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IN VIVO EVALUATION OF 7α-[11-(4-[^{125}I]IODOPHENOXY)UNDECYL]-17β-ESTRADIOL: A POTENTIAL VECTOR FOR THERAPY OF ADRENAL AND ESTROGEN RECEPTOR-POSITIVE CANCERS

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Summary—7α-[11-(4-[^{125}I]Iodophenoxy)undecyl]-17β-estradiol ([^{125}I]IPUE$_2$) was synthesized and its tissue distribution studied in immature female Fischer rats. Upon intravenous administration, [^{125}I]IPUE$_2$ was shown to accumulate in the adrenals and, to some extent, in the uterus and the ovaries. Coinjection with estrogen receptor (ER)-saturating quantities of unlabeled 17β-estradiol did not significantly reduce the uptake of [^{125}I]IPUE$_2$ in these tissues. The high adrenal uptake of [^{125}I]IPUE$_2$ is most likely associated with the lipophilic nature of the 7α-substituted estradiol. The potential to use the 7α-undecylestradiol as a vector to direct therapeutic groups to adrenal and ER-positive cancers is discussed.

INTRODUCTION

Endocrine therapy, together with estrogen-ablative therapy such as ovariectomy, hypophysectomy and adrenalectomy, is used beneficially in the treatment of estrogen receptor (ER)-positive breast tumors [1–5]. Patients with tumors lacking ER are not responsive to hormonal therapy and are treated by chemotherapy [1–5]. Antitumor drugs used in conventional chemotherapy are usually void of selectivity [6, 7], and in order to increase the effectiveness of the latter treatment in the case of ER-positive breast cancer, cytotoxic agents may be coupled to an ER-based vector. Selective delivery of the drugs to mammary carcinoma cells would provide for a means to lower effective drug doses and diminish side-effects to healthy tissues.

7α-Undecylestradiol derivatives were shown to bind to calf uterine ER [8, 9] and the resulting complexes are believed to bind tightly to nuclear DNA of target cells [10]. The use of a long linear spacer, at the 7α-position of 17β-estradiol, allows the attachment of relative bulky groups at the end of the side chain while assuring minimal interference with the ER binding process [11, 12]. Accordingly, such a ligand could be used for directing different agents to DNA of ER-rich cells. The relative binding affinity of 7α-undecylestradiol derivatives for ER are between 0.5 and 8.4% compared to that of natural occurring 17β-estradiol [9]. Albeit low, this value is in the same order of magnitude as clinically used antiestrogen drugs [13], such as tamoxifen [14].

The synthesis of 7α-undecylestradiol labeled directly with ^{125}I at the end of the spacer chain, e.g. 7α-(11-[^{125}I]iodoundecyl)-17β-estradiol ([^{125}I]IUE$_2$), has previously been reported and this compound showed a high binding affinity ($K_d = 4.2 \times 10^{-9}$ M) for calf uterine ER [11]. Thus, this type of carrier molecule could have a potential use in the transport of cytotoxic agents [15–18] or γ-emittors [19–24] to the nuclei of ER-positive human tumor cells, to provide for more selective chemotheraphy and diagnostic imaging. Tissue distribution of [^{125}I]IUE$_2$ in immature female Fischer rats revealed rapid deiodination [11]. In this report, we describe the tissue distribution of the 7α-undecylestradiol carrier labeled with a bulkier, but more stable *p*-[^{125}I]iodophenoxy group. The stability of this tracer molecule allowed us to evaluate the *in vivo* potential of 7α-undecylestradiol as an ER-based vector in therapy and imaging of ER-positive human cancers.

EXPERIMENTAL

Chemistry

Solvents and reagents were of the highest commercial grade available. Carrier-free [^{125}I]-

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NaI (1625–2125 Ci/mmol) was purchased from Amersham Canada Ltd and 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (iodogen) was obtained from Sigma. High-performance liquid chromatography (HPLC) analyses were carried out on a Beckman HPLC, utilizing a Chromosorb LC-6 normal phase HPLC column (10 μm, 25 × 0.94 cm, CSC, Montréal) at a flow rate of 2 ml/min. Eluting compounds were detected by their u.v. absorption at 254 nm with an Altex u.v. detector and in the case of ^{125}I-labeled compounds, simultaneously by their γ-radiation via a sodium iodide detector (Ortec). 7α-(11-bromoundecyl)-17β-estradiol was prepared from the 7α-hydroxyundecyl derivative [8], and [^{125}I]IUE$_2$ and 16α-[^{125}I]iodo-17β-estradiol ([^{125}I]-IE$_2$) were synthesized as previously reported [11].

