Drugs of the Future 2009, 34(7): 555-563

CCC: 0377-8282/2009
DOI: 10.1358/dof.2009.34.7.1380625

REVIEW ARTICLE

INHIBITORS OF 17-β-HYDROXYSTEROID DEHYDROGENASE TYPE 3 (17-β-HSD 3)

M.S. Olusanjo[1], S. Ahmed[2]*

[1]School of Pharmacy and Chemistry, Kingston University, Penrhyn Road, Kingston, Surrey, U.K.; [2]School of Science, Faculty of Science and Technology, University of West of Scotland, Paisley, Scotland, U.K. *Correspondence: sabbir.ahmed@uws.ac.uk

CONTENTS

ABSTRACT

The specific conversion of androstenedione to testosterone is catalyzed by 17-β-hydroxysteroid dehydrogenase type 3 (17-β-HSD 3), an enzyme which has been shown to be localized in the microsomal fraction of the Leydig cells of the testis. As such, this enzyme has become a major target in the treatment of hormone-dependent prostate diseases, since the biosynthesis of testosterone allows the formation of the more potent androgen dihydrotestosterone. Here we report some of the steroidal and nonsteroidal inhibitors that have been considered for development as potent and specific inhibitors of 17-β-HSD 3.

INTRODUCTION

The 17-β-hydroxysteroid dehydrogenase (17-β-HSD) family of enzymes catalyze the activation and deactivation of sex steroids involving redox reactions centered about the C(17) area of the steroid backbone (Fig. 1) (1). As such, this family of enzymes has a direct impact on diseases such as breast and prostate cancers that have been shown to be hormone-dependent. More specifically, both diseases depend on the long-term exposure to potent estrogens (e.g., estradiol [E2]) and androgens (e.g., testosterone), which are biosynthesized as a result of the action of different types of 17-β-HSD enzymes.

To date, some 15 forms of this enzyme have been reported (2), with each form catalyzing a substrate- and cofactor-specific reaction (Figs. 2A and 2B) (3). The specific conversion of androstenedione to testosterone is catalyzed by type 3 (17-β-HSD 3) utilizing NADPH as the cofactor, and this enzyme has been shown to be localized in the microsomal fraction of the Leydig cells of the testis (4).

The crystal structure of 17-β-HSD 3 has yet to be elucidated, although Ghosh et al. (5) have shown that, in comparison to the previously determined crystal structure of type 1 17-β-HSD (17-β-HSD 1), an additional 46 amino acid residues have been shown to exist within the primary structure of 17-β-HSD 3, thereby totaling some 310 amino acid residues. Furthermore, comparison of the two structures shows that the substrate specificity of 17-β-HSD 1 in comparison to 17-β-HSD 3 is due to the former amino acid sequences (residues 192-228) at the portion of the helix αG′ of the enzyme, while the latter shows amino acid sequence residues at 235-261 which could therefore be associated with substrate specificity and preferential androgen catalysis by 17-β-HSD 3 (5, 6).

STEROIDAL INHIBITORS OF 17-β-HSD 3

As previously mentioned, the crystal structure of 17-β-HSD 3 is unknown, and therefore a number of workers within the field have used the substrate as a template for the design of novel inhibitors (7, 8). An early attempt to map the active site of 17-β-HSD 3 with the view of determining a pharmacophore so as to aid in the design of novel inhibitors was undertaken by Pittaway (9). From that study of numerous steroid-based compounds, the author concluded that the C(17) carbonyl moiety, together with a nonaromatic A-ring, were two important features of potential inhibitors of 17-β-HSD 3. Numerous androgen-based compounds were evaluated using enzyme derived from canine testis, but only two compounds were found to possess inhibitory activity. More specifically, compounds **1** and **2** (Fig. 3) were found to competitively inhibit the enzyme and were found to possess K_i values of 2.4 and 6.8 μM, respectively. However, due to the steroidal nature of compounds **1** and **2**, in particular due to their structural similarity to androstenedione, they were found to possess a strong androgenic effect.

Lombardo et al. (10) undertook the evaluation of atamestane (Fig. 3) for activity against 17-β-HSD 3. This compound is a substrate analogue of androstenedione, for both aromatase and 17-β-HSD 3, and has previously been reported to be an irreversible inhibitor of aromatase (11). However, it was found to possess an identical K_m value to androstenedione for 17-β-HSD 3.

Poirier et al. (12) screened 80 steroids of different classes and based their inhibitor design on the C(19) steroid androsterone. The various

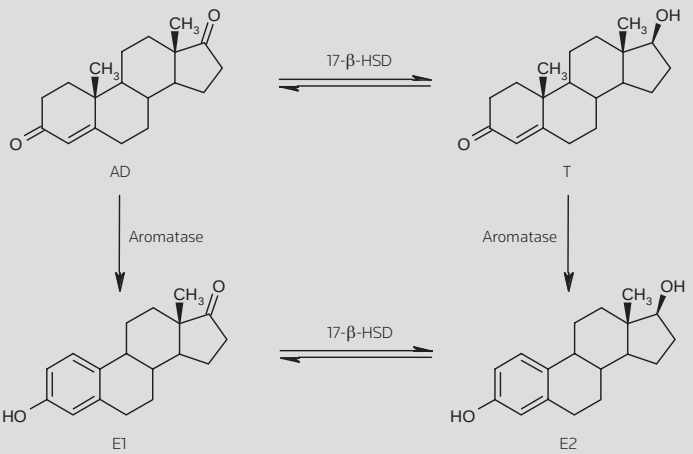


Figure 1. General mechanism catalyzed by the 17-β-HSD family of enzymes on the C(17) position of the steroid backbone.

