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Optimization of a series of 2,4-diaminopyridines as neuropeptide Y Y1 receptor antagonists with reduced hERG activity

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ABSTRACT

The synthesis and evaluation of a series of 2,4-diaminopyridine-based neuropeptide Y Y1 (NPY Y1) receptor antagonists are described. Compound **1** was previously reported by our laboratory to be a potent and selective Y1 antagonist; however, **1** was also found to have potent hERG inhibitory activity. The main focus of this communication is structure–activity relationship development aimed at eliminating the hERG activity of **1**. This resulted in the identification of compound **3d** as a potent and selective NPY Y1 antagonist with reduced hERG liability.

Neuropeptide Y (NPY) is a 36-amino acid peptide widely distributed in the central nervous system and peripherals.[1–3] NPY has been implicated in the central regulation of feeding behavior and energy homeostasis.[4,5] Chronic administration of NPY into the brain results in body weight gain with hyperphagia, reduced energy expenditure, and increased lipogenic activity in the liver and adipose tissue.[5,6] In addition, NPY-deficient *ob/ob* mice are less obese and have reduced food intake compared with *ob/ob* mice.[7] Five distinct NPY receptor subtypes have been characterized (Y1, Y2, Y4, Y5 and y6).[8] From pharmacological data, the Y1 receptor is considered to be a major feeding receptor;[8,9] therefore antagonism of the Y1 receptor might have considerable therapeutic benefits in treating obesity. Over the past decade, significant effort has been devoted to this matter, and a number of potent Y1 antagonists have been identified and evaluated by pharmaceutical companies for their potential as anti-obesity agents.[10]

Previously, we reported a series of 2,4-diaminopyridine-based NPY Y1 antagonists.[11] Compound **1** (Fig. 1) inhibits food intake after intraperitoneal administration in rodents. These effects were shown to be Y1 specific.[11a] Although a promising lead class was identified, further evaluation revealed that compound **1** has potent inhibitory activity (IC_{50} = 36 nM) for I_{kr} potassium channel hERG (human Ether-a-go-go Related Gene).[12,13] Accordingly, we focused our modification efforts on identifying hERG attenuated potent Y1 antagonists by modifying this promising 2,4-diaminopyridine series. Although compound **1** is potent and shows Y1-specific anti-obesity effects, it was thought that only limited modifications could be made due to its large molecular weight (M_W, 572) and high lipophilicity ($\log D_{7.4} > 4$). Therefore, 2,4-diaminopyridine derivatives in our compound library were re-evaluated to find those with reduced molecular weight, lipophilicity and hERG activity. Compound **2a** was identified as a good starting candidate with appreciably potent Y1 activity and reduced hERG activity, and with a lower molecular weight and decreased lipophilicity compared to **1** (Fig. 1). This Letter is focused on SAR development aimed at eliminating the hERG activity of **2a**.

2,4-Diaminopyridine-based Y1 receptor antagonists **2**–**4** were prepared according to the general synthesis illustrated in Scheme 1. Bromination of chelidamic acid (**5**) followed by substitution with morpholine afforded the morpholine intermediate **6**. After esterification of **6**, the corresponding two symmetrical ester groups were differentiated by half-reduction using sodium borohydride in the presence of calcium chloride to give **7**. The