7α-[11-(4-[^{125}I]Iodophenoxy)undecyl]-17β-estradiol ([^{125}I]-IPUE$_2$). The inside wall of a 1 ml Eppendorf plastic tube was coated with a thin layer of iodogen (80 μg), via evaporation of a methylene chloride solution (10 mg/ml) under a stream of nitrogen. A solution of Na^{125}I (40 μl, 4.0 mCi) dissolved in water, together with an aqueous solution of phenol (60 μg, 6.4×10^{-7} mol), was added to the coated tube. The reaction mixture was agitated in an ultrasonic bath for 1 h at room temperature, in order to increase the ^{125}I incorporation yield. After evaporation of the water under a stream of nitrogen at 90°C, 2.8 mCi of the initial radioactivity was solubilized in 400 μl of methylene chloride (resolubilization yield, 70%) and injected onto the HPLC column (*n*-heptane–2-propanol, 99.3:0.7), to give 1.1 mCi of *p*-[^{125}I]iodophenol (radiochemical yield 39%, based on redissolved radioactivity, 28% overall yield). *p*-[^{125}I]Iodo-phenol [25] eluted at 41 min and was well separated from the starting material (the retention time, t_R, of phenol is 24 min, under the same HPLC conditions).

p-[^{125}I]Iodophenol (0.9 mCi) in methylene chloride was transferred to a Pierce screw cap reacti-vial and the solvent was evaporated under a stream of nitrogen. To the residue was added 7α(11-bromoundecyl)-17β-estradiol (120 μg, 2.4×10^{-7} mol) in 40 μl DMF, approximately 0.5 mg sodium bicarbonate and 10 μl DMF. The resulting mixture was heated at 90°C for 15 h and taken to dryness under a stream of nitrogen. The [^{125}I]IPUE$_2$ was then purified by HPLC (*n*-heptane–2-propanol, 49:1, t_R: 32 min) and was well separated from *p*-[^{125}I]-iodophenol and the bromoundecyl starting material (radiochemical yield 0.4 mCi, 45%).

In vivo distribution studies

Animals. Immature female Fischer rats (24 days old, 30–35 g) were obtained from Charles River Breeding Farm, and the animals were allowed free access to food and water throughout the experiment. The animal experiments were conducted in accordance with the recommendations of the Canadian Council on Animal Care.

Tissue distribution. The [^{125}I]IPUE$_2$ was dissolved in an isotonic sterile NaCl solution (0.9%, Squibb Canada Inc.) containing 4% ethanol. All injections were carried out under aseptic conditions. Before injection, the animals were warmed for a few minutes under an infrared lamp to ensure dilation of the tail vein [22]. They were then injected, under ether anesthesia, through the lateral tail vein with 0.2 ml of the radiopharmaceutical solution (approximately 0.5 μCi). Four animals were sacrificed by cardiac puncture, while under ether anesthesia, 30 min, 3 and 6 h after administration of [^{125}I]-IPUE$_2$. Six animals were used for the 1 h time point, and were treated with or without co-injected unlabeled estradiol (E$_2$, 18 μg/injection), in order to establish specific estrogen receptor-mediated uptake. Samples of blood were collected, and uterus, ovaries, thyroid, liver, spleen, lungs, kidney, muscle, eye and brain samples were excised, washed with 0.154 M KCl, blotted dry and placed in sealed preweighed test tubes. The radioactivity in each sample was counted in a γ-counter (LKB, Model 1282), together with a reference sample of the injected preparation. Radioactivity levels are expressed as percentage of the injected dose per g of tissue (%ID/g) or as tissue-to-blood ratios. The statistical incertitude is given as the percentage coefficient of variation (% c.v.) corrected for small sample size effect [26].

