



In vitro and in vivo models for the evaluation of potent inhibitors of male rat 17α -hydroxylase/ $C_{17,20}$ -lyase

I. Duc^{a,*}, P. Bonnet^a, V. Duranti^a, S. Cardinali^a, A. Rivière^a, A. De Giovanni^a,
J. Shields-Botella^a, G. Barcelo^b, N. Adje^b, D. Carniato^b, J. Lafay^b,
J.C. Pascal^b, R. Delansorne^a

^a Preclinical R&D Department, Théramex, 6 Avenue Prince Héritaire, Albert 98000, Monaco

^b Chemical R&D Department, Théramex, 6 Avenue Prince Héritaire, Albert 98000, Monaco

Received 19 August 2002; accepted 27 January 2003

Abstract

The $C_{17,20}$ -lyase is a key enzyme in the biosynthesis of androgens by both the testes and adrenals. A complete inhibition of this enzyme would provide an alternative means of androgen suppression for the treatment of prostatic cancers. In the present study, the inhibitory effects of new non-steroidal compounds were tested in vitro on rat $C_{17,20}$ -lyase versus abiraterone, a reference steroidal inhibitor. Their activities were also evaluated in vivo on plasma testosterone (T) and luteinizing hormone (LH) levels and on testes, adrenals, seminal vesicles (SV) and ventral prostate (VP) weights after 3 days of oral treatment to adult male rats (50 mg/kg per day p.o.).

Inhibition in the nanomolar range was obtained with TX 977, the lead racemate product in this series, and optimization is ongoing based on a slight dissociation observed between its two diastereoisomers, TX 1196-11 (S) and TX 1197-11 (R). These non-steroidal compounds (including YM 55208, a reference competitor) proved to be more active in vivo than abiraterone acetate in this model, but the observed impact on adrenal weight suggests that the specificity of lyase inhibition versus corticosteroid biosynthesis deserves further investigations with this new class of potentially useful agents for the treatment of androgen-dependent prostate cancer.

© 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Steroidogenesis; Androgen; P450C17; Inhibitor of $C_{17,20}$ -lyase; Rat

1. Introduction

The 17α -hydroxylase/ $C_{17,20}$ -lyase plays a key part in the pathways of steroid hormone biosynthesis [1,2]. This enzyme catalyses two reactions: 17α hydroxylation of C_{21} steroids and cleavage of $C_{17,20}$ bond of C_{21} steroids. The 17α hydroxylation activity is a required step in cortisol biosynthesis whereas the $C_{17,20}$ bond side chain cleavage is essential for the biosynthesis of androgens. The 17α -hydroxylase/ $C_{17,20}$ -lyase is a cytochrome P450-dependent microsomal enzyme (P450C17), which is expressed in testicular and adrenal tissues and catalyses the conversion of pregnenolone or progesterone into dehydroepiandrosterone (DHEA) or androstenedione ($\Delta 4A$), respectively, two precursors of testosterone (T) [3–7]. The use of effective and selective inhibitor of P450C17 appears as a possible alternative to orchidectomy or other endocrine therapy,

to lower circulating androgens in patients with prostate cancer.

In the rat and other rodents, 17α -hydroxylase/ $C_{17,20}$ -lyase is not expressed in zona fasciculata of adrenal cortex, consequently corticosterone and not cortisol as in human, is the major glucocorticoid. Moreover, the rat P450C17 can convert both 17α -hydroxy-pregnenolone and 17α -hydroxy-progesterone (17OHP) into DHEA and $\Delta 4A$, respectively, as compared to human and bovine enzyme that can only cleave the $C_{17,20}$ bond of 17α -hydroxy-pregnenolone [8,9]. Even so, due to the lack of availability of human tissue and variability among samples, the rat remains, in spite of differences in localization and substrate specificity of this enzyme, a suitable model for the in vitro and in vivo evaluation of inhibitory potential of new compounds [10,11].

The first aim of this study was, to compare in vitro, the inhibitory activity of new steroidal and non-steroidal compounds on $C_{17,20}$ -lyase from rat testis microsomes. 17OHP was chosen as substrate, and the inhibitory potential of test compounds was determined by measuring the end product of the reaction, $\Delta 4A$, by radioimmunoassay (RIA).

* Corresponding author. Tel.: +377-92-05-08-58;

fax: +377-92-05-08-81.

