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Synthesis and evaluation of substituted 4-alkoxy-2-aminopyridines as novel neuropeptide Y1 receptor antagonists

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Abstract—A series of substituted 4-alkoxy-2-aminopyridines **2**, which were formally derived from neuropeptide Y1 antagonist **1** by replacing the morpholino portion with alkoxy groups, were synthesized and evaluated as neuropeptide Y Y1 receptor antagonists. Primary structure–activity relationships and identification of potent 4-alkoxy derivatives are described.

Neuropeptide Y (NPY) is the most potent orexigenic peptide identified to date.[1–3] Chronic administration of NPY into the brain results in body weight gain with hyperphagia, reduces energy expenditure, and increases lipogenic activity in the liver and adipose tissue.[4,5] In addition, NPY-deficient *ob*/*ob* mice were less obese and exhibited reduced food intake when compared with *ob*/*ob* mice.[6] From these findings, NPY has been inferred to be a major regulator of energy balance. Accumulating evidence shows that NPY-mediated feeding might be regulated by multiple hypothalamic NPY receptor subtypes.[7–13] The Y1 receptor is considered to be a major feeding receptor. Compound **1** is one of the most promising ligands (Fig. 1), with in vivo Y1 specificity demonstrated by feeding experiments using Y1 receptor deficient mice.[13] With this promising structure in hand, we attempted to further extend the structural diversity of this series. The structure–activity relationships (SAR) for the 6- and *N*-substituents of the morpholino-2-aminopyridine core of compound **1** have been investigated in detail over the past few years;[14] however, the 4-morpholino portion remains to be explored. Exploration of the 4-substituent was initiated by the replacement of the morpholino group with various alkoxy groups. In this work, we report the synthesis and the preliminary SAR of the 4-alkoxy-2-aminopyridine derivatives **2**.

Two possible precursors **3** and **4** (Scheme 1) were considered on the basis of three reliable chemical reactions (A, B, and C). The alkylation of hydroxypyridine **3** (A) appeared not be suitable due to the limited availability

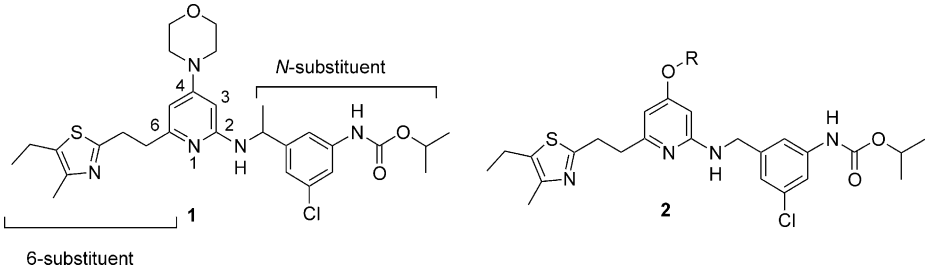

Figure 1. Structures of compound **1** and **2**.

