P225 PREPARATION, BIOLOGICAL EVALUATION AND PHARMACOKINETICS OF 86Y-CHX-A''-DTPA-PANITUMUMAB FOR QUANTITATIVE PET IMAGING OF HER1-EXPRESSING CANCER

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Objectives: Panitumumab was conjugated to the bifunctional chelating agent, CHX-A''-DTPA and radiolabeled with 86Y (half-life= 14.7 h). Immunoreactivity was evaluated to determine the in vitro specificity of the radioimmunoconjugate (RIC). In vivo biodistribution, blood clearance, area under the curve (AUC), area under the moment curve (AUMC) and mean residence time (MRT) were determined on mice bearing HER-1 over-expressing human colorectal (LS174T), human prostate (PC-3) and human epidermoid (A431) xenografts. Receptor-specificity was demonstrated by co-injection of 0.1 mg panitumumab with the RIC. Longitudinal PET imaging was also performed to determine target-specific uptake.

Methods: Panitumumab was conjugated to the bifunctional chelating agent, CHX-A''-DTPA and radiolabeled with ^{86}Y (half-life= 14.7 h). Immunoreactivity was evaluated to determine the in vitro specificity of the radioimmunoconjugate (RIC). In vivo biodistribution, blood clearance, area under the curve (AUC), area under the moment curve (AUMC) and mean residence time (MRT) were determined on mice bearing HER-1 over-expressing human colorectal (LS174T), human prostate (PC-3) and human epidermoid (A431) xenografts. Receptor-specificity was demonstrated by co-injection of 0.1 mg panitumumab with the RIC. Longitudinal PET imaging was also performed to determine target-specific uptake.

Results: ^{86}Y-CHX-A''-DTPA-Panitumumab was successfully prepared with specific activity exceeding 2 GBq/mg and yields over 70%. The immunoreactivity ranged from 68-78%. Biodistribution and PET imaging studies demonstrated high specific tumor uptake of the RIC. In mice bearing LS174T, PC-3 or A431 tumors, the tumor uptake at 3d pi were 34.65, 22.1 and 22.74 % ID/g, respectively. The corresponding tumor uptake in mice coinjected with 0.1 mg cold panitumumab were 9.28, 8.80 and 10.04 % ID/g, respectively at the same time point, demonstrating specific blockage of the receptor. The LS174T, A431 and PC-3 tumors were clearly visualized by PET imaging after injecting 1.8-2.0 MBq ^{86}Y-CHX-A''-DTPA-panitumumab. Organ uptakes quantified by PET were closely related (r^2= 0.95, P= 0.87, n=30) to values determined by ex vivo biodistribution studies. From non-compartmental pharmacokinetic analysis in tumor bearing mice, the alpha-half life of blood clearance ranged from 2.9-3.8 h and the beta-half life was 76-81 h. LS174T tumor had the highest AUC (96.8 %ID·d·g^{-1}) and AUMC (262.5 %ID·d^2·g^{-1}), however the tumor MRT were identical for all three tumors (2.7-2.8 d). The LS174T tumor AUC: blood AUC ratio of 3.1 was greater than the PC-3 and A431 tumor: blood AUC ratios of 2.0.

Conclusions: This preclinical study of ^{86}Y-CHX-A''-DTPA-panitumumab demonstrates the potential of the radioimmunoconjugate for non-invasive assessment of the HER-1 status of tumors. It represents the first step towards clinical translation of ^{86}Y PET imaging of HER-1 using the full human monoclonal antibody, panitumumab.

Research Support: Intramural NIH

P226 DEVELOPMENT OF [67GA]-DTPA-GONADORELIN IN NORMAL RATS

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Objectives: The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH) plays a key role in the regulation of mammalian reproduction. The aim of this study is to prepare and evaluate the biodistribution of ^{67}Ga-DTPA-Gonadorelin (GnRHagonists) in normal rat.

Methods: Gonadorelin was successively labeled with [^{67}Ga]-gallium chloride after residulation with freshly prepared cyclic DTPA-dianhydride. The best results of the conjugation were obtained by the addition of 1 mg of a Gonadorelin to a glass tube pre-coated with DTPA-dianhydride (0.33 mg) at 25 ^{C} with continuous mild stirring for 1 hour. Radio thin layer chromatography showed an overall radiochemical purity of >90% at optimized conditions after labeling. HPLC showed a radiochemical purity more than 95% (specific activity =400-450 GBq/M). The stability of the radioconjugate was tested in presence of human serum at 37 C.

Results: Preliminary in vivo studies in normal rats were performed to determine the biodistribution of the conjugate up to 48hr. The breast and ovaries uptake were significantly high in first 15 minutes post injection, which is in agreement with the other reports regarding the presence of specific GnRH receptors. Cell binding assay with ^{67}Ga-DTPA-Gonadorelin demonstrated more than 99% binding affinity to MCF-7 cells. The presence of specific receptor on MCF-7 was assayed with a near complete presence of buserelin. No specific binding to MCF-7 cells could be detected.

Conclusions: the activity is accumulated in ovaries, breast as well as kidneys, all in accordance with reported GnRH receptor biodistribution and metabolized peptides. This tracer can be used in various malignancies associated with over-expression of gonadorelin receptors 15 minutes post injection.

P227 TARGETING OF CCK2 RECEPTOR EXPRESSING TUMOURS USING AN 111IN-LABELLED MINIGASTRIN DIMER

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Objectives: The gastrin/CCK2 receptor is overexpressed in several tumour types and is thus a potential target for peptide receptor radionuclide imaging or therapy of cancer. A variety of radiolabelled gastrin and CCK analogues have been investigated both in animals and humans, however none to date combine the high tumour uptake and low kidney retention required for effective PRRT. We have previously studied several gastrin monomer analogues which displayed low kidney retention but also relatively low tumour uptake. In this study, peptide dimerisation has been investigated as a means of increasing the in vivo uptake of the radiolabelled compound in CCK2 receptor expressing tumours.

Methods: A DOTA-conjugated minigastrin dimer with a maleimide linkage was synthesised: DOTA-GSC(succinimidopropionyl-EAYGWNleDF-NH2)-EAYGWNleDF-NH2 (MGD5). The in vitro stability, receptor binding and internalisation of 111In-MGD5 were studied in comparison with a minigastrin monomer compound, 111In-APH070. In vivo biodistribution and imaging using a NanoSPECT-CT camera were also performed.

Results: This maleimide linked dimer had higher binding affinity on CCK-2 receptor expressing AR42J cell membranes than 111In-APH070 and was internalised into AR42J cells at twice the rate of 111In-APH070. In vivo biodistribution studies in nude AR42J tumour bearing mice showed the tumour uptake of 111In-MGD5 was more than doubled in relation to that of 111In-APH070 with an increase in tumour to kidney ratio. This increase in tumour uptake of 111In-MGD5 over 111In-APH070 was also demonstrated by imaging using a NanoSPECT-CT camera.

Conclusions: Dimerisation of the C-terminal sequence of minigastrin seems to be effective in increasing tumour uptake while maintaining acceptable kidney retention

P228 RADIOSYNTHESIS AND IN VIVO EVALUATION OF [11C]-(R)-N-(1-CYCLOHEXYLETHYL-N-METHYL-1H-PYRROLE-2-CARBOXAMIDE

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Objectives: Monoamine oxidase (MAO) is a flavin-containing enzyme that occurs in brain and peripheral organs. It catalyzes the oxidative deamination of amines from endogenous and exogenous sources. Both isoforms of MAO, termed MAO-A and MAO-B are important for regulation of monoaminergic transmission and are involved in psychiatric and neurological disorders such as depression and Parkinson disease as well as in tobacco addiction. Only a few PET tracers for MAO-A have been designed and they all have their own drawbacks which justifies the search for new ligands with optimal kinetics. This study reports the radiolabeling of [^{11}C]-(R)-N-(1-cyclohexylethyl-N-methyl-1H-pyrrole-2-carboxamide ([^{11}C]-RS 2360) and the characterization of its in vivo properties.

Methods: Male NMRI mice (n=3) were injected i.v. with approximately 3.7 MBq [^{11}C]-RS 2360. At various time points p.i., the mice were sacrified and dissected. Results were expressed as % injected dose/gram tissue. Specificity of [^{11}C]-RS 2360 for MAO was investigated at 10 and 30 min p.i. by preinjection of clorgyline or R-(-)-deprenyl. At 1, 10 and 30 min (n=3) p.i., metabolite analysis was performed. Mice were injected with 29.6 - 37 MBq [^{11}C]-RS 2360. Blood and brain were removed and extracted with CH_3CN. Supernatant was drawn off and analyzed by RP HPLC.

Results: [^{11}C]-RS 2360 was prepared in a total synthesis time of 35 min. Based on $^{11}CH_3I$, [^{11}C]-RS 2360 was obtained in a decay-corrected RCY of 30 % with a radiochamical purity of > 98 % and specific activity of 41 - 106 GBq/μmol. After injection in mice, [^{11}C]-RS 2360 showed high brain uptake (7.08 %ID/g at 1 min p.i.) and rapid brain clearance (0.51 %ID/g at 30 min p.i.). Additionally, the liver (7.45 %ID/g at 10 min p.i.) and the kidneys (6.56 %ID/g at 1 min p.i.) showed a high uptake, indicating metabolims in the liver and urinary clearance of radioactivity. Preinjection with clorgyline or (R)-deprenyl lowered the lung uptake but has only a minor effect on brain uptake. Blood metabolite studies showed 29 % and 21 % intact product at 10 and 30 min p.i. respectively. In the brain, 81 % intact product was found at 10 min p.i. whereas at 30 min p.i. 66 % was still present as unchanged [^{11}C]-RS 2360. At 1 min p.i. no degradation products occured.

Conclusions: These results indicate that [^{11}C]-RS 2360 passess the blood-brain barrier. In plasma, the tracer is very rapidly metabolized whereas in brain the tracer is more stable. Further research is ongoing to determine the selectivity and usefulness of [^{11}C]-RS 2360 in vivo.

P229 [11C]AF150(S), AN AGONIST PET LIGAND FOR IN VIVO IMAGING OF THE M1 MUSCARINIC ACETYLCHOLINE RECEPTOR

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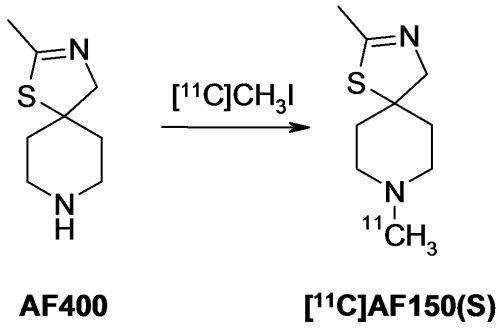
Objectives: The M1 muscarinic acetylcholine receptor (M1ACh-R) is the subtype with the highest density in the brain and belongs to the class of G-protein coupled receptors (GPCRs). The usually applied antagonist PET ligands label the total pool of GPCRs and do not distinguish between the G-protein-coupled activated receptor and the uncoupled inactive receptor. The aim is to investigate the labelled functionally selective M1ACh-R agonist AF150(S), [^{11}C]AF150(S), as an agonist PET ligand for the M1ACh-R, that would label the activated GPCR pool, in rats in vivo.

Methods: [^{11}C]AF150(S) was obtained via methylation of AF400 with [^{11}C]CH_3I (Scheme 1). An incorporation yield of 90% of [^{11}C]CH_3 was achieved in 5 minutes reaction time at 60°C in CH_3CN. The product was purified by HPLC and recovered by solid phase extraction, yielding 3237±2056 MBq of [^{11}C]AF150(S), SA of 39±16 GBq/μmol, radiochemical purity > 99%. The regional distribution of [^{11}C]AF150(S) in rat brain was assessed by dynamic PET imaging with an HRRT (Siemens) after IV administration of [^{11}C]AF150(S). Next to that PET imaging was performed in the same rat after pre-treatment with relatively selective M1ACh-R ligands pirenzepine and trihexyphenidyl, xanomeline, an M4/M1ACh-R agonist, and the neuroleptic and δ ligand haloperidol to asses the specificity of binding of [^{11}C]AF150(S).



Scheme 1. Radiosynthesis of [^{11}C]AF150(S)

Results: Uptake of [^{11}C]AF150(S) was higher in M1ACh-Rs rich areas, striatum and cerebral cortex, compared to M1ACh-Rs poor regions, cerebellum and medulla oblongata.[1] Highest brain region/cerebellum ratios occurred at 7.5 min post-injection and were 1.36±0.02, 1.34±0.05 and 1.2±0.03 for left/right striatum and cerebral cortex, respectively. Pirenzepine and trihexyphenidyl caused the largest reductions in cerebral cortex (24-28%) and striatum (19-22%). The reductions by xanomeline were less pronounced, possibly due to an increased heart rate or altered brain kinetics. Haloperidol, produced the largest reductions in striatum (22%), probably due to haloperidol-mediated enhanced acetylcholine release as a consequence of dopamine D_2 receptorblockade.[2] Statistical comparison of the effects in brain regions compared to cerebellum revealed that pre-treatment with selective M1ACh-R ligands afforded a statistically significant decrease in [^{11}C]AF150(S) binding compared to baseline in the striatum as well as in the cerebral cortex. The findings suggest that [^{11}C]AF150(S) possibly labels M1ACh-R in vivo.

Conclusions: The agonist ligand [^{11}C]AF150(S) provides a small but significant M1ACh-R related signal in vivo, warranting further evaluation as a putative PET tracer for in vivo imaging of the G-protein coupled M1ACh-R with PET.

Research Support: The A.J. Coops foundation for granting this research. S. Berndsen and P.J. Klein for their assistance. The BV Cyclotron VU for providing [^{11}C]CO_2.

References: [1]Mash et al Neuroscience 1986 19 551 [2]Bianchi et al Eur J Pharmacol 1979 58 235

P230 FLUORINE-18 LABELED SCH 442416 DERIVATIVE FOR IMAGING ADENOSINE A2A RECEPTORS

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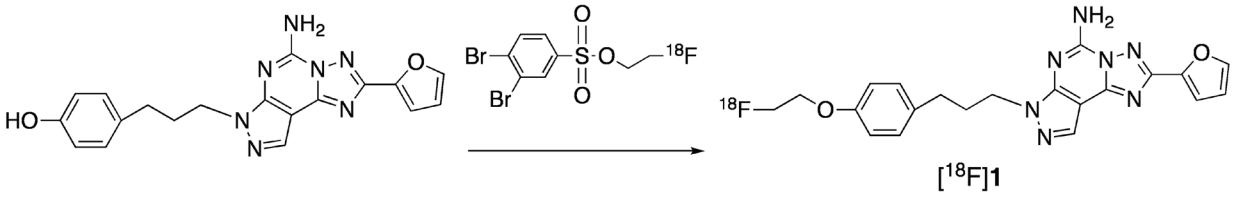
Objectives: The A_{2A} adenosine receptor, highly expressed in the striatum, is a therapeutic target for treating movement disorders including Parkinson's disease. Molecular imaging agents specifically targeting this receptor can be an important research and diagnostic tools for such movement disorders. Several carbon-11 labeled high affinity PET ligands, for A_{2A} adenosine receptor, have been developed including carbon-11 labeled selective A_{2A} antagonist SCH 442416. However, the main drawback of this carbon-11 labeled compound is the short half-life that limits their use as a PET imaging agent. The purpose of this project is to develop fluorine-18 labeled SCH 442416 derivatives with a longer half-life that may have advantages over the carbon-11 analog.

Methods: The fluoroethyl derivative (1) of SCH 442416 was prepared in two steps by first removing the phenolic O-methyl group from commercially available SCH 442416 using boron tribromide, and then O-alkylation with fluoroethyl tosylate. The affinity of this compound was measured using an in vitro adenosine receptor binding assay. Two separate radiochemical procedures were evaluated to produce [^{18}F]1 with one procedure using directing labeling and the other one through a two-step procedure. The directing labeling used mesylate as the leaving group and the two-step procedure included O-alkylation of phenol with [^{18}F]fluoroethyl 3,4-dibromobenzenesulfonate, which was prepared from ethane-1,2 diyl bis(3,4-dibromobenzenesulfonate). In vitro autoradiography was performed using rat brain slices incubated with radioligand with or without cold SCH 442416 and visualized with a phosphorimager.



Results: The relative affinity of the fluoroethyl derivative of SCH 442416 is 4 times lower than that of SCH 442416. However, we expect this affinity to be sufficient because the Bmax in striatum is quite high. The radiochemical yields for both procedures ranged from 1% to 5% which require further optimization. The in vitro autoradiography image clearly showed specific binding in the striatum as binding could be blocked by pre- or co-incubation with unlabeled SCH 442416.

Conclusions: The fluorine-18 labeled fluoroethyl derivative of SCH 442416 can be achieved by direct labeling or through a two-step procedure. Our preliminary affinity and in vitro autoradiography results suggest that this radioligand has the potential for the study of A_{2A} adenosine receptor in human disease.

Research Support: This work was supported by the intramural programs of the National Institute of Biomedical Imaging and Bioengineering and the National Institute on Diabetes and Digestive and Kidney Diseases.

P231 ORAL METHYLPHENIDATE SIGNIFICANTLY OCCUPIES NOREPINEPHRINE TRANSPORTERS AT CLINICALLY RELEVANT DOSES: A PET STUDY WITH (S,S)-[11C]MRB IN HEALTHY SUBJECTS

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Objectives: Attention deficit hyperactivity disorder (ADHD) is the major psychiatric disorder in children, but effective treatments remain elusive and the neurochemical mechanisms of this disorder are poorly understood. Although methylphenidate (Ritalin, MP) has been used for the treatment of ADHD for over 50 years, its mechanism(s) of action have not been well characterized. Oral MP (60 mg) significantly blocked dopamine transporter (DAT) [1]; however, MP binds to NET with an even higher inhibitory effect on NE uptake than on DA uptake (IC_{50} 37.7 vs. 193 nM) [2]. The in vivo investigation of ED_{50} and the duration of action by oral MP in blocking NET is therefore crucial. Here we report the first PET imaging study in healthy subjects using (S,S)-[^{11}C] methylreboxetine (MRB), a promising NET ligand, to determine the duration and occupancy of oral MP on NET [3].

Methods: For the duration study, on three different days, subjects received placebo and oral MP (40 mg) 75, 150, and 225 min before each scan. For the occupancy study, each subject had four PET scans after oral administration of single-blind placebo or MP 2.5 mg, 10 mg, and 40 mg. After injection of 740 MBq of ^{11}C-MRB, 120 min dynamic PET acquisition using HRRT was performed. Parametric images were computed using the multilinear reference tissue model (MRTM2) with occipital cortex as the reference region. Regions of interest (ROIs) from the AAL template were analyzed. In addition, ROIs for small brain regions including locus coeruleus (LC), brainstem nuclei, hypothalamus, and thalamic subnuclei were also defined. BP_{ND} (non-displaceable binding potential) and IC_{50} values were estimated.

Results: 1. There was no significant difference in the BP_{ND} values after 75 min compared to 150 or 225 min; thus, we chose 75 min as the timing for the occupancy study; 2. BP was reduced by MP in a dose-dependent manner in all NET-rich regions; 3. The avg. IC_{50} was 9.7 mg (range 6-15 mg); 4. At 40 mg MP, complete displacement in LC, raphe and hypothalamus, but 50-70% in thalamus and its subnuclei, was observed.

Conclusions: Oral MP reached peak NET occupancy approx. at 75 min, lasting for at least 3 hours, and it occupied NET in a dose-dependent manner. Our data indicate that oral MP significantly occupies NET at clinically relevant doses, strongly suggesting the role of NET in ADHD.

References: [1]. Volkow ND et al., Am J Psychiatry 155:1325-133, 1998; [2]. Eshleman AJ et al., J Pharmacol Exp Ther, 289:877-885, 1999; [3]. Ding Y-S et al., Curr Pharm Design, 12:3871-45, 2006. Review.

P232 COMPARISON OF BREAST TUMOR TARGETING WITH 99mTc RADIOLABELED PR81 AND ITS F(AB')2 FRAGMENT

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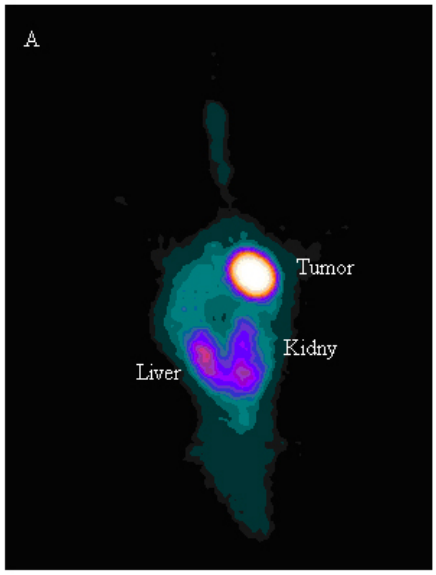
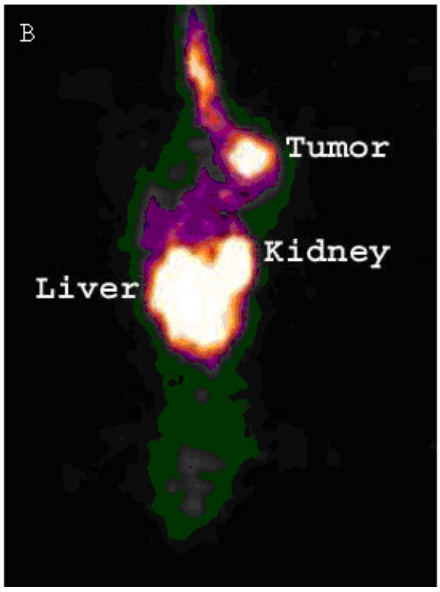
Objectives: Compared to intact IgG, $F(ab')_2$ and Fab exhibit significantly improved tumor specificity and intratumor penetration in animal models. Generally, lower molecular-weight agents provide better target to nontarget ratios due to their rapid background clearance. In this study we compared the biodistribution and localization characteristics of ^{99m}Tc labeled intact PR81 and its $F(ab')_2$to identify potentially more useful radiopharmaceutical for diagnosis of human breast cancer.

Methods: Purified monoclonal antibody PR81 was digested with 5% (w/w) pepsin for 28 hours at 37ºC in 0.1 M sodium acetate buffer Ph 4.2. The $F(ab')_2$ fragments were purified by protein A column chromatography followed by elution with PBS. The purity of $F(ab')_2$preparation was evaluated by SDS-PAGE under nonreducing conditions and proved to be more than 95%. ^{99m}Tc Radiolabeling of PR81 and $F(ab')_2$ fragment were performed using the HYNIC as a chelator and tricine as a co-ligand. The immunoreactivity of the complexes was assessed by radioimmunoassay using MCF7 cells. Biodistribution and imaging studies were performed in female BALB/c mice with breast tumor xenograft after 4 and 24 hs after the preparations injection.

Results: Labeling of PR81 and $F(ab')_2$ fragment with ^{99m}Tc resulted in a specific activity of 89.2%±4.7 and 70.1%±5.2 respectively. The immunoreactivity of the ^{99m}Tc-HYNIC-PR81 was 83.2%±4.7 and the immunoreactivity of its ^{99m}Tc labeled fragment was 65.2%±5.1. Tumor to liver ratio at 4 hrs was 0.73 for PR81 and 3.67 for $F(ab')_2$ and reached at 24 hr to 1.30 and 0.64 respectively. The tumors were visualized with high sensitivity after 4 (figure A) and 24 (figure B) hrs injections of radiolabeled PR81 and its fragment respectively.

Conclusions: Our comparative study showed that $F(ab')_2$ fragment of PR81 is much more suitable, rapid and reliable than intact PR81 for diagnosis of breast tumors.





P233 PHENYTOIN IS A WEAK MODULATOR OF P-GLYCOPROTEIN TRANSPORTERS: EVALUATION WITH 11C-DESMETHYLLOPERAMIDE IN MICE

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Objectives: 30 % of epilepsy patients are resistant to treatment with antiepileptic drugs. The cause of this refractory epilepsy is not fully understood. A possible hypothesis is the overexpression of the efflux transporters at the blood-brain barrier (BBB), particularly P-glycoprotein (Pgp). In vitro studies already demonstrated a Pgp inhibitory potency of phenytoin, but in doses higher than the therapeutic ones. The aim of this study was to investigate the in vivo interaction of phenytoin on Pgp transporters both in therapeutic dose as in higher dose, by ^{11}C-desmethylloperamide (^{11}C-dLop). As a control, we used Cyclosporine A (CsA), a known inhibitor of Pgp as well as mdr1a knock-out (KO) mice.

Methods: ^{11}C-dLop was synthesized by methylation of didesmethylloperamide with $^{11}CH_3I$. The mixture was heated for 10 min at 30 °C, purified on a RP-C18 kolom and concentrated on a Seppak. WT mice (n = 3 per time point) were injected i.v. with ^{11}C-dLop, 120 min after i.p. administration of phenytoin (25mg/kg and 100 mg/kg). As a control experiment WT pre-injected with CsA (50 mg/kg), 30 min before tracer administration and KO mice were performed. Mice were killed at 1 and 10 min after tracer injection. Brain and blood were isolated and counted for radioactivity. Blood was centrifuged for 15 min, plasma was collected and analyzed for phenytoin. To study the influence of phenytoin and CsA on metabolisation pattern of ^{11}C-dLop, plasma and brain were analysed by HPLC.

Results: Mean RCY of ^{11}C-dLop was 36.2% +/- 4.6%. Radiochemical purity was always > 95%. S.A. averaged around 72 GBq/μmol.

Type	Blood (%ID/G)	Brain (%ID/g)	Phenytoin concentration (μg/mL)
WT CsA (50 mg/kg)	1.92 +/- 0.51	2.86 +/- 0.50	/
WT phenytoin (25mg/kg)	1.07 +/- 0.31	0.15 +/- 0.02	10.25 +/- 2.48
WT phenytoin (100mg/kg)	1.37 +/- 0.53	0.28 +/- 0.03	31.29 +/- 7.15
KO	1.46 +/- 0.38	2.33 +/- 0.04	/

KO mice and CsA pretreated WT mice showed a 7.4 fold increase in brain uptake in ^{11}C-dLop compared to non pretreated WT mice both at 1 and 10 min after injection of ^{11}CdLop while the blood activity was not significantly different. The brain/blood ratio was not significant difference between the CsA pretreated group and the KO mice. Phenytoin pretreatment at 25 mg/kg was significantly different from 100 mg/kg, brain uptake was increased, while blood activity remains the same. Phenytoin (25 mg and 100 mg/kg) pretreatment did not change the metabolisation pattern both in brain and blood. Plasma concentrations of phenytoin at 25 mg/kg were within the therapeutic range (10 - 20 μg/ml), while at 100 mg/kg there were 3.1 times higher.

Conclusions: The results demonstrate that phenytoin is a weak modulator of Pgp transporters at the BBB. Within the therapeutic range of phenytoin (10 – 20 μg/mL) the influence on the brain uptake of ^{11}C-dLop could not be demonstrated but at concentrations three times higher a small but significant difference increase could be observed.

P234 NEW HYNIC-BOMBESIN ANALOGUE FOR TARGETING PROSTATE TUMOURS: TC-99M LABELLING AND PRECLINICAL EVALUATION

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Objectives: BN(Bombesin)-like peptides have very high binding affinity for the gastrin-releasing peptide (GRP)receptor which has proved to be highly expressed on prostate cancer. In a previous study[1,2], we found that Tricine/TPPTS were outstanding coligands for ^{99m}Tc labelling of HYNIC-peptide including HYNIC-BN(7-14). In this study, we made a new radiotracer ^{99m}Tc(HYNIC-Aca-BN(7-14))(Tricine)(TPPTS) for prostate cancer diagnosis.

Methods: HYNIC-Aca-BN(7-14) and Aca-BN(7-14) were studied in vitro for their binding affinity with GRP-R using PC-3 cells. The full sequence of the bombesin peptide(1-14) was set as a standard. ^{99m}Tc(HYNIC-Aca-BN(7-14))(Tricine)(TPPTS)was synthesized as described previously[2]. After labelling, a sample of the resulting solution was analyzed by ITLC and radio-HPLC. The internalization and efflux properties of the respective radioligands were tested in vitro. The biodistribution profiles and the imaging characteristics were determined in athymic mice bearing human PC-3 xenografts.

Results: The radiochemical yield and purity of ^{99m}Tc-HYNIC-Aca-BN(7-14) were 90% and >95% after purification respectively . Aca-BN(7-14) and BN(1-14) displayed a comparable binding affinity in PC-3 cell with IC_{50} in the lower nanomolar range (3.27±0.08nM and 3.48±0.08nM).The IC_{50} of HYNIC-Aca-BN(7-14) was 12.8±0.14 nM. The attachment of HYNIC group seems to reduce the GRPR binding affinity of Aca-BN(7-14). Figure 1 shows the internalization (left) and efflux (right) kinetics of ^{99m}Tc-HYNIC-Aca-BN(7-14). The internalization occurred during 30 min after start of incubation, maximum was reached with 79±8 % of the internalized radioactivity at 15 min postincubation.^{99m}Tc-HYNIC-Aca-BN(7-14) shows a long retention time in the PC-3 tumour cells in efflux experiment. More than 50% of the internalized radioactivity remained after 4 h p.i.

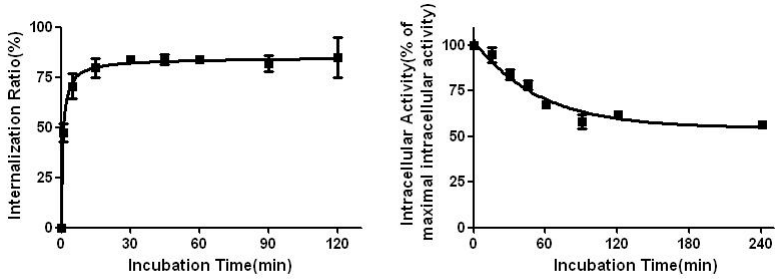

Figure 1 Internalization(left) and efflux(right) kinetics of ^{99m}Tc(HYNIC-Aca-BN(7-14))(Tricine)(TPPTS)

In general, ^{99m}Tc-HYNIC-Aca-BN(7-14) (Figure 2) had a rapid clearance, predominantly through the renal route. In the biodistribution study a relatively high uptake of ^{99m}Tc-HYNIC-Aca-BN(7-14)(2.24 ± 0.64 %ID/g) in human PC-3 xenografts was found at 0.5 h pi with a steady decrease over the 4 h study period. Generally, tumour-to-normal tissues ratio increased over time because of the long retention time of the radiotracer in tumour. In vivo blocking experiments (Figure 2, bottom) with HYNIC-Aca-BN(7-14) showed a significant reduction in the tumour uptake of radiotracer and several other organs, such as pancreas and intestine, indicating that uptake was GRPR-mediated.

Conclusions: The present study indicated that ^{99m}Tc(HYNIC-Aca-BN(7-14))(Tricine)(TPPTS) is a suitable tracer for in vivo tumour targeting. Work is in progress to implement this radiotracer for clinical studies.

References: [1]. Jia B, Shi J, Yang Z, Xu B, Liu Z, Zhao H et al.Bioconjugate Chem. 2006.17, 1069-1076 [2]. Shi J, Jia B, Liu Z, Yang Z, Yu Z, Chen K et al.Bioconjugate Chem. 2008; 19, 11701178.

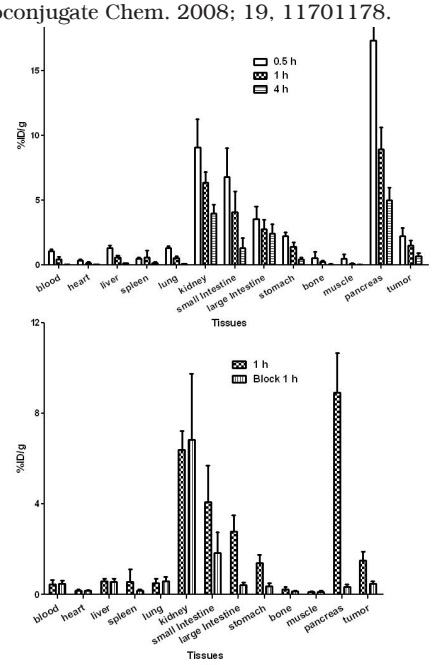

Figure 2 Organ uptake of ^{99m}Tc(HYNIC-Aca-BN(7-14))(Tricine)(TPPTS) in athymic mice bearing PC-3 human prostate cancer xenografts

P235 PET IMAGING OF VEGF-A TUMOR ANGIOGENESIS WITH 86Y-CHX-A''-DTPA-BEVACIZUMAB

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Objectives: The vascular endothelia growth factor (VEGF) is an important angiogenesis target for the development of cancer therapeutics. Bevacizumab, a humanized mAb binds to tumor-secreted VEGF-A and therefore inhibits tumor angiogenesis. In 2004, Bevacizumab was approved by the FDA for the treatment of metastatic colorectal carcinoma in combination with chemotherapy. In this report, we describe the preclinical evaluation of ^{86}Y-CHX-A''-DTPA-bevacizumab for potential use in quantitative PET imaging of VEGF-A tumor angiogenesis.

Methods: Bevacizumab was conjugated to the bifunctional chelating agent, CHX-A''-DTPA and radiolabeled with ^{86}Y (half-life= 14.7 h). Immunoreactivity was evaluated to determine the in vitro specificity of the radioimmunoconjugate (RIC). In vivo biodistribution and PET imaging studies were performed on mice bearing VEGF-A positive human colorectal (LS174T), human ovarian (SKOV3) and VEGF-A negative human mesothelioma (MSTO-211H) xenografts. Receptor-specificity was demonstrated by co-injection of 0.05 mg bevacizumab with the RIC.

Results: ^{86}Y-CHX-A''-DTPA-bevacizumab was successfully prepared with specific activity exceeding 2 GBq/mg and yields over 70%. Biodistribution and PET imaging studies demonstrated high specific tumor uptake of the RIC. In mice bearing VEGF-A +ve LS174T, SKOV3 and VEGF-A –ve MSTO-211H tumors, the tumor uptake at 3d pi were 13.6, 17.4 and 6.8 % ID/g, respectively. The corresponding tumor uptake in mice coinjected with 0.05 mg cold bevacizumab were 5.8, 8.9 and 7.4 % ID/g, respectively at the same time point, demonstrating specific blockage of the target in VEGF-A +ve tumors. The LS174T, SKOV3 were clearly visualized by PET imaging after injecting 1.8-2.0 MBq ^{86}Y-CHX-A''-DTPA-bevacizumab. Organ uptakes quantified by PET were closely related (r^2= 0.87, P= 0.64, n=18) to values determined by ex vivo biodistribution studies.

Conclusions: This preclinical study demonstrates the potential of the 86Y-CHX-A''-DTPA-bevacizumab radioimmunoconjugate for non-invasive assessment of the VEGF-A tumor angiogenesis status.