E-mail address: iduc@theramex.mc (I. Duc).

Secondly, the inhibitory potential of the compounds was evaluated *in vivo* in adult male rats after 3 days of oral treatment. This *in vivo* model allowed a rapid screening of new compounds as compared to the classic 15-day model [12]. The weights of androgen-target organs such as ventral prostate (VP) and seminal vesicles (SV) were measured as well as circulating levels of T and LH. The weights of androgen-sensitive organs such as testes and adrenal glands were also monitored as an index of side-effects. Abiraterone (CB7598), abiraterone acetate (CB7630), steroid derivatives [13] and YM 55208 [14], a non-steroid compound derived from YM 116 [15], were used as references as well as the anti-fungal bifonazole [16–18].

2. Materials and methods

2.1. Chemical inhibitors and radioactive steroids

TX 977 (racemate), TX 977-11 (salt of TX 977), TX 1196-11 (*S*-enantiomer of TX 977), TX 1197-11 (*R*-enantiomer of TX 977), abiraterone (3 β -hydroxy-17-(3-pyridyl)-androsta-5,16-diene), abiraterone acetate (3 β -acetoxy-17-(3-pyridyl)-androsta-5,16-diene), and YM 55208 (2-(1-(¹H-imidazol-1-yl)ethyl)-⁹H-carbazole) were synthesized by Théraxem (Monaco). Bifonazole (1-(*p*, α -diphenylbenzyl)-imidazole) was purchased from Sigma (St. Louis, MO, USA). [1,2,6,7-³H]-Androt-4-ene-3,17-dione(³H- Δ 4A) was purchased from NEN Life Science Products (Boston, MA, USA).

Other not listed or not specified compounds and reagents were from Sigma (St. Quentin Fallavier, France).

2.2. Animals

Adult Wistar male rats were selected for the present study. They were purchased from Iffa Credo (Elevage des Oncins, L'Arbresle, France). They were housed under conditions of 12 h light–dark, maintained in an air-conditioned room and provided with a standard diet of AO4C pellets from UAR (Villemoisson-sur-Orge, France) and filtered mains water *ad libitum*.

2.3. Preparation of rat testis microsomes

Testes from 230 to 310 g rats were obtained by scrotal castration. They were washed with an isotonic solution of NaCl, pooled, weighed and then blended with an ultra-turrax homogenizer in 0.25 M sucrose, 20 mM Tris–HCl (pH 7.4). The homogenate was centrifuged at 10 000 \times g for 30 min at 4 °C. The supernatant obtained was centrifuged at 100 000 \times g for 60 min at 4 °C. The microsomal pellet [19,20] thus obtained was resuspended in 5 mM MgCl₂, 50 mM Tris–HCl, pH 7.4 buffer, treated briefly with a dual/kontes homogenizer to ensure full dispersion and divided into 400 μ l por-

tions for storage at –70 °C. The protein concentration of the suspension was determined by the method of Bradford [21] with the Bio-Rad protein assay kit.

2.4. C_{17,20}-lyase activity assay

Microsomes were diluted to a final protein concentration of 50 μ g/ml in the reaction mixture which contained 0.25 M sucrose, 20 mM Tris–HCl (pH 7.4), 10 mM G6P and 1.2 IU/ml G6PDH. After equilibration at 37 °C for 10 min, the reaction was initiated by addition of β NADP to obtain a final concentration of 0.6 mM. Prior to the distribution of 600 μ l of the reaction mixture in each tube, test compounds were evaporated to dryness under a stream of nitrogen and then were incubated at 37 °C for 10 min.

After incubation with inhibitors, 500 μ l of the reaction mixture was transferred to tubes containing 1 μ M of the enzyme substrate, 17OHP. After a further 10 min incubation, tubes were placed on ice and the reaction was stopped by addition of 0.1 ml NaOH 1N. Tubes were deep-frozen and stored at –20 °C until assayed for Δ 4A levels.