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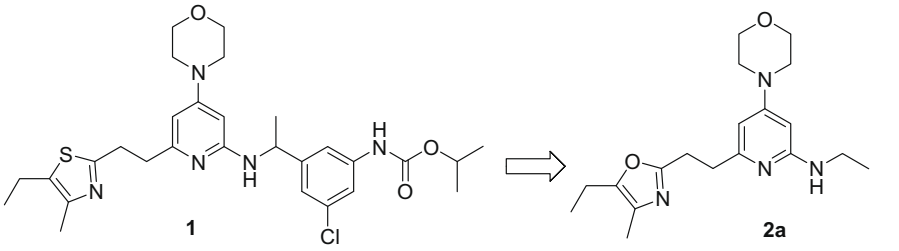
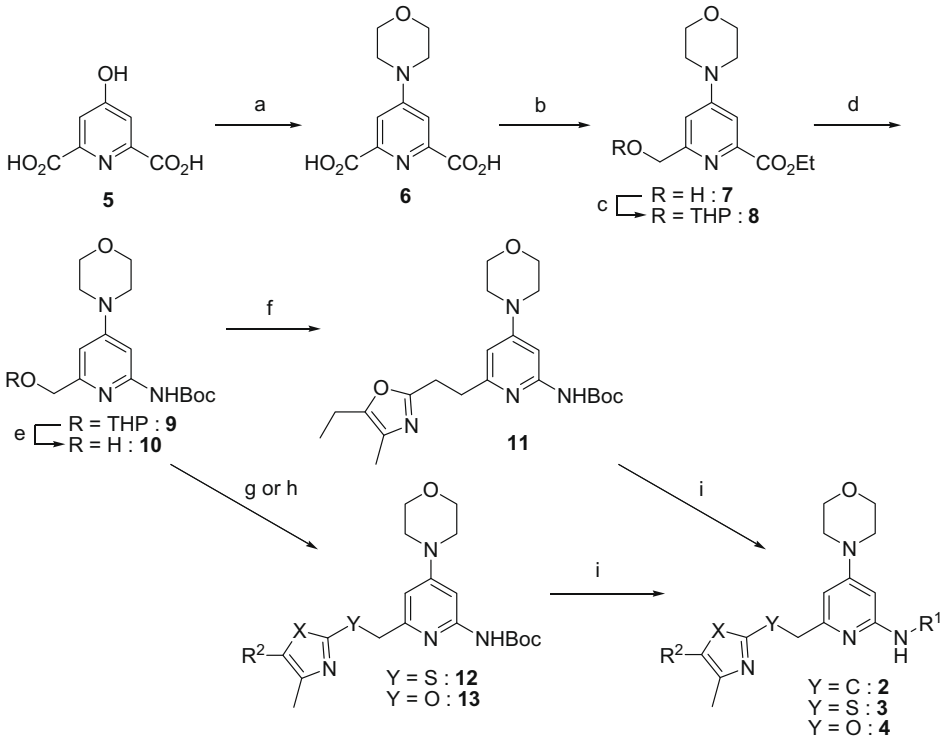


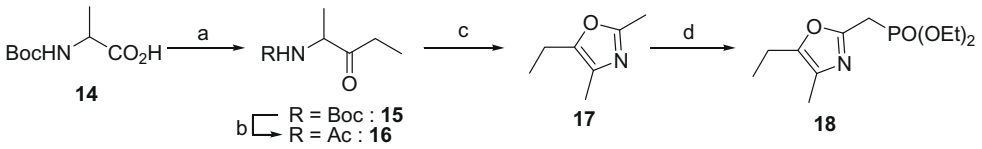
Figure 1. Structures of compounds **1** and **2a**.



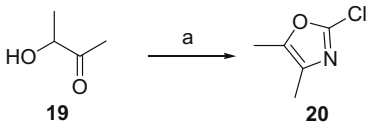
Scheme 1. Synthesis of 2,4-diaminopyridine derivatives **2**–**4**. Reagents and conditions: (a) (i) P_2O_5, *n*-Bu_4NBr, toluene, 130 °C (87%); (ii) morpholine, 130 °C (51%); (b) (i) concd H_2SO_4, EtOH, reflux (95%); (ii) $NaBH_4$, $CaCl_2$, EtOH, 0–10 °C (69%); (c) DHP, *p*-TsOH, $CHCl_3$, rt; (d) (i) 1 N aqueous NaOH, MeOH, 40 °C; (ii) DPPA, Et_3N, 1,4-dioxane, rt; (iii) *t*-BuOH, 1,4-dioxane, reflux (60% from **7**); (e) PPTS, EtOH, 40 °C (99%); (f) (i) SO_3Py, DMSO, Et_3N, rt; (ii) **18**, NaH, THF, 0 °C (71%); (iii) H_2, Pd/C, MeOH, rt (75%); (g) (i) MsCl, Et_3N, AcOEt, 0 °C; (ii) ArSH, K_2CO_3, DMF, rt (90–96%); (h) NaH, **20**, THF, 0 °C to rt (55%); (i) (i) R^1X, NaH, DMF, rt; (ii) TFA, $CHCl_3$, rt (40–93%).