RESULTS

Radiochemistry

Our synthetic approach involved a two step reaction, in which *p*-[^{125}I]iodophenol, initially prepared, undergoes a nucleophilic bromide-displacement on the bromoundecyl to yield no carrier added (n.c.a.) [^{125}I]IPUE$_2$. *p*-[^{125}I]-Iodophenol was prepared by electrophilic substitution on phenol with ^{125}I$_2$, using iodogen as oxidizing agent. The reaction is fast and the use of n.c.a. ^{125}I and unsilynized plastic tubes [27, 28], resulted in high resolubilization yields

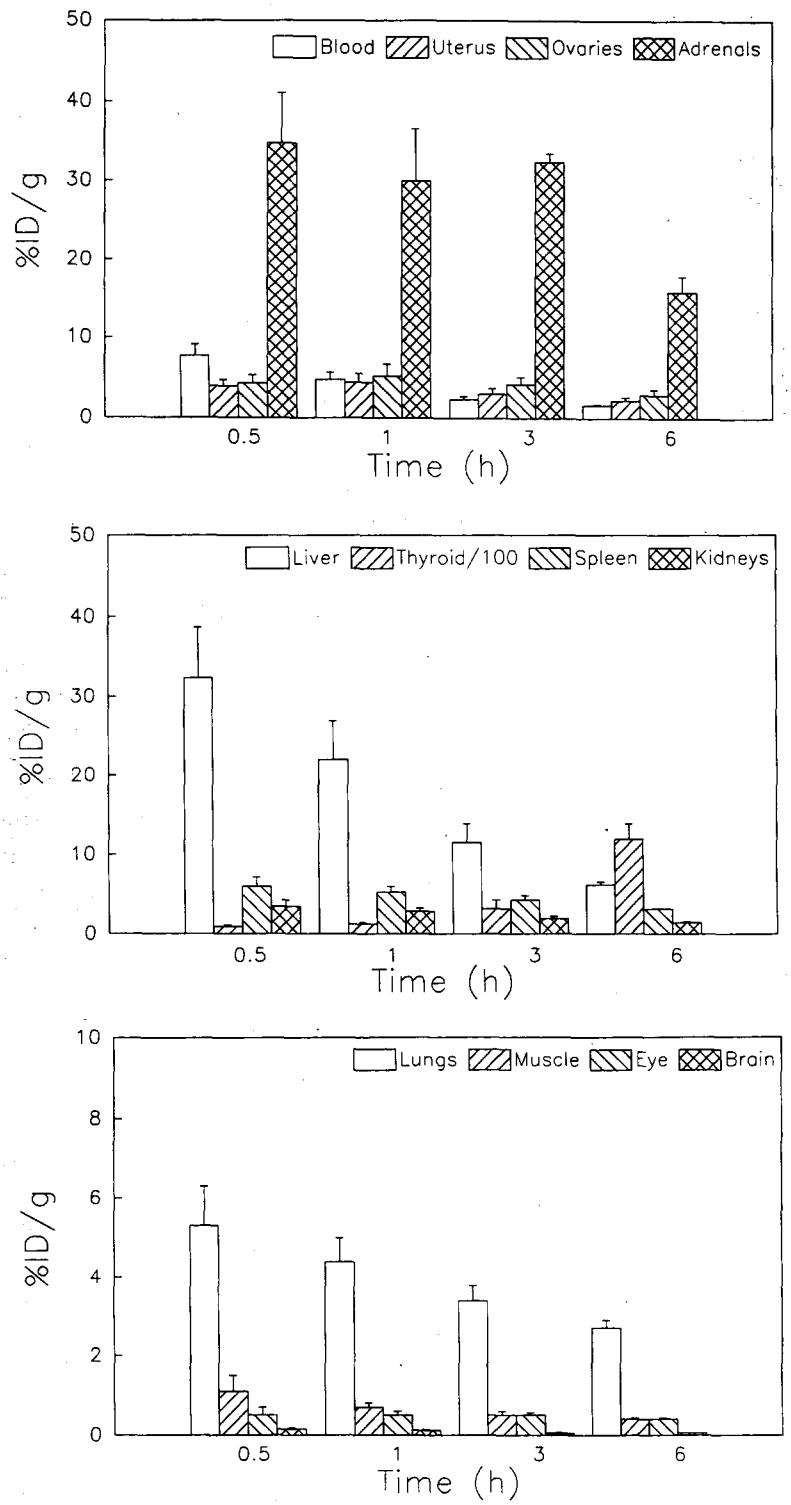

Fig. 1. Tissue distribution in % ID/g (error bars represent standard deviation) of ^{125}I-activity after i.v. administration of 7α-[11-(4-[^{125}I]iodophenoxy)undecyl]-17β-estradiol ([^{125}I]IPUE$_2$) in immature female Fischer rats.

