Design and validation of specific inhibitors of 17β-hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis

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Abstract

17β-Hydroxysteroid dehydrogenases (17β-HSDs) are enzymes that are responsible for reduction or oxidation of hormones, fatty acids and bile acids in vivo, regulating the amount of the active form that is available to bind to its cognate receptor. All require NAD(P)(H) for activity. Fifteen 17β-HSDs have been identified to date, and with one exception, 17β-HSD type 5 (17β-HSD5), an aldo–keto reductase, they are all short-chain dehydrogenases/reductases, although overall homology between the enzymes is low. Although named as 17β-HSDs, reflecting the major redox activity at the 17β-position of the steroid, the activities of these 15 enzymes vary, with several of the 17β-HSDs able to reduce and/or oxidise multiple substrates at various positions. These activities are involved in the progression of a number of diseases, including those related to steroid metabolism. Despite the success of inhibitors of steroidogenic enzymes in the clinic, such as those of aromatase and steroid sulphatase, the development of inhibitors of 17β-HSDs is at a relatively early stage, as at present none have yet reached clinical trials. However, many groups are now working on inhibitors specific for several of these enzymes for the treatment of steroid-dependent diseases, including breast and prostate cancer, and endometriosis, with demonstrable efficacy in in vivo disease models. In this review, the recent advances in the validation of these enzymes as targets for the treatment of these diseases, with emphasis on 17β-HSD1, 3 and 5, the development of specific inhibitors, the models used for their evaluation, and their progress towards the clinic will be discussed.

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Introduction

Steroid-dependent diseases

Breast cancer, a major cause of death in both European and American women, occurs most frequently in post-menopausal women. After menopause, a low level of oestrogen is produced, mainly from the local conversion of androstenedione (Adione; Fig. 1a) to oestrone (E1; Fig. 1b) by aromatase in adipose and normal and malignant breast tissues, despite the cessation of ovarian function. This oestrogen has a crucial role in supporting the growth of hormone-dependent breast cancer in these women. Much of the E1 formed by aromatase is stored in an inactive form as E1 sulphate, which can be reactivated within breast cells by steroid sulphatase (Stanway et al. 2006). Presently, various methodologies are used in the clinic to inhibit the stimulation of hormone-dependent breast cancer by oestrogen. These include oestrogen receptor (ER) antagonists, such as tamoxifen (Heel et al. 1978, Jordan 2003), and inhibitors of steroid synthesis, such as letrozole, an aromatase inhibitor (Bhatnagar et al. 1990, Bhatnagar 2006, Scott & Keam 2006) or more recently 667-COUMATE, a steroid sulphatase inhibitor (Purohit et al. 2003, Stanway et al. 2006, 2007).

Treatment of hormone-dependent prostate cancer by androgen ablation is initially usually successful, reducing primary tumour burden and increasing 5-year...
survival rates. Presently, androgen ablation is achieved using various approaches that include orchidectomy, androgen receptor (AR) blockers such as bicalutamide (Fradet 2004), agonists of luteinising hormone-releasing hormone such as goserelin (Akaza 2004), finasteride, an inhibitor of 5α-reductase, the enzyme that converts testosterone (Fig. 1d) to the more active androgen, 5α-dihydrotestosterone (DHT; Fig. 1e) (Rittmaster 1997), or a combination of the above.

However, prostate cancer is the third highest cause of cancer-related death in men, because tumours are often unnoticed for several years, presenting at an advanced stage in older men, by which time they have often progressed to hormone independency. At present, there are many efforts to understand the mechanisms by which prostate cancer cells develop hormone independence. These are thought to include AR up-regulation, an adaptation to the low levels of androgen present during ablation therapy or mutations to the pathways involved in the activation of the AR, such as mutations in the receptor itself, or in its co-regulators, allowing enhanced activation by the low-level androgens (Mizokami et al. 2004) or ligands other than androgens to activate the proliferative pathways (Rau et al. 2005, Pienta & Bradley 2006). It has also been suggested that many cases of recurrent androgen-independent prostate cancer may not actually be independent of androgen signalling, as high levels of testosterone and DHT have been found in the prostates of patients with recurrence during ablation therapy, suggesting that surgical or chemical castration treatments may not result in complete removal of active androgens, and that in situ formation of testosterone from adrenal production of androgens may continue (Titus et al. 2005).

Endometriosis is one of the most common causes of pelvic pain and infertility in women. In this condition, endometrial tissue grows abnormally outside the uterus, often in locations such as the ovaries, fallopian tubes and abdominal cavity. It causes adhesions and scarring, pain and heavy bleeding, and can damage reproductive organs, interfere with ovulation and inhibit implantation of the embryo. Although the specific causes of endometriosis are still undetermined, it appears that inheritable defects (Barlow & Kennedy 2005) allow for the peritoneal implantation and survival of endometrial tissue displaced by retrograde menstruation, a process initially proposed by Sampson (1927).

Treatments for endometriosis include the use of contraceptives, progestins and gonadotrophin-releasing hormone analogues (Farquhar 2007) to inhibit menstruation, a source of much of the pain associated with endometriosis, and to suppress the growth of the endometriotic tissue. Additional treatments are also necessary to relieve the effects of endometriosis, including drugs such as clomiphene citrate to improve fertility, and non-steroidal anti-inflammatory drugs (NSAIDs) and other analgesics to relieve the pain. However, although these treatments provide relief from the symptoms of endometriosis, none provides a cure, and those which alter hormone balance can result in side effects such as hot flushes, weight gain and acne.

The clinical success of inhibitors of steroid synthesis or action in both hormone-dependent breast cancer (including tamoxifen, letrozole and 667-COMUATE) and hormone-dependent prostate cancer (including bicalutamide, goserelin and finasteride) has provided justification for this approach in the treatment of these cancers. As more is becoming understood about the altered expression of steroidogenic enzymes in endometriosis, and also in endometrial cancer, it is envisaged that inhibitors of steroid action may also have a role in the clinical treatment and possible

Figure 1  Steroid structures. (a) 4-Androstene-3,17-dione (Adione; with carbon positions numbered), (b) oestrone (E1; with ring positions labelled), (c) 17β-oestradiol (E2), (d) testosterone, (e) 5α-dihydrotestosterone (DHT), (f) 5-androstene-3β,17β-diol (Adiol) and (g) dehydroepiandrosterone (DHEA).
future cure of diseases of the endometrium (Hompes & Mijatovic 2007).

In all of these diseases, the clinical effect of the inhibition of the one step of hormone activation remains to be investigated (Purohit et al. 2006). This is the reduction of the steroids at the 17β-position, catalysed by specific 17β-hydroxysteroid dehydrogenases (17β-HSDs), to form the active steroid that binds to its specific receptor to stimulate cell proliferation. In oestrogenic activation, this results in the formation of active oestradiol (E2; Fig. 1c) from E1, and to a lesser degree, the production of androstenediol (Adiol; Fig. 1f) from dehydroepiandrosterone (DHEA; Fig. 1g). In androgenic activation, 17β-HSDs reduce Adione to form testosterone, and this is finally metabolised by 5α-reductase enzymes (Tindall & Rittmaster 2008) to form DHT, the active androgen.

17β-HSDs

17β-HSDs are enzymes that are responsible for reduction or oxidation of hormones, fatty acids and bile acids in vivo. All require NAD(P)H for activity. Fifteen 17β-HSDs have been identified to date, and with one exception, 17β-HSD type 5 (17β-HSD5), an aldo–keto reductase (AKR), they are all short-chain dehydrogenases/reductases (SDRs).

The major substrates for these enzymes are hormones, and the reduction or oxidation of hormones by 17β-HSDs regulates the amount of active steroid available to bind to a particular receptor. Although named as 17β-HSDs, reflecting the major redox activity at the 17β-position of the steroid, several of the 17β-HSDs are able to convert multiple substrates at multiple sites, such as at the 3 position on the steroid ring. Most also have bidirectional capabilities, catalysing either the oxidative or reductive reaction in the presence of NAD(P)⁺ or NAD(P)H respectively, but in vivo appear to function unidirectionally. Although they are generally of a similar size (250–350 amino acids) and contain highly conserved motifs, such as those within the Rossman fold, overall homology across the 17β-HSDs is low (Duax et al. 2000, 2005, Lukacik et al. 2006) and the intracellular location of the enzymes is diverse. Different 17β-HSDs have been found specifically in the cytosol (17β-HSD1), microsomes (17β-HSD3), mitochondria (17β-HSD10) and peroxisomes (17β-HSD4), and many have specific expression patterns across tissues and organs. These observations, along with kinetic studies, have demonstrated that although the enzymes have multifunctional capabilities, most have preferential substrate usage and directionality in vivo.

The 17β-HSDs: nomenclature, substrate profile and expression patterns

17β-HSD type 1 (17β-HSD1/HSD17B1; Fig. 2a) is the most well characterised of the 17β-HSDs and catalyses the reduction of E1 to form active E2 (Miettinen et al. 1996, Peltoketo et al. 1996), and also the reduction of DHEA to form Adiol (Lin et al. 2006). The reverse of these reactions, the inactivation of E2 to E1 and Adiol to DHEA, as well as that of testosterone to Adione, is mediated by 17β-HSD2 (HSD17B2; Fig. 2). 17β-HSD2 also activates 20α-progesterone to form progesterone but may also be involved in the oxidation of retinoids (Zhongyi et al. 2007). 17β-HSD3 (HSD17B3; Fig. 2b) is expressed in the testes and catalyses the reduction of Adione to testosterone (Luu-Thai et al. 1995). It is not the only 17β-HSD thought to be responsible for the formation of testosterone in vivo, as 17β-HSD5 (AKR1C3; Fig. 2c), which is expressed more ubiquitously, forms the testosterone produced in other steroidal tissues, such as prostate, breast, ovary and endometrium (Pelletier et al. 1999, Ji et al. 2005). However, 17β-HSD5 is a multifunctional enzyme, with additional 3α- and 20α- steroid reductase activities, including the conversion of DHT to 3α-androstenediol (Penning et al. 2000), and progesterone to 20α-hydroxyprogesterone (Dufort et al. 1999). It is also known as prostaglandin (PG) F synthase (PGFS), its 11-ketoreductase activity preferentially reducing PGD2 to 9α,11β-PGF2 (Matsuura et al. 1998), although it also forms 9α,11α-PGF2 (PGF₂α) from PGH₂ (Komoto et al. 2004, Penning et al. 2006). These 17β-HSDs, 17β-HSD1, 17β-HSD2, 17β-HSD3 and 17β-HSD5, will be discussed in greater detail in later sections of this review.

