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Synthesis and evaluation of a series of 2,4-diaminopyridine derivatives as potential positron emission tomography tracers for neuropeptide Y Y1 receptors

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ABSTRACT

A series of 2,4-diaminopyridine derivatives was synthesized and evaluated as potential candidates for neuropeptide Y (NPY) Y1 receptor positron emission tomography (PET) tracers. Derivatives bearing substitutions allowing reliable access to radiolabeling were designed, focusing on Y1 binding affinity and lipophilicity. The advanced derivatives **2n** and **2o** were identified as promising PET tracer candidates.

Neuropeptide Y (NPY) is a 36-amino acid peptide abundantly distributed in the central nervous systems.[1–3] NPY is implicated in the regulation of a variety of physiological functions including feeding behavior, energy homeostasis,[4,5] cardiovascular function,[6] hormone secretion,[7] and pain.[8] The biological effects of NPY are mediated by a family of G-protein-coupled receptors consisting of five distinct receptor subtypes of which Y1, Y2, Y4, Y5, and Y6 have been characterized.[9] NPY is one of the most potent orexigenic substances when directly administrated into the brain, and a number of studies have suggested that Y1 and Y5 receptors play a role in NPY-induced food intake and development of obesity.[9,10]

Over the past decade, many pharmaceutical companies have devoted significant efforts towards discovering potent and selective NPY Y1 antagonists to probe the physiological roles of the Y1 receptor.[11] To better understand NPY Y1 biology in vivo, a suitable NPY Y1 positron emission tomography (PET) tracer would be a powerful tool allowing non-invasive Y1 receptor imaging and determination of receptor occupancy. In addition, PET tracers would be valuable tools for designing and testing promising drug candidates. In theory, a successful PET tracer targeting a receptor in the central nervous system needs to have high affinity for the target receptor, ideally with a $B_{max}/K_d > 10$. Reasonable lipophilicity with log P or log $D = 1-3.5$ is also necessary for appropriate brain penetrability and achievement of an optimal specific/non-specific binding ratio.[12,13] Another critical criterion for the selection of a brain-targeting tracer candidate is lack of susceptibility to P-glycoprotein (P-gp), which is an efflux transporter expressed at the blood–brain barrier.[14] To our knowledge, there are no reports of PET tracers available for in vivo studies of the NPY Y1 receptor.[15] Here we report the synthesis and optimization of 2,4-diaminopyridine derivatives as potential candidates for NPY Y1 PET tracers.

Previously, we reported the discovery of the potent and selective 2,4-diaminopyridine-based NPY Y1 antagonist **1** (Fig. 1).[16a] Compound **1** showed excellent selectivity over other NPY receptor subtypes (Y2, Y4, Y5 > 10 μM) and demonstrated food intake inhibition in rodents. During the course of structure–activity relationship (SAR) studies of the 2,4-diaminopyridine class, we identified the 2-fluoropyridine derivative **2a** as a potential lead for PET tracer development. Compound **2a** has appropriate lipophilicity and is amenable to radiolabeling with ^{18}F,[17] although its Y1 binding affinity is moderate. Accordingly, we directed our efforts towards modifying compound **2a** by specifically focusing on improving the Y1 binding affinity and lipophilicity of **2a**.

Preparation of 2,4-diaminopyridine derivatives **2a–l**, **3a**, **3b**, **4a**, and **4b** is illustrated in Scheme 1. Esterification of chelidamic acid (**5**) followed by protection of the 4-hydroxy group as its benzyl ether produced **6**. The two symmetrical ester groups were differentiated by half-reduction using sodium borohydride in the presence of calcium chloride to give **7**. The hydroxyl group of **7** was protected as its tetrahydropyranyl (THP) ether. The ester of **8** was hydrolyzed to the corresponding carboxylic acid, which was trea-