(70% in CH_2Cl_2) of *p*-[^{125}I]iodophenol. The use of water to solubilize the latter did not increase the yield. The formation of the *p*-[^{125}I]iodophenoxyundecyl derivative proceeded as previously reported for the unlabeled IPUE$_2$ [9]. The radiochemical purity of [^{125}I]IPUE$_2$ was in excess of 99% after HPLC purification, no other radioactivity material could be detected on the

Table 1. Effect of coinjected unlabeled 17β-estradiol on the biodistribution of [^{125}I]IPUE$_2$ 1 h after injection[a]

Tissue	% ID/g Control	Coinjected[b]	% Change[c]
Adrenals	30.00 (22%)[d]	26.12 (17%)[d]	−13
Uterus	4.41 (25%)	3.68 (23%)	−17
Ovaries	5.24 (28%)	4.53 (13%)	−14
Blood	4.76 (19%)	4.88 (18%)	+3
Liver	21.97 (22%)	21.47 (25%)	−2
Thyroid	118.06 (19%)	127.59 (25%)	+8
Spleen	5.18 (14%)	5.41 (17%)	+4
Lungs	4.44 (13%)	5.06 (9%)	+14
Kidney	2.79 (13%)	3.15 (9%)	+13
Muscle	0.65 (9%)	0.68 (9%)	+5
Eye	0.53 (17%)	0.50 (12%)	−6
Brain	0.12 (25%)	0.18 (17%)	+50

[a]Immature female Fischer rats received 0.5 μCi of [^{125}I]IPUE$_2$ via the tail vein.
[b]Coinjected with 18 μg of unlabeled E_2.
[c]Percentage change between animals coinjected with unlabeled E_2 and animals injected with [^{125}I]IPUE$_2$ only.
[d]Mean values of 6 rats. The incertitude is given as % c.v., e.g. the percentage coefficient of variation corrected for small sample size effect [26].

chromatogram. The identity of the *p*-[^{125}I]-iodophenol and [^{125}I]IPUE$_2$ was confirmed by the identical chromatographic mobilities (TLC and HPLC) of the unlabeled analogs. Specific activities could not be calculated for these compounds since no u.v. absorption at the most sensitive detector setting (254 nm), was observed in the region of the radioactive HPLC peaks. Similar observations were made with the analogous 7α-(11-[^{18}F]fluoroundecyl)-17β-estradiol prepared with n.c.a. [^{18}F]KF [29] as well as other ^{125}I-labeled steroids [24]. Based on the specific activity of the n.c.a. [^{125}I]NaI employed (2125 mCi/mmol) the specific activity of [^{125}I]IPUE$_2$ probably exceeds 500 Ci/mmol.

Tissue distribution studies

Tissue distribution patterns of [^{125}I]IPUE$_2$ in immature female Fischer rats are shown in Fig. 1. Animals were injected with 0.5 μCi of the ^{125}I-labeled steroid and sacrificed at 0.5, 1, 3 and 6 h after injection. The usual estrogen nontarget tissues such as liver, spleen, lungs, kidney and muscle showed radioactivity retention curves parallel to the washout of [^{125}I]IPUE$_2$ from the blood, in accord with the absence of ER in these tissues. Muscle, eye and brain showed low radioactivity uptake. The uterus and ovaries gave a different accumulation pattern, e.g. the ^{125}I levels increased during the first 60 min and then decreased progressively. Moreover, these ER-rich tissues exhibited higher radioactivity