Research Support: Intramural NIH

P236 PRECLINICAL EVALUATION OF A CARBON-11 LABELLED 2-OXOQUINOLINE FOR CANNABINOID TYPE 2 RECEPTOR PET IMAGING

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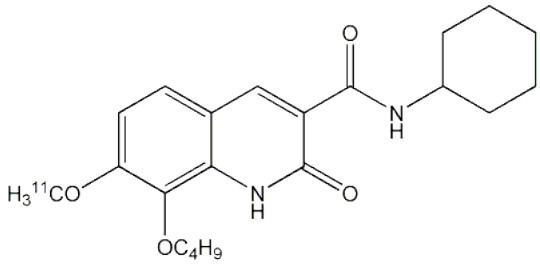
Objectives: The type 2 cannabinoid receptor (CB2R) is part of the endocannabinoid system and is predominantly expressed in cells related to the immune system. In contrast to the type 1 cannabinoid receptor, there is no significant expression of the CB2R in the cerebrum in control conditions. However, elevated CB2R expression is present in activated microglia, near senile amyloid plaques in Alzheimer patients and plaques of demyelination in multiple sclerosis patients. Furthermore, selective CB2R agonists/antagonists are being evaluated for the treatment of pain, inflammation and cancer and may provide neuroprotection. We reported (radio)synthesis and biological evaluation in mice of 7-[^{11}C]methoxy-2-oxo-8-butyloxy-1,2-dihydroquinoline-3-carboxylic acid cyclohexylamide ([1], Evens et al., Nucl Med Biol 2009, in press) showing in vivo CB2R affinity and high brain uptake, indicating promise for CB2R PET imaging in neuroinflammation. Here, we extended evaluation of [1] in rat and monkey brain in vivo.



[1]

Methods: Biodistribution studies were performed in normal rats in control conditions or after pretreatment with either 'cold' [1] or a CB2R specific inverse agonist at 2 and 60 min pi. Ex vivo autoradiography studies of rat spleen tissue were carried out in the same three conditions at 30 min pi. Blood was isolated to determine the proportion tracer in plasma and blood cells. μPET studies of normal mice, rat and monkey were performed.

Results: Specific CB2R binding in spleen was confirmed both by biodistribution studies and autoradiography studies. Brain uptake in rats was two times lower (0.8 % ID at 2 min pi) compared to mouse values. Tracer blood values were initially high (7.7 % ID at 2 min pi) and persistent (5.5 % ID at 60 min pi) suggesting binding of [1] to CB2R expressing blood cells. This was confirmed by preliminary blood tests where blood cell associated activity was decreased by 10-20 % by pretreatment of the animal with 'cold' [1] or a CB2R specific inverse agonist. Pilot monkey μPET studies show good initial brain uptake of the tracer (SUV = 1.7 at 1.5 min pi in frontal cortex) and fast wash-out (SUV = 0.2 at 27 min pi in frontal cortex).

Conclusions: [1] is a promising candidate for in vivo CB2 imaging in animal and human. The tracer thus deserves further evaluation in animal models for neuroinflammation (multiple sclerosis) and human studies.

Research Support: Research funded by a Ph.D. grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen).

P237 [^{11}C]GO6976 AS A POTENTIAL RADIOLIGAND FOR IMAGING PROTEIN KINASE C WITH PET

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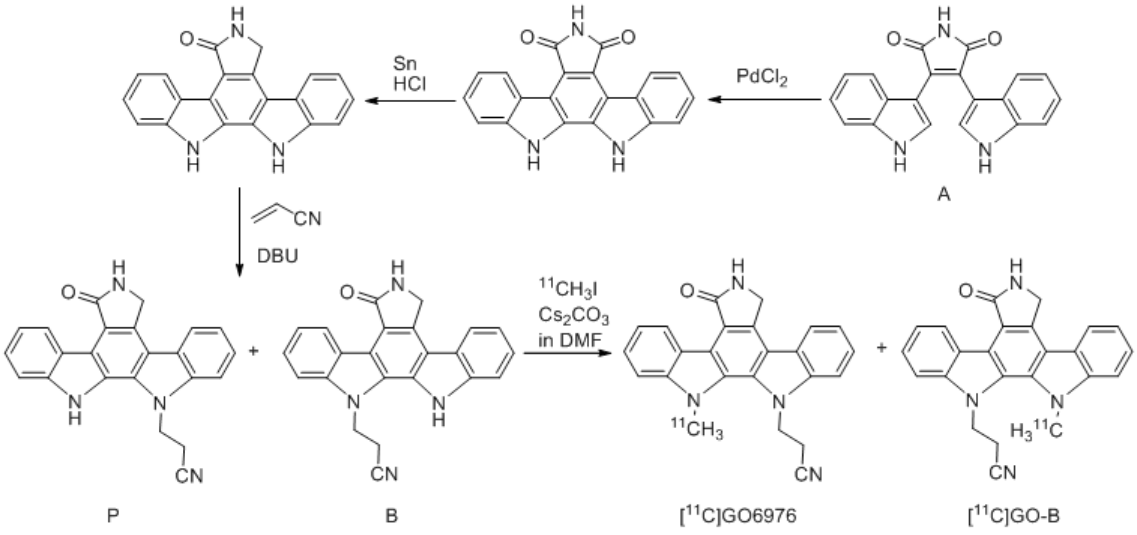
Objectives: Inhibition of protein kinase C (PKC) may become a new strategy for the treatment of bipolar disorder [Zarate C.A. et al., Bipolar Disord. 2007, 9, 561]. PET imaging of brain PKC may also prove useful for the investigation and diagnosis of bipolar disorder and the development of new therapeutics. GO6976 is a potent and selective PKC inhibitor [Martiny-Baron G. et al., J. Biol. Chem. 1993, 268, 9194]. Here we aimed to prepare [^{11}C]GO6976 (Scheme) for evaluation as a potential PET radioligand for imaging brain PKC.

Methods: An N-desmethyl precursor (P) was synthesized in three steps from a commercially available starting material (A, Scheme). P, also containing its unseparated isomer B, was treated with [^{11}C]CH_3I and Cs_2CO_3 in DMF at room temperature for 5 min, and the generated [^{11}C]GO6976 and its unseparated isomer ([^{11}C]GO-B, Scheme) were isolated with reverse phase HPLC. A rat (295 g) was injected with [^{11}C]GO6976/[^{11}C]GO-B (1.08 mCi; 3 Ci/μmol). Brain uptake of radioactivity was monitored for 70 min with an HRRT PET camera. A solution (0.30 mL, 2 mg/mL) of GO6976 in DMSO/PEG 400 (1: 2 v/v) was administered at 1 mg/kg body weight at 30 min after radioligand injection.

Results: Conditions for the preparative separation of the precursor (P) from its isomer (B) remain to be established. Analytical HPLC showed that the obtained mixture contained P and B in 2: 1 ratio. A variety of bases were evaluated for the radiolabeling of this mixture. However, only dry Cs_2CO_3 gave [^{11}C]GO6976 consistently. Low temperature was critical for successful radiolabeling. At 80 °C, acrylonitrile was eliminated from the obtained N-[^{11}C]methyl labeled product. Decay-corrected radiochemical yields of [^{11}C]GO6976/[^{11}C]GO-B were very low (~ 1%). The uptake of radioactivity in brain after i.v. injection of [^{11}C]GO6976 into rat was modest (1.0 SUV at ~ 9 min, n = 1). Administration of GO6976 displaced about 50% of the brain radioactivity.

Conclusions: The radioactivity in rat brain displaceable by carrier after administration of [^{11}C]GO6976/[^{11}C]GO-B may represent specific binding to PKC. Our initial PET findings warrant further improvement to precursor synthesis and [^{11}C]GO6976 radiosynthesis to allow further investigation of this radioligand behavior. This work is in progress.



Scheme. Synthesis of [^{11}C]GO6976

P238 SYNTHESIS AND BIOLOGICAL EVALUATION OF [11C]D617, A METABOLITE OF [11C]VERAPAMIL

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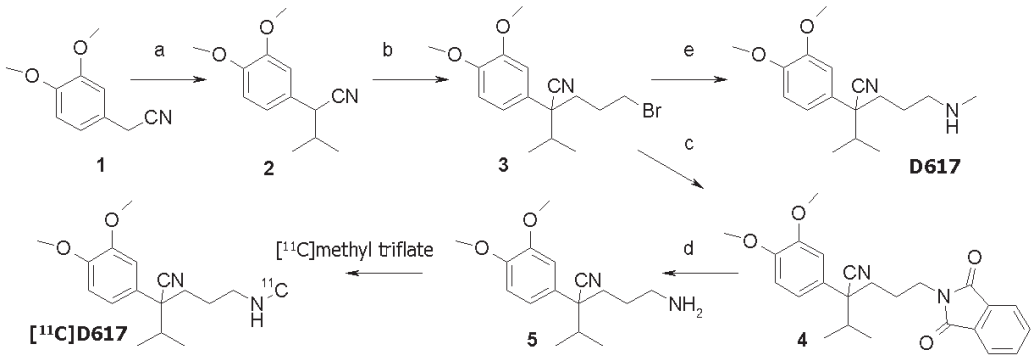
Objectives: Using [^{11}C]verapamil and positron emission tomography, it is possible to measure P-glycoprotein activity. In the optimal tracer kinetic model, however, it is assumed that the main radioactive metabolite of [^{11}C]verapamil, [^{11}C]D617, has similar pharmacokinetics as [^{11}C]verapamil itself[1]. To assess whether this assumption is correct, the pharmacokinetic properties of [^{11}C]D617 need to be evaluated. The aim of this study is to develop the synthesis of [^{11}C]D617 and to study its biodistribution in rats.

Methods: The precursor for labelling D617 with C-11, compound 5, was synthesized in 4 steps and subsequently reacted with [^{11}C]methyl triflate to give [^{11}C]D617 (scheme 1). Purification was achieved using preparative HPLC and the isolated product was reformulated with solid phase extraction in 10% ethanol and 90% 7.09 mM NaH_2PO_4 in 0.9% saline. The biodistribution of [^{11}C]D617 was determined in male Wistar rats (N=4) at 5, 15, 30 and 60 min after injection. Tissues of interest were dissected, counted for radioactivity and weighed. The biodistribution was also assessed at 30 min following injection of [^{11}C]D617 in a group of rats (N=4), pre-treated with 15 mg/kg tariquidar (PgP inhibitor) in order to determine whether [^{11}C]D617 is a PgP substrate.



Scheme 1. Syntheses of [^{11}C]D617; a: DMF, NaH, isopropyl bromide RT 5 hours 77%; b: THF, n-BuLi, 1,3-dibromopropane -78°C 2 hours 83%; c: toluene, 18-Crown-6-ether potassium phtalimide reflux 6 hours 86%; d: THF, EtOH, hydrazine·H_2O RT 2 hours 74%; e: methylamine, toluene/H_2O 56%.

Results: Precursor, 5, was synthesized in 41% overall yield. Reference D617 was synthesized in >98% purity and 56% yield starting from 3. The labelled product, [^{11}C]D617, was synthesized in 62-68% yield and with >99% (radio)chemical purity. Synthesis time was 50 minutes and specific activity was 70-94 GBq/μmol at end of synthesis. Brain uptake of [^{11}C]D617 was very low (<0.1% ID/g) and homogeneous. Peripheral biodistribution showed high clearance via liver and urinary tract. Pre-treatment with tariquidar did not alter the distribution of [^{11}C]D617, nor its uptake in the brain.

Conclusions: [^{11}C]D617 was synthesized with good yield and SA. Brain uptake was low and could not be enhanced by pre-treatment with tariquidar, indicating that [^{11}C]D617 is not a PgP substrate in this rat model. Whether metabolism of [^{11}C]D617 is causing this effect is currently under investigation.

Research Support: This work was funded by the EU 7th framework programme EURIPIDES, grant nr HEALTH-F5-2007-201380. The authors like to acknowledge H.J.C. Buiter, R.P. Klok, R.C. Schuit and J.D.M. Herscheid for assistance during the experiments, and the BV Cyclotron VU for providing [^{11}C]CO_2.

References: 1. Lubberink M, et alJ. Cereb.Blood Flow Metab. 2007;27, 1-10

P239 DEVELOPMENT OF NOVEL FLUORINE-18 LABELED PET TRACERS FOR IMAGING THE METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 5 (MGLUR5)

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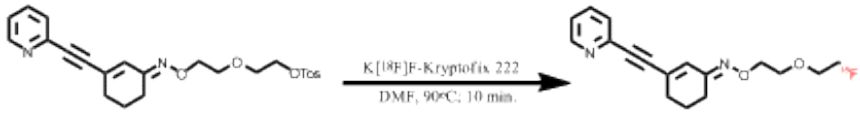
Objectives: The metabotropic glutamate receptor subtype 5 (mGluR5) is one of eight known subtypes of mGluRs. It is recognized to be involved in numerous neurodegenerative diseases such as M. Parkinson, M. Alzheimer and M. Huntington as well as in other central nervous system disorders such as neurogenic pain, epilepsy, depression, drug addiction and Fragile X. The neurobiological basis of these impairments is, however, still not well understood. Our group reported the first successful imaging of mGluR5 in humans using a carbon-11 labelled mGluR5 antagonist, [^{11}C]-ABP688 [1]. The physical half-life of carbon-11 is 20 minutes and, consequently, the use of [^{11}C]-ABP688 is limited to nuclear medicine centres with a radiochemistry facility. To obtain a widely applicable mGluR5 PET tracer, we decided to develop fluorine-18 (physical half-life = 110 min.) labeled derivatives of ABP688.

Methods: Based on the core structure of ABP688, several new compounds were designed. The derivatives were designed such that the compounds are amenable to ^{18}F labeling. Each compound was evaluated in vitro for its binding affinity using rat brain homogenates without cerebellum. For the most promising candidate, F-DEG-PECO, the radiolabeling of the appropriate precursor was carried out (Scheme 1). The LogP value of [^{18}F]-DEG-PECO was determined using the shake-flask method. Plasma stability tests were carried out in human and in mouse plasma. In vitro autoradiography using rat brain slices and a PET study on an SD rat were performed.

Results: A total number of 20 derivatives bearing the core structure of ABP 688 were successfully synthesized and characterized. The evaluation of these molecules in competition binding experiments using rat brain homogenate resulted in six candidates exhibiting K_i values below 10 nM. The highest binding affinity with a K_i value of 3.8 ± 0.4 nM was observed for F-DEG-PECO. Its corresponding tosyl-precursor was synthesized in eight steps. [^{18}F]-DEG-PECO was obtained in a radiochemical yield of 33 % and the radiochemical purity was higher than 97%. The specific activity ranged from 152 to 370 GBq/μmol. The compound showed a LogP of 1.68 ± 0.07. The plasma stability tests showed no radiometabolites up to 120 minutes of incubation. The in vitro autoradiography showed a heterogeneous uptake consistent with the distribution of mGluR5 in the rat brain. The preliminary data of the first in vivo imaging showed no in vivo defluorination.

Conclusions: Six novel mGluR5 antagonists were identified as promising candidates. The most promising compound, F-DEG-PECO, was successfully radiolabeled with fluorine-18 and the evaluation of its potential as an mGluR5 imaging agent in rodents is currently underway. The evaluation of the remaining promising candidates for their suitability as PET tracers for mGluR5 is also planned.

References: [1] S. M. Ametamey et al. Human PET studies of mGluR5 with [^{11}C]-ABP688 J. Nucl. Med., 2007, 48, 247-252.



Precursor [18F]-DEG-PECO

P240 SYNTHESIS AND EVALUATION OF N-METHYL-2-(2-AMINO-4-[18F]FLUOROPHENYLTHIO)BENZYLAMINE AS SERT IMAGING AGENT

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Objectives: Abnormalities in serotonin transporters (SERTs) have been implicated in several neuropsychiatric disorders and are the target for antidepressants. Several ^{11}C and ^{18}F labeled N,N-dimethyl-2-(arylthio)benzylamines have been developed as SERT imaging agents, with varying specific-to-non-specific binding ratios in vivo and with different brain uptake kinetics (1-5). The N-methyl-2-(arylthio)benzylamines, the metabolites of the N,N-dimethyl parent compounds, have been found to be a potent serotonin reuptake inhibitor, are less lipophilic, but have slightly higher and more enduring uptake in rat brain, and have higher binding affinity toward SERT than the parent N,N-dimethyl parent compounds (6-8). Thus, this class of compounds may also be potent SERT imaging agents. We reported herein the synthesis and evaluation of N-methyl-2-(2-amino-4-[^{18}F]fluorophenylthio) benzylamine (2a) as a SERT imaging agent.

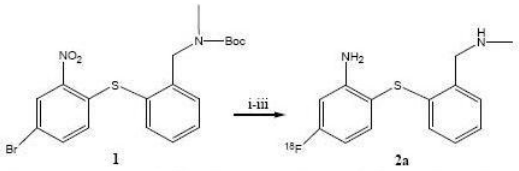
Methods: The bromo-precursor, N-methyl-N-tert-butoxycarbonyl-2- (2-nitro-4-bromophenylthio)benzylamine (1) was synthesized from 2,5-dibromonitrobenzene and thiosalicylic acid in 4 steps. The authentic N-methyl-2-(2-amino-4-fluorophenylthio) benzylamine (2) was synthesized from 2-chloro-5-fluoronitrobenzene and thiosalicylic acid in 3 steps. N-methyl-2-(2-amino-4-[^{18}F]fluorophenylthio)benzylamine (2a) was synthesized by nucleophilic substitution of the bromo-precursor (1) with K[^{18}F]/$K_{2.2.2}$ followed by reduction with $NaBH_4$/$Cu(OAc)_2$, de-protection with 6.5N HCl and purification with HPLC (Phenomenex Luna (2) C_{18}, 4.6 × 250 mm; CH_3CN:0.1 M HCO_2NH_4 [30:70] containing 0.3%, by volume, of acetic acid, 3.75 ml/min) and C_{18} Sep-Pak extraction (Scheme 1). The PET scanner used for this study was a micro-PET R4 scanner (Concorde MicroSystems, Knoxville, TN). Dynamic sinograms were produced with 12 x 10 sec, 6 x 30 sec, 5 x 300 sec, 3 x 600 sec and 4 x 900 sec frames. The data were expressed as %ID/g and the Specific Uptake Ratios (SURs) were expressed as (target – cerebellum)/cerebellum.

Results: The overall chemical yields of the bromo-precursor 1 and authentic 2 were 17 and 11%, respectively. The radiochemical yield of 2a was ~3% in a synthesis time of 120 min from EOB. The radiochemical purity was >98% and the specific activity was 2.08 Ci/μmol (EOS). μ-PET study in rat showed rapid and high influx of 2a into rat brain (1.2 ID%/g), but declined rapidly thereafter (Fig.1). The max SUR for midbrain, thalamus, hypothalamus, striatum, hippocampus and frontal cortex were 0.15, 0.33, 0.27, 0.51, 0.53 and 0.41, respectively, 30 min post-injection of 2a.

Conclusions: Compound 2a has been synthesized by aromatic nucleophilic fluorination. μ-PET study in rat showed that 2a has low specific binding to SERT and is not a suitable SERT imaging agent. This underscores the necessity to have two methyl groups substituted at benzylamine moiety of 2-(arylthio)benzylamines in order to be a suitable SERT imaging agent.

Research Support: This research was supported, in part, by the National Science Council of Taiwan, Grants NSC 95-2811-B-016-004, NSC 95-2321-B-016-001-MY2, NSC 97-2811-B-016-005, and NSC 97-2321-B-016-002.

References: 1. Shiue, G.G., Choi, S.R., Fang, P et al., (2003) J Nucl Med. 44, 1890-1897. 2. Huang, Y., Bae, S.A., Zhu, Z et al., (2005) J Med Chem. 48, 2559-2570. 3. Wang, J.L., Parhi, A.K., Oya, S et al., (2008) Nucl Med Biol. 35, 447-458. 4. Wilson, A.A., Ginovart, N., Schmidt, M., et al., (2000) J Med Chem. 43,3103-311. 5. Lundberg, J., Odano, I., Olsson, H et al., (2005) J Nucl Med. 46, 1505-1515. 6. Emond, P., Vercouillie, J., Innis, R et al., (2002) J. Med. Chem. 45:1253-1258. 7. Silver, I.S., Wurm, R.M., Welch, R.M et al., (1996) J. Liq. Chromatogr. Relat. Technol. 19:1117-26. 8. Wilson, A.A. and Houle, S. (1999) J. Labelled Cpd. Radiopharm. 42:1277-1288.



Scheme 1. Radiosynthesis of 2a. i. K[^{18}F]/$K_{2.2.2}$, DMSO, 120℃; ii. $NaBH_4$/$Cu(OAc)_2$, EtOH, 78℃, 20 min; iii. 6.5N $HCl_{(aq)}$, 120℃, 5 min.

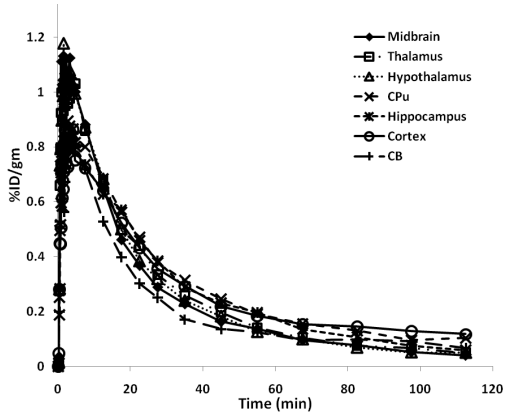


Fig. 1. TACs of **2a** in rat brain.

P241 DEVELOPMENT AND PRELIMINARY EVALUATION OF A 11C LABELED SULFONAMIDE DERIVATES AS POTENTIAL PET TRACER FOR BLOOD POOL IMAGING

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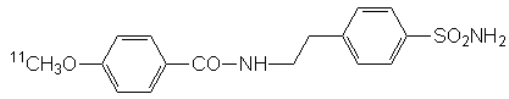
Objectives: Radionuclide blood pool imaging is applied for investigation of heart function, gastrointestinal hemorrhage and localization of intramuscular hemangioma. Carbonic anhydrase II (CA II), a metalloenzyme found in erythrocytes (RBC), can specifically be targeted by a sulfonamide derivative[1], which could be the basis for development of a radiotracer for in vivo labelling of RBC. In this study we present a preliminary evaluation of N-{2-[4-(aminosulfonyl) phenyl]ethyl}-4-[^{11}C]methoxy-benzamide ([^{11}C]-3) as a potential PET-tracer for in vivo visualization of erythrocytes.



N-{2-[4-(aminosulfonyl)phenyl]ethyl}-4-[^{11}C]methoxy-benzamide ([^{11}C]-3)

Methods: The labeling precursor (N-{2-[4-(aminosulfonyl)phenyl]ethyl}-4-hydroxybenzamide 2) was synthesized starting from 4-acetoxybenzoic acid, which was first converted to the acid chloride and then conjugated with [4-(2-aminoethyl-benzene) sulfonamide (AEBS; 1) in pyridine. Finally, the acetyl protective group was removed with CH_3ONa. The cold reference compound 3 was made by acylation of 1 with 4-methoxybenzoyl chloride in pyridine. [^{11}C]-3 was synthesized by heating 2 with [^{11}C]CH_3I and Cs_2CO_3 in DMF at 90 °C for 15 min, followed by purification through RP- HPLC. The biodistribution of the tracer was evaluated in normal mice at 2 and 60 min p.i. In vitro studies were carried out by incubating [^{11}C]-3 tracer with mixed blood cells in plasma free or plasma rich medium at RT for 10 or 20 min followed by washing with PBS and counting of the wash and cell fractions using a gamma counter.

Results: [^{11}C]-3 was synthesized with a radiochemical yield of 30% and a radiochemical purity >99%. The identity of the tracer was confirmed by co-injection of authentic 3. After IV injection in mice, the majority of tracer uptake was observed in blood (80% and 67% of ID at 2 and 60 min p.i., respectively) and clearance was mainly through the hepatobilary system (9% and 12% of ID in respectively liver and intestines at 60 min p.i.). After incubation with mixed blood cells >94% of activity was associated with red blood cells, both in plasma rich and plasma poor medium. The retention of [^{11}C]-3 in red blood cells was reduced by 20% by addition of 5 μmol AEBS which has a Ki of 160 nM for CAII[2], indicating that retention of [^{11}C]-3 in erythrocytes is caused by binding to CAI/II.

Conclusions: We successfully synthesized a new benzenesulfonamide derivative and labeled it with [^{11}C]. Biodistribution studies in normal mice and in vitro studies using human blood cells showed a high tracer uptake in erythrocytes, suggesting this new tracer can be used for in-vivo labeling of erythrocytes for blood pool imaging with PET or μPET. Further research is ongoing to validate in vivo binding of similar compounds for blood pool imaging.

References: [1] Scozzafava A. et.al., Expert Opin. Ther. Pat. 2006, 16, 12, 1627-1664. [2] Vullo D. et.al., Bioorg. Med. Chem. Lett. 2003, 13, 6, 1005-1009.

P242 PREPARATION OF THE NOVEL FLUORINE-18-LABELED T140 ANALOG FOR PET IMAGING

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Objectives: The chemokine receptor CXCR4 and its endogenous ligand CXCL12 (stromal cellderived factor-1, SDF-1) are partners in multiple important functions in normal physiology involving the leukocyte chemotaxis in the immune system and progenitor cell migration during embryologic development of the cardiovascular, hemopoietic,and central nervous systems. It is highly expressed on the surface of malignant primary breast cancer cells and plays an important role in tumor metastasis. To develop a radiopharmaceutical for the imaging of CXCR4-expressing tumors in vivo,we choose a 14-residue peptidic CXCR4 inhibitor, Ac-TZ14011, as a precursor for radiolabeled peptides.

Methods: For ^{18}F labeling, N-succinimidyl-4-[^{18}F] fluorobenzoate (SFB) was synthesized and conjugated with the side chain of d-Lys^{8} which is distant from the residues indispensable for the antagonistic activity. The stability of the product N-4-[^{18}F] fluorobenzoyl-ACTZ14011 (1) was determined in vitro. The biodistribution of (1) was evaluated in normal mice at 10min,60min and 120min.

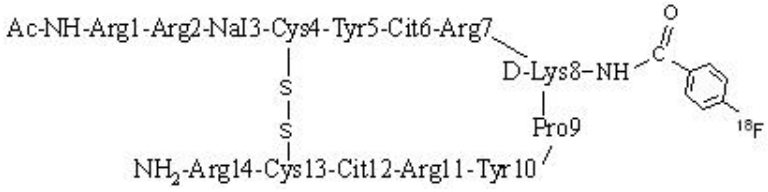

Fig. 1. The structure of N-4-[^{18}F]fluorobenzoyl-ACTZ14011.Nal: l-3-(2-naphthyl)alanine, Cit: l-citrulline

Results: (1) was produced in a decay-corrected radiochemical yield (RCY) of 25%±3% within 3h in four steps (n=5), the specific radioactivity of (1) was >5GBq/umol and the radiochemical purity (RCP) was >99%. The identity of (1) was confirmed by co-elution with the authentic non-radioactive compound on RP-HPLC. After 6 hours,the RCP of (1) in PBS is more than 90%. In mice, bone uptake was decreased from 3.5 %ID/g to 0.2% ID/g at 10mins and 120mins after iv injection. Clearance from the blood was rapid (<1% ID at 60 min p.i.) and proceeded mainly by the renal system.

Conclusions: Ac-TZ14011 inhibited the binding of SDF-1 to CXCR4 in a concentration-dependent manner with an IC_{50} of 1.2 nM as reported[1].(1) was shown good stability in PBS and good defluorination stability. Further evaluation of this tracer as potential PET probe in animal models is warranted.

Research Support: This study was funded in part by the project 06DZ19506 of Science and Technology Commission of Shanghai Municipality.

References: 1. Hirofumi Hanaoka et all .Nuclear Medicine and Biology 2006;33: 489C494.

P243 THE PREPARATION AND IN VITRO EVALUATION OF CARBORANE SELECTIVE ESTROGEN RECEPTOR MODULATORS

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Objectives: The objective of this study was to synthesize carborane containing Selective Estrogen Receptor Modulators (SERMs) to be evaluated for their ability to bind to the estrogen receptor (ER). Of particular interest was the preparation and in vitro evaluation of the closo species and the iodinated (^{125}I) analogues as potential imaging agents.

Methods: Promising in vitro binding assay results from a series of Re(I) and Tc(I) di-aryl carboranes,[1] has led to the synthesis of an iodinated mono-phenol carborane as well as a stereoselective synthesis to incorporate a closo carborane into the backbone of Tamoxifen, creating a novel and potentially catabolism resistant SERM. The radioactive iodine carboraneanalogues and their corresponding closo carboranes were subsequentlyevaluated for their ability to bind the ER by means of an uptake and inhibition assay respectively.

Results: All three closo carboranes were shown to inhibit breast cancer cell growth as much as or more effectively than Tamoxifen between 0.001 - 0.1μM in the presence of estradiol. The Z-carborane Tamoxifen isomer showed the best results in an estrogen free environment, while at low concentrations the E isomer and the mono-phenol carborane were ideal with estradiol present. Degradation of the closo carborane Tamoxifen analogue to the nido species under basic and aqueous conditions, using traditional or microwave heating, resulted in E-Z isomerization. In the presence of aprotic solvents, the nido species was prepared and halogenated with iodine. The radioiodinated mono-phenol carborane analogue and the two geometric carborane Tamoxifen analogues (Z and E) were successfully synthesized.

Conclusions: Three novel closo carborane SERMs showed superior growth inhibition to Tamoxifen in the presence of estradiol, while the optimal performance of each SERM was found to be dependent on the presence of estradiol.

References: Causey, P. W.; Besanger, T. R.; Valliant, J. F. J. Med. Chem. 2008, 51, 2833-2844.

P244 PRELIMINARY PET EVALUATION AND RADIOMETABOLISM OF A 18F-LABELED NR2B NMDA RECEPTOR ANTAGONIST

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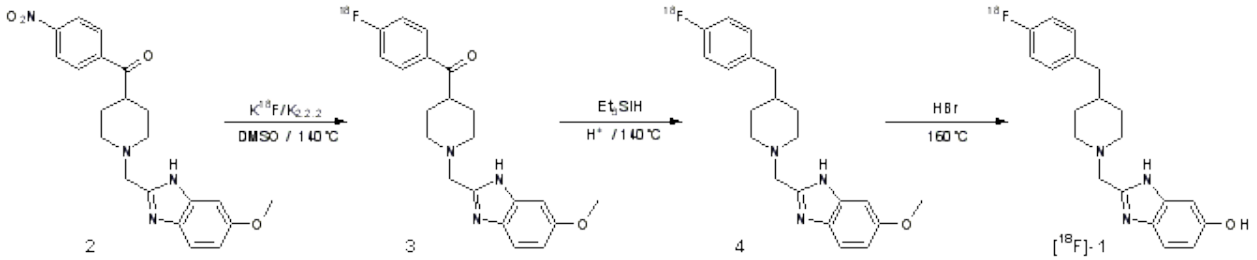
Objectives: The NR2B subtype of N-Methyl-D-aspartate (NMDA) receptors has been demonstrated to be of importance in physiopathological processes and the imaging of those receptors in vivo by PET would allow understanding their involvement in stroke or in neurodegeneratives disorders. Several PET radioligands have been developed to image NR2B receptors but poor brain penetration, high non-specific binding and/or fast biodegradation were observed. A series of benzimidazolol was described as potent NR2B antagonist (McCauley et al. J. Med. Chem. 2004, 2089-96). The compound 2-[(4-(4-fluorobenzyl)piperidin-1-yl) methyl]benzimidazol-5-ol (1) showed a high affinity (IC_{50}=1.1 nM) for NR2B receptors and selectivity towards NR2A subunit. We considered the ^{18}F-labeling of this compound 1 to evaluate its radiopharmacological properties.

Methods: [^{18}F]-1 was prepared by a three steps radiosynthesis from the 4-nitrobenzoylprecusor 2. In vitro autoradiographic studies were realized on rat brain sections incubated with the radiotracer during 90 min. Small animal PET experiments were performed in anesthetized rats during 90 min. The radiometabolite study was realized by TLC and HPLC analyses of plasma samples.



Results: The radiofluorination of 2 using $K^{18}F/K_{222}$ gave the 4-[^{18}F]-fluorobenzoylpiperidine intermediate 3 in 15-20% radiochemical yield after C18 solid phase extraction. Reduction using triethylsilane and triflic acid led to the benzylic compound 4 within 71% radiochemical yield. The deprotection of the methoxy group by HBr at 160°C afforded the final product [^{18}F]-1 within 69% yield. Reversed phase HPLC purification and formulation gave [^{18}F]-1 with an overall radiochemical yield about 5% and a radiochemical purity >99%. Incubation of rat brain sections in presence of [^{18}F]-1 showed specific binding in NR2B receptor rich regions. PET studies in rats demonstrated no passage through the blood brain barrier and high uptake in liver as well as in bones, jaw and cartilage. Plasmatic radiometabolite studies showed the presence of the parent compound (75% at 10 min, 50% at 35 min and 16% at 90 min) and polar compound. To assess the defluorination of the 4-(4-[^{18}F]-fluorobenzyl)piperidine moiety, we labeled this substructure in two steps from the 4-(4-nitrobenzoyl)-N-Boc-piperidine. PET scans on rats showed the same uptake profile for 4-(4-[^{18}F]-fluorobenzyl)piperidine than for [^{18}F]-1. Plasma metabolite studies showed faster plasmatic degradation (20% of intact product at 20 min) and presence of [^{18}F]-fluoride.

Conclusions: Despite specific binding observed with in vitro autoradiographic studies on rat brain sections, preliminary in vivo PET scans on rats demonstrated poor brain incorporation and metabolisation of [^{18}F]-1. Rapid defluorination was confirmed by 4-(4-[^{18}F]-fluorobenzyl)piperidine injections in rats. Those results opposed the use of the 4-(4-[^{18}F]-fluorobenzyl)piperidine moiety for PET radiotracers design.

P245 EVALUATION OF DOPAMINE RECEPTOR AGONISTS, 18F-5-OH-FPPAT, 18F-5-OH-FHXPAT and 18F-7-OH-FHXPAT

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Objectives: Dopamine D2/D3 receptor agonists are being used for PET imaging studies of schizophrenia, Parkinson's disease, substance abuse and other applications. Several carbon-11 labeled D2/D3 agonists have been successfully evaluated in animal and human studies. These include aminotetralins ^{11}C-5-OH-DPAT, ^{11}C-PPHT, ^{11}C-ZYY 111 (Shi et al., 1999; Mukherjee et al., 2000; 2004), ^{11}C-NPA (Hwang et al., 2000), ^{11}C-PHNO (Wilson et al., 2005) and ^{11}C-MNPA (Finnema et al., 2005). Efforts towards development of fluorine-18 labeled agonists have been fewer (Shi et al., 2004, Vasdev et al., 2007), but only (R,S)-^{18}F-5-OH-FPPAT has demonstrated potential as a PET imaging agent. We report further evaluation of the synthesis and evaluation of (R,S)-^{18}F-5-OH-FPPAT (1), (R,S)-^{18}F-5-OH-FHXPAT (2) and (R,S)-^{18}F-7-OH-FHXPAT (3).