A Δ 4A RIA was developed and automated on a microplate format in our laboratory using a specific antibody against Δ 4A and instructions provided by Biogenesis (Poole, England). The separation of free and bound antigen was achieved with a dextran-coated charcoal suspension. After centrifugation, aliquots of the clear supernatant were counted in duplicates in a 1450 MicrobetaPlus liquid scintillation counter (Perkin-Elmer Instruments, Courtaboeuf, France). The Δ 4A concentrations of unknown samples were determined from the standard curve. The detection limit was 0.5 ng/ml and the within and between assay coefficients of variation were 10.7 and 17.6%, respectively at an assay value of 13 ng/ml. The rate of enzymatic reaction was expressed as pmol of Δ 4A formed per 10 min and per mg of protein. The value of maximum activity without inhibitor (control) was set at 100%. The IC₅₀ values were calculated using non-linear analysis from the plot of enzyme activity (%) against log of inhibitor concentration (GraphPad Prism, version 3.0).

2.5. *In vivo* assays

Adult male Wistar rats weighing 220–240 g were used. All the tested compounds were prepared in distilled water with a few drops of Tween 80 (polyoxyethylene sorbitan monoleate) and administered by oral route at 10 ml/kg per day to animals once daily for 3 days. Control group was dosed with vehicle (water-Tween 80) and test compounds were given orally at 50 mg/kg per day. On the day following the last treatment, animals were anaesthetized with isoflurane. VP, SV, testes and adrenals were removed, dissected and weighed. Arterial blood was withdrawn and collected into heparinized tubes. Plasma was stored at –20 °C until required.

2.6. T and LH RIA assays

Plasma was used for the determination of T as described in the ^{125}I -T assay kit supplied by Diagnostic Systems Laboratories Inc. (Webster, TX, USA). The detection limit was 0.1 ng/ml and the within and between assay coefficients of variation were 13.9 and 13.7%, respectively at an assay value of 0.5 ng/ml.

The assay for LH used the rat specific ^{125}I -LH assay system from Amersham Biosciences Europe GmbH (Orsay, France). The detection limit was 0.8 ng/ml and the within and between assay coefficients of variation were 5.2 and 1.9%, respectively at an assay value of 3.1 ng/ml.

2.7. Statistical analysis

Statistical analysis was performed using the SAS software version 6.12 (SAS Institute, Grégy-Sur-Yerres, FR). For IC_{50} , organ weights and plasma hormone levels, homogeneity of variances was checked by Levene's test. Results were analyzed using the parametric analysis of variance followed by multiple range tests or the non-parametric Kruskal–Wallis analysis and Wilcoxon tests, depending on the homogeneity of the variances. A *P* value less than 0.05 was considered as statistically significant.

3. Results

3.1. Inhibition of rat testicular $\text{C}_{17,20}$ -lyase

As shown in Fig. 1, a concentration-related inhibition of the rat testicular $\text{C}_{17,20}$ -lyase activity was obtained with

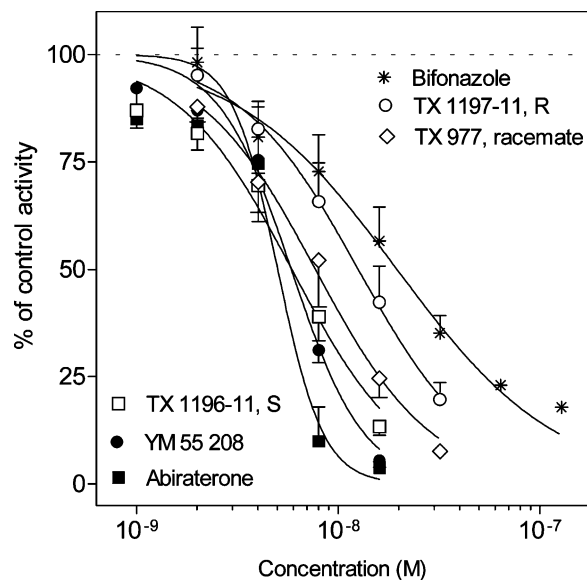


Fig. 1. Inhibition of rat testicular $\text{C}_{17,20}$ -lyase activity. Rat testes microsomes were prepared as described in Section 2. $\text{C}_{17,20}$ -lyase activity was measured after incubation with 1 μM of 17OHP and the indicated concentrations of compounds. Each point represents the mean \pm S.E.M. of four determinations each made in duplicate in individual experiments.

Table 1

IC_{50} for the inhibition of lyase activity in rat testis microsomes

Compound	IC_{50} (nM)
Abiraterone	5.8 \pm 0.8 ^a
YM 55208	6.2 \pm 0.5
TX 1196-11, <i>S</i> -enantiomer	6.4 \pm 1.0 ^a
TX 977 racemate	8.7 \pm 2.0 ^a
TX 1197-11, <i>R</i> -enantiomer	13.6 \pm 3.4 [*]
Bifonazole	21.5 \pm 5.6 [*]

Means \pm S.E.M. for four determinations.

