Radiolabeled Glucocorticoids as Molecular Probes for Imaging Brain Glucocorticoid Receptors by Means of Positron Emission Tomography (PET)

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Abstract: Over the last two decades, numerous attempts have been made to develop ^{11}C- and ^{18}F-labeled radiotracers in order to study glucocorticoid receptor (GR)-mediated abnormalities of the hypothalamus-pituitary-adrenocortical (HPA) axis function and regulation *in vivo* by means of positron emission tomography (PET). The present review addresses the research efforts dealing with the design, radiosynthesis and radiopharmacological evaluation of PET radiotracers for brain GR imaging. The underlying problems such as metabolic instability, insufficient blood-brain-barrier penetration and/or high non-specific binding will be discussed.

Key Words: Glucocorticoid receptor, PET, radioligands, carbon-11, fluorine-18.

INTRODUCTION

Positron emission tomography (PET) is a powerful non-invasive imaging technique which provides functional information on physiological, biochemical and pharmacological processes *in vivo* [1, 2]. PET allows the detailed study of the pathophysiological background of cellular function behind diseases in laboratory animals and humans. The possibility to observe molecular interactions in living organisms and to determine absolute values of physiological parameters places PET in a unique position among other radiotracer and stable isotope tracer kinetic analysis techniques in biomedical research. In a typical PET study the PET radiotracer, a compound labeled with a short-lived positron emitter, is injected intravenously into man or animal. Tissue concentrations of the radiotracer are measured over time, and these data are combined with information on plasma concentration of the radiotracer to assay metabolism. Mathematical methods for the evaluation of PET measurements within the framework of compartment models are well established [3]. The result is an image which reflects the 3-dimensional distribution of the radiotracer concentration in terms of spatial and temporal resolution.

During the last 30 years PET has become an indispensable tool in scientific research. It has reached clinical imaging where it has found numerous applications in the field of oncology, cardiology and neurology. Moreover, PET also has been recognized and applied as a valuable research tool in the process of drug development and evaluation [4-9].

As a non-invasive imaging methodology, PET allows the assessment of novel drug action in man at a very early stage in the drug development and evaluation process. These non-invasive studies span pharmacokinetic/pharmacodynamic evaluation of potential drug candidates, receptor occupancy studies to determine drug efficacy, proof of potential biochemical mechanisms of drug action, and characterization of disease-associated physiological parameters.

The success of PET as part of the molecular nuclear medicine field in the era of functional genomics and proteomics research stems largely from the availability of suitable PET radiotracers. The choices of the appropriate radionuclide and the labeling position are among the most important aspects for the design of novel PET radiotracers. The half-life of the radionuclide should match the time window of the biological process to be studied and the labeling position should address the metabolic pathway of the compound. The most widely used PET radionuclides are ^{11}C ($t_{1/2}$ = 20.4 min), ^{13}N ($t_{1/2}$ = 9.9 min), ^{15}O ($t_{1/2}$ = 2 min) and ^{18}F ($t_{1/2}$ = 109.8 min). The short half lives of these radionuclides require their in-house production, usually accomplished by a dedicated small biomedical cyclotron. Carbon, nitrogen and oxygen are the main constituents in most molecules of biological importance. Hence, isotopic labeling with ^{11}C, ^{13}N and ^{15}O will lead to radiotracers undistinguishable from their unlabeled counterparts, with the exception of very small kinetic isotope effect. The lack of a positron-emitting isotope of hydrogen can be compensated in many cases by using ^{18}F as a bioisosteric replacement for a hydrogen atom in a given molecule. A fluorine atom may also imitate a hydroxyl group as exemplified by the most important PET radiotracer 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG). Moreover, many novel drugs contain a fluorine atom which also can isotopically be replaced with ^{18}F.

Radiosyntheses involving short-lived positron emitters require the application of synthetic methods and techniques apart from those used in conventional organic synthesis. First of all, the synthesis must be carried out within a certain time frame compatible with the half-life of the radionuclide. As a rule of thumb the synthesis of a radiotracer (including purification and formulation) should be accomplished within three

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half-lives. As a consequence, ^{11}C and ^{18}F are especially suitable for radiolabeling enabling to some extent multi-step synthesis sequences.

Another important characteristic of PET radiochemistry represents the use of a very small amount of labeled compounds. Typically 10^{13}-10^{15} labeled molecules are administered into the living body. Compounds labeled with the short-lived positron emitters ^{11}C, ^{13}N, ^{15}O and ^{18}F may be obtained at high specific radioactivity. Specific radioactivity expresses the amount of radioactivity per gram or mole. In the case of receptor binding radiotracers, a high specific activity is essential to avoid receptor saturation and, therefore, perturbation of the receptor-ligand interaction.

The present review addresses the status of radiotracer development for imaging brain glucocorticoid receptors by means of PET. This mini-review is organized to give a brief introduction into general aspects of the glucocorticoid receptor (GR) and its role in the central stress response. Based on the basic concepts for the design and synthesis of PET radiotracers for imaging brain receptors, the main part of the review will deal with the presentation of various ^{11}C- and ^{18}F-labeled compounds as potential ligands for brain GRs. The underlying problems such as metabolic instability, insufficient blood-brain-barrier penetration and/or high non-specific binding will be discussed.

THE GLUCOCORTICOID RECEPTOR AND ITS ROLE IN THE CENTRAL STRESS RESPONSE

Corticosteroid hormones regulate a variety of essential physiological functions, such as energy metabolism, mineral balance and the body`s stress response. Corticosteroids are produced in the adrenal cortex, and their production is controlled *via* the hypothalamo-pituitary-adrenocortical (HPA) axis, a classical closed-loop endocrine system. The release of corticosteroids occurs in high amounts after periods of stress. In the late 1960s, McEwen and colleagues could show that corticosteroids not only exert their effects on peripheral organs but also enter the brain [10]. In the brain, they bind to two different intracellular receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). MRs and GRs are co-located in brain regions that are involved in the regulation of fear and anxiety, such as hippocampus, septum, and amygdala [11, 12]. Moreover, GRs are widely distributed in other parts of the brain, both in neurons and glia cells. Areas with particularly high GR expression are found in regions involved in the feedback regulation of the hormonal stress response, such as the paraventricular nucleus of the hypothalamus, hippocampal formation, thalamic nuclei, central amygdala, striatum, and cortical layers [11, 12].

There is accumulating evidence that corticosteroids, in addition to corticotropin releasing factor, play an important role in stress and the pathophysiology of anxiety disorders and depression [11, 12]. It has been suggested that a disturbed HPA axis regulation may be a primary causal factor in depression. Glucocorticoids participate in the shut-off of the stress response through negative feedback. Since feedback inhibition of glucocorticoids is decreased in many depressive patients, it has been hypothesized that disturbances in the MR/GR balance may have deleterious consequences for regulation of the stress response, and it may increase vulnerability to depression.

The molecular basis for physiological actions of glucocorticoids is their recognition by and binding to the GR. The GR belongs to the nuclear receptor superfamily, which also include the MR, progesterone receptor (PR), androgen receptor (AR), and estrogen receptor (ER), as well as peroxisome proliferator-activated receptors (PPARs), vitamin D (VDR), and thyroid hormones (TR) [13]. All these receptors function as ligand-dependent transcription factors to regulate and control gene expression. Like most other nuclear receptors, the GR is a multifunctional domain protein consisting of an *N*-terminal activation function domain, a central DNA binding domain containing two zinc-fingers, and a *C*-terminal ligand binding domain. The GR consists of 777 amino acids [14, 15] The steroid hormone receptors (ER, AR, PR and GR) share over 50% identity in their amino acid sequence and a similar three-dimensional structure. In the cytoplasm, free GR is associated with heat-shock proteins (HSPs) and other proteins. This multi-protein complex is considered to keep the GR in a high-affinity configuration.

