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Discovery and SAR of cyclic isothioureas as novel NPY Y_1 receptor antagonists

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ABSTRACT

A class of novel 2-aminobenzothiazoles have been identified as NPY Y_1 antagonists. Various N-heterocyclic substituted aminophenethyl-2-aminobenzothiazole analogs were synthesized to explore the SAR. Isothiourea analogs and ligands with high potency (K_i 30 nM) have been identified.

Neuropeptide Y (NPY), a 36 amino acid polypeptide, is a member of a peptide family that includes peptide YY (PYY) and pancreatic polypeptide (PP).[1] NPY is widely distributed in the central and the peripheral nervous system, and has been implicated in the regulation of food intake, energy metabolism, cardiovascular function, neuronal excitability and endocrine function.[2] There are five NPY receptors that have been cloned (Y_1, Y_2, Y_4, Y_5 and Y_6), and more than one receptor subtype seems to be involved in the regulation of feeding.[3] Among these, NPY Y_1 is thought to be an important major receptor, since various NYP Y_1 antagonists are found to inhibit food intake.[4] Therefore, identification of NPY Y_1 antagonists would have implications in the treatment of obesity. After the identification of BIBP3226, an arginine derivative as the first non-peptide NPY Y_1 antagonist,[5] a number of small molecule NPY Y_1 antagonists, including dihydropyridines,[6] indoles,[7] benzimidazoles,[8] pyrazolopyrimidines,[9] aminopyridines,[10] benzazepines[11] and tetrahydrocarbazoles,[12] have been described. Here we report another novel structural series of selective NPY Y_1 antagonist.

Isothiourea **1a** with a K_i of 248 nM for NPY Y_1 binding was identified through a high throughput screening campaign. Compound **1a** displayed an antagonist profile determined by a functional assay with a K_b of 58.5 nM (Table 3), and was highly selective against NPY Y_2, Y_4, and Y_5 receptors (Table 3). This molecule has a 2-aminobenzothiazole on one end and a five-membered cyclic isothiourea group on the other, bridged with a flexible phenethyl linker (Fig. 1).

Isothiourea **1a**, with its multiple points for solid phase attachment, holds potential for efficient solid phase synthesis. Therefore, we developed multiple mild solid phase methodologies to explore a diverse SAR space that varied both ends of the molecules (**2** and **3**). Therefore, 2-aminobenzothiazole (**5**) was loaded onto *p*-nitrophenol carbonate Wang resin (**4**) to form the resin bound carbamate **6**. The acidity of the benzothiazole amino group, activated by the carbamate linker, is high enough to undergo Mitsunobu alkylation[13] with 2-(*p*-aminophenyl)ethanol to give the resin bound *p*-aminophenethyl-2-aminobenzothiazole **7**. The resin loading level was 0.46 mmol/g based on the recovered *p*-aminophenethyl-2-aminobenzothiazole (**7a**) obtained from resin cleavage using TFA. The resin bound benzoyl thiourea **8**, thiourea **9** and isothiocyanate **10** were obtained by treating **7** with benzoyl isothiocyanate, with Fmoc-isothiocyanate followed by Fmoc de-protection, and with di-2-pyridylthionocarbonate,[14] respectively (see Scheme 1).

We first attempted the replacement of the thiazoline in **1a** with other groups such as amines, amides, ureas or sulfonamides without success (data not shown). We then turned our attention to heterocycles structurally related to the cyclic isothiourea in **1a**, such as imidazolines, imidazolones, thiazoles and 4-thiazolones represented by **11**, **12**, **13** and **14** in Schemes 2 and 3.