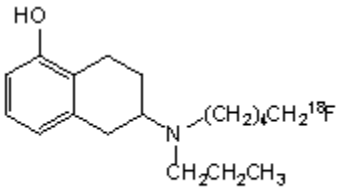
Methods: (R,S)-^{18}F-5-OH-FHXPAT and (R,S)-^{18}F-7-OH-FHXPAT were synthesized using modifications of our previously reported procedures for (R,S)-^{18}F-5-OH-FPPAT (Shi et al., 2004). Respective bromo precursors were radiolabeled with ^{18}F-Kryptofix-K_2CO_3 in CH_3CN at 96 °C for 30 min followed by 15 min reduction with lithium aluminum hydride (LAH) and deprotected with 1N HCl at 80 °C for 15 min. In vitro binding on 10 μm brain slices treated with 1-3 μCi/cc at 37 °C were carried out followed by autoradiographic analysis. Competition with sulpiride (10 mM), dopamine (1nM to 100μM), haloperidol (10 μM), SKF10047 (10μM) and GppNHp (10-50μM) were carried out to demonstrate selectivity of binding to dopamine D2/D3 high affinity states. Ex vivo imaging was carried out on brain slices.

Results: ^{18}F-Aminotetralins (1-3) were made in modest yields (approx 5-10%); the 3-step procedure makes the synthesis tedious and efforts are underway to optimize synthesis of precursors that will avoid the reduction step. Purification by HPLC (60%CH3CN-40% water containing 0.1% Et3N; flow rate 2.5 ml/min-C18 semiprep) provided specific activities of approx 2 Ci/mmol. Striata was clearly visualized by the three tracers; in vitro ratios were striata/cerebellum=10 (for 1) and 5 (for 2) and 3 (for 3). Pretreatment with GppNHp significantly reduced the striatal binding, suggesting high-affinity state binding of the radiotracers. Binding of 3 was also observed in other brain areas such as the cerebellum, consistent with D3 receptor localization. Nonspecific binding of the tracers, particularly 3 (to sigma receptors, displaced by SKF10047) was high.

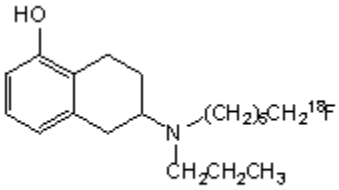
Conclusions: ^{18}F-Aminotetralins are suitable agonist imaging agents for dopamine D2 and D3 receptors. Five carbon chain provides higher ratios than the 6-carbon chain. 7-Hydroxy derivatives are more suitable for D3 receptors compared to the 5-hydroxy derivatives. Use of pure isomers with MicroPET imaging is currently underway.

Research Support: Research Supported by NIH R01 EB006110

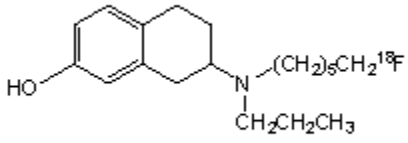
References: 1. Shi et al.,Nucl. Med. Biol., 26: 725-735, 1999; 31: 303-311, 20042. Mukherjee et al., Synapse, 37: 64-70, 2000; 54: 83-91, 2004. 3. Hwang et al., Nucl. Med. Biol., 27:533-539, 2000. 4. Wilson et al., J. Med. Chem., 48:4153-4160, 2005. 5. Finnema et al., Nucl. Med. Biol., 32:353-360, 2005. 6. Vasdev et al., Nucl. Med. Biol., 34:195-203, 2007.



^{18}F-5-OH-FPPAT,1



^{18}F-5-OH-FHXPAT,2



^{18}F-7-OH-FHXPAT,3

P246 COMPARISONS OF 2-[18F]-ADAM, 4-[18F]-ADAM AND [18F]AFM AS SERT IMAGING AGENTS IN RATS USING μPET

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Objectives: Abnormalities in serotonin transporters (SERTs) have been implicated in several neuropsychiatric disorders and are the target for antidepressants. Several ^{11}C and ^{18}F labeled N,N-dimethyl-2-(arylthio)benzylamines have been developed as SERT imaging agents, with varying specific-to-non-specific binding ratios in vivo and with different brain uptake kinetics [1-5]. Both 2-[2-(dimethylaminomethyl)-phenylthio]- 5-[^{18}F]fluoromethylphenylamine ([^{18}F]AFM, 1) and N,N-dimethyl-2-(2-amino-4- [^{18}F] fluorophenylthio)benzylamine (4-[^{18}F]-ADAM, 2) are potent SERT imaging agents (1,2). We report herein the direct comparisons of 1, 2 and the newly developed N,N-dimethyl-2-(2-[^{18}F]fluoro-4-aminophenylthio)benzylamine (2-[^{18}F]-ADAM, 3) as SERT imaging agents in rats using μPET.

Methods: Compound 1 was synthesized by the reported method [1]. Compound 2 was synthesized by the reaction of N,N-dimethyl-2-(2,4-dinitrophenylthio)- benzylamine with K[^{18}F]/$K_{2.2.2}$ @ 120°C for 10 min followed by reduction with $NaBH_4$/$Cu(OAc)_2$ at 80°C and purification with HPLC (Phenomenex Luna (2) C_{18}, 4.6 × 250 mm; CH_3CN:0.1 M HCO_2NH_4 [30:70] containing 0.3%, by volume, of acetic acid, 3.75 ml/min) and C_{18} Sep-Pak extraction (Scheme 1) [2]. Compound 3 was synthesized by the same reaction conditions as that described for the synthesis of compound 2. The authentic N,N-dimethyl-2-(2-fluoro-4-aminophenylthio)-benzylamine (3) was synthesized from 3-fluoro-5-bromoaniline and thiosalicylic acid in 3 steps. Male S-D rats and micro-PET R4 scanner (Concorde MicroSystems, Knoxville, TN) were used for the experiments. Dynamic sinograms were produced with 12 x 10 sec, 6 x 30 sec, 5 x 300 sec, 3 x 600 sec and 4 x 900 sec frames. The data were expressed as %ID/g and the Specific Uptake Ratios (SURs) were expressed as (target – cerebellum)/cerebellum.

Results: The overall chemical yield of the authentic 3 was 12%. The radiochemical yields of compounds 1, 2 and 3 were ~1, 6 and 14%, respectively, in a synthesis time of 120 min from EOB. Specific activity was ~3 Ci/μmol. The log P of 1, 2 and 3 were 2.44, 2.73 and 1.34, respectively. μPET studies showed that the uptake of 1, 2 and 3 in midbrain were 1.08, 0.87 and 0.43 %ID/g, respectively, and both 1 and 2 had high specific uptake in rat brain regions rich in SERT while 3 had low specific uptake in rat brain (Fig. 1). The maximum SUR for midbrain, hypothalamus, striatum, hippocampus and frontal cortex were 2.28, 2.20, 1.85, 1.21 and 1.22, respectively, at 55 min post-injection of compound 1. The corresponding values forcompound 2 were 3.95, 3.81, 3.83, 2.36 and 2.36, respectively, 83 min post-injection. In contrast, the maximum SUR for midbrain, hypothalamus, striatum, hippocampus and frontal cortex were only 0.50, 0.60, 0.74, 0.47 and 0.44, respectively, at 12 min post-injection of compound 3.

Conclusions: Both 1 and 2 are potent SERT imaging agents, although the radiochemical yield of 1 is too low for practical applications. In contrast, 3 is not a useful SERT imaging agent.

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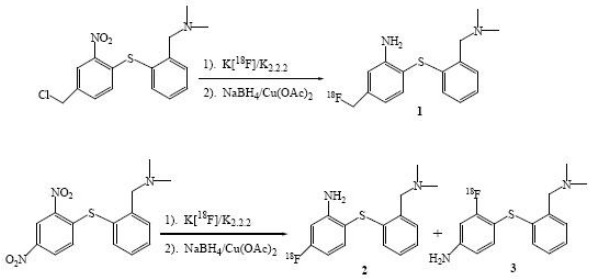
References: 1. Huang, Y., Bae, S.A., Zhu, Z et al., (2005) J Med Chem. 48, 2559-2570. 2. Shiue, G.G., Choi, S.R., Fang, P et al., (2003) J Nucl Med. 44, 1890-1897. 3. Wang, J.L., Parhi, A.K., Oya, S et al., (2008) Nucl Med Biol. 35, 447-458. 4. Wilson, A.A., Ginovart, N., Schmidt, M., et al., (2000) J Med Chem. 43,3103-311. 5. Lundberg, J., Odano, I., Olsson, H et al., (2005) J Nucl Med. 46, 1505-1515.



Scheme 1. Synthesis of **1, 2** and **3**.

P247 LABELLING AND IN-VIVO EVALUATION IN BABOONS OF A NOVEL [11C]-CARBON LABELLED IMIDAZOPYRIDINE, FOR THE STUDY OF THE PERIPHERAL BENZODIAZEPINE RECEPTORS USING PET

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Objectives: Peripheral benzodiazepine receptors (PBR) are implicated in a number of neurodegenerative and inflammatory disorders as well as in cancer. Hence the development of specific PBR tracers for imaging these conditions using PET and SPECT would be highly desirable. Limitations with the use of [^{11}C]-PK11195 in imaging has seen a considerable effort in the development of new PBR ligands that could be radiolabelled with both PET and SPECT isotopes. The aim of this study was to radiolabel the novel high affinity and selective imidazopyridine 1 and evaluate its suitability as a probe for imaging PBR receptors in vivo.

Methods: The radiolabelling of 1 with [^{11}C]-methyl iodide was investigated in a variety of solvents and conditions. Reactions in acetonitrile, DMF or acetone using NaH or NaOH at temperatures ranging from room temperature to 80^{o}C were investigated. The crude reaction mixtures were purified by HPLC followed by trapping on a Sep-Pak® C18 light and elution with ethanol. Dilution with saline gave [^{11}C]-1 ready for biological evaluation. The distribution kinetics and selectivity of [^{11}C]-1 in non-human primates (Papio Hamadryas) is currently being investigated by PET/CT.

Results: In-vitro binding studies indicated that 1 is a selective PBR ligand (IC_{50} = 4.5 nM) with low affinity for the central benzodiazepine receptors (IC_{50} = >10 000 nM). N-methylation of the desmethyl precursor 2 with [^{11}C]-methyl iodide in acetonitrile or DMF, using NaH or NaOH, at temperatures ranging from 30-80^{o}C gave either low yields or complex reaction mixtures. The optimum [^{11}C]-1 methylation conditions were achieved by bubbling [^{11}C]-methyl iodide into a solution of precursor 2 in acetone in the presence of aqueous sodium hydroxide at RT for 2 mins then heated at 80°C for 3 mins. HPLC purification followed by Sep-Pak® C18 light trapping and reconstitution in saline gave [^{11}C]-1 in 55-65% (n = 15) radiochemical yield with > 99% radiochemical purity and a specific activity > 55 GBq/mmol after approximately 40 mins of synthesis time.

Conclusions: The imidazopyridine 1 is a novel, high affinity and selective ligand for the PBR with a very low affinity for CBR. Consequently [^{11}C]-1 could be prepared in one step using aqueous sodium hydroxide with good radiochemical yield and radiochemical purity suitable for biological evaluation. The investigation of [^{11}C]-1 in baboons as a potential marker for the PBR using PET/CT will be presented. The presence of the fluoropyridine side chain also allows for the potential radiolabelling of 1 with F-18.

P248 SYNTHESIS AND BIOLOGICAL EVALUATION OF CYCLIC RGD-MONO-TO HEXADECIMERS FOR ENHANCED avb3 INTEGRIN IMAGING

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Objectives: RGD peptides have gained widespread interest in diagnostic imaging for cardiologic as well as oncologic purposes. Recently, several studies investigated the use of the multimerization of RGD peptides for enhanced binding affinities enabling a higher quality of the imaging results. Mainly mono-, di- and tetramers have been investigated, but also octamers have been synthesized recently. The aim of this study was to determine the limit of RGD-multimerization (regarding complete substitution of the scaffold structure and high homogeneity) and to investigate the obtained multimers regarding their binding affinities to avb3 integrin and their in vivo properties.

Methods: For the multimerization reactions, PAMAM dendrimer scaffolds of different size and number of derivatization sites and high homogeneity were used. The application of dendritic scaffolds also results in the extension of the distance between two RGD moieties which has been shown to further enhance the binding affinities of the multimers. A main problem in the synthesis of multimeric structures is an incomplete derivatization of the scaffold structure. To obtain highly homogeneous target structures, the amino functions of the dendritic PAMAM systems had to be derivatized thoroughly with RGD moieties. Competitive receptor binding assays were performed using immobilized avb3 integrin and human glioblastoma cells (U87MG) with 125I-echistatin. Biodistribution and μPET studies were carried out using a U87MG cell xenograft nude mice model.

Results: By using maleimide-thiol-coupling, highly homogeneous and completely RGD-derivatized dendritic structures could be synthesized and the products could be identified via mass spectrometry. The obtained RGD-multimers contained 1, 2, 4, 8 and 16 cyclic RGD peptides and a DOTA moiety for radiolabeling. The synthesis of larger multimeric systems has shown to be intricate due to the low solubility of multimeric systems containing more than 20 RGD peptides. The RGD-multimers were labeled with 68Ga and their avb3 binding affinities were investigated, revealing that the affinities increased from the monomer (Ki=32 nM) to the hexadecimer (Ki=0.26 nM). First in vivo experiments using U87MG tumor-bearing mice showed very promising results for the large multimeric systems with specific tumor uptake of 4 %ID/g at 60 min p.i.

Conclusions: RGD-multimers of different size and number of RGD moieties were synthesized on dendritic scaffolds to determine the limit of RGD-multimerization. Multimers containing from one to 16 RGD peptides were successfully synthesized and their homogeneity was shown by mass spectrometry. The multimers expectedly showed an increase of avb3 binding affinity with increasing number of RGD moieties. Together with the promising in vivo results, the large RGD-multimers suggest high potential for use in cardiologic and oncologic imaging applications.

P249 SYNTHESIS AND EVALUATION OF A NEW RADIOLABELED BOMBESIN ANALOGUE FOR DIAGNOSIS OF BOMBESIN RECEPTOR EXPRESSING TUMORS

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Objectives: Bombesin a 14-amino acid shows high affinity for BN/ GRP receptors, which are overexpressed by a variety of cancers, including prostate, breast, pancreas, gastrointestinal, and small cell lung cancer. In this study we synthesis and evaluate a ^{99m}Tc-Bombesin analogue as an imaging agent for BN/ GRP receptor-positive tumors.

Methods: Synthesis of the HYNIC peptide was carried out on Rink Amide MBHA (4-Methylbenzhydrylamine) resin. ^{99m}Tc labeling was performed in the presence of Coligand Tricine/EDDA. Radiochemical evaluation was carried out by Reversed phase HPLC and ITLC-SG. In-vitro internalization was tested using human prostate cancer PC-3 cells with blocked and non-blocked receptors.Biodistribution was determined in rats.

Results: [^{99m}Tc-EDDA-HYNIC^{0}, D-Tyr^{6}, D-Trp^{8}] Bombesin (6-14) was obtained with radiochemical purities >95%. Results of in-vitro studies demonstrated a high stability in serum and suitable internalization. Biodistribution data showed a rapid blood clearance, with renal excretion and binding towards BN /GRP receptor-positive tissues such as pancreas.

Conclusions: This novel Bombesin conjugate with ^{99m}Tc has promising Characteristics for the diagnosis of malignant tumors.

Research Support: 1-Nuclear Science Research School, Nuclear Science & Technology Research Institute (NSTRI), Atomic Energy Organization of Iran, Tehran, Iran 2-Department of Medicinal Chemistry and nuclear pharmacy, School of Pharmacy, Medical Sciences University of Tehran, Tehran, Iran

References: 1-Okarvi S.M. (2004) Peptide-based radiopharmaceuticals: future tools for diagnostic imaging of cancers and other diseases. Med. Res. Rev. 24,357. 2.Okarvi S.M., Jammaz I. (2003). Synthesis, radiolabelling and biological characteristics of a bombesin peptide analog as a tumor imaging agent. Anticancer. Res. 23, 2745. 3.Lin K.S., Luu A., Baidoo K.E., et al. (2005).A new high affinity technetium- 99m-bombesin analogue with low abdominal accumulation. Bioconj. Chem. 16, 43. 4.Gandomkar, M., Najafi, R., Shafiei, M., Mazidi, M., SadatEbrahimi, S.E. (2007) Preclinical evaluation of^{99m}Tc/EDDA/tricine/HYNIC^{0}, 1-Nal^{3}, Thr^{8}]-octreotide as a new analogue in the detection of Somatostatin-receptor positive tumors. Nucl. Med. Biol .34, 651 5.Smith C.J., Volkert W.A., Hoffman T.J. (2005). Radiolabeled peptide conjugates for targeting of the bombesin receptor superfamily subtypes. Nucl .Med. Biol. 32,733.

P250 SYNTHESIS OF A PROSPECTIVE ^{18}F-LABELED TRACER FOR IMAGING P-GLYCOPROTEIN FUNCTION

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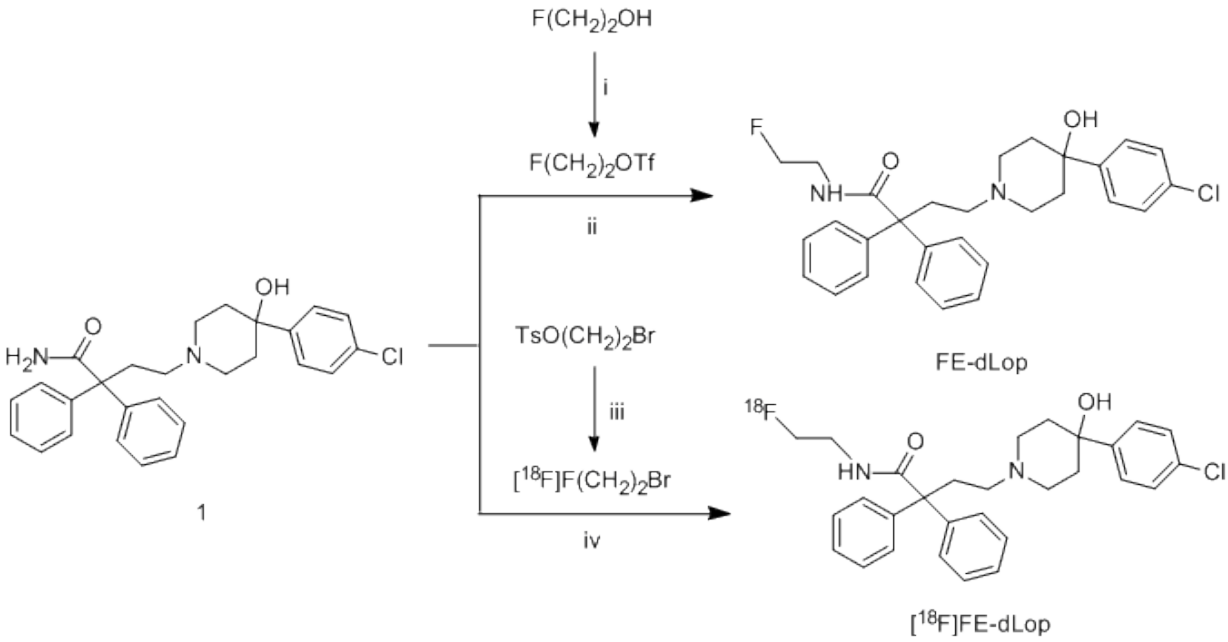
Objectives: P-Glycoprotein (P-gp) functions as a drug efflux pump at the blood-brain barrier and some other tissues, including some tumors. Radiotracers for imaging P-gp function in vivo could be valuable, for example, in assessing the role of P-gp in neuropsychiatric disorders and multi-drug resistance during cancer chemotherapy. Loperamide is a potent μ-receptor agonist which is nevertheless a safe antidiarrheal drug because it is excluded from brain by P-gp. Both [N-methyl-^{11}C]loperamide and its primary metabolite, [N-methyl-^{11}C]N-desmethyl-loperamide ([^{11}C]dLop), have been evaluated as radiotracers for imaging P-gp function. [^{11}C]dLop shows greater promise because of its more favorable metabolic profile [Lazarova et al., J. Med. Chem., 2008, 51, 6034]. We considered that an ^{18}F-labeled analog of [^{11}C]dLop ([^{18}F]FE-dLop; Scheme) might also behave as a prospective radiotracer for imaging P-gp function and potentially offer an advantage of greater availability for wider application. Here we aimed to prepare [^{18}F]FE-dLop.

Methods: Synthesis of FE-dLop: Triflic anhydride (10 mmol) was added slowly to a solution of 2-fluoroethanol (10 mmol) and Et_3N (10 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 1 h at room temperature, concentrated, and transferred to a mixture of the amide 1 (0.36 mmol) and NaH (0.39 mmol) in DMF (5 mL). This mixture was then stirred for 12 h at 80 °C. Chromatography (silica gel; hexane/EtOAc, 1: 3 v/v; then EtOAc) of the crude mixture, followed by HPLC on a Luna C18 column (250 × 10 mm) eluted at 8 mL/min with 0.025% aq. NH_4OH (A)-MeCN (B), with B increased from 30 to 100% over 30 min, gave FE-dLop (t_R = 16.8 min) in high purity. Radiosynthesis of [^{18}F]FE-dLop: Cyclotron-produced [^{18}F]fluoride ion solution (100–120 mCi) was mixed with Kryptofix 2.2.2 (5 mg) and K_2CO_3 (0.5 mg) in MeCN-H_2O (95: 5 v/v; 0.1 mL) and dried by two cycles of addition-evaporation of MeCN (2 mL). 2-Bromoethyl tosylate (30 μL) in t-butanol plus 1,2-dichlorobenzene (1 mL; 1: 9 v/v) was added and then heated at 90 °C for 10 min. [^{18}F]2-Fluoroethyl bromide (20–25 mCi) was distilled out, passed through a silica Sep-Pak cartridge, and trapped in a sealed V-vial containing amide 1 (2 mg) and NaH (0.5 mg) in DMF (250 μL). The reaction mixture was heated at 110 °C for 10 min, cooled, and diluted with MeCN-H_2O (1: 1 v/v). A sample was injected onto a Prodigy column (250 × 4.6 mm) eluted at 1 mL/min with mobile phase A-B (3: 7 v/v). The decay-corrected radiochemical yield (RCY) of [^{18}F]FE-dLop (t_R = 11.5 min) from labeling agent was estimated from the radio-chromatogram.

Results: FE-dLop was synthesized in 20% yield from 1. The RCY of [^{18}F]FE-dLop is low but consistent (~ 5–7%). The low RCY may be due to product decomposition through cyclization.

Conclusions: [^{18}F]FE-dLop was obtained in low RCY. Improvement of the procedure through use of other conditions and labeling reagents is in progress.



Scheme. Syntheses of FE-dLop and [^{18}F]FE-dLop. (i) Et_3N, $(CF_3SO_2)_2O$, rt, 1 h; (ii) NaH, DMF, 80 °C, 12 h, 20%; (iii) [^{18}F]fluoride ion, K_2CO_3, K 2.2.2, t-butanol and 1,2-dichlorobenzene, 90 °C, 10 min; (iv) NaH, DMF, 110 °C, 10 min, RCY 7%.

P251 RADIOFLUORINATION AND PHARMACOLOGICAL EVALUATION OF [18F]FLUOROPHENYLSULFONYL- AND [18F]FLUOROPHENYLSULFINYL-PIPERIDINES AS SEROTONIN 5-HT2A RECEPTOR ANTAGONISTS

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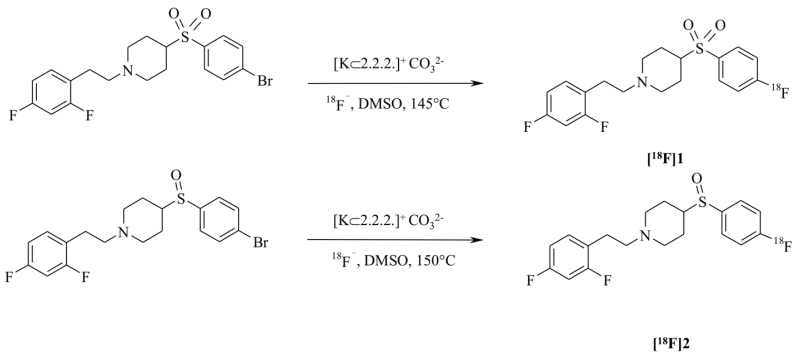
Objectives: In psychiatric disorders 5-HT_{2A} receptors play an important role. For studying this receptor system in vivo using PET, there is an increasing interest in obtaining a metabolically stable, subtype selective and high affinity radioligand. Thus, 1-(2,4-difluorophenethyl)-4-(4-fluorophenylsulfonyl)piperidine 1, which has shown high affinity and selectivity for 5-HT_{2A} receptors in in vitro studies [Fletcher et al. J. Med. Chem. 2002, 45, 492] and its analogue 1-(2,4-difluorophenethyl)-4-(4-fluorophenylsulfinyl) piperidine 2 were chosen for ^{18}F-labeling and pharmacological evaluation.

Methods: The radiosynthesis of both, 1-(2,4-difluorophenethyl)-4-(4-[^{18}F]fluorophenylsulfonyl)piperidine [^{18}F]1 and 1-(2,4-difluorophenethyl)-4-(4-[^{18}F]fluorophenylsulfinyl)piperidine [^{18}F]2, was performed by a direct ^{18}F-for-Br exchange using a Kryptofix2.2.2/K_2CO_3 mediated reaction in DMSO at 145 °C and 150 °C, respectively. The lipophilicity of 1-(2,4-difluorophenethyl)-4-(4-fluorophenylsulfonyl)piperidine 1 and 1-(2,4-difluorophenethyl)-4-(4-fluorophenylsulfinyl)piperidine 2 was determined using the HPLC method corresponding to the OECD guideline for the testing of chemicals. Furthermore, in vitro autoradiography studies with 1 and 2 in comparison with (+)-MDL 100907 competing [^{3}H]ketanserine, and assays with fluorine-18 labeled 1 and 2 with (+)-MDL 100907 for blocking on horizontal rat brain slices were performed.

Results: The time of preparation and isolation for both radiotracers was 45 min, [^{18}F]1 with a RCY of 34.5 ± 8 % and [^{18}F]2 with 9.5 ± 2.5 %. The $logD_{7.4}$ values of 2.47 for 1 and of 2.67 for 2 are comparable to MDL 100907 with 1.99. The K_i values of 1 and 2 towards the 5-HT_{2A} receptor were determined using [^{3}H]ketanserine to be 1.9 ± 0.6 nM and 198 ± 8 nM, respectively. In vitro autoradiography of [^{18}F]1 on horizontal rat brain slices showed high non-specific binding (about 80 %) in frontal cortex, while for [^{18}F]2 this was only 43 %.

Conclusions: Even though the K_i values of 1 were very promising with regard to the affinity and selectivity of this compound, [^{18}F]1 exhibits an intolerable high non-specific binding. This is much lower with [^{18}F]2 but can not compensate for its low affinity. Thus, both tracers are unsuitable as in vivo imaging agents for the serotonin 5-HT_{2A} receptor studies using PET.



[^{18}F]1

[^{18}F]2

P252 EVALUATION OF A Cu-64-LABELED SarAr CONJUGATED BOMBESIN ANALOG FOR DETECTION OF GASTRIN-RELEASING PEPTIDE RECEPTOR POSITIVE TUMORS

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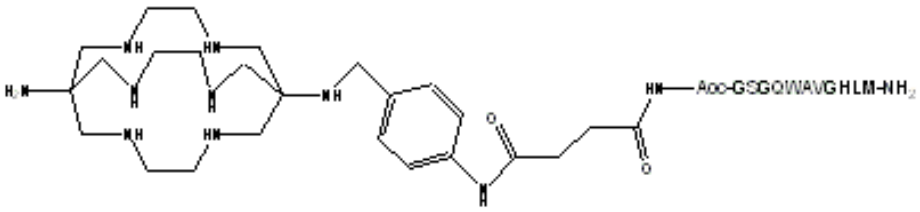
Objectives: Bombesin (BN) is a fourteen amino acid amphibian peptide that binds with high affinity to the gastrin-releasing peptide receptor (GRPR), which is overexpressed on a variety of solid tumors. It has been demonstrated that BN analogs can be radiolabeled with a variety of radiometals for potential diagnosis and treatment of GRPR-positive tumors. In this regard, a number of studies have utilized different chelators conjugated to the eight C-terminal amino acids of BN (BN(7-14)) for radiolabeling with ^{64}Cu. These analogs have demonstrated GRPR-specific microPET imaging of tumors, but have various advantages and disadvantages. The objective of this study is to conjugate the previously described (1-N-(4-aminobyenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-eicosane-1,8-diamine) (SarAr) chelator to BN(7-14), radiolabel the conjugate with ^{64}Cu, and evaluate in vitro and in vivo.

Methods: SarAr was synthesized as previously published and conjugated to BN(7-14) by solid phase peptide synthesis using standard Fmoc chemistry. Succinic acid (SA), 8-aminooctanoic acid (Aoc), and Gly-Ser-Gly (GSG) were used as linkers between SarAr and BN(7-14) to yield the resulting SarAr-SA-Aoc-GSG-BN(7-14) conjugate (Figure). The unlabeled peptide was evaluated in a competitive binding assay using human PC-3 androgen independent prostate cancer cells and ^{125}I-Tyr^4-BN to determine an IC_{50} value. SarAr-SA-Aoc-GSG-BN(7-14) was radiolabeled with ^{64}Cu by incubating at room temperature for 30 min in 0.1M NH_4OAc pH 5.5 and radioactive purity was determined to by TLC. ^{64}Cu-SarAr-SA-Aoc-GSG-BN(7-14) was added to PC-3 cells and incubated for 1 h at room temperature with or without an excess of Tyr^4-BBN as an inhibitor.

Results: SarAr-SA-Aoc-GSG-BN(7-14) was > 95% pure by HPLC and confirmed by electrospray mass spectrometry. Calculated mass ($C_{83}H_{134}N_{26}O_{16}S$), 1784.20; (ES+MS) m/z, 892.95 (M+2), 595.60 (M+3). The radiochemical purity of ^{64}Cu-SarAr-SA-Aoc-GSG-BN(7-14) was > 95% with a specific activity of ~200 mCi/mg and the product was used without further purification. The competitive binding assay (n = 3) demonstrated that SarAr-SA-Aoc-GSG-BN(7-14) had an IC_{50} value of 1.9 ± 0.5 nM. Binding of ^{64}Cu-SarAr-SA-Aoc-GSG-BN(7-14) to PC-3 cells showed that 4,700 ± 760 cpm bound without inhibitor compared 2,060 ± 510 cpm in the presence of inhibitor.

Conclusions: These studies demonstrate that SarAr-SA-Aoc-GSG-BN(7-14) bound with high affinity to GRPR expressing cells. In addition, it could be radiolabeled with ^{64}Cu at room temperature and the resulting radiolabeled product bound specifically to GRPR expressing cells. ^{64}Cu-SarAr-SA-Aoc-GSG-BN(7-14) will be evaluated for tumor uptake in vivo using biodistribution and imaging studies.



P253 SYNTHESIS OF [3H]FALLYPRIDE USING [3H]METHYL NOSYLATE

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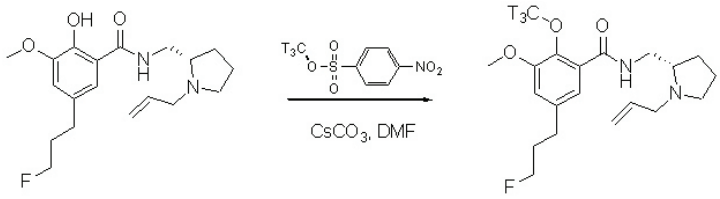
Objectives: Dopamine is the predominant catecholamine-neurotransmitter in the human brain. Malfunctions in the dopaminergic neurotransmission are associated to several neuropsychiatric diseases. Various radio-labelled benzamides which are highly selective towards the D_2/D_3-receptors have been developed and are used to visualize these receptors in vivo via PET/SPECT. One of the most promising structures is the [^{18}F]-labelled benzamide [^{18}F]Fallypride. Due to its excellent affinities and selectivity it is an ideal tracer for visualizing receptor availabilities in brain regions with high and low receptor density. However, long-term experiments as e.g. autoradiographies and replacement studies are limited to the short half-life of [^{18}F]fluorine. The introduction of tritium into a molecule provides a way to accomplish the desired experiments. Consequently, it was the aim to introduce a tritium label into the original Fallypride structure.

Methods: Tritium can be introduced via many different routes into organic molecules. One of the most favoured synthetic pathways is the halogen/tritium-exchange using tritium gas and catalysts like palladium. Due to the allyl-group and the obliged reduction of the double bond this pathway cannot be implemented. Another possibility of introducing the desired tritium label is the methylation via a tritium methylating agent such as [^{3}H]methyl iodide. The 2-methoxy group in the benzamide structure offers a good approach for the introduction of the tritiated methyl-group. Based on the published procedure [1] for [^{11}C]Fallypride, the 2-hydroxy-precursor was synthesized with slight modifications. After optimisation of the labelling reaction using [^{1}H]-analogues of the tritium methylating agents, the final tritium labelling was carried out with 40 mCi [^{3}H]methyl nosylate by RC Tritec AG.

Results: The desired labelling precursor was synthesised starting from 2-hydroxy-3-methoxy-benzoic acid using a benzyl-protecting group in 10 steps with an over all yield of 14%. First labelling for optimisation of the labelling with [^{1}H]methyl iodide resulted in yields below 50%. The use of [^{1}H]methyl nosylate, Cs_2CO_3 and DMF at room temperature resulted in 98% yield. These conditions were chosen for the [^{3}H]-labelling. The [^{3}H]methyl nosylate was synthesized starting from tritium gas over 5 steps, semi-preparative HPLC provided 15 mCi of [^{3}H]Fallypride with a radiochemical purity of >99 % (HPLC). Starting from [^{3}H]methyl nosylate the radiochemical yield was 38%.



Conclusions: A multi-step synthesis starting from 2-hydroxy-3-methoxy-benzoic acid provided the 2-hydroxy-labelling precursor. After optimisation of the labelling reaction, the precursor was reacted with [^{3}H]methyl nosylate to give [^{3}H]Fallypride. HPLC purification afforded [^{3}H]Fallypride with a radiochemical purity of >99 % (HPLC).Further experiments will focus on in vitro and ex vivo autoradiographic tests analysing local high-resolution D2-like receptor distributions and densities.