The 17β-HSD4 enzyme (HSD17B4), also known as peroxisomal multifunctional protein-2 (MFP2), is a 736 amino acid protein of ~79 kDa. It comprises an N-terminal dehydrogenase domain, and a larger C-terminal domain, of around 79 kDa. It comprises both hydratase and lipid carrier moieties (Breitling et al. 2001, Huysge et al. 2006). Although it can have E₂ oxidoreductase activity (Adamski et al. 1995), in vivo it is involved in peroxisomal fatty acid β-oxidation (Breitling et al. 2001), with defects in its expression causing d-specific MFP deficiency (Huysge et al. 2006). It is present in many tissues, with highest concentrations in the liver, heart, prostate and testis, and is up-regulated in prostate cancer (Zha et al. 2005).

17β-HSD6, initially in humans designated as 3(α→β)-hydroxysteroid epimerase (also known as RoDH-like 3α-HSD/RL-HSD), is a 36 kDa enzyme with both oxidoreductase and epimerase activities involved in androgen catabolism. The oxidoreductase
activity can oxidise 3α-Adiol to form DHT (Bauman et al. 2006a), while the epimerase activity can convert androsterone (ADT) to epiandrosterone (Huang & Luu-The 2000, 2001, Belyaeva et al. 2007). Despite the major, and possibly the sole, site of action of this enzyme being at the 3 position of the androgens, it is classified as 17β-HSD6 in humans as it is 71.4% homologous to rat hsd17b6 (Huang & Luu-The 2000) which does oxidise steroids at the 17 position (Biswa & Russell 1997). Recently, data have indicated that polymorphisms in the HSD17B6 gene are associated with errors in androgen metabolism in polycystic ovary syndrome (PCOS; Jones et al. 2006).

When prolactin receptor-associated protein (PRAP; Duan et al. 1996) was also identified as 17β-HSD7, it was thought to be involved predominantly in the reduction of E1 to E2 (Nokelainen et al. 1998, Krazesien et al. 1999). However, sequence and promoter analysis indicate that the major role of this enzyme may be as a 3-ketosteroid reductase in cholesterol biosynthesis, reducing zymosterone at the 3 position to form zymosterol (Marijanovic et al. 2003, Ohnesorg et al. 2006). Despite this, a recent study has indicated that 17β-HSD7, along with 17β-HSD1, 17β-HSD5 and other steroidogenic enzymes, is significantly up-regulated in ovarian tissue of patients with ovarian endometriosis (Šmuc et al. 2007).

17β-HSD8, also known as FabG (beta-ketoacyl-[acyl-carrierprotein] reductase, E. coli)-like (FABGL), HZ-K region expressed gene 6 (HKE6) and ring finger protein 2 (RING2), is preferentially an oxidative enzyme. Although assays of the mouse enzyme in vitro have demonstrated that it can use both E2 and testosterone as substrates (Fomitcheva et al. 1998), sequence analysis again suggests that this enzyme may primarily be involved in the regulation of fatty acid metabolism (Pletnev & Duax 2005). It is highly expressed in murine kidney and spleen, with some expression in the ovary and testes, but is significantly down-regulated in mouse models of polycystic kidney disease (Fomitcheva et al. 1998). In humans, it is expressed in tissues including the liver, pancreas, kidney and skeletal muscle (Ando et al. 1996). A study of human genes expressed in the polymorphic human leukocyte antigen (HLA) region of chromosome 6 has indicated that 17β-HSD8 is also down-regulated in oral cavity tumour tissue compared with surrounding normal tissue (Reinders et al. 2007).

Mouse hsd17b9 has 17β- and 3α-HSD activities, recognising both steroids and retinols as substrates (Napoli 2001). Although it has closest homology to rat hsd17b6, it is more homologous to members of the retinol dehydrogenase family than to other 17β-HSDs (Su et al. 1999). A human 17β-HSD9 homologue has not been identified.

Human 17β-HSD10 was initially identified and named by several groups before its 17β-HSD activity and homology to 17β-HSD4 was recognised, resulting in its recategorisation as 17β-HSD10 (He et al. 1999). It is also

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**Figure 2** Activity of (a) 17β-HSD1, (b) 17β-HSD3 and (c) 17β-HSD5 (AKR1C3/PGFS/20α-HSD/3α-HSD2).
known as short-chain \( l\)-3-hydroxyacyl coenzyme A dehydrogenase II (SCHAD/HCD2/HADH2) and endoplasmic reticulum-associated binding protein (ERAB), as well as amyloid beta peptide-binding alcohol dehydrogenase (ABAD) and 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD). 17β-HSD10 is a mitochondrial protein, initially isolated from rat liver (Luo et al. 1995) and subsequently from other species and tissues such as human brain (He et al. 1998). It has a wide substrate profile (Nordling et al. 2001, Shafqat et al. 2003), being involved in isoleucine degradation, \( \beta \)-oxidation of fatty acids and oxidation of steroids, inactivating E2, but converting 5\( \alpha \)-androstanediol to DHT (Yang et al. 2005a,b). It has been implicated in the development of Alzheimer’s disease as it is overexpressed in neurones of patients with the disease, and associates with the neurotoxic peptide amyloid-\( \beta \) (Yan et al. 1997). It is expressed in other tissues including the prostate (Bauman et al. 2006a) and has been seen to be overexpressed in primary prostate cancer cell cultures (He et al. 2003).

17β-HSD11 (HSD17B11), also known as Pan1b, retSDR2 and DHR58, was initially isolated by a group attempting to find enzymes homologous to the 11β-HSDs (Li et al. 1998). It was found to have oxidative 17\( \beta \)-steroid activity, metabolising 5\( \alpha \)-androstan-3\( \alpha \),17\( \beta \)-diol to the less androgenic ADT; but despite also binding retinoids, it has no retinoid-metabolising activity (Brereton et al. 2001). It is expressed in both steroidogenic and non-steroidogenic tissues, including the pancreas, kidney, liver, lung, small intestine and heart, and the adrenal glands, ovary, endometrium and Leydig cells, and its expression can be down-regulated by the steroidogenic combination of cAMP with all-\textit{trans}-retinoic acid (Chai et al. 2003). However, a physiological role for the enzyme in lipid metabolism is implicated: agonists of peroxisome proliferator-activated receptor-\( \alpha \) (PPAR\( \alpha \)) induce a rapid increase in 17\( \beta \)-HSD11 expression in the endoplasmic reticulum and lipid droplets of mouse liver and intestine (Motojima 2004, Yokoi et al. 2007), and 17β-HSD11 has been identified as one of the three major proteins in lipid droplets of human liver cells that accumulate in fatty liver disease, along with adipose differentiation-related protein and acyl-CoA synthetase 3 (Fujimoto et al. 2004, 2006).

3-ketoacyl reductase (KAR), a protein in the membrane of the endoplasmic reticulum that uses NADPH to reduce 3-ketoacyl-CoA to 3-hydroxyacyl-CoA during the second step of fatty acid elongation (Moon & Horton 2003), has also been identified as 17β-HSD12 and has high homology to 17β-HSD3. Some recent studies have suggested that it may also be important in the reduction of E1 to form E2 (Luu-The et al. 2006, Blanchard & Luu-The 2007), although other studies have indicated that it does not efficiently catalyse this reaction (Day et al. 2008), despite its up-regulation in the tumours of breast cancer patients (Song et al. 2006). However, investigation of its expression in normal human tissues indicated that it is highly expressed in many active lipid-metabolising tissues, including liver, kidney, heart and skeletal muscle, as well as in placenta and testis, with additional expression in other steroidogenic tissues such as adrenal gland, ovary and prostate (Sakurai et al. 2006). This seems to support a primary role for 17β-HSD12 as a regulator of lipid biosynthesis.

Presently, little is known about 17β-HSD13, 14 and 15, as they have only recently been identified. 17β-HSD13 is found at 4q22.1 and is also known as short-chain dehydrogenase/reductase 9. It has 78% homology with 17β-HSD11, also located at 4q22.1, and is highly expressed in the liver, apparently within the cytoplasm (Liu et al. 2007). 17β-HSD14 was originally named retSDR3 as it was first discovered in the retina, and is also known as DHR10. It is a cytosolic enzyme with high levels of expression in the brain, liver and placenta, and can oxidise E2, testosterone and Andiol using NAD\(^+\) as cofactor (Lukacik et al. 2007). Transfection of human MCF-7 and SKBR3 breast cancer cell lines with 17β-HSD14 significantly decreased E2 levels, and in an RT-PCR study of 131 breast tumours, patients with ER-positive tumours that highly expressed 17β-HSD14 showed significantly better recurrence-free survival and breast cancer-specific survival prognoses (Jansson et al. 2006) indicating that 17β-HSD14 may well have a role in E2 inactivation \textit{in vivo}. 17β-HSD15, the most recently discovered of the 17β-HSDs, may play a role in androgen biosynthesis (Luu-The et al. 2008).