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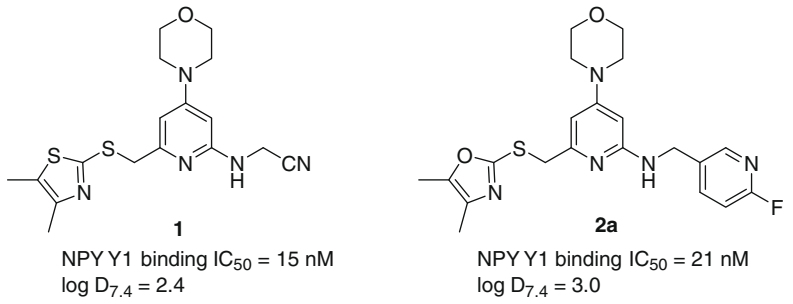
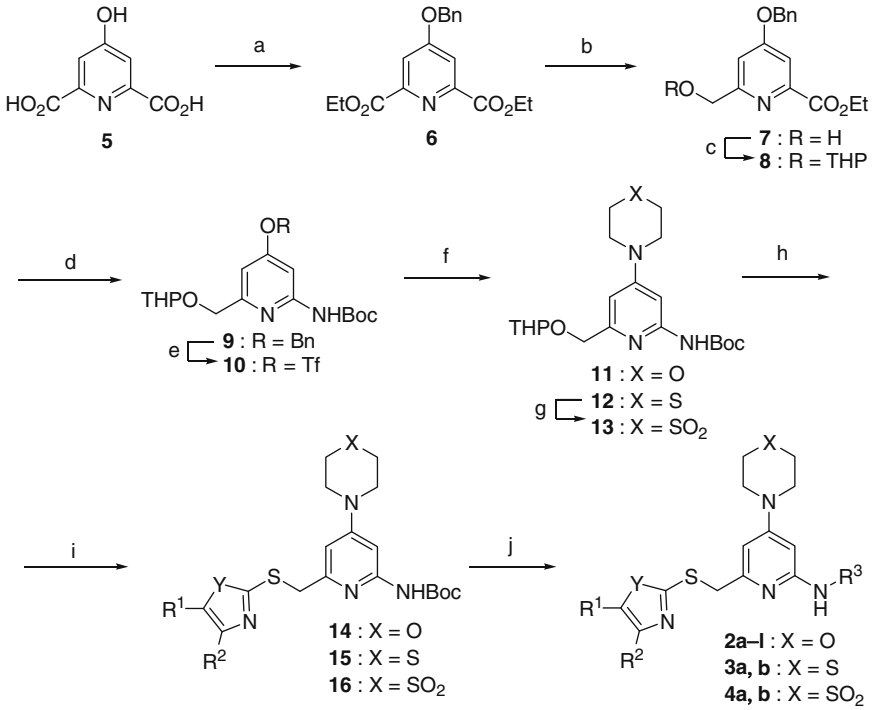
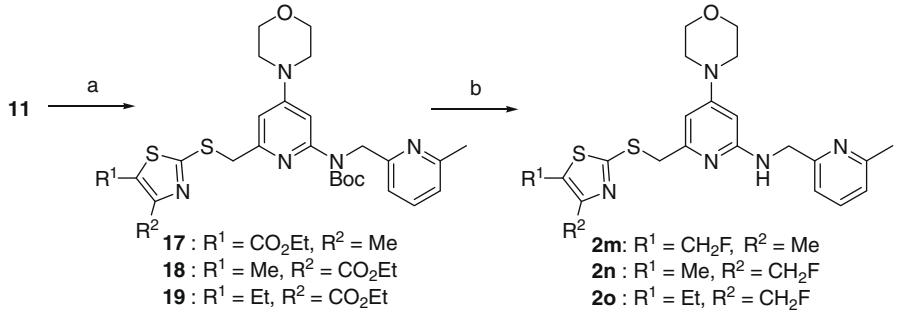


Figure 1. Structures of NPY Y1 antagonists **1** and **2a**.