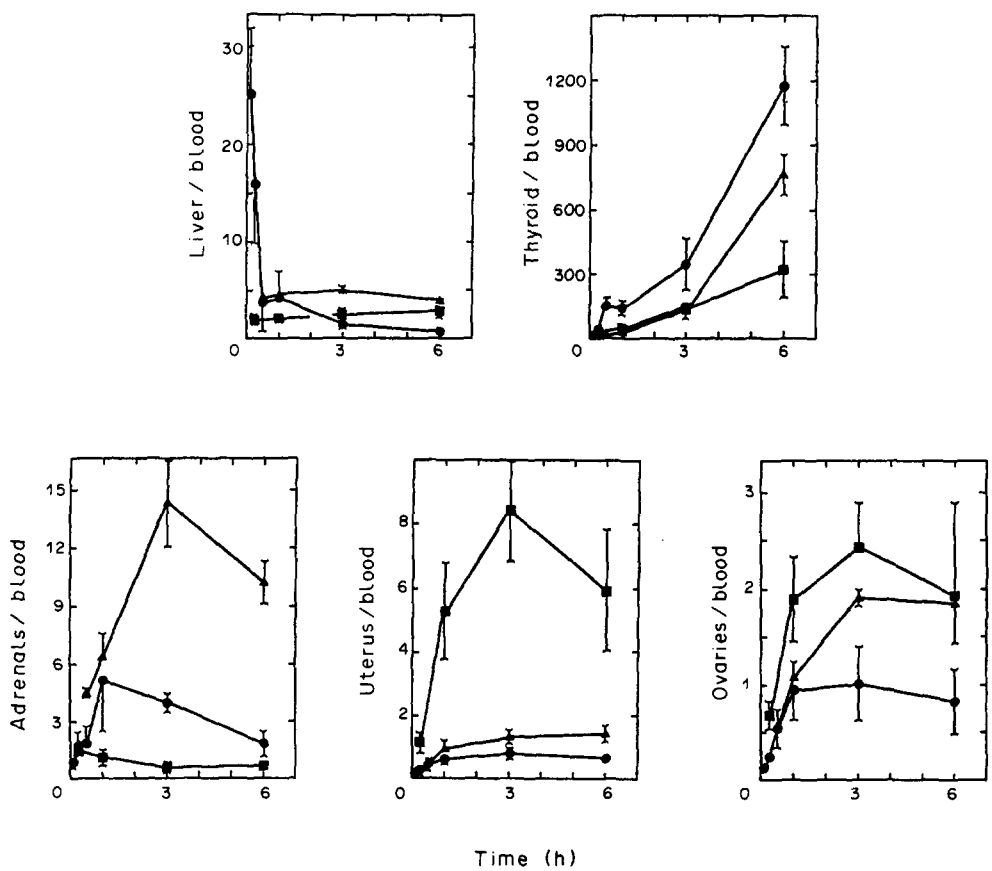

Fig. 2. Tissue-to-blood ratios of radioactivity in immature female Fischer rats, following i.v. administration of [^{125}I]IPUE$_2$ (▲), [^{125}I]IUE$_2$ (●) and [^{125}I]IE$_2$ (■).

retention than the nontarget tissues such as muscle, eye and brain, suggesting a specific uptake mechanism. Surprisingly, apart from the thyroid, the adrenals exhibited the highest retention of [^{125}I]IPUE$_2$, reaching maximum levels about 3 h after injection. Metabolism and deiodination likely accounts for the radioactivity in the thyroid.

Tissue distribution of [^{125}I]IPUE$_2$ in immature female Fischer rats, 1 h after i.v. administration with and without 18 μg of nonradioactive E_2, is summarized in Table 1. A reduction in the ^{125}I uptake in the ovaries, uterus and adrenals was observed upon coadministration of E_2, however, variations were not found to be statistically significant. No change was observed in the ^{125}I uptake by nontarget tissues, derived from animals treated either with or without coinjected nonradioactive E_2.

Figure 2 compares tissue uptake of [^{125}I]-IPUE$_2$ with earlier reported biodistribution data on [^{125}I]IUE$_2$ and [^{125}I]IE$_2$ [11]. Immature female Fischer rats were injected with 0.5 μCi of the radiotracers, and animals were sacrificed between 5 min and 6 h after injection. The liver displayed early retention of [^{125}I]IUE$_2$, indicative of rapid metabolism of the labile C–I bond by liver enzymes, resulting in turn, in high ^{125}I-uptake by the thyroid [11, 30]. Compared to [^{125}I]IUE$_2$, [^{125}I]IPUE$_2$ and [^{125}I]IE$_2$ exhibit better *in vivo* stability, e.g. less deiodination. The uptake of radioactivity by the thyroid 3 h after the administration of [^{125}I]IPUE$_2$ may reflect slow liberation of free ^{125}I. The adrenal gland shows high accumulation of the 7α-undecylestradiol radiotracers, particularly [^{125}I]IPUE$_2$, whereas the reference [^{125}I]IE$_2$ exhibits no adrenal uptake. Furthermore, the adrenal uptake of [^{125}I]IPUE$_2$ is higher than the uterus uptake of [^{125}I]IE$_2$, while their retention curves exhibit similar accumulation pattern. The uterus and ovaries display low uptake of the 7α-undecylestradiol derivatives, which contrasts the high uptake of the [^{125}I]IE$_2$ in these organs. The *p*-[^{125}I]iodophenoxyundecyl derivative [^{125}I]-IPUE$_2$ shows better accumulation in the adrenals, uterus and ovaries than [^{125}I]IUE$_2$.