References: [1] Mukherjee J., Shi B., Christian B.T.; Chattopadhyay S., Narayanan T.K., Bioorganic and Medicinal Chemistry, Volume 12 (1), 2004, pp. 95-102

P254 IN VITRO EVALUATION OF [18F]AH110690 (GE-067) FOR BETA-AMYLOID PLAQUE IMAGING OF ALZHEIMER'S DISEASE

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Objectives: New PET-radiopharmaceuticals for imaging beta-amyloid plaques of Alzheimer's disease are being continuously developed (Rowe et al., Lancet Neurol 2008, Qu et al. Bioorg. Med. Chem. Lett. 2008, Stephenson et al., Bioconjug Chem. 2007). The preclinical evaluation of these pharmaceuticals usually includes ex vivo and in vivo testing with rodents and in vitro studies with brain homogenates of Alzheimer's patients. Infrequently in vitro binding studies with post mortem brain sections of Alzheimer's patients are performed. These autoradiographic studies are useful to evaluate if binding of the radiopharmaceutical is specific to human β-amyloid plaques. A novel β-amyloid agent [^{18}F]AH110690 (GE-067) was therefore studied to assess β-amyloid plaque binding in human post mortem brain sections in vitro.

Methods: [^{18}F]AH110690 was synthesized at Turku PET Centre using nucleophilic ^{18}F-fluorination. The non optimized yield of [^{18}F]AH110690 was 10.0 ± 1.8 % (EOB, n=10), radiochemical purity exceed 99 % and the specific radioactivity was >900 GBq/μmol (EOS). Deparaffinized post mortem, formalin fixed frontal cortical brain sections of Alzheimer's disease and age matched control subjects were incubated for 30 minutes in a 0.35 MBq/ml solution of [^{18}F]AH110690. For competition binding studies on adjacent sections excess amounts of the β-amyloid binding agent 6-OH-BTA-1 (Klunk et al., Ann Neurol 2004) were added to the incubation solution. Radioactivity distribution was determined with digital autoradiography using 10 μm resolution (Fuji FLA 5100, Fujifilm, Japan). The digital autoradiography images were analyzed using image analysis software (AIDA, Raytest Inc, Germany).

Results: In this study [^{18}F]AH110690 showed high binding in cortical areas of brain sections from Alzheimer's disease patients. Binding to the cortical, and white matter of age matched controls and to the white matter of Alzheimer's disease patients was only 10% of the binding to amyloidal plaques of Alzheimer's patients.

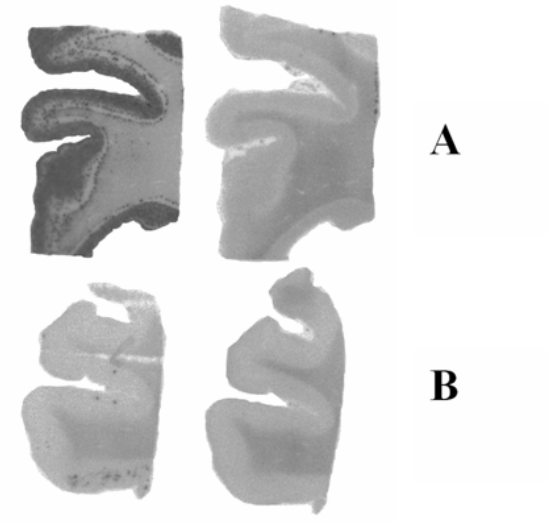

Figure 1. *In vitro* binding of [^{18}F]AH110690 to *post mortem* human brain sections.
A. Alzheimer's patient on left, on right the tracer displaced with 6-OH-BTA-1.
B. Normal control on left, on right the tracer displaced with 6-OH-BTA-1.

In brain sections of the Alzheimer's disease patient the cortical binding of [^{18}F]AH110690 was displaceable by adding excess 6-OH-BTA-1, whereas white matter binding was not changed. This suggests that both [^{18}F]AH110690 and 6-OH-BTA-1 bind specifically to β-amyloid plaques.

Conclusions: [^{18}F]AH110690 shows appropriate in vitro behaviour to be a useful tracer to be investigated as a potential in vivo radiopharmaceutical for β-amyloid imaging. It shows specific binding to brain β-amyloid considered to be one of the hallmarks of Alzheimer's disease pathology and as a result of experiments such as these the agent has progressed into clinical development as an in vivo PET tool.

P255 OPTIMIZATION OF THE BOMBESIN PEPTIDE SEQUENCE FOR IN VIVO PRECLINICAL IMAGING OF GRP RECEPTOR POSITIVE TUMORS

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Objectives: The purpose of this study was to determine what effect small changes to the truncated bombesin antagonist sequence would have on tumor uptake and non-tumor clearance rates in hopes of optimizing the target:nontarget ratios for imaging of gastrin releasing peptide receptor (GRPr) positive tumors.

Methods: DOTA-8-AOC-(X) –BBN(7-13)(NHC_2H_5) where BBN(7-13) is W-A-V-G-H-L and X = Q, (D)F-Q, (D)A-Q, (D)S-Q, (D)Y-Q, (D)W-Q, (D)F-N-Q, (D)F-G-N-Q were synthesized using standard Fmoc protected solid phase peptide synthesis and labeled with Ga-nat/67. IC_{50} values were determined for each Ga labeled peptide in the GRPr expressing cell line in PC-3. In vivo biodistributions were performed in SCID mice bearing PC-3 tumor xenografts and tissues evaluated to determine %ID and %ID/g in each organ/tissue for peptides with IC_{50} values< 20 nM. SPECT imaging was also conducted in SCID mice bearing PC-3 tumor xenografts.

Results: Solid phase peptide synthesis yields of the peptides investigated ranged from 2.87% to 24.5%. The highest yields came from the X= (D)W-Q conjugate and the lowest yield came from the longest peptide x=(D)F-G-N-Q, which would be expected. All natural Ga conjugates were purified using RP-HPLC. IC_{50} analysis of the Ga conjugates exhibited increased binding affinity when the unnatural $(D)F^6$ was present (3.34 ±0.39) as compared to the analogue with no unnatural amino acids present (5.44 ± 1.56). When the position of the (D)F was altered from the 6^{th} position to the 4^{th} position a loss of binding affinity was observed, ranging from IC_{50} values of 3.34 to 29.53. The 6^{th} position amino acid was then altered to determine effects of structure in that position on binding affinity. Results showed a two-fold increase in binding affinity with aromatic amino acids (IC_{50}'s ranging from 3.3 to 5.9) when compared to the aliphatic amino acid (IC_{50} of 105). Due to the high binding affinities observed when X=Q, (D) F-Q, (D)W-Q, and (D)Y-Q , biodistribution studies in SCID mice bearing PC-3 tumor xenografts were studied with the Ga-67 labeled peptides at 15 min, 1 h, and 4 h. All peptide derivatives showed high and specific uptake in tumors with varied clearance rates and pathways. Of the analogues studied the X=(D)W-Q analogue showed the highest tumor uptake at 4 hours (7.31 ± 0.81) with the highest tumor: pancreas ratio (19:1) as well. SPECT imaging results showed tumor visualization in all cases.

Conclusions: Modification to the truncated bombesin sequence exhibited highest binding affinity when an unnatural D aromatic amino acid was substituted into the sixth position of the sequence. Based on the current results Ga-DOTA-8-AOC-(D) W-BBN(7-13)(NHC_2H_5) showed the highest uptake in PC-3 tumors and therefore warrants further investigation as a molecular imaging agent for the gastrin releasing peptide receptor.

Research Support: U.S. Department of Veteran's Affairs Merit Review

P256 PIG BRAIN & PLASMA METABOLITES OF [11C]CIMBI-5, A NOVEL 5HT2A AGONIST PET LIGAND

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Objectives: Receptor agonist PET tracers have a better potential than antagonist tracers to reflect displacement under endogenous neurotransmitter release, however, only antagonistic PET tracers targeting the 5-HT_{2A} receptor are currently known. We have validated the binding of a novel 5-HT_{2A} agonist PET tracer, [^{11}C]Cimbi-5, in the pig brain[1]. Here we report an analysis of radiolabelled metabolites in pig plasma and brain tissue.

Methods: The high-affinity 5-HT_{2A} receptor selective agonist [^{11}C]Cimbi-5 (N-(2-[^{11}C-OCH_3]methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine) was radiolabelled by methylation of the N-Boc-protected precursor using [^{11}C]methyl triflate, followed by deprotection with TFA and HPLC purification. Following IV bolus injection of [^{11}C]Cimbi-5, arterial blood samples were taken at regular intervals up to 90 min post injection. In a second experiment, the pig brain was removed 25 min after IV bolus injection of [^{11}C]Cimbi-5. Brain tissue was extracted by homogenising with perchloric acid and, after centrifugation, filtration and pH adjustment, the brain extracts were analysed using column switching HPLC[2]. Unadulterated plasma samples were analysed directly by HPLC in a similar fashion.

Results: [^{11}C]Cimbi-5 showed a moderate rate of metabolism, with ca. 30% parent compound present in plasma after 10 min and ca. 10% at 60 min post injection. At least 3 radioactive metabolites were detected, one being fairly lipophilic though slighly less so than the parent compound (Figure 1). The amount of this lipophilic metabolite increased during the first 20 min then remained fairly constant. Analysis of brain tissue from frontal cortex and cerebellum revealed predominately the presence of [^{11}C]Cimbi-5 (> 90% in both areas).

Conclusions: The novel agonist PET tracer [^{11}C]Cimbi-5 is metabolised in pig to give a lipophilic metabolite which could enter the brain and confound the analysis of the PET data. HPLC analysis revealed, however, the absence of significant amounts of this metabolite in both frontal cortex and cerebellum. Thus, the less than optimal cortex/cerebellum ratio of 1.4 found for [^{11}C]Cimbi-5 appears not be a consequence of the presence of radiolabelled metabolites in brain. Attempts to improve the PET images by changing the ^{11}C-labeling site to alter the metabolite profile or changing the chemical structure to alter affinity and/or lipophilicity are underway.

References: 1. A Ettrup, M Palner, N Gillings, LK Rasmussen, K Nagren, S Lehel, S Keller, M Sibomana, J Madsen, M Begtrup and GM Knudsen. [^{11}C]Cimbi-5: A novel 5-HT_{2A} agonist PET tracer, Brain PET 2009 and SNM 2009 (submitted abstracts). 2. NM Gillings, L Marner and GM Knudsen. A rapid, robust and fully automated method for analysis of radioactive metabolites in plasma samples from PET studies, J. Labelled Compd. Radiopharm. 2007, 50, P416.

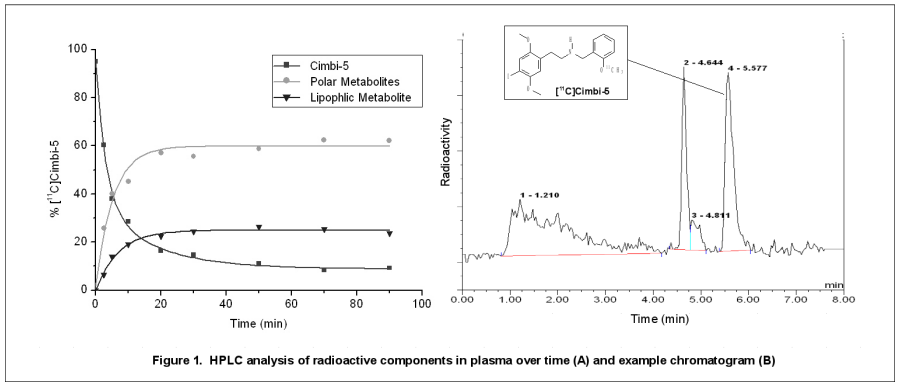

Figure 1. HPLC analysis of radioactive components in plasma over time (A) and example chromatogram (B)

P257 BINDING RESEARCH ON A SERIES OF NON-RADIOFLAVONE DERIVATIVES AS IMAGING PROBES FOR β-AMYLOID PLAQUES (1～40) IN SOLUTION VIA FLUORESCENCE TITRATION

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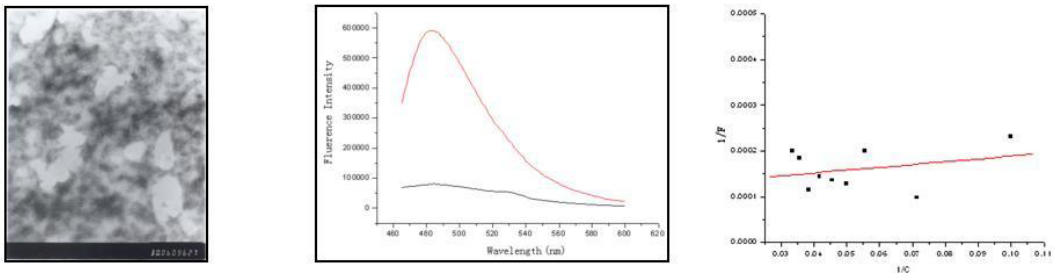
Objectives: To develop the non-invasive diagnostic agents for Alzheimer's disease, a series of 26 flavone analogues were afforded to be as probes for β-amyloid peptides (1～40) in solution using the approach of fluorescence titration. As a result, almost all of the flavone species associated rapidly with aggregated fibrils of Aβ(1～40), In addition, reaction between flavone derivatives and β-amyloid aggregates demonstrated high affinities, which were in nano-molar ranges of dissociation constants (Kd).

Methods: Brief Aβ(1～40) was dissolved in PBS to result in a visibly cloudy solution (Figure 1: a), confirmed by a Jeol 100CX transmission electron microscope. Additional test for fibrils formation used Thioflavine-T (Figure 1: b). Fresh solution flavone analogues ware obtained in a final concentration range of 10～30 nM probes in 500 μL PBS, with 5 μL Aβ(1～40) fibrils.

Results: Almost all of the flavone analogues showed high affinities in binding to fibrils, with 9.1 nM of formononetin (Figure 1: c). we speculated that the cause of the high quantum yield of fluorescence of flavone incorporated in aggregates is because of the steric restriction of the rotation of the rings.

Conclusions: The selected formononetin is an appropriate candidate for Aβ(1～40) aggregates imaging agent as a labeling precursor. It is in process of the experiment of 99mTc/Re complexed with formononetin and QSAR, primary docking studies of flavone analogues with Aβ(1～40) peptide.

Research Support: This work was supported by the Natural Science Foundation of China (No. 20671013) ; National Basic Research Program of China (No. 2006CB500705); The Natural Key Technologies R&D Program (No. 2008BAI49B04)



a b c

Figure 1: a, Aβ(1～40) fibrils under transmission electron microscope; b, red line: Thioflavine T + Aβ(1～40) fibrils, black line: Thioflavine T only; c, double reciprocal representation of formononetin binding to Aβ(1～40) at 423 nm

P258 RADIOSYNTHESIS OF [18F] MK-0518 (ISENTRESS) FOR PET IMAGING STUDIES

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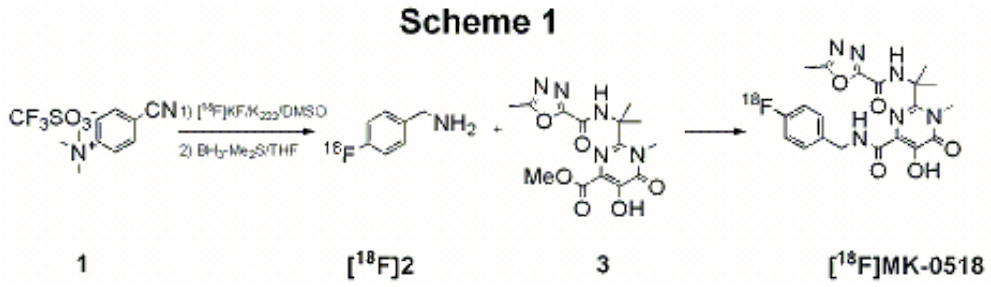
Objectives: Human immunodeficiency virus (HIV) infection has been transformed over the past two decades from a fatal to a chronic disease because of combination antiretroviral therapy. ISENTRESS (MK-0518) is the first integrase inhibitor approved for the treatment of HIV-1 infection in combination with other antiretroviral agents. [^{18}F]MK-0518 was synthesized to enable the non-invasive study of MK-0518 biodistribution in rhesus monkeys using PET.

Methods: [^{18}F]MK-0518 was radiolabeled via a three step reaction sequence shown in scheme 1. 4-Trimethylammonium triflate 1 was converted to [^{18}F]4-fluorobenzonitrile and reduced to [^{18}F]4-fluorobenzyl amine 2 in one pot in 40 min[1]. Removal of the THF and coupling of [^{18}F]2 with ester 3 in DMSO at 80°C for 10 min gave [^{18}F]MK-0518 after purification by preparative HPLC. [Image]

Results: [^{18}F]Fluorobenzonitrile was obtained in 85-90% radiochemical yield (RCY), and the reduction to [^{18}F]2 had a RCY of ~25%. The final step, the coupling of [^{18}F]2 with ester 3, achieved an overall decay corrected RCY of ~ 2% after HPLC purification. The total synthesis time was about 90 minutes to give 0.37-1.74 GBq (10- 47 mCi) of [^{18}F]MK-0518 , starting from 66.6 GBq (1.8 Ci) [^{18}F]fluoride batch . Dynamic whole body PET imaging studies were carried out in two male rhesus monkeys (3 h total scan time). [^{18}F]MK-0518 was excreted primarily via the hepatobiliary excretion pathway with approximately 50% of the injected dose being eliminated from the body at 3 h post injection. The radioactivity in the brain was low (standard uptake value peaked at 0.5 within 10 min) and washed out rapidly, suggesting that [^{18}F]MK-0518 does not efficiently cross the blood brain barrier.

Conclusions: A convenient three step, one-pot radiosynthesis of [^{18}F]MK-0518 via [^{18}F]fluorobenzyl amine has been developed giving quantities of [^{18}F]MK-0518 needed for animal PET studies.

References: Haradahira T., Hasegawa Y., Furuta K., Suzuki M., Watanabe Y. and Suauki K. Synthesis of a F-18 labeled analog of antitumor prostaglandin ^{7} PGA_1 methyl ester using -[^{18}F] fluorobenzylamine. Appl. Radiat. Isot.. 49: 1551-1556, 1998.



P259 BIODISTRIBUTION AND METABOLIC STUDIES OF A RADIOIODINATED VESAMICOL ANOLOG AS A SIGMA RECEPTOR LIGAND FOR TUMOR IMAGING

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Objectives: It has been reported that sigma receptors are highly expressed in a variety of human tumors. Previously, we investigated the binding affinities for sigma receptors of vesamicol analogs introducing iodine into the 4-phenylpiperidene moiety of vesamicol. In this study, we selected (+)-2-[4-(4-iodophenyl)piperidino] cyclohexanol [(+)-pIV] as a sigma receptor ligand and evaluated the potential of radioiodinated (+)-pIV as a tumor imaging agent.

Methods: (+)-[^{125}I]pIV was prepared by the iododestannylation reaction under no-carrier-added conditions.Biodistribution experiments were performed by the intravenous injectionof (+)-[^{125}I]pIV into male nude mice bearing human prostate tumor (DU-145) xenograft.Blocking studies were performed by intravenously injection of (+)-[^{125}I]pIV mixed with an excess amount of unlabeled sigma ligand into DU-145 tumor-bearing mice. For metabolite analysis, at 1 and 24 hours postinjection of (+)-[^{125}I]pIV into DU-145 tumor-bearing mice, interested tissues were removed and homogenized. After centrifugation, the supernatants of homogenized samples were analyzed by TLC.

Results: (+)-[^{125}I]pIV was prepared with radiochemical purity over 99% after HPLC purification. (+)-[^{125}I]pIV showed high uptakes and long residence in tumor. High tumor to blood and muscle ratios were achieved because radioactivity levels of blood and muscle were low. However, the accumulations of radioactivity in non-target tissues, such as liver and kidney, were high. The radioactivity in the non-target tissues slowly decreased as time passed. Co-injection of (+)-[^{125}I]pIV with excess amount of unlabeled sigma ligand resulted in a significant decrease in the tumor / blood ratio, indicating sigma receptors-mediated tumor accumulation. In the metabolic analysis experiments, at 24 hours postinjection, the ratios of intact form in all tissues were much lower than those at 1 hour postinjection. At this point, radioactivity in tumor showed relatively high ratio as intact form compared with that in other non-target tissues.The difference of elimination rate of radioactivity between tumor and non-target tissues may be derived from the difference of intact form ratio in tissues.

Conclusions: (+)-[^{125}I]pIV has high potential for a sigma receptor imaging in tumor because it showed high tumor uptake via sigma receptor in the animal model. These results may help to develop a new agent to reduce the radioactivity levels in the non-target organs.

P260 SYNTHESIS AND FLUORINE-18 LABELING OF A SERIES OF BP897-ANALOGUES FOR D3 RECEPTORS IMAGING WITH PET

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Objectives: Recent pharmacological developments have underlined a role of D3 receptors in several CNS disorders and addictive behaviours [1]. PET could clearly play a key-role in elucidating, non-invasively, the involvement of these receptors in the brain. The discovery of selective D3 radiotracers labeled with a positron-emitter still remains today highly challenging. Several chemical families of D3 receptor ligands have been described but the most often reported derivative is undoubtedly the partial agonist compound coded BP897 (N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-2-naphthamide) (K_i: 1.4 nM, D2/D3 > 150). In the present study, we report the synthesis of six fluorine-containing BP897 analogues (N-(4-(4-(ortho/meta/para-(fluoro-ethoxy/propoxy)phenyl)piperazin-1-yl)butyl)-2-naphthamides, 1a-c/2a-c) and their radiolabeling with fluorine-18.

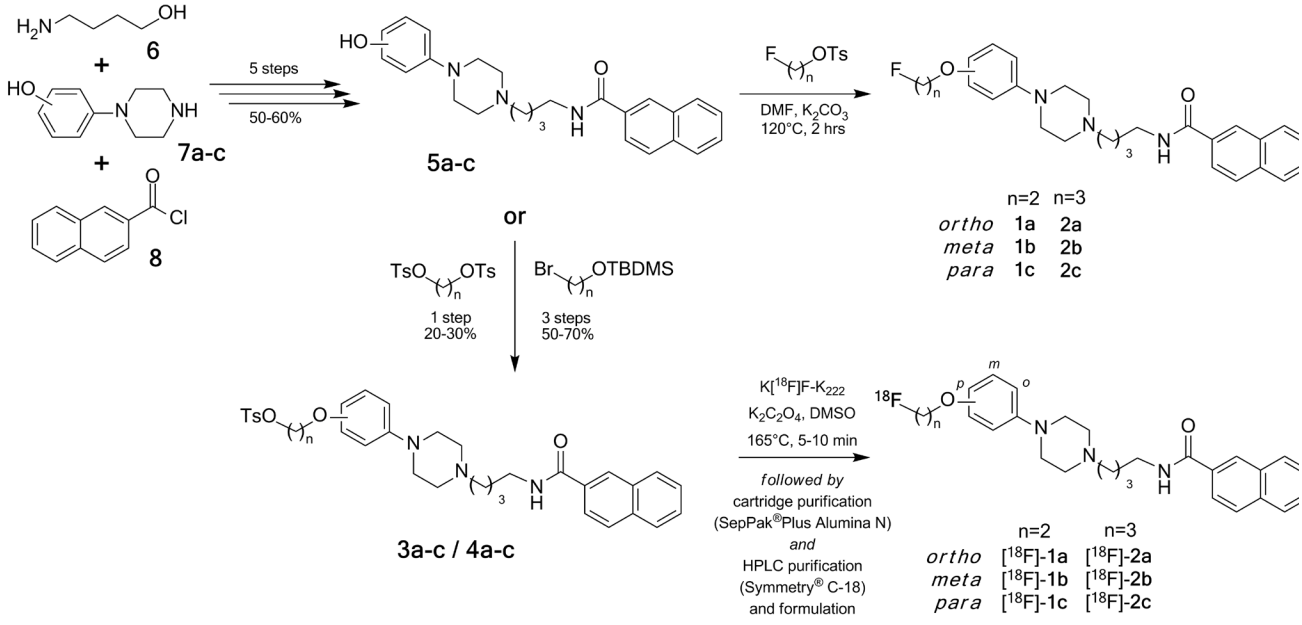
Methods: Chemistry. Key-intermediates 5a-c were prepared in 5 steps starting from 4-aminobutan-1-ol (6), naphthoyl chloride (8) and 2-, 3- and 4-hydroxyphenyl-1-piperazine (7a-c), respectively. Reaction of 5a-c with either fluoroethyltosylate or fluoropropyltosylate in DMF at 120°C for 2 hrs (with K_2CO_3) gave the reference compounds 1a-c/2a-c. For the preparation of the tosylates 3a-c/4a-c as precursors for the labelling with fluorine-18, two synthetic pathways were explored (a) a one-step pathway consisting in the reaction of derivatives 5a-c with ethyl- or propylditosylate in DMF with NaH at 60°C for 3 hrs or (b) a three-step pathway including alkylation of derivatives 5a-c using a silyloxyalkylbromide, removal of the silyl-protective group and finally tosylation. Radiochemistry. Radiofluorination of 1a-c/2a-c was performed using a TRACERLab™ FX-FN synthesizer. The procedure involves (A) preparation of the K[^{18}F]F-Kryptofix®222 complex, followed by (B) reaction with the tosyl-precursors (3a-c/4a-c, 5-6 mg) at 165°C for 5-10 min in DMSO (0.7 mL), (C) SepPak®Plus Alumina N cartridge pre-purification, (D) purification using semi-preparative reversed-phase HPLC (Waters Symmetry® C18) and finally (E) SepPak®Plus C-18-based formulation for i.v. injection. The process was programmed in one single "method" divided in three "time-lists".

Results: Compounds 5a-c were obtained in about 50-60% overall yield. Alkylation of 5a-c provided the reference compounds 1a-c/2a-c in 90% yield. The one-step pathway used for the preparation of the tosylates 3a-c/4a-c as precursors for the labelling with fluorine-18 (from 5a-c) provided the desired compounds in moderate 20% to 30% yields whereas the use of the alternative three-step pathway provided compounds 3a-c/4a-c in 50% to 70% overall yields. Ready-to-inject [^{18}F]1a-c/2a-c (>95% radiochemically pure) were prepared within 50 minutes and in non-decay-corrected yields ranging from 2% to 32%. Specific radioactivities ranged from 26 to 74 GBq/μmol.

Conclusions: Six fluorine-containing analogues of the lead D3-ligand BP897 have been successfully synthesized and labeled with fluorine-18 using a TRACERLab™ FX-FN synthesizer. MicroPET evaluation in rats of these compounds is currently in progress.

Research Support: RH is supported by a CIFRE contract (Promedical, France).

References: [1] Sokoloff et al. CNS & Neurogical Disorders Drug targets (2006), 5, 25-43.



P261 RADIOSYNTHESIS OF [11C]A000571499, A NOVEL SELECTIVE CB1 ANTAGONIST

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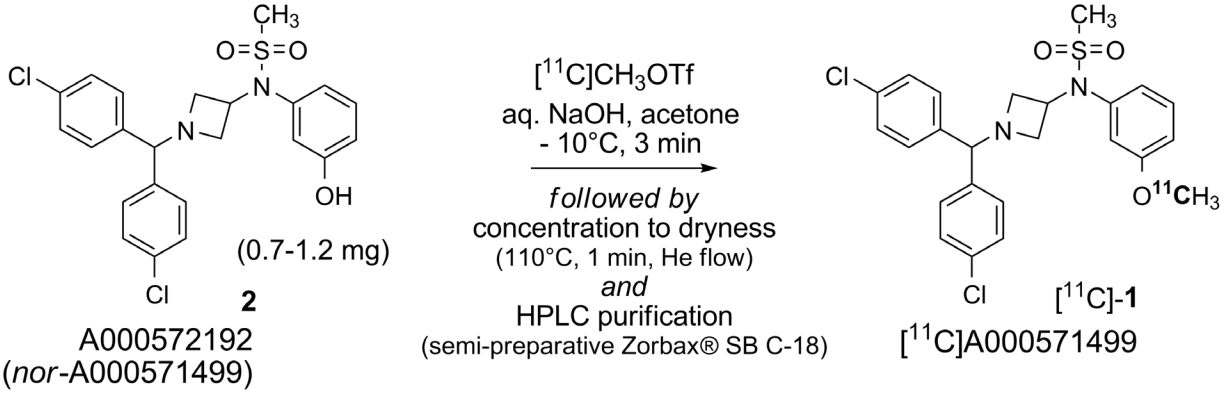
Objectives: Cannabinoid receptors type 1 (CB_1) are found in the brain, where they are thought to be the most widely expressed G-protein coupled receptors, but also in many peripheral tissues (such as heart, lung, prostate, testis and spleen). Within the brain, the highest densities of CB_1 are found in the globus pallidus, the substania nigra, the cerebellum, the cerebral cortex, the striatum and the hippocampus whereas the pons, thalamus and brain stem are almost devoid of CB_1 receptor expression. Abnormalities in regional brain CB_1 receptor densities or function may be involved in an array of neuropsychiatric and/or neurodegenerative disorders. Noninvasive and quantitative molecular imaging of these receptors with positron emission tomography (PET) could not only play a significant role in clinical research on neuropsychiatric conditions but clearly affect ongoing drug discovery and development processes. Within a novel series of N-[1-[bis(4-chlorophenyl)methyl]-3-azetidinyl]methanesulfonamides, developed by Sanofi-Aventis laboratories as highly selective CB_1-antagonists [1,2], A000571499 (1) was selected based on its pharmacological characteristics as a potent candidate for PET imaging and was labeled with carbon-11 ($t_{1/2}$: 20.38 min).

Methods: A000571499 (1) was labelled at its methoxy group from the corresponding nor-derivative 2 (A000572192) and the highly efficient methylation reagent [^{11}C]methyl triflate. Optimized conditions for the preparation of [^{11}C]-1 were the following: (1) trapping at -10°C of [^{11}C]methyl triflate in 300 μL of acetone containing 0.7 to 1.2 mg of precursor 2 (1.4 to 2.5 μmol) and 5 μL of a 3M solution of NaOH in water (15 μmol, about 6-10 eq.) ; (2) concentration to dryness of the reaction mixture (at 110°C, using a helium stream for 1 minute) ; (3) taking up the residue with 0.5 mL of HPLC mobile phase and (4) purification using semi-preparative HPLC (semi-preparative Zorbax® SB C-18, Hewlett-Packard (250 x 9.4 mm)). Formulation of [^{11}C]A000571499 ([^{11}C]-1) for i.v. injection includes a Waters SepPak®Plus C-18 cartridge-based removal of the HPLC solvents followed by a simple dilution with aq. 0.9% NaCl (physiological saline) to an ethanol concentration below 10%.

Results: Typically, starting from 55 GBq of a [^{11}C]CO_2 production batch, 9.5-10.0 GBq of [^{11}C]A000571499 ([^{11}C]-1, 55-148 GBq/μmol) were obtained within a total synthesis time of 25 to 30 minutes (non-decay-corrected radiochemical yields : 17-18%). No attempts were made to further optimise the process, the yields producing sufficient material for further evaluation.

Conclusions: [^{11}C]A000571499 ([^{11}C]-1) was labeled with carbon-11. Dynamic PET studies in baboons are currently underway to evaluate the potential of this radioligand to image CB_1-receptors in vivo.

References: [1] Black et al. WO2007067617. [2] Achard et al. WO2001064634.



P262 FLUORINE-18 LABELING OF S43473 FOR IMAGING NICOTINIC ACETYLCHOLINE RECEPTORS WITH PET

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1. CEA, I2BM Service Hospitalier Frederic Joliot, Orsay, France; 2. Institut de Recherche Servier, Suresnes, France

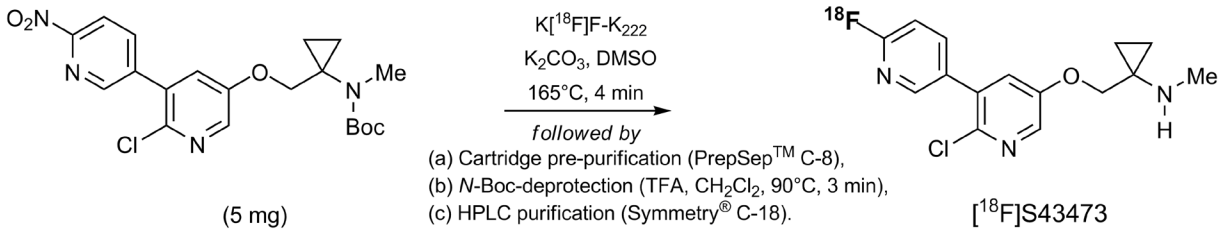
Objectives: Central nicotinic acetylcholine receptors (nAChRs) are implicated in learning-memory processes and neuropsychiatric disorders. Over the last few years, several radioligands labeled with the positron-emitter fluorine-18 (half-life : 109.8 minutes) have been developed, as for example 2-[^{18}F]F-A-85380, to specifically image the nAChR α4β2 subtype using Positron Emission Tomography (PET). Within a novel series of highly potent α4β2-selective ligands recently developed by Servier Laboratories, S43473 was selected as a potent candidate for PET imaging based both on its pharmacological and biological characteristics and its chemical structure, the latter displaying a fluoropyridine moiety. Its two-step labeling with fluorine-18 using heteroaromatic nucleophilic substitution with [^{18}F]fluoride is reported herein.

Methods: Fluorine-18 labeling of S43473 used the following conditions : (1) reaction of K[^{18}F]F-Kryptofix®222 at 165°C for 4 min in DMSO (0.6 mL) containing the N-Boc-protected nitro-precursor for labeling (5 mg) ; (2) PrepSep C-8 cartridge pre-purification; (3) N-Boc-removal using TFA in dichloromethane (90°C, 3 min) and (4) purification using semi-preparative reversed-phase HPLC (Waters Symmetry® C-18 - eluent : 0.9% aq. NaCl / EtOH / AcOH : 100 / 30 / 0.1 (v/v/v) - flow rate : 4 mL/min - detection at 254 nm). Two PET studies (control and blocking : 0.4 mg/kg nicotine, 30 min before radiotracer injection) were performed in two different baboons on EXACT HR+ tomograph (Siemens). The dynamic PET data were acquired for 180 min after i.v. injection of 225 ± 103 MBq of [^{18}F]S43473. Femoral arterial blood was withdrawn for plasma kinetic determination.

Results: Starting from a 37 GBq cyclotron-produced [^{18}F]fluoride batch, 5.5-9.2 GBq of [^{18}F]S43473, > 99% radiochemically pure and ready-to-inject, were obtained within 70 min (including HPLC-purification, R_t : 10-11 min). Specific radioactivities ranged from 111-296 GBq/μmol (3-8 Ci/μmol). In PET experiments, brain radioactivity peaked early after tracer injection (15 min) and then decreased rapidly. Blocking induces a reduction of the SUV (about 40%) at the peak. However after 60 min the brain uptake is similar to that obtained with the control experiment.

Conclusions: The decay-corrected overall yields for the preparation of [^{18}F]S43473 were ranging from 23% to 39%. Despite its promising in vitro characteristics, the in vivo results obtained in baboons clearly demonstrated that [^{18}F]S43473 does not possess the required characteristics for imaging the nAChRs with PET.

References: [1] Goldstein et al. WO 2007085750.



