P193 MICRO-PET IMAGING AND TUMOR CELL BINDING STUDIES OF SIGMA RECEPTOR LIGANDS: A COMPARISON WITH METABOLIC PET TRACERS

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Introduction: The common PET tracer FDG is not tumor-specific, but can also accumulate in inflammatory cells leading to false-positive lesions. Sigma receptors are strongly overexpressed in most tumors. We therefore examined whether radiolabeled sigma ligands are more tumor-specific than FDG, and quantified their uptake in comparison to established oncological PET tracers. We also checked whether endogenous steroids can reduce the binding of sigma ligands to tumor cells.

Experimental: ^{11}C-Choline, ^{11}C-methionine,^{18}F-FLT, and ^{18}F-FDG were prepared by literature procedures. ^{11}C-SA4503 (sigma-1 ligand) and ^{18}F-FE-SA5845 (non-subtype-selective sigma ligand) were made by reaction of ^{11}C-methyl iodide and ^{18}F-fluoroethyl tosylate with the appropriate 4-O-methyl compound. The tracers were administered to male Wistar rats that bore a C6 glioma in the right shoulder and also had sterile inflammation in the left calf muscle, induced by injection of 0.1 ml of turpentine. Twenty-four hours after administration of turpentine, the rats received an i.v. bolus of PET tracer. ^{18}F-FLT animals were pretreated with i.v. fosforylase (1000 U, infused from 30 to 10 min before tracer administration). Whole body images were made with a microPET Focus 220 scanner, 20-60 min after tracer injection. Binding of ^{11}C-SA4503 to C6 cells grown in monolayers was quantified after 60 min at 37°C in the presence and absence of various steroids.

Results and Discussion: ^{18}F-FDG was taken up avidly both by the tumor and by the inflamed leg. ^{11}C-choline accumulated less in tumor and inflammatory tissue than ^{18}F-FDG and provided no selectivity advantage. ^{11}C-Methionine showed marginally better tumor selectivity than ^{18}F-FDG but poor tumor-to-muscle contrast. ^{18}F-FLT, ^{11}C-SA4503 and ^{18}F-FESA5845 visualized the tumor but not the inflammation. Unfortunately, tumor-to-muscle contrast of these three novel tracers was much lower than that of ^{18}F-FDG. In vitro binding studies with intact C6 cells indicated that various steroid hormones compete with sigma ligands for their binding sites in tumor tissue. Progesterone was the most potent competitor, followed (ex aequo) by testosterone, the progesterone metabolite allopregnanolone and the testosterone metabolite androstanolone. Dehydroeepiandrosterone sulfate was ineffective.

Conclusion: In this animal model, sigma ligands and the nucleoside ^{18}F-FLT are more tumor-selective but less sensitive than ^{18}F-FDG. Our measurements of ^{11}C-SA4503 binding to intact tumor cells suggest that steroid hormones and their metabolites bind to sigma receptors and can reduce the tumor uptake of sigma ligands in vivo.

P194 EARLY CHANGES OF FDG AND SIGMA LIGAND UPTAKE IN RAT GLIOMAS AFTER IN VIVO TREATMENT WITH DOXORUBICIN

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Introduction: Metabolic changes induced by chemotherapy precede the morphological changes, therefore quantitative imaging of tissue biochemistry may be used to predict tumor response to treatment at a relatively early stage. Sigma receptors are strongly over-expressed in most rodent and human tumors and are proliferation markers. To evaluate the potential of a radiolabeled sigma ligand for therapy monitoring, we compared early changes of ^{11}C-SA4503-binding and ^{18}F-FDG uptake in rat gliomas after in vivo chemotherapy.

Experimental: C6 cells ($2.5x10^6$) were s.c. injected in the right shoulder of male Wistar rats. After 7 d, the tumor mass was 0.60 ± 0.08 g. Control animals were then injected with saline (carrier, i.p.), whereas treated animals received doxorubicin (8 mg/kg). SA4503 and doxorubicin were selected on the basis of in vitro experiments in C6 cells. One control and one treated rat were scanned simultaneously, 24 or 48 h after treatment, under pentobarbital anesthesia (60 mg/kg). The total number of animals was 20 (5 for each time point and treatment group). A list mode protocol was run on a microPET Focus 220 camera (60 min, tumors in field of view). Animals were maintained in a fixed position and scanned first with ^{11}C-SA4503 (25 MBq, i.v.), followed after > 5 half-lives of ^{11}C by ^{18}F-FDG (20 MBq, i.v.). Finally, the biodistribution of ^{18}F was assessed. Tumor homogenates (10% w/v in 50 mM Tris-HCl, pH 7.4 containing 0.32 M sucrose) were prepared and stored at –80°C for sigma receptor assays.

Results and Discussion: Body weight of saline-treated rats showed a steady increase (from 337 ± 6 to 365 ± 6 g in the last 7 days, mean ± s.e.m.). Doxorubicin-treated rats reduced their food intake. Here, body weight increased until treatment (from 333 ± 6 to 348 ± 5 g in 5 days) but remained steady thereafter (345 ± 6 g at day 7). In both groups, tumors appeared 4-5 days after inoculation and grew logarithmically. No significant reduction of tumor growth was visible within 48 h after doxorubicin treatment. Tumors were visualized by both PET tracers and their uptake was reduced following chemotherapy (PET data ^{11}C-SA4503: -26.5 ± 6.5% at 24h, -26.5 ± 7.5% at 48h; ^{18}F-FDG: -21.3 ± 3.6% at 24h, -27.1 ± 4.7% at 48h; ex vivo ^{18}F-FDG: -22.4 ± 5.4% at 24h, -31.7 ± 12.7% at 48h, mean ± s.d.). Assays of sigma receptor density in the tumors are still in progress.

Conclusion: Uptake of the sigma ligand ^{11}C-SA4503 and of the glucose analog ^{18}F-FDG both declined in C6 gliomas following chemotherapy. Changes in tracer uptake preceded the morphological changes by at least 48 h.

Keywords: Sigma Receptors, FDG, Tumor, Chemotherapy, MicroPET

P195 5-CHLORO-2-(2'-((DIMETHYLAMINO)METHYL)-4'-IODOPHENYLTHIO)BENZENAMINE: A NEW SEROTONIN TRANSPORTER LIGAND

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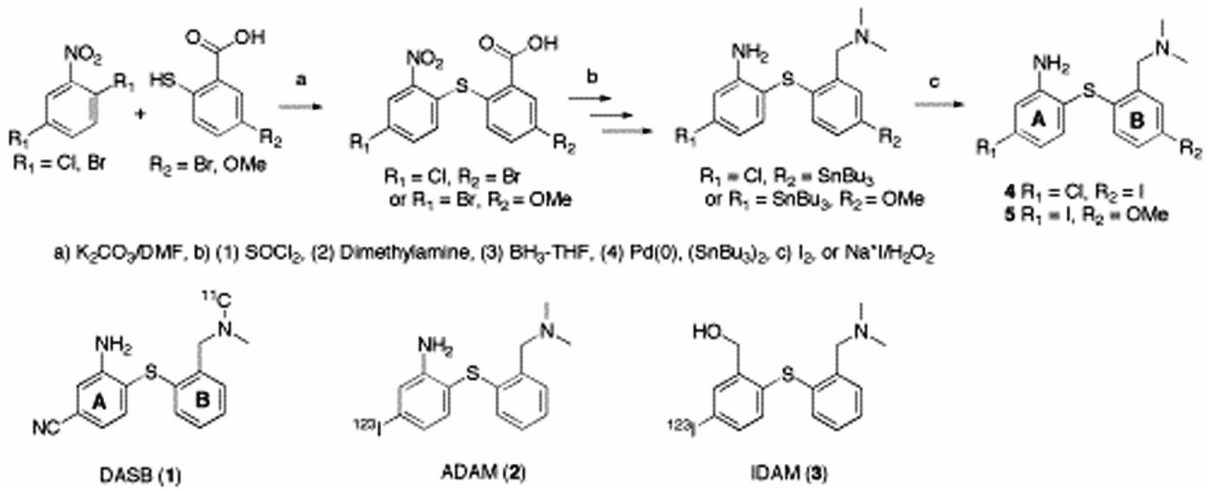
Introduction: Imaging serotonin transporter (SERT) binding sites may provide a useful tool to study mechanisms of drug actions and improve patient management. *In vivo* imaging of SERT binding sites has been significantly improved by utilizing diphenyl sulfide derivatives. Radiolabeled [^{11}C]DASB (**1**), and [^{123}I]ADAM (**2**) for PET and SPECT imaging, respectively, have been tested for studying drug occupancies in human brain. Most of the diphenyl sulfides reported, including IDAM (**3**) contain substitutions mainly on the phenyl ring A. We report herein novel diphenyl sulfides, **4** and **5**, containing 4'-substitutions on the phenyl ring B.

Experimental: Compounds **4**, **5** were prepared as shown in scheme. ^{125}I labelings were achieved by iodo-destannylation reaction (radiochemical yield >95%). The cold compounds **4**, **5** were used to measure inhibition constants (K_i). Biodistribution and *ex-vivo* autoradiography in rats, and baboon SPECT study were performed.



Results and Discussion: Inhibition constants (K_i) of **4** and **5** showed high affinities to SERT (K_i = 0.22 nM, **4**, 0.11 nM, **5**). Biodistribution study of [^{125}I]**4** in rats displayed excellent brain uptakes and prolonged retention in hypothalamus, a region with high SERT concentration. (Brain uptakes were 1.77, 1.21, 0.98, 0.75, 0.57 and 0.26%dose/g and the hypothalamus/cerebellum ratios were 0.89, 2.90, 4.24, 7.10, 8.24 and 12.6 for 2, 60, 120, 240, 360 and 720 min, respectively). The localization of [^{125}I]**4** in the hypothalamus region in rats brain was blocked by pretreatment with SERT ligands. *Ex-vivo* autoradiography in rat brain and initial SPECT imaging of baboon brain demonstrated excellent localizations of **4** in the hypothalamus.

Conclusion: A novel diphenyl sulfide derivative, **4**, with a 4'-iodo-group on the phenyl ring B, displayed excellent binding affinity and selectivity. Moreover, the 4'-iodo-diphenyl sulfide derivative dramatically changed the kinetics of *in vivo* biodistribution resulting in improved hypothalamus uptake and retention, a property desirable for a SPECT imaging agent. This novel ligand **4**, or its analogs, may be potential candidates for studying SERT binding sites by *in vivo* SPECT imaging.

Acknowledgement: This work was supported by a grant awarded from the National Institutes of Health (R01-MH068782, H.F.K.).

Keywords: Serotonin Transporter, SPECT, Brain Imaging, Autoradiography

P196 5-BROMO-2'-(2-((DIMEYHYLAMINO)METHYL)-4'-(2-(^{18}F)FLUOROETHOXY)-PHENYLTHIO)BENZENAMINE: A HIGHLY SELECTIVE SEROTONIN TRANSPORTER IMAGING AGENT

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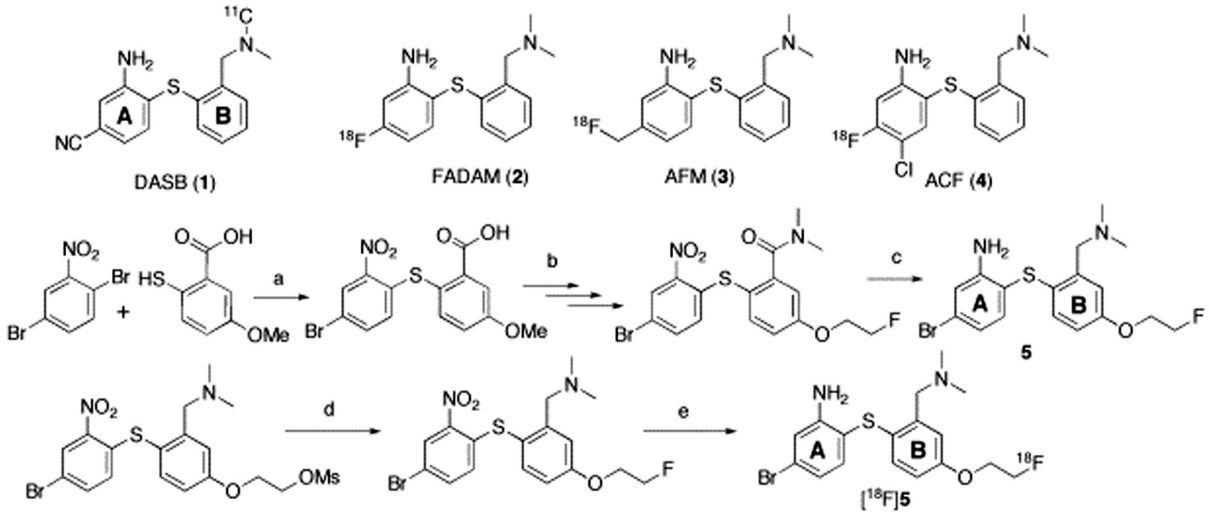
Introduction: The association between the serotonergic functions and depression is well documented. Selective serotonin reuptake inhibitors (SSRIs) are currently being widely prescribed to treat patients with depression. Imaging serotonin transporter (SERT) binding sites may be useful to improve the management of patients on SSRIs. For PET, ^{11}C labeled DASB (**1**) has been tested in humans to study drug occupancy. Several ^{18}F labeled SERT imaging agents, such as FADAM (**2**), AFM (**3**), ACF (**4**) have been developed. Those compounds are diphenyl sulfide derivatives containing substitutions on the phenyl ring A. In this report, a novel ^{18}F labeled ligand with a 4'-fluoroethoxy group on the phenyl ring B, [^{18}F]**5**, was tested as a SERT imaging agent.

Experimental: Compound **5** was prepared as shown in scheme. ^{18}F labeling was performed from corresponding mesylate precursor by ^{18}F/K222 reaction followed by a reduction of nitro group (overall radiochemical yield ~10%). Inhibition constant (K_i), biodistribution in rats and baboon PET study were performed.



Results and Discussion: Compound **5** showed a high binding affinity to SERT (K_i=0.09 nM). Biodistribution of [^{18}F]**5** in rats displayed excellent brain uptakes and prolonged retention in hypothalamus a region with high concentration of SERT. (Brain uptakes were 2.05, 1.88, 1.92, 2.16, 1.57 and 1.36%dose/g and the hypothalamus to cerebellum ratios were 0.95, 2.04, 2.97, 3.70, 4.26 and 4.32 for 2, 30, 60, 120, 240 and 360 min, respectively). The localization of [^{18}F]**5** in the hypothalamus in the rat brain was blocked by a pretreatment with (+)McN5652, escitalopram or ADAM, all selective SERT ligands. Initial PET imaging of baboon brain demonstrated an excellent localization of [^{18}F]**5** in the hypothalamus.

Conclusion: A novel diphenyl sulfide derivative, [^{18}F]**5**, was prepared and tested as a SERT imaging agent for PET. This new ligand, with a 4-(2'-fluoroethoxy)-group on the phenyl ring B, displayed excellent binding affinity and selectivity. It dramatically changed the kinetics of *in vivo* biodistribution and improved the hypothalamus uptake and retention. This novel ligand and its analogs are potential PET imaging agents for studying SERT.

Acknowledgement: This work was supported by a grant awarded from the National Institutes of Health (R01-MH068782, H.F.K.).

Keywords: Serotonin Transporter, F-18, Brain Imaging, Diphenyl Sulfide

P197 CYCLEN-BASED COPPER COMPLEXES AS POTENTIAL ESTROGEN RECEPTOR LIGANDS: SYNTHESIS, BINDING AFFINITY, AND COMPUTER MODELING

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Introduction: The estrogen receptor (ER), which is over-expressed in ER-positive breast tumors, can be imaged by positron emission tomography (PET) using ^{18}F-labeled steroidal estrogen ligands such as [^{18}F]FES. Compared to Cu-64 ($t_{1/2}$ = 12.7 h), the half-life of F-18 ($t_{1/2}$ = 1.8 h) is relatively short, and radio-fluorination yields are usually low compared to those for Cu-64 complexation to a suitable chelate system. 1,4,7,10-Tetraazacyclododecane (cyclen) is a widely used chelate that forms stable metal complexes with copper, indium, gallium, and gadolinium. Molecular modeling studies suggested that a suitably derivatized cyclen could mimic estrogen. With this in mind, we incorporated two phenolic hydroxyl groups in cyclen-based Cu complexes to mimic estradiol.

Experimental: 1,7-Diprotected cyclen, 1,7-bis(benzyloxycarbonyl)-cyclen, was synthesized according to the reported procedure. After introducing two 4-(benzyloxy)benzyl groups at 4,10-positions, the benzyloxycarbonyl and benzyl groups were both removed by hydrogenation over Pd/C to give 1,7-bis(4-hydroxybenzyl)-cyclen (**1**). 1,7-Bis(*tert*-butoxycarbonylmethyl)-4,10-bis(benzyloxybenzyl)-cyclen was prepared from 1,7-bis(*tert*-butoxycarbonylmethyl)-cyclen by a similar method. After hydrolysis in 6 M HCl, the final product, 1.7-bis(carboxylmethyl)-4,10-bis(4-hydroxybenzyl)-cyclen (**2**) was obtained as the HCl salt.

Results and Discussion: The novel chelates **1** and **2** were fully characterized by ^{1}H, ^{13}C-NMR and mass spectrometry, and they were then reacted with copper ions to give complexes Cu(**1**)Cl_2, Cu(**1**)$(ClO_4)_2$, and Cu(**2**), whose structures were confirmed by HR-mass spectrometry (FAB). The relative binding affinities of these copper complexes were quite low, being 0.0011, 0.0018 and 0.0019 for ER_α and 0.0013, 0.0024 and 0.0033 for ER_β, respectively [estradiol = 100].

Conclusion: In conclusion, we successfully synthesized two cyclen derivatives bearing two phenol groups at trans N-positions of cyclen. Three Cu(II) complexes, of +2, +1, and neutral overall charge, were prepared as a potential PET tracer for ER imaging, but they showed low relative binding affinities for the ERs, presumably because the two phenol groups are either too bulky or are not properly disposed on the cyclen core. Ongoing Cu-64 labeling and biodistribution studies of these ligands will be reported.

Acknowledgement: This work was supported by the Brain Korea 21 Project in 2007.

Keywords: Estrogen Receptor Ligand, Cu-64, PET, Cyclen, Molecular Modeling

P198 STRUCTURE-SELECTIVITY INVESTIGATIONS OF D2-LIKE RECEPTOR LIGANDS BY CoMFA AND CoMSIA GUIDING THE DISCOVERY OF D3 SELECTIVE PET RADIOLIGANDS

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Introduction: Elucidation of the physiological role of the D3 receptor and its distribution in brain using positron emission tomography (PET) is hampered by the lack of subtype selective tracer ligands. To efficiently approach suitable D3 radioligands, we expected an integrative procedure involving the elucidation of structural features determining D3 selectivity by comparative molecular analysis to be highly beneficial. Extending our recent work on the 3D-QSAR analyses of dopaminergic agents, we describe the generation of selectivity contour maps by CoMFA and CoMSIA displaying the molecular origins for a subtype specific recognition of D3 over D2 and D4. Exploiting this information, the synthesis and receptor binding studies directed us to the synthesis two new fluorinated subtype selective PET tracers with high D3 affinities.

Experimental: We have successfully developed CoMFA and CoMSIA models based on the difference between binding activities (ΔpKi) of a series of 63 ligands representing a very broad range of selectivities (ΔΔpKi(D3/D2) = 5.0 and ΔΔpKi(D3/D4) = 8.1). These models yielded highly significant cross-validations (CoMSIA: q2 cv(D3/D2) = 0.860; CoMFA: q2 cv(D3/D4) = 0.917) and excellent predictions of a 14 ligand test set (r2 pred between 0.790 and 0.932). 4-(4-(2-Methoxyphenyl)piperazinyl)butylamine was coupled with 4-(6-fluoropyridin-2-yl)- or 4-(6-fluoropyridin-3-yl)-benzoic acid with DCC, to give the new compounds (A, B). For the nucleophilic radiofluorination, precursors were synthesized with 4-(6-bromopyridin-2-yl)- or 4-(6-nitropyridin-3-yl)-benzoic acid, described above.

Results and Discussion: The synthesis of the fluorinated compounds A and B, featuring substantial subnanomolar D3 affinities and considerable selectivities for D3 over D2 (A: ΔpKi = 1.86; B: ΔpKi = 1.53) and for D3 over D4 receptors (A: ΔpKi = 1.88; B: ΔpKi = 1.88) and, subsequently, to the subtype selective PET tracers [F-18]A and [F-18]B. Applying aromatic F-18-for-Br(NO2) substitution, high radiochemical yields between 73-86% were obtained for [F-18]A and [F-18]B.

Conclusion: The 3D-QSSR analysis makes it possible to relate chemical structures of ligands with their binding selectivity with respect to different subtypes of a target receptor when using the CoMFA or CoMSIA techniques. The CoMFA and CoMSIA models showed high cross-validation correlation coefficient q2 values for D3/D2 and D3/D4 selectivity, respectively. This was extended to the successful application of our analyses in guiding the synthesis of novel PET tracers for D3 receptors, both revealing high affinity and subtype selectivity.

Keywords: 3D-QSSR, D2-Like Dopamine Receptors, CoMFA/CoMSIA Model, PET

P199 SYNTHESIS AND BIOLOGICAL EVALUATION OF A NOVEL ^{99m}Tc COMPLEX AS POTENTIAL RADIOLIGAND FOR 5-HT$_{1A}$ RECEPTOR IMAGING

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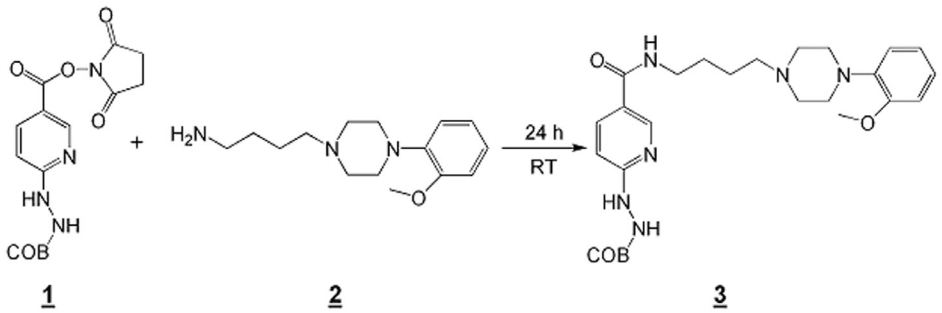
Introduction: The goal of this study is to develop a new ^{99m}Tc-complex as potential 5-HT$_{1A}$ receptor imaging agent. A designed 5-HT$_{1A}$ receptor ligand, HYNIC-MPP (**3**), was synthesized, which containing the MPP ((2-methoxyphenyl)piperazine) motif of WAY-100635 that is known to have high affinity to the 5-HT$_{1A}$ receptor.

Experimental: The synthesis route of complex **3** was showed in Scheme 1. The radiolabeling conditions of complex **3** with ^{99m}Tc were optimized. ^{99m}Tc(HEDTA)-HYNIC-MPP was prepared with coligand HEDTA (Hydroxyethyl Ethylenediamine Triacetic Acid). The characterization of ^{99m}Tc(HEDTA)-HYNIC-MPP was tested by electrophoresis and octanol/water partition coefficient experiments. The in vitro stability was also evaluated under room temperature. Biodistribution of ^{99m}Tc(HEDTA)-HYNIC-MPP was investigated in normal mice.



Scheme 1

Results and Discussion: Complex **3** was synthesized in the yield of 17.2%. Its m.p. was determined as 106-108°C. This brown-yellow powder was conformed by IR, ^{1}H-NMR, and EI-MS. ^{99m}Tc(HEDTA)-HYNIC-MPP was prepared in high yield (>90% by TLC) under the optimized labeling conditions. This labeled complex remained stable over 6 h at RT. ^{99m}Tc(HEDTA)-HYNIC-MPP is a hydrophilic complex (lg*P*=-1.8). The charge property of this labeled complex was observed as neutral (~70%) mainly and with partially negative charge (~30%) from electrophoresis experiments. Biodistribution of ^{99m}Tc(HEDTA)-HYNIC-MPP showed that this complex had moderate brain uptake (0.49%ID/g at 2 min and 0.26%ID/g at 120 min post-injection) and good retention (>50% of the radioactivity was retained in the brain at 120 min p.i.). Unfortunately, this ^{99m}Tc-complex had high blood uptake at 2 min p.i. (15.51%ID/g), although it was cleared very fast from blood, the uptake was still 4.68%ID/g at 120 min p.i.

Conclusion: HYNIC-MPP was designed as a potential 5-HT$_{1A}$ receptor ligand. ^{99m}Tc(HEDTA)-HYNIC-MPP was obtained with high radiochemical purity. Based on the results of the above experiments, we concluded that the ^{99m}Tc(HEDTA)-HYNIC-MPP is a hydrophilic and partially neutral complex. Biodistribution resulted that this complex had moderate brain uptake and very good retention. The specific binding of this radiotracer to 5-HT$_{1A}$ receptor will be tested in near future. On the other hand, improvement of this novel ^{99m}Tc-complex needs further ligand modification to decrease the blood uptake.

Acknowledgement: This project (20401004) was supported by National Natural Science Foundation of China.

Keywords: Technetium-99m, 5-HT1A Receptor Imaging Agent, 6-Hydrazinonicotinic (HYNIC), 2-Methoxyphenyl-Piperazine (MPP), Biodistribution

P200 SYNTHESIS OF A I-123 LABELLED TIAGABINE ANALOGUE. A POTENTIAL RADIOLIGAND FOR THE GABA TRANSPORTER GAT-1

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Introduction: GABA is released at approximately 40% of brain synapses. GABA transporters (GATs) are a key player in fine-tuning the homeostatic balance of the GABAergic inhibitory tone. These high-affinity plasma membrane proteins clear GABA from the synaptic cleft, thereby ensuring the high signal-to-noise ratio that is necessary for proper neurotransmission. The principal neuronal GAT (GAT-1) is responsible for the majority of GABA clearance.

Targeting and modulation of GATs is of therapeutical interest in GABA-related neurological (epilepsy, neuropathic pain and painful tonic spasms in multiple sclerosis) and psychiatric disorders (schizophrenia, bipolar disorders). Gabitril (tiagabine) is a potent selective inhibitor of GAT-1 that has recently been approved as antiepileptic drug, while other clinical applications are currently under investigation.

Temporal lobe epilepsy (TLE) is one of the most prevalent forms of epilepsy. TLE can begin at any time but usually occurs in late childhood. The pathologic lesion that can be found in up to 65% of the TLE-patients has been defined as mesial temporal sclerosis. This pathology is characterized by a loss of neurons and gliosis in the hippocampus, amygdala and parahippocampal structures.

A novel radioligand for SPECT imaging in human brain can be a valuable diagnostic tool to identify and to localize, as early as possible, a brain lesion in patients with epilepsy, which could lead to earlier neurosurgical treatment and seizure relief and consequently improvement of quality of life.

Results and Discussion: In principal, tiagabine might be labelled in one of the thiophene moieties. However, to circumvent Z,E formation we decided to label the vinylic part (Fig. 1). Bromination of tiagabine(**1**) in CCl_4 was straightforward to give **2** in 70% yield. Radioiodination was performed using the Cu(I) mediated non-isotopical exchange reaction generating the Cu(I) *in situ* with gentisic acid. As far as we know, this is the first time this reaction is applied on a vinylic position. The overall radiochemical yield after preparative HPLC (R_t **2** = 42 min., R_t **3** = 48 min.) was 50%, with a radiochemical purity of > 99%.

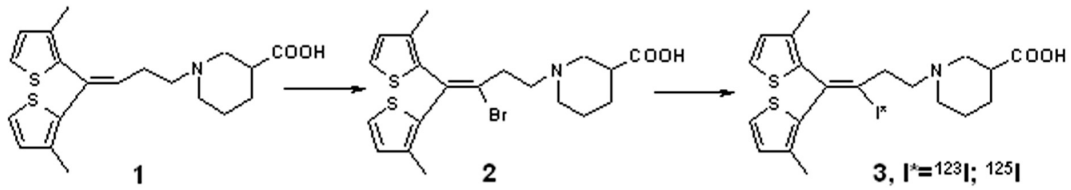

Fig. 1

Initial SPECT imaging showed uptake of **3** in designated rat brain areas. Studies on the selectivity of **3** for GAT-1 as well as its biodistribution in rats are in progress.

Keywords: Tiagabine, GAT-1, I-123

P201 EVALUATION OF GENE TRANSFER USING AN ADENOVIRAL VECTOR ENCODING SOMATOSTATIN RECEPTOR SUBTYPE 2 FUSED TO ENHANCED GREEN FLUORESCENT PROTEIN

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Introduction: Clinical gene therapy trials have traditionally used tissue biopsies for assessing the efficacy of gene transfer. Non-invasive imaging offers a distinct advantage over tissue biopsies in that the magnitude and duration of gene transfer can be monitored repeatedly. Our group and others have evaluated the somatostatin receptor subtype 2 (SSTr2) as a platform for the nuclear imaging of gene transfer. To extend this concept, we have developed a somatostatin receptor-enhanced green fluorescent protein fusion (SSTr2:eGFP) for nuclear and fluorescent imaging.

Experimental: An adenovirus containing SSTr2:eGFP (AdSSTr2:eGFP) was constructed by using pαSSTr2 and mNLS-TK-eGFP as template DNA for two separate PCRs that introduced complementary 7-amino acid linker sequences for subsequent linkage of the two imaging genes. The PCR products were purified and used as templates for a third PCR that produced the fused SSTr2:eGFP fragment. SSTr2:eGFP was excised and ligated into the pShuttle-CMV plasmid to produce pShuttle-CMVHASSTr2:eGFP. The adenovirus was then constructed using the AdEasy system. AdSSTr2 was constructed from pαSSTr2 in a similar manner to serve as a control. DU-145 human prostate carcinoma cells were infected with either virus at 500 plaque forming units (pfu) per cell in vitro. Saturation binding and internalization assays were performed using ^{111}In-DTPA-Y3-octreotate. For binding assays, membrane preparations were made and various concentrations (~0.01 nM to ~30 nM) of ^{111}In-DTPA-Y3-octreotate were used. For internalization assays, ^{111}In-DTPA-Y3-octreotate was added (~1.5nM) and incubated for 15, 30, 60, 120, and 240min. The cells were then acid washed to remove the surface bound radioactivity and harvested to determine the amount internalized.

Results and Discussion: The saturation binding studies showed that ^{111}In-DTPA-Y3-octreotate had a K_d of 0.11 ± 0.02 nM for SSTr2 and 0.13 ±0.01nM for SSTr2:eGFP. The B_{max} values for SSTr2 and SSTr2:eGFP were 1753 ± 282 and 1746 ± 173 fmol/mg, respectively. The internalization studies showed that ^{111}In-DTPA-Y3-octreotate had a maximum internalization of 2101 ± 157 fmol/mg for SSTr2 and 2401 ± 243 fmol/mg for SSTr2:eGFP. The initial velocities of internalization were 29.0 and 30.2 fmol/mg/min for SSTr2 and SSTr2:eGFP, respectively.

Conclusion: This study shows that the fusion of eGFP does not have an adverse effect on ^{111}In-DTPA-Y3-octreotate binding or internalization to SSTr2. These adenoviral vectors will be further evaluated in small animal SPECT and fluorescent imaging studies.

Acknowledgement: The authors greatly acknowledge tissue culture support from Rebecca Andrews. This work was supported by NIH grant R01 EB004533 (BER).

Keywords: Somatostatin Receptor, Indium-111, Green Fluorescent Protein, Multimodality Imaging

P202 METABOLISM OF ^{18}F- AND ^{11}C-ANALOGUES OF THE PBR RADIOTRACER, (^{11}C)-PBR28, IN RAT BRAIN

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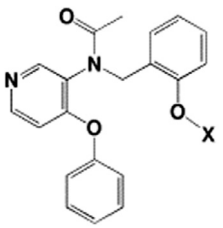
Introduction: [^{11}C]-PBR28 (X=$^{11}CH_3$) has been reported as a promising radiotracer for imaging the peripheral benzodiazepine receptor (PBR) and has recently been taken into man (1). It had been reported that 10-15% of the radioactivity in rat brain, was due to labeled metabolite species (1). Were this result carried over to human PET imaging it could introduce complications in the interpretation of PET studies. Since the radioactive metabolite(s) from [^{11}C]-PBR28 were highly polar, we hypothesised that they could be C1 species resulting from cleavage of the O-[^{11}C]CH_3 bond. Consequently we synthesized [^{11}C]-PBR28 and two analogues in order to see if this metabolic route could be eliminated or reduced.

Experimental: [^{11}C]-PBR28 was efficiently synthesized from its normethyl precursor and [^{11}C]-CH_3I using the "LOOP" method. Similarly [^{11}C]-D_3PBR28 (X=$^{11}CD_3$) was synthesized using from [^{11}C]-CD_3I –prepared from [^{11}C]CO_2, $LiAlD_4$, and HI. [^{18}F]-MS15 (X=$CH_2CH_2{}^{18}$F) was synthesized by [^{18}F]-fluoride displacement of the tosylate group in MS14 (X=CH_2CH_2OTs). Incorporation of [^{18}F]-fluoride in this reaction was remarkably efficient at >93% after 5 mins at 90°C. Following iv administration of the radiotracers, rats were killed at various timepoints; whole brain was excised, homogenized in 70% ethanol, and centrifuged. Radio-HPLC was used to determine the extent of metabolism in the samples.



Results and Discussion: The extent of metabolism of [^{11}C]-PBR28 in rat brain was similar to that previously reported (11-15% after 40 min). Introduction of deuterium into the O-methyl position had, at best, a minor influence on the extent of radioactive metabolites in brain tissue (Table). The ^{18}F-labelled analogue, [^{18}F]-MS15, showed a modest decrease in the presence of radioactive metabolites in brain, even though it was metabolised in plasma to a greater extent.

Time of Kill	% unmetabolised [^{11}C]-PBR28		% unmetabolised [^{11}C]-D_3PBR28		% unmetabolised [^{18}F]-MS15	
	Plasma	Brain	Plasma	Brain	Plasma	Brain
Control	>99	>99.5	>99	>99	97	>99.5
5 min	53.8	98.1	37.6	98.5	33.1	98.5
15 min	50.7	91.9			11.9	95.5
40 min	20.7	84.6, 84.8, 90.9	20.8	89.5, 90.5, 87.5, 89.1	7.2	93.3, 95.4, 92.7

Conclusion: There seems little to be gained by introducing deuterium into [^{11}C]-PBR28. However, given the efficiency of its synthesis, the modest reduction in radioactive brain metabolites, and the advantages of ^{18}F, [^{18}F]-MS15 is worth exploring as a potential PBR imaging radiotracer.

Reference: [1] Briard et al (2005) JLCR 48 S71.

Keywords: Peripheral Benzodiazepine Receptor, Fluorine-18, Carbon-11, Methylation, LOOP

P203 SYNTHESIS AND EVALUATION OF (*S*, *S*)-(^{11}C)METREBOX AS A NOREPINEPHRINE TRANSPORTER IMAGING AGENT

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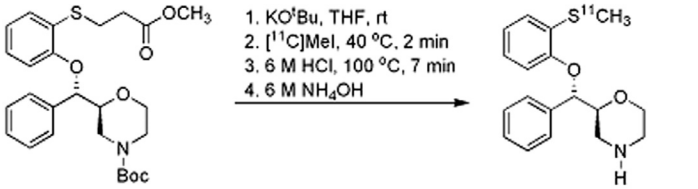
Introduction: The norepinephrine transporter (NET) is involved in several neuropsychiatric disorders and is a molecular target for the treatment of depression, ADHD, and anxiety disorder. However, many of these important findings have resulted from *in vitro* studies using post-mortem tissues. The successful development of a NET imaging agent would be very valuable in better defining the role of the noradrenergic system in neuropsychiatric disorders, as well as evaluating potential drugs targeting the NET. Here we report the synthesis, radiolabeling and characterization of a new ^{11}C-labeled reboxetine analog, (*S*,*S*)-2-[α-(2-(methylthio)phenoxy)benzyl]morpholine ((*S*,*S*)-METREBOX), as a radioligand for imaging NET with microPET.

Experimental: (*S*,*S*)-METREBOX was synthesized stereospecifically in eight linear steps starting from commercially available (*S*)-3-amino-1,2-propanediol. *In vitro* competition assays of (*S*,*S*)-METREBOX were performed in cells transfected to express human NET, SERT and DAT. Radiolabeling of (*S*,*S*)-[^{11}C]METREBOX was accomplished by treatment of N-*t*-Boc-S-methyl propanoate precursor (**1**) with potassium *t*-butoxide and $^{11}CH_3I$ in THF followed by deprotection with 6 M HCl and HPLC purification. The in vivo regional brain uptake of (*S*,*S*)-[^{11}C]METREBOX was determined in anesthetized rhesus monkeys after administration of ~15 mCi with a Concorde microPET P4.



1 **(S,S)-[^{11}C]METREBOX**

Results and Discussion: (*S*,*S*)-METREBOX exhibited high affinity and selectivity for the NET (K_i = 2.06 nM) over the SERT (K_i = 90.88 nM) and the DAT (K_i >5000 nM). (*S*,*S*)-[^{11}C]METREBOX was obtained in quantitative decay-corrected radiochemical yield with a radiochemical purity of >98%, and a log $P_{7.4}$ of 1.91. (*S*,*S*)-[^{11}C]METREBOX showed high uptake in NET-rich regions (thalamus, locus ceruleus, midbrain, and pons) with peak achieved between 32.5-37.5 min. Ratios of radioactivity in thalamus, locus ceruleus, and midbrain to that in cerebellum at 95 min were 1.35, 1.4, 1.43, respectively. A significant reduction of radioactivity from the NET-rich regions was observed with the desipramine pretreatment (0.25 & 0.5 mg/kg).

Conclusion: compound **1** is an effective precursor for ^{11}C-labeling of (*S*,*S*)-[^{11}C]METREBOX suggesting S-methyl propanoate protecting group may have more value in the labeling of other aryl alkyl sulfides. Additionally, the preliminary studies suggest that (*S*,*S*)-[^{11}C]METREBOX could be a potential agent for mapping the human NET by PET.

Acknowledgement: Research supported by Wyeth Ayerst.

Keywords: Norepinephrine Transporter, Reboxetine Analog, C-11 Labeling, PET Imaging

P204 SYNTHESIS OF ^{18}F RADIOLABELLED 5-HT$_7$ RECEPTOR ANTAGONIST FOR PET IMAGING

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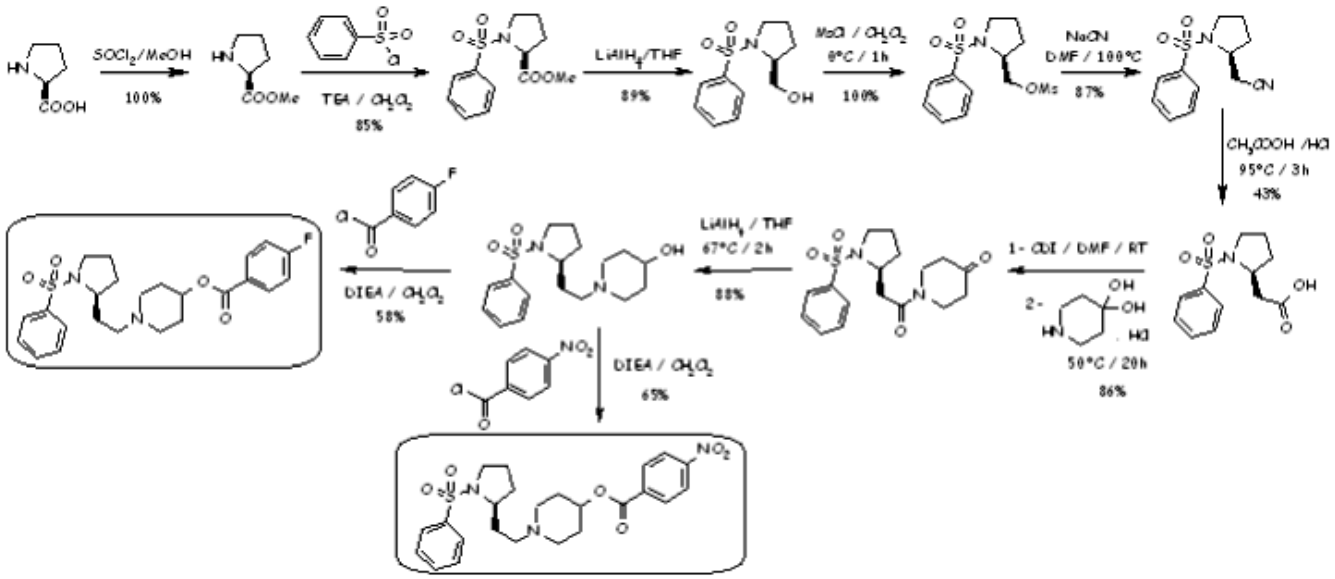
Introduction: 5-HT$_7$ receptor is the most recent identified serotonin receptor. Although physiological functions of these 5-HT$_7$ receptors are not completely known, they seem involved in some physiological disorders of sleep, depression and schizophrenia.

Thus, it is interesting to synthesize specific radiolabelled ligands of these 5-HT$_7$ receptors in order to realize in vivo studies by PET to acquire new informations on their localization and on their biological roles.

Starting from SB-269970 (Lovell et al. J. Med. Chem. 2000) known to present a 5-HT$_7$ activity, we modified the structure for easy introduction of fluorine-18 by fluoro-for-nitro exchange.

Experimental: 2ST36 (nitro) and 2ST46 (fluoro) were obtained in multistep synthesis starting from proline (fig).



Starting from 10 mg nitro precursor, [^{18}F]2ST46 was obtained with a reprogrammed Coincidence module (fluorination step 10 min 150°C), HPLC C18 prep column THF/H_2O pH 5/MeOH 4/9/1, with a 15% radiochemical yield.

Results and Discussion: First biological studies (rat autoradiography) suggest a poor brain penetration and probably a fast urine excretion.

Conclusion: A new potential 5-HT$_7$ ligand has been synthesized and radiolabelled with fluorine-18 but seems to lack pharmacological requirements for a successful tracer.

Acknowledgement: Support of Region Rhone-Alpes (ST) is gratefuly acknowledged.

Keywords: Fluorine-18, 5-HT7, Autoradiography

P205 FLUORINE-18 LABELLING AND PRELIMINARY BIOLOGICAL EVALUATION OF A N-(PHENYL SULFONYL)INDOLE DERIVATIVE, 12ST05 AS 5-HT$_6$ RECEPTOR ANTAGONIST

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Introduction: Recent molecular biology results led to discovery of serotonin 5-HT$_6$ receptors, with a distribution nearly exclusive in the central nervous system (striatal, limbic and cortical regions), suggesting a major role in learning and memory.

Some 5-HT$_6$ receptor ligands have been proposed and labelled with carbon-11, such as GSK215083 and GSK224558 (Huiban et al, NeuroImage, 2006).