The detailed molecular basis behind the ligand-dependent regulation of the GR has been illustrated by crystal structure determination of the GR ligand binding domain (LBD). The crystal structure of the human GR-LBD was reported in complex with the agonist dexamethasone (DEX) and a co-activator peptide [14, 15]. Despite the similar three-dimensional structure of the ER, AR, PR, and GR, subtle differences in the secondary structure and the topology of the LBD result in the formation of an additional side pocket in the GR-LBD [14]. Compared with structurally related LBDs of the ER, AR and PR, this side pocket is unique for the GR. Endogenous steroid hormones such as estradiol, testosterone, progesterone or cortisol possess a similar chemical core structure based on a cyclopentano-perhydrophenantren skeleton, but mediate different biological functions. Structural comparison of ER, AR, PR, and GR has revealed how functional specificity is achieved by the steroid receptors. The additional side pocket of the GR can accommodate larger substituents at position 17α of the steroid skeleton as typically found in glucocorticoids like cortisol. Therefore, the distinct GR binding pocket might explain its selectivity for glucocorticoids and its diversity of responses [14]. Besides this unique binding pocket the crystal structure has revealed several other distinct features not found in other nuclear receptors, such as a novel dimerization interface and a second charge clamp that is thought to be important in determining co-activator binding selectivity [15].

The development of GR ligands which are appropriately labeled with the short-lived positron-emitting radioisotopes carbon-11 or fluorine-18 would allow the non-invasive *in vivo* imaging and mapping of brain GRs by means of positron emission tomography (PET). In this context, PET studies of brain GRs would provide important information on the neurobiological basis of GR-mediated functional abnormalities of HPA axis function in depression. Hence, during the last two decades considerable efforts have been made for the design, radiolabeling and radiopharmacological evaluation of ligands for brain GRs.

GENERAL ASPECTS FOR THE DESIGN OF PET RADIOTRACERS FOR BRAIN GLUCOCORTICOID RECEPTOR IMAGING

Positron emission tomography (PET) as a non-invasive clinical and research imaging technique has witnessed increasing acceptance as a powerful imaging methodology to study physiological and biochemical processes at the molecular level *in vivo*, particularly for the central nervous system (CNS). The successful development of PET radiotracers for imaging binding sites in the brain (receptors, active site of an enzyme or transporter binding sites) is an iterative process. The potential compound selected for PET radiotracer development usually shows appropriate *in vitro* characteristics, such as high affinity and selectivity for the binding site. However, *in vivo* various other parameters, such as protein binding, metabolism, flow and delivery, come into play. These *in vivo* parameters are for the most part unknown, but may have contributed to a great extent to the failure of numerous PET radiotracers. Therefore, all PET radiotracers should meet some basic requirements (1) to achieve a reasonable target-to-non-target ratio; (2) to exhibit high *in vivo* affinity, specificity and selectivity to cellular targets of interest; to (3) posses an appropriate lipophilicity (*logP* between 2 to 3) to enable sufficient blood-brain-barrier penetration, while avoiding high nonspecific binding, e.g. to plasma proteins; (4) to demonstrate reasonable metabolic stability to avoid formation of radioactive metabolites capable of penetrating the blood-brain-barrier as it complicates image interpretation and kinetic analysis. Lastly, (5) the potential radiotracer should be not a substrate for the P-glycoprotein efflux pump [16].

The binding potential B_{max}/K_d, where B_{max} represents the receptor concentration [fmol/mg] of the target site and K_d the dissociation constant [nM] of the radiotracer, can be used as a general guideline whether a potential target site could be imaged by a radiotracer. Thus, receptor imaging is dependent on the ratio of receptor density (B_{max}) over the affinity of the radioligand for the receptor, and the ratio B_{max}/K_d should be >>1. The calculated B_{max}/K_d value was shown to be a valuable predictor to assess various inhibitors for the dopamine transporter (DAT), serotonine transporter (SERT) and norepinephrine transporter (NET) for *in vivo* imaging purposes. For very low density binding sites such as NET and nicotinic cholinergic receptors (B_{max} in the range of 10 to 100 fmol/mg protein (~1-10 nM) successful *in vivo* imaging was only accomplished with very high affinity (< 1 nM) radioligands [17-19].

For the design of suitable radiolabeled ligands for PET imaging, brain GRs also have to be considered as very low density binding sites, reaching concentrations of approximately 30-40 fmol/mg protein (3-4 nM) in the human brain [20]. A slightly higher concentration is found in the rodent brain. This very low receptor concentration is comparable to that of other steroid hormone receptors. For example, the average number of receptors per cell for ER and AR is reported to be 10,000, which equals only to 1 ppm of all cellular proteins [21].

As a consequence of this very low intracellular receptor concentration, very high affinity ligands in the low nanomolar or even picomolar range are a necessary criterion for the design of appropriate radiotracers. However, a favourable value of the binding potential B_{max}/K_d is by far not a sufficient criterion. Other factors, such as affinity of the radiotracer for other receptors and proteins, an appropriate lipophilicity, and chemical and metabolic characteristics of the radiotracer complete the criteria for the development of radiotracers for imaging brain GRs. The brain uptake of a selective radiotracer for brain GRs depends on the combination of all these factors. Glucocorticoids are small (MW <500) and lipophilic compounds (*logP* >3). The high lipophilic nature of glucocorticoids should enable BBB penetration, but a high lipophilicity is also often associated with high nonspecific binding. Glucocorticoids as low molecular weight compounds should preferentially be labeled with the short-lived positron emitters fluorine-18 and carbon-11. Both radionuclides enable the preparation of radiotracers at high specific activity. The specific activity of receptor binding radiotracers, especially for very low density sites such as GRs, should be sufficiently high to avoid significant receptor occupancy through the parent compound. This is of special importance in the case of radiotracers for the GR, since competition of the binding sites with endogenous glucocorticoids such as corticosterone (in rats) and cortisol (in humans), has to be considered. Moreover, the specific activity of the radiotracer is particularly important for small rodent PET imaging with respect to the mass effect of the injected dose. The mass effect is particularly crucial in mice [22]. The very low acceptable mass for receptor binding studies in mice may limit the activity injectable into mice to such an extent that small animal PET imaging of brain GRs is not feasible anymore.

PET RADIOTRACERS FOR IMAGING BRAIN GLUCOCORTICOID RECEPTORS

Research on the development of PET radiotracers for imaging of brain glucocorticoid receptors (GRs) began more than 20 years ago. However, compared with the extensive research efforts focused on radiotracers for other steroid hormone receptors such estrogen receptor (ER), progesterone receptor (PR) and androgen receptor (AR), comparatively little work has been done on the development of radiotracers for imaging GRs. The design, synthesis and radiopharmacological evaluation of GR-binding PET radiotracers were mainly focused on compounds labeled with the positron emitter fluorine-18 ($t_{1/2}$ = 109.8 min) as the preferred radionuclide for radiolabelings. Only a few attempts have used carbon-11 ($t_{1/2}$ = 20.4 min) to prepare radiotracers for the GR.

Compounds which have been envisaged as GR-binding radiotracers can basically be categorized into three different classes: (1) Compounds based upon endogenous and potent synthetic steroids, (2) steroids containing an aryl-[3,2-c]pyrazole moiety, and (3) non-steroidal compounds consisting of heterocyclic core structures.