Therefore, resin bound benzoyl thiourea **8**, activated by the benzoyl group on thiourea nitrogen, reacted with α-aminoalcohols using EDCI as a coupling reagent to form the corresponding benzoyl guanidines,[15] which were then cyclized under Mitsonobu reaction condition to give the corresponding *N*-benzoyl imidazolines (**11**) after TFA cleavage and purification (Scheme 2). Similarly, when α-aminoesters were used in this scheme, the intermediate benzoyl guanidines readily cyclize after treatment with NaOEt in

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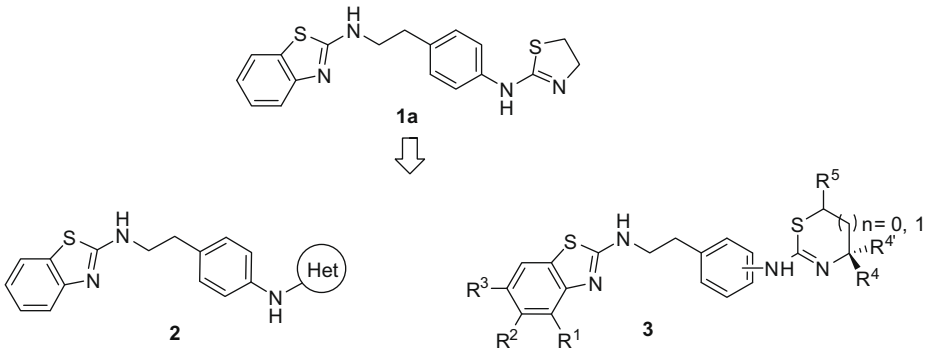
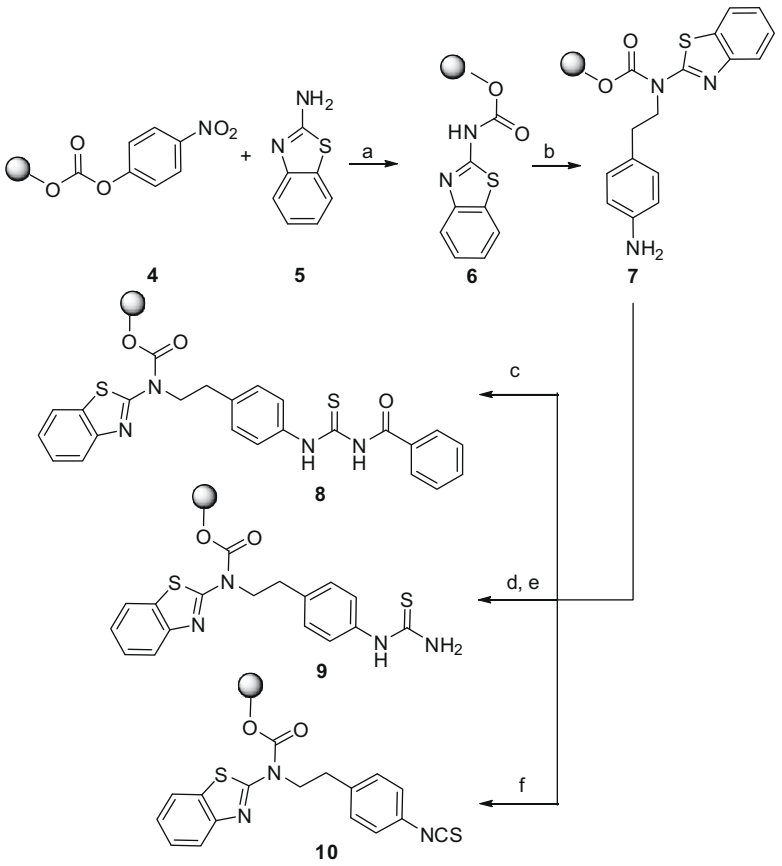
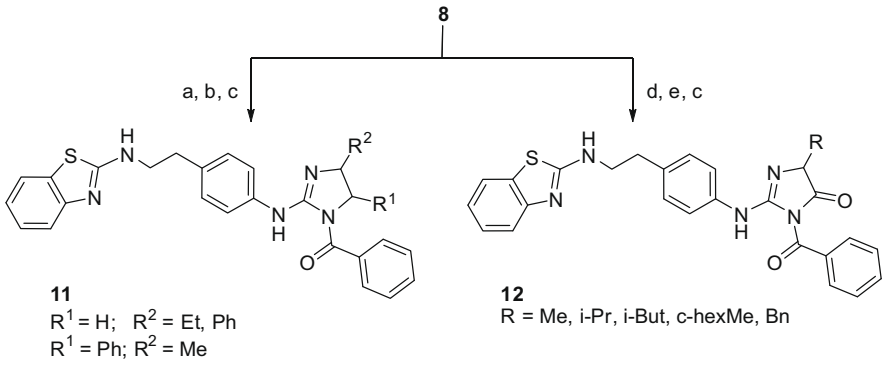