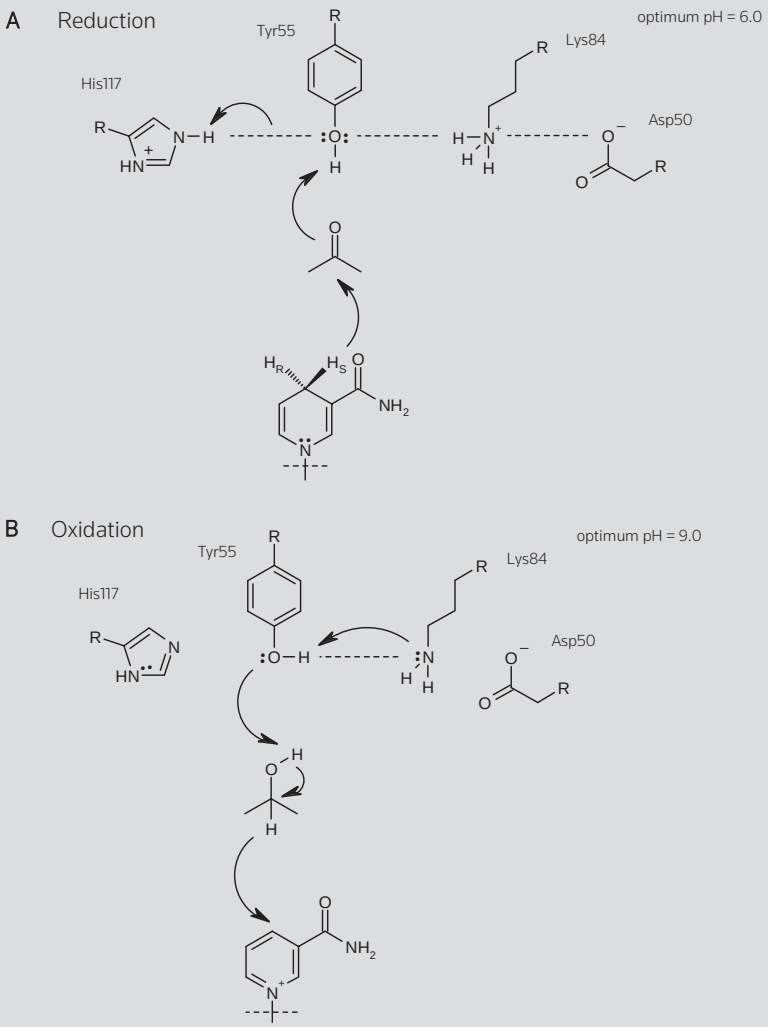


Figure 2. A) Reduction and **B**) oxidation reactions catalyzed by the various forms of the 17-β-HSD family of enzymes (3).

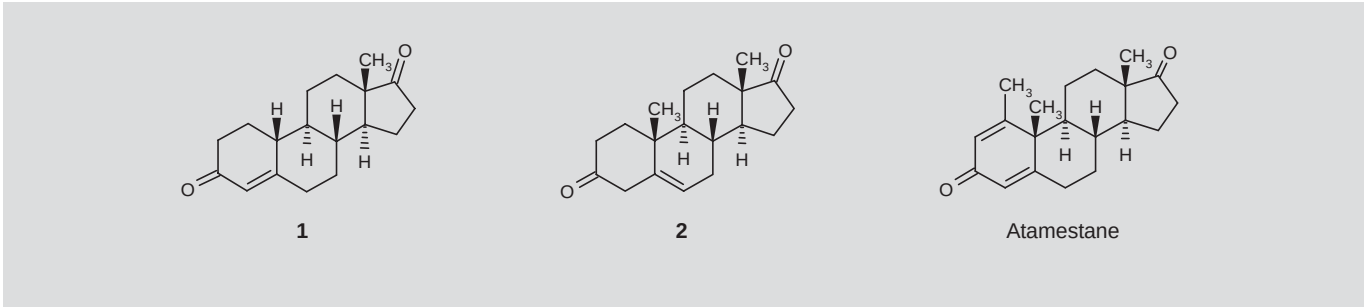


Figure 3. Steroidal inhibitors of 17-β-HSD 3.

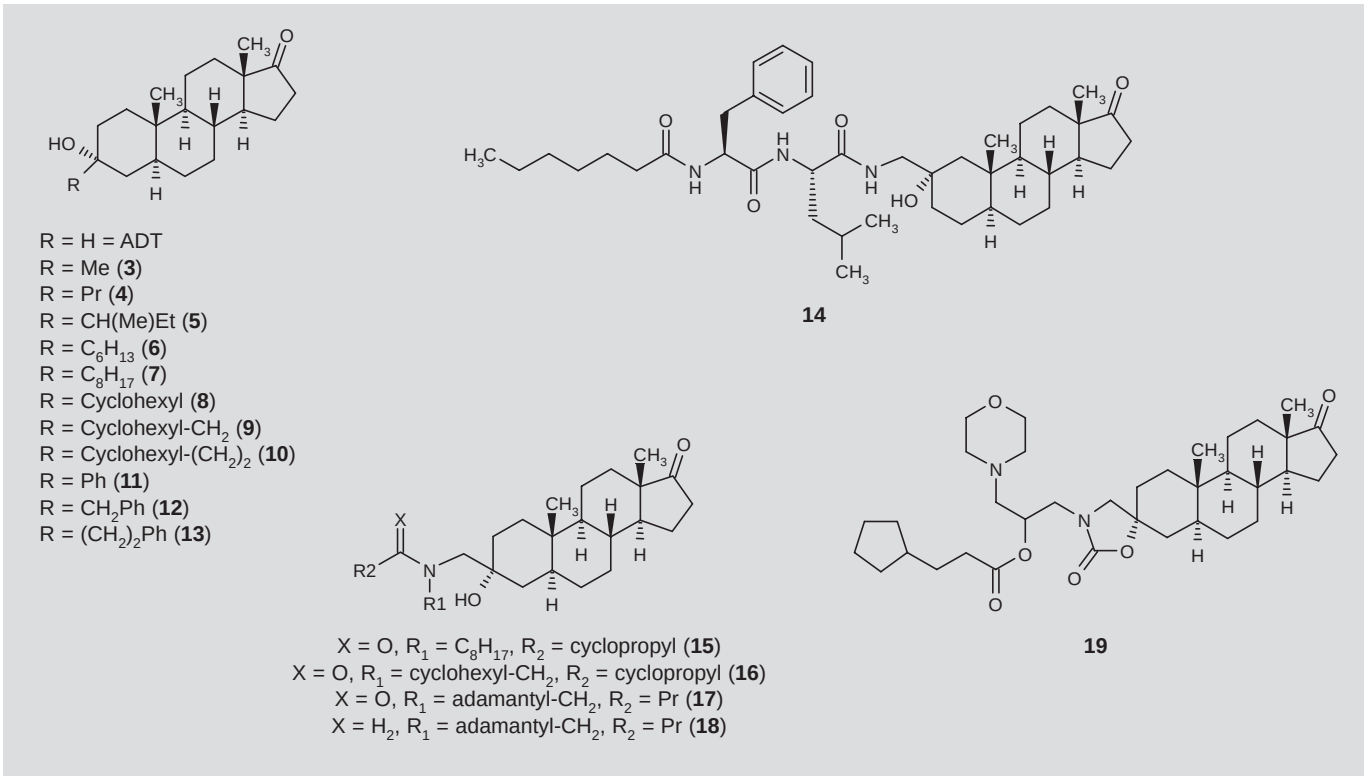
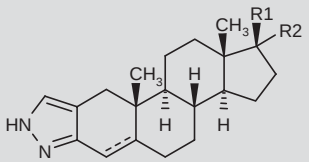