1. Design and Synthesis of GR-Binding Radiotracers Based Upon Endogenous and Potent Synthetic Steroids

The design and synthesis of radiotracers for imaging of brain GRs commenced in the 1980s with the preparation of

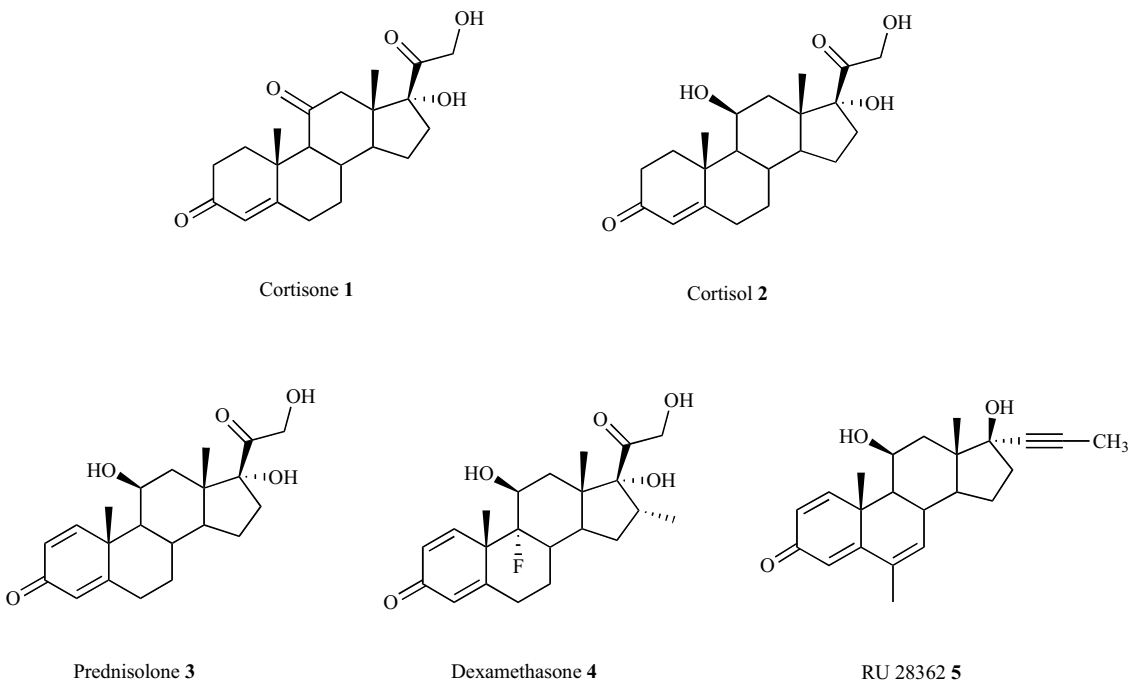


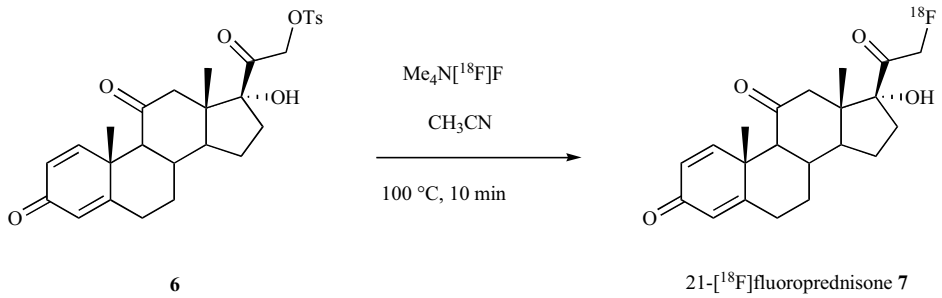
Fig. (1). Selected endogenous and synthetic steroids.

various ^{18}F-labeled derivatives of endogenous and synthetic corticosteroids. Natural occurring corticosteroids such as cortisone **1** and cortisol **2**, as well as some highly potent synthetic steroids like prednisolone **3** and dexamethasone **4**, possess a characteristic 17α,21-dihydroxy-20-keto substitution pattern as typically found in steroids of the pregnane series. Moreover, several reports on corticosteroids like RU 28362 **5** have shown that the D-ring is tolerant toward different alkynyl substitution patterns at position C17 of the steroid skeleton while retaining high binding affinity to the GR [23]. Examples of endogenous and synthetic steroids containing a 17α,21-dihydroxy-20-keto side chain and a 17α-alkynyl substitution are shown in Fig. (**1**).

The design and synthesis of ^{18}F-labeled radiotracers was based on the bioisosteric substitution of either the 21-hydroxy group or a hydrogen atom in the 17α-alkynyl side chain with the positron emitter fluorine-18. The introduction of fluorine-18 was accomplished *via* an aliphatic nucleophilic substitution with cyclotron-produced [^{18}F]fluoride on steroids equipped with an appropriate leaving group, such as sulfonic acid esters or halogens like bromine and iodine.

One of the first examples for a ^{18}F-labeled GR radiotracer was reported by the radiosynthesis of 21-[^{18}F]fluoroprednisone **7** [24]. 21-[^{18}F]Fluoroprednisone **7** was prepared in a single step starting from the corresponding 21-tosylate precursor **6** at 100 °C within 10 min in decay-corrected radiochemical yields of 2-8% at a specific activity reaching up to 92.5 GBq/μmol (Fig. **2**).

The radiopharmacological evaluation of the radiotracer involved *in vitro* stability, biodistribution, and PET imaging in normal male Wistar rats. Although 21-[^{18}F]fluoroprednisone **7** was completely stable in rat plasma at 37 °C for 1 h, the radiotracer underwent transformation with a half-life of 53 min to a new species *in vivo* and in heparinized rat whole blood. The amount of radio-defluorination was determined to be 20% after 1.5 h *in vitro*. Metabolite analysis using mass spectrometry identified a metabolite with a reduced C20 keto group as consistent with the common metabolic pathway for steroids of the pregnane series. Experimental *logP* determination revealed an increase in lipophilicity through introduction of the 21-fluorine group (21-fluoroprednisone: *logP* = 2.2, prednisone: *logP* = 1.4), mak-



6 21-[^{18}F]fluoroprednisone **7**

Fig. (2). Synthesis of 21-[^{18}F]fluoroprednisone **7** [24].

ing 21-[^{18}F]fluoro-prednisone **7** more lipophilic than the flow tracer *n*-butanol (*logP* = 0.9). Therefore, the authors suggested, that the brain uptake of 21-[^{18}F]fluoroprednisone **7** should be flow- rather than diffusion-limited. Biodistribution data were collected for one single time point (60 min p.i.). Only little activity uptake was found in the brain (less than 0.05 %ID/g). Brain activity was lower than activity in the muscle. Most activity was found in the liver (1.7 %ID/g), small intestine (1.0 %ID/g), kidney (0.6 %ID/g), and bladder (0.4 %ID/g). The authors conclude that high endogenous steroid capacity has led to GR saturation, hence resulting in low uptake of the radiotracer in GR target tissues.

Various 21-[^{18}F]fluoro-substituted steroids containing a 9α-fluorine atom as radiotracers for the GR were reported by Pomper *et al*. [25] and Visser *et al*. [26]. The incorporation of fluorine-18 at position 21 of the steroid backbone was achieved *via* displacement of appropriate leaving groups (tosylate and triflate) with [^{18}F]fluoride (Fig. **3**).