DISCUSSION

We have previously shown that 7α-undecylestradiol derivatives form stable ligand–ER complexes, with RBA values in the range of 0.1–8.4 [9]. In order to evaluate the *in vivo* potential of the 7α-undecylestradiol moiety as a vector to direct cytotoxic or γ-emitting groups to ER-rich tissues we used an analog in which radioiodine was directly attached onto the alkyl substituent, e.g. [^{125}I]IUE$_2$ [11]. However, under *in vivo* conditions this compound was found to undergo rapid deiodination thus masking the pharmacokinetics of the parent compound [11]. In the present study we used instead the more stable 4-[^{125}I]iodophenoxyundecyl analog [^{125}I]-IPUE$_2$. Substantial less hepatic deiodination is observed (Fig. 2) and this is in line with the higher chemical stability of the aromatic C–I bond in [^{125}I]IPUE$_2$ as compared to the primary C–I bond in [^{125}I]IUE$_2$. *In vivo* deiodination is reflected in elevated ^{125}I-thyroid levels (Fig. 2) and results in an underestimation of the potential [^{125}I]IUE$_2$ uptake by target tissues. In fact, apart from its chemical stability, [^{125}I]IUE$_2$ should exhibit higher target tissue uptake than [^{125}I]IPUE$_2$, since IUE$_2$ is less lipophilic and exhibits somewhat higher affinity to ER [9]. The use of the [^{125}I]iodophenoxy group provides for a convenient ^{125}I-labeling procedure which could be applied to many other types of radioactive ligands and radiopharmaceuticals.

The biodistribution of [^{125}I]IPUE$_2$ in immature female Fischer rats showed particularly good uptake by the adrenals and, to some extent, by the uterus and ovaries. The uptake kinetics in these tissues suggest a selective retention mechanism (Fig. 1), although coinjected unlabeled 17β-estradiol did not significantly reduce uptake of the radiopharmaceutical (Table 1). The higher uptake levels, in the adrenals, likely reflect the lipophilic nature of this compound [31]. The retention mechanism could involve association of [^{125}I]IPUE$_2$ with plasma lipoproteins, which facilitates uptake by steroid-secreting tissues, e.g. adrenal cortex and ovaries, via a lipoprotein receptor-mediated process similar to that observed with cholesterol [32, 33]. In fact, both undecyl derivatives [^{125}I]IPUE$_2$ and [^{125}I]IUE$_2$ exhibit better uptake in adrenals and ovaries than in uteri, which is contrary to observations made with the highly ER-affinic [^{125}I]IE$_2$ (Fig. 2).

The low levels of uptake of [^{125}I]IPUE$_2$ in the uterus indicate that the 7α-undecylestradiol is not an appropriate vector for diagnostic imaging of ER-positive cancers with γ-emitters. However, selected 7α-alkyl amide analogs of estradiol, which are similar to our 7α-undecylestradiol derivatives, have recently been shown *in vivo* to be devoid of estrogenic activity while exhibiting potent antiestrogenic proper-

ties [34, 35]. We are currently evaluating the antiestrogenic activities of our 7α-undecylestradiol derivatives. If active, such compounds could provide an alternative to hormonal therapy of ER-positive cancers by combining antiestrogenic and cytotoxic activity in a single carrier molecule.

