **3** (left above in grey) is depicted docked into the active site of the homology model with some key hydrophobic residues highlighted (magenta). In a similar fashion compound **5** (right above in grey) is also shown in which a hydrophobic region is occupied by the fused benzo ring. The acetyl group in compounds **3** and **5** is in the region of the nicotinamide group of the cofactor (green) where hydrogen bond interactions are likely. The aromatic ring of the benzazepine in compound **5** may have additional π-stacking effects with Trp153, Phe151 and Tyr159.

4. Results and Discussion

The development of 17β-HSD3 inhibitors is at a relatively early stage and no clinical candidates have been reported. Both Schering-Plough and BMS have reported potent selective non-steroidal inhibitors as promising leads (Guzi *et al.*, 2004; Fink *et al.*, 2006). Based on highly preferred structures from the Schering–Plough patents a pharmacophore was constructed and used in virtual screening of the Maybridge database to discover potential lead templates for further modification. The pharmacophore consisted of hydrophobic aromatic groups based on the diphenylmethylene moiety, a hydrophobic group based on the *t*-butyl substituent and a distal hydrogen bond acceptor representing the *N*-acetyl moiety. This resulted in the identification of a potential hit with a diphenylether 'hydrophobic head' that could mimic the diphenylmethylene moiety in SCH-451659 and related structures (Scheme 3). Key features of SCH-451659 are

thought to be the aromatic hydrophobic head, a functionalised spacer or linking group and a hydrogen bond acceptor distal from the hydrophobic head.

Insert Scheme 3

From the structure of SCH-451659 and our database hit, compound **1** (Scheme 1) was proposed as an initial target. It was very encouraging that compound **1** showed a good inhibition of 17β-HSD3 with an IC_{50} = 770 nM and was selective over both 17β-HSD1 and 17β-HSD2 making it a and novel promising new lead. Further substitution in one of the aromatic rings of the diphenylether with a chlorine gave **2** which resulted in a ~ 3 fold loss of activity with an IC_{50} = 2.4 μM. However the loss was not dramatic and indicates that further substitutions in the hydrophobic head are tolerated. When compound **1** was docked into our homology model (not shown) it was clear that there was additional space available in the active site which could be exploited to achieve further inhibitor interactions the region of the piperidine moiety. As the active site is very hydrophobic in nature (Fig. 3) an initial proposed change was to ring expand compound **1** to give compound **3**. This change would give a more flexible 7-membered ring system and add some more hydrophobicity to the molecule. Compound **3** had an IC_{50} = 1.8 μM and is ~ 2 fold less potent than **1** and thus shows similar potency. Importantly, the ring expansion from piperidine to azepine is tolerated and gives rise to another novel series of 17β-HSD3 inhibitors for further optimisation. Substitution in one of the aromatic rings of the diphenylether with a chlorine to give **4** resulted in a compound with ~ 2 fold more activity than **3** with an IC_{50} = 900 nM. This again showed that substitution in the hydrophobic head is tolerated and this may be a way to design compounds with different physicochemical properties. When compound **3** was docked into our homology model (Fig. 4) it was apparent that there was scope for substitution on the azepine ring to potentially give rise to further interactions with lipophilic residues in the active site. Docking studies (Fig. 4) indicated that fusion of a benzene ring to the azepine may be capable of filling space in the active site region to achieve such interactions in a similar fashion to

the *t*-butyl group in SCH-451659. Indeed, compound **5** proved to be a potent selective inhibitor of 17β-HSD3 with an IC_{50} ~ 200 nM in our whole cell assay, giving a 9 fold improvement over compound **3**. This result is very encouraging as docking to the homology model had given an insight as to the structural modifications required to improve a ligand's potency and hence showed the usefulness of the model as an aid to structure based drug design.

5. Conclusions

We have built and tested a 17β-HSD3 homology model that can, at least partially, explain the trends in activity displayed for both Schering-Plough and BMS compounds as 17β-HSD3 inhibitors. A new series of novel potent and selective inhibitors of human 17β-HSD3 has been discovered by the application of structure based drug design encompassing pharmacophore generation, database mining, homology model construction and docking. Compound **5** shows an encouraging profile *in vitro* and initial *in vivo* studies indicate inhibition of androstenedione-stimulated tumour growth in a mouse model (Day et al., 2008). Further lead optimisation is currently ongoing in this promising series.