21-Fluoro-21-deoxytriamcinolone acetonide **8** and 21-fluoro-21-deoxydexa methasone **9** exhibited good relative binding affinities (RBAs) towards the GR (RBA = 174% and 136%, respectively; RBA (dexamethasone) = 100%). 21-Fluoro-ORG 6141 **10** exhibited very high affinity for the GR with a reported K_D value of 0.6 nM [26]. The radiosynthesis of 21-[^{18}F]fluoro-21-deoxytriamcinolone acetonide **8** was carried out starting from the corresponding 21-triflate precursor in THF at 0°C for 20 min. Two consecutive HPLC purification steps were required to separate the product from co-eluting mass. Thus, the radiochemical yield was very low (0.6%), and the specific activity was determined to be 1.5 GBq/μmol. Radiosynthesis of 21-[^{18}F]fluoro-21-deoxydexamethasone **9** proceeded best with 21-triflate precursor in THF at room temperature for 20 min. to afford the radiotracer in radiochemical yield of 34% at a specific activity of 1 GBq/μmol. The 21-tosylate precursor was used for the radiosynthesis of 21-[^{18}F]fluoro-ORG 6141 **10**. 21-[^{18}F] Fluoro-ORG 6141 **10** was obtained in 10% radiochemical yield at a specific activity of 8.2 to 37 GBq/μmol after a reaction time of 5 min at 70 °C.

Data on biodistribution have been reported for 21-[^{18}F] fluoro-21-deoxytriamcinolone acetonide **8** and 21-[^{18}F] fluoro-ORG 6141 **10** in adrenalectomized male rats. For both radiotracers significant activity accumulation was found in the bones (1.5 %ID/organ and 31 %ID/organ at 1 h p.i., respectively), which is indicative for *in vivo* radiodefluorination. Radiodefluorination seems to be a general problem for compounds possessing a 21-[^{18}F]fluoro-20-keto substitution pattern. Chemical enolisation or enzymatic hydroxylation leads to highly unstable [^{18}F]fluorohydrins, which easily release [^{18}F]fluoride, which then is rapidly accumulated in the bones.

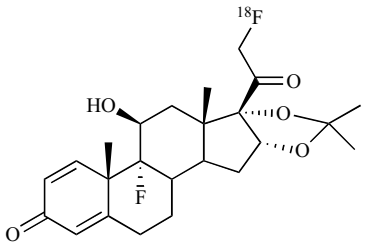
21-[^{18}F]Fluoro-21-deoxytriamcinolone acetonide **8** and 21-[^{18}F]fluoro-ORG 6141 **10** showed high radioactivity uptake in the pituitary as a known GR target site outside the blood-brain-barrier. Only little uptake or retention of the radiotracers was observed in GR-containing brain target tissues like hippocampus and cortex.

Two independent studies described the radiosynthesis and radiopharmacological evaluation of [^{18}F]RU 52461 **13**, a potent synthetic glucocorticoid containing a 17α-alkynyl side chain (RBA = 59%) [25, 27]. The radiosynthesis of [^{18}F] RU 52461 **13** was accomplished *via* two different synthetic routes starting either from the corresponding mesylate **11** (method A) or bromo precursor **12** (method B) (Fig. **4**).

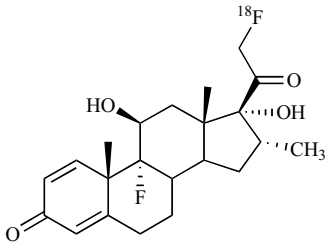
Method A afforded the radiotracer in low radiochemical yield of 5% at a specific activity of 16 GBq/μmol, whereas method B gave the radiotracer in much better radiochemical yields of 12-30% at higher specific activities of 33-55 GBq/μmol. However, method B required two successive HPLC purifications to provide radiochemically and chemically pure product.

Radiopharmacological evaluation of [^{18}F]RU 52461 **13** involved tissue distribution studies in rats and a PET study in baboon brain. Biodistribution studies in rats showed high uptake in the adrenals and retention in the pituitary. All other brain regions showed only low uptake of radioactivity. Except for the pituitary, blocking experiments with RU 28362 **5** or dexamethasone **4** in adrenalectomized rats showed no evidence of receptor-mediated uptake. Increasing bone uptake over time, which culminated in a nearly 90% accumulation of the injected activity in bone after 3 h, is indicative of substantial radiodefluorination *in vivo* [25].

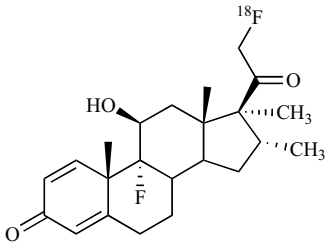
The PET studies showed very low levels of radioactivity in baboon brain, suggesting that [^{18}F]RU 52461 **13** is not capable of penetrating the BBB sufficiently. The lack of BBB penetration in baboon and the high degree of radiode-



21-[^{18}F]fluoro-21-deoxy-triamcinolone acetonide **8** (RBA = 174%)



21-[^{18}F]fluoro-21-deoxy-dexamethasone **9** (RBA = 136%)



21-[^{18}F]fluoro-ORG 6141 **10** (K_D = 0.6 nM)

Fig. (3). 21-[^{18}F]fluoro-substituted steroids as GR radiotracers [25, 26].

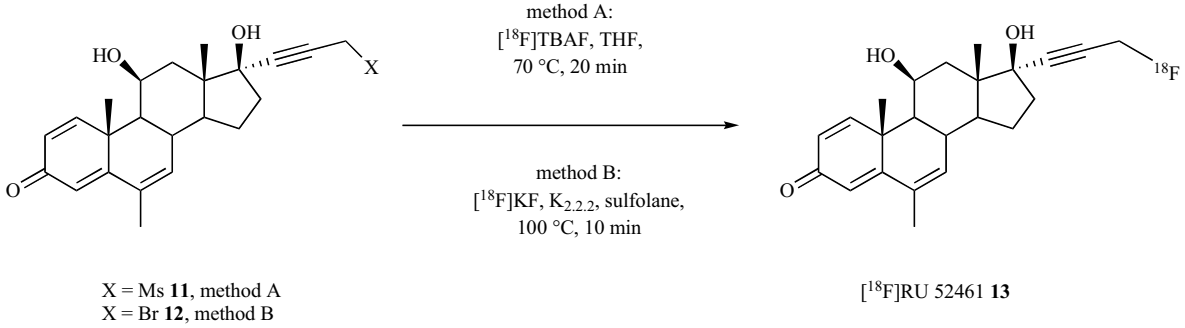


Fig. (4). Two different synthesis routes for the radiosynthesis of [^{18}F]RU 52461 **13** [25, 27].

fluorination in rodents make [^{18}F]RU 52461 **13** not suitable for imaging brain GRs.

Various carbon-11 labeled radiotracers for GRs based upon endogenous and highly potent synthetic steroids have also been described [28, 29].

A first approach dealt with the radiosynthesis and radiopharmacological evaluation of [^{11}C]RU 40555 **15**, a derivative of the potent progesterone antagonist RU 486 with high affinity toward the GR (K_i = 2.4 nM) [30]. The radiosynthesis is based on methylation of the desmethyl precursor **14** with [^{11}C]methyl iodide in dimethylimidazolidinone (DMI) or [^{11}C]methyl triflate in acetone as the labeling precursors (Fig. **5**).

