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Identification of positron emission tomography ligands for NPY Y5 receptors in the brain

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ABSTRACT

A series of *trans*-3-oxospiro[(aza)isobenzofuran-1(3*H*),1′-cyclohexane]-4′-carboxamide derivatives were synthesized and profiled for NPY Y5 binding affinity, brain and CSF penetrability in rats, and susceptibility to human and mouse P-glycoprotein transporters in order to develop a PET ligand. Compound **12b** exhibited an acceptable profile for a PET ligand, and [^{11}C]**12b** was successfully utilized in clinical settings as a Y5 PET ligand.

Neuropeptide Y (NPY) is a highly conserved C-terminus amidated peptide consisting of 36 amino acid residues which has been shown to have potent, centrally mediated orexigenic effects.[1–4] At least six receptor subtypes of the NPY family have been characterized based on cloning and/or their pharmacological properties.[5–19] Various pharmacological studies, employing receptor deficient mice and/or subtype-selective agonists and antagonists, have suggested that the Y1 and Y5 receptors are involved in body weight regulation.[14,16,20–32] In diet-induced obese (DIO) mice, treatment with Y5 antagonists reduced body weight in a fat mass-selective manner, implying that Y5 receptor antagonists are useful for the treatment of obesity. Ex vivo receptor occupancy data showed that long-term (>15 h) Y5 receptor occupancy (>90%) is required for efficacy in the DIO mouse model.[32] We developed potent and orally bioavailable Y5 receptor antagonists,[33–37] some of which advanced to clinical evaluation. In order to support clinical proof-of-concept studies, it would be useful to develop a method for assessing Y5 receptor occupancy in the human brain.

Positron emission tomography (PET) is a noninvasive clinical and research imaging technique that is recognized as a powerful tool for establishing clinical dosages of CNS drugs. Specifically designed radiotracers with appropriate physical properties as well as high affinity and selectivity for targeted receptors can be used to obtain tomography images. In vivo competition studies using PET tracers and unlabeled drug candidates are performed to determine clinical dosages required to achieve targeted blockade of the receptor (i.e., occupancy). Studies of this type are done using radiotracers labeled with short-lived positron-emitting radionuclides, such as ^{11}C or ^{18}F ($t_{1/2}$ = 20.4 min and 109.8 min, respectively). The desirable properties for a candidate Y5 radioligand at least include (1) high binding affinity for the Y5 receptor and selectivity versus other NPY receptor subtypes; (2) acceptable lipophilicity generally falling in the log $D_{7.4}$ range of 1–3 for good brain penetration and low nonspecific binding; and (3) low susceptibility to P-glycoprotein (P-gp) transporters.

The signal to background window for a PET ligand is approximated by the ratio of B_{max}/K_d, and a B_{max}/K_d ratio of more than 10 is generally required. A relatively low concentration of the Y5 receptor was presumed in the brain,[38] suggesting that acceptable ligands should have high affinity of at least a low nM range. In the course of our medicinal chemistry effort aimed at development of drug candidates, we identified various high affinity Y5 antagonists. The majority of the antagonists, however, suffered from high lipophilicity, and some of them were good substrates for P-gp transporters. Of the Y5 antagonists we developed, the spiro[3-oxoisobenzofurane-1(3*H*),4′-piperidine] derivatives, exemplified by **1a** and **1b**, were particularly attractive for their high Y5 binding affinities (Fig. 1). They inhibited Y5-agonist [D-Trp^{34}]NPY-induced food intake in rats with a minimum effective dose of 3 mg/kg po and showed high brain Y5 receptor occupancy in mice, demonstrating that these antagonists were active in in vivo.[37] In addition, the 3-oxoisobenzofurane structure can be labeled with ^{11}C by a CO insertion reaction employing palladium chemistry (Fig. 2). On top

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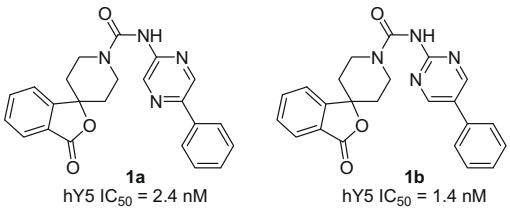


Figure 1.

of that, incorporation of a fluorine atom into the structure may provide additional opportunity for ^{18}F-labeled compounds. Here we describe the synthesis and evaluation of 3-oxoisobenzofurane derivatives to identify Y5 PET ligands.

