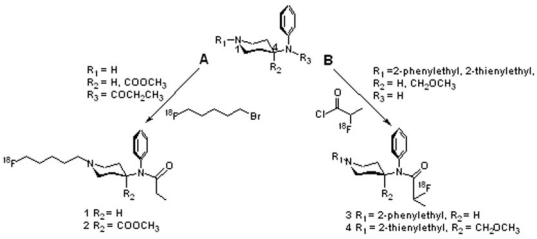
μ-OPIOID RECEPTOR TARGETING WITH ^{18}F-LABELED 4-ANILIDOPIPERIDINES

G. Henriksen,[1] S. Platzer,[2] A. Berthele,[2] A. Hauser,[1] M. Schwaiger,[1] H.-J. Wester.[1]
[1]Department of Nuclear Medicine, Technical University of Munich, Munich, Germany; [2]Department of Neurology, Technical University of Munich, Munich, Germany.

Background: Despite being involved in several clinically relevant diseases and syndromes, the function of the μ-opioid receptor (μOR) is incompletely understood. [^{11}C]carfentanil ([^{11}C]CAF) ($t_{½}$ = 20.3 min) is the only available radioligand for positron emission tomography (PET) imaging of the (μOR) in humans. To improve the feasibility of kinetic data analysis and to provide useful alternative PET tracers, we investigated two series of ^{18}F-fluorinated ($t_{½}$ = 109.7 min) 4-anilidopiperidines (4-APs) as potential μOR selective PET-ligands. These compounds have been developed either by (A) N_1-5-[^{18}F]fluoropentylation of *nor*-4-APs or (B) 2-[^{18}F]fluoroacylation of *des*-propionyl 4-AP precursors.

Results: Although the radiochemical yields are not optimized, all compounds were obtained in an isolated radiochemical yield of > 30 % and a specific activity of > 37 GBq/μmol. All syntheses are completed in less than 2h. As demonstrated by regional brain distribution studies in mice, derivatives with no substituent on the C_4 ([^{18}F]**1** and ([^{18}F]**3**) show low specific binding in regions with high μ-opioid receptor density. In contrast, [^{18}F]**2** with a C_4-carboxy methyl and [^{18}F]**4** with a C_4-methoxymethyl show distribution pattern similar to the known μ-opioid receptor distribution, i.e. high binding in striatum, thalamus and cerebral cortex and low binding in cerebellum. Moreover, the activity concentration of [^{18}F]**2** and [^{18}F]**4** at 5 and 30 min after injection in regions with high binding relative to that in cerebellum revealed specificity of binding similar to that of [^{11}C]CAF at both time points. [^{18}F]**2** and [^{18}F]**4** reached maximum uptake ratios already at 20 min after injection. Quantification of the radiolabeled species extracted from mice brains at 40 min after injection revealed that more than 93 and 91 % of the total radioactivity corresponded to intact [^{18}F]**2** and [^{18}F]**4**, respectively, indicating a high metabolic stability of the compounds. Moreover, extra-cereberally generated metabolites are not taken up significantly and do not accumulate in brain. In separate experiments the binding pattern of [^{18}F]**2** and [^{18}F]**4** to rat brain sections under naive and receptor blocking conditions *in vitro* was measured by means of binding autoradiography. The two ligands show highly selective binding to brain regions with known high μOR density. Co-incubation with naloxone (blocking of μ-,δ and κOR) or unlabeled sufentanil (blocking of μOR) nearly completely inhibited binding of [^{18}F]**2** and [^{18}F]**4** further demonstrating their selectivity for the μOR. In other experiments, the binding affinity to the human μOR was determined to be 0.7 and 0.1 nM for [^{18}F]**2** and [^{18}F]**4**, respectively. **Conclusion:** The study reported here is part of an ongoing effort to explore new series of radiolabeled 4-anilidopiperidines by systematic modifications of the 4-AP lead structure and the labeling position. Two radiofluorination strategies resulting in compounds [^{18}F]**2** and [^{18}F]**4** have been identified and key features of the ^{18}F-labeled 4-APs governing regional brain uptake, μOR-affinity, metabolic stability and specificity of binding have been investigated. Based on the current data and ongoing experiments, [^{18}F]**2** and [^{18}F]**4** are promising new tracers for the in vivo imaging of the μ-opioid receptor with PET.



Keywords: mu-Opioid Receptors, Fluorine-18, PET

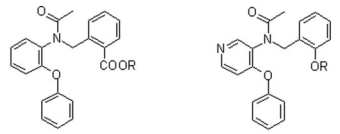
SYNTHESIS AND EVALUATION OF TWO CANDIDATE ^{11}C-LABELED RADIOLIGANDS FOR BRAIN PERIPHERAL BENZODIAZEPINE RECEPTORS

E. Briard, J. Hong, J.L. Musachio, S.S. Zoghbi, M. Fujita, M. Imaizumi, V. Cropley, R.B. Innis, V.W. Pike.

Molecular Imaging Branch, National Institute of Mental Health, NIH, Bethesda, MD, United States.

Introduction: [^{11}C](*R*)-PK 11195 has long been used for imaging brain 'peripheral benzodiazepine receptors' (PBR) with PET.[1] However, this method has low sensitivity (*i.e.* a low ratio of PBR-specific to non-specific binding) and difficulty in quantitation. Quite recently a new class of high affinity PBR ligands has been developed based on aryloxyanilides. Some promising PET radioligands have already been developed from this class[2]. We also sought to develop sensitive brain-penetrant PBR ligands based on aryloxyanilides. Here we report the radiosynthesis and evaluation of one especially high affinity ^{11}C-labeled ligand, **1,** and a less lipophilic alternative, **2**.

Methods. Ligand **1** was synthesized as reported[3] and the corresponding acid precursor (**3**) for labeling obtained by basic hydrolysis. Ligand **2** was prepared as reported[4] and the *O-desmethyl* precursor (**4**) prepared from the corresponding 2-acetoxy compound (synthesized analogously) by ester hydrolysis. Each ligand was assessed for affinity at PBR in a radioligand binding assay. Ligand **1 or 2** was labeled by methylation of **3 or 4**, respectively, with [^{11}C]iodomethane and purified by reverse phase HPLC. [^{11}C]**1** and [^{11}C]**2** were each evaluated as radioligands by i.v. injection into rhesus monkey with PET monitoring of regional brain radioactivity (baseline experiments) and in similar experiments in which PK 11195 (12 mg/kg i.v.) or DAA 1106 (1 mg/kg i.v.) was given at 30 min after the radioligand (displacement experiments). In one experiment DAA 1106 (3 mg/kg i.v.) was given 24 min before [^{11}C]**2** (pre-block experiment). Radioligand metabolism was investigated by HPLC of plasma. In vitro blood parameters were also measured.



1, R = Me; IC_{50} = 0.06 nM; cLogP = 4.2
3, R = H

2, R = Me; IC_{50} = 0.6 nM; cLogP = 3.0
4, R = H

Results. [^{11}C]**1** and [^{11}C]**2** entered monkey brain well with peak SUV (SUV% = injected dose/ g_{tissue} x $g_{bodyweight}$) values appearing in putamen (340% at 17.5 min and 400% at 27.5 min after injection, respectively). These values declined to 280% and 380% SUV, respectively at 1 h. In PBR-containing regions (*e.g.* cortical regions, radioactivity was well retained in baseline experiments and largely (66% for [^{11}C]**1** and 60% for [^{11}C]**2**) released by PK 11195 or DAA 1106 in the displacement experiments. In the pre-block experiment with [^{11}C]**2,** early regional maximal radioactivity uptake declined sharply to about 100% SUV in all PBR-containing regions over 40 min. [^{11}C]**1** and [^{11}C]**2** were stable in whole blood for 1 h while the free fractions in plasma were 1.8% for [^{11}C]**1** and 2.8% for [^{11}C]**2**. [^{11}C]**1** distributed 68% into cellular blood elements. Less lipophilic radioactive metabolites in plasma at 30 min after radioligand injection represented about 87% and 80% of total plasma radioactivity for [^{11}C]**1** and [^{11}C]**2**, respectively.

Discussion: The displacement and blocking experiments provide strong evidence for a high degree of PBR-selective binding in monkey brain. The less lipophilic [^{11}C]**2** has somewhat greater brain penetration than [^{11}C]**1** and provides a similar PBR receptor-specific signal. [^{11}C]**1** shows faster kinetics for uptake and washout of radioactivity.

Conclusion: [^{11}C]**1** and [^{11}C]**2** are promising radioligands for PET imaging brain PBR.

1. Shah F et al. *Nucl Med Biol* 1994; **21**: 573-581.
2. Zhang MR et al. *Nucl Med Biol* 2003; **30**: 513-519.
3. Nakazato A et al. *US Patent* 6,476,056 B2, 2002.
4. Nakazato A et al. *JP Patent* 2000001476.

Keywords: Periphal Benzodiazepine Receptor, Radioligand, Carbon-11

EVALUATION OF [*O-METHYL*-^{11}C]S14506 AS AN AGONIST RADIOLIGAND FOR BRAIN 5-HT$_{1A}$ RECEPTORS IN RAT AND MONKEY

S.Y. Lu,[1] J.S. Liow,[1] M. Ichise,[1] J. Hong,[1] J.L. Musachio,[1] S.S. Zoghbi,[1] L. Nichols,[1] J. Seidel,[2] E.S. Vermeulen,[3] H.V. Wikstrom,[3] R.B. Innis,[1] V.W. Pike.[1]

[1]Molecular Imaging Branch, National Institute of Mental Health, NIH, Bethesda, MD, United States; [2]Department of Nuclear Medicine, Warren Grant Magnuson Clinical Center, NIH, Bethesda, MD, United States; [3]Department of Medicinal Chemistry, University of Groningen, Groningen, Netherlands.

Introduction: S14506[1] is one of the most potent and selective agonists at 5-HT$_{1A}$ receptors (K_i = 0.98 nM). Under *in vitro* conditions[2] the specific binding of [^{3}H]S14506 is similar to that of the antagonist radioligand, [^{3}H]WAY 100635, *i.e.* it binds to both the G-protein coupled and uncoupled forms of the 5-HT$_{1A}$ receptor with a nanomolar affinity in contrast with 8-OH-DPAT, which has a nanomolar affinity for the G-protein coupled form of the receptor only. Because of these unusual binding properties, S14506 could be a potential agonist PET radioligand for imaging a subset of 5-HT$_{1A}$ receptors. Here we aimed to evaluate [*O-methyl*-^{11}C]S14506 as a radioligand in monkey and rats. The early finding of a quite low brain uptake was postulated to be due to Pgp-mediated efflux across the BBB and was investigated using PET imaging in rats.

Methods: [*O-methyl*-^{11}C]S14506 (**1**) was synthesized by treating the *O-desmethyl* precursor with [^{11}C]iodomethane in the presence of TBAH followed by reverse phase HPLC purification and formulation in 10% EtOH in saline for monkey and 5% EtOH in saline for rats. For monkey PET imaging, a rhesus monkey (15 kg) was administered with a bolus i.v. injection of 3.8 mCi of **1** alone or with 3.9 mCi of **1** followed by 0.3 mg/kg of WAY 100635 at 40 min after radioligand injection. Emission data were acquired for 90 min on a GE Advance PET camera. For small animal PET imaging, Sprague-Dawley rats were given (a) a bolus tail vein injection of ~1 mCi of **1** (baseline experiment); (b) cyclosporine A (CsA) (in EtOH plus Cremophore EL, 25-50 mg/kg) 30 min before ~ 1 mCi of **1** injection, for Pgp inhibition; and (c) CsA 30 min before injection of **1** (~1 mCi) and *desmethyl*-WAY 100635 (2 mg/kg) 30 min after radioligand injection (displacement experiment). Data were acquired on ATLAS (Advanced Technology Laboratory Animal Scanner).

Results and Discussion: In the monkey experiments, radioligand concentrations in areas of high 5-HT$_{1A}$ receptor density, such as temporal cortex (TEM), cingulate gyrus (CG) and thalamus (TH), were higher than in receptor-devoid cerebellum (CE) from 10 min after radioligand injection (Figure 1). WAY 100635 given at 40 min displaced **1** from regions of high 5-HT$_{1A}$ receptor density. Both the transient nature of agonist binding to G-coupled receptors and Pgp are thought to influence radioligand uptake. The low brain uptake in rat was significantly enhanced (peak SUV from 200 to 300%) after the Pgp inhibitor, CsA, was administered (Figure 2). A discrete regional distribution of radioactivity in rat brain was only discernible after the Pgp pump blockade, with 5-HT$_{1A}$ receptor-rich regions showing appreciably higher radioactivity concentration than CE.

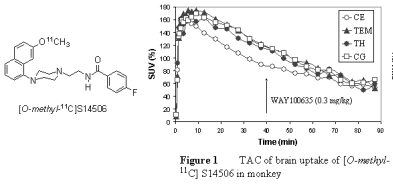


Figure 1 TAC of brain uptake of [*O-methyl*-^{11}C] S14506 in monkey

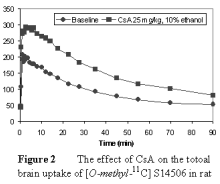


Figure 2 The effect of CsA on the totoal brain uptake of [*O-methyl*-^{11}C] S14506 in rat

Conclusion: These results show that [^{11}C]S14506 is a Pgp substrate in rat and is able to show a small, but distinct receptor-specific signal from 5-HT$_{1A}$ receptors in monkey and rat brain *in vivo*.

1. Colpaert FC, et al. *Drug Dev Res* 1992; 26: 21-48.

2. Lima L, et al. *J Neural Transm* 1997; 104: 1059-1075.

Keywords: 5-HT$_{1A}$ Agonist, Pgp Substrate, PET Imaging

5-HT_{1A} RECEPTOR IMAGING WITH [^{11}C](*S*)-RWAY IN RAT BRAIN SHOWS A STRIKING DIFFERENCE IN PGP EFFECT COMPARED TO IMAGING IN MONKEY

J.A. McCarron,[1] S.S. Zoghbi,[1] J.S. Liow,[1] J. Hong,[1] S.Y. Lu,[1] E.S. Vermeulen,[2] H.V. Wikstrom,[2] J. Seidel,[3] R.B. Innis,[1] V.W. Pike.[1]

[1]Molecular Imaging Branch, NIMH, NIH, Bethesda, MD, United States; [2]Department of Medicinal Chemistry, University of Groningen, Groningen, Netherlands; [3]Department of Nuclear Medicine, Warron G Magnuson Clinical Center, NIH, Bethesda, MD, United States.

Introduction. [*O-methyl*-^{11}C](*S*)-RWAY (**1**) has been shown to be an effective radioligand for imaging 5-HT_{1A} receptors in monkey brain [1]. **1** can be prepared by simple methylation of its *O-desmethyl* analog with [^{11}C]iodomethane and may become a useful radioligand for clinical PET studies. To gain more understanding on the fate of the radiometabolites of **1**, particularly with regard to their brain uptake and receptor binding, we set out to examine **1** in rat brain by PET imaging and *ex-vivo* dissection. A finding of low uptake of **1** into rat brain was unexpected and further experiments were carried out to determine whether this was a Pgp effect.

Experimental. Radioligand **1** and its enantiomer, [^{11}C](*R*)-RWAY (**2**), were prepared as before [1]. Two rats were injected with **1** alone and another with **2**. A fourth was treated with cyclosporine A (CsA; 50 mg/kg) before **1** and two others with CsA (50 mg/kg) before **1** plus WAY-100635 (0.5 mg i.v.) at 20 min after **1**. Dynamic PET images were obtained for each rat. The biodistributions of **1** and possible radiometabolites in brain tissue, with and without CsA pretreatment, were determined in frontal cortex, occipital cortex, hippocampus and cerebellum by dissection and radio-HPLC analysis. Plasma sampled at 30 min after administration of **1** was analyzed similarly.

Results and Discussion. Following injection of **1** alone, uptake of radioactivity into rat brain was very low (maximally 76% SUV at 1 min). The summed PET images did not distinguish any receptor-specific binding. This contrasts sharply with the high uptake of **1** in receptor-rich regions of monkey brain (800% SUV) [1]. To determine whether the poor rat brain entry of **1** was a Pgp effect, the experiments were repeated in rats predosed with CsA (50 mg/kg i.v.). The uptake of the **1** increased markedly (*e.g.* to 300% SUV in whole brain) (Figure 1A). The PET data showed very clear specific binding particularly in the frontal cortex, occipital cortex and hippocampus with tissue to cerebellum ratios of 4.8, 4.6 and 4.0 at 90 min, respectively (Figure 1B). Injection of the rat with CsA before **1** and with WAY-100635 at 20 min after **1** reduced ratios to unity at 110 min (Figure 1C), showing the receptor selectivity of **1**. In the rat injected with **2** alone, only a very low uptake of radioactivity (maximally 113% SUV at 0.5 min) was observed. Analysis of rat plasma at 30 min after injection of **1** showed that 20% of the radioactivity was **1** and the remainder composed of two radiometabolites. Both radiometabolites were found in all sampled brain tissues at the same level as that found in cerebellum, showing that they bind non-specifically.

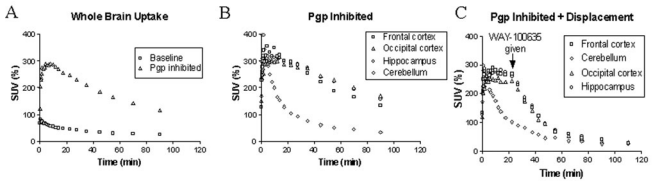


Figure 1. Brain time radioactivity curves after injection of **1** into rat under different circumstances.

Conclusion. These results support our previous finding in monkey, that **1** is an effective PET radioligand for brain 5-HT_{1A} receptors. However, the remarkable difference in Pgp effects between rat and monkey highlights the importance of not relying on a single animal species in the development of PET radioligands. The radiometabolites of **1** appear to bind non-specifically only and are not expected to obstruct 5-HT_{1A} receptor quantification with **1**.

Reference

1. McCarron JA. et al. *NeuroImage* 2004; 22 (Suppl. 2): T34-T35.

Keywords: [^{11}C](S)-RWAY, PET, Rat

EVALUATION OF [^{18}F]FPWAY AND [^{18}F]FBWAY, 5-HT$_{1A}$ RECEPTOR PET LIGANDS, FOR SENSITIVITY TO ENDOGENOUS 5-HT IN RAT

E.M. Jagoda,[1] L. Lang,[1] J. Tokugawa,[1] Y. Ma,[1] C. Contoreggi,[2] W.C. Eckelman.[3]
[1]PET Radiochemistry Group, NIBIB, Bethesda, MD, United States; [2]IRP, NIDA, Baltimore, MD, United States; [3]PET Dept, Clinical Center, National Institutes of Health, Bethesda, MD, United States.

