Pergamon

0960-0760(94)00176-6

J. Steroid Biochem. Molec. Biol. Vol. 52, No. 3, pp. 281–286, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0960-0760/95 $9.50 + 0.00

Estrogenicity, Antiestrogenicity and Estrone Sulfatase Inhibition of Estrone-3-amine and Estrone-3-thiol

Kyle W. Selcer[1]* and Pui-Kai Li[2]

[1]Department of Biological Sciences and [2]Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15282, U.S.A.

Estrogen levels in breast tumors of post-menopausal women are at least 10 times higher than estrogen levels in plasma. The high level of estrogen in these tumors is postulated to be due to *in situ* formation of estrogen, possibly through conversion of estrone sulfate to estrone by the enzyme estrone sulfatase. Thus, inhibitors of estrone sulfatase are potential agents for the treatment of hormone-dependent breast cancers. We designed and synthesized a series of estra-1,3,5(10)triene-17-one, 3-amino and estra-1,3,5(10)triene-17-one, 3-thio derivatives. We have shown previously that several of these compounds substantially inhibit estrone sulfatase, exceeding Danazol in their inhibitory activity. However, little is known about the metabolism of these compounds and the possible effects of their metabolites *in vivo*. Two probable metabolites of the synthetic estrone analogs are estra-1,3,5(10)triene-17-one, 3-amine (E_1-NH_2), and estra-1,3,5(10)triene-17-one, 3-thiol (E_1-SH). We tested these two compounds for estrogenicity, antiestrogenicity and inhibition of estrone sulfatase activity using a combination of *in vivo* and *in vitro* assays. The ovariectomized rat uterine weight gain assay was used to test for estrogenicity. Neither E_1-NH_2 nor E_1-SH were estrogenic, as indicated by a lack of uterine weight gain when given at 25 μg/day for 7 days. The test compounds also were not antiestrogenic, in that they did not block estrone-induced uterine weight gain when given (100 μg/day) simultaneously with estrone (2 μg/day). Both compounds showed low affinity for the estrogen receptor. Using rat uterine cytosol as a source of estrogen receptor, the compounds displaced only a small percentage of [^{3}H]estradiol binding, even when present at 1000-fold excess. Inhibition of estrone sulfatase activity was tested using human placental microsomes as a source of estrone sulfatase. E_1-NH_2 and E_1SH showed very low levels of estrone sulfatase inhibition (15.1 and 9.8%, respectively) under conditions where Danazol showed more than 60% inhibition. Our results indicate that neither of these two compounds would present significant problems if they were the primary metabolite in a treatment involving estrone sulfatase inhibition of estrogen-dependent breast cancer.

J. Steroid Biochem. Molec. Biol., Vol. 52, No. 3, pp. 281–286, 1995

INTRODUCTION

Estrogen levels in breast tumors of post-menopausal women are more than 10 times higher than estrogen levels in plasma from the same individuals [1, 2]. The high concentration of estrogen in breast tumors is postulated to be due to *in situ* formation of estrogen [3, 4]. One possible source of estrogen in breast tumors is through aromatization of androgens. Indeed, aromatase is present in breast tumors and conversion of androgen to estrogen has been demonstrated in tumor cells [5, 6]. However, recent studies indicate that the main source of estrogen in breast tumor cells may be through conversion of estrone sulfate to estrone by the enzyme estrone sulfatase. Estrone sulfate is the most abundant circulating estrogen in women; furthermore, estrone sulfatase activity has been demonstrated in breast cancer cells by several research groups [3, 4, 7–17]. Thus, inhibitors of estrone sulfatase have potential as agents to treat estrogen-dependent breast cancers.

Recently, several groups of compounds have been reported to be inhibitors of estrone sulfatase, most of

*Correspondence to K. W. Selcer.
Received 22 Aug. 1994; accepted 28 Oct. 1994.

which are synthetic steroid analogs. Reed and coworkers reported on sulfatase inhibitory activities of the synthetic analogs estrone-3-*O*-methylthiophosphonate, estrone 3-*O*-phosphonate and thiophosphonates, and estrone sulfamates [18–20]. These compounds inhibited estrone sulfatase activity in placenta, breast tumors and in MCF-7 cells. We also have reported the synthesis and sulfatase inhibitory activities of sulfonate and its analogs that contain the estrane nucleus [21–23]. One of the conclusions from these studies is that an oxygen atom, or at least a sterically or electronically similar link between the steroid ring and sulfonate moiety, is essential for high affinity towards the sulfatase. Therefore, we designed and synthesized a series of estra-1,3,5(10)triene-17-one, 3-amino and estra-1,3,5(10)triene-17-one, 3-thio derivatives as potential estrone sulfatase inhibitors.