**Inhibition of 17β-HSD enzymes**

The selectivity of each of the 17β-HSD enzymes for their preferred substrates and directional redox activities, combined with their tissue-specific localisations, contributes greatly to the fine tuning of the endocrine system. This selectivity of action also suggests that many of the 17β-HSDs would provide good targets for modulation of the endocrine response in disease states, especially in those diseases in which they or other steroidogenic enzymes are being abnormally expressed.

Early work on inhibitors of these enzymes was reviewed by Penning & Ricigliano (1991), again by Penning (1996), and most recently and...
comprehensively by Poirier (2003). Several of these enzymes have now been validated as targets for the treatment of endocrine-related diseases. Progress in the design and development of inhibitors of specific 17β-HSD enzymes for use in the treatment of various disorders, including steroid-dependent diseases such as breast and prostate cancer, and endometriosis, has advanced greatly in recent years, and will be discussed in the course of the rest of this review.

17β-HSD1 inhibition

Application of 17β-HSD1 inhibitors in breast cancer and endometriosis

Oestrogens have a crucial role in supporting the growth of hormone-dependent breast cancer in post-menopausal women. Although both 17β-HSD1 and 17β-HSD2 are present in healthy pre-menopausal subjects, several studies have indicated that the ratio of 17β-HSD1 to 17β-HSD2 is increased in the tumours of post-menopausal patients with hormone-dependent breast cancer (Suzuki et al. 2000, Miyoshi et al. 2001). This results in an increased level of E2 that drives the proliferation of the tumour tissue via the ER. Several studies have indicated that patients with tumours that have high 17β-HSD1 expression have significantly shortened disease-free and overall survival (Gunnarsson et al. 2005, Salhab et al. 2006, Vihko et al. 2006), suggesting that compounds which inhibit the activity of this enzyme may be of therapeutic benefit in the treatment of hormone-dependent breast cancer in post-menopausal patients (Reed & Purohit 1999, Purohit et al. 2006, Sasano et al. 2006).

In endometriotic tissue, although data are conflicting, there seems to be a change in the expression of steroidal enzymes, resulting in the presence of a high concentration of E2 that stimulates proliferation of the tissue (Bulun et al. 2000). Aromatase, responsible for the formation of E1 from Adione, is negligible in normal endometrium, but is up-regulated in endometriotic tissue (Gurates & Bulun 2003). It has been suggested that the expression of 17β-HSD2, the enzyme that inactive E2 to E1, is down-regulated in endometriosis (Bulun et al. 2006), although in two studies of mRNA expression in the endometriotic tissue down-regulation of 17β-HSD2 was not seen (Matsuzaki et al. 2006, Carneiro et al. 2007). A recent study, however, indicated that there is a down-regulation of 17β-HSD2 mRNA expression in endometriotic samples, while both aromatase and 17β-HSD1 are up-regulated in comparison with normal endometrium (Dassen et al. 2007).

The down-regulation of 17β-HSD2 in endometriosis is thought to be due to a lack of progesterone receptor B (PR-B) expression and a very low level of PR-A expression in the endometriotic tissue, resulting in the resistance to progesterone, which in normal endometrium stimulates the expression of 17β-HSD2 (Bulun et al. 2006). In another recent RT-PCR study, the authors found no change in the expression of 17β-HSD2 mRNA between normal and endometriotic tissue (Šmuc et al. 2007), but did find an increase in the expression of 17β-HSD1, 17β-HSD7, steroid sulphatase and ERβ mRNA. It has also been suggested that there is a link between a 17β-HSD1 polymorphism, Ser312Gly, and endometriotic risk and severity (Tsuchiya et al. 2005). This polymorphism has previously been associated with higher E2 levels in some women (Setiawan et al. 2004).

Inhibitors of 17β-HSD1

As both hormone-dependent breast cancer and endometriosis are oestrogen-dependent diseases, with an increase in the ratio of 17β-HSD1 to 17β-HSD2 expression implicated in many studies, it has been suggested that this enzyme is a good target for inhibition in the treatment of both of these diseases. A recent 17β-HSD1 transgenic mouse study indicated that 17β-HSD1 is also capable of causing a significant amount of androgen activation in vivo, suggesting that 17β-HSD1 inhibitors may also have a role to play in women with diseases related to androgenic dysfunction (Saloniemi et al. 2007). There are now many different groups working to find selective inhibitors of 17β-HSD1.

Until recently, there have only been two major methods by which the activity and inhibition of 17β-HSD1 are assayed. In whole cells and lysates, as for many other steroidal enzymes, 17β-HSD1 activity is usually measured using radiometric assays, often with tritiated substrate at physiological concentrations (Singh & Reed 1991). Substrate and product require extraction, followed by separation by either thin layer chromatography (TLC) or reverse phase high performance liquid chromatography (HPLC). In assays using purified enzyme, however, the redox state of the cofactor, NAD(P)(H), can be used to determine the progress of the reaction using spectrophotometric measurement at 340 nm (Chin & Warren 1975). The radiometric TLC and HPLC methods allow for sensitive determination of changes in activity, but are very time consuming; whereas the spectrophotometric method, though less sensitive, is faster, but cannot be used for analysis of 17β-HSD1 activity in tissue...
samples as it requires purified enzyme. ELISA-based assays are also used for determination of steroid levels in blood and other tissues, but again there are problems with the sensitivity requirements for their use. Recently, novel methods have been developed to improve the determination of 17β-HSD1 activity. These include homogeneous proximity (Kokko et al. 2006) and fluorescence resonance energy transfer (FRET)-based assays (Kokko et al. 2007) for high throughput in vitro screening, and sensitive HPLC-based methods for tissue sample analysis, such as that of Delvoux et al. (2007), which can be used to determine the activity of several different steroidogenic enzymes in one tissue sample.

The elucidation of the crystal structure of 17β-HSD1 provided a good basis for the design of initial inhibitors. After optimisation of the crystallisation conditions for 17β-HSD1 (Zhu et al. 1993, 1994), a homodimer, structural data revealed a Rossman fold and an active site containing a Tyr-X-X-X-Lys sequence, both characteristic of short-chain dehydrogenases (Duax et al. 2000). 17β-HSD1 also contains three α-helices and a helix-turn-helix motif, which along with a histidine residue, influence active site availability and thus substrate specificity (Ghosh et al. 1995). When crystallised in the presence of E2 (Azzi et al. 2003), 17β-HSD1 also contains three α-helices and a helix-turn-helix motif, which along with a histidine residue, influence active site availability and thus substrate specificity (Ghosh et al. 1995). Crystallisation studies with the enzyme in complex with 20α-hydroxyprogesterone, DHT and DHEA (Lin et al. 1999, Han & Lin 2000, Han et al. 2000), indicated that although all of these steroids can be accommodated in the binding site of 17β-HSD1, they bind in the reverse orientation to E2 (Gangloff et al. 2003), and it is Leu149 that is involved in the discrimination between E2 and C19 steroids, resulting in a far greater affinity for E2. Val225 has been shown to act on the α-face of E2, in concert with Leu149 on the β-face, to sandwich the A-ring in place and sterically hinder binding of C19 steroids. The carboxamide group of NADPH forms a hydrogen bond with the peptidic amide group of Val188, stabilising the binding of the cofactor, but in C19 steroid complexes this bonding is disturbed, destabilising the ternary structure (Shi & Lin 2004).

Modelling studies of 17β-HSD1 place E2 in the same binding area within the cleft as the crystallographic data, with His221 and Glu282 forming hydrogen bonds to the C3-OH end of E2, and Ser142 and Tyr155 binding to C17-OH, and also interacting with the cofactor. These mechanisms of selectivity over C19 steroids, steric hindrance at the A-ring and hydrogen bonding of the hydroxyl groups at either end of the molecule, are also seen in the binding of E2 to ERα (Nahoum et al. 2003). Modelling has also confirmed that cofactor and substrate may bind in either order (Zhorov & Lin 2000), as was seen in early kinetic activity studies (Betz 1971).

Before 17β-HSD1 was successfully crystallised, the inhibitory potency of other steroids and similar non-steroidal compounds was assessed to study the binding characteristics and specificity of the enzyme (Blomquist et al. 1984). Substrate and cofactor analogues were developed to explore the involvement of amino acid residues of the active site in the binding mechanism. These included alkylating agents such as 3-chloroaacetylpuridine-adenine dinucleotide, an NAD+ analogue (Fig. 3a; Bielmann et al. 1976), 16z-bromoacetoxyestradiol 3-methyl ether, an E2 analogue (Fig. 3b; Chin & Warren 1975) and 6β-bromoacetoxyprogesterone, a progesterone analogue (Fig. 3c; Thomas & Strickler 1983). C16,C17-substituted pyrazole and isoxazole E1 derivatives (Fig. 3d) have also been shown to be competitive inhibitors of 17β-HSD1 (Sweet et al. 1991).
of flavonoids, the chalcones, also inhibit 17β-HSD1 and aromatase when a hydroxyl substitution is present on position 4 of the A-ring (e.g. 4-hydroxychalcone, IC_{50}=16 μM; Fig. 3g), equivalent to position 7 of the flavonoids (Le Bail et al. 2001). Phytoestrogens have also been found to inhibit fungal Cochliobolus lunatus 17β-HSD (Kristan et al. 2005, Sova et al. 2006), used as a model enzyme for the SDR family, as do cinnamic acid (Fig. 3h) and its derivatives (Gobec et al. 2004, Kristan et al. 2006, Sova et al. 2006), suggesting that they would be good inhibitors of 17β-HSD1. Two residues that appear crucial for binding of inhibitors in the fungal enzyme active site, Asn154 and Tyr212, can be matched by their counterparts, Arg258 and Tyr218, in 17β-HSD1. The cofactor NADP also occupies the same position in both enzymes. However, there are no residues in the fungal enzyme which correspond to Glu282 and His221 of human 17β-HSD1, and these appear to determine selectivity for 4′-hydroxyflavones.

