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STRUCTURE–ACTIVITY RELATIONSHIPS OF A SERIES OF BENZOTHIOPHENE-DERIVED NPY Y1 ANTAGONISTS: OPTIMIZATION OF THE C-2 SIDE CHAIN

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Abstract: A series of benzo[*b*]thiophene-derived NPY-1 receptor antagonists is described. Systematic modification of the C-2 substituent afforded a 1000-fold range in Y1 receptor affinity. Appropriate substitution at the *ortho* and *para* positions of the C-2 phenyl ether produced a synergistic effect on Y1 binding affinity, which led to the discovery of the most active ligands, **12t** (K_i = 15 nM), **12u** (K_i = 11 nM), and **12v** (K_i = 13 nM).

Keywords: Antagonists; Benzothiophenes; Receptors; Neurologically active compds

Introduction

Neuropeptide Y (NPY), a 36 amino acid polypeptide that is widely distributed throughout the central and peripheral nervous system, is involved in the regulation of a variety of neuroendocrine functions.[1] These include glucose metabolism, stress reponse, circadian rhythms, central autonomic functions, eating and drinking behavior, sexual function, and motor behavior. These effects are mediated through activation of a family of G-protein-coupled receptors, at least six subtypes of which (Y1–Y6) are currently recognized.[2] When directly administered into the CNS, NPY is the most potent feeding stimulant known.[3] This has generated considerable interest in the therapeutic potential of selective NPY antagonists for the treatment of obesity and Type 2 diabetes. Both the Y1 and the more recently identified Y5 receptors have been implicated in mediating the feeding reponse,[4,5] but the full characterization of this pharmacology awaits the discovery of appropriate selective NPY antagonists.

In previous reports from these laboratories, we have described the synthesis and structure-activity relationships (SAR) of related series of selective NPY Y1 receptor antagonists derived from the indole[6] and benzimidazole[7,8] platforms. In this report we describe the development of a complementary series based on a benzo[*b*]thiophene (BT) core that evolved concurrently. Our objective was to exploit several properties of the BT ring system that could prove advantagous. First, benzo[*b*]thiophene is more lipophilic than either indole or benzimidazole,[9] which could facilitate CNS penetration.[10] Second, BT is somewhat less electron rich than indole and therefore may be less prone to chemical and metabolic degradation.[11] Finally, the BT nucleus lends itself to alternative synthetic strategies which were complementary to those being employed for the nitrogen heterocycles. Unlike the indole and benzimidazole platforms, which required elaboration of the C-2 aryloxymethyl substituent prior to installation of the C-3 (indole) or N-1 (benzimidazole) side chain, we

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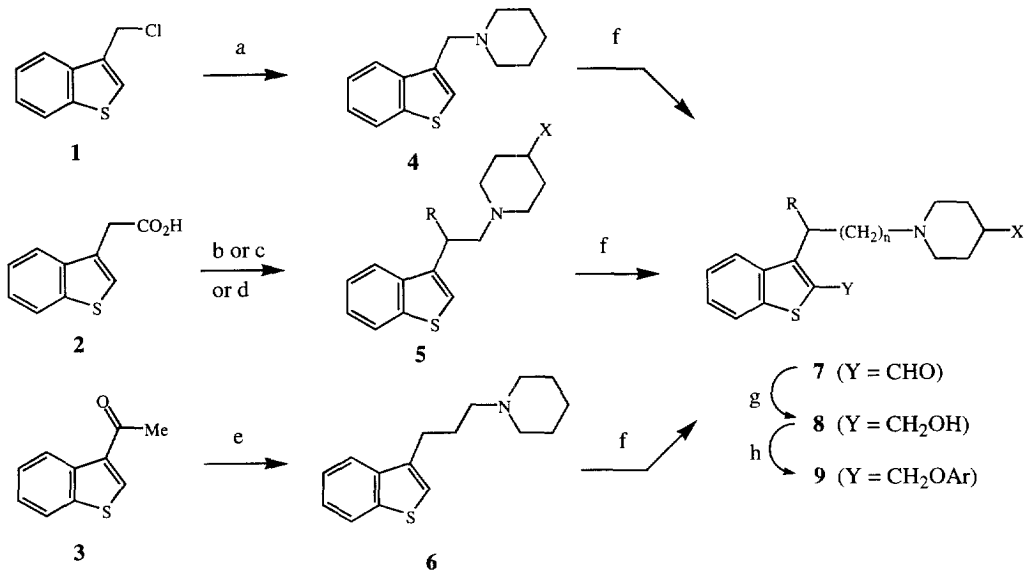
envisioned that introduction of the C-2 substituent on the benzothiophene platform could be conveniently accomplished via direct lithiation after construction of the C-3 side chain. This strategy greatly facilitated development of the C-2 SAR, and complemented activities on the nitrogen containing platforms.