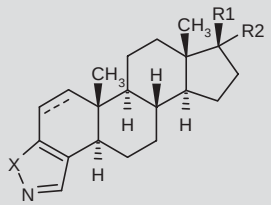
Figure 4. Androsterone derivatives as inhibitors of 17-β-HSD 3.

inhibitors designed were biologically evaluated in the microsomal fraction of human embryonic kidney HEK-293 cells transfected with 17-β-HSD 3. The initial derivatization undertaken involved substitutions at the 3β-position of androsterone. Indeed, the 3β-substituted compounds **3**-**13** (Fig. 4) showed good inhibitory activity and were found to possess IC_{50} values between 57 and 147 nM. More specifically, of the compounds evaluated against 17-β-HSD 3, 3β-propylandrosterone (**4**), 3β-*sec*-butylandrosterone (**5**), 3β-2(cyclohexylethyl)-androsterone (**10**) and 3β-benzylandrosterone (**12**) were the most potent, showing IC_{50} values of 67, 73, 60 and 57 nM, respectively; in comparison, androsterone was found to have an IC_{50} value of 330 nM. Furthermore, the compounds were found to be specific inhibitors of 17-β-HSD 3 compared to 17-β-HSD 1 and 17-β-HSD 5, even at an inhibitor concentration of 0.3 μM. However, as with previous androgen-based compounds, they were all found to possess strong androgenic effects (13). In an effort to reduce the androgenicity of the compounds, derivatization at the C(16) position of the steroid backbone was undertaken, but these compounds showed weak inhibitory activity. For example, the use of a 3′-bromopropyl side-chain at the C(16β) position resulted in a compound that was found to possess 39% inhibitory activity against 17-β-HSD 3 at a concentration of 3 μM (14).

Maltais et al. (15), using combinatorial chemistry, undertook the solid-phase synthesis of derivatives of 3β-peptido-3α-hydroxy-5α-androstan-17-one. The compounds were derivatized so as to produce a number of levels of molecular diversity in an effort to optimize the inhibitory activity while decreasing their androgenic properties. The compounds were evaluated against 17-β-HSD 3 and the most potent inhibitors were found to be those containing a trisubstituted amino acid residue at the 3β side-chain of androsterone. The most potent was compound **14** (Fig. 4), which was found to have an IC_{50} value of



R_1 = OH, R_2 = Me (**20**)
R_1 = CH(Me)CH_2OH, R_2 = H, Δ^4 (**21**)



R_1 = OH, R_2 = Me, X = NH (**22**)
R_1 = CH(Me)CH_2OH, R_2 = H, X = NH (**23**)
R_1 = CH(Me)CH_2OH, R_2 = H, X = NH, Δ^1 (**24**)
R_1 = CH(Me)CH_2OH, R_2 = H, X = O (**25**)

Figure 5. Azoles as specific inhibitors of 17-β-HSD 3.

227 nM, being twice as potent as androsterone (IC_{50} = 489 nM). Furthermore, compound **14** showed weak androgenic activity at 1 μM and was devoid of any androgenic properties at 0.1 μM. The synthesis of further 3β-substituted derivatives of androsterone resulted in four inhibitors that showed extremely potent inhibitory activity against Shionogi AR^+ 17-β-HSD 3 cells, namely compounds **15** (IC_{50} = 57 ± 13 nM), **16** (IC_{50} = 85 ± 9 nM), **17** (IC_{50} = 35 ± 6 nM) and **18** (IC_{50} = 80 ± 18 nM) (Fig. 4). The synthesis of a library based on 3-carbamate-*N*-substituted-5α-androstan-17-one was also undertaken and resulted in compound **19** (IC_{50} = 74 ± 12 nM), which was found to possess good inhibitory activity and was devoid of androgenic activity (Fig. 4) (16, 17).

Some saturated and unsaturated azole-containing steroidal inhibitors (Fig. 5) have also been synthesized and on subsequent evaluation were found to possess inhibitory activity against 17-β-HSD 3. Ferrari and Arnold (18) showed that pyrazole-containing steroidal compounds were potent and selective inhibitors of bacterial 17-β-HSD 3 obtained from *Pseudomonas testosteroni* and were found to inhibit the enzyme via a reversible and competitive mode of action. Although the enzyme from *P. testosteroni* is not directly comparable to the human enzyme, these compounds were shown to selectively inhibit the bacterial 17-β-HSD 3. Furthermore, Levy et al. (19) examined the specificity of pyrazole-fused steroidal compounds and their derivatives against 17-β-HSD 3 obtained from *P. testosteroni* and showed that the isoxazole-fused steroidal compounds were less potent than the previously reported pyrazole-fused compounds. For example, consideration of the 2,3-steroidal fused pyrazoles shows that compounds **20** and **21** were found to possess K_i values of 20 ± 4 and 15 ± 3 nM, respectively, while the 3,4-derivatives (compounds **22** and **23**) were found to be more potent, possessing K_i values of 6 ± 2 and 7 ± 2 nM, respectively. In comparison, the introduction of a double bond functionality at the C(1) position of the steroid backbone resulted in a decrease in inhibitory activity, with compound **24** showing a K_i value of 100 ± 20 nM. The isoxazole derivative **25**, however, was found to possess the weakest inhibitory activity against 17-β-HSD 3, with a K_i value of 190 ± 30 nM.

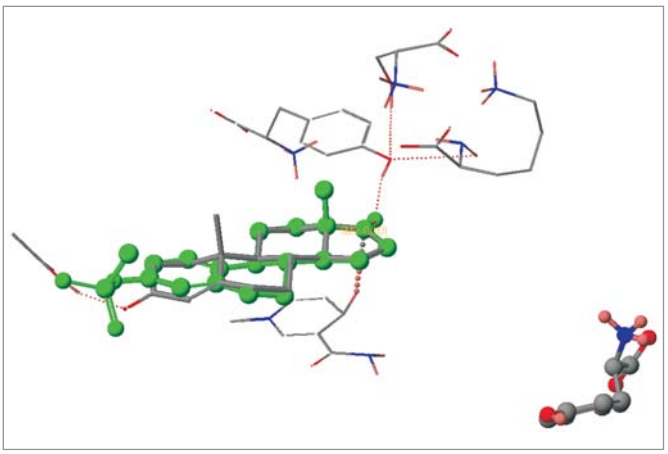


Figure 6. Compound **26** (in green) superimposed onto the transition state for the reaction catalyzed by 17-β-HSD 3 (20, 21).