Unfortunately, although many of these steroids and phytoestrogens are potent inhibitors of 17β-HSD1, they are not useful as therapeutic inhibitors as they are often oestrogenic, or are not specific for 17β-HSD1 inhibition, having inhibitory effects on other steroidal enzymes and receptors (Deluca et al. 2005). These include other 17β-HSDs, for example, 17β-HSD5 (Brožič et al. 2006); aromatase, as the 7-hydroxy group of the flavonoids mentioned above is also essential for aromatase inhibition (Le Bail et al. 2001); 3β-HSD (Arlt et al. 2004) and the ER, often resulting in stimulatory effects (Usui 2006, Turner 2007). However, as we have seen, their use has been invaluable in the understanding of the mechanism of catalysis and inhibition of 17β-HSD1.

From these studies, several important factors have been determined for the design of potent inhibitors: a planar hydrophobic ring core structure, such as E2, lacking a C19 group, to fit into the narrow hydrophobic binding region; β-oriented electron withdrawing groups to form hydrogen bonds with catalytically essential amino acids such as Tyr155 and Ser142; α-oriented hydrophobic groups at C17 or C16 to block the cofactor from binding; and the availability of space to accommodate substituents at the 7α-position of the steroid (Han et al. 2000, Owen & Ahmed 2004, Alho-Richmond et al. 2006).

A series of compounds designed as pure antioestrogens for the treatment of hormone-dependent breast cancer had additional 17β-HSD1 inhibitory properties in in vivo mouse studies (Labrie et al. 1992). The competitive inhibitors, steroidal derivatives possessing both a 7α-undecanamide group and either a halogen atom at C16 or a double bond at C14–C15 or

Figure 3 17β-HSD1 inhibition. (a) 3-chloroacetylpyridine adenine dinucleotide, (b) 16β-bromoacetoxy-E2 3-methyl ether, (c) 6β-bromoacetoxyprogesterone, (d) 16,17-pyrazole-D-isoxazole-E1, (e) equilin, (f) apigenin, (g) 4-hydroxychalcone, (h) cinnamic acid, (i) EM139, (j) 6β-(thiaheptanamide)-E2, (k) EM1745, (l) 16β-m-carbamoyl benzyl-E2, (m) C5′-pyridylethy lamide-16,17-pyrazole-E1, (n) STX1040 (2-ethyl-16β-m-pyridyl methyl amido-methyl-E1), (o) non-steroidal STX1040 mimic, (p) pyrimidinone core and (q) 3-benzyl-2-(2-bromo-3,4,5-tri methoxy-phenyl)-8-hydroxy-3H-benzo[4,5]thieno[2,3-d]pyrim idin-4-one.

Later, equilin (Fig. 3e), an equine oestrogen that is a major component of Premarin, used in hormone replacement therapy, was also shown to inhibit 17β-HSD1 (IC_{50}<1 μM), and the crystal structure of the 17β-HSD1 homodimer in a ternary complex with NADP^+ and equilin was solved (Sawicki et al. 1999). Equilin binds at the substrate binding site, with the substrate entry loop in a closed conformation. In the equilin complex, the 17-keto group is a greater distance from the C4 atom of the cofactor due to the C7=C8 double bond of equilin, and this results in inhibition of the catalytic hydride transfer.

Many flavonoids and other phytoestrogens also inhibit 17β-HSD1 (Mäkelä et al. 1998, Hoffrén et al. 2001). Flavonoids with a hydroxyl group in position 7 of the A-ring, which mimics the D-ring of steroids, such as apigenin, chrysins, genistein and naringenin, inhibit 17β-HSD1, apigenin being most potent (IC_{50}<1 μM; Fig. 3f; Le Bail et al. 1998). Derivatives
C15–C16, inhibited both E1-stimulated uterine growth, an anti-oestrogenic effect, and the conversion of E1 to E2, and A to T, in the mice, when dosed at concentrations as low as 3 μg twice daily. 17β-HSD1 was crystallised with one of these dual-site inhibitors, EM139 (Fig. 3i), which although larger than E2, binds at the same position (Zhu et al. 1999). These first dual-site inhibitors had only weak 17β-HSD1 inhibitory activity but were optimised with the aims of improving 17β-HSD1 inhibitory potency and specificity and reducing intrinsic oestrogenicity, while also maintaining the ER antagonist activity (Tremblay & Poirier 1998).

Many steroid derivatives, including those of E2, E1, progesterone and Adione substituted at C16 with reactive halogenated functional groups, such as bromoacetoxy and bromoacetamido groups, were found to be irreversible inhibitors of 17β-HSD1 as the halogenated group covalently bonds with an amino acid residue, permanently inactivating the enzyme (Poirier et al. 1998). Several oestratriene derivatives with fluorine substitutions at C17 inhibit 17β-HSD1 with micromolar potencies, but most have activities against other 17β-HSD enzymes, including types 2, 5 and 7 (Deluca et al. 2006). 16α-Iodopropyl and bromopropyl substituted E2 derivatives are potent inhibitors, with IC50 values of 420 nM (Sam et al. 1998) and 460 nM (Sam et al. 1998, Tremblay & Poirier 1998) respectively in assays using partially purified 17β-HSD1 from human placenta.

Reversible E2 derivatives with β-oriented thia-alkanamide side chains at C6 show enhanced inhibitory activity, with the most potent, 6β-(thiaheptanamide) E2 (Fig. 3j), having an IC50 of 170 nM in assays using 17β-HSD1 partially purified from human placenta (Poirier et al. 1998). This compound, however, was found to be oestrogenic, and attempts to improve it by removing the 3-hydroxy group, changing the 6β-substitution for a 6α-substitution, changing the amide group of the side chain for a methyl or changing the thio-ether for an ether bond, all reduced the potency of the compound, despite the ether bond improving the oestrogenic profile (Tremblay et al. 2005).

These structure-activity relationships (SAR) studies indicate that 17β-HSD1 potency and specificity may be optimised by the rational design of compounds that interact with both the cofactor-binding and substrate-binding regions of the enzyme. A series of E2 derivatives with 16β-propylaminoacyl substitutions were designed containing hydrophilic and hydrophobic moieties to interact with the cofactor- and substrate-binding regions of the enzyme respectively (Tremblay et al. 2001). Although these compounds are non-oestrogenic, having no interaction with the ER, they also fail to inhibit 17β-HSD1. Derivatives of gossypol, which potently inhibits lactate dehydrogenase by targeting the Rossmann fold, inhibit 17β-HSD1 in the micromolar range, also by binding in the Rossmann fold from the cofactor binding site across to the substrate-binding region (Brown et al. 2003). Active site modelling suggested that their potency and specificity may be improved by the incorporation of a substrate analogue into their structure.

E2-adenosine-based compounds were designed to specifically target both the substrate and cofactor binding sites. The most potent of these hybrid inhibitors is EM1745 (Fig. 3k), a reversible competitive inhibitor with an IC50 of 52 nM, in which the structure is linked to the adenosine moiety by a 16β-oriented side chain containing eight methylene groups, allowing it to bind to Leu96 and Val196 (Qiu et al. 2002). Two compounds, which each have only one of the components of EM1745, 16β-nonyl-E2 and 5-nonanoyl-o-adenosine, do not inhibit the enzyme (Poirier et al. 2005). Simplified versions of these hybrids, containing adenosine mimics to improve the stability and bioavailability of these compounds, are less potent than EM1745 (Bérubé & Poirier 2004), as are C16-substituted aryl E1 and E2 derivatives, such as 16β-benzyl E2, which has an IC50 of ~800 nM using purified enzyme (Poirier et al. 2006). However, further modification of this structure resulted in 16β-m-carbamoylbenzyl-E2, an inhibitor with an IC50 of 44 nM (Fig. 3l) in which the m-carbamoylbenzyl group mimics the nicotinamide ring of the cofactor. Although this compound is weakly oestrogenic (Laplante et al. 2008), its oestrogenic profile is improved by modifications at the C2, C3 and C7 positions; however, these substitutions lead to a decrease in potency.

Other inhibitors have also been designed to bind across the substrate binding site towards that of the cofactor by substituting the steroid scaffold at the C16 position, both alone and in combination with C2, C6 and C17 substitutions. One class of compounds resulting from this approach is the E-ring pyrazole amides (e.g. C5′-pyridylethylamide-16,17-pyrazole-E1; Fig. 3m), inhibitors that selectively inhibit 17β-HSD1 with submicromolar IC50 values in a whole cell assay (Fischer et al. 2005, Allan et al. 2006a). Of the others, which include C16-substituted alkenyl, alkyl and carboxyl, C6-oxo and C6 and C16 and C17-oxime E1 and E2 derivatives, the most active inhibitors are those containing an m-methylene carboxamide functionality extending from the C16β position (Lawrence et al. 2005, Allan et al. 2006b, Vicker et al. 2006).
The most potent of the \( m \)-methylene carboxamide compounds, with an IC\(_{50} \) of 27 nM in a whole cell assay, is STX1040 (2-ethyl-16\( b \)-\( m \)-pyridyl methyl amido-methyloestrone; Fig. 3n; Lawrence et al. 2005). Fig. 4 shows STX1040 docked in place of E\(_2\) in the 17\( \beta \)-HSD1 crystal structure, protein database (PDB) entry ‘1FDT’, in complex with NADP\(^+\) using the docking programme GOLD (Jones et al. 1997). The high potency of STX1040 may be explained by its interactions with the cofactor. In 1FDT, the nicotinamide carbonyl and amide nitrogen of the cofactor form hydrogen bonds with Val188 and Thr140 respectively. In the inhibitor complex, it appears that there may be an interaction between the nicotinamide amide moiety and the amide carbonyl of the 16\( \beta \) side chain. The pyridyl nitrogen of the 16\( \beta \) side chain may interact with an oxygen atom 3.16 Å away in the phosphate group of the cofactor. A C2 ethyl group, included to eliminate oestrogenicity as it interferes with hydrophobic interactions with the ER (Vicker et al. 2006), also contributes to inhibitory activity by interacting with Leu262 and Phe259. Hydrophobic interactions with Leu149, Val225, Phe226 and Phe259, observed when substrate is docked into the enzyme, are maintained in the inhibitor complex.

