Pergamon

0960-0760(94)00092-1

J. Steroid Biochem. Molec. Biol. Vol. 50, No. 5/6, pp. 261–266, 1994

0960-0760/94 $7.00 + 0.00

Inhibition of Human Placental Sterylsulfatase by Synthetic Analogs of Estrone Sulfate

Leif Dibbelt,[1]* Pui-Kai Li,[2] Radhakrishnan Pillai[2] and Rudolf Knuppen[1]

[1]Institut für Biochemische Endokrinologie, Medizinische Universität Lübeck, 23538 Lübeck, Germany and Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15282, U.S.A.

Synthetic analogs of estrone sulfate which carry differently substituted sulfonyl groups at position 3 of an invariable 3-desoxyestrone (dE1) moiety were tested *in vitro* as inhibitors of the human placental sterylsulfatase. Using both placental microsomes and a highly purified placental sterylsulfatase preparation as the enzyme source and dehydroepiandrosterone [^{35}S]sulfate or estrone [^{35}S]sulfate as the substrate, the following order of inhibitory potencies was observed: dE1-3-sulfonylchloride > dE1-3-sulfonylfluoride ≈ dE1-3-sulfonate > dE1-3-sulfonamide ≈ 3-methylsulfonyl-dE1. According to the results, the association of enzyme and inhibitor appears to be favored by an electronegative substituent at the sulfur atom (-Cl, -F, -O$^-$). Since, however, even the most potent synthetic inhibitor was bound by the enzyme with significantly lower affinity than was the natural substrate estrone sulfate, an oxygen function between the aromatic ring and the sulfur atom may be necessary for high affinity binding towards the sterylsulfatase. In addition to its fast reversible association with the enzyme, dE1-3-sulfonylchloride further affected the sulfatase activity in a time-dependent manner. This latter inhibitory activity which may be due to a covalent modification (alkylation) of sterylsulfatase by the analog was partially prevented in the presence of substrate.

J. Steroid Biochem. Molec. Biol., Vol. 50, No. 5/6, pp. 261–266, 1994

INTRODUCTION

The transformation of steroid sulfates, the major metabolites of steroids present in human blood and certain tissues, into unconjugated steroids is initiated by the membrane-bound enzyme sterylsulfatase (STS; EC 3.1.6.2) [1, 2]. STS activity has been found in many normal and malignant human tissues and cells where it is thought to control the local production of free and possibly active steroids from sulfoconjugated precursors [1–7] but is extraordinarily high in the placenta where it is involved in the synthesis of estrogens from sulfoconjugated C-19 steroids of fetal and maternal origin [1, 2, 8]. Therefore, most data concerning the physico-chemical and kinetic properties of the human STS have been obtained with the placental enzyme that has been solubilized and purified to homogeneity by several groups including ours ([9] and literature cited therein).

Using the membrane-bound as well as the isolated enzyme, STS inhibition by natural or synthetic steroids and steroid sulfates has been extensively studied; from the results of these studies it has been concluded that an almost planar ring system as present in phenolic, 5-en or 5α steroids, an oxygen function in the D ring, and above all the presence of a sulfate group at position 3 of the steroid ring favor high affinity binding towards the enzyme [9–11]. Part of the present authors recently synthesized analogs of estrone sulfate which are characterized by an invariable 3-desoxyestrone (dE1) moiety carrying different substituents at the 3-position [12]. In the present study, these synthetic analogs were tested *in vitro* as inhibitors of the human placental STS in order to evaluate the contribution of distinct components of the sulfate ester group or its related 3-substituents to the binding affinity of the substances towards the enzyme.

EXPERIMENTAL

Chemicals

^{35}S-labeled dehydroepiandrosterone sulfate (DHEA-^{35}S) and estrone sulfate (E1-^{35}S) were prepared and purified according to Dibbelt and Kuss [13]. The

*Correspondence to L. Dibbelt.
Received 22 Feb. 1994; accepted 9 May 1994.

following derivatives of dE1 [estra-1,3,5(10)-trien-17-one] were synthesized as described in detail elsewhere [12]: desoxyestrone-3-sulfonylfluoride (dE1-3-SO_2F), -3-sulfonylchloride (dE1-3-SO_2Cl), -3-sulfonamide (dE1-3-SO_2NH_2), -3-sulfonic acid (dE1-3-SO_3^-, potassium salt), and 3-methylsulfonyl-desoxyestrone (dE1-3-SO_2CH_3). Unlabeled dehydroepiandrosterone sulfate (DHEA-S), estrone sulfate (E1-S), and all other chemicals were commercially obtained at the highest purity available.