Chemistry

We initially set out to identify a C-3 side chain that would confer high Y1 receptor binding affinity to the BT platform. Guided by our previous studies in the indole series,[6] we targeted a homologous series in which the piperidine N1 was tethered to the BT C-3 position by hydrocarbon linkers of varying length. Following the strategy outlined above, the C-3 side chains were elaborated from the commercially available precursors **1**, **2**, and **3** as illustrated in the scheme. In the case of the two carbon tether, the effect of β-branching was also probed by methylation of the enolate derived from the precursor piperidyl acetamide prior to its reduction to the corresponding amine. The 3-carbon homolog was conveniently prepared from the Mannich base derived from 3-acetylbenzothiophene, piperidine, and formaldehyde,[12] followed by stepwise reduction of the carbonyl to the hydrocarbon by treatment with $NaBH_4$ followed by TFA-Et_3SiH.

Scheme.



(a) piperidine, K_2CO_3, CH_3CN (b) 1. $(COCl)_2$, cat. DMF 2. piperidine 3. Red-Al®, 25 °C (c) 1. CDI, CH_2Cl_2; 4-dimethylaminopiperidine 2. Red-Al®, 25 °C (d) 1. CDI, 4-dimethylaminopiperidine 2. 1.15 eq LDA, THF, 0 °C, 1 h; 1.2 eq CH_3I, 0-15 °C. 3. Red-Al®, 25 °C. (e) 1. piperidine, 37% aq HCl, $(CH_2O)_n$, EtOH, Δ (Ref. 12). 2. $NaBH_4$, EtOH 3. Et_3SiH, CF_3CO_2H, 25 °C (f) 1. 1.1 eq *n*-BuLi, THF, -78 °C, 30 min. 2. DMF, -78 °C (g) $NaBH_4$, EtOH (h) Method a: NaH, ArF, DMF, 25-100 °C. Method b: ArOH, *i*-$PrO_2CN{=}NCO_2$-*i*-Pr, Ph_3P, THF, 0-25 °C.

As anticipated, C-2 lithiation of **4–6** on treatment with n-BuLi in THF was rapid and complete at -78 °C, and subsequent quenching with DMF followed by $NaBH_4$ reduction provided the corresponding 2-

hydroxymethyl derivatives **8** in high yield. Conversion of these alcohols to their desired aryl ethers **9** was achieved either by S_NAr displacement of an appropriate fluorobenzene with the derived alkoxide (Method a), or Mitsunobu coupling with the corresponding phenol (Method b). Method a was generally preferred for preparing aryl ethers containing electron withdrawing substituents (e.g., Cl, Br, CN, CF_3, or phenyl) whereas Method b was generally preferred for aryl ethers bearing electron donating groups (e.g., methyl or *t*-butyl). The complementarity of the two methods provided access to aryl ethers having a wide diversity of substituents.

Quenching of these 2-lithio derivatives with other electrophiles (e.g., 2,4-dichlorobenzadehyde for the preparation of **10b**) also allowed us to conveniently and efficiently prepare benzothiophenes having a wide array of C-2 substituents. This allowed us to quickly probe the C-2 SAR quite generally, including a study of the effect of the -CH_2O- linker on Y1 binding affinity (vide infra).

Structure Activity Relationships

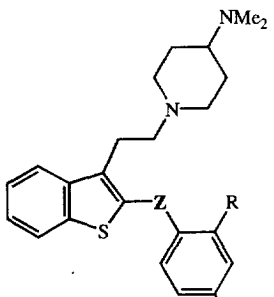
In our preliminary studies we established that optimal binding affinity to cloned human Y1 receptors[13] resulted when the piperidine ring was tethered to the BT C-3 position by a 2-carbon (ethylene) linker (i.e., **9b**, Table 1). Similar to the indole series, introduction of a dialkylamino substituent at the piperidine C-4 position resulted in a significant sixfold increase in Y1 binding. A methyl branch at the benzylic position of the C-3 side chain (**9e**) proved to be somewhat detrimental to Y1 activity. This limited study led to the discovery of **9d**, a 0.14 μM (K_i) Y1 antagonist having the 4-(dimethylamino)piperidyl ethyl C-3 side chain. This C-2 aryl ether showed 600-fold greater activity than its precursor alcohol **8c**, illustrating the importance of the C-2 moiety for high Y1 receptor affinity.