^a NS: *P* > 0.05.

^{*} 0.01 < *P* < 0.05 as compared with YM 55208.

all the test compounds. The residual activity expressed as the percentage of total activity obtained in presence of test compounds, decreased within a range of 115–1% from 1 to 128 nM. All test compounds were shown to be potent inhibitors of the $\text{C}_{17,20}$ -lyase with IC_{50} values in the nanomolar range as summarized in Table 1. The decreasing sequence of the inhibitory activity was as follows:

$$\begin{aligned} \text{abiraterone} &\geq \text{YM 55208} \geq \text{TX 1196-11}(\textit{S}\text{-enantiomer}) \\ &\geq \text{TX 977}(\text{racemate}) \\ &\geq \text{TX 1197-11}(\textit{R}\text{-enantiomer}) \geq \text{Bifonazole.} \end{aligned}$$

The racemate product and its *S*-enantiomer, were as potent as abiraterone and YM 55208, the steroidal and non-steroidal references, respectively. There was a slight but not statistically significant difference between the inhibitory potencies of the two diastereoisomers. Bifonazole and the *R*-enantiomer were the less potent compounds in this in vitro model.

3.2. Organ weights

As shown in Fig. 2, after 3 days of oral treatment at 50 mg/kg per day, abiraterone acetate, markedly inhibited VP (–14%) and SV weights (–37%) without affecting adrenal weight (–7%). YM 55208 induced a more potent reduction in VP (–37%) and SV (–48%) but exhibited a harmful effect on adrenals by increasing their weight by 17%. The racemate compound and its *S*-enantiomer, produced similar notable reductions in VP (–24 and –22%, respectively) and SV (–42 and –41%, respectively) but the *S*-enantiomer only caused a slight but statistically significant increase in adrenal weight (+11%).

The *R*-enantiomer caused a marked reduction in VP (–24%) which was comparable to that obtained with its two related compounds. However, it induced a weaker inhibition of SV (–22%), while the increase observed in adrenal weight (15%) was not statistically significant. None of the tested compounds modified testis weight.

3.3. Plasma hormone levels

Fig. 2 shows that when administered orally at 50 mg/kg per day to adult male rat, abiraterone acetate significantly