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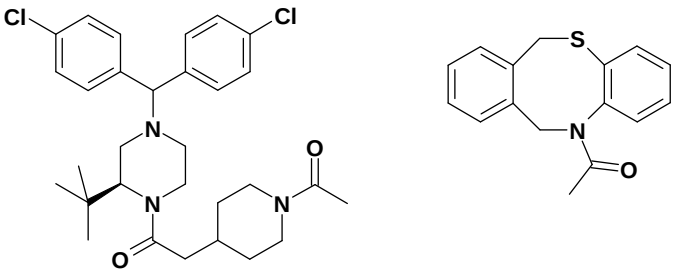
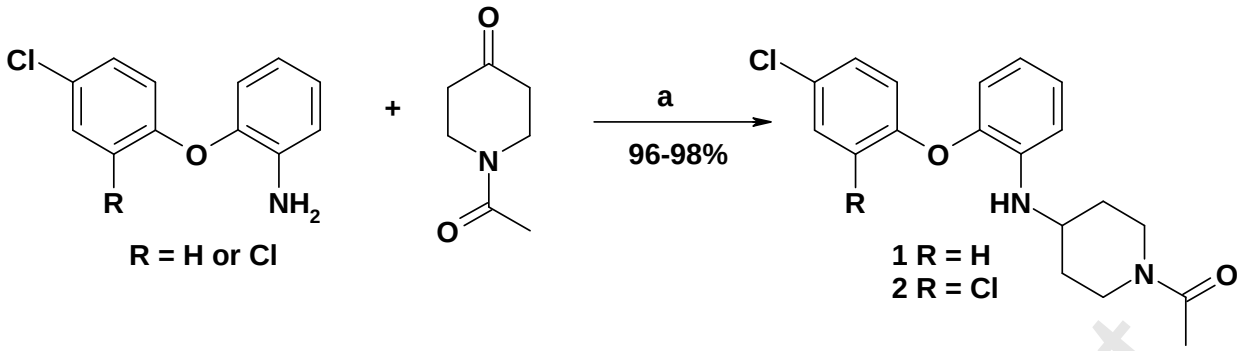
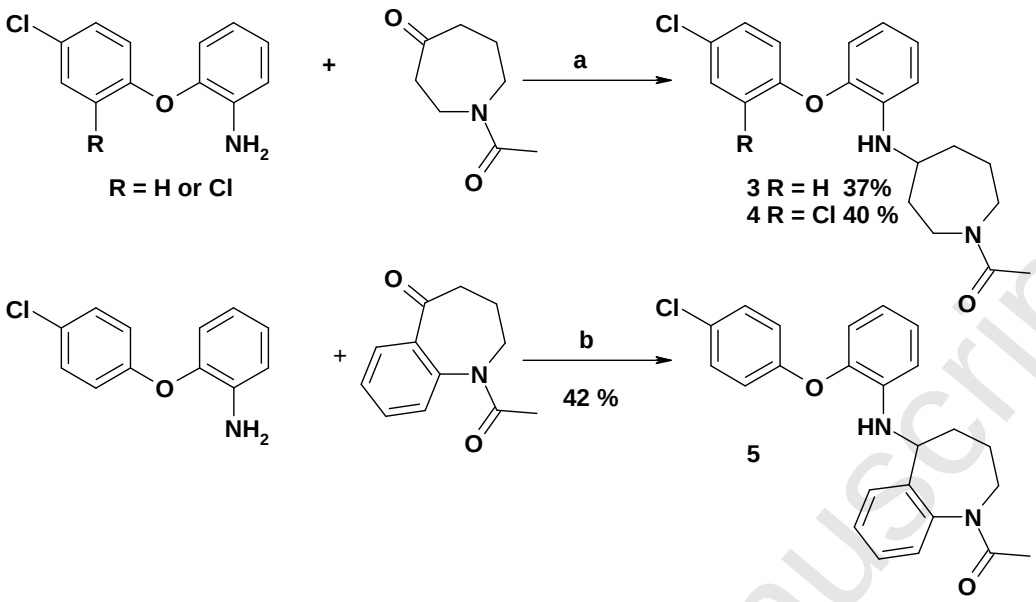