We have previously shown that several of the estrone-3-amino and estrone-3-thio derivatives have substantial sulfatase inhibitory activity [24], with some showing inhibition greater than Danazol, a known sulfatase inhibitor. However, little is known about the metabolism of these compounds and the possible effects of their metabolites *in vivo*. Two probable metabolites of the synthetic estrone analogs are estra-1,3,5(10)triene-17-one, 3-amine (E_1-NH_2), and estra-1,3,5(10)triene-17-one, 3-thiol (E_1-SH). In the present study, we tested these two compounds for estrogenicity, antiestrogenicity and inhibition of estrone sulfatase activity, using a combination of *in vivo* and *in vitro* assays.

EXPERIMENTAL

Chemicals and reagents

[^{3}H]estradiol ([17β-estradiol, 1,2,6,7-^{3}H(N)]-; 99 Ci/mmol), [^{3}H]estrone-sulfate (estrone sulfate, ammonium salt, [6,7-^{3}H(N)]-; 49 Ci/mmol) and [^{14}C]estrone ([4-^{14}C]-; 51 Ci/mmol) were obtained from DuPont/New England Nuclear (Boston, MA). Radioinert steroids were obtained from Sigma Chemical Co. (St Louis, MO). TEMG buffer contained 25 mM Tris–HCl, pH 7.5, 10 mM EDTA, 1 mM monothioglycerol and 10% (w/v) glycerol. Dextran-coated charcoal contained 0.5 g Norit A (Sigma) and 50 mg Dextran-70 (Pharmacia, Piscataway, NJ) in 100 ml of 25 mM Tris–HCl and 1 mM EDTA, pH 7.5. Liquid scintillation cocktail was Ecolume (ICN, Costa Mesa, CA).

Synthesis of E_1-NH_2 and E_1-SH

Estra-1,3,5(10)triene-17-one, 3-amine, and estra-1,3,5(10)triene-17-one, 3-thiol were synthesized according to published procedures [25, 26].

Estrogenicity and antiestrogenicity

Estrogenicity and antiestrogenicity were assessed using an ovariectomized rat uterine weight gain assay. Ovariectomized adult female rats (225–250 g) were purchased from Zivic-Miller Laboratories, Inc. (Zelienople, PA), and housed in the Duquesne University animal care facility. Ovariectomized rats were maintained for 2 weeks prior to treatment to allow their uteri to regress to basal size. Treatments were given by i.p. injection in a 1% gelatin, 0.9% NaCl solution at a volume of 0.5 ml. Steroids were dissolved in ethanol to 1 mg/ml then further diluted into the injection solution to the desired concentration. All treatments were continued for 7 days. At the termination of the experiments, animals were sacrificed by cervical dislocation under ether anesthesia. Body weight was taken, then uteri were excised, stripped of fat, and weighed to the nearest 0.1 mg.

For determination of estrogenicity, five treatment groups were established: vehicle control, estradiol-17β, estrone, E_1-NH_2, and E_1-SH. Steroids were administered at 25 μg/day. For determination of antiestrogenicity, four treatment groups were established: vehicle control, estrone, estrone + E_1-NH_2, and estrone + E_1-SH. Estrone was administered at 2 μg/day while E_1-NH_2 and E_1-SH were given at 100 μg/day.

Estrogen receptor binding

Estrogen receptor binding was measured using an [^{3}H]estradiol binding assay of rat uterine cytosol. Uteri were weighed, minced with scissors, then homogenized in TEMG buffer with a Tissue Tearor homogenizer (Biospec Products, Bartlesville, OK) using three bursts of 30 s each. The homogenate was centrifuged at 1000 *g* for 10 min at 4°C, after which the supernatant was decanted. Supernatant from the low-speed nuclear centrifugation was transferred to polyallomer tubes and centrifuged at 170,000 *g* for 1 h at 4°C. The resulting supernatant was diluted 1:1 (v/v) with TEMG buffer, and this constituted the cytosol preparation.