P263 CARBON-11 LABELING OF S38695 AND S39628, TWO NEW α4β2-SELECTIVE LIGANDS FOR PET IMAGING OF NICOTINIC ACETYLCHOLINE RECEPTORS

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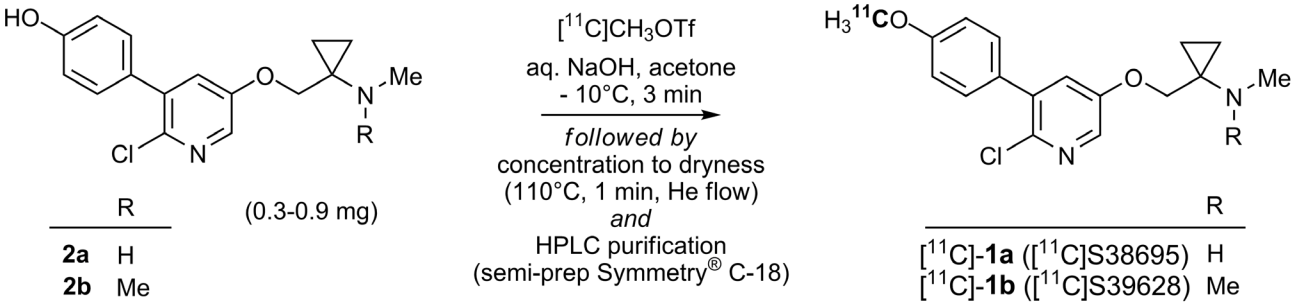
Objectives: There is considerable evidence that a variety of functions and disorders (e.g. Alzheimer's and Parkinson's disease) of the central nervous system, is linked to the neuronal nicotinic acetylcholine receptors and particularly to the subtypes containing α4 and β2 subunits. Recently, a novel series of highly potent α4β2-selective [1-(pyridin-3-yloxymethyl)-cyclopropyl]-amines has been developed by Servier Laboratories [1]. Within this series, S38695 (1a) and S39628 (1b) were selected on the basis of their pharmacological and biological characteristics as potent candidates for PET imaging and labeled with carbon-11 using [^{11}C]methyl triflate.

Methods: Carbon-11 labeling of S38695 (1a) and S39628 (1b) comprises (a) trapping at -10°C of [^{11}C]MeOTf in acetone (0.3 mL) containing the appropriate nor-derivative (O-demethylated, 0.3-0.9 mg) and aq. 3N NaOH (3 μL) ; (b) concentration to dryness of the reaction mixture (at 110°C, using a helium stream for 1 min) ; (3) taking up the residue in 0.5 mL of the HPLC mobile phase and (4) purification using semi-preparative reversed-phase HPLC (Waters Symmetry® C-18 - eluent : 0.9% aq. NaCl / EtOH / AcOH : (1a) : 70 / 30 / 0.5 (v/v/v) ; (1b) 60 / 40 / 0.5 (v/v/v) - flow rate : 5-6 mL/min - detection at 295 nm). Two PET studies (one control experiment and one blocking study with nicotine (0.4 mg/kg, 30 min before radiotracer injection) were performed in two different baboons with each radiotracer on an EXACT HR+ tomograph (Siemens). The dynamic PET data were acquired for 120 min after i.v. injection of 165 ± 85 MBq of the radiotracer. Femoral arterial blood was withdrawn for plasma kinetics determination.

Results: Starting from a 74 GBq cyclotron-produced [^{11}C]carbon dioxide batch, 2.6 to 3.3 GBq of [^{11}C]-1a or [^{11}C]-1b, > 99% radiochemically pure and ready-to-inject, were obtained within 30 min (including HPLC-purification, R_t : 7-8 min). Specific radioactivities ranged from 37 to 74 GBq/μmol. In PET experiments, brain radioactivity peaked early after tracer injection (17 and 7 min for [^{11}C]-1a and [^{11}C]-1b respectively) and decreased very rapidly. Blocking study did not modify significantly the uptake and the kinetics of the compounds.

Conclusions: The decay-corrected overall yields for the preparation of [^{11}C]S38695 and [^{11}C]S39628 ranged from 9.7% to 12.4% (n=5). Despite their promising in vitro characteristics, our in vivo results obtained in baboons clearly demonstrated that both labeled compounds do not possess the required characteristics for imaging the nAChRs with PET.

References: [1] Goldstein et al. WO 2007085750.



P264 ZW-102 AND ZW-104, TWO NOVEL FLUORINE-18 LABELED RADIOLIGANDS FOR IMAGING NICOTINIC ACETYLCHOLINE RECEPTORS WITH PET

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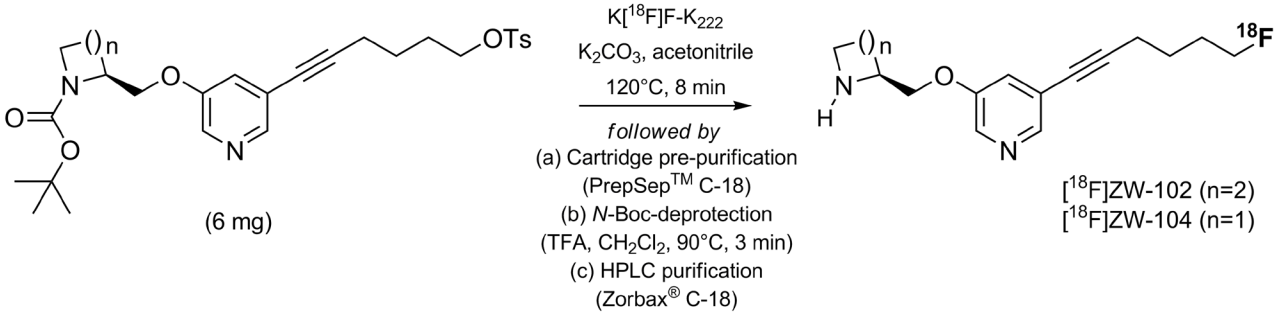
Objectives: Based on the hypothesis that cholinergic dysfunction contributes to cognitive impairments in patients with senile dementia of the Alzheimer type or Parkinson disease, considerable efforts have been engaged in the design, synthesis and pharmacological characterization of Positron Emission Tomography (PET) radioligands, in order to visualize and quantify nicotinic acetylcholine receptors (nAChRs) in human brain. Among those labeled with fluorine-18, one of the most attractive positron-emitting radioisotopes for radiopharmaceutical chemistry (half-life : 109.8 minutes), 2-[^{18}F]F-A-85380 is the only PET probe currently used in humans for quantitative brain imaging of the nAChRs. In the course of the development of this radioligand, new azetidinyl-based and pyrrolidinyl-based structures have recently been designed and of particular interest are compounds showing a fluoroalkyl chain linked to the 5'-position of the pyridine ring through an alkyne motive [1]. Within this series, ZW-102 (5-(6-fluorohexyn-1-yl)-3-[2(S)-2-pyrrolidinylmethoxy]pyridine, 1a) and ZW-104 (5-(6-fluorohexyn-1-yl)-3-[2(S)-2-azetidinylmethoxy]pyridine, 1b) have been selected as potent candidates for PET imaging based on their pharmacological and biological characteristics and labeled with fluorine-18.

Methods: Fluorine-18-labeling of ZW-102 and ZW-104 used the following conditions : (1) reaction of K[^{18}F]F-Kryptofix®222 at 120°C for 8 min in ACN (0.6 mL) containing the N-Boc-protected tosyloxy-precursor for labeling (6 mg) ; (2) PrepSep C-18 cartridge pre-purification; (3) N-Boc-removal using TFA in dichloromethane (90°C, 3 min) and (4) purification using semi-preparative reversed-phase HPLC (Hewlett-Packard Zorbax® C-18 - eluent : 0.9% aq. NaCl / EtOH / AcOH : 800 / 200 / 1 (v/v/v) - flow rate : 6 mL/min - detection at 254 nm).

Results: Starting from a 37 GBq cyclotron-produced [^{18}F]fluoride batch, 0.6-0.8 GBq (22-30 mCi) of [^{18}F]ZW-102 (n=5) and 1.6-2.6 GBq (44-70 mCi) of [^{18}F]ZW-104, > 99% radiochemically pure and ready-to-inject, were obtained within 100 min (including HPLC-purification, R_t : 17-18 min). Specific radioactivities ranged from 37-111 GBq/μmol (1-3 Ci/μmol).

Conclusions: The decay-corrected overall yields for the preparation of [^{18}F]ZW-102 and [^{18}F]ZW-104 were 2.8%-4.1% and 8.3%-13.2%, respectively. Dynamic PET studies in baboons (including pre-saturation experiments with nicotine and non labeled ZW-102/104) are currently underway to evaluate the potential of these ligands to image central $\alpha_4\beta_2$ nicotinic acetylcholine receptors in vivo.

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



P265 SYNTHESIS AND EVALUATION OF METHOXY-SUBSTITUTED DESCHLOROMAZINDOLS AS POTENTIAL PET RADIOLIGANDS FOR IMAGING THE NOREPINEPHRINE TRANSPORTER

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Objectives: 5-(4-Chlorophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]isoindole (mazindol) is currently one of the most potent ligands to the norepinephrine transporter (NET). The fact that mazindol has not been considered as a good pharmacophore candidate for the development of a NET-selective PET radioligand is perhaps due to its poor binding selectivity (high binding affinity towards the dopamine transporter (DAT) as well as the serotonin transporter (SERT)), and the lack of suitable positions for labeling with C-11. Houlihan and co-workers (J Med Chem 2002; 45: 4097-4109) reported that (see Table 1): (1) the deschloro analog of mazindol (deschloromazindol) preserves high binding affinity towards NET, though with a substantially decreased binding affinity towards DAT and SERT; (2) 6-, 7-, or 9-methoxy substituted mazindol increases its binding affinity towards NET, but with a much decreased binding affinity towards SERT. Based on this observation, we have synthesized eight methoxy-substituted deschloromazindols and evaluated their potential for labeling with C-11 as NET-selective PET radioligands.

Methods: Methoxy-substituted deschloromazindols were synthesized via the coupling of methyl benzoate with methoxy-substituted 2-phenylimidazolines which were prepared from the condensation of methoxy-substituted benzaldehydes or benzonitriles with ethylenediamine in the presence of phosphorus pentasulfide or N-bromosuccinimide, respectively. The lipophilicity CLogP values were calculated using the Chemdraw software (CambridgeSoft, Cambridge, MA). The binding affinities to NET, DAT and SERT were measured as inhibition constants (Ki) against tritium-labeled nisoxetine, WIN35428, and citalopram, respectively, through in vitro binding assays using membrane proteins prepared from transfected cell lines.

Results: Methoxy-substituted 2-phenylimidazolines were synthesized in 20 - 92 % yields from benzonitriles, or in 41 - 81 % yields from benzaldehydes. The final coupling reaction with methyl benzoate provided the methoxy-substituted deschloromazindols in 5 - 60 % yields. The CLogP values (Table 1) of deschloromazindol analogs are in the range of 2.59 to 3.63 indicating that they are moderately lipophilic and are likely to cross the blood-brain barrier. In vitro binding assays (Table 1) showed that all methoxy-substituted deschloromazindols have low binding affinity to SERT (Ki > 2400 nM). Only compounds 2, 3 and 6 have submicromolar binding affinity (Ki = 76, 6.8 and 75 nM, respectively) to NET, but they are not selective as compounds 2, 3 and 6 also have similar binding affinity (Ki = 69, 18 and 121 nM, respectively) to DAT.

Conclusions: We have synthesized eight methoxy-substituted deschloromazindols for potential labeling with C-11. However, these compounds do not have high binding affinity to NET as well as high selectivity for NET over DAT as we have expected. Therefore, they are not suitable for labeling with C-11 as NET-selective PET radioligands.

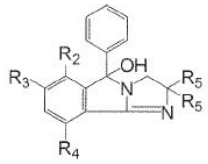

Table 1: Structures, binding affinities (Ki) and lipophilicities (CLogP) of mazindol analogs

Compound	R_1	R_2	R_3	R_4	R_5	Ki (nM)			CLogP
						NET	DAT	SERT	
Mazindol	Cl	H	H	H	H	4.9*	43*	94*	3.39
Deschloromazindol	H	H	H	H	H	2.8*	730*	2140*	2.67
6-OCH_3-Mazindol	Cl	OCH_3	H	H	H	1.7*	60*	3600*	3.30
7-OCH_3-Mazindol	Cl	H	OCH_3	H	H	1.5*	17*	1960*	3.30
9-OCH_3-Mazindol	Cl	H	H	OCH_3	H	4.7*	42*	280*	3.30
1	H	OCH_3	H	H	H	4430	624	>10000	2.59
2	H	H	OCH_3	H	H	76	69	4570	2.59
3	H	H	H	OCH_3	H	6.8	18	2406	2.59
4	H	OCH_3	H	H	CH_3	1507	>10000	>10000	3.63
5	H	H	OCH_3	H	CH_3	>10000	4415	>10000	3.63
6	H	CH_3	H	OCH_3	H	75	121	5050	3.09
7	H	OCH_3	H	OCH_3	H	>10000	3760	>10000	2.68
8	H	OCH_3	H	F	H	>10000	8040	>10000	2.87

*IC_{50} values from literature (Houlihan et al. J Med Chem 2002; 45: 4097-4109).

P266 UPTAKE INHIBITION OF 3-I-125-IODO-ALPHA-METHYL-L-TYROSINE INTO COLON CANCER DLD-1 CELL BY AMINO ACID LIKE DRUGS

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Objectives: We examined 3-[^{123}I]iodo-alpha-methyl-L-tyrosine ([^{123}I]IMT) uptake and inhibition by amino acids and amino acid-like drugs in the human DLD-1 colon cancer cell line, to discuss correlation between the inhibition effect and structure.

Methods: The time course of [^{125}I]IMT uptake, contributions of transport systems, concentration dependence, and inhibition effects by amino acids and amino acid-like drugs (1 mM) on [^{125}I]IMT uptake were examined.

Results: Expression of system L (4F2hc, LAT1), system A (ATA1, ATA2), and system ASC (ASCT1) was detected by PCR. [^{125}I]IMT uptake in DLD-1 cells involved Na^+-independent system L primarily and Na^+-dependent system(s). Uptake of [^{125}I]IMT in Na^+-free buffer followed Michaelis-Menten kinetics, with a K_m of 78 mM and Vmax of 333 pmol/10^6 cells/min. Neutral D- and L-amino acids with branched or aromatic large side chains inhibited [^{125}I]IMT uptake. Tyrosine analogues, tryptophan analogues, L-phenylalanine and p-halogeno-L-phenylalanines, and gamma amino acids (including 3,4-dihydroxy-L-phenylalanine (L-DOPA), DL-threo-beta-(3,4-dihydroxyphenyl)serine (DOPS), 4-[bis(2-chloroethyl)amino]-L-phenylalanine, and 1-(aminomethyl)-cyclohexaneacetic acid) highly inhibited [^{125}I]IMT uptake, but weakly for L-tyrosine methyl ester and R(+)/S(-)-baclofen. The substrates of system ASC and A did not inhibit [^{125}I]IMT uptake except L-serine and D/L-cysteine.

Conclusions: [^{125}I]IMT uptake in DLD-1 cells involves mostly LAT1 and its substrates' (including amino acid-like drugs derived from tyrosine, tryptophan and phenylalanine) affinity to transport via LAT1. Whether transport of gamma amino acid analogues is involved in LAT1 depends on the structure of the group corresponding to the amino acid residue. Beta-hydroxylation may confer reduction of transport affinity of tyrosine analogues via LAT1.

P267 SERUM PROTEIN BINDING DISPLACEMENT: THEORETICAL ANALYSIS USING A HYPOTHETICAL RADIOPHARMACEUTICAL

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Objectives: If a drug binding to serum protein is displaced competitively at the binding site, the free fraction of this drug will increase, thereby producing a more potent biological effect. In the case of radiopharmaceutical, total clearance and tissue distribution are increased in such circumstances. We calculated the effect of inhibitors of serum protein binding using a hypothetical radiopharmaceutical. ^{123}I-N-isopropyl-p-iodoamphetamine (IMP) is widely used for investigating cerebral blood flow in clinical settings, and has high protein binding activity. To compare the results, in vitro experiments and protein binding inhibitor-loaded monkey scintigraphy were then conducted using IMP as the radiopharmaceutical.

Methods: Free fraction ratios of radiopharmaceutical were calculated with one radiopharmaceutical, two serum proteins and two specific inhibitors in the steady state at various serum protein concentrations. In vitro protein binding inhibition studies using human, rat and monkey sera were performed with site selective displacers of specific binding sites: 400 mM 6-methoxy-2-naphthylacetic acid (6MNA; a major nabumeton metabolite) as a serum albumin site II inhibitor, and 400 mM erythromycin (ETC) as a α_1-acid glycoprotein (AGP) site inhibitor. Scintigraphy with or without 6MNA loading of monkeys was performed.

Results: The theoretical findings roughly corresponded to the experimental results. Approximately 75% of IMP bound to serum albumin site II and AGP in the species examined. The free fraction of IMP (25.0 ± 0.6% for human, 22.8 ± 0.4% for monkey, 23.7 ± 0.3% for rat) increased with loading of specific protein binding inhibitors (6MNA: 28.0 ± 0.3% for human, 24.5 ± 0.7% for monkey, 24.3 ± 0.2% for rat; ETC: 26.3 ± 0.4% for human, 29.5 ± 1.1% for monkey, 26.0 ± 0.7% for rat), and was serum protein concentration-dependant based on the results of calculations. Simultaneous administration of 6MNA and ETC produced a higher free fraction ratio of IMP (31.9 ± 1.0% for human, 34.6 ± 0.4% for monkey, 27.0 ± 0.3% for rat) than summation of the single administrations of 6MNA and ETC (domino effect) in human, rat, and monkey sera. Rapid cerebral accumulation was observed with 6MNA loading in monkey scintigraphy.

Conclusions: Although the principle was provisionally proven by the calculation, the appropriate binding constants should be determined experimentally in future research. 6MNA appears to change the pharmacokinetics and brain accumulation of IMP in monkeys. Further studies in human are required. Displacement methods could therefore help to more rapidly produce better diagnostic images with lower radiation doses.

P268 PRECLINICAL AND INITIAL HUMAN PET STUDIES ON C-11 LABELED CHIBA-1001 FOR MAPPING ALPHA-7 NICOTINIC ACETYLCHOLINE RECEPTORS IN THE BRAIN

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Objectives: α7 nicotinic acetylcholine receptor (nAChR) is one of the predominant nAChR subtypes in the brain and is suggested to play an important role in the pathologic states of neurological and psychiatric disorders, such as Alzheimer's disease, dementia, and schizophrenia. 4-[^{11}C]methylphenyl 2,5-diazabicyclo[3.2.2]nonane-2-carboxylate ([^{11}C]CHIBA-1001) was recently developed as a novel PET tracer for α7nAChR in the brain. In the present study, we performed preclinical and initial clinical PET studies using [^{11}C]CHIBA-1001 for imaging α7 nAChRs in the human brain.

Methods: [^{11}C]CHIBA-1001 was synthesized by methylation of the tributylstannyl precursor with [^{11}C]CH_3I in a palladium-promoted Stille cross-coupling reaction. The radiation absorbed-dose by [^{11}C]CHIBA-1001 in humans was calculated from distribution data in mice. The acute toxicity of CHIBA-1001 at a dose of 3.20 mg/kg body weight, which is more than 41,000-fold the clinical equivalent dose of [^{11}C]CHIBA-1001, was evaluated. The mutagenicity of CHIBA-1001 was studied by a reverse mutation test in S. typhimurium (Ames test). Metabolite analysis in the mouse brain was carried out by high-performance liquid chromatography. The initial clinical PET imaging of α7 nAChRs with [^{11}C]CHIBA-1001 in a normal volunteer was also performed.

Results: A suitable preparation method for [^{11}C]CHIBA-1001 injection was established. The decay-corrected radiochemical yields of [^{11}C]CHIBA-1001 based on [^{11}C]CH_3I were 67.2±7.6% (range, 56.0–83.8)(n = 10). The radiochemical purity of [^{11}C]CHIBA-1001 was always greater than 99%. The specific activities were 54.8±8.7 TBq/mmol (range 41.3–66.8), at 30 min after EOB. The radiation absorbed-dose by [^{11}C]CHIBA-1001 in humans was low enough for clinical use, and no acute toxicity or mutagenicity of CHIBA-1001 was found. Most radioactively in the mouse brain was detected as an unchanged form, although peripherally [^{11}C]CHIBA-1001 was degraded. We successfully performed brain imaging by [^{11}C]CHIBA-1001 PET in a normal volunteer. A 90-minute dynamic scan showed a rapid accumulation and gradual washout of radioactivity in the brain. The highest distribution volume of [^{11}C]CHIBA-1001 was found in the thalamus; however, regional differences in brain radioactivity were small. Peripherally, [^{11}C]CHIBA-1001 was stable in human: >80% of the radioactivity in plasma was detected as the unchanged form for 60 min.

Conclusions: These results demonstrate that [^{11}C]CHIBA-1001 is a suitable radioligand to use in clinical trials for imaging α7 nAChRs in the human brain, providing acceptable dosimetry and pharmacological safety at the dose required for adequate PET imaging.

References: [1] Hashimoto K, et al., PLoS ONE 2008; 3:e3231.

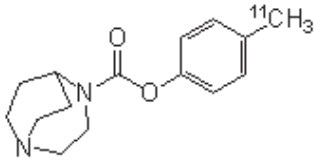

Fig. 1 Structure of [^{11}C]CHIBA-1001.

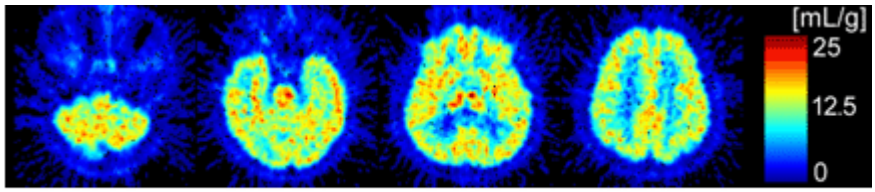

Fig. 2 V_T images of [^{11}C]CHIBA-1001 in health human subject.

P269 TRANS-STIMULATION OF 3-I-125-IODO-ALPHA-METHYL-L-TYROSINE UPTAKE INTO CHINESE HAMSTER OVARY CELL BY AMINO ACID ESTERS

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1. Ibaraki Prefectural University of Health Sciences, Dept of Radiological Sciences, Ibaraki, Japan; 2. Ibaraki Prefectural University of Health Sciences, Center for Medical Science, Kanazawa, Japan; 3. Ibaraki Prefectural University of Health Sciences, Center for Humanities and Sciences, Ibaraki, Japan; 4. Kanazawa University, School of Health Sciences, Kanazawa, Japan

Objectives: ^{123}I-3-iodo-alpha-methyl-L-tyrosine (^{123}I-IMT), an amino acid analog for SPECT tumor imaging, is mainly mediated by amino acid transport system L in most tissues. To propose a method to stimulate ^{123}I-IMT uptake for improving amino acid transport imaging, a basic study on the effects of amino acid esters ^{125}I-IMT uptake into Chinese hamster ovary cells was conducted.

Methods: CHO-K1 cellswere incubated at 37 °C for 10 min with 18.5 kBq ^{125}I-IMT in uptake medium. To verify ^{125}I-IMT uptake mechanisms in CHO-K1 cells, the following inhibitors were tested at 1.0 mM: 2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH, a system L specific inhibitor); 2-(methylamino)isobutyric acid (MeAIB, a system A specific inhibitor); p-aminohippurate (PAH, an organic anion transporter specific inhibitor); tetraethylammonium chloride (TEA, an organic cation transporter specific inhibitor); 2,4-dinitrophenol (DNP, inhibitor of anaerobic mitochondrial energy production); and sodium azide (NaN_3, inhibitor of aerobic energy production); 20 D- or L-amino acids. Uptake in the Na^+-free and low temperature (4°C) condition was tested. L-Gly, L-Ser, L-Leu, L-Phe, L-Met, L-Tyr, D-Tyr, L-Val, and L-Lys ethyl/methyl esters were tested in combination with ^{125}I-IMT at 1.0 mM with 1-hr incubation. Time course studies were conducted for 3 hrs with L-Tyr ethyl and methyl esters.

Results: Both Na^+-dependent and -independent uptake were observed. Uptake was temperature-dependent. BCH inhibited uptake of ^{125}I-IMT ($p<0.001$), whereas MeAIB, PAH, TEA, DNP, and NaN_3 did not. Uptake was inhibited by D- and L-isomers of Cys, Leu, Ile, Phe, Met, Tyr, His, Trp, and less strongly by Val, Ala, Ser and Thr, suggesting involvement of the L-type amino acid transporter-1 (LAT1). Accumulation of ^{125}I-IMT was stimulated significantly (2-3 fold, $p<0.001$) by Tyr ethyl and methyl esters.

Conclusions: Efficiently enhanced ^{125}I-IMT uptake into CHO-K1 cells in vitro suggested that L-Tyr ethyl ester is a candidate compound.

P270 SYNTHESIS AND EVALUATION OF C-11 LABELED DUAL MODULATOR FOR P-GP AND BCRP AS A PET PROBE

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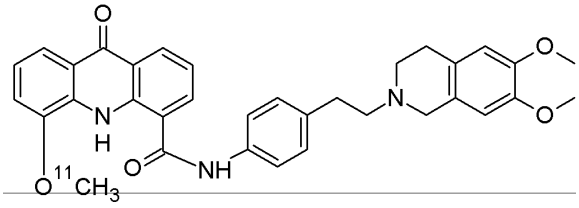
Objectives: P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) are drug efflux transporters of the ATP binding cassette family proteins. Both P-gp and BCRP are located in the blood-brain barrier. Therefore, P-gp and BCRP modulator improve brain penetration of P-gp or BCRP substrate drugs. GF120918 (elacridar) is a third-generation P-gp inhibitor that was shown to also have an inhibitory effect on BCRP-mediated transport. To evaluate for brain penetration-mediated P-gp and BCRP, we synthesized [^{11}C]GF120918 as a PET probe.

Methods: [^{11}C]GF120918 was synthesized by the methylation of 5-O-desmethyl GF120918 with [^{11}C]methyl iodide. The metabolite study in brain tissue and plasma of mice was investigated 30 min after injection of [^{11}C]GF120918. The PET study with [^{11}C]GF120918 was performed in P-gp and Bcrp knockout mice and wild-type mice.

Results: In the radiosynthesis of [^{11}C]GF120918, radiochemical yields based on [^{11}C]CO_2 was 18–41%. The specific activity at 30 min from EOB was 41–58 TBq/mmol. The radiochemical purity was >99%. In the metabolite study of mice 30 min after [^{11}C]GF120918 injection, the percentages of unchanged formwas 95±1.7% (n=4) in the brain tissue, and 96±1.9% (n=4) in the plasma. In the PET study of mice brain, the radioactivity level in wild-type decreased immediately after initial uptake, and remained constant level. In the P-gp and Bcrp knockout mice, the radioactivity level in the brain was six- to ninefold higher than that in wild-type mice after initial uptake.

Conclusions: [^{11}C]GF120918 was reliably prepared, showed the P-gp and BCRP modulation in the brain, and also showed high metabolic stability in mice. It is considered that [^{11}C]GF120918 is a potential PET probe for evaluating brain penetration-mediated P-gp and BCRP.

Research Support: This study was partially supported by a consignment grant for the Molecular Imaging Program on Research Base for PET Diagnosis from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japanese Government.



P271 PREPARATION AND IN VIVO EVALUATION OF 99mTc-FA-HSA

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Objectives: Folate receptor is over expressed in a wide variety of human tumors, such as nasopharyngeal, ovarian, endometrial, renal cancers.In this study, a novel ^{99m}Tc labelled conjugate of folate, ^{99m}Tc-FA-HSA, was prepared for diagnosis as it may be selectively taken up by tumor cells via folate receptor. Human serum albumin (HSA) was used as drug carrier due to its favorable physiologic properties. On the other hand, there are many sulfhydryl groups which allow it to conjugate with ^{99m}Tc core easily.

Methods: FA-HSA was prepared with NHS activated FA and HSA in buffer solution, then the conjugate was purified by Sephadex G100 and coupling degree was determined by spectrophotometry. The corresponding ^{99m}Tc-complex had been successfully obtained by adding buffer solution and $^{99m}TcO_4^-$ to FA-HSA aqua solution at room temperature for 30 min, using $SnCl_2 \cdot 2H_2O$ as reducing agent. The radiochemical purity of the product was above 95% as measured by TLC. Biological study of ^{99m}Tc-FA-HSA was performed in Kunming mice bearing S180 tumor.

Results: Coupling degree of the FA-HSA showed that 1 molecule of HSA was loaded with about 3 folic acid molecules. Biodistribution showed that ^{99m}Tc-FA-HSA had a selective accumulation (4.37±1.12%ID/g at 1h) and good retention (3.40±0.69%ID/g at 4h) in tumor. The ratios of tumor/blood and tumor/muscle rose with time and were 0.45 and 4.28 at 1 h p.i., and reached 0.76 and 5.00 at 4 h p.i., respectively. Comparison of the organ uptake of ^{99m}Tc-FA-HSA, ^{99m}Tc- HSA and ^{99m}Tc-FA-HSA with cold ligand (5mg/kg) was showed in Table 1.

Conclusions: Biodistribution of ^{99m}Tc-FA-HSA was different from ^{99m}Tc-HSA in mice. As a kind of targeted carrier, folate increase the targeting ability of HSA in tumor. After blocking with the cold ligand, the uptake by kidney and tumor were lower than the control. The results indicated the specific binding of this radiotracer to the folate receptor. Further investigation of biological evaluation of the complex is in progress.

Research Support: Thiswork was supported by the National Natural Science Foundation of China (No.20701004).

Table1 Comparison of the biodistribution of ^{99m}Tc-FA-HSA, ^{99m}Tc-HSA and ^{99m}Tc-FA-HSA with cold ligand (5 mg/kg) in mice bearing S180 tumor 2 h after injection (%ID/g ± SD, n = 5)

Complex	^{99m}Tc-FA-HSA	^{99m}Tc-HSA	^{99m}Tc-FA-HSA with cold ligand (5 mg/kg)
Heart	2.16 ± 0.41	0.69 ± 0.04	0.28 ± 0.19
Liver	7.86 ± 1.13	1.10 ± 0.11	33.42 ± 6.97
Lungs	3.70 ± 0.71	1.06 ± 0.13	1.54 ± 0.45
Kidney	22.35 ± 1.60	10.62 ± 1.62	2.70 ± 0.87
Muscle	0.89 ± 0.35	0.29 ± 0.08	0.07 ± 0.02
Blood	7.08 ± 1.44	2.02 ± 0.27	1.15 ± 0.10
Tumor	3.70 ± 0.40	1.36 ± 0.18	0.49 ± 0.07

P272 SYNTHESIS OF F-18 LABELLED AMMONIUM SALTS AS INHIBITOR FOR hEAG1 CHANNELS

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Objectives: Ion channels are known for their critical role in a diverse physiological function such as excitability, contraction, progression.[1] Especially, potassium channels family was highlighted as a marker for different cancer.[2] Recent studies revealed that hEAG1 potassium channels are abundantly expressed in a variety of tumor cells.[3] Since hEAG1 channels play a role in cell proliferation, specific markers for hEAG1 channels could be used as a imaging agent for oncological research by labeling with radioactive isotopes. Recently, ammonium salt was shown to be a blocker of hEAG1 channels.[4] Thus, we synthesized several ammonium salts and tested their block efficacy towards hEAG1 channels. This results show that hEAG1 channels have a specific heptyl-binding moiety. Finally, we synthesized F-18 labeled heptyl ammonium salt to investigate propriety as tumor imaging agent for hEAG1 channels.

Methods: Ammonium salts (AMS) were synthesized through 5 steps with alkanediols and 4-(4-nitrophenyl)butanol. The structures of AMS were confirmed with ^{1}H-NMR spectroscopy and Mass spectrometry. hEAG1 wild type and A453S mutant were subcloned in pGEM-HE. Inside-out recordings were performed at room temperature (20-23°C) using an EPC 9 patch clamp amplifier. [^{18}F]fluoride was produced on a GE PET trace cyclotron by irradiation of a $H_2{}^{18}O$ water target. In F-18 labeling reaction, tosyl-AMS was reacted with dried [^{18}F]fluoride in Kryptofix 2,2,2 / K_2CO_3 solution in HMPA for 30 min at 90℃. The purification was achieved by HPLC (Vydac C18, 300*3.9 mm, 4 μm; ammonium formate/CH_3CN, 60/40; flow rate, 2 mL/min).

Results: Wild type hEAG1 and A453S-hEAG1 channels were expressed in Xenopus oocytes and inhibition by AMS with different alkyl-chain length was measured in the inside-out configuration of the patch-clamp technique. Our results clearly show that the length of the alkyl chain strongly has an impact on the hEAG1 blocking efficacy of AMS (up to 24.3-fold for heptyl-AMS compared to pentyl-AMS) and that heptyl-AMS is optimal for binding. F-18 labeling reaction was carried out using hexamethylphosphoramide (HMPA) and acetonitrile as a solvent, respectively. HMPA (35%) is more suitable for F-18 nucleophilic substitution than CH_3CN (15%) due to polar aprotic property. The F-18 labeled compounds was coinjected with the reference compound into HPLC to identify with [^{18}F]F-heptyl-AMS . The overall time was 65 min and specific activity was 502 GBq/mMol.

Conclusions: We found that heptyl-AMS has a good block efficacy to hEAG1 channels. [^{18}F]F-heptyl-AMS (N-4-(4-nitrophenyl) butyl-N,N-diethyl-7-[^{18}F]fluoroheptylammonium salt) was synthesized in 25% of radiochemical yield and a radiochemical purity of > 95% to be investigated as potential imaging agent due to blocking hEAG1 channels. The biological evaluation of [^{18}F]F-heptyl-AMS is ongoing.

References: 1. R. Schoenherr, J. Membrane Biol. 2005, 205, 175184. 2. W. Sthmer, F. Alves, F. Hartung, M. Zientkowska, L.A. Pardo, FEBS Lett. 2006, 580, 2850-2852. 3. B. Hemmerlein, R.M. Weseloh, F. Mello de Queiroz, H. Knotgen, A. Sanchez, M.E. Rubio, S. Martin, T. Schliephacke, M. Jenke, H.J. Radzun, W. Stuhmer, L.A. Pardo, Mol. Cancer. 2006, 5,41. 4. G. Gessner, M. Zacharias, S. Bechstedt, R. Schnherr, S.H. Heinemann, Mol. Pharmacol. 2004, 65, 1120-1129.