Developing a PET imaging agent to monitor changes in 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) density or occupancy in vivo would aid in understanding the pathophysiology of various psychiatric disorders associated with uncontrolled aggression, anxiety and stress. [^{18}F]FPWAY (4-fluoro-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-N-(pyrimidin-2-yl)benzamide) and [^{18}F]FBWAY (4-fluoro-N-(2-(4-(2-methoxyphenyl)piperazino)ethyl)-N-(2-pyridinyl)benzamide) exhibited weaker K_as and lower hippocampus (HI) to cerebellum (CB) ratios of 6.5 and 5.7, respectively compared to WAY 100635. With these lower affinity 5-HT$_{1A}$R ligands, the binding maybe reversible and sensitive to changes in endogenous 5-HT.

The reversibility of [^{18}F]FPWAY and [^{18}F]FBWAY binding to 5-HT$_{1A}$R containing brain regions were assessed in awake (AW) rats using a fenfluramine-treated group (FEN) compared to AW controls (CON). A "chase paradigm" was used in which both groups were injected with [^{18}F]FPWAY or [^{18}F]FBWAY and killed after 1 h; the FEN was injected 20 min following the tracer. Decreases in 5-HT$_{1A}$R regional uptake in the FEN compared to the CON would be expected due to the increase of endogenous 5-HT caused by the fenfluramine (Zimmer, *J. Neurochem,* 2002). The [^{18}F]FPWAY and [^{18}F]FBWAY uptakes in all brain regions examined were decreased 17% to 29% and 19% to 39%, respectively, for the FEN vs CON. The data were analyzed using specific binding ratios [SR= (tissue uptake/CB uptake)-1] to attempt to control for blood flow effects. The HI exhibited the highest SRs of 5.8 and 3.4 for [^{18}F]FPWAY and [^{18}F]FBWAY CONs, respectively; cortex (CX) SRs were 1.8 and 1.3 for [^{18}F]FPWAY and [^{18}F]FBWAY CONs, respectively. SRs in the caudate (CD), thalamus (TH) and brain stem (BS) were < 1 indicating very low specific binding to 5-HT$_{1A}$R for both ligands. These regional brain SRs are consistent with known 5-HT$_{1A}$R densities. [^{18}F]FPWAY SRs of the FEN were significantly decreased 24% and 29% in TH and BS, respectively, compared to CON; [^{18}F]FBWAY SRs of the FEN were significantly decreased 66%, 40%, 27%, and 25% in CD, TH, BS and CX. With both ligands no significant differences were observed in HI SRs of the FEN vs the CON.

Further studies were done with urethane anesthetized (AN) rats using the same "chase paradigm" and an AW CON group. Uptakes of [^{18}F]FPWAY and [^{18}F]FBWAY were increased in all brain regions of the AN vs the AW CON with the largest increases in HI of 107% and 42%, respectively. In contrast HI uptake in the AN-FEN vs the AN was significantly decreased ~30% for both ligands. With these peripheral effects from the urethane and fenfluramine the data were analyzed using SRs also. The [^{18}F]FPWAY SRs of the AN-FEN were significantly increased 111% and 32% in TH and BS, respectively, compared to AN CON. The [^{18}F]FBWAY SRs were significantly decreased 37% in TH only of the AN-FEN vs the AN CON. For both ligands no significant differences were observed in HI SRs of the AN-FEN vs the AN CON.

Analysis of this data using SRs (>2) appears to identify differences in uptake due to specific 5-HT$_{1A}$R binding rather than flow induced uptake due to anesthesia and/or drug interactions. The TH, BS, CD, and CX SRs exhibited sensitivity to these induced blood flow changes which might be expected since the ratios are < 2 indicating low 5-HT$_{1A}$R concentrations with high non-specific binding. The HI, which has the highest SRs and therefore the highest specific binding for 5-HT$_{1A}$R for both [^{18}F]FPWAY and [^{18}F]FBWAY, failed to exhibit any sensitivity to changes in endogenous 5-HT whether in AW or AN rats.

Keywords: PET Radioligands, 5-HT'1A Receptors, Serotonin

EX VIVO EVALUATION OF p-[^{18}F]D-MPPF FOR THE STUDY OF 5-HT$_{1A}$ RECEPTORS

C. Defraiteur, C. Lemaire, A. Luxen, A. Plenevaux.
Cyclotron Research Center, Liege University, Liege, Belgium.

The development of PET radioligands for the study of the 5-HT$_{1A}$ receptors is an active research area. We have previously reported (1) the radiosynthesis of the p-[^{18}F]D-MPPF, the desmethylated analogue of p-[^{18}F]MPPF (5-HT$_{1A}$ antagonist). This molecule was considered in order to improve the amount of radioactivity getting into the brain (cf desmethylated WAY100635 (2)). This work presents the ex vivo autoradiography, tissue distributions and plasma metabolism results obtained in rats with p-[^{18}F]D-MPPF.

Methods: Tissue distribution studies were conducted in Sprague-Dawley male rats (200 - 300 g) kept under anesthesia with an IP injection of ketamine (80 mg/kg) xylazine (10 mg/kg). p-[^{18}F]D-MPPF (0.5 mL, 18 - 37 MBq) was IV injected in the tail vein, rats were killed 15, 30, 45 and 60 min after injection. The results, based on five replicates, are expressed as means ± SD % of injected dose per gram of tissue (%ID/g). Ex vivo autoradiography where conducted under the same conditions except the radioactivity injected (~ 0.5 mL, 78 MBq for p-[^{18}F]D-MPPF and 315 MBq for p-[^{18}F]MPPF) and time: 30 min. Metabolism was assessed in plasma with HPLC. These results are expressed as means ± SD % of parent (unmetabolized) compound compared to whole blood radioactivity.

Results: Figure 1 shows some of the results obtained with tissue distributions: striatum/cereb, hippocampus/cereb and time-%ID/g curves for striatum and hippocampus at 15, 30, 45 and 60 min for p-[^{18}F]D-MPPF and p-[^{18}F]MPPF. For the 5-HT$_{1A}$ specific brain tissue hippocampus the %ID/g obtained with p-[^{18}F]D-MPPF were much higher (~300%) than the one observed with p-[^{18}F]MPPF. For the cerebellum and striatum (non specific), the increase of radioactivity was around 200%. These results lead to hippo/cereb and frontal cortex/cereb of 6.4 and 3 at 30 min respectively. Compared to the ratio obtained with p-[^{18}F]MPPF at the same time: 4 and 1.7, p-[^{18}F]D-MPPF gives an easier detection and a better contrast. **Figure1:**

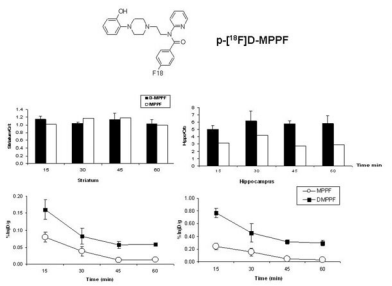
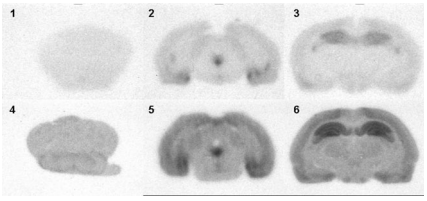


Figure 2 shows a direct qualitative comparison, with ex vivo autoradiography, between p-[^{18}F]MPPF (1-3) and p-[^{18}F]D-MPPF (4-6) for cerebellum (1, 4) raphe (2, 5) and hippocampus (3, 6). The distribution patterns are exactly the same but the contrast is much better with p-[^{18}F]D-MPPF although the amount of radioactivity injected is much lower. These autoradiography results confirm the one obtained with tissue distributions.

Figure 2:



As shown in table 1, the metabolism of p-[^{18}F]D-MPPF seems to be slower.

Table 1:

% of parent compound in plasma

p-[^{18}F]D-MPPF	n	p-[^{18}F]MPPF	Time (min)
50 ± 10	4	62 ± 12	15
50 ± 14	7	33 ± 10	30
40 ± 12	5	20 ± 13	45
39 ± 10	2	15 ± 11	60

Conclusion: Compared to p-[^{18}F]MPPF, p-[^{18}F]D-MPPF leads, in rats, to an easier detection with better contrast of 5-HT$_{1A}$ specific brain structures. The specificity for 5-HT$_{1A}$ receptors appeares comparable to the one of p-[^{18}F]MPPF. This molecule can be considered as a potential radiopharmaceutical candidate.

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(2) V.W. Pike et al. *Eur. J. Nucl. Med.* (1998) 25: 338.

Keywords: 5-HT$_{1A}$ Receptors, Autoradiography, ^{18}F Radioligand

RADIOSYNTHESIS OF ^{18}F-LABELLED PYRAZOLO[1,5*a*]PYRIDINES AND 5-CYANOINDOLES AS SUBTYPE SELECTIVE D4 RECEPTOR LIGANDS FOR PET

O. Prante,[1] C. Hocke,[1] R. Tietze,[1] S. Lober,[2] H. Hubner,[2] P. Gmeiner,[2] T. Kuwert.[1]
[1]Laboratory of Molecular Imaging, Clinic of Nuclear Medicine, Friedrich-Alexander University, Erlangen, Germany; [2]Department of Medicinal Chemistry, Emil Fischer Center, Friedrich-Alexander University, Erlangen, Germany.

The exploration of the dopamine D4 receptor subtype in living human brain is of great interest as alterations of the D4 receptor (D4R) have been implicated in the genesis and treatment of a broad range of neurobehavioral and psychiatric disorders such as novelty seeking, attention-deficit hyperactivity disorder and schizophrenia. As yet, there is no suitable radioligand for the non-invasive assessment of the dopamine D4 receptor by positron emission tomography (PET). As a part of our drug discovery and SAR investigations on selective D4R ligands, we characterized the pyrazolo[1,5*a*]pyridine FAUC113 and cyanoindole derivatives as high affinity D4R ligands (1,2). Thus, the aim of this study is the development of selective D4R radioligands for PET derived from these lead compounds.

The radiosyntheses of 3-[4-(2-[^{18}F]fluoroethoxyphenyl)-piperazin-1-ylmethyl]pyrazolo [1,5*a*]pyridine (2-[^{18}F]FPP), the corresponding 4-[^{18}F]fluoroethoxy-substituted compound (4-[^{18}F]FPP), 2-[4-(2-[^{18}F]fluoroethoxyphenyl)-piperazin-1-ylmethyl]indole-5-carbonitrile (2-[^{18}F]FIC) and the corresponding 4-[^{18}F]fluoroethoxy-substituted indole (4-[^{18}F]FIC) are presented. [^{18}F]Fluoroethytosylate was used for ^{18}F-alkylation of the corresponding hydroxyl labelling precursors. Authentic ^{19}F-substituted reference compounds were synthesized by Mannich reaction or reductive amination of indole-2-aldehyde. Radio-HPLC was used to determine radiochemical yields (RCY) and to confirm identity of ^{18}F-labelled compounds. Receptor binding assays were performed using human D2R, D3R and D4R expressed in CHO-cells and porcine striatal membranes (D1R) with [^{3}H]spiperone and [^{3}H]SCH23390. Affinities to the adrenergic α1 receptor were evaluated utilizing [^{3}H]prazosin and porcine cortical membranes.

Radiosyntheses of 2-[^{18}F]FPP, 4-[^{18}F]FPP, 2-[^{18}F]FIC and 4-[^{18}F]FIC by the use of [^{18}F]fluoroethyltosylate and sodium methylate in DMF at 120°C gave RCYs of 45-60% within 50min. The molar activity of ^{18}F-labelled ligands was about 2.5 Ci/μmol, radiochemical purity for all radioligands was >95% (HPLC). In-vitro binding assays demonstrated that 2-[^{18}F]FPP and 2-[^{18}F]FIC were highly D4 subtype-selective displaying nanomolar receptor affinities (Ki(D4R)=1.6 and 2.4nM). However, in comparison with the corresponding 2-hydroxyl precursor compounds the dopamine receptor subtype selectivity was not improved by ^{18}F-fluoroethylation (Ki(D2R)/Ki(D4R)=160). Interestingly, ^{18}F-fluoroethylation of the 4-hydroxyphenyl moiety to obtain 4-[^{18}F]FPP and 4-[^{18}F]FIC, respectively, led to good D4 receptor affinities (Ki(D4R)= 28 and 9.6nM) and a significant increase in subtype selectivity, simultaneously (Ki(D2R)/Ki(D4R)=2600). Moreover, 4-[^{18}F]FPP and 4-[^{18}F]FIC explicitly showed lower affinity for α1 receptors (Ki=2000-4500nM) when compared to 2-[^{18}F]FPP, 2-[^{18}F]FIC or the 4-hydroxyl precursor.

In conclusion, ^{18}F-fluoroethylation in the 4-position of the hydroxyphenylpiperazinyl moiety of the lead structures led to putative D4 receptor radioligands under retention of high D4 receptor affinity and subtype selectivity in vitro. These characteristics are promising for studying the biodistribution and metabolic stability of 4-[^{18}F]FPP and 4-[^{18}F]FIC as D4 radioligands in vivo.

Acknowledgement

This work is supported by the Deutsche Forschungsgemeinschaft (DFG, grant PR 677/2-2).

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Keywords: D4 Receptor, Subtype Selectivity, Fluorine-18

RECENT DEVELOPMENT IN NEW GENERATION RADIOTRACERS FOR PET IMAGING OF THE NOREPINEPHRINE TRANSPORTER

Y.-S. Ding, K.-S. Lin, J. Logan.
Chem., Brookhaven National Laboratory, Upton, NY, United States.

Objectives: We have synthesized and evaluated several new ligands for imaging the norepinephrine transporter (NET) system in baboons with PET, including C-11 labeled (R)-nisoxetine (Nis), oxaprotiline (Oxap), and lortalamine (Lort), as well as our previously reported C-11 and F-18 derivatives of reboxetine (RB), methylreboxetine (MRB) and their individual (R,R) and (S,S) enantiomers. Based on these characterization studies in baboons, we were encouraged by the promising properties of the MRB analogs as potential NET ligands for PET. In search of optimal tracers for in vivo imaging of the NET system, we have prepared more new ligands and comparative studies in baboon will be presented. **Methods**: Multi-step synthetic procedures were developed to prepare the corresponding precursors, which were used to synthesize C-11 and F-18 labeled NET radioligands. PET imaging studies were then carried out in baboons. Ligands possessing high brain penetration, high affinity and selectivity, appropriate lipophilicity (log P = 1.0-3.5), high plasma free fraction and reasonable stability in plasma were selected as candidates for further studies. A critical examination of the kinetic properties of radioligands has been a crucial part of our radiotracer development strategy. **Results**: We have synthesized and evaluated several new ligands for imaging NET system in baboons with PET. Our comparative studies in baboons demonstrate that in addition to the high uptake in striatum (higher than thalamus), Nis, Oxap and Lort displayed high non-specific binding and poor signal to noise ratio, in contrast to more desirable properties with C-11 and F-18 derivatives of reboxetine (RB) and methylreboxetine (MRB) analogues. We have identified the superiority of (S,S)-[^{11}C]MRB and the suitability of the MRB analogs ((S,S)-[^{11}C]MRB >(S,S)-[^{11}C]3-Cl-MRB >(S,S)-[^{18}F]FRB) as NET ligands for PET. (R)-[^{123}I]iodoNis, which has high affinity as a promising in vitro ligand, also has high non-specific binding and lack of selectivity in vivo as indicated by Kiyono et al. 2004. Thus, (S,S)-[^{11}C]MRB remains by far the most promising NET ligand for PET studies. In our first paper we reported the DV rather than the DVR since we had not yet identified a reference region. We have now advanced our graphical analysis methods for quantification of NET by choosing an average of the occipital (Occ) and striatal (ST) regions (ST + Occ) for the reference region. In fact, using the data from our Synapse paper (Ding et al., 2003), we calculated the DVR for the thalamus/(ST + Occ) for SS, racemic, and RR compounds of [^{11}C]MRB, and the value for the racemic compound (1.38) fell between those of the SS (1.83) and the RR enantiomer (1.13), which is consistent with expectations. **Conclusions**: (S,S)-[^{11}C]MRB remains by far the most promising NET ligand for PET studies. With our new approach for quantification of NET availability, we should be able to study the role of NET in various neuropsychiatric disorders, such as ADHD, substance abuse, depression and anxiety disorders.

SUPPORTED BY DOE-OBER, ONDCP AND NIH.

Keywords: Norepinephrine Transporter, Reboxetine, PET

DESIGN OF HALOGENATED PIPERIDINES AS SELECTIVE PROBES FOR IMAGING OF MONOAMINE TRANSPORTERS

M.P. Smith, D.F. Keith, F.J. Femia, J.W. Babich.
R&D, Molecular Insight Pharmaceuticals, Cambridge, MA, United States.

We describe our efforts toward the development of monoamine transporter ligands containing halogens which can be radiolabeled with positron or single photon emitting isotopes. These ligands will provide for PET or SPECT imaging of neurological disorders. The phenylpiperidine pharmacophore presents an excellent starting point for this design.

The chlorophenyl piperidines are readily prepared as previously reported. This methodology was extended to afford the 4-bromophenyl piperidines. The related 4-iodophenyl piperidines were prepared via a copper catalyzed halogen exchange reaction. A series of 3-fluoroalkyl piperidines were prepared by reduction to the alcohols followed by fluorination using DAST. A series of *N*-fluoroalkyl piperidines were also prepared. This involves the *N*-demethylation of **1** using ACE-Cl to afford **5**, followed by reaction with the 1-bromo-ω-fluoroalkanes to afford **6**.

Scheme 1: Synthesis of Halophenyl Piperidines.

These compounds were examined for affinity at the monoamine transporters. Several exhibited affinities in the low nanomolar range at the DAT with the 3β,4β-isomers exhibiting the highest activity. These isomers also exhibited the highest affinity for the SERT and the NET, leading to limited selectivity. The enantiomers, (+)-**1,** exhibit reduced affinity. For the 3α,4β-isomers moderate affinity is observed for the DAT and NET, with the SERT affinity being low leading to improved selectivity for the DAT and NET. The enantiomer, (-)-**2**, exhibits a reduction in binding to all three transporters, with the reduction at the DAT and NET being greater than that for the SERT. This results in (-)-**2c** which exhibits affinity at the SERT (96 nM) and selectivities of >10 and >20 over the DAT and NET respectively.