Enzyme preparation

Microsomal membranes were prepared by differential centrifugation of homogenates (40% w/v) of normal human term placentas obtained immediately after delivery [13]. The STS was solubilized from these microsomes and purified to apparent homogeneity as described previously [14, 15]. The enzyme preparation eventually obtained was concentrated by membrane filtration and was stored at −20°C until assayed.

STS assay

STS activity was determined radiometrically using ^{35}S-labeled DHEA-S or E1-S as substrate [13]. In brief, placental microsomes or the isolated STS appropriately diluted with 100 mmol/l Tris–acetate, pH 7.0, were incubated at 37°C with labeled substrate dissolved in the same buffer for time intervals of 2 to 10 min. The incubation volume (100 µl) additionally contained 5 µl of the inhibitor dissolved in buffer (unlabeled E1-S, dE1-3-SO_3^-), in dried acetone (dE1-3-SO_2Cl) or in dimethyl sulfoxide (all other analogs) or of the solvent without inhibitor, respectively. In some experiments, the enzyme was preincubated for 10 to 90 min in the presence of inhibitor solution or solvent in a volume of 90 µl prior to the addition of 10 µl of the substrate solution. The enzymatic reaction was terminated by adding citrate-buffered charcoal to the incubation mixture. After centrifugation, inorganic [^{35}S]sulfate was quantified in the supernatant by liquid scintillation counting. Blank values were measured by adding charcoal to the incubation mixture prior to enzyme. Kinetic evaluation of the results was performed graphically by the Lineweaver–Burk plot as well as by a statistic computer program utilizing non-linear least-squares curve fitting [16]. Chemical stability of all analogs in the absence and presence of enzyme were checked by thin layer chromatography on silica using each of three different solvent systems [benzene–acetone–methanol (2:1:1, by vol), ethyl acetate–acetic acid (9:1, v/v) and chloroform–ethyl acetate (4:1, v/v)] as the mobile phase.

RESULTS

The synthetic analogs of E1-S tested as STS inhibitors differently affected the initial velocity of DHEA-S hydrolysis catalyzed by placental microsomes (Table 1). At a concentration of 50 µmol/l, dE1-3-SO_2Cl caused a more than 50% reduction of the reaction rate, whereas dE1-3-SO_2F and dE1-3-SO_3^- exhibited slightly lower inhibitory potencies; dE1-3-SO_2NH_2 and dE1-3-SO_2CH_3 markedly affected the microsomal STS activity only at a threefold higher concentration. However, even the most potent inhibitor dE1-3-SO_2Cl affected the hydrolysis of DHEA-S less strongly than did the natural substrate E1-S. Similar results were obtained when the STS activity of placental microsomes was tested with E1-S instead of DHEA-S as the substrate (not shown). While the highly polar sulfonate derivative of dE1 was readily soluble in the aqueous incubation buffer, the solubility of the non-polar analogs appeared to be rather limited, in particular that of dE1-3-SO_2F as was indicated by the concentration dependence of its inhibitory effect (Fig. 1). Except for dE1-3-SO_2Cl which in the presence of water was spontaneously transformed into the sulfonate derivative, all analogs turned out to be stable when they were incubated at nominal concentrations of 100 and 500 µmol/l in the absence and presence of microsomes (1 mg protein per ml) for time intervals of up to 2 h at 37°C as was demonstrated by thin layer chromatography of the incubation mixtures.