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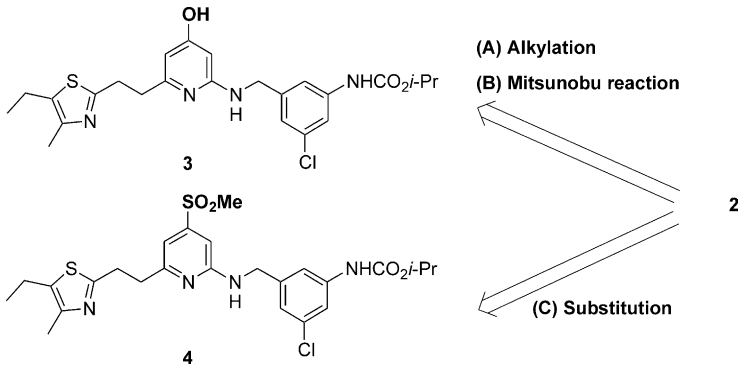
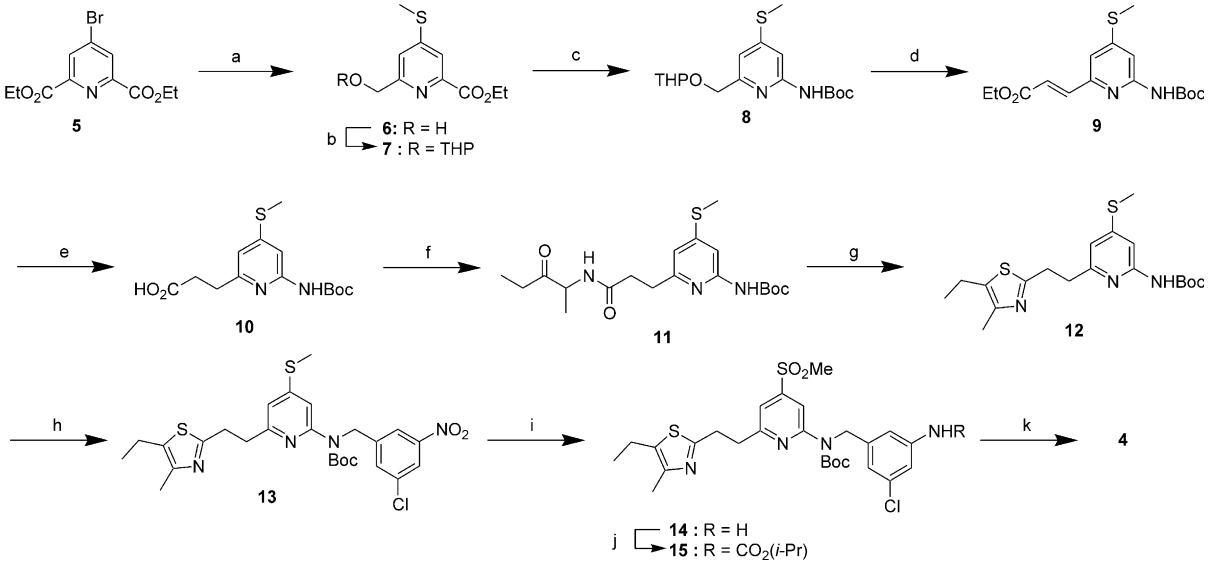
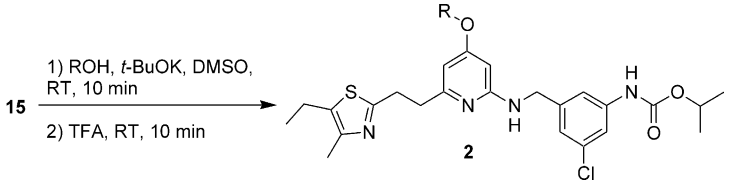
of alkylating reagents. For the Mitsunobu reaction (B) and the aromatic nucleophilic substitution reaction (C), commercially available alcohols can be utilized as the source of the alkyl group.[15] In the substitution reaction (C), a methanesulfonyl group was considered to be a better leaving group than the frequently utilized chloro group, since the reactive methanesulfonyl group can be masked as a sulfide group in the course of the synthesis. Considering operational simplicity and ease of purification associated with arising side products, the substitution reaction (C) was thought to be ideal for the present study. Thus, we decided to develop a synthesis of the 4-methanesulfonylpyridine **4** (Scheme 2). The starting bromide **5** smoothly reacted with sodium thiomethoxide to give the corresponding thiomethoxy ether. The two symmetrical esters were differentiated by half-reduction using $NaBH_4$ in the presence of $CaCl_2$ to afford the hydroxy ester **6** in 72% yield over 2 steps. The hydroxy group of **6** was protected as its tetrahydropyranyl (THP) ether. The ester **7** was hydrolyzed to the corresponding carboxylic acid, which was treated with diphenylphosphoryl azide (DPPA) followed by thermal



Scheme 1.