Aliquots (300 μl) of cytosol were incubated in a total volume of 500 μl with 1 nM of [^{3}H]estradiol for determination of total binding. A parallel set of samples was incubated with 1 μM radioinert estradiol for determination of nonspecific binding. Specific binding was calculated as total binding minus nonspecific binding. Another parallel set of samples was incubated with varying concentrations (1, 0.1 and 0.01 μM) of competitors. Samples were incubated for 16–20 h at 4°C. After incubation of cytosol samples, free steroid was removed by addition of 500 μl of dextran-coated charcoal for 3 min, followed by centrifugation at 1500 *g* for 3 min. The supernatant was decanted into scintillation vials and 4 ml of scintillation cocktail was added. Samples were counted in a Packard Tri-carb liquid scintillation spectrometer at 50% efficiency. Data for binding in the presence of competitors is expressed as a percentage of specific binding.

Placental microsome preparation

Human placentas were obtained immediately upon delivery from Mercy Hospital (Pittsburgh, PA) and

stored on ice during transportation to the laboratory. Preparation of microsomes was performed according to the method of Reed and Ohno [27]. All procedures were carried out at 0–4°C. The placenta was cut free of connective tissue and large blood vessels with scissors. The tissue was then homogenized in a Waring blender with two parts of tissue to one part of homogenization buffer consisting of 0.05 M sodium phosphate, 0.25 M sucrose, and 0.04 M nicotinamide, pH 7.0. The homogenate was centrifuged at 10,000 *g* for 30 min. The debris was discarded and the supernatant was centrifuged at 105,000 *g* for 1 h. The centrifugation procedure was repeated once again and the resulting pellets were stored at −80°C. The pellets were used within 6 weeks after preparation.

Estrone sulfatase inhibition assay

[^{3}H]estrone sulfate (20 μM/tube; 300,000 dpm/tube) in ethanol and the experimental compounds (10 μM/tube in ethanol) were added to a 5 ml test tube. The ethanol was removed with a stream of nitrogen. Tris–HCl buffer (0.05 M, pH 7.2, 0.2 ml) was added to each tube. Placental microsomes were then diluted with Tris–HCl buffer to 80 μg/ml buffer. The microsomes and assay tubes containing steroids were preincubated for 5 min at 37°C in a shaking water bath. The assay was initiated by addition of the microsomes (0.8 ml) to the tubes. After 20 min of incubation at 37°C, 4 ml of toluene was added to quench the assay. [^{14}C]estrone (10,000 dpm/tube) was added concurrently with the toluene as an internal standard for the determination of extraction efficiency. Control samples with no inhibitor were incubated simultaneously. Blank samples were obtained by incubating with boiled microsomes. The quenched samples were vortexed for 45 s and centrifuged at 1000 *g* for 10 min. One ml of toluene was removed from the organic phase of the centrifuged samples and added to 5 ml of scintillation cocktail. The aliquots were counted for determination of product formation. All samples were run in triplicate. Variation between tubes was less than 5%. Product formation for samples containing an inhibitor was compared to that of the control samples, and are reported as percent inhibition of control samples.