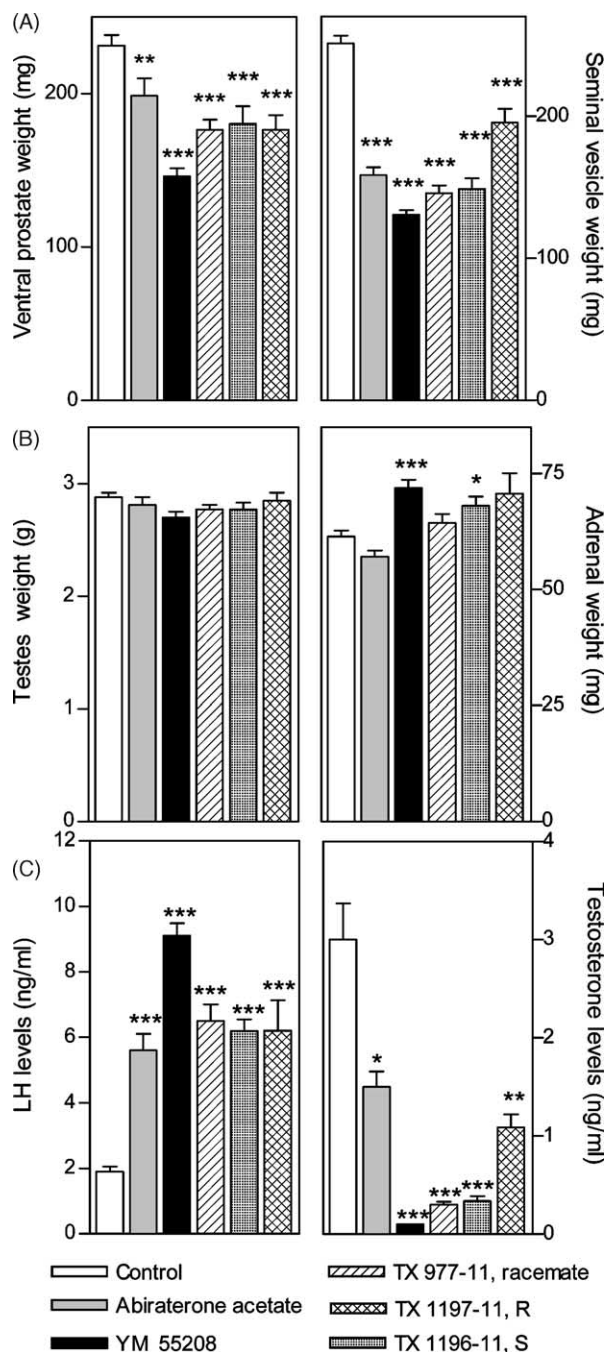


Fig. 2. Anti-androgenic activity in the rat. Adult rats were treated as described in Section 2. They were treated orally with 50 mg/kg per day for 3 days. On the day following the last treatment, ventral prostate seminal vesicles (A), testes and adrenal glands (B) were removed and weighed. Arterial blood was collected and stored at -20°C till T and LH assays (C). Each bar represents the mean \pm S.E.M. of at least eight determinations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ as compared with control group.

inhibited T secretion (-48%) and in turn increased LH concentration (192%). YM 55208 was shown to totally inhibit plasma T secretion (more than -97%) with levels lower than the LOQ. This inhibitory effect, more potent than that of abiraterone acetate was also associated with a feedback

rise in LH secretion (378%). The racemate product and its *S*-enantiomer induced a 89% inhibition on T secretion and increased LH secretion by 240 and 225%, respectively. The *R*-enantiomer significantly inhibited T levels (-63%) and increased LH levels (225%) but its effect on plasma T appeared to be less marked than that of its related compounds.

4. Discussion

In the search of potent inhibitors of $\text{C}_{17,20}$ -lyase, the key enzyme in androgen biosynthesis, a chemical series of new non-steroidal inhibitors was synthesized and tested in vitro and in vivo on the rat $\text{C}_{17,20}$ -lyase.

In the in vitro assay reported here, the HPLC product isolation [22–25] or the classical measurement of the ^3H -acetic acid released [26–28] were substituted with a RIA of $\Delta 4\text{A}$ produced from the substrate 17OHP. Optimum conditions for rat testicular microsome $\text{C}_{17,20}$ -lyase activity measurement with respect to substrate, cofactor, and protein concentrations, as well as time and compatibility with the range of $\Delta 4\text{A}$ in the RIA were determined during preliminary experiments, and are described in details in Section 2. The activity of the control reaction (substrate without enzyme) was found to be less than 4% of the enzyme activity. The kinetic study of the $\text{C}_{17,20}$ -lyase in our conditions indicated that the K_m was 230 nM and the V_{max} was 260 pmol/(min mg) of protein (results not shown).

With this in vitro assay, a chemical series of new non-steroidal compounds was investigated for its inhibitory potency on rat $\text{C}_{17,20}$ -lyase activity (results not shown). One of the compounds was isolated as a lead, TX 977 a racemate product, and its salt TX 977-11 was used in in vivo assays. Its two diastereoisomers, TX 1196-11 (*S*) and TX 1197-11 (*R*), were then synthesized and evaluated in comparison with abiraterone [29] and YM 55208 [14], as a steroidal and a non-steroidal references, respectively, and the anti-fungal bifonazole [16]. In vitro, a slight difference was observed between the two enantiomers, in favor of the *S*-compound. However, the three compounds were as potent as abiraterone and YM 55208 with IC_{50} in the nanomolar range, while bifonazole was the less active compound.

In order to obtain a fast evaluation of the in vivo activity of our new compounds, a short model based on a 3-day treatment by oral route and a sacrifice 24 h after the last administration was set-up. In this model, the known inhibitory effects of abiraterone acetate [30] and YM 55208 [12] on VP and SV weights were reproduced. They were directly related to the decrease in circulating T levels obtained by inhibition of the $\text{C}_{17,20}$ -lyase activity. This decrease in T levels was associated with a rise in plasma LH, illustrating the absence of the negative feedback of T on the pituitary gland. Despite this LH increase, which should stimulate the testicular androgen production, YM 55208 was able to maintain very low levels of T, below the LOQ of the assay that is 0.1 ng/ml. The lead compound and its two enantiomers

were shown to be more potent than abiraterone acetate on VP weights and T levels. As in vitro, a slight dissociation in favor of the *S*- versus the *R*-enantiomer was observed for SV weights and T levels. No side-effects were observed for none of the test compounds on testis (Fig. 2), liver or body weights (results not shown).