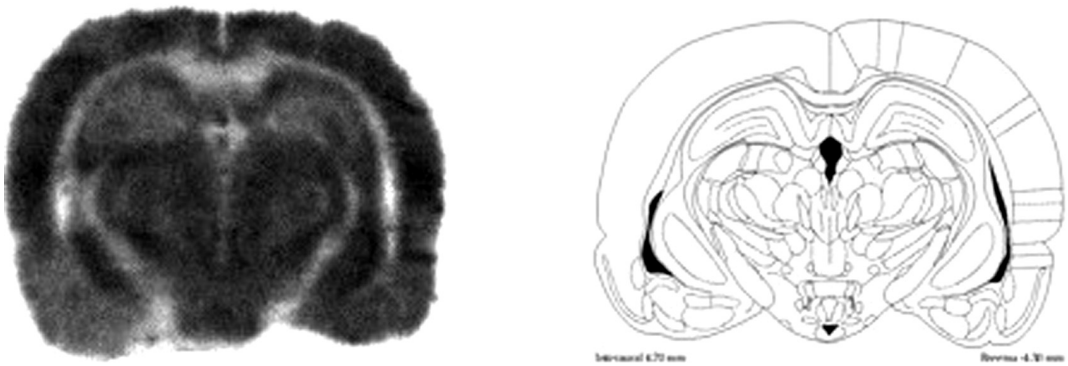
Recently a novel class of N-(phenysulfonyl)indoles has been identified as potent and selective 5-HT$_6$ ligands (Zhou et al, Bioorg. Chem. Lett., 2005). We report here the labelling and biological evaluation of 12ST05, [^{18}F]N-[2-(1-[(4-fluorophenyl)sulfonyl]-1H-indol-4-yloxy)ethyl]-N,N-dimethylamine.

Experimental: Nitro precursor was obtained from 4-hydroxyindole and dimethylaminoethanol followed by nitrobenzenesulfonyl introduction. Fluorine-18 was obtained via the ^{18}O(p,n)^{18}F reaction on IBA Cyclone 18/9 cyclotron. The nitro/fluoro exchange was realized on a Coincidence synthesizer with sequence modification: after initial fluoride preparation 10 mg of nitro precursor were introduced and the reaction mixture was heated at 150°C for 10 minutes. After dilution with water, the reaction mixture was passed through an activated C18 cartridge. Final product was obtained after separation on a preparative HPLC (C18 SymetryPrep Waters 7.8x300 mm) eluted with THF/H_2O pH5/MeOH 4/9/1.

Rat ex-vivo autoradiographies were obtained 20 min after intracaudal injection of 75 MBq of radiotracer (formulated via SPE as 5% ethanol in saline).

Results and Discussion: Labelling of [^{18}F]12ST05 from its nitro precursor was straightforward, with a 30% radiochemical yield corrected for decay, 100 GBq.μmol-1 specific activity and 70 minutes radiosynthesis time.

Autoradiography demonstrated heterogeneous fixation with localization of radioactivity in regions known to present high densities of 5-HT$_6$ receptors (hippocampus, thalamus, cortex), as shown by immunolocalisation (Gerard et al, Brain Research, 1997).



Conclusion: 12ST05 was succesfully labelled with fluorine-18 and preliminary biological results are encouraging. Further pharmacological work is in progress to demonstrate the usefulness of this molecule as a 5-HT$_6$ radiotracer.

Acknowledgement: Support of Region Rhone-Alpes (ST) is gratefuly acknowledged.

Keywords: 5-HT6, Fluorine-18, Antagonist, Autoradiography

P206 VEGFR-TK INHIBITORS AS IMAGING AGENTS

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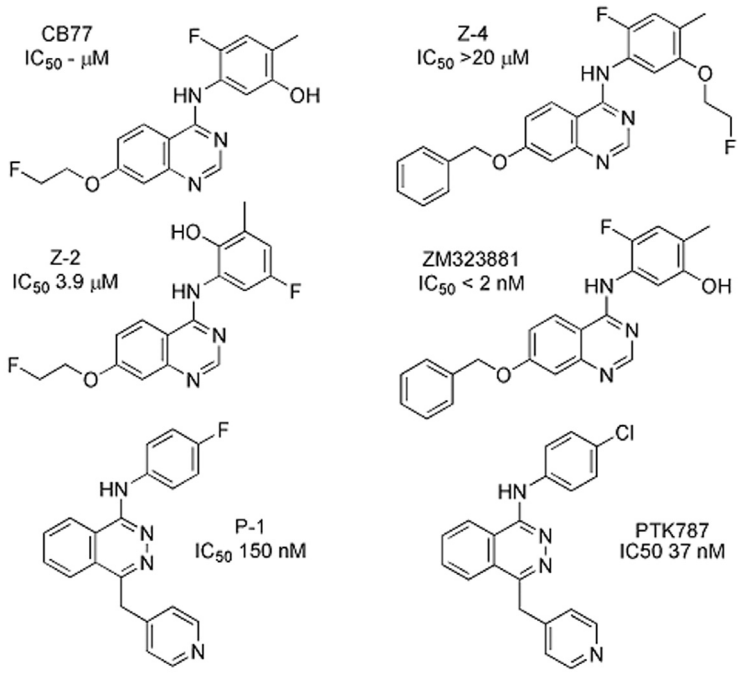
Introduction: In tumors, the vasculature is haphazard, disorganized and comprises of leaky blood vessels and excessive branching. This functional abnormality leads to poor drug delivery and hypoxic areas within the tumor. In response to hypoxia, tumors secrete angiogenetic growth factors to stimulate vessel growth and oxygen delivery. VEGF is a potent angiogenetic growth factor with a key role in tumour progression and metastasis. All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors (VEGFRs) on the cell surface, leading to receptor dimers and activation through transphorylation. VEGF binds to the three receptor tyrosine kinases, flt-1 (VEGFR-1), Flk-1/KDR (VEGFR-2) and flt-4 (VEGFR-3). Our current research is focussed on ^{18}F labeled VEGFR-2 inhibitors for positron-emission-tomography (PET) to monitor responders during e.g. PTK787 therapy.

Experimental: The unlabeled compounds shown here were synthesized to evaluate their IC_{50} values for a series of kinases: CB77, Z2 and Z4 are based on the skeletal structure of ZM323881. P1 is based on the structure of PTK787. The most important part of the multi-step syntheses is the coupling of the two building blocks, the aniline derivatives with the heterocycles. Until now, IC_{50} determinations with Z-2, Z-4 and P-1 were performed with 39 recombinant tyrosine and other kinases.

Results and Discussion: The indicated compounds were designed to accomplish straightforward radiofluorination. The unlabeled congeners of Z-2, Z-4, P-1 and CB77 were successfully synthesized and characterized by MS and NMR. They were screened for their inhibitory potential using a series of kinases. Only two compounds showed remarkable VEGFR-2 kinase inhibition: P-1 (IC_{50} 150 nM) and Z-2 (IC_{50} 3.9 μM). The parent compounds showed an IC_{50} of <2 nM (ZM323881) and 37 nM (PTK787). Modifications at the molecular structure of ZM323881 led to a dramatic decrease of affinity. The decrease was less pronounced for Z-2 and the inhibition was diminished only by a factor of four after replacement of Cl by F in PTK787. All other 38 kinases played a minor role indicating selectivity of the compounds.



Conclusion: P-1 and Z-2 are candidates for the synthesis of analogues radiofluorinated derivatives. The resulting [^{18}F]Z-2 and [^{18}F]P-1 will be subjected to biodistribution studies and clinical studies if applicable.

Keywords: VEGF Receptor, Tyrosine Kinase Inhibitor, PTK787, ZM323881, Fluorine-18

P207 SYNTHESIS OF A NEW IMAGING AGENT FOR A CENTRAL NICOTINIC ACETYLCHOLINE RECEPTOR α7 SUBTYPE

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Introduction: Nicotinic acetylcholine receptor (nAChR) α7 subtype is one of the major nAChR subtypes in the brain. Recent in vitro autoradiographic investigations have suggested that α7 nAChR is implicated in Alzheimer's disease, schizophrenia, etc. However, there is no appropriate ligand for in vivo imaging. On these bases, we designed new α7 nAChR ligands and radiolabelling with C-11 was carried out in this paper.

Experimental: Benzoic acid 1-aza-bicyclo[2.2.2]oct-3-yl ester (**1**), 2-Amino-benzoic acid 1-aza-bicyclo[2.2.2]oct-3-yl ester (**2**), 2-Methylamino-benzoic acid 1-aza-bicyclo[2.2.2]oct-3-yl ester (**3**) and 2-(3-Methyl-2,5-dioxo-pyrrolidin-1-yl)-benzoic acid 1-aza-bicyclo[2.2.2]oct-3-yl ester (**4**) were synthesized. The affinity for α7 nAChRs were evaluated by receptor binding study using [^{125}I]α-bungarotoxin. Radiosynthesis of [^{11}C]**3** was tested in various conditions using [^{11}C]CH_3I or [^{11}C]CH_3OTf. As a precursor, **2** or 2-amino-benzoic acid 1-aza-bicyclo[2.2.2]oct-3-yl ester-borane complex (**5**) was used.

Results and Discussion: The Ki value of the synthesized compounds for α7 nAChRs were 1.4 (**1**), 0.14 (**2**), 0.17 (**3**) and 13.0 (**4**) μM. Since **3** was revealed to have high affinity to this receptor, we tested radiolabelling this with C-11. The results were summarized in the table. The optimal labeling condition selected was as follows: **5** was treated with [^{11}C]CH_3OTf and NaOHaq in MEK. When NaH or LDA was used as a base, the precursor was decomposed.

Radiolabeling of [^{11}C]**3** in various conditions.

Precurcer	[^{11}C]Methylation	Solvent	Alkaline	Condition	Radiochemical yield*
2 (5 mg)	[^{11}C]CH_3I	DMF	NaH	100°C, 3min	0%
2 (5 mg)	[^{11}C]CH_3OTf	MEK	non	80°C, 5min	0%
2 (2 mg)	[^{11}C]CH_3OTf	MEK	NaH	100°C, 5min	< 1%
2 (10 mg)	[^{11}C]CH_3I	THF	LDA	0°C, 3min	3%
2 (10 mg)	[^{11}C]CH_3I	THF	LDA	60°C, 5min	< 1%
5† (1 mg)	[^{11}C]CH_3I	DMF	K_2CO_3	130°C, 5min	0%
5† (2mg)	[^{11}C]CH_3OTf	MEK	K_2CO_3	80°C, 8min	3%
5† (2 mg)	[^{11}C]CH_3OTf	MEK	LDA	80°C, 8min	< 1%
5† (2 mg)	[^{11}C]CH_3OTf	MEK	NaOHaq	20°C, 7min	15%
5† (2 mg)	[^{11}C]CH_3OTf	MEK	NaOHaq	60°C, 5min	28%

*Radiochemical yields were determined based on [^{11}C]CH_3I or [^{11}C]CH_3OTf and normalized at EOB with decay correction. †When 5 was used as a precursor, the reactant was treated with 2N HCl after methylation for removing BH_3 (80°C, 5min).

Conclusion: We synthesized a new C-11 labeled α7 nAChR ligand. In vivo studies are currently underway.

P208 SYNTHESIS AND EVALUATION OF A SEROTONIN TRANSPORTER (SERT) PET IMAGING AGENT: ^{11}C-BrHOMADAM

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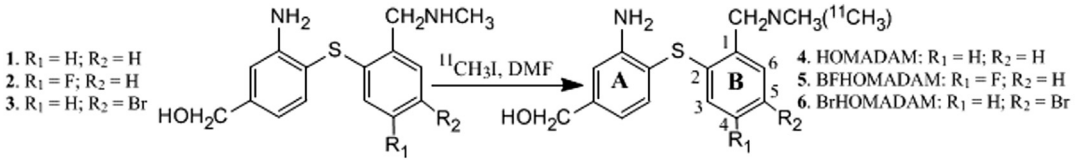
Introduction: [^{11}C]-*N*,*N*-dimethyl-2-(2'-amino-4'-hydroxymethylphenylthio)benzylamine (^{11}C-**4**) displayed potent binding affinity and selectivity for the human brain SERT[1]. [^{11}C]-*N*,*N*-dimethyl-2-(2'-amino-4'-hydroxymethylphenylthio)-4-fluoro-benzylamine (^{11}C-**5**) was previously reported as a HOMADAM derivative with a fluorine on the 4-position of ring B[2]. In order to further explore the influence of a halogen on the HOMADAM SERT binding affinity, a bromine was incorporated onto the 5-position of ring B. *N*,*N*-dimethyl-2-(2'-amino-4'-hydroxymethylphenylthio)-5-bromo-benzylamine (**BrHOMADAM**) was prepared as a new SERT PET imaging agent. This ligand was radiolabeled with C-11 and evaluated in monkeys by microPET.



Experimental: **6** was synthesized in a six-step reaction sequence starting from coupling of 4-bromo-3-nitro-toluene with 5-bromo-2-mercaptobenzoic acid. The *in vitro* competition assays of **6** were performed in cells expressing human SERT, DAT and NET. ^{11}C-**6** was prepared by C-11 methylation of the monomethylbenzylamine precursor **3** with $^{11}CH_3I$ using a loop injector. Uptake and kinetics of ^{11}C-**6** in brain regions of interest were determined in anesthetized cynomolgus monkeys using a Concorde microPET P4.

Results and Discussion: **6** exhibited high affinity and selectivity for the SERT (Ki = 0.29 nM) over the DAT (Ki = 221 nM) and NET (Ki = 725nM). After HPLC purification, ^{11}C-**6** was obtained in 17% radiochemical yield, radiochemical purity of >99% and a log $P_{7.4}$ of 2.63. The total synthesis time was 50 min. The microPET images of ^{11}C-**6** showed that this ligand displayed high uptake in the midbrain followed by the pons, medulla, putamen, thalamus, caudate, occipital cortex and frontal cortex with tissue to cerebellum ratios of 2.72, 2.48, 2.27, 2.11, 2.02, 1.53, 1.36 and 1.02 respectively at 55 min post-injection. A quasi-equilibrium was established at 20 min after ^{11}C-**6** injection. *In vivo* binding of ^{11}C-**6** was shown to be specific to the SERT by displacement with citalopram (a potent SERT ligand) that reduced radioactivity in SERT-rich regions such as midbrain and thalamus to the level of cerebellum.

Conclusion: These results suggest that the new radiotracer **^{11}C-BrHOMADAM** is an attractive candidate for further validation in monkeys for translation to image SERT in humans.

Acknowledgement: Research supported by NIMH.

References: [1] M.M. Goodman, N. Jarkas, J.R. Votaw et al. *J. Nucl. Med.*, 2006; 47; 136P. [2] N. Jarkas, R.J. Voll, L.A. Williams et al. *J. Nucl. Med.*, 2005; 46; 137P.

Keywords: BrHOMADAM, Benzylamine, MicroPET, C-11, SERT

P209 (^{99m}Tc)DEMOGASTRINS IN CCK-2/GASTRIN-R-TARGETED IMAGING

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Introduction: The expression of cholecystokinin-2/gastrin receptors (CCK-2/gastrin-R) in human tumors (e.g. MTC) provides the opportunity to develop radiolabeled CCK or gastrin analogs for their scintigraphic detection. We report herein on a small library of [(D)Glu^1]MG derivatives carrying acyclic tetraamines for stable binding of ^{99m}Tc, [^{99m}Tc]Demogastrin 1–9. This group of compounds comprises Met^{11}-substituted and/or *des*-(Glu)$_5$ [(D)Glu^1]MG members in order to select radiotracers displaying the most suitable profile for CCK-2/gastrin-R-targeted diagnostic imaging of human MTC.

Experimental: The [N_4-$Y_{AA}{}^0$,(D)Glu^1,$X_{AA}{}^{11}$]MG and truncated [N_4-(D)Glu^6,$X_{AA}{}^{11}$]MG(6-13) sequences, Y_{AA}= 0 or Gly, X_{AA}= Met(S=O), Nle, Mox, were assembled on the solid support. Labeling with ^{99m}Tc was performed at ambient temperature in alkaline medium using $SnCl_2$ as reductant in the presence of citrate. Internalization of [^{99m}Tc]Demogastrin 1–9 was tested by incubation with AR4-2J cells at 37°C. Biodistribution of [^{99m}Tc]Demogastrin 1–9 was studied in Swiss nu/nu mice implanted with AR4-2J tumors.

Results and Discussion: [^{99m}Tc]Demogastrin 1–9, with the exception of the sulfoxide-Met^{11} analog, [^{99m}Tc]Demogastrin 7, internalized rapidly in AR4-2J cells. When injected in AR4-2J tumor-bearing mice, radiopeptides, with the exception of [^{99m}Tc]Demogastrin 7, were able to specifically target the stomach and the AR4-2J tumor with differing efficacy, depending upon peptide chain length as well as on position 11 substitution. Full-length Met^{11} and Nle^{11} analogs displayed the highest uptake, but also substantial accumulation in mouse kidney. Although truncated analogs resulted in low renal values they displayed inferior targeting of CCK-2/gastrin-R-expressing tissues. Most promising results were exhibited by the [Gly^0,(D)Glu^1]MG analog, [^{99m}Tc]Demogastrin 2, which is currently undergoing clinical evaluation in MTC patients.

Conclusion: Structural modifications of tetraamine coupled [(D)Glu^1]MG analogs affected both CCK-2/gastrin-R targeting of the corresponding ^{99m}Tc-radiopeptides and their accumulation in mouse kidney. Substitution with polar residues or oxidation of Met^{11} deteriorated receptor interaction. On the other hand, *des*-(Glu)$_5$ analogs exhibited inferior receptor targeting capacity despite their low renal uptake in mice. As a result, [^{99m}Tc]Demogastrin 2 seems to be the agent of choice for the diagnosis of human MTC, as recently supported by clinical data from a first group of MTC patients.

Acknowledgement: This work has been supported by Biomedica Life Sciences, S.A. and by Project "Functional and Functionalized Biomolecules in Biodiagnosis and Radiopharmacy" (EPAN 3.3. Excellence in Research).

Keywords: Targeted Imaging, CCK-2/gastrin Receptor, ^{99m}Tc-Labeled Gastrin

P210 FLUORINE-18-LABELLING OF S40584 AND S42877, TWO NEW $\alpha_4\beta_2$-SELECTIVE LIGANDS FOR PET IMAGING OF NICOTINIC ACETYLCHOLINE RECEPTORS

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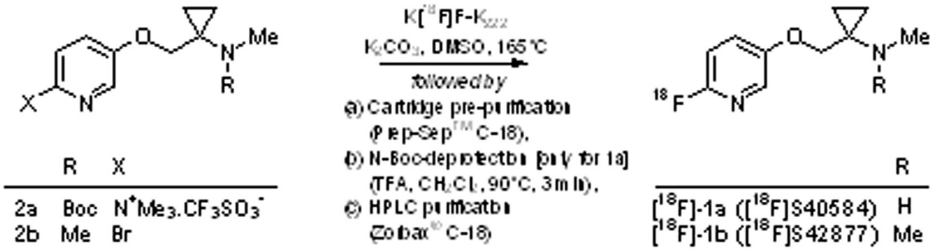
Introduction: There is considerable evidence that a variety of functions and disorders of the central nervous system (CNS), such as Alzheimer's and Parkinson's disease, is linked to the neuronal nicotinic acetylcholine receptors and particularly to the subtypes containing α4 and β2 subunits. Among the [^{18}F]ligands already developed for imaging these receptors with PET, 2-[^{18}F]F-A-85380 is the only one currently used in humans. However, this radioligand displays rather slow brain kinetics and is characterized by a relatively high non-specific binding. Recently, a novel series of highly potent α4β2-selective [1-(pyridin-3-yloxymethyl)-cyclopropyl]-amines has been developed by Servier Laboratories (1). Within this series, fluoropyridinyl-containing compounds were selected based on their pharmacological and biological characteristics as potent candidates for PET imaging and labelled with fluorine-18 using *hetero*aromatic nucleophilic substitution with [^{18}F]fluoride. The labelling of two candidates, S40584 (**1a**) and S42877 (**1b**) is reported herein.



Experimental: The Fluorine-18-labelling of S40584 (**1a**, two-step process) and S42877 (**1b**, one-step process) is described in the figure above (incorporation step: **2a** (3.5-4.5 mg), 165°C, 5 min; **2b** (4.5-9.0 mg) 165°C, 15 min). The decay-corrected overall yields for the preparation of [^{18}F]-**1a** and [^{18}F]-**1b** were 25-35% and 9-13%, respectively.

Results and Discussion: Starting from a 500 mCi aliquot of a cyclotron-produced [^{18}F]fluoride production batch, 80-100 mCi of [^{18}F]-**1a** but only 25-40 mCi of [^{18}F]-**1b**, radiochemically pure (> 99%) and ready-to-inject, were obtained after semi-preparative HPLC (Zorbax® C18, eluent: 0.9% aq. NaCl/EtOH/AcOH: 500/50/0.5 (v:v:v)) in 80-85 min.

Conclusion: The decay-corrected overall yields for the preparation of [^{18}F]-**1a** and [^{18}F]-**1b** were 25-35% and 9-13%, respectively. Dynamic PET studies in baboons (including pre-saturation experiments with nicotine) are currently underway to evaluate the potential of these ligands to image central $\alpha_4\beta_2$ nicotinic acetylcholine receptors *in vivo*.

Reference: [1] Goldstein S. et al., Eur. Pat. Appl. (2002).

Keywords: AChR, Fluorine-18, S40584, S42877, Fluoropyridines

P211 ONE-STEP RADIOSYNTHESIS OF (^{18}F)LBT-999, A SELECTIVE RADIOLIGAND FOR THE VISUALISATION OF THE DOPAMINE TRANSPORTER WITH PET

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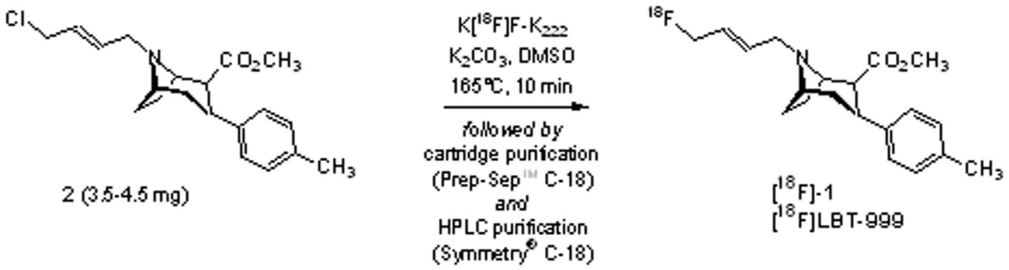
Introduction: LBT-999 (**1**, 8-((*E*)-4-fluoro-but-2-enyl)-3b-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2b-carboxylic acid methyl ester) is a recently developed cocaine derivative belonging to a new generation of highly selective DAT ligands (1). First labelled with carbon-11 (2) then with fluorine-18 (3), LBT-999 shows very promising *in vivo* pharmacological characteristics (1,2,4) and is currently further evaluated in non-human primates. Initial fluorine-18-labelling (3) was based on the robust and reliable two-step radiochemical pathway often reported for such tropane derivatives, involving first the preparation of (*E*)-1-[^{18}F]fluoro-4-tosyloxybut-2-ene followed by its coupling to the appropriate nor-tropane moiety. In order to facilitate both the automation and the purification process, a simple one-step fluorine-18-labelling of **1** has been developed.



Experimental: The conditions used were the following: (a) reaction of K[^{18}F]F-Kryptofix®222 at 165°C for 10 min in DMSO (0.6 mL) containing **2** (3.5-4.5 mg); (b) C-18 Sep-Pak cartridge pre-purification and (c) purification using semi-preparative HPLC (Symmetry C-18).

Results and Discussion: The RCYs of fluorine-18 incorporation (TLC-calc.) were about 30-40%.

Conclusion: Typically, 70-100 mCi of [^{18}F]LBT-999 ([^{18}F]-**1**, 2.59-3.07 GBq, > 95% chemically and radiochemically pure) could be obtained (specific radioactivities: 1-3 Ci/micromol (37-111 GBq/micromol)) within 80-85 min (HPLC purification and Sep-pak-based formulation incl.), starting from a 1 Ci (37.0 GBq) [^{18}F]fluoride batch (overall RCY: 7.0-10.0%, non decay-corrected).

Reference: [1] Dolle F. et al., *Bioorg. Med. Chem.* 2006, *14*, 1115-1125. [2] Chalon S. et al., *J. Pharm. Exp. Ther.* 2006, *317*, 147-152. [3] Dolle F. et al., *J. Label. Compounds Radiopharm.* 2006, *49 (8)*, 687-698. [4] Saba W. et al., *Synapse* 2007, *61*, 17-23.

Acknowledgement: Supported in part by the EC - FP6-project DiMI (LSHB-CT-2005-512146) and the RNTS 03B243 FLUOPARK programme.

Keywords: Fluorine-18, LBT-999, DAT

P212 FLUORINE-SUBSTITUTED 6-ARYL-1,4-DIHYDROBENZO(*d*)(1,3)OXAZINE-2-THIONES FOR BREAST TUMOR IMAGING AND RADIOTHERAPY: SYNTHESIS AND RECEPTOR BINDING AFFINITY

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Introduction: The progesterone receptor (PR) is present in many breast tumors, and measurement of tumor PR levels is used to predict the success of certain endocrine therapies. Tanaproget (**1**), recently described by Wyeth Pharmaceuticals, is a non-steroidal progestin agonist with very high PR binding affinity. When appropriately radiolabeled,[1] it might be useful for PET imaging of PR-positive breast tumors.

Experimental: We synthesized and determined the PR binding affinities of a series of fluorine-substituted 6-aryl-1,4-dihydrobenzo[*d*][1,3]oxazine-2-thiones (**2** and **3**), analogs of tanaproget; synthesis of the most promising compound **3a** is detailed in Scheme 1. Hydroboration and oxidation of **4** gave the primary alcohol **5**, which was converted to the fluoropropyl derivative **6** by treatment with (diethylamino)sulfur trifluoride (DAST). Compound **6** was then coupled under standard conditions with the protected pyrrole-2-boronic acid to afford the compound **7**. The 5-cyano group was installed by treatment with chlorosulfonyl isocyanate, followed by DMF quench to give **8**. Thermolytic deprotection afforded the pyrrole **9**. The target compound **3a** was prepared by thionation of **9** using Lawesson's reagent.

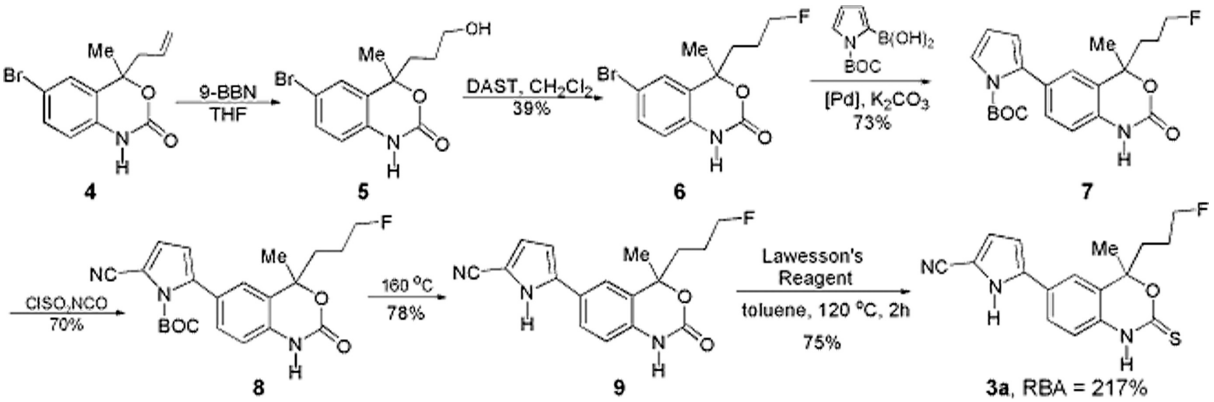

Fig. 1. Synthesis of the analog of tanaproget **3a**.

Results and Discussion: Some of the synthesized compounds showed binding affinities comparable to or even higher than that of the known high affinity PR ligand R5020. The structures of these compounds and their relative binding affinity [RBA] values (as a percent that of R5020) are given in Scheme 2.

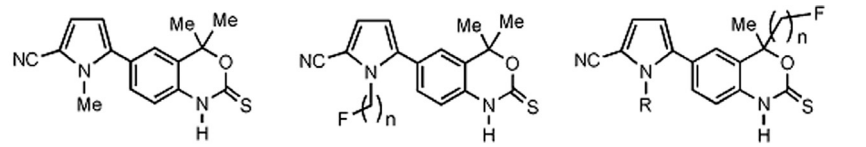

Fig. 2. Structure and binding affinities of the tanaproget and its analogs.

Conclusion: We have synthesized a series of fluorine-substituted analogs of tanaproget, some of these compounds have showed high binding affinities for progesterone receptor and will be evaluated as potential diagnostic imaging agents for breast cancer.

References: [1] Fensome, A.; Bender, R.; Chopra, R.; Cohen, J.; Collins, M.A.; Hudak, V.; Malakian, K.; Lockhead, S. et al. *J. Med. Chem.* **2005,** 48, 5092-5.

Acknowledgement: Supported by grants from the Department of Energy (DE FG02 86ER60401) and the National Institutes of Health (PHS 5R37 CA25836).

Keywords: Progesterone Receptor Ligand, Analogs of Tanaproget, Fluorination, Breast Tumor Imaging, Radiotherapy

P213 BROMINE-SUBSTITUTED ESTROGEN RECEPTOR BETA SELECTIVE BENZOXAZOLES FOR BREAST TUMOR IMAGING AND RADIOTHERAPY: SYNTHESIS AND RECEPTOR BINDING AFFINITY

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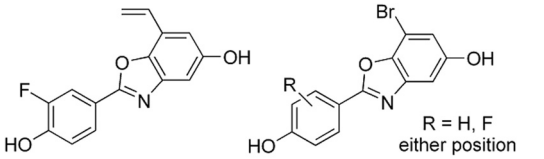
Introduction: The estrogen receptors, ER alpha and ER beta, are important regulators of estrogen action in target tissues and breast cancer. In our search for subtype-selective estrogen receptor (ER) ligands to be radiolabeled with bromine-76 for PET imaging of ER-positive breast tumors, we investigated ERB-041, a novel benzoxazole under clinical development by Wyeth Pharmaceuticals (Scheme 1). ERB-041 is reported to have an affinity for ERβ of 5.0 nM and ERα of 1216 nM, and in animal models, ERB-041 is effective in various models of inflammation, inflammatory bowl disease and rheumatoid arthritis that appear to be ERβ-mediated.

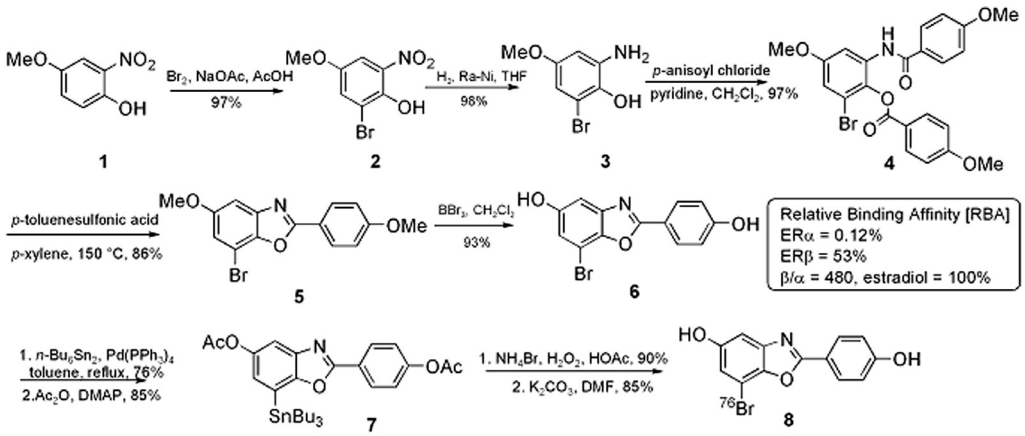


ERB-041	***ERB-041 Bromine Analogs***
ERα = 1216 nM	ERα = 44-260 nM
ERβ = 5.0 nM	ERβ = 1.4-3 nM
β/α = 226	β/α = 32-81

Scheme 1. Binding values (K_i) of ERB-041 and its analogs.

Experimental: Interestingly, ERB-041 has an aromatic fluorine substituent, so–in principle–it could be F-18 labeled itself by the diaryliodonium approach. Other compounds reported by Wyeth during the development of ERB-041 bear bromine substituents and bind with even higher affinity and good ERβ selectivity; these molecules could be labeled with Br-76 at this site. It is also likely that fluoroalkyl groups could be introduced at certain sites with preservation of the ERβ-selective binding characteristics of the parent compound. As described by Wyeth, these benzoxazoles are easily prepared by condensing benzoates with *o*-aminophenols, which in turn are derived from *o*-nitrophenols.

Results and Discussion: As outlined in Scheme 2, we have synthesized the bromine analog of ERB-041 **6**; this compound has affinities for ERα and β of 0.12% and 53%, respectively, comparable to that of estradiol (estradiol = 100%), with ERβ affinity selectivity ~500. The radio-bromination was conducted by electrophilic aromatic substitution of the tributylstannyl derivative **7**, which was prepared by coupling using hexabutylditin and the bromo derivative **6** in the presence of a catalytic amount of the palladium(0) complex.



Scheme 2. Synthesis of analogs of ERB-041.

Conclusion: The evaluation of the radio-bromo-76 compound **8** as potential diagnostic imaging agents for breast cancer is in progress.

Acknowledgement: Supported by a grant from the National Institutes of Health (PHS 5R37 CA25836).

Keywords: Estrogen Receptor Beta Ligands, Benzoxazoles, Bromination, Breast Tumor Imaging, Radiotherapy

P214 DETERMINATION OF LIGAND BIODISTRIBUTION AT TRACER DOSES USING LC-MS: VALIDATION WITH THE 5-HT_{1A} LIGAND FPWAY

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Introduction: The LC/MS method is valuable in evaluating novel radiopharmaceuticals. The highly sensitive Waters Q-TOF coupled with UPLC Acquity system allowed increased sensitivity and reduced analysis time. Using this tool, we are able to quantitate no-carrier-added level radiopharmaceuticals in biological tissues without the need to develop complicated radiolabeling schemes. We compared this technique in the analysis of Differential Uptake Ratio (DUR) and the brain tissue specific binding ratio of [^{18}F]FPWAY {4-Fluoro-*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyrimidinyl)benzamide} to the value obtained by standard radioactivity measurements.

Experimental: Rats were injected intravenously with [^{18}F]FPWAY (~2.0 mCi, 2-4 nmol or plus 4.3nmol FPWAY) and sacrificed after 5 and 30 min. The brains were removed and dissected; the dissected regions (caudate, thalamus, hippocampus, brain stem, cerebellum, cortex) were weighed and counted to determine the radioactivity. The total activity in each region was expressed as DUR (%ID/g * body weight/100). The specific binding ratios were defined as [(DURtissue/DURcerebellum)-1].

The same brain samples were homogenized in pH 12.5 buffer containing Internal Standard FBWAY (4 ng). The mixture was extracted with 1 ml of Hex:EtOAc (4:1) and the organic phase evaporated to dryness. The residue was taken up in ACN:50 mM NH4OAc(1:1)and analyzed by LC/MS. The plasma was extracted with same method. LC/MS analysis employed an Acquity BEH Shield RP18 column (150 X 2.1 mm, gradient with 25 mM ammonium acetate and acetonitrile) interfaced to the Waters Q-TOF MS.

Results and Discussion: The brain tissue specific uptake ratios determined by the two methods were differed by less than 10% in most brain tissues (table). No differences were observed between the LC/MS and radioactivity method in determine of Differential Uptake Ratio and the specific binding ratio in the rat brain indicating the high sensitivity LC/MS is an indispensable tool in evaluating the presence of tracer in target tissue in the development of new molecular imaging probes.

DUR of FPWAY in Rat Brain

DUR	Radioactivity	LC/MS	% Difference
Hippocampus	0.832 ±0.032	0.790 ±0.032	5.21
Cortex	0.506 ±0.044	0.521 ±0.049	-2.98
Cerebellum	0.225 ±0.018	0.233 ±0.033	-3.65

Conclusion: With compounds for which high sensitivity detection is possible by LC-MS and if the specific brain uptake is appropriate, preliminary biodistribution studies to predict brain uptake and tissue ratios can be determined without the need to synthesize the radiolabeled product.

Keywords: LC/MS, Radiotracer, 5-HT1A, Biodistribution, FPWAY

P215 TARGETING THE ESTROGEN RECEPTOR WITH METAL-CARBONYL DERIVATIVES OF ESTRADIOL

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[1]Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA, USA; [2]Deaprtment of Chemistry, Augsburg College, Minneapolis, MN, USA; [3]Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT, USA

Introduction: Based upon the current understanding of the steroidal ligand-estrogen receptor binding process, we designed and synthesized novel metal-carbonyl derivatives of estradiol as a potential imaging agents. Although simple 17α-(hetero)arylvinyl derivatives of estradiol can be prepared via palladium(0) catalyzed reactions, the incorporation of metal carbonyl groups imposes more challenges.

Experimental: The unsymmetrical 5-bromo-2,2'-bipyridine was generated by Stille coupling of 5-bromo-2-trimethylstannyl pyridine with 2-bromopyridine. The $Re(CO)_3$-complex was obtained by complexing the bipyridine and $[Net_4]_2[Re(CO)_3Br_3]$ in methanol. The synthesis of the 3-pyridylvinyl estradiol proceeded without difficulty, but our efforts to couple the stannylvinyl estradiol with 5-bromo-2,2'-bipyidine proved to be unsuccessful. When Stille coupling was done with the preformed $Re(CO)_3$-bipyridyl complex, the desired product was obtained in a good overall yield. The receptor binding affinity of the $Re(CO)_3$-bipyridyl-vinyl estradiol complex for the ERα-LBD was determined by radiometric assays with [H-3] estradiol. The binding results indicate that by appending the second pyridyl ring para to the first and introducing the metal carbonyl moiety reduces the RBA value to 4% that of estradiol.

Results and Discussion: The preparation of the bipyridyl rhenium carbonyl complex represents the first example of Stille coupling with these reagents, however, the receptor binding properties.of the products were low, suggesting that the binding pocket into which the chelating moiety fits has limited steric capacity.

Conclusion: The synthesis constitutes the first report of a Stille coupling between a metallated complex and a vinylstannane. The final product retains significant estrogen receptor binding properties suggesting that structural modifications of the ligand that enhance affinity may lead to breast cancer imaging agents. Although we were successful in ultimately introducing the metal in a late stage, the receptor binding properties of the ligands were not sufficient to undertake preparation of the ligands in their radiolabeled form.

Acknowledgement: Acknowledgments. This work was supported in part by a grant from the US Army Medical Research and Materiel Command W81XWH-04-1-0544 (RNH).

Keywords: Rhenium Tricarbonyl, 17-Alpha-Substituted Estradiol Derivatives, Estrogen Receptor, Synthesis, Binding Affinity

P216 (^{18}F)AFFIBODY CONJUGATE FOR IN VIVO DETERMINATION OF HER2 EXPRESSION

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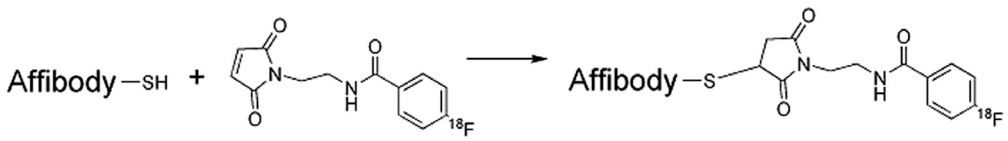
[1]NIBIB, NIH, Bethesda, MD, USA; [2]NCI, NIH, Bethesda, MD, USA

Introduction: Tumor expression of HER2 (human epidermal growth factor receptor) is correlated with poor prognosis [Witton, 2003]. HER2 expression has been shown to be heterogeneous among the primary and metastatic tumors in the same patient [Zidan, 2005]. Thus the development of an imaging agent that can assay the HER2 receptor in a patient would be valuable for treatment planning. Small α-helical, stable, highly soluble proteins, called Affibody® molecules have been developed. His6-$Z_{HER2:342}$ Affibody that displays binding specificity for HER2 [Orlova, 2006] was modified with a terminal cysteine and radiolabeled with F-18 using maleimide chemistry.

Experimental: We prepared N-([2-(4-[^{18}F]fluorobenzamide)ethyl]maleimide by coupling of [^{18}F]benzoic acid with N-aminoethyl maleimide using diethylcyanophosphonate. This isolated intermediate was incubated with the Affibody under reducing conditions. Conjugated Affibody molecule was purified by passage through a NAP-5 column. Purity was assessed by HPLC and PAGE. In vitro binding studies were conducted using SKBR-3 human breast cancer cell line. Tumor uptake in vivo was examined in SKOV-3 xenograft-bearing nude mice using both PET imaging using the ATLAS small animal PET scanner and tissue dissection.

Results and Discussion: The radiochemical synthesis was completed with an overall yield of 3-6% based on starting fluoride with a total time of 108-140 min. The radiochemical purity was >98% measured by HPLC and 86% by PAGE. We could not separate conjugated from unconjugated protein. Saturation analysis with SKBR-3 cells indicated a single high affinity site that could be inhibited with unlabeled Affibody in a dose dependent manner. High specific binding and low non-specific binding was apparent. ATLAS PET images demonstrated a rapid clearance of radioactivity to the urinary bladder and clearly showed an increased uptake in tumor relative to surrounding tissue within 50 min.



Conclusion: This F-18 Affibody has promising properties for the in vivo imaging of HER2 receptors for planning of patient therapy.

References: [1] Witton CJ, et al. J. Pathol, 2003, 200:290-297. [2] Zidan J, et al. Br. J. Cander, 2005, 93: 552-556. [3] Orlova, A, et al. Cancer Res. 2006, 66: 4339-4348.

Acknowledgement: Funding provided by the Intramural Research Program of the NIH.

Keywords: Fluorine-18, HER2 Receptor, Affibody

P217 SYNTHESIS AND *IN VIVO* CHARACTERIATION OF HIGH AFFINTY ANDROGENS TO EVALUTE THE ROLE OF SHBG IN RADIOSTEROID DELIVERY

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Introduction: Sex hormone-binding globulin (SHBG) is an important steroid hormone binding protein circulating in human plasma. While it is generally believed that binding of testosterone to SHBG prevents the bound hormone from diffusing out of the bloodstream, thereby hindering cellular uptake of testosterone and its access to the intracellular pool of ARs, alternative views hold that SHBG facilitates androgen uptake through a specific cell-surface receptor for the SHBG-steroid complex on target cells.

Experimental: We synthesized two F-18 labeled compounds: 7α-fluoromethyl-dihydrotestosterone (7α-FM-DHT) and 7α-fluoromethyl-nortestosterone (7α-FM-norT). Both 7α-FM-DHT and 7α-FM-norT have high affinities for the AR; however, 7α-FM-norT has a significantly lower affinity for SHBG (Figure 1). 7α-[^{18}F]FM-DHT and 7α-[^{18}F]FM-norT were injected into androgen-depleted and androgen-blocked adult male rats and tissue distribution studies were done (Tables 1 and 2). Additionally, studies were done to determine the metabolic fates of both compounds.

7α-Fluoromethyl-dihydrotestosterone

AR (R1881) 1.3 ± 0.2 nM
SHBG (E2) 0.94 ± 0.04 nM

7α-Fluoromethyl-nortestosterone

AR (R1881) 1.5 ± 0.08 nM
SHBG (E2) 18 ± 0.6 nM

Fig. 1. Structure of ^{18}F-labeled androgens and their binding affinities for the androgen receptor (AR) and sex hormone binding globulin (SHBG).

Table 1. Tissue distribution of 7α-[^{18}F]FM-DHT in DES-pretreated rats

Organ	1 h	1 h block	2 h	2 h block
Blood	0.056 ± 0.019	0.056 ± 0.036	0.027 ± 0.0068	0.028 ± 0.015
Prostate	0.29 ± 0.21	0.19 ± 0.072	0.22 ± 0.059	0.12 ± 0.051

Table 2. Tissue distribution of 7α-[^{18}F]FM-nor-T in DES-pretreated rats

Organ	1 h	1 h block	2 h	2 h block
Blood	0.11 ± 0.030	0.080 ± 0.020	0.057 ± 0.0086	0.055 ± 0.017
Prostate	0.53 ± 0.17	0.19 ± 0.061	0.35 ± 0.12	0.066 ± 0.020

Results and Discussion: Both 7α-[^{18}F]FM-DHT and 7α-[^{18}F]FM-norT demonstrate selective AR-mediated uptake into AR rich tissues. Both compounds demonstrate lowered levels of metabolic degredation.