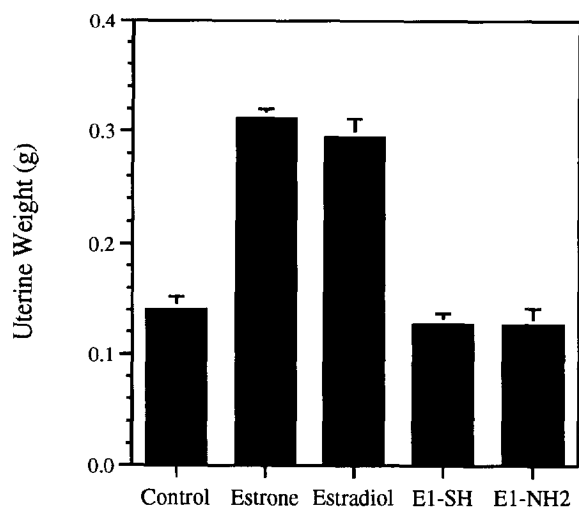

Fig. 1. Estrogenicity of E_1-NH_2 and E_1-SH as determined by rat uterine weight gain assay. Ovariectomized rats were treated for 7 days with 25 μg/day of estradiol, estrone, E_1-NH_2 or E_1-SH. Data are means ± SEM, $n = 5$ for all groups. Control animals received vehicle only (0.9% NaCl, 1% gelatin, 500 μl, i.p. injection). ANOVA indicated significant differences between groups ($F = 54.77$, 4, 20 df, $P < 0.001$). Uterine weights of rats from estrone and estradiol groups were significantly ($P < 0.05$) higher than those in all other groups as determined by Student–Newman–Keuls test. Uterine weights of E_1-NH_2 and E_1-SH groups were not significantly different from the control group.

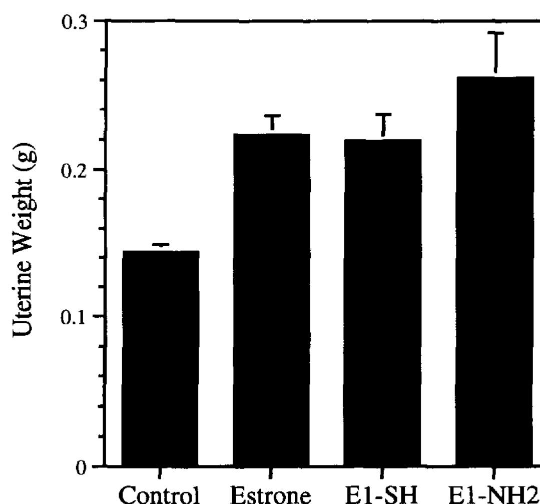

Fig. 2. Antiestrogenicity of E_1-NH_2 and E_1-SH as determined by rat uterine weight gain assay. Data are means ± SEM, $n = 5$ for all groups. Ovariectomized rats were treated for 7 days with 100 μg/day of estrone-3-amine or estrone-3-thiol in combination with 2 μg/day estrone. Control animals received vehicle only (0.9% NaCl, 1% gelatin, 500 μl, i.p. injection). ANOVA indicated significant differences between groups ($F = 6.71$, 3, 16 df, $P < 0.005$). Uterine weights of rats from E_1-NH_2 and E_1-SH groups were not significantly different from the estrone group.

Statistical analysis

Rat body weights and uterine weights were compared among treatment groups using one way analysis of variance (ANOVA). Comparisons between specific groups were made using Student–Newman–Keuls test. Probabilities of less than 0.05 were considered significant.

RESULTS

Estrogenicity and antiestrogenicity in vivo

A rat uterine weight gain assay was used to determine if either of the estrone-3 derivatives had estrogenic or antiestrogenic activity. Estrogenicity was assessed by measuring increases in uterine weight of ovariectomized rats after treatment with the compounds.

Neither compound caused a significant increase in uterine weight over control levels when given at 25 μg/day for 7 days (Fig. 1). In contrast, both estradiol and estrone significantly increased uterine weight above control levels.

Antiestrogenicity was assessed as the ability of the compounds to block estrone-induced uterine weight gain in ovariectomized rats. When the compounds (100 μg/day) were given simultaneously with estrone (2 μg/day), there was a significant increase in uterine weight of both treatment groups (Fig. 2). The increase in uterine weight for the two groups was of the same magnitude as the increase observed for the estrone-only treatment group, indicating that the compounds did not block estrone-induced uterine growth.

Estrogen receptor binding

The ability of the compounds to bind to the estrogen receptor was determined using a [^{3}H]estradiol binding assay. Figure 3 shows the percentage of specific [^{3}H]estradiol binding in the presence of the test compounds at three concentrations. Neither compound competed for estrogen receptor at the same level as estrone, which displaced 100% of the specific [^{3}H]estradiol binding at a 1000-fold excess. E_1-NH_2 displaced 60% of [^{3}H]estradiol binding at 1000-fold excess, while E_1-SH displaced only 10%.

Estrone sulfatase inhibition in placental microsomes

Using 20 μM estrone sulfate as substrate and 10 μM of test inhibitors, E_1-NH_2 inhibited 15.1% of estrone sulfatase activity and E_1-SH inhibited 9.8%, as compared to control samples with no inhibitor (Fig. 4). Under the same conditions, Danazol resulted in 60.5% inhibition and estrone-3-sulfamate resulted in 99% inhibition.