Scheme 1. Synthesis of 2,4-diaminopyridine derivatives **2a–l**, **3a**, **3b**, **4a**, and **4b**. Reagents and conditions. (a) (i) *p*-TsOH, EtOH, reflux, 98%, (ii) BnBr, K_2CO_3, DMF, rt, 75%; (b) $NaBH_4$, $CaCl_2$, EtOH, 0 to 10 °C, 70%; (c) DHP, PPTS, $CHCl_3$, rt, 99%; (d) (i) 1 N aqueous NaOH, MeOH, 40 °C, (ii) DPPA, Et_3N, 1,4-dioxane, rt, (iii) *t*-BuOH, 1,4-dioxane, reflux, 78%; (e) (i) cyclohexene, Pd/C, reflux, (ii) Tf_2O, Et_3N, $CHCl_3$, 0 °C, 84%; (f) morpholine or thiomorpholine, DMSO, 50 °C, 58–71%; (g) $KMnO_4$, 20% AcOH–acetone, rt, 96%; (h) *p*-TsOH, EtOH, 40 °C, 99%; (i) (i) MsCl, Et_3N, AcOEt, 0 °C, (ii) ArSH, K_2CO_3, DMF, rt, 73–96%; (j) (i) R^3X, NaH, DMF, rt, (ii) TFA, $CHCl_3$, rt, 40–93%.

ted with diphenylphosphoryl azide followed by thermal rearrangement in the presence of *tert*-butylalcohol to yield *tert*-butoxycarbonyl (Boc)-protected aminopyridine **9**. After removal of the benzyl protecting group under transfer hydrogenation conditions using cyclohexene and palladium on carbon, the resulting hydroxyl group was converted to triflate **10**. Substitution reaction of the triflate **10** with morpholine or thiomorpholine produced the corresponding 2,4-diaminopyridine intermediates **11** and **12**. The thiomorpholinedioxide intermediate **13** was prepared by oxidizing **12** with potassium permanganate. After removal of the THP group of **11–13**, the resulting hydroxyl group was mesylated and displaced by appropriate heterocyclic thiol in the presence of potas-



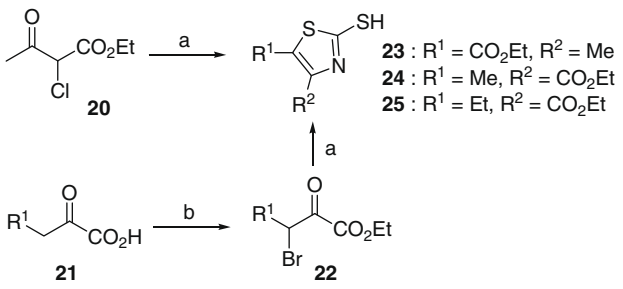
Scheme 2. Synthesis of 2,4-diaminopyridine derivatives **2m–o**. Reagents and conditions. (a) (i) 2-(Chloromethyl)-6-methylpyridine hydrochloride, NaH, DMF, rt, 94%, (ii) *p*-TsOH, EtOH, 40 °C, 99%; (iii) MsCl, Et_3N, AcOEt, 0 °C, (iv) **23**, **24** or **25**, K_2CO_3, DMF, rt, 90–96%; (b) (i) LAH, THF, 0 °C, 91–95%, (ii) DAST, THF, 0 °C, (iii) TFA, $CHCl_3$, rt, 56–70%.

sium carbonate to give thioethers **14–16**.[18] Alkylation of **14–16** followed by deprotection of the Boc group provided the target compounds **2a–l**, **3a**, **3b**, **4a**, and **4b**. Synthesis of **2m–o** is described in Scheme 2. Alkylation of the right-hand Boc-protected amine group of **11** with 2-(chloromethyl)-6-methylpyridine followed by introduction of the desired thiazoles **23–25** yielded **17–19**. After reduction of the ethoxycarbonyl group of **17–19**, the resulting hydroxyl group was fluorinated by diethylaminosulfur trifluoride, followed by removal of the Boc group to produce **2m–o**. The substituted thiazoles **23–25** were synthesized by the treatment of α-haloketones **20** or **22** with ammonium carbamodithioate, as illustrated in Scheme 3.