Conclusion: Further studies are planned to evalute 7α-[^{18}F]FM-DHT and 7α-[^{18}F]FM-norT in humans to determine the role of SHBG in radiosteroid delivery to target tissue.

Acknowledgement: We thank Kathryn Carlson for her assistance in binding affinity work. This work was supported by DOE grants (FG02 86ER60401 to J.A.K.) and (FG02 84ER60218 to M.J.W.) and NIH (PHS 1R24 CA86307 to M.J.W.).

Keywords: Prostate Cancer, 18-F, SHBG

P218 SYNTHESIS OF THE NEW α_2 RECEPTOR ANTAGONIST (O-METHYL-^{11}C)(1*R*.12b*R*)-(-)-1,3,4,6,7,12b-HEXAHYDRO-1-METHOXYMETHYL-1-METHYL-2*H*-BENZOFURO(2,3-a)QUINOLIZINE

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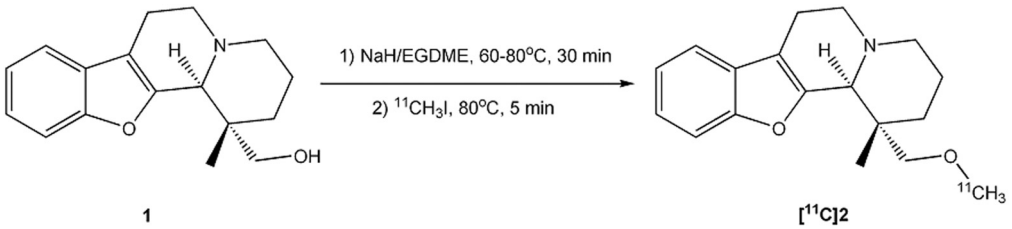
[1]Radiopharmaceutical Chemistry Laboratory, Turku PET Centre, Turku, Finland; [2]Department of Medicinal Chemistry, Orion Corporation Orion Pharma, Espoo, Finland

Introduction: α_2 receptors are localized throughout the CNS and play a regulatory role in neurotransmitter release. Of particular interest is the role of α_2 receptors in psychiatric diseases such as depression. Although several α_2 selective radiopharmaceuticals for PET have been developed, none of these have fulfilled the criteria of a successful brain radiotracer. Compound **2** is a new α_2 antagonist with a high affinity (K_i <10nM) and a high selectivity for the α_2 receptors. The aim of this study has been to prepare [^{11}C]**2** for further characterization as a PET tracer of α_2 receptors.

Experimental: The sodium alcoholate of the starting material **1**, (1*R*,12b*R*)-(-)-1,3,4,6,7,12b-hexahydro-1-hydroxymethyl-1-methyl-2*H*-benzofuro[2,3-a]quinolizine was generated *in situ* using NaH in THF or EGDME (ethyleneglycol dimethylether) for 30 min at 60-80°C. [^{11}C]Methyl iodide was trapped in this solution at 4°C followed by 5 min heating at 80°C. 600±100 µl of 0.4M HCl (MeOH/H2O, 1/1) was added and the crude product purified by HPLC on a semi-preparative µ-Bondapak C18 using 28% CH_3CN in 10 µM phosphoric acid as mobile phase. After evaporation of the HPLC solvent, the purified product was dissolved in sterile propylene glycol/ethanol/0.1M phosphate, 7/3/50 and filtered through a 0.22 µm sterile filter.



1 [^{11}C]2

Results and Discussion: Methylation of the sodium salt of **1**, generated from 8 mg NaH (60%, oil) and 5 mg of **1** in 250 µl of THF or EGDME, with [^{11}C]methyl iodide for 5 min at 80°C gave [^{11}C]**2** in a moderate crude radiochemical yield (RCY) of 15-40%. The large variation in RCY is due to difficulties in handling small amounts of NaH. HPLC purification was greatly improved by the use of EGDME as reaction solvent and addition of 0.4M HCl in MeOH/water before purification.The final product [^{11}C]**2** was obtained in 10-30% RCY 40-45 min after EOB in >99.5% radiochemical purity and >95% chemical purity with specific radioactivity of 50-160 MBq/nmol.

Conclusion: The new α_2 receptor antagonist **2** have been successfully labelled with ^{11}C in moderate RCY, high purity and specific radioactivity. *In vitro* and *in vivo* characterization of this new radiopharmaceutical is ongoing and will be reported elsewhere.

Keywords: α2 Receptors, [^{11}C]Methyl Iodide, α2 Antagonist, PET

P219 SYNTHESIS OF ^{18}F-LABELLED CUBYL-WAY

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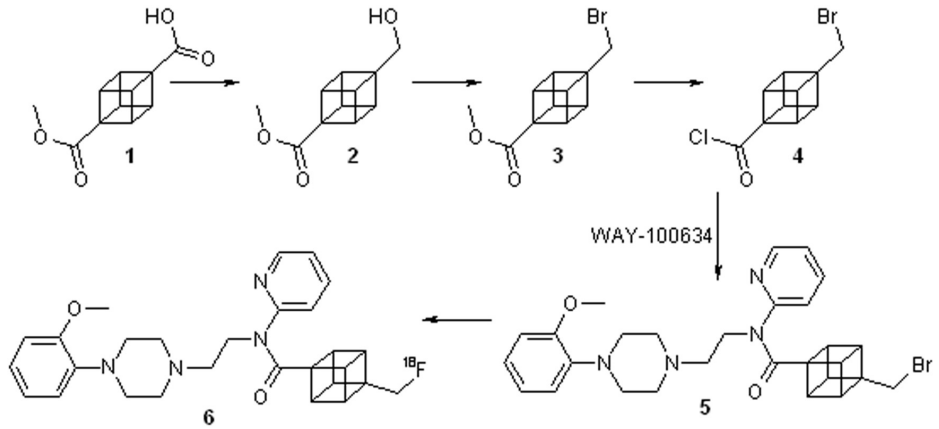
Introduction: Earlier we have found that substitution of the cyclohexyl group in WAY-100635 by a cubyl moiety does not really alter the affinity for the HT_{1A}-receptor. Furthermore we have shown that [^{123}I]iodocubyl-WAY binds to this receptor in rat brain. Since we have recently found that the hydrolysis rate of this compound when incubated with human hepatocytes is much lower than for e.g. MPPF, we decided to prepare an analogous fluorine-18 labelled derivative.

Although direct substitution of a bridgehead iodine with [^{18}F]XeF_2 is possible, this would lead to a compound with low specific activity. Therefore we have introduced a –$CH_2{}^{18}$F function on the bridgehead for which elimination of HF is also impossible.

Experimental: Reduction of **1** with $BH_3.SMe_2$ in dry THF under argon gave **2** in 70% yield. Further bromination with CBr_4/triphenylposphine in THF under argon gave **3** in 80% yield. The ester was then saponified with NaOH and subsequently treated with $SOCl_2$ in dry MeCN, to give the acid chloride **4** in 60% yield. WAY 100634 and TEA in MeCN where added to **4**, to give **5** in 90% yield.



Radiofluorination of **5** with ^{18}F in dry MeCN using kryptofix and K_2CO_3 gave **6** in a labelling yield of 80% in ten minutes. Separation from the precursor is easily performed using reversed phase HPLC yielding a chemically and radiochemically pure product.

Results and Discussion: The ease of which the nucleophilic labelling occurs is surprising. Probably this is due to the fact that the exocyclic carbon-carbon bond has a higher degree of S-character than in normal aliphatic bromides. Compound **6** was further found to be thermally stable on heating for 60 minutes at 130°C in acetonitrile in a closed vial, in contrast to its precursor **5** which has to be stored in a refrigerator. The stability of **6** towards serum or hepatocytes is still not known.

Conclusion: A simple high-yield synthesis for a nca ^{18}F-labelled analogue of WAY-100635 has been developped.

Affinity and selectivity of this compound *in vitro* as well as *in vivo* is now under investigation.

Acknowledgement: This research has in part been made possible by financial support of the Dutch Technology Foundation (STW).

Keywords: WAY-100635, Cubane, 5-HT1A, Fluorination

P220 SYNTHESIS AND IN VIVO EVALUATION OF A NOVEL (^{123}I) INDOLGLYOXYLAMIDE FOR THE PERIPHERAL BENZODIAZEPINE BINDING SITES

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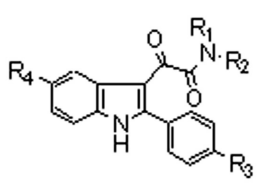
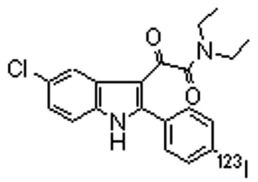
Introduction: The Peripheral Benzodiazepine Binding Sites (PBBS) are significantly upregulated in Huntington's and Alzheimer's diseases, multiple sclerosis, and several types of tumours. The development of specific PBBS radiotracers for imaging with PET and SPECT provide a means by which these changes can be monitored and correlated to disease. *N,N*-Dialkyl-2-phenylindolyl-3-glyoxylamides represent a new class of potent PBBS ligands which offer the potential for radiolabelling with radionuclides for both PET and SPECT imaging. Here, we report the synthesis and binding affinities of new indolglyoxylamides, and the radiolabelling and in vivo evaluation of [^{123}I]-*N,N*-diethyl-[5-chloro-2-(4-iodophenyl)indol-3-yl]glyoxylamide, [^{123}I]PBR200.

Experimental: A series of compounds modifying groups R_1-R_4 (Fig 1) were synthesised, and the binding affinity (IC_{50}) of all new compounds for the PBBS and CBR (central benzodiazepine receptor) were determined. Radioiodination of PBR200 was achieved via oxidative iododestannylation reaction using Na^{123}I and peracetic acid. Biodistribution studies with n.c.a [^{123}I]PBR200 was performed in Sprague-Dawley rats up to 24 h. Drug competition studies with PK11195, Ro5-4864 and flumazenil were used to assess the in vivo specificity of [^{123}I]PBR200 to PBBS. The in vivo stability of [^{123}I]PBR200 in the kidney, heart, adrenals, brain, and plasma was investigated by radio-TLC.



R_1 and R_2 = hexyl, propyl, ethyl, or methyl
R_3 = Br or I
R_4 = Cl or H



[^{123}I]-PBR200

Results and Discussion: All compounds displayed high selectivity for PBBS vs CBR. The new compounds displayed PBBS affinities (IC_{50}) ranging from 7.86 - 618 nM and for CBR, IC_{50}> 5000 nM. Radiolabelling of PBR200 was achieved in 55-60% RCY and >98% RC purity. Biodistribution showed high uptake of the [^{123}I]PBR200 in all organs known to contain PBBS, including kidneys, lungs, heart, and adrenals at 30 min (2.3, 4.2, 3.8 and 6.7% ID/g respectively). Uptake in the blood decreased from 0.1-0.01% ID/g throughout the 24 h. PBR drugs PK11195 and Ro 5-4864, and PBR200 significantly decreased the uptake of [^{123}I]PBR200 in peripheral organs and brain. The CBR drug, flumazenil showed no significant decrease in uptake of the tracer. The tracer was found to be >95% intact in the kidney, heart, adrenals and brain after 3 h, and >65% intact in the plasma after 3 h.

Conclusion: The results obtained demonstrate the potential of [^{123}I]PBR200 for SPECT imaging of PBBS.

Acknowledgement: This work was partially funded by AINSE.

Keywords: Peripheral Benzodiazepine Binding Sites, Radioiodination, In Vivo Evaluation, SPECT, PBBS

P221 SYNTHESIS AND BIODISTRIBUTION OF A RADIOFLUORINATED LIGAND FOR IMAGING THE AT1 ANGIOTENSIN RECEPTOR WITH PET

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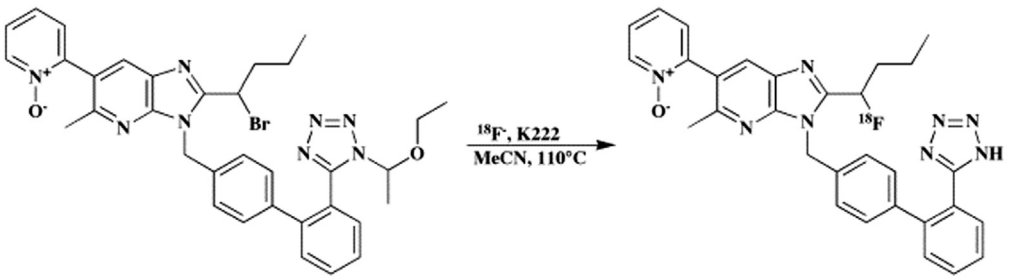
Introduction: The renin-angiotensin system plays a major role in the regulation of blood pressure and in the pathogenesis of hypertension. Although carbon-11 labeled tracers have been used successfully to image AT1 receptors in the kidneys, the density of the receptor in the heart is 50 times lower. The development of a radiofluorinated AT1 angiotensin receptor ligand would provide a complementary and more clinically useful tool for examining the role of these sites.

Experimental: A fluoro analog of the well-characterized AT1 antagonist SK-1080 was prepared and designated KR33943. [^{18}F]KR33943 was synthesized from a protected bromo precursor under standard conditions using a GE TRACERlab box.



[^{18}F] KR33943

Results and Discussion: There was no need for a separate deprotection step. The time for synthesis, purification, and formulation was 97 minutes (n=3) with an average radiochemical yield of 2.6% and an average specific activity of 1,241 mCi/μmol at end-of-synthesis. An initial ex vivo metabolite assay in mice showed that 32% of the tracer was metabolized after 40 minutes. A biodistribution study in mice using [^{18}F]KR33943 showed high initial uptake in the liver which rapidly washed out over 30 minutes. After 30 minutes, the highest uptake was in the adrenal glands. Although the adrenal uptake is in accordance with the known density of AT1 receptors, kidney and heart uptake remained lower than expected. A peak heart to blood ratio of 2.1 was observed at 60 minutes. Throughout the study the liver to heart uptake ratio was greater than 11. There was no uptake in the brain. An inhibition study using 1 mg/kg of SK-1080 showed 96% specific binding in the adrenal glands at 60 minutes. The specific binding of [^{18}F]KR33943 in other organs was 65% in kidneys, 72% in lungs, 67% in the heart, and 64% in the liver.

Conclusion: These results indicate that other fluoro analogs may be more suitable for imaging AT1 receptors with PET.

Acknowledgement: This work was supported by NIH grants DK050183 and CA092871.

Keywords: Angiotensin, AT1, Fluorine-18, Mice, PET

P222 OPTICALLY RESOLVED 9-FLUOROPROPYL-DIHYDROTETRABENAZINE AS AN IMAGING AGENT TARGETING VESICULAR MONOAMINE TRANSPORTERS

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Introduction: F-18 labeled derivatives of dihydrotetrabenazine (DTBZ) may provide useful agents for imaging vesicular monoamine transporters (VMAT2). We report the results on a study of the optically resolved active ligand 9-fluoropropyl-(+)-DTBZ (FP-(+)-DTBZ), which may have more promising characteristics.

Experimental: The inhibition constant (K_i) was estimated for FP-(+)-DTBZ (using [^{3}H](±)-DTBZ as the labeled ligand in rat striatal homogenates). Biodistribution and autoradiography studies were performed in normal and unilateral-lesioned mice (treated with 6-hydroxydopamine).

Results and Discussion: Optically resolved FP-(+)-DTBZ showed a lower Ki value as compared to the racemic FP-(±)-DTBZ (0.10 ± 0.01 vs 0.19 ± 0.04 nM). The inactive isomer, FP-(-)-DTBZ, displayed a much lower binding affinity with a K_i value >3000 nM. Biodistribution studies in mice after an iv injection of [^{18}F]FP-(+)-DTBZ exhibited a ratio of striatum (ST, target) to cerebellum (CB, background) of 4.51 at 30 minutes post-injection, which is a higher value than previously obtained with the racemic ligand [^{18}F]FP-(±)-DTBZ (ST/CB = 2.95). Brain extraction at 30 minutes after the tracer injection in mice showed that >95% of the radioactivity corresponded to the parent, non-metabolized, compound remaining in the striatum, suggesting that the tracer has an excellent *in vivo* stability. Furthermore, localization of the tracer in the brain examined with *ex vivo* autoradiography displayed a typical distribution pattern consistent with VMAT2 sites. The highest labeling was observed in monoaminergic neuron regions (caudate putamen, olfactory tubercle, nucleus accumbens, substania nigra, dorsal raphe and locus coerules). We also tested the selective labeling of this tracer at the dopamine neurons in unilateral-lesioned mice. When [^{18}F]FP-(+)-DTBZ and [^{125}I]IPT ((N-(3'-iodopropen-2'-yl)-2-beta-carbomethoxy-3-beta-(4-chlorophenyl)tropane, a selective marker for dopamine transporters in dopaminergic neurons) were simultaneously injected into lesioned mice, we observed an excellent correlation (r = 0.95) for these tracers.

Conclusion: These findings support the conclusion that [^{18}F]FP-(+)-DTBZ is a sensitive and selective tracer for VMAT2 binding sites and it may be useful for *in vivo* evaluation of diseases relating to changes of monoamine neuronal integrity.

Acknowledgement: This work was supported by a grant awarded from the National Institutes of Health (EB-002171 for H.F.K. and NS-015655 for M.R.K.). We are grateful to the National Institute of Mental Health's Chemical Synthesis and Drug Supply Program for providing the samples of resolved (+)9-O-desmethyl-DTBZ, (-)-TBZ and [^{3}H](±)-TBZ used in this project.

Keywords: Brain Imaging, 6-Hdroxydopamine, PET, In Vitro Binding, Biodistribution

P223 DEVELOPMENT OF ^{18}F-LABELLED EGFRvIII TARGETING PEPTIDES

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[1]PET & Cykloton Unit, Copenhagen University Hospital, Copenhagen, Denmark; [2]Department of Natural Sciences, Royal Veterinary and Agricultural University, Frederiksberg, Denmark; [3]Department of Radiation Biology, Copenhagen University Hospital, Copenhagen, Denmark; [4]Département de Recherche Médicale, Service Hospitalier Frédéric Joliot, Orsay, France

Introduction: One of the few known cancer specific surface markers is the epidermal growth factor tyrosine kinase receptor mutation (EGFRvIII). EGFRvIII has never been identified in normal tissues, and is therefore a promising target for imaging and treatment of various types of cancer; ovarian, glioblastomas and breast cancer.

A novel EGFRvIII targeting peptide, PEPHC1 (HFLIIGFMRRALCGA) has previously been reported [Campa, MJ. 2000 Biochem Biophys Res Com, 275 631-636]. However, this peptide has low affinity and displays considerable non-specific binding when tested in cells. We used this sequence as a starting point for the design of peptide sequences which may show improved specific binding to EGFRvIII. The novel peptides will be radiolabelled using 1-[3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione ([^{18}F]FPyME).

Experimental: Using alanine substitution in the sequence of PEPHC1 (an alanine-scan), the contribution of each amino acid residue towards the binding to EGFRvIII was established. Binding assays were performed by using the Biotin-streptavidin method on NR6M, N6RW-A and NR6 cells, expressing EGFRvIII, EGFR and either of the receptors, respectively. Based on these results a number of truncated peptides were synthesised and modified by adding a cysteine residue in either the N- or C-terminal, to allow the labelling with a fluorine-18 pyridine based prosthetic group. PEPHC1 and the truncated analogs were radiolabelled using [^{18}F]FPyME.

Results and Discussion: The Ala-scan showed that substitution of N-terminal residues His^{1}, Phe^{2}, Leu^{3}, Ile^{4} and Ile^{5} decreased the binding affinity towards EGFRvIII, indicating that these residues directly interact with the mutated receptor and are crucial for the binding.

Truncated analogs of PEPHC1 which still contained these 5 residues showed binding affinity towards EGFRvIII similar to PEPHC1. Radiolabelling of PEPHC1 and the cysteine-modified truncated analogs was achieved in 40-50% radiochemical yield by reaction with [^{18}F]FPyMe in DMSO at ambient temperature for 10 min.

Conclusion: The Ala-scan results have allowed us to understand which residues of PEPHC1 are required for binding, and a series of peptides which can be easily labelled with fluoride-18 have been synthesised. Full results of binding assays and optimised results of radiolabelling and purification will be presented.

Acknowledgement: The Danish Cancer Society for fundings.

Keywords: EGFRvIII, Targeting Peptides, 18-Fluorine, Positron Emission Tomography

P224 RADIOLABELLING WITH CARBON-11 OF A 2-OXAMIDE BENZIMIDAZOLONE, A POTENT NR2B SELECTIVE NMDA RECEPTOR ANTAGONIST

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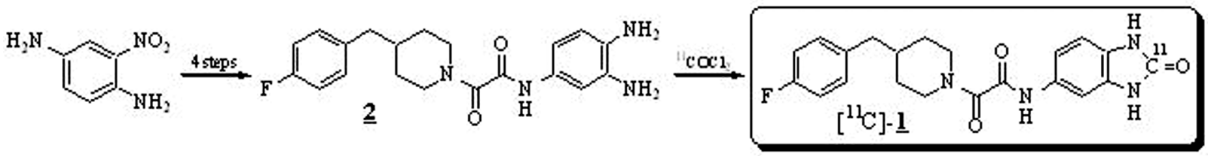
Introduction: N-Methyl-D-aspartate (NMDA) receptors are implicated in neurotoxicity associated with stroke and in neurodegenerative pathologies. The NR2B subtype of NMDA receptors has been demonstrated to be of importance in these physiopathological processes and imaging those receptors *in vivo* by Positron Emission Tomography would allow studies to understand their involvement in neurological diseases.

Several radioligands developed from NR2B selective NMDA receptor antagonists have been labelled with positron emitters but poor brain penetration, high non-specific binding and/or fast biodegradation have been observed [1]. Recently, a novel series of oxamides was identified as potent NR2B selective NMDA receptor antagonists [2] where the compound **1** showed a high affinity (IC_{50} = 5 ± 1 nM vs. [^{3}H]Ro-25,6981) and selectivity towards NR2A subunit. The benzimidazolone ring of **1** allowed its labelling with carbon-11 using [^{11}C]-phosgene.

Results and Discussion: The labelling precursor **2** was obtained by an original four-step synthesis. A selective reaction of ethyl oxalyl chloride with 2-nitro-1,4-phenylenediamine followed by alkaline hydrolysis, coupling with 4-(4-fluorobenzyl) piperidine and reduction afforded compound **2** in 16% overall yield.

The radiosynthesis was developed from [^{11}C]-phosgene obtained from cyclotron-produced [^{11}C]-methane via [^{11}C]-carbon tetrachloride and oxidation[3]. Optimized labelling conditions consisted in trapping $^{11}COCl_2$ at room temperature in a solution of **2** (1.6 mg, 4μmol) in THF (300μL) or acetonitrile (500 μL) and in presence of Et_3N (7μL). HPLC purification occurred using a semi-preparative column (Symmetry C18, Waters). Starting from a typical 750 mCi (27 GBq) batch of [^{11}C]-methane, up to 100 mCi (3.7 GBq) of [^{11}C]-**1** could be obtained within 30 minutes with a specific radioactivity ranging from 2 to 3.5 mCi μmol^{-1} EOS (75–130 GBq μmol^{-1}). The radiochemical purity was >99% and the decay-corrected yield on starting [^{11}C]-methane was 40%.



a) Ethyl oxalyl chloride, Et_3N, $CHCl_3$, RT, 67% ; b) KOH, EtOH, H_2O, RT, 80% ; c) 4-(4-Fluorobenzyl) piperidine, HBTU, DMF, RT, 84% ; d) Fe, HCl, H_2O, reflux, 36% ; e) [^{11}C]-Phosgene, THF, Et_3N, RT.

Conclusion: Preliminary *in vivo* μPET scans on rats are under investigations and will be presented.

References: [1] Årstad E. et al., *Bioorg. Med. Chem.*, **2006**, 14, 6307-6313 and ref. cited. [2] Domány G. et al., *Bioorg. Med. Chem. Lett.*, **2004**, 14, 3953-3956. [3] Link, J.M. et al., *J. Label. Compd. Radiopharm.*, **1997**, 40, 306-308.

Keywords: PET, Carbon-11, NR2B NMDA Receptors, [^{11}C]-Phosgene

P225 (^{11}C)AC-5216: RADIOSYNTHESIS AND EVALUATION AS A NOVEL PET LIGAND FOR THE PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR IN BRAIN

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[1]Department of Molecular Probes, National Institute of Radiological Sciences, Chiba, Japan; [2]Department of Molecular Neuroimaging, National Institute of Radiological Sciences, Chiba, Japan

Introduction: The peripheral-type benzodiazepine receptor (PBR, TSPO) density was increased in the injured brain, and this increase has been used as an indicator of neuronal damage and several neurodenerative disorders such as Alzheimer's disease. We have previously developed two PET ligands [^{11}C]DAA1106[1] and [^{18}F]FEDAA1106[2] for PBR imaging in the human brain. Here, we report radiosynthesis and evaluation of [^{11}C]AC-5216 as a novel ligand for the PBR imaging. AC-5216 shows a higher affinity for PBR (ki=0.297 nM) than PK11195 (0.602 nM).[3]

Experimental: A desmethyl analogue of AC-5216, a precursor for radiolabeling with C-11, was synthesized via 6 steps starting from the commercially available materials. The radiolabeling was successfully accomplished by reaction of the precursor with [^{11}C]CH_3I and NaH in DMF. The total synthesis time was 22 min from EOB and the specific activity of [^{11}C]AC-5216 in isotonic saline was averaged to be 93 GBq/μmol (n=12) at EOS.

Results and Discussion: After intravenous injection of [^{11}C]AC-5216 into mice, high accumulation of radioactivity was found in the lung, heart, kidney and other PBR-rich regions. The ex vivo autoradiography showed [^{11}C]AC-5216 had a considerably high uptake in the rat brain. In the brain, a significantly high radioactivity was observed in the olfactory bulb and choroids plexus, two high PBR density areas in the rodent brain. Co-injection with PK11195 exhibited a statistically significant reduction of [^{11}C]AC-5216 radioactivity in the brain regions. Metabolite analysis for the brain homogenate displayed that [^{11}C]AC-5216 was in vivo stable. PET study determined [^{11}C]AC-5216 had a similar radioactivity level as [^{11}C]DAA1106 in the occipital cortex, a high PBR density area in the primate brain. Co-injection with non-radioactive AC-5216 and PK11195 reduced the radioactivity level of [^{11}C]AC-5216 in the brain, suggesting high specific binding with PBR in the brain. This result suggests that [^{11}C]AC-5216 could serve as an in vivo imaging agent for PBR in the brain.

References: [1] Zhang M.-R. et al. Nucl. Med. Biol. 30, 513-519 (2003). [2] Zhang M.-R. et al. J. Med. Chem. 47, 2228-2235 (2004). [3] Kita A. et al. Br. J. Pharmacol., 142, 1059-1072 (2004).

Keywords: Peripheral-Type Benzodiazepine Receptor, [^{11}C]AC-5216, [^{11}C]DAA1106, Autoradiography, PET

P226 SYNTHESIS AND EVALUATION OF (^{11}C)-MeJDTic AS A SELECTIVE PET RADIOTRACER FOR IMAGING THE KAPPA OPIOID RECEPTOR

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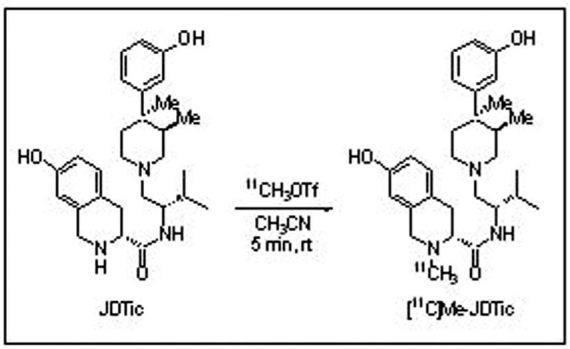
GDM-TEP, UMR CEA 2E, CEA/DSV, Université de Caen-Basse Normandie, Cyceron, Caen, France

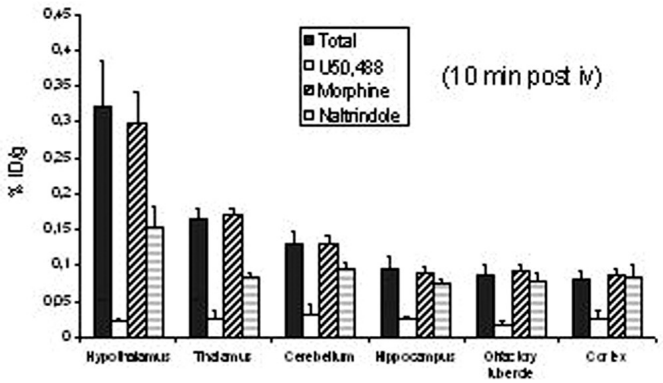
Introduction: PET imaging of κ receptor could provide important information on the assessment of the opioidergic system in healthy volunteers and patients with clinical brain disorders including addiction, epilepsy, schizophrenia, Tourette's syndrome and Alzheimer's disease.[1] To our knowledge, [^{11}C]GR 103545 is the sole promising radiotracer for κ receptor imaging reported so far.[2] However, its poorly efficient radiosynthesis, its lack of selectivity as well as its agonist properties that may be responsible for sedation, cardiac and respiratory depression as unwanted effects post iv, could constitute limitations to the studies in humans. Recently, the *N*-methylated analogue of JDTic we named MeJDTic, has been described as a potent and selective antagonist for the κ receptor (Ki = 0.053 nM; μ/κ = 700; δ/κ > 10000).[3] Here we report its radiosynthesis and its evaluation in mouse.

Results and Discussion: [^{11}C]-MeJDTic was prepared by *N*-methylation of JDTic using [^{11}C]-CH_3OTf in CH_3CN for 5 min at rt with a radiochemical yield ranging from 78 to 92% (decay corrected and calculated from [^{11}C]-CH_3OTf). In production mode, batches of [^{11}C]-MeJDTic (6-30 mCi, 222-1110 MBq) formulated in an aqueous solution of 2-hydroxypropyl-β-cyclodextrin (2%, pH 6.5) were obtained in a total synthesis time of 55 min. Specific activities ranged from 40-120 mCi/μmol (1480-4440 MBq/μmol, EOS) and radiochemical purities were greater than 97%.

Ex vivo biodistribution studies in mouse brain demonstrated that [^{11}C]-MeJDTic crossed the blood brain barrier readily (the brain uptake reaching a maximum value of 0.15% ID/g at 5 min post iv then decreasing from 20 min), and localized, at 10 min post iv, in brain regions known to contain κ receptors. Radioactive metabolite analysis carried out at this time showed that [^{11}C]-MeJDTic was 90 and 60% of the total radioactivity in brain and plasma respectively. Blocking studies were consistent with selective binding to κ receptor e.g. U50,488 (a κ referring agonist) induced an important blockade of specific binding while morphine (a μ agonist) and naltrindole (a δ antagonist) had no or little effect.



Radiosynthesis of [^{11}C]-MeJDTic



Biodistribution of [^{11}C]-MeJDTic in mouse brain regions

Conclusion: [^{11}C]-MeJDTic is a leading candidate radiotracer for the imaging of the κ receptor using PET.

References: [1] Machulla HJ et al *J. Nucl. Med.* 2005; **46**: 386-387. [2] Talbot PS et al *J. Nucl. Med.* 2005; **46**: 484-494. [3] Thomas JB et al *J. Med. Chem.* 2003; **46**: 3127-3137.

Keywords: Kappa Opioid Receptor Antagonist, MeJDTic, [^{11}C]-Methyl Triflate, Carbon-11, Positron Emission Tomography

P227 IN VITRO AND IN VIVO CHARACTERIZATION OF DIFFERENT CCK2R BINDING PEPTIDES COUPLED TO DTPAGLU AND LABELED WITH In-111

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Introduction: We are evaluating ligands aimed at targeting the CCK2 or CCKB receptor. We have previously described the labeling and biological properties of the CCK8 peptide (sequence DYMGWMDF-NH2) labeled through coupling to a novel DTPA derivative (DTPAGlu) at the N terminus using a glycine spacer (DTPAGlu-G-CCK8). Given the success of gastrin based ligands in targeting CCK2 receptor expressing tissues and tumors both in animal models and in clinical studies, we have now evaluated binding and biodistribution properties of two DTPAGlu coupled minigastrin derivatives.

Experimental: CCK2 receptor overexpressing A431 and control cells were utilized for in vitro binding experiments and in vivo biodistribution studies in animal models. The DTPAGlu chelating moiety was coupled to the N terminus of long minigastrin (LMG, sequence eEEEEEAYGWMDF-NH2) or short minigastrin (SMG, eAYGWMDF-NH2) in solid phase giving DTPAGlu-LMG and DTPAGlu-SMG. Reversed Phase HPLC was utilized to evaluate labeling efficiency with In-111 and quality control.

Results and Discussion: Saturation experiments with DTPAGlu-LMG and DTPAGlu-SMG on receptor expressing cells showed similar affinity to that observed with DTPAGlu-G-CCK8 (Kd $\sim$ 10-8 M). In biodistribution experiments, LMG gave higher level targeting of CCK2 receptor overexpressing xenografts (2.8 $\pm$ 1.0%ID/g at 1h) compared to the control tumor (0.2 $\pm$ 0.1%ID/g). These values are higher than those observed for SMG (1.2 $\pm$ 0.6%ID/g in positive xenograft versus 0.1 $\pm$ 0.0%ID/g in control) and for previously published values for DTPAGlu-G-CCK8 (1.6 $\pm$ 0.5%ID/g in positive xenograft versus 0.2 $\pm$ 0.2%ID/g in control). However, high level retention of the compound was observed in the kidneys for DTPAGlu-LMG (48.1 $\pm$ 18.6%ID/g) compared to that observed with DTPAGlu-SMG and DTPAGlu-G-CCK8 (2.6 $\pm$ 0.3%ID/g and 4.1 $\pm$ 1.4%ID/g, respectively) at 1h.

Conclusion: DTPAGlu-LMG accumulation in the CCK2R expressing tumors relative to the control tumor is very favorable compared to the other two compounds. However, kidney accumulation is much higher for DTPAGlu-LMG compared to DTPAGlu-SMG and DTPAGlu-G-CCK8. Tumor to kidney ratios are therefore much less favorable for DTPAGlu-LMG while they are better and very similar for DTPAGlu-SMG and DTPAGlu-G-CCK8. Our findings suggest that for diagnostic use LMG derivatives may be more suitable for the higher receptor targeting however for therapeutic use the lower level targeting to the kidneys of SMG and CCK8 would make use of these derivatives more desirable.

Keywords: Cholecystokinin Receptors, Radiolabeled Peptides, Indium-111, Affinity, Biodistribution

P228 4-FLUORO-*N*-(2-(4-(2,3-DIHYDROBENZO(B)(1,4)DIOXIN-8-YL)PIPERAZIN-1-YL)-ETHYL)BENZAMIDE, A NEW HIGH AFFINITY 5-HT_{1A} RECEPTOR AGONIST – SYNTHESIS AND ^{18}F-LABELING AS A PROSPECTIVE RADIOLIGAND

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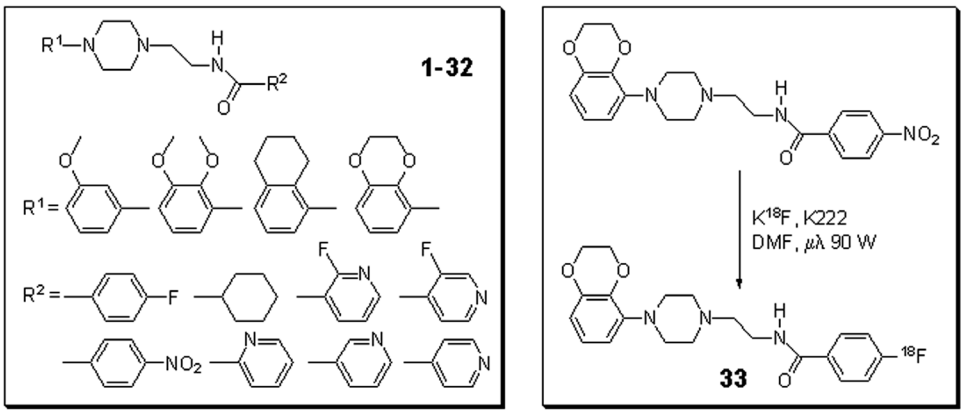
Introduction: Agonist radioligands for imaging brain 5-HT_{1A} receptors continue to attract interest because they may be useful for investigation of this G-protein coupled receptor in the 'high affinity' state. In our search for effective agonist PET radioligands for the 5-HT_{1A} receptor, we synthesized new ligands (**1**-**32**) by coupling one of four selected aryl substituted piperazines with one of eight alkyl/aryl amide moieties and tested their 5-HT_{1A} receptor affinities and intrinsic activities. Here we report the discovery of one high affinity agonist from this group and its labeling with fluorine-18 for PET evaluation.



Experimental: All syntheses began with the preparation of the aryl piperazine, followed by alkylation with bromoacetonitrile, then reduction with LAH. The 2'-aminoethyl arylpiperazines were then coupled with the appropriate acid chloride (Method A) or acid (Method B) to produce **1**-**32** for assessment of binding affinity and intrinsic activity in a $GTP_{\gamma}S$ binding assay. Microwave enhanced drying of [^{18}F]fluoride ion and reaction were performed in a modified Synthia radiochemistry module equipped with a RI 521 microwave cavity. Typically, the nitro precursor (2.0 mg) in DMF (0.5 mL) was irradiated (90 W; 5 × 2 min) with dried [^{18}F]fluoride ion and K2.2.2. The reaction mixture was diluted with water (0.7 mL) and injected onto preparative HPLC using isocratic MeCN-10 mM $HCOONH_4$ (28:72) as mobile phase at 6 mL/min. The ^{18}F-labeled product (**33**) was collected with retention time between 38.5 and 41.5 min.

Results and Discussion: The highest affinity compounds comprised 2,3-dihydrobenzo[*b*][1,4]dioxin-8-yl substituted piperazine and 4-fluorobenzamide (K_i = 0.44 nM) or cyclohexanecarboxamide (K_i = 0.71 nM) moieties. Both are full 5-HT_{1A} receptor agonists. ^{18}F-labeling of **33** (12-62% decay-corrected radiochemical yield) was achieved in DMF heated at >120°C under microwave irradiation. **33** was not obtained in DMSO below 110°C; the precursor decomposed at higher temperature in this solvent. Optimizations of reaction and formulation conditions are in progress.

Conclusion: The title compound is a high affinity, high efficacy 5-HT_{1A} receptor agonist and can be labeled with fluorine-18 in DMF for future evaluation as a PET radioligand.

Acknowledgement: This research was supported by the Intramural Research Program of the NIH, NIMH. We thank H. Lundbeck A/S for performing the *in vitro* evaluation.

Keywords: Fluorine-18, Agonist, 5-HT_{1A} Receptor, Microwave

P229 PREPARATION AND IN VITRO AND IN VIVO EVALUATION OF ^{99m}Tc-MAG$_3$-FOLATE

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Introduction: The cell-membrane folic acid (FA) receptors are known to be responsible for cellular accumulation of FA and FA analogs, and are over-expressed on several tumor cells. Folate possesses high affinity for the folate-receptor positive cells and tissues and several ^{99m}Tc radiolabeled folate were deemed useful for diagnostic imaging of folate-receptor positive tumors. Herein, we report synthesis of S-acetyl-MAG$_3$-Folate, and the radiolabeling of ^{99m}Tc-MAG$_3$-Folate and *in vitro* and *in vivo* evaluation of this conjugate.

Experimental: A bifunctional chelator S-acetyl-mercaptoacetyltrigylcine N-hydroxysuccinimide ester (S-acetyl-MAG$_3$-NHS) was synthesized. Folic acid was reacted with ethylenediamine (EDA) to yield folate-EDA. The product was reacted with S-acetyl-MAG$_3$-NHS. After purification by RP-HPLC, MAG$_3$-Folate was obtained. The MAG$_3$-Folate conjugate was then radiolabeled with ^{99m}Tc by using sodium tartrate as a transchelator. The ^{99m}Tc-MAG$_3$-Folate radiolabeling conditions and its *in vitro* stability were investigated in detail. The biodistribution and blood clearance of ^{99m}Tc-MAG$_3$-Folate was studied in normal mice. The preliminary studies on tumor uptake and imaging property of ^{99m}Tc-MAG$_3$-Folate were evaluated in nude mice bearing HeLa cell.

Results and Discussion: Under optimum conditions, the radiolabeled yield of ^{99m}Tc-MAG$_3$-Folate was in excess of 70% and the radiochemical purity was more than 95% after purification with a C18 Sep-Pak Cartridge. The *in vitro* stability of ^{99m}Tc-MAG$_3$-Folate was satisfied. In mice, the radioconjugate showed rapid clearance from blood and excretion mainly through the renal/urinary system. Except for the kidney, uptake by other tissues was rather low. ^{99m}Tc-MAG$_3$-Folate was localized moderately in tumor at 4 h imaging.

Conclusion: The MAG$_3$-Folate was successfully labeled with ^{99m}Tc in high yield and stability. The studies to evaluate the receptor binding affinity of radiolabeled conjugates and their biodistribution in animal tumor xenograft models are in progress.

Acknowledgement: This work was supported in part by the National Natural Science Foundation of China (No. 30370421, No.30670584).

Keywords: Folate, MAG3, Technetium-99m

P230 SYNTHESES AND PRE-CLINICAL EVALUATION OF ^{18}F-LABELED BICYCLIC ANALOGUES OF VESAMICOL AS POTENTIAL *VAChT* IMAGING AGENTS

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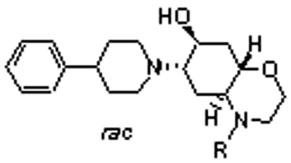
Introduction: ^{18}F-labeled vesamicol analogues are potential radioligands to visualize cholinergic transmission deficits in brain by PET. They bind with high affinity to the vesicular acetylcholine transporter *(VAChT)*, located in central cholinergic nerve terminals. Here, we report on the radiosyntheses of bicyclic octahydrobenz[1,4]oxazin derivatives, a novel class of vesamicol analogues with appropriate lipophilicity and binding properties.

Experimental: Binding affinities of **1** and **2** to *VAChT* and σ receptors were determined on *rVAChT* transfected cells. [^{18}F]**1** was obtained from **1a** by nucleophilic substitution (DMF, 145°C, 15 min) followed by SPE and HPLC. [^{18}F]**2** was obtained from **2a** using microwave-assisted synthesis (DMF, 154°C, 15 min). Five anesthetized piglets were injected with [^{18}F]**2** (~120 MBq). One animal was preinjected with vesamicol. Two animals received the σ receptor ligand *DTG*. Dynamic PET was acquired for 7 h. Plasma samples were withdrawn for metabolite analysis with HPLC.

Results and Discussion: **1** and **2** reveal high affinity *VAChT* binding (K_i; **1**: 42 ± 7 nM, **2**: 27,5 ± 8.8 nM) and selectivity towards σ receptors (**1**: 6175 ± 100 nM, **2**: 775 ± 60 nM). [^{18}F]**1** was synthesized with a radiochemical purity > 99%, a specific activity of 50-150 GBq/μmol and a yield of 35 ± 7% (75 min, n=31). Precursor **2a** displays *VAChT* affinity as well. A separation of [^{18}F]**2** and **2a** by HPLC was not successful. To reduce **2a**, the best results were obtained with Pd/ammonium formate (MeOH, 60°C). [^{18}F]**2** was synthesized with a radiochemical purity > 99%, a specific activity of 50-250 GBq/μmol and a yield up to 15% (150 min, n=21). [^{18}F]**1** and [^{18}F]**2** were stable in 0.9% NaCl, PBS, and inactivated plasma up to six hours. PET studies in pigs showed a high brain uptake of [^{18}F]**2** and enrichment in regions with high *VAChT* densities. The binding kinetics is slow. Application of vesamicol blocked the specific binding. However, also *DTG* reduced the specific binding, indicating partial σ receptor binding of [^{18}F]**2**.