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REFERENCES

1. McGuire W. L.: Current status of estrogen receptors in human breast cancer. *Cancer* **36** (1975) 638–644.
2. Walt A. J., Singhakowinta A., Brooks S. C. and Cortez A.: The surgical implications of estrophile protein estimations in carcinoma of the breast. *Surgery* **80** (1976) 506–512.
3. McGuire W. L., Horwitz K. B., Zava D. T., Garola R. E. and Chamness G. C.: Hormones in breast cancer: update 1978. *Metabolism* **27** (1978) 487–501.
4. Osborne C. K., Knight W. A. III, Yochmowitz M. G. and McGuire W. L.: Modern approaches to the treatment of breast cancer. *Blood* **56** (1980) 745–752.
5. Henderson C. and Canellos G. P.: Cancer of the breast. The past decade. *New Engl. J. Med.* **302** (1980) 17–30.
6. Hurwitz E., Levy R., Maron R., Wilchek M., Arnon R. and Sela M.: The Covalent binding of daunomycin and adriamycin to antibodies, with retention of both drug and antibody activities. *Cancer Res.* **35** (1975) 1175–1181.
7. Levy R., Hurwitz E., Maron R., Arnon R. and Sela M.: The specific cytotoxic effects of Daunomycin conjugated to antitumor antibodies. *Cancer Res.* **35** (1975) 1982–1986.
8. Bucourt R., Vignau M., Torelli V., Richard-Foy H., Geynet C., Secco-Millet C., Redeuilh G. and Baulieu E. E.: New biospecific adsorbents for the purification of estradiol receptor. *J. Biol. Chem.* **253** (1978) 8221–8228.
9. DaSilva J. N. and van Lier J. E.: Synthesis and structure-affinity of a series of 7α-undecylestradiol derivatives: a potential vector for therapy and imaging of estrogen receptor-positive cancers. *J. Med. Chem.* **33** (1990) 430–434.
10. Spelsberg T. C., Ruh T., Ruh M., Goldberger A., Horton M., Hora J. and Singh R.: Nuclear acceptor sites for steroid hormone receptors: comparisons of steroids and antisteroids. *J. Steroid Biochem.* **31** (1988) 579–592.
11. DaSilva J. N. and van Lier J. E.: Evaluation of an 7α-undecyl substituted estradiol derivative as a carrier for breast tumor imaging and therapy. In *Current Applications in Radiopharmacology* (Edited by M. W. Billinghurst). Pergamon Press, Toronto (1986) pp. 80–87.
12. DaSilva J. N. and van Lier J. E.: Synthesis of 7α-undecyl substituted estradiol derivatives for breast tumor imaging. *J. Labelled Compd Radiopharm.* **23** (1986) 1414–1416.
13. Pasqualini J. R., Sumida C. and Giambiagi N.: Pharmacodynamic and biological effects of anti-estrogens in different models. *J. Steroid Biochem.* **31** (1988) 613–643.
14. Furr B. J. A. and Jordan V. C.: The pharmacology and clinical uses of tamoxifen. *Pharmac. Ther.* **25** (1984) 127–205.
15. Leclercq G., Devleeschouwer N. and Heuson J. C.: Guidelines in the design of new antiestrogens and cytotoxic-linked estrogens for the treatment of breast cancer. *J. Steroid Biochem.* **19** (1983) 75–85.
16. Delbarre A., Oberlin R., Roques B. P., Borgna J. L., Rochefort H., LePecq J. B. and Jacquemin-Sablon A.: Ellipticine derivatives with an affinity to the estrogen receptor, an approach to develop intercalating drugs with a specific effect on the hormone-dependent breast-cancer. *J. Med. Chem.* **28** (1985) 752–761.
17. Pillai K. M. R., McLaughlin W. H., Lambrecht R. M. and Bloomer W. D.: Carrier-free astatination of steroid hormones. *J. Labelled Compd Radiopharm.* **24** (1987) 1117–1122.
18. DeSombre E. R., Mease R. C., Hughes A., Harper P. V., DeJesus O. T. and Friedman A. M.: Bromine-80m-labeled estrogens: auger electron-emitting, estrogen receptor-directed ligands with potential for therapy of estrogen receptor-positive cancers. *Cancer Res.* **48** (1988) 899–906.
19. Hochberg R. B.: Iodine-125-labeled estradiol: a gamma-emitting analog of estradiol that binds to the estrogen receptor. *Science* **205** (1979) 1138–1140.