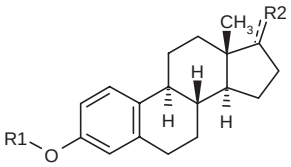
Recently, a number of esters of both androgens and estrogens have been synthesized and evaluated against rat testis microsomal 17-β-HSD 3 and were found to be potent and specific inhibitors of the enzyme (20, 21). Within the same study, the synthesis of a series of sulfonate derivatives of estrone (E1), including estrone-3-*O*-sulfamate (EMATE) and compounds **26**-**35,** was undertaken, and on subsequent evaluation the compounds were found to possess potent inhibitory activity against 17-β-HSD 3 (Table I). Furthermore, the compounds were found to be weak inhibitors of both 17-β-HSD 1 and 3-β-HSD, suggesting that they are specific inhibitors of 17-β-HSD 3. Detailed modeling of the compounds within the representation of the active site of 17-β-HSD 3 shows that the inhibitors are good mimics of the substrate and that the sulfonate moiety undergoes conformational change so as to avoid steric hindrance, with the area of the active site corresponding to the C(3) area of the substrate backbone (Fig. 6). These compounds, containing an estrogenic backbone, would therefore be expected to possess weak androgenic properties, and as such, they provide an alternative route to the design and synthesis of novel steroidal inhibitors of 17-β-HSD 3.

NONSTEROIDAL INHIBITORS OF 17-β-HSD 3

In general, steroidal inhibitors have been shown to possess androgenic effects, and therefore the synthesis of nonsteroidal compounds has increased in pace to decrease this effect. That is, due to the structural differences between both steroidal and nonsteroidal compounds, the latter compounds should not be able to mimic the androgens and therefore would not elicit an androgenic effect.

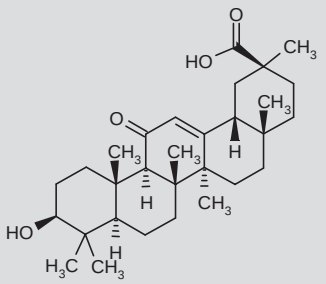
For example, glycyrrhetinic acid (Fig. 7), produced from the hydrolysis of the licorice component glycyrrhizic acid, has been shown to inhibit the in vitro conversion of androstenedione to testosterone using rat testis microsomal enzyme, giving an IC_{50} value of 4 μM (22). To determine the effect of licorice on gonadal function, male volunteers were administered licorice (7 g daily containing 0.5 g of

Table I. *Estrone-based inhibitors of 17-β-HSD 3.*



Compound	R1	R2	% Inhibition of 17-β-HSD 3 at 100 μM	IC_{50} (μM)
EMATE	SO_2NH_2	O	82.4 ± 0.09	22.15 ± 1.57
26	SO_2Me	O	87.7 ± 0.22	1.45 ± 0.08
27	SO_2CF_3	O	80.8 ± 2.32	1.64 ± 0.01
28	4-NO_2-$PhSO_2$	O	29.5 ± 4.85	ND
29	4-Ph-$PhSO_2$	O	60.7 ± 0.61	ND
30	4-Br-$PhSO_2$	O	68.1 ± 0.70	ND
31	4-Cl-$PhSO_2$	O	71.9 ± 1.91	ND
32	4-CF_3-$PhSO_2$	O	65.6 ± 0.56	ND
33	4-I-$PhSO_2$	O	68.4 ± 1.96	ND
34	$PhSO_2$	O	71.3 ± 1.36	ND
35	SO_2Me	OSO_2Me	4.1 ± 1.68	ND

ND = not determined.



Glycyrrhetinic acid

Figure 7. Licorice components as inhibitors of 17-β-HSD 3.

glycyrrhizic acid) and it was found that serum testosterone levels decreased by 35% while 17α-hydroxyprogesterone levels increased by 21%, thereby suggesting that licorice was able to inhibit both 17-β-HSD 3 and 17,20-lyase enzymes; as such, it lacked specificity and therefore was not developed further (23, 24).

Le Lain et al. (25) screened a series of derivatives of *p*-benzoquinones, flavones, isoflavones and triphenylethene (Fig. 8), in human testicular microsomes in the presence of androstenedione (K_m = 0.28 ± 0.06 μM; substrate concentration = 2 μM) and NADPH as cofactor; these compounds were found to give IC_{50} values within the range of 2.7-100.5 μM. Diphenyl-*p*-benzoquinone and phenyl-*p*-benzoquinone were found to be the most potent inhibitors, with IC_{50} values of 2.7 and 5.7 μM, respectively.

Coumarin-based compounds have also been evaluated against human testis microsomal 17-β-HSD 3, with umbelliferone and 4-methylumbelliferone (Fig. 8) found to be potent inhibitors, possessing IC_{50} values of 1.4 and 0.91 μM, respectively (26).

However, Olusanjo (20, 21) showed that substituted 4-methylumbelliferone-based compounds (compounds **36**-**38**) possessed low inhibitory activity against rat testis microsomal 17-β-HSD 3 (Table II). These compounds were found to show greater inhibitory activity against estrone sulfatase; for example, coumate showed 97% and ~45% inhibition, respectively, of estrone sulfatase and 17-β-HSD 3 (27). In this study, 4-methylumbelliferone (**36**) was found to show 59% inhibition of 17-β-HSD 3 at 100 μM, much less than that observed by Le Lain et al. (26) using human microsomal enzymes.