In the case of [^{11}C]methyl iodide as the labeling precursor the radiochemical yield was 8%, whereas the use of more reactive [^{11}C]methyl triflate gave significantly improved radiochemical yields of up to 19%. The specific activity was determined to be 75.5±14 GBq/μmol for the compound prepared with [^{11}C]methyl triflate [28]. According to the authors, the isopropyl group at the amine should stabilize the carbon-11 label in [^{11}C]RU 40555 **15** against metabolism. PET studies in normal and adrenalectomized rats showed low brain uptake, and maximum accumulation of radioactivity in the brain was observed after 20 min. Radioactivity concentration in plasma was in the same range as found in the brain. In normal rats the percentage of injected dose (%ID/g) in selected brain regions like cortex, striatum, hippocampus, and cerebellum was reported to be 0.03. In adrenalectomized rats the brain uptake of radioactivity was increased by 20% compared to normal rats. After 20 min, 51% of parent [^{11}C]RU 40555 **15** was found in normal rat plasma.

Recently, a novel class of glucocorticoids containing a modified C-17 side chain has been identified in a screening effort to discover functionally dissociated GR modulators [29]. These steroids bind to the GR causing a conformational change in the receptor that allows it to transrepress gene transcription but have little or no transactivation activity [31, 32]. Within a series of 21-methylthioethers, one compound (**17**) derived from triamcinolone acetonide showed promising binding toward the GR (RBA = 144%, RBA (dexamethasone) = 100%), and was chosen for ^{11}C labeling (Fig. **6**).

The radiolabeling was carried out starting from corresponding 21-*S*-thiobenzoate **16** and *in situ* formation of the corresponding sodium thiolate, which was further reacted with [^{11}C]methyl iodide. The obtained radiochemical yield was 20-30%. The specific activity was determined to be 20-40 GBq/μmol at the end-of-synthesis, and the radiochemical purity exceeded 98%. No data on the radiopharmacological evaluation of radiotracer **17** has been reported.

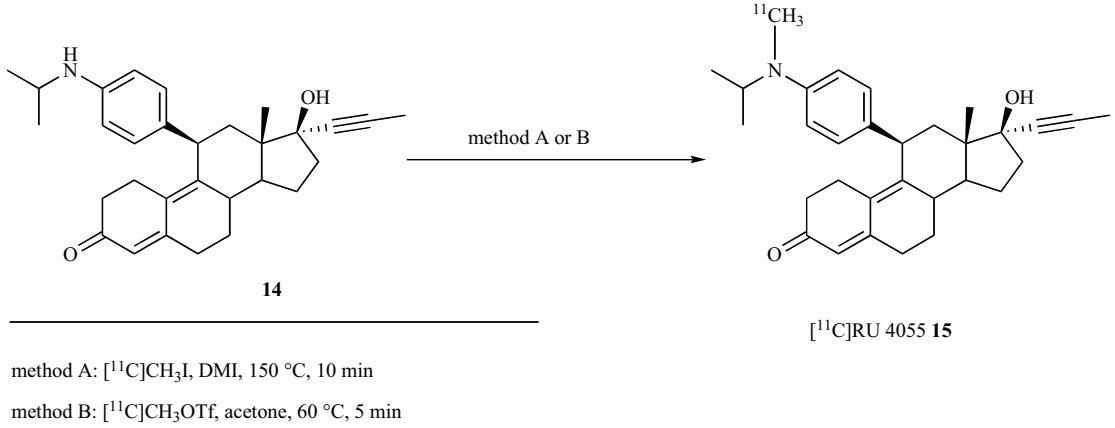


Fig. (5). Radiosynthesis of [^{11}C]RU 40555 **15** [28].

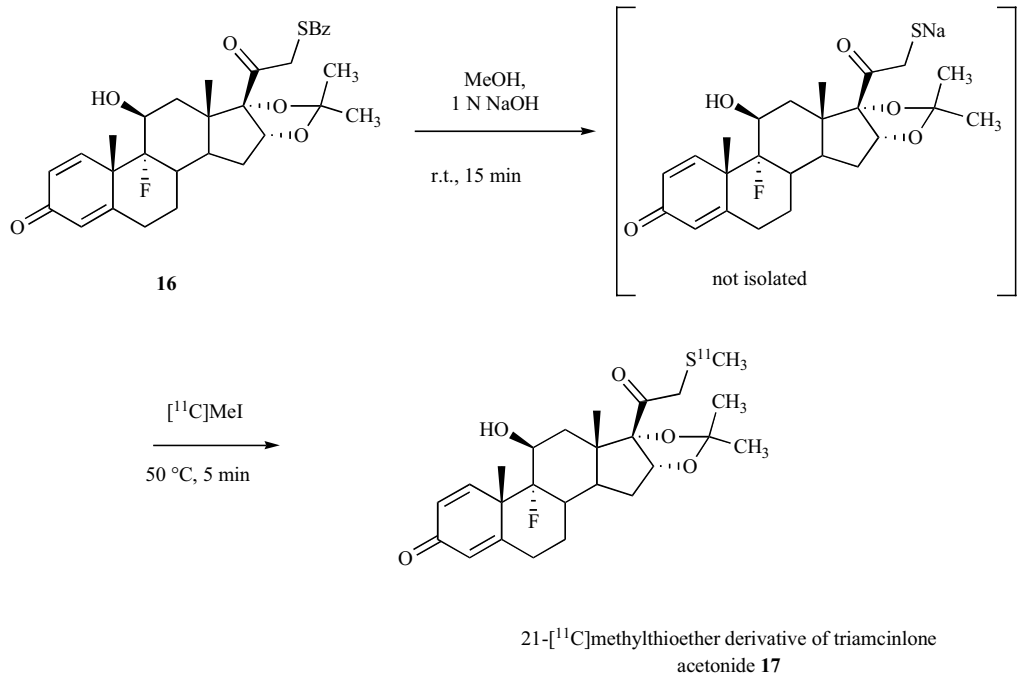


Fig. (6). Radiosynthesis of 21-[^{11}C]methylthioether derivative **17** [29].

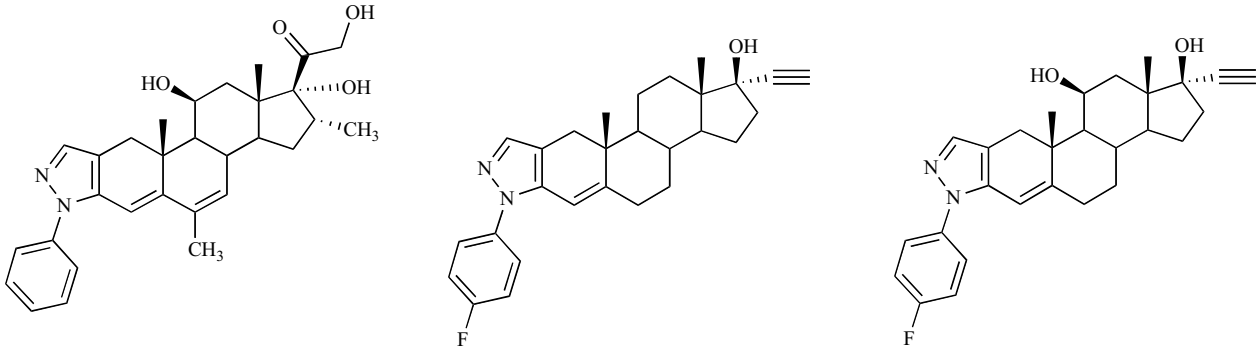
2. Design and Synthesis of GR-Binding Radiotracers Based Upon Steroids Containing an aryl-[3,2-c]pyrazole Moiety

A second approach for the development of PET radiotracers for imaging brain GRs is based upon a unique set of synthetic aryl-[3,2-c]pyrazolo steroids, exemplified by deacylcortivazol **18**, nivazol **19** and WIN 44577 **20**. Deacylcortivazol **18**, nivazol **19**, and WIN 44577 **20** are very high-affinity ligands (RBA (dexamethasone) = 100%, K_D = 3.8 nM [25]) for the GR (Fig. **7**).

