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Steroid Receptor Ligands for Breast Cancer Targeting: An Insight into Their Potential Role As Pet Imaging Agents

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Abstract: The design and development of radiolabelled steroid derivatives has been an important area of research due to their well-known value in breast cancer targeting. The estrogen receptor (ER) and progesterone receptor (PR) are important biomarkers in the diagnosis, prognosis and follow-up of the therapeutic response of breast tumours. Thus, several radioligands based on estrogens and progestins have been proposed for targeted functional ER imaging. The aim of this review is to survey and analyze the developments in this field, which have led to the design of a number of PET steroid-based imaging agents, a few of which seem to be promising as radiopharmaceuticals for detection of ER-positive breast tumours.

Keywords: Breast cancer, estradiol, estrogen receptor, progesterone, progesterone receptor, PET imaging, tumour targeting.

1. INTRODUCTION

Breast cancer is a major cause of premature death among women in the Western World, in spite of a decline in mortality rate in recent years [1]. Although the causes of cancer are still poorly understood, it is well known that the process of carcinogenesis includes several stages. The first step, *initiation*, begins with a start-off event, which involves some sort of damage to the genetic code carried in the cell, the DNA. It also involves *promotion*, a process that enables those damaged cells to grow. During the next stage of *tumour growth*, there is a period of further proliferation of the cells into a lump that can be recognised. Then, the cells are no longer copies of each other, and this is called *tumour progression*. Afterwards, tumour cells can be spread through the blood stream or through the lymphatic system. Breast carcinoma cells are mainly transported via the lymphatic system. These cells migrate to the lymph nodes under the arm and this is often the first place where *metastasis* is detected [2].

The link between body physiology and cancer was first reported in 1896, when Beatson [3] observed a remarkable regression of advanced breast cancers in several pre-menopausal women after ovariectomy. The idea that some substance within these organs might contribute to the growth of cancer came up, and the search for the factors affecting tumour growth and death began. Further advances in different scientific areas as well as the advent of novel assay techniques were decisive for a better understanding of cancer and for the development of novel therapeutic approaches. During the 1930's, the first preparation of non-steroidal estrogens brought a new insight into the study of estrogens, antiestrogens and their receptors [4]. The production of labelled ^{14}C and ^{3}H steroids during the 1940's led to a more detailed investigation of the function and effects of steroid hormones and their analogues on receptors and cancer. In the 1960's, estrogen receptors were first purified from rat uteri [5, 6] and some of their physical properties were identified [7]. The first physical evidence of a mechanism to concentrate estrogen in a target organ was reported by Jensen *et al.* [8] who demonstrated that [^{3}H]-estradiol was selectively taken up by target tissues. In the 1960's, tamoxifen, a non-steroidal antiestrogen, was shown to be effective in breast cancer treatment, opening up routes to the use of endocrine therapies in the management of all stages of breast cancer [9].

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This manuscript is dedicated to Dr. Luciana Patricio, former Head of our group, who initiated in our laboratory the work with steroid receptor ligands twenty years ago.

Approximately 70–80% of all breast tumours are estrogen receptor (ER) positive [10-14]. Here, ER is over-expressed in the cell nucleus. These tumours tend to grow more slowly, are better differentiated, and are associated with a slightly better overall prognosis. Thus, today, ER expression has become one of a few prognostic factors, along with axillary lymph node status, tumour size, and histological grade. Low levels of estrogen receptors in breast tumours are indicative of a poor response to endocrine therapy. More important, the detection of ER in breast carcinoma cells is a main indicator of potential response to endocrine therapy. Similar to ER, progesterone receptor (PR) status is a good predictor of tumour responsiveness to therapy. Nearly 50% of all ER tumours are also reported to be PR positive, and about 75% of these hormone responsive tumours (ER/PR+) respond positively to endocrine therapy [15]. Hence, knowledge of ER and PR expression can help to identify the patients that may benefit from endocrine therapy. Endocrine therapy response rates in advanced disease average 33 % in tumours positive for one hormone receptor and 50-70% in tumours positive for both estrogen and progesterone receptors [14, 16, 17].

The hormone responsive tumours, which constitute one of the major forms of cancer among women in the age group 26-55, are characterised by their growth and spread in the presence of estradiol and progesterone [18-21]. The effective management of breast cancer requires its early detection and accurate staging. Among the diagnostic techniques often used, such as mammography, computed tomography, ultrasonography and bone scintigraphy, the non-invasive detection of ER/PR status provides relevant information for the most effective treatment of primary and metastatic breast cancers. The overexpression of ER and PR in human tumour cells as well as their binding characteristics, which involve a prolonged retention of the hormone compared to non-hormone compounds, provide a favourable mechanism for localisation of tumours [22, 23].

The design of molecular probes for tumour receptor imaging as well as the imaging approaches for external detection are challenging issues in the management of cancer due to the particular nature of the receptors. Most receptors have high affinities for their cognate ligands and are even active at very low concentrations of these ligands. For this reason, radiopharmaceuticals with high specific activity are mandatory. Even small molar quantities of an imaging agent may saturate the receptor and limit the ability to visualize receptor expression [23, 24].

In general, molecular imaging modalities include molecular magnetic resonance imaging (MRI), magnetic resonance spectroscopy, optical imaging using fluorescence or bioluminescence, targeted ultrasound, single-photon emission computed tomography (SPECT), and positron emission tomography (PET) [25-28]. How-

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ever, in the case of tumour receptors the radionuclide-based imaging modalities (PET and SPECT) appear to be the only feasible approach due to the limited capacity of the receptor binding system that demands a tracer with high specific activity. PET and SPECT are the most sensitive molecular imaging techniques since they are able to determine concentrations of specific biomolecules as low as in the picomolar range.

The development of a receptor-based breast cancer imaging method requires a receptor ligand labelled with an appropriate radionuclide for imaging steroid receptors by SPECT (*e.g.* ^{123}I or ^{99m}Tc) or by PET (*e.g.* ^{18}F). These imaging agents, as long as they have a high binding affinity for the target receptor and a low binding affinity for non-specific binding sites as well as a suitable chemical and metabolic stability, can provide a non-invasive method to localise primary and metastatic tumours that will help in predicting the chances of the patient's survival and in predicting the response to various therapies [25].

Several classes of ER and PR targeting compounds, potentially useful as chemotherapeutic/chemopreventive agents and as radioimaging agents, have been designed and evaluated over the years. Non-invasive imaging of breast tumours based on their hormonal receptor's status can be used for hormone therapy follow-up by SPECT or PET. While SPECT still remains the more practical imaging modality for routine clinical studies in nuclear medicine due to the use of medium to long-lived radionuclides, it is well-recognized that PET imaging has higher resolution, higher sensitivity and better quantitation capability. The search for selective agents to image *in vivo* receptor densities in steroid-sensitive tumours, as well as the need for probes to study hormonal action, has led to the synthesis and evaluation of a series of halogenated derivatives of estradiol and progesterone labelled with PET radionuclides [29-35]. However, few of these agents have reached the clinical stage [36-39].

Many excellent reviews have been written concerning the radiosynthesis and clinical application of diverse PET radiotracers in various clinical areas [40-45]. Herein, we will mainly focus on steroid receptor ligands for ER/PR targeting as potential PET breast cancer imaging agents. Labelled non-steroidal molecules (*e.g.* ^{18}F-hexestrols, ^{18}F-fluorocyclofenils) [46-48] and non-steroidal antiestrogens such as ^{18}F-tamoxifen [49] for PET imaging of ER have also been reported in the literature, but will not be considered within the scope of this review.

In the first part of this manuscript we will discuss the molecular mechanisms underlying the action of steroid hormone receptors. In the second part, an outline on molecular imaging with PET, including PET methodology, PET radionuclides and radiolabelling strategies will be presented. In the third part, a brief overview concerning the biological evaluation of ER and PR expression with estradiol- and progesterone-based ligands will be given. The main preclinical and clinical achievements in the field will also be addressed. Finally this review will give an outlook on the future perspectives of the application of steroid receptor ligands for breast cancer PET molecular imaging.

2. MOLECULAR BIOLOGY OF STEROID HORMONE RECEPTORS

The estrogens estradiol and estrone are, together with progesterone, the most important female hormones. These estrogens are responsible for the development and maintenance of the sexual characteristics of the female. Although the intrinsic activity of estradiol is much higher than that of estrone, these compounds can be converted into each other by the estradiol-17β-dehydrogenase present in the body. In a concerted action with progesterone, the estrogens are also responsible for the progress of the normal menstrual cycle and have a role in the maintenance of pregnancy.

Estrogen biosynthesis occurs not only in the female reproductive tissues, starting from androstenedione, but also in adipose tissues *via* the androgens produced in the adrenal gland, depending on the menopausal status. In premenopausal women, the ovary actively produces high basal estrogens. In postmenopausal women, ovarian function has ceased and estrogen is primarily produced in peripheral tissues such as fat and muscle. Progesterone is produced in the ovary and in placenta from pregnenolone but also in the adrenals by the enzyme 3β-hydroxy-Δ^5-steroid dehydrogenase Fig. (**1**).

Estrogen Receptor

The steroid hormone estradiol manifests its biological activity through the specific high affinity ERs located within the target cell [50, 51]. The ERs are nuclear proteins belonging to a superfamily of transcription factors, known as nuclear receptors that mediate cell response to estrogens by the regulation of the synthesis of specific RNAs and proteins [52].

The great importance of ER lies in the significant activities that its ligands have on the development and function of the reproductive system, including the mammary gland and the uterus. In addition, estrogens also exert crucial actions in other tissues, including the pituitary, hypothalamus, bone, liver and cardiovascular system [51, 53]. The effect of estrogen on the mammary gland is particularly interesting, because of its association to breast cancer. Estrogens stimulate the proliferation and metastatic activity of nearly 40% of breast cancers, specifically the hormone-responsive tumours, containing significant ER levels.

ER was first identified in the rat uterus by Toft *et al.* in 1966 [7]. Twenty years later, the human ER was cloned and sequenced from MCF-7 human cancer cells [54]. This ER is now known as ERα to distinguish it from a second ER subtype, ERβ, which was cloned in 1996, first from the rat [55] and subsequently from the human [56].

Isoforms of both ER subtypes have also been described [57, 58]. ERα and ERβ exhibit differences in their tissue distribution patterns and are expressed in different ratios in brain, breast, cardiovascular system, urogenital tract, and bone [52, 57]. In normal breast tissue, ERβ predominates. However, in premalignant lesions, ERα is overexpressed, promoting cell proliferation under estrogen stimulation, and decreasing ERβ expression, which is associated with lower proliferation and pathological grade, and thought to downregulate ERα, acting as a tumour suppressor [57, 59, 60]. ERα is the therapeutic target of choice in breast cancer and is routinely quantified *in vitro* by immunohistochemistry on breast cancer biopsy specimens for treatment planning. However, the clinical value of ERβ in cancer in general and breast carcinoma in particular still needs to be determined [57, 60].

ERα and ERβ have the same basic structure, divided into six functional specific domains (designated A-F) responsible for functions leading to the transcription of target genes, such as ligand binding, DNA binding and transactivation Fig. (**2**) [61, 62] Although their overall sequence homology is only approximately 30% there is high homology of 95% and 55% within the DNA and hormone-binding domains, respectively [55]. This domain-specific homology suggests that ERα and ERβ are likely to share similar DNA and ligand-binding function, but the low overall homology indicate that their overall effects differ.

The ERα gene is located on chromosome 6q25.1 [63] and encodes a 595 amino acid long protein, composed of six domains, [61, 64], whereas the ERβ gene is located on chromosome 14q23.2 [64] and encodes a 530 amino acid long protein [65]. The two receptor subtypes exhibit differences in their ligand selectivity and transcriptional activities [66].

The estrogen receptor contains two distinct, non-acidic activation functions, AF-1 and AF-2. AF-1 is localised at the amino ter-

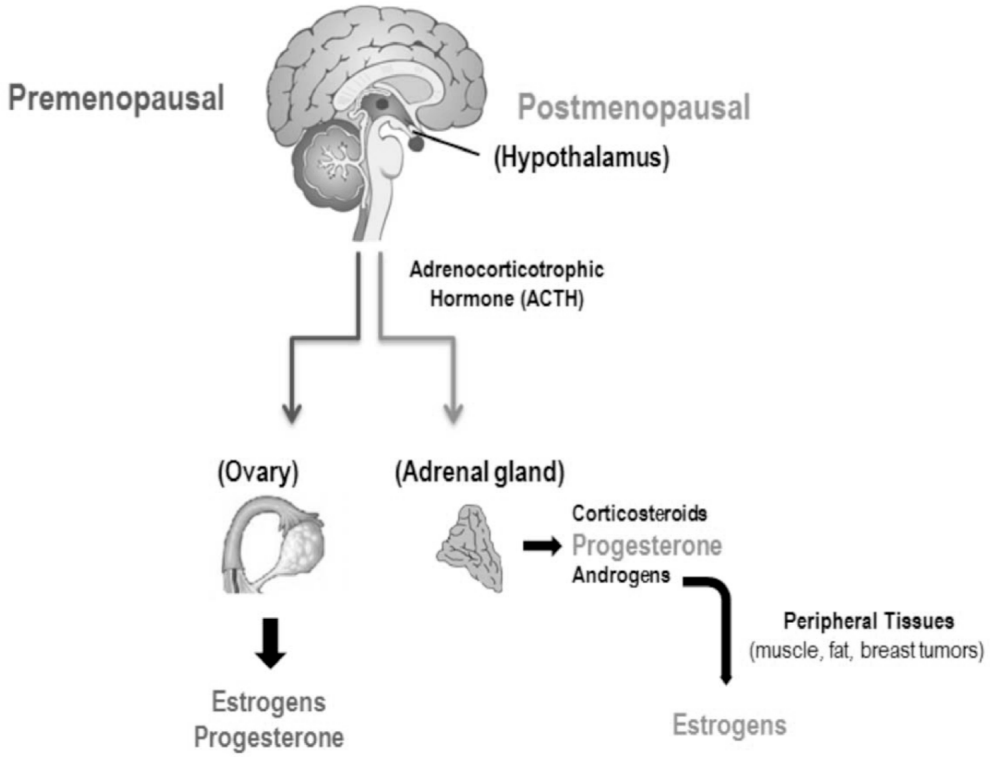


Fig. (1). Biosynthetic routes to estrogen and progesterone in pre-menopausal and post-menopausal women.

Fig. (2). Domain structures of human ERα and ERβ isoforms.

minal A/B domain [61]. However, functional studies have indicated that ERβ lacks AF1 activity [67]. AF-2, a hormone-dependent activation function, is located at the carboxyl terminus in the ligand-binding domain [68-70]. AF-2 is highly conserved among different species and other nuclear hormone receptors [50, 69] whereas the AF-1 function is less well conserved [50, 71]. The activity of each activation function is cell and gene promoter-dependent. AF-1 can exhibit transcriptional activity in the absence of AF-2 in some cell contents [72] but, in most cell and promoter contexts, both AF-1 and AF-2 function in a synergistic way and are required for full estrogen receptor activity [51, 62, 72]. The binding of estrogen promotes an interaction between AF-1 and AF-2 that seems to be an essential requirement for the ability of ER-ligand complexes to induce transcription [73]. Also different regions of AF-1 are required for antiestrogen- and estradiol-dependent transcriptional activation [74].