Table 1. Binding affinity of Benzothiophenes **8**, **9** at cloned human NPY1 receptors.[a]

Compound	n	R	X	Ar	K_i(μM)[b] AV12-C2
9a	0	H	H	4-Cl-Ph	11.1 ± 2.9
9b	1	H	H	4-Cl-Ph	1.9 ± 0.05
8c	1	H	NMe_2	—	88 ± 3.1
9c	1	H	NMe_2	4-Cl-Ph	0.31 ± 0.004
9d	1	H	NMe_2	2,4-di-Cl-Ph	0.14 ± 0.002
9e	1	CH_3	NMe_2	2,4-di-Cl-Ph	0.69 ± 0.007
9f	2	H	H	4-Cl-Ph	2.6 ± 0.06

[a] Ref 13. [b] Values represent mean ± SEM (n = 2).

In subsequent studies the C-3 side chain of **9d** was held constant while the effect of the C-2 substituent on Y1 affinity was probed in greater depth. We first studied the effect of the C-2 linker (-CH_2O- in **9d**). Of the 15 modifications examined, which included O-deletion (**10a**) or replacement with NH (**11f**), methylene insertion (**10o**), and methylene substitution (**10l–10n**), all afforded a 10 to 100-fold reduction in Y1 receptor binding affinity (Table 2).



10 (R = Cl)
11 (R = Me)

Table 2. Binding affinity of Benzothiophenes **10**, **11** at cloned human NPY1 receptors.[a]

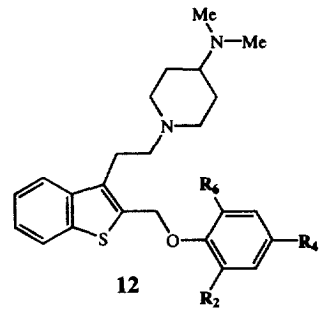
Compound	Z	$K_i(\mu M)$[b] AV12-C2	Compound	Z	$K_i(\mu M)$[b] AV12-C2
10a	$-CH_2-$	4.2 ± 0.5	**10i**	—	6.5 ± 0.3
10b	-CH(OH)-	3.8 ± 0.4	**10j**	-C≡C-	7.9 ± 1.0
10c	-CH(OMe)-	4.0 ± 0.2	**10k**	-CH=CH-	12.4 ± 0.6
10d	-C(=O)-	5.6 ± 0.2	**10l**	-CH(Me)O-	1.7 ± 0.04
10e	-C(=O)NH-	2.3 ± 0.1	**10m**	-CH(Ph)O-	5.8 ± 0.03
11f	$-CH_2NH-$	1.7 ± 0.6	**10n**	$-C(Me)_2O-$	12.0 ± 0.8
11g	-CH=N-	12.6 ± 0.3	**10o**	$-CH_2CH_2O-$	2.8 ± 0.02
10h	-CH=NNH-	4.5 ± 0.3			

[a] Ref 13. [b] Values represent mean ± SEM (n = 2).

Once we had established the apparent necessity of the $-CH_2O-$ linker for high Y1 receptor affinity, we next developed the SAR of the C-2 phenyl ether substituents. Since previous studies had established that *meta* substitution was detrimental to activity, we focused on *ortho* and *para* substitution (Table 3). Neither the unsubstituted phenyl ether nor any of the *ortho*-mono substituted derivatives displayed comparable Y1 affinity to that of the 2,4-dichloro derivative **9d**. Of the *ortho* mono-substituted derivatives prepared, the $-CH_3$, $-CH_2OH$, Br, and I groups afforded a similar threefold improvement in Y1 affinity relative to the unsubstituted compound. Methyl and Cl substitution at the para position had a similarly beneficial effect. Only the para mono Br derivative had comparable Y1 affinity to that of the 2,4-dichloro compound **9d**. Some of the 2,4-disubstituted phenyl ethers exhibited approximate additivity of the beneficial effects of their *ortho* and *para* substituents, as exemplified by the dimethyl and dibromo derivatives. Introduction of a second *ortho* substituent, as in the 2,4,6-tribromo and trimethyl derivatives, was clearly detrimental, resulting in a 20- to 30-fold reduction in Y1 affinity. However, some notable deviations from substituent effect additivity were observed. For example, the near 100-fold improvement in affinity (relative to **12a**) that resulted from the 2-hydroxymethyl-4-bromo derivative **12t** (K_i = 15 nM) was significantly greater than would have been predicted from the effects of the individual substituents. Both the corresponding methyl ether **12u** (K_i = 11 nM) and the 2-cyano-4-bromo analog **12v** (K_i = 13 nM) showed similar Y1 affinities. Since **12p** and **12q**, the regioisomers of **12t** and **12v**, respectively, were more than 10-fold less active, we have speculated that the *ortho* substituents in these "anomalously potent" binders may be engaged in a specific H-bond accepting interaction with the receptor, which might also involve the ether oxygen of the $-CH_2O-$ linker.