SCH-451659

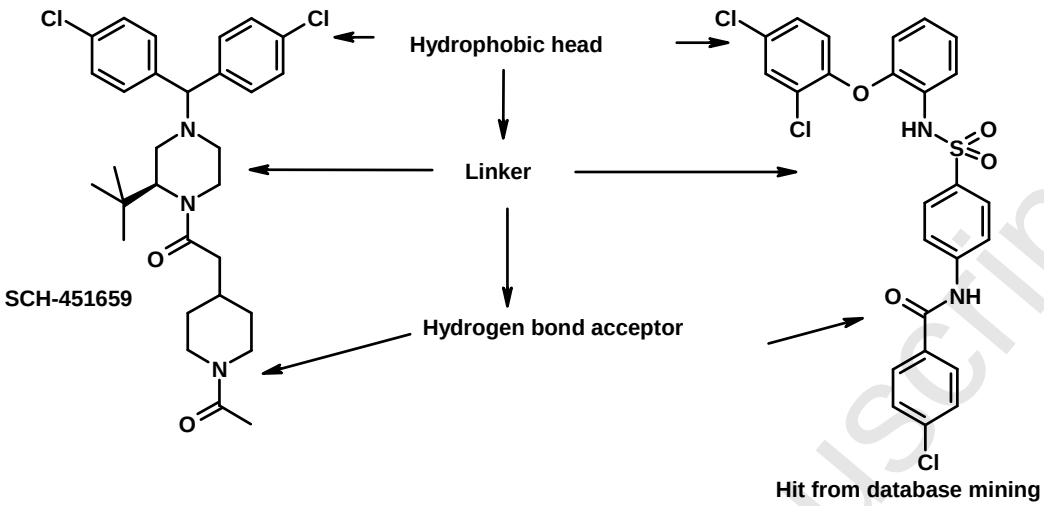
Fig.1. Structures of Schering-Plough (SCH-451659) and BMS inhibitors of 17β-HSD3.



Scheme 1. Synthesis of substituted piperidine compounds **1** and **2**. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, Dichloroethane, µW 140°C 300 W for 10 min.

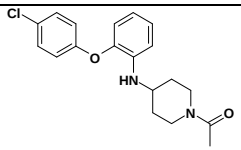
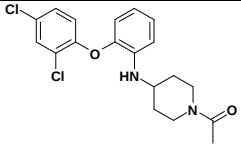
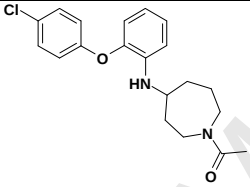
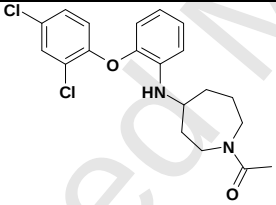
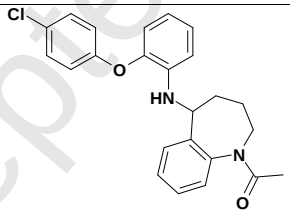