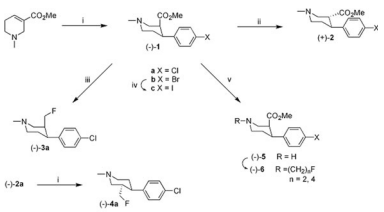

Table 1: *In vitro* Pharmacology of 4-Halophenyl and 3-Fluoroalkyl Piperidines.

Compound	K_i (nM) DAT	SERT	NET	SERT/DAT	NET/DAT	NET/SERT
(-)-**1**	27.2	256	690	9.4	25	2.7
(-)-**1**	19.3	72.9	829	3.8	43	11
(-)-**1**	13	23.4	1338	1.8	100	57
(+)-**2**	235	>10000	3460	43	15	0.35
(+)-**2**	76.9	2098	2583	27	34	1.2
(+)-**2**	102	1169	1839	11	18	1.6
(+)-**1**	728	1256	>10000	1.7	14	8.0
(+)-**1**	3154	506	>10000	0.16	3.2	20
(+)-**1**	2448	201	>10000	0.082	4.1	50
(-)-**2**	1099	1492	1820	1.4	1.7	1.2
(-)-**2**	917	452	1776	0.49	1.9	3.9
(-)-**2**	1072	95.6	2371	0.089	25	2.2
(-)-**4**	33.1	940	326	28	9.8	0.35
(+)-**4**	546	858	4138	1.6	7.6	4.8
(-)-**5**	888	804	1106	0.91	1.2	1.4
(-)-**5**	105	107	837	1.0	8.0	8.0
(+)-**5**	1741	29.2	4920	0.017	2.8	170
(-)-**6a2**	196	2470	>10000	13	51	4.0
(+)-**6a2**	1508	>10000	>10000	6.6	6.6	1.0
(-)-**6a4**	1604	>10000	>10000	6.2	6.2	1.0
(+)-**6a4**	>10000	>10000	>10000	-	-	-
(-)-**6b2**	101	1250	>10000	12	99	8.0
(-)-**6b4**	474	2357	>10000	5.0	21	4.2
(+)-**6b4**	>10000	>10000	>10000	-	-	-

With respect to the halogen on the aromatic ring a divergent set of structure activity relationships is readily apparent. For the (-)-**1** series, the activity at the DAT and SERT increase in the series Cl < Br < I while the NET activity is the opposite. For the other cis enantiomer, (+)-**1**, the affinity at the DAT increases over the series Br > I > Cl, and the SERT affinity increases significantly over the series Cl < Br < I and for all halogens the NET affinity is low. For the trans isomer, (+)-**2** the affinity at the DAT increases over the order of Cl < I < Br, while that for both the SERT and NET increase over the order Cl < Br < I leading to improved selectivity for the DAT. The other trans isomer (-)-**2**, shows low affinity at the DAT or NET that is not effected by the halogen, while the SERT affinity strongly increases over the order Cl < Br < I.

For the 3-fluoroalkyl series the affinity was generally greater than the 3-methyl ester analogs. It is expected that this substitution provides a valuable means of increasing the affinity and metabolic stability of this pharmacophore. The removal of the *N*-methyl results in distinct changes in the binding affinity of the piperidines. The affinity at the DAT and NET are both decreased while that at the SERT is significantly increased. In looking at the effect of substitution of the piperidine nitrogen with a fluoroalkyl all such modifications result in a decrease in affinities. The magnitude of these changes is greater for the SERT and NET than the DAT and can allow a means for improving the selectivity for the DAT. The affinity at the DAT for several of these is sufficient to allow for examination as potential imaging agents.

Keywords: Radiohalogenated, Monoamine Transporter, Ligands

SYNTHESIS AND COMPARATIVE EVALUATION OF TWO NOVEL F-18 LABELLED OCTAHYDRO-BENZO[1,4]OXAZINE DERIVATIVES OF VESAMICOL

J. Vercouillie,[2] D. Sorger,[1] U. Grossmann,[1] M. Scheunemann,[2] R. Schliebs,[3] O. Sabri,[1] J. Steinbach.[2]
[1]Department of Nuclear Medicine, University of Leipzig, Leipzig, Saxonia, Germany; [2]Institute of Interdisciplinary Isotope Research, Leipzig, Leipzig, Saxonia, Germany; [3]Paul Flechsig Institute of Brain Research, University of Leipzig, Leipzig, Saxonia, Germany.

The vesicular acetylcholine transporter (VAChT) is thought to be an appropriate target for the detection of changes in brain cholinergic transmission. PET radioligands for *in vivo* imaging of VAChT are based on the chemical structure of vesamicol which binds with high affinity to VAChT. We have shown, that the binding specificity of vesamicol is improved, when the cyclohexanol moiety is conformationally stabilized in a bicyclic ring system such as in benzooxazine derivatives (Fig.1).

Aim: Preparation of **Fluoroa**cetyl-(**[^{18}F]FAMV**) and **Fluorob**enzoyl**m**orpholine**v**esamicol (**[^{18}F]FBMV**) and evaluation of their potentials as PET tracer for imaging VAChT with respect to binding affinity and specificity, brain accumulation and pharmacokinetic properties in rats.

Methods: [^{18}F]FAMV) and **[^{18}F]FBMV** were synthesized in one step from **2b** resp. **2c,** using K[^{18}F]/Kryptofix$_{2,2,2}$ in DMF and microwave (50 W, 20 min). K_i-values of **FAMV**, **FBMV** and unsubstituted **MV** (range from 10^{-11} to 10^{-5} M) were determined by competitive binding assays a) using PC12 cells stably expressing VAChT and [^{3}H]Vesamicol (2 nM) for affinity testing to VAChT b) using rat liver membranes and [^{3}H]DTG (4 nM) for affinity testing to sigma-1,2 receptors. 3 MBq **[^{18}F]FAMV** resp. **[^{18}F]FBMV** were injected in female Sprague Dawley rats (n = 30). Decapitation 5, 15, 30, 60, 120 and 180 min p.i. Half-life corrected radioactivity of samples of blood, organs and different brain sections was expressed as % injected dose (i.d.) per gram.

Results: [^{18}F]FAMV (110 min) resulted in a radiochemical yield (decay corrected) of 21 ± 3%,.Classical heating methods or acetonitrile as solvent resulted in lower yields (< 10%). The use of DMSO and heating or microwave activation was unsuccessful. The radiosynthesis of **[^{18}F]FBMV** (120 min) resulted in a 10 - 15 % yield. This reaction was successful using DMF, wheras using acetonitrile or DMSO with or without using microwave did not afford the radioligand. Like for the fluoroacetyl derivative, heating gave lower yields (5%). After HPLC purification (16 x 250 mm, Nucleosil 100-7 C18; H_2O/MeOH/triethylamine, 5 mL∗min^{-1}) the tracer were obtained with a radiochemical purity higher than 99%.

The affinity to VAChT clearly increased when the benzoxazine N-atom was substituted: K_i values: 331 nM (**MV**), 19 nM (**FAMV**), 11 nM (**FBMV**). The binding affinity of the compounds to sigma receptors was low (K_i > 4000 nM). The accumulation of **[^{18}F]FBMV** in brain was 2.5 times that of **[^{18}F]FAMV** (0,12 vs. 0,05 % i.d per gram., 30 min p.i.). Both compounds were quickly eliminated from blood. Radioactivity in the kidneys decreased continuously. **[^{18}F]FBMV**: The radioactivity of striatum and cortex related to that of cerebellum increased with time (2,6 resp. 2,0) at 180 min p.i. Accumulation was highest in bowel, liver and lung (2/ 1,6/ 1.2) % i.d./gram.

Conclusion: The specific accumulation of **[^{18}F]FBMV**) in brain is higher than that for **[^{18}F]FAMV;** however, further studies are necessary to evaluate its sensitivity for imaging cholinergic deficits.

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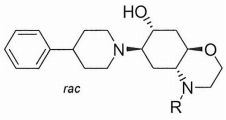
Keywords:
VAChT,
Vesamicol
Anlogues,
Radiosynthesis



R = H **MV** R = $BrCH_2CO$- **2b**
FCH_2CO- **FAMV** 4-NO_2-C_6H_4CO- **2c**
4-F-C_6H_4CO- **FBMV**

Fig.1

SYNTHESIS AND EVALUATION OF ^{11}C-LABELED METHYLVESAMICOLS AS A PET LIGAND FOR VESICULAR ACETYLCHOLINE TRANSPORTERS OR SIGMA RECEPTORS

K. Kawamura,[1] K. Shiba,[2] K. Yajima,[3] K. Ishiwata.[1]

[1]Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; [2]Advanced Science Research Center, Kanazawa University, Kanazawa, Ishikawa, Japan; [3]Basic Research Division, The Medical and Pharmacological Research Center Foundation, Hakui, Ishikawa, Japan.

Several radioligand based on vesamicol have been proposed as the probes to detect vesicular acetylcholine transporter (VAChT) by PET and SPECT. Substituted positions and optical isomerization of the vesamicol derivatives altered their affinities for VAChT and sigma receptors [ref.]. Here we synthesized (-)-[^{11}C]o-methylvesamicol {(-)-[^{11}C]OMV} and (+)-[^{11}C]p-methylvesamicol {(+)-[^{11}C]PMV}, and evaluated their properties as PET radioligands.

The affinities of methylvesamicols for VAChT and sigma receptors were investigated by membrane binding assay. (-)-[^{11}C]OMV and (+)-[^{11}C]PMV were synthesized by a palladium-promoted cross-coupling reaction with [^{11}C]CH_3I and (-)-*o*-trimethylstanylvesamcol and (+)-*p*-tributylstanylvesamcol, respectively, in the presence of $Pd_2(dba)_3$, (*o*-tol)$_3$P, CuCl and K_2CO_3. Biodistribution of two radioligands were investigated in male Wistar rats by tissue dissection and *ex vivo* autoradiography. Specific uptake in the rat brain was evaluated in blocking studies with cold (-)-OMV or (+)-PMV (0.5 μmol/kg coinjection), (-)-vesamicol (0.5 μmol/kg coinjection) as a high affinity VAChT ligand, and (+)-pentazocine (0.5 μmol/kg coinjection) or (±)-pentazocine (50 μmol/kg pretreatment) as a sigma$_1$ receptor ligand.

In the in vitro binding assay, (-)-OMV exhibited a high and selective affinity for VAChT, and (+)-PMV exhibited a high and selective affinity for sigma$_1$ receptor (Table 1). Radiochemical yields of (-)-[^{11}C]OMV and (+)-[^{11}C]PMV were 31-44% and 7.7-19%, respectively, based on [^{11}C]CH_3I. The specific activity at 30 min from EOB was 2.6-19 TBq/mmol for the (-)-[^{11}C]OMV and 41-160 TBq/mmol for (+)-[^{11}C]PMV. In *in vivo* distribution studies, the brain uptakes of (-)-[^{11}C]OMV and (+)-[^{11}C]PMV in rats showed similar time course: the levels were 1.1 %ID/g at 5 min postinjection, and retained a high level for 60 min. The (-)-[^{11}C]OMV uptake in all brain regions was significantly inhibited (60-65% reduction) by coinjection with (-)-OMV and (-)-vesamicol, but not with (+)-pentazocine. However, the pretreatment with an excess amount of (±)-pentazocine reduced the uptake in the different manner in the brain regions; 25% reduction in the striatum with a high density of VAChT, and 50-55% reduction in the other regions with lower densities of VAChT. The (+)-[^{11}C]PMV uptake was significantly inhibited (45-55% reduction) in all brain regions by coinjection with (+)-PMV and (-)-vesamicol and by prereatment with (±)-pentazocine. *Ex vivo* autoradiography showed different regional brain distribution patterns between (-)-[^{11}C]OMV and (+)-[^{11}C]PMV.

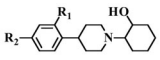
In conclusion, (-)-[^{11}C]OMV mainly bound to VAChT but the binding to sigma$_1$ receptor may not be disregarded. On the other hand, (+)-[^{11}C]PMV is a radioligand for sigma$_1$ receptor.

[Reference] Shiba K, Yano T, Sato W, et al; Life Sci 71:1591-1598 (2002).

Table 1. Affinity of vesamicol derivatives and (+)-pentazocine for VAChT and sigma receptors

	VAChT Ki (nM)	Sigma$_1$ receptor Ki (nM)	Sigma$_2$ receptor Ki (nM)
(-)-OMV	6.7 ± 1.6	34 ± 5.9	280 ± 30
(+)-PMV	200 ± 32	3.0 ± 0.2	41 ± 2.9
Vesamicol	4.4 ± 0.6	74 ± 11	380 ± 68

Mean ± S.D. (n = 3)



	R_1	R_2
(–)-[^{11}C]OMV	[^{11}C]CH_3	H
(+)-[^{11}C]PMV	H	[^{11}C]CH_3

Figure 1. Structures of (–)-[^{11}C]OMV and (+)-[^{11}C]PMV

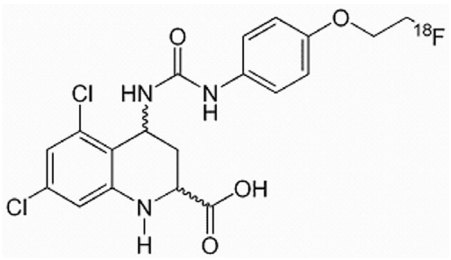
Keywords: Vesicular Acetylcholine Transporters, sigma Receptors, Vesamicols

SYNTHESIS, LABELLING AND EVALUATION OF A NEW FLUORINATED NMDA LIGAND TO STUDY GLUTAMATERGIC NEUROTRANSMISSION *IN VIVO*

M. Piel,[1] S. Hoehnemann,[1] M. Jansen,[2] U. Schmitt,[3] G. Dannhardt,[2] F. Roesch.[1]
[1]Institute of Nuclear Chemistry, Johannes Gutenberg-Universitaet, Mainz, Germany; [2]Institute of Pharmacy, Johannes Gutenberg-Universitaet, Mainz, Germany; [3]Department of Psychiatry, Johannes Gutenberg-Universitaet, Mainz, Germany.

Introduction:

Glutamate is the most abundant excitatory amino acid in the central nervous system of mammals and plays an important role in neurodevelopment, synaptic plasticity and neurotoxicity [1]. Glutamate is recognized by metabotropic and ionotropic receptors, of which the N-methyl-D-aspartate (NMDA) receptor is the most important, due to their involvement in neurological disorders such as Morbus Parkinson, Alzheimer's disease and schizophrenia [2]. The glycine binding site of this NMDA receptor is an important target for the development of new ligands. Therefore the [^{18}F]fluorine labelled molecule trans-5,7-dichloro-4-(3-{4-[4-(2-[^{18}F]fluoroethyl)-piperazin-1-yl]-phenyl}-ureido)-1,2,3,4-tetra-hydroquinoline-2-carboxylic acid (fig. 1) was synthesised to visualize the NMDA receptor *in vivo:*



Experimental:

At first the synthesis for the reference compound and the precursor was developed. This synthetic route also includes a separation of the trans-isomers from the cis-isomers, because the trans-isomers are known for a higher affinity to this binding site than the cis-derivatives. With this compound the *in vitro*-affinities were determined in [^{3}H]MDL-105,519 binding assays, resulting in a very high K_i of 2.9 nM [3]. The lipophilicity of the molecule was determined using the HPLC method, resulting in a logD of 1.3. Afterwards the ^{18}F-fluorinated ligand was synthesised and its labelling optimised. The highest radiochemical yields (RCY) of 95 % were achieved in DMSO at 100 °C using 2 equivalents of 5 M NaOH and 2-[^{18}F]fluoroethyl tosylate as labelling agent. In first animal studies in Sprague Dawley rats (250-300 g) the biodistribution of the ligand was examined *ex vivo*. The tracer showed a maximum brain uptake of about 0.1 %ID/g which was reached after 5 min. Excretion was primarily via the urine, while kidney and liver showed an uptake of up to 1.8 % ID/g and 1.7 % ID/g within 60 minutes. The low ^{18}F-activity in the bone, with a maximum of 0.1% ID/g, indicates a small metabolic hydrolysis of the ligand under release of ^{18}F-fluoride.

Conclusion:

The synthesis for the reference compound and the precursor for this new NMDA-ligand were developed. The ^{18}F-labelling reaction was examined and optimised, resulting in a radiochemical yield of about 95%. Preliminary pharmacological experiments were performed with the ligand, showing a logD of 1.3 and a very high affinity for the glycine binding site with a K_i of 2.9 nM. First ex vivo biodistribution studies showed a maximum brain uptake of 0.1 %ID/g within 60 minutes. Further studies are underway to evaluate the ligand in vivo.

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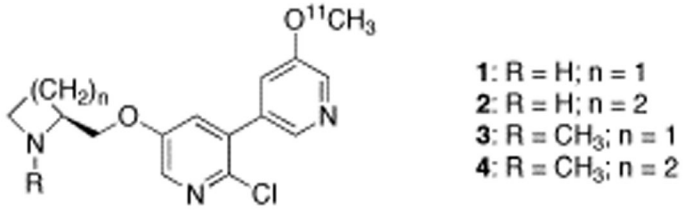
Keywords: NMDA, Glutamate, ^{18}F-Fluoroalkylation

C-11 LABELED 6-CHLORO-5-(PYRIDIN-3-YL) ANALOGS OF AZETIDINYL AND PYRROLIDINYL 3-PYRIDYL ETHERS: SYNTHESIS AND EVALUATION AS *IN VIVO* TRACERS OF NICOTINIC ACETYLCHOLINE RECEPTOR

H. Fan,[1] A.G. Horti,[1] H.T. Ravert,[1] U.A. Scheffel,[1] D.P. Holt,[1] P.A. Rauseo,[1] Y. Xiao,[2] K.J. Kellar,[2] D.F. Wong,[1] R.F. Dannals.[1]

[1]Department of Radiology, Division of Nuclear Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States; [2]Department of Pharmacology, Georgetown University, Washington, DC, United States.