hydroxyl group of **7** was protected as its tetrahydropyranyl (THP) ether. The ester **8** was hydrolyzed to the corresponding carboxylic acid, which was treated with diphenylphosphoryl azide followed by thermal rearrangement in the presence of *tert*-butylalcohol to yield *tert*-butoxycarbonyl (Boc)-protected aminopyridine **9**. The THP group was removed using pyridinium *p*-toluenesulfonate to furnish the alcohol intermediate **10**. The hydroxyl group of **10** was oxidized to the corresponding aldehyde, which was subjected to the Horner–Emmons reaction with diethyl phosphonate **18** and subsequently hydrogenated in the presence of palladium on carbon to furnish **11**. Alternatively, the alcohol **10** was mesylated and displaced by the desired five-membered heterocyclic thiol in the presence of potassium carbonate to give thioether **12**. Regarding the ether derivatives, the alcohol **10** was coupled with 2-chlorooxazole **20** in the presence of sodium hydride to provide **13**. Alkylation of the Boc-protected amine of **11**–**13** followed by deprotection of the Boc group with trifluoroacetic acid afforded target compounds **2**–**4**. Synthesis of diethyl phosphonate **18** is described in Scheme 2. The starting *N*-Boc alanine (**14**) was converted to the corresponding Weinreb amide and treated with ethyl magnesium bromide to afford α-aminoketone **15**. Swapping of the Boc with an acetyl group was followed by cyclization under acidic conditions to afford the 2-methyloxazole **17**. The 2-methyl group of **17** was lithiated by lithium diisopropylamide, followed by treatment with diethyl chlorophosphonate to furnish phosphonate **18**. Preparation of 2-chlorooxazole **20** is illustrated in Scheme 3. The reaction of acetoin (**19**) with potassium cyanate under acidic conditions, followed by chlorination with phosphorus oxychloride, afforded **20**.

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.[14] Inhibitory activity for hERG potassium channel was evaluated using the [^{35}S]



Scheme 2. Synthesis of diethyl phosphonate **18**. Reagents and conditions: (a) (i) MeNH(OMe)·HCl, WSC, HOBt, Et_3N, $CHCl_3$, 0 °C to rt; (ii) EtMgBr, THF, 0 °C to rt (88%); (b) (i) 4 N HCl/AcOEt, 0 °C; (ii) AcCl, Et_3N, $CHCl_3$, 0 °C to rt (58%); (c) concd H_2SO_4, 100 °C (90%); (d) (i) *n*-BuLi, *i*-Pr_2NH, THF, −78 °C; (ii) $ClPO(OEt)_2$, THF, −78 °C to rt (65%).

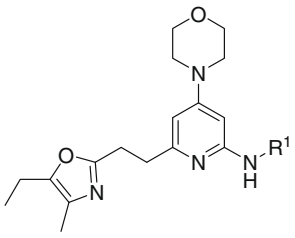


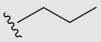
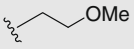
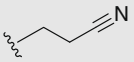
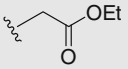
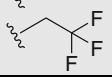
Scheme 3. Synthesis of 2-chlorooxazole **20**. Reagents and conditions: (a) (i) KCNO, concd HCl, DMF, 120 °C (27%); (ii) $POCl_3$, pyridine, 120 °C (57%).