	R
1	F-acetyl
1a	Br-acetyl
2	4-F-benzoyl
2a	4-NO_2-benzoyl

Conclusion: Radiosyntheses of ^{18}F-labeled bicyclic derivatives of vesamicol were established. As suggested by *in vivo* studies further structural modifications are needed to obtain radiotracers with higher selectivity and faster binding kinetics.

Keywords: Vesamicol, ^{18}F-Labelling, Animal Studies, PET

P231 SYNTHESIS AND RADIOFLUORINATION OF THE PUTATIVE AMPA RECEPTOR LIGAND *N*-2-(4-*N*-(4-(^{18}F)FLUOROBENZAMIDO)PHENYL)-PROPYL-2-PROPANESULFONAMIDE

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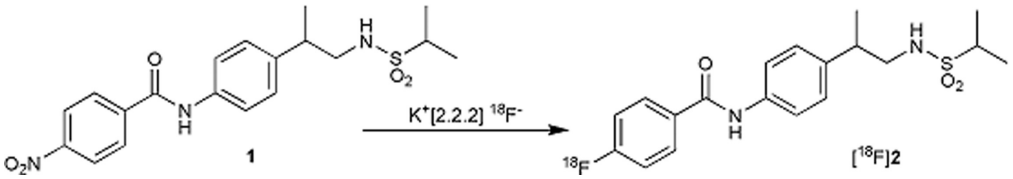
Introduction: The search for new AMPA receptor ligands lead to a series of arylpropylsulfonamides which showed promising results. With *N*-2-(4-(*N*-benzamido)phenyl)-propyl-2-propanesulfonamide (LY395153), that has previously been labeled with tritium[1], a benzamide with sulfonamide functionality came into the focus of research. This structure served as lead structure for the design of a fluorine-18 labeled radioligand of the AMPA receptor for potential application in cerebral imaging with positron emission tomography.

Experimental: The standard compound *N*-2-(4-*N*-(4 fluorobenzamido)phenyl)propyl-2-propanesulfonamide (**2**) and the labeling precursor *N*-2-(4-*N*-(4-nitrobenzamido)phenyl)propyl-2-propanesulfonamide (**1**) were synthesized in six steps, following a published method of synthesis.[1] Using the labeling precursor **1** in a Kryptofix 2.2.2®/K_2CO_3 activated nucleophilic radiofluorination, the putative AMPA receptor ligand *N*-2-(4-*N*-([^{18}F]-4-fluorobenzamido)phenyl)-propyl-2-propanesulfonamide ([^{18}F]**2**) was obtained after semipreparative separation by HPLC (cf. Scheme).

Results and Discussion: After optimization of the reaction parameters time, temperature, solvent and concentration, the radiochemical yield obtained was about 38% at 180°C in DMSO within 30 minutes reaction time. After solid phase extraction followed by semipreparative HPLC-separation and a final solid phase extraction, the product was isolated in radiochemical yields of about 5%. Radiochemical purity was higher than 95% and the specific activity amounted to 77 GBq/μmol after 120 minutes of synthesis.

First *in vitro* assays with rat brain slices however revealed a high nonspecific binding and a uniform distribution of [^{18}F]**2** not lending it for *in vivo* imaging purposes.



Conclusion: A Kryptofix 2.2.2®/K_2CO_3 activated nucleophilic radiofluorination reaction for the putative AMPA potentiator *N*-2-(4-*N*-(4-[^{18}F]fluorobenzamido)-phenyl)-propyl-2-propanesulfonamide ([^{18}F]**2**) was developed and its radiochemical yield was optimized. It was produced reliably in radiochemical yields of 5% within 120 minutes with a specific activity of 77 GBq/μmol. From the first *in vitro* assays can be concludeded that [^{18}F]**2**, due to its high nonspecific binding and the uniform distribution, is rather unsuitable for imaging AMPA potentiator binding sites.

Reference: [1] Zarrinmayeh H, Bleakman D, Gates MR, Yu H, Zimmerman DM, Ornstein PL, McKennon T, Arnold MB, Wheeler WJ, Skolnick P. *J Med Chem* 2001; **44**: 302-304.

Keywords: AMPA Receptor, Potentiator, Radioligand, N.C.A. Radiofluorination, Fluorine-18

P232 SYNTHESIS OF 5-FLUOROINDOL-3-YL CYCLOBUTYLAMINES AS SEROTONIN TRANSPORTER LIGANDS FOR ^{18}F LABELING

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Introduction: Several mental illnesses are associated with disorders of the serotonergic neurotransmission. The serotonin transporter (SERT) is crucial for the regulation of the synaptic concentration of serotonin and is a primary target in the development of antidepressants. Indolylcycloalkanyl amines (C_nH_{2n-2}, n=6,5,3), considered as conformationally constrained analogues of 5-hydroxytryptamine (5-HT, serotonin), have been introduced as a class of candidates holding highly potent SERT inhibitors.

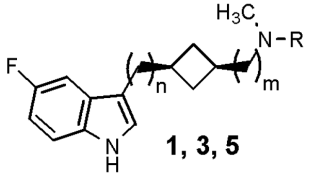
In the search for new SERT ligands for PET with improved binding profiles we turned our interest towards rigid structures containing a 1,3-disubstituted cyclobutane (C_nH_{2n-2}, n=4).

The present work describes the synthesis and initial biological evaluation of the mono-fluorinated (**1a,2a,3a,6a**) and double-fluorinated target molecules (**1b,3b**).

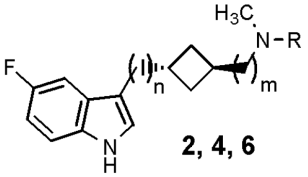
Experimental: Suitable cyclobutanone precursors were obtained *via* 2+2 cycloaddition starting from corresponding olefines and dichloroketene under ultrasonic irradiation followed by dechlorination using Zn-acetic acid.

Results and Discussion: The carbonyl group of both 3-(2-benzyloxy-ethyl)- and 3-(3-benzyloxy-propyl)-cyclobutanone was processed to yield the N-methyl, N-Boc protected *cis*- or *trans*-cyclobutyl amines and -methylenamines respectively. After *O*-debenzylation the alcohol was oxidized to the aldehyde applying the known TEMPO procedure. The secondary amines (**1a,2a,3a,6a**) were then directly obtained from the cyclobutyl aldehyde precursor via Fischer indole synthesis; for the tertiary amines **1b, 3b** a reaction of **1a** and **3a** with 3-fluoropropyliodide was appended.

Displacement experiments on hSERT-HEK293 cells labeled with [^{3}H]citalopram have shown that **1b** and **3a** have nanomolar affinity for human SERT (IC50 (**1b,3a**) <10 nM). By contrast, parallel studies applying [^{3}H]paroxetine as radioligand indicated significantly lower SERT affinities (100 nM < IC50 (**1b,3a**) <1000 nM). Thus, potential radiotracers based on the new derivatives may be able to selectively label the citalopram binding site of the SERT.



1, 3, 5



2, 4, 6

R	n	m	*cis*	*trans*
H	0	0	**1a**	**2a**
3-F-propyl	0	0	**1b**	2b
H	1	0	**3a**	4a
3-F-propyl	1	0	**3b**	4b
H	0	1	5a	**6a**
3-F-propyl	0	1	5b	6b

Conclusion: We have developed a general access to 5-fluoroindol-3-yl cyclobutylamines both in *cis*- (**1,3,5**) and *trans*-configuration (**2,4,6**) obtained from readily accessible starting materials. First compounds synthesized displayed promising affinities for SERT. Appropriate candidates will be labeled for *in vivo* distribution studies.

Acknowledgement: We thank the Deutsche Forschungsgemeinschaft for financial support.

Keywords: Serotonin Transporter, 5-Fluoroindole, Cyclobutylamine, Fluorine-18

P233 SYNTHESIS AND ^{18}F-FLUORINATION OF R91150 – A 5-HT$_{2A}$ ANTAGONIST

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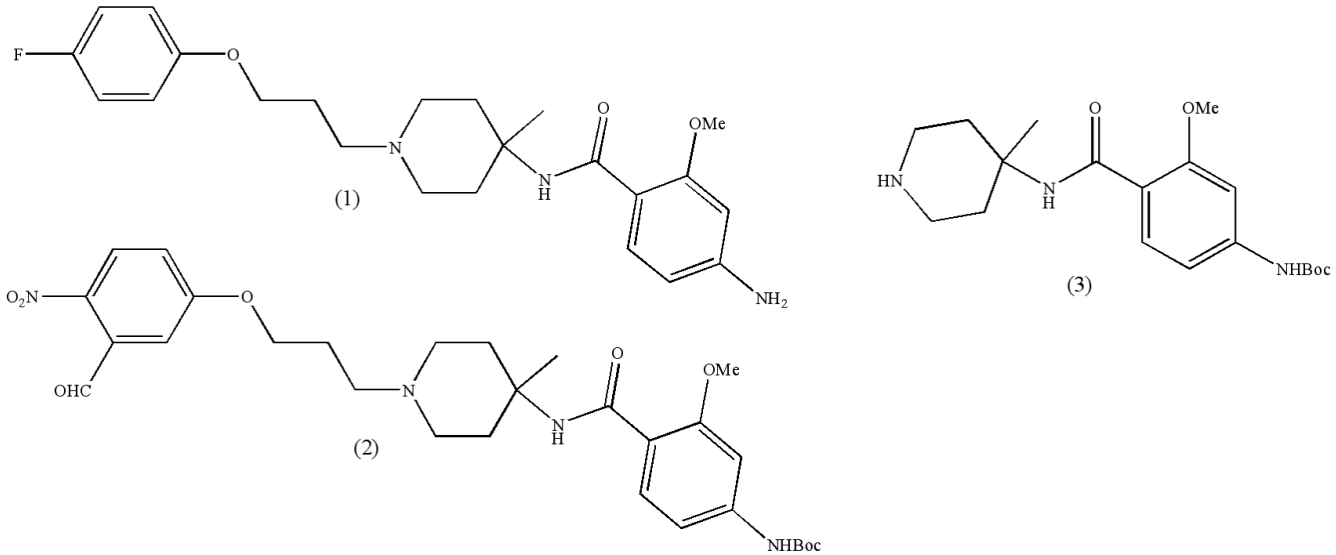
Introduction: In psychiatric disorders such as depression and schizophrenia 5-HT$_{2A}$ receptors seem to play an important role. In order to investigate them there is an increasing interest in obtaining a selective and high affinity radiolabelled ligand for receptor binding studies using PET or SPECT. For PET studies several 5-HT$_{2A}$ antagonists are utilized like [^{11}C]MDL100907 or [^{18}F]altanserin, both of which have some disadvantages. The binding of [^{11}C]MDL100907 to 5-HT$_{2A}$ is very specific[1] but due to the short half life of C-11 use is limited. [^{18}F]Altanserin exhibits relatively high affinities for α_1, H_1, and D_2 receptors[1]. For SPECT studies 5-[^{123}I]R91150 has been employed but also shows relatively high unspecific binding[2]. Therefore finding a highly selective 5-HT$_{2A}$ antagonist labelled with F-18 is desirable. In comparison to 5-[^{123}I]R91150 the parent compound R91150 possesses a somewhat lower IC_{50} value of 0.18 nM vs. 0.60 nM[3].

Experimental: For the development of methods for identification and purification of the radioactively labelled compounds the respective non-radioactive standard compounds such as R91150 (1) had to be synthesized. A first labelling precursor was the activated nitro-derivative (2) for direct radiofluorination. Another approach to radiofluorination was the preparation of 1-(3-bromopropoxy)-4-[^{18}F]fluorobenzene and conjugation to the piperidine-derivative (3).



Results and Discussion: The non-radioactive standard compounds and labelling precursors were synthesized successfully. Radiofluorination of the nitro-derivative did not result in the desired [^{18}F]R91150. 4-[^{18}F]Fluorophenol[4] was obtained in 57–69% RCY and a reaction time of 55 min starting with [^{18}F]fluoride. 1-(3-bromopropoxy)-4-[^{18}F]fluorobenzene was synthesized and isolated in 45–70% RCY in 60 min starting with 4-[^{18}F]fluorophenol. Reaction of this compound with piperidine-derivative (3), deprotection, and isolation afforded [^{18}F]R91150 in 52–65% RCY within 60 min, with a radiochemical purity of >99.9% and specific activity between 303–698 MBq/μmol.

Conclusion: The new 5-HT$_{2A}$ antagonist [^{18}F]R91150 was prepared via 4-[^{18}F]fluorophenol and 1-(3-bromopropoxy)-4-[^{18}F]fluorobenzene which allows the *in vitro* and *in vivo* evaluation of this compound for its suitability as a selective ligand for PET studies of 5-HT$_{2A}$ receptors.

References: [1] Kristiansen, H. et al. *Synapse* 2005; **58:** 249–257. [2] Busatto, G.F. et al. *Eur. J. Nucl. Med.* 1997; **24:** 119-124. [3] Leysen, J. et al. Patent WO 94/02462. [4] Ludwig, T. et al. *Nucl. Med. Biol.* 2002; **29:** 255-262.

Keywords: Fluorine-18, 5-HT$_{2A}$ Receptor, Radioligand, R91150, 4-[^{18}F]Fluorophenol

P234 RADIOSYNTHESIS OF ^{18}F-LABELLED PYRAZOLO(1,5-a)PYRIMIDINE AS POTENTIAL α1-GABA$_A$ IMAGING AGENTS FOR PET

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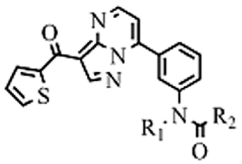
Introduction: Novel non-benzodiazapine sedative-hypnotic agents for the treatment of insomnia act as GABA$_A$ receptor agonists. The therapeutic effects are based on high affinity and specificity to α1 subunit-containing γ-aminobutyric acid (GABA)$_A$ receptors in the brain, involved in numerous brain disorders. Therefore, ^{18}F labelled pyrazolo[1,5-a]pyrimidine were developed as new specific tracers for neuroimaging.

Experimental: Four candidates for ^{18}F labelling were selected by in vitro affinity, and nucleophilic ^{18}F labelling strategies were developed. Properties such as stability against defluorination, partition coefficients and affinity to plasma proteins were analyzed.

Results and Discussion: Due to sensitivity of compound [^{18}F]**1**, the fixation of K^{18}F on Kryptofix K222 was replaced by 18-crown-6. The bromoacetyl precursor was reacted to the ^{18}F-labelled derivative in good yield (MeCN, 80°C, 20 min) followed by multiple-stage purification (SPE, preparative HPLC). The final product was analyzed by HPLC and TLC. Radiochemical yield (RCY) of 50-60% (85 min), radiochemical purity of >99%, and a specific activity of >250 GBq/μmol were achieved. [^{18}F]**2** was obtained from the 4-nitrobenzoyl precursor by microwave-assisted synthesis (DMF, 140-150W, 155°C, 12 min). However, defluorination of [^{18}F]**2** and numerous decomposition products were found. Labelling yields of up to 12% were achieved, and a time-consuming purification was necessary. Analytical data of [^{18}F]**2**: radiochemical purity >95%, specific activity >5 GBq/μmol, total preparation time ~ 3 h. [^{18}F]**3** and [^{18}F]**4** were prepared in a single radiofluorination step of the tosylate precursors. The reaction was performed in DMF at 155°C within 8-10 min, followed by HPLC purification combined with SPE. Both compounds were obtained with RCYs of 65-70%, radiochemical purity >98.5% and specific activity >300 GBq/μmol. Radiotracers [^{18}F]**1-4** were stable in 0.9% NaCl, PPS, and inactivated plasma at 37°C up to five hours. No decomposition or defluorination was observed. Low partition coefficients (in n-octanol/water) result in a moderate binding to plasma proteins.



	1	2	3	4
R_1	CH_3	CH_3	$(CH_2)_3F$	$(CH_2)_4F$
R_2	CH_2F	C_6H_4F	CH_3	CH_3
K_i [nM]	7.18	14.0	2.15	4.83
log D, pH 7.4	1.49	–	2.07	2.43

Conclusion: Radiosyntheses of ^{18}F-labelled pyrazolo[1,5-a]pyrimidines **1-4** were established. Apart from compound **2**, good RCY, high radiochemical purity and specific activity were achieved. First biological data prompt us to continue with animals studies.

Keywords: α1-GABA(A) Agonists, Pyrazolo[1,5-a]Pyrimidine Derivatives, [F-18] Labelling, Brain Imaging, PET

P235 (^{11}C)REMIFENTANIL: A NOVEL PET TRACER FOR VISUALIZATION OF μ-OPIOID RECEPTORS

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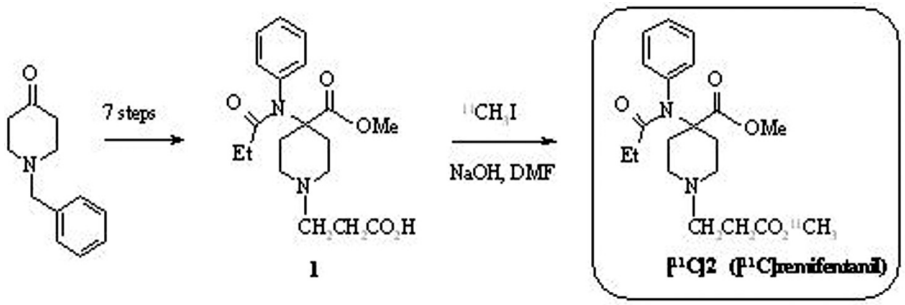
GDM-TEP, UMR CEA 2E, CEA/DSV – Université de Caen-Basse Normandie, Cyceron, Caen, France

Introduction: Opioid receptors are classified into three major subtypes: mu, delta and kappa and play an important role in analgesia. They are also involved in several clinically relevant diseases such as addiction, Parkinson's, Alzheimer's, analgesia and seizure disorders.

[^{11}C]Carfentanil,[1] the only available radioligand for μ-opioid receptors in human PET studies, produces side-effects at very low doses. In order to overcome these disadvantages, we have developed the radiosynthesis of [^{11}C]remifentanil[2], a μ selective receptor agonist which belongs also to the family of 4-anilinopiperidine derivatives. Remifentanil has the pharmacokinetic advantage of an ultra-short acting opioid agent and is used for clinical pratice as a part of general balanced anesthesia for conscious sedation and as an analgesic adjunct to local or regional anesthesia.

Results and Discussion: We have developed the carboxylic acid precursor **1**[3-5] for C-11 incorporation in a 7 step synthesis starting from *N*-benzyl-4-piperidinone in 12.5% yield. [^{11}C]Remifentanil was prepared by O-methylation of the precursor **1** with [^{11}C]methyl iodide in the presence of sodium hydroxide at room temperature for 5 min followed by HPLC purification (Scheme 1).



Scheme 1

Conclusion: After formulation and sterile filtration, [^{11}C]**2** ([^{11}C]remifentanil) was obtained in a synthesis time of 50 min with a radiochemical yield of 30-55% (based on initial [^{11}C]methyl iodide), specific activities (at end of the synthesis) ranged from 1300-1500 mCi/μmol, and radiochemical purities greater than 99%.

References: [1] Dannals RF, Ravert HT, Frost JJ, Wilson AA, Burns HD, Wagner HN, *Int. J. Appl. Radiat. Isot.*, **1985**, 36, 303-306. [2] Peter SA. Glass., *J. Clin. Anesth.*, **1995**, 7, 558-563. [3] Feldman PL, James MK, Brackeen MF, Johnson MR, Leighton HJ, *Eur. Pat. Appl.*, **1990**, 90-301586. [4] Van Dael PGH, De Bruyn MFL, Boey JM, Sanczuk S, Agten JTM,.Janssen PAJ, *Arzeneim-Forsch Drug Res.*, **1976**, 26, 1521-1531. [5] Feldman PL, James MK, Brackeen MF, Bilotta JM, Schuster SV, Lahey AP, Lutz MW, Johnson MR, Leighton HJ, *J. Med. Chem.*, **1991**, 34, 2202-2208.

Keywords: Carbon-11, Opiate Receptors, Remifentanil

P236 SYNTHESIS AND IN VITRO METABOLISM STUDIES OF THE VEGFR-2-TK INHIBITOR (R,S) *N*-(4-BROMO-2-FLUOROPHENYL)-6-METHOXY-7-{(1-(^{11}C)METHYL-3-PIPERIDINYL)METHOXY}-4-QUINAZOLINAMINE

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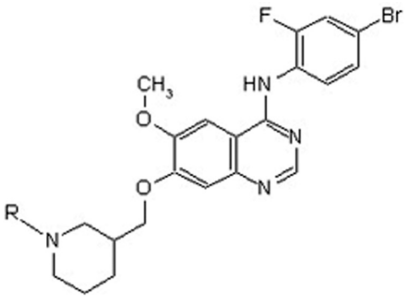
Introduction: The vascular endothelial growth factor receptor (VEGFR)-2 plays a pivotal role in angiogenesis in normal and malignant tissue. Receptor expression is increased in many forms of cancer (Ferrara). Compound **1**, (R,S) *N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methyl-3-piperidinyl)methoxy]-4-quinazolinamine, exerts inhibitory effects on VEGFR-2 by competitively blocking the ATP-binding site of the intracellular tyrosine kinase domain, (IC_{50}=1 nM and 10 nM for the R- and S-isomers, respectively, at ATP levels of 2 μM) (Hennequin). It was chosen here as a lead candidate for PET studies of VEGFR-2. **1** R = CH_3 **2** R = H **3** R = $^{11}CH_3$.



Experimental: Compounds **1** and **2** were synthesized starting from methyl vanillate and 3-chloromethyl-N-methylpiperdine (**1**) or 3-(hydroxymethyl)-piperidine (**2**) (Hennequin, Rivera). They were identified using LC-MS spectrometry. Compound **3** was synthesized by the reaction of **2** with [^{11}C]CH_3I produced by standard methods at the Karolinska Hospital/Institutet. [^{11}C]CH_3I was trapped in DMF containing the precursor and K_2CO_3 and the mixture was allowed to react at 120°C. Reaction yield, radiochemical purity and metabolite formation was assessed on HPLC (μ-Bondapak C18 (300x3.9 mm); CH_3CN:H_2O=60:40 NH_4OAc (aq) 0.025 mM), UV- (λ 254 nm) and radio-detectors in series. In vitro metabolism studies were performed using rat liver microsomes (male, Spraque Dawley) in PBS, NADPH, 37°C.

Results and Discussion: The decay corrected amount of **3** represented 30% of the total activity after 3 min reaction time with only minor increases at longer reaction times according to radio-HPLC. In the in vitro screening one major radioactive metabolite was observed in increasing amounts up to 60 min. Its elution near the void volume is consistent with metabolites arising from *N*-demethylation of **3**, though further studies are needed for identification.

Conclusion: This radiotracer will be subjected to further in vitro and in vivo evaluation of its capabilities for quantifying VEGFR-2 and the degree of angiogenesis in vivo. Pending positive results, the enantiomers will be also be separated.

Acknowledgement: Financial support by the Karolinska Institutet, Swedish Cancer Society (03 0367) and EU FP6 (LSHC-CT-2004-505785).

References: [1] Ferrara N. *The Oncologist*. Suppl 1:2-(2004). [2] Hennequin LF,.et al. *J Med Chem.* 45; 1300-1312 (2002). [3] Rivera J, et al. *Tetrahedron letters*. 43; 8917-8919 (2002).

Keywords: VEGFR-2-tk, Angiogenesis, Carbon-11, Positron Emission Tomography

P237 SYNTHESIS AND POSITRON EMISSION TOMOGRAPHY STUDIES OF C-11 LABELED GTS-21, A PARTIAL α7 NICOTINIC CHOLINERGIC AGONIST DRUG

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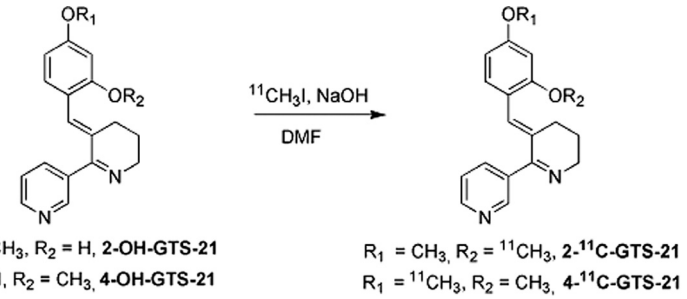
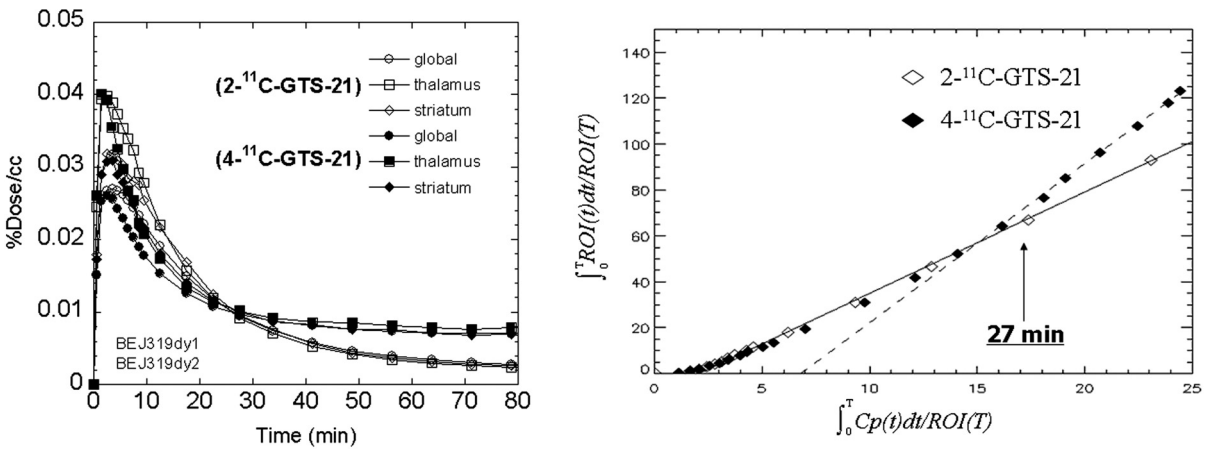
Introduction: (*E*)-3-[2, 4-dimethoxybenzylidene]anabaseine (GTS-21) is a partial α7 nicotinic acetylcholine receptor agonist drug which has been shown to improve cognition in schizophrenia and Alzheimer's disease (Olincy A. et al. *Arch Gen Psychiatry.* 2006;63:630.). GTS-21 has an active metabolite, 4-OH-GTS-21 which has been suggested to contribute to its therapeutic effects. Here, we labeled GTS-21 with C-11 in two positions for pharmacokinetic studies with PET and microPET.

Experimental: Two isotopomers of GTS-21 (2-^{11}C-GTS-21 and 4-^{11}C-GTS-21) were synthesized from [^{11}C]methyl iodide and their respective nor-precursors and compared in baboon brain. The pharmacokinetics of 2-^{11}C-GTS-21 in peripheral organs was also measured. MicroPET studies were performed in the mice followed by *ex vivo* analysis.



Results and Discussion: Radiochemical yields and purities were 20-43% and >98%, respectively, for both isotopomers and specific activities ranged from 0.8 to 2.9 Ci/μmol. Initial C-11 global brain uptake was high and rapid peaking from 1-3.5 min. Clearance was also very rapid ($t_{1/2}$=15 min from peak). The time-activities curves and distribution volume of two isotopomers were identical until 27 minutes, after which C-11 uptake was higher for 4-^{11}C-GTS-21 than 2-^{11}C-GTS-21. The chemical form in the mouse brain was largely GTS-21. The main route of excretion was through the gallbladder for both mouse and baboon.



Conclusion: Although initial brain uptake of GTS-21 is high and widespread, it shows rapid clearance resulting in low brain retention by 30 min. Differences between the 2 and 4-^{11}C isotopomers in brain at later times suggest different concentration of labeled metabolites. These results may be important in understanding mechanism of action and in designing dosing of GTS-21 for treatment of neurocognitive disorders.

Acknowledgement: Supported by DOE-OBER and NIDA K05 DA 020001.

Keywords: [^{11}C]GTS-21, α7 Nicotinic Acetylcholine Receptor, Drug Pharmacokinetics

P238 SYNTHESIS AND BIOLOGICAL EVALUATION OF ^{18}F-LABELED RALOXIFENE DERIVATIVE FOR ESTROGEN RECEPTOR IMAGING AGENT

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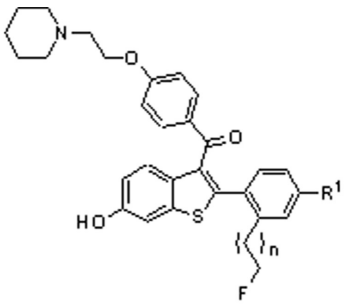
Introduction: Estrogen receptor (ER) is one of a nuclear receptor for estrogens, which regulate the production of proteins that modulate various biological events. Of numerous ER ligands, raloxifene, classified as a selective estrogen receptor modulator, is currently under clinical evaluation for the prevention and treatment of postmenopausal osteoporosis and has been found to have as effective as tamoxifen to breast cancer.We previously synthesized compounds **1**, **2**, and **3**, and their relative binding affinity (RBA) showed 89%, 60%, and 45%.

Experimental: The precursor of **1** that has the highest RBA value was synthesized by Suzuki coupling reaction from 6-methoxybenzo[*b*]thiophene, followed by Friedel-Crafts acylation. [^{18}F]**1** was prepared using [^{18}F]TBAF in CH_3CN at 120°C for 15 min, followed by deprotection using 1 N HCl at 120°C for 10 min. [^{18}F]**1** was purified by HPLC (4 mL/min, 0 min to 30 min: 34% CH_3CN:66% 0.1 M formate buffer solution, 30 min to 35 min: 40% CH_3CN:60% 0.1 M formate buffer solution, R_t = 18-20 min). The radiochemical yield was achieved in 15-25% with radio-decay correction and >95% of radiochemical purity.



1, R^1 = H, n = 1; RBA = 89 %
2, R^1 = H, n = 2; RBA = 60 %
3, R^1 = OH, n = 1; RBA = 45 %

Results and Discussion: The normal biodistribution result was obtained after 2 h after post-injection in tail vein of normal female rate (Figure 1). [^{18}F]**1** showed good uptake in the uterus (1.99%ID/g) and ovaries (2.17%ID/g). The uterus to muscle and ovaries to muscle uptake ratio were highly 5.21 and 5.68 after 2 h, respectively.

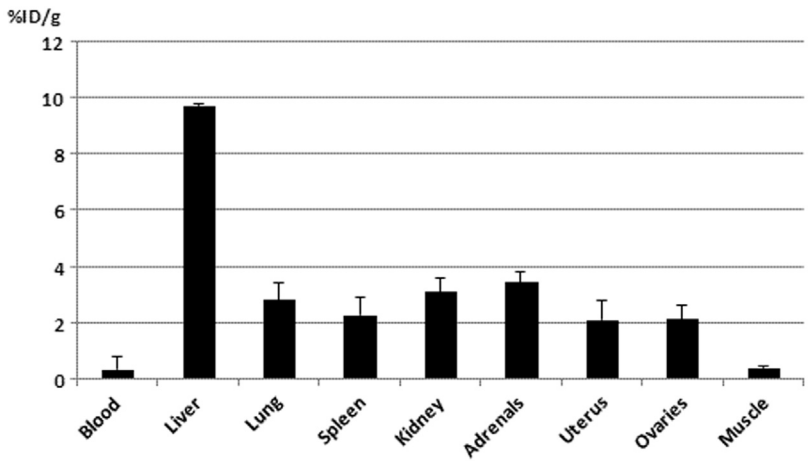


Fig. 1. Normal tissue biodistribution of 2-fluoroethyl raloxifene derivative [^{18}F]**1**.

Conclusion: These results propose that [^{18}F]**1** could be having potential as a positron emission tomography (PET) imaging agent for estrogen receptor-positive tumors.

Keywords: Raloxifene, Estrogen Receptor, F-18

P239 NOVEL TACN COMPLEXES AS ESTROGEN RECEPTOR LIGANDS FOR TUMOR IMAGING

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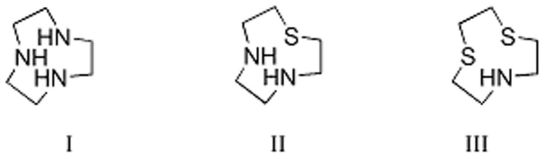
Introduction: In vivo imaging of the estrogen (ER) levels in breast tumors can assist in the selection of a therapy most likely to benefit a breast cancer patient. While in vivo imaging of ER levels in breast tumors can be done using F-18 labeled estrogens, receptor imaging agents labeled with the widely available and less expensive technetium-99m radionuclide would greatly expand the availability of such agents. We are exploring the design and evaluation of metal carbonyl complexes of cyclic tridentate ligands as potential ligands for the estrogen receptor.

The chelates we have explored are based on 1,4,7-triazacyclononane (tacn) (**I**), its sulfur analogs, 1,4-diaza-7-thiacyclononane (**II**) and 7-aza-1,4-dithiacyclononane (**III**) (Figure 1).



I II III

Fig. 1

Experimental: Ligands **I**, **II** and **III** have been extensively studied for their strong chelating ability to a variety of metals and are promising candidates for radiometal labeling. The tridentate character of these ligands provides a unique way of incorporating tricarbonyl forms of the technetium radionuclides, such as ^{94m}Tc or ^{99m}Tc, into ER through analogs in which the metal complex has been added via a functionalizable part of a high affinity receptor ligand (pendent design). The two ligands that have been chosen to be functionalized are 17a-ethynyl estradiol and a bicyclononane ligand, which was developed in our lab. Both of these ligand systems have very high affinity for ER and can be functionalized for the pendent attachment of tacn (Figure 2).

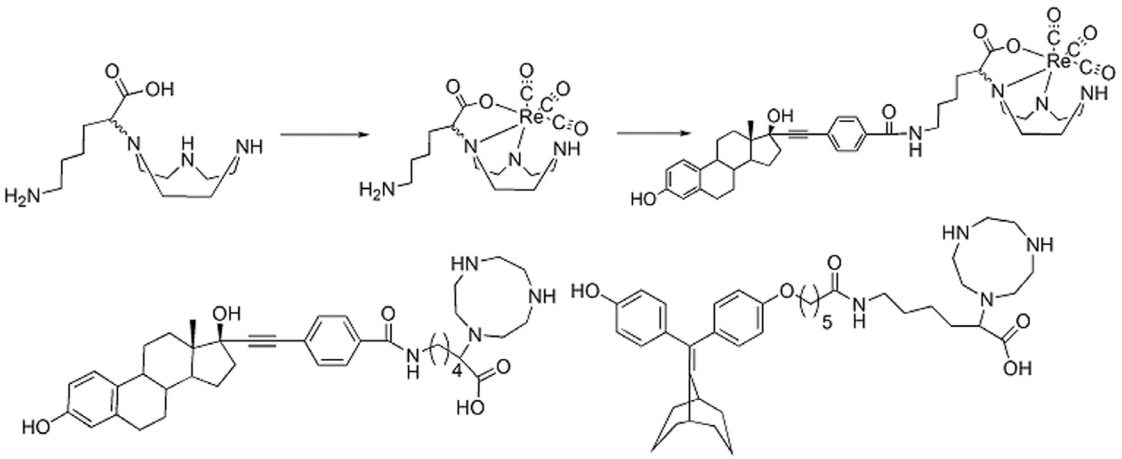


Fig. 2

Results and Discussion: The chelate systems shown have been prepared, and their binding affinities for ERα are 37% (for the ethynyl estradiol) and 24% (for the bicyclononane system) that of estradiol.

Conclusion: Radiometal-complexation methods to produce these ER ligands are currently under development and have thus far produced adequately potent ligands.

Acknowledgement: Supported by the Department of Energy.

Keywords: Metal Chelate, Estrogen Receptor Ligands

P240 PRECLINICAL EVALUATION OF ^{99m}Tc-HISTIDINE-FOLATE FOR FOLATE RECEPTOR-POSITIVE TUMOR TARGETING

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Introduction: Using folic acid as a "Trojan Horse" is a promising strategy to carry radionuclides into folate receptor (FR)-positive tumors. The goal of this work was the preclinical evaluation of a new folate-conjugate **1** with a histidine-chelator for coordination of the $M(CO)_3^+$-core (M = ^{99m}Tc, ^{188}Re). The data obtaind with **1** were compared with those previously found with ^{99m}Tc-picolylamine monoacetic acid (PAMA)-folate (**2**). The effects of the antifolate pemetrexed (PMX), which showed a favorable effect on the tumor-to-kidney ratio of radiofolate **2** were also investigated for compound **1**.

Experimental: $^{99m}Tc(CO)_3$-labeling was carried out in two steps via the Isolink method. *In vitro* stability was tested in PBS and human plasma for 24 h at 37°C. For *in vivo* studies athymic nude mice, bearing human KB-tumor xenografts were used. Biodistribution studies were performed 1 h, 4 h and 24 h after intravenous administration of ^{99m}Tc-His-folate **1**, with or without i.v. pre-administration of PMX (400 μg). SPECT/CT-scans were acquired 20 h after injection of radioactivity.

Results and Discussion: $^{99m}Tc(CO)_3$-labeling of His-folate (**1**) was more efficient even at low ligand concentration (10^{-5} M; yield > 98%) compared to **2** (10^{-5} M; yield > 85%). It was stable in PBS and human plasma (> 95%) over 24 h. Tumor uptake of **1** (4.35 ± 0.71% IA/g; 4 h p.i.) was almost twice that of radiofolate **2** (2.33 ± 0.36% IA/g; 4 h p.i). Radioactivity in non-targeted tissues and organs was low. Only in FR-positive kidneys we found high accumulation of radioactivity (**1**: 24.6 ± 3.2% IA/g, 4 h p.i.) resulting in unfavorably low tumor-to-kidney ratios (**1**: 0.18 ± 0.03, 4 h p.i.). Administration of PMX resulted in a significant reduction of renal retention (**1**: 3.4 ± 1.1% IA/g; 4 h p.i.) as demonstrated for **2**, while tumor accumulation was not significantly different (**1**: 4.21 ± 1.11% IA/g). Imaging via SPECT/CT confirmed the *ex vivo* findings of FR-specific accumulation.

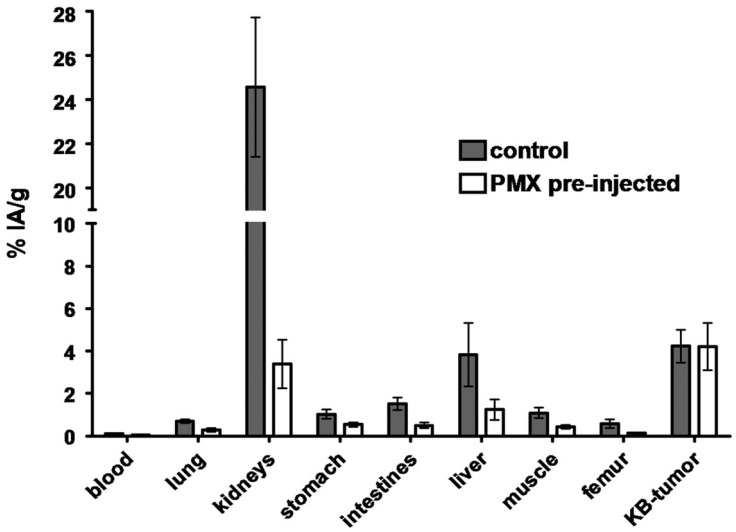

Fig. 1. Biodistribution in mice 4 h p.i. of ^{99m}Tc-His-folate.

Conclusion: The properties of the new radiofolate **1** revealed to be clearly superior to those of compound **2**. Since tumor uptake was significantly increased, a therapeutic application with the particle-emitting ^{188}Re-radionuclide would become accessible in particular if combined with PMX preinjection for selective kidney blockade.

Keywords: Folate Receptor, Technetium-99m, Folate-Based Radiotracer, Pemetrexed (alimta), Kidney Blockade

P241 SYNTHESIS, *IN VITRO* EVALUATION AND 3D-QSAR STUDY ON NEW DERIVATIVES OF DIPHENYL SULFIDE AS SEROTONIN TRANSPORTER (SERT) LIGANDS

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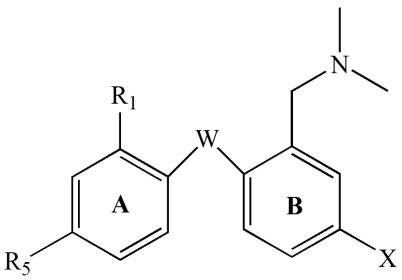
[1]Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education, College of Chemistry, Beijing Normal University, Beijing, China; [2]Institute of Interdisciplinary Isotope Research, Leipzig, Germany; [3]Universite F. Rabelais de Tours, France

Introduction: Imaging of SERT with PET and SPECT in humans could provide a useful tool for understanding how alterations of this system are related to depressive illnesses and other psychiatric disorders, as well as to monitor the treatment of depressed patients[1]. Recently, [^{123}I]ADAM showed excellent binding affinity and selectivity toward SERT *in vitro* and *in vivo*[2]. To improve the biokinetics of this imaging agent in the brain, we report herein the synthesis and biological evaluation *in vitro* of several new *O*,*S*-bridged diphenyl derivatives. Furthermore, molecular modeling using CoMFA and CoMSIA are employed for 44 analogs of diphenyl sulfide including our newly synthesized compounds.

Experimental: We have designed and synthesized **1**-**8** (Figure) as new analogs of diphenyl sulfide. The affinities of these compounds were evaluated by inhibiting the binding of [^{3}H]paroxetine in rat striatal membranes. 3D-QSAR models were derived on the basis of protonated molecules with pK_i values spreading over a range of four logarithmic units. All molecular modeling studies were performed using SYBYL 7.0. Gasteiger-Hueckel charges were calculated for all compounds. We used common atoms of two aromatic rings and the nitrogen atom of substitution at **B** ring for alignment.



Compound	R_1	R_5	X	W
1	CH_2NH_2	Br	H	S
2	CH_2NHCH_3	Br	H	S
3	CH_2NHCH_3	I	H	S
4	CN	Br	H	S
5	NH_2	Br	F	S
6	NH_2	I	F	S
7	NH_2	Br	F	O
8	NH_2	I	F	O

Results and Discussion: Compounds **1**-**4** were included in the training set and **5**-**8** were selected as test set. The CoMFA model generated from both steric and electrostatic fields exhibited q^2 0.616 with four components, r^2 0.909, F-value 69.887, with 64.9% steric and 35.1% electrostatic field contributions. The CoMSIA model for steric, electrostatic and hydrophobic fields yielded q^2 0.535 with five components, r^2 0.923, F-value 64.561, with 21.4% steric, 25.6% electrostatic and 52.9% hydrophobic field contributions. The preliminary experimental and predicted results demonstrated **5**-**8** had desirable *Ki* values in nanomolar or subnanomolar range. Further radiolabelling and *in vivo* evaluation are in progress.

Acknowledgement: This work was supported by NSFC (20471011).

References: [1] Brust P., et al. *Current Psychiatry Reviews 2*, 111 (2006). [2] Oya S., et al. *Nucl. Med. Biol.* 27, 249 (2000).