The interaction of STS with its inhibitors was studied in detail using a solubilized and highly purified preparation of the placental enzyme. Since the sulfatase was inhibited by substrate at concentrations higher than about 10 µmol/l, all kinetic experiments were

Table 1. Effect of E1-S and its synthetic analogs on the initial rate of DHEA-S hydrolysis catalyzed by placental microsomes

Inhibitor (concentration)	Residual STS activity (%) DHEA-^{35}S concentration 1 µmol/l	10 µmol/l	*n*
dE1-3-OSO_3^- (E1-S)			
(50 µmol/l)	2 ± 2	5 ± 3	2
dE1-3-SO_2Cl			
(50 µmol/l)	31 ± 3	48 ± 1	2
(150 µmol/l)	24 ± 4	43 ± 1	2
dE1-3-SO_2F			
(50 µmol/l)	48 ± 9	60 ± 6	5
(150 µmol/l)	40 ± 11	56 ± 6	5
dE1-3-SO_3^-			
(50 µmol/l)	54 ± 11	67 ± 11	5
(150 µmol/l)	32 ± 9	47 ± 11	5
dE1-3-SO_2NH_2			
(50 µmol/l)	78 ± 5	89 ± 4	2
(150 µmol/l)	56 ± 8	79 ± 3	2
dE1-3-SO_2CH_3			
(50 µmol/l)	72 ± 10	92 ± 6	2
(150 µmol/l)	58 ± 7	74 ± 7	2

The residual STS activity in the presence of nominal inhibitor concentrations of 50 and 150 µmol/l is given as percentage of the activity measured in the presence of the respective solvent without inhibitor (mean ± SD of results from *n* independent experiments).

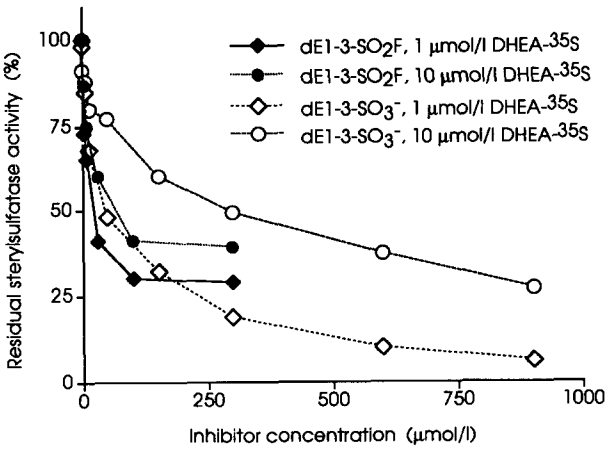


Fig. 1. Concentration dependence of inhibition of the microsomal STS by dE1-3-SO_2F and dE1-3-SO_3^-. The residual enzyme activity measured at two different substrate concentrations in the presence of inhibitor solution is given as percentage of the activity measured in the presence of solvent without inhibitor (means of duplicate measurements).

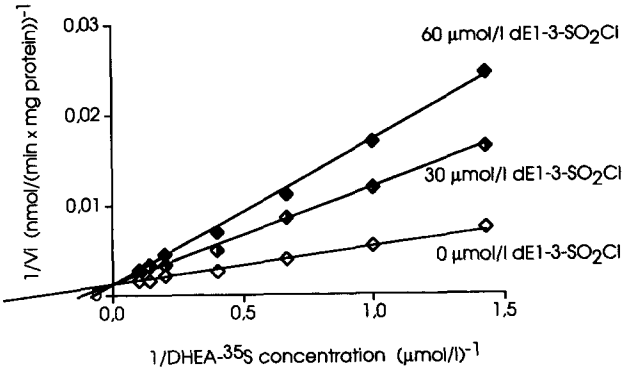


Fig. 3. Inhibition of the purified STS by dE1-3-SO_2Cl. Initial velocities (V_i, means of triplicate determinations) of DHEA-S hydrolysis measured at the indicated substrate concentrations in the absence and presence of the inhibitor are given in the double-reciprocal plot.