The preparation of cold versions of PET ligand candidates comprises the synthesis of *trans*-3-oxospiro[(aza)isobenzofuran-1(3*H*),1′-cyclohexane]-4′-carboxylic acid portions (**9a**–**e**) and subsequent coupling with aromatic amine partners. The carboxylic acids (**2**, **4**, and **5**), the carboxamide (**3**), and the benzonitrile (**6**) were treated with *n*-BuLi or lithium tetramethylpiperidide to generate anions, which were subsequently reacted with 1,4-cyclohexanedione mono-ethyleneketal followed by acid-mediated hydrolysis to afford the spiro[(aza)benzofurane-1(3*H*),1′-cyclohexane]-3,4′-diones (**7a**–**e**). Stereoselective reduction of the ketone by $NaBH_4$ or $LiAl(OtBu)_3H$ yielded the *cis*-alcohols (**8a**–**e**). The alcohols were reacted with MsCl in the presence of Et_3N, and the resulting mesylates were reacted with Et_4NCN followed by hydrolysis to produce the desired *trans*-3-oxospiro[(aza)isobenzofuran-1(3*H*),1′-cyclohexane]-4′-carboxylic acids (**9a**–**e**). The carboxylic acids were reacted with 2-amino-5-(substituted)phenylpyrimidines in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in pyridine to give the desired cold versions of PET ligand candidates (Scheme 1).

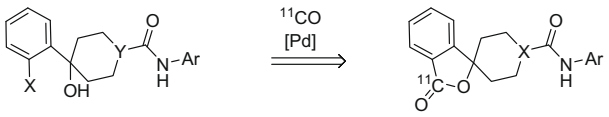
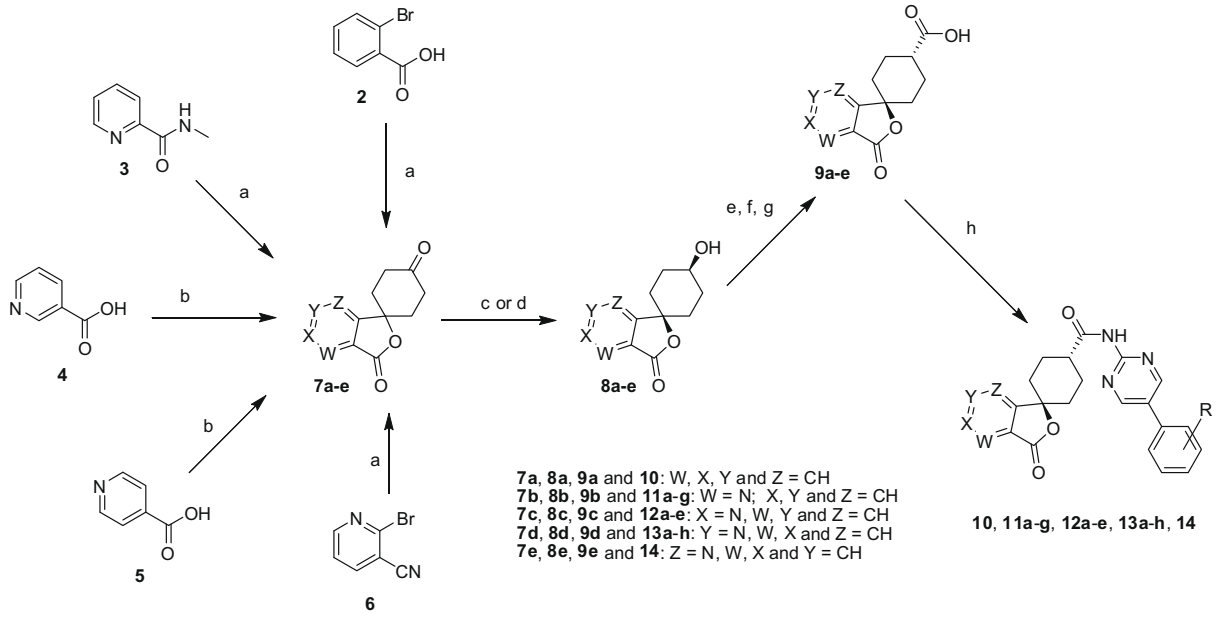


Figure 2. Possible synthesis of a ^{11}C-labeled ligand.