Keywords: Serotonin Transporter, 3D-QSAR, Diphenyl Sulfide

P242 DEVELOPING FLOURINE-18 LABELED PPAR GAMMA ANTAGONISTS FOR PET IMAGING

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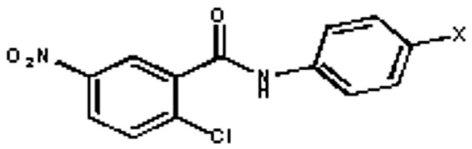
Introduction: Peroxisome proliferator activated-receptor gamma (PPARγ) has been shown to inhibit cellular growth and promote differentiation in some cancerous and non-cancerous cells. In addition, PPARγ agonists have been used for cancer treatments, making PPARγ-targeting compounds good candidates for tumor imaging. Previous reports have demonstrated that the agonists, ^{18}F-SB213068 or ^{11}C-GW7845, do not exhibit receptor-mediated uptake. In our previous study, the antagonist 2-bromo-5-nitro-*N*-phenyl-benzamide was radiolabeled and assessed. In tumor transplanted mice, the tumor/tissue ratios of radiolabeled compounds increased over time. However, stability studies suggested a rapid compound degradation. The strategy for developing 2nd generation compounds is to preserve the benzoate moiety of GW9662 and substitute different halogens at the para-position of the aniline ring.

Experimental: All chemicals were purchased from Aldrich Chemical Co. The compound identities were confirmed with both ^{1}H NMR spectra and Mass-Spec. Affinity screening was performed with a cell-free system using histidine-tagged PPARγ, copper and scintillant coated beads, and ^{3}H-rosiglitazone, a PPARγ agonist.

Results and Discussion: The structures are shown in Scheme 1. The IC_{50} values of the compounds were all in the nanomolar range. As compounds **6** and **7** both contain fluorine, they can be labeled with fluorine-18 by displacement using the mesylated precursors. Two methods were attempted. Whereas microwave heating yielded no desired products, heating at 90°C in the presence of $CaCO_3$ and K_{222} gave a mixture of radioactive peaks. When the peaks were separated by HPLC, in both cases, three major peaks were obtained. They were F-18 fluoride, the F-18-labeled target compounds and an unidentified compound with a retention time close to the precursor. It is likely that the strong electron withdrawn 2-nitro group makes the 5-chloro substituent labile. Therefore, the unknown radioactive peaks are believed to be the 5-F-18 compound. The specific activities were calculated as approximately 600-1400 mCi/mmol.



X= F (**1**); Br (**2**); I (**3**); OCH_3 (**4**); SCH_3 (**5**); CH_2CH_2F (**6**); OCH_2CH_2F (**7**)

Scheme 1

Conclusion: Both PPARγ analogs were successfully radiolabled with F-18. More studies are needed to evaluate the in vitro and in vivo stabilities of these compounds and biodistribution studies are in progress.

Acknowledgement: The work is supported by grants HL13851 and CA86307 from the National Institute of Health and grant ER60218 from the Department of Energy.

Keywords: PPAR gamma, Tumor Imaging, Receptor Binding

P243 CHARACTERIZATION OF A NOVEL F-18 LABELLED RADIOLIGAND FOR VMAT2

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Introduction: The vesicular monoamine transporter type 2 (VMAT2) has been implicated in movement disorders such as Parkinson's disease. Recently, VMAT2 has been indicated as a potential marker for imaging beta-cell mass (*J Clin Invest*, **2006**, 116:1506). Beta-cells regulate insulin production, and the progressive loss of beta-cell mass leads to the development of type 2 diabetes. It is of great interest to monitor beta cell mass, as an accurate measure would facilitate the diagnosis and therapeutic control of diabetic patients. [^{11}C]DTBZ is an established, useful PET tracer for imaging VMAT2 (*Nuc Med Biol*, **1997**, 24:197). We wished to develop an F-18 analog of DTBZ in order to facilitate the execution of more time-consuming in vitro and ex vivo experiments. For these reasons, [^{18}F]fluoro-DTBZ ([^{18}F]F-DTBZ) was synthesized and characterized.

Experimental: [^{18}F]F-DTBZ was synthesized via reaction of a phenol precursor with [^{18}F]FCH_2Br. Briefly, (+)desmethyl DTBZ (0.2mg, ABX) was dissolved in DMF (0.25mL) and Cs_2CO_3 (2mg) was added. The suspension was cooled to 0°C, and [^{18}F]FCH_2Br was distilled into the suspension. The mixture was transferred to a vial containing Cs_2CO_3 (2mg) and heated at 80°C for 10 min. Water was added and the solution was purified via semi-preparative HPLC to provide [^{18}F]F-DTBZ as the major product in >98% radiochemical and chemical purity. For autoradiography studies, tissue slices were incubated for 120 min at rt followed by 3x3 min washes with saline at 0°C and then 2x2 sec dip in H_2O at 0°C. For saturation binding studies, homogenates were incubated for 2h @ rt before filtration of GF/B filters presoaked in 0.5% PEI. Nonspecific binding for all studies was defined by 10μM tetrabenazine.

Results and Discussion: The regional brain uptake of [^{3}H]DTBZ and [^{18}F]F-DTBZ was compared by performing autoradiography on rat, rhesus, and human brain slices. For both tracers, regional binding was characterized by highest uptake in the caudate and putamen, with lower specific binding in the hippocampus and brain stem. Saturation binding studies with [^{18}F]F-DTBZ in striatum homogenate estimated that [^{18}F]F-DTBZ has a K_d of as 0.87nM, 0.52nM, and 2.4nM for VMAT2 in rat, rhesus, and human, respectively. This is comparable to the reported K_d of 3nM for [^{3}H]DTBZ binding to VMAT2 (*Biochem Pharmacol*, **1989**, 38:2395). Preliminary imaging studies indicate [^{18}F]F-DTBZ is stable in rat, with no significant defluorination observed.

Conclusion: [^{18}F]F-DTBZ shows similar regional brain uptake and binding affinity compared to [^{11}C]DTBZ. One would expect [^{18}F]F-DTBZ and [^{11}C]DTBZ to show similar utility as PET tracers for VMAT2. Experiments are in progress to determine the ability of [^{18}F]F-DTBZ to measure beta-cell mass.

Keywords: VMAT2, DTBZ, Beta-Cell Mass, Islet, Pancreas

P244 DESIGN AND SYNTHESIS OF POTENT AND SELECTIVE FLUORINATED DOPAMINE D_3 RECEPTOR LIGANDS

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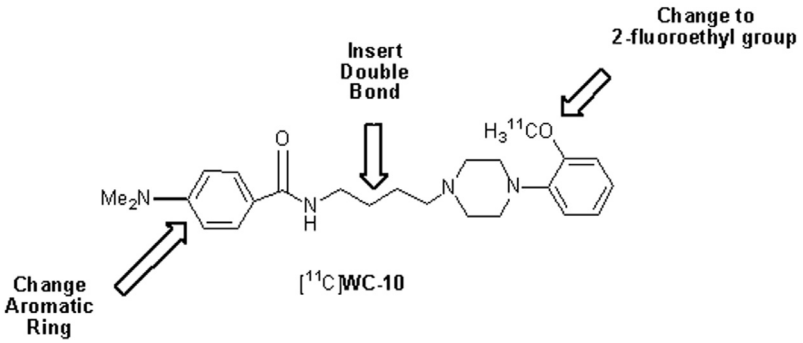
Introduction: Dopamine receptor subtypes D_2 and D_3 share structural homology and have similar pharmacological properties. These two dopamine subtypes have fundamental differences in expression, regulation and second messenger activation. D_3 dopamine receptor selective compounds may have therapeutic potential for the treatment of neuropsychiatic diseases, Parkinson's disease, and the abuse of psychostimulants. The lack of highly-selective D_3 receptor ligands in vivo has hampered the pharmacological investigation of the D_3 receptor as well as limited the development of PET radiotracers for imaging this receptor. We previously reported the synthesis and in vitro characterization of a number of conformationally-flexible benzamide analogs having a high affinity for dopamine D_3 versus D_2 and D_4 receptors.[1] This led to the evaluation of the carbon-11 labeled tracer, [^{11}C]**WC-10**, which has yielded promising results from microPET imaging studies in macaque monkeys.[2] The goal of the present study was to continue our investigation of the structure-activity relationship of this class of compounds with the aim of preparing a fluorine-18 labeled radiotracer for imaging dopamine D_3 receptors.

Experimental: WC-10 was modified by 1) replacing the methoxy group with a 2-fluoroethoxy group; 2) replacing the benzene ring in the benzamide moiety with other heteroaromatic ring systems; and, 3) addition of a double bond in the four carbon spacer to produce rigid analogues.



Results and Discussion: The results of this study resulted in the identification of a number of fluorinated compounds that displayed a high affinity for D_3 receptors (Ki: 0.52 - 5 nM) with reduced affinity affinity for D_2 receptors (15–375 nM). The D_2:D_3 affinity ratio of these analogs ranged from 10 to 65. Additionally, the fluorinated compounds have similar log P values as the corresponding N-(2-methoxylphenyl)piperazine analogs. Function assays determined that these compounds are antagonists or partial antagonists at D_3 receptors.

Conclusion: Several of these fluorinated compounds have the potential to be labeled with F-18 and serve as PET tracers for studying D_3 receptors in non-human primate brain.

Acknowledgement: This research was supported by NIH grants DA16181 and NS04056.

References: [1] *Bioorganic & Medicinal Chemistry* 13: 77-87, 2005. [2] *J. Nucl. Med.* 2006; 47: 27P.

Keywords: PET Imaging, F-18 Labeling, Dopamine D_3 Receptors

P245 SYNTHESIS AND EVALUATION OF A CARBON-11 LABELLED TRIARYL BIS-SULFONE AS A PET RADIOLIGAND WITH AFFINITY FOR THE CB2 RECEPTOR

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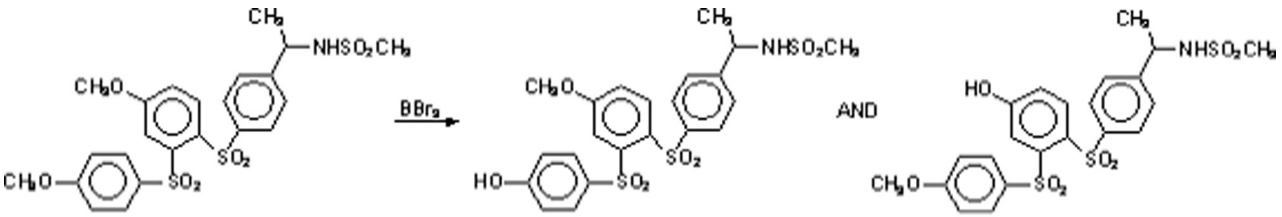
Introduction: Recent data indicate that the CB2 receptor participates in the control of peripheral pain, inflammation and cancer proliferation. At this moment, there is no PET tracer available to study the receptor in vivo. The aim of this study was the development and evaluation of a ^{11}C-labelled triaryl bis-sulfone as a potential CB2 receptor tracer agent.

Experimental: Starting compound for this study was N-[(1s)-1-[4-[[4-methoxy-2-[(4-methoxyphenyl)sulfonyl]-phenyl]sulfonyl] phenyl]ethyl] methyl sulfonamide **(1)**. Demethylation of **(1)** was performed using BBr_3 at -70°C. A mixture of two mono-methoxy derivatives (**(2)** and **(3)**) was obtained, as proven by MS analysis. The structure of isomer **(3)** was confirmed by ^{1}H-NMR. Labelling of **(3)** with carbon-11 was done by heating 200 μg **(3)** with $^{11}CH_3I$ in 200 μl DMF at 90°C for 4 min in the presence of 2-4 mg Cs_2CO_3. The reaction product was purified by RP-HPLC. The log $P_{oct/buff}$, biodistribution and metabolism of ^{11}C-**(1)** in mice were studied.



(1) (2) (3)

Results and Discussion: Mono-methoxy derivatives **(2)** and **(3)** were obtained in a proportion 25:75. They were separated by preparative RP-HPLC. **(3)** was efficiently alkylated with $^{11}CH_3I$, yielding 12 to 46% of ^{11}C-**(1)**, as shown by co-elution with authentic **(1)**. The compound has a log P value of 2.15. After IV injection in normal mice the brain uptake was low (0.05% of ID at 2 min). Clearance from the blood, defined as the 2 min/60 min activity ratio, was 4.2. Excretion proceeds mainly through the hepatobiliary pathway with 22.2% of ID in the liver and 42.5% of ID in the intestines after 60 min. Blood metabolite studies showed 93.2% intact product after 2 min; 63.0% after 10 min and 60.1% after 30 min.

Conclusion: A triaryl bis-sulfone compound was successfully demethylated and labelled with a carbon-11 methyl group. The labelled compound showed only limited passage over the blood brain barrier. In view of the established high and selective affinity for human CB2 receptors (0.4 nM) [1], ^{11}C-**(1)** will be further evaluated as a PET tracer for the visualisation of peripheral CB2 expression.

Reference: [1] Shankar B.B. et al. *Bioorg. Med. Chem. Lett.*, 2005.

Acknowledgement: This study was funded in part by the EC - FP6-project DiMI, LSHB-CT-2005-512146.

Keywords: CB2 Receptor, PET Radioligand, Carbon-11

P246 (^{18}F)DPA-714 AS A NOVEL PET TRACER FOR PBR: A COMPARISON WITH (^{11}C)PK11195 IN A RAT MODEL OF HSV ENCEPHALITIS

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Introduction: Many neurological diseases, including Parkinson's disease and herpes simplex encephalitis (HSE), are associated with neuroinflammation. Expression of the peripheral benzodiazepine receptor (PBR) is increased during neuroinflammation and can be visualised by positron emission tomography (PET) with [^{11}C]PK11195. However, [^{11}C]PK11195 shows low brain uptake and high non-specific binding and may not be sensitive enough to visualise mild inflammation. Recently, [^{18}F]DPA-714 was developed as a more sensitive PET tracer than [^{11}C]PK11195. In this study, [^{18}F]DPA-714 was evaluated in a rat model of HSE and compared to [^{11}C]PK11195 in the same model.

Experimental: DPA-714, the 2-fluoroethyl analog of DPA-713, was prepared by reacting of the corresponding tosylate precursor with K^{18}F/kryptofix in acetonitrile at 100°C for 10 min. The product was passed through an Alumina N seppak and purified by reversed phase HPLC. The stability of [^{18}F]DPA-714 was tested ex vivo and in vivo by TLC analysis. Male Wistar rats were intranasally inoculated with the herpes simplex virus type-1 (10^7 PFU in 100μl PBS) or PBS (control). Within a week after inoculation, replicating virus migrated into the brain and induced neuroinflammation. At day 6 or 7 following inoculation the rats received an i.v. injection of [^{18}F]DPA-714 (55±9 MBq) or [^{11}C]PK11195 (78±22 MBq) and dynamic PET scans (MicroPET Focus 220) were performed for 2 h and 1 h respectively, followed by ex vivo biodistribution.

Results and Discussion: [^{18}F]DPA-714 was obtained in 20±5% radiochemical yield, with a specific activity of 104±28 MBq/nmol and a radiochemical purity >99%. Ex vivo, [^{18}F]DPA-714 was stable in rat plasma: 95± 1% unchanged [^{18}F]DPA-714 after 2 h at 37°C. In vivo, [^{18}F]DPA-714 was converted into more polar metabolites, with 78±1% of the radioactivity in rat plasma consisting of the parent compound 2 h after tracer injection. The PET images of [^{18}F]DPA-714 showed a low tracer uptake in control rats (n=3), which was significantly lower than [^{11}C]PK11195 uptake (n=5) at 1 h (p=0.01), and a slow tracer clearance from the brain ($T^{1/2}$>100 min). [^{18}F]DPA-714 uptake in HSE rats (n=3) was significantly increased (90-150%) in olfactory and retrograde brain areas where HSV-1 accumulates, whereas [^{11}C]PK11195 uptake (n=5) was not significantly increased in these areas.

Conclusion: [^{18}F]DPA-714 is a promising tracer to visualise neuroinflammation, which is more sensitive than [^{11}C]PK11195 because of a better contrast between inflamed and noninflamed areas.

Acknowledgement: Stanley Medical Research Institute.

Keywords: Neuroinflammation, PBR, HSV-1, MicroPET

P247 COMFA STUDY OF SPIROPIPERIDINES LIGANDS FOR σ1 RECEPTOR IMAGING

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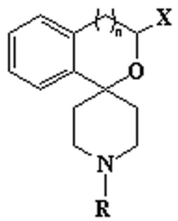
Introduction: At present no radiotracers for PET or SPECT are available for clinical application in σ1 receptor imaging. Among many different structural classes of σ1 receptor ligands, the spiropiperidines offer the best potential σ1 receptor affinity and selectivity towards σ2 and other brain receptors[1,2]. In order to design potential σ1 receptor imaging agents, a 3D-QSAR/CoMFA model was performed with sybyl 7.0 software for 35 spiropiperidines in this paper. The experimental pKi values of two newly synthesized derivatives have validated this model.

Experimental: Computational methods: The general structure of spiropiperidines is shown in figure 1. All the molecules were generated and optimized with the sybyl 7.0 software. The phenyl ring and the O in benzopyran (furan) as well as the N in piperidine were the reference atoms for alignment.



a: n=0, R=4-F-benzyl, X=OH
b: n=0, R=4-F-benzyl, X=OCH_3

Fig. 1

Results and Discussion: The CoMFA model provided the following values: q^2 0.709, r^2 0.991, s 0.111, and F 508, with 6 components. Based on this model, the reference compounds for ^{18}F- and ^{123}I-radiotracers were designed. Two of them (a, b) were synthesized and used as the test set. The experimental and predicted pKi values for a were 8.00 and 8.23, for b 8.82 and 8.71, respectively. Experimental versus predicted pKi values of above compounds contained the training set and test set of the CoMFA model as shown in figure 2. The solid line is the ideal correlation line, whereas the dotted lines indicate the ±0.2 log point error margins. The above results suggest that the obtained model is a useful tool for the prediction of test set as well as newly designed structures for σ1 receptor ligands.

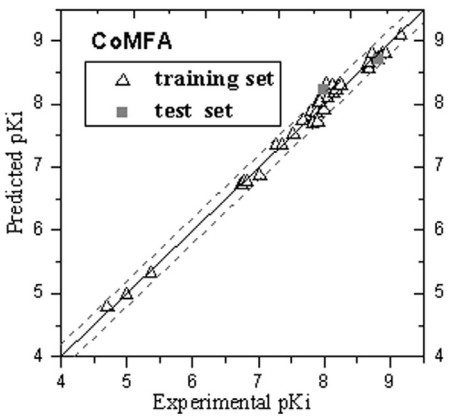


Fig. 2

Acknowledgement: This work is supported by NSFC (Grant No.20501004).

References: [1] C.A. Maier and B. Wuensch. J. Med. Chem. 2002, 45, 438-448. [2] C.A. Maier and B. Wuensch. J. Med. Chem. 2002, 45, 4923-4930.

Keywords: σ1 Receptor, CoMFA, Spiropiperidine, 3D-QSAR

P248 PET IMAGING OF THE DOPAMINE TRANSPORTER WITH (^{18}F)FBFNT

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Introduction: The human dopamine transporter (DAT) is a 620 amino acid protein on presynaptic neurons and functions to remove dopamine from the synapse. The DAT is the target of cocaine, amphetamine, and Ritalin, and has been implicated in the pathophysiology of Parkinson's Disease, Huntington's Disease, and ADHD. The DAT is found in high density in the striatum and can be used as a marker of the integrity of presynaptic dopamine-producing neurons in this brain region. Hence, the ability to image the DAT with PET will allow for the measurement of DAT density and thus may enable the diagnosis of neurodegenerative disease and the monitoring of neurodegeneration as disease progresses. We have previously reported the synthesis, radiolabeling, and PET evaluation of several *N*-((*E*)-4-fluorobut-2-en-1-yl)-2β-carbomethoxy-3β-(4'-substituted-phenyl)nortropanes for imaging the DAT.[1] Additionally, the 3β-tolyl analogue has recently been reported.[2] We report here the synthesis, radiolabeling, biological evaluation, and PET imaging of *N*-((*E*)-4-fluorobut-2-en-1-yl)-2β-carbomethoxy-3β-(4'-fluorophenyl)nortropane (FBFNT).

Experimental: FBFNT was synthesized by *N*-alkylating nor-CFT with (*E*)-4-fluoro-1-tosyloxy-but-2-ene. [^{18}F]FBFNT was synthesized by reacting nor-CFT with (*E*)-4-[^{18}F]fluoro-1-tosyloxy-but-2-ene in DMF followed by semi-prep HPLC purification. The octanol/H_2O partition coefficient of [^{18}F]FBFNT was measured and found to be $\log P_{7.4} = 1.95$.

Results and Discussion: FBFNT was screened against transfected human DAT, SERT, and NET, and afforded the following inhibition constants: K_i (nM) = 1.70 (DAT), 85.5 (SERT), >10,000 (NET). Brain imaging with [^{18}F]FBFNT was performed with a Concorde microPET P4 in an anesthetized monkey. High uptake was observed in the putamen with peak uptake achieved after 18 min followed by a steady washout. Peak uptake in the caudate was achieved after 55 min and remained nearly steady throughout the study. Lesser uptake was observed in the substantia nigra and negligible uptake was observed in the cerebellum. Brain imaging in an awake rhesus monkey was performed with a Siemens 951 PET scanner. High uptake was observed in the striatum with peak uptake achieved after 15 min followed by a steady washout.

Conclusion: In conclusion, FBFNT has a high affinity for the DAT with a 50-fold higher affinity for the DAT vs. the SERT. MicroPET imaging in an anesthetized monkey with [^{18}F]FBFNT showed rapid and high uptake and favorable kinetics in the putamen with lesser uptake in the caudate. The uptake and kinetics were reproducible in an awake monkey.

References: [1] Chen, P.; *et al.*, *J. Labelled Cpd. Radiopharm.* **1999**, *42*, Suppl. 1, S400. [2] Dollé, F.; *et al.*, *Bioorg. Med. Chem.* **2006**, *14*, 1115.

Acknowledgement: Funded by NIMH.

P249 PREPARATION AND EVALUATION OF (^{99m}Tc) maEEE-$Z_{HER2:342}$ AFFIBODY MOLECULE FOR IMAGING OF HER2 EXPRESSING TUMOURS

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Introduction: Imaging of a HER2-expression in breast cancer may help to select patients who will benefit from a treatment with trastuzumab. The anti-HER2 Affibody molecule $Z_{HER2:342}$ (7 kDa) binds to HER2 with a subnanomolar affinity. Previous experiments have demonstrated that ^{125}I- and ^{111}In-labeled $Z_{HER2:342}$ can be used for imaging of HER2 expression in tumour xenografts. Earlier, we have shown that the use of mercaptoacetyl-containing chelators (MAG3 and MAS3) enables stable labelling of $Z_{HER2:342}$ with ^{99m}Tc, and provides specific imaging of HER2-expressing xenografts. However, hepatobiliary excretion reduced contrast in the abdominal area. The aim of this study was to evaluate if the use of mercaptoacetyl tri-glutamic acid (maEEE) as a chelator can reduce hepatobiliary excretion.

Experimental: $Z_{HER2:342}$ was prepared using solid phase peptide synthesis and maEEE was incorporated as the N-terminus in a single peptide synthesis procedure. Affinity of HER2 binding was measured using BIAcore. Labelling was performed at alkaline pH. Biodistribution of the tracer was studied in normal and tumour-bearing mice.

Results and Discussion: The synthetic maEEE-$Z_{HER2:342}$ possessed an affinity to HER2 of 0.41 nM. A direct ^{99m}Tc labelling provided a yield of 90 ± 1%, and, after purification on a NAP-5 column, the radiochemical purity was more than 97%. [^{99m}Tc] maEEE-$Z_{HER2:342}$ demonstrated good stability in vitro. The capacity for specific binding to HER2-expressing SKOV-3 cells *in vitro* was retained after labelling. A biodistribution study of [^{99m}Tc] maEEE-$Z_{HER2:342}$ in normal mice demonstrated an appreciable reduction of a radioactivity accumulation in intestine content in comparison with both [^{99m}Tc] MAG3-$Z_{HER2:342}$ and [^{99m}Tc] MAS3-$Z_{HER2:342}$. In tumour-bearing mice, the tumour uptake was 7.9 ± 1.0% 4 h pi, the tumour-to-blood ratio 40 and the tumour-to-liver ratio 15. Gamma-camera imaging (6h pi) showed a clear visualisation HER2-expressing xenografts using [^{99m}Tc] maEEE-$Z_{HER2:342}$. Only kidneys were visualised among normal organs. No interfering radioactivity in a gastrointestinal tract was seen.

Conclusion: The use of maEEE chelator provides reduction of hepatobiliary excretion of ^{99m}Tc-labelled Affibody molecules. The results of this study demonstrate that [^{99m}Tc] maEEE-$Z_{HER2:342}$ can be used for imaging of HER2-expressing tumours, including abdominal ones, at the day of injection.

Acknowledgement: This study was supported by grants from Swedish Cancer Society and Swedish Research Council.

Keywords: HER2, Affibody Molecules, Tc-99m, Molecular Imaging

P250 NON-SPECIFIC BINDING OF POSITRON EMISSION TOMOGRAPHY (PET) LIGANDS AS SEEN FROM AB-INITIO COMPUTATIONAL STUDY

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Introduction: In developing new PET radiotracers, a common reason for candidate failure is that high non-specific and sub cellular binding obscures the specific binding to the molecular target and, thus, reduces the quality of the PET scan data. Non-specific binding is thought to be correlated in part to a molecule's lipophilicity, typically estimated by measuring (or calculating) octanol-water partition coefficient. This is, however, a gross simplification of a complex phenomenon. The purpose of this *ab-initio* computational study is to increase our understanding of non-specific binding by investigating the molecular basis of ligand-membrane interactions.

Experimental: Ten well-characterized central nervous system PET radiotracers acting on a variety of molecular targets were used as primary set. Quantum mechanical methods were used to estimate accurately the strength of the electronic interactions between individual drug molecules and a single phospholipid molecule commonly present in brain membranes. This was achieved by finding the lowest optimized ground state energy of several drug-lipid complexes with Hartree-Fock and B3LYP density functional theory/6-31g** electronic structure calculations, without using any experimental reference.

Results and Discussion: The computed energies showed a correlation with the *in vivo* non-specific binding distribution volumes relative to the free tracer plasma concentration, as evaluated by kinetic analysis. Significantly, the drugs with stronger lipid interaction possessed, in general, a higher non-specific binding value, Fig. 1. However, DASB, the only serotonin transporter ligand in our set, was an outlier. The representation of hypervalent sulphur atoms is a known problem at the chosen level of theory and may be an explanation for DASB poor correlation. It is also possible that the relative high non-specific binding value of DASB is not dominated by the formation of highly stable lipid complexes.

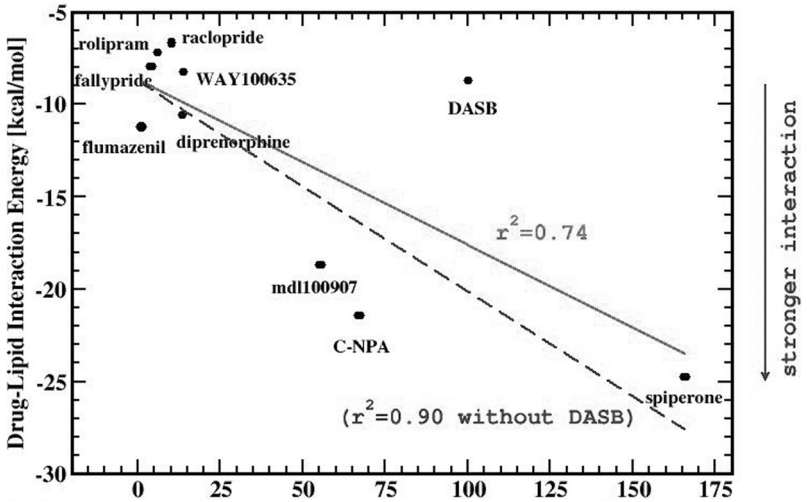

Fig. 1. *In vivo* non-specific distribution volume relative to free fraction in plasma (mL/g).

Conclusion: The usefulness of this method for prospectively identifying PET radioligand candidates with low non-specific binding characteristics will be tested with a blinded set of radiotracers.

Acknowledgement: GlaxoSmithKline for supporting the GSK-Imperial College Non-Specific Binding Initiative.

Keywords: Positron Emission Tomography PET, Kinetic Analysis, Quantum Chemical Predictor, Brain Imaging, QSAR

P251 EVALUATION OF A NEW SERIES OF COPPER-64-NOTA-BOMBESIN TARGETED RADIOPHARMECUTICALS WITH PET IMAGING POTENTIAL

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Introduction: Recently, we have begun the search for a new bifunctional chelating agent that will sufficiently stabilize Copper-64 radionuclide under *in vivo* conditions. Receptor-specific peptide conjugates containing the chelating agents DOTA and TETA have shown some promise. However, $^{64}Cu^{2+}$-complexes of these chelators are only moderately stable under *in vivo* conditions, resulting in demetallation and subsequent accumulation in non-target tissues such as the liver. Thus, there is some impetus to improve the *in vivo* kinetic stability of ^{64}Cu-macrocyclic bioconjugates to reduce accumulation in collateral tissue. Herein we report the design of new Copper-64-NOTA-Bombesin conjugates having high selectivity and affinity for the GRP receptor.

Experimental: Bombesin conjugates were derived of the form, NOTA-X-BBN (X = GGG, SSS, βAla, 5Ava, and 8Aoc). The unligated BBN [7-14] peptides with spacer groups were synthesized by Fmoc-protected solid-phase peptide synthesis (SPPS) and purified by reverse phase-high performance liquid chromatography (RP-HPLC). NOTA chelator was conjugated to the N-terminal primary amine of BBN in the presences of stoichiometric amounts of N-Hydroxysulfosuccinimide)and 1-Ethyl-3-(dimethylaminopropyl)carbodiimide in buffered aqueous solution. The final NOTA-X-BBN derivatives were purified by RP-HPLC and their chemical constitution confirmed *via* electrospray ionization-mass spectrometry. [^{64}Cu-NOTA-X-BBN]-conjugates were prepared by addition of $^{64}CuCl_2$ to a buffered solution containing the conjugate followed by purification *via* RP-HPLC.

Results and Discussion: *In vivo* studies of [^{64}Cu-NOTA-8-Aoc-BBN]-conjugates in normal CF-1 mice showed receptor-specific uptake in normal pancreatic tissue, an organ known to express the GRPr in very high numbers. For example, uptake of conjugate in normal pancreas was 17.4 ± 2.99% ID/g (n = 5) at 1h post-intravenous injection. Furthermore, there appears to be little or no *in vivo* dissociation of $^{64}Cu^{2+}$ from the NOTA chelator as evident by absence of liver accumulation of radioactivity at 1h post-intravenous injection (i.e., Uptake in liver at 1h post-intravenous injection was found to be 1.00 ± 0.63% ID/g (n = 5). Kidney accumulation at 1h post-intravenous injection was 1.28 ± 0.25% ID/g.

Conclusion: These studies show that the NOTA chelator provides sufficient *in vivo* kinetic inertness to ^{64}Cu-radiolabeled peptides giving some impetus for conjugates of this type to be used as site-directed PET or therapeutic agents for diagnosis or treatment of specific human cancers.

Keywords: Bombesin, Copper-64, NOTA

P252 DEVELOPMENT OF ^{34m}Cl-LABELED DOPAMINE D1 AGONISTS AS PET IMAGING AGENTS

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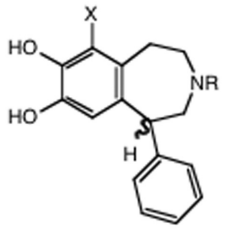
Introduction: Dopamine D1 (D1R) and D2 (D2R) receptor subtypes exist in either high or low affinity states for binding agonists. The high affinity states are the functional states. PET tracers for D1R are less developed compared to those for D2R. The first reported D1 agonists as PET tracers were [^{11}C]SKF 82957 and [^{11}C]SKF 75670 (1). The goals of this project are (a) to develop routine production of ^{34m}Cl ($t_{1/2}$=32 m) and (b) to prepare [^{34m}Cl]SKF 81297 and [^{34m}Cl]SKF 82957 for comparison with [^{11}C]SKF 82957 and [^{11}C]SKF 75670 in imaging high affinity D1R. The affinities and selectivities of these D1R agonists (2) are shown below.

Experimental: Natural sulfur (4% ^{34}S) was bombarded with 11 Mev protons in a RDS 111 cyclotron to produce ^{34m}Cl via the ^{34}S(p,n)^{34m}Cl reaction. The reactivity of [^{34m}Cl]chloride was tested by K222-catalyzed nucleophilic labeling of chloro-deoxyglucose (ClDG) and chloromethane ($ClCH_3$). To test the feasibility of imaging of ^{34m}Cl positrons ($E_{\beta max}$=4.5 Mev), a micro-Derenzo phantom filled with ^{34m}Cl was imaged using a Concorde MicroPET P4 scanner. Chlorination of SKF 75670 was done using $NaIO_4$ as oxidant to produce SKF 82957.

Results and Discussion: Extrapolated thick target saturation yield for 100% enriched ^{34}S was found to be 12 mCi/μA (3). [^{34m}Cl]Chloride reactivity was shown by the successful syntheses of [^{34m}Cl]ClDG and [^{34m}Cl]$ClCH_3$ as verified by HPLC and GC, respectively. MicroPET phantom imaging of ^{34m}Cl showed adequate image resolution using maximum a posteriori (MAP) reconstruction algorithm. Initial chlorination of SKF 75670 possibly via electrophilic route gave modest amounts of SKF 82957. Further optimization experiments are underway.



Compound	X	R	D1 affinity	D1/D2 selectivity
SKF 38393	H	H	26.6 nM	>376
SKF 81297	Cl	H	1.9 nM	669
SKF 75670	H	CH_3	8.8 nM	16
SKF 82957	Cl	CH_3	0.9 nM	85

Conclusion: [^{34m}Cl] can be produced in a 11Mev proton cylotron and its utility in PET imaging is feasible using MAP image reconstruction. [^{34m}Cl] labeling to prepare PET agents is possible via nucleophilic or electrophilic reactions. This will allow development of [^{34m}Cl] labeled D1R agonists and the PET evaluation of other chloro-drugs of interest. SKF 82957 labeled with either [^{34m}Cl] or [^{11}C] can elucidate the in vivo metabolism of this compound and aid in developing optimal D1R agonists as PET agents.

Acknowledgement: Support of NIH Grant NS054933 is gratefully acknowledged.

References: [1] DaSilva et al., Appl. Radiat. Isot. 47:279-284, 1996. [2] Neumeyer et al., Eur. J. Pharmacol. 474:137-140, 2003. [3] Nickles, J. Label. Comp. Radiopharm. 46:1-27, 2003.

Keywords: Dopamine D1 Agonists, Cl-34m Production, PET Imaging of Dopamine Receptors

P253 SYNTHESIS, RADIOLABELING AND BIOLOGICAL EVALUATION OF NEW STRUCTURAL CLASS OF ESTROGEN-RECEPTOR TARGETED NEUTRAL TRIDENTATE Re/Tc(I) PYRIDIN-2-YL HYDRAZINE DERIVATIVES

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Introduction: Breast and genital cancer are sometimes classified into two subtypes based upon the expression of estrogen receptor (ER). Previously, we had demostrated that the steroid linkage affected the affinity and selectivity of estrogen binding with these receptors.In this study, we describe a new structural class of neutral tridentate pyridin-2-yl hydrazine chelates with alkyne linkage for labeling with tricarbonyl Re/Tc(I) under aqueous conditions and investigate the in vitro and in vivo receptor binding of synthetic estradiol derivatives.

Experimental: The Re/99mTc(I)-estradiol-pyridin-2-yl hydrazine derivative was synthesized using the Suzuki-Miyaura cross-coupling reaction and tricarbonyl approach. The radiochemical purity was assessed by HPLC and stability and transchelation studies were performed. Cell binding studies were performed on ER-expressing human breast adenocarcinoma MCF-7 cells. In vivo biodistribution of Tc-E2 was done in female C57BL/6 immature mice in different phases of the estrus cycle. To study mass effects, increasing amounts of radiotracer were injected and biodistribution studies were done. Biodistribution and NanoSPECT/CT imaging studies were performed on ER-expressing tumor bearing mice.

Results and Discussion: The radiochemical purity of Tc-estradiol derivative assessed by HPLC was $\geq$ 95% and excess of ligand was removed using solid phase extraction. The radiolabeled derivative exhibited high stability in serum and less than 30% transchelation was observed with 100 fold excess histidine solution after 24 hours of incubation at 37°C. The log P value determined was 3.9 $\pm$ 0.5. The cellular Kd was 10.97 $\pm$ 1.47 nM and EC50 was 15.00 $\pm$ 1.40 nM. In vivo studies showed highest receptor-mediated uptake in reproductive organ during the diestrus phase of the estrus cycle. There was dose-dependent uptake in the liver and the bone demonstrating the effect of mass injected. Highly selective uptake into reproductive tissues and high target/muscle ratios were observed, however the uptake in the liver and blood was also high. Tc-derivative was slowly excreted mainly via the hepatobiliary system. No radioactive metabolites were observed in HPLC plasma and urine analysis. The imaging studies showed relatively low uptake in the tumor (< 1.2% ID/g) and high liver and gut uptake.

Conclusion: We have demonstrated the role of estrus cycle and mass effects on target tissue uptake. Although Tc-derivatives showed great promise for standard biodistribution studies, further structural modifications are needed to optimize for better imaging characteristics.

Acknowledgement: WM Keck Foundation.

Keywords: Estrogen-Receptor, 99mTc, Tricarbonyl, NanoSPECT/CT Imaging, Estradiol

P254 ([11]C)DPA713: RADIOSYNTHESIS AND EVALUATION FOR CEREBRAL PERIPHERAL BENZODIAZEPINE RECEPTOR IMAGING

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Introduction: The Peripheral Benzodiazepine Receptor, expressed both peripherally and in the central nervous system, is involved in several biological processes. Despite low sensitivity and difficult quantification, [^{11}C]PK11195 was, until recently, the only PET tracer available for imaging brain PBR. Alternative radioligands have emerged: some aryloxyanilides (DAA1106 family) and a pyrazolopyrimidine ([^{11}C]DPA713). DPA713 shows higher affinity for PBR than PK11195 (K_i=4.7 vs 9.3 nM). Its calculated lipophilicity suggests lower non-specific binding than PK11195 ($cLogD_{7.4}$ = 2.6 vs 4.6). Here we report an efficient radiosynthesis of [^{11}C]DPA713 and its evaluation in the pig.

Experimental: [^{11}C]DPA713 was prepared in high yield from *nor*-DPA713 using [^{11}C]CH_3I with TBAF in DMF (3 min, RT). The radiotracer (5-24 GBq) was obtained with specific activities of 30-238 GBq/umol.

Results and Discussion: The observed rank order of regional brain concentration was thalamus>frontal cortex~occipital cortex~cerebellum. The plasma free fraction was ~0.25. Specific to non-specific binding ratio was ~3 (60 min). Occipital cortex TACs shown here represent a blocking study with escalating doses of PK11195 (0.05-5mg/kg). A dose dependant response of brain uptake and volume of distribution was observed. The plasma to whole blood radioactivity ratios were ~0.1 for baseline and ~1 for blocking studies, suggesting [^{11}C]DPA713 binds to a non-plasma blood component. [^{11}C]DPA713 was rapidly metabolised, with parent representing 40% of the total arterial plasma radioactivity after 30 min.

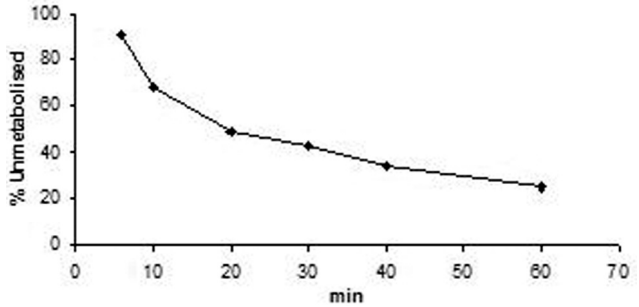

Fig. 1

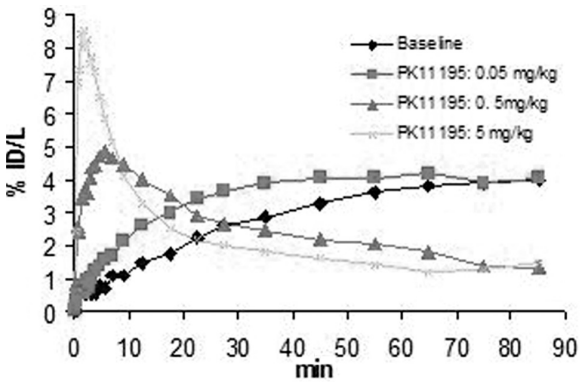

Fig. 2

Conclusion: These studies showed specific uptake of [^{11}C]DPA713 in porcine brain. Further work is being carried out to assess the value of this molecule as a probe for cerebral inflammation.

P255 SYNTHESIS AND COMPARISON OF ^{11}C-LABELED TZTP DERIVATIVES AS POTENTIAL RADIOTRACERS FOR IMAGING MUSCARINIC M2 RECEPTORS WITH PET

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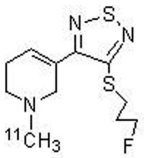
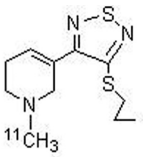
Introduction: ^{18}FP-TZTP is a musacrinic M2 selective agonist radiotracer. In healthy subjects with APOE-ε4 alleles (risk gene for Alzheimer's disease), ^{18}FP-TZTP binding is increased [Cohen, R; et al. *Synapse* 2003, 49, 150]. Here we labeled FP-TZTP and two other TZTP derivatives with C-11, for multiple PET studies at short time intervals, to compare the effect of small structural changes on pharmacokinetics (PK).

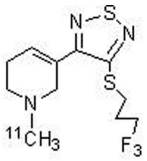
Experimental: Derivatives **1**, **2** and **3** were prepared form $^{11}CH_3I$ and the corresponding precursor compounds. LogD, plasma free fraction, kinetics in brain and arterial plasma, distribution volumes (DV) and pharmacological blockade in baboons were compared for derivatives **1**, **2** and **3**.



[^{11}C] FP-TZTP, 1



[^{11}C] P-TZTP, 2



[^{11}C] F_3P-TZTP, 3

Results and Discussion: Radiochemical yields were 75-85%; specific activities were 8-14 Ci/μmol at EOB; radiochemical purities were >99%. Values for Log D were 2.4, 2.8 and 2.9 and % plasma free fractions were 8.6, 2.9 and 5.0 for **1**, **2** and **3** respectively. The fraction of parent radiotracer in plasma was higher and the AUC lower for **3** than for **1** and **2** (Table 1). Time activity curves in brain were very different with **1** showing PK similar to the F-18 tracer while **2** showed slowed uptake and clearance and 3 showed very slow uptake and no clearance over 90 min (Figure 1). DV's were higher for striatum and cortical regions than cerebellum (data not shown). The uptake of ^{11}C-**1** and ^{11}C-**3** (but not ^{11}C-**2**) was strongly inhibited by coinjection of unlabeled **1** or **2** suggesting that binding is saturable, reversible and specific (data not shown).

Table 1

Tracer	Plasma Integral (AUC) (60 min)		% Parent tracer				
	Uncorrected	Metabolite Corrected	1 min	5 min	10 min	30 min	60 min
1	2508.2	1456.8	98	83	68	33	18
2	2646.1	1822.1	95	85	76	50	31
3	718.4	645.0	97	92	89	76	58

Conclusion: Small structural variations on the TZTP structure greatly changed the brain PK and behavior in blood but not the log D or plasma protein binding. Studies are underway to confirm the specificity of derivatives **2** and **3**.

Acknowledgement: DOE-OBER and NIH (K05-DA020001).