P273 SYNTHESIS AND EVALUATION OF A NOVEL C-11 LABELED I2 IMIDAZOLINE BINDING SITE LIGAND AS A PET PROBE

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Objectives: Imidazoline binding site (IBS) were proposed to explain certain actions of imidazoline containing agents that could not be accounted for by their interaction with adrenoceptor. IBS have been categorized at least as two different subtypes, I_1-IBS and I_2-IBS. While the I_1-IBS participate in the regulation of cardiovascular function, the I_2-IBS appears to be involved in the Parkinson's disease, depression, and other central nervous systems disorders. Recently, a novel selective ligand, 2-(3-fluoro-4-tolyl)imidazoline (FTIMD), for I_2-IBS with high selectivity (Ki for I_2, 3.0 nM; Ki for I_1, >10,000 nM; Ki for adrenoceptor α_2, >10,000 nM), was developed [ref.1]. Previously, a few PET probes for IBS was developed [ref.2,3]; however, a selective PET probe for I_2-IBS was not evaluated in vivo. We synthesized ^{11}C-labeled FTIMD and evaluated in vivo as a PET probe for I_2-IBS.

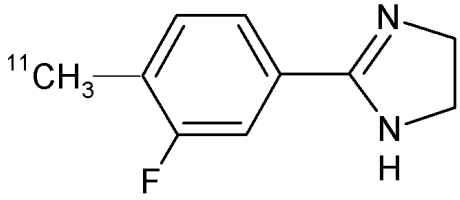
Methods: [^{11}C]FTIMD was synthesized by a palladium-promoted cross-coupling reaction with tributylstannyl-precursor and [^{11}C]methyl iodide in the presence of tris(dibenzylideneacetone)dipalladium(0) and tri(o-tol)phosphine. Biodistribution was investigated in male SD rats by tissue dissection methods. Specific uptake of [^{11}C]FTIMD in rats brain was evaluated in blocking studies with cold FTIMD and BU224 as a selective I_2 ligand (0.1 mg/kg co-injection).The metabolite study in brain tissue and plasma of rats was investigated 15 min after injection.

Results: In the radiosynthesis, radiochemical yields based on [^{11}C]CO_2 was 5.4±2.0% (n=7). The specific activity at 30 min from EOB was 68–163 TBq/mmol. The radiochemical purity was >95%.In rats, the uptake in the brain was gradually decreased after initial uptake. In the blocking study, the uptake in the brain was significantly decreased by co-injection of BU224 (27% of control) and of cold FTIMD (26 % of control) 15 min after injection.In the metabolite study of rats 15 min after injection, the percentages of unchanged formwas 99±0.4% (n=3) in the brain tissue, and 53±5.4% (n=3) in the plasma.

Conclusions: [^{11}C]FTIMD was reliably prepared, and showed specific binding to I_2-IBS of the brain in rats. It is considered that [^{11}C]FTIMD is a promising PET probe for imaging I_2-IBS in the brain.

Research Support: This study was partially supported by a consignment grant for the Molecular Imaging Program on Research Base for PET Diagnosis from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japanese Government.

References: 1. Anastassiadou M. et al., Bioorg Med Chem 2001; 9: 585-92. 2. Roeda D. et al. J Label Compd Radiopharm 2003; 46: 11419. 3. Hudson AL. at al. Ann NY Acad Sci 2003; 1009: 3028.



P274 PRODUCTION OF HIGH SPECIFIC ACTIVITY NCA Lu-177g BY CYCLOTRON IRRADIATION

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Objectives: ^{177g}Lu is a low energy negatron emitter that, thanks to its favourable decay properties ($t_{1/2}$ = 6.734 d, negatron emission 100 %, $E_{\beta,max}$ = 489.3 keV, $<E_\beta>$ = 163 keV, E_γ = 208.4 keV), is one of the most promising radionuclide to be used in nuclear medicine, especially in metabolic radiotherapy of cancer of small dimensions. This RN is mainly produced in thermal nuclear reactor in two different ways: the first in carrier added (CA) form by (n,γ) reaction on enriched target of ^{176}Lu leading to a lower specific activity A_S, compared with the theoretical carrier free value A_S(CF) = 4.05 GBq•μg^{-1}, the second in no carrier added (NCA) form by (n,γ) reaction on enriched target of ^{176}Yb followed by negatron decay leading, after selective separation of Lu from Yb, to a higher A_S. This latter case shows no evidence of production of the long-lived impurity ^{177m}Lu. An alternative method is to produce ^{177}Lu by the deuteron activation of natural or enriched in ^{176}Yb targets. In this case, the routes of interest are the indirect reaction ^{176}Yb(d,p)^{177}Yb that decays by negatron emission to ^{177g}Lu and the direct reaction ^{176}Yb(d,n)$^{177(g+m)}$Lu.

Methods: In order to optimize the ^{177g}Lu production the thin target yields (ttys) of the nuclear reactions involved were measured as a function of the projectiles energy by the stacked-foil technique irradiating Yb targets of natural composition at the MC40 cyclotron of the JRC, Ispra, Italy, that can deliver deuterons with energies up to 19 MeV. The measurements, done at the radiochemistry laboratory of LASA by high resolution gamma spectrometry (HPGe detectors), were started few hours from the EOB (end of bombardment) and were carried on for many months, till more than one year, after the irradiation.

Results: The excitation functions of all radionuclides produced were measured and compared with the data, if present, published in the unique previous literature publication. It was determined the decay curve of ^{177}Yb and the growth curve of the cumulative (direct and indirect) production of ^{177g}Lu. The analysis of these curves conduct to the evidence that the predominant route for the production of ^{177g}Lu is the indirect reaction ^{176}Yb(d,p)^{177}Yb, that decays to ^{177g}Lu. The direct reaction ^{176}Yb(d,n)$^{177(g+m)}$Lu is observable only above 13 MeV and contributes for only 4% of the total in correspondence of 14.6 MeV. In the spectra acquired one year from the EOB the γ lines of ^{177m}Lu are not presented. By detection limit method the activity of ^{177m}Lu at the EOB is been evaluated, in the worst case, less than 0.07% of total activity of ^{177}Lu.

Conclusions: The production of ^{117g}Lu by deuteron irradiation of Yb targets will be competitive with neutron activation. The deuteron activation for 12 hours of a thick target of ^{176}Yb (100% enriched) with E_{in} = 12.5 MeV, ΔE = 10.0 MeV, I = 100 μA can produce up to 10 GBq of cumulative ^{177g}Lu.

Research Support: The present research work was funded in the mainframe of the experiment LUTHETIUM-177 of the Commission V of INFN and the MIUR, Italy. The contributions of JRC-Ispra of European Community was substantial.

P275 EVALUATION OF THE CONVERSION RATE OF 6-HALOGENOPURINE DERIVATIVES AS A PROBE FOR ASSESSING MRP1 FUNCTION

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Objectives: Multidrug resistance-associated protein 1 (MRP1), energy-dependent efflux pump, serves as defense mechanisms limiting tissue accumulation of naturally occurring toxins, xenobiotics, and drugs. 6-Bromo-7-[^{11}C]methylpurine has been developed as a probe to assess the function of MRP1 in the brain. Based on the concept of Metabolite Extrusion Method (MEM), the probe enters the brain after intravenous injection and is rapidly converted to a MRP1 substrate of a glutathione (GSH) conjugate in situ. In MEM, the conversion process is extremely important for the assessment; however, there would be a species difference in the conversion rate. In this study, 6-halogeno-9-[^{14}C]methylpurines (9Xs) and 6-halogeno-7-[^{14}C]methylpurines (7Xs) (X = F, Cl, Br or I) were designed, and their spontaneous and enzymatic reactivity with GSH was examined for choosing a probe applicable to different kinds of species including human.

Methods: All purine derivatives were synthesized by N-methylation of 6-halogenopurine with [^{14}C]CH_3I in acetone and were subsequently purified by preparative TLC. The spontaneous and enzymatic reaction rates were examined by the radiometric TLC analysis in phosphate buffer and rat brain homogenate supplemented with 2 mM GSH, respectively. The partition coefficients (PC) of purine derivatives were determined in octanol/phosphate buffer systems.

Results: The radiochemical purities of all purine derivatives were greater than 98%. The radiochemical yields of 9Xs and 7Xs were 35-50% and 8-20%, respectively. The steric hindrance between halogen atom and methyl group probably causes the reduction of 7Xs formation. The difference in the spontaneous reaction rate with GSH among 7Cl, 7Br and 7I was small, and the reaction rate of 7F was much higher than that of other 7Xs. The relative reactivity of 9Xs was very similar to that of 7Xs. The spontaneous reaction rates of 7Xs were much higher than those of the corresponding 9Xs These results suggested that the rate-limiting step would be the nucleophilic attack of GSH on 6-halogenopurine derivatives and that methyl-group introduction into 7-position caused the increase in 6-halogenopurine electrophilicity. Similarly to the spontaneous reactivity, the enzymatic reaction rate of 7Xs was higher than that of 9Xs. The relative reactivity was < 0.05 (9Xs), 0.5 (7F), 0.3 (7Cl), 1 (7Br), and 2.8 (7I). The enzymatic reaction rate of 7F was not so much fast despite the high spontaneous reactivity. Given that the PC rank order of 7Xs was 7I > 7Br > 7Cl > 7F, 7F may have the low affinity for GSH S-transferase, the enzyme catalyzing GSH conjugation, due to the low hydrophobicity. These results indicate that two factors of the spontaneous reactivity and hydrophobicity are determinant for enzymatic reaction rates of 6-halogenopurine derivatives.

Conclusions: We obtained probes with wide-ranging conversion rates. The reactivity of 7I was found to be higher than that of 7Br, which is a desirable feature for the concept of MEM. The use of these probes may allow the assessment of MRP1 function in different kinds of species including human. These findings would be useful for the chemical design of a radioprobe for MRP1 assessment using a purine platform.

P276 SYNTHESIS, 18F-LABELING AND IN VIVO EVALUATION OF rac-4-FLUOROBENZOYL-AZASPIROVESAMICOL AS POTENTIAL LIGAND FOR THE VESICULAR ACETYLCHOLINE TRANSPORTER

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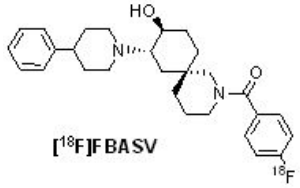
Objectives: Azaspirovesamicols are a novel class of vesamicol analogs and were developed as potential ^{18}F-labeled radiotracers for the vesicular acetylcholine transporter (VAChT). Located in presynaptic cholinergic neurons in the brain, VAChT is intensely investigated as target for imaging of neurodegenerative disorders such as Alzheimer's Disease. So far, insufficient selectivity, especially due to affinity towards sigma receptors, and other limitations such as low brain uptake or metabolite accumulation in the brain has precluded the clinical application of most of the known VAChT radioligands. Therefore, we have synthesized eight novel vesamicol analogs. One of them (FBASV) was selected as candidate for radiofluorination and further biological evaluation.

Methods: Nucleophilic epoxide ring-opening was applied to synthesize the new azaspirovesamicol derivatives. Using (-)-[^{3}H]vesamicol and [^{3}H]DTG as radioligands, the affinities of the new compounds for the VAChT and for sigma-receptors were determined by competitive binding assays on PC12 cells, stably transfected with the rat VAChT. The selected candidate (±)-[^{18}F]FBASV was obtained by microwave assisted synthesis from the corresponding nitro-precursor and subsequent purification using SPE and semi-preparative HPLC. Testing several chiral stationary phases, (±)-FBASV was enantioseparated via chiral HPLC using a chiral detector for identification of the enantiomers.



Results: On the basis of its appropriate VAChT affinity (K_i = 28 nM) and comparably low sigma receptor affinity (K_i = 696 nM) in vitro, (±)-FBASV was selected for radiosynthesis. The ^{18}F-labeling of the nitro-precursor was achieved with a labeling yield of 20%, a radiochemical purity > 99% and a specific activity of 30-75 GBq/mmol. In vivo studies in mice showed a maximum brain uptake of 1.7% ID/g. No radio-metabolites were found in the rat brain at 60 min. p.i. Ex vivo autoradiography of the rat brain revealed accumulation of the radiotracer in typical cholinergic regions such as the striatum, hippocampus and cortex. Enantioseparation of (±)-FBASV was successful for three different chiral stationary phases (PIRKLE phase, amylose and cellulose type). Best separations were achieved with the two cellulose based chiral columns in normal phase mode and in reversed phase mode.

Conclusions: (±)-[^{18}F]FBASV is a promising new radioligand for the vesicular acetylcholine transporter. As preliminary studies in rats have shown, this radiotracer is stable in vivo and accumulates in cholinergic brain regions. Further in vivo studies are needed to confirm these preliminary results. Furthermore, a FBASV enantiomer, with higher affinity to VAChT, has yet to be selected and determined in vitro and evaluated in vivo.

P277 RADIOSYNTHESIS AND IN VITRO EVALUATION OF F-18-LABELED CYANOINDOLE-CYCLOHEXYLAMINE DERIVATIVES AS RADIOLIGANDS FOR THE SEROTONIN TRANSPORTER

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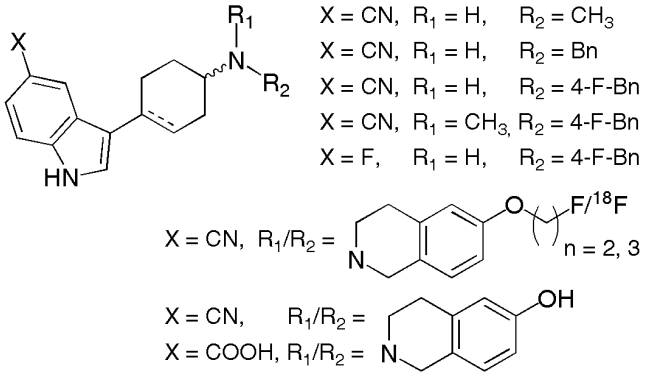
Objectives: Aminocyclohexyl indoles bind with high affinity and specificity to the serotonin transporter (SERT). Based on this new structural lead, we have recently designed 5-cyanoindole-3-yl cyclohex(en)ylamines for future application as ^{18}F-labeled tracers for SERT imaging with PET. Here we report on ^{18}F-labeled 6-alkoxydihydroisoquinoline derivatives as potential PET radiotracers.

Methods: Non-radioactive reference compounds (see fig.) were prepared by multi-step syntheses and tested for their affinity and selectivity towards the SERT in radioligand displacement studies. K_i values for the SERT, dopamine transporter (DAT) and the norepinephrine transporter (NET) were determined on HEK293 cells transfected with the corresponding human genes. Affinities towards the serotonin $5HT_{1A}$ receptor were determined on membranes from rat brain cortex. 6-Hydroxydihydroisoquinoline-cis- and -trans-derivatives, c1 and t1 respectively, have been used as precursors for radiosynthesis via fluoroalkylation. Accordingly, 1,3-bistosyloxypropane 2 was converted into the propyltosylate [^{18}F]3 and used directly for etherification of c1 and t1 (~2 mg) to 6-[^{18}F]fluoropropoxydihydroisoquinoline-cis- and -trans-derivatives c[^{18}F]4 and t[^{18}F]4, respectively. Purification by SPE (Sep-Pak C18 Plus) was followed by semi-preparative radio-HPLC. Lipophilicity (logD) was determined for c[^{18}F]4 and t[^{18}F]4 in batch experiments as well as by HPLC methods.



Results: Saturated cyclohexyl-compounds bound selectively to the hSERT with K_i values between 4 and 330 nM. Notably, cis-isomers showed a higher selectivity than trans-isomers with high affinity to the hSERT and a significant lower affinity to hNET and $r5HT_{1A}$. N.c.a. [^{18}F]3 was obtained with a labeling yield (LY) of 60–80% within 10–15 minutes. Etherification to c[^{18}F]4 and t[^{18}F]4 could be performed in 8–10 minutes with a LY of 40–50% and a radiochemical yield of 11–22% within a total synthesis time of about 2.5 h. Radiochemical and chemical purities were determined to be ≥99% (HPLC, TLC). The specific activity was ~50 GBq/μmol (HPLC). Comparable results were achieved for ^{18}F-labeled fluoroethoxy derivatives (LY 50–60%), starting from 1,2-bistosyloxyethane. This procedure is potentially suitable for an automated module synthesis. LogD values within a range of 2.37 to 3.27 indicate a good potential for brain penetration of c[^{18}F]4 as well as t[^{18}F]4.

Conclusions: Radiochemical, physicochemical and in vitro data suggest that ^{18}F-labeled 6-alkoxydihydroisoquinoline derivatives are promising radiotracers for SERT imaging. Further evaluation of c[^{18}F]4 will be performed to determine radiotracer affinity and to investigate biodistribution and brain uptake.

Research Support: This work has been supported by a grant of the Deutsche Forschungsgesellschaft.

P278 SYNTHESES AND BIOLOGICAL EVALUATION OF NEW COMPOUNDS AS POTENTIAL IMAGING AGENTS FOR THE NMDA-RECEPTOR

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Objectives: It is investigated, that the NMDA receptor is involved in several disorders in the CNS. To provide effective ligands for the glycine binding site for PET imaging, a new series of indole-2-carboxylate derivatives have been synthesized**.**

Methods: Based on the indole-2-carboxylate GV150526 (1), a series of ethyl esters and free acids were synthesized, which are conjugated with a fluoroethoxy group in the terminal phenyl ring. These new compounds, namely ethyl-3-((E)-2-((2/3/4-fluoroethoxy)phenylcarbamoyl)vinyl)-4,6-dichloro-1H-indole-2-carboxylate (1, 3, 5) and the corresponding carboxylic acids (2, 4, 6) were synthesized, cf. Figure 1. Their fluorine-18 labelled analogues could be used for imaging the NMDA receptor by PET. The preliminary IC_{50} values of the ^{19}F-inactive compounds were determined using [^{3}H]MDL-105,519 receptor binding assay (2).

Results: The affinity data of the ethyl esters show affinities about 300 μM. The affinities improve dramatically for the free acids in the order ortho (1438 nM) > meta (595 nM) > para (0.23 nM) for the substitution of the terminal phenyl ring.

Conclusions: The data demonstrate that affinity depends on the variation of the fluoroethoxy group. Especially the para substituted compound shows affinity in the low nanomolare range. The radiolabelled analogue could be used as imaging agent. The synthesis of the corresponding precursor molecule for this promising compound is in progress.

Research Support: Research was supported by Deutsche Forschungsgemeinschaft (DFG RO985/20-1).

References: (1) Di Fabio, R. et al.; J. Med. Chem. 1997, 40, 841-850 (2) Jansen, M.; Potschka, H., Brandt, C.; Loescher, W.;Dannhardt, G.; J. Med. Chem. 2003, 46, 64-73

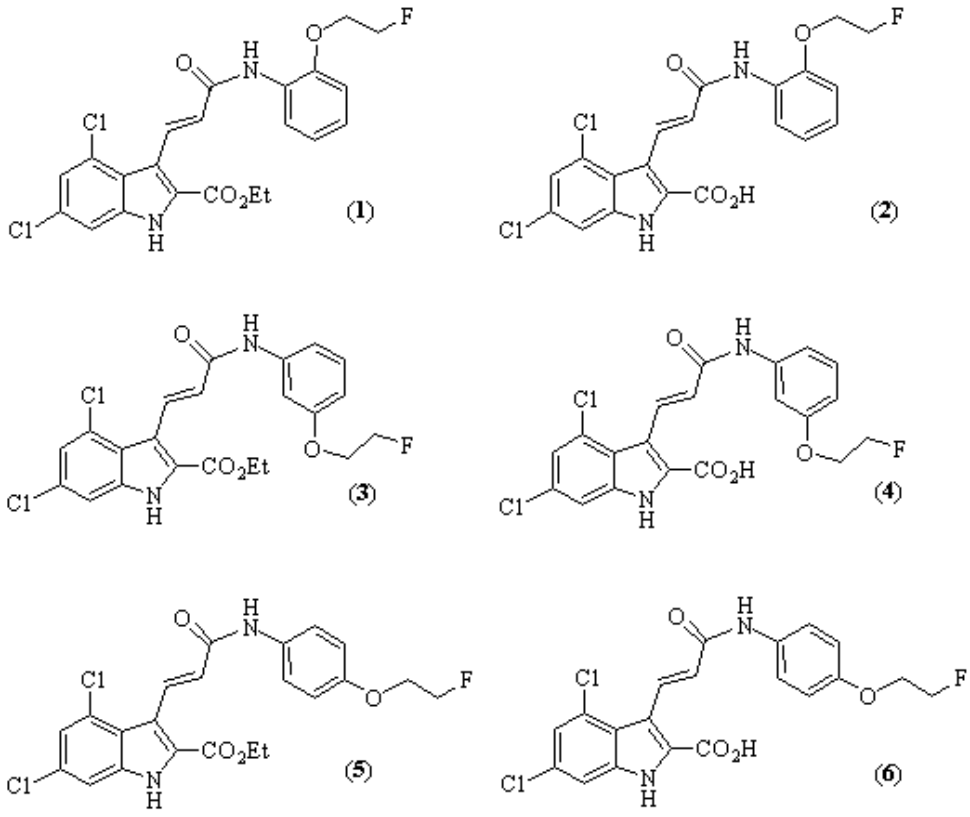

Figure 1: Structures of synthesized NMDA-ligands

P279 [11C]NS-12857: A NOVEL PET LIGAND FOR ALPHA7-NICOTINERGIC RECEPTORS

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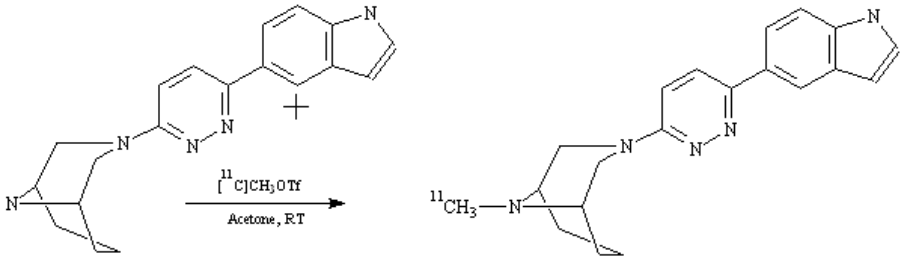
Objectives: The α7-nicotinergic acetylcholine receptors (α7-nAChRs) are pentameric, cationic, ligand gating calcium channels, which are predominantly found in the central nervous system. They are proposed to play an important role in the pathophysiology of different neuropsychiatric diseases, such as schizophrenia, Alzheimer's disease, and drug addiction, thus the α7-nAChR is a promising therapeutic drug target [1]. The clinical relevance of α7-nAChR makes it also an attractive target for radionuclide imaging, e.g. positron emission tomography (PET). Recently promising α7-nAChR ligands, such as [^{11}C]CHIBA-1001, [^{76}Br]SSR180711 [2] and [^{18}F]NS10743 [3] were synthesised. Here we present the synthesis of [^{11}C]NS12857, a novel α7-nAChR agonist PET ligand (Scheme 1.), and the first results from in vivo PET-imaging studies in the pig brain are discussed.



Scheme 1. Preparation of ^{11}C-labelled NS12857

Methods: Synthesis of [^{11}C]NS12857. 0.5mg precursor was dissolved in 300μl acetone, and [^{11}C]MeOTf was bubbled through the solution for 3 minutes at room temperature. The reaction mixture was diluted with 4.5ml 0.1v/v% phosphoric acid and was injected onto the HPLC column (Luna C18(2) 5μm 10×250 mm, eluent: 0.1v/v% phosphoric acid/ethanol 96% = 92/8, flow rate 6.0ml/min). The product fraction was collected in 30 seconds (3.0ml) and transported through a sterile filter to 20ml sterile glass contained 12.0ml 0.1M phosphate buffer pH=7. In vivo PET-imaging. Approx. 500MBq bolus IV injection of [^{11}C]NS12857 was given to a young Danish Landrace pig under propofol anaesthesia, and 90 min baseline scan was performed. After the baseline PET scan, SSR180711, a α7-nAChR partial agonist [4] was injected (5mg/kg bolus + 2mg/kg×h constant infusion). After 30 min, approx. 500 MBq bolus IV injection of [^{11}C]NS12857 was administrated in the blocked condition, and a second scan was performed for 90 minutes.

Results: [^{11}C]NS12857 was obtained in >99% radiochemical purity within 30 min, and the specific radioactivity of the product was up to 550 GBq/μmol. The use of non-toxic eluent in the preparative RP-HPLC method provides injectable grade of [^{11}C]NS12857 without further purification or formulation steps. The yield of the final product was between 3 – 5 GBq EOS. The in vivo PET-studies demonstrated that [^{11}C]NS12857 was widely distributed in the pig brain resembling earlier reports for [^{11}C] CHIBA1001. However, brain uptake of [^{11}C]NS12857 (0.14% dose/ml) generally seems to be higher compared to [^{11}C]CHIBA1001 (0.04% dose/ml).

Conclusions: We synthesised [^{11}C]NS12857, a novel PET-ligand for α7-nAChR receptors. [^{11}C]NS12857 is demonstrated to be a promising radiopharmaceutical for in vivo imaging of α7-nAChRs in the mammalian brain.

References: [1.] K. Brejc et al.,Nature 411., 269 (2001) [2.] K. Hashimoto et al., Plosone, 3.(9) e3231 (2008) [3.] W. Deuther-Conrad et al., Eur. J. Nucl. Med. Mol. Imaging, publised on line 10 January 2009 [4.] B. Biton et al., Neuropsychopharmacology, 32., 1 (2007)

P280 [F-18]-SUBSTITUTED SPIROPIPERIDINES TARGETING SIGMA-1 RECEPTORS: RADIOLABELLING AND BIOLOGICAL EVALUATION

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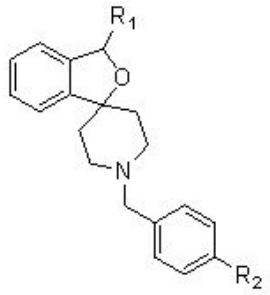
Objectives: Central σ_1 receptors are involved in several neurodegenerative and psychiatric diseases. Proven therapeutic effects of σ_1 receptor agonists and first data on impaired σ_1 receptor properties in Parkinson's and Alzheimer's disease suggest σ_1 receptor imaging as a tool for target validation in drug development as well as for clinically applied research. However, clinical σ_1 receptor imaging by PET is restricted by the availability of only one ^{11}C-labelled tracer. A recently reported new class of F-substituted spirocyclic piperidines has shown very high σ_1 affinity and selectivity. Therefore, potent derivatives containing either a p-fluorobenzyl residue at the piperidine or a fluoropropyl substituent at the isobenzofuran system were selected as candidates for ^{18}F-labelling and first evaluation in vivo.



Compound	R_1	R_2
1	OCH_3	F
2	CN	F
3	n-C_3H_6F	H

Methods: Compound [^{18}F]1 was intended to be obtained by radiofluorination of the trimethylanilinium triflate precursor and subsequent reduction of the 4-fluorobenzamide moiety. [^{18}F]2 should be prepared via reductive amination of the secondary amine with 4-[^{18}F]benzaldehyde. The radiosynthesis of [^{18}F]3 was performed by direct aliphatic nucleophilic substitution of the tosyl precursor. First ex vivo data on brain uptake, organ distribution, and metabolic stability of the radiotracer were obtained in CD-1 mice. The brain distribution of the radiotracer was investigated by ex vivo autoradiography, and its target specificity by in vivo blocking with haloperidol.

Results: The radiosynthesis of [^{18}F]1–3 was considered because of the high σ_1 receptor affinity and selectivity of the compounds 1–3 (K_i in nM and σ_1/σ_2 selectivity: 0.79 and 1205, 0.86 and 311, 1.35 and 620, resp.). The designed radiosynthetic approach towards 1 failed due to difficulties with the required reduction of the 4-fluorobenzamide to the 4-fluorobenzylamine moiety. The reductive amination of the secondary amine with 4-[^{18}F]fluorobenzaldehyde yielded only 2% of [^{18}F]2, not acceptable for further experiments. [^{18}F]3 was obtained with high labelling efficiencies of 60-70%. The following radiochemical parameters were achieved: RCY 35-48%, radiochemical purity >99.5%, specific radioactivity 150-238 GBq/μmol within 90-120 min (n=7). An apparent distribution coefficient of log D=3.03 was determined experimentally, and no radiometabolites permeated into the brain of CD-1 mice up to 60 min p.i. High radiotracer uptake and a long retention were observed in the brain (4% ID/g at 5 min p.i., >50% of the initial uptake at 120 min p.i.). The [^{18}F]3 distribution pattern in the brain corresponds to the σ_1 receptor expression. Furthermore, uptake in the brain and σ_1 receptor expressing organs were sensitive for haloperidol pretreatment.

Conclusions: [^{18}F]3 is a new promising radioligand for imaging σ_1 receptors. Brain uptake kinetics, target specificity, and favorable metabolic properties make [^{18}F]3 suitable for further in vivo investigations.

P281 RADIOSYNTHESIS AND IN VITRO EVALUATION OF NOVEL HISTAMINE H3 TRACERS

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Objectives: The histamine H3 receptor is thought to be implicated in numerous neurological and psychological disorders including Alzheimer's disease and attention-deficit hyperactivity disorder. We are working in collaboration with the pharmaceutical industry to develop a single photon emission computed tomography (SPECT) brain imaging radiolabelled tracer for the H3 receptor in order to facilitate the development of selective histamine H3 antagonists as novel treatments for these disorders.

Methods: The tracer candidates were identified from a library of compounds that have high affinity for the histamine H3 receptor. The radiosynthesis of three lead compounds was investigated, via iododestannylation and iododesilylation with radioiodide, for SPECT imaging. The K_d and B_{max} of the radiolabelled compounds were determined in vitro in rat brain tissue.

Results: All three compounds were successfully radiolabelled with an isolated radiochemical yield of greater than 39% and a radiochemical purity of greater than 99%. The stability of the isolated ^{125}I-labelled compounds is such that the radiochemical purity is >95% after three days. The results from the in vitro evaluation of the all three candidates in rat brain will be presented.

Conclusions: Further investigation of the lead candidate using in vivo animal imaging studies aims to determine the suitability of the tracer as a clinical imaging tool, which could then be used as biomarker of drug action in vivo.

Research Support: This work was supported by an award (Ref: NS_AU_084) from the Translational Medicine Research Collaboration – a consortium made up of the Universities of Aberdeen, Dundee, Edinburgh and Glasgow, the four associated NHS Health Boards (Grampian, Tayside, Lothian and Greater Glasgow & Clyde), Scottish Enterprise and Wyeth Pharmaceutical. Dr S Champion is funded by the Scottish Imaging Network: A Platform for Scientific Excellence (SINAPSE).

P282 IMAGING OF THE EGF RECEPTOR IN A431 TUMOR BEARING MICE USING C-11 AG1478

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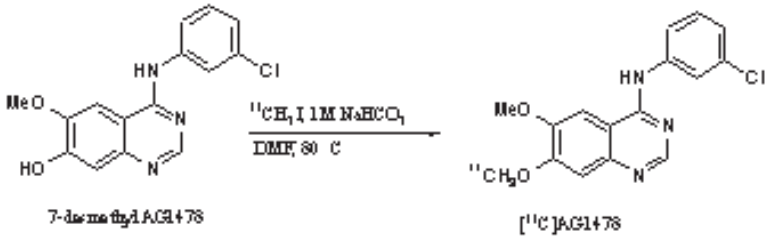
Objectives: The objective of this project is to determine, whether C-11 AG 1478 can be used to image the EGF receptor in A431 tumor bearing mice and to measure pharmacokinetics using dynamic small animal imaging.

Methods: Synthesis of C-11 AG1478 In a reactor vial, 3 mg of 7-desmethyl AG1478 were dissolved in 250 μL of DMF and 10 μL of a 1M $NaHCO_3$ solution were added. C-11 methyl iodide was distilled into the reactor vial and the vial heated to 80 °C for 5 minutes.



The radiotracer was then purified by semi-preparative HPLC and reformulated in 10% ethanol/saline for injection into A431 tumor bearing mice. The decay corrected radiochemical yield of C-11 AG1478 was 58-72% with a specific activity of 21 MBq/mmol. Xenograft model A431 tumor xenografts were established in the right shoulder of Balb/c nude mice via subcutaneous injection of a suspension of 6×10^6 A431 cells. Tumor sizes at the time of scanning ranged between 1 cm^3 and 600mm^3. Animals were scanned for 60 minutes from the time of injection. Twelve 5 minute frames were recorded.

Results: For most major organs such as liver, brain and bowel, the pharmacokinetics observed in the small animal PET scan are very similar to the biodistribution measured by classical excising of organs. However, the cardiac and tumor uptake observed in the biodistribution studies could not be confirmed with small animal PET studies.

Conclusions: The measurement of EGFR density in tumors does not seem possible with C-11 AG1478. Due to the rapid clearance of C-11 AG 1478 from the bloodstream, we do not believe that this is due to the short halflife of the C-11 isotope but more likely due to the reversible nature of this compound's binding to the EGF receptor. The good correlation between biodistribution and small animal imaging studies should result in a reduction of animal numbers required to measure general biodistribution of future EGF receptor imaging agents.

P283 COMPETITIVE BINDING OF RADIOLABELED NEUROTENSIN AGONIST AND ANTAGONIST TO NEUROTENSIN RECEPTORS

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Objectives: Both central and peripheral actions of neurotensin are initiated by association of the peptide to specific receptors located on the plasma membrane of target cells. The aim of our studies was to evaluate the emergent paradigm which measures the preference expressed of radiolabeled neurotensin agonist and antagonist to neurotensin receptors

Methods: In the present study, we have compared the bioaffinity of 177Lu-DOTA-Neurotesin analogue agonist and 177Lu-DOTA-SR48692 nonpeptide neurotensin antagonist by competitive binding methods of their to neurotensin receptors as well as the influence of SR48692 on the affinity and sensitivity of neurotensin receptors, using an ascending dose paradigm. The binding affinity were determined by evaluation of receptor binding affinity of the cold and radiolabeled conjugates by a competitive respectively direct binding assay.Competition binding assay was performed using rat brain cortex membrane. 35 000- 40 000 cpm of radiolabeled conjugate were added in each test tube in the presence of increasing concentration of neurotensin agonist and antagonist cold conjugates. After incubation the samples were processed and measured on NaI (Tl) Gamma counter . We analysed the experimental results on PRISM 4, fitting the total binding data to determine the values of IC50 and K_d

Results: The obtained results for IC50 and $K_d$clear show the influence of SR48692 neurotensin antagonist in the binding of 177Lu-DOTA-Neurotensin to the neurotensin receptors, challenging NT responses.