The promising GR binding affinity of compounds like WIN 44577 **20** make this class of compounds make this class of compounds attractive for the development of novel fluorine-18 labeled ligands for PET studies of brain GR.

A first radiosynthesis of a ^{18}F-labeled aryl-[3,2-c]pyrazolo steroid was reported by Feliu [33]. The laborious five step synthesis sequence is depicted in Fig. (**8**).

The challenging radiosynthesis of [^{18}F]WIN 44577 **20** commenced with the introduction of [^{18}F]fluoride into 1,4-dinitrobenzene **21** *via* nucleophilic aromatic substitution. The obtained decay-corrected radiochemical yield was 85%, and the resulting 4-[^{18}F]fluoronitrobenzene **22** was further converted into 4-[^{18}F]fluoroaniline **23** (90% radiochemical yield). Treatment of 4-[^{18}F]fluoroaniline **23** with HCl and $NaNO_2$ gave diazonium salt **24**, which was further reacted with an excess of cyanoborohydride to give 4-[^{18}F]fluorophenyl hydrazine hydrochloride **25**. 4-[^{18}F]Fluorophenyl hydrazine **25** was condensed with a 1,3 dicarbonyl compound **26** to yield the desired 4-[^{18}F]fluorophenyl-[3,2-c]pyra-



Deacylcortivazol **18**
RBA = 175%

Nivazol **19**
RBA = 39%

WIN 44577 **20**
RBA = 97%

Fig. (7). High-affinity aryl-[3,2-c]pyrazolo glucocorticoids.

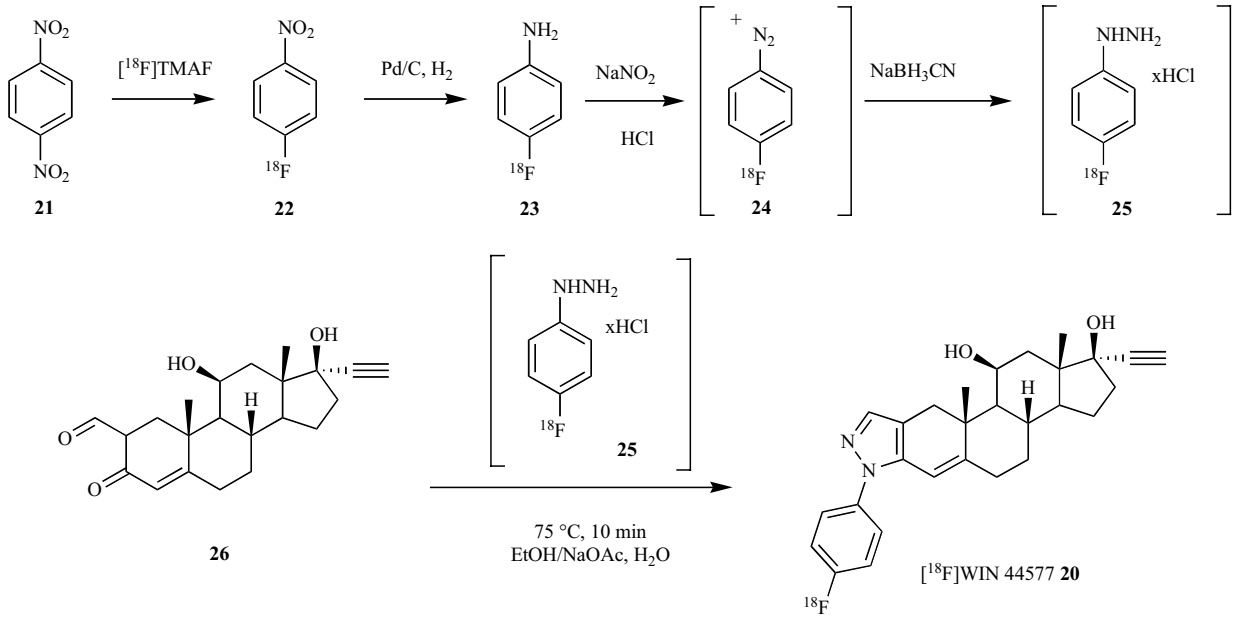


Fig. (8). Radiosynthesis of [^{18}F]WIN 44577 **20** [33].

zole [^{18}F]WIN 44577 **20**. The total radiochemical yield for the challenging five step synthesis sequence was remarkably good, being 6% after a synthesis time of 100 min. The specific activity was 48-111 GBq/μmol (related to the diazonium salt intermediate). No radiopharmacological data were published.

Another set of compounds possessing the characteristic 4-fluorophenyl pyrazole motif showed RBA values towards the GR of up to 56% (related to dexamethasone, RBA = 100%) [34]. Selected compounds were chosen for ^{18}F labeling.

A first approach dealt with the introduction of [^{18}F] fluoride into position 21 of the 17α,21-dihydroxy-20-keto side chain, or into a 17α-alkynyl side chain in the D-ring of the steroid skeleton. The radiosyntheses are given in Fig. (**9**).

The radiosynthesis of 21-[^{18}F]fluoro- and 17α-propynyl-substituted compounds **28** and **30** as shown in Fig. (**9**) pro-

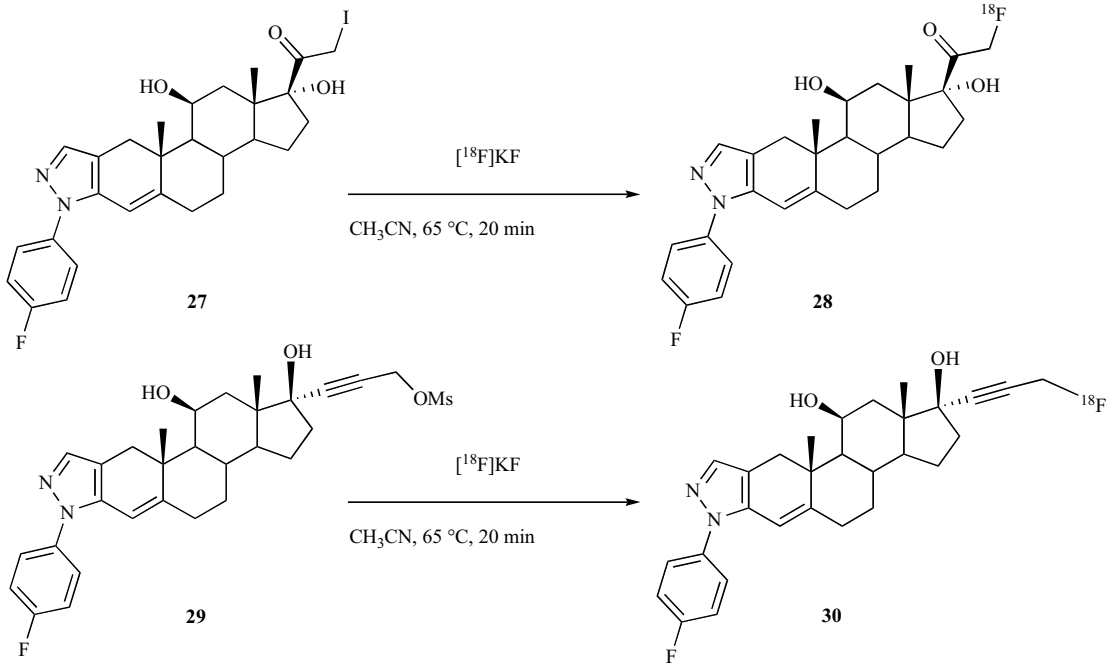


Fig. (9). Radiosynthesis of 4-fluorophenyl-[3,2-c]pyrazolo steroids [34].