carried out using a limited range of substrate concentrations from 0.3 to 10 µmol/l. Under the experimental conditions applied, K_m values of 2.2 and 0.9 µmol/l and maximal velocities of 1000 and 3100 nmol min^{-1} (mg protein)$^{-1}$ were obtained with DHEA-S and E1-S as substrates, respectively; these values did not differ markedly from the ones measured in the presence of organic solvents used to dissolve the lipophilic inhibitors. When DHEA-S was applied as the substrate and dE1-3-SO_3^- as the inhibitor, the analog appeared to affect the STS activity according to a mixed inhibitor type (Fig. 2) with inhibition constants of approx. 45 and 140 µmol/l. Similar results were observed when DHEA-S was replaced by E1-S as the substrate (not shown). Likewise, a mixed-type inhibition was derived from experiments with DHEA-S as the substrate and unlabeled E1-S as the inhibitor (K_i values about 0.9 and 15 µmol/l) (not shown). The data obtained from kinetic experiments with all other analogs more likely approximated a classical competitive type of inhibition as demonstrated in Fig. 3 (dE1-3-SO_2Cl as inhibitor). Inhibition constants derived from experiments with purified STS are summarized in Table 2; for clarity, only the lower K_i value is given in cases where a mixed-type inhibition was observed. In agreement with results obtained with the microsomal STS, dE1-3-SO_2Cl was found to strongly inhibit the isolated enzyme according to a K_i value of about 30 µmol/l, whereas K_i values obtained with dE1-3-SO_2F and dE1-3-SO_3^- were slightly and with dE1-3-SO_2NH_2 and dE1-3-SO_2CH_3 were markedly higher.

When the isolated enzyme was preincubated with the various inhibitors prior to the addition of labeled substrate, the extent of enzyme inhibition by the analogs was essentially independent of the length of the preincubation period with the exception of dE1-SO_2Cl which turned out to largely inactivate the enzyme after preincubation for 30 min (Fig. 4). The rate of the

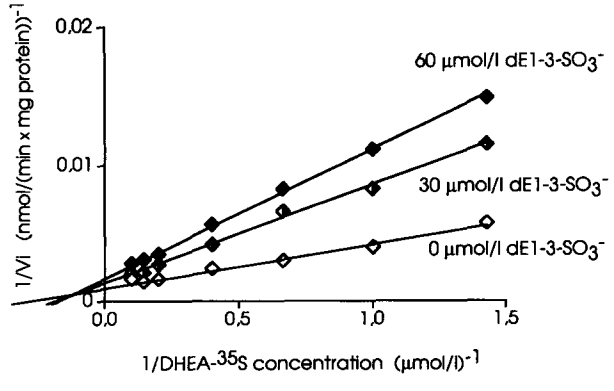
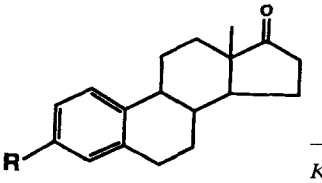


Fig. 2. Inhibition of the purified STS by desoxyestrone-3-sulfonate. Initial velocities (V_i, means of triplicate determinations) of DHEA-S hydrolysis measured at the indicated substrate concentrations in the absence and presence of the inhibitor are given in the double-reciprocal plot.

Table 2. Inhibition constants derived from kinetic experiments with purified STS



R	DHEA-^{35}S K_i (µmol/l)	(n)	E1-^{35}S K_i (µmol/l)	(n)
R: -OSO_3^- (E1-S)	0.9 ± 0.2	2	—	—
-SO_2Cl	26 ± 4	3	28	1
-SO_2F	43 ± 14	4	35	1
-SO_3^-	45 ± 10	4	40	1
-SO_2NH_2	140 ± 30	2	110	1
-SO_2CH_3	160 ± 40	3	130	1

DHEA-^{35}S or E1-^{35}S were used as the substrate applying unlabeled E1-S or its synthetic analogs as inhibitors at concentrations of 3 to 150 µmol/l (means from n independent measurements).

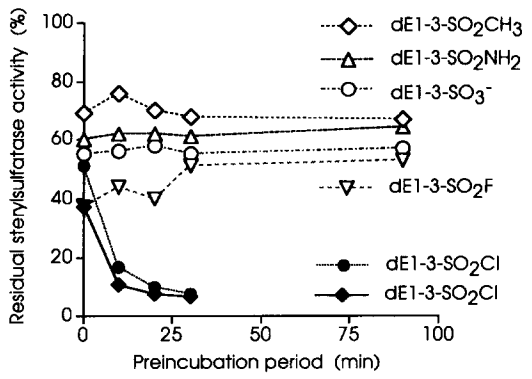


Fig. 4. Time dependence of STS inhibition by E1-S analogs. The purified enzyme was preincubated at room temperature with the inhibitor in a volume of 90 µl. At the end of the preincubation period, 10 µl substrate were added and the mixture [containing 1 (◆) or 10 µmol/l DHEA-^{35}S (all other symbols) and 100 µmol/l inhibitor] was further incubated at 37°C. STS activity measured after enzyme preincubation in the presence of inhibitor is given as percentage of the respective activity measured in the absence of inhibitor (means of triplicate determinations, in the case of dE1-3-SO_2Cl means of data derived from three independent experiments).

time-dependent inactivation of STS by dE1-3-SO_2Cl appeared to slightly depend on the enzyme concentration in the preincubation mixture, being in fact lower at higher STS concentrations (not shown), but was markedly reduced when unlabeled substrate was present in the preincubation mixture (Fig. 5).