A series of 2,4-diaminopyridine compounds was tested in a [^{125}I]PYY binding assay using CHO (NFAT-bla) cell membranes expressing human recombinant Y1 receptors.[19] The log $D_{7.4}$ values of the derivatives were measured using the protocol previously reported by our laboratory.[20]

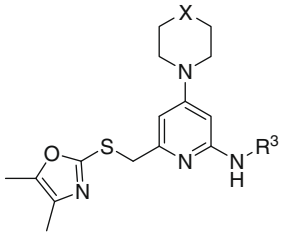
Variation of the right-hand 2-amino group was initially examined (Table 1). Among the various 2-fluoropyridine derivatives (**2a–d**), the 2-fluoro-6-methylenepyridine derivative **2d** exhibited the most potent Y1 affinity. Based on this result, the 2-fluorine group of compound **2d** was substituted with functional groups that are amenable to radiolabeling. Introduction of alkyloxymethyl, fluoromethyl, or fluoroethoxy groups provided no improvement in Y1 activity as in **2e–h**. However, replacement of the fluorine with a methyl group as in **2i** resulted in a twofold increase in Y1 binding and a slight reduction of lipophilicity, as compared to **2d**.[21] Next, we modified the 4-amino group. Remarkable enhancement of Y1 affinity was observed when the 4-morpholine was replaced with thiomorpholine as in **3a** and **3b**, resulting in IC_{50} values for **3a** and **3b** of 0.27 and 0.16 nM, respectively. The thiomorpholinedioxide derivatives **4a** and **4b** exhibited substantially reduced lipophilicity, although their Y1 binding affinities were substantially decreased. The thiomorpholine derivatives **3a** and **3b** displayed potent Y1 activity; however, their log $D_{7.4}$ values were relatively high for further optimization aimed at PET ligand identification. Therefore, further SAR studies were pursued using the less lipophilic derivative **2i** as a template.

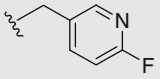
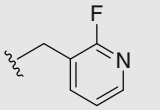
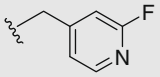
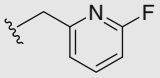
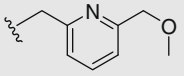
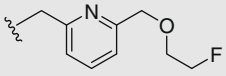
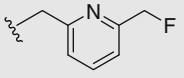
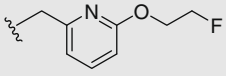
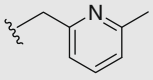
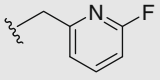
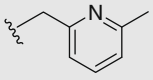
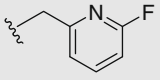
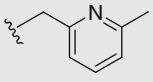
Optimization of the left-hand heterocycle portion of **2i** is summarized in Table 2. The 5-ethyl-4-methyloxazole or tetrahydrobenzoxazole derivatives **2j** and **2k** displayed increased Y1 binding affinity. Replacement of the 4,5-dimethyloxazole ring of **2i** with a 4,5-dimethylthiazole ring as in **2l** led to a further enhancement of Y1 activity while the log $D_{7.4}$ value is below 3. Attachment of a fluorine atom to the 5-methyl group of the thiazole ring of **2l** showed decreased Y1 binding as in **2m**. However, moving the fluorine from the 5-methyl to the 4-methyl group of the thiazole ring produced **2n,** which displayed improved Y1 activity and reduced lipophilicity relative to **2l**. Importantly, this fluorine substitution provides an additional labeling option for



Scheme 3. Synthesis of substituted thiazoles **23–25**. Reagents and conditions. (a) Ammonium carbamodithioate, EtOH, rt, 20–61%; (b) (i) concd H_2SO_4, EtOH, reflux, (ii) $CuBr_2$, AcOEt, $CHCl_3$, reflux, 29–58%.