Scheme 2. Reagents and conditions: (a) (i) CH_3SNa, DMF, 0 °C, 10 min, (ii) $NaBH_4$, $CaCl_2$, EtOH, rt, 3 h, 72%; (b) DHP, PPTS, $CHCl_3$, 50 °C, 1.5 h; (c) (i) 1 N aqueous NaOH–MeOH, 40 °C, 1 h, (ii) DPPA, Et_3N, 1,4-dioxane, rt, 1 h, (iii) *t*-BuOH, 1,4-dioxane, reflux, 0.5 h, 57% from **6**; (d) (i) *p*-TsOH, EtOH, 40 °C, 1 h, (ii) SO_3·Py, DMSO, Et_3N, rt, 0.5 h, (iii) $(MeO)_2P(O)CH_2CO_2Me$, NaH, THF, 0 °C, 0.5 h, 70%; (e) (i) $NaBH_4$, $NiCl_2$, THF–MeOH, rt, 0.5 h, (ii) 4N aqueous NaOH, THF–MeOH, 40 °C, 2 h, 83%; (f) 2-amino-3-pentanone, EDCI, HOBt, *N*-methylmorpholine, DMF, rt, 15 h, 87%; (g) Lawesson's reagent, toluene, reflux, 6 h, 79%; (h) NaH, DMF, 0 °C, 0.5 h, then 1-chloro-3-(chloromethyl)-5-nitrobenzene, 0 °C, 1 h, 100%; (i) (i) $KMnO_4$, 20% aqueous AcOH–acetone, rt, 10 min, (ii) Fe, NH_4Cl, MeOH–H_2O, reflux, 2.5 h, 77%; (j) *i*-PrOCOCl, DMAP, $CHCl_3$, rt, 2 h, 99%; (k) TFA, rt, 10 min, 99%.

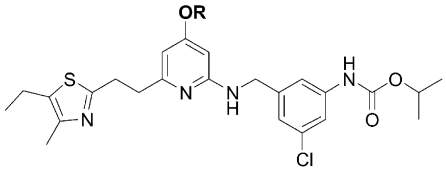


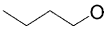
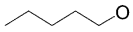
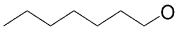
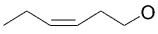
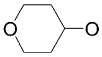
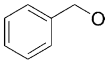
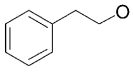
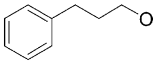
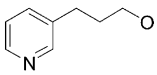
Scheme 3.

rearrangement in refluxing 1,4-dioxane in the presence of *tert*-butylalcohol to furnish *tert*-butoxycarbonylaminopyrdine **8** in 57% yield over 4 steps. The hydroxy group of **8** was unmasked with pyridinium *p*-toluenesulfonate (PPTS) and oxidized to the corresponding aldehyde with sulfurtrioxide–pyridine complex in DMSO, which was subjected to the Horner–Emmons reaction with trimethyl phosphonoacetate and NaH to provide the α,β-unsaturated ester **9** in 70% yield from **8**. The reduction of the double bond of **9** with a combination of $NaBH_4$ and $NiCl_2$ followed by saponification gave **10** in 83% yield. The carboxylic acid **10** and 2-amino-3-pentanone were coupled, and treatment of the resulting ketoamide **11** with Lawesson's reagent effected cyclization, affording thiazole **12** in 79% yield. 1-Chloro-3-(chloromethyl)-5-nitrobenzene, which was derived from chlorination of 1-chloro-3-(hydroxymethyl)-5-nitrobenzene with thionyl chloride, was coupled with **12** in the presence of NaH to furnish **13** in quantitative yield. Oxidation of the thiomethoxy group of **13** to the corresponding methanesulfonyl group was best-accomplished using potassium permanganate, and the subsequent reduction of the nitro group with Fe afforded **14** in 77% yield in 2 steps. Treatment of **14** with isopropyl chloroformate in the presence of 4-(dimethylamino)pyridine (DMAP) followed by cleavage of the *tert*-butoxycarbonyl (Boc) group under acidic conditions afforded the key intermediate **4**. With methanesulfonylpyridine **4** in hand, we examined the substitution of the methanesulfonyl group by alkoxide anions. The substitution reaction of **4** turned out to be sluggish; the desired substituted product was obtained in at most 20% yield. It was subsequently determined that the protected precursor **15** participates in the substitution reaction under mild conditions (Scheme 3). The desired alcohol (5 equiv) and **15** were combined in anhydrous DMSO, which was treated with 5 equiv of *t*-BuOK at ambient temperature. The starting 4-methanesulfonylpyridine **15** was completely consumed within 10 min, giving the desired 4-alkoxypyridine with 30–80% yield. Deprotection of the Boc group with TFA afforded the target alkoxy compound **2**. The crude products were purified by HPLC (YMC-Pack Pro C18 (50×30 mm I.D.) with liner gradient system of H_2O–CH_3CN–TFA 75:25:0.1 to 25:75:0.1 over 8 min and at a flow rate of 40 mL/min) and tested as TFA salts.