Objectives: Previous studies have demonstrated that density changes in neuronal nicotinic acetylcholine receptors (nAChRs) are associated with the normal aging process, as well as neurological diseases such as Alzheimer's disease and several movement disorders. Along with the α7-nAChR subtype, the α4β2-nAChR represents the most abundant nAChR in the brain. Herein, four potential α4β2-nAChR ligands, 6-chloro-5-(pyridin-3-yl) analogs of azetidinyl and pyrrolidinyl 3-pyridyl ethers have been synthesized and labeled with carbon-11. These targets were selected since the 5-position of the pyridine nucleus in this series is known to be tolerant to substitution. Moreover, previous reports suggested that the additional of heterocyclic ring might play an important role on the binding affinity of the ligands with nAChRs. **Methods**: The tracers in unlabeled forms and precursors were prepared via Stille coupling reaction of the bromopyridyl ethers, which were resulted from Mitsunobu reactions, with the corresponding trimethyltin derivatives. The trimethyltin heteroaromatic intermediates were obtained via palladium-assisted reactions between bromoheteroarene substrates and hexamethylditin. C-11 labeled tracers were prepared by based-promoted methylation with [^{11}C]-iodomethane. Affinities of the four ligands at nAChRs were determined from binding competition experiments with [^{3}H]-epibatidine in rat forebrain. The *in vivo* kinetic studies are under investigation at the present time. **Results**: [^{11}C]-6-chloro-5-(pyridin-3-yl) analogs of azetidinyl and pyrrolidinyl 3-pyridyl ethers were successfully synthesized in reasonable radiochemical yield (7.8-16% non-decay corrected) and high specific activities (7520-20720 Ci/mmol). *In vitro* assays gave K_i values ranged from 0.019 to 12.4 nM, with compound **1** having a 3 fold lower K_i than epibatidine. Preliminary *in vivo* mouse studies with compound **1** indicated a brain biodistribution profile consistent with high-affinity nAChRs. **Conclusion**: Novel nAChR imaging agents were synthesized with reasonable yields.



1: R = H; n = 1
2: R = H; n = 2
3: R = CH_3; n = 1
4: R = CH_3; n = 2

Keywords: Nicotinic Acetylcholine Receptor, PET, Carbon-11

SYNTHESIS AND *IN VIVO* EVALUATION OF ARYLALKYL 4-BENZYL PIPERAZINES AS RADIOLIGAND SIGMA RECEPTORS

M. Kassiou,[1,2] T. Pham,[3] R. Fulton,[1] F. Mattner,[3] D. Henderson,[1] A. Katsifis,[3] X. Liu,[3] S. Eberl,[1] I. Greguric,[3] M. Fulham,[1] S. Thompson.[1]

[1]PET & Nuclear Medicine, Royal Prince Alfred Hospital, Sydney, NSW, Australia; [2]Pharmacology, University of Sydney, Sydney, NSW, Australia; [3]Radiopharmaceuticals Divison R&D, ANSTO, Sydney, NSW, Australia.

Since the discovery of sigma receptors, research has been ongoing in an attempt to understand the functional roles of these sites. There are 2 identified subtypes, namely sigma-1 and sigma-2 sites which are involved in several CNS disorders. Recently, 1-benzofuran-2-ylmethyl-4-benzylpiperazine was reported as a high affinity selective sigma ligand (IC_{50}=0.4nM) compared to $5HT_{1A}$ (IC_{50}=5000nM) and D_2 (IC_{50}=1000nM) receptors (1). Based on this lead compound, we synthesised the iodinated and methoxy ether analogues which serve as radioligands for use in PET and SPECT. Here we report the synthesis, radiosynthesis, *in vitro* and *in vivo* evaluation of 1-benzofuran-2-ylmethyl-4-(4-[^{123}I]iodo-benzyl)piperazine [^{123}I]**1** and 1-benzofuran-2-ylmethyl-4-(4-[^{11}C]methoxy-benzyl)piperazine [^{11}C]**2** in rat and baboon brain respectively.

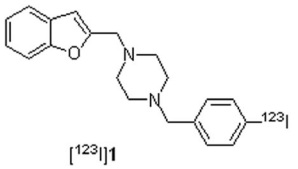
Compounds **1 and 2** were synthesised from benzofuran-2-carboxylic acid with the trimethyl stannane derivative serving as precursor for labelling using iodine-123 and the phenolic precursor for labelling using [^{11}C]CH_3I. [^{123}I]**1** was prepared by iododestannylation in ethanol using CAT at pH 1-2 while [^{11}C]**2** was prepared by *O*-alkylation in DMF with TBAH. Purification for both [^{123}I]**1** and [^{11}C]**2** was performed using C-18 semi-preparative HPLC whilst radiochemical purity and specific activity (SA) were calculated using analytical HPLC. The biodistribution of [^{123}I]**1** was evaluated in Sprague Dawley rats following tail vein injection at specified times up to 6hrs p.i. [^{11}C]**2** was imaged for 1hr on a Siemens PET/CT scanner in 1 healthy male *Papio hamadryas* baboon in 2 separate experiments. The first experiment being baseline whilst the other involved pre-treatment with haloperidol (1 mg/kg) 5min prior to injection of [^{11}C]**2**.

In vitro binding revealed sigma-1 selectivity for **1** (IC_{50} sigma-1=1.2nM, sigma-2=<65% inhibition) while **2** displayed marginal selectivity (IC_{50} sigma-1=1.1nM, sigma-2=5.9nM). [^{123}I]**1** was synthesised in 81% RCY while [^{11}C]**2** was obtained in 22% yield with SA of >76 GBq/μmol and 73 GBq/μmol respectively. The radiochemical purity was >98% for both radioligands. The *in vivo* biodistribution of [^{123}I]**1** in rats showed high uptake in the posterior and frontal cortex and cerebellum (1.2%ID/g) at 5 min. The uptake remained in a plateau and started to decline by 6hrs. Pre-treatment with pentazocine (1 mg/kg) or unlabelled **1** (1 mg/kg) reduced radioligand uptake by 40% and 70% in most brain structures. Following i.v. injection of [^{11}C]**2** in baboon, significant accumulation of radioactivity was seen in cortical areas (25KBq/ml) and cerebellum (20KBq/ml) at 5 min p.i. which remained in a plateau. Compared to the baseline study, pre-treatment with haloperidol reduced [^{11}C]**2** uptake by 80% in both these regions at 1hr p.i.

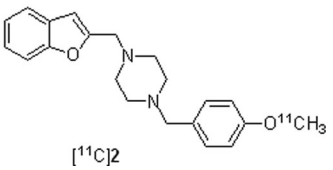
These results clearly demonstrate that 4-benzylpiperazines, [^{123}I]**1** and [^{11}C]**2** demonstrate specific sigma receptor binding *in vivo* and should serve as templates for the development of subtype selective sigma receptor radioligands for imaging using PET and SPECT.

1. Younes S, Labssita Y, et.al. Eur. J. Med. Chem. 2000; 35: 107-121.

Keywords: sigma Receptors, Arylalkyl Benzylpiperazines, PET and SPECT



[^{123}I]**1**



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

SYNTHESIS, *IN VITRO* TESTING AND RADIOLABELLING OF 2-ARYL APOMORPHINES AS POTENTIAL CANDIDATES AS DOPAMINE D2 AGONIST LIGANDS FOR PET

K. Sondergaard,[1,2,3] J. Madsen,[1] S. Lehel,[1] M. Palner,[2] G.M. Knudsen,[2] M. Begtrup,[3] N. Gillings.[1]
[1]PET & Cyclotron Unit, Copenhagen University Hospital, Copenhagen, Denmark; [2]Neurobiology Research Unit, Copenhagen University Hospital, Copenhagen, Denmark; [3]Department of Medicinal Chemsitry, Danish University of Pharmaceutical Sciences, Copenhagen, Denmark.

Introduction

The alkaloid apomorphine displays a number of interesting biological effects toward various disorders such as erectile dysfunction and Parkinson's Disease. The pronounced efficacy of apomorphine for agonistic binding to the dopamine receptors has made it an object of intense research. Since apomorphine is not dopamine receptor subtype selective (k_i = 101 nM, 32 nM, 26 nM, 2.6 nM, 10 nM for D_1-D_5 respectively), modifications have been made to address the selectivity and binding affinity of apomorphine derivatives by varying the substituents at the 2-position. Many of these modifications equal or increase binding to the dopamine D_2 receptor suggesting the presence of a receptor-excluded cavity at the 2-position. It has been suggested that this cavity in the dopamine D_2 receptor is delimited by lipophilic residues. To the best of our knowledge no derivatives of apomorphine with carbon substituents at the 2-position have been prepared so far. We set out to explore the potential hydrophobic interactions near the 2-position of the apomorphine skeleton by introduction of aryl substituents, with a view to the development of novel D_2 agonist ligands for PET. The corresponding 2-substituted norapomorphines are amenable to labelling with carbon-11 to give the *N*-alkyl norapomorphines (methyl, ethyl or propyl).

Methods

From codeine, 2-aryl substituted apomorphines were synthesised in 6 steps each. Oxidation of codeine with IBX gave codeinone which underwent an acid catalysed rearrangement to give morphothebaine. This was selectively triflylated at the 2-position and subsequently *O*-acetylated at the 11-position. The resulting triflate was coupled in a Suzuki-Miyaura type reaction with 4-substituted arylboronic esters**,** to give, after deprotection, the desired 2-aryl apomorphines, **6a-d**. These four compound were tested in *in vitro* binding assays using cloned human receptors and rat brain homogenates, and autoradiography of rat brain slices (Table 1).

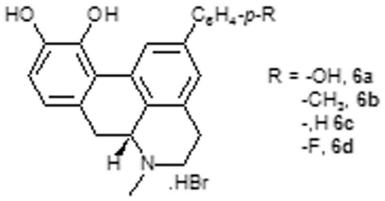


TABLE 1. ki values (nM) from in vitro binding assays

	Human						Rat
Compd.	D1	D2	D3	D4	D5	D2 (homogenate)	D2 (autoradiography)
6a	167	4	3	5	313	2	1
6b	1129	346	879	57	4255	-	-
6c	755	88	16	21	2359	29	7
6d	1217	145	831	18	4936	-	3

Results and Conclusions

The most promising candidate for further testing was **6a** with an affinity for dopamine D_2 receptors of 2-4 nM. Apomorphine **6a** has been labelled with carbon-11 by *N*-methylation of the corresponding norapomorphine, as previously described for apomorphine. In vivo testing in animals is scheduled and results of this will be presented.

Keywords: Apomorphines, Dopamine D2 Agonist

TOWARDS A METABOLICALLY STABLE FLUORINE-18 PET RADIOTRACER FOR SEROTONIN 5HT$_{1A}$ RECEPTORS

N. Saigal, D. Collins, R. Pichika, J. Mukherjee.
Psychiatry and Human Behavior, University of California, Irvine, Irvine, CA, United States.

Objectives:

Extensive research shows involvement of 5-HT$_{1A}$ receptor in Alzheimer's disease, dementia, anxiety, schizophrenia, and depression. While PET imaging is currently underway with ^{11}C-WAY-100635 (WAY) ^{18}F-FCWAY and ^{18}F-MPPF there is further need for a superior radioligand for in vivo studies. A radioligand is needed that does not get quickly metabolized or cleared from the plasma and can be synthesized in adequate yield. Our goal is to stabilize breakdown of a radiotracer based on the WAY-100635 backbone by using two general approaches: 1. Stabilize the fluorine-18, by including it on a primary carbon, e.g.,(fluoromethyl group on cyclohexyl ring of WAY, and 2. Stabilize breakdown of the amide in WAY by converting it into a thioamide, which are known to be more stable in vivo.

Towards goal #1, we prepared *N*-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-^{18}F-fluoromethylcyclohexane)carboxamide (^{18}F-Mefway; Fig-1a). ^{18}F-Mefway was synthesized and evaluated for its potential as a radioligand for labeling 5-HT$_{1A}$ receptors. Towards goal #2 we are examining the thio analogs of MPPF (Fig-1b) and WAY (Fig-1c).

Methods:

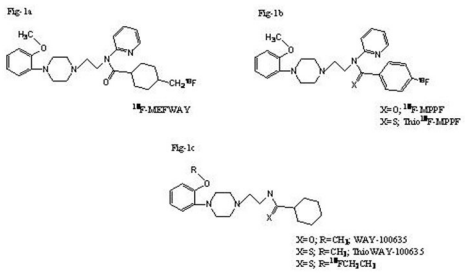
Mefway was prepared in three steps from WAY-100634. Radiolabeling with fluorine-18 (from an MC-17 Cyclotron) and a tosylate precursor of Mefway in the CPCU (96 ^{0}C; CH_3CN, 30 min.) Product was purified in a reverse-phase HPLC with 60% CH_3CN:40% 0.1% Et_3N flow rate of 2.5ml/min. Binding studies were carried out in rat brains at 37^{o}C. Brain slices were incubated with 3.5 Ci/cc at 37 ^{o}C for 1 hr. Nonspecific binding was measured in the presence of 10µ of WAY-100635. Competition with 10µ serotonin was measured. Autoradiographs were generated using the Packard Phosphor Cyclone Imager. Thioamides of WAY, MPPF, and the Nitro precursor for MPPF were made by refluxing with Lawsons Reagent in xylene. Radiolabeling of the nitro-MPPF and the thio analog was done in the CPCU (125 ^{0}C; DMSO, 30 min.) Product was purified in reverse-phase HPLC C-18 column with 60% CH_3CN:40% 0.1% Et_3N flow rate of 2.5ml/min. Binding studies were performed in rat brain tissues.

Results:

Towards our first goal, tosylate to fluorine-18 exchange gave modest radiochemical yield of ^{18}F-Mefway (=5% decay corrected after HPLC). The retention time of ^{18}F-mefway was 10.5 min and specific activity was >2000Ci/mmol. A methyl/fluoromethyl group at the 4-position maintain <2 nM affinity in these compounds. *In vitro* binding of ^{18}F-mefway showed exceptional binding in hippocampus (Hp), entorhinal cortex, dentate gyrus and cortex (Ctx) with limited binding in the cerebellum (Cer). Ratios of Hp/Cer = 27 and Ctx/Cer = 9 were measured which are comparable to ^{11}C-WAY. WAY displaced >90% in Hp and Ctx and serotonin displaced >85% in Hp and >70% in Ctx of ^{18}F-mefway binding. Radiochemical yields of ^{18}F-thioMPPF were very poor. Radiosynthesis optimization of ^{18}F-fluoroethylthioWAY is currently in progress.

Conclusions:

^{18}F-Mefway binding to 5HT$_{1A}$ regions indicates its potential as a PET imaging agent. The addition of flourine-18 on a primary carbon is expected to make the compound more stable to deflourination. *In vivo* studies with ^{18}F-mefway are currently ongoing to demonstrate stability and selectivity of binding. The synthesis and radiosynthesis of the thio analog of mefway is currently underway. The in vitro evaluation of the thioanalogs of WAY, MPPF, and MEFWAY are in progress.



Keywords: Serotonin Receptors, Receptor-Binding Radiopharmaceutical, PET

SYNTHESIS AND EVALUATION OF THIONAPHTHENE DERIVATIVES AS LIGANDS FOR IMAGING β-AMYLOID PLAQUES IN THE BRAIN

Y.S. Chang,[1] J.M. Jeong,[1] Y.-S. Lee,[1] J.H. Cho,[2] C.H. Oh,[2] D.S. Lee,[1] J.-K. Chung,[1] M.C. Lee.[1]
[1]Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Medicinal Chemistry Research Center, Korea Institute of Science and Technology, Seoul, Korea.

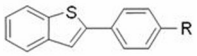
Formation and accumulation of aggregates of β-amyloid (Aβ) peptides in the brain is knowwn as a major factor in the pathogenesis of Alzheimer's disease. *In vivo* assessment of Aβ plaques in the brain will be useful in early diagnosis and monitoring the progression of the disease. A series of novel Aβ aggregate-specific lignads based on thionaphthene was synthesized and evaluated.

Thionaphthene derivatives, 2-(4′-hydroxyphenyl)thionaphthene (HPT) and 2-(4′-methoxyphenyl)thionaphthene (MPT), 2-(4′-aminophenyl)thionaphthene (APT), 2-(4′-*N*-methylaminophenyl)thionaphthene (MAPT), and 2-(4′-*N,N*-dimethylaminophenyl)thionaphthene (DMAPT) were synthesized by a literature method. Fluorinated thionaphthene derivatives, 2-(4′-O-(2"-fluoroethyl)hydroxyphenyl)thionaphthene (FEHPT), 2-(4′-O-(3"-fluoropropyl)hydroxyphenyl) thionaphthene (FPHPT), 2-(4′-N-(2′-fluoroethyl)hydroxyphenyl)thionaphthene (FEAPT), 2-(4′-N-(3"-fluoropropyl)hydroxyphenyl)thionaphthene (FPAPT) were synthesized by alkylation of 2-hydroxy and 2-amino thionaphthene with appropriate bromofluoroalkane and sodium hydride. All compounds were comfirmed by ^{1}H-NMR. 2-(4′-methylaminophenyl)benzothiazole (BTA-1) was labeled with ^{125}I using chloamine-T method and purified with HPLC. The Kd value of [^{125}I]2-(3′-iodo-4′-methylaminophenyl)benzothiazole ([^{125}I]3′-I-BTA-1) was evaluated with $A\beta_{(1\text{-}40)}$ aggregates. The Ki values of the synthesized thionaphthene derivatives were evaluated by competition against [^{125}I]3′-I-BTA-1 binding to $A\beta_{(1\text{-}40)}$ aggregates. Fluorescence staining using the frozen brain sections of a 24-month-old Tg2576 mouse with HPT and MPT was achieved.

The labeling efficiency of [^{125}I]3′-I-BTA-1 was 74±9%, radiochemical purity after purification was over 97% and specific activity was 2.175×0^{6}Ci/mol. The log P value of [^{125}I]3′-I-BTA-1 was 2.3. The values of Kd and Bmax of [^{125}I]3′-I-BTA-1 were 0.032×0^{-9} M and 37.1 pmol/mg protein, respectively. The Ki values of BTA-1 and 3′-I-BTA-1 were 0.64 and 4.70 nM, respectively. All the synthesized thionaphthene derivatives showed high binding affinity to $A\beta_{(1\text{-}40)}$ aggregates. *O*-methylation or *N*-methylation of HPT or APT led to increase in the binding affinity. Introduction of fluoroehtyl- or fluoropropyl- to HPT or APT also increased the affinity of the products to β-amyloid aggregates. HPT and MPT showed weak fluorescence after binding with β-amyloid aggregates.