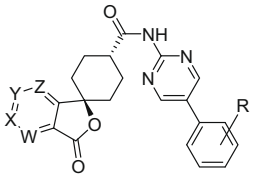
In the course of our structure activity studies of the spiropiperidine Y5 antagonists,[36,37] we noticed that urea derivatives of phenylpyrimidine produced a relatively high binding affinity as well as less lipophilicity. Compound **1b** exhibited a high Y5 binding affinity (IC_{50} = 1.4 nM) and acceptable lipophilicity (log $D_{7.4}$ = 2.90), thus this structure was highlighted as a starting point for further derivatization. Replacement of the urea linkage with an amide produced **10** with enhanced Y5 binding affinity; however, the urea to amide transformation resulted in an increase in lipophilicity (log $D_{7.4}$ = 3.73). Incorporation of a nitrogen atom in the benzene ring of the 3-oxoisobenzofurane moiety was effective to decrease the lipophilicity as shown in **11a**, **13a**, and **14**, and those compounds showed Y5 binding affinities in the low nM range. Introduction of a fluorine atom into the molecules was accomplished by incorporation of F, CH_2F, or OCH_2F groups on the phenyl group of the phenylpyrimidine moiety, and those compounds generally showed acceptable Y5 binding affinities in the low nM range as well as acceptable lipophilicity with log $D_{7.4}$ of less than 3 (Table 1).

Based on their relatively high binding affinities and structural diversity, **12b**, **12c**, **12f**, **13f**, and **13g** were chosen for further evaluation. The compounds were evaluated for their brain and cerebrospinal fluid (CSF) penetrability in rats as well as susceptibility to human and mouse P-gp transporters, and the results are summarized in Table 2. The compounds showed relatively high CSF concentrations compared with the brain concentrations. We hypothesized that the high CSF levels were brought about by high free fraction levels of the compounds, which may be evidence of low nonspecific binding in the brain. The compounds seemed to be attractive candidates for PET ligands in this aspect. In contrast, the compounds showed brain/plasma ratios in a range of 0.1–0.5, indicating that these compounds were good to moderate substrates for the rat P-gp transporter. We examined the extent of



Scheme 1. Synthesis of cold versions of Y5 PET ligand candidates. Reagents and conditions: (a) *n*-BuLi, 1,4-cyclohexanedione mono-ethyleneketal, THF, −70 °C; then concd HCl, H_2O, acetone, 80 °C; (b) *n*-BuLi, 2,2,6,6,-tetramethylpiperidine, 1,4-cyclohexanedione mono-ethylene ketal, THF, −40 °C; then 6 N HCl, 80 °C; (c) $NaBH_4$, THF, H_2O, 0 °C; (d) $LiAl(OtBu)_3H$, THF, 0 °C; (e) MsCl, Et_3N, 0 °C; (f) Et_4NCN, DMF, 100 °C; (g) 30% H_2SO_4, 100 °C; (h) 2-amino-5-(substituted)phenylpyrimidine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, pyridine.