Smith et al. (28), using 17-β-HSD 3 from human testicular microsomes, evaluated the inhibitory effect of a series of novel benzyltetralin-based inhibitors (compounds **39**-**47**), which were found to possess good inhibitory activity (biochemical evaluation was undertaken at pH 7.45 with a substrate concentration of 2.0 μM and an inhibitor concentration of 200 μM) (Fig. 9). Compounds **39**, **40** and **41** were found to possess IC_{50} values of 1.8, 8.3 and 7.0 mM, respectively. The compounds were also evaluated against 17-β-HSD 3 obtained from rat testicular microsomes at a concentration of 100 μM; under these conditions, the compounds showed inhibitory activity within the range of 58-79%. More specifically, compounds **39** and **41** were found to give IC_{50} values of 46.9 and 48.6 μM, respectively. The most potent compound against rat 17-β-HSD 3 was compound **42**, which showed an IC_{50} value of 32.7 μM. In an effort to determine specificity, the compounds were also evaluated against both 17-β-HSD 1 and 17-β-HSD 2 enzymes and were found to inhibit these enzymes; for example, compound **41** showed an IC_{50} value of 21.0 μM against 17-β-HSD 1. Compound **41** was more potent against 17-β-HSD 1 than 17-β-HSD 3, and the compounds were therefore not further developed. Derivatives of **47** and the novel benzofuranone **48** (Fig. 9) have also been considered and were found to possess inhibitory activity against 17-β-HSD 3, and were therefore considered to have potential therapeutic use against prostate cancer (29, 30). However, all of these compounds showed potent inhibitory activity against other members of the 17-β-HSD family of enzymes, especially 17-β-HSD 1, and therefore have not been further developed.

Lota et al. (31, 32) undertook the design and synthesis of a series of 4-hydroxyphenylketones (**49-62**). The inhibitors were designed with a ketone functionality so as to mimic the C(17) carbonyl of the steroid backbone and a hydroxyl moiety that would form hydrogen bonds

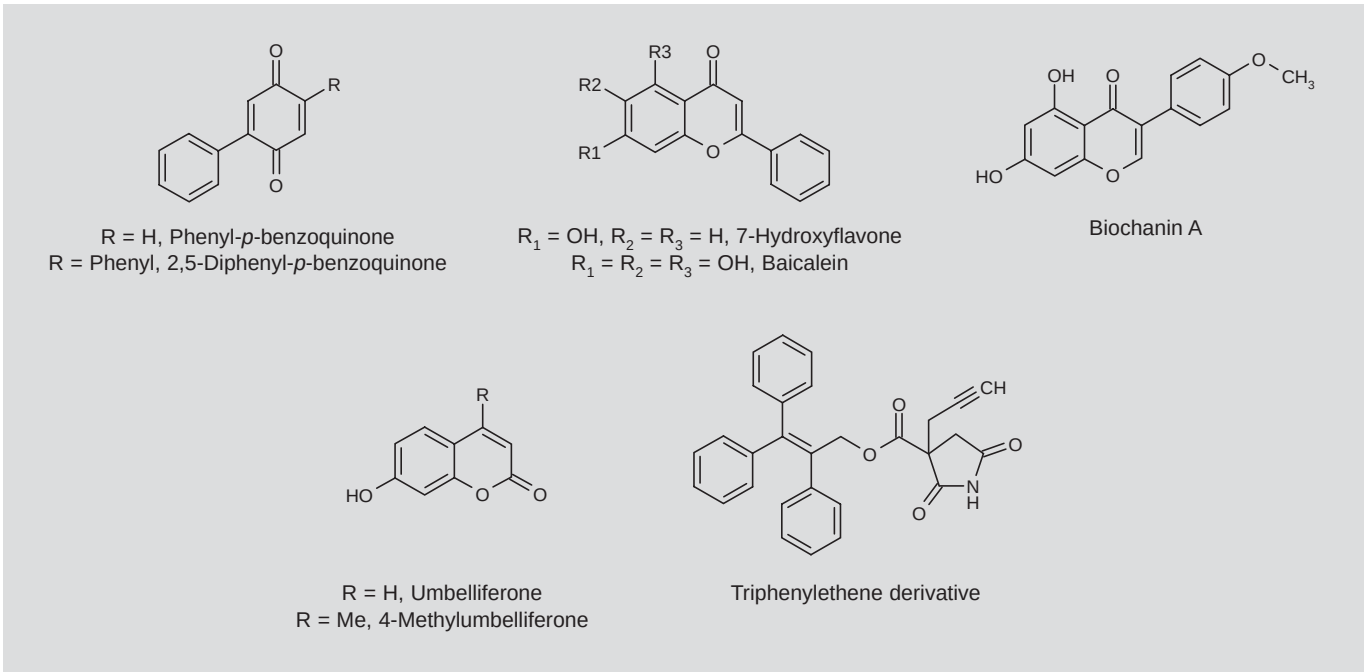
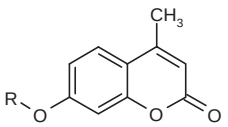


Figure 8. Nonsteroidal inhibitors of 17-β-HSD 3.

Table II. *Coumarin-based inhibitors of 17-β-HSD 3.*



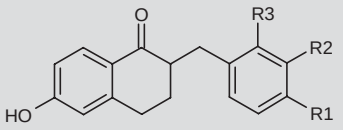
Compound	R	% Inhibition of 17-β-HSD 3 at 100 μM
36	H	59.2 ± 0.08
Coumate	SO_2NH_2	44.6 ± 2.93
37	SO_2Me	12.9 ± 1.59
38	SO_2CF_3	12.2 ± 3.59

with potential hydrogen-accepting groups about the C(16) area of the steroid backbone (Table III). Biochemical evaluation using rat testis microsomal enzyme showed that a number of compounds were, in general, more potent than previously reported standard inhibitors such as baicalein or 7-hydroxyflavone, except for compounds **49** and **50**. The most potent compounds were found to be **54** (IC_{50} = 7.8 μM), **55** (IC_{50} = 6.5 μM), **56** (IC_{50} = 2.9 μM), **57** (IC_{50} = 5.0 μM) and **58** (IC_{50} = 7.6 μM), with **56** being the most active, while **52** (IC_{50} = 60.5 μM) was equipotent to 7-hydroxyflavone (IC_{50} = 67.0 μM); compound **56** was approximately 65 and 23 times more potent, respectively, than baicalein and 7-hydroxyflavone. Consideration of increasing alkyl chain length with inhibitory activity showed that the calculated partition coefficient (log*P*) was an important factor in the determination of the overall inhibitory activity of the compounds. Indeed, a good correlation was observed when the log*P* was plotted against IC_{50} values of the inhibitors, with the optimum log*P* being 4.1.