Keywords: Muscarinic M2 Receptors, Pharmacokinetics

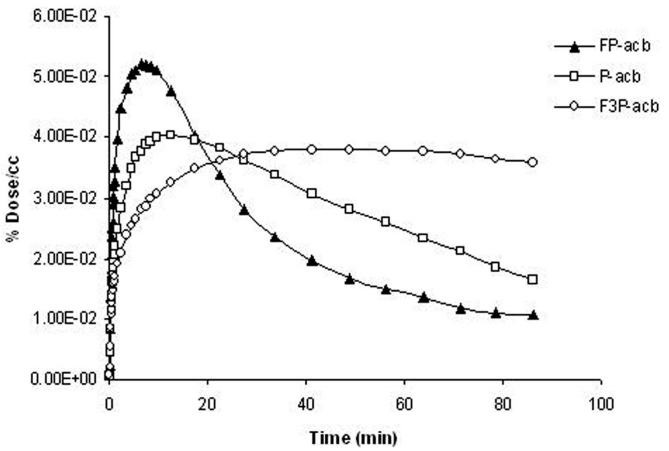


Fig. 1. TAC-cerebellum, same animal.

P256 E-((R, R) AND (S, S))-5-(^{125}I)-AOIBV: POTENTIAL SPECT TRACERS FOR IN VIVO STUDIES OF THE VESICULAR AcetylCholine TRANSPORTER (VAChT)

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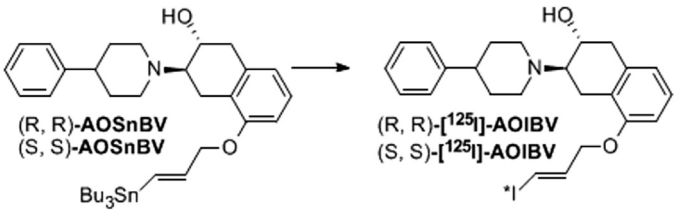
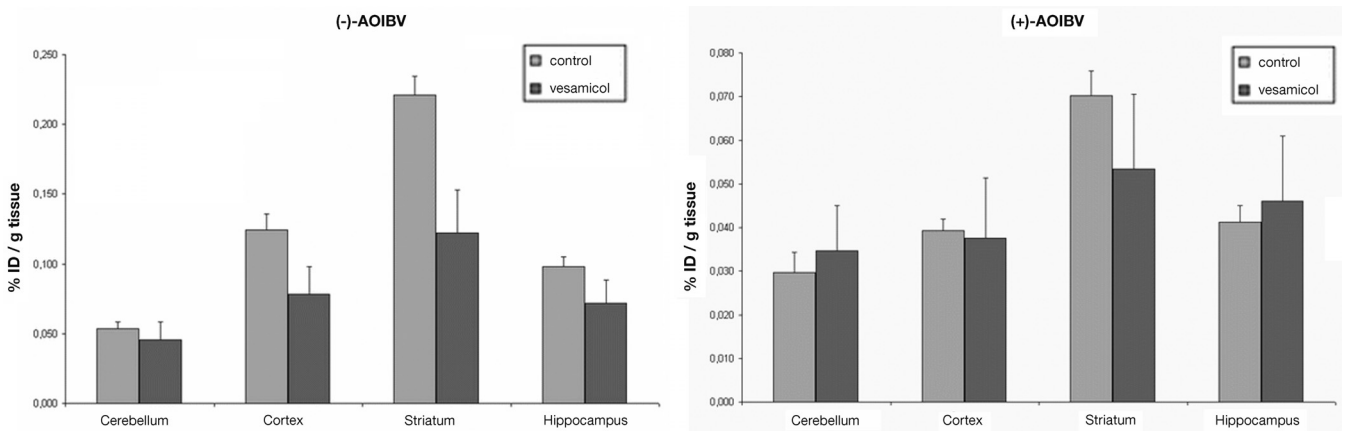
Introduction: The applicability of benzovesamicol probes in imaging studies of Alzheimer's Disease (AD) is based on the premise that over the course of the disease, VAChT levels change in parallel with other cholinergic marker proteins. Our goal was to synthesize radioiodinated benzovesamicol derivatives, which could potentially be used as SPECT tracers for VAChT exploration in AD.

Experimental: Radioiodinated [R, R]-AOIBV and [S, S]-AOIBV were obtained by iododestannylation of their corresponding tin precursors using Chloramine T as oxidant. Rat ex-vivo biodistribution and competition experiments (0.5 μmol/kg of vesamicol) were carried out 2 hours post injection.

Results and Discussion: Radioiodinated [R, R]-AOIBV and [S, S]-AOIBV were obtained in better than 90% yield. The average radiochemical and optical purity were greater than 97%.



Kd values were 4.3 ± 1.4 nM and 0.45± 0.11 nM for (R, R)-AOIBV and (S, S)-AOIBV, respectively. The (R, R) enantiomer accumulated in all brain regions in a homogeneous manner. In contrast, (S, S)-AOIBV accumulated in brain areas containing VAChT (striatum, cortex, hippocampus) whereas the cerebellum displayed a low level of radioactivity. Moreover, this uptake is specific to the VAChT as the (S, S)-AOIBV binding is inhibited by pre-administration of vesamicol.



Conclusion: These results demonstrate that (S, S)-AOIBV is a highly potent VAChT ligand which can be labelled using radioiodine. The ex vivo biodistribution profile in rat suggestes that this compound is a good candidate for further evaluation in primate to determine its potential use as imaging agent for studies involving the cholinergic system.

Keywords: Vesicular AcetylCholine Transporter, Benzovesamicol Derivatives, Iodine

P257 SYNTHESIS, ^{18}F-LABELING AND EVALUATION OF α_5-SUBTYPE-SELECTIVE GABA$_A$-RECEPTOR-LIGANDS

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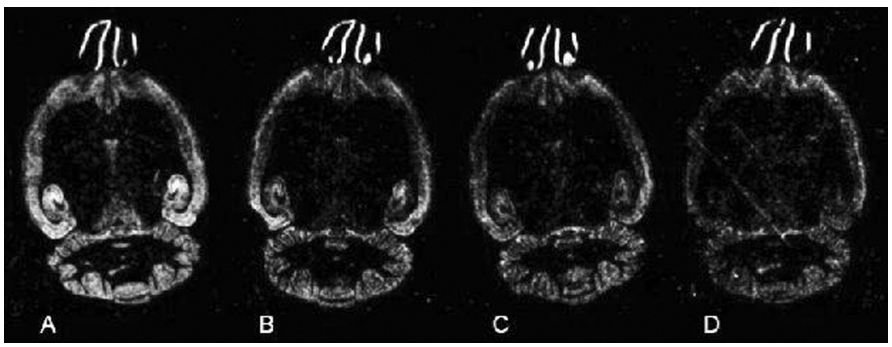
Introduction: The visualization and quantification of the α_5-subunit of the GABA$_A$-receptor by PET may allow a better diagnosis and therapy control of miscellaneous neurological disorders, e.g. Alzheimer's disease (AD) and post-traumatic stress disorder (PTSD). α_5-Subtype-selective GABA$_A$-receptor ligands also provide an opportunity to give a deeper understanding of the important processes of learning and memory. 7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-a]phthalazine [L.J. Street et al., J Med Chem 47 (2004), 3642-57] seems to be a promising lead structure for those new ligands, especially for PET tracers, which allow non-invasive measurement of ligand biodistribution and accumulation kinetics related to GABA$_A$-receptor studies in the living brain.

Experimental: A novel series of 6-(6-fluoro-pyridine-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-a]phthalazine derivatives TC 07-TC12 were synthesized in a modular organic synthesis. The reference substances were evaluated in receptor binding assays and by autoradiography of [^{3}H]Ro15-4513 binding against increasing concentrations of the synthesized derivatives. The corresponding precursors for ^{18}F-syntheses were built in a multi-step-synthesis. The subsequent ^{18}F-labeling was achieved by direct ^{18}F-fluorination via nucleophilic substitution using [^{18}F]fluoride.

Results and Discussion: For the fluoro-reference compounds, both binding assays and autoradiographic data showed nanomolar affinities (K_i) and a very high selectivity for the α_5-subunit of the GABA$_A$-receptor. Autoradiographic data indicate a dose dependent selective displacement of the radioligand from α_5-subunit containing GABA$_A$-Receptors. The figure below shows the displacement of [^{3}H]Ro15-4513 with increasing concentrations of compound TC12 (A: total binding of radioligand, B: 20nM TC12, C: 200nM TC12, D: 2000nM TC12). For the most relevant compounds, the ^{18}F-labeling reactions were optimized in terms of temperature, time of reaction and precursor concentration.



Conclusion: The experiments identified the synthesized substances to be potent substrates concerning the α_5-subtype of the GABA$_A$-receptor. Based on the obtained results so far, *ex vivo* and *in vivo* small-animal-studies using PET will be carried out next. Thus, new and highly selective PET-ligands for imaging the α_5-subunit in cell studies and in *ex vivo* and *in vivo* small-animal-studies using PET might soon be available.

Keywords: PET, GABAA, Subtype, Alpha5, [^{18}F]Fluoride

P258 DEVELOPMENT, SYNTHESIS AND *IN VITRO* EVALUATION OF NOVEL ET-A RECEPTOR PET-RADIOLIGANDS BASED ON PD 156707

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Introduction: Endothelin (ET) receptor distribution is dysregulated in many cardiovascular diseases. It is also known that a number of human cancer cell lines exhibit a pathologically upregulated density of ET-A receptors, influencing tumour growth and aggressiveness. Radiolabelled ET receptor antagonists offer the possibility to noninvasively assess ET receptor distribution *in vivo* by SPECT and PET. Therefore, these diagnostic tools are invaluable for the evaluation of disease progression and therapy effects. Here, the development and synthesis of highly affine ET-A receptor selective F-18-labelled radioligands based on lead structure PD 156707 is presented.

Experimental: Based on previous work dealing with radioiodinated derivatives of the ET-A receptor specific antagonist PD 156707, we accomplished the synthesis of two F-18-labelled ET-A receptor radioligands. Suitable precursor compounds were prepared by multistep organic syntheses. These were radiofluorinated by F-18-fluoride/Kryptofix in acetonitrile. Purification was performed by reversed phase radio-HPLC. The related non-radioactive counterparts were also sythesised and tested in *in vitro* assays for their affinity towards endothelin receptors.

Results and Discussion: The radiofluorination of two suitable tosylate precursors using F-18-fluoride/Kryptofix was accomplished with 10-15% radiochemical yield (decay-corrected) and with a radiochemical purity > 98%. Specific radioactivities were 20-50 GBq/μmol (EOS) after a total preparation time of 50 min after EOB. In *in vitro* assays the affinities of the non-radioactive reference compounds towards endothelin receptors were determined. Both compounds displayed high ET-A affinity (K_i=0.14 nM - 0.16 nM) and ET-A selectivities > 1000.

Conclusion: The two fluorinated derivatives of the known antagonist PD 156707 are potent ET A receptor ligands *in vitro*. We developed a practicable radiosynthesis of F-18 labelled analogues of these compounds. In further studies we plan to evaluate the *in vivo* potential of these radioligands using animal models with locally enhanced ET-A receptor density and small animal PET.

Acknowledgement: Financial support of the DFG (SFB 656/A1) is gratefully acknowledged.

Keywords: Endothelin Receptors, PET-Imaging, Radiofluorination

P259 RADIOCHEMISTRY AND PRELIMINARY PET EVALUATION OF A 5-HT1A AGONIST IN *CYNOMOLGUS* MONKEY

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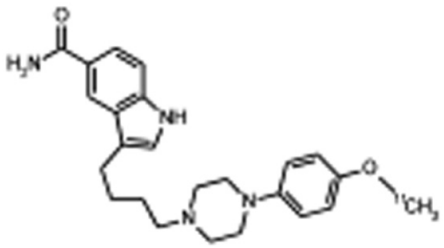
Introduction: The development of radiolabeled agonists as imaging tools will be crucial for the study of certain aspects of serotonergic neurotransmission such as measuring the endogenous serotonin levels. A group of arylpiperazine-butylindoles has been presented as potential 5-HT1A radioligands for PET [1]. We have radiolabeled and evaluated one highly potent and selective compound, 3-(4-(4-(4-methoxyphenyl)piperazin-1-yl)butyl)-1H-indole-5-carboxamide, (**1**) in that group.

Experimental: Compound **1** was radiolabeled by base promoted O-methylation using carbon-11 methyl iodide. One baseline and one pre-treatment experiment with WAY-100635 (0.5mg/kg injected 10 minutes prior to radioligand injection) were performed. Metabolites were measured at four and thirty minutes after injection of the radioligand in both studies.



Results and Discussion: Compound **1** was successfully radiolabeled by O-methylation using carbon-11 methyl iodide. The radiochemical purity was >98% and specific radioactivity was >11000 Ci/mmol. [^{11}C]**1** has a relatively low brain uptake (1% of injected dose). The radioactive uptake increases during the entire study. No high uptake was observed in the high-density 5-HT1A areas. No significant effect was observed after pre-treatment with WAY-100635. Two labeled metabolites, both more hydrophilic than the mother compound were observed. Four minutes after injection less than 20% of the radiolabeled material was attributed to the mother compound and after 30 minutes all [^{11}C] **1** was metabolized.

IC50 (nM) for compound **1**

5-HT1A	D2	D2/5-HT1A
0.09	140	1555

Conclusion: The present findings suggest that [^{11}C]**1** is not useful as a radioligand for PET studies. One possible explanation may be that [^{11}C] **1** is a substrate for P-glycoprotein.

Reference: [1] Heinrich, T., H. Bottcher, R. Gericke, G.D. Bartoszyk, S. Anzali, C.A. Seyfried, H.E. Greiner, and C. Van Amsterdam, Synthesis and structure–activity relationship in a class of indolebutylpiperazines as dual 5-HT(1A) receptor agonists and serotonin reuptake inhibitors. J Med Chem, 2004. 47(19): p. 4684-92.

Keywords: Serotonin, Agonist, PET, Carbon-11

P260 HRRT AND ECAT PET SCAN USING (^{11}C)YOHIMBINE

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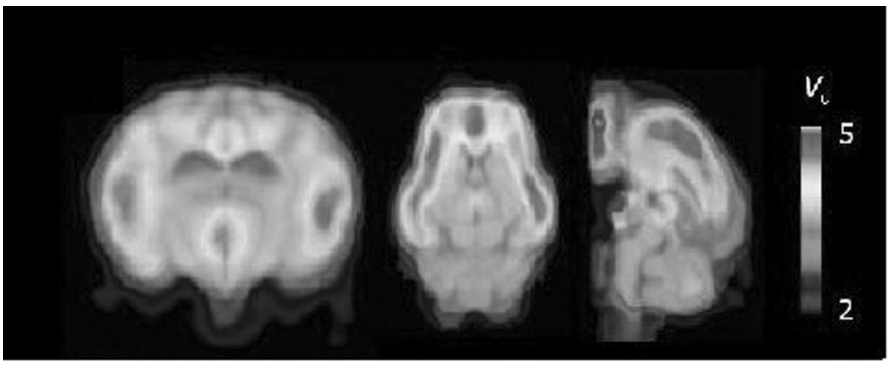
Introduction: Yohimbine is a potent α2-adrenoceptor antagonist and we have shown that carbon-11 labelled yohimbine image α2-adrenergic receptors in vivo in the brain of pigs using PET. Here we explore the selectivity and the sensitivity of yohimbine binding towards endogenous noradrenaline using either the ECAT PET scanner or the high-resolution research tomograph (HRRT) scanner.

Experimental: Female pigs (38-41 kg) were used in the study and we maintained body temperature and blood chemistry (gases, glucose and pH) of the pigs within the normal range, infused isotonic saline intravenously at a rate of ca. 3 ml/min and continuously monitored heart rate and blood pressure. Pigs received [^{11}C]yohimbine first in a baseline condition and again 120 min later after different drug challenges: WAY-100635 (0.5 mg/kg, n=2), amphetamine (10 mg/kg, n=3), HEAT (1 mg/kg, n=2) and reboxetine (0.5 mg/kg, n=1) as intravenous infusion, beginning 10-15 min before the initiation of dynamic PET recordings.

Results and Discussion: Figure 1 shows [^{11}C] yohimbine Vd maps superimposed on the MR pig atlas in the baseline condition. The brain regions which show highest binding in the baseline condition include cortical structures, mesencephalon, striatum, putamen (Vd of 4-5 ml g-1), somewhat lower binding in diencephalon and thalamus (Vd of 3-4 ml g-1) and lowest in pons and cerebellum (Vd of 2); in agreement with our previous findings. After administration of WAY-100635 (a selective 5HT1A antagonist) no changes in binding was observed. Treatment with the norepinephrine transporter inhibitor reboxetine resulted in a global reduction of Vd by 50% (results obtained in a single pig). The amphetamine challenge resulted in a reduction of the binding by 60% throughout the pig brain.



Conclusion: The current data obtained so far show that performing pig [^{11}C] yohimbine baseline scan in the HRRT gives similar values of regional distribution volumes as compared to baseline Vd obtained with the ECAT scanner. A more complete comparison including kinetic information will appear shortly. It remains to be explored, if the spacial resolution of the HRRT scanner allows for delineating the white matter of the cerebellum, such that this brain region potentially could serve as a reference tissue region in PET experiments with 11C yohimbine.

Keywords: PET, HRRT, 11C Yohimbine, Adrenergic Receptor

P261 RAT DISTRIBUTION OF (^{18}F)-FLUOROPROPOXY- AND (^{18}F)-FLUOROETHOXYBENZOVESAMICOL, AS PET RADIOLIGANDS FOR THE VESICULAR ACETYLCHOLINE TRANSPORTER (VAChT)

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Introduction: Attempts to image the VAChT using PET and F-18 have focused on the vesamicol scaffold. Several radioligands including [^{18}F]NEFA, [^{18}F]FBT and [^{18}F]FEOBV (1) have been reported but have never been used in human PET studies. We described benzovesamicol derivatives among which (2R,3R)-5-FPOBV (2) (Kd=0.77nM) appeared as a suitable candidate for in vivo VAChT imaging agent.

We present the labelling and rat biodistribution studies of (1) and (2).

Experimental: [^{18}F]-1 and [^{18}F]-2 were labelled with F-18 using [^{18}F]-kryptofix-K222 and the corresponding tosylate precursors. The radiolabelling was performed using a GE TRACERlab MXFDG synthesiser and following HPLC purification.

Ex vivo biodistribution of [^{18}F]-1 and [^{18}F]-2 were performed in rats (male Wistar, weighing 300 g). Ten rats were used for each tracer, 5 injected with radiolabelled compound and 5 pre-injected with L(-)-vesamicol 0,5 μmol/kg. Each animal was sacrificed 2 h post injection of radiolabelled compound and tissue samples were removed and the radioactivity measured.

Results and Discussion: Radiolabelling yields of 1.7% for [^{18}F]-1 and 2.6% (range: 1-5.4) for [^{18}F]-2 were obtained, with good specific activity: 124-338 GBq/μmol and radiochemical purity greater than 98%.

For both compounds, we observed a low and homogenous brain uptake (less than 0.12%ID/g tissue) and a low and non significant displacement in animals pre injected with L(-)-vesamicol. These results were in accordance with non-specific brain uptake. Furthermore, with compound (2) we observed high bone uptake (0.8%ID g tissue) indicating defluorination of [^{18}F]-2.

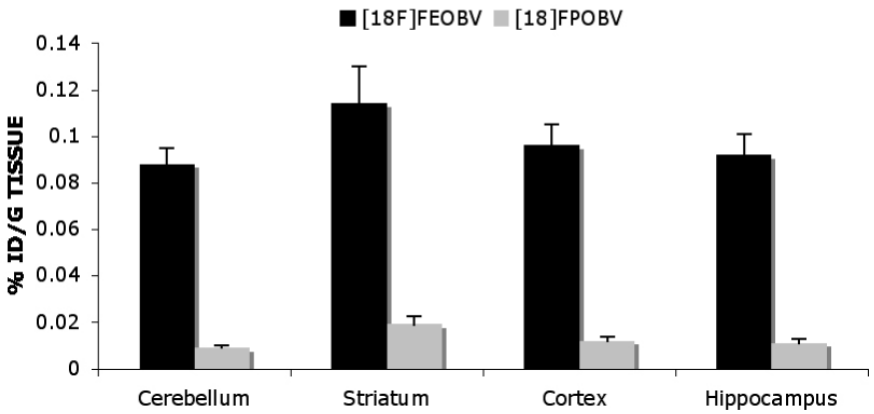

Fig. 1. Biodistribution of [^{18}F]-FEOBV and [^{18}F]-FPOBV.

Conclusion: As no preferential localisation was observed in regions known to contain high levels of VAChT, the uptake of [^{18}F]-1 and [^{18}F]-2 appear mainly as non specific. Furthermore, defluorination and rapid accumulation of radioactivity in the gastrointestinal tract indicated a fast metabolism of both compounds in rat. From these results [^{18}F]-1 and [^{18}F]-2 appear not suitable as radioligands for the VAChT in PET studies.

Acknowledgement: This study was funded in part by the EC - FP6-project DiMI, LSHB-CT-2005-512146.

Keywords: Vesicular AcetylCholine Transporter, Benzovesamicol, Fluorine

P262 PRELIMINARY EVALUATION OF NEW ^{99m}Tc-LABELED BOMBESIN-LIKE PEPTIDES FOR PROSTATE CANCER DETECTION

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Introduction: Bombesin (BN) is a 14-amino acid neuropeptide with high affinity for the gastrin-releasing-peptide (GRP) receptors. BN and its mammalian counterpart GRP, a 27-amino acid peptide, have respective biological properties and similar C-terminal amino acid sequence. Specific receptors for BN, also known as GRP receptors, are expressed on a variety of human tumors including prostate, gastric, colon, breast, lung and pancreatic cancers. Aim of the present work is the evaluation of the ^{99m}Tc-complexes of the two following new BN-like peptides: Gly-Gly-Cys-*Aca*-BN[2-14], (BN1.1), and Gly-Gly-Cys-*Aca*-BN[7-14] (BN1.1p), where *Aca*: 6-amino-hexanoic acid. Pyroglutamic acid in the bombesin molecule has been replaced by the chemical moiety *Gly-Gly-Cys-Aca*.

Experimental: The new BN derivatives were synthesized, purified and characterized via the respective $^{185/187}$Re-complexes. ^{99m}Tc-labeling was performed via the precursor ^{99m}Tc-gluconate. The stability of the radiolabeled species obtained was examined with time and in human plasma. Metabolic studies were performed in kidney and liver homogenates. IC_{50} values were determined, comparatively, for the BN-derivatives, their $^{185/187}$Re complexes and Tyr^4-BN. Internalization was investigated in PC-3 cells at 37°C. The degree of the residualization of ^{99m}Tc into the cells was examined with time. Tissue distribution of the radiopeptides was evaluated in normal mice and prostate cancer experimental models.

Results and Discussion: Chromatographic analysis showed for both derivatives, that ^{99m}Tc labeling led to the formation of a single radioactive species in high yield (>98%), stable with time. ^{99m}Tc-BN1.1 and ^{99m}Tc-BN1.1p were practically stable in human plasma, whereas they degraded rapidly in kidney and liver homogenates. Both unlabeled and labeled peptides demonstrated high binding affinity for the human prostate adenocarcinoma PC-3 cell line and were internalized rapidly into the prostate cancer cells. Significant uptake of radioactivity was observed in the pancreas of normal mice. Receptor-blocking studies with native BN confirmed the specificity of ^{99m}Tc-BN1.1 and ^{99m}Tc-BN1.1p toward the GRP receptors. Excretion took place mainly via the urinary system for ^{99m}Tc-BN1.1 and through the hepatobiliary in the case of ^{99m}Tc-BN1.1p. Satisfactory tumor images were obtained with both radiolabeled peptides.

Conclusion: The above preliminary results indicate that these new Bombesin derivatives are promising for human cancer studies.

Keywords: Bombesin, Tc, Prostate Cancer, Imaging

P263 NEW ^{68}Ga- AND ^{18}F-LABELED GNRH-I ANALOGS FOR IN VIVO PET IMAGING OF GNRH-R-EXPRESSING TUMORS

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Introduction: A large majority of tumors of the reproductive system express the gonadotropin releasing hormone receptor (GnRH-R) at a high level [1]. Addressing this receptor with various (ant)agonistic analogs of native GnRH-I (pGlu^1-His^2-Trp^3-Ser^4-Tyr^5-Gly^6-Leu^7-Arg^8-Pro^9-Gly^{10}-NH_2) has shown high therapeutic impact. In this study, new GnRH-I derivatives with structural analogy to the highly active cytotoxic GnRH-analog AN-152 [2] were radiolabeled with the PET nuclides ^{18}F and ^{68}Ga and evaluated in vitro with respect to their GnRH-R targeting potential.

Experimental: The reference peptide Triptorelin (DTrp^6-GnRH-I) and DLys^6-GnRH-I were synthesized via standard Fmoc-SPPS. For ^{68}Ga-labeling, the latter was coupled with DOTA at DLys^6 [3]. To allow ^{18}F-labeling via chemoselective oxime formation [4], DLys^6-GnRH-I was also conjugated with Ahx (aminohexanoic acid) or β-Ala, which in turn were coupled with Boc-aminooxyacetic acid. ^{18}F-labeling via oxime formation with 4-[^{18}F]fluorobenzaldehyde was performed using the Boc-protected precursors. Labeling with ^{68}Ga and ^{125}I was carried out according to standard protocols. The internalization kinetics of [^{68}Ga]DOTA-GnRH-I, DLys^6-Ahx([^{18}F]FBOA)-GnRH-I and DLys^6-βAla([^{18}F]FBOA)-GnRH-I (FBOA = fluorobenzyloxime acetyl) into GnRH-R-expressing EFO-27 and OVCAR-3 ovarian cancer cells at 37°C was determined by dual tracer studies using [^{125}I]Triptorelin as an internal reference.

Results and Discussion: Generally, absolute ligand internalization within 120 min was low (≤2% of added dose), and internalization was slow, as indicated by a high and slowly decreasing fraction of receptor bound (acid releasable) activity (app. 50% of total binding after 120 min). However, of the compounds investigated, DLys^6-Ahx([^{18}F]FBOA)-GnRH-I showed the highest relative radioligand uptake in EFO-27 and OVCAR-3 cells (89.1±14.9% and 84.7±5.6% of the internalization found for [^{125}I]Triptorelin after 120 min, respectively), indicating a similar binding affinity. DLys^6-βAla([^{18}F]FBOA)-GnRH-I showed reduced internalization (41.2±4.5% of [^{125}I]Triptorelin (EFO)), whereas complete loss of binding affinity was found for [^{68}Ga]DOTA-GnRH-I (2.2±0.5% of [^{125}I]Triptorelin). To evaluate the suitability of DLys^6-Ahx([^{18}F]FBOA)-GnRH-I for in vivo studies, its serum half-life in human serum was determined and found to be sufficiently long (>2h).

Conclusion: In summary, DLys^6-Ahx([^{18}F]FBOA)-GnRH-I is the first ^{18}F-labeled GnRH analog with high GnRH-R-targeting efficiency and might have certain potential for GnRH-R-imaging in vivo.

References: [1] G.S. Harrison et al. *Endocrine-Related Cancer* **2004**, *11*, 725. [2] A. Nagy et al. *Proc.Natl.Acad.Sci. USA* **1996**, *93*, 7269. [3] M. Schottelius et al. *Tet.Lett.* **2003**, *44*, 2393. [4] T. Poethko et al. *J.Nucl.Med.* **2004**, *45*, 892.

Keywords: GnRH, Gonadotropin, Fluorine-18, PET

P264 CYCLIC RGD PEPTIDE BEARING TWO DIFFERENT CHELATORS FOR THE *FAC*-^{99m}Tc(CO)$_3$ CORE: A COMPARATIVE STUDY

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Introduction: Radiolabeled RGD peptides are currently investigated as possible angiogenesis detection agents, due to their ability to attach to the $\alpha_v\beta_3$ integrin. The α_v integrins are the most important integrins involved in tumor induced angiogenesis which play a vital role in the growth and metastasis of tumors. In the present study we perform a radiochemical and radiobiological evaluation of two cyclic RGD derivatives labeled with ^{99m}Tc(I), in normal mice and in pathological models bearing MDA-MB 435 human breast carcinoma.

Experimental: The study was performed, comparatively, on two derivatives of the cyclic peptide cRGDfK: namely cRGDfK-His and cRGDfK-CPA [CPA: (3)-L-Cysteine Propionic Acid]. cRGDfK was synthesized by employing the SPPS method, according to the F-moc strategy. The two chelators, His and CPA, were linked to the peptide moiety via the N^{ε}-amino group of Lys on the resin. Both derivatives were identified with RP-HPLC and ESI-MS. Labeling was performed *via* the precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$. The effect of heating time and peptide concentration on the labeling was studied. The *in vitro* stability of both labeled derivatives was investigated at different time-points, in the presence of an excess of His and Cys and in human plasma. *In vitro* internalization and efflux studies in cancer cells were also performed. Their *in vivo* behavior was assessed in normal and tumor bearing mice. Gamma-camera images of the injected tumor bearing mice were obtained 30min p.i.

Results and Discussion: Labeling of both derivatives led to the formation of a single, stable product and takes place in high yield (>98%) even at low peptide concentrations. His-Cys challenge showed that both products remain stable (>95% at 24h). Incubation with human plasma showed satisfactory stability for both compounds which also internalize rapidly in cancer cells. The study of their *in vivo* behavior in normal mice showed that cRGDfK-His-^{99m}Tc$(H_2O)(CO)_3$ is mainly cleared *via* the hepatobiliary route while cRGDfK-CPA-^{99m}Tc$(CO)_3$ shows elimination primarily *via* the kidneys. From the evaluation of tumor uptake in scid mice it is assumed that experimentally induced breast cancer can be imaged at early times.

Conclusion: The *in vitro* and *in vivo* characteristics of both, ^{99m}Tc(I) labeled RGD derivatives under study are satisfactory. Thus, both compounds can be considered as promising agents for breast cancer imaging.

Keywords: RGD, Integrins, Carbonyls, Cancer, Tc

P265 EVALUATION OF NEW α_1-ADRENOCEPTOR ANTAGONIST (^{11}C)LU AA27122 AS POTENTIAL PET RADIOLIGAND FOR BRAIN IMAGING

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Introduction: α_1-Adrenoceptors belong to the family of G-protein coupled receptors. In the CNS, α_1-adrenoceptors are known to facilitate locomotion, arousal and higher cerebral functions. Nanomolar affinity for α_1-adrenoceptors is also a common feature of several atypical antipsychotics. However, lack of blood-brain barrier penetrating PET radioligands has limited evaluation of the role of α_1-adrenoceptors in neuropsychiatric disorders and in relation to antipsychotic drug effects in man.

A sertindole analogue Lu AA27122 (1-(4-fluorophenyl)-3-(1-methylpiperidin-4-yl)-5-pyrimidin-5-yl-1*H*-indole) is a selective α_1-adrenoceptor antagonist with high *in vitro* affinity ($K_i[\alpha_{1A}]$ = 0.52 nM, $K_i[\alpha_{1B}]$ = 1.9 nM, $K_i[\alpha_{1D}]$ = 2.5 nM) and high Caco-2 permeability (P_{app}/Ratio= 13.4/0.7).

Experimental: The desmethyl precursor of Lu AA27122, was methylated with [^{11}C]methyl iodide with 30% incorporation yield and with high specific radioactivity (>10 000 Ci/mmol). *In vitro* binding of the synthesized [^{11}C]Lu AA27122 was measured with autoradiography in post mortem human brain. Specific binding was examined with 10 μM phentolamine. Regional brain distribution of the radioligand *in vivo* was evaluated in cynomolgus monkey in baseline conditions and after two different doses of prazosin (0.1 mg/kg and 0.3 mg/kg). Metabolite analysis was performed using gradient HPLC. Plasma protein binding was measured with ultra-filtration method.

Results and Discussion: Autoradiographic examination revealed high binding in regions known to have high α_1-adrenoceptor concentration, i.e. in cortical layers and in thalamus, caudate head and dentate gyrus. However, only minor decrease in binding was observed, when the sections were co-incubated with phentolamine.

In cynomolgus monkey, total brain uptake of [^{11}C]Lu AA27122 was high, 3.9%ID, 36 minutes after the tracer injection. Highest uptake was observed in the occipital cortex (region over cerebellum ratio 1.33), cingulate cortex (ratio 1.30) and thalamus (ratio 1.23). However, pretreatment with prazosin had no influence on regional binding. Metabolite analysis demonstrated that over 30% of the parent compound was left after 45 minutes. Plasma protein binding was 87.8 ± 0.8%.

Conclusion: [^{11}C]Lu AA27122 demonstrates high brain uptake and high binding in regions enriched with α_1-adrenoceptors. However, both autoradiographic and PET examination indicated too high level of non-specific binding, which could be caused by the relative high lipophilicity of the compound.

Keywords: Alpha1-Adrenoceptor, Antagonist, Brain, Autoradiography, PET

P266 IMPACT OF PKM LINKERS ON *IN VITRO* AND *IN VIVO* CHARACTERISTICS OF ^{111}In-LABELED CYCLIC RGDFK DIMER

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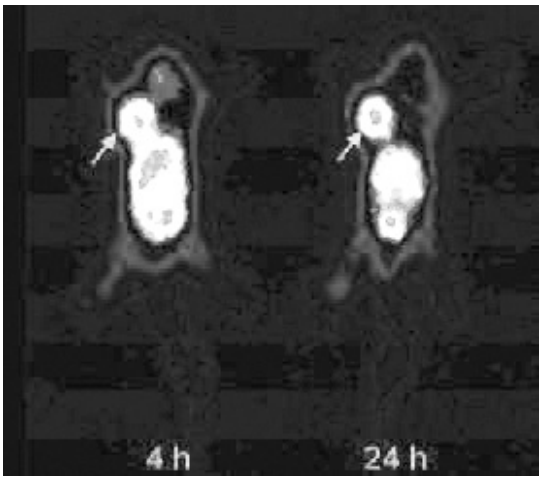
Introduction: In the last several years, a variety of pharmacokinetics modifying (PKM) linkers have been used to improve excretion kinetics of radiolabeled cyclic RGD peptides. In this study, DTPA-Bz-SU016, DTPA-Bz-E-SU016 and DTPA-Bz-Cys-SU016 (SU016 = E[c(RGDfK)]$_2$, E = glutamic acid, Cyc = cysteic acid) were labeled with In-111, and their *in vitro* and *in vivo* characteristics were evaluated. The main objective of this study is to select an optimal PKM linker for In-111-labeled small biomolecules.

Experimental: Receptor binding experiments were performed on U87MG human glioma cells for three compounds. For the labeling, to a clean Eppendorf tube were added 20 mL of RGD peptides (0.5 mg/mL in 0.2M NaOAc buffer, pH=5.0), 180 mL of 0.2M NaOAc buffer (pH=5.0) and 15 mL of $^{111}InCl_3$ solution (222 MBq, in 0.05M HCl). The reaction mixture was kept at room temperature for 20 min. The labeling yield and radiochemical purity (RCP) after purification of C-18 column were analyzed by radio-HPLC and ITLC. The solution stability of three radiotracers was determined in saline and 6 mM EDTA solution (pH=5.0). In order to compare the lipophilicity of three radiotracers, Log P values were measured. The biodistribution and γ imaging were performed in nude mice with U87MG human glioma xenografts.

Results and Discussion: The IC_{50} values of DTPA-Bz-SU016, DTPA-Bz-E-SU016 and DTPA-Bz-Cys-SU016 were 7.86 nM, 5.76 nM and 6.86 nM, respectively. There was no significant difference on integrin $\alpha_V\beta_3$ binding. The labeling yield of three In-111 complexes was more than 95%, and the RCP after purification was more than 99%. The stability of three In-111 complexes in saline and 6 mM EDTA solution was excellent with RCP > 95% during 24 h after labeling. The Log P values were -3.42, -2.72 and -3.31 for three radiotracers, respectively. At 4 h postinjection, the ratios of tumor/liver and tumor/kidney for ^{111}In-DTPA-Bz-E-SU016 were higher than that of other two radiotrvers (0.87 vs 0.66 and 0.64, 0.49 vs 0.27 and 0.14). The same results were obtained at 24 h postinjection (1.64 vs 0.88 and 1.22, 0.65 vs 0.28 and 0.20). The clear images of xenografted tumors were obtained at 4 h and 24 h postinjection for ^{111}In-DTPA-Bz-E-SU016.



Conclusion: ^{111}In-DTPA-Bz-E-SU016 is better for integrin $\alpha_V\beta_3$-positive tumors imaging.

Keywords: PKM Linker, RGD Dimer, In Vitro, In Vivo, Glioma

P267 SYNTHESIS AND EVALUATION OF THE TRANSLOCATOR PROTEIN (18 kDa) (TSPO) LIGAND ([11]C)DPA-715 IN RAT AND NON-HUMAN PRIMATE

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Introduction: The translocator protein (18kDa) (TSPO), formerly known as the peripheral benzodiazepine receptor (PBR), is over expressed upon microglial activation. Such activation is present in a number of neurodegenerative conditions suggesting that imaging may provide disease location, chronicity and progression. This study involved the evaluation of the pyrazolopyrimidine DPA-715 (TSPO Ki = 16.4 nM) in behavioural studies and the radiolabelled form, [^{11}C]DPA-715, in healthy non-human primate and AMPA-lesioned rats as a model of activated microglia using PET.

Experimental: The *in vivo* anxiolytic effects of DPA-715 were assessed using the social interaction test which represents social anxiety in humans. DPA-715 was administered at 0.5 mg/kg, 5 mg/kg and 20 mg/kg doses. A vehicle only rat was used as a behavioural control. [^{11}C]DPA-715 was prepared using [^{11}C]CH_3I as the labelling intermediate from the phenolic precursor of DPA-715 using TBAH and DMF followed by HPLC. The non-human primate distribution studies were performed using a clinical PET scanner, and AMPA-lesioned rats using microPET. Blocking studies were conducted using PK11195 (5 mg/kg).

Results and Discussion: In the social interaction test a significant overall effect for the duration of time spent in general investigation, adjacent lying and rearing was observed. Post hoc analysis revealed a significantly greater time spent in general investigation and adjacent lying in the 20 mg/kg DPA-715 treatment group compared to vehicle treated rats. In PET radiolabelling experiments, the average non-decay corrected RCY of [^{11}C]DPA-715 was 0.27 ± 0.05% with an average s.a. of 16.32 ± 4.01 GBq/μmol. The baboon PET distribution studies revealed poor brain uptake. Pre-treatment with PK11195 resulted in no change of radioligand uptake, which suggests that this brain uptake is representative of non-specific binding. In agreement with these results, the brain uptake in the AMPA lesioned model, depicted no significant differences between the lesioned striatum and the non-lesioned contralateral striatum (Fig. 1).

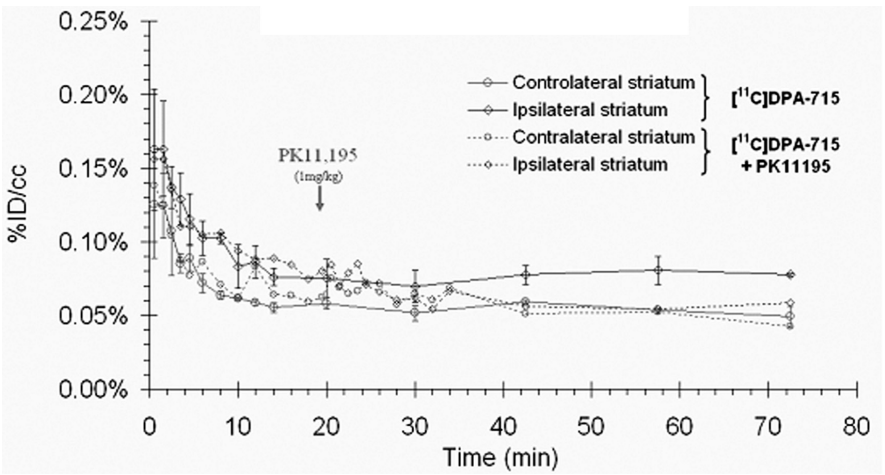

Fig. 1. Time-activity curve for [^{11}C]DPA-715 (n = 2) and [^{11}C]DPA-715 + PK11195 (n = 1).

Conclusion: Although DPA-715 does possess anxiolytic properties in vivo, [^{11}C]DPA-715 does not possess the required properties for further development as a PET radioligand for imaging the TSPO (18 kDa).

Keywords: TSPO (18kDa), PBR, Carbobn-11, DPA-715, Anxiolytic

P268 ATTEMPT OF RADIOLABELING α-MSH ANALOGUE WITH F-18 AS MELANOMA TUMOR IMAGING AGENT

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Introduction: α-melanocyte-stimulating hormone regulates melanoma cell proliferation and function. The presence of high affinity MSH receptor on melanoma cell lines drove researchers towards the development of radiolabeled MSH analogues. Because of the instability of α-MSH, Vaidyanathan et al. labeled the more stable [Nle^4, D-Phe^7]-α-MSH with ^{18}F. However, its actual imaging property was not determined in melanoma tumor bearing mice. Until now, mainly DOTA conjugates, e.g. DOTA-ReCCMSH and DOTA-NAPamide, have been reported with good in vivo imaging results. Presently, we radiolabeled [Ac-Cys^4, D-Phe^7, Cys^{10}]-α-MSH (4-13) with good resistance to in vivo proteolysis and high affinity for MC1R by ^{18}F and its in vivo bio evaluation was carried out.

Experimental: [^{18}F]SFB was prepared according to the published reaction conditions. Then, the peptide was reacted with [^{18}F]SFB to get [Ac-Cys^4, D-Phe^7, Cys^{10}, Lys^{11}-(^{18}F)PFB]-α-MSH (4-13) ([^{18}F]FB-MSH$_{4\text{-}13}$) in DMF/TEA and it was purified by HPLC finally. Its corresponding ^{19}F compound was prepared as an HPLC standard. Biodistribution studies were carried out on female C57BL/6 mice implanted with 1×10^6 cultured B16/F1 murine melanoma cells. Whole body PET images were also obtained on a micro-PET system. Metabolic stability was analyzed in normal mice by HPLC.

Results and Discussion: A typical experiment was started with 1.1 GBq of $^{18}F^-$ and yielded about 56 MBq [^{18}F]FB-MSH$_{4\text{-}13}$ within about 3h. Its radiochemical purity was more than 98% and the specific activity was more than 37 GBq/μmol. It was identified with [^{19}F]FB-MSH$_{4\text{-}13}$ by radio/UV-HPLC. Biodistribution showed that its tissue clearance was rapid and a high activity concentration was found in urine. The tumor uptake was 2.82±0.45%ID/g and the ratio of tumor to muscle was 3.7 at 30 min p.i. Unfortunately, block experiment showed [^{18}F]FB-MSH$_{4\text{-}13}$ had no specific accumulation in the melanoma tumor and there were no clear images of melanoma tumor from the micro-PET results. HPLC analysis of serum and urine showed no whole peptide was present at 30 min p.i., which could explain the unsuccessful results. Although the peptides were prepared as the amides and the N-terminus was acetylated besides the exchange from L-Phe^7 to D-Phe^7, this peptide was still susceptible to proteolysis in vivo.

Conclusion: We successfully radiolabeled α-MSH analogue with high labeling yield and specific activity. However, the bio-evaluation showed its no successful melanoma tumor imaging properties. In vivo stability of the labeled α-MSH analogues might be much more critical than specific activity for good imaging. To design new α-MSH analogues with high in vivo stability would be our future aim.

Acknowledgement: The authors thank the crew of Molecular Probe Group of Molecular Imaging Center (NIRS) for their support.