Conclusions: The obtained results in this work constitute the database for the in vivo researches regarding the pharmacokinetic and efficiency treatment of 177Lu-DOTA-Neurotensin in the neurotensin antagonist presence on pathological animal models, in the hypothesis that targeting neuromodulatory systems, such as the NT systems may offer new strategies in the targeted radionuclide therapy of cancer

P284 SYNTHESIS AND EVALUATION OF N-(3-[11C]METHOXYPHENYL)-4-CHLOROCINNAMIDE: A RADIOLIGAND FOR IN VIVO VISUALIZATION OF THE TRANSIENT RECEPTOR VANILLOID SUBFAMILY TYPE 1 RECEPTOR WITH PET

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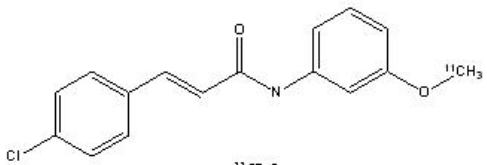
Objectives: The transient receptor potential vanilloid subfamily type 1 (TRPV1) receptor is a non-selective cation channel, mainly expressed on primary sensory neurons, which plays a key role in the integration of noxious stimuli of chronic inflammatory pain or tissue injury. However, the exact role of this receptor in the brain remains elusive. TRPV1 has been visualized using in vitro autoradiography, but there is currently no radioligand available that allows in vivo visualization of this receptor using PET. Therefore, we have synthesized and evaluated a carbon-11 labeled analog of N-(3-methoxyphenyl)-4-chlorocinnamide which was reported as a specific high affinity (18 nM) antagonist for TRPV1.

Methods: N-(3-methoxyphenyl)-4-chlorocinnamide (1) was synthesized as previously described (Gunthorpe MJ, Neuropharmacology 2004, 46:133-149). The labeling precursor (N-(3-hydroxyphenyl)-4-chlorocinnamide (2) was synthesized in four steps starting from 3-acetamidophenol. Both compounds were purified by column chromatography on silica gel and their structures were confirmed by MS and ^{1}H NMR.Carbon-11 was produced by a $^{14}N(p,\alpha)^{11}C$ nuclear reaction in a Cyclone 18/9 cyclotron (IBA, Louvain-la-Neuve, Belgium) yielding $[^{11}C]CH_4$, which was converted to [^{11}C]MeI or [^{11}C]MeOTf in a home-built recirculation module. The obtained [^{11}C]MeI or [^{11}C]MeOTf was then bubbled through a solution of 2 in DMF in the presence of Cs_2CO_3. The reaction mixture was heated at 70 °C, diluted with water and purified with RP-HPLC. The biodistribution of [^{11}C]-2 was evaluated in normal mice at 2 and 60 min p.i.

Results: The log $P_{octanol/buffer}$ value of [^{11}C]-2 was 1.82, suggesting that it may cross the BBB. After intravenous injection in mice, brain uptake was high (at 2 min p.i. 2.3% ID) and wash-out was rapid (at 60 min p.i. 0.2% ID). [^{11}C]-2 was also efficiently cleared from plasma (2 min: 4.7% ID; 60 min: 0.5% ID), mainly by the hepatobiliary pathway.

Conclusions: N-(3-hydroxyphenyl)-4-chlorocinnamide (2) was synthesized and efficiently labelled with ^{11}C to obtain compound [^{11}C]-2, which has favourable biodistribution characteristics in normal mice. Work is in progress to further evaluate the biological properties of [^{11}C]-2. The results of a μPET study and metabolite study are in progress.



[^{11}C]-2

P285 METABOLISM OF DPA-714, A NEW PERIPHERAL BENZODIAZEPINE RECEPTOR PET LIGAND

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Objectives: The translocator protein, formerly known as the peripheral benzodiazepine receptor (PBR) is highly upregulated during neuroinflammatory processes. [^{18}F]DPA-174 (N,N-diethyl-2-(2-[4-(2-[^{18}f]fluoroethoxy)phenyl]-5,7-dimethyl-pyrazolo[1,5-a] pyrimidin-3-yl)-acetamide), is a recently developed radioligand for PET-imaging the PBR [1,2], currently under investigation in non human primate. Understanding the metabolic fate of this radiotracer is essential for an accurate analysis and interpretation of PET measurements. In the present study, the main oxidative metabolites of DPA-714 and the P450 enzymes responsible for these biotransformations were identified. In vivo, the time-course of unchanged [^{18}F]DPA-714 in plasma, required for the determination of the input function of the tracer was measured.

Methods: In vitro study was conducted in rat, human, baboon liver microsomes and cDNA-expressed human P450s for structural elucidation of the main metabolites by LC/ESI/MS/MS. The main isoforms of rat-P450 involved were identified by using specific P450 inducers. The ability of individual human P450 to catalyse the oxidation of DPA-714 was tested with cDNA-expressed human CYPs. In vivo studies were performed following i.v. injection of [^{18}F]DPA-714 in baboon. Plasma samples were analysed by radioactive HPLC. The radioactivity due to unchanged [^{18}F]DPA-714 was expressed as a fraction of the total peak areas. In plasma, the main radiometabolites were identified by coelution with microsomal incubates.

Results: Eight metabolites were produced by rat liver microsomes by the following metabolic pathways: methyl and/or phenyl hydroxylations leading to three different metabolites M1, M2, M4 (m/z=415), N-dealkylation M3 (m/z=371), O-dealkylation M5 (m/z=353) and multiple oxidation reactions M6, M7, M8 (m/z=387, 335,337). In baboon microsomes, M3, M4 and M5 (and M6 detected in low amounts) were generated. The same metabolites were produced by human liver microsomes and recombinant CYP3A4. In rat liver microsomes, the main metabolite was M1 whereas in baboon and human liver microsomes the main metabolite was M3. In rats, induction studies suggested that P4503A was involved in N-dealkylation (M3), whereas P4501A was mainly responsible for phenyl hydroxylation (M1). In vivo in baboons, [^{18}F]DPA-714 was extensively metabolized into 3 main radiometabolites. Two radiometabolites coeluted with M3 and M6 respectively whereas one highly polar radiometabolite was also detected. The latter could arise from further oxidation in plasma of one hydroxylated compound to form an acid derivative. As O-dealkylation of DPA-714 occurred in vitro, its potential formation in vivo would result in the loss of the radioactive fluorine atom. Time-courses of unchanged radioligand in baboon plasma showed that 40% of the tracer remained intact 30 min after administration.

Conclusions: The major routes of DPA-714 metabolism were identified as N-dealkylation, methyl and/or phenyl hydroxylations and O-dealkylation. Species differences were observed in the in vitro oxidative metabolism of DPA-714. In vivo studies in baboon suggested an extensive metabolism with predominant formation of [^{18}F] N-dealkylated metabolites.

References: [1] James et al. J. Nucl. Med. (2008), 49, 814-822. [2] Damont et al. J. Label. Compds Radiopharm. (2008), 51, 286-292.

P286 NOVEL DERIVATIVES CONTAINING N-METHYL-4-PHENYLSULFANYLPHTHALIMIDE CORE AS POTENTIAL IMAGING AGENTS FOR β-AMYLOID PLAQUES

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Objectives: One of the key pathological features in the Alzheimer's disease (AD) brain is the presence of β-amyloid plaques. Therefore, non-invasive detection of them using imaging agents will be a powerful strategy for accurate and early diagnosis of AD. Recently, we developed and evaluated a ^{125}I-labelled DAPH derivative termed N-methyl-4-(4-iodoanilino)phthalimide as a new potential SPECT tracer for AD (Kd=0.21nM). So, we have now used it as a candidate in our continued efforts to develop analogs suitable for Aβ plaque imaging studies. These efforts led to the synthesis of 1 and 2 containing N-methyl-4-phenylsulfanylphthalimide core (Fig.1). The binding studies using post mortem human AD brain homogenates were carried out and 1 was also investigated for its fluorescent staining of senile plaques in human AD brain sections (Fig. 2).

Methods: Synthesis of 1 and 2 is shown in Fig. 1

Results: Ki values of 1 and 2 were 10.2 nM and 1.1 nM for AD brain homogenates in vitro, respectively. Compound 1 showed excellent fluorescent staining of β-amyloid plaques in the brain section. In a qualitative way, this image supported the specificity of both compounds for β-amyloid plaques.

Conclusions: The preliminary results are encouraging, further studies are planned to develop this core-based ^{125}I-labelled derivatives as new potential SPECT tracers for Aβ plaque imaging studies.

Research Support: This work was funded by NSFC (20871021).

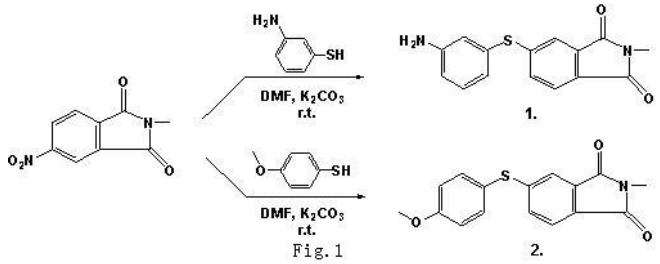
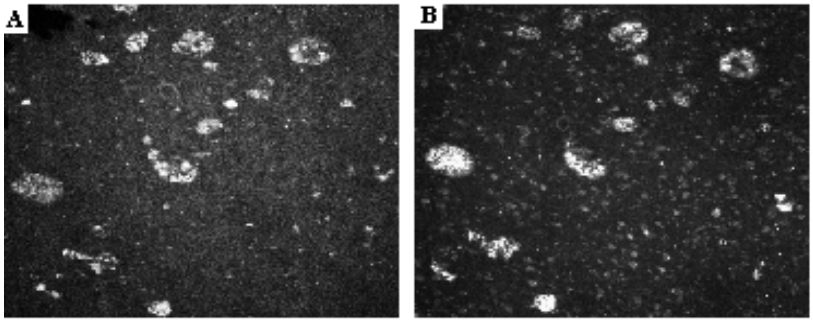


Fig. 1



P287 LABELING OF SP203 WITH CARBON-11 FOR EVALUATION AS AN MGLUR5 RADIOLIGAND IN MONKEY WITH PET

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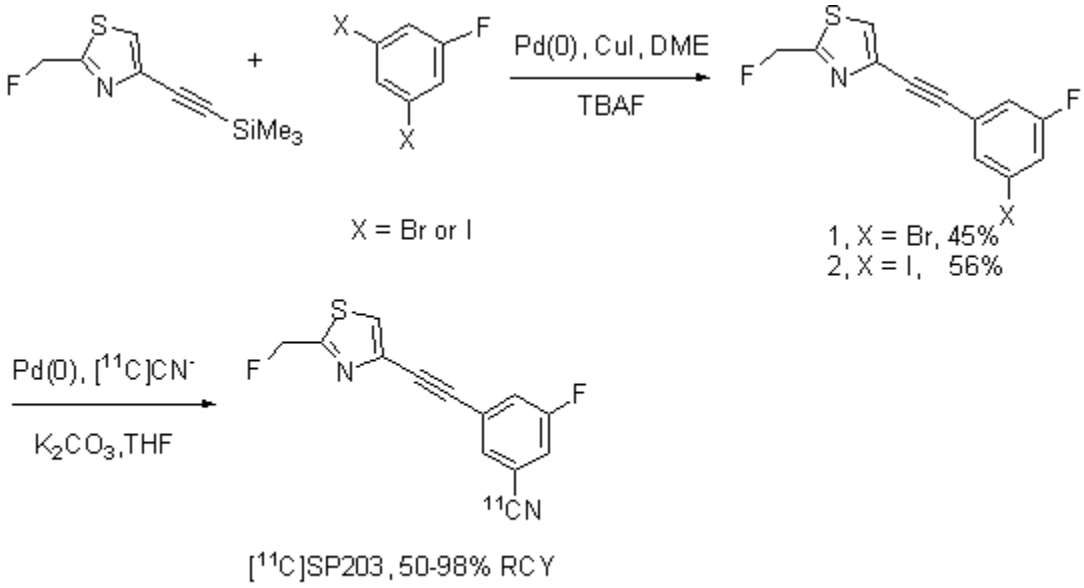
Objectives: [^{18}F]SP203 (3-fluoro-5-[[2-([^{18}F]fluoromethyl)thiazol-4-yl]ethynyl]benzonitrile), labeled in its 2-fluoromethyl group, has proven to be an effective radioligand for imaging brain mGluR5 receptors in human subjects in vivo, without complications from radiodefluorination [Brown et al., J. Nucl. Med. 2008, 49, 2042]. However, [^{18}F]SP203 is defluorinated in monkey and shows high uptake in jaw bone and skull [Siméon et al., J. Med. Chem. 2007, 50, 3256]; this uptake potentially confounds measurement of mGluR5 receptors in monkey brain. Labeling of SP203 with carbon-11 might avoid problems from troublesome radiometabolites. Here, we aimed to prepare [^{11}C]SP203 for evaluation as a new mGluR5 radioligand in monkey.

Methods: Treatment of 2-fluoromethyl-4-((trimethylsilyl)ethynyl)-1,3-thiazole with either 3,5-di-bromo- or 3,5-di-iodo-benzonitrile in 1,2-dimethoxyethane with CuI (1%), $Pd(PPh_3)_2Cl_2$ (2%) and t-butylammonium fluoride at 80 °C under argon overnight gave bromo precursor (1) or iodo precursor (2) in 45 or 56% yield, respectively (Scheme). Cyclotron-produced [^{11}C]HCN was bubbled into a solution of K_2CO_3 (1 mg) and kryptofix 2.2.2 (4 mg) in acetonitrile (500 μL). Then the solvent was evaporated off and the residue treated with either precursor 1 or 2 (1 mg) and tetra-kis(triphenylphosphine)palladium(0) (1 mg) in THF (0.5 mL) at 80 °C for 4 min. The radioligand (t_R = 8.0 min) was purified on a semi-preparative size Luna C18 column eluted with acetonitrile: aq. 10 mM ammonium formate (60: 40, v/v) at 6 mL/min. Kinetic brain images were acquired on an HRRT PET camera for 120 min after intravenous injection of [^{11}C]SP203 (~ 4 mCi; 560 mCi/μmol) into a rhesus monkey.



Scheme. Synthesis of [^{11}C]SP203 from [^{11}C]cyanide.

Results: The iodo precursor gave almost quantitative decay-corrected radiochemical yield of [^{11}C]SP203 (250–560 mCi/μmol; n = 10). The bromo precursor, gave lower radiochemical yield (≤ 52%). After injection of [^{11}C]SP203 into monkey, the uptake of radioactivity into brain was similar to that after injection of [^{18}F]SP203 [Siméon et al., J. Med. Chem. 2007, 50, 3256], with the exception that uptake of radioactivity into bone was almost absent; uptake in the mandible was only 1.5 standardized uptake value (SUV) and decreased rapidly with time. Radioactivity reached 6.7 SUV in the striatum and 4.8 SUV in the hippocampus at 27 min compared to radioactivity concentrations of about 5.5 SUV in the same two regions after injection of [^{18}F]SP203.

Conclusions: [^{11}C]SP203 was readily prepared from [^{11}C]cyanide ion. [^{11}C]SP203 appears promising for imaging brain mGluR5 receptors in monkey. Further radioligand evaluation is in progress.

P288 AUTOMATISATION AND FIRST EVALUATION OF [18F]FE@SUPPY:2, AN ALTERNATIVE PET-TRACER FOR THE ADENOSINE A3 RECEPTOR: A COMPARISON WITH [18F]FE@SUPPY

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Objectives: Since the Adenosine-A_3-receptor was identified in the late 1990´s, there is little data available describing its distribution in vivo. Recently, we introduced [^{18}F]FE@SUPPY as the first PET-tracer for this receptor. In the present investigation we translated this fluoroethyl-ester into the fluoroethyl-thioester [^{18}F]FE@SUPPY:2 (5-ethyl 2,4-diethyl-3-((2-[^{18}F]fluoroethyl) sulfanylcarbonyl)-6-phenylpyridine-5-carboxylate). Aims of the present study were the evaluation of (1) the automatized preparation of both [^{18}F]FE@SUPPY-derivatives, (2) the biodistribution of [^{18}F]FE@SUPPY:2, (3) the lipophilicity and (4) the comparison of the findings of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2.

Methods: The automated preparations of both [^{18}F]FE@SUPPY-analogues were performed on a GE TRACERlab Fx_{FN} synthesizer using suitable precursors. Biodistribution experiments were performed using Sprague-Dawley rats/Him:OFA. Lipophilicity of the compounds was determined using an HPLC assay.

Results: 22 automated radiosyntheses were performed for both radiotracers. Specific radioactivity was 70±26GBq/μmol for [^{18}F]FE@SUPPY and 340±140GBq/μmol for [^{18}F]FE@SUPPY:2. Biodistribution experiments evinced bowels and liver as organs with highest uptake and intermediate uptake in kidney, lung and heart. LogP values of both molecules ranged from 3.99 to 4.12 at different pH.

Conclusions: From a radiopharmaceutical perspective, drastically better specific radioactivities would militate in favour of [^{18}F]FE@SUPPY:2; preclinical evaluations, so far, do not permit the decision upon the selection of the optimum [^{18}F]FE@SUPPY-derivative. With [^{18}F]FE@SUPPY:2, we are able to provide a second potential tracer that could help to further characterize the still quite unexplored Adenosine-A_3-receptor.

Research Support: This project is partly sponsored by the Austrian Academy of Sciences (DOC-fFORTE 22347) awarded to D. Haeusler and by the Austrian Science Fund (FWF P19383-B09) awarded to M. Mitterhauser. The authors thank Drs Kvaternik and Bornatowicz at the ARC for providing their facilities. Sylvia Hiessberger at BSM Diagnostica is acknowledged for organizing talents.

Table 1 – Biodistribution values of [^{18}F]FE@SUPPY:2 in rats at different time points.

Tissue	5min	15min	30min	60min	120min
blood	0,18 ± 0,05	0,15 ± 0,05	0,18 ± 0,05	0,14 ± 0,02	0,12 ± 0,03
liver	1,08 ± 0,39	0,76 ± 0,34	0,79 ± 0,24	0,40 ± 0,06	0,21 ± 0,04
femur	0,27 ± 0,10	0,20 ± 0,10	0,31 ± 0,10	0,48 ± 0,08	0,67 ± 0,10
lung	0,48 ± 0,14	0,31 ± 0,11	0,25 ± 0,06	0,12 ± 0,04	0,07 ± 0,05
heart	0,85 ± 0,24	0,40 ± 0,07	0,31 ± 0,08	0,15 ± 0,03	0,10 ± 0,03
thyroid	0,41 ± 0,13	0,24 ± 0,09	0,30 ± 0,13	0,21 ± 0,03	0,14 ± 0,09
kidney	1,06 ± 0,37	0,56 ± 0,16	0,61 ± 0,13	0,43 ± 0,11	0,26 ± 0,05
testes	0,10 ± 0,04	0,08 ± 0,04	0,13 ± 0,05	0,11 ± 0,02	0,09 ± 0,03
fat	0,05 ± 0,02	0,08 ± 0,04	0,18 ± 0,17	0,19 ± 0,03	0,25 ± 0,08
muscle	0,26 ± 0,10	0,15 ± 0,09	0,18 ± 0,08	0,12 ± 0,04	0,08 ± 0,04
colon	0,19 ± 0,09	0,21 ± 0,15	0,66 ± 0,84	0,69 ± 1,00	0,16 ± 0,11
ileum/jejunum	0,78 ± 0,41	1,45 ± 0,97	0,50 ± 0,32	0,45 ± 0,28	0,21 ± 0,11
spleen	0,33 ± 0,13	0,18 ± 0,05	0,17 ± 0,05	0,09 ± 0,02	0,07 ± 0,03
brain	0,34 ± 0,13	0,18 ± 0,02	0,21 ± 0,06	0,09 ± 0,02	0,07 ± 0,01
carcass	0,31 ± 0,09	0,39 ± 0,13	0,33 ± 0,13	0,26 ± 0,05	0,15 ± 0,05

Values represent percentage of injected dose per gram of tissue (% I.D./g; arithmetic means ± SD

P289 IN VIVO EXAMINATION OF 99mTc(I) TRICARBONYL LABELED HYNIC-D-Phe1-OCTREOTIDE AS AN IMAGING AGENT FOR PANCREATIC TUMOR

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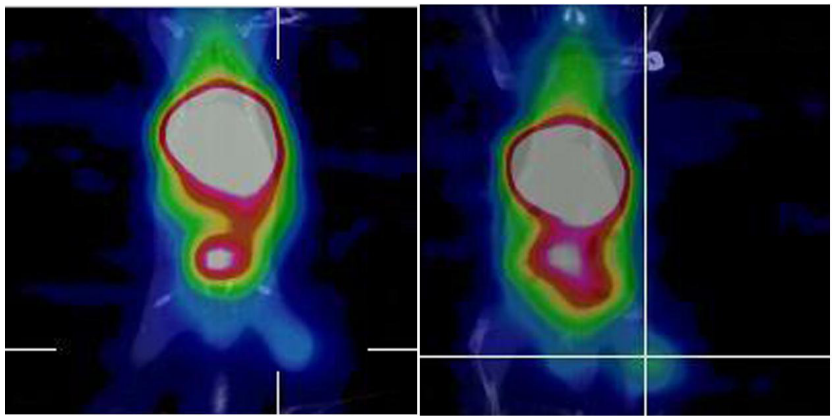
Objectives: We have attempted to produce a novel agent as ^{99m}Tc(I)-hydrazinonicotinyl(HYNIC)-D-Phe^1-octreotide from the reaction of $[^{99m}Tc(CO)_3\ (OH_2)_3]^+$ with HYNIC-D-Phe^1-octreotide. In vitro and in vivo studies were carried out for assessing its potential as an imaging agent for detecting pancreatic tumor.

Methods: $[^{99m}Tc(CO)_3\ (OH_2)_3]^+$ was prepared by using IsoLink kit (Mallinckrodt). D-Phe^1-octreotide was prepared by a solid phase peptide synthetic method. HYNIC in the form of tri-t-butyl ester was coupled to the Lys^5(BOC) protected D-Phe^1-octreotide in N, N'-dimethylformamide followed by decoupling and S-S cyclization. The HYNIC-D-Phe^1-octreotide conjugate obtained was purified by HPLC and identified with ESI/MS. For ^{99m}Tc labeling, $[^{99m}Tc(CO)_3\ (OH_2)_3]^+$ and HYNIC-D-Phe^1-octreotide were mixed and reacted under pH 7 solution at 75~80℃. The yield of ^{99m}Tc(I)-HYNIC-D-Phe^1-octreotide was assayed by HPLC. Stability of ^{99m}Tc(I)-HYNIC-D-Phe^1-octreotide was tested by incubation in PBS and FBS solution, respectively followed by HPLC assay for its radiochemical purity. Receptor binding was tested using AR42J rat pancreatic tumor cell membranes. The binding affinity of HYNIC-D-Phe^1-octreotide was tested in a competition assay against ^{125}I-Tyr^1-somatostatin . Biodistribution and tumor uptake were determined in AR42J tumor-bearing nude mice via tail vein injection. A micro-SPECT/CT imaging was simultaneously conducted.

Results: The molecular weight of the purified HYNIC-D-Phe^1-octreotide was determined to be 1154.6 Da, in accord with its calculated value. The labeled yield of ^{99m}Tc(I)-HYNIC-D-Phe^1-octreotide could reach ≧90%. The ^{99m}Tc(I) labeled octreotide was found to be stable in PBS solution but decrease its radiochemical purity about 40~45% after incubation in FBS solution at 37℃ for 1 h. In displacement studies using ^{125}I-Tyr^1-somatostatin as the radioligand, the IC_{50} value of HYNIC-D-Phe^1-octreotide was determined to be close to that of Sandostatin, i.e., 0.35 nM. Biodistribution showed tumor uptakes with tumor to muscle ratios from 2.10 at 0.5 h postinjection to 4.76 at 4.0 h postinjection. Correspondingly, the tumor scintigraph appeared apparently in the imaging at 1 h postinjection and became distinct in the imaging at 4 h postinjection (see Fig. 1). However, the accumulations of radioactivity remained high in the blood and in the liver at ca. 6~7 %ID/g and ca. 32~35 %ID/g through the whole period of postinjection time studied. This could be also correspondingly seen from micro-SPECT/CT imaging (see Fig. 1).

Conclusions: The proposed ^{99m}Tc(I) labeled HYNIC-octreotide agent showed good stability in PBS and high receptor binding affinity to the AR42J cell membranes. However, the proposed agent was not sufficiently stable in blood, most probably owing to protein binding; the resulted high accumulations in the blood and in the liver might limit its possible clinical use, although the uptake in the pancreatic tumor could be still clearly displayed.



P290 DETERMINATION OF POSSIBLE P-GLYCOPROTEIN INTERATION OF A NOVEL 5-HT2A LIGAND [18F] MH.MZ

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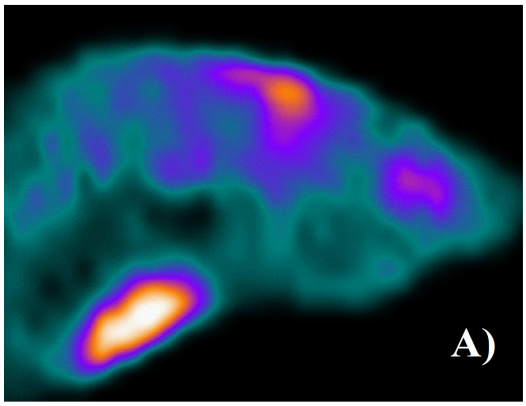
Objectives: To date in vivo studies for imaging the serotinin signal cascades have been performed with several 5-HT_{2A} selective antagonists such as [^{11}C]MDL 100907 or [^{18}F]altanserin. Based on the advantage of MDL 100907 in terms of its high affinity and selectivity we recently developed an ^{18}F-analog of MDL 100907, [^{18}F]MH.MZ (K_i=3.0 nM for 5-HT_{2A}) resulting in a superior PET-tracer of studying the 5-HT_{2A} receptor status at least in rats.[1,2] [^{18}F]altanserin, another 5-HT_{2A} tracer, has recently been shown to be a substrate of efflux transporters, namely the P-glycoprotein (P-gp) of the blood-brain-barrier, limiting its availability in the CNS.[3] This structure is very similar to that of [^{18}F]MH.MZ. The objective of this study was to determine the influence of the receptor binding of [^{18}F]MH.MZ regarding its P-gp activity.

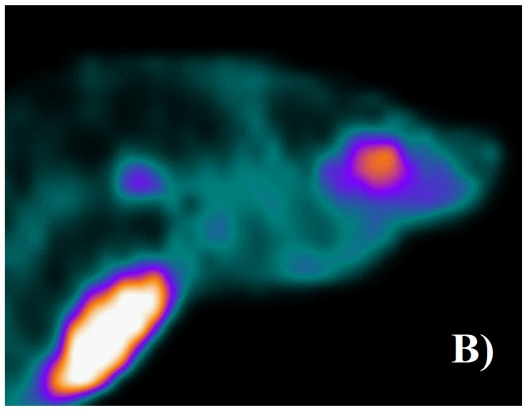
Methods: [^{18}F]MH.MZ was applied as a putative substrate to measure changes in 5-HT_{2A} receptor binding in transgenic P-gp KO and wild-type mice. Moreover, μPET was used to study the uptake profile of [^{18}F]MH.MZ within the brain, whereas the brain to plasma concentrations of [^{18}F]MH.MZ and MH.MZ were determined ex vivo.

Results: μPET imaging in KO mice showed a global increase of radioactivity (Figure 1), whereas ex vivo analysis showed an entry of MH.MZ into the brain which clearly is P-gp dependent. Moreover, these studies indicated that the brain to plasma concentration was increased 4 fold in P-gp KO vs. wild-type mice using a dose of 10 mg/kg of MH.MZ.

Conclusions: In summary, the brain to plasma concentration ratios were higher in P-gp KO transgenic mice treated with MH.MZ vs. wild-type mice. However, the frontal cortex-to-cerebellum ratio of [^{18}F]MH.MZ showed to be in both KO- and WT mice to be equal. Therefore, the cerebellum can be used as a reference region for the 5-HT_{2A} antagonist, [^{18}F]MH.MZ. Thus, regional quantification of P-gp is not necessary for accurate PET assessment of 5-HT_{2A} receptor density. These findings are very similar to the observed behaviour of [^{18}F]altanserin (not published data).

References: 1. Herth, M.M.; Piel, M.; Debus, F.; Schmitt, U.; Lddens H.; Rsch, F.; Preliminary in vivo and ex vivo evaluation of the 5-HT_{2A} imaging probe [^{18}F]MH.MZ. Nuclear Medicine and Biology 2009, (accepted) 2.Herth, M.M., Debus, F.; Piel, M.;Palner, M.; Knudsen, G.M.; Lddens, H.; Rsch, F.; Total synthesis and evaluation of [^{18}F]MH.MZ. Bioorg. Med. Chem. Lett. (2008) 18, 1515 - 1519. 3. Palner, M; Syvnen, S:, Kristoffersen; U.S.; Gillings, N.; Kjaer, A.; Knudsen, G.M. The effect of aP-glycoprotein inhibitor on rat brain uptake and binding of[18F]altanserin: a micro-PET study. Abract Brain and Mind Forum 2007





P291 SYNTHESIS AND IN VITRO AFFINITIES OF VARIOUS MDL 100907 DERIVATIVES AS POTENTIAL 18F-RADIOLIGANDS FOR 5-HT2A RECEPTOR IMAGING WITH PET

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Objectives: Radiolabelled piperidine derivatives such as [^{11}C]MDL 100907 and [^{18}F]altanserin have played an important role in diagnosing malfunction in the serotonergic neurotransmission. Concerning molecular imaging, the advantage of [^{18}F] altanserin (b) over [^{11}C]MDL 100907 (a) is the possibility to perform equilibrium scans lasting several hours and to transport the tracer to other facilities based on the 110 minute half-life of ^{18}F-fluorine. A drawback of [^{18}F]altanserin is its rapid and extensive metabolism. Four metabolites are formed in humans that cross the blood-brain-barrier,whereas metabolites of [^{11}C]MDL 100907 do not enter the brain to any larger extent.[1,2,3] The aim of this study was to synthesize a ligand combining the reported better selectivity and in vivo stability of MDL 100907 as compared to altanserin and the superior isotopic properties of an ^{18}F-label as compared to an ^{11}C-label.

Methods: A variety of novel piperidine MDL 100907 derivatives, possible to label with ^{18}F-fluorine, were synthesized to improve molecular imaging properties of [^{11}C]MDL 100907. Their in vitro affinities to a broad spectrum of neuroreceptors and their lipophilicities were determined and compared to the clinically used reference compounds MDL 100907 and altanserin.

Results: The novel compounds MA-1 and (R)-MH.MZ show K_i-values in the nanomolar range towards the 5-HT_{2A} receptor and insignificant binding to other 5-HT receptor subtypes or receptors. Interestingly, compounds MA-1, MH.MZ and (R)-MH.MZ provide a receptor selectivity profile similar to MDL 100907. These compounds could possibly be preferable antagonistic ^{18}F-tracers for visualisation of the 5-HT_{2A} receptor status. Medium affine compounds (e.g. VK-1) were synthesized and have K_i values between 30 and 120 nM. All promising compounds show logP values between 2 and 3, i.e. within range of those for the established radiotracers altanserin and MDL 100907. The novel compounds MA-1 and (R)-MH.MZ thus appear to be promising high affine and selective tracers of ^{18}F-labelled analogues for 5-HT_{2A} imaging with PET.

Conclusions: A series of novel MDL 100907 derivatives containing a fluorine atom were synthesized and evaluated for their in vitro behaviour. Structure-Activity Relationships (SAR) studies suggested that the tested compounds had affinities to the 5-HT_{2A} receptor in the nanomolar range. Several ^{18}F-analogues are being synthesised for further pharmacologic characterisation.

References: 1. Meltzer, C. C.; Smith, G.; DeKosky, S. T.; Pollock, B. G.; Mathis, A. M.; Moore, R. Y.; Kupfer, D. J.; Reynolds, C.F. Neuropsychopharm 1998, 18, 407 2. Heinrich, T., Bttcher, H., Prcher, H., Gottschlich, R., Ackermann, K-A.,van Amsterdam, C. Chem. Med. Chem. 2006, 1, 245 3. Schmidt, C.J.; Kehne, J.H.; Carr, A.A. CNS Drug Reviews 1997, 3, 49

P292 RADIOLABELING AND EVALUATION OF MDL 100,907 DERIVATES AS POTENTIAL 18F-RADIOLIGANDS TO DETERMINE CHANGES IN ENDOGENOUS SEROTONIN

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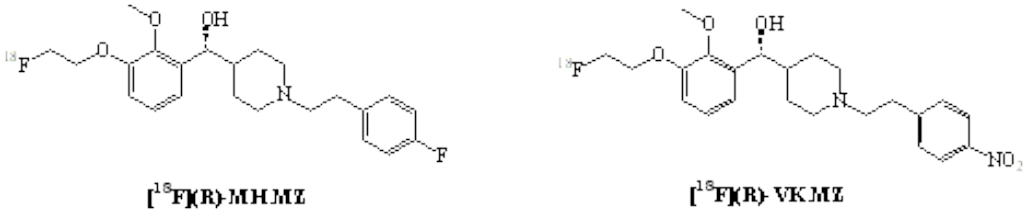
1. Johannes Gutenberg University of Mainz, Institute of Nuclear Chemistry, Mainz, Germany; 2. Johannes Gutenberg University of Mainz, Department of Psychatry, Mainz, Germany; 3. Rigshospitalet, Center for Integrated Molecular Brain Imaging, Copenhagen, Denmark

Objectives: PET ligands that are able to detect changes in the concentration of endogenous serotonin are a valuable tool to study the pathophysiology of depressions and the effects of its pharmacotherapies. The purpose of this study was to explore the effect of paroxetine-induced increased serotonin levels on the binding of the 5-HT_{2A} antagonist [^{18}F](R)-MH.MZ and its nitroderivate [^{18}F](R)-VK1.MZ.



Structure of [^{18}F](R)-MH.MZ and [^{18}F](R)-VK1.MZ

Methods: The in vitro-affinity for the inactive fluoro-compound (R)-VK1.MZ was determined in a [^{3}H]MDL 100,907 binding assay. Both radioligands were labeled with ^{18}F by fluoroethylation of the corresponding phenolic precursors using [^{18}F]fluorethyltosylate. The radiolabeling procedure for [^{18}F](R)-VK1.MZ was optimized due to time, temperature and solvent. Purification was carried out by HPLC and cartridge separation. Competition studies with serotonin were performed by autoradiography and a first μPET-study was carried out.

Results: Both ligands demonstrate good affinities in the nanomolar range and a high selectivity for the 5-HT_{2A} receptor. Optimization of the radiochemical reaction conditions for (R)-VK1.MZ gave radiochemical yields of about 80 % for the fluoroethylation. The final formulation took no longer than 80 minutes and provided the labeled compound in a radiochemical yield of 50 % with a purity > 96 % and a typical specific activity of about 10 GBq/μmol. Autoradiographic studies of [^{18}F](R)-MH.MZ showed excellent binding properties (BP = 8.3), whereas [^{18}F](R)-VK1.MZ showed a lower specific binding (BP = 2.4). This is probably due to the decreased affinity. For both ligands the specific binding could be reduced significantly by the addition of 100 nM serotonin.