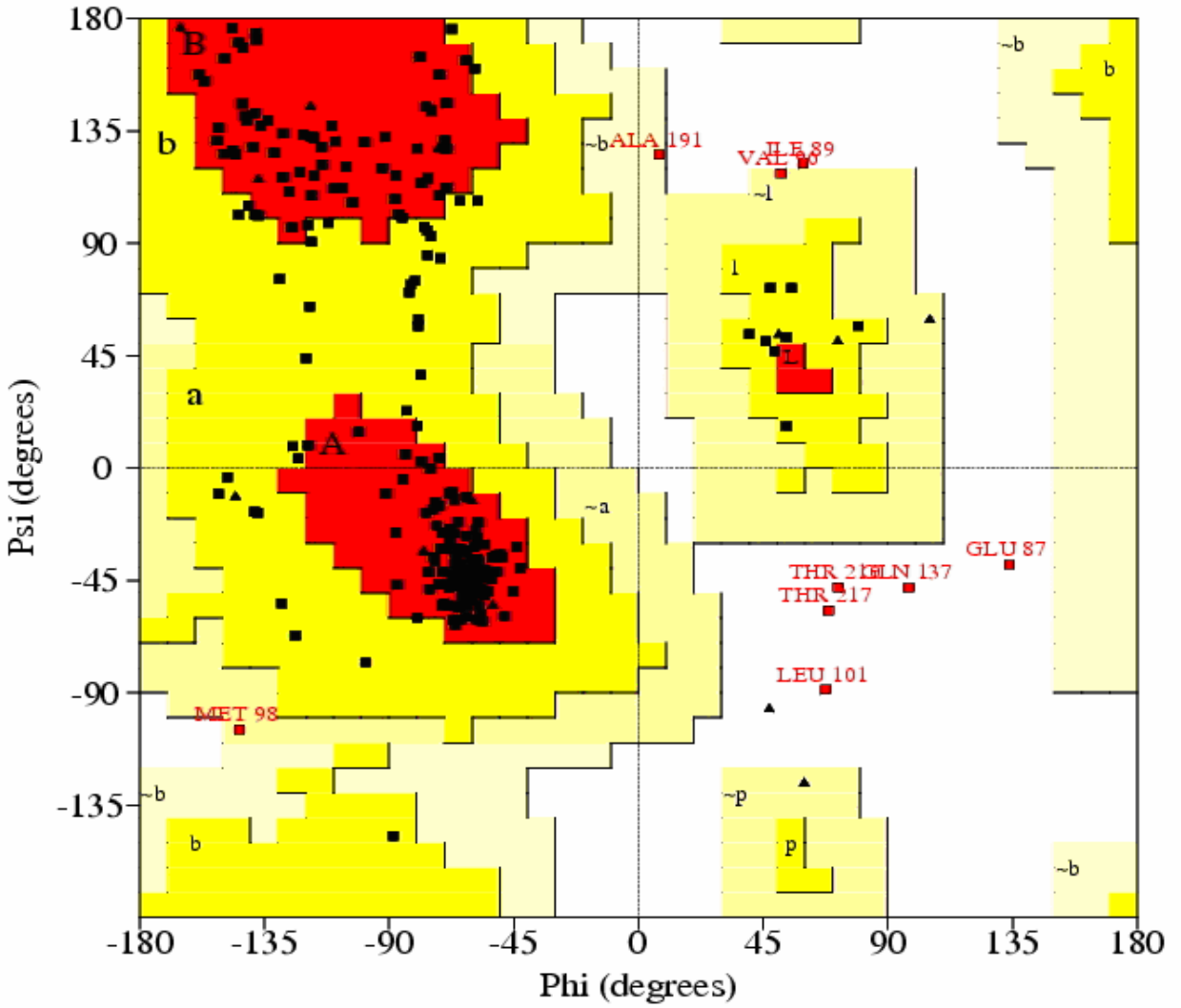
Scheme 2. Synthesis of substituted azepine compounds **3**-**5**. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, Dichloroethane, room temperature for 10 days. (b) $TiCl(OiPr)_3$, toluene followed by 1M BH_3 in THF.



Scheme 3. Structure of the hit compound from database mining.

Table 1. Inhibition of 17β-HSD3 by compounds **1-5** in human 293-EBNA[HSD3] cells.

Compound	Structure	IC_{50} 293-EBNA [HSD3] cells (nM)
1		**770**
2		**2400**
3		**1800**
4		**900**
5		**200**



Plot statistics

Residues in most favoured regions [A,B,L]	190	79.2%
Residues in additional allowed regions [a,b,l,p]	41	17.1%
Residues in generously allowed regions [~a,~b,~l,~p]	3	1.2%
Residues in disallowed regions	6	2.5%
Number of non-glycine and non-proline residues	240	100.0%
Number of end-residues (excl. Gly and Pro)	3	
Number of glycine residues (shown as triangles)	17	
Number of proline residues	8	
Total number of residues	268	

Figure 2: Ramachandran plot for the final homology system.