Our synthetic route allowed us to prepare the substituted 4-alkoxy derivatives incorporating a variety of commercially available alcohols (Table 1).[16–18] The normal alkyl derivatives (**2a**–**g**) were first prepared in order to estimate the size of the binding pocket. The propoxy and butoxy derivatives (**2c** and **d**) displayed the highest binding affinities. The derivatives with longer or shorter chain length showed a decrease in the binding affinity. It should be noted that the hexenyloxy derivative **2h** had a considerably higher binding affinity (IC_{50} = 4.7 nM), despite its rather long chain length. The structural constraint imparted by the internal double bond might be responsible, which may provide an opportunity for further optimization. With the approximate size of the binding pocket in mind, the branched alkoxy (**2i**–**m**) and cycloalkyloxy (**2n**–**q**) derivatives were

Table 1. Y1 Binding affinities of 4-alkoxy-2-aminopyridine derivatives[a]



Compd	RO	Binding affinity[b] (IC_{50}, nM)	$[Ca^{2+}]_i$ response[c] (IC_{50}, nM)
1	Morpholino	1.4±0.2	2.9±0.2
2a	MeO	234±9	[d]
2b	EtO	33±6	[d]
2c		17±2	[d]
2d		16±4	[d]
2e		35±4	[d]
2f		63±3	[d]
2g		72±10	[d]
2h		4.7±1.4	64±7
2i		12±1	[d]
2j		8.4±1.0	59±9
2k		17±3	[d]
2l		55±3	[d]
2m		5.5±1.7	33±3
2n		11±1	[d]
2o		11±2	[d]
2p		29±1	[d]
2q		4.4±2.2	42±2
2r		237±27	[d]
2s		30±3	[d]
2t		20±2	[d]
2u		6.5±1.2	46±6

[a] The values represent the mean±SE for n = 3.
[b] Human recombinant Y1 receptors in CHO/dhFr$^-$ cell membranes, [^{125}I]PYY; see ref 13.
[c] Antagonistic activities (human recombinant Y1 receptors in CHO/dhFr$^-$ cells) at 1 nM NPY stimulation; see ref 13.
[d] Not determined.

examined. Branching was found to be effective to enhance potency. The 1-ethylpropoxy derivative **2m** was 3-fold more potent than the parent propoxy derivative **2c**. The cyclization strategy was not found to result in significant improvement with respect to potency; however, the pyranyloxy derivative **2q** was found to have a high binding affinity ($IC_{50}=4.4$ nM), a 7-fold improvement over the cyclohexyloxy derivative **2p**. The significantly improved binding affinity is attributed to the oxygen atom of the pyranyl ring that is probably acting as a hydrogen bonding acceptor. Finally phenyl substituted derivatives were examined. It is important to note that phenoxy derivatives could not be prepared since phenoxide anions did not participate in the current substitution reaction. Substitution in the phenyl moiety was not observed to contribute to the enhanced binding affinity (**2r**–**t**); however, the binding affinity of the pyridyl derivative **2u** was relatively higher than the corresponding phenyl derivative **2t**. This slight increase in binding affinity is probably ascribed to electrostatic effects, rather than the hydrogen bonding acceptor property of the pyridine ring. The antagonistic activities of the selected high-affinity compounds were measured by their ability to inhibit NPY-induced $[Ca^{2+}]i$ increases in CHO/dhFr$^-$ cells, which expressed the recombinant human Y1 receptor.[16] In this $[Ca^{2+}]i$ functional assay, **2h**, **j**, **m**, **q**, and **u** dose-dependently inhibited the $[Ca^{2+}]i$ increase (See Table 1 for their IC_{50} values).