DISCUSSION

The potential importance of STS activity as a major factor controlling the production of unconjugated steroids from sulfoconjugated precursors in numerous normal and malignant human cells and tissues has been addressed by many authors (see, for instance [1–8].

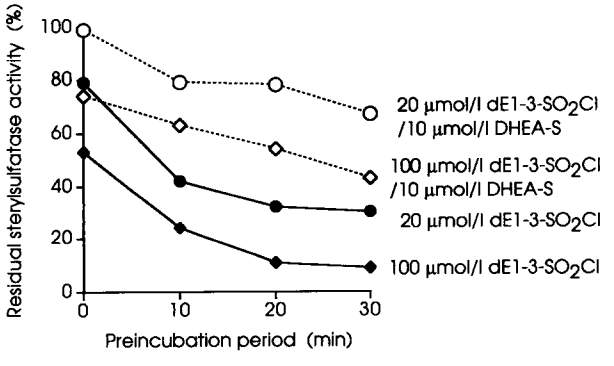


Fig. 5. Effect of substrate on the time-dependent inactivation of STS by dE1-3-SO_2Cl. The purified enzyme was preincubated without or with inhibitor in the absence or presence of unlabeled DHEA-S (10 µmol/l) prior to the addition of ^{35}S-labeled DHEA-S (10 µmol/l). STS activity obtained in the presence of inhibitor is given as percentage of the respective activity measured in the absence of inhibitor (mean of duplicate determinations).

Like other groups [11, 17–21], we thus started a still ongoing search for a specific and effective STS inhibitor that may be applied to modulate the enzyme activity *in vivo*. Since it is well known that sulfoconjugated steroids are bound with much higher affinity by the sulfatase than are their unconjugated counterparts [9–11], this search concentrated on synthetic compounds which structurally resemble the sulfate ester of estrone. In a first series of experiments reported in the present communication, the inhibitory potencies of 3-desoxyestrone-3-sulfonate and several closely related derivatives thereof were studied using both a crude membrane-bound and a solubilized and highly purified STS from human term placenta as the enzyme source. These enzyme preparations were chosen because the human term placenta is a plentiful and easily available supply of STS activity. Since, however, a single functional STS gene is known to exist in the human genome (Xp22.32) [2], the enzyme expressed in the human placenta can be expected to essentially share its properties with the STS present in other human tissues.

The kinetic properties of the isolated placental STS found in the present communication are identical to results reported earlier [9]: the enzyme hydrolyzes both phenolic (E1-S) and neutral (DHEA-S) steryl sulfates, binds E1-S with slightly higher affinity (K_m 0.9 µmol/l) than DHEA-S (K_m 2.2 µmol/l), and is inhibited by excess substrate concentrations. With the exception of about 2-fold higher K_m values for E1-S and DHEA-S, kinetic properties of the crude membrane-bound STS [13, 14] are nearly identical to the ones of the solubilized and purified enzyme described above. This is also demonstrated by the finding that the order of inhibitory potencies of the various E1-S analogs tested in the present study does not depend on whether this inhibition was measured using placental microsomes or the isolated STS as the enzyme source.