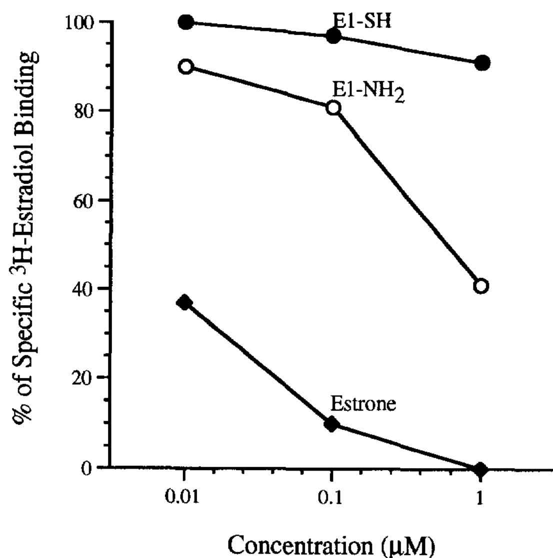

Fig. 3. Displacement of specific [^{3}H]estradiol binding to the rat uterine estrogen receptor. Rat uterine cytosol was used as a source of estrogen receptor. [^{3}H]estradiol was added at 1 nM and competitors were added at 0.01, 0.1, and 1 μM. Data are expressed as a percentage of specific binding (total binding minus binding in the presence of 1 μM radioinert estradiol).

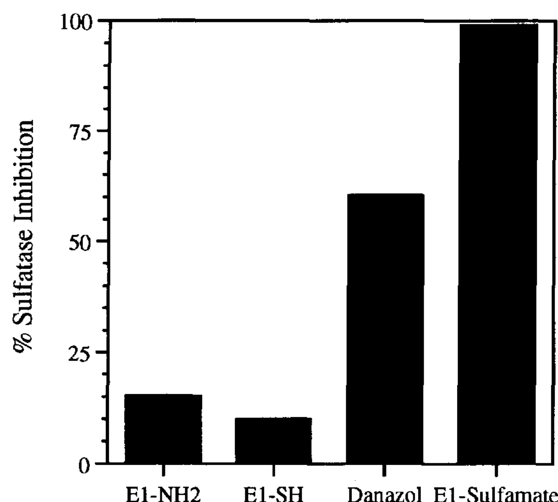

Fig. 4. Inhibition of estrone sulfatase activity in human placental microsomes by E_1-NH_2, E_1-SH, Danazol and E_1-sulfamate. Data are the average of two separate experiments with each treatment run in triplicate. Interassay variation was less than 7% for any treatment. Data are expressed as percent of control (enzyme activity in the absence of inhibitor).

DISCUSSION

Estrone sulfatase inhibitors may prove to be important in the treatment of estrogen-dependent breast tumors of post-menopausal women. Recent studies indicate that some estrone analogs are effective inhibitors of estrone sulfatase [18–23]. Our rationale for studying E_1-NH_2 and E_1-SH is that these two compounds are probable metabolites of newly designed estrone-3 derivatives that have shown promise as estrone sulfatase inhibitors [24]. Little is known of the biological properties of estrone-3 derivatives, and our study provides new data on estrogenicity, antiestrogenicity, and sulfatase inhibition of two of these compounds.

We used an *in vivo* approach to test for estrogenicity and antiestrogenicity. While we did not specifically test for toxicity of the two compounds, these experiments provide an indication of the potential toxicity of E_1-NH_2 and E_1-SH. Neither treatment group showed significantly different body weights from the control group at the termination of the experiments, indicating that the rats did not suffer any ill effects from the treatments that were manifested in loss of body weight. Further, upon necropsy, there were no obvious signs of morbidity in the treated rats. Thus, it appears that the two estrone-3 derivatives are not overtly toxic at up to 100 μg/day.

The ovariectomized rat uterine weight gain assay is a sensitive and reliable method of assessing the ability

of a compound to elicit estrogen-dependent actions under physiological conditions [28]. We tested both E_1-NH_2 and E_1-SH at 25 μg/day and found no significant increase in uterine weight. Given that estrone caused significant increases in uterine weight at less than 2 μg/day, it is clear that E_1-NH_2 and E_1-SH are not estrogenic. This is an important finding since it would be disadvantageous for an estrone sulfatase inhibitor used in treatment of estrogen-dependent cancer to be metabolized to a compound that was estrogenic. The rat uterine weight gain assay also revealed that the two compounds were not antiestrogenic since they did not block estrone-induced uterine weight gain.