Keywords: Fluorine-18, α-MSH Analogue, Melanoma

P269 SYNTHESIS AND *IN VIVO* EVALUATION OF ([11]C)p-PVP-MEMA AS A PET RADIOLIGAND FOR IMAGING NICOTINIC RECEPTORS

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Introduction: Nicotinic acetylcholine receptors (nAChR) are involved in a wide range of diseases of the brain making them attractive targets for tomographic imaging. Substituted analogues of the nAChR agonist 3-(2-amino-ethoxy)pyridine have been reported with around 100-fold higher affinity than their parent structure. Of particular interest, ({(R)-2-[6-chloro-5-((*E*)-2-pyridin-4-yl-vinyl)-pyridin-3-yloxy]-1-methyl-ethyl}-methyl-amine) (p-PVP-MEMA) displayed an affinity of Ki = 0.077 nM for nAChR when using [^{3}H]cytisine and whole rat brain membrane (1).

Experimental: p-PVP-MEMA and its corresponding nor-methyl derivative where obtained using a multistep synthesis. [^{11}C]p-PVP-MEMA was prepared from the nor-methyl derivative and labelled with [^{11}C]CH_3I. The reaction was conducted in DMF using tetrabutyl ammonium hydroxide (TBAH) as base and allowed to react at RT for 2 min, followed by heating at 80°C for 5 min (Fig. 1). The reaction mixture was diluted with 0.5 mL HPLC mobile phase consisting of 0.1 M NH_4Ac (pH 10):ACN (70:30; v:v) and injected onto a semi preparative C-18 HPLC column. Using a flow rate of 6.0 mL/min, the retention time of [^{11}C]p-PVP-MEMA was 8.6 min. *In vivo* assessment of [^{11}C]p-PVP-MEMA was performed in baboon using PET.

Fig. 1

Results and Discussion: [^{11}C]p-PVP-MEMA was isolated in a 1.5% (n = 4) non-decay corrected RCY based on [^{11}C]CH_3I in an average synthesis time of 33.6 min. The radiochemical and chemical purity was greater than 99% with a specific activity of 86.4 GBq/μmol. Following i.v. administration of [^{11}C]p-PVP-MEMA, significant accumulation was observed in thalamus (Fig. 2). However, high accumulation was also observed in the cerebellum, a region low in nAChR density, with a thalamus to cerebellum ration of 1 throughout the 60 min time course of the imaging experiment. This is indicative of a high level of non-specific binding.

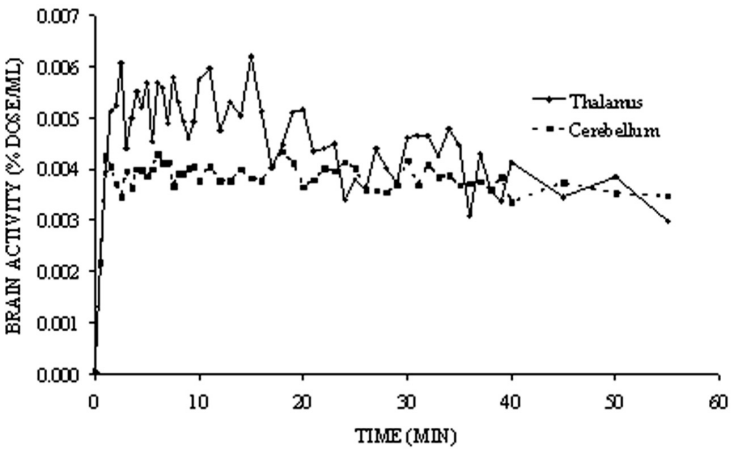

Fig. 2. [^{11}C]p-PVP-MEMA in baboon brain.

Conclusion: [^{11}C]p-PVP-MEMA does not posses the required properties for further development as a PET radioligand for imaging nAChRs.

Reference: [1] Lin NH et al. Bioorg Med Chem Lett 2002; 12: 3321-3324.

Keywords: Carbon-11, nAChR, PET, p-PVP-MEMA

P270 RADIOSYNTHESIS AND PRELIMINARY EVALUATION OF ZW-90 AND ZW-110, TWO NOVEL ACETYLENIC PYRIDINES FOR IMAGING NICOTINIC RECEPTORS

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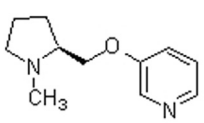
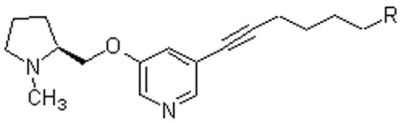
Introduction: Several radioligands have been developed to image the nicotinic acetylcholine receptor (nAChR) $\alpha4\beta2$ subtype using PET. Recently it was reported that introduction of a substituted alkynyl group into the C-5 position of the pyridinyl ring of A-84543, significantly increased the selectivity for nAChRs containing $\beta2$ subunits over nAChRs containing $\alpha4$ subunits (1). Two selected candidates, ZW-90 (**1**) and ZW-110 (**2**) were labelled with carbon-11 and evaluated *in vivo*.

Experimental: ZW-90 (**1**) and ZW-110 (**2**) were labelled with $[^{11}C]CH_3I$ using standard procedures with the addition of the weak base diisopropylethylamine (DIPEA) to aid in the *N*-$[^{11}C]$methylation. PET evaluation of $[^{11}C]$-**1** and $[^{11}C]$-**2** was performed in baboon.



A-84543



R = OH, ZW-90 (**1**)
R = F, ZW-110 (**2**)

Results and Discussion: $[^{11}C]$-**1** and $[^{11}C]$-**2** were prepared in an average non-decay corrected RCY, based on starting $[^{11}C]CH_3I$ of 7.1 ± 2.6% (n = 5) in a synthesis time of 26 min. The s.a was 37.9 ± 5.2 GBq/μmol with radiochemical purity >98% for both radioligands. In PET experiments $[^{11}C]$-**1** rapidly entered the baboon brain, with maximum uptake in the thalamus and cerebellum 2 min p.i. (0.013% dose/mL). The washout from cerebellum was faster than that from thalamus, suggesting that specific binding can be optimally measured at 20 min p.i. Pretreatment with nicotine (5 mg/kg) resulted in decreased uptake in thalamus (60%) and cerebellum (10%). $[^{11}C]$-**2** also rapidly entered the baboon brain, with a high uptake in the thalamus within 5 min with surprisingly a similar upatke in the striatum (0.03% dose/mL). Pretreatment with nicotine (5 mg/kg) resulted in inhibition of uptake of 8% and 1%, in the thalamus and cerebellum respectively, while no change was observed in the striatum. Using unlabelled **2** (5 mg/kg), 32% inhibition of radioligand uptake was observed in the thalamus and striatum while uptake in the cerebellum was reduced by 24%.

Conclusion: While further work will be necessary in the development of optimal imaging agents for nAChRs, efforts will be made to examine the potential of these newly developed radioligands to serve as diagnostic agents in the early detection of neurological disorders.

Reference: [1] Wei et al., J. Med. Chem. 2005, 48, 1721-1724.

Keywords: Carbon-11, nAChR, PET, Acetylenic Pyridines

P271 ^{99m}Tc-BOMBESIN (BN1.1) ASSESS THE INVOLVEMENT OF AMYGDALA IN FEEDING BEHAVIOUR: PRELIMINARY RESULTS IN RAT

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Introduction: There are increasing evidences that the neuropeptide Bombesin (BN) is involved in the mechanisms of satiety. The presence of the BNRs into the amygdala has been demonstrated by immunohistochemical and autoradiographic investigations. The present study has been carried out to assess the usefulness of the Bombesin-like peptide 1.1 labelled with technetium-99m (^{99m}Tc-BN1.1) as tool for the in vivo evaluation of the involvement of amygdala in feeding behaviour.

Experimental: BN 1.1 provided by NCR Demokritos (Athens, GR) was prepared by Fmoc synthesis as previously described (Varvarigou et al. 2002). For the study six female Wistar rats were kept in temperature- and light-controlled room for two weeks. Labelled peptide was prepared by dissolving the dry kit containing 20 μg of BN1.1, stannous chloride, and mannitol in 1 ml of freshly eluted sodium pertechnetate solution containing 370 MBq (10.0 mCi) of Tc-99m. After 10 min of incubation at room temperature, the LE was evaluated by ITLC strip using saline as mobile phase. An aliquote of 0.1 ml of ^{99m}Tc-BN1.1 (37 MBq, 2 mg of BN1.1) was intravenously injected in tail vein of 250 g food-deprived and non-deprived rats, intra-peritoneally anaesthetized. Cerebral SPECT images were acquired by using an high resolution camera (HRC) with 1 mm spatial resolution 30 min after injection, with 36 steps over 180° on a circular orbit. The uptake in the amygdale was evaluated by calculating the T/B ratio in food-deprived and non-deprived rats.

Results and Discussion: The Bombesin-like peptide 1.1 was labelled with technetium-99m with a high LE and radiochemical purity (99%), measured by ITLC. ^{99m}Tc-BN1.1 showed a fast accumulation in the rat brain with an increasing concentration during the time in the amygdale. A ratio of 1,5 in uptake for ^{99m}Tc-BN1.1 was found in the amygdale of food-deprived rats compared to non-deprived.

Conclusion: In these preliminary targeting studies on rats, we have obtained promising results for the use of ^{99m}Tc-BN1.1 in the imaging of CNS structures in vivo. Our results demonstrate in vivo the involvement of amygdala in feeding behaviour. The different uptake between food-deprived and non-deprived rat could be due to a differential expression of the BNRs in the rat CNS. The scintigraphic images confirm that the bombesin-like peptides may act as a satiety signal at level of amygdale and encourage further investigations on BNRs role in the control of feeding-satiety mechanisms using ^{99m}Tc-BN1.1.

Keywords: Bombesin-Like Peptide, Technetium-99m, Amygdala

P272 EVALUATION OF THE PERIPHERAL BENZODIAZEPINE RECEPTOR AS A MARKER OF PROLIFERATION

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Introduction: [*N*-methyl-^{11}C]PK 11195 ([^{11}C]PK) is a peripheral benzodiazepine receptor (PBR) ligand used as a tracer for PET imaging of neuroinflammation. However, literature suggests that PBR holds promise as an imaging target outside of the central nervous system. Increased expression of PBR has been measured in peripheral tumor tissues and cell lines, such as breast cancer, and a positive relationship between proliferation and PBR expression was reported in human breast cancer lines (1,2).

This led us to explore the utility of [^{11}C]PK for the detection of PBR-expressing tumors outside of the CNS. Our previous studies show specific uptake *in vitro* and preliminary biodistribution data (3). This study was designed to examine the capability of [^{11}C]PK to measure proliferation by comparing tumor uptake *in vivo* in breast cancer cell lines to two gold standards, the metabolic tracer FDG and the proliferation marker FLT.

Experimental: Two human breast cancer cell lines were selected for implantation: MDA-MB-435 and ER(-) MCF-7. In a previous *in vitro* cell study, MCF-7 cells showed very little specific uptake of [^{11}C]PK while MDA-MB-435 exhibited high specific uptake (3). Female athymic mice were implanted bilaterally in the mammary fat pad, and maintained according to standard animal care protocol. Tumors were imaged when they grew to approximately 100-350 μL. [^{18}F]FDG and [^{18}F]FLT were robotically produced in high specific activity. [^{11}C]PK was synthesized based on methods described in literature; specific activity exceeded 3600 Ci/mmol (EOS). Animals were imaged in a three day sequence, ending with [^{11}C]PK and [^{11}C]PK biodistribution measurement. Image analysis was performed to quantify microPET data.

Results and Discussion: FLT uptake did not differ significantly between the two cell lines. Average uptake for FDG was higher for both cell lines in tumors that grew more rapidly, and had the highest degree of variability. MCF-7 tumors had slightly higher FDG uptake values. However, the data for the [^{11}C]PK was perplexing. First, despite their *in vitro* expression differences, the two cell lines had similar uptake values, confirmed in biodistribution data. This could be due to a change in the expression of PBR. Secondly, the uptake values were 3-fold lower than FDG and FLT.

Conclusion: These data show that the two cell lines possess differential uptake of [^{18}F]FDG, [^{18}F]FLT and [^{11}C]PK. Additionally, imaging with [^{11}C]PK fails to identify the cells that express high and low levels of PBR *in vitro*.

Acknowledgement: This work was financially supported by NIH Grant 1R24CA83060-06.

References: [1] Beinlich, A. et al. (2000) Biochem. Pharm. 60, 397. [2] Hardwick, M. et al. (1999) Cancer Res. 59, 831. [3] Shockley, S. et al. (2005) J Label Compd. Radiopharm. 48, S264.

Keywords: [11-C]PK 11195, Peripheral Benzodiazepine Receptor, Tumor Imaging

P273 EVALUATION OF (^{123}I)MNI-200 AS A RADIOLIGAND FOR IMAGING ADENOSINE 2A RECEPTORS IN PRIMATE BRAIN

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Introduction: Recent interest in therapy of Parkinson's disease (PD) with drugs acting through adenosine 2a (A2a) receptors has emphasized the need for non-invasive scintigraphic methods to assess these biological targets for both new drug development and elucidating pathophysiological changes in PD patients. There are only a few early-stage radioligands available for imaging adenosine receptors. We have radiolabeled MNI-200, a promising candidate with low subnanomolar affinity for A2a, with I-123 and evaluated it as a SPECT imaging agent in non-human primates.

Experimental: Dynamic SPECT acquisitions were performed following a bolus injection of 8 to 10 mCi of [^{123}I]MNI-200 in 3 ovariectomized female baboons (*Papio anubis*) in a series of studies to evaluate: 1) the specific accumulation and washout properties in brain, and 2) the effects of displacing doses of caffeine or ZM241385, both of which bind to A2a and compete with the radioligand for the A2a receptor binding site.

Results and Discussion: Having *log*D of 2.4 at pH 7.4, [^{123}I]MNI-200 demonstrated low blood protein binding (10% free) with no lipohilic metabolites detectable by reverse phase HPLC. Highest accumulation of radioactivity occured in the striatum with peak striatal-to-cerebellar ratios ranging from 1.5 to 2.0 at 60 min post injection. Administration of caffeine in separate studies at doses of 2.5 and 10 mg/kg i.v. at 60 minutes post radioligand injection decreased the specifically-bound activity in the striatum by 52% and 69%, respectively. Similarly, challenge with a more specific A2a agent ZM241385, administered at 60 minutes post [^{123}I]MNI-200 injection, produced 24 to 56% displacement of the specifically-bound striatal activity.

Conclusion: These preliminary studies suggest that [^{123}I]MNI-200 is a promising agent for *in vivo* imaging of A2a receptors in the brain. This radioligand could prove useful in assessing occupancy at A2a receptors for putative therapeutic agents in human studies.

Keywords: SPECT, Adenosine Receptors, Parkinson's Desease

P274 SYNTHESIS AND EVALUATION OF A NOVEL PET RADIOLIGAND FOR IMAGING BRAIN I_2 RECEPTORS

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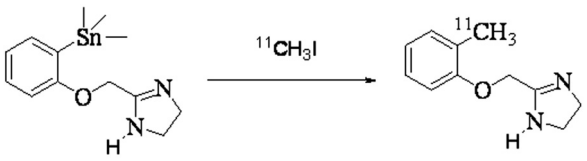
Introduction: Three types of imidazoline receptors, I_1, I_2 and I_3, are recognized [1]. Changes in brain I_2 receptor density are implicated in various neuropsychiatric conditions, such as eating disorders, depression and Huntington's disease [2]. Currently, there is no effective radioligand available to study these receptors with PET. 2-(2'-Methylphenoxymethyl)-imidazoline (**1**) is a high affinity antagonist (pK_i = 9.05) and is selective over α_1 and α_2 receptors (pA_2 = 3.76 and 6.67, respectively).[2] **1** has moderate lipophilicity (calc'd Log*P* = 2.12). We aimed to prepare [^{11}C]**1** for evaluation as a prospective PET radioligand.

Experimental: [^{11}C]**1** was prepared from a synthesized trimethyltin precursor (**2**) via a Stille coupling reaction (Scheme 1)[3]. [^{11}C]Iodomethane was trapped in a solution of $Pd_2(dba)_3$ (1.2 mg) and *tri*-o-tolylphosphine (6.8 mg) in DMSO (500 mL) and heated at 80°C for 2 min. The solution was then added to **2** (2.5 mg) containing CuI (1.0 mg) in DMSO (200 mL) and heated at 80°C for 5 min. Purified [^{11}C]**1** was injected into a rhesus monkey and dynamic PET images obtained for 90 min. In a second PET experiment, the monkey was dosed with **1** (2 mg/kg) at 20 min before [^{11}C]**1** and scanned for 90 min**.** Dynamic PET image were also acquired in rats after injection of [^{11}C]**1** alone and following dosing with **1** (3 mg).



2 [^{11}C]**1**

Scheme 1. Radiosynthesis of [^{11}C]2-(2'-methylphenoxymethyl)-imidazoline.

Results and Discussion: 11-15 mCi of radiochemically and chemically pure [^{11}C]**1** (7.2–7.5 Ci/μmol) were obtained at 40 min from end of radionuclide production. Only low radioactivity entered monkey brain after *i.v.* administration of [^{11}C]**1** (SUV's for frontal cortex, temporal cortex, area postrema, occipital cortex, pineal gland < 100%). Pre-dosing the monkey with **1** increased radioactivity in all brain regions to 100-200% SUV. No I_2-receptor specific binding was observed. Evaluation of [^{11}C]**1** in rats also showed low brain radioactivity uptake with no evidence of specific binding.

Conclusion: [^{11}C]**1** was prepared in useful radioactivities for evaluation in rodents and monkeys. Low brain uptake, however, does not render this radioligand useful for imaging I_2 receptors. Further experiments will determine whether low brain uptake is due to the effects of efflux pumps. These results will guide our further development of I_2 receptor PET radioligands.

References: [1] Eglen R.M. *Trends Pharmacol Sci* 1998, **19**, 381. [2] Pigini M.L. *et al. Bioor Me Chem Lett* 1998, **6**, 2245. [3] Karimi F. *et al. J Label Compd Radiopharm* 2002, **45,** 423.

Keywords: PET, Imidazoline Type 2, Carbon-11, Radioligand, Imaging

P275 COMPARISON OF 3 RADIOLABELLED PEPTIDES FOR CCK2 RECEPTOR SCINTIGRAPHY IN PATIENTS WITH MEDULLARY THYROID CARCINOMA

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Introduction: Radiolabelled peptides are in development for CCK2 receptor targeting. Aim was to optimise radiolabelling and study metabolism and diagnostic potential of ^{99m}Tc-N_4-Gly-DGlu-$(Glu)_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (Demogastrin 2), ^{111}In-DOTA-DGlu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (DOTA-MG11) and ^{111}In-DOTA-DAsp-Tyr-Nle-Gly-Trp-Asp-Phe-NH_2 (DOTA-CCK) in patients. The 3 analogs showed similar uptake in CCK2 receptor-positive cell cultures. All 3 analogs were studied in 6 patients (5F) with evidence of recurrence or metastases of medullary thyroid cancer (MTC) after thyroidectomy.Calcitonin levels ranged from 0.23 - 131 μg/L (median 34 μg/L, n=6).

Experimental: Radiolabelling DOTA-peptides was optimised for t, T and presence of quenchers, while measuring % incorporation and RCP by HPLC. Oxidation of Met in DOTA-MG11 was provoked and the Met-oxidized form isolated for CCK2 receptor binding in vitro. Patient doses of 740 MBq ^{99m}Tc-Demogastrin 2, 220 MBq ^{111}In-DOTA-MG11 or ^{111}In-DOTA-CCK (5-7 nmol per ligand) and administered during 5 min.

Results and Discussion: Demogastrin 2 labeling could be labelled up to a specific activity of >200 MBq per nmol, with a RCP of >85%. Radiolabelling DOTA-MG11 was completed after 5 min at 80C, in the presence of i.e.ascorbate, with a RCP of 85-90%. Prolonging t results in a drop of RCP, even in the presence of other quenchers. ^{111}In-DOTA-MG11 with oxidized Met has <10% of ^{111}In-DOTA-MG11's in vitro CCK2 receptor binding. Serum samples showed slower breakdown of Demogastrin 2 than the other 2 analogs at 10 min p.i: 64%, 38% and 9% intact (HPLC) radiolabelled Demogastrin 2, DOTA-CCK and DOTA-MG11 resp. The radiometabolites of the DOTA-analogs in blood contained ≥2 amino acids. Radioactivity cleared rapidly from blood ≃ 30% at 20 min and 15% at 1 h p.i. Urinary excretion was high for all, already ≃ 70% at 3 hr p.i. and 90% at 24 h p.i. In urine at 1 hr p.i. of Demogastrin 2 14% was intact, and <2% for the DOTA-analogs. Demogastrin 2 scintigrams showed pathologic retention in all 6 patients, e.g. in neck, brain, liver and bone. In 4 patients the other analogs missed (some of) the lesions.

Conclusion: Radiolabelling the DOTA-MG11 with minimal oxidation of Met is possible in the presence of ascorbate, and completed in 5 min at 80C with a RCP of 85-90%. In vivo stability of ^{99m}Tc-Demogastrin 2 is superior to the ^{111}In-DOTA-analogs. In this small group of patients CCK2 receptor imaging with Demogastrin 2 seems to be a very promising diagnostic tool. Further study has to show its value in patient management.

Keywords: CCK-2 Receptor, Receptor-Targeted Scintigraphy, Medullary Thyroid Carcinoma

P276 SYNTHESIS AND *IN VITRO* EVALUATION OF A NOVEL FLUORINE-18 LABELED GLYBURIDE DERIVATIVE FOR IMAGING PANCREATIC β-CELLS

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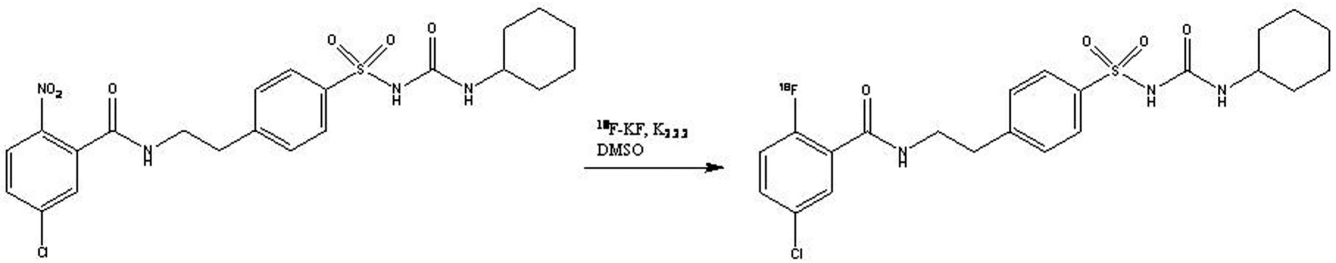
Introduction: Diabetes results from an absolute or relative reduction in pancreatic beta cell mass (BCM) leading to insufficient insulin secretion and hyperglycemia. Thus, noninvasive assessment of β-cell mass would provide an important tool for both therapeutic interventions and better understanding of the etiology of diabetes.

Glyburide, a drug used for the treatment of type 2 diabetes, has already been labeled and evaluated as β-cell imaging agent. However, its high uptake into the liver and the high plasma protein binding led to a relatively low signal-to-noise ratio. Our investigations were prompted by the need to develop new imaging agents that lack these disadvantages. In this study, we report on the synthesis and radiolabeling of a new F-18 labeled glyburide analog.

Experimental: The radiosynthesis of the F-18 labeled glyburide derivative was accomplished by a nucleophilic aromatic substitution on a nitro precursor as depicted in Scheme 1. The appropriate starting material in DMSO was added to dry K_{222}-$K^{18}F$ and heated at 160°C for 10min. The crude mixture was purified by reversed-phase HPLC using a Waters C-18 Bondapak column with a mobile phase consisting of H_2O/MeCN (50/50) with 1% (v/v) H_3PO_4 at a flow of 3ml/min. The pure compound was collected and passed over a C18 SepPak cartridge. The desired labeled tracer was eluted from the cartridge with 2ml of EtOH and formulated using physiological saline. The total synthesis time was 50min from EOB. The labeled compound was tested for its ability to bind *in vitro* to rat pancreatic β-cells (Ins1).



Results and Discussion: The overall radiochemical yield was on average 20% and the specific activity was 50–60GBq/μmol. Using the shake-flask method a logD value of 1.2 was obtained for F-18 labeled glyburide derivative. The binding of the radioligand to rat pancreatic β-cells (Ins1 cells) *in vitro* was clearly inhibited by co-incubation with cold glyburide suggesting that radioligand uptake into Ins1 cells and binding to SUR1 receptor is specific.

Conclusion: These preliminary results suggest that our new F-18 labeled glyburide derivative with its lower lipophilicity and *in vitro* good binding properties may be superior to the already existing β-cell imaging agents. Further *in vitro* and *in vivo* experiments are in progress.

Keywords: 18F-Glyburide, B-Cell Imaging Agents, Diabetes, Lipophilicity

P277 PREPARATION OF THE ADENOSINE A_3 RECEPTOR LIGAND, (^{18}F)FE@SUPPY

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Introduction: Changes of the adenosine A3 receptor subtype (A3AR) expression have been shown in a variety of pathologies, especially (1) neurological and affective disorders, (2) cardiac diseases, (3) oncological diseases and (4) inflammation processes.

Preliminary evaluations of FE@SUPPY (5-(2-fluoroethyl) 2,4-diethyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate) on adenosine receptors showed high affinity (Ki 4.22 nM) and selectivity for the A3AR. Thus, our aim was the preparation of [^{18}F]FE@SUPPY, the first PET ligand for the A3AR.

Experimental: A multistep organic synthesis including a Hantzsch condensation as the central step was performed. There, 3 components were condensed: the β-enaminoester, the β-ketoester and propionaldehyde. Then the resulting 1,4-dihydropyridine derivative was oxidized to give the corresponding pyridine. Finally, the precursor molecule, Tos@SUPPY (5-(2-tosyloxy-ethyl) 2,4-diethyl-3-(ethylsulfanylcarbonyl)-6-

phenylpyridine-5-carboxylate), and the reference standard, FE@SUPPY, were easily obtained.

For the preparation of [^{18}F]FE@SUPPY, cyclotron produced [^{18}F]fluoride was activated with kryptofix 2.2.2. and potassium carbonate and azeotropically dried in the presence of acetonitrile. Then, Tos@SUPPY was added and the vial was sealed. Radiochemical investigations regarding the influence of the amount of precusor, reaction time and temperature were performed. Purification of the crude product was achieved using semi-preparative HPLC and SPE.

Results and Discussion: Precuror and reference standard were synthesized straight forward in satisfactory yields. Purified [^{18}F]FE@SUPPY was prepared within 40 min (EOB to EOS). Influences of the amount of precusor, reaction time and temperature were obeserved. Optimized reaction conditions used 3 mg/mL precursor in acetonitrile at >75°C for 20 min. HPLC purification on a monolithic column was achieved within 5 min and subsequent SPE purification within 8 min. Radiochemical purity was checked with TLC and HPLC and always exceeded 95%. Overall yields were 10-20% (based on [^{18}F]fluoride; not corrected for decay), so far.

Conclusion: We herewith present the successful preparation of Tos@SUPPY, FE@SUPPY and [^{18}F]FE@SUPPY, the first A3AR PET ligand, so far. Hence, preclinical and clinical investigations targeting the A3AR are enabled using state of the art techniques. This work was sponsored by the Austrian Science Fund (FWF), project number P19383-B09.

Keywords: Neuroimaging, PET, Fluorine-18, Adenosine, Receptor

P278 THE NEW NOREPINEPHRINE TRANSPORTER IMAGING AGENT FOR THE ASSESSMENT OF CARDIAC SYMPATHETIC NERVOUS FUNCTION

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Introduction: The norepinephrine transporter (NET) is located presynaptically on sympathetic nerve terminals and plays a critical role in the regulation of the synaptic norepinephrine (NE) concentration via the reuptake of NE. Changes of NET have been recently reported in several cardiac failures. Therefore, a NET-specific radioligand is useful for in vivo assessment of changes in sympathetic nervous function in various cardiac disorders. Recently, we developed radioiodinated reboxetine analogue, (2S,αS)-2-(α-(2-iodophenoxy)benzyl)morpholine ((S,S)-IPBM) for NET imaging. Therefore, we assessed the applicability of radioiodinated (S,S)-IPBM to cardiac sympathetic nervous function imaging.

Experimental: (S,S)-IPBM was synthesized via a 10-step synthetic procedure. The synthesis of no-carrier-added (S,S)-[^{125}I]IPBM was carried out by bromine-radioiodine exchange reaction. The NET affinity and selectivity were measured from the ability to displace specific [^{3}H]nisoxetine and (S,S)-[^{125}I]IPBM binding to rat myocardial membrane respectively. To evaluate the distribution of (S,S)-[^{125}I]IPBM in vivo, biodistribution experiment was performed in rats.

Results and Discussion: The radiochemical yield of (S,S)-[^{125}I]IPBM was about 65% and the radiochemical purity was greater than 98%. In vitro binding assays showed that the affinity of (S,S)-IPBM (IC_{50}=10.8 nM) to NET was similar to those of the well-known NET inhibitors, nisoxetine and desipramine (IC_{50}=9.0 and 6.0 nM). Furthermore, (S,S)-[^{125}I]IPBM binding was inhibited by NET inhibitors, nisoxetine and desipramine (IC_{50}=2.3 and 6.9 nM), but not by dopamine or serotonin transporter inhibitors (both $IC_{50} > 1000$ nM). These data indicated that (S,S)-IPBM had high affinity and selectivity for NET in vitro. Biodistribution studies in rats showed rapid and high uptake of (S,S)-[^{125}I]IPBM by the myocardium and rapid clearance from the blood. The myocardium-to-blood ratio was 28.9 at 180 min after the injection. The level of radioactivity in the thyroid was low, which indicated high stability of (S,S)-[^{125}I]IPBM to in vivo deiodination.

Conclusion: Radioiodinated (S,S)-IPBM is a potential radioligand for the assessment of cardiac sympathetic nervous function.

Keywords: Norepinephrine Transporter, Radioiodination, Cardiac Sympathetic Nervous Function

P279 SYNTHESIS AND PRELIMINARY IN VIVO EVALUATION OF (^{123}I)-(1-(4-FLUOROPHENETHYL)PIPERIDIN-4-YL)(4-IODOPHENYL)METHANONE AS A POSSIBLE SPECT TRACER FOR THE 5-HT_{2A} RECEPTOR

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Introduction: The serotonin 5-HT_{2A} receptor is an important target in psychiatry and nuclear medicine. Changes in central 5-HT_{2A} receptors have been reported in several psychiatric conditions. In vivo quantification of these receptors with SPECT provides valuable, objective information of brain receptor status. (1-(4-fluorophenethyl)piperidin-4-yl)(4-iodophenyl)methanone is an analogue of ketanserin with nanomolar affinity for the 5-HT_{2A} receptor and good selectivity over other receptor types. We present here the synthesis, radiosynthesis and preliminary in vivo evaluation of [^{123}I]-(1-(4-fluorophenethyl)piperidin-4-yl)(4-iodophenyl)methanone as a possible new 5-HT_{2A} receptor tracer.

Experimental:: [^{123}I]-(1-(4-fluorophenethyl)piperidin-4-yl)(4-iodophenyl)methanone was synthesized from the corresponding bromo precursor. The bromo precursor was synthesized in 3 steps from 4-fluorophenylacetic acid. Radiolabelling was performed using a Cu^{+}-assisted nucleophilic halogen exchange reaction in aqueous medium. Radiosynthesis proceeded at 140° C during 1 h, in the presence of appropriate amounts of precursor, citric acid, gentisinic acid, cuprous sulphate and stannous sulphate. The tracer was purified by reversed-phase HPLC and formulated in sterile saline containing 10% ethanol. A biodistribution study on male NMRI mice was performed. Approximately 500 kBq of the tracer was injected in the tail vein of NMRI mice. At various time points post injection, the mice were sacrificed by decapitation and blood was collected. The organs were rapidly dissected, weighed and counted for radioactivity. Results were expressed as a percentage of the injected dose per gram of tissue (% ID/g). A metabolite analysis was also performed with HPLC.

Results and Discussion: The bromoprecursor was synthesized in 40% yield. Radiochemical yield was 60% after purification, and radiochemical purity was >95%. Biodistribution studies in male NMRI mice showed good penetration of the tracer in the brain: 5% ID/g in brain and 1% ID/g in blood at 60 min post injection. No lipophilic radiolabelled metabolites could be detected in blood or brain of NMRI mice.

Conclusion: [^{123}I]-(1-(4-fluorophenethyl)piperidin-4-yl)(4-iodophenyl)methanone was synthesized with a radiochemical yield of 60%. Radiochemical purity was always >95%. The tracer showed good uptake in mouse brain. No radiolabelled metabolites could be detected in blood or brain. The potential of [^{123}I]-(1-(4-fluorophenethyl)piperidin-4-yl)(4-iodophenyl)methanone as a 5-HT_{2A} tracer will be evaluated further by regional biodistribution and displacement studies with specific 5-HT_{2A} antagonists (for example ketanserin) in larger animals such as rats or rabbits.

Keywords: 5-HT2A, SPECT, Tracer, Psychiatry, Radiosynthesis

P280 MULTIMODAL IMAGING OF CXCR4 CHEMOKINE RECEPTORS

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Introduction: A key role in metastasis and organ specific homing of tumor cells is attributed to the chemokine receptor CXCR4 and its endogenous ligand SDF-1α. The aim of this study was a) to evaluate newly developed small radiolabeled cyclic peptides; b) to establish animal models suitable to evaluate the in vivo imaging properties of these peptides; c) to compare CXCR4 expression as detected by PET with optical imaging and d) to investigate animal models for the investigation of functional metastasis.

Experimental: On the basis of a newly developed cyclic peptide I-125-CPCR4 that binds with low nM affinity to CXCR4 receptors on Jurkat cells, the receptor affinity of a set of >100 newly synthesized peptide analogues were tested. A new tumor model for co-expression of CXCR4 and luciferase (Luc) as reporter gene or only for eGFP as control was established by stable transduction of CMS5 cells. The cells were characterized in vitro by FACS analyses and in radioligand binding assays. For multimodal tumor imaging studies, CXCR4+ cells and controls were injected s.c. into nude mice. CXCR4 expression was analysed by IHC in human small cell lung cancer cells grown in vitro and in vivo in pfp/rag2 mice.

Results and Discussion: CXCR4+ CMS5 sarcoma cells showed high expression of CXCR4 and high affinity for I-124-CPCR4. Compared to SDF-1α, unspecific uptake was significantly lower. Functional expression of Luc was ascertained in bioluminescence cell assays. FACS analyses revealed similar growth rates for different mixtures of CXCR4+ and CXCR4- cells in vitro. I-124-CPCR4 and μ-PET allowed a clear in vivo delineation of CXCR4+ tumours in animals bearing CXCR4+/- CMS5 tumours. PET Imaging was validated by optical imaging and ex vivo studies. CXCR4 expression was confirmed on different human SCLC cell lines grown in vitro. However, the CXCR4 expression in the xenograft tumours varied. The battery of newly synthesized peptides included further promising candidates with low nM affinity suitable for labelling with PET radioisotopes.

Conclusion: This approach allows for the first time multimodal imaging of CXCR4 expression. We hypothesize that this new class of tracers will be very promising probes for early imaging of the metastatic processes.

Keywords: Peptide Receptor, Peptide, Chemokine, Metastasis, Molecular Imaging

P281 INFLUENCE OF POLAR SPACERS ON THE BIODISTRIBUTION OF RADIOLABELED BOMBESIN ANALOGUES

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Introduction: The over expression of gastrin-releasing peptide (GRP) receptors in several human cancers, e.g. breast and prostate, makes them interesting targets for *in vivo* imaging and therapy with radiolabeled bombesin (BBS) analogues. The introduction of Tc- and Re-tricarbonyl complexes provides a valuable tool for the labeling of biomolecules, but in combination with bombesin a rather lipophilic compound with high liver and only moderate tumour uptake was obtained. Since introduction of hydrophilic spacers into the molecule showed an improvement in biodistribution, we investigated the influence of different polar spacers on the biodistribution of new stabilized BBS analogues labeled with ^{99m}Tc and ^{188}Re.

Experimental: Different groups with increasing polarity, namely βhomoSer, βLys, βhomoGlu and glucose or shicimic acid linked to the ε-aminogroup of Lys, were inserted between the stabilized binding sequence BBS(7-14) and an (N^{α}His)Ac-chelating system. The new analogues were labeled with the [^{99m}Tc(CO)$_3$]-core and tested *in vitro* with GRP-receptor expressing human tumor cell lines PC-3 (prostate) and T47D (breast) for binding affinity, internalization, externalization, as well as plasma stability. *In vivo* biodistribution studies were carried out in mice bearing tumor xenografts. SPECT/CT images were acquired with an X-SPECT™ system.

Results and Discussion: The labeled and unlabeled analogues showed a specific binding to the GRP receptors with a high affinity and K_d and IC_{50} values in nanomolar range. However, in biodistribution studies the positively charged βLys led to high accumulation in both the kidneys and the liver. The hydroxyl groups brought about a clear decrease in liver accumulation, but negative or no effects on tumor to kidney ratios. The negatively charged βhomoGlu spacer significantly enhanced the tumor to background ratios due to a combination of both, low kidney uptake and increased accumulation in the receptor expressing pancreas, colon and tumor xenografts. All peptides showed a fast clearance from the blood. In the SPECT/CT images a specific uptake into the tumor xenografts could be clearly visualized 1.5 h after i.v. injection.

Conclusion: While the *in vitro* characteristics of the new analogues were not significantly affected, the polar spacers show a crucial influence on the biodistribution. The negatively charged spacer enhances the already high potential of stabilized BBS analogues as radiopharmaceuticals for targeting GRP receptor expressing tumors. Ongoing studies will show whether increasing the number of charges leads to further improvement.

Keywords: Bombesin, Prostate Tumor, Biodistribution, 99m-Tc, Spacer

P282 SYNTHESIS AND RADIOFLUORINATION OF SELECTIVE LIGANDS FOR THE DOPAMINE D3 RECEPTOR SUBTYPE

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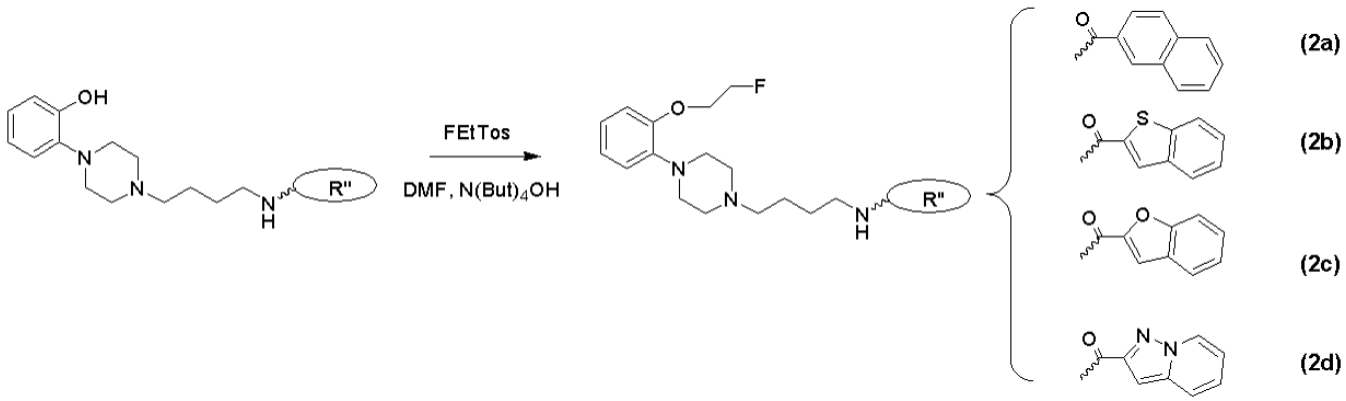
Introduction: The dopamine D3 receptor is supposed to be implicated in the pathogenesis of several diseases of the brain such as schizophrenia, Parkinson's disease or addiction. The aim of this study was to synthesize new fluorine-18-labeled D3 subtype selective ligands for in vivo investigation of this receptor with PET. The leading structures FAUC 346 and BP 897 were modified as part of our investigation.

Experimental: The reference compounds and the precursors were prepared by condensing 2-methoxyphenylpiperazinyl-substituted butylamine (1) with naphthyl-(a), benzothiophenyl- (b), benzofuranyl- (c) and pyrazolo[1,5-a]pyridinyl (d) -2-carboxylic acid chlorids to give the benzamides (1a-d) in 54-86% yield, respectively. Demethylation of the methoxygroup and subsequent fluoroalkylation of the received hydroxygroup with fluoroethyltosylate led to, 4-[4-(2-fluoroethylphenyl)-piperazine-1-yl]-butyl)-naphthyl- (2a), benzothiophene- (2b), benzofuran- (2c), and pyrazolo[1,5-a]pyridinyl (2d)-2-carboxamids as derivatives of FAUC 346 and BP 897. The radiofluorinated compounds [F-18]2a-d were prepared in DMF at 100°C using 1.4 eq. $N(But)_4OH$ and 2-[^{18}F]fluoroethyltosylate as labelling agent. Binding data were generated by measuring their ability to compete with [^{3}H]spiperone for the cloned human dopamine receptor subtypes D2long, D2short, D3 and D4.4 stably expressed in Chinese hamster ovary cells (CHO).

Results and Discussion: All new radiotracers could be isolated in high radiochemical purity and in good radiochemical yields (RCY). The RCY's of compounds 2a-d ranged between 24-65%. The D3 receptor affinities of all radioligands were below 1nM (see Table 1).

Binding Affinities (Ki [nM]) and RCY of [F-18]2a-d

Compd.	$D2_l$	$D2_s$	D3	D4.4	RCY [%]
2a	18	14	0.16	33	56
2b	16	14	0.12	34	65
2c	28	24	0.35	64	53
2d	32	33	0.68	150	24



Conclusion: All compounds under investigation showed high affinities and a good subtype selectivity. These radiotracers displayed promising binding data and stimulate further studies to determine their biodistrubution and metabolic stability in vivo.

Keywords: PET, Receptor Ligand, D3

P283 SYNTHESIS AND RADIOLABELLING OF N^5-(^{18}F)FLUOROETHYL-PIRENZEPINE AND ITS METABOLITE N^5-(^{18}F)FLUOROETHYL-LS 75

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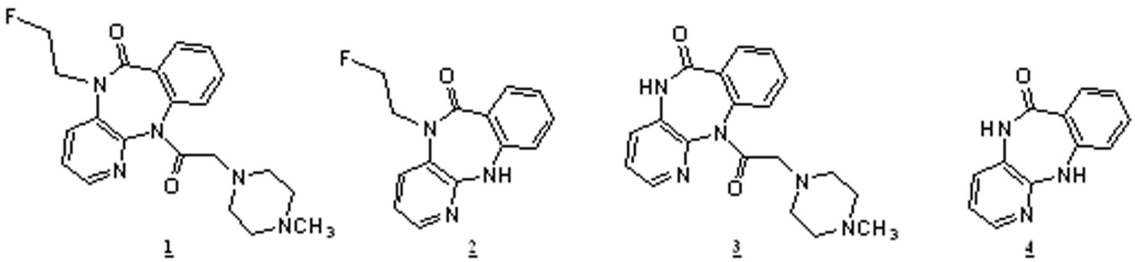
Introduction: Pirenzepine **3**, namely 11-[2-(4-methyl-piperazin-1-yl)-acetyl]-5,11-dihydro-benzo[e]pyrido[3,2-b][1,4]diazepin-6-one has originally been developed as M_1 selective muscarinergic antagonist. In vivo, **3** is metabolised to LS-75 **4** 5,11-dihydro-benzo[e]pyrido[3,2-b][1,4]diazepin-6-one. The latter compound was found to be a moderate inhibitor of PARP, an enzyme directly related to e.g. neuronal signal transduction and in particular to the regulation of key events in apoptotic cascades. Moreover we were interested to investigate this second Pirenzepine-related mode of action on a physiological level[2]. Our aim was to synthetise appropriate ^{18}F-fluorinated analogues of **3** and **4** in order to provide the tools for an in vivo PET-study in healthy Sprague-Dawley rats of these potentially beneficial side effects of **3**, which are beyond pure M_1 antagonism.