The thionaphthene derivatives displayed excellent binding affinity for $A\beta_{(1\text{-}40)}$ aggregates. Especially, introduction of fluoroehtyl- or fluoropropyl- residues to HPT or APT showed increased affinity. These compounds may be useful PET agents to imaging Aβ plaques in the brain.

Comp	R	Ki (nM)
1. HPT	OH	2.55
2. MPT	OCH_3	0.75
3. APT	NH_2	5.14
4. MAPT	$NHCH_3$	1.27
5. DMAPT	$N(CH_3)_2$	1.04
6. FEHPT	$O(CH_2)_2F$	0.09
7. FPHPT	$O(CH_2)_3F$	0.12
8. FEAPT	$NH(CH_2)_2F$	0.28
9. FPAPT	$NH(CH_2)_3F$	0.10



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Keywords: Thionaphthene Derivatives, Imaging β-Amyloid Plaques, PET Agent

SYNTHESIS AND RADIOIODINATION OF HALOGENATED BENZAMIDE DERIVATIVES FOR THE NON-INVASIVE VISUALIZATION OF D2-LIKE DOPAMINE RECEPTORS

D. Stark,[1] M. Piel,[1] D. Bier,[2] M. Holschbach,[2] U. Schmitt,[3] H. Huebner,[4] P. Gmeiner,[4] H. Lueddens,[3] F. Roesch.[1]

[1]Institute of Nuclear Chemistry, University of Mainz, Mainz, Germany; [2]Institute of Nuclear Chemistry, Forschungszentrum Juelich GmbH, Juelich, Germany; [3]Department of Psychiatry, University of Mainz, Mainz, Germany; [4]Department of Medicinal Chemistry, University of Erlangen, Erlangen, Germany.

Aim: Halogenated benzamides especially F-18-Fallypride (F-18-FP) and F-18-Desmethoxyfallypride (F-18-DMFP) have been established as D2-selective PET-radioligands visualizing dopamine receptors in the brain [1, 2]. In order to make the favourable properties of these F-18-labelled allylic derivatives available to SPECT and to offer an interesting alternative to I-123-IBZM, the iodinated analogues N-allylepidepride (NAE) [3] and N-allyldesmethoxyepidepride (NADE) were synthesized.

Methods: The fluoropropyl moiety in the 5-position of the aromatic ring was replaced by iodine and bromine. Precursors and reference compounds were synthesized by coupling the respective benzoic acid derivative with (S)-2-aminomethyl-1-allylpyrrolidine. To obtain (S)-2-aminomethyl-1-allylpyrrolidine, a stereoconservative route described by Hoegberg et al. was followed [3]. The benzoic acid derivatives were synthesized by oxidizing the corresponding aldehydes. Chloro-ethylformiate was employed to activate the benzoic acid derivatives before reacting them with (S)-2-aminomethyl-1-allylpyrrolidine [5].

The stannous precursors were obtained by reacting the reference compounds with hexabutyldistannane using tetrakis(triphenylphosphine)palladium(0) as catalyst [5].

Lipophilicities were determined using the HPLC-method with Soerensenbuffer as eluent [6].

In vitro affinities to dopamine receptor subtypes of the synthesized benzamides were examined. In order to determine their selectivity to dopaminergic receptors their affinity to serotonergic (p5-$HT_{1A}$and p5-HT_2) and adrenergic receptor subtypes (pα1) was tested as well.

For the radioiodinations I-131-NaI was used due to easier handling and longer half-life. The radioiodination was carried out in a phosphate buffer system using Chloroamine-T as the oxidizing agent. Reaction parameters such as reaction time, buffer volume, precursor concentration, etc. were varied. Radiochemical yields for both compounds, I-131-NAE and I-131-NADE, were moderate to good.

Radioiodination experiments with I-123-NaI were conducted as well.

Results: K_i-values for the D2-receptor (hD2short) were found to be 0.7 nM for NAE and 19 nM for NADE. $LogD_{7.4}$-values for NAE and NADE are 2.81 and 2.70 respectively.

Radiochemical yields for the radioiodination with I-131-NaI in a phosphate buffer / Chloroamine-T system ranged from 50 % to 70 % for I-131-NAE and from 20 % to 60 % for I-131-NADE.

Autoradiography in rat brain showed excellent visualization of the dopamine receptor regions.

Conclusions: The promising results of the *in vitro* studies for NAE and the new compound NADE as well as the brominated derivatives (NABrE and NaBrDE) justify further investigations. *Ex vivo* biodistribution studies and *in vivo* small animal SPECT studies are in preparation.

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Keywords: D2-Receptors, Benzamide / Epidepride, SPECT

EVALUATION OF CLINDE, A RADIOLABELLED PERIPHERAL BENZODIAZEPINE RECEPTOR LIGAND IN A MURINE MODEL OF NEURO-INFLAMMATION: POTENTIAL PROBE FOR ASSESSING NEURODEGENERATION

A. Katsifis,[1] F. Mattner,[1] P. Mana,[2] D. Linares,[2] P. Ballantyne,[1] P. Berghofer,[1] T. Pham,[1] D. Willenborg.[2]

[1]Radiopharmaceuticals Research Institute, ANSTO, Sydney, NSW, Australia; [2]Neuroscience Research Unit, Australian National University Medical School, Canberra, ACT, Australia.

Introduction: Peripheral benzodiazepine receptor (PBR) has been proposed as a sensitive marker to visualize and measure microglial cell activation associated with various forms of brain injury and inflammation. Recently we have shown that the PBR density increased in a murine model of neuroinflammation. When C57Bl/6 mice are fed cuprizone, mature oligodendroglia in the corpus callosum die. Oligodendrocyte death is followed by astrogliosis and recruitment and activation of microglia and peripheral macrophages leading to phagocytosis of myelin. T cells are thought not to be involved and the blood brain barrier remains intact. Within weeks the entire corpus callosum is demyelinated. When cuprizone is withdrawn complete remyelination takes place. The aim of this study was to evaluate the iodine-123-labelled imidazopyridine: N,N-diethyl-6-chloro-(4′-iodophenyl)imidazo(1,2-a)pyridine-3-acetamide ([^{123}I]-CLINDE), as a potent radioligand for the PBRs in assessing microglial/macrophage activation in the CNS. CLINDE is a high affinity (IC_{50} = 1.7 nM) ligand for the PBR whilst it exhibits significantly lower binding to the central benzodiazepine receptors (IC_{50} = 450 nM).

Methods: [$^{123/125}$I]-CLINDE has been prepared by classical iododestannylation reactions with peracetic acid in > 80% radiochemical yield and >98% radiochemical purity. Groups of male C57BL/6J were fed a powdered diet containing 0.2% cuprizone or control diet. Mice were sacrificed 1, 2, 3, 4 weeks following diet administration and brain tissue was harvested and frozen for receptor in vitro autoradiography. PBR receptors were labelled with [^{125}I]-CLINDE and non-specific binding was determined in the presence of PK11195. Futhermore for *in vivo* studies, groups of 4 weeks cuprizone fed and controls were injected with [^{123}I]-CLINDE (0.7 MBq in 100 µl) and the time course distribution of the tracer in the brain and other tissues was evaluated.

Results: *In vitro* autoradiography with [^{125}I]-CLINDE revealed increase PBR levels mainly in the corpus callosum area of the brain and this increase is proportional to the duration of the cuprizone diet. There are an increase of receptor levels in corpus callosum of the brain of cuprizone fed mice compared to controls of 60, 87, 127 and 250% for 1, 2, 3 and 4 weeks diet respectively. Other areas of the brain showed increased PBR levels compared to controls and quantitative data will be presented. The *in vivo* biodistribution of [^{123}I]-CLINDE in cuprizone fed and controls mice showed peak uptake in the brain at 30 min (2 and 1% ID/g respectively) and by 24 h (0.27 and 0.18% ID/g respectively). High uptake was also observed in the mouse adrenals, heart and kidney in both controls and cuprizone fed mice.

Conclusion: These results demonstrates the specific PBR receptor uptake of [^{123}I]-CLINDE *in vivo* of cuprizone fed mice. Futhermore it provides evidence that neuronal PBR receptors are increased in the brain of cuprizone fed mice. This suggests that [^{123}I]-CLINDE warrants further investigation as a potential SPECT marker for the study of neuroinflammation.

Keywords: Peripheral Benzodiazepine Receptor, Microglia, Demyelination

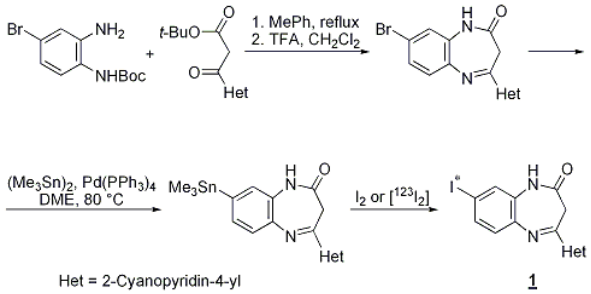
RADIOIODINATION OF BENZODIAZEPINE DERIVATIVES AS LIGANDS FOR GROUP II METABOTROPIC GLUTAMATE RECEPTORS

A.O. Koren,[1] H. Da Costa,[2] K.P. Cosgrove,[2] J.K. Staley,[2] L.A. Amici,[2] F. Bois,[2] R.M. Baldwin,[2,3] G.D. Tamagnan.[1,2]

[1]Chemistry, Institute for Neurodegenerative Disorders, New Haven, CT, United States; [2]Psychiatry, Yale University, VAMC, West Haven, CT, United States; [3]Medical School Radiology, Vanderbilt University, Nashville, TN, United States.

Group II metabotropic glutamate receptors (mGluR2/mGluR3) appear uniquely positioned to play a major role in the treatment of neuropsychiatric and neurological disorders.[1] These receptors have attracted considerable attention as novel targets for drug development. As a result, there is an urgent need for selective antagonist-based mGluR radioligands for *in vivo* drug occupancy studies. Such probes would also clarify if the density of group II mGluRs is affected in any psychiatric or neurological disorder, nicotine or opiate addiction, and other pathological conditions.

Recently, 4-(het)aryl-1,3-dihydro-2*H*-1,5-benzodiazepin-2-ones have been identified as high-affinity antagonists at mGluR2s.[2] It was also demonstrated that bulky substituents (including iodo) at positions 7 and 8 of the benzodiazepine system were well tolerated in terms of mGluR2 binding. We, therefore, selected structure **1** as a promising candidate for radioligand development. The compound was synthesized as shown in the Scheme.



Surprisingly, attempts to carry out ^{123}I-iododestannylation of the trimethylstannyl precursor with Na[^{123}I]I and commonly used oxidizers (peracetic acid, H_2O_2, Chloramine-T) resulted in negligible to no yield of the target compound, while the iodide was consumed by side processes yielding several by-products. Use of trifluoroethanol (TFE) as a co-solvent[3] promoted formation of [^{123}I]**1**. Furthermore, decreasing concentration of peracetic acid also appeared to increase yield of the target product. Based on these observations, the desired reaction was accomplished using a weaker oxidizer, *t*-BuOOH, in the presence of phosphoric acid and TFE. Radiolabeling yield was in the range of 20 to 60%. Compound [^{123}I]**1** was readily purified by reverse-phase HPLC, its radiochemical purity exceeded 95%. It should be noted that, to the best of our knowledge, the use of combination of *t*-BuOOH and TFE in radioiodination has never been reported previously.

A preliminary SPECT study in baboon showed that [^{123}I]**1** penetrated the blood-brain barrier and accumulated in the brain. The synthesis, radiolabeling, *in vitro* and *in vivo* evaluation of **1** and related compounds will be presented in detail.

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Keywords: Iododestannylation, mGluR2, SPECT

NORCHLORO-[^{18}F]FLUOROHOMOEPIBATIDIN: PET EVALUATION OF TRACER PROPERTIES IN PIGS

J.T. Patt,[1] W. Deuther-Conrad,[1] P. Brust,[1] M. Patt,[2] O. Sabri,[2] J. Steinbach.[1]
[1]Radiopharmacy, IIF, Leipzig, Germany; [2]Klinik und Poliklinik fur Nuklearmedizin, University Leipzig, Leipzig, Germany.

Introduction: 2-[F-18]fluoro-A-85380 (2-FA) is currently used as radiopharmaceutical for imaging of $\alpha4\beta2$ neuronal nicotinic receptors in the brain. Comparison of the biodistribution of both enantiomers of F-18 labeled norchloro-fluorohomoepibatidine (NCFHEB) (6-β-(2-fluoro-5-pyridinyl)-8-azabicyclo[3.2.1]octane) with 2-[F-18]fluoro-A-85380 in mice revealed superior properties of the enantiomers of NCFHEB. Besides higher brain uptake of both enantiomers of NCFHEB compared to 2-FA, especially (+)-NCFHEB seems to exhibit a faster kinetics in the brain of awake mice (3.7 %ID/g (5 min), 7.5 %ID/g (20 min), 1.7 %ID/g (60 min) for (+)-[F-18]NCFHEB; 4.1 %ID/g (5 min), 4.4 %ID/g (20 min), 2.6 %ID/g (60 min) for (-)-[F]NCFHEB; and 2.3 %ID/g (5 min), 3.2 %ID/g (20 min), 2.3 %ID/g (60 min) for 2-[^{18}F]fluoro-A-85380). After i.v. application of 120 nmol/kg of (-)- or (+)-NCFHEB in mice no toxic effects were observed. We have started the comparison of these tracer compounds by PET studies in pigs.

Experimental: The enantiomers of the radiolabeling precursor NCBrHEB (6-β-(2-bromo-5-pyridinyl)-8-azabicyclo[3.2.1]octane) were separated on a chiral HPLC column with methanol/trimethylamine (0.2 v%)/acetic acid (0.2 v%) as mobile phase. Radiolabeling was performed by reacting a solution of Kryptofix222/potassium carbonate/[F-18]fluoride complex (ca. 10 mg Kryptofix222 and the corresponding amount of potassium carbonate) with 0.3 to 0.7 mg of enantiomerically pure precursor in a microwave (CEM Discover) (90 W, 5 min). After removal of the solvent the residue was dissolved in eluent (NaH_2PO_4 (10 mM) adjusted to pH 4 with HCl (1 M), 5 % ethanol) and injected on a semipreparative HPLC column (Multospher 120 RP 18 AQ, 10 x125 mm). The product peak was collected and (-)-[F-18]NCFHEB injected into a pig (20 kg bodyweight).

Juvenile mixed breed female pigs were anesthetized with 1.0% isoflurane in nitrous oxide and oxygen. A central venous catheters was inserted through a branch of the left and right external jugular veins for radiotracer and drug administration. Catheters were also inserted in both saphenous arteries for withdrawal of arterial blood samples. The plasma was deproteinized by addition of aqueous perchloric acid (30 %,15 µL/ 100 µL of plasma), the precipitate was removed by centrifugation (10000 g, 10 min) and the supernatant injected on a semipreparative HPLC column (Multospher 120 RP 18 AQ, 150 x 10 mm) and analysed (92.5 % aqueous acetic acic (0.5 %),7.5 % acetonitrile). Fractions were counted together with aliquots in the γ counter. Results and discussion: The enantiomers of NCBrHEB were well separated by chiral HPLC, the (-)-enantiomer eluting after 18 min, the (+)-enantiomer after 23 min. However, while the (-)-NCBrHEB eluted as a sharp peak, the peakshape of the (+)-NCBrHEB showed a strong tailing. Therefore, to establish the method the first PET-experiments in a anaesthetized pig were performed with the (-)-enantiomer. Reaction of NCBrHEB in the microwave yielded small (1.5 - 3.5 %) but for these experiments sufficient amounts of radiotracer with low consumption of the enantiomerically pure precursor. Precursor and product were well separated by semipreparative HPLC, the product eluting after 10.5 min. (-)-[F-18]NCFHEB showed a high uptake in the brain displaying the typical distribution of $\alpha4\beta2$ nAChR subtypes in the brain with very high accumulation in the thalamus, high accumulation in the cortex and low activity concentration in the cerebellum. The time activity curve for the thalamus showed a maximum at 115 min. In the deproteinized plasma the amount of unchanged [F-18]NCFHEB was > 40 % at 45 min.

Keywords: nAChR, PET, Homoepibatidine

2α,3β-TROPANE-DERIVATIVES: NEW FLUORINATED LIGANDS FOR THE DOPAMINE TRANSPORTER

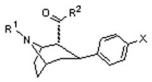
B. Drewes, W. Sihver, H.H. Coenen.
Institut fur Nuklearchemie, Forschungszentrum Julich GmbH, Julich, Germany.

The present study describes the syntheses and first *in vitro* evaluation of new fluorinated tropane derivatives as potential dopamine transporter ligands (DAT ligands, see Figure). Several known DAT ligands were also synthesized for comparative evaluation.

From the present study a compound having an unusual 2α,3β-configuration (2α,3β-CIT-amide **1**) with unexpected high nanomolar affinity for the DAT has emerged. The configuration of **1** was verified by its X-ray structure which will be presented.[1]

Among the new compounds, **1** shows the highest affinity in pig brain both in membrane and autoradiographic *in vitro* studies.

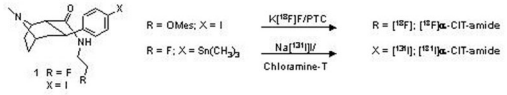
These results and exemplarily *ex vivo* data of the ^{131}I-labeled compound **1** will be presented.



Structure	R^1	R^2	X	No.	Ki [nM]
2α,3β	CH_3	NH-C_2H_4-F	I	1	19 ± 5
2α,3β	CH_3	NH-C_2H_4-F	CH_3	2	374 ± 68
2α,3β	(E)-I-CH=CH-CH_2	NH-C_2H_4-F	CH_3	3	1062 ± 136
2α,3β	(E)-I-CH=CH-CH_2	O-C_2H_4-F	CH_3	4	705 ± 260
2β,3β	CH_3	O-C_2H_4-F	CH_3	5[2]	137 ± 15
2β,3β	F-C_3H_6	CH_3	I	FP-CIT[3]	2.7 ± 0.8
2β,3β	(E)-I-CH=CH-CH_2	CH_3	CH_3	PE2I[4]	7.1 ± 2.7
2β,3β	CH_3	CH_3	I	β-CIT[5]	0.07 ± 0.01

Figure: Synthesized cocaine derivatives; K_i-values versus [^{131}I]β-CIT in pig striatum homogenate

2α,3β-CIT-amide was radiofluorinated and radioiodinated (see Scheme).