The **A/B** domain, a domain where deletion mutations have no effect on either estradiol or nuclear binding, is the least conserved between ERα and ERβ [61]. DNA binding occurs through a helix-loop-helix motif in the highly conserved **C** domain, located approximately in the middle of the receptor [61]. The DNA binding domain (DBD) mediates specific binding to estrogen responsive elements (ERE) on DNA. The **D** domain is a hypervariable region that corresponds to a hinge domain between the DBD and the ligand-binding domain **E** and contains the nuclear localization signal [71]. Amino acid deletions or insertions in this region can abolish tight nuclear binding [61]. The ligand binding **E/F** domain (LBD) located in the carboxyl terminal region of the receptor is relatively large. The **E** region is mainly hydrophobic and consists of 12 short helices that form an open pocket in the absence of the ligand [75, 76]. When agonist ligands such as estradiol binds to ER, helix 12 goes over the ligand, nestling the ligand in a tight fitting pocket.

The estrogen hormones mediate their effects on target gene transcription *via* ERs that, in the absence of ligand, are in a transcriptionally inactive form. The binding of a ligand to the latent receptor induces its conformational change, whereby it becomes "activated", an event that facilitates receptor dimerization and promotes its interaction with specific DNA sequences called estrogen response elements (ERE) [77, 78]. Depending on the cellular and promoter context, the DNA-bound receptor can regulate positively or negatively the target gene transcription, interacting with the transcription system [79]. Finally, these interactions stabilise the transcription complex and enhance RNA polymerase activity.

Progesterone Receptor

Progesterone has a major role in regulating expression of specific gene networks in the female reproductive tract and other target tissues [80-82]. One of the main functions of progesterone is to prepare the uterus for pregnancy. The physiological effects of progesterone are mediated by its nuclear receptor, the progesterone receptor (PR). These receptor proteins, that specifically bind progesterone and are induced by estrogen, were initially characterized in the mammalian uterus and chick oviduct in the early 1970s [83-86].

The progesterone receptor belongs to the type-1 nuclear receptor subfamily, which includes the androgen receptor, the estrogen receptor, the glucocorticoid receptor and the mineralocorticoid receptor. The human PR is expressed as two protein isoforms encoded by the same gene, full-length PR-B and N-terminally truncated PR-A that have distinct transcription activities [87]. PRs are found in the uterus and many other organs, including the brain. Usually, both subtypes are found in the normal breast tissue [59]. However, in malignant tumours, the PR-A:PR-B ratio is altered and the dominance of both isoforms has been reported [59, 88, 89]. On the other hand, loss of PR expression in breast cancer is associated with more aggressive tumours and worse prognosis [59].

Like other members of the nuclear receptor family, PR contains three main functional domains [90-92]. In humans, the N-terminal 164 amino acids of PR-B are missing in isoform PR-A. Detailed molecular dissection has identified two distinct activation function domains (AFs) within both PRs: AF-1, which is located in the N-terminal region, is ligand independent; AF-2, which is ligand dependent, is contained in the ligand-binding domain that is located in the C-terminal region. A DNA-binding domain and the hinge region are mapped to the central region of both receptors. Furthermore, a unique activation function domain, AF-3, is contained in the upstream segment of PR-B that is missing in PR-A. The centrally located DNA-binding domain is not only involved in DNA binding but also in receptor dimerization. The C-terminal domain, or ligand-binding domain (LBD), is involved in both ligand binding and transcriptional activation Fig. (**3**).

Like other steroid receptors, PRs are ligand-activated nuclear transcription factors that regulate transcription by interaction with protein cofactors and binding to specific response elements in target genes. Binding of agonist molecules to PR induces a major conformational change within the LBD that is thought to promote receptor dimerization, and its interaction with DNA at specific response elements located in the regulatory regions of target genes. The agonist-induced conformational change also promotes the recruitment of transcriptional coactivators and the ordered assembly of multiprotein complexes with chromatin-modifying activities [90, 93].

3. PET IMAGING

PET is a nuclear medicine modality with high sensitivity and good spatial resolution that can provide precise, quantitative analysis of dynamic biochemical processes *in vivo*. This technique is based on the administration and detection of the biodistribution of radiopharmaceuticals labelled with positron-emitting radionuclides, allowing better quality imaging than conventional SPECT. The interest in PET as a clinical and medical research imaging tool has gradually matured during the last three decades, and PET has currently been widely applied in diverse clinical fields such as oncology, cardiology and neurology [94-99]. Moreover, PET plays an important role in the process of drug development and evaluation [100-102].

PET requires a tracer that is labelled with a positron-emitting radionuclide and a PET camera for imaging the subject. Once the tracer is prepared and administered to the subject, the PET radionuclide decays in the body by positron emission Fig. (**4**). The emitted positron (β+) travels a short distance and collides with a nearby electron. This collision results in an annihilation event producing two γ-rays photons of 511 keV that travel at 180 degrees to each other. Two scintillation detectors that are separated by 180 degrees transmit a coincident signal, when they are hit by the γ-ray photons simultaneously.

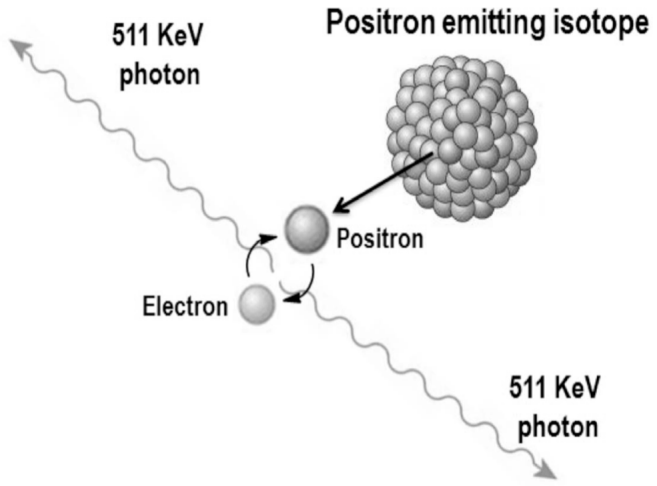


Fig. (4). A positron and an electron *annihilation* process with production of two 511 keV photons travelling in opposite directions.

In the last few years a significant progress in scanner technology has been the introduction of the integrated PET/CT technique, which combines the functional information of the PET scan with the detailed anatomical images from the CT scan [103-105].

3.1. Radionuclides for PET Imaging

PET has evolved around the use of short-lived radioactive tracers of natural organic elements such as ^{11}C ($T_{1/2}$=20 min), ^{13}N ($T_{1/2}$=10 min) and ^{15}O ($T_{1/2}$=2 min). The technical advantages offered by medium half-life radionuclides (approximately 1-100 hours) to study prolonged biochemical interactions, that are not possible even with the longest half-life organic radionuclide ^{11}C, led to the use of other positron emitters, such as ^{18}F, ^{94m}Tc, $^{120,124}I$, ^{110}In, $^{66,68}Ga$ and $^{61,64}Cu$. These radionuclides show a wide range of

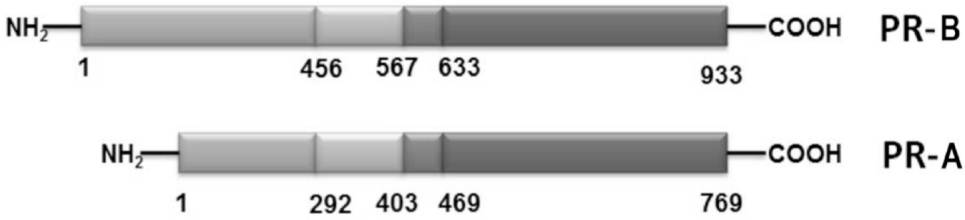


Fig. (3). Domain structures of human PR-A and PR-B isoforms.

half-lives and a spectrum of chemical and biochemical properties that justify their use as PET imaging agents. In addition to the practical benefits of medium half-life radiotracers, certain applications, particularly in oncology, require tracer kinetics to be followed for periods exceeding the limits of conventional short-lived PET radionuclides. PET facilities are expanding nowadays, and concern has been raised with respect to the cost effectiveness of the technique. To this end, the use of radiotracers based on medium half-life and generator-produced radionuclides enables positron emission tomography to be carried out even at centres remote from a cyclotron. The longer half-life of such radionuclides allows regional distribution of a number of radiotracers, and the goal of significantly reducing the cost of PET can finally be achieved. Extensive literature concerning the radiosynthesis of diverse PET radiotracers has been reported in the past few years [40-43]. Therefore, only a brief outline regarding the most used PET radionuclides and the main aspects of PET radiochemistry will be addressed below.

Carbon-11 can be used to label molecules without introducing foreign atoms, making their structure closer, if not identical to endogenous ligands. Its relatively short half-life presents a challenge to its widespread use, particularly in the labelling of complex molecules. Rapid decrease of radioactivity can be an attractive property in a radiopharmaceutical, especially when PET scans have to be repeated within short periods of time and there is concern about patient radiation exposure. Otherwise, the time limitations imposed by the short half-life of carbon-11 are a matter of concern.

The radiohalogens are particularly attractive as radiolabels for PET radiophamaceuticals. While positron-emitting chlorine has not been utilized, radioiodines, radiobromines and radiofluorine have great importance in PET (Table **1**).

The chemistry of radioiodine and radiobromine has some common features, whereas the chemistry of radiofluorine is sufficiently unique to deserve extensive discussion [40, 106-108]. Several positron-emitting radiobromines and radioiodines are not included in (Table **1**), as there is little literature regarding their routine production and use in PET imaging. ^{75}Br [109, 110] and ^{122}I [111, 112] will be not discussed further in this review since ^{75}Br production and purification are complicated procedures and the need of a high energy cyclotron to produce ^{122}I has limited its application.

In spite of its adequate nuclear properties, the positron emitter ^{124}I has not yet been extensively explored in PET imaging, because of a complex decay scheme that includes several high energy gamma rays, and will not be discussed further in this review. However, the favorable 4.2 day half-life combined with its ease of production in cyclotrons makes ^{124}I a promising radiohalogen for the development of novel probes for molecular imaging [113].

3.2. Radiochemistry

One of the main challenges of PET for chemists is the development of rapid synthetic methods for introducing the short-lived positron-emitting isotopes into the molecule of interest. The labelled probe has to be synthesized, purified, analyzed, and formulated usually within a few half-lives to ensure that there is enough radiolabelled material to administer to a subject undergoing the PET scan. Ideally, the synthesis and purification period should not exceed 2 to 3 times the physical half-life of the radionuclide in use, and the radiolabelling strategies should be designed in a way that the label would be introduced in the last steps of the synthetic sequence. The extremely short half-lives of the radionuclides demand that the labelled probes are prepared close to radionuclide production facilities and used almost immediately after their synthesis. A number of modern PET facilities hold cyclotrons, radiosynthetic laboratories, and PET scanners to allow efficient production and transport of short-lived PET probes from the laboratory to the scanner. All PET radiopharmaceuticals, whether for human or animal use, should have a high radiochemical purity (>95%), which is frequently achieved by high pressure liquid chromatography or solid phase extraction. Since inhalation or more commonly intravenous injection is required to administer PET radiopharmaceuticals, it is essential to check for sterility, apyrogenicity and all other quality control parameters that may have an adverse effect on an animal or a human subject under study.

3.2.1. Labelling with Fluorine-18

Fluorine-18 is the most commonly used positron-emitting radionuclide for the development of PET imaging agents. It presents favourable physical properties, including a half-life of 110 min, high positron decay (97%) and low positron energy (0.635 MeV). The adequate half-life makes it convenient not only for chemical manipulations, but also allows time for biological distribution of agents, while retaining sufficient activity for imaging. Since its atomic size is close to that of hydrogen, fluorine can substitute hydrogen in many molecular structures without introducing changes in activity and in some cases even with an increase in the desired biological activity.

The chemical methods by which ^{18}F can be introduced into target molecules are rather limited. The main synthetic strategies behind ^{18}F labelling can be roughly divided into direct fluorination, where ^{18}F is introduced in one step "directly" into the target molecule of interest, and indirect fluorination, which exploits the so-called ^{18}F prosthetic group approach, requiring a multistep synthetic strategy. These prosthetic groups are used to react with more complex biological molecules, which may not be suitable or stable enough to tolerate direct fluorination. Direct ^{18}F-labelling can be

Table 1. Cyclotron-Produced PET Radiohalogens

Radionuclide	Half-life	E_β max (MeV)	Mode of Decay (%)	Production Reaction
^{18}F	109.8 min	0.635	β^+ (97)	^{18}O(p,n)^{18}F
			EC (3)	^{20}Ne(d,α)^{18}F
^{76}Br	16.1 h	3.98	β^+ (57)	^{75}As(3He,2n)^{76}Br
			EC (43)	^{76}Se(p,n)^{76}Br
^{75}Br	98 min	1.74	β^+ (76)	^{75}As(3He,3n)^{75}Br
			EC (24)	^{78}Kr(p,α)^{75}Br
^{124}I	4.2 days	2.13	β^+ (25)	^{124}Te(p,n)/^{124}I
			EC (75)	^{124}Te(d,2n)^{124}I
^{122}I	3.6 min	3.12	β^+ (77)	^{127}I(p,6n)$_{122}$Xe/^{122}I
			EC (23)	

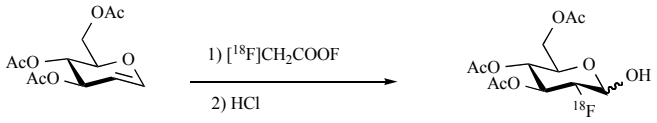
EC: Electron capture.

achieved via either electrophilic or nucleophilic radiofluorination [42, 43].

3.2.1.1. Electrophilic Radiofluorination

Electrophilic fluorinations use ^{18}F-labelled fluorine ($[^{18}F]F_2$) and other less reactive derivatives such as [^{18}F]acetyl hypofluorite [40]. $[^{18}F]F_2$ gas can be generated by bombardment of neon with deuterons, however, $[^{18}F]F_2$ is currently produced via the ^{18}O(p,n) process using [^{18}O]water.

Nowadays, electrophilic ^{18}F fluorinations are less favoured since they generally give labelled products with low specific activity due to the carrier-added method of $[^{18}F]F_2$ production. Moreover, the highly reactive $[^{18}F]F_2$ usually reacts unspecifically, leading to mixtures of ^{18}F-labelled products. These major limitations can restrict its application in the synthesis of PET imaging agents. However, electrophilic fluorination has played an important and historic role in the development of ^{18}F-labelled molecules for PET imaging. Thus, the first synthesis of 2-[^{18}F]fluoro-2-deoxy-D-glucose **2** ([^{18}F]FDG) was carried out by electrophilic fluorination of glycal **1** using [^{18}F]acetyl hypofluorite (Scheme **1**) [114]. PET cancer imaging routinely employ this radiolabelled glucose analogue since nearly all malignant tumours exhibit increased [^{18}F]FDG uptake, reflecting an enhanced rate of glycolysis in tumour tissue [115]. [^{18}F]FDG-PET is a reliable means for distinguishing many benign from malignant tumours.