ceeded by [^{18}F]fluoride ion displacement of the corresponding iodo precursor **27** or mesylate precursor **29**. The reactions were carried out in acetonitrile at 65°C for 20 min. The radiochemical yield was 14% and 9%, respectively. Despite the relatively moderate binding affinity (RBA = 11%) of 21-[^{18}F]fluorosubstituted compound **28**, the radiotracer was used in radiopharmacological studies in normal male rats [35]. The radiotracer showed promising brain uptake (0.24±0.02% ID/g) at 5 min p.i., which remained constant over 60 min. The biodistribution profile of the radiotracer is consistent with its high lipophilicity (*logP* = 3.9). Interestingly, the biodistribution data showed only little radioactivity uptake in bone (0.33±0.04 %ID/g at 5 min; 0.33±0.07 %ID/g at 60 min), which reflects little radiodefluorination *in vivo*. The systemic clearance was predominatly governed by hepatobiliary elimination. Radioactivity uptake in pituitary and thymus as the potential GR-containing target organs was not increased compared to other main tissues. Blocking experiments with hydrocortisone did not confirm receptor-mediated uptake. Small animal PET images clearly visualized the brain. Ex vivo autoradiography showed higher radioactivity accumulation in the pituitary gland, cerebellar nuclei, plexus chorioideus and inferior colliculi than in surrounding tissue.

21-[^{18}F]fluorodeacylcortivazol **32** is another example for a radiotracer possessing an aryl-[3,2-c]pyrazole motif [36]. The radiosynthesis is depicted in Fig. (**10**).

Treatment of triflate precursor **31** with tetrabuylammonium [^{18}F]fluoride ([^{18}F]TBAF) gave the radiotracer **32** at a specific activity of 13.7 GBq/µmol. The obtained radiochemical yield was not reported. Radiopharmacological evaluation of the radiotracer in adrenalectomized rats showed only little radiodefluorination *in vivo* (bone: 0.17±0.05% ID/g at 2 min; 0.49±0.05 % ID/g at 2 h). This finding agrees with the results obtained by Wuest *et al*. [35]. The brain to blood ratios at 2 h and 8 h post injection were 6.71±0.26 and 5.43±0.64, respectively.

In order to incorporate the ^{18}F label into the metabolically stable position of the aromatic ring, a nucleophilic aromatic substitution of [^{18}F]fluoride on diaryliodonium salts has been explored (Fig. **11**) [34].

The used diaryliodonium salts differ in their functionalization of the aryl ring, being phenyl **33** and tolyl **34**, respectively. Radiochemistry was performed using the powerful nucleophilic radiofluorinating agent [^{18}F]KF in DMF as the solvent at 120 °C for 40 min. The use of phenyl-functionalized iodonium salt **33** gave the desired product in only 0.2% yield. Since the attack of the fluoride ion preferentially occurs on the more electron-deficient aromatic ring, the use of the more electron-donating tolyl-fuctionalized iodonium salt **34** was envisaged to favor the formation of the desired radiotracer. Indeed, by using the corresponding tolyl-functionalized iodonium salt **34** the radiochemical yield of compound **35** could be increased up to 2%. Despite the determined binding affinity (RBA = 56%) no pharmacological data of compound **35** are available, which was probably due to the very low radiochemical yields.

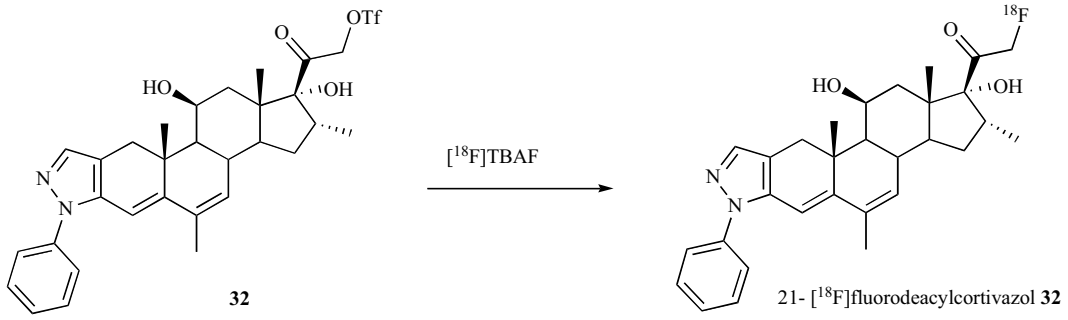


Fig. (10). Radiosynthesis of 21-[^{18}F]fluorodeacylcortivazol **32** [35].

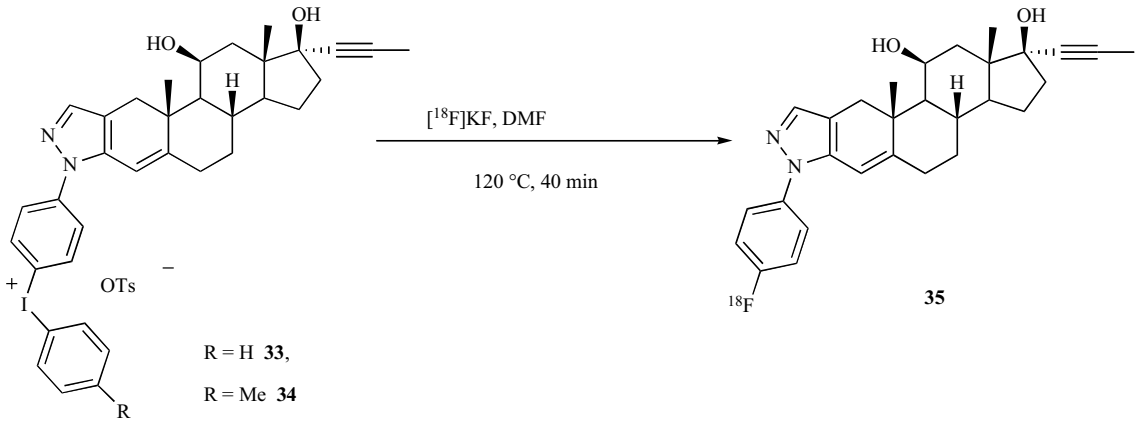


Fig. (11). Radiochemical synthesis involving iodonium salts [34].

Related papers on aryl-[3,2-c]pyrazolo-corticosteroids described a set of compounds possessing a pyridyl or pyrimidyl ring in the structure (Fig. **12**) [37, 38].

Some of the compounds, in particular, fluoropyridyl derivatives **36** and **37**, showed high affinity for the GR. The corresponding chloropyridyl precursors are very useful for the incorporation of fluorine-18 through a nucleophilc aromatic substitution with [^{18}F]fluoride. The incorporation could drastically be enhanced by microwave activation. However, no radiosynthesis, and, therefore, no radiopharmacological data were reported.

3. Design and Synthesis of GR-Binding Radiotracers Based Upon Non-Steroidal Compounds Consisting of Heterocyclic Core Structures

Recently identified non-steroidal compounds with high affinity and receptor selectivity towards the GR provide an interesting alternative for the design and synthesis of PET radiotracers for imaging brain GRs. Non-steroidal GR-binding ligands possess a broad structural variety, and prominent examples are based on dibenzyl anilines, *N*-arylpyrazolo-based ligands or benzopyrano-quinolines (Fig. **13**) [39].