Non-steroidal E\(_1\) mimics are also active as 17\( \beta \)-HSD1 inhibitors (Allan et al. 2008). In these inhibitors, the E\(_1\) moiety is replaced by aryl ring-containing scaffolds that retain both a hydroxyl group equivalent to that at the 3 position of E\(_1\) and a ketone functionality at the 17-position equivalent. The most potent of these, with IC\(_{50}\) values of 3.7 and 1.7 \( \mu \)M in a whole cell assay, are the biphenyl ethanones and biphenyl indanones respectively. Substitution of the biphenyl ethanone scaffold to form mimics of STX1040 results in improved activity, with the most potent compound having an IC\(_{50}\) of 1.8 \( \mu \)M (Fig. 3o).

The potency of STX1040 was assayed using T47D cells to measure 17\( \beta \)-HSD1 inhibition, while the high selectivity of STX1040 for the 17\( \beta \)-HSD1 enzyme over 17\( \beta \)-HSD2 was determined using MDA-MB-231 cells, as these breast cancer cell lines have high 17\( \beta \)-HSD1 and 17\( \beta \)-HSD2 activities respectively (Day et al. 2006a, Purohit et al. 2006). The T47D cell line was also used to develop an \textit{in vitro} proof of concept assay for the inhibition of E\(_1\)-stimulated proliferation of hormone-dependent breast cancer cells by 17\( \beta \)-HSD1 inhibitors such as STX1040, as it is an ER-positive breast cancer cell line whose proliferation is dependent on oestrogens in the medium. For this reason, it is also an appropriate cell line to use to establish whether such inhibitors are oestrogenic \textit{in vitro} (Day et al. 2006b, 2008, Laplante et al. 2008). Using this model, and dosing E\(_1\) from 1 nM to 1 \( \mu \)M, STX1040 at 5 \( \mu \)M is seen to be non-oestrogenic while significantly inhibiting the E\(_1\)-dependent proliferation of T47D cells (Day et al. 2006b, 2008).

The low inter-species homology of the 17\( \beta \)-HSD1 enzyme has made the development of animal models for the assessment of the inhibitors difficult. Mouse 17\( \beta \)-HSD1 is only 63\% homologous to human 17\( \beta \)-HSD1 at the amino acid level, and this is reflected in its different substrate affinity, as, in addition to E\(_1\) to E\(_2\) activity, it also converts Adione to testosterone (Nokelainen et al. 1996). The rat enzyme has 93\% homology to that of the mouse, but only 68\% to human 17\( \beta \)-HSD1 (Ghersevich et al. 1994). STX1040 has an IC\(_{50}\) of \( \sim \)100 \( \mu \)M against the rat enzyme when it is expressed in 293-EBNA (Invitrogen, Paisley, UK) cells (unpublished results), indicating that inhibition by STX1040 is specific to the human enzyme. In female rodents, the major expression of 17\( \beta \)-HSD1 is in the ovary (Peltoketo et al. 1999), with low levels in the uterus and some expression in the sebaceous glands of the skin (Nokelainen et al. 1996, Pelletier et al. 2004).

To limit endogenous steroid enzyme expression and oestrogen synthesis ovariectomised mice are often used in animal models of steroidogenic enzyme

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**Figure 4** STX1040 docked in place of E\(_2\) in the 17\( \beta \)-HSD1 crystal structure (reproduced with permission from: Day et al. 2008).
inhibition, resulting in similar oestrogen levels to those of post-menopausal women (Yue et al. 1994). Human tumour xenograft growth can then be stimulated by exogenous doses of steroid, dependent on expression of the required enzyme by the human tumour cells for conversion to the more active steroid.

STX1040 was shown to be efficacious in vivo using a novel ovariectomised nude mouse model based on the principles above (Day et al. 2006b, 2008). As T47D cells had been successful in in vitro model development, this cell line was also used to develop the in vivo model in which growth of the T47D tumours was stimulated by low doses of E1, dependent on the human 17β-HSD1 expressed by the tumours for conversion to E2, the more active oestrogen. Tumours stimulated with E1, either daily by s.c. injection (0.05–0.1 μg/day) or by use of a time-release pellet (0.1–0.28 μg/day), as unconjugated oestrogens have a short half-life in vivo (Ruder et al. 1972), were significantly larger than those of the control animals. Treatment of the mice with STX1040 caused a significant decrease in their tumour volumes and plasma E2 levels in comparison with those dosed with E1 alone. STX1040 was confirmed to be non-oestrogenic in vivo using a standard rat uterotrophic model.

Despite the number of groups working towards the development of 17β-HSD1 inhibitors for clinical use, only one other group has reported activity in vivo using inhibitors optimised from those with core structures which mimic those of the natural inhibitors, coumestrol and kaempferide. From various non-steroidal flavone-, tetrahydrochromanoquinoline-, chromone- and pyrimidinone-based structures, the pyrimidinone core was selected for further optimisation (Fig. 3p; Messinger et al. 2006) as selectivity and solubility problems were observed with the other classes of compounds. Several compounds were selected for synthesis and evaluation from those modelled using published crystal structures. The most potent of these (Fig. 3q) has an IC_{50} of 5 nM in assays using purified recombinant 17β-HSD1, although in whole cell assays, its efficacy is much lower than that of STX1040 (inhibition at 1 μM <67%). It is selective for 17β-HSD1 over 17β-HSD2, is non-oestrogenic and is efficacious in vivo using an intact nude mouse model bearing MCF-7 cells transfected with 17β-HSD1 (Husen et al. 2006a,b).

Although there are differences between the two in vivo models, in both studies treatment of the mice with E1 caused mouse uterine weight to increase significantly. Use of the inhibitors, however, although resulting in a decrease in E1-stimulated tumour size, did not significantly decrease the E1-stimulated uterine weight. Despite this, E2 levels in the T47D tumour study (Day et al. 2006b, 2008) were shown to be significantly decreased after treatment with STX1040, suggesting that tumour 17β-HSD1 expression is a major E2 source in these models. The lack of an effect of the 17β-HSD1 inhibitors on uterine weight in both models may therefore be due to either the higher sensitivity of the uterus than the tumour to circulating oestrogens or maximal stimulation of uterine growth over the initial few weeks.

The demonstration of E1-dependent tumour growth inhibition in vivo by 17β-HSD1 inhibitors provides justification for the many years of research into inhibition of the enzyme, and suggests that it may indeed be a valid target for the treatment of hormone-dependent breast cancer. Although models for the use of these inhibitors in other hormone-dependent diseases, such as endometriosism, are yet to be developed, the observed decrease in the plasma level of active hormone after STX1040 treatment of animals carrying the human enzyme indicates that these inhibitors may well be effective for the treatment of these other diseases of hormone metabolism. The successful in vivo application of inhibitors of 17β-HSD1, the most studied of the 17β-HSDs, also suggests that inhibition of other 17β-HSDs known to be involved in disease states may well prove clinically beneficial in the future.

17β-HSD3 inhibition

Application of 17β-HSD3 inhibitors in prostate cancer

DHT is the main intracellular androgen in the prostate and stimulates the growth of hormone-dependent prostate tumours via its interaction with the AR. It is formed from testosterone by 5α-reductases 1 and 2. 17β-HSD3, microsomally expressed almost exclusively in the testes (Geissler et al. 1994, Luu-The et al. 1995), specifically converts non-androgenic Adione (Laplante & Poirier 2008) to active circulating testosterone in the presence of NADPH. It has not been reported to have any other activities. However, it is not the only enzyme that provides testosterone to the body, as 17β-HSD5 (AKR1C3), whose activities and inhibition will be discussed later in this review, converts Adione to testosterone in the prostate and other tissues.

A defect in the expression of 17β-HSD3 causes the autosomal recessive genetic disorder, male pseudohermaphroditism (Geissler et al. 1994), in which the individual is usually reared as a female, often having been born with female external genitalia and the
absence of a prostate, despite having testes and Wolffian duct-derived male internal genitalia (Andersson & Moghrabi 1997). Diagnosis is assisted by the presence of a high Adione to testosterone ratio, distinguishing the disorder from the clinically similar androgen insensitivity syndrome. The phenotype can vary in severity, even in individuals with the same mutation (Lee et al. 2007), but subjects often become virilised at puberty. The mechanism by which this occurs is not fully understood, although it has been suggested that either this is due to incomplete impairment of testes testosterone production, or due to peripheral conversion of Adione to testosterone (Andersson et al. 1996), perhaps by the action of 17β-HSD5.

Although 17β-HSD3 is expressed almost exclusively in the testes, there have been some reports of its expression in other tissues. One report indicated that expression of 17β-HSD3 mRNA increased over 30-fold in cancerous prostate biopsies (Koh et al. 2002). In this study, the authors also found a corresponding decrease in 17β-HSD2 mRNA expression, indicating that the reductive formation of testosterone is favoured, but found no change in the expression of AKR1C3 mRNA. Expression of 17β-HSD3 was up-regulated in an AR-positive prostate cell line, LNCaP, after it was treated for 48 h with dutasteride (Biancolella et al. 2007), an inhibitor of 5α-reductases 1 and 2. A polymorphism in the HSD17B3 gene, G289S, has also been linked to an increased susceptibility to prostate cancer (Margiotti et al. 2002). Microarray and subsequent RT-PCR and functional analysis indicated that 17β-HSD3, along with 17β-HSD12, is also expressed in human blood platelets and megakaryocytes. While 17β-HSD12 is up-regulated >25-fold in essential thrombocythemia, a rare myeloproliferative disorder, 17β-HSD3 is down-regulated ~4.5-fold (Gnatenko et al. 2005).