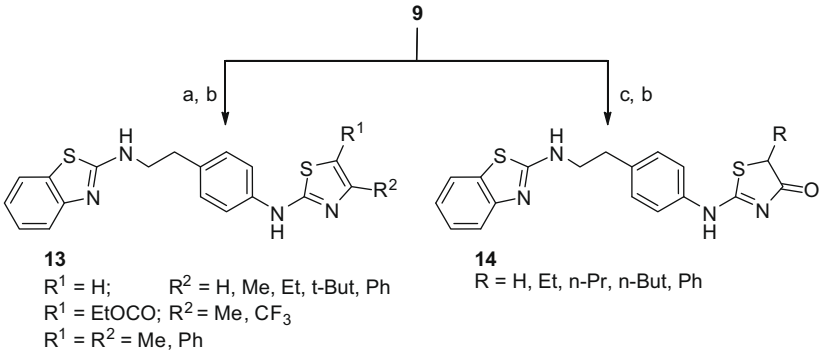
Figure 1. SAR exploration on the basis of the lead **1a**.



Scheme 1. Reagents and conditions: (a) *p*-nitrophenol carbonate Wang resin (**4**)/2-aminobenzothiazole (**5**)/DIEA/60 °C; (b) *p*-aminophenylethanol/ADDP/Ph_3P; (c) benzoyl isothiocyanate; (d) Fmoc isothiocyanate; (e) piperidine; (f) di-2-pyridylthionocarbanate.



Scheme 2. Reagents: (a) α-amino alcohols/EDCI/TEA; (b) Ph_3P/TMAD; (c) TFA cleavage; (d) α-amino acid methyl esters/DCI/TEA; (e) NaOEt.



Scheme 3. Reagents: (a) α-bromoketones/pyridine; (b) TFA cleavage; (c) α-bromoesters/pyridine.

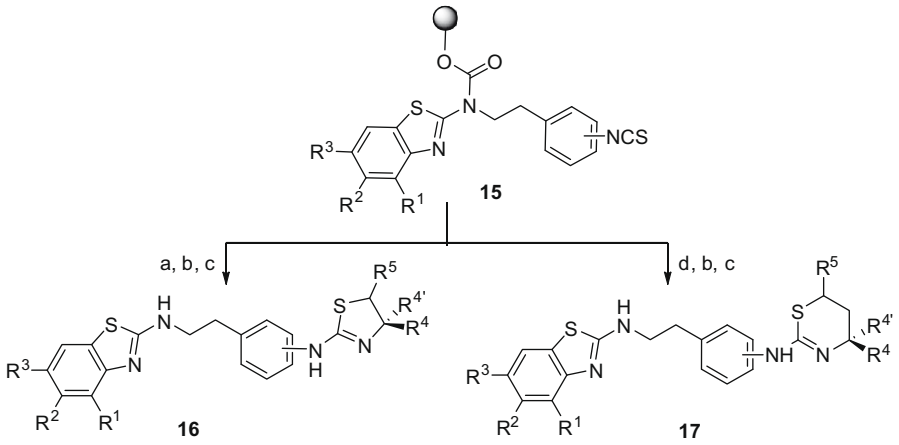
EtOH to give *N*-benzoyl imidazolones (**12**) after TFA cleavage (Scheme 2). Reaction of resin bound thiourea **9** with α-bromoketones and α-bromoesters led to the corresponding thiazoles (**13**) and 4-thiazolones (**14**) after cleavage and purification (Scheme 3).