However, the *S*-enantiomer was found to induce an increase on adrenal weight. This side-effect although weaker than that observed with YM 55208 may indicate a lack of specificity in inhibition. Then, since in rat the biosynthesis of corticosterone does not require the cytochrome P450C17 enzyme, a selective inhibitor should not have an effect on adrenal function and particularly no effect on corticosterone levels. In an attempt to explore this effect on adrenals, corticosterone levels were measured after a 1-week treatment with the *S*-enantiomer at the dose of 10 mg/kg per day (result not shown). There was no statistically significant difference observed in corticosterone levels as compared with the intact control group, indicating that this new compound is probably devoid of inhibitory activity on other enzymes involved in corticosterone biosynthesis.

In conclusion, optimization is ongoing based on the nanomolar range inhibition obtained with TX 977-11 the lead of our new chemical series of non-steroidal compounds and the slight dissociation observed between its two diastereoisomers, TX 1196-11 (*S*) and TX 1197-11 (*R*). Together with YM 55208 in our model, they proved to be more active in vivo than abiraterone acetate. However, the impact observed on adrenal weight suggests that the specificity of lyase inhibition versus corticosteroid biosynthesis deserve further investigations with this new class of potentially useful agents for the treatment of androgen-dependent prostate cancer. Specificity, pharmacokinetic and long-term models (4 weeks at least) are now in progress.