The K_m value for E1-S obtained with the isolated enzyme preparation represents the lower end of a wide range of published data which may reflect *in vitro* methodological differences between different laboratories rather than *in vivo* kinetic differences between STS from different human tissues. As compared to this K_m value for E1-S (or its K_i value measured using E1-S as an inhibitor of DHEA-S hydrolysis), the K_i value for dE1-3-SO_3^- is about 50-fold higher. Since E1-S differs from dE1-3-SO_3^- solely by an oxygen atom between the phenolic ring and the sulfur atom, this oxygen or at least a sterically or electronically similar link between the steroid ring and the sulfonate moiety appears to be essential for high affinity binding towards the sulfatase. E1-S and dE1-3-SO_3^- both affected the hydrolysis of DHEA-S according to a mixed type of inhibition. Like the enzyme's inhibition by high substrate concentrations mentioned above, this observation indicates non-linear kinetics of STS under our experimental conditions; possible reasons for those non-linear

kinetics have already been discussed [9]. If the negatively charged oxygen of the sulfonate moiety of dE1-3-SO_3^- is replaced by a non-charged substituent, the respective derivatives appeared to inhibit the STS according to a classical competitive rather than to a mixed-type mechanism; this finding parallels earlier results which showed a mixed-type inhibition of STS by negatively charged steryl sulfates but a competitive inhibition by non-charged unconjugated steroids [9]. The K_i value and thus the binding affinity of the inhibitor towards the sulfatase is not changed markedly if the negatively charged oxygen of the sulfonate moiety is replaced by a non-charged electronegative substituent as, for instance, a fluorine atom; however, the K_i value is increased significantly if the oxygen anion is replaced by an amino or methyl group. Therefore, one may speculate that binding towards the sulfatase is favored by an electronegative substituent at the sulfur atom which may, for instance, act as a hydrogen bond acceptor. As compared to the sulfonylfluoride derivative, dE1-3-SO_2Cl turned out to act in a more complex manner that renders the interpretation of the kinetic results more difficult. In addition to its associative binding to the sulfatase, the substance slowly reacted with both the enzyme and water. Whereas the latter reaction results in the formation of 3-desoxyestrone-3-sulfonate which could be detected by thin layer chromatography, the time-dependent inactivation of STS by dE1-3-SO_2Cl at present can only be hypothesized to represent the covalent modification of the enyzme by alkylation of reactive amino acids, a reaction that probably takes place at the active site of the enzyme because its rate is markedly reduced in the presence of substrate. Our observation that the extent of STS inhibition by dE1-3-SO_2Cl at the beginning of the preincubation period clearly depends on the concentration of labeled substrate during the subsequent determination of enzyme activity but becomes totally independent of this substrate concentration after 30 min of preincubation (Fig. 4) supports the concept of an irreversible reaction between enzyme and inhibitor, but unequivocal experimental evidence is needed to prove this hypothesis.

Taken together, our results indicate that the various synthetic analogs of E1-S tested as effectors of the human placental STS act as competitive or, in the case of dE1-3-SO_3^-, mixed-type inhibitors and thus can be considered as chemical probes of the active site of the enzyme. All these inhibitors, however, are bound by the STS with significantly lower affinity than is the natural substrate E1-S. This lower binding affinity is probably due to the absence of an oxygen or a similar atom between the A-ring of the steroid and the sulfonate/sulfonyl group. Future research will mainly be directed towards the synthesis and testing of compounds which carry this apparent structural prerequisite for high affinity binding to the human sterylsulfatase.

Acknowledgements—The authors thank Ms H. Nagel for expert technical assistance.