Resin bound isothiocyanate **10** or **15**, obtained with different aminobenzothiazoles and aminophenethyl alcohols, were converted to hydroxyalkyl thioureas upon treatment of α- and β-aminoalcohols. These resin bound thioureas were cleaved from resin with 20% TFA followed by intramoleclular cyclization in neat TFA by heating[13] to form the corresponding cyclic isothioureas **16** and **17** (Scheme 4).[16]

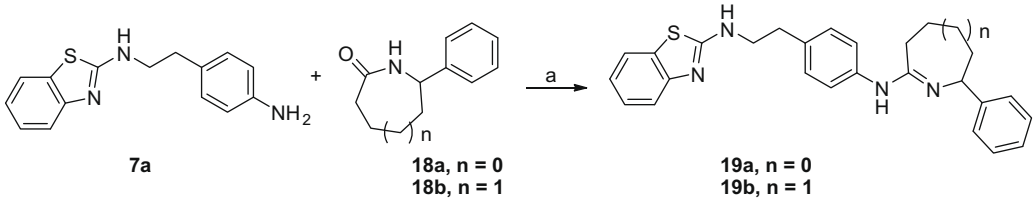
Finally, we replaced the cyclic isothiourea group with cyclic amidines as isosteres (**19a**, **19b**), which have an estimated pK_a range of ~8.[17] These two compounds were accomplished by condensations of intermediate **7a** with **18a** and **18b** (Scheme 5).

All compounds were screened for NPY Y_1 receptor binding affinity[18] and the K_i values or % inh. at 2 μg/mL are shown in Tables 1 and 2. Imidazolines (**11**), imidazolones (**12**), thiazoles (**13**) and 4-thiazolones (**14**) were found to be poor replacements for the cyclic isothiourea in **1a** (data not shown). The substitution pattern of the phenethyl group is important for thiazolines (**20**, **21**) and dihydrothiazines (**23**, **24**) with both *meta*- and *ortho*-positions on the phenyl group giving low NPY Y_1 binding affinity in contrast to the corresponding *para*-substituted analogs **1a** and **22a** (Table 1). Compounds with five or six-member cyclic thioisourea scaffolds were found to have comparable NPY Y_1 binding affinity (**22a,** K_i 221 nM vs **1a,** K_i 248 nM in Table 1). The dihydrothiazine core is a novel pharmacophore having NPY Y_1 activity.

The SAR for the substitutions on the aminothiazole group is shown in Table 1. In the compounds with a thiazoline ring, all substitutions investigated on the aminothiazole seem to reduce the activity, for example, **1b** through **1j** (4–24-fold). Substitution on the aminobenzothiazole seems to be much more tolerated in the six-membered dihydrothiazine ring series with C6-methyl (**22d**), C6-fluoro (**22f**), C6-chloro (**22g**), and C5–C6 dichloro (**22j**) having comparable K_i range (318–616 nM). However, large substituents at C6-position reduced the activity, that is, methoxy (**22e**), nitro (**22h**) and methylsulfonyl (**22i**) groups.

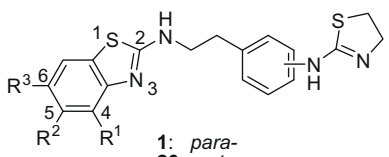


Scheme 4. Reagents and conditions: (a) α-aminoalcohols; (b) 20% TFA cleavage; (c) neat TFA/heat; (d) β-aminoaminoalcohos.

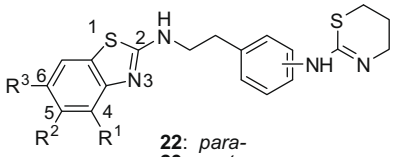


Scheme 5. Reagents and conditions: $TiCl_4$/toluene/DIEA/heat.