Scheme: Radiolabelling of 2α,3β-CIT-amide

Preliminary radiofluorination of the corresponding mesylate of **1** resulted only in poor yields of [^{18}F]**1**. Radioiodination of the corresponding $Sn(CH_3)_3$-precursor leads to a ≥ 80 % radiation yield of [^{131}I]α-CIT-amide.

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Keywords: 2-alpha,3-beta-Tropanes, DAT, Radiohalogenation

IN VITRO AND *IN VIVO* BINDING CHARACTERISTICS OF C-11 LABELED ANTAGONISTS FOR NR2B SUBUNIT OF NMDA RECEPTORS

T. Haradahira,[1] T. Fuchigami,[2] N. Fujimoto,[2] T. Okauchi,[1] J. Maeda,[1] K. Suzuki,[1] T. Suhara,[1] F. Yamamoto,[2] T. Mukai,[2] M. Maeda.[2]

[1]Department of Medical Imaging & Brain Imaging Project, National Institute of Radiological Sciences, Chiba, Japan; [2]Faculty of Pharmaceutical Sciences, Kyusyu University, Fukuoka, Japan.

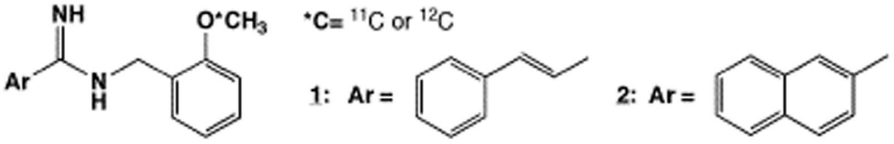
NR2B subunit of NMDA receptors exclusively exist in mammalian forebrains and has been implicated in modulating functions such as learning and memory formation, as well as being involved in a number of human disorders. We have previously synthesized [^{11}C]Methoxy-CP-101,606 as an NR2B-selective PET radioligand (Nucl. Med. Biol., 29, 517, 2002). Although this radioligand showed an extremely high *in vitro* specific binding to the NR2B subunit in rodent brains, it showed no NR2B selectivity after intravenous injection. It was further suggested that NMDA-related ligands, polyamines and divalent cations, might behave as endogenous inhibitors for the specific binding of [^{11}C]Methoxy-CP-101,606 *in vivo*. In this study, we have synthesized two C-11 labeled NR2B-selective antagonists with structurally unrelated to CP-101,606, and evaluated their *in vitro* and *in vivo* binding characteristics including effects of the endogenous ligands.

The amidine analogs (1 and 2) have recently been reported as NR2B-selective NMDA receptor antagonists with extremely high binding affinities (ki of 1 and 2 for [^{3}H]ifenprodil binding is 0.7 nM and 1.3 nM, respectively) (Bioorg. Med. Chem. Lett., 13, 693, 2003). These amidines were easily labeled with carbon-11 by conventional methylation of the corresponding des-methyl precursors with [^{11}C]CH_3I. *In vitro* binding was assessed on rat brain cryosections in 50 mM Tris HCl buffer. *In vivo* brain distribution was assessed by dissections of mouse brain regions (cortex, hippocampus, thalamus, striatum, and cerebellum) after intravenous injection of [^{11}C]amidine.

In vitro bindings of [^{11}C]amidines were the highest in the NR2B-rich forebrains and the lowest in the NR2B-poor cerebellum. Specific bindings in the forebrains were 70-80 % of total bindings, slightly lower than that (95 % of total) of [^{11}C]Methoxy-CP-101,606, and strongly inhibited by NR2B-selective antagonists (CP-101,606, Ifenprodil, 10 μM), indicating high NR2B selectivity *in vitro*. Among the polyamines (spermine, spermidine, putrescine) and divalent cations (Mg, Ca, Zn), spermine and zinc showed the highest inhibition (80% and 70% inhibition of total, respectively) at 1 mM. These binding characteristics were similar to those of [^{11}C]Methoxy-CP-101,606.

In vivo studies in mice showed low initial brain uptakes of radioactivity (0.2-0.3 %dose/g at 1 min). The radioactivities slightly increased with time and became 0.3-0.4 %dose/g for [^{11}C]1 and 0.6-0.7 %dose/g for [^{11}C]2 at 30 min. There was no regional difference in uptake and the ratios to cerebellum were close to unity in any regions, indicating no NR2B selectivity *in vivo*.

The present studies suggest that [^{11}C]amidines bind with the same site as [^{11}C]Methoxy-CP-101,606 on NR2B subunit, in which some polyamines and divalent cations might be highly inhibitory to radioligand binding under physiological conditions. The metabolite and monkey PET studies of [^{11}C]amidines are now in progress.



Keywords: NR2B Subuint, NMDA Receptor, Amidine Analogs

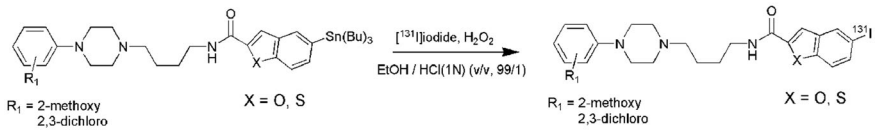
SYNTHESIS AND RADIOIODINATION OF SELECTIVE LIGANDS FOR THE DOPAMINE D3 RECEPTOR SUBTYPE

C. Hocke,[1] S. Lober,[2] H. Hubner,[2] P. Gmeiner,[2] T. Kuwert,[1] O. Prante.[1]
[1]Laboratory of Molecular Imaging, Clinic of Nuclear Medicine, Friedrich-Alexander University, Erlangen, Germany; [2]Department of Medicinal Chemistry, Emil Fischer Center, Friedrich-Alexander University, Erlangen, Germany.

A variety of disorders are believed to be associated with the D3 receptor signaling pathway, including schizophrenia, Parkinson's disease and cocaine craving. Interestingly, the preferential D3 receptor partial agonist BP-897 was found to inhibit cocaine-seeking behavior without revealing any intrinsic, primary rewarding effect. Although several radioligands are available to evaluate the D2 receptor mediated neurotransmitter system by singlephoton emission tomography (SPET), there is still need for a suitable selective radioligand to explore the CNS located dopamine D3 receptors in vivo. Recently, we adopted an interactive drug discovery process leading to the super-potent benzothiophene and benzofuran lead compounds FAUC 346, FAUC 365 and to the pyrazolo[1,5-*a*]pyridine analog FAUC 329, which proved to be protective against MPTP-induced dopamine depletion (1,2). This study aimed at utilizing these type of potent D3 ligands for the development of suitable selective D3 receptor radioligands for SPET.

A series of iodine substituted benzofuran, benzothiophene and thiophene carboxamides containing a dichloro- or methoxyphenylpiperazine moiety was synthesized. Radioiodinated ligands were obtained by iododestannylation reactions using hydrogen peroxide as oxidant (scheme 1). Stannyl precursors were prepared from the corresponding bromo precursors by the use of Pd(0) as the catalyst and hexabutyldistannane via tin-for-bromo exchange reaction. Radio-HPLC was used to determine radiochemical yields (RCY) and to confirm identity of radioiodinated compounds. Receptor binding assays were performed using human D2R, D3R and D4R expressed in CHO-cells and porcine striatal membranes (D1R) with [^{3}H]spiperone and [^{3}H]SCH23390. Affinities to the serotonin receptors 5HT1A, 5HT2, and the adrenergic α1 receptor were evaluated utilizing [^{3}H]8-OH-DPAT, [^{3}H]ketanserin, and [^{3}H]prazosin and porcine cortical membranes, respectively.

The RCYs of radioiodinated benzofuranes and benzothiophenes were about 55% when significant differences in yields between both bicyclic systems could not be observed. The 2,3-



dichlorophenylpiperazinyl substituted derivatives exhibited substantial D3 selectivity when binding only in high nanomolar or micromolar concentrations to D2long, D2short and D4, but showing D3 affinities of 5.7 and 4.5 nM, respectively. In comparison with the methoxy substituted radioligands, the dichlorophenylpiperazine moiety induced clear selectivity for the D3 receptor (560- and 100-fold over D2 and 390- and 120-fold over D4).

In conclusion, highly selective dopamine D3 receptor radioligands were synthesized as potential SPET imaging agents. The 2,3-dichlorophenylpiperazinyl substituted benzofuran derivative displayed promising binding data and stimulate further studies to determine its biodistribution and metabolic stability in vivo.

Acknowledgement

This work is supported by the Deutsche Forschungsgemeinschaft (DFG, grant PR 677/2-2).

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Keywords: Dopamine D3 Receptors, SPET, Radioligand

SYNTHESIS AND EVALUATION OF [^{11}C]EMMP AS A BRAIN NPY-Y1 RECEPTOR IMAGING AGENT FOR POSITRON EMISSION TOMOGRAPHY

M. Ogawa,[1] K. Hatano,[2] J. Abe,[2] K. Ito,[2] Y. Magata.[1]
[1]Photon Medical Research Center, Hamamatsu University School of Medicine, Hamamatsu, Japan; [2]Department of Brain Science and Molecular Imaging, National Institute for Longevity Sciences, Obu, Japan.

Objectives: Neuropeptide Y (NPY) is a 36-amino acid peptide that is widely distributed in the CNS. NPY plays an important role in food intake, cognition and mood disorders (1,2). At present, five NPY receptor subtypes have been cloned. NPY-Y1 receptor is distributed in the cerebral cortex, thalamus and hippocampus. Because this subtype is involved in a variety of NPY-induced pathways, PET imaging of NPY-Y1 receptors is of great interest. Recently, an NPY-Y1 receptor antagonist with high affinity and selectivity, 6-(5-ethyl-1,3-thiazol-2-ylthiomethyl)-2-[3-methoxy-5-(2-propenyloxycarbonylamino)benzylamino]-4-morpholinopyridine (EMMP), has been reported (3). We labeled this ligand with carbon-11, by *O*-methylation of 6-(5-ethyl-1,3-thiazol-2-ylthiomethyl)-2-[3-hydroxy-5-(2-propenyloxycarbonylamino)benzylamino]-4-morpholinopyridine (EHMP) and evaluated its potential for in vivo imaging of NPY-Y1 receptors.

Methods: [^{11}C]EMMP was synthesized by *O*-[^{11}C]methylation reaction using [^{11}C]methyl triflate at 40ºC for 3 min in acetone and purified by reversed-phase HPLC (Fig. 1). The partition coefficient (logD) of [^{11}C]EMMP was measured with octanol and phosphate buffer. Biodistribution studies were undertaken in male ddY mice by tail vein injection of [^{11}C]EMMP. For metabolic study, the mice were injected with [^{11}C]EMMP and the radioactivities in the blood and the brain were extracted with methanol. The extracts were analyzed with TLC. In vitro autoradiographic studies were performed using brain slices of Wistar rats. The blocking effects of non-labelled EMMP and NPY were also investigated.

Results: The overall synthesis time for [^{11}C]EMMP was 36 min after the end of bombardment and the radiochemical yield was 85% (from EOB). The specific radioactivity was over 101 GBq/μmol at the end of synthesis. The log D was 1.82 at pH 7.0, 1.91 at pH 7.4 and 1.99 at pH 8.0, respectively. High radioactivity was initially highly distributed to the liver and kidney but was cleared rapidly from these regions. The radioactivity in the blood rapidly decreased. The uptake to the brain was low (0.18 %ID/g at 5min and 0.15 %ID/g at 15min post-injection) and regional difference was not observed. No metabolite was observed in the brain. In the blood, 86 % of the radioactivity remained intact at 5 min and 76% at 15 min post-injection of [^{11}C]EMMP. The in vitro autoradiogram showed a high non-specific binding of [^{11}C]EMMP. The binding was slightly reduced with non-labeled EMMP, however no effect was observed with NPY.

Discussions: [^{11}C]EMMP was obtained with a high radiochemical yield. Although the lipophilicity was thought to be ideal, the uptake to the brain was low. The relatively high molecular size (M.W. 592) might be one of the causes of the low penetration through the BBB. In conclusion, although EMMP showed a good in vitro affinity to NPY-Y1, [^{11}C]EMMP is not suitable imaging agent for NPY-Y1 receptor because of the low brain uptake and the high non-specific binding.

Acknowledgements: The authors thank Mr. Haga, Banyu Pharmaceutical Co., Ltd. for providing EMMP and EHMP.

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Keywords: NPY Receptor, Carbon-11, Brain

N-[^{18}F]FLUOROETHYLPIPERIDIN-4-YLMETHYL ACETATE, A ^{18}F-LABELLED PROBE ANALOGOUS WITH [^{11}C]MP4A FOR MEASURING CEREBRAL ACETYLCHOLINESTERASE ACTIVITY

T. Kikuchi, S. Obayashi, Y. Nagai, Z. Ming-rong, T. Okamura, K. Suzuki, S. Tanada, K. Fukushi, T. Irie.
Medical Imaging, National Institute of Radiological Sciences, Chiba, Japan.

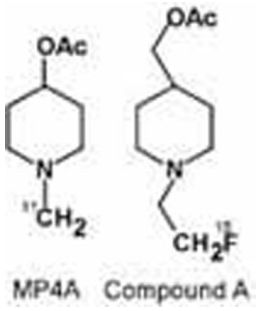
Decrease of acetylcholinesterase (AChE) activity is known to occur in the brain of patients with Alzheimer's disease compared with age-matched controls. For quantitative measurement of brain AChE activity using positron emission tomography (PET), lipophilic acetylcholine analogs, *N*-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]MP4A) and propionate ([^{11}C]MP4P) were developed and applied to PET studies of dementia disorders.^{18}F-labelled analogues of MP4A and MP4P would have advantages such as possibility of long-distance transportation to other PET institutions, due to the longer half-life (^{18}F, 110 min; ^{11}C, 20 min) of ^{18}F compared to ^{11}C.

We had developed a potential ^{18}F-labeled MP4A analogue, *N*-[^{18}F]fluoroethylpiperidin-4ylmethyl acetate (compound **A)** by screening of *N*-alkyl piperidinemethanol esters *in vitro*, and the compound **A** showed high specific reactivity to AChE and the moderate hydrolysis rate comparable to MP4A and MP4P. In this study, the chemical form and the time-activity curves in the rat brain (Wistar, male, 8 wo.) after intravenous injection (1, 5, 15, 30, 60 min) of **A** were evaluated to verify the behavior of the compound as a metabolic-trapping tracer *in vivo*. Furthermore, the compound **A** was evaluated whether the uptake corresponds to regional AChE activity in the brain by the simultaneous measurement of regional AChE activity, blood flow and MP4A uptake.

The chemical form analysis by TLC indicate that almost radioactivity in the brain (> 89%) was composed to compound **A** and the hydrolyzed metabolite in the brain. The fraction of compound **A** was rapidly decreased as time courses, and only 2.9% of radioactivity was remained at 5 min after injection. A small amount of radioactivity observed at the TLC origin is under discussion at present.

The initial uptake (1 min) in the cerebral cortex after intravenous injection of **A** was much higher (2.7% dose/g) than that of MP4A (approx. 1% dose/g). When the hydrolyzed metabolite of **A** was injected, the brain uptake of the radioactivity remained low (0.031-0.053% dose/g) during 1-60 min after injection, indicating that the transfer of the metabolite from blood to brain is limited. After an initial incorporation of **A** in which mainly the radioactivity of metabolite exist, the ^{18}F radioactivity in the brain gradually eliminated with half-lives of approximately 20 min. The elimination rate was 3-times higher than that of metabolite of MP4A, due to the lipophilicity of the metabolite of **A** (log P=-1.3) is higher than that of the metabolite of MP4A (-2.2). Since there is a large species difference between rat and human regarding the elimination from the brain, that is, the elimination rate of the metabolite of MP4A is almost 10-fold slower in human than that in rat. Considering such a species difference, the metabolite of compound **A** formed in the human brain might have sufficiently long residence time for human PET study. Moreover, from the result of the simultaneous measurement, the regional brain uptake of compound **A** correlated well to the regional AChE activity and the MP4A uptake.

We conclude that the novel compound **A** would be applicable to human PET study for measuring cerebral AChE activity by PET. The evaluation of the compound **A** with monkey PET study is in progress.



Keywords: Fluorine-18, Acetylcholinesterase, Alzheimer's Disease

EVALUATION OF A C-11 LABELED CONFORMATIONALLY-FLEXIBLE BENZAMIDE ANALOG AS A DOPAMINE D_3 RECEPTOR RADIOTRACER

Z. Tu,[1] W. Chu,[1] J. Xu,[1] S. Li,[1] L.A. Jones,[1] C.S. Dence,[1] R.R. Luedtke,[2] R.H. Mach.[1]
[1]Radiology Department, Washington University School of Medicine, St. Louis, MO, United States; [2]Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX, United States.

Over the past decade there has been interest in developing agents that can function as agonists, partial agonists, and antagonists of the dopamine D_3 receptor. Receptor autoradiography studies have shown that both D_2 and D_3 receptors are widely distributed in striatal regions of human and nonhuman primate brain.

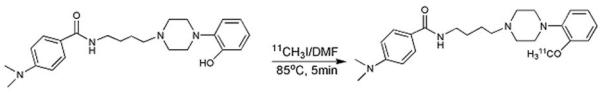
However, the high density of D_3 receptors in limbic regions suggests that this receptor may play an important role in the pathological abnormalities associated with many dopaminergic-based CNS disorders. Autoradiography studies have also revealed a decrease of D_3 receptors in the frontal cortex and an increase in expression in the ventral striatum of schizophrenics compared to normal individuals.[1] Dopamine D_3 receptors are also believed to play a role in the dyskinesias associated with Parkinson's disease.[2] The activation of dopamine D_3 receptors is currently believed to be involved in the sensitization/rewarding properties of psychostimulants, such as cocaine.[3] Therefore, partial agonists or antagonists that can reduce the interaction of psychostimulant-induced increases in synaptic dopamine levels with the D_3 receptor may be useful in the pharmacological treatment of cocaine abuse.

We previously reported that conformationally flexible benzamide, 4-dimethylamino-N-{4-[4-(2-methoxy-phenyl)-piperazin-1-yl]-butyl}-benzamide, **1**, had a high affinity of D_3 receptors (Ki = 0.8 ± 0.1 nM) vs. D_2 receptors (Ki = 34.4 ± 4.7 nM).[4] Functional assays indicate that this compounds is either an antagonist or a weak partial agonist.[4] The results of in vitro study indicate that [^{11}C]**1** could be a useful radiotracer for PET studies of the dopamine D_3 radiotracer.