The lack of estrogenic and antiestrogenic activity for E_1-NH_2 and E_1-SH suggests that these compounds do not interact to any great extent with the estrogen receptor. We confirmed this using a radioligand binding assay for rat uterine estrogen receptor. Both compounds showed only moderate displacement of radiolabeled estradiol from cytosolic estrogen receptor when present at a 1000-fold excess. E_1-NH_2 competed better for the receptor than E_1-SH, but neither competed as effectively as estrone.

Estrone-3 derivatives have been shown to be effective inhibitors of estrone sulfatase activity [18–24]. We tested E_1-NH_2 and E_1-SH for their ability to inhibit estrone sulfatase activity using human placental microsomes as a source of estrone sulfatase. In this assay, the two compounds showed 10–15% inhibition of estrone sulfatase activity. In contrast, two known estrone sulfatase inhibitors, Danazol [29] and estrone-3-sulfamate [20], showed substantially greater levels of estrone sulfatase inhibition (60 and 99%, respectively). Thus, it appears that E_1-NH_2 and E_1-SH are only weak inhibitors of estrone sulfatase.

The results in our study indicate that E_1-NH_2 and E_1-SH are not substantially estrogenic or antiestrogenic, nor do they exhibit marked affinity for the estrogen receptor. Further, they do not substantially inhibit estrone sulfatase. These properties taken together indicate that neither compound would cause significant problems if it were the primary metabolite of an estrone sulfatase inhibitor used for the treatment of estrogen dependent breast cancer.

Acknowledgements—We thank Sharada Jagannathan, Deepali Gaitonde and Heather Kuzma for their technical assistance. This work was supported in part by the Hunkele Foundation.