**Inhibitors of 17β-HSD3**

Because of its unique expression and substrate specificity, 17β-HSD3 provides a good target for the inhibition of testosterone formation in the treatment of androgen-dependent diseases such as hormone-dependent prostate cancer. Although its crystal structure has not yet been solved, 17β-HSD3 has undergone various mutational analyses as pseudohermaphroditism results from the effects of deleterious mutations on 17β-HSD3 activity (Lee et al. 2007). This has given an insight into the catalytic importance of various residues, such as ARG80, which are involved in the binding and selectivity of the enzyme for the cofactor, NADPH (McKeever et al. 2002).

The first demonstrated inhibition of 17β-HSD3 activity was in canine testicular microsomes by two steroids, 4-estrene-3,17-dione (Fig. 5a) and 5-androstene-3,17-dione (Fig. 5b), which are structurally very similar to the substrate, Adione (Pittaway 1983). These studies indicated that a non-aromatic A-ring and C17 carbonyl group were important for inhibition. Using human testicular tissue, the similarly structured atamestane (1-methyl-3,17-dione-androsta-1,4-diene; Fig. 5c), a potent aromatase inhibitor, was also shown to inhibit 17β-HSD3 (Lombardo et al. 1993).

As expression of 17β-HSD3 is specific to the testes, attempts have been made to find a more readily available source of the enzyme for the screening of inhibitors. (a) 4-estrene-3,17-dione, (b) 5-androstene-3,17-dione, (c) atamestane, (d) 1,4-androsta-diene-3,6,17-trione, (e) androsterone (ADT), (f) 3β-phenylmethyl-ADT, (g) 3β-amidomethyl-ADT derivatives, (h) 3-carbamate-ADT derivatives, (i) 3R-spiro-[3′-[3′′-N-morpholino-2′-(3′′-cyclopentylpropionyloxy) propyl]-2′-oxo-oxazolidin-5′-yl]-5α-androstan-17-one, (j) 3-O-benzyl-androsterone, (k) BMS 856, (l) 8/9-substituted tetrahydrobenzazocine (THB), (m) diphenyl-p-benzoquinone, (n) umbelliferone, (o) 1-(4-hydroxyphenyl)-nonan-1-one, (p) tributyltin chloride (TBT) and triphe-nyltin chloride (TPT).
potential inhibitors of 17β-HSD3. Microsomes isolated from rat testicular tissue were tested, but the enzyme was found to differ from the human enzyme in optimum reaction pH, in sensitivity to inhibition by candidate compounds, and in substrate specificity, efficiently reducing E1 as well as Adione (Le Lain et al. 2001). Most groups working on the inhibition of 17β-HSD3 now use enzyme from cells transfected with 17β-HSD3 cDNA, in purified form, as microsomes, or in whole cell assays. Recently, the pig 17β-HSD3 enzyme has been sequenced, cloned and expressed. As it has a similar substrate profile to the human enzyme and a higher amino acid homology to the human enzyme, at 82%, than that of rats and mice, microsomes from pig testicular homogenates may also have the potential to be used as a ready source of 17β-HSD3 for inhibitor studies (Ohno et al. 2006).

Using transfected cell microsomes, 1,4-androstadiene-3,6,17-trione (Fig. 5d) potently and selectively inhibited 17β-HSD3 activity (Luu-The et al. 1995). Another steroid, the weak androgen ADT (Fig. 5e), was found to be twice as potent as the substrate Adione, with an IC50 of 330 nM in transfected cell microsomes. Several libraries of ADT derivatives were synthesised, and their inhibitory potencies and androgenicity compared (Tchédam Ngatcha et al. 2000, 2005, Maltais et al. 2001, 2002). ADT derivatives substituted at the 16 position, although proving non-androgenic, demonstrated only weak inhibition of 17β-HSD3 activity (Tchédam Ngatcha et al. 2002). 3β-Substituted alkyl and aryl derivatives of ADT were potent inhibitors, with IC50 values of 57–147 nM, and were found to be specific for 17β-HSD3 over 17α-HSD1 and 17β-HSD5. 3α-Ether-3β-substituted ADT derivatives had a lower inhibitory activity than the 3β-substituted ADT analogues, with the exception of 3β-phenylethyl-3α-methyl-O-ADT and 3β-phenylethyl-ADT, which had IC50 values of 73 and 99 nM respectively. The most potent of these 3β derivatives was 3β-phenylmethyl-ADT (IC50 = 57 nM; Fig. 5f; Tchédam Ngatcha et al. 2000, 2005), but unfortunately it was found to be as androgenic as DHT. However, a somewhat less potent (IC50 = 227 nM) ADT derivative synthesised using parallel solid-phase techniques, 3β-peptido-3α-hydroxy-5α-androstan-17-one, was found to be tenfold less androgenic (Maltais et al. 2001).

Further understanding of the SAR for the ADT derivatives was achieved using 3β-amidomethyl-ADT (Fig. 5g) and 3-carbamate-ADT (Fig. 5h) libraries generated by liquid-phase parallel syntheses. Long alkyl chains at R1 and R2 were found to be badly tolerated, with preference for a shorter R2 chain than that at R1, and tertiary amides were more active than secondary amines. Adding polarity to the chains did not generally improve their inhibitory potential, but adding rigidity at R1 did. The most potent of nearly 300 amidomethyl compounds, with an IC50 of only 35 nM, was 3β-[(N-adamantylmethyl-N-butanoxy)aminomethyl]-3α-hydroxy-5α-androstan-17-one, but this compound was found to be almost as androgenic as 3β-phenylmethyl-ADT. The most potent of the 25 3-carbamate-ADT compounds was 3R-spiro-[3′-[3′′-morpholino-2″-(3′″-cyclopropyl propionyloxy)-propyl]-2′-oxo-oxazolidin-5″-yl]-5α-androstan-17-one (Fig. 5i), with an IC50 of 74 nM, and this compound was not significantly androgenic, even at 1 μM (Maltais et al. 2002).

Bisubstrate compounds from the same group, incorporating components that bind in both the substrate and cofactor binding sites, similar to the hybrid inhibitors developed for the inhibition of 17β-HSD1, were also tested as inhibitors of 17β-HSD3. Activity in homogenated cells overexpressing 17β-HSD3 was 78% inhibited by 1 μM Adione substituted at the 17α-position with a 12 methylene spacer esterified to adenosine, although this compound was less potent in whole cells (Bérubé et al. 2006). Attempts to improve the potency of this class of compound by phosphorylation of the adenosine group, to mimic the preferred cofactor NADPH over NADH, did not prove successful, suggesting that either this moiety is not interacting with the cofactor binding site, possibly due to prior binding of the cofactor itself, or that its phosphate group interaction is not optimised (Bérubé & Poirier 2007)

A 3-substituted ADT derivative, 3-O-benzylandrosterone (Fig. 5j), was used by Bristol Myers Squibb (BMS) to initiate a large-scale screening program (Spires et al. 2005). Inhibition of 17β-HSD3 activity in transfected cell microsomes was assayed using a 96-well format scintillation proximity assay (SPA) with a testosterone-specific antibody for higher throughput of compounds than the usual radiolabelled TLC-based assay (Luu-The et al. 1995). 3-O-benzylandrosterone and 18β-glycyrrhetinic acid both had inhibitory IC50s of ~90 nM in the SPA assay. Inhibition in whole cells was measured using an AR stimulation-dependent chemiluminescent secreted alkaline phosphatase (SEAP) reporter assay in AR-positive cell lines transfected stably with the AR stimulation-dependent chemiluminescent secreted alkaline phosphatase (SEAP) reporter plasmid. The inhibitory IC50 values for 3-O-benzylandrosterone and 18β-glycyrrhetinic acid were, as expected, much higher in this whole cell assay, at 1.6 and 3.8 μM respectively (Spires et al. 2005).
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Of over 200 000 compounds screened by BMS, a series of anthranilamide-based compounds substituted at position 1 with an amide-linked chlorophenol group were identified as inhibitors of 17β-HSD3 activity, and these were most potent when substituted at the 4 position, further carboxamide substitutions at the 2 position (e.g. BMS-856; Fig. 5k; IC₅₀ values of 60 and 300 nM respectively in the enzyme and whole cell assays). Dibenzothiazocines (DBTs) and tetrahydridobenzothiazocines (THBs) were also discovered to be highly active in these assays (Fink et al. 2006). Although DBTs were most potent, especially when substituted with chlorine at position 2 or 3, the THBs were chosen for further SAR study as the aryl sulphur atom of the DBT compounds renders them susceptible to metabolic degradation. Unlike the DBTs, substitution of the THBs at the A-ring did not improve their potency, although substitution at positions 8 and 9 of the C-ring did (Fig. 5l), with an aryl ring substitution at position 8 providing significant improvement. Ortho-substitution of the 8-aryl-substituted ring with electron donating groups, including methyl ester, methyl ketone and trifluoromethyl and aldehyde groups, resulted in the highest potencies in both the enzyme and cellular assays (IC₅₀ values of around 20 and 500 pM respectively). However, no data on the selectivity of these inhibitors for 17β-HSD3 over other 17β-HSDs has been published.