1 **[^{18}F]FDG (2)**

Scheme 1. Radiosynthesis of [^{18}F]FDG as a typical example of electrophilic ^{18}F fluorination [114].

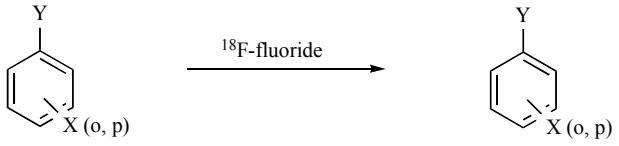
3.2.1.2. Nucleophilic Radiofluorination

Nucleophilic ^{18}F-fluorination reactions are routinely used to efficiently produce some of the most important ^{18}F PET radiotracers because of their greater selectivity and capability to give highly specific radioactive compounds. The [^{18}F]fluoride ion is generally produced by irradiation of enriched [^{18}O]water and is obtained in aqueous solution [41, 116]. Fluoride ion is a poor nucleophile in aqueous medium. Consequently, reactions involving nca (no-carrier-added) [^{18}F]fluoride ions require strict exclusion of water, which is removed by azeotropic distillation with acetonitrile at high temperatures under a stream of nitrogen. Further activation of the [^{18}F]fluoride ion is achieved through the use of cryptands in combination with alkali (K, Cs, Rb) or tetra-*n*-butylammonium cations. The aminopolyether Kryptofix 2.2.2 (K_{222}) complex is the most commonly used cryptand [117, 118]. Nucleophilic substitution reactions are performed mainly in dipolar aprotic solvents. For aliphatic systems the reaction mechanism proceeds via an S_N2 mechanism, and most often precursors bearing bromo, iodo, triflate, tosylate, nosylate, and sulfonate functionalities as leaving groups are employed. This type of radiofluorination has been the most common method to prepare radiofluorinated steroids. An example is the radiosynthesis of 16α-[^{18}F]fluoroestradiol **4** ([^{18}F]FES) starting from the corresponding triflate precursor **3** (Scheme **2**) [119].

3 **[^{18}F]FES (4)**

Scheme 2. Radiosynthesis of [^{18}F]FES as a typical example of nucleophilic ^{18}F fluorination [119].

Aromatic nca ^{18}F nucleophilic substitutions are only viable with activated aromatic systems. Strong electron-withdrawing substituents such as CN, CHO, NO_2, COOR, and RCO in *ortho* or *para* position are suitable for activation, and the leaving groups are usually the nitro or the trimethylammonium group (Scheme **3**) [120, 121]. Nca nucleophilic substitution reactions involving heterocyclic aromatic systems such as the electron-poor pyridine series, however, do not necessarily require activating groups [122-125].

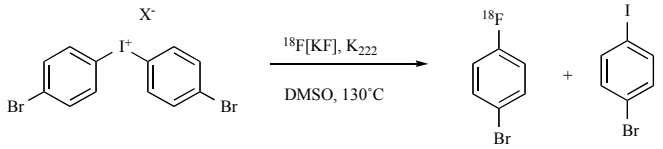


X (activating groups) = NO_2, CN, CHO, COR, COOR

Y (Leaving groups) = NO_2, $(CH_3)_3N^+$

Scheme 3. Aromatic nca ^{18}F nucleophilic substitutions: examples of the most generally used activating and leaving groups [120, 121].

More recently, efforts have been directed towards the use of diaryliodonium salts, which allows the fluorination of both electron-deficient and electron-rich arenes [42, 126]. The diaryliodonium salts are easily prepared by reaction of Koser's reagent, hydroxy (tosyloxy)-iodobenzene, with either aryltrialkylsilanes, aryl boronic acids or organostannanes [42, 127, 128]. These diaryliodonium salts are then reacted with [^{18}F]fluoride ion under standard conditions (Scheme **4**) [129]. Encouraging results have been achieved for simple molecules using these precursors, but the aromatic nca nucleophilic substitution of more complex molecules still remains a challenge [130].



X = Br, OTf, OTs

Scheme 4. One-step synthesis of 1-bromo-4-[^{18}F]fluorobenzene as a typical example of the use of diaryliodonium salts [129].

In most cases, more complex biological molecules such as oligonucleotides, peptides, and antibodies are not amenable to direct fluorination with nca ^{18}F-fluoride ion due to denaturation of the sensitive organic substrates or the presence of reactive functional groups in the substrates [131]. Thus, radiofluorination methods employing prosthetic groups chemoselectively coupled to the biomolecules have also been developed Fig. (**5**). [132-141]. Usually, these prosthetic groups are small ^{18}F-labelled alkyl or aryl groups bearing reactive functionalities, which are incorporated into the biomolecules via alkylation, acylation, amidation, or photochemical reactions [142, 143].

In the past few years, "click chemistry" has also been applied in PET chemistry to synthesise ^{18}F-labelled tracers. The application of this methodology is still in its early stages of development, but shows great promise for preparing ^{18}F-labelled peptides (Scheme **5**) [144].

3.2.2. Labelling with Bromine-76

Bromine-76 decays with a significant amount of positron emission (Table **1**), a characteristic that allows for diagnostic PET imaging in adition to radiotherapy. It can be produced conveniently via the ^{76}Se(p,n)^{76}Br reaction in the majority of medical cyclotrons [145]. ^{76}Br has a half life of 16.2 hours, which is long enough to

permit target tissue-selective distribution, while being sufficiently short, so that the bulk of the dose can be delivered to the tissue prior to metabolism and elimination of the radiopharmaceutical. Because the natural abundance of bromine is low, organic compounds can be labelled with very high specific activity, which is a general requirement for the study of receptor-binding related biological processes.

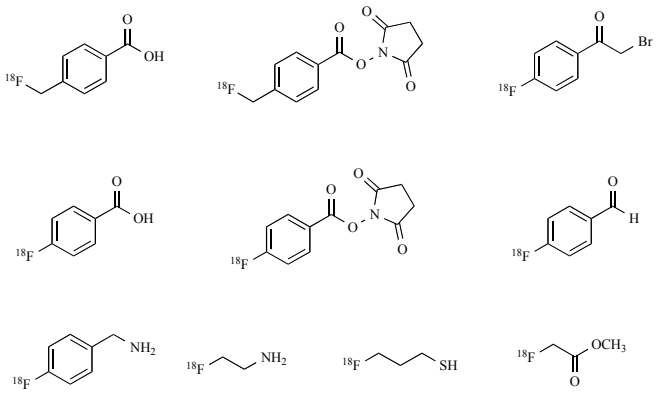
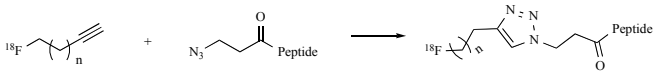
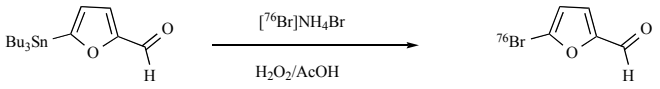


Fig. (5). Examples of some prosthetic groups frequently used for radiofluorination of biomolecules [132-141].



Scheme 5. Labelling of peptides using 1,3-dipolar cycloaddition as a typical example of "click chemistry" [144].

As with most halogen chemistry, [^{76}Br]bromide can be used either as a source of nucleophilic bromide or as a source of electrophilic bromine upon oxidation. The radiochemistry of bromine, analogous to that of iodine, relies essentially on oxidative electrophilic substitution to afford a relatively stable radiolabel. The radiobromination of small molecules normally involves the oxidative substitution of a tri-alkyltin leaving group, using various oxidizing agents (Scheme **6**) [146]. Large molecular weight proteins, such as antibodies, can be labelled directly at their tyrosine residues or through a prosthetic group that reacts with the amino function of lysine residues. The direct labelling method involves either enzymatic oxidation [147] or chemical oxidizing agents such as chloramine-T [148]. Prosthetic methods using N-succinimidyl-3-(4-hydroxyphenyl) propionate [149], N-succinimidyl-4-(tri-n-butyl stannyl)benzoate [40] or N-succinimidyl-4-(tri-methylstannyl) benzoate [150] have also been reported.

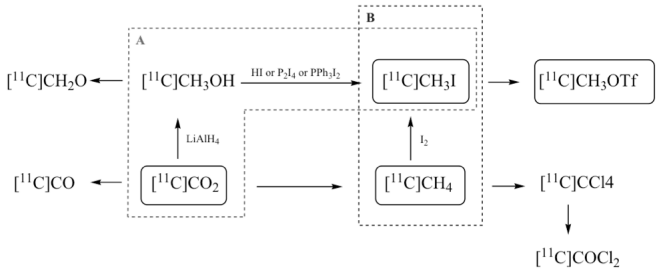


Scheme 6. Typical example of ^{76}Br bromination via an electrophilic destannylation reaction [151].

3.2.3. Labelling with Carbon-11

As carbon is one of the main constituents of biomolecules isotopic labelling with ^{11}C will result in radiotracers indistinguishable from their non-labelled counterparts, with the exception of very small kinetic isotope effects. This is particularly important since the introduction of an "artificial" PET tag (such as ^{18}F or others) may change the biological properties of the compound of interest as they have different chemical structures.

The production of ^{11}C is commonly carried out through the ^{14}N(p,α)^{11}C nuclear reaction. Despite the short half-life of carbon-11 ($t_{1/2}$ = 20.4 min), this radionuclide has been successfully applied to the development of ^{11}C labelled biomolecules [42, 152, 153]. The synthesis involving ^{11}C starts with [^{11}C]CO_2 or [^{11}C]CH_4 as the primary product. These two radiochemical precursors are the base for the production of secondary ^{11}C-labelling reagents such as [^{11}C]methyl triflate [154] or [^{11}C]methyl iodide [155, 156], generally used as alkylating agents for the radiolabelling of biomolecules (Scheme **7**). [^{11}C]Methyl iodide can be produced by two distinct methods, referred to as "wet" or "gas phase" chemistry routes. In the "wet" chemistry route, the [^{11}C]CO_2 is reduced to [^{11}C] methanol, which is then converted to [^{11}C]methyl iodide (Scheme **7**, A) [157, 158]. The "gas phase" chemistry route involves the conversion of [^{11}C]methane into [^{11}C]methyl iodide by radical iodination with elemental iodine (Scheme **7**, B) [159].



Scheme 7. Schematic representation of ^{11}C-labelled primary products and most common secondary ^{11}C-labelled precursors.

Due to its short half-life, ^{11}C should be incorporated in the last step of the synthetic route to a radiopharmaceutical. Obviously, the introduction of the radionuclide at the final synthetic step requires as well that the reaction proceeds quickly and, as a general rule, that the synthesis and purification should not take more than one half-life, since long-lasting syntheses not only reduce the radiochemical yield, but also decrease the specific activity of the final product. Even with specific activities as high as 5000 Ci/mmole, most of the mass of the carbon-11 radiotracer is from the carrier carbon (CO_2) and as a consequence, every half-life reduces the specific activity of the radiotracer 2-fold [106].

4. BIOLOGICAL EVALUATION OF ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION

Estrogen and progesterone receptors provide a system with favourable binding characteristics for the uptake and selective retention of imaging agents [22, 23]. Radiolabelled estrogens and progestins with appropriate biological properties have been shown to be taken up by target sites with high efficiency and great selectivity [30, 32, 35, 160-162]. An effective breast tumour-imaging ligand should present high specific activity, high binding selectivity, which is associated to the ligand's ability to differentiate the target receptor from other receptors and non-specific proteins, and also an adequate metabolic and pharmacokinetic profile [23, 163].

Several good reviews have appeared in the literature that highlight the application of ER and PR imaging agents for breast cancer detection and management by SPECT and PET modalities [29, 32, 33, 161, 164]. Hence, only a short overview concerning structure affinity relationship (SAR) studies will be presented below.

4.1. Structure Affinity Relationship Studies

The biological response of a cell to a steroid hormone reflects the binding of the steroid to the receptor, since without binding there will be no effect on the cell. Also, it is recognized that the *in vitro* receptor affinity of a steroid is well correlated with its *in vivo* biological behaviour. Nevertheless, receptor binding affinity (RBA) is a function of many experimental parameters (*e.g.* temperature and equilibrium time). Other factors such as animal species and different sources of receptor (tissue minces and cytosol homogenate preparations) used in the assays can affect the values of relative

affinities. Beyond the PR and ER ligands' non-specific binding to albumin, estrogens also bind with very high affinity to the sex human binding globulin (SHBG). The binding specificity for these plasmatic proteins is, however, quite different from that of the receptor, so that it is possible to design receptor ligands with high affinity for the specific receptor and low affinity for the serum binders. It is also important that the eventual metabolization of the receptor ligand should not lead to the accumulation of circulating labelled metabolites that enter tissues but no longer bind to the receptor.

There is a significant structural diversity among the steroids that bind to the ER and PR. Generally, the selection of the substituent groups and their site of attachment to the steroid molecule, have been based on substitutions that are known to increase the *in vivo* biological activity or to improve the uptake selectivity either by enhancing receptor binding, reducing non-specific or serum binding, or by altering the route of metabolism or reducing its rate.

The introduction of a *substituent* in the ligand's molecule can affect the binding to the receptor in two different ways. The substituent can alter the binding by favourable or unfavourable interaction with the receptor and/or alter the binding by a conformational effect, *i.e.* by changing the overall shape of the ligand and accordingly, the positions of the other groups in the molecule relative to the receptor [165]. The position of a substituent in the steroid molecule will determine the extent to which it will interact directly with the receptor and/or modify the shape of the steroid [166]. These two substituent effects can deal in antagonism to each other: a group may induce favourable conformational changes in the molecule, while its nature (*e.g.* electronegativity, selective binding via hydrogen bonding, etc.) can negatively affect the binding affinity.

Although there is a considerable structural diversity among steroid derivatives that have affinity to estrogen and progesterone receptors, for the purpose of this review we have limited our analysis to relatively simple systems encompassing estradiol, progesterone and nor-progesterone skeletons, as most of the labelled tracers developed for ER and PR targeting belong to these series. Other ligand systems with affinity for PR, such as testosterone, nortestosterone and dihydrotestosterone rings, that also might create the basis of PET imaging agents, will not be considered.