Experimental: **3** and **4** were prepared via a modified procedure published elsewhere[1]. Alkylation with 2-fluoroethyl bromide afforded reference compounds **1** and **2**. **3** and **4** were labeled with 2-[^{18}F]fluoroethyl tosylate. [^{18}F]-**1** and [^{18}F]-**2** were isolated, purified by HPLC and formulated in PBS prior to application. For autoradiography, brain sections from adult, male Sprague-Dawley rats were used. Sections were preincubated in assay buffer (50 mM Tris/HCl buffer, pH 7.5, containing 120 mM NaCl). Nonspecific binding was determined using 100 μM of pirenzepine.

Results and Discussion: **3** and **4** were synthetised and labeled with 2-[^{18}F]fluoroethyltosylate in radiochemical yields of 30 ± 5% after 20 min. Autoradiographic studies on rat brain sections showed high unspecific binding. Assay conditions therefore need further refinement. Nonetheless specific uptake in the thalamus could be detected. Displacement of [^{18}F]-**1** by 100 μM pirenzepine hints on no significant deviation in binding by fluoroalkyl-derivatives compared to the parent compound.



Conclusion: After autoradiographic evaluation, [^{18}F]-**1** and [^{18}F]-**2** can now be utilised in small animal PET studies using Sprague-Dawley rats.

Acknowledgement: The authors wish to thank Dr. Kurt Hamacher for his advice regarding separation of [^{18}F]-**1** and [^{18}F]-**2** from DMSO on cation exchange resins.

References: [1] Holzgrabe U. et al. Tetrahedron 59 (2003) 781 ff. [2] Schrattenholz A. et al. Current Topics in Medicinal Chemistry 6 (2006) 663 ff.

Keywords: Pirenzepine, PARP, LS-75, M1 Antagonists, PET

P284 RADIOSYNTHESIS AND IN VIVO AND VITRO EVALUATION OF AN ^{18}F-LABELED RADIOTRACER FOR IMAGING DOPAMINE D_3 RECEPTORS IN THE CNS

Z. TU [1], J. XU [1], S. LI [1], L.A. JONES [1], D. CHEN [1], R.R. LUEDTKE [2], M.A. MINTUN [1], J.S. PERLMUTTER [1] and R.H. MACH [1]

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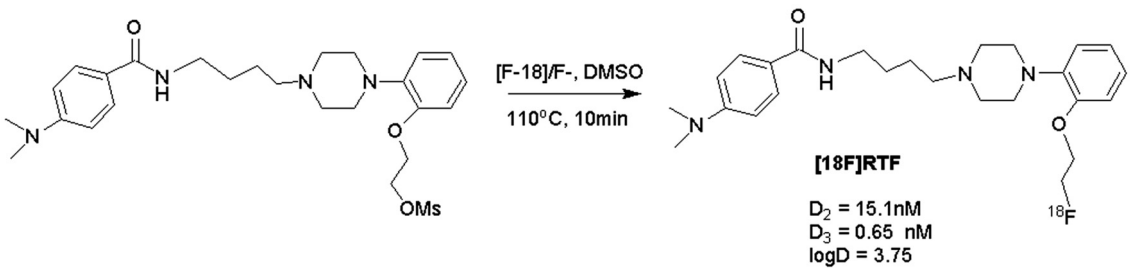
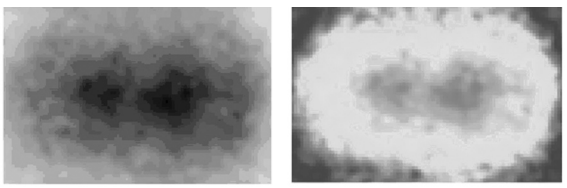
Introduction: We reported the in vitro and vivo evaluation of [^{11}C]**WC-10** as a potential PET radiotracer for imaging D_3 receptors.[1,2] The initial evaluation of [^{11}C]**WC-10** showed that it is possible to image D_3 receptors in the CNS of nonhuman primate brain.[2] In order to take advantage of ideal properties of fluorine-18 ($t_{1/2}$ = 110 min) and its widespread commercial availability, we synthesized analogues of **WC-10** that have the potential to be labeled with F-18.4-Dimethylamino-N-{4-[4-(2-fluoroethoxyphenyl)piperazin-1-*yl*]butyl}benzamide (**RTF**) is one of them with structurally similar to [^{11}C]**WC-10**, and can be labeled with F-18. **RTF** has a high affinity of D_3 receptors (Ki = 0.65 ± 0.1 nM) reduced affinity for D_2 receptors (Ki = 15.1 ± 4.7 nM) and is moderately selectivity for D_3 vs. D_2 receptors (23-fold selective). Functional assays indicate that it is an antagonist or a weak partial agonist of D_3 receptors.

Experimental: The radiosynthesis of [^{18}F]**RTF** was readily achieved via nucleophilic replacement of the mesylate precursor with [^{18}F]fluoride in DMSO by microwave irradiation (Scheme 1). The labeling yield was ~40% and [^{18}F]**RTF** was obtained in a specific activity of >2,000 mCi/μmol (EOS). The biodistribution studies was conducted on sprague dawley rats sprague dawley(S.D.) rats (250 ~ 350 mg). The aurodiography was performed on S.D. rat brain and Rhesus monkey brain slices (20 μM).

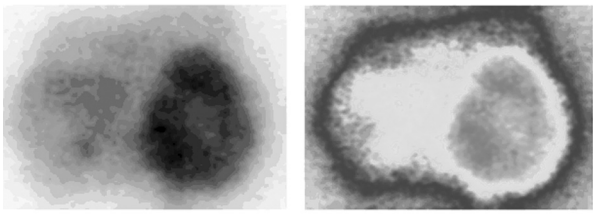


Scheme 1

Results and Discussion: In vitro autoradiography studies in rat and rhesus monkey brain slices revealed that [^{18}F]**RTF** has a high uptake in the striatum of rat brain and caudate and putamen of rhesus monkey brain. MicroPET imaging studies are currently under investigation.



Rat striatum



Monkey Striatum

Conclusion: [^{18}F]**RTF** has the potential to be a useful PET tracer for imaging dopamine D_3 receptors in vivo with PET. Further evaluation of [^{18}F]**RTF** is currently under investigation.

Acknowledgement: This research was supported by NIH grants DA16181 and NS04056.

References: [1] *Bioorg. Med. chem.*13: 77-87, 2005. [2] *J. Nucl. Med.* 2006; 47: 27P.

Keywords: PET, F-18 Labeling, Dopamine D3 Receptors

P285 LABELING OF PipISB WITH ^{11}C OR ^{18}F FOR EVALUATION AS PROSPECTIVE PET RADIOLIGANDS FOR CB_1 RECEPTORS

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Introduction: PipISB (**1,** Fig. 1) is a structurally unique CB_1 receptor antagonist/inverse agonist. Certain properties of **1** (CB_1 K_B = 0.99 nM; CB_2 K_B > 7,000 nM; *cLog P* = 5.14, Pallas 3.0) make it appealing for development as a PET radioligand. Here we report the labeling of **1** with ^{11}C ($t_{1/2}$ = 20.4 min) or ^{18}F ($t_{1/2}$ = 109.7 min) for initial evaluation as PET radioligands.

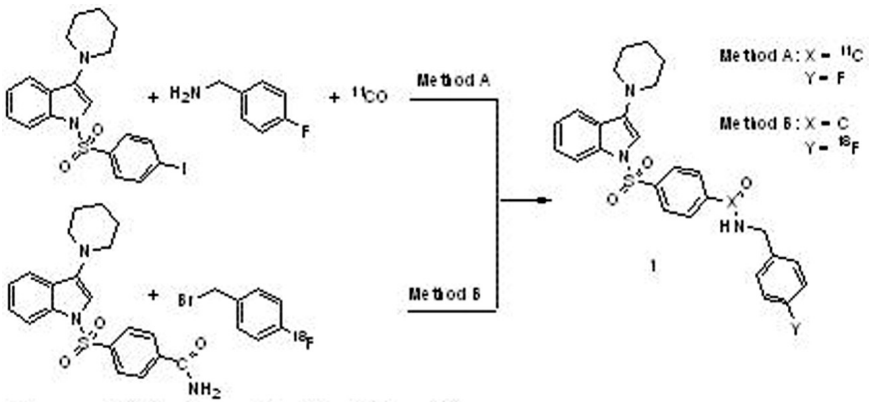

Fig. 1. Labeling of **1** with ^{11}C or ^{18}F.

Experimental: [^{11}C]**1** was prepared in one step from [^{11}C]carbon monoxide and [^{18}F]**1** in four steps with [^{18}F]4-fluorobenzyl bromide as labeling agent (Fig. 1). [^{11}C]**1** or [^{18}F]**1** was injected into monkey under baseline or pretreatment conditions (**1**; 1 mg/kg, i.v.) and evaluated with PET. Regions of interest were drawn on striatum, cerebellum, neocortex, limbic region and pons. Metabolism was investigated with HPLC on plasma samples.

Results and Discussion: [^{11}C]**1** was obtained after 44 min in 3 to 12% decay-corrected radiochemical yields (RCYs) with specific activities (SAs) from 21 to 67 GBq/μmol. [^{18}F]**1** was obtained after 115 min in 1.5 to 5.6% RCY with SAs from 200 to 348 GBq/μmol. Following injection of [^{11}C]**1** or [^{18}F]**1** into monkey, maximal radioactivity uptakes were in striatum and cerebellum (160% SUV at 120 min or 190% SUV at 180 min respectively; Fig. 2). Brain radioactivity distributed according to CB_1 receptor density. No reference region was identified. Under the pretreatment condition, all regional brain radioactivity diminished together, either to 60% SUV at 120 min ([^{11}C]**1**) or 20% SUV at 180 min ([^{18}F]**1**) (Fig. 2). Defluorination of [^{18}F]**1** was low.

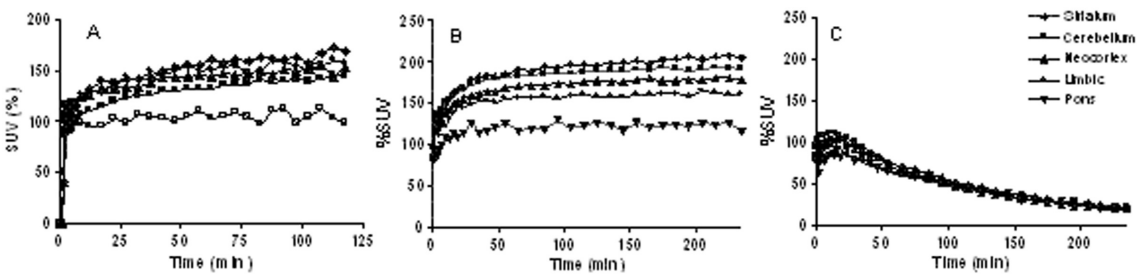

Fig. 2. PET imaging of [^{11}C]**1** under baseline (A) conditions and [^{18}F]**1** under baseline (B) and pretreatment (C) conditions

Conclusion: [^{11}C]**1** and [^{18}F]**1** behaved as CB_1 receptor-selective radioligands *in vivo*, with a high ratio of specific to non-specific binding; they merit further investigation in humans.

Acknowledgement: We are grateful to Ell Lilly & Co. for provision of materials under a CRADA agreement. This research was supported in part by the Intramural Program of the NIH.

Keywords: CB1 Receptors, PET, Arylsulfonyl-Substituted Indoles

P286 NOVEL SYNTHESIS OF (^{18}F)FE1-MDL 100907, A POTENTIAL 5-HT$_{2A}$ ANTAGONIST

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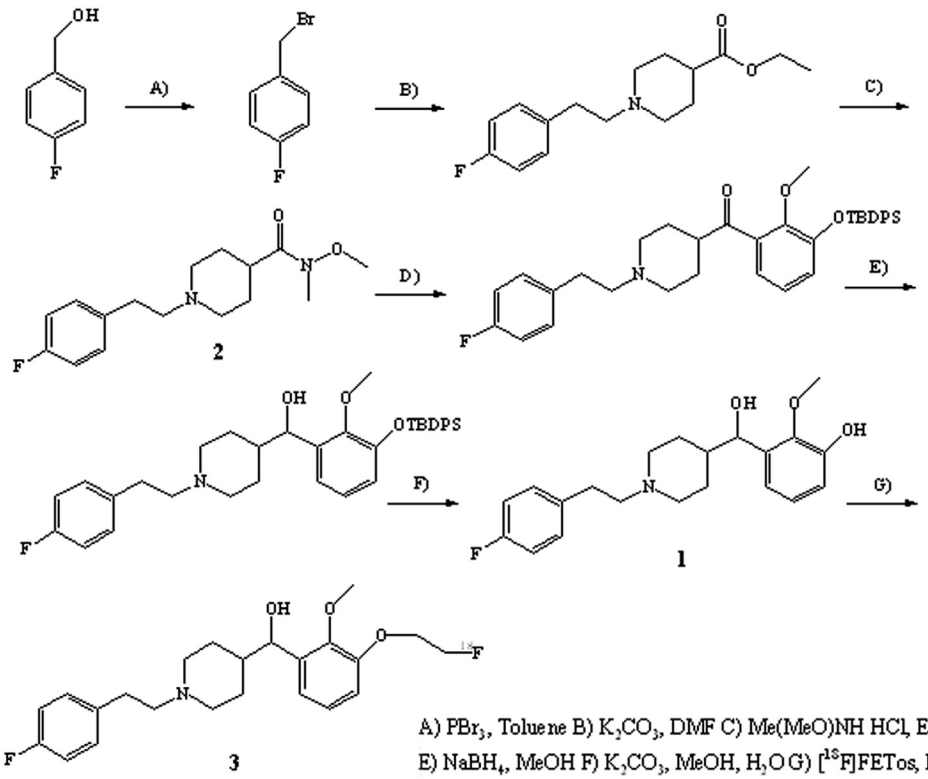
Introduction: Serotonergic 5-HT$_{2A}$ receptors are of central interest in the pathophysiology of schizophrenia and other diseases. The serotonergic receptor antagonist [^{11}C]MDL 100907 shows high affinity and selectivity for 5-HT$_{2A}$ receptors *in vitro* and *in vivo*. The K_i's are more than 100 fold higher for other receptors such as 5-HT$_{2C}$, α_1, D_1, D_2. It has also been proposed that the selectivity of [^{11}C]MDL 100907 is better than the selectivity of [^{18}F]altanserin. Nevertheless, up to now any ^{18}F-derivatives of MDL 100907 exist. The aim of this study was to develop ^{18}F-analogs of MDL 100907.

Experimental: The synthetic route for the ^{18}F-labelling precursor MDL 105725 (**1**) via a key transformation of an ester to a keton via a Weinreb-amide intermediate (**2**) was carried out as published by Huang et al. (1999) with some modifications. The fluoroalkylation of the precursor was conducted in dry DMF by addition of sodium hydride and 1-bromo-2-fluoroethane (Fig. 1).

The [^{18}F]fluoroalkylation of MDL 105725 was carried out with [^{18}F]FETos, produced in a self-made module, in try DMF at 100°C in less than 15 minutes. The final compound (**3**) [^{18}F]FE1-MDL 100907 ([3-(2-[^{18}F]fluoro-ethoxy)-2-methoxy-phenyl]-{1-[2-(4-fluoro-phenyl)-ethyl]-piperidin-4-yl}-methanol) was purified by HPLC (ET 250/8/4 Nucleosil ® 5 $C_{18;}$ MeCN/H_2O 40:60, R_f = 8.68 min).

Results and Discussion: Synthesis of the precursor MDL 105725 was carried out in yields of > 15%, whereas the final product FE1-MDL 100907 was obtained in 40% yield. The labelled compound [^{18}F]FE1-MDL 100907 (**3**) could be obtained in > 80% yield. Separation of the compounds, i.e. MDL 105725 (R_f = 4.6 min), [^{18}F]FETos (R_f = 19.2 min) and [^{18}F]FE1-MDL100907 (R_f = 8.7 min) via HPLC was very efficient.



A) PBr_3, Toluene B) K_2CO_3, DMF C) Me(MeO)NH HCl, EtMgBr, THF D) n-BuLi, THF, TBDPS-guajacol
E) $NaBH_4$, MeOH F) K_2CO_3, MeOH, H_2O G) [^{18}F]FETos, DMF, 100°C

Conclusion: The new labelled compound [^{18}F]FE1-MDL 100907 was synthesized in high radiochemical yields of about 80% and high purity. In addition, an enantioselective synthesis is underway as in the case of MDL 100907 the R-enantiomer shows a 100-fold higher affinity to the 5-HT$_{2A}$ receptor subtype. Autoradiograhic experiments and evaluation of FE1-MDL 100907 and [^{18}F]FE1-MDL 100907 are planed.

Keywords: [^{18}F]FE1-MDL 100907, 5-HT2A Antagonist, Radiosynthesis

P287 COMPARISON OF ([18]F)β-CFT AND ([18]F)β-CFT-FP FOR IMAGING DOPAMINE TRANSPORTERS WITH POSITRON EMISSION TOMOGRAPHY (PET) IN RAT

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Introduction: Structural modifications change the biological properties of cocaine-like compounds such as phenyltropanes, and their interactions with the dopamine transporter (DAT), in particular, different substituents attached at the 2β, 3β or nitrogen (N) positions. This work compares the DAT-labelling properties of [^{18}F]β-CFT [2β-carbomethoxy-3β-(4-[^{18}F]fluorophenyl)tropane] and its fluoropropyl analogue, [^{18}F]β-CFT-FP [N-(3-[^{18}F]fluoropropyl)-2β-carbomethoxy-3β-(4-fluorophenyl)nortropane] in rat.

Experimental: [^{18}F]β-CFT was synthesized according to [1] and [^{18}F]β-CFT-FP according to [2]. Rats were injected intravenously with [^{18}F]β-CFT or [^{18}F]β-CFT-FP and sacrificed at various time points from 5 to 120 min postinjection (pi). Brains were cut into sections for autoradiography and tissue samples measured for ^{18}F-radioactivity. The DAT selectivity of the tracers was assessed by pre-treatment of rats with a selective DAT inhibitor GBR12909. The *in vivo* biodistribution was studied by dynamic imaging with an HRRT PET scanner.

Results and Discussion: [^{18}F]β-CFT accumulated with a maximum striatum-to-cerebellum uptake ratio of 9.6±0.7 at 2 h pi. [1]. The labelling kinetics of [^{18}F]β-CFT-FP was faster; the striatum-to-cerebellum uptake ratio reaching a maximum of 3.1±0.6 within 5 minutes. Preliminary PET data also confirmed this difference. GBR12909 significantly decreased the uptake of both tracers, suggesting that the labelling was DAT-specific. No uptake of [^{18}F]β-CFT-FP was seen in brain regions rich in serotonin (SERT) or noradrenalin (NET) transporters, whereas [^{18}F]β-CFT accumulated in locus ceruleus, a brain region with high densities of NET. Thus, [^{18}F]β-CFT-FP has lower affinity for SERT and NET and higher selectivity for DAT. In all soft tissues, the highest uptake values for [^{18}F]β-CFT-FP were recorded at 5 min. For [^{18}F]β-CFT, the highest uptake in several organs was reached at 20 min pi. The uptake of [^{18}F]β-CFT radioactivity in bone at 2 h was 0.2±0.2%ID/g, and the corresponding value for [^{18}F]β-CFT-FP was 1.4±0.5%ID/g, reflecting moderate defluorination.

Conclusion: [^{18}F]β-CFT has high specific uptake but slow kinetics. [^{18}F]β-CFT-FP has lower binding potential but fast kinetics.

References: [1] Haaparanta M, Bergman J, Laakso A, Hietala J, Solin O. Synapse 1996:23:321-7. [2] Koivula T, Perhola O, Kämäräinen E-L, Lipponen T, Vepsäläinen J, Solin O. J Label Compd Radiopharm 2005:48:463-71.

Keywords: [^{18}F]Beta-CFT, [^{18}F]Beta-CFT-FP, DAT, PET

P288 FUNCTIONALIZATION AND MODIFICATION OF BOMBESIN ANALOGUES USING 'CLICK'-CHEMISTRY

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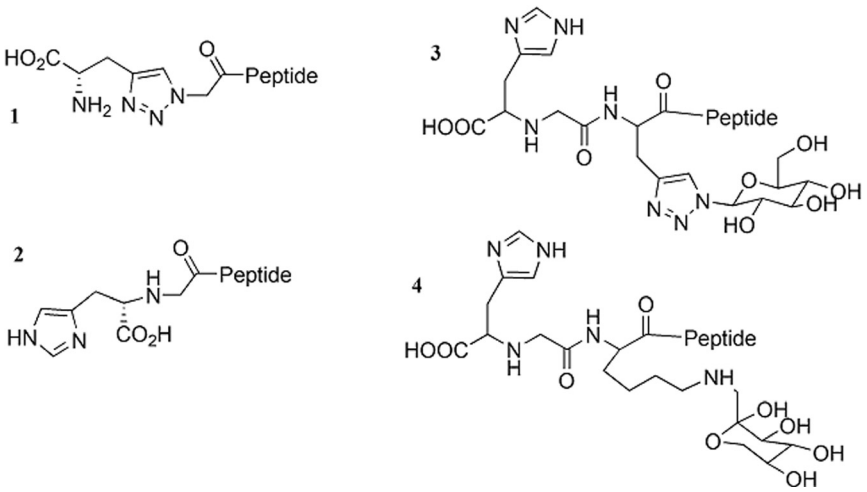
Introduction: The Cu(I)-catalyzed [3+2] cycloaddition of alkynes and azides ('Click'-chemistry) has found a wide variety of applications for the coupling of chemical entities via formation of a stable triazole linkage.In this study we compared the properties of a novel triazole-chelating system (**1**) and the established (N^{α}His)Ac-chelator (**2**) for ^{99m}Tc-labeling of bombesin (BBS) analogues, known to be promising peptides for targeting of GRP-receptor expressing tumors. Furthermore click chemistry was employed to facilitate the introduction of a hydrophilic group (glucose) into the rather lipophilic BBS derivatives to potentially improve their biodistribution profile.

Experimental: New BBS analogues were synthesized manually on a Rink amide resin by solid phase peptide synthesis using Fmoc-strategy. The click reaction for **1** was carried out on the resin at RT using 0.2 eq CuBr, 2 eq DIPEA and 2 eq *N*-Fmoc protected propargyl glycine. A BBS with (N^{α}His)Ac-chelator was glycated via click chemistry (**3**) and compared to an analogue glycated at the ε-aminogroup of an inserted Lys (**4**). All peptides were labeled with the [^{99m}Tc(CO)$_3$]-core and tested *in vitro* with GRP-receptor expressing human tumor cell lines PC-3 (prostate). *In vivo* biodistribution studies were carried out in nude mice bearing PC-3 tumor xenografts including the performance of SPECT/CT experiments.



Results and Discussion: Labeling of **1** with ^{99m}Tc resulted with a high yield in a stable tricarbonyl complex similar to **2**. Both peptides showed comparable *in vitro* and *in vivo* properties and the binding proofed to be specific and with high affinity (K_d = 0.2 ± 0.06). Biodistribution studies showed similar tumor uptake for compounds **1** (1.2 ± 0.8%ID/g) and **2** (0.8 ± 0.4%ID/g) in combination with a clearly decreased accumulation in the liver and similar uptake in the kidneys. The ^{99m}Tc-labeled compounds **3** and **4** showed comparable high affinity for the BBS receptor with K_d values in subnanomolar range.

Conclusion: Click chemistry provides the opportunity to establish a new stable chelating system for BBS with improved tumor to liver ratio. Additionally the triazole linkage offers the possibility for single step coupling of polar groups to bombesin without negative influence on the binding affinity.

Keywords: Bombesin, Click Chemistry, 99m-Tc, Triazole, Chelator

P289 MODELING OF PROPERTIES RELATED TO SPECIFIC VERSUS UNSPECIFIC UPTAKE RATIO FOR SERT RADIOLIGANDS

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Introduction: We use the tools of molecular modeling to investigate one of the issues that often disqualify radiopharmaceutical leads – the specific versus unspecific uptake. At present, we relate the issue of unspecific uptake primarily with LogP (calculated or measured), which is not particularly good and clearly not sufficient. The ultimate aim of the modeling is to obtain a better way to pick out compounds with good potential for high ratio of specific vs. unspecific uptake.

Results and Discussion: We have surveyed the literature concerning serotonin reuptake transporter (SERT) radioligands and established a small learning set for molecular modeling of the properties of good radioligands. Some potential issues regarding the available data are discussed, such as absent affinity data or that the available affinity data are not truly comparable, which may mean that we are de facto modeling properties more related to affinity rather than the desired specific vs. unspecific uptake. This issue arises because we cannot be sure that the compounds that do not work actually have sufficient affinity. Several computed properties are evaluated for correlation with the ability to discriminate between a group of good radioligands and a group of poor radioligands.

Acknowledgement: Pfizer Inc. is acknowledged for financial support.

Keywords: Molecular Modeling, Serotonin Reuptake Transporter Ligands, Specific Versus Unspecific Uptake

P290 SYNTHESIS AND EVALUATION OF (^{18}F) LABELLED IMIDAZOPYRIDINES, FOR THE STUDY OF PERIPHERAL BENZODIAZEPINE BINDING SITES USING PET

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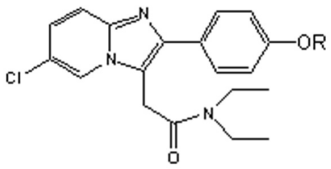
Introduction: Peripheral benzodiazepine binding sites (PBBS) are promising targets for development as imaging agents in the study of neurodegeneration, inflammation and cancer. Consequently, we have prepared a number of iodinated and fluorinated imidazopyridines, imidazopyridazines, pyrazolopyrimidines and indolglyoxylamides as potential tracers for PET and SPECT imaging. The aim of this study was to prepare and evaluate two F-18 imidazopyridines **1** and **2** as tracers for the study of PBBS using PET.

Experimental: Tracers **1** and **2** were prepared by nucleophilic substitution of the corresponding ethyl and propyl tosylates with ^{18}F-fluoride in the presence of K_{222}, K_2CO_3 in ACN at 100°C for 5 mins.

The biodistribution of **1** and **2** were performed in SD rats and brain and peripheral tissues analysed at 15, 30 min, 1, and 4 h p.i. The specificity and selectivity of the tracers were assessed by pre-treatment with the PBBS ligands PK11195 and Ro 5-4864 and with Flumazenil for CBR at 1 mg/kg 5 min prior to injection of the tracers.



1 R = $CH_2CH_2{}^{18}F$

2 R = $CH_2CH_2CH_2{}^{18}F$

Results and Discussion: The IC_{50} of **1** and **2** are 13.2 and 7.5 for the PBBS and >1500 nM for the CBR. **1** and **2** were synthesised in 40-55% radiochemical yield uncorrected and with > 95% radiochemical purity. The biodistribution of **1** and **2** showed uptake in tissue rich in PBBS. The uptake of **1** in the olfactory bulbs ranged from 0.56% at 15 min to 0.40% ID/g at 4 h p.i compared to 0.64% to 0.41% at 15 min and 4 h for **2**. Blood activity levels for **1** remained constant (0.20%) over the 4 h period whilst **2** showed a decrease from 0.4% at 15 min to 0.1% at 4 h. In adrenals **1** showed high uptake, increasing from 9% at 30 min p.i to 11% at 4 h whereas, **2** increased from 6.5 at 30 min to 16% at 4 h. In kidneys, the activity peaked at 15 min for both tracers, 4.5% for **1** and 3.2% for **2,** decreasing to 1.3 and 2.3% at 4 h. Bone uptake for **1** ranged from 0.4 at 15 min to 1.0% at 4 h whereas for **2** was 0.87 to 4.3% for the same period. Pre-treatment with PK 11195 and Ro 5-4864 decreased the uptake of **1** and **2** in peripheral organs except in the adrenals which showed an increase. In the olfactory regions a non-significant decrease was observed with **2**. Flumazenil had no effect in the uptake of **1** or **2**.

Conclusion: These results demonstrate that **1** and **2** are suitable for development as PBBS imaging agents. The uptake of **2** showed a higher target to non-target ratio in the brain.

Keywords: Imidazopyridines, PET, Fluorine-18, Peripheral Benzodiazepine Binding Site

P291 FURTHER CHARACTERIZATION OF THE DOPAMINE D_1 RECEPTOR PARTIAL AGONIST RADIOLIGAND (*S*)-(^{11}C)N-METHYL-NNC 01-0259

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Introduction: Typically the dopamine D_1 receptor has been studied with PET using antagonist radioligands as [^{11}C]SCH23390 and [^{11}C]NNC 112. The dopamine D_1 receptor *in vivo* selectivity of these radioligands was recently more thoroughly investigated, after contradictory findings of prefrontal cortex D_1 binding in patients with schizophrenia were reported. This new study suggested that 20-30% of [^{11}C]SCH23390 and [^{11}C]NNC 112 binding in the neocortex is due to $5HT_{2A}$ binding [1]. We here report a further characterization of the enantiomers of the partial dopamine D_1 receptor agonist radioligand [^{11}C]N-Methyl-NNC 01-0259 ([^{11}C]***1***).

Experimental: Binding affinity and intrinsic activity to the D_1 receptor for the enantiomers of ***1*** and the reference compounds SCH23390 and NNC112 were determined *in vitro*. The configuration of the enantiopure distomer was evaluated with X-ray analysis. Both enantiomers *R*- and *S*-[^{11}C]***1*** were prepared by N-methylation of the bromide salt of the corresponding secondary amine precursor NNC 01-0259 with [^{11}C]methyl iodide and were evaluated in a cynomolgus monkey.

Results and Discussion: *In vitro* binding affinity measurements indicated that the distomer has a 7 times lower affinity (60 nM) to the D_1 receptor than the eutomer (9 nM) and that both enantiomers are partial agonists (see table 1). X-ray analysis demonstrated that the distomer is configured as (*R*)-***1.*** Both *R*- and *S*-[^{11}C]***1*** were obtained in good yield and with high specific radioactivity (>5000 Ci/mmol). Radioactivity in brain peaked at 2.5% of ID, 5 minutes after radioligand administration. After *S*-[^{11}C]***1*** iv injection, radioactivity accumulated in neocortex and striatum. The neocortex and striatum to cerebellum ratios were at 45 and 66 minutes 1.5 and 3.5, respectively. Specific binding peak equilibrium was reached in neocortex and striatum after approximately 24 and 45 minutes, respectively.

Table 1. In vitro binding data to the dopamine D1 receptor

Compound	D1 Binding, Ki (nM)	D1 Functional Assay*
(S)-N-methyl NNC 01-0259	9	35%
(R)-N-methyl NNC 01-0259	60	29%
SCH 23390	2.1	0%
NNC 112	1.5	0%

*Relative to dopamine that is defined to give 100%

Conclusion: (*S*)-[^{11}C]N-Methyl-NNC 01-0259 has promising properties as a radioligand for PET imaging of the D_1 receptor. Further characterization of *in vitro* and *in vivo* selectivity to the D_1 over the $5HT_{2A}$ receptor is in progress.

References: [1] Ekelund et al. (**2006**) *Neuroimage* 31/S2, T111.

Keywords: Dopamine, Agonist, PET, Carbon-11

P292 (^{18}F)FE-PE2I, PREPARATION AND PRELIMINARY *IN VITRO* CHARACTERIZATION OF A NEW DOPAMINE TRANSPORTER RADIOLIGAND

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Introduction: Several radioligands have been developed for brain imaging of dopamine transporters (DATs) using positron emission tomography (PET). Although most ligands show high signal to DAT, some of these ligands give rise to blood brain barrier (BBB) permeable radiometabolites (e.g. [^{18}F]FECNT), which may hinder an accurate quantification of the obtained PET data.

To investigate the potential influence on metabolism by introducing a fluoroethyl group in the ester moiety of the DAT ligand PE2I, we here report the synthesis, radiosynthesis and preliminary in vitro characterization of (E)-*N*-(3-iodoprop-2-enyl)-2β-carbofluoroethoxy-3β-(4'-methylphenyl)nortropane, FE-PE2I (**2**, Fig. 1).

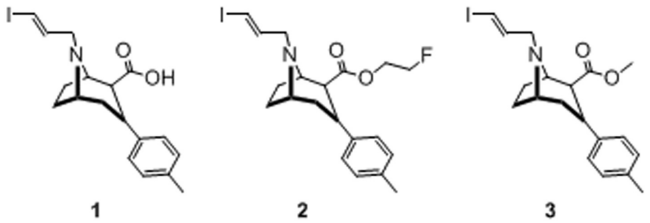

Fig. 1. Structures of acid precursor (**1**), FE-PE2I (**2**) and PE2I (**3**).

Experimental: Reference compound **2** was prepared in two steps from acid **1**. The K_i of **2** at DAT was determined using [^{125}I]PE2I on membrane fractions from rat brain striatum homogenates. Aqueous ^{18}F-fluoride ion was trapped on-line from the target on an anion excheange cartridge, eluted, dried and reacted with bromoethyl tosylate to produce [^{18}F]fluoroethyl bromide, which was purified by distillation and subsequently reacted with **1** to produce [^{18}F]**2**. *In vitro* autoradiography was performed on whole hemispheres from the human brain post mortem. Sections containing caudate and putamen were selected for the experiments. Co-incubation experiments were performed with the two DAT inhibitors, GBR12909 and β-CIT (each at a concentration of 10 μM).

Results and Discussion: Compound **2** was obtained in 30% yield from **1**. The K_i of **2** at DAT was 12 ± 1.7 nM (*n*=3). The radiochemical yield of [^{18}F]fluoroethyl bromide and [^{18}F]**2** from [^{18}F]fluoride ion was 38 and 7%, respectively (uncorrected for decay). In the autoradiography experiments, a high accumulation of [^{18}F]**2** was observed in caudate and putamen, which are both well known high density DAT regions. The accumulation of [^{18}F]**2** in these regions was abolished in co-incubation experiments with either GBR12909 or β-CIT, suggesting that [^{18}F]**2** binds specifically to DAT. There was also a non-specific accumulation of [^{18}F]**2** in white matter.

Conclusion: The new ligand [^{18}F]FE-PE2I ([^{18}F]**2**) binds to DAT with high affinity *in vitro*. Further experiments are required to characterize its distribution and metabolism *in vivo*.

Acknowledgement: The authors are grateful to DIMI for financial support. Siw Eriksson is also gratefully acknowledged for excellent technical assistance in the autoradiography experiments.

Keywords: Dopamine Transporter, Radioligand, Fluorine-18, PE2I

P293 FIRST DOPAMINE D_2 AGONIST RADIOLIGAND FROM THE BENZO(*g*)QUINOLINE SCAFFOLD – RADIOLABELING AND PET EVALUATION OF THE PRODRUG (^{11}C)-6,7-di-OAc-PBGQ

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Introduction: The dopamine D_2 receptor agonist radioligands developed so far originate mainly from three chemical classes, the apomorphines, the hydroxyaminotetralins and the hydroxynaphthoxazines [1-5]. We now report the first attempt at using the di-hydroxy-benzo[*g*]quinoline scaffold, as *N*-propyl-6,7-di-hydroxy-benzo[*g*]quinoline (6,7-di-OH-PBGQ) is a potent dopamine D_2 receptor agonist. We labeled the prodrug of 6,7-di-OH-PBGQ, *N*-propyl-6,7-di-acetoxy-benzo[*g*]quinoline ([^{11}C]-6,7-di-OAc-PBGQ), with carbon-11 and performed a preliminary PET evaluation in a cynomolgus monkey.

Experimental: [^{11}C]-6,7-Di-OAc-PBGQ was synthesized by ^{11}C-propylation of racemic *N*-H-6,7-di-acetoxy-benzo[*g*]quinoline in DMF and $NaHCO_3$. After trapping of [^{11}C]propyl iodide in the reaction vial, the mixture was heated for 10 minutes at 160°C. Semi-preparative reversed phase HPLC was used to purify the product and the final product was formulated in 7 ml PBS and 1 ml Ethanol.

Results and Discussion: The synthesis yield of [^{11}C]-6,7-di-OAc-PBGQ was about 30% and total synthesis time was 50-55 minutes. Radiochemical purity was >99% and specific radioactivity was 72 Ci/mmol. After i.v. injection of 55 MBq [^{11}C]-6,7-di-OAc-PBGQ into a cynomolgus monkey, total brain uptake was maximal 5 min after injection at which time about 4.0% of the total injected radioactivity was in brain. Plasma metabolite analysis indicated almost total conversion of [^{11}C]-6,7-di-OAc-PBGQ into [^{11}C]-6,7-di-OH-PBGQ and other labeled metabolites, already at 4 minutes. The peak in specific binding in the striatum was reached after about 30 minutes. The striatum to cerebellum ratio approached a value of 3 by the end of the study (70 min). The binding potential calculated with MRTM 2 was 1.7 in the striatum.

Conclusion: This preliminary study suggest that [^{11}C]-6,7-di-OAc-PBGQ is a promising prodrug agonist radioligand for the dopamine D_2 receptor. Fast *in vivo* conversion of [^{11}C]-6,7-di-OAc-PBGQ into [^{11}C]-6,7-di-OH-PBGQ was observed in plasma. The measured BP value was higher than that previously shown for [^{11}C]MNPA.

References: [1] Halldin et al. (**1992**) *J. Labelled Compd. Radiopharm.* 35, S265-S266. [2] Hwang et al. (**2000**) *Nucl. Med. Biol.* 27, 533-539. [3] Finnema et al. (**2005**) *Nucl. Med. Biol.* 32, 353-360. [4] Hwang et al. (**2003**) *J. Nucl. Med.* 44, 294P. [5] Wilson et al. (**2005**) *J. Med. Chem.* 48, 4153-4160.

Keywords: Dopamine, Agonist, PET, Carbon-11

P294 SYNTHESIS, RADIOLABELING AND ANIMAL BIODISTRIBUTION OF IOZAPINE AN ANALOG OF CLOZAPINE AS A POTENTIAL DOPAMINE D4 IMAGING AGENT

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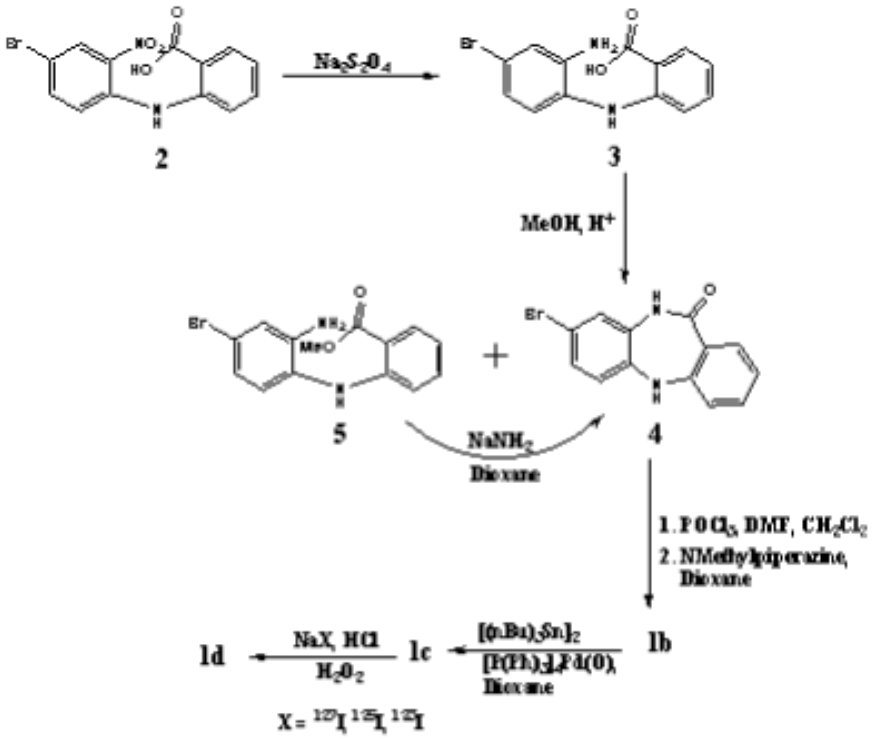
Introduction: Clozapine (8-chloro-11-(4-methylpiperazino)-5H-dibenzo (b, e) (1, 4) - diazepine (1a) is an effective antipsychotic agent which is devoid of extrapyramidal side effects. Although it has considerable value in the treatment of schizophrenia, its therapeutic use has been restricted by the relatively high incidence of agranulocytosis.

Experimental: From receptor binding studies it has been shown that Clozapine, in addition to blocking the D_2 receptor, also blocks other dopamine and serotonin receptors in the human brain and exerts potent antagonist effects on the adrenergic, cholinergic and histaminergic receptors. The atypical antipsychotic profile of Clozapine has been suggested to arise from its preferential blockade of the dopamine D_1 or D_4 receptors. It has also been reported that Clozapine shows a 10-fold higher selectivity for D_4 over D_2 receptors. Although D_4 receptors represent a relatively minor proportion of the total dopamine receptor population in the basal ganglia of the normal human brain, Seeman and associates found there is a six-fold increase in the population of these receptors in the brain of schizophrenics. Therefore a radiolabeled imaging agent structurally related to Clozapine and exhibiting comparable receptor affinities may be of value in the investigation of schizophrenia.

Results and Discussion: In this paper we describe the synthesis of iozapine, an iodinated (^{127}I, ^{125}I, ^{123}I) analog of Clozapine and a study of its biodistribution in animals.

The title compound was prepared using a modification of the Hunziker synthesis for (1b). Ullman synthesis from 2,5-dibromonitrobenzene and anthranilic acid in the presence of potassium carbonate and copper catalysis gave the diphenylamine derivative (2) in good yield (76%). Reduction of the nitro group with sodium dithionite furnished the amino acid (3) in 88% yield. Heating (2) in methanol under sulfuric acid catalysis afforded a mixture of the lactam (4) in 68% yield and the amino-ester (5) in 24% yield, which were readily separated by silica gel chromatography. The amino ester (5) was readily converted to the lactam (4) in high yield by treatment with sodamide in dioxane. The lactam (4) was converted to (1b) first, by treatment with phosphorus oxychloride and DMF in dichloromethane to form the imido-chloride which was treated with N-methylpiperazine in dioxane to form (1b). Reaction of (1b) with bis(tributyltin) in dioxane under tetrakis(triphenyl phosphino) palladium catalysis gave (1c). Oxidative iodo-destannylation with KI and hydrogen peroxide under acid conditions gave the target compound (1d).



Scheme 1

Conclusion: Radio-iodination of (1c) was conducted using sodium iodide (^{123}I and ^{125}I) under similar conditions in greater than 90% radiochemical yield and the product was purified by hplc. The biodistribution was studied in mice and rabbits using ^{125}I and ^{123}I. Initial brain activity in mice was high (3.5%) but quickly decreased by 2 hours to 0.1%. The hepatobiliary clearance was relatively rapid with 35% in the intestine at 1-2 hours post injection. The product appeared to be de-iodinated in mice as indicated by increasing thyroid and stomach activity over time with concomitant slow blood clearance. Lung uptake was high and persistent in the rabbit studies but the thyroid uptake was minimal suggesting that de-iodination was not as prevalent in the rabbit. In addition, the brain uptake in rabbits was slower and more persistent than observed in mice.

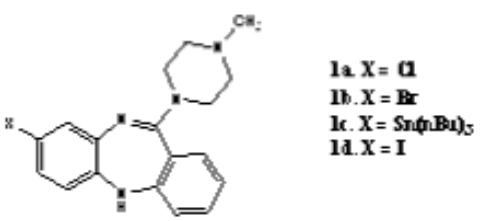


Fig. 1