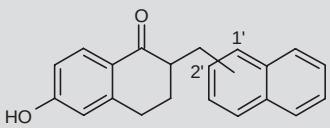
Day et al. (33) recently reported a range of novel compounds as potent and selective inhibitors of 17-β-HSD-3 (Fig. 10). When **STX-2171** and **STX-2624** were evaluated against 17-β-HSD 3, these inhibitors were shown to possess IC_{50} values of 200 and 441 nM, respectively, compared to a dibenzothiazocine standard (**STX-2622**; IC_{50} = 201 nM), which was previously shown to be a potent inhibitor by Bristol-Myers Squibb (34). STX-2171 and STX-2624 were found to be selective inhibitors, as both showed no activity against 17-β-HSD 1 or 17-β-HSD 2 when evaluated in vitro and in vivo using radiometric assays. When evaluated against wild-type and transfected LNCaP prostate cancer cell lines, STX-2171 showed cytotoxicity at 5 μM, but both STX-2171 and STX-2624 inhibited androstenedione-stimulated transfected cell lines at 500 nM, without toxicity (33, 35).

Owen et al. (36) recently reported the inhibitory activity of a series of imidazole-based compounds (**63**-**79**) against 17-β-HSD 3. These compounds were originally designed to inhibit the 17β-hydroxylase/17,20-lyase ($P450_{17\alpha}$) enzyme (Table IV). The compounds were evaluated against rat testis microsomal 17-β-HSD 3 and $P450_{17\alpha}$ in comparison to ketoconazole, a standard that has previously been shown to be a potent inhibitor of $P450_{17\alpha}$. Compounds **68**, **71** and **73** were found to provide ~40% maximal inhibition of 17-β-HSD 3, whereas ketoconazole produced 15% inhibitory activity and **63** 7% inhibition at a concentration of 100 μM. However, these compounds were found to show potency and specificity for $P450_{17\alpha}$ rather than 17-β-HSD 3.

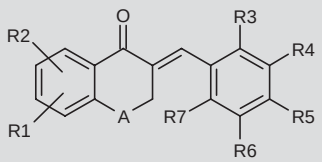
The use of known commercially available nonsteroidal compounds prescribed for other diseases to inhibit steroid biosynthesis in rats was first reported by Ghosh and Dasgupta (37). Gentamicin (Fig. 11), an aminoglycoside used in the treatment of Gram-negative bacterial infections, was administered as a short-term treatment to male Wistar rats for 7 days at doses of 40, 60, 80 and 100 mg/kg. The results showed that 17-β-HSD 3 enzyme activity was reduced to the greatest extent at higher doses, with 34% and 37% inhibition, respectively, at 80 and 100 mg/kg. Sesquiterpene *S*-petasin (Fig. 11),



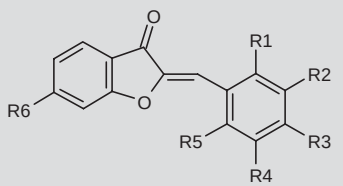
R_1 = Cl, R_2 = R_3 = H (**39**)
R_1 = Br, R_2 = R_3 = H (**40**)
R_1 = F, R_2 = R_3 = H (**41**)
R_1 = R_3 = Cl, R_2 = H (**42**)
R_1 = R_3 = H, R_2 = Me (**43**)
R_1 = R_3 = H, R_2 = CF_3 (**44**)



1'-Naph (**45**)
2'-Naph (**46**)



47



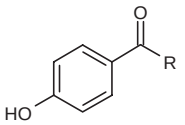
48

Figure 9. Novel nonsteroidal inhibitors of 17-β-HSD 3 (where A = O/CH_2).

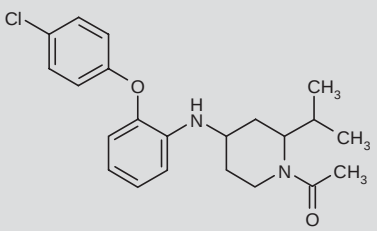
an anti-inflammatory and analgesic agent, was also evaluated both in vitro and in vivo against 17-β-HSD 3 by Lin et al. (38). For the in vivo assay, adult male Sprague–Dawley rats were administered an i.v. injection of 1 μg/kg, which produced a decrease in plasma testosterone levels of 38% after 30 min. For the in vitro assay, rat testicular interstitial cells were incubated with the drug at concentrations of 0-43 μM in the presence of androstenedione (1 nM), with the greatest inhibition being observed at concentrations of 0.43 and 43 μM, equivalent to 0.14 and 14.4 μg/mL, respectively, *S*-petasin dose.

Andric et al. (39) studied the use of a mixture of poly (tri-, tetra- and penta-) chlorinated biphenyls (combination named arochlor-1248) for inhibition of rat testicular androgenesis using both in vitro and in vivo assays. For the in vivo studies single i.p. (10 mg/kg) or bilateral intratesticular (25.5 mg/testis) injections were used and resulted in a significant decrease in serum testosterone after 24 h. When rat interstitial cells were incubated with arochlor-1248 in the presence of androstenedione (2 μM) for 2 h in vitro, weak inhibitory activity was observed against 17-β-HSD 3. Furthermore, the conversion of

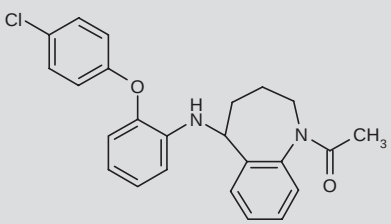
Table III. *Some 4-hydroxyphenylketones as potent inhibitors of 17-β-HSD 3.*



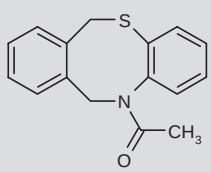
Compound	R	IC_{50} (μM)
49	Me	1708.9 ± 170.7
50	Et	150.6 ± 12.2
51	Pr	89.5 ± 6.7
52	Bu	60.5 ± 5.8
53	C_5H_{11}	18.0 ± 0.9
54	C_6H_{13}	7.8 ± 0.3
55	C_7H_{15}	6.5 ± 0.1
56	C_8H_{17}	2.9 ± 0.0
57	C_9H_{19}	5.0 ± 0.2
58	$C_{11}H_{23}$	7.6 ± 0.3
59	Cyclobutyl	27.2 ± 2.2
60	Cyclopentyl	33.2 ± 1.6
61	Cyclohexyl	36.2 ± 2.5
62	Cycloheptyl	29.7 ± 0.7
Baicalein		185.9 ± 12.7
7-Hydroxyflavone		67 ± 0.9



STX-2624



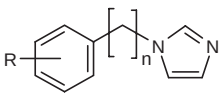
STX-2171



STX-2622

Figure 10. Potent inhibitors of 17-β-HSD 3 (33).