Conclusions: The reaction parameters for the radiolabeling of [^{18}F](R)-VK1.MZ were optimized. [^{18}F](R)-MH.MZ and [^{18}F](R)-VK1.MZ could be obtained as an injectable solution in good radiochemical yields. Both tracers showed good binding properties in vitro and their specific binding could be reduced by the addition of physiological amounts of serotonin. μPET-studies with male rats under the influence of paroxetine are being performed in the close future.

P293 SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS OF NEW 5-HT2A RECEPTOR ANTAGONISTS COMBINING THE STRUCTURE OF (R)-MH.MZ, ALTANSERINE AND SR 46349B

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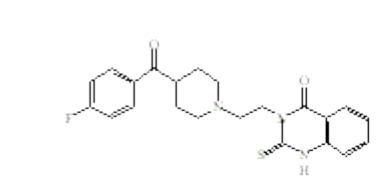
Objectives: ^{18}F-labeled 4-benzoylpiperidine derivatives such as [^{18}F]MH.MZ and [^{18}F]altanserin play an important role as imaging agents to determine the 5-HT$_{2A}$ receptor status in vivo using positron emission tomography (PET). Both radioligands can be well accommodated to the binding model published by Anderson et al [1]. This binding model provides two possible directions in which 4-benzoylpiperidine derivatives can bind to the receptor binding site. The aim of this work was to develop new derivatives containing structure elements of both ligands, [^{18}F]MH.MZ and [^{18}F]altanserin, to identify in which orientation they bind to the binding site. This information should provide the possibility to deduce structures for new high affine ligands or to optimize established tracers.

Methods: Three new ligands were synthesised containing a 4-benzoylpiperidine moiety as lead structure, cf. Fig. 1.. For compound 1 the p-fluorphenyl ring of altanserin was replaced by the 3-fluorethoxy-2-methoxyphenyl ring present in the structure of MH.MZ. Compound 2 contains a quinazolinon ring instead of the p-fluorphenyl substituent in the structure of MH.MZ. The O-dimethylaminoethyloxim residue, present in the structure of SR 46349B, was introduced in compound 3 to study SAR. The in vitro affinity for compounds 1-3 was determined by [^{3}H]MDL 100,907 competition binding assays with GF-62 cells, expressing high amounts of the 5-HT$_{2A}$ receptor.

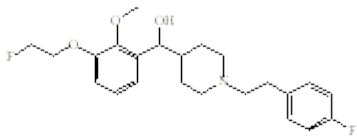
Results: Altanserin binds to the 5-HT$_{2A}$ receptor with the p-fluorbenzoyl moiety in a hydrophobic binding pocket with subnanomolar affinity. The remarkable reduced affinity of compound 1 and 2 indicates, that the additional space required by the fluorethoxy group and the methoxy group is not tolerated. These results demonstrate that [^{18}F]MH.MZ can only bind to the 5-HT$_{2A}$ receptor with the p-fluorphenylethyl residue in the hydrophobic binding pocket. By varying size and hydrophobic properties of the substituent in the para position it should be possible to improve the binding characteristics of the radioligand.

Conclusions: This work demonstrates that [^{18}F]MH.MZ binds to the 5-HT$_{2A}$ receptor with the p-fluorphenylethyl residue in a sterically restricted hydrophobic binding pocket. Structure-Activity Relationships (SAR) studies of derivatives with different p-substituents of [^{18}F]MH.MZ are currently being performed.

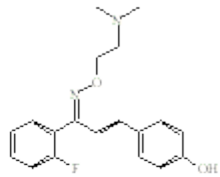
References: [1]Andersen K, Liljefors T, Gundertofte K, Perregaard J, Berges KP; (1994); Development of a Receptor-Interaction Model for Serotonin 5-HT$_2$ Receptor Antagonists. Predicting Selectivity with Respect to Dopamine D2 Receptors; J. Med. Chem. 37, 950



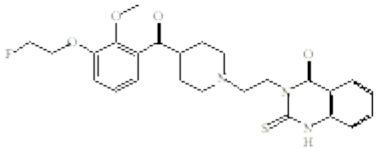
Altanserin



MH.MZ



SR 46349B



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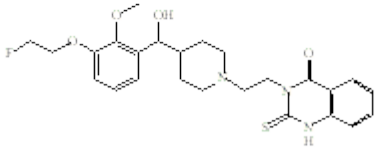

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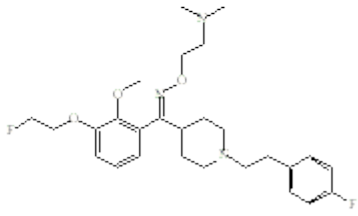

3

P294 IMAGING OF CHANGES IN P-GLYCOPROTEIN ACTIVITY IN VIVO WITH 68GA-SCHIFF BASE DERIVATIVES

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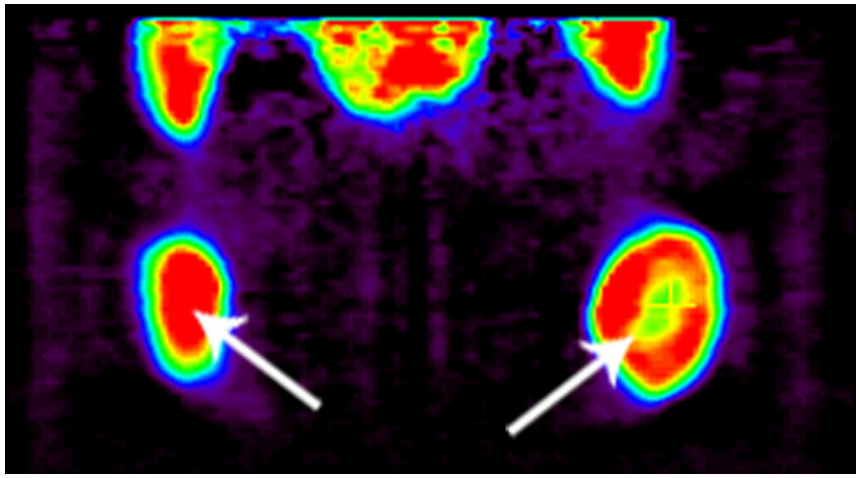
Objectives: P-glycoprotein (pGP) is an active drug transporter of the ABC family pumping a wide number of xenobiotics out of the cell. Since many tumours overexpress pGP and several chemotherapeutics are substrate, the cytotoxicity of these drugs is reduced leading to multidrug resistance. Recent studies showed that the metabolic microenvironment of tumour affects the functional pGP-activity. Especially an extracellular acidosis (pH 6.6) leads to more than doubling of the transport rate resulting in a reduced cytotoxicity of chemotherapeutics [1]. In this mechanism, MAP kinases (p38 and ERK1/2) play an important role in the signal pathway. With Gallium-68 Schiff base complexes [2], in particular ^{68}Ga-MFL6.MZ, it became possible to visualize the functional activity of the pGP in vivo [3]. This compound allows the analysis of alterations of the tumour microenvironment (acidosis) as well as interrupting the signal pathway (inhibition of p38 and ERK1/2) on the pGP-transport activity non-invasively.

Methods: The ^{68}Ge/^{68}Ga generator provides the positron emitter Gallium-68 as an inexpensive source of a PET nuclide. Using a published purification method [4] the ligand MFL6.MZ was labelled in a fast and easy process, directly ready for injection. Tumours were induced by subcutaneous injection of R3327-AT1 cells into the hind foot dorsum of male Copenhagen rats. Tumours were used when they had reached 1-2 mL. Acidification of the tumour was achieved by injection of small amounts (20 μL) of lactic acid directly into the tumour. The same amount of sodium lactate was injected into the second tumour as control. The MAP kinases were inhibited by intratumoural injection of SB203580 (p38) and U0126 (ERK1/2).

Results: Acidifying the tumour led to a local reduction of the tracer accumulation in the tumour. A reduced tissue concentration indicates a higher pGP transport activity. The tracer concentration of acidified tumours was only 80% of controls. In contrast, MAP kinase inhibitors lead to a reduced pGP transport rate which should result in a higher tracer accumulation in the tissue. Inhibition of p38 led to almost a doubling of the tracer activity as compared to the contralateral control tumour, whereas with the ERK1/2 inhibitor the concentration increased by 30%.



Conclusions: The Schiff base derivative MFL6.MZ labelled with ^{68}Ga allows a non-invasive monitoring of the functional pGP-activity in tumours. The results confirm previous in vitro studies that the transport rate in acidic tumour is markedly increased and that the MAP kinases p38 and ERK1/2 play a central role in the signalling pathway. The new tracer will be helpful in the development of new pGP-inhibitors in order to overcome multidrug resistance. In addition, this tracer will allow identifying patients overexpressing pGP, eventually needing a more aggressive treatment or other therapy modalities (e.g. radiotherapy).

References: [1] Sauvant et al, Int J Cancer 123: 25322542 [2] Sharma et al, J Nucl Med 46: 354-364 [3] Fellner et al, in preparation [4] Zhernosekov et al, J Nucl Med 48: 1741-1748

P295 EVALUATION OF [18F]FEPPA FOR IMAGING THE PERIPHERAL BENZODIAZEPINE RECEPTOR IN A BREAST CANCER XENOGRAFT MOUSE MODEL

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Objectives: The peripheral benzodiazepine receptor (PBR) has established roles in breast cancer,[1] however, no attempts have been made to image this target with PET. Our objective was to explore the feasibility of imaging PBRs in a human breast cancer xenograft mouse model using [^{18}F]FEPPA. We here report the automated radiosynthesis of [^{18}F]FEPPA, in vivo imaging and ex vivo biodistribution of this radiotracer in MDA-MB-231 xenograft mouse models, and a comparison of imaging and biodistribution with [^{18}F]FDG.

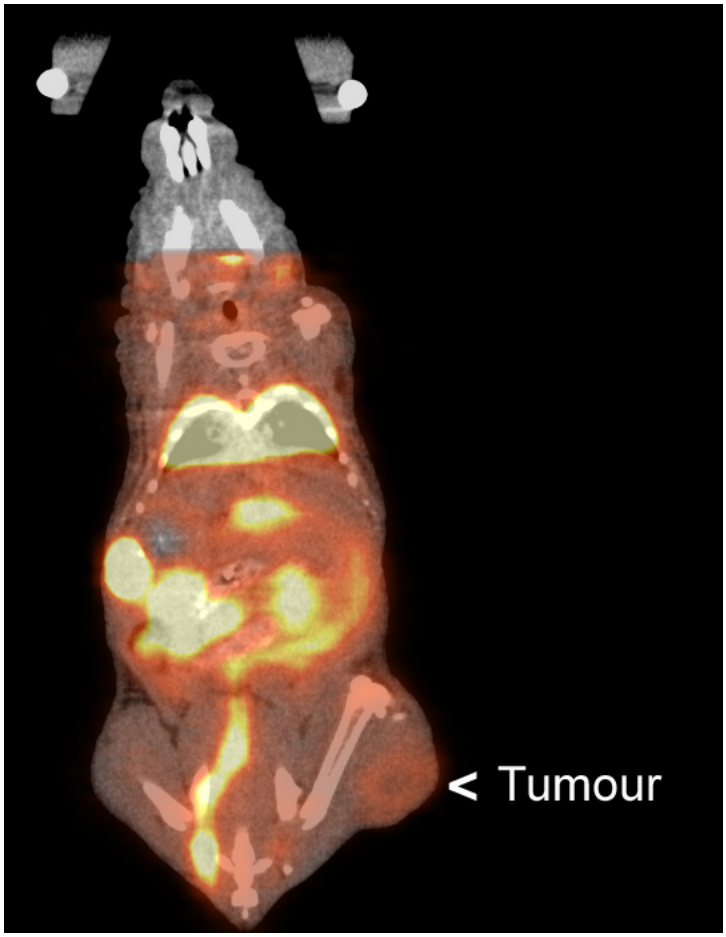
Methods: [^{18}F]FEPPA was prepared as recently reported by our group,[2] with modifications to automate this radiosynthesis using a GE FX_{FN} synthesis module.[3] The MDA-MB-231 (Bmax = 8.7 pmol/mg protein) tumor xenografts were established in female athymic CD1 nu/nu mice. Mice were inoculated subcutaneously with 5×10^6 MDA-MB-231 cells in 200 μL of a 1:1 mixture of Matrigel and serum-free culture medium. After 10-15 days groups of 4-5 mice were selected for imaging. Mice were injected (i.v.; conscious) with 10-20 MBq of the radiotracer and imaged using a Focus 220 microPET scanner (Siemens) under isoflurane. CT scans for anatomical reference and ex vivo biodistribution studies were carried out immediately following the PET scans.

Results: Automated radiosyntheses of [^{18}F]FEPPA were routinely achieved with high isolated radiochemical yields (40-50%, uncorrected) and high specific activities (2-5 Ci/mmol) in less than 1 hour. Dynamic imaging studies in MDA-MB-231 xenograft mice revealed maximal uptake of [^{18}F]FEPPA in the tumour at 90 min post-injection of the radiotracer. Low tumour uptake (1% i.d./g) was observed in static scans and biodistribution studies (Figure). Blocking of this signal was not achieved with pre-administration using 2 mg/kg of FEPPA, 1 hour prior to radiotracer injection. Imaging and biodistribution of [^{18}F]FDG in the same mouse model showed a tumour uptake of 5% i.d./g.

Conclusions: The synthesis of [^{18}F]FEPPA was successfully automated. [^{18}F]FEPPA did not show specific uptake in the MDA-MB-231 human breast cancer xenograft mouse model, in contrast to [^{18}F]FDG, which showed significantly higher uptake.

Research Support: Ontario Institute for Cancer Research

References: 1.Hardwick, M.; Ferikh, D.; Culty, M.; Li, H.; Vidic, B.; Papadoopoulos, V. Cancer Res. 1999, 59, 831-842. 2. Wilson, A.A.; Garcia, A.; Parkes, J.; McCormick, P.; Stephenson, K.A.; Houle, S.; Vasdev, N. Nucl. Med. Biol. 2008, 35, 305-314. 3.van Oosten, E.M.; Wilson, A.A.; Stephenson, K.A.; Mamo, D.C.; Pollock, B.G.; Mulsant, B.H.; Yudin, A.K.; Houle, S.; Vasdev, N. Appl. Radiat. Isot. 2009, 67, 611-616.



P296 C-Met RECEPTOR INHIBITOR WITH CLICK POCKET FOR Tc-99m LABELING AS A SMALL PEPTIDE PROBE FOR SPECT STUDY

D. KIM, E. KIM*, E. PARK, S. KALME, H. JEONG, S. LIM and M. SOHN
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Objectives: c-Met is a receptor tyrosine kinase involved in tumor cell growth, invasion, metastases, and angiogenesis. Overexpression of c-Met is frequently observed in several tumor types. Here, we present the in vitro cell-binding properties and biodistribution and SPECT/CT imaging in glioma (U87MG) xenograft-bearing mice of ^{99m}Tc labeled c-Met-binding peptides (cMBPs). In this study, we evaluated the use of the Cu(I)-catalyzed Huisgen cycloaddition, also known as a click reaction, for labeling ^{99m}Tc with βAK- (G0cMBP), βAKGGG- (G3cMBP) or βAKGGGGGG- (G6cMBP) containing KSLSRHDHIHHHpeptides.

Methods: Azidoacetic acid was coupled with β-alanine terminal HMP and reacted with L-propargyl glycine. Receptor binding affinity of peptides was tested in 96 well-plates coated with cMet/Fc chimera protein. Click peptides were labeled with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ using Isolink kit. In vitro study was performed in U87MG cells. Gamma images were acquired with a small animal SPECT/CT camera after injection of $^{99m}Tc(CO)_3$cMBP (3 nmol, 15 MBq/mouse) in U87MG xenograft model.

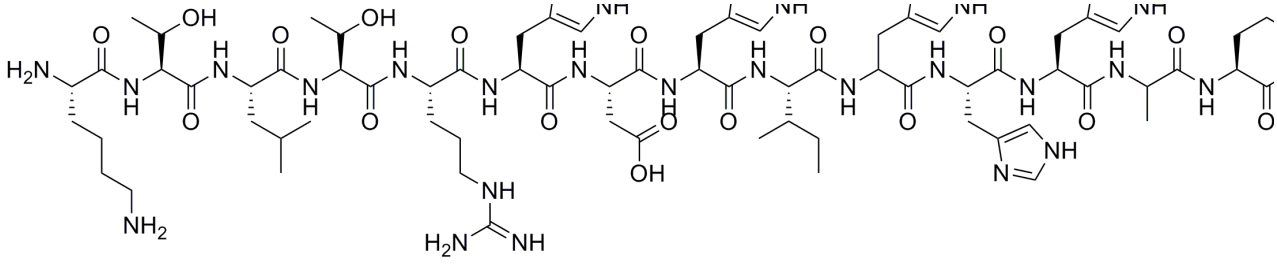
Results: The purity of peptides were 90%. The binding affinities of G0cMBP, G3cMBP, and G6cMBP were determined to be 0.68 μM, 0.57 μM, and 0.41 μM, respectively. Labeling efficiencies were above 93%. Cellular binding activities were significantly inhibited by non-radiolabeled cMBP (> 80%). Imaged based tumor to muscle ratio was 3.1, 4.4, and 4.8 of G0cMBP, G3cMBP, and G6cMBPrespectively.

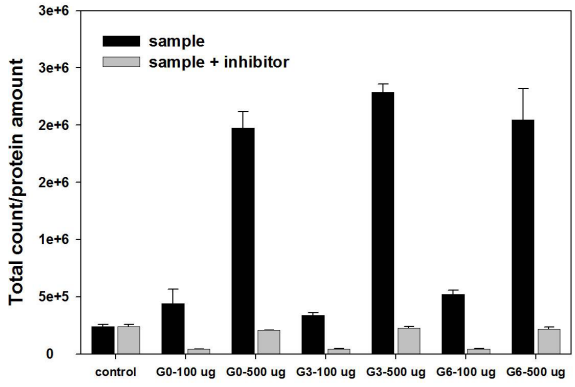
Conclusions: The linker modification slightly improved the in vivo characteristic. These results showed $^{99m}Tc(CO)_3$cMBP might be a good candidate for detecting c-Met expressed tumor.

References: Fig. 1. Structure of cMBP Receptor Inhibitor with Click Pocket for Tc-99m Labeling Fig. 2. In vitro Cellular Uptake in U87MG Cells

Table 1. Binding Affinity of cMBPs

entry	peptide	IC_{50}(M)
1	cMBP-G3	2.739×10^{-7}
2	cMBP-Re-Click-G0	6.843×10^{-7}
3	cMBP-Re-Click-G3	5.754×10^{-7}
4	cMBP-Re-Click-G6	4.149×10^{-7}





P297 SYNTHESIS, RADIOIODINATION AND IN VITRO AUTORADIOGRAPHIC EVALUATION OF A NOVEL HIGH-AFFINITY LIGAND FOR IMAGING BRAIN CANNABINOID SUBTYPE-1 (CB1) RECEPTORS

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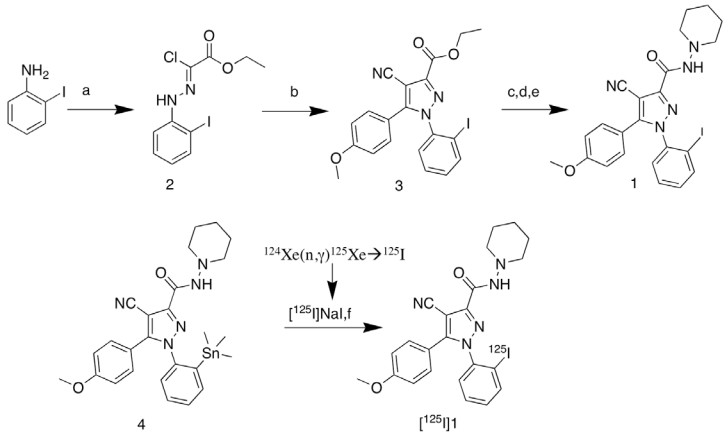
Objectives: Currently, there is a strong interest to image brain cannabinoid subtype-1 (CB_1) receptors with single-photon emission tomography (SPET) to study their role in neuropsychiatric disorders. However, a suitable SPET radioligand does not yet exist. Here we report the initial development of a promising candidate radioligand, [^{125}I]1-(2-iodophenyl)-4-cyano-5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxylate ([^{125}I]1, Scheme).

Methods: Ligand 1 was synthesized in four steps (Scheme) starting from commercially available 2-iodoaniline (Fan H., et al. J. Label. Compd. Radiopharm. 2006, 46, 1021). Its lipophilicity was calculated with Advanced Chemistry Development (ACD) 9.2. Ligand 1 was assayed in vitro at > 40 receptor systems for binding affinity and selectivity. [^{125}I]1 was prepared by classical ^{125}I-iodostannylation of a trimethylstannyl precursor (4), which was itself prepared by palladium-catalyzed coupling using hexamethylditin, with [^{125}I]NaI plus chloramine-T (oxidizer) under basic conditions (Scheme). In vitro autoradiography of [^{125}I]1 was performed on post-mortem whole-hemisphere cryosections of normal human brain.



Scheme. Synthesis and radioiodination**.** Reagents and conditions: a) concentrated HCl, $NaNO_2$, e thyl 2-chloroacetoacetate, NaOAc, EtOH–H_2O; b) DI PEA, *tert*-BuOH, 4-methoxybenoylacetonitrile, Δ; c) *aq*-LiOH, THF, 65 °C; d) $DMF_{(cat)}$, $(COCl)_2$, DCM; 1-aminopiperidine, DIPEA, DCM;. f) chloramine-T, *aq*-HCl, MeOH.

Results: Ligand 1 showed adequate computed lipophilicity (cLogD = 4.14 at pH 7.4) and selective, high CB_1 receptor affinity (K_i = 3.40 ± 0.43 nM, n = 3). [^{125}I]1 was prepared in 48–59% yield (decay–corrected). The specific radioactivity was 81.4 GBq/μmol and radiochemical purity > 98%. Autoradiography showed a distinct regional distribution of radioactivity, in accord with known brain regional CB_1 receptor densities.

Conclusions: [^{125}I]1 behaved as a selective, high-affinity CB_1 radioligand that now merits labeling with iodine-123 and further evaluation with SPECT imaging in non-human primate.

Research Support: We thank the Psychoactive Drug Screening Program (PDSP) for performing binding assays.

P298 NOVEL IMAGING AGENTS FOR BETA-AMYLOID PLAQUES BASED ON THE N-BENZOYLINDOLE CORE

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Objectives: Alzheimer's disease (AD) is a kind of neurodegenerative disease characterized by dementia, congnitive impairment and memory loss. Formation of β-amyloid (Aβ) plaques in the brain is one of the pivotal clinical pathological features of AD. Therefore, in vivo imaging agent for Aβ would be very useful for early diagnosis of AD. Now, design of radiotracers with novel core structures to image Aβ in vivo will be a great challenge. Recently, we have reported that a variety of Aβ probe molecules were derived from small-molecule Aβ ihbitors[1]. Indomethacin, a non-steroidal anti-inflammatory drug (NSAID), has been known to inhibit β-amyloid, and is believed to bind to Aβ[2]. In our present study, we have developed Indomethacin analogs containing N-Benzoylindole core as potential ligands for Aβ plaques imaging.

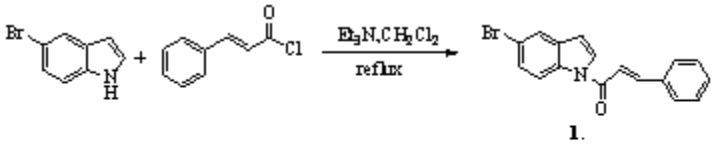
Methods: The novel N-Benzoylindole derivatives were synthesized from proper 5-subsituted indole and m-substituted benzoyl chloride. In an attempt to further development of novel imaging agents, we replaced benzoyl chloride with propenoyl chloride, 1 was synthesized from 5-bromoindole (1 equiv) and trans-3-phenyl-2-propenoyl chloride (1 equiv) (Fig.1). The affinities to postmortem human AD brain homogenates were measured by competitive radioligand binding studies using ^{125}I-IMPY as the radiolabeled standard. After the reaction mixture was incubated at 37℃ for 2 h, the bound radioactivity to the human brain homogenates was collected on Whatman GF/B filters via a cell harvester and then counted. Datas were analyzed by software Prism 5.0 and K_i values were calculated.

Results: 1 was synthesized in 42% yields. In competitive radioligand binding studies, the K_i value of it is 31 nM.

Conclusions: The data demonstrates that 1 has high binding affinity for Aβ plaques. This novel N-Benzoylindole compound shows promise as a new core structure targeting β-amyloid plaques. Preparation and in vivo evaluation of ^{125}I-labelled new derivatives based on this core structure are currently underway.

Research Support: The authors will thank Jiang-Ning Zhou from University of Science and Technology for his kindness to provide us postmortem human AD brain homogenates. This work was supported by NSFC(NO.20871021).

References: [1]Duan XinHong, et al. Science in China Series B: Chemistry (2008),51,801-807.[2] Mie Hirohata, et al. Neuropharmacology (2005),49,1088-1099.



1.

Fig 1

P299 PREPARATION AND EVALUATION OF 99MTC-LABELED ARGININEGLYCINEASPARTATE (RGD) DERIVATIVES FOR INTEGRIN TARGETING

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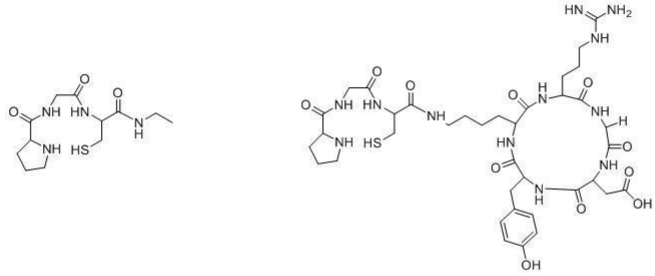
Objectives: Integrin is a valuable tumor marker, since it is frequently over-expressed on various cancer types. The purpose of this work was to synthesize and evaluate a novel bifunctional chelating agent(BFCA) having N3S1 coordination core and comprising of amino acids, Pro-Gly-Cys (PGC) for radiolabeling of RGD peptide with 99mTc for integrin targeting (Fig. 1).



a) P-G-C-(N-ethyl) amide b) PGC-c[RGDyK]

Fig. 1. Structure of novel chelator having N3S1 coordination core for 99mTc and its RGD-conjugate derivative

Methods: The 99mTc-PGC-cyclic[RGDyK] radiotracer was prepared by kit-like procedure. The efficiency of radiolabeling yield was analyzed using HPLC. The in vitro stability was studied by incubation of 99mTc-PGC-cyclic[RGDyK] at different time points at 37°C. In Calu6 tumor bearing mice, the tumors, blood and various organs were removed, weighed and the biodistribution of 99mTc-PGC-c[RGDyK] was evaluated using scintillation counter.

Results: The 99mTc-PGC-c[RGDyK] had high radiolabeling yield (>98%). This radiolabeled compound was stable in 37°C upto 24hrs. In vivo, the RGD derivatives showed significantly better tumor uptake (0.5±0.19%ID/g, 2hr p.i.) than control group, 99mTc-PGC (0.29±0.11%ID/g, 2hr p.i.). Initial blocking with c[RGDyK] prior to injection of the compound showed comparable results to that of the control (0.21±0.02%ID/g, 2hr p.i.). The tumor/blood ratio for control, blockage and 99mTc-PGC-c[RGDyK] group are 1.43±0.73, 5.04±1.30, and 10.84±2.45, 2hr p.i., respectively. The clearance of the radiotracer from the blood and other non-targeted tissues was efficient (tumor to blood ratio approx. 17.35, 24hr p.i.).

Conclusions: Radiotracer which comprised of amino acids to make the coordination core for 99mTc was developed. Results revealed high labeling yield using kit vial formulation. Its biological activity was evaluated in vivo and showed better tumor uptake than control group .Pre-treatment with c[RGDyK] prior to injection of the novel 99mTc-PGC-c[RGDyK], showed that the radiotracer was able to specifically target integrin-positive tumors. Further investigations with various biomolecules for tumor specific targeting are needed to expand the potential use of the novel BFCA for radiolabeling with 99mTc.

P300 METABOLISM OF THE A1 ADENOSINE RECEPTOR PET LIGAND [18F]CPFPX IN HUMANS

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Objectives: The receptor ligand [^{18}F]8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine ([^{18}F]CPFPX) 1* is used to image the A_1 adenosine receptor (A_1AR) in human brain (Holschbach et al. 2002: J Med Chem 45:5150, Bauer et al. 2003: NeuroImage 19:1760). Because this ligand does not undergo degradation in the CNS, specifically bound ligand accounts for a very large fraction of brain radioactivity. However, this is not the case in peripheral tissues. Therefore, the development of second generation radioligands for in vivo PET brain imaging of the A_1 adenosine receptor subtype necessitates the knowledge of metabolism of CPFPX in vivo. The literature contains little information about the metabolism of synthetic xanthines.

Methods: In the present work the main metabolite M1 of CPFPX in human blood 30 min p.i. was identified by means of liquid chromatography, mass spectrometry and chemical synthesis.

Results: Because the mass of injected tracer is ≤ 5 nmol, concentrations in plasma are too low to analyze. However, in vitro metabolism by human liver microsomes (HLM) seems to be a reasonable alternative for generating quantities of metabolites sufficient for analyzing their structures. HML generate main metabolites having HPLC retention times identical to those in human plasma. MS-Experiments using "in source" fragmentation identified the cyclopentyl moiety as the most functionalized moiety by liver microsomes and in vivo oxidations. Except for two metabolites, hydroxylated at the N-1 propyl chain, all oxidative modifications took place here. An [M+H]+ ion corresponding to a N-dealkylated derivative was not detected. Thus, like the natural methylxanthines, CPFPX appears to undergo oxidation by liver microsomes, but, unlike those methylxanthines, dealkylation does not occur. Mass fragmentation of the main metabolite and comparison of the fragmentation spectrum with those of CPFPX and DPCPX revealed the positions of functionalisation (Bier et al. 2006: Drug Metab Dispos 34:570).

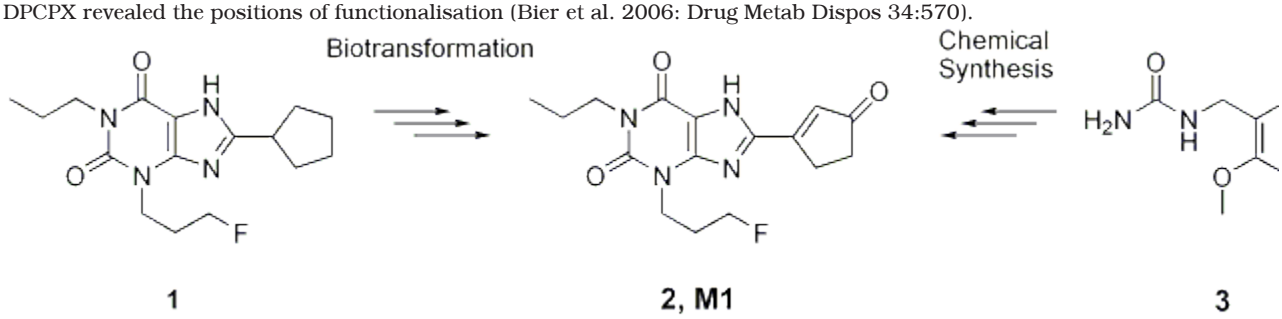

Figure 1: In vivo metabolism of CPFPX to M1 and starting compound 3 for the chemical synthesis of the main metabolite 2. Once LC-MS tentatively identified 8-(3-oxocyclopent-1-enyl)-3-(3-fluoropropyl)-1-propylxanthine 2 as the likeliest structure of M1 (Figure 1) it was necessary to synthesize it as a proof of structure.

Conclusions: A first synthetic approach was based on the synthesis of the parent ligand CPFPX 1 by substituting the original cyclopentyl moiety by a (functionalized) cyclopentenyl derivative, but all efforts to isolate even traces of the desired enone 2 were unsuccessful. Finally a completely different synthetic strategy to compound 2 was followed, the key step being a Pd-catalyzed Stille coupling between a brominated xanthine and a cyclopentenyl stannane. The need for many protection-deprotection steps needed to accomplish this synthesis accounts for its length of 15 steps. The retention time of 2 corresponds to that of the main metabolite in human blood. Moreover the fragmentation patterns of M1 and that of the main metabolite made by human liver microsomal preparations coincide precisely.

P301 RADIOSYNTHESIS, IN VIVO, AND EX VIVO EVALUATION OF [18F]-1-DEOXY-1-FLUORO-SCYLLO-INOSITOL AS A NEW APPROACH FOR IMAGING AMYLOID PLAQUES

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Objectives: To develop a novel PET imaging agent based on the promising Alzheimer's therapeutic scyllo-inositol, a compound that is known to cross the blood-brain barrier, enter neurons and astrocytes, as well as interact with all types of amyloid plaques. Given the promise of deoxy-fluoro-scyllo-inositol in vitro,[1] in both the mouse model and the astrocytoma inositol transport assay, the objective of this work is to radiolabel this compound with ^{18}F and carry out a biological evaluation of the radiotracer in rodent models.

Methods: 1,6:3,4-bis-[O-(2,3-dimethoxybutane-2,3-diyl)]-2-O-trifluoromethanesulphonyl-5-O-benzoyl-myo-inositol,[1] was reacted with potassium cryptand fluoride ($[K_{222}][^{18}F]$) in CH_3CN, and deprotected with TFA, followed by 2N NaOH, in 15 min at 90 °C for each step (Scheme). The product was purified and isolated by solid phase extraction and formulated in saline in an overall synthesis time of 1 hour. Male Sprague-Dawley rats were administered the [^{18}F]-scyllo-inositol derivative and sacrificed at 5 and 30 min post-injection of the radiotracer, prior to biodistribution studies, in conjunction with in vivo SIC probe characterization.

Results: A key element in achieving selective [^{18}F]-fluorination was the use of a multi-functionalized precursor, an acetal and benzoate-protected scyllo-inositol derivative bearing a triflate leaving group. Fluorination proceeded via nucleophilic displacement of the triflate group, followed by sequential deprotection of the acetal and benzoate groups with acid and base, respectively. The radiosynthesis was monitored by radio-HPLC and radio-TLC, and carrier-added reactions with KF further confirmed the identity of the final compound following ^{19}F NMR spectroscopic characterization of the purified product. The radiochemical yield of the formulated product was 17 ± 3% (uncorrected for decay, n > 5), and had > 98% radiochemical purity, with a specific activity >10 Ci/μmol. Unfortunately, ex vivo biodistribution, and in vivo SIC probe studies, in rat models revealed radioactivity in all brain regions were <0.2 %ID/g at all time points.

Conclusions: A fluorine-18 labelled scyllo-inositol derivative was successfully prepared, however, low brain penetration of this compound will preclude its use for studies of the CNS. This work provides an ideal starting point for the evaluation of inositol-based imaging agents for PET.

References: 1. Sun, Y.; Zhang, G.; Hawkes, C.A.; Shaw, J.E.; McLaurin, J.; Nitz, M. Bioorg. Med. Chem. 2008, 16, 7177-7184.