In summary, a number of substituted 4-alkoxy-2-aminopyridine derivatives **2** were synthesized and evaluated for their binding affinities to the human NPY Y1 receptor. Several potent antagonists were identified among them. The primary SAR was elucidated, revealing that a number of alkoxy substituents are capable of replacing the morpholino portion of compound **1**. In addition, the activities of the potent derivatives **2h**, **j**, **m**, **q**, and **u** are of interest for use in in vivo studies. The pharmacokinetics and brain penetrability of these derivatives in rodents remain to be addressed for further in vivo evaluation of the present alkoxy type of Y1 antagonists.

Acknowledgements

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16. See ref 13 for the experimental conditions for the binding and functional assays described herein.
17. All of the compounds tested for Y1 binding are >95% pure. Analytical HPLC analyses were performed under the following conditions: Wakopak combi ODS fast (30×2.0 mmI.D.) with liner gradient system of H_2O–CH_3CN–TFA 95:5:0.1 to 5:95:0.1 over 6 min and at a flow rate of 0.8 mL/min.
18. Selected spectral data: **2q** TFA salt: ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (3H, t, $J=7.4$ Hz), 1.30 (6H, d, $J=6.3$ Hz), 1.70 (2H, m), 1.87 (2H, m), 2.34 (3H, s), 2.74 (2H, q, $J=7.4$ Hz), 3.19 (2H, t, $J=7.6$ Hz), 3.43 (2H, t, $J=7.6$ Hz), 3.54 (2H, ddd, $J=2.8$, 8.8, 12.2 Hz), 3.91 (2H, td, $J=4.4$, 12.2 Hz), 4.37 (2H, d, $J=5.4$ Hz), 4.46 (1H, m), 4.99 (1H, sept, $J=6.3$ Hz), 5.69 (1H, d, $J=2.2$ Hz), 6.23 (1H, d, $J=2.2$ Hz), 6.97 (1H, s), 7.22 (1H, s), 7.28 (1H, s), 7.54 (1H, s), 10.38 (1H, t, $J=5.4$ Hz); MS (ESI) m/z 573.2 $[M+H]^+$. **2u** TFA salt: ^1H NMR (400 MHz, $CDCl_3$) δ 1.28 (6H, d, $J=6.3$ Hz), 1.33 (3H, t, $J=7.4$ Hz), 2.14 (2H, m), 2.23 (3H, s), 2.80 (2H, q, $J=7.4$ Hz), 2.98 (2H, t, $J=7.2$ Hz), 3.21 (2H, dd, $J=6.7$, 9.1 Hz), 3.63 (2H, dd, $J=6.7$, 9.1 Hz), 4.06 (2H, t, $J=6.0$ Hz), 4.41 (2H, d, $J=5.6$ Hz), 4.97 (1H, sept, $J=6.3$ Hz), 5.71 (1H, d, $J=2.2$ Hz), 6.37 (1H, d, $J=2.2$ Hz), 6.97 (1H, s), 7.25 (1H, s), 7.40 (1H, s), 7.52 (1H, s), 7.77 (1H, dd, $J=5.6$, 8.0 Hz), 8.13 (1H, d, $J=8.0$ Hz), 8.66 (1H, dd, $J=1.4$, 5.6 Hz), 8.74 (1H, d, $J=1.4$ Hz), 10.24 (1H, t, $J=5.6$ Hz); MS (ESI) m/z 608.2 $[M+H]^+$.