A number of studies have demonstrated that estrogens with a variety of different structures, labelled with radioisotopes of fluorine and iodine, retain high ER binding affinity and show good target site-selective *in vivo* uptake [23, 167]. Several of these estrogens have proven to be effective in humans, where clear images of primary and metastatic ER positive tumours have been observed [36, 38, 168-172]. The situation with the PR-based agents, however, is less satisfactory. Although they have been neither as intensively studied as ER ligands nor for such a long time, some PR agents, that showed good results *in vitro* and *in vivo* in animal models [22, 173-176], have given disappointing results in humans [177, 178]. The reason for this divergence might be the low PR affinity of the ligands, together with their high metabolic lability and high lipophilicity, which results in high non receptor binding.

4.1.1. Influence of Structural Substitutions in Estradiol on ER Binding

Estradiol is a non-polar and hydrophobic molecule, except at its molecular termini. Hydroxyl groups at C-3 and C-17 of the estradiol molecule are necessary for effective binding to the estrogen receptor [165, 179]. The hydrocarbon structure that separates the two oxygen functions confers an appropriate configuration to these functionalities, allowing for their interaction with the amino acid residues of the ER ligand-binding domain. The hydrocarbon structural framework itself also plays a very important role in the binding of the ligand to the receptor [179]. Modifications of the spatial configuration of the hydrocarbon structure of the ligands usually lead to different interactions resulting in a decreased ER binding affinity [165, 166]. The effects of diverse substituents on the binding of estradiol to the ER, obtained from *in vitro* competitive binding assays, have been reported extensively in the literature and are summarized below Fig. (**6**).

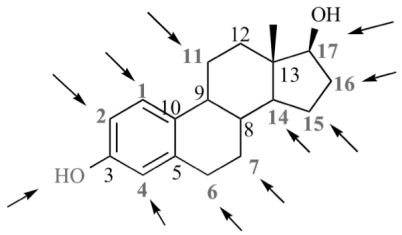


Fig. (6). Chemical structure of estradiol showing where different substituents or modifications have been introduced.

Studies of the effect of A-ring substituents on the ER binding affinity of estradiol suggests a negative influence of groups of any size at C-1 [179, 180]. Small groups are reasonably well tolerated at C-2 and C-4, whereas large groups and those that might involve an intramolecular hydrogen bond substantially reduce binding affinity [179, 181-186].

The ER is intolerant to both polar and non-polar groups at C-6 [179, 187]. Groups at the 7α-position are well tolerated, even if they are of considerable length [186, 188-192]. Polar functions at this position are not well tolerated unless they are positioned away from the core of the steroid structure [188, 190, 191, 193].

The 11β-position of estrogens is a site, where relatively large non-polar substituents, such as ethyl or chloromethyl groups, are well tolerated by the receptor [194-196]. By contrast, polar substituents at 11β-position not sufficiently far-away from the ligand core reduce receptor binding [195, 196]. While a 7α-methyl group is well tolerated by the estrogen receptor [197, 198] the combined effect of both 7α-methyl and 11β-methoxy substituents leads to compounds that have lost virtually all their ER affinity [197].

A 17α-ethynyl substituent enhances receptor-binding affinity and would appear to act in synergy with certain other substituents, such as the 11β-methoxy or 11β-ethoxy group [199]. Substituents larger than the ethynyl group and/or the extension of the 17α-moiety to a substituent containing three or more carbons are usually poorly tolerated by the ER. However, despite the apparent steric intolerance at the 17α-position, several organometallic 17α-substituted derivatives have shown to retain considerable ER affinity suggesting the presence of a binding site in this region that can accept species of hydrophobic character [200-202]. Also, groups linked to an ethynyl or vinyl group are well tolerated. The 17α-iodoethynyl estradiol, despite of the larger volume of the iodine relative to a proton, shows quite favourable binding, probably due to the greater polarizability of the iodine [203, 204]. 17α-vinyl estradiols have also been investigated in considerable detail [205-210]. The presence of the iodovinyl group does not significantly alter the ER binding affinity [211-213]. Nevertheless, with iodovinylestradiols, the binding behaviour is strongly dependent on the stereochemistry of the vinyl group. Preliminary binding assay studies from Ali *et al.* with (17α,20E/*Z*)-iodovinylestradiol have shown that the *Z* isomer possesses a slightly higher ER binding affinity than the *E* isomer [205]. Further work on the effect of stereochemistry at C20, in the series of 17α-halovinyl estradiols, has shown a marked difference between the *E* and Z isomer. Thus, the *Z*-isomer showed much higher binding affinity than the *E* isomers, with bulky substituents enhancing affinity at this position. The opposite pattern was observed in the *E* series [206]. The behaviour of the related phenyl, phenylthio, and phenylseleno vinyl estradiols has been shown to follow the same pattern suggesting that the orientation of the substituent on the vinyl group towards the receptor differs considerably depending on the *E*- and *Z*-configuration of the double

bond [208, 214]. The lower affinity of the 17α-*E*-vinyl isomers has been explained as being attributable to their structural similarity to the 17α-ethynyl series that is sensitive to small changes in molecular volume [206]. For the *E* isomers and 17α-iodoethynyl derivatives the iodo-group is oriented in a similar way, outward from below the α-face of the steroid and qualitatively similar binding affinities are observed. The *Z* isomers have the substituent directed towards a different portion of the receptor, inward toward the α-face, and this could explain the observed different values for the binding affinity, suggesting an additional hydrophobic pocket that can accommodate small to moderately sized lipophilic moieties in this region. The ER is moderately intolerant of polarity in D-ring area, except for the specific 17α-position. Small, non-polar substituents are well tolerated at the 16α-position, whereas large substituents, especially those with increased polarity, result in poor affinity [215-217]. In contrast, the 16β-position is sterically less permissive [183, 218]. The 14- and the 15-position can accept small, non-polar groups without appreciable loss of binding affinity [219].

Correlated quantitative structure-activity relationships (QSAR) could also be inferred for several halogenated estradiol derivatives from comparative molecular field analyses (CoMFA) of their interaction with the ER [220].

4.1.2. Influence of Progesterone Structural Substitutions on PR Binding

Binding studies with progesterone derivatives have shown that hydrophobic interactions between the lipophilic steroid and the PR contribute for the major part of the binding energy. A minor contribution comes from hydrogen bond formation between the receptor and the 3- and 20-carbonyl groups. Unexpectedly, the 19-nor derivative of progesterone shows a stronger PR affinity than progesterone itself [221-223]. The effects of various substituents of the progesterone skeleton on the binding to its receptor have been described extensively in the literature and are summarized below Fig. (**7**).

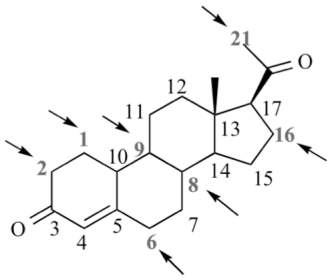


Fig. (7). Chemical structure of progesterone showing where different substituents or modifications have been introduced.

A 3-keto-4-ene moiety within the molecule is necessary, though not sufficient, for high PR binding. Testosterone, for example, binds poorly to PR [224]. All the structural modifications that enhance binding are found in the B, C and D rings of the steroid suggesting that these regions can accommodate structural substituents more readily than the A-ring. Data from the literature suggest that interactions between receptor and ligand are mainly hydrophobic and thus far, at no position in progesterone has a hydroxyl group substitution been noted to increase binding [221, 225, 226]. Moreover, acylation of a hydroxyl group always enhances binding, relative to the hydroxy parent [225, 227]. Hydrophilic interactions such as hydrogen bonding occur at C-3 and C-20, also in the analogues of progesterone. The conformational effects of certain substituents, as well as their direct polar or hydrophobic interactions with PR, can be used to impart possible changes in binding affinity. The introduction of a double bond at C-1 in progesterone causes a decrease in binding affinity [228]. A substituent at C-2 (*e. g* methyl) also lowers the binding affinity [222]. The effect of an alteration at C-6, such as the introduction of a substituent or a double bond, seems to depend basically on the parent steroid to which the derivative is compared [229]. However, such kind of alteration in the progesterone scaffold moderately decreases its binding affinity [230]. As with modifications at C-6, the introduction of a double bond at C9 has a varying effect on the binding affinity of the molecules [222]. Modification of the B/C ring junction by a C8-C9 double bond moderately decreases the affinity for the receptor. This modification flattens the molecule, introduces some strain into the C ring, and reorients the axial hydrogen on carbon 11 to a pseudoaxial conformation. However, double bonds between C9 and C11 or between C11 and C12 have little effect on the affinity of the steroid. This relationship is interesting since 9(11)-dehydro steroids have decreased biological activity, whereas 11-dehydro progesterone is quite potent. Large, bulky groups at various positions in the C and D ring, such as an ethyl group at C-13 or alkyl groups at C-16α, C-17α or C-21, often enhance binding. This has been explained to result from direct hydrophobic interaction between the substituents and the protein surface [221, 230] or, alternatively, from the introduction (by the large substituents) of molecular strain that is transmitted to the flexible A-ring. Possibly the enhanced binding is due to a synergy between these two factors. Removal of the C-19 methyl group, as in the case of 19-norprogesterone itself, leads to an enhanced affinity for PR. The steric bulk of the angular methyl-group is thought to be detrimental to the binding of the ligand [222, 231].

Taking together all available data from the literature, it seems that the possibilities of developing progesterone derivatives with increased PR affinity are limited. This is also illustrated by the two steroids Org2058 and R5020 (promegestone), currently used for PR radioimmunoassay (see Fig. **17**); their affinity for PR is only 5 to 15 times higher than that of progesterone itself [175, 232-235].

5. PRE-CLINICAL AND CLINICAL STUDIES OF RADIOLIGANDS FOR STEROID RECEPTOR PET IMAGING

The growth of breast epithelial cells is a process mediated by estrogens, which manifest their biological activity through a specific high binding affinity to the estrogen receptor, and this action results in the induction of progesterone receptors, since in target tissues the synthesis of the progesterone-receptor is an estrogen controlled process [18, 236]. Research on specific tumour receptor imaging has mainly been directed to the measurement of the estrogen receptor status, since breast cancer patients in whom these receptors are present, may benefit from hormonal therapy. In breast cancer tumours patients on hormonal therapy, the imaging of progesterone receptor-rich tumours with a progestin-based imaging agent may be better suited, since the response to endocrine therapy can be more accurately predicted after quantification of both estrogen and progesterone receptor status [19, 237]. Estrogen receptor ligands are useful tools for imaging prior to hormonal therapy. However, during antiestrogen hormonal therapy, such as with tamoxifen, the circulating levels of the antiestrogen and its metabolites are sufficiently high to fully occupy the estrogen receptor sites resulting in decreased uptake of the estrogen-based radioligand. In contrast, progesterone receptors in tumours of patients on tamoxifen regimen should be unoccupied, and their levels might even be increased by the antiestrogen therapy [238-240]. Thus, to evaluate the tumour response to endocrine therapy based on changing tumour size or receptor content, a radiolabelled progestin analogue would permit imaging of progesterone receptor rich tumours, while the antiestrogen therapy is carried out at the same time. Therefore, the tumour size and the number and density of progesterone receptors could be compared over the course of treatment.

Generally, a ligand with affinity to either ER or PR might be used for breast tumour imaging and therapy of a previously un-

treated patient. However, a PR-based radioligand has some potential advantages over an ER-based one, since there is a better correlation between PR status and hormonal responsiveness than there is with ER status [241-244]. Moreover, a PR-based ligand could be used after the initiation of anti-estrogen hormonal therapy, whereas an ER based ligand would not be useful, when the tumour ER is saturated by the therapeutic hormonal agent [245]. Additionally, PR-based ligands may benefit from the increased PR levels induced by a transient agonistic effect of tamoxifen during the initial course of tamoxifen-based treatment of breast tumours [151, 238-240].

Next, the reported pre-clinical/clinical data for the most relevant estradiol- and progesterone-based radioligands will be provided.

5.1. Estrogen Receptor Ligands

5.1.1. Fluorine-18 Ligands

16α-[^{18}F]Fluoroestradiol ([^{18}F]FES)

In the1980´s, Kiesewetter *et al.* [119] described the synthesis and evaluation of [^{18}F]FES **4**, together with other fluorine-18 labelled estrogens (16β-[^{18}F]fluoroestradiol **7**, 1-[^{18}F]fluoropentestrol **8** and 1-[^{18}F]fluorohexestrol **9** Fig. (**8**). The four ^{18}F-labelled estrogens were prepared with high chemical and radiochemical purity and adequate specific activity by a fluoride-displacement reaction on triflate precursors as depicted in (Scheme **2**).

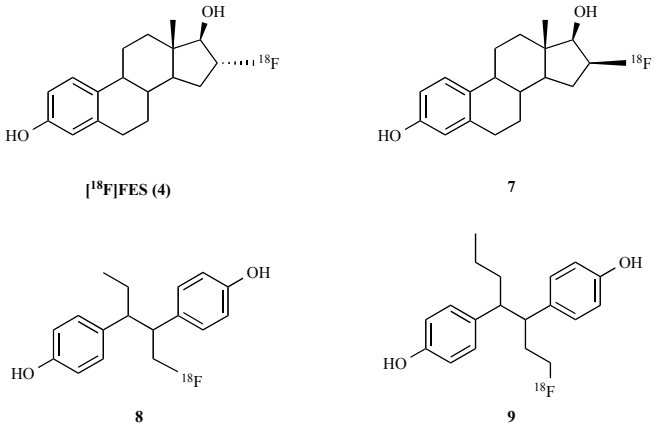
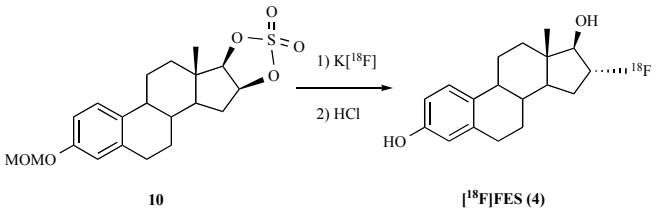


Fig. (8). Chemical structures of [^{18}F]FES, 16β-[^{18}F]fluoro estradiol, 1-[^{18}F]fluoropentestrol and 1-[^{18}F]fluorohexestrol [119].

All four fluoroestrogens displayed high ER binding affinities (80%, 30%, 129% and 127%, respectively, relative to estradiol=100%). In biodistribution studies, in immature female rats, all compounds exhibited selective uterus uptake, which was blocked by co-administration of unlabelled estradiol indicating a receptor mediated mechanism. [^{18}F]FES exhibited the highest uptake selectivity, with uterus/blood and uterus/non-target tissues ratios of 83 and 45, respectively, at 2hr [119].

Since then, [^{18}F]FES has been studied extensively as a potential radiolabelled probe for breast cancer imaging. The radiosynthesis of [^{18}F]FES was improved further by using the more reactive cyclic sulfate **10** as a precursor (Scheme **8**) [246]. An automated radiosynthesis, developed by Romer *et al.* [247], is currently used as a routine procedure.

Further *in vivo* studies from other groups, in rat and mouse models, confirmed the high uptake of [^{18}F]FES in tissues with ER expression, such as the uterus and ovaries - [248-250]. Studies conducted by Yoo *et al.* [251], in ERα and ERβ knock-out mice, showed that [^{18}F]FES is a subtype selective tracer binding preferentially to ERα. As far as we know efforts to develop an ERβ selective tracer have been unsuccessful to-date.