Screening of a range of compounds by another group (Le Lain et al. 2001) revealed that other non-steroidal compounds, such as diphenyl-p-benzoquinone, phenyl-p-benzoquinone (Fig. 5m), 7-hydroxyflavone, baicalein and biochanin A, with IC₅₀ values of 2.7, 5.7, 9.0, 9.3 and 10.8 µM respectively, are also able to inhibit 17β-HSD3. The 7-hydroxycoumarins, umbelliferone (Fig. 5n) and 4-methylumbelliferone are more potent inhibitors of human testes microsomal 17β-HSD3, with IC₅₀ values of 1.4 and 1.9 µM respectively (Le Lain et al. 2002). However, many of these compounds, such as the flavones, as already discussed in regard to 17β-HSD1 inhibition, are non-selective for 17β-HSD3 inhibitory activity, having effects on other 17β-HSDs, other steroidal enzymes and on steroid receptors. 4-Hydroxyphenyl ketones with attached straight chain alkyl groups inhibit 17β-HSD3 activity in rat testicular microsomes with IC₅₀ values in the low micromolar range. The best of those tested was 1-(4-hydroxyphenyl)-nonan-1-one (IC₅₀ = 2.86 µM; Fig. 5o; Lota et al. 2006), containing an octyl chain, thought to mimic the steroid backbone of Adione while the 4-hydroxy group forms hydrogen bonds at the active site. In this study, baicalein was shown to be far less potent (IC₅₀ value of 186 µM) than in the human microsome study discussed above (IC₅₀ = 9.3 µM; Le Lain et al. 2001), suggesting that the alkyl-substituted 4-hydroxyphenyl ketones may prove more potent if tested on the human enzyme.

Organotin compounds are known to be endocrine-disrupting marine pollutants. Two of these compounds, tributyltin chloride and triphenyltin chloride (TBT and TPT respectively; Fig. 5p), were found to be potent inhibitors of 17β-HSD3 in microsomes from pig testes, with IC₅₀ values of 7.2 and 2.6 µM respectively. The compounds did not affect expression of the enzyme, suggesting that these effects are due to direct inhibition of 17β-HSD3 activity. Surprisingly, however, in whole cultured Leydig cells, the potency of both compounds increased ~60-fold to an IC₅₀ of 114 nM for TBT and 48 nM for TPT. The unusual increased activity in the whole cell assay was suggested to be either due to the fat solubility of organotins resulting in their high concentration throughout the membrane structure of Leydig cells, in which 17β-HSD3 is located, or due to effects on other enzymes involved in the production of testosterone, such as those involved in transcription and signal transduction (Ohno et al. 2005).

Despite the many groups now working on inhibitors of 17β-HSD3, the crystal structure of the enzyme has not been published and there are no formal reports of the efficacy of these inhibitors in in vivo models. Groups such as Schering–Plough (WO2004060488) and Sterix Ltd (WO2007003934) have published patents pertaining to the development of 17β-HSD3 inhibitors, with Schering–Plough indicating efficacy of non-steroidal inhibitors in mouse Shionogi tumour (Minesita & Yamaguchi 1965) and monkey models in conference abstracts (American Association of Cancer Research annual meeting 2005); however, data from these studies have not yet been published in peer-reviewed journals.

The development of successful cost-effective in vivo models to ascertain the efficacy of 17β-HSD3 inhibitors may prove complex due to the low homology between the human and mouse enzymes. Although use of the Shionogi model, a transplantable androgen-dependent AR-positive mouse breast tumour (Minesita & Yamaguchi 1965) may give an indication of efficacy, the effect would depend on inhibition of testosterone formation by mouse 17β-HSD3, and thus would not truly reflect the use of inhibitors against the human enzyme. Despite the more accurate representation of the human situation by primate models, the cost and regulations involved in their use are prohibitive for most groups and for screening of more than a few compounds. To overcome the problem of the lack of homology between human and rodent...
17β-HSD3, the development of models using castrated mice with human 17β-HSD3-expressing androgen-dependent tumour xenografts may prove useful.

17β-HSD5 inhibition

Application of 17β-HSD5 inhibitors in prostate cancer

17β-HSD5 (AKR1C3/PGFS) also catalyses the reduction of Adione to form testosterone, but unlike 17β-HSD3 it is expressed far more ubiquitously in tissues including the prostate, breast, ovary and endometrium (Pelletier et al. 1999, Penning et al. 2000, Ji et al. 2005). It has additional 3α- and 20α-steroid reductase activities (Dufort et al. 1999, Penning et al. 2000), including the protective inactivation of deoxycorticosterone in mineralocorticoid tissues (Sharma et al. 2006). However, its other major activity is as PGFS: it has PGD2 11-ketoreductase activity that reduces PGD2 to 9α,11β-PGF2α (Matsuura et al. 1998) and also PGI2 9,11-endoperoxide reductase activity that reduces unstable PGI2 to PGF2α (Komoto et al. 2004). AKR1C3 is the only 17β-HSD that is not a short-chain dehydrogenase. It is an AKR and is highly homologous to three other AKR enzymes, AKR1C1, AKR1C2 and AKR1C4. Although multifunctional, AKR1C2 and AKR1C4 primarily have 3α-HSD activities, while the major activity of AKR1C1 is as a 20α-HSD (Penning et al. 2004).

17β-HSD5/AKR1C3 has been seen to be up-regulated in prostate cancer. Elevated expression of 17β-HSD5 protein has been demonstrated in the epithelium of malignant prostate tissue as well as in non-neoplastic processes such as benign prostatic hyperplasia and inflammation (Bauman et al. 2006b, Fung et al. 2006). Its expression is increased in advanced prostate cancer (Nakamura et al. 2005) and has been correlated with Gleason grade (Wako et al. 2008), a measure of tumour aggressiveness. In a microarray analysis of bone marrow metastases of hormone-independent prostate cancer, the expression of 17β-HSD5 was more than fivefold higher than in primary androgen-dependent tumours, and this was confirmed by both RT-PCR and immunoblotting (Stanbrough et al. 2006). Remarkably however, although other androgen synthesis genes were also up-regulated, and expression of the AR was 5.8-fold that in primary tumours, there was no correlation between the increased expression of 17β-HSD5 and AR in the samples, suggesting the involvement of different mechanisms of androgen independence.

Although there are no reports of increased expression of 17β-HSD5 in the ovaries of hyperandrogenised individuals with PCOS, in one study a single nucleotide polymorphism in the AKR1C3 promoter that increases its affinity to nuclear transcription factors, SNP-71G was found in around 10% of affected individuals (Qin et al. 2006). Conversely, in a separate study, there was no association between PCOS or testosterone levels and the occurrence of either SNP-71G or four other AKR1C3 polymorphisms (Goodarzi et al. 2008). There was also no association between four common AKR1C3 polymorphisms and precocious puberty, a hyperandrogenic condition thought to be a risk factor for PCOS (Petry et al. 2007). 17β-HSD5 is also expressed in normal (Pelletier et al. 1999, 2001) and malignant breast tissue (Amin et al. 2006). Its expression is significantly up-regulated in breast cancer and is associated with a poor prognosis (Vihko et al. 2005).

However, as previously mentioned, 17β-HSD5 is also known as PGFS. It is thought that it may also exert a proliferative signal in cancer by reducing PGD2 to 9α,11β-PGF2α. 9α,11β-PGF2α is a proliferative PG that stimulates the MAP kinase pathway, preventing the formation of the PPARγ ligand, PGJ2, a PG involved in differentiation signalling (Desmond et al. 2003).

Inhibitors of 17β-HSD5

The earliest patents for the inhibition of AKR1C1–AKR1C4 (US5439943, US5399790, US5187187, US5118621 and US5068250) were published by the University of Pennsylvania from 1992–1995. Novel non-steroidal suicide inhibitors were described for potential therapeutic use in potentiating the action of androgens, in androgen replacement therapy for hypogonadism of pituitary and testicular origin, and in the maintenance of male fertility. Suicide substrates mimic the physiological substrate and are transformed by the enzyme to highly reactive alkylating agents which then inactivate it by forming a covalent bond at the active site. They can be highly selective as they are themselves inactive until they are transformed by the target enzyme. These patents described the activity of monocyclic-aromatic allylic and acetylenic alcohols as highly selective inhibitors that are transformed by the 3α-HSD activity to monocyclic-aromatic vinyl and acetylenic ketones that alkylate the pyridine nucleotide binding site of the enzyme (e.g. 1-(4′-nitrophenyl)-2-propen-1-ol is transformed to 1-(4′-nitrophenyl)-2-propen-1-one by the action of the 3α-HSDs; Fig. 6a).

Since then, as 17β-HSD5 has been shown to have activities other than as a 3α-HSD, interest in its
inhibition for anti-cancer therapy has been growing. Several environmental dietary phytoestrogens, such as zearalenone, coumestrol, quercetin and biochanin A (IC\textsubscript{50} = 2–14 μM; Fig. 6b–e), have been shown to inhibit 17β-HSD5 (Krazeisen et al. 2001). The potency of these compounds increases with increasing number of hydroxylated sites, apparently due to binding to the hydrophilic cofactor-binding pocket of the enzyme. Derivatives of \textit{trans}\textendash cinnamic acids, \(\alpha/\beta\)-unsaturated plant carboxylic acids that are natural precursors of structurally related 17β-HSD5-inhibiting flavonoids, have also been evaluated as 17β-HSD5 inhibitors (Brožič et al. 2006). The best inhibitor in the series tested was \(\alpha\)-methylcinnamic acid (Fig. 6f; IC\textsubscript{50} = 6.4 μM). Parallel tests indicated that these compounds have no inhibitory effects on fungal 17β-HSD, suggesting that they may be selective for 17β-HSD5 over the 17β-HSDs from the short-chain dehydrogenase family. A C17-difluorinated oestratriene derivative, J2404 (Fig. 6g), inhibited 17β-HSD5 by 72% at 2 μM, with no effect on the other SDR 17β-HSD enzymes tested, including types 1, 2, 4 and 7 (Deluca et al. 2006).