Table IV. *Substituted phenylalkylimidazole-based compounds as inhibitors of 17-β-HSD 3.*



Compound	R	n	% Inhibition of 17-β-HSD 3 at 100 μM
Ketoconazole	N/A	N/A	15.5
63	H	1	6.6
64	H	2	0
65	H	3	0
66	H	4	14.9
67	H	5	25.8
68	H	6	40.3
69	H	7	37.2
70	H	8	28.2
71	H	9	40.8
72	3-I	1	15.2
73	3-F	1	41.1
74	3-Cl	1	0
75	3-Br	1	0
76	3,4-F_2	1	11.6
77	3,5-$(CF_3)_2$	1	17.7
78	3,4-Cl_2	1	29.8
79	4-Me	1	9.3

N/A = not applicable.

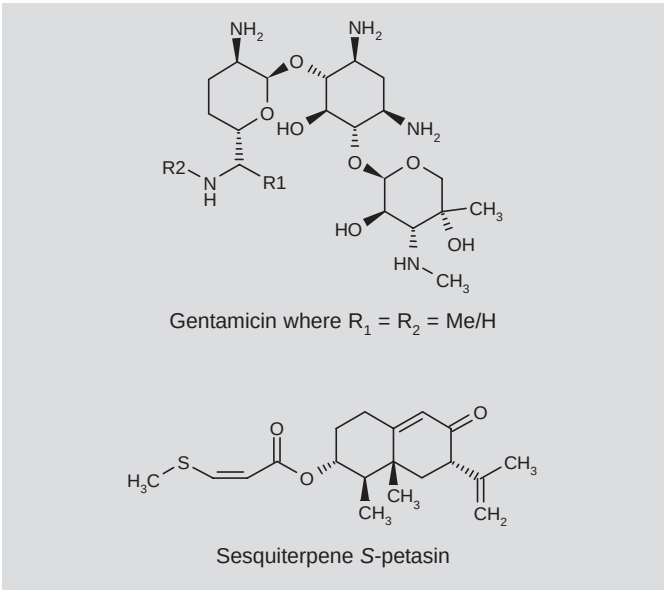


Figure 11. Inhibitors of 17-β-HSD 3.

progesterone to testosterone and dihydrotestosterone was inhibited in a concentration-dependent fashion, with IC_{50} values in the sub-nanomolar range, suggesting that arochlor-1248 is specific for the $P450_{17\alpha}$ enzyme and not 17-β-HSD 3.

CONCLUSIONS

In conclusion, the design and synthesis of selective inhibitors of 17-β-HSD 3 has progressed considerably since the initial synthesis of androgen-based compounds, which were shown to possess androgenic effects. Indeed, recently, with the development of molecular models for the active site of 17-β-HSD 3, considerable progress has been made towards the synthesis of potent and specific (both steroidal and nonsteroidal) inhibitors of this enzyme.

DISCLOSURE

The authors state no conflicts of interest.

REFERENCES

1. Mindnich, R., Möller, G., Adamski, J. *The role of 17beta-hydroxysteroid dehydrogenases*. Mol Cell Endocrinol 2004, 218(1-2): 7-20.
2. Luu-The, V., Belanger, A., Labrie, F. *Androgen biosynthetic pathways in the human prostate.* Best Pract Res Clin Endocrinol Metab 2008, 22(2): 207-21.
3. Penning, T.M. *Molecular determinants of steroid recognition and catalysis in aldo-keto reductases. Lessons from 3α-hydroxysteroid dehydrogenase.* J Steroid Biochem Mol Biol 1999, 69(1-6): 211-25.
4. Moeller, G., Adamski, J. *Multifunctionality of human 17β-hydroxysteroid dehydrogenases*. Mol Cell Endocrinol 2006, 248(1-2): 47-55.
5. Ghosh, D., Pletnev, V.Z., Zhu, D.-W. et al. *Structure of human estrogenic 17β-hydroxysteroid dehydrogenase at 2.20Å resolution*. Structure 1995, 3(5): 503-13.
6. Moghrabi, N., Andersson, S. *17β-Hydroxysteroid dehydrogenases: Physiological roles in health and disease*. Trends Endocrinol Metab 1998, 9(7): 265-70.
7. Poirier, D. *Inhibitors of 17β-hydroxysteroid dehydrogenases*. Curr Med Chem 2003, 10(6): 453-77.
8. Day, J.M., Tutill, H.J., Purohit, A., Reed, M.J. *Design and validation of specific inhibitors of 17β-hydroxysteroid dehydrogenase for therapeutic appli-*

cation in breast and prostate cancer, and in endometriosis. Endocr Relat Cancer 2008, 15(3): 665-92.