Table 1
Human Y5 binding affinities



Compounds	W	X	Y	Z	R	hY5 binding IC_{50}[a] (nM)	Log $D_{7.4}$
10	CH	CH	CH	CH	H	0.64 ± 0.28	3.73
11a	N	CH	CH	CH	H	2.1[b]	2.32
11b	N	CH	CH	CH	*o*-F	3.0[b]	NT
11c	N	CH	CH	CH	*m*-F	2.2[b]	2.48
11d	N	CH	CH	CH	*p*-F	7.5[b]	NT
11e	N	CH	CH	CH	*o*-OCH_2F	4.5[b]	NT
11f	N	CH	CH	CH	*m*-OCH_2F	3.1[b]	NT
11g	N	CH	CH	CH	*m*-CH_2F	2.3[b]	NT
12a	CH	N	CH	CH	H	2.0[b]	NT
12b	CH	N	CH	CH	*o*-F	1.5 ± 0.3	2.79
12c	CH	N	CH	CH	*m*-F	1.5 ± 0.2	2.78
12d	CH	N	CH	CH	*p*-F	2.2[b]	NT
12e	CH	N	CH	CH	*o*-OCH_2F	2.2[b]	NT
12f	CH	N	CH	CH	*m*-OCH_2F	1.8 ± 0.4	2.71
13a	CH	CH	N	CH	H	1.8[b]	2.65
13b	CH	CH	N	CH	*o*-F	1.8 ± 0.4	2.79
13c	CH	CH	N	CH	*m*-F	1.8 ± 0.3	2.80
13d	CH	CH	N	CH	*p*-F	2.7[b]	NT
13e	CH	CH	N	CH	*o*-OCH_2F	2.6[b]	2.71
13f	CH	CH	N	CH	*m*OCH_2F	1.9 ± 0.1	2.75
13g	CH	CH	N	CH	*o*-CH_2F	2.0 ± 0.3	2.54
13h	CH	CH	N	CH	*m*-CH_2F	1.8 ± 0.2	2.65
14	CH	CH	CH	N	H	2.9[b]	2.71

[a] [^{125}I]PYY binding to human recombinant Y5 receptors in LMtk-cells (see Ref. 39). Values represent mean ± standard deviation of at least three separate experiments.
[b] $n = 2$ IC_{50} determination.

P-gp-mediated transport by using the polarized pig kidney epithelial cell lines (LLC-PK1) transfected with human P-gp cDNA (L-MDR1) or mouse P-gp (mdr1a) cDNA. For P-gp substrate, the transcellular transport of compound in the direction of basolateral-to-apical (B-to-A) compartment exceeds that in the opposite direction (A-to-B), since P-gp is located inside cell on the apical membrane catalyzing efflux transport of substrate from inside cell to apical compartment. Therefore, the value of relative rate of polarized transport (B-to-A/A-to-B) serves as an index for the extent of P-gp substrate susceptibility in P-gp-expressing cell monolayers.[40] In the in vitro P-gp transporter assay, these compounds exhibited relatively high basal-to-apical (B-to-A)/apical-to-basal (A-to-B) ratios of >4 in mouse L-mdr1a, which suggested that these compounds were good substrates for the mouse P-gp transporter. Assuming the rat and mouse P-gp transporters are highly similar, the in vivo and in vitro data are consistent with each other. In contrast, the compounds exhibited B-to-A/A-to-B ratios of between 1 and 2 in the human L-MDR1 assay, which suggested that these compounds were poor substrates for the human P-gp transporter. In particular, **12b** showed a B-to-A/A-to-B ratio of 1.11, which indicated that **12b** was effectively not a substrate for the human P-gp transporter. In addition, compound **12b** was selective for Y5 over Y1, Y2, and Y4 receptors (human Y1, Y2, and Y4 IC_{50} >10 μM). Thus, **12b** was selected as a primary candidate for a PET ligand.

[^{11}C]**12b** can be synthesized by the reaction of a tertiary alcohol precursor **19** and ^{11}CO in the presence of $Pd(PPh_3)_4$.[41,42] The tertiary alcohol precursor **19** was synthesized according to the method described in Scheme 2. 3-Bromopyridine was reacted with LDA followed by 1,4-cyclohexanedione mono-ethyleneketal and acid-mediated hydrolysis to afford **16**. The ketone was stereoselectively reduced by $NaBH_4$ to afford the *cis*-alcohol **17**. Mesylation of the alcohol, followed by reaction with NEt_4CN and acid-mediated hydrolysis gave the *trans* carboxylic acid **18**, which was coupled with 2-amino-5-*o*-fluorophenylpyrimidine to produce the precursor **19**.