AL-438, a benzopyrano-quinoline was identified as one of the first compounds exhibiting both, high affinity (K_i = 2.5 nM) and selectivity for the GR [40]. Moreover, the methoxy group at position 10 of AL-438 makes this compound an attractive candidate for labeling with carbon-11. The radiolabeling was accomplished *via O*-methylation of the corresponding desmethyl precursor **38** with [^{11}C]methyl iodide (Fig. **14**).

The radiotracer **39** was obtained in radiochemical yields of 30±4% within 35 min at a specific activity ranging from 10-15 GBq/μmol at the end-of-synthesis [41].

Compound **39** was tested in an *in vitro* competitive GR and PR binding assay. The inhibition constant (pEC_{50}) was determined to be 8.1 for the GR and 6.9 for the PR; which is indicative of a favourable GR/PR selectivity.

Radiopharmacological evaluation of the radiotracer involved biodistribution and small animal PET imaging in normal rats. The radiopharmacological evaluation was completed with autoradiography studies using rat brain slices. Biodistribution studies demonstrated high radioactivity uptake in pituitary and brain of 2.49 %ID/g and 1.52 %ID/g after 5 min, and 1.77 %ID/g and 0.56 %ID/g after 60 min, respectively. However, the inability of high dose corticosterone to block binding suggested that the radioactivity accumulation in the brain was not receptor-mediated. The biodistribution pattern of [^{11}C]AL-438 **39** corresponded with the high lipophilicity (*logP* = 6.1) of the compound. Especially high radioactivity concentration was found in the brown fat.

However, the ease of synthesis, the high *in vitro* GR binding affinity and selectivity, and the promising brain uptake of [^{11}C]AL-438 **39** make non-steroidal GR-binding ligands interesting candidates for the further design and synthesis of novel radiotracers for imaging GRs.

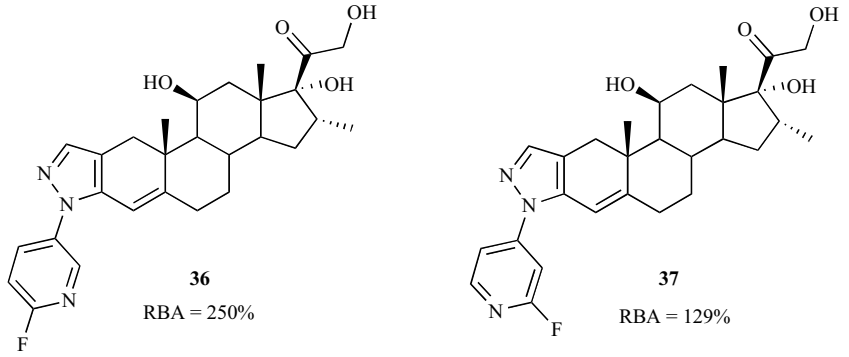


Fig. (12). Structure of fluoropyridyl derivatives of [3,2-c]pyrazolocorticosteroids [37, 38].

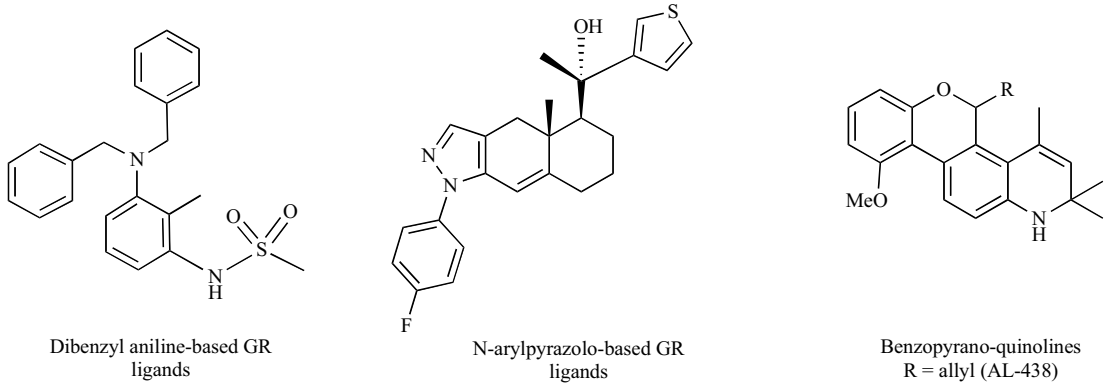
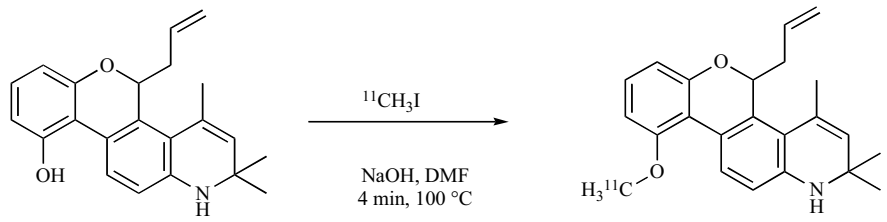


Fig. (13). Structures of non-steroidal GR ligands [39].



38 [^{11}C]AL-438 **39**

Fig. (14). Radiosynthesis of [^{11}C]AL-438 **39** [41].

SUMMARY AND CONCLUSION

Over the last 20 years, many research efforts have been made to develop radiotracers for imaging brain GRs by means of PET. Based on the general prerequisites for the design and synthesis of PET radiotracers for imaging brain receptors, numerous compounds have been prepared and subjected to preclinical evaluation. However, to date, no suitable PET radiotracer for imaging brain GRs has been developed to enter the clinic for application in humans. The inability to penetrate the blood-brain-barrier, high non-specific binding and/or *in vivo* instability, mainly governed by radiodefluorination, are the major obstacles which have prevented successful development of GR-binding radiotracers.

The GR is a challenging molecular target for the radiotracer design. The low concentration of the receptor protein inside the cell requires ligands, which bind to the GR with high affinity and selectivity. In this line, a broad variety of structurally different compounds has been developed. Starting from endogenous and highly potent synthetic steroids, first attempts to develop radiotracers for the GR dealt with the incorporation of the short-lived positron emitter fluorine-18 into the steroid backbone. The rational behind this approach was the bioisosteric substitution of a hydroxyl group or a hydrogen atom with fluorine-18 within a series of compounds possessing the characteristic 17α,21-dihydroxy-20-keto substitution pattern or a 17α-alkynyl side chain. The inauguration of aryl-[3,2-c]pyrazoles as novel lead structures for the design of highly selective GR ligands has prompted further radiopharmaceutical research on GR-binding radiotracers. However, although some of the prepared compounds showed promising brain uptake, the observed high non-specific binding due to high lipophilicity has made this class of GR ligands not suitable for further radiotracer development efforts.

The recently reported non-steroidal GR ligands open a novel and promising avenue towards GR-binding radiotracers. The non-steroidal compounds comprise a structural variety ideal for the fine tuning of receptor binding affinity, selectivity, and lipophilicity.

The lessons learnt from more than 20 years of research on GR-binding radiotracers have clearly shown that ligands with sub-nanomolar (<1 nM) binding affinity towards the GR and moderate lipophilicity (*logP* between 2 and 3) will be needed to prepare PET radiotracers with a radiopharmacological profile suitable for imaging brain GRs *in vivo*.

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