Scheme 8. Radiosynthesis of [^{18}F]FES using a cyclic sulfate precursor, 3-*O*-methoxymethyl-16,17-O-sulfuryl-16-epiestriol **10** [247].

The possibility of quantitation of ER binding parameters *in vivo* with [^{18}F]FES-PET using both equilibrium and dynamic kinetic analyses was demonstrated in rats by Moresco *et al.* [252]. However, in ER-rich tissues such as the uterus and ovaries, [^{18}F]FES uptake is limited by blood flow and tissue permeability and may not exactly reflect the receptor expression [253]. In human breast tumours, receptor concentrations appear to be in the range 0.3-3 pmol/g tissue, so that here the flow limitation should not be encountered, and [^{18}F]FES uptake by primary tumours seems to parallel their receptor distribution, allowing to quantify ER density [168]. Actually, a good correlation was found between ER expression, as determined *in vitro* by immunochemistry, and [^{18}F]FES uptake in different animal models of estrogen positive tumours [250]. In addition to receptor density, [^{18}F]FES-PET also allows to determine the receptor occupancy of a drug. *In vivo* optimization of tamoxifen dosing schedule was achieved, in animal model, by an *in vivo* titration method as described by Katzenellenbogen *et al.* [253].

[^{18}F]FES-PET has also been applied successfully to determine ER status of human breast tumours and the first clinical study with [^{18}F]FES in 13 patients with primary breast cancer was reported in 1988, by Mintun *et al.* [168]. PET imaging demonstrated [^{18}F]FES uptake at sites of primary carcinomas and in several foci of axillary lymph node metastases, as well as in one distant metastatic site. An excellent correlation of 0.96 between uptake within the primary tumour, measured by PET imaging, and the tumour ER status, measured *in vitro* in biopsy specimens, was found in 9 patients. These results were supported further in a different PET study by the same authors [170]. In a series of 16 patients with metastatic breast cancer, in which treatment with antiestrogen drugs was planned, [^{18}F]FES accumulation was observed in 14 patients and in 93% (53/57) of the known metastatic lesions. In 7 of the patients, PET imaging was performed both before and after initiation of antiestrogen therapy. In all cases, there was a decrease in [^{18}F]FES uptake after initiation of antiestrogen therapy. These results indicated that [^{18}F]FES accumulation within metastatic lesions of breast carcinoma was a receptor-mediated process that could be blocked by antiestrogen therapy. This study also demonstrated the high sensitivity and specificity of [^{18}F]FES for detecting breast carcinoma in nodal and distant metastases.

In a subsequent study, including in 53 patients with breast cancer, of whom 32 had a diagnosis of primary breast tumours and 31 had metastatic breast cancer or recurrent disease Dehdashti *et al.* [254] found an agreement of 88% (35/40) between the ER content determined by PET imaging and measured *in vitro* in biopsy samples reconfirming, in a larger series of patients, their earlier observation that [^{18}F]FES-PET reliably assesses the ER status of breast cancer lesions [168, 170]. In patients with advanced breast cancer prior to initiation of systemic therapy, the authors also compared tumour [^{18}F]FDG uptake and tumour ER status (assessed both by *in vitro* assay and by [^{18}F]FES-PET) to determine if [^{18}F]FDG uptake could be used as a surrogate marker of ER status. However, since information on the ER status is not obtainable with [^{18}F]FDG, no correlation was observed visually or quantitatively between [^{18}F]FDG and [^{18}F]FES tumour uptake.

To predict the response to systemic therapy, Mortimer *et al.* [255] correlated results of [^{18}F]FES uptake with ER status. This

study involved 43 women with locally advanced or metastatic breast cancer, who underwent [^{18}F]FES-PET prior to antiestrogen treatment. All the tumours were submitted for ER determination by quantitative measurement (20 patients) and by immunohistochemical assay (25 patients) The ER status was positive (ER+) in 21 patients, negative (ER-) in 20 patients, and unknown in 2 patients. All 20 ER- tumours on *in vitro* assays were [^{18}F]FES- as well. The tumours were considered sensitive to hormonal therapy, [^{18}F]FES positive ([^{18}F]FES+), when the standardized uptake value (SUV) of the lesion on [^{18}F]FES-PET was >1.0, and resistant to hormonal therapy, [^{18}F]FES negative ([^{18}F]FES-), when SUV was < 1.0. The information obtained by [^{18}F]FES-PET was compared to the results of the ER assays. Of the 21 ER+ tumours, 16 were [^{18}F]FES+ and 5 were [^{18}F]FES-. Thirteen patients with ER+ and [^{18}F]FES+ tumours initiated hormonal therapy and 61% (8/13) responded to the treatment. When compared with *in vitro* assays of the ER status [^{18}F]FES-PET demonstrated a sensitivity of 76% and a specificity of 100%. The sensitivity of [^{18}F]FES-PET was high in ER+ lesions, and the specificity was high in ER- patients. Although the number of patients evaluated in this study was small the authors hypothesised that [^{18}F]FES positivity might predict hormone responsiveness more accurately than do the results of *in vitro* ER assays.

The findings from Mortimer that [^{18}F]FES uptake might predict response of locally or advanced metastatic cancer to tamoxifen therapy were further confirmed in a different series of patients by Linden *et al.* [256]. In a study conducted on 46 patients with ER+ primary and recurrent breast cancer the authors showed that [^{18}F]FES uptake could predict response to hormonal therapy (mostly aromatase inhibitors) depending on the HER2/*neu* status of the tumors. [^{18}F]FES uptake was predictive of response in HER2/*neu*-negative patients, but in HER2/*neu*-positive patients the outcome was not predictable.

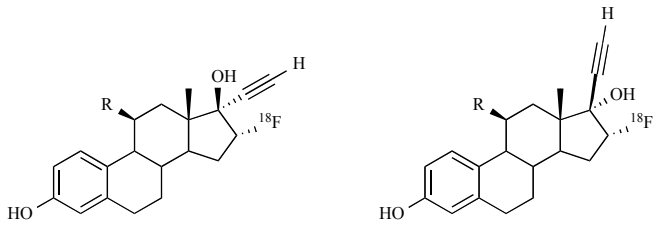
The radiation dosimetry of [^{18}F]FES was established by Mankoff *et al*. [257] in a PET study involving 49 patients. The effective dose equivalent was 0.022 mSv/MBq corresponding to 4.8mSv for a typical injected dose of 220 MBq. The radiation burden was within the normal range of other nuclear medicine procedures.

Mathias *et al.* [258] investigated clearance and metabolism of [^{18}F]FES in rats and found significant levels of protein binding and circulating labelled metabolites that do not bind to ER. Mankoff *et al.* [259] have extended these studies to patients, with the goal of optimizing the imaging protocol for detecting ER-containing breast lesions. [^{18}F]FES was rapidly cleared from the blood and metabolized (at 20 min only 20% of the circulating radioactivity was non metabolized [^{18}F]FES). The detectable metabolites in either blood or urine were sulfate and glucuronidate conjugates that were excreted through the kidneys at a rate comparable to their introduction into the circulation. After 20 min post injection the blood levels of radioactivity remained fairly constant. Since [^{18}F]FES clearance was rapid and metabolite background nearly constant, the authors suggested that a good visualization of ER-containing tissues could be achieved if imaging was performed at 20 to 30 min after injection.

Current data suggests that [^{18}F]FES is sensitive to image ER+ tumours, and complements the information given by [^{18}F]FDG to assess or predict the response of the cancer cells to therapy. [^{18}F]FES is currently in phase II of a study to predict response to first line hormone therapy in women with ER+ metastatic breast cancer [260]. Although the utility of [^{18}F]FES-PET in predicting therapy response still needs to be established, recent data suggests that a subset of patients that will not respond to endocrine therapy can be selected by [^{18}F]FES-PET.

16β-[^{18}F]Fluoromoxestrol (β[^{18}F]FMOX)

To improve the *in vivo* biological behaviour of [^{18}F]FES, Katzenellenbogen *et al.* [195, 261] have prepared a number of analogues bearing additional 11- and 17-substituents, namely 17α-ethynyl-FES **11** and 17β-ethynyl-FES **14**, and the 11β-methoxy-**12**/**15** and 11β-ethyl- **13**/**16** derivatives Fig. (**9**). The 11β- and 17α-substituents are known to increase the *in vivo* potency [194, 262-264] or to improve the *in vivo* uptake selectivity [265-268] by altering either the binding properties or the estrogen metabolism pathway. All members of this series were initially labelled with fluorine at the 16α position, since the 16α epimer has shown a higher ER affinity than the 16β epimer [119].



11: R = H
12: R = OCH_3
13: R = CH_2CH_3

14: R = H
15: R = OCH_3
16: R = CH_2CH_3

Fig. (9). Chemical structures of 16α-[^{18}F]fluoroestradiol derivatives [195, 269].

All of these 11β- and 17α-substituted 16α-[^{18}F]fluoroestradiols have shown a broad range of estrogen receptor and non-specific binding affinities, consistent with the nature of the substituents [195, 269]. The 11β-methoxy moiety lowered the ER binding affinity, which is consistent with former studies from Raynaud *et al.* [262], while the 11β-ethyl increased the affinity for the receptor. The 17α-ethynyl substituent also raised the binding affinity, but less markedly than the 11β-ethyl functionality. The 17-epimeric series with 17α-hydroxyl and 17β-ethynyl groups had significantly lower binding affinity. However, the 11β-ethyl moiety appeared to confer enough binding character to **16**, since it still showed a RBA value 1.5 times higher than that of estradiol (159%).

Selective uptake of all 17α-ethynyl compounds **11**-**13** and of 11β-ethyl-17β-ethynyl compound **16** was observed in target uterus and ovaries of female Sprague-Dawley immature rats. With the exception of the 11β-ethyl **16** the 17β-ethynyl-fluoroestradiols displayed little or no selective uterine uptake. The decreased target tissue uptake in animals treated with a blocking dose of estradiol indicated a receptor-mediated mechanism. The biodistribution of the four promising ethynyl compounds **11**-**13** and **16** was also compared with that of [^{18}F]FES. The uterine uptake was higher for all the new compounds with the exception of the 11β-ethyl-17β-ethynyl-derivative **16**.

The criteria, followed for the development of these new derivatives, has been to prepare a radiopharmaceutical with good contrast between target and non-target tissues, which has to be achieved by increasing the agent's receptor binding and simultaneously decreasing its non-specific binding [23]. However, these two parameters are often difficult to put together. For instance, the introduction of an 11β-ethyl substituent, as in **13** and **16**, will increase receptor binding, but being non-polar, will also increase the non-specific binding, although to a lesser extent. Conversely, a polar 11β-methoxy substituent, as in **12** and **15**, reduces non-specific binding considerably, though in this case the receptor binding also decreases.

Although target tissue uptake selectivity correlates directly with ER affinity and correlates inversely with the lipophilicity, as reported by Katzenellenbogen *et al.* [270], in this study this correlation was not found in all the cases [195, 269]. On the other hand, as demonstrated by Katzenellenbogen *et al.* [270], in previous biodistribution studies in immature rats involving *in vivo* titrations, the uptake of [^{18}F]FES by the target uterus may not be a good predictor

of uptake by other target tissues and tumours with low ER concentrations, since the uterine uptake is limited by blood flow and tissue permeability, and consequently might not reflect directly the compound's ER affinity, nor its potential for efficient and selective uptake by tissues, but instead could reflect the overall biological properties of the molecules, including their suitability to cross the biological barrier to reach the target organ [253]. Thus, seeking further optimization of the *in vivo* properties of [^{18}F]FES the same group [167] has extended its investigation to the 16β-fluoro estradiol series with substituents on the 11β-, on the 17α- and on both 11β- and 17α-positions of the 16β-fluoroestradiol framework (**17-20**, Fig. **10**).

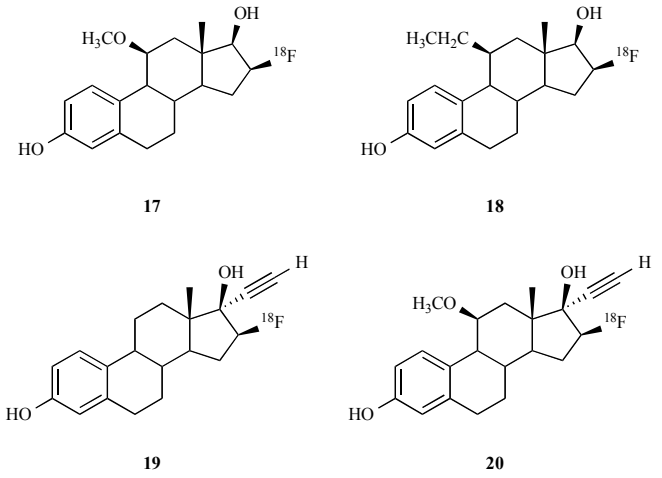


Fig. (10). Chemical structures of 16β-[^{18}F]fluoroestradiol derivatives [167].

In biodistribution studies in immature female rats, all the labelled analogues of the 16β-fluoro series demonstrated ER-selective uptake in the principal target tissues (uterus and ovaries) as well as in organs with low ER concentrations (kidney, thymus, fat and muscle). Although factors other than specific and non-specific binding obviously affect the tissue biodistribution of these 16β-[^{18}F]fluoroestradiols, in most cases a reasonable correlation between their ER selective uptake by both principal and secondary target tissues and their binding selectivity index (BSI) values was found [167]. The most promising compound appeared to be the 17α-ethynyl-11β-methoxy-16β-[^{18}F]fluoroestradiol **20** (16β-[^{18}F] fluoromoxestrol, β[^{18}F]FMOX) with a very good binding affinity for the ER (78%). The uterus uptake was nearly four times greater than that of [^{18}F]FES with higher uterus/blood and uterus/muscle ratios. The reason for this elevated uptake seemed to be its greater *in vivo* metabolic stability over [^{18}F]FES [167, 269]. Contrary to [^{18}F]FES, β[^{18}F]FMOX demonstrated considerable uptake in tissues with relatively low ER concentration, suggesting that it might be more effective in imaging human breast tumours with low ER concentrations.

Contrary to expectations that β[^{18}F]FMOX could be a potential imaging agent for human breast cancers due the improved *in vitro* characteristics and the very encouraging preliminary biodistribution and metabolic studies in animal model, human clinical PET imaging studies revealed its unsuitability as a human ER imaging agent. Actually, in a preliminary PET imaging study with β[^{18}F]FMOX involving 12 patients, 3 of whom had ER-positive breast cancer, no tumour localization by β[^{18}F]FMOX was found [271]. In fact, β[^{18}F]FMOX, which is a good ligand for ER but has a low affinity for the sex hormone binding globulin (SHBG), displays poor uptake in human ER-positive breast tumours when compared to [^{18}F]FES. While [^{18}F]-FES possesses a lower ER affinity, its affinity for SHBG is 260–fold greater than that of β[^{18}F]FMOX [167].