REFERENCES

1. Hobkirk R.: Steroid sulfotransferases and steroid sulfate sulfatases: characteristics and biological roles. *Can. J. Biochem. Cell. Biol.* **63** (1985) 1127–1144.
2. Shapiro L. J.: Steroid sulfatase deficiency and X-linked ichthyosis. In *The Metabolic Basis of Inherited Disease* (Edited by C. R. Scriver *et al.*) McGraw-Hill, New York, 6th Edn, Vol. II (1989) pp. 1945–1964.
3. Miller R. W. and O'Neill J. S.: The significance of steroid metabolism in human cancer. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 317–325.
4. Haning R. V., Flood C. A., Hackett R. J., Loughlin J. S., McClure N. and Longcope C.: Metabolic clearance rate of dehydroepiandrosterone sulfate, its metabolism to testosterone, and its intrafollicular metabolism to dehydroepiandrosterone, androstenedione, testosterone, and dihydrotestosterone *in vivo*. *J. Clin. Endocr. Metab.* **72** (1991) 1088–1095.
5. Pasqualini J. R., Schatz B., Varin C. and Nguyen B.-L.: Recent data on estrogen sulfatases and sulfotransferases activities in human breast cancer. *J. Steroid Biochem. Molec. Biol.* **41** (1992) 323–329.
6. James V. H. T., Reed M. J. and Purohit A.: Inhibition of oestrogen synthesis in postmenopausal women with breast cancer. *J. Steroid Biochem. Molec. Biol.* **43** (1992) 149–153.
7. Evans T. R. J., Rowlands M. G., Silva M. C., Law M. and Coombes R. C.: Prognostic significance of aromatase and estrone sulfatase enzymes in human breast cancer. *J. Steroid Biochem. Molec. Biol.* **44** (1993) 583–587.
8. Dibbelt L., Kuss E. and Zander J.: Die Hormone der Plazenta. In *Gynäkologie und Geburtshilfe* (Edited by O. Käser, V. Friedberg, K. G. Ober, K. Thomsen and J. Zander). G. Thieme Verlag, Stuttgart, 2nd Edn, Vol. I/1 (1987) pp. 4.138–4.167.
9. Dibbelt L. and Kuss E.: Human placental sterylsulfatase. Interaction of the isolated enzyme with substrates, products, transition-state analogues, amino-acid modifiers and anion transport inhibitors. *Biol. Chem. Hoppe-Seyler* **372** (1991) 173–185.
10. Townsley J. D.: Further studies on the regulation of human placental steroid 3-sulfatase activity. *Endocrinology* **93** (1973) 172–181.
11. Evans T. R. J., Rowlands M. G., Jarman M. and Coombes R. C.: Inhibition of estrone sulfatase enzyme in human placenta and human breast carcinoma. *J. Steroid Biochem. Molec. Biol.* **39** (1991) 493–499.
12. Li P.-K., Pillai R., Young B. L., Bender W. H., Martino D. M. and Lin F.-T.: Synthesis and biochemical studies of estrone sulfatase inhibitors. *Steroids* **58** (1993) 106–111.
13. Dibbelt L. and Kuss E.: Human placental steroid sulfatase. Kinetics of the in vitro hydrolysis of dehydroepiandrosterone 3-sulfate and of 16α-hydroxydehydroepiandrosterone 3-sulfate. *Hoppe-Seyler's Z. Physiol. Chem.* **364** (1983) 187–191.
14. Dibbelt L. and Kuss E.: Human placental steroid sulfatase solubilized with a cholic acid derivative: molecular mass, kinetic properties and susceptibility to glycosidases. *Hoppe-Seyler's Z. Physiol. Chem.* **365** (1984) 1154–1153.
15. Dibbelt L. and Kuss E.: Human placental sterylsulfatase: enzyme purification, production of antisera, and immunoblotting reactions with normal and sulfatase-deficient placentas. *Biol. Chem. Hoppe-Seyler* **367** (1986) 1223–1229.
16. Lutz R. A. and Rodbard D.: A BASIC computer program for use in evaluating enzyme-substrate-inhibitor kinetics. *Clin. Chem.* **31** (1985) 656.
17. Bedin M., Fournier T., Tanguy G., Cedard L., Zorn J. R. and Sureau C.: *In vivo* and *in vitro* study of the mechanism of action of a synthetic progestin in lowering unconjugated estradiol and total estriol in plasma during normal human pregnancy. *Am. J. Obstet. Gynec.* **145** (1983) 373–375.
18. Birnböck H. and von Angerer E.: Sulfate derivatives of 2-phenylindols as novel steroid sulfatase inhibitors. An *in vitro* study on structure-activity relationship. *Biochem. Pharmac.* **39** (1990) 1709–1713.

19. Prost-Avallet O., Oursin J. and Adessi G. L.: *In vitro* effect of synthetic progestogens on estrone sulfatase activity in human breast carcinoma. *J. Steroid Biochem. Molec. Biol.* **39** (1991) 967–973.
20. Purohit A. and Reed M. J.: Oestrogen sulphatase activity in hormone-dependent and hormone-independent breast-cancer cells: modulation by steroidal and non-steroidal therapeutic agents. *Int. J. Cancer* **50** (1992) 901–905.
21. Chetrite G., Varin C., Delalonde L. and Pasqualini J. R.: Effect of promegestone, tamoxifen, 4-hydroxytamoxifen and ICI 164,384 on the oestrone sulphatase activity of human breast cancer cells. *Anticancer Res.* **13** (1993) 931–934.