20. Kiesewetter D. O., Kilbourn M. R., Landvatter S. W., Heiman D. F., Katzenellenbogen J. A. and Welch M. J.: Preparation of four fluorine-18-labeled estrogens and their selective uptakes in target tissues of immatures rats. *J. Nucl. Med.* **25** (1984) 1212–1221.
21. Hanson R. N. and Franke L. A.: Preparation and evaluation of 17α-[^{125}I]iodovinyl-11β-methoxyestradiol as a highly selective radioligand for tissues containing estrogen receptors: concise communication. *J. Nucl. Med.* **25** (1984) 998–1002.
22. Ali H., Rousseau J., Ghaffari M. A. and van Lier J. E.: Synthesis, receptor binding, and tissue distribution of (17α,20E)- and (17α,20Z)-21-[^{125}I]iodo-19-norpregna-1,3,5(10),20-tetraene 3,17-diol. *J. Med. Chem.* **31** (1988) 1946–1950.
23. Zielinski J. E., Larner J. M., Hoffer P. B. and Hochberg R. B.: The synthesis of 11β-methoxy-[16α-^{123}I]iodo-estradiol and its interaction with the estrogen receptor *in vivo* and *in vitro*. *J. Nucl. Med.* **30** (1989) 209–215.
24. Hanson R. N., Franke L. A. and Kaplan M. L.: Synthesis and evaluation of (17α,20E)21-[^{125}I]iodo-11-substituted-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols: the influence of 11-stereochemistry on tissue distribution of radioiodinated estrogens. *Nucl. Med. Biol.* **16** (1989) 3–9.
25. Kometani T., Watt D. S., Ji T. and Fitz T.: An improved procedure for the iodination of phenols using sodium iodide and tert-butyl hypochlorite. *J. Org. Chem.* **50** (1985) 5384–5387.
26. Sherrer B. *Biostatistique*. Editions G. Morin, Chicoutimi, Quebec, Canada (1984).
27. Brodack J. W., Kilbourn M. R., Welch M. J. and Katzenellenbogen J. A.: NCA 16α-[^{18}F]fluoroestradiol-17β: the effect of reaction vessel on fluorine-18 resolubilization, product yield, and effective specific activity. *Int. J. Appl. Radiat. Isot.* **37** (1986) 217–221.
28. Irie T., Fukushi K., Ido T., Nozaki T. and Kasida Y.: ^{18}F-fluorination by crown ether-metal fluoride: II. Non-carrier-added labeling method. *Int. J. Appl. Radiat. Isot.* **35** (1984) 517–520.
29. DaSilva J. N., Crouzel C. and van Lier J. E.: Synthesis of NCA 7α-(11-[^{18}F]fluoroundecyl)-estradiol: evaluation of a vector for estrogen receptor based agents. *J. Labelled Compd Radiopharm.* **26** (1989) 342–343.
30. Landvatter S. W., Katzenellenbogen J. A., McElvany K. D. and Welch M. J.: (2R*,3S*)-1-[^{125}I]Iodo-2,3-bis(4-hydroxyphenyl) pentane ([^{125}I]-iodonorhexestrol) and (2R*,3S*)-1-[^{77}Br]bromo-2,3-bis(4-hydroxyphenyl)-pentane ([^{77}Br]bromonorhexestrol), two γ-emitting estrogens that show receptor-mediated uptake by target tissues *in vivo*. *J. Med. Chem.* **25** (1982) 1307–1312.

31. McManaway M. E., Jagoda E. M., Kasid A., Eckelman W. C., Francis B. E., Larson S. M., Gibson R. E., Reba R. C. and Lippman M. E.: [^{125}I]17α-Iodovinyl 11β-methoxyestradiol interaction *in vivo* with estrogen receptors in hormone-independent MCF-7 human breast cancer transfected with the v-ras oncogene. *Cancer Res.* **47** (1987) 2945–2949.
32. Seevers R. H., Schwendner S. W., Swayze S. L. and Counsell R. E.: Potential organ- or tumor-imaging agents. 22. Acyl-labeled cholesterol esters. *J. Med. Chem.* **25** (1982) 618–621.
33. Counsell R. E., Schappa L. W., Korn N. and Huler R. J.: Tissue distribution of high-density lipoprotein labeled with radioiodinated cholesterol. *J. Nucl. Med.* **21** (1980) 852–858.
34. Wakeling A. E. and Bowler J.: Biology and mode of action of pure antiestrogens. *J. Steroid Biochem.* **30** (1988) 141–147.
35. Wakeling A. E.: Comparative studies on the effects of steroidal and non-steroidal oestrogen antagonists on the proliferation of human breast cancer cells. *J. Steroid Biochem.* **34** (1989) 183–188.