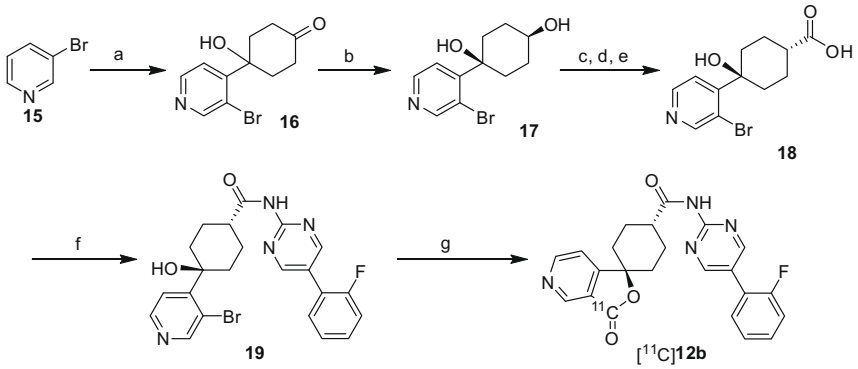
In preliminary studies in rhesus monkeys, images of [^{11}C]**12b** binding were obtained in the brain, and a Y5 receptor-specific signal was achieved in the striatum region as reported separately.[42] Therefore, the ligand subsequently was studied in humans where it also produced the Y5 receptor-specific signal in the human brain. In clinical trials of the Y5 receptor antagonist MK-0557, [^{11}C]**12b** was used to assess the receptor occupancy produced by oral administration of MK-0557.[43] The receptor occupancy data assessed by using the PET ligand [^{11}C]**12b** was consistent with the efficacy data obtained in the dose-ranging study, confirming the dosage employed in the long-term weight-loss study. Thus, this ligand was useful to support clinical proof-of-concept studies of the Y5 antagonist MK-0557.

In summary, we synthesized a series of *trans*-3-oxospiro[(aza)isobenzofuran-1(3*H*),1′-cyclohexane]-4′-carboxamide derivatives as candidates for a Y5 PET ligand. The compounds were evaluated for their Y5 binding affinities as well as lipophilicity, brain and CSF penetrability in rats, and in vitro P-gp susceptibility in humans and mice. Of the compounds synthesized, **12b** was potent as a Y5 receptor and selective over Y1, Y2, and Y4 receptors. Compound **12b** had acceptable lipophilicity and moderate brain penetrability as well as excellent CSF penetrability. In addition, **12b** was not a substrate for the human P-gp transporter although it was a good substrate for the mouse P-gp transporter. Thus, **12b** was selected as a candidate for the Y5 PET ligand, and [^{11}C]**12b** proved to be an acceptable PET ligand for imaging of brain Y5 receptors in humans.

Table 2
Plasma, brain, and CSF level data in rats and susceptibility to P-gp transporters

Compounds	Plasma, brain and CSF levels after 3 mg/kg iv in rats[a]					Susceptibility to P-gp transporters[b] (B-to-A/A-to-B ratio)	
	Plasma (μM)	Brain (μM)	CSF (μM)	Brain/plasma ratio	CSF/brain ratio	Human L-MDR1[c]	Mouse L-mdr1a[d]
12b	3.10	1.55	0.288	0.50	0.19	1.11	4.43
12c	3.45	1.42	0.308	0.41	0.22	1.41	7.78
12f	1.50	0.15	0.064	0.11	0.42	1.52	9.51
13f	2.04	0.74	0.095	0.37	0.13	1.37	8.23
13h	1.22	0.37	0.079	0.30	0.22	1.69	7.90

[a] Results represent the average of three animals.
[b] Transcellular transport across the monolayers of L-MDR1 or L-mdr1a. Values represent the ratio of basal-to-apical (B-to-A) versus apical-to-basal (A-to-B) at 3 h.
[c] Human MDR1 transfectants.
[d] Mouse mdr1a transfectants.



Scheme 2. Synthesis of [^{11}C]**12b**. Reagents and conditions: (a) LDA, 1,4-cyclohexanedione mono-ethyleneketal, MTBE-DME, <−70 °C; then *p*-TsOH, H_2O, acetone, reflux; (b) $NaBH_4$, THF, H_2O, <0 °C; (c) MsCl, NEt_3, THF, 0 °C; (d) NEt_4CN, 1,4-dioxane, 100 °C; (e) 30% H_2SO_4, 95 °C; (f) 2-amino-5-(2-fluorophenyl)pyrimidine, DMC, pyridine, $CHCl_3$; (g) See Refs. 41 and 42.

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