To better understand this unsuccessful trial, Jonson *et al*. [271] studied the rates of metabolism of [^{18}F]FES and β[^{18}F]FMOX in isolated hepatocytes of the rat, baboon, and human, and examined the effect that SHBG, which is present in humans and primates but not in rats, might have on these rates and possibly in the relative target tissue uptake of these two compounds.

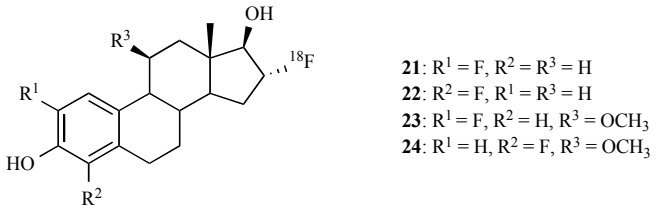
The trend seen in the metabolic rate differences of β[^{18}F]FMOX and [^{18}F]FES, between the different species, provided some explanation for the poor results of the clinical PET studies. In immature female rat hepatocytes, the animal model in which β[^{18}F]FMOX displays better target tissue uptake efficiency, [^{18}F]FES is metabolized 31 times faster than β[^{18}F]FMOX. However, in other the animal models, this differential in metabolism rate is not so large: Baboon and human hepatocytes metabolized [^{18}F]FES 2 times faster than β[^{18}F]FMOX. Thus, based on these hepatocyte studies, the authors have concluded that in human, where β[^{18}F]FMOX does not act as an ER imaging agent, its metabolic rate is not much different than that of [^{18}F]FES, whereas in rats, where its target tissue uptake is very high, β[^{18}F]FMOX metabolism is much slower than that of [^{18}F]FES.

Initial studies on factors affecting target site uptake of estrogens anticipated that binding to non-receptor binding proteins, such as to SHBG, would reduce target uptake and most of the steroid-based imaging agents have been designed in a way to fulfil this pattern [272]. However, subsequent findings have shown that actually SHBG facilitates the delivery of estrogens to ER-rich tissues, while protecting them from metabolism [273-275]. Also, an inverse relationship between a compound's affinity for SHBG and its rate of metabolic clearance has been observed, suggesting that a high binding affinity for this protein might in fact be desirable [276]. According to Jonson the very high target tissue uptake observed with β[^{18}F]FMOX in the rat, which lacks SHBG, is the result of its slower metabolism in this species. However, this differential is lost in primates and humans, where the presence of SHBG selectively protects [^{18}F]FES from metabolism and, presumably facilitates the delivery of this compound to target tissues in a more effective way [271].

4,16α-[^{18}F]Difluoro-11β-methoxyestradiol (4-F[^{18}F]MFES)

Introduction of fluorine at C-2 or C-4 of the estradiol A-ring decreases the rate of metabolism by blocking the formation of catechol estrogens, one of the major metabolic pathways of estradiol [277]. Seimbille *et al*. [249] hypothesized that adding a fluorine at either the 2- or 4-position of [^{18}F]FES should prolong its blood circulation time, and, consequently, improve its localization in ER-rich target tissues. Thus, a series of [^{18}F]FES derivatives, substituted with a fluorine atom at C2 or C4, with or without an 11β-OMe group (**21-24,** Fig. **11**) were prepared, and their binding affinities for the ER and for different serum proteins, including SHBG and rat alphafeto-protein (AFP), were evaluated. Labelling at the 16α-position was accomplished via nucleophilic substitution with [^{18}F]F$^-$ on the reactive 16β,17β-cyclic sulfate intermediates described for the preparation of [^{18}F]FES (Scheme **8**).

Substitutions with 4-F showed little effect on binding affinities. Addition of a 2-F decreased ER and AFP-binding affinities while augmenting the affinity for SHBG. 11β-OMe substitution decreased all binding affinities, particularly to AFP and to SHBG. In contrast, biodistribution of the corresponding 16α-[^{18}F]fluoro analogs in immature female rats revealed that the presence of the 11β-OMe group improved ER-mediated uterus uptake, with the 4,16α-[^{18}F]difluoro-11β-methoxyestradiol **24** (4-F[^{18}F]MFES) showing the highest uterus uptake and the highest uterus/blood and uterus/nontarget ratios at 1h post injection. These data suggested that the concomitant addition of 4-F and 11β-OMe group might improve the potential of [^{18}F]FES for ER imaging of breast tumours by PET [249].



21: R^1 = F, R^2 = R^3 = H
22: R^2 = F, R^1 = R^3 = H
23: R^1 = F, R^2 = H, R^3 = OCH_3
24: R^1 = H, R^2 = F, R^3 = OCH_3

Fig. (11). Chemical structures of 2/4-fluoro-16α-[^{18}F]fluoroestradiols derivatives [249].

Subsequent biodistribution studies conducted by Benard´s group with 4-F[^{18}F]MFES in ER+ tumour-bearing mice also demonstrated its high uptake by mammary tumours. Tumour uptake was blocked by co-injection with estradiol suggesting a receptor-mediated mechanism [278]. Due to the favourable biodistribution of 4-F[^{18}F]MFES observed in animal model and the higher target/nontarget uptake ratios achieved when compared to [^{18}F]FES [249, 278], the same group carried out subsequent biodistribution, dosimetry and safety studies in healthy women [38]. 4-F[^{18}F]MFES exhibited significant, ER-mediated, uterus uptake in pre- and post-menopausal patients This finding which is in agreement with small-animal experiments, further highlights the potential of 4-F[^{18}F] MFES for ER imaging in breast cancer patients. 4-F[^{18}F]MFES might be considered safe for human use and its effective dose, calculated at 4.2 mSv for a typical injected dose of 185 MBq, is within the acceptable radiation dose limits and should allow good image quality using standard whole-body PET acquisition protocols. The critical organ was the gallbladder (0.80 mGy/MBq), but the relatively high absorbed dose could potentially be reduced if administered to a non fasting patient.

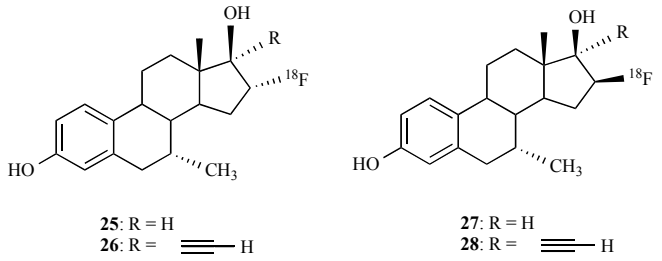
Preliminary data from a Phase II trial suggest that 4-F[^{18}F]MFES can successfully detect ER-positive breast cancer in patients, with higher tumor-to-background ratio relative to [^{18}F] FES-PET [279].

Other Fluorinated Ligands

To develop better PET scanning agents, also many other fluorine-18 labelled steroidal and non-steroidal molecules (*e.g.* ^{18}F-hexestrols, ^{18}F-fluorocyclofenils) have been synthesised for ER imaging [46-48].

It is well known from previous studies that a 7α-methyl substituent may increase the binding affinity of estradiol to the estrogen receptor [280]. Thus, to evaluate whether a 7α-methyl would improve the *in vitro* binding characteristics and the *in vivo* tissue distribution of [^{18}F]-labelled estrogens, VanBrocklin *et al.* [281] prepared four ^{18}F labelled analogues of 7α-methylestradiol, namely 7α-methyl-16α-[^{18}F]fluoro-17β-estradiol **25**, 7α-methyl-16β-[^{18}F] fluoro-17β-estradiol **26**, 7α-methyl-16α-[^{18}F]-17α-ethynyl-17β-estradiol **27** and 7α-methyl-16β-[^{18}F]-17α-ethynyl-17β-estradiol **28** Fig. (**12**). In this series, the 7α-methyl substituent did raise the ER binding affinity and also decreased non-specific binding considerably. The tissue biodistribution profile of these compounds was similar to that of the corresponding fluoroestradiols lacking the 7α-methyl substituent. However, the ethynyl analogues might even be more useful for imaging tissues or tumours with low receptor concentrations, due to the unusually high specific uptake observed in secondary target tissues.

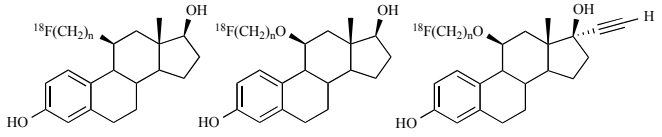
Based on previous investigations that demonstrated a marked influence of an 11β-ethyl substituent on estrogen receptor binding [195, 282, 283] Hanson *et al.* [285] prepared 11β-fluoroethylestradiol **29** Fig. (**13**), which has shown a much greater ER affinity than estradiol itself (1820% *vs* 100%). Furthermore, placement of the fluoro substituent on the 11β-ethyl moiety in the estradiol molecule avoids the moderate reduction of estrogen receptor binding observed, when the fluoro substituent is introduced in the 16α position.



25: R = H
26: R = ≡—H

27: R = H
28: R = ≡—H

Fig. (12). Chemical structures of 7α-methyl-16α/β-[^{18}F]fluoroestradiols and the corresponding ethynyl analogues [281].



29: n = 2
30: n = 3
31: n = 4

32: n = 2
33: n = 3

34

Fig. (13). Chemical structures of 11β-[^{18}F]fluoroalkylestradiol derivatives [249].

These encouraging results led French *et al.* [285] to prepare a number of [^{18}F]-labelled 11β-fluoroalkyl- and 11β-fluoroalkoxy substituted estradiols to investigate the effect of the nature of the steroid-radiolabel linkage at the substituent-tolerant 11β-position (by an alkyl or an ether function) on the biological behaviour of these compounds Fig. (**13**). The 11β-fluoroethyl **29**, 11β-fluoro propyl- **30** and 11β-fluorobutyl estradiol **31** analogues all possessed higher ER binding affinities than [^{18}F]FES itself, which reflects the tolerance of the 11β-position for bulky, lipophilic substituents. The more hydrophilic substituents, as present in 11β-fluoroethoxy **32** and in 11β-fluoropropoxy **33**, led to reduced receptor binding affinities when compared to the lipophilic derivatives. However, the combination of the 11β-fluoroethoxy with a 17α-ethynyl group as in **34** was well tolerated by the estrogen receptor. Tissue biodistribution data revealed that, apart from the 11β-fluoropropoxy derivative, all the compounds showed uterus selective uptake. This lack of selectivity could be explained by its rapid metabolism and low ER affinity. In general, the uterus uptake of the fluoroalkyl estrogens was greater than that of their fluoroalkoxy counterparts. Nevertheless, the inherent lipophilicity of the alkyl estradiols led to some undesirable uptake in non-target tissues as well as in adipose tissue. The level of bone uptake, as index of metabolic defluorination, varied greatly among the series, suggesting that structural and functional features appear to affect the rate and/or extent of metabolic defluorination. Taking together all available data from this study, it can be assumed that the nature and length of the linkage affects the uterus uptake and metabolic defluorination in a way that can be correlated with their ER binding affinity, lipophilicity and susceptibility to defluorination.

To improve the pharmacokinetics of fluorine-18 labelled estrogens, Hostetler *et al.* [136] synthesised 2-[^{18}F]fluoroestradiol, known to have a reasonable ER affinity and also to bind very well to SHBG. However, [^{18}F]F^- incorporation into the electron-rich phenolic A-ring of estradiol proceeded in low radiochemical yield. As far as we know, no further studies with this tracer have been reported to date.

The syntheses of three fluorine-18 labelled analogues of fulvestrant Fig. (**14**), a pure ER antagonist, have also been reported [286]. The labelling was performed at the 16-position of fulvestrant, in a similar manner as reported for the synthesis of [^{18}F]FES [146, 246]. Each derivative corresponded to a different oxidation state of the sulfur atom within the chain at the 7-position, including sulfox-

ide **35**, sulfone **36** or sulphide **37**, where the former was identical to fulvestrant. The potential of these new mimics of fulvestrant to serve as improved radiopharmaceuticals for PET imaging of ER+ target tissues and to predict the response of breast cancer to hormonal therapy was evaluated in an animal model. Although 16α-[^{18}F]fulvestrant and its sulphur and sulphide analogues were prepared in good yields and high specific activity, the addition of a 16α-fluorine to fulvestrant resulted in a dramatic decrease in the ER binding affinity (16α-fluoro-fulvestrant=1.01%, fulvestrant=89%, relative to estradiol) [287]. Changes in the oxidation state of the 7-side-chain sulphur atom of fulvestrant to give **36** and **37** did not significantly affect the apparent RBA of **35** for the ER (0.46 for **36** and 1.40 for **37**). Since these tracers likely bind to a specific, saturable and relative high-affinity binding site other than the classical ER binding site, these low binding values may in fact be misleading as to their potential affinity for the ER [288]. However, tissue biodistribution data of the three PET-mimics of fulvestrant in immature Sprague-Dawley female rats revealed low ER-rich target tissue uptake (1.83–3.37 %ID/g), *i.e*., only one-third or less of the uterine uptake previously observed with [^{18}F]FES, whereas the nonspecific uptake values were definitely higher than those of [^{18}F]FES.

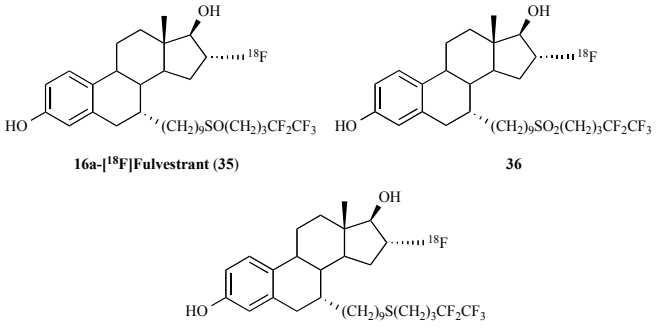


Fig. (14). Chemical structure of ^{18}F-fulvestrant and its sulphur and sulphide analogues [285, 287].

Also, non-steroidal antiestrogens have also been synthesised for ER imaging by means of PET. Thus, tamoxifen **38** Fig. (**15**), the most commonly used drug for endocrine therapy, has been radiofluorinated for ER imaging by PET [289, 290]. Inoue *et al.* [291] in a preliminary clinical trial within ER+ breast cancer patients, have found a good correlation (>71.4%) between tumour imaging with [^{18}F]fluorotamoxifen **39** and tumour response to antiestrogen therapy with tamoxifen. Therefore, this tracer has proven to be a valuable tool through PET imaging to predict the responsiveness of ER+ breast tumours to tamoxifen therapy. However, further studies are still needed to establish its clinical usefulness.