In order to establish functional Y1 receptor antagonism, two of these compounds were assayed for their ability to reverse NPY-induced inhibition of forskolin-stimulated cyclic AMP production in SK-N-MC cells.[14,15] Both **9d** and **12t** proved to be full antagonists in this assay, having respective K_i's of 4.7 ± 0.3 μM (n = 3) and 1.2 ± 0.3 μM (n = 2).

To summarize, through systematic SAR, the structural requirements for high NPY-1 receptor affinity for a series of BT-derived ligands was determined. Activity at the Y1 receptor proved to be critically dependent on both the linker (-CH_2O-) and phenyl substituents of the C-2 aryloxymethyl side chain. The three most potent 2,4-disubstituted phenyl ethers discovered, **12t**, **12u**, and **12v**, displayed ~10-fold greater Y1 affinity than the corresponding dichloro analog **9d**. These BT-derived NPY antagonists compared favorably to similarly substituted indole[6] and benzimidazole[8] derivatives.



12

Table 3. Binding affinity of Benzothiophenes **12** at cloned human NPY1 receptors.[a]

Compound	R_2	R_4	R_6	K_i(μM)[b] AV12-C2
12a	H	H	H	1.26 ± 0.1
12b	CH_3	H	H	0.42 ± 0.04
12c	Br	H	H	0.45 ± 0.04
12d	I	H	H	0.38 ± 0.05
12e	$CH_2CH{=}CH_2$	H	H	0.98 ± 0.07
12f	CHO	H	H	2.76 ± 0.02
12g[c]	CH_2OH	H	H	0.45 ± 0.03
9c	H	Cl	H	0.31 ± 0.004
12h	H	Br	H	0.16 ± 0.016
12i	H	CH_3	H	0.37 ± 0.007
12j	H	CF_3	H	0.74 ± 0.02
12k	H	Ph	H	3.70 ± 0.36
9d	Cl	Cl	H	0.14 ± 0.002
12l	Br	Br	H	0.12 ± 0.004
12m	CH_3	CH_3	H	0.12 ± 0.014
12n	CMe_3	CH_3	H	2.83 ± 0.62
12o	Br	CH_3	H	0.078 ± 0.007
12p[c]	Br	CH_2OH	H	0.19 ± 0.03
12q	Br	CN	H	0.38 ± 0.01
12r	CH_3	Br	H	0.42 ± 0.005
12s	CF_3	Br	H	0.72 ± 0.02
12t[c]	CH_2OH	Br	H	0.015 ± 0.001
12u	CH_2OMe	Br	H	0.011 ± 0.001
12v	CN	Br	H	0.013 ± 0.001
12w[c]	$CONH_2$	Br	H	0.172 ± 0.016
12x	CH_2NHMe	Br	H	0.66 ± 0.006
12y	CH_2NMe_2	Br	H	2.43 ± 0.4
12z	CH_3	CH_3	CH_3	2.42 ± 0.3
12aa	Br	Br	Br	3.60 ± 0.5

[a] Ref 13. [b] Values represent mean ± SEM (n = 2). [c] Prepared as described in Ref 16.

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16. **12g** was obtained by reduction of **12f** with $NaBH_4$ in EtOH. **12p** was prepared by S_NAr condensation of **8c** with 3-bromo-4-fluorobenzaldehyde (Method a, 25 °C), followed by reduction of the resultant crude aldehyde with $NaBH_4$ in EtOH. **12t** was prepared by S_NAr condensation of **8c** with 5-bromo-2-fluorobenzaldehyde (Method a, 25 °C), followed by reduction of the resultant crude aldehyde with $NaBH_4$ in EtOH. **12w** was prepared by hydrolysis of **12v** with NaOH-H_2O_2 according to the general method described in *Organic Synthesis*; Blatt, A. H., Ed.; John Wiley & Sons, New York, 1943; Coll. Vol. 2, pp 586–587.