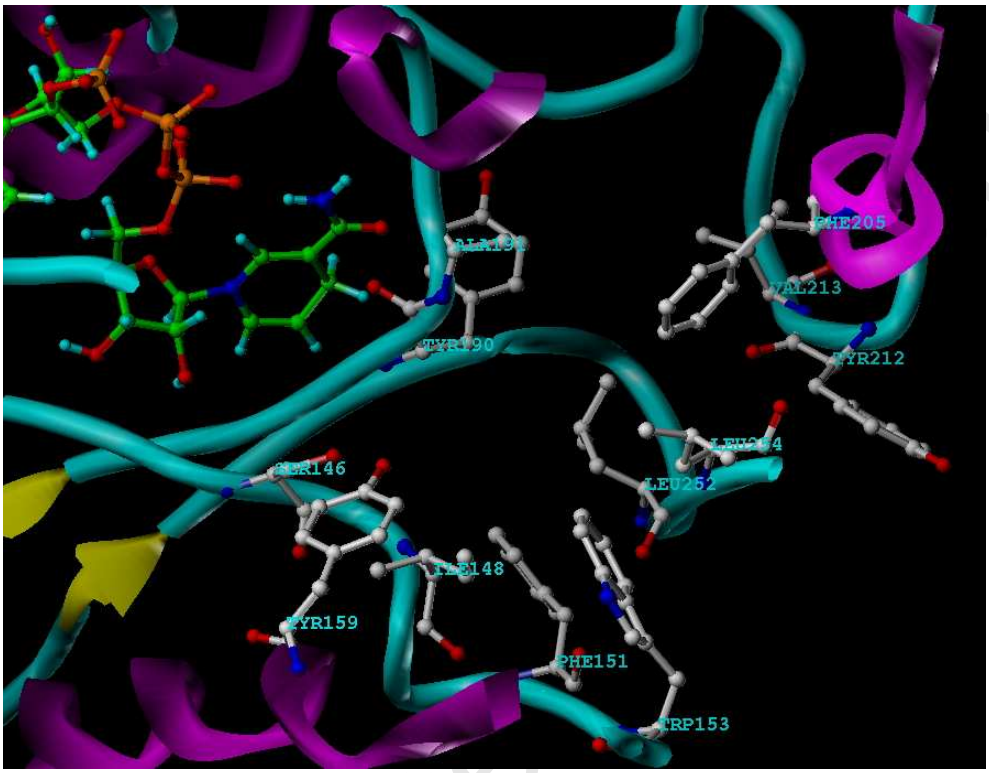
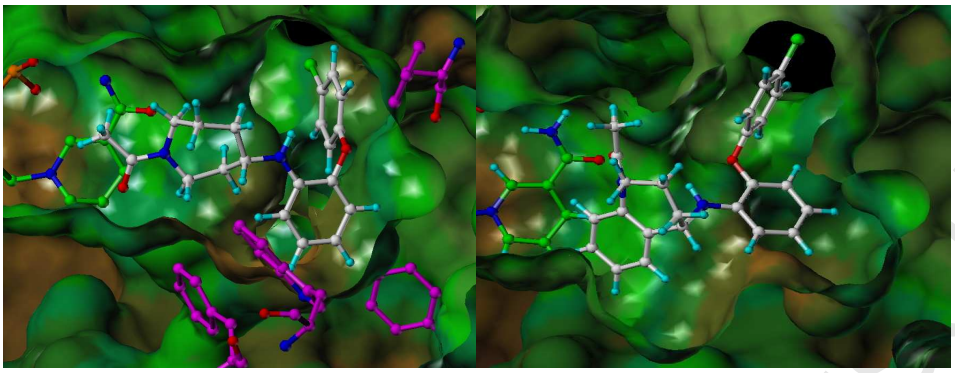


Fig. 3. Active site of 17β-HSD3 of the homology model with hydrophobic residues in grey and the nicotinamide residue of the cofactor in green.



Panel (a) Panel (b)

Fig. 4. Panel (a) and panel (b) depict compounds **3** and **5** docked in the active site of the homology model of 17β-HSD3.