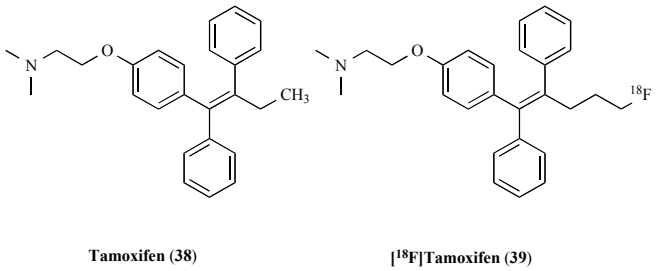


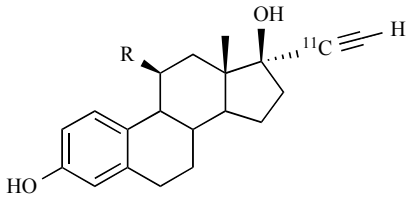
Fig. (15). Chemical structure of tamoxifen and [^{18}F]fluorotamoxifen [289, 290].

Although a number of estradiols labelled with the SPECT radionuclide bromine-77 have been prepared and evaluated, especially in terms of their potential for selective radiotherapy [292-295], their use in imaging studies, particularly in humans, has been more limited [293]. While promising early results in animals and humans have suggested the potential of 16α-[^{77}Br]bromoestradiol as a SPECT imaging agent [292, 293, 295], to the best of our knowledge, attempts to develop a labelling approach for PET imaging using ^{76}Br have not yet been reported in the literature.

5.1.2. Carbon-11 Ligands

The perspective of using the shorter-lived, positron-emitting radionuclide carbon-11 for labelling estradiol derivatives has also been considered. However, the logistical problems encountered in the preparation of estrogens labelled with a short-lived isotope are considerable, so that this route has not been extensively explored. The half-life and achievable specific activity of carbon-11, as well as the demands of detection *in vivo* must be taken into account to evaluate the capacity of carbon-11-labelled analogues as estrogen receptor binding radiopharmaceuticals.

The preparation of 17α-[^{11}C]ethynylestradiol **40** and 11β-methoxy-17α-[^{11}C]ethynylestradiol (17α-[^{11}C]moxestrol, **41** Fig. (**16**) was reported by Vaalburg *et al.* [296, 297]. 17α-Ethynyl estradiol and moxestrol are known to be very potent estrogens that bind to the estrogen receptor with high affinity and stimulate uterine growth [262]. Feenstra *et al.* [266] used the titriated analogues to investigate the potential of carbon-11 labelled 17α-ethynylestradiol and moxestrol as ER-binding radiopharmaceuticals. Because of the low half-life of carbon-11, the tissue distribution of the tritiated compounds was determined at 15-45 min after administration. Within this period both compounds showed an ER-specific biodistribution in immature female rats and in rats bearing DMBA-induced mammary tumours, which suggested that the use of carbon-11 labelled estrogens for *in vivo* studies would not be prevented by its low half-life. However, the achieved low specific activity of these ^{11}C radiopharmaceuticals (5-10 Ci/mmol) was still too low for a reliable detection of tumours.

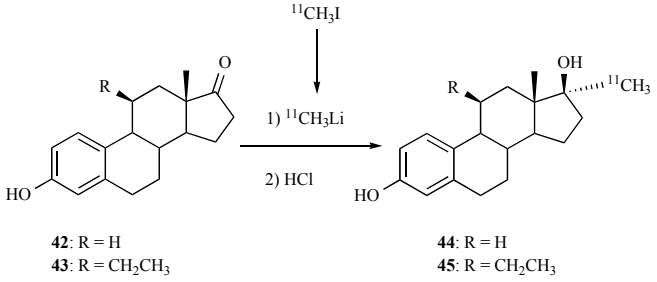


40: R = H
41: R = OCH_3

Fig. (16). Chemical structures of ^{11}C-labelled ethynylestradiol and moxestrol [296, 297].

Napolitano *et al.* [206, 298] investigated the structure-affinity relationship of a number of estrogen derivatives that could be labelled readily with carbon-11. This, together with previous studies, has shown that small groups are well tolerated in the 17α-position of the estradiol framework, and with some of them enhanced ER binding affinity has been found [30]. 17α-[^{11}C]Methylestradiol **44** (Scheme **9**) has been prepared [299], albeit with low specific activity, and its tritiated analogue was investigated, as a model compound, in mature female rats and in rats bearing DMBA-induced mammary tumours [300]. Even with a low specific activity, the tritiated compound showed high uterine uptake and high uterus/blood ratio, suggesting that the ^{11}C-labelled analogue could be regarded as a potential radiopharmaceutical for *in vivo* imaging of estrogen receptors. Therefore, 17α-methylestradiol was selected for labelling with C-11 at high specific activity [301]. Subsequently, 17α-[^{11}C]methylestradiol **44** and its derivative, 11β-ethyl-17α-[^{11}C]methylestradiol **45** (Scheme **9**) were prepared, in high specific activities (300-1000 Ci/mmol) [302]. The choice of labelling a derivative bearing an ethyl group at this position was made, because it is well known that this substitution increases the ER binding affinity [177, 298]. Both compounds showed favourable RBA values, comparable to FES. In as short as 20 min, both com-

pounds accumulated selectively in the uterus and ovaries of immature female rats, and this uptake was found to be receptor-mediated. At 40 min, both compounds showed an even higher total uptake and increased uptake selectivity by the target tissues than at 20 min. The animal biodistribution studies indicated the potential use of these ligands for estrogen receptor targeting.

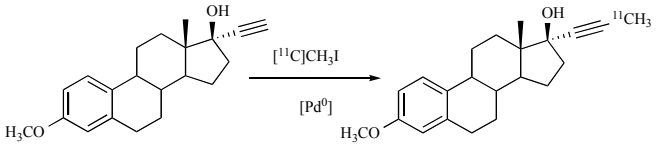


42: R = H
43: R = CH_2CH_3

44: R = H
45: R = CH_2CH_3

Scheme 9. Chemical structures of 17α-[^{11}C]methylestradiol and 11β-ethyl-17α-[^{11}C]methylestradiol [301].

These results were sufficiently encouraging to prompt further evaluation of these and related compounds as tumour imaging agents of estrogen-rich malignant tissues. However, the clinical potential of these ligands will require the consistent preparation of products with higher specific activity, since the highest specific activity possible may be required to optimise the contrast between tumour and non-tumour tissue over relatively short imaging times. Later, the radiosynthesis of 17α-(3'-[^{11}C]prop-1-yn-1-yl)-3-me thoxy-3,17β-estradiol **47** was accomplished via a modified Sonogashira-like reaction for the labelling of the terminal alkyne group of mestranol **46** with [^{11}C]methyl iodide (Scheme **10**). This reaction allows for the convenient coupling of readily available [^{11}C]methyl iodide to terminal alkynes and proceeds in sufficient radiochemical yield and in short reaction times. However, the achieved specific activity was still low (between 220 and 513 Ci/mmol), and no biological data has been presented [303].



46 **47**

Scheme 10. Radiosynthesis of 17α-(3'-[^{11}C]prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol [303].

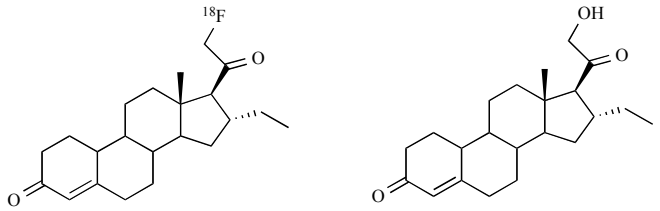
5.2. Progesterone Receptor Ligands

5.2.1. Fluorine-18 Ligands

21-[^{18}F]Fluoro-16α-ethyl-19-norprogesterone ([^{18}F]FENP)

Progesterone receptor-based agents have not been as intensively studied as estrogen receptor ligands, but a number of fluorine-18-labelled progestins have been prepared and evaluated. 21-[^{18}F] fluoro-16α-ethyl-19-norprogesterone **48** ([^{18}F]FENP) was the first progestin to be labelled and has been the most studied to date Fig. (**17**). [^{18}F]FENP is a fluoro substituted analogue of the potent high affinity progestin ORG2058 **49** (16α-ethyl-21-hydroxy-19-nor pregn-4-ene-3,10-dione) in which the 21-hydroxyl group is replaced by a radiofluorine atom [234]. [^{18}F]FENP is a potent progestin, possessing a high binding affinity (60 times that of progesterone) for the progesterone receptor (700% relative to R5020=100%) [304].

Biodistribution studies in estrogen-primed rats showed highly selective and receptor-mediated uterine uptake and excellent uterus/blood and uterus/muscle ratios. Also, considerable activity was observed in the liver and kidney, which is consistent with organs associated with metabolism and routes of excretion of steroids in rodents [175]. Furthermore, significant uptake was found in bone, resulting from the metabolic generation of the [^{18}F] fluoride ion, and in fat. Fat uptake, which is observed with lipophilic compounds, is considerably greater with [^{18}F]FENP than that previously encountered with the less lipophilic ^{18}F-labelled estrogens [119]. Since adipose tissue constitutes a significant fraction of the breast tissue, this high fat uptake might reduce the selectivity of the receptor-mediated uptake in progesterone receptor-positive breast tissue.



48 **49**

Fig. (17). Chemical structures of [^{18}F]FENP and ORG2058 [234].

Owing to its favourable features, Dehdashti *et al.* [177] used [^{18}F]FENP for imaging progesterone receptors in eight patients with primary breast carcinoma. Despite of the good results obtained in the animal model [175], only 50% of PR-positive tumours were identified. Moreover, it appeared that the uptake of the tracer was not correlated with the tumour progesterone-receptor levels. These findings, together with the low target-to background ratio and metabolic defluorination leading to high bone uptake, excluded the possibility of using [^{18}F]FENP for PR imaging in humans.

The main issues associated with [^{18}F]FENP were found to be correlated with its lipophilicity and high metabolic liability. The high lipophilicity resulted in elevated uptake in adipose tissue and increased liver uptake in animal biodistribution studies leading to greater metabolite production in human studies. The susceptibility of the C-21 position to metabolic scission of the radiolabel and the reduction of the C-20 keto group, which occurs rapidly in humans, but is not observed in rats, and results in an inactive 20-hydroxy steroid metabolite [177, 178], also contributed to its failure to give satisfactory images of progesterone receptor-positive tumours in humans. Moreover, considerable defluorination was observed, suggesting that the C-21 position was also prone to hydroxylation.

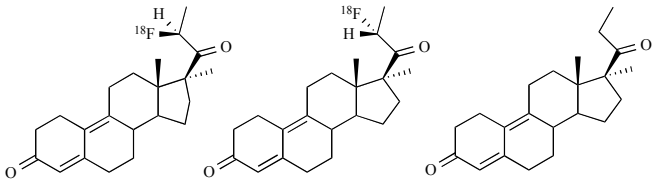
(21S)-[^{18}F]Fluoro-R5020 and (21R)-[^{18}F]fluoro-R5020

Progesterone does not show selective uptake and retention by target tissues, such as the estrogen-primed tissue of the rat [305], seemingly because its PR affinity is too low (13% relative to R5020=100%) and it is high lipophilic (log $P_{o/w}$=3.87) [306]. However, Carlson *et al.* [305] found that some high affinity synthetic progestins, such as ORG2058 **49** (200%, relative to R5020) and R5020 **52** (promegestone, 17α,21-dimethyl-19-nor-4,9-pregna diene-3,20-dione) did show selective uptake and retention by target tissues. These findings indicated clearly that in order to obtain adequate contrast between target and non-target tissues in dynamic uptake studies PR ligands with affinities significantly greater than that of the natural progesterone were required.

Thus, two analogues, (21*S*)-[^{18}F]fluoro-R5020 **50** and (21*R*)-[^{18}F]fluoro-R5020 **51,** of the potent, high affinity progestin R5020 [233] were also prepared in ^{18}F labelled form [176] Fig. (**18**). The epimeric fluoro analogues (21*S*)- and (21*R*)-fluoro R5020 displayed binding affinities lower than that of ORG2058 and R5020 (45% and 11%, respectively, relative to R5020=100%).

The tissue biodistribution of the labelled compounds was evaluated in estrogen-primed immature rats, with the high affinity 21*S*-fluoro-R5020 ***50*** showing some uterine selective uptake, but little prolonged retention, while the low affinity 21*R*-stereoisomer **51** led

to no selective uptake. Both compounds displayed high bone uptake, resulting from metabolic defluorination.

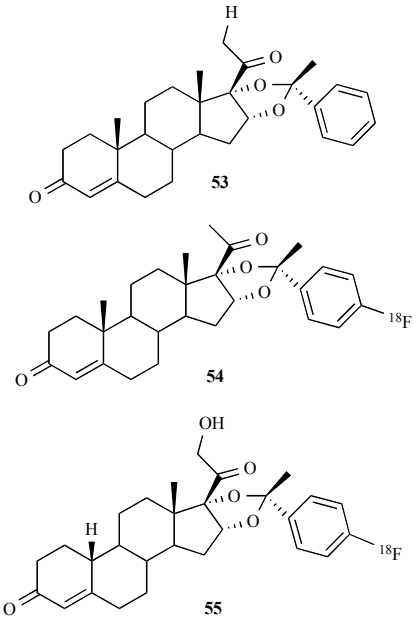


50 **51** **52**

Fig. (18). Chemical structures of (21*S*)-[^{18}F]fluoro-R5020, (21*R*)-[^{18}F]-fluoro-R5020 and R5020 [233].

Radiofluorinated 16α,17α-fluoroacetophenone ketals of 16α,17α-dihydroxy progesterone and 16α,17α,21-trihydroxy-19-norprogesterone

The failure of [^{18}F]FENP in human model has suggested the use of more metabolically stable imaging agents in order to prevent defluorination. Early reports by Fried *et al.* [307] have shown that the addition of a 16α,17α-dioxalane system into the pregnadienone nucleus gave compounds that were potent progestins. In particular, 16α,-17α-dihydroxyprogesterone acetophenide **53** Fig. (**19**) was reported to be twice as potent as progesterone in parenteral activity [308].



53 **54** **55**

Fig. (19). Chemical structure of 16α,-17α-dihydroxyprogesterone acetophenide and of 16α,17α-fluoroacetophenone ketals of 16α,17α-dihydroxyprogesterone and 16α,17α,21-trihydroxy-19-norprogesterone labelled with fluorine-18 [304].

The aromatic ring of **53** provides an attractive site for radiolabelling, as literature precedent suggested that the aryl carbon-fluorine may be metabolically stable [309, 310]. This, together with the findings from Kym *et al.* [311] that progestin derivatives having bulky substituents at the 16α and 17α-positions bound to the PR with comparable or higher affinities than the natural ligand, progesterone, determined that the progesterone receptor might be tolerant to substituents projecting behind the D-ring.