References

- [1] W.L. Miller, Molecular biology of steroid hormone synthesis, *Endocrinol. Rev.* 9 (1988) 295–318.
- [2] I. Hanukoglu, Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis, *J. Steroid Biochem. Mol. Biol.* 43 (8) (1992) 779–804.
- [3] H. Vanden Bossche, Inhibitors of P450-dependent steroid biosynthesis: from research to medical treatment, *J. Steroid Biochem. Mol. Biol.* 43 (8) (1992) 1003–1021.
- [4] D.F.V. Lewis, P. Lee-Robichaud, Molecular modelling of steroidogenic cytochromes P450 from families CYP11, CYP17, CYP19 and CYP21 based on the CYP102 crystal structure, *J. Steroid Biochem. Mol. Biol.* 66 (4) (1998) 217–233.
- [5] W.L. Miller, R.J. Auchus, D.H. Geller, The regulation of 17,20 lyase activity, *Steroids* 62 (1997) 133–142.
- [6] M. Namiki, M. Kitamura, E. Buczko, M.L. Dufau, Rat testis P-450(17)alpha cDNA: the deduced amino acid sequence, *Biochem. Biophys. Res. Commun.* 157 (2) (1988) 705–712.
- [7] R.J. Auchus, W.L. Miller, Molecular modeling of human P450c17 (17 α -hydroxylase/17,20-lyase): Insights into reaction mechanisms and effects of mutations, *Mol. Endocrinol.* 13 (7) (1999) 1169–1182.
- [8] A.J. Conley, I.M. Bird, The role of cytochrome P450 17 α -hydroxylase and 3 β -hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the Δ 5 and Δ 4 pathways of steroidogenesis in mammals, *Biol. Reprod.* 56 (1997) 789–799.
- [9] B.J. Brock, M.R. Waterman, Biochemical differences between rat and human cytochrome P450c17 support the different steroidogenic needs of these two species, *Biochemistry* 38 (5) (1999) 1598–1606.
- [10] R.W. Hartmann, M. Hector, B.G. Wachall, A. Paluszczak, M. Palzer, V. Huch, M. Veith, Synthesis and evaluation of 17 α -aliphatic heterocycle-substituted steroidal inhibitors of 17 α -hydroxylase/C17-20-lyase (P450 17), *J. Med. Chem.* 43 (23) (2000) 4437–4445.
- [11] I.P. Nnane, K. Kato, Y. Liu, B.J. Long, Q. Lu, X. Wang, Y.-Z. Ling, A. Brodie, Inhibition of androgen synthesis in human testicular and prostatic microsomes and in male rats by novel steroidal compounds, *Endocrinology* 140 (6) (1999) 2891–2897.
- [12] J. Li, Y. Li, C. Son, P. Banks, A. Brodie, 4-Pregnene-3-one-20 β -carboxaldehyde: a potent inhibitor of 17 α -hydroxylase/C17,20-lyase and of 5 α -reductase, *J. Steroid Biochem. Mol. Biol.* 42 (3/4) (1992) 313–320.
- [13] S.E. Barrie, M. Jarman, G.A. Potter, British Technology Group Limited, UK, 17-Substituted steroids useful in cancer treatment, UK Patent Applic. 2,265,624 (1993).
- [14] M. Okada, T. Yoden, E. Kawaminami, Y. Shimada, T. Ishihara, M. Kudoh, Yamanouchi Pharmaceutical Co. Ltd., Japan. Preparation ofazole derivatives as steroid 17–20 lyase inhibitors, WO Patent 9,509,157 (1994).
- [15] Y. Ideyama, M. Kudoh, K. Tanimoto, Y. Susaki, T. Nanya, T. Nakahara, H. Ishikawa, T. Yoden, M. Okada, T. Fujikura, H. Akaza, H. Shikama, Novel nonsteroidal inhibitor of cytochrome P450_{17 α} (17 α -hydroxylase/C17-20 lyase), YM 116, decreased prostatic weights by reducing serum concentrations of T and adrenal androgens in rats, *Prostate* 37 (1998) 10–18.
- [16] M. Ayub, M.J. Levell, Inhibition of testicular 17 α -hydroxylase and 17,20-lyase but not 3 β -hydroxysteroid dehydrogenase-isomerase or 17 β -hydroxysteroid oxidoreductase by ketoconazole and other imidazole drugs, *J. Steroid Biochem.* 28 (5) (1987) 521–531.
- [17] M. Ayub, M.J. Levell, Inhibition of human adrenal steroidogenic enzymes in vitro by imidazole drugs including ketoconazole, *J. Steroid Biochem.* 32 (4) (1989) 515–524.
- [18] H. Vanden Bossche, P. Marichal, J. Gorrens, M.-C. Coene, G. Willemsens, D. Bellens, I. Roels, H. Moereels, P.A.J. Janssen, Biochemical approaches to selective antifungal activity. Focus onazole antifungals, *Mycoses* 32 (Suppl. 1) (1989) 35–52.
- [19] P.B. Kan, M.A. Hirst, D. Feldman, Inhibition of steroidogenic cytochrome P-450 enzymes in rat testis by ketoconazole and related imidazole anti-fungal drugs, *J. Steroid Biochem.* 23 (6A) (1985) 1023–1029.
- [20] M. Ayub, M.J. Levell, Inhibition of rat testicular 17 α -hydroxylase and 17,20-lyase activities by anti-androgens (flutamide, hydroxyflutamide, RU23908, cyproterone acetate) in vitro, *J. Steroid Biochem.* 28 (1) (1987) 43–47.
- [21] M.M. Bradford, A rapid and sensitive method for the quantitation of μ g quantities of protein utilizing the principle of protein dye binding, *Anal. Biochem.* 72 (1976) 248–254.
- [22] S.E. Barrie, M.G. Rowlands, A.B. Foster, M. Jarman, Inhibition of 17 α -hydroxylase/C17-C20 lyase by bifuranol and its analogues, *J. Steroid Biochem.* 33 (6) (1989) 1191–1195.
- [23] M.E. Lombardo, S.I. Hakky, M.K. Hall, P.B. Hudson, A study of androgen biosynthesis by the human testis in vitro, *J. Steroid Biochem. Mol. Biol.* 44 (2) (1993) 191–198.
- [24] G.T. Klus, J. Nakamura, J.-S. Li, Y.-Z. Ling, C. Son, J.A. Kempainen, E.M. Wilson, A.M.H. Brodie, Growth inhibition of human prostate cells in vitro by novel inhibitors of androgen synthesis, *Cancer Res.* 56 (1996) 4956–4964.
- [25] J.-S. Li, Y. Li, C. Son, A.M.H. Brodie, Synthesis and evaluation of pregnane derivatives as inhibitors of human testicular 17 α -hydroxylase/C17,20-lyase, *J. Med. Chem.* 39 (1996) 4335–4339.
- [26] S.L. Miller, J.N. Wright, D.L. Corina, M. Akhtar, Mechanistic studies on pregnene side-chain cleavage enzyme (17 α -hydroxylase-

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.