Initial radiolabeling studies have focused on preparing [^{11}C]**1** labeled at the methoxy position. This was readily achieved via O-alkylation of the des-methyl precursor with [^{11}C]methyl iodide. The labeling yield was ~70% and [^{11}C]**1** was obtained in a specific activity of ~8,000 mCi/μmol.

PET studies were conducted in the same nonhuman primate comparing the uptake of [^{11}C]**1** vs. that of the known D_2 radiotracer, [^{11}C]raclopride. The results of this imaging study indicated that [^{11}C]**1** did initially localize in regions of the brain expressing D_3 receptors. However, the low density of D_3 receptor, high nonspecific binding of [^{11}C]**1**, and rapid wash out from brain prevented the prolonged visualization of D_3 receptors.

Tritiated **1** ([^{3}H]**1**) vitro binding studies indicated that [^{3}H]**1** had rapid K_{on} (0.04 $nM^{-1}min^{-1}$) and K_{off}(0.16 min^{-1}) values. The rapid k_{off} value from the vitro study is consistent with the rapid washout of [^{11}C]**1** from D_3-rich areas observed in the microPET imaging study. These data indicate that [^{11}C]**1** may not be a useful ligand for imaging dopamine D_3 receptors in vivo with PET.



[^{11}C]**1**
D_2 = 34.7 ± 4.7nM
D_3 = 0.8 ± 0.1 nM
D_4 = 29.0 ± 6.2 nM
logD = 3.09

Acknowledgement. The authors would like to thank Jo Ann V. Antenor, John Ohm, Susan Donovan for their excellent technical assistance. This research was supported by NIH grants DA 12647 and DA16181.

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Keywords: Dopamine D3 Receptors, C-11 Labeling, PET

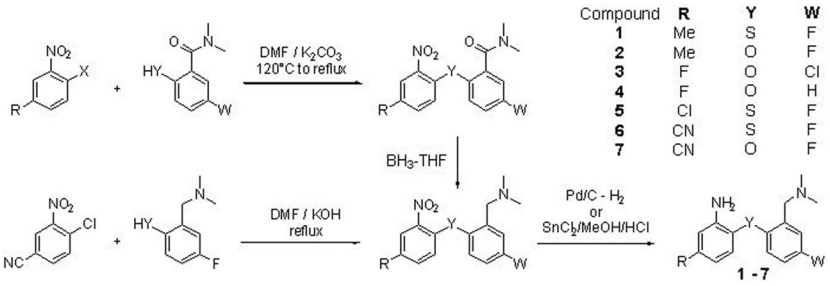
NEW FLUORO-DIPHENYL CHALCOGEN DERIVATIVES TO EXPLORE THE SERT BY PET

J. Vercouillie, W. Deuther-Conrad, M. Scheunemann, P. Brust, J. Steinbach.
Radiopharmaceutical Division, Institute of Interdisciplinary Isotope Research, Leipzig, Germany.

Abnormalities of the serotonin transporter (SERT) are implicated in several brain disorders such as depression, schizophrenia, obsessive-compulsive disorder and Alzheimer's disease. The SERT is also the primary target of antidepressant drugs. Therefore, there has been a considerable interest to image the SERT either by PET or SPECT.

During the last decade various PET and SPECT tracers were developed. All of them have some limitations such as slow kinetics, low brain uptake or high non-specific binding. Recently, several C-11 labelled diphenylsulfides were studied and demonstrated promising properties (good to high brain uptake, low non-specific binding) (1). Nevertheless, there is a lack of a F-18 labelled tracers which allows longer scan duration, full kinetic data analysis and application even outside of a PET centre.

For theses reasons we focused our work on the development of fluorinated aromatic diphenyl chalcogen derivatives to increase the stability of radiotracers and limit the in vivo defluorination (2). We report herein the synthesis and in vitro evaluation of fluoro-diphenylsulfide and fluoro-diphenylether derivatives.



Compound	R	Y	W
1	Me	S	F
2	Me	O	F
3	F	O	Cl
4	F	O	H
5	Cl	S	F
6	CN	S	F
7	CN	O	F

Using tin chloride in methanolic/HCl solution performed the reduction of the nitro functions to get compound **3**. When using the hydrogenation method, with palladium/charcoal as catalyst, hydrodechlorination was observed and afforded derivative **4**. All compounds were assayed for their SERT affinity using rat cerebral cortex. For competitive binding assay experiments, the membrane fraction of rat cortex was diluted with incubation buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl; pH 7.4 at room temperature). It was added to glass tubes containing [H-3]citalopram as specific SERT ligand and different concentrations of the test compounds. The Cheng-Prussof equation was applied to calculate Ki values from the estimated IC_{50} values. Ki values (mean ± SD) are the average of 3 determinations.

The compounds were prepared in good yields and characterised by NMR and mass spectrum. All of the synthesised compounds display high SERT affinities in a nanomolar to subnanomolar range (Ki: 0.11 ± 0.054 nM; 1.32 ± 0.05 nM; 1.91 ± 0.30 nM respectively for derivatives **1, 2** and **3**).

The in vitro evaluation demonstrated that all fluoro derivatives present high SERT affinity. The syntheses of the correspondent F-18 precursors are in process and they will be soon radiolabelled and evaluated to determine if they can be suitable F-18 radiotracers to image the SERT in vivo by PET.

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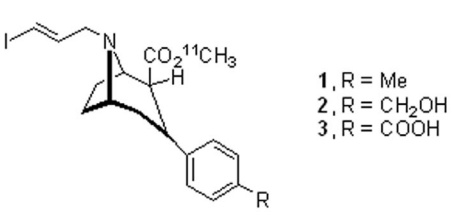
Keywords: Serotonin Transporter, Diphenyl-(thio-)ether Derivatluorineives, Fluorine-18

THE DOPAMINE TRANSPORTER PROBE [^{11}C]PE2I SHOWS ACTIVE AND INACTIVE RADIOACTIVE METABOLITES IN RAT BRAIN

S.S. Zoghbi,[1] U.H. Shetty,[1] J.-S. Liow,[1] M. Ichise,[1] J. Hong,[1] J.L. Musachio,[1] N. Seneca,[1] C. Halldin,[2] J.L. Seidel,[3] V.W. Pike,[1] R.B. Innis.[1]
[1]MIB, NIMH, Bethesda, MD, United States; [2]Clinical Neuroscience, Karolinska University Hospital, Stockholm, Sweden; [3]Nuclear Medicine, NIH, Bethesda, MD, United States.

Introduction*:* [^{11}C]PE2I (*N*-(3-iodoprop-2*E*-enyl)-2ß-carbomethoxy-3ß-(4′-methylphenyl)nortropane) (**1**) is a PET radioligand for the dopamine transporter (DAT) giving high ratios of specific to nonspecific uptake in human and nonhuman primate brain (1). This radioligand might also be useful for imaging in rodents. However, the metabolism of xenobiotics is often more rapid and diverse in rodents than in primates. Any radiometabolites of **1** that may enter rodent brain might confound the imaging results. The aims of this study were to quantify the parent radioligand **1** and any radiometabolites that enter rat brain *ex vivo,* and to measure their respective concentrations in receptor-rich or receptor-poor brain regions.



Methods: Three Sprague-Dawley rats (304 ± 75 g) were used. One was injected (bolus i.v.) with **1** (940 µCi; mean specific activity: 1900 mCi/µmol; radiochemical purity: 99%) resulting in a brain DAT occupancy of < 5%. Subsequent imaging data were acquired on an Advanced Technology Laboratory Animal Scanner (ATLAS) up to 110 min after injection. Two rats were injected (bolus i.v.) with 3.5 or 4.5 mCi of either no-carrier-added **1 or 1** plus carrier (150 µg) under 1.5% isoflurane anesthesia. After 30 min the plasma and brains of the latter two rats were removed, excised and dissected into regions and placed in acetonitrile. Tissues were counted in a calibrated automatic γ-counter. Then tissues were homogenized with acetonitrile and analyzed by reverse phase HPLC. In the carrier-added experiment, radiochromatographic peaks corresponding to the radiometabolites and **1** were collected separately and analyzed by LC-MS and MS-MS.

Results: At 30 min after injection of **1** into rat there was a sizeable ratio (8) of radioactivity in striatum to cerebellum at 30 min. However, at this time two radiometabolites were detected in the striatum, cortex, frontal cortex and cerebellum, while **1** was 93, 74, 81 and 74% of the total radioactivity, respectively. In the same tissues, the intermediate radiometabolite was a constant 4%, while the polar radiometabolite was the remainder (3, 22, 15 and 23% in the above tissues, respectively). The plasma was 17% parent, 1% intermediate and 81% polar radiometabolite. Normalizing brain tissue radioactivity compositions for their weights, revealed that the intermediate radiometabolite had a distribution consistent with high affinity binding to DAT. LC-MS identified the bioactive radiometabolite as the benzyl alcohol (**2**) and the polar radiometabolite as the benzoic acid (**3**).

Conclusions: Analysis of rat brain after i.v. administration of **1** showed that the radioactivity in brain consisted of parent radioligand (**1**) plus two radiometabolites (**2 and 3**). The concentration of the polar radiometabolite (**3**) was equal in the various brain sections and hence **3** may be considered an 'inactive' metabolite (*i.e.* one that does not bind avidly to DAT). However, the intermediate radiometabolite (**2**) showed affinity to striatal DAT with its own nonspecific binding to other brain tissues. Hence, **3** may be considered an 'active' metabolite. Therefore, the rat striatum was contaminated with both active and inactive radiometabolites of **1**.

Reference:

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Keywords: [^{11}C]PE2I, Dopamine Transporter, Metabolism

SYNTHESIS AND *IN VIVO* EVALUATION OF [^{11}C]SIB-1553A, FOR β-4

SUBTYPE NICOTINIC ACETYLCHOLINE RECEPTOR IMAGING BY POSITRON EMISSION TOMOGRAPHY

F. Sobrio, D. Debruyne, M. Dhilly, T. Quentin, S. Tymciu, L. Barre.
CEA/DSV - UMR CEA FRE CNRS 2698, Centre Cyceron, Caen, France.

Neuronal nicotinic acetylcholine receptors (nAChRs) are involved in neurodegenerative disorders, nicotine addiction, cognitive functions and control of pain[1]. (±)-4-[2-(1-Methyl-2-pyrrolidinyl)ethyl]-thiophenol hydrochloride (SIB-1553A) is a neuronal nicotinic acetylcholine receptor (nAChR) agonist[2]. *In vitro*, SIB-1553A, in HEK cell lines expressing recombinant human nAChRs, increased calcium responses in a sub-type selective manner and was more potent on human β4 subunit[3]. *In vivo*, SIB-1553A stimulated the release of acetylcholine in both prefrontal cortex and hippocampus in young adult rats and improved both attention and memory performance in non-human primates[4]. In clinical studies, SIB-1553A was presented as a potential therapeutic agent to treat cognitive dysfunction[5], especially in Alzheimer disease[6].

In order to develop a radioligand selective for β4-subunit containing nAChRs for *in vivo* PET studies, we have developed the radiosynthesis of [^{11}C]-SIB-1553A, and evaluated its *in vivo* pharmacological properties in rat.

The labelling was achieved by *N*-methylation reaction using [^{11}C]-CH_3I (Figure 1). The desmethyl precursor was prepared within 5 steps with overall yields of 45-56% from *N*-Boc-prolinal. N-[^{11}C]-Methylation occurred in DMF without any base at 25°C with a radiochemical yield about 75%. The radiosynthesis was achieved into 32 min (EOB) leading to 2.6-4.8 GBq of [^{11}C]-SIB-1553A. Radiochemical purity was higher than 97% and the specific radioactivity ranged from 7.5 to 30 GBq/μmol (EOS).

Results for peripheral biodistribution are reported in Figure 2. The rat brain radiotracer uptake reached a maximum value of 0.49 % I.D./g at 10 min and decreased slowly to 0.25 % I.D./g at 40 min. The radiometabolite TLC analysis achieved on plasma samples at 30 min showed about 72 % of radioactive degradation products but the TLC analysis from brain homogenates attested that the entire radioactivity present in brain was due to the parent compound.

The cerebral distribution in rat brain of [^{11}C]-SIB 1553A was studied over 18 structures by autoradiography at 15 min and 30 min after tracer i.v. injection with or without pre-treatment with SIB-1553A. Although [^{11}C]-SIB-1553A was properly synthesized and rapidly crossed the blood brain barrier without undisturbing metabolites, the regular cerebral distribution and the unquantifiable specific binding observed in the rat model could not incite further development as a PET radiotracer for nAChRs investigation in non human primate or human.

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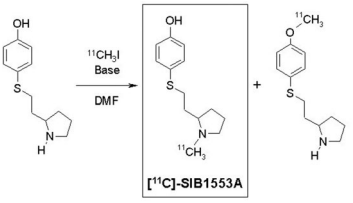

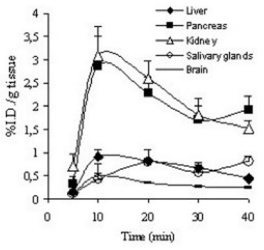

Keywords: Carbon-11, SIB-1553A, Nicotinic Acetylcholine Receptor

SYNTHESIS, LABELLING AND EVALUATION OF 4,6-DICHLORO-3-((3-(4-(2-FLUOROETHOXY)PHENYL)2,4-DIOXOIMIDAZOLIDINE)-1*H*-INDOLE-2-CARBOXYLIC ACID AS RADIOTRACER FOR IMAGING THE NMDA-RECEPTOR STATUS *IN VIVO*

A. Bauman,[1] M. Piel,[1] S. Hoehnemann,[1] M. Jennewein,[1] M. Jansen,[2] A. Krauss,[2] U. Schmitt,[3] G. Dannhardt,[2] F. Roesch.[1]

[1]Institut fur Kernchemie, Johannes Gutenberg - Universitat, Mainz, Germany; [2]Institut fur Pharmazie, Johannes Gutenberg - Universitat, Mainz, Germany; [3]Psychiatrische Klinik und Poliklinik, Johannes Gutenberg - Universitat, Mainz, Germany.

Aim:

L-Glutamate is the most important and a widely represented excitatory neurotransmitter in the central nervous system (CNS). It is the endogenous ligand for the N-methyl-D-aspartat (NMDA) receptor. This receptor is together with the AMPA- and Kainate subtypes, part of ionotropic receptors which belong to the family of glutamate receptors. The NMDA-receptor complex plays a great role in long time and learning processes and is also involved in many neurological diseases, such as morbus Parkinson and morbus Alzheimer. For investigating these neurological disorders in early diagnosis, the imaging of the NMDA-receptor status could be very helpful.

Methods:

For imaging the NMDA-receptor status with Fluorine-18 labelled tracers and PET, the non-radioactive reference compound and the precursors were synthesized following a modified pathway, reported in literature (1). For the non-radioactive compound the IC_{50} value for the inhibition of [^{3}H]MDL-105,519 was determined. The radiolabelling was performed using 2-[^{18}F]fluoroethyl tosylate ([^{18}F]FETos) and [^{18}F]FETos/LiI, respectively (2). Purification and analysis was performed using radio-HPLC and radio-DC.

Results:

The non-radioactive reference compound and the precursors for [^{18}F]fluoroethylation were synthesized successfully. The IC_{50} value of 4,6-dichloro-3-((3-(4-(2-fluoroethoxy)phenyl)2,4-dioxoimidazolidine)-1*H*-indole-2-carboxylic acid **2a** was 54 nM. The radiosynthesis of **2b** could be realized in 7 % respectively 21 % yield by labelling the precursor **1b** with [^{18}F]FETos and [^{18}F]FETos/LiI in DMF at 100 °C and 5N NaOH as base. Due to the higher alkylating properties of *in situ* generated 2-iodo-1-[^{18}F]fluoroethane ([^{18}F]IFE) the radiochemical yields were significantly increased. Following this synthetic strategy, high amounts of a not further characterized sideproduct was detected, which probably is the radiolabelled carboxylic acid. Higher radiochemical yields of about 35 % were achieved by labelling the precursor **1a** with [^{18}F]FETos and NaOH as base. The labelling reaction has to be followed by the cleavage of the protecting ester group. Using a 1N NaOH solution this cleavage was performed quantitatively after a reaction time of 20 minutes. First *in vivo* animal studies with Sprague Dawley rats showed a dynamic accumulation of activity in the hypocampus.

Conclusions:

In summary, a novel NMDA ligand was developed and labelled with fluorine-18. *In vitro* the IC_{50} value was determined to be 54 nM. First *in vivo* studies using small animal PET in rats showed that the ligand permeates the blood-brain-barrier and visualized the hypocampus. In further experiments radiochemical yields will be optimized and detailed *in vivo* studies will be performed.

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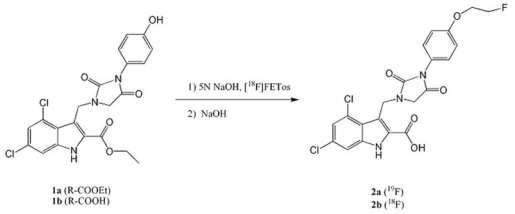

Keywords: PET, NMDA, [^{18}F]FETos

FLUORINE-18 LABELED BENZAZEPINES FOR IMAGING DOPAMINE D1 RECEPTORS

C.S. Wang, D. Collins, J. Mukherjee.
Psychiatry and Human Behavior, University of California, Irvine, Irvine, CA, United States.

Objectives: Dopamine D1 receptors are present in high concentrations in various parts of the brain and are implicated in working memory and drug addiction. We have reported high affinity fluorinated derivatives of SCH 38548 (Fig-1a). The most potent *N*-(4-^{18}F-fluorobenzoyl)SCH 38548 (Ki = 0.55 nM; Fig-1b) showed rapid breakdown *in vivo* whereas the potential utility of *N*-(3-^{18}F-fluoropropyl)SCH 38548 (^{18}F-Profazepine, Ki = 4.8 nM; Fig-1c) for imaging D1 receptors was demonstrated. However, the difficult radiosynthesis has precluded further studies with this radiotracer. Our goals are two-fold (1). Optimize radiosynthesis of ^{18}F-profazepine for *in vitro* and *in vivo* studies, and (2). Develop radiosynthesis for the new tracer, *N*-(4-^{18}F-fluorobenzyl)SCH 38548 (Ki = 1.53 nM; Fig-1d) which has a higher affinity than profazepine and is likely to be metabolically stable *in vivo*.