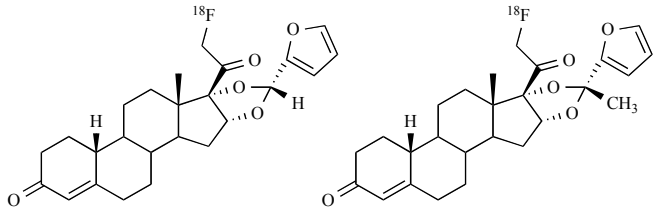
Hence, Kochanny *et al.* [304] described the preparation of two fluorine-substituted progestins, the 16α,17α-fluoroacetophenone ketals of 16α,17α-dihydroxyprogesterone, (16α,17α-[(*R*)-1'-(4-[^{18}F]fluorophenyl)-(ethylenodioxy)]-pregn-4-ene-3,20-dione **54** and 16α,17α,21-trihydroxy-19-norprogesterone (16α,17α-[(*R*)-1'-(4-[^{18}F]fluorophenyl)-(ethylenodioxy)]-21-hydroxy-19-norpregn-4-ene-3,20-dione **55** Fig. (**19**), that were designed to avoid defluorination by placing the radiolabel on the phenyl ring of the acetophenone ketal system.

Both compounds demonstrated high binding affinity for the progesterone receptor (53% and 240%, respectively, relative to R5020=100%). In tissue biodistribution studies in estrogen-primed immature female rats, both ketals showed selective uterine uptake, which was blocked by coinjection of a saturating dose of the unlabelled progestin ORG 2058. Moreover, the low bone radioactivity levels suggested metabolic stability. Both compounds exhibited high fat uptake in agreement with their relative high lipophilicities (log $P_{o/w}$=4.05 and 5.71, respectively). However, the selective uptake and the metabolic stability of these 16α,17α-fluoroaceto phenone ketals suggested that analogs of these compounds with reduced lipophilicities might be promising for *in vivo* imaging of progesterone-positive breast tumours.

21-[^{18}F]fluoro-16α,17α-α-[(R)-1'α-furylmethylidene)-dioxy]-19-norpregn-4-ene-3,20-dione ([^{18}F]FFNP)

While the 16α,17α-fluoroacetophenone ketals **54** and **55** were rather inert towards metabolic defluorination, their lipophilicity was still quite high, and consequently the efficiency and selectivity of their *in vivo* target tissue uptake were only moderate. The incorporation of a bulky substituent, such as the 16α,17α-dioxolane system in this series of compounds, suggested, however, that this structure might block the metabolism, presumably by protecting the C-20 ketone from reduction by 20-dehydrogenases [178, 312].

Based on these observations, Buckman *et al.* [306] prepared a series of seven novel 16α,17α-(furanyldioxalanes) that offered the possibility of reduced lipophilicity compared to the acetophenone ketals. Two progestins incorporating a furanyl acetal and a furanyl ketal at the 16α,17α-position, 21-[^{18}F]fluoro-16α,17α-α-[(*R*)-1'α-furylmethylidene)-dioxy]-19-norpregn-4-ene-3,20-dione **56** ([^{18}F] FFNP) and 21-[^{18}F]fluoro-16α,17α-α-[(*R*)-1'α-furylethylidene)-dioxy]-19-norpregn-4-ene-3,20-dione **57** respectively Fig. (**20**) were chosen for labelling with fluorine-18 at the 21-position on the basis of the high PR relative binding affinity (190% and 173%, respectively, relative to R5020=100%) of their fluorinated counterparts.



56 **57**

Fig. (20). Chemical structures of 21-^{18}F-labelled progestins incorporating a furanyl acetal ([^{18}F]FFNP) and a furanyl ketal at the 16α,17α-position [306].

From *in vitro* studies, these compounds were also shown to have low lipophilicity (log $P_{o/w}$=3.87 and 4.13, respectively) and low non-specific binding. The observed reduction in lipophilicity due to the change from phenyl to furan compensates for the loss of lipophilicity resulting from radiolabelling at the C-21 position by replacing the C-21 hydroxyl with the fluorine-18 (log $P_{o/w}$ ORG 2058=4.02 and log $P_{o/w}$ FENP=4.66).

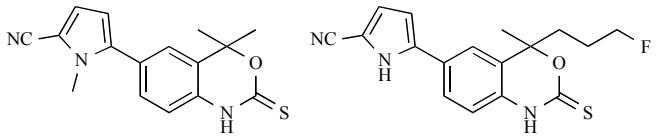
In tissue distribution studies in estrogen-primed immature female rats, both compounds demonstrated high progesterone receptor-selective uptake in the uterus and ovaries, and relatively low uptake in fat and bone. The 21-position in these progestins appeared to be less prone to metabolism than that in other 21-fluoro pro-

gestins [177, 178], which might reflect a sterically induced inhibition of metabolism at this site due to the bulk of the furan-substituted dioxolane ring at the 16α,17α-position.

Lately, a first-in-human study was designed to evaluate the safety and dosimetry of [^{18}F]FFNP as well the feasibility of imaging progesterone receptors in breast cancer patients [39]. Twenty patients with 22 primary breast cancers (16 PR-positive [PR+] and 6 PR-negative [PR-]) were evaluated by whole-body PET, and the results were correlated with ER and PR status. The maximum standardized uptake value of [^{18}F]FFNP in the tumour was not significantly different in PR+ and PR- cancers (2.5 ± 0.9 *vs.* 2.0 ± 1.3), but the tumour-to-normal breast (T/N) ratio was significantly greater in the PR+ cancers (2.6 ± 0.9 *vs.* 1.5 ± 0.3). This study determined that PET imaging with [^{18}F]FFNP might be a safe, non-invasive means for evaluating tumour PRs *in vivo* in breast cancer patients. The relatively small doses absorbed by normal organs allow for the safe injection of up to 440 MBq of the radiopharmaceutical.

4-[^{18}F]Fluoropropyl-Tanaproget ([^{18}F]FPTP)

Recently, a fluorine-18 labelled analogue of Tanaproget **58**, a nonsteroidal progestin initially developed by Wyeth Pharmaceuticals [313], with very high PR binding affinity (151% relative to R 5020=100%) and low affinity for androgen and glucocorticoid receptors [314] was prepared, and its biological profile was studied in estrogen-primed rats to evaluate its potential for imaging PR status in breast cancer patients [315] Fig. (**21**).



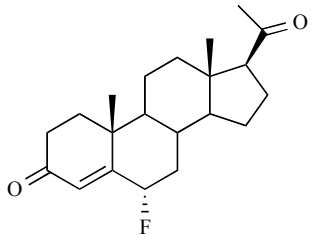
58 **59**

Fig. (21). Chemical structures of Tanaproget and [^{18}F]FPTP [315].

In biodistribution studies, the target tissue uptake of 4-[^{18}F]fluoropropyl-Tanaproget **59** ([^{18}F]FPTP**)** was high at both 1 and 3 h (uterus, 4.55 and 5.26%ID/g; ovary, 2.32 and 2.20%ID/g, respectively) and was blocked by co-injection of excess unlabelled compound. Uterus/blood and uterus/muscle activity ratios were 9.2 and 5.2 at 1 h and 32 and 26 at 3 h, respectively. The target tissue distribution of [^{18}F]FPTP compared favourably to that of labelled steroidal progestins [^{18}F]FENP and [^{18}F]FFNP. Its high target tissue uptake efficiency and selectivity, and prolonged retention, suggested its potential as a PET imaging agent for PR-positive breast tumours. Although [^{18}F]FPTP does not have biodistribution features in rat model that are significantly better than those of [^{18}F]FENP and [^{18}F]FFNP, it shows other favorable characteristics that should be considered for its further development. Being a nonsteroidal progestin [^{18}F]FPTP would not be a substrate for the 20-hydroxysteroid dehydrogenase found in human blood and responsible for the failure of [^{18}F]FENP in humans [178] despite its favourable biodistribution in the rat model [175]. [^{18}F]FPTP is a chiral compound and thus far has been prepared and studied only as a racemate. Probably one of the [^{18}F]FPTP enantiomers will have higher binding affinity than the other and thus the high-affinity isomer might have more favourable biodistribution properties. The authors are currently developing methods to separate and/or prepare selectively the [^{18}F]FPTP enantiomers and further studies are planned with these compounds in other animal models.

6α-[^{18}F]fluoroprogesterone

The C-6 position is a site where alkyl and halogen substitution in high potency progestins (*e.g.*, DU41165, chlormadinone, meges trol, medroxyprogesterone acetate) is well tolerated. Based on previous observations that 6α- and 21-fluoroprogesterones have equal or even higher relative binding affinity values than progesterone [228], Choe *et al.* [316] have synthesised 6α-[^{18}F]fluoro progesterone **60** in high specific activity Fig. (**22**).



60

Fig. (22). Chemical structure of 6α-[^{18}F]fluoroprogesterone [316].

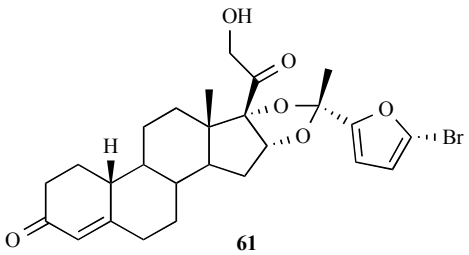
Tissue distribution studies in estrogen-primed female rats demonstrated low uptake by the uterus, low target tissue uptake selectivity, and high uptake in fat. These findings, together with a high uptake observed in the bone, which indicates extensive metabolic defluorination, suggested that in the design of potential imaging agents for the progesterone receptor, the C-6 position of a progestin may not be a good site for labelling with fluorine-18.

5.2.2. Bromine-76 Ligands

Although a number of steroids labelled with bromine radioisotopes have been prepared [265, 292-295] their use as imaging agents, particularly in humans, has been more limited [293]. However, these agents have been well studied in terms of their potential in selective radiotherapy due to their favourable characteristics as Auger electron-emitters [317-319]. Auger electron-emitting halogens, such as bromine-76, decay with a significant amount of positron emission, a characteristic that allows for diagnostic PET imaging to be used to complement their use in radiotherapy. The half-life of bromine-76 is sufficiently long to permit target tissue-selective distribution, while being sufficiently short so that the bulk of the dose can be delivered to the tissue prior to metabolism and elimination of the radiopharmaceutical.

Based on what is known about the structure-affinity relationships of PR ligands, a good candidate molecule for labelling with bromine or iodine should have a skeleton related to [^{18}F]FFNP **56** Fig. (**20**) [306]. Due to its favourable pharmacokinetic and pharmacodynamic features [^{18}F]FFNP appeared to have promise as a PET imaging agent, and continues being developed for that purpose.

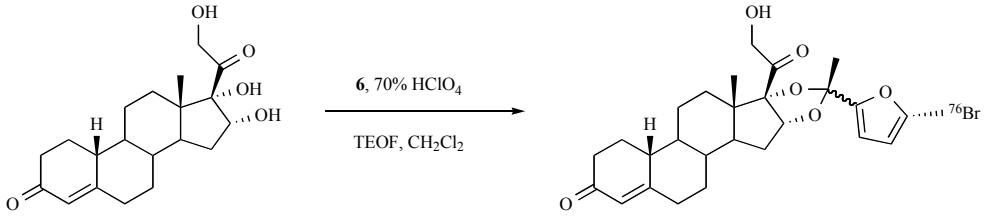
Thus, a series of bromine-substituted 16α,17α-dioxolane progestins, based on [^{18}F]FFNP **56** Fig. (**20**) [306] and on its analogue **57** Fig. (**19**) [304], have been synthesized by Zhou *et al.* [320]. Among this series, 16α,17α-[(*R*)-1'-α-(5-bromofurylmethylidene) dioxyl]-21-hydroxy-19-norpregn-4-ene-3,20-dione **61** appeared to be the most promising with good relative binding affinity to PR (65% relative to R5020=100%) and moderate lipophilicity (log $P_{o/w}$ = 5.09).



61

Fig. (23). Chemical structure of 16α,17α-[(*R*)-1'-α-(5-bromofuryl-methylidene)dioxyl]-21-hydroxy-19-norpregn-4-ene-3,20-dione [320].

16α,17α-[(*R*)-1'-α-(5-[^{76}Br]Bromofurylmethylidene)dioxyl]-21-hydroxy-19-norpregn-4-ene-3,20-dione **63** (Scheme **11**) was



62 **63**

Scheme 11. Radiosynthesis of 16α,17α-[(*R*)-1'-α-(5-[^{76}Br]bromofurylmethylidene) dioxyl]-21-hydroxy-19-norpregn-4-ene-3,20-dione via acetalization with a prosthetic group [151].

successfully synthesized via a two-step reaction and its tissue biodistribution and metabolic stability were evaluated in estrogen-primed immature female Sprague-Dawley rats. The synthesis of the tributyltin precursor for direct labelling via electrophilic substitution failed to provide the desired product. Thus, a two-step synthesis was adopted from a previously reported method [304] which relies on the use of a radiobrominated furfural prosthetic group **6** (Scheme **6**) and further acetalization with the triol **62** (Scheme **11**). Under acid catalysis a mixture of two radiobrominated furanyldioxalanes was obtained, corresponding to both endo and exo isomers.

The radiobrominated analogue demonstrated high PR-mediated uptake in the target tissues, uterus and ovaries, that was blocked by excess of unlabelled progestin. Uptake in non-PR target tissues, however, was not low, especially at early times. This high, initial non-specific uptake is probably due its high lipophilicity compared to that of [^{18}F]FFNP. The radioactivity in blood and muscle was also relatively high because of its high lipophilicity and the subsequent formation of metabolites and may cause problems for imaging and radiotherapy. Also, **63** was metabolized quickly in the liver, but not in the blood, to form a more polar radioactive unidentified metabolite and free [^{76}Br]bromide.

Thus, despite the favourable initial biodistribution, the subsequent metabolism of **63** precluded its application as a potential imaging agent and as a radiotherapeutic agent, which would require specific and prolonged target tissue retention [151].

CONCLUDING REMARKS

PET imaging is a clear advance in the approach to staging and monitoring breast cancer, offering better accuracy than conventional imaging in the identification of metastatic disease, both in the initial staging and in follow-up of the disease. While evaluation of ER or PR levels in human breast tumours is currently obtained *in vitro* by immunohistochemical assays, there is great interest in developing methods to determine these levels by *in vivo* PET imaging, which could potentially assess receptor densities at all tumour sites, simultaneously and noninvasively. This data could be used to select patients most likely to benefit from endocrine therapies, thereby sparing some the morbidity of radiation and chemotherapy. Forthcoming refinements in scanner technology and development of novel radiopharmaceuticals will probably result in better detection of smaller lesions. Dedicated breast PET/CT or PET/mammography units show promise in improved detection in primary breast cancer, while also providing a method for image guided biopsy. Current research on the molecular basis of cancer will likely provide new agents that may better identify tumours not well imaged by FDG.

In conclusion, taken together, the studies described to date, indicate that radiolabelled estradiol derivatives are suitable tracers to determine the ER status in breast cancer patients. A variety of ER imaging agents have been and continue to be developed and tested, and clinical PET imaging of ER in breast cancer using [^{18}F]FES is quite advanced. An advantage of [^{18}F]FES over iodinated tracers is that it allows quantitative measurement of ER density and occupancy, which may be useful for therapy evaluation and drug development. Thus far, however, only limited progress has been made with PET imaging of PR.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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