Table 1
SAR of compounds **2a–i**, **3a**, **3b**, **4a,** and **4b**[a]

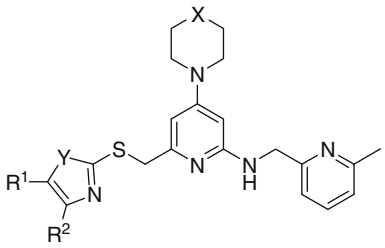


Compound	X	R^3	Y1 binding IC_{50}[b] (nM)	Log $D_{7.4}$[c]
2a	O		21	3.0
2b	O		7.7	3.0
2c	O		5.2	3.1
2d	O		1.5	3.1
2e	O		4.3	2.8
2f	O		10	2.6
2g	O		1.8	3.0
2h	O		19	3.9
2i	O		0.69	2.8
3a	S		0.27	3.5
3b	S		0.16	3.6
4a	SO_2		8.0	2.3
4b	SO_2		1.7	2.2

[a] Values represent the mean for $n \geqslant 2$ experiments.
[b] [^{125}I]PYY binding assay using CHO (NFAT-bla) cell membranes expressing human recombinant Y1 receptors.
[c] Octanol–water distribution coefficient at pH 7.4; see Ref. 20.

incorporation of ^{18}F. Furthermore, the 4-fluoromethyl-5-ethylthiazole derivative **2o** exhibited a further improvement in Y1 bind-

Table 2
SAR of compound **2i–o**[a]



Compound	X	Y	R^1	R^2	Y1 binding IC_{50}[b] (nM)	Log $D_{7.4}$[c]
2i	O	O	CH_3	CH_3	0.69	2.8
2j	O	O	CH_2CH_3	CH_3	0.39	3.1
2k	O	O	R1 = R^2 = $(CH_2)_4$		0.46	3.5
2l	O	S	CH_3	CH_3	0.27	2.9
2m	O	S	CH_2F	CH_3	0.56	2.3
2n	O	S	CH_3	CH_2F	0.20	2.7
2o	O	S	CH_2CH_3	CH_2F	0.13	3.2

[a] Values represent the mean for $n \geqslant 2$ experiments.
[b] [^{125}I]PYY binding assay using CHO (NFAT-bla) cell membranes expressing human recombinant Y1 receptors.
[c] Octanol–water distribution coefficient at pH 7.4; see Ref. 20.

ing with an IC_{50} of 0.13 nM and applicable lipophilicity with a log $D_{7.4}$ value of 3.2. Overall, compounds **2n** and **2o** appeared to be the best candidates in this series for PET tracers. Compounds **2n** and **2o** showed good selectivity over other NPY receptor subtypes (Y2, Y4, Y5; $IC_{50} > 10$ μM).[22] In addition, **2n** and **2o** have low or negligible human P-gp susceptibility (the transcellular transport ratios (B-to-A/A-to-B) for **2n** and **2o** are 1.8 and 1.4 for human P-gp, respectively).[23]

In summary, a series of 2,4-diaminopyridines was synthesized and evaluated for the development of novel NPY Y1 PET tracers. Our SAR studies were focused on increasing the Y1 affinity of lead compound **2a** while maintaining reasonable lipophilicity, and resulted in the identification of the potent and selective compounds **2n** and **2o** as promising candidates for Y1 PET tracers. Further evaluation and in vivo studies with radiolabeled compounds are ongoing.

Acknowledgment

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18. For the preparation of 4,5,6,7-tetrahydrobenzoxazole-2-thiol for compound **2k**, see: Bobosik, V.; Piklerova, A.; Martvon, A. *Collect. Czech. Chem. Commun.* **1983**, *48*, 3421. 4,5-Dimethyloxazole-2-thiol for **2a–i** and 5-ethyl-4-methyloxazole-2-thiol for **2j** were prepared in a similar manner. 4,5-Dimethylthiazole-2-thiol for **2l** is commercially available (Wako Pure Chemical Industries, Ltd).
19. Inhibitory effects of compounds on [^{125}I]PYY binding to membranes overexpressing human NPY Y1 receptors were examined using a minor modification of the method described in Ref. 16b. In brief, the membranes were incubated in 0.2 ml of 20 mM HEPES buffer (pH 7.4), containing 0.1% bacitracin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.5% BSA and Hank's balanced salt solution (HBSS) in the presence of various concentrations of the test compound with [^{125}I]PYY (25 pM) at 25 °C for 120 min. Following three washes, the membrane-bound radioactivity was measured using a TopCount™ microplate scintillation counter (Packard, Meriden, CT). Non-specific binding was determined in the presence of an excess amount of cold porcine PYY (1 μM).
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