17β-HSD5 is also found in the brain, where it is believed to be involved with AKR1C1 and AKR1C2 in the metabolism of neurosteroids, which act on the \(\gamma\)-aminobutyric acid type A (GABA\textsubscript{A}) receptors. Benzodiazepines are sedatives that modulate the activity of the GABA\textsubscript{A} receptors. However, several of them have been found to inhibit AKR1C1–AKR1C3 (17β-HSD5), and one, cloxazolam (Fig. 6h) is a potent and specific inhibitor of 17β-HSD5 with an IC\textsubscript{50} of 2.5 μM (Usami et al. 2002).

Studies of the catalytic mechanism of the enzyme by Penning et al. (2001), based on studies of a 69% homologous rat 3α-HSD (AKR1C9), indicated that an ordered bi-mechanism operates in which the substrate binds to the enzyme once the pyridine nucleotide cofactor, NADPH, is bound. After the reduction of the substrate through an oxyanion transition state, the product is released before the release of the cofactor. This mechanism suggests that steroidal-based inhibitors that compete with the steroid product are desirable as they would act as uncompetitive inhibitors. This led to the suggestion that transition state analogues, such as steroid carboxylates and pyrazoles, and mechanism-based inactivators, such as \(3\alpha\)-, 17β- or 20α-spiro-oxiranyl steroids (Fig. 6i–k) and oxiranyl non-steroids, may be potent 17β-HSD5 inhibitors. Endorecherche, Inc. (Quebec, Canada) has published several patents pertaining to the inhibition of 17β-HSD5 by steroidal and spirolactone compounds for the treatment of androgen-dependent diseases including prostate cancer (e.g. US2004082556, AU2004200173, MXPA00008868, ZA9901924, EP1321146).

One of the most potent of the Endorecherche, Inc. compounds is an E\textsubscript{2} derivative, EM1404 (Fig. 6l), a strong competitive inhibitor with an IC\textsubscript{50} of 3.2 ± 1.5 nM using 100 nM Adione as substrate, a higher potency than that of any other reported inhibitor. The lactone ring of EM1404 is located at the base of the substrate binding site, and the amide group is oriented towards the surface of the enzyme, despite the fact that the inhibitor occupies only part of the binding cavity (Qiu et al. 2007). Although this compound is specific for 17β-HSD5 over 17β-HSD1, 17β-HSD2 and 17β-HSD3 (IC\textsubscript{50} > 10 μM; patent WO9946279), its specificity over other members of the AKR1C family and cyclooxygenase 1 and 2 (COX1/COX2) remains to be assessed.

17β-HSD5 is also inhibited by NSAIDs, including indomethacin (Fig. 6m) and flufenamic acid (Fig. 6n),
at similar concentrations and the same order of potency as for the inhibition of COX1/COX2 (Bauman et al. 2005, Byrns et al. 2008). In HL-60 promyelocytic leukaemia cells, the inhibition of 17β-HSD5 by indomethacin is thought to lead to a decrease in proliferation and a corresponding increase in differentiation via the PPARγ signalling pathway due to the presence of higher concentrations of PGJ2 and lower concentrations of PGF2α (Desmond et al. 2003). Using the extensive SAR knowledge generated during the development of COX1 and COX2 inhibitors, derivatives of NSAIDs with increased 17β-HSD5 potency and selectivity for 17β-HSD5 inhibition over COX1 and COX2 inhibition are being developed, with structures based on N-phenylanthranilic acids, cholanic acids, and on N-acylanthranilic acids, 2-benzoylbenzoic acids, benzophenones, and phenoxybenzoic acid (Bauman et al. 2005, Gobec et al. 2005, Penning et al. 2006). One of the most specific is N-(4-chlorobenzoyl)-melatonin (Fig. 6o), a derivative of indomethacin that inhibits the reduction of 5 μM Adione to testosterone by 17β-HSD5 with similar potency (IC50 = 11.4 μM) to indomethacin (IC50 = 8.5 μM), but which does not inhibit COX1, COX2, AKR1C1 or AKR1C2 (Byrns et al. 2008).

The rational design of these inhibitors has been greatly assisted by the elucidation of the crystal structure of 17β-HSD5, complexed as a ternary structure with the cofactor NADP(H), and with either the substrates PGD2 (Komoto et al. 2004) or Adione (Qiu et al. 2004), the product testosterone (Qiu et al. 2004) or potential inhibitors, such as the NSAIDs, indomethacin and flufenamic acid (Lovering et al. 2004), rutin (Fig. 6p; Komoto et al. 2004), a PGF2α analogue, bimatoprost (Fig. 6q; Komoto et al. 2006) and EM1404 (Qiu et al. 2007).

A patent for the use of N-sulphonylindole derivatives as selective 17β-HSD5 inhibitors was recently published by Astellas Pharma Inc. (Tokyo, Japan, WO2007100066). Derivatives in which a carbon atom in the indole group is substituted by a carboxy group, a carboxy-substituted lower alkyl group or a carboxy-substituted lower alkenyl group were found to potently inhibit 17β-HSD5 with selectivity over 17β-HSD3 inhibition (IC50 > 10 μM). The most potent of these inhibitors have IC50 values below 100 nM (e.g. Ex144; Fig. 6r).

In all of these studies, the potency of the inhibitors has been assayed using cell lines transfected with 17β-HSD5. The expressed enzyme has then either been assayed in situ in whole cell assays using radiolabelled steroid substrate or purified for direct enzyme assay, measuring the decrease in absorbance at 340 nm as NADPH is oxidised to NADP++, using either steroid substrate or 9,10-phenanthrenequinone (Fig. 6s) as a test substrate. The purified enzyme has also been used for crystallisation studies, as previously mentioned, to determine the structure of the active site and the residues that are important to its activity and inhibition. Although there are several groups presently working on these inhibitors, there have been no reports of the development of in vivo models of 17β-HSD5 inhibition. This may well be due to the ubiquitous expression of 17β-HSD5 throughout the tissues, its substrate plasticity and the initial lack of specificity of these inhibitors. However, in order to understand the contribution of the various catalytic activities of 17β-HSD5 to the development of hormone-dependent prostate cancer and its progression to hormone independence, and to other cancers, analysis of the effect of inhibition of 17β-HSD5 in vivo is necessary. Until these models have been developed and the effects of these inhibitors on both androgen and PG production have been tested in vivo, the value of the development of these inhibitors and their future application in the treatment of prostate or other cancers cannot be fully established.

### Inhibition of other 17β-HSDs

#### Applications and inhibitor development

17β-HSD2 is the enzyme responsible for the oxidation of active E2 and testosterone to their inactive forms, E1 and Adione. As it is involved in inactivation of active steroids, there has been less interest in the development of 17β-HSD2 inhibitors than in those of 17β-HSD1, 17β-HSD3 and 17β-HSD5. However, partly for use as a research tool and partly as inhibitors of 17β-HSD2 may prove beneficial for conditions in which the concentration of active steroid is too low, some effort has been made to find inhibitors of this enzyme. In 2002, Bayer Pharmaceuticals published patent WO0226706 for the use of pyrrolidinones, pyrroli-thiones and 1-methyl-4-phenylpyrrolidin-2-ones (Fig. 7a i) in the treatment of osteoporosis. Another group has explored the use of 17-spiro-lactones attached to either an E2 nucleus (Sam et al. 2000, Bydal et al. 2004) or other steroid nuclei (Tremblay et al. 1999, Poirier et al. 2001) as 17β-HSD2 inhibitors. These compounds are selective for 17β-HSD2 inhibition over 17β-HSD1 and 17β-HSD3, with IC50 values in the nM range. However, the most active of these compounds, the spiro-δ-lactone C17β-O/C17α-δ-lactone (Fig. 7a ii), which has an IC50 of 6 nM for 17β-HSD2 inhibition, also inhibits 17β-HSD5 with an IC50 of ~10 nM and has some oestrogenicity (Bydal et al. 2004).
Presently, there is little research into the development of inhibitors of the remaining, less well characterised, 17β-HSDs. However, as their number continues to increase, and the substrate specificity, directional activity and expression pattern of each of the enzymes are elucidated, the potential of specific inhibitors of each of these enzymes for the treatment of various diseases, of both steroidal and non-steroidal origin, will become clear. Indeed, the inhibition of 17β-HSD10 is already being explored for the treatment of Alzheimer’s disease, and a potent inhibitor, AG18051 (1-azepan-1-yl-2-phenyl-2-(4-thioxo-1,4-dihydropyrazolo[3,4-d]pyrimidin-5-yl)-ethanone; Fig. 7b), with an IC₅₀ of 92 nM, has been identified (Kissinger et al. 2004). As this enzyme also converts 5α-androstanediol to DHT (Yang et al. 2005a,b) and has been seen to be overexpressed in primary prostate cancer cultures (He et al. 2003), it would also be interesting to see whether inhibitors of 17β-HSD10 may also have a role in the treatment of prostate cancer.

Summary

Despite the success of inhibitors of steroidogenic enzymes in the clinic, such as those of aromatase and steroid sulphatase, the development of inhibitors of 17β-HSDs is at a relatively early stage. At present, none of these inhibitors has reached clinical trials, and only recently has efficacy been demonstrated for the first of the enzymes in in vivo disease models. However, great advances in both the understanding of the function of these enzymes, and in techniques for elucidation of protein structure and computer-aided SAR have been made over the last decade. As the precise substrate and cofactor specificity, directional activity and expression pattern of each of the 15 17β-HSDs in normal and diseased states becomes clearer, it can be seen that the development of specific inhibitors could provide a great opportunity to fine-tune pathways in the therapeutic intervention of steroidogenic and other metabolic disorders. Presently, the development of inhibitors of 17β-HSD1 for the treatment of hormone-dependent breast cancer and endometriosis, and 17β-HSD3 for the treatment of hormone-dependent prostate cancer, are most advanced.

Declaration of interest

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