REFERENCES

1. Edery M., Goussard J., Dehennin L., Scholler R., Reiffsteck J. and Drosdowsky M. A.: Endogenous oestradiol-17β concentration in breast tumors determined by mass fragmentography and by radioimmunoassay: relationship to receptor content. *Eur. J. Cancer* 17 (1981) 115–120.
2. Millington D. S.: Determination of hormonal steroid concentrations in biological extracts by high resolution fragmentography. *J. Steroid Biochem.* **6** (1975) 239–245.
3. Santner S. J., Feil P. D. and Santen R. J.: *In situ* estrogen production via the estrone sulfatase pathway in breast tumors: relative importance versus the aromatase pathway. *J. Clin. Endocrinol. Metab.* 59 (1984) 29–33.
4. Santen R. J., Leszczynski D., Tilson-Mallet N., Feil P. D., Wright C., Manni A. and Santner S. J.: Enzymatic control of estrogen production in human breast cancer: relative significance of aromatase versus sulfatase pathways. *Ann. N. Y. Acad. Sci.* **464** (1986) 126–137.
5. Adams J. B. and Li K.: Biosynthesis of 17β-oestradiol in human breast carcinoma tissue and a novel method for its characterization. *Br. J. Cancer* **31** (1975) 429–433.
6. Tilson-Mallet N., Santner S. J., Feil P. D. and Santen R. J.: Biologic significance of aromatase activity in human breast tumors. *J. Clin. Endocrinol. Metab.* 57 (1983) 1125–1128.
7. Tseng L., Mazella J., Lee L. Y. and Stone M. L.: Estrogen sulfatase and estrogen sulfotransferase in human primary carcinoma. *J. Steroid Biochem.* **19** (1983) 1413–1417.
8. Prost O., Turrel M. O., Dahan N., Craveur C. and Adessi G.: Estrone and dehydroepiandrosterone sulfatase levels in human breast carcinoma. *Cancer Res.* 44 (1984) 661–664.
9. Wilking N., Carlstrom K., Gustafsson S. A., Skoldefors H. and Tollborn O.: Oestrogen receptors and metabolism of oestrone sulphate in human mammary carcinoma. *Eur. J. Cancer* **16** (1980) 1339–1344.
10. Vignon F., Terqui M., Westley B., Derocq D. and Rochefort H.: Effect of plasma estrogen sulfate in mammary cancer cells. *Endocrinology* **106** (1980) 1079–1086.
11. Pasqualini J. R., Gelly C. and Nguyen B-L.: Metabolism, biological effects and morphological responses of estrogen-3-sulfates and estrogen-17-sulfates in MCF-7 and R-27 human mammary cancer cell lines. *Endocrinology* **118** [suppl.] (1986) 246.
12. Dao T. L., Hayes C. and Libby P. R.: Steroid sulfatase activity in human breast tumors. *Proc. Soc. Exp. Biol. Med.* **146** (1974) 381–384.
13. Pasqualini J. R. and Gelly C.: Estrogen sulfates: biological and ultrastructural responses and metabolism in MCF-7 human breast cancer cells. *Breast Cancer Res. Treat.* **8** (1986) 233–240.
14. Pasqualini J. R. and Gelly C.: Effect of tamoxifen and tamoxifen derivatives on the conversion of estrone sulfate to estradiol in the MCF-7 mammary cancer cell lines. *Cancer Lett.* **40** (1988) 115–121.
15. MacIndoe J. H.: The hydrolysis of estrone sulfate and dehydroepiandrosterone sulfate by MCF-7 human breast cancer cells. *Endocrinology* 123 (1988) 1281–1286.
16. Pasqualini J. R., Gelly C., Nguyen B-L. and Vella C.: Importance of estrogen sulfates in breast cancer. *J. Steroid Biochem.* 34 (1989) 155–163.
17. Santner S. J., Levin M. C. and Santen R. J.: Estrone sulfate stimulates growth of nitrosomethylurea-induced breast carcinoma *in vivo* in the rat. *Int. J. Cancer* **46** (1990) 73–78.
18. Duncan L., Purohit A., Howarth N. M., Potter B. V. L. and Reed M. J.: Inhibition of estrone sulfate activity by estrone-3-methylthiophosphonate. *Cancer Res* 53 (1993) 298–303.
19. Howarth N. M., Cooper G., Purohit A., Duncan L., Reed M. J. and Potter B. V. L.: Phosphonates and thiophosphonates as sulfate surrogates: synthesis of estrone-3-methylthiophosphonate, a potent inhibitor of estrone sulfatase. *Bioorg. Med. Chem. Lett.* 3 (1993) 313–318.
20. Howarth N. M., Purohit A., Reed M. J. and Potter B. V. L.: Estrone sulfamates: potent inhibitors of estrone sulfatase with therapeutic potential. *J. Med. Chem.* 37 (1994) 219–221.
21. 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.
22. Dibbelt L., Li P. K., Pillai R. and Knuppen R.: Inhibition of human placental sterylsulfatase by synthetic analogs of estrone sulfate. *J. Steroid Biochem. Molec. Biol.* 50 (1993) 261–266.
23. Li P. K., Pillai R. and Dibbelt L.: Estrone sulfate analogs as estrone sulfatase inhibitors. *Steroids* (1995) (in press).
24. Selcer K. W., Jagannathan S. and Li P. K.: *Inhibition of MCF-7 Breast Cancer Cell Proliferation by Estrone Sulfatase Inhibitors.* The Endocrine Society, 76th Annual Meeting, Abstract #747 (1994).
25. Conrow R. B. and Bernstein S.: Some fused diaza-heterocycles of estra-1,3,5(10)-triene-17-one. *Steroids* 2 (1968) 151–164.

26. Newman M. S. and Karnes H. A.: The conversion of phenols to thiophenol via dialkylthiocarbamates. *J. Org. Chem.* **31** (1966) 3980–3984.
27. Reed K. C. and Ohno S.: Kinetic properties of human placental aromatase: application of an assay measuring ^3H_2O release from 1β, 2β-3H-androgens. *J. Biol. Chem.* **251** (1976) 1625–1631.
28. Turner C. D.: *General Endocrinology*. W. B. Saunders, PA (1966) p. 579.
29. Nguyen B-L., Ferme I., Chetrite G. and Pasqualini J. R.: Action of Danazol on the conversion of estrone sulfate to estradiol and on the sulfatase activity in the MCF-7, T-47D and MD-MB-231 human mammary cancer cells. *J. Steroid Biochem. Molec. Biol* **46** (1993) 17–23.