N-[(*4R*)-17-[(*2R*)-6-cyano-1,2,3,4,-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[*2H*-1-benzopyran-2,4′-piperidin]-6-yl] methanesulfonamide ([^{35}S]MK-499) binding assay to assess cardiac QT prolongation liability.[15]

The effects of substituents on the 2-amino group were initially investigated (Table 1). Replacement of the ethyl group of **2a** with substituted methyl groups as in **2b–e** displayed Y1 affinity and hERG inhibition comparable to **2a**. Introduction of an electron-withdrawing cyanomethyl group, as in **2f,** resulted in significantly reduced hERG binding affinity while retaining Y1 activity. The calculated pK_a value[16] of **2f** is significantly lower than those of **2a**–**e**, while their log *D* values[17] are not significantly different.[18] At this point, we hypothesized that reduction of basicity might be a favorable strategy for attenuating hERG binding affinity. Consequently, we focused on exploring electron-withdrawing substituents, specifically targeting derivatives with reduced basicity. The cyanoethyl and ester derivatives (**2g** and **2h**) have higher pK_a values

Table 1
SAR of compounds **2a**–**j**, variation of the R^1 group



Compound	R^1	Y1 binding IC_{50}[a,b] (nM)	hERG IC_{50}[a,c] (μM)	Log $D_{7.4}$[d]	Calculated pK_a[e]
2a		16	0.40	2.2	9.8
2b		32	0.83	1.7	9.8
2c		9.0	0.48	2.5	9.8
2d		25	0.54	2.4	9.6
2e		13	0.90	1.9	9.3
2f		21	4.3	2.4	7.9
2g		22	1.6	2.0	9.0
2h		15	0.91	2.8	8.6
2i		320	7.0	2.3	6.7
2j		4.3	1.6	3.5	8.3

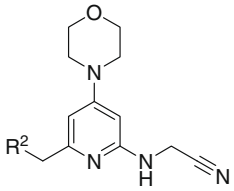
[a] The values represent the mean for $n \geqslant 2$.
[b] [^{125}I]PYY binding assay using CHO (NFAT-bla) cell membranes expressing human recombinant Y1 receptors.
[c] Inhibition of [^{35}S]MK-499 binding to hERG K^+ channel in HEK293 cells.
[d] Octanol–water distribution coefficient at pH 7.4; see Ref. 17.
[e] See Ref. 16 for details of the calculation and software.

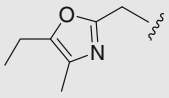
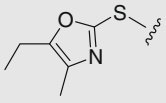
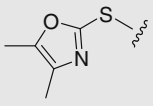
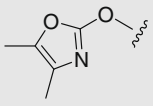
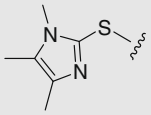
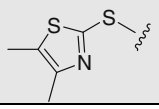
than **2f**. As anticipated, the hERG activities of **2g** and **2h** were more potent than that of **2f**. The acetyl derivative **2i**, which has an order of magnitude lower pK_a value than **2f**, showed improved hERG activity. Unfortunately, compound **2i** displayed a significant loss of Y1 activity. Although introduction of an electron-withdrawing trifluoroethyl group, as in **2j**, was not effective in reducing basicity and hERG activity, noticeably improved Y1 activity was observed. From this SAR study, the cyanomethyl substitution was identified to be the most suitable for the 2-amino moiety.

Next, the left-hand portion of the molecule was modified using compound **2f** as a template (Table 2). Replacement of the ethylene linkage with a thiomethylene linkage as in **3a** showed retained Y1 potency and a further reduction in pK_a, which led to substantial attenuation of hERG affinity. The dimethyloxazole derivative **3b** displayed negligible hERG inhibitory activity, although its Y1 activity was decreased fourfold. Surprisingly, replacement of the thioether linkage with an ether linker as in **4** resulted in a complete loss of Y1 activity. Replacement of the oxazole ring of **3b** with a substituted imidazole ring as in **3c** showed improved Y1 activity, while negligible hERG activity was retained. The thiazole derivative **3d** was more potent than the parent **2f** and displayed negligible hERG activity. Compound **3d** showed potent antagonistic activity in a [^{35}S]GTPγS binding assay (IC_{50} = 45 nM).[19] In addition, **3d** showed good selectivity over other NPY receptor subtypes (Y2, Y4, Y5; IC_{50} > 10 μM).[20]