9. Pittaway, D.E. *Inhibition of testosterone synthesis in the canine testis in vitro*. Contraception 1983, 27(4): 431-6.
10. Lombardo, M.E., Hakky, S.I., Hudson, P.B. *In vitro studies on the inhibition of testosterone synthesis in the human testis by atamestane*. J Steroid Biochem Mol Biol 1993, 44(3): 287-90.
11. Etreby, M.F., Nishino, Y., Habenicjt, U.F., Henderson, D. *Atamestane, a new aromatase inhibitor for the management of benign prostatic hyperplasia*. J Androl 1991, 12(6): 403-14.
12. Poirier, D., Labrie, F., Luu-The, V. *Inhibition de la 17β-hydroxysteroide dehydrogenase (17β-HSD) type 3 par des derives steroidiens: Etude exploratoire*. Medecine-Sciences 1995, 11(Suppl. 2): 24.
13. Tchedam-Ngatcha, B., Luu-The, V., Poirier, D. *Androsterone 3β-substituted derivatives as inhibitors of type 3 17β-hydroxysteroid dehydrogenase*. Bioorg Med Chem Lett 2000, 10(22): 2533-6.
14. Tchedam-Ngatcha, B., Luu-The, V., Poirier, D. *Androsterone derivatives substituted at position 16: Chemical synthesis, inhibition of type 3 17β-hydroxysteroid dehydrogenase, binding affinity for steroid receptors and proliferative/antiproliferative activity on Shionogi (AR+) cells.* J Enzyme Inhib Med Chem 2002, 17(3): 155-65.
15. Maltais, R., Luu-The, V., Poirier, D. *Parallel solid-phase synthesis of 3β-peptido-3α-hydroxy-5α-androstan-17-one derivatives for inhibition of type 3 17b-hydroxysteroid dehydrogenase*. Bioorg Med Chem 2001, 9(12): 3101-11.
16. Maltais, R., Poirier, D. *A solution-phase combinatorial parallel synthesis of 3β-amido-3α-hydroxy-5α-androstane-17-ones.* Tetrahedron Lett 1998, 39(24): 4151-4.
17. Maltais, R., Luu-The, V., Poirier, D. *Synthesis and optimisation of a new family of type 3 17β-hydroxysteroid dehydrogenase inhibitors by parallel liquid-phase chemistry*. J Med Chem 2002, 45(3): 640-53.
18. Ferrari, R.A., Arnold, A. *Inhibition of β-hydroxysteroid dehydrogenase I. Structural characteristics of some steroidal inhibitors*. Biochim Biophys Acta 1963, 77: 349-56.
19. Levy, M.A., Holt, D.A., Brandt, M., Metcalf, B.W. *Inhibition of 3(17)β-hydroxysteroid dehydrogenase from Pseudomonas testosteroni by steroidal A ring fused pyrazoles.* Biochemistry 1987, 26(8): 2270-9.
20. Olusanjo, M.S. *Synthesis and biochemical evaluation of potential steroidal and non-steroidal inhibitors of 17β-hydroxysteroid dehydrogenase (17β-HSD) in the treatment of hormone-dependent cancers*. Thesis, Kingston University, 2008.
21. Olusanjo, M.S., Shahid, I., Owen, C.P., Ahmed, S. *Inhibition of 17β-hydroxysteroid dehydrogenase (17β-HSD) by imidazole-based compounds.* Lett Drug Des Discov 2008, 5(1): 48-51.
22. Sakamoto, K., Wakabayashi, K. *Inhibitory effect of glycyrrhetinic acid on testosterone production in rat gonads.* Endocrinol Jpn 1988, 35(2): 333-42.
23. Armanini, D., Bonnani, G., Palermo, M.N. *Reduction of serum testosterone in men by licorice*. N Engl J Med 1999, 341(15): 1158.
24. Farese, R.V. Jr., Biglieri, E.G., Shackleton, C.H.L., Irony, I., Gomez-Fontes, R. *Licorice-induced hypermineralocorticoidism*. N Engl J Med 1991, 325(17): 1223-7.
25. Le Lain, R., Nicholls, P.J., Smith, H.J., Maharlouie, F.H. *Inhibitors of human and rat testes microsomal 17β-hydroxysteroid dehydrogenase (17β-HSD) as potential agents for prostatic cancer.* J Enzyme Inhib 2001, 16(1): 35-45.
26. Le Lain, R., Barrell, K.J., Saeed, G.S., Nicholls, P.J., Simons, C., Kirby, A., Smith, H.J. *Some coumarins and triphenylethene derivatives as inhibitors of human testes microsomal 17β-hydroxysteroid dehydrogenase (17β-HSD type 3): Further studies with tamoxifen on the rat testes microsomal enzyme*. J Enzyme Inhib Med Chem 2002, 17(2): 93-100.
27. Cartledge, T. *Synthesis, biochemical evaluation and physicochemical property determination of a range of potential estrone sulfatase inhibitors in the treatment of hormone-dependent breast cancer*. Thesis, Kingston University, 2008.
28. Smith, H.J., Mason, P., Ahmadi, M., Nicholls, P.J., Greer, V. (University College Cardiff Consultants Ltd.). *Benzyl tetralins, formulations and uses thereof*. WO 2001042181.
29. Yoshihama, M., Nakakoshi, M., Nakamura, J., Nakayama, S. (Snow Brand Milk Product Co. Ltd.). *Benzofuranone derivatives and a method for producing them*. WO 1998030556.
30. Yoshihama, M., Nakakoshi, M., Nakamura, J., Nakayama, S. (Snow Brand Milk Product Co. Ltd.). *Tetralone or benzopyranone derivatives and a method for producing them.* WO 1998032724.
31. Lota, R.K., Dhanani, S., Owen, C.P., Ahmed, S. *Synthesis, biochemical evaluation and rationalisation of the inhibitory activity of a series of 4-hydroxyphenyl ketones as potential inhibitors of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3).* Bioorg Med Chem Lett 2006, 16(17): 4519-22.
32. Lota, R.K., Olusanjo, M.S., Dhanani, S., Owen, C.P., Ahmed, S. *Synthesis, biochemical evaluation and rationalisation of the inhibitory activity of a range of 4-hydroxyphenyl ketones as potent and specific inhibitors of the type 3 of 17β-hydroxysteroid dehydrogenase (17β-HSD).* J Steroid Biochem Mol Biol 2008, 111(1-2): 128-37.
33. Day, J.M., Tutill, H.J., Foster, P.A. et al. *Development of hormone-dependent prostate cancer models for the evaluation of inhibitors of 17β-hydroxysteroid dehydrogenase type 3.* Mol Cell Endocrinol 2009, 301(1-2): 251-8.
34. Fink, B.E., Gavai, A.V., Tokarski, J.S. et al. *Identification of a novel series of tetrahydrodibenzazocines as inhibitors of 17β-hydroxysteroid dehydrogenase type 3*. Bioorg Med Chem Lett 2006, 16(6): 1532-6.
35. Vicker, N., Sharland, C.M., Heaton, W.B. et al. *The design of novel 17β-hydroxysteroid dehydrogenase type 3 inhibitors*. Mol Cell Endocrinol 2009, 301(1-2): 259-65.
36. Owen, C.P., Shahid, I., Olusanjo, M.S., Patel, C.H., Dhanani, S., Ahmed, S. *Synthesis, biochemical evaluation and rationalisation of the inhibitory activity of a range of phenyl alkyl imidazole-based compounds as potent inhibitors of the enzyme complex 17α-hydroxylase/17,20-lyase (P45017a).* J Steroid Biochem Mol Biol 2008, 111(1-2): 117-27.
37. Ghosh, S., Dasgupta, S. *Gentamicin induced inhibition of steroidogenic enzymes in rat testis.* Indian J Physiol Pharmacol 1999, 43(2): 247-50.
38. Lin, H., Chien, C.H., Lin, Y.L., Chen, C.F., Wang, P.S. *Inhibition of testosterone secretion by S-petasin in rat testicular interstitial cells*. Chin J Physiol 2000, 43(3): 99-103.
39. Andric, S.A., Kostic, T.S., Stojilkovic, S.S., Kovacevic, R.Z. *Inhibition of rat testicular androgenesis by a polychlorinated biphenyl mixture Arochlor 1248.* Biol Reprod 2000, 62(6): 1882-8.