Methods: The radiosynthesis of ^{18}F-profazepine was carried out by reacting SCH 38548 with either ^{18}F-fluoropropyl iodide or ^{18}F-fluoropropyl tosylate, K_2CO_3 in DMF at 110-120 °C. Alternatively, reaction with ^{18}F-fluoropropyl tosylate in DMF in the absence of base at 100-110 °C, reaction of ^{18}F-fluoropropyl tosylate in HMPA at 160 °C for 30-60 mins in the presence $NaHCO_3$ or K_2CO_3 was carried out. SCH 38548 was reacted with $BrCH_2CH_2COCl$ in acetone at 50 °C to give ($BrCH_2CH_2CO$)-SCH 38548 (Fig-1e). Attempts were made to protect the phenol group using a tetrahydropyranyl ether or using a BOC. Direct radiolabeling on ($BrCH_2CH_2CO$)-SCH 38548 using ^{18}F-fluoride in CH_3CN, 96 °C, 15 min was also carried out. ^{18}F-Fluorobenzylation of SCH 38548 done by first preparing ^{18}F-fluorobenzaldehyde (4-nitrobenzaldehyde, ^{18}F-fluoride, DMSO, 126 °C, 30 mins) followed by reductive coupling with SCH 38548 (methanol-acetic acid, mol. sieves, 80 °C, 30 mins; $NaCNBH_3$, 60 °C, 15 min).

Results: ^{18}F-profazepine was synthesized in very low radiochemical yields using SCH 38548 with ^{18}F-fluoropropyl iodide in the presence of K_2CO_3 in DMF at 110-120 °C. The presence of side-products (*O*-^{18}F-fluoroalkylation and other side products) made HPLC purification extremely difficult. In the absence of base or using a weak base, the reaction was not successful either in DMF or HMPA at high temperatures. Bromopropionyl SCH 38548 (Fig-1e) was efficiently synthesized in high yields (>80%). Direct radiolabeling of this precursor did not provide the desired product due to potential interference by the free phenol group. Our attempts to protect the phenol group with either a tetrahydropyranyl ether or a *O*-BOC have not been successful due to the high reactivity of the bromine to the reaction conditons, and is easily displaced in the presence of mild acids/bases. Radiosynthesis of 4-^{18}F-fluorobenzaldehyde proceeded smoothly and was coupled to SCH 38548. Radiochemical yields of *N*-(4-^{18}F-fluorobenzyl)SCH 38548 were <2% with low apparent specific activity due to contamination of the nitro precursor.

Conclusion: We have prepared ^{18}F-profazepine and a new fluorine-18 benzazepine, *N*-(4-^{18}F-fluorobenzyl)SCH 38548 in low radiochemical yields (<2%). The poor basicity of the "aniline nitrogen" in SCH 38548 and the presence of a phenolic group makes ^{18}F-fluoroalkylation methods challenging. Thus, an appropriate precursor containing the "functionalized propyl group" is essential for ^{18}F-profazepine. Reaction conditions are being optimized for the radiosynthesis of *N*-(4-^{18}F-fluorobenzyl)SCH 38548.

Keywords: Dopamine D1 Receptors, Fluorine-18 Benzazepines, PET

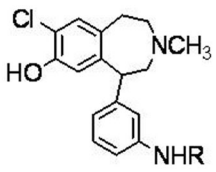

Fig-1a: R= H; **SCH 38548**
Fig-1b: R= $COC_6H_4{}^{18}F$
Fig-1c: R= $CH_2CH_2CH_2{}^{18}F$; **Profazepine**
Fig-1d: R= $CH_2C_6H_4{}^{18}F$
Fig-1e: R= $COCH_2CH_2Br$

RADIOCHEMICAL SYNTHESIS OF OCH_2F-[^{18}F]MPPF A NEW ANALOGUE OF p-[^{18}F]MPPF FOR THE STUDY OF 5-HT_{1A} RECEPTORS

C. Defraiteur, C. Lemaire, A. Luxen, A. Plenevaux.
Cyclotron Research Center, Liege University, Liege, Belgium.

Many studies, related to the serotonergic neurotransmission system, were conducted. Serotonin (5-HT) is a central neurotransmitter and neuromodulator involved in many physiological functions and pathological disorders. The 5-HT_{1A} receptors are of particular interest as they may be involved in various physiological processes. A new generation of specific ligands for the 5-HT_{1A} receptors is currently under consideration. We have set out to investigate the fluoromethoxy-MPPF identified as OCH_2F-MPPF, a new analogue of the 5-HT_{1A} antagonist MPPF (1), for the study of 5-HT_{1A} receptors.

Results: the reference compounds OCH_2F-MPPF and OCH_2F-$MPPNO_2$ were obtained through alkylation of the desmethylated p-MPPF and of its nitro derivative with CH_2BrF (Scheme 1). The pure compounds were isolated with yields ranging around 50-70 % and were fully characterized (^{1}H and ^{13}C NMR, LC-MS, HPLC).

No-carrier-added (n.c.a.) [^{18}F]F^- was classically produced via the ^{18}O(p,n)^{18}F reaction on enriched [^{18}O]water. At the end of the bombardement, activity was transferred with nitrogen pressure through 30 m of teflon tubing to the laboratory. Activity was trapped and the enriched water was recovered. Fluorine-18 was then eluted from the column with 500 µL of a CH_3CN/H_2O solution containing Kryptofix 222 (660 mg) and potassium carbonate (220 mg). The water was evaporated under a steam of nitrogen at 110°C and coevaporated to dryness with acetonitrile (3 × 100 µL). The dried fluorinating agent [K/222]$^+$[^{18}F]$^-$ complex was then used.

The radiolabeling of OCH_2F-$MPPNO_2$ consisted of the nucleophilic substitution of the nitro group with [^{18}F]F^-. Typically, the nitro derivative (10 mg) dissolved in DMSO (500 µL) was allowed to react with activated [K/222]$^+$[^{18}F]$^-$ for 2 min (time recorded from DMSO boiling). The raw OCH_2F-[^{18}F]MPPF was then extracted and roughly purified with SPE using a tC18 Sep-Pak™. The raw compound was then eluted (with 1.5 mL of a methanol/THF solution (50/50)). The OCH_2F-[^{18}F]MPPF was purified with the help of preparative HPLC (XTerra Prep RP18 10 x 250 mm 10 µm, 5 mL/min, 50 mM NH_4Ac pH 6.6: 50% – THF: 19% – MeOH: 31%) and the final compound was obtained with an overall radiochemical yields (from [^{18}F]fluoride) of about 10 % (n = 3) corrected to EOB within 60 min. Further optimizations are underway.

Conclusions: the new compounds OCH_2F-MPPF and OCH_2F-$MPPNO_2$ were isolated and characterized. The radiochemical pathway starting from [^{18}F]F^-, leads to OCH_2F-[^{18}F]MPPF ready for injection. This new radiopharmaceutical can now be evaluated in animals.

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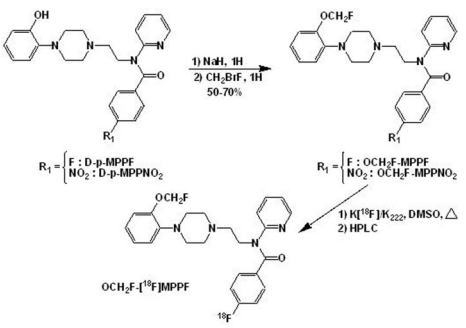
Scheme 1



Keywords: 5-HT_{1A} Receptors, ^{18}F Radioligand, p-MPPF

SUITABILITY OF A RAT ANIMAL MODEL OF CHOLINERGIC DEFICIENCY FOR THE EVALUATION OF NEW RADIOLIGANDS FOR THE VESICULAR ACETYLCHOLINE TRANSPORTER (VACHT) AS REVEALED BY IMMUNOCYTOCHEMISTRY

U. Grossmann,[1] W. Hartig,[2] D. Sorger,[1] E. Kouznetsova,[2] R. Schliebs,[2] M. Scheunemann,[3] J. Steinbach,[3] O. Sabri.[1]

[1]Department of Nuclear Medicine, University of Leipzig, Leipzig, Saxonia, Germany; [2]Paul Flechsig Institute of Brain Research, University of Leipzig, Leipzig, Saxonia, Germany; [3]Institute of Interdisciplinary Isotope Research, Leipzig, Leipzig, Saxonia, Germany.

The basal forebrain cholinergic system is known to play an important role in normal cognitive function, and cortical cholinergic dysfunction has been implicated in cognitive deficits that occur in Alzheimer's disease and other demential disorders. Massive loss of basal forebrain cholinergic neurons was demonstrated by reductions in number of cholinergic markers such as choline acetyltransferase, muscarinic and nicotinic acetylcholine receptor binding as well as levels of acetylcholine.

Early diagnosis of brain cholinergic deficits by imaging techniques is important for designing effective treatment strategies. The vesicular acetylcholine transporter (VAChT) specifically localized in the membrane of vesicles of acetylcholine storage and transport is a possible target molecule for vesamicol derived radioligands to in vivo image these deficits by Nuclearmedicine methods like SPET and PET.

Aim:

To evaluate novel fluorine-18 labelled octahydro-benzo[1,4]oxazine derivatives of vesamicol for their sensitivity to detect cholinergic dysfunction an animal model of a specific cholinergic lesion in the rat basal forebrain is used. It is the aim of this study to validate immunhistochemically that a quantitative reduction of VAChT occurs following a single dose of intracerebroventricular applicated cholinergic immunotoxin to degenerate cholinergic terminals in cholinoceptive target regions.

Methods:

The immunocytochemical labelling of VAChT was performed with free-floating, coronal frozen sections from perfused paraformaldehyde-fixed rat brains 6 weeks after stereotaxic injections of 2 μg 192 IgG-saporin into both the left and right ventricle. Controls received a saline injection. VAChT immunoreactivity was visualized both by immunoperoxidase and carbocyanine immunofluorescence staining, respectively, based on commercially available antisera (rabbit anti VAChT (rat), Weihe et al. 1996).

Results:

Following immunolesion a drastic loss of VAChT-immunoreactivity in the magnocellular basal forebrain nuclei such as in the medial septum/diagonal band complex was observed. In parallel, the vast majority of VAChT immunopositive fibres projecting to neocortex and hippocampus was apparently eliminated by the immunotoxin. The VAChT immunoreactivity in the cholinergic striatal interneurons was obviously not affected.

Conclusion:

The cortical reduction of the VAChT was immunocytochemically demonstrated in the rat animal model. It was directly shown that the VAChT represents a potential target molecule to reflect cholinergic deficits in brain. The animal model of cholinergic immunolesion is an appropriate tool to test novel radiolabelled analogues of vesamicol for their sensitivity to detect cholinergic reductions.

Acknowledgements

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Keywords: VAChT, Immunocytochemistry, Rat Animal Model

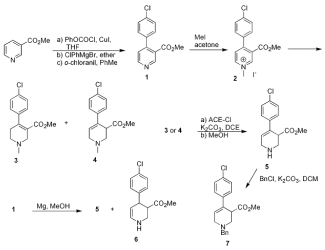
IDENTIFICATION OF A NOVEL TETRAHYDROPYRIDINE PHARMACOPHORE FOR DESIGN OF SELECTIVE PROBES FOR IMAGING OF THE SEROTONIN TRANSPORTERS

M.P. Smith, D.F. Keith, F.J. Femia, J.W. Babich.
R & D, Molecular Insight Pharmaceuticals Inc., Cambridge, MA, United States.

We describe the identification of a tetrahydropyridine pharmacophore for the design of monoamine transporter ligands with selectivity for the serotonin transporter. These ligands were designed with an eye for the development of novel ligands that can readily be radiolabeled with positron or single photon emitting elements. Towards this end all of the compounds incorporated a halophenyl substituent that could be radiohalogenated, or a *N*-methyl that can be labeled with ^{11}C-methyl. These ligands will enable PET or SPECT imaging of neurological and psychiatric disorders related to serotonergic dysfunction.

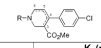
The synthetic route employed in this study is shown in Scheme 1 below. This synthesis involves the two step arylation of methyl nicotinate to afford **1**.[1] Methylation, followed by reduction of the pyridinium salt **2** afforded a 1:1 mixture of the 3,4-olefin **3** and the 4,5-olefin **4**. The demethylation of either **3 or 4** afforded **5**. This product could be converted to the corresponding *N*-benzyl analog **7**. Additionally, the direct reduction of pyridine **1,** using magnesium in methanol afforded a mixture of **4** and the 5,6-olefin **6**. All of these compounds were fully characterized and screened for their affinity at the monoamine transporters.

Scheme 1: Synthesis of Tetrahydropyridines



The compounds prepared were examined to determine their affinity for the monoamine transporters (shown in Table 1 above). This preliminary investigation into a novel pharmacophore, the 4-aryl-tetrahydronicotinates, represents an extension of our work using the 4-arylpiperidines.[2] The 1,2,3,6-tetrahydropyridines (**4 and 5**) exhibit significant affinity and selectivity for the SERT. The comparison of this series with the 4-arylpiperidines[3] shows the structure activity relationships developed in that series can be extended to this tetrahydronicotinate pharmacophore. Examining the effect of nitrogen substitution demonstrates that the removal of the methyl group in **4** to afford **5**, results in an increase in the SERT affinity with a decrease in the DAT and NET affinity, leading to improved affinity and selectivity for the SERT. Additionally, the introduction of a more bulky substituent, such as the benzyl results in a decrease in the affinities at all three monoamine transporters with this increase being smaller for the DAT. It is important to note that the **4**, and **5** are racemic compounds and no efforts were made to separate the enantiomers. As is noted for the 4-arylpiperidines and serotonin selective reuptake inhibitors such as paroxetine[4] a significant enantioselectivity is noted in the affinity of these ligands at the monoamine transporters. Based on this, it is anticipated that the 3-S enantiomer (corresponding to the configuration of paroxetine) would exhibit significantly increased affinity and selectivity for the SERT.

Table 1: In vitro Pharmacology of Tetrahydorpyridine Pharmacophores.



			K_i (nM)		
	R	Δ	DAT	SERT	NET
3	Me	3,4	961	389	4136
4	Me	4,5	283	20.5	3699
5	H	4,5	479	13.7	3221
6	H	5,6	704	1865	4389
7	Bn	4,5	450	4698	8676

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Keywords: Monoamine Transporter Ligands, Serotonin Selective, Tetrahydropyridines

SYNTHESIS OF 5-[^{11}C]ETHYL-A85380 ([^{11}C]5EA) AS A BRAIN NICOTINIC ACETYLCHOLINE RECEPTOR IMAGING AGENT FOR POSITRON EMISSION TOMOGRAPHY

M. Ogawa,[1] S. Nishiyama,[2] H. Tsukada,[2] Y. Iida,[3,4] H. Saji,[3] Y. Magata.[1]
[1]Photon Medical Research Center, Hamamatsu University School of Medicine, Hamamatsu, Japan; [2]Central Research Laboratory, Hamamatsu Photonics K.K., Hamamatsu, Japan; [3]Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan; [4]School of Medicine, Gunma University, Maebashi, Japan.

Objectives: Nicotinic acetylcholine receptors (nAChRs) in the CNS have been implicated in a variety of brain functions and behavioral states, including learning, memory, attention and anxiety [1]. Visualization of nAChRs in human brain has thus been of great interest for the evaluation of brain functions and diagnosis of neurodegenerative diseases. We previously synthesized the C-11 labeled ligand for $\alpha_4\beta_2$ nAChR subtype, 5-[^{11}C]methyl-A85380 ([^{11}C]5MA) and evaluated its potential for in vivo imaging agent by monkey PET study [2]. Although the nAChR was clearly imaged with [^{11}C]5MA, the brain uptake was not sufficient because of its low lipophilicity. Therefore, in this study, we tried to synthesize the [^{11}C]ethylated derivative, 5-[^{11}C]ethyl-3-(2-(S)-azetidinylmethoxy)pyridine, (5-[^{11}C]ethyl-A85380, [^{11}C]5EA), expecting the improvement of blood-brain barrier permeability by elevating the lipophilicity.

Methods: The affinity of 5EA for central nAChRs was evaluated by receptor binding study using [^{3}H]cytisine. [^{11}C]5EA was synthesized by the incorporation of [^{11}C]ethyl moiety into 5-(Tri-n-butylstannyl)-3-((S)-1-(tert-butoxycarbonyl)-2-azetidinylmethoxy) pyridine, using a Pd-catalyzed coupling reaction. [^{11}C]Ethyl iodide was prepared by a three-step reaction, that is, the Grignard reaction, reduction and iodination, according to previously reported method [3]. The ethylation was carried out by two-step reaction. At first, the obtained [^{11}C]ethyl iodide was bubbled into tris(dibenzylideneacetone)dipalladium and Tri-o-tolylphosphine containing DMF to form complex. Then the [^{11}C]ethylation was accomplished by treating this complex with butylstannyl precursor, CuCl and K_2CO_3. Cleavage of the tert-Boc protection was achieved by treating with hydrochloric acid. The labeled product was purified and analyzed by the reverse-phase HPLC system. The reaction was investigated under low (25ºC) and high temperatures (80ºC).

Results: In vitro receptor binding assays demonstrated that 5EA has a high binding affinity for nAChRs (Ki = 0.38nM), being equivalent to 5MA (Ki = 0.27 nM). However, the radiochemical yield of [^{11}C]5EA was considerably low, 0% for the high temperature and 0.1% for the low temperature reaction. More than 90% radioactivities were volatile and were lost away during the purification. In the high temperature condition, the rather low boiling point compound which can be easily volatilized at room temperature comprised 70% of the total volatile radioactivity. On the other hand, in the low temperature condition, this component was only 7%. In this condition, most of the volatile radioactivity has higher boiling point and it was non-reacted [^{11}C]ethyl iodide.

Discussions: The rather volatile product in the high temperature condition was considered to be [^{11}C]ethylene which was produced through b-elimination from the [^{11}C]palladium-complex. This b-elimination hardly occurred in the low temperature condition, however, the [^{11}C]palladium-complex could not be generated sufficiently and lead to the low yield of [^{11}C]5EA. Other labeling method should be explored such as stabilization of [^{11}C]palladium-complex by changing the phosphate ligand.

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Keywords: Nicotinic Acethylcholine Receptor, 5-[^{11}C]ethyl-A85380, Pd-Catalyzed Coupling Reaction