Compound **3d** was evaluated in vivo. Compound **3d** is a significant substrate for mouse P-gp, so brain penetration by **3d** in mice is limited by P-gp mediated efflux. However, **3d** is a weak or negligible human P-gp substrate.[21,22] We therefore used P-gp-deficient mice to evaluate **3d**. Compared with wild-type mice, this mouse model is considered to more accurately predict drug action in humans. Accordingly, P-gp-deficient *mdr1a* (−/−) mice was utilized in the present study.[23] Intraperitoneal administration was selected to achieve required exposure for the present in vivo study since low oral bioavailability associated with low metabolic stability is an issue of this series. After intraperitoneal administration of compound **3d** at 30 mg/kg, the brain-to-plasma ratio was 0.9 in *mdr1a* (−/−) CF-1 mice (Table 3). In contrast, the brain-to-plasma ratio of **3d** in C57BL/6J mice was only 0.27, which clearly suggests that P-gp mediated efflux has a considerable influence on brain penetration of **3d**. Compound **3d** was tested in a starvation-induced food intake model using *mdr1a* (−/−) CF-1 mice.[24] After intraperitoneal administration at 30 mg/kg, compound **3d** exhibited significant

Table 2
SAR of compounds **2f**, **3a**–**d**, and **4**



Compound	R^2	Y1 binding IC_{50}[a,b] (nM)	hERG IC_{50}[a,c] (μM)	log $D_{7.4}$[d]	Calculated pK_a[e]
2f		21	4.3	2.4	7.9
3a		21	8.3	2.6	6.9
3b		70	>10	2.0	6.9
4		>1000	NT[f]	2.1	6.7
3c		38	>10	1.0	7.1
3d		15	>10	2.4	7.0

[a] The values represent the mean for $n \geqslant 2$.
[b] [^{125}I]PYY binding assay using CHO (NFAT-bla) cell membranes expressing human recombinant Y1 receptors.
[c] Inhibition of [^{35}S]MK-499 binding to hERG K^+ channel in HEK293 cells.
[d] Octanol–water distribution coefficient at pH 7.4; see Ref. 17.
[e] See Ref. 16 for details of the calculation and software.
[f] NT: not tested.

Table 3
Brain penetrability of **3d** in *mdr1a* (−/−) CF-1 and C57BL/6J mice[a]

	Concentration		Ratio
	Plasma (μM)	Brain (nmol/g)	Brain/plasma
mdr1a (−/−) CF-1	5.2 ± 1.7	4.7 ± 2.4	0.9 ± 0.24
C57BL/6J	3.4 ± 0.5	0.9 ± 0.2	0.27 ± 0.01

[a] The values represent the means ± SD for $n = 3$. The concentrations were measured 30 min after 30 mg/kg intraperitoneal administration.

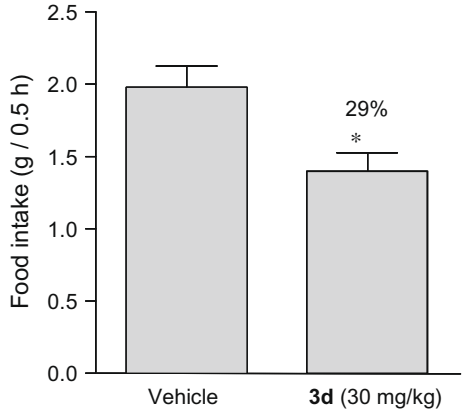


Figure 2. Effect of **3d** on starvation-induced food intake in *mdr1a* (−/−) CF-1 mice. Values are means ± SE for $n \geqslant 7$. $^{*}P < 0.05$ compared with the vehicle control.

suppression of food intake compared with the vehicle-treated group in this feeding model (Fig. 2).

In summary, we have designed a series of 2,4-diaminopyridine derivatives which have potent NPY Y1 antagonistic activity. The major focus of this study was the elimination of hERG activity from lead compound **2a**. A potent and selective derivative, **3d**, was identified which demonstrates suitable brain exposure and food intake inhibition in P-gp deficient *mdr1a*(−/−) CF-1 mice. Further evaluation of this class of compounds is currently underway.

Acknowledgments

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References and notes

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14. 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. 11a. 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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