Table 1
NPY Y_1 receptor binding affinity of the *para*-, *meta*- and *ortho*-substituted five- and six-member cyclic isothiourea analogs with substituted benzothiazole moieties



1: *para*-
20: *meta*-
21: *ortho*-

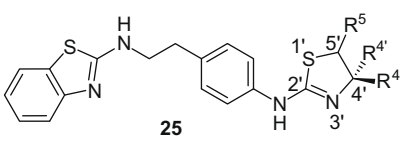


22: *para*-
23: *meta*-
24: *ortho*-

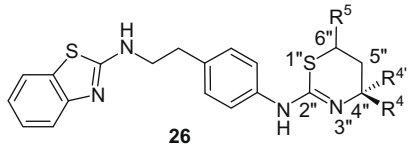
Compound	R^1	R^2	R^3	K_i[a] (nM)	%Inh[b]
1a	H	H	H	248	
1b	Me	H	H	3460	
1c	MeO	H	H	3990	
1d	H	H	Me	1060	
1e	H	H	MeO	1670	
1f	H	H	F	1120	
1g	H	H	Cl	1240	
1h	H	H	O_2N	5250	
1i	H	H	MeO_2S	5920	
1j	H	Cl	Cl	3640	
20	H	H	H		27
21	H	H	H		27
22a	H	H	H	221	
22b	Me	H	H		77
22c	MeO	H	H		71
22d	H	H	Me	616	
22e	H	H	MeO	1250	
22f	H	H	F	318	
22g	H	H	Cl	342	
22h	H	H	O_2N	3300	
22i	H	H	MeO_2S	5200	
22j	H	Cl	Cl	316	
23	H	H	H		34
24	H	H	H		24

[a] NPY Y_1 receptor binding affinity K_i values were determined as described in Ref. 18.
[b] Percentage inhibition at 2 μg/mL concentration of testing compounds.

Table 2
NPY Y_1 receptor binding affinity of the five- and six-membered cyclic isothiourea analogs with substituted aminothiazole/aminothiazine moieties



25



26

Compound	R^4	$R^{4'}$	R^5	K_i[a] (nM)	%Inh[b]
25a	Me	H	H	379	
25b	Et	H	H	153	
25c	*n*-Pr/H[c]	H	482		
25d	*n*-Bu/H[c]	H		69	
25e	*i*-Bu	H	H	712	
25f	Ph	H	H	175	
25g	H	Ph	H		0
25h	H	H	Me		61
25i	H	H	OMe	2550	
25j	H	H	Ph		17
26a	Me/H[c]	H	50		
26b	*i*-Pr/H[c]	H	53		
26c	*i*-Bu/H[c]	H	62		
26d	PhEt/H[c]	H	195		
26e	*c*-Hex/H[c]	H	332		
26f	Ph/H[c]	H	55		
26g	*p*-OMePh/H[c]	H	55		
26h	*p*-ClPh/H[c]	H	122		
26i	*m*-ClPh/H[c]	H	107		
26j	*o*-ClPh/H[c]	H	956		
26k	Ph	H	H	30	
26l	H	Ph	H	464	

[a] NPY Y_1 receptor binding affinity K_i values were determined as described in Ref. 18.
[b] Percentage inhibition at 2 μg/mL concentration of testing compounds.
[c] Racemate.

Table 3
Functional data and selectivity of **1a** and **26k**

	K_b[a] (nM)	K_i[b] (nM)			
		NPY Y_1	NPY Y_2	NPY Y_4	NPY Y_5
1a	58.5	248	>10,000	>10,000	>10,000
26k	15.4	30	>10,000	>10,000	5978

[a] Functional assay K_b values were determined as described in Ref. 19.
[b] Binding affinity K_i values for NPY Y_1, Y_2, Y_4, and Y_5 receptors were determined as described in Ref. 18 using the cells expressing the human NPY Y_1, Y_2, Y_4 and Y_5 receptors, respectively.

Substituent effects on the thiazoline and dihydrothiazine rings are shown in Table 2. At the C4′ position of the five membered thiazoline series (**25**), small aliphatic groups, such as methyl (**25a**) or ethyl (**25b**), and aromatic groups, such as phenyl (**25f**), were tolerated with a range of K_i 153–379 nM, comparable to **1a** (K_i 248 nM). In contrast, the C5′ substitutions, that is, methyl (**25h**), methoxy (**25i**) and phenyl (**25j**), all had much reduced NPY Y_1 binding affinity. The SAR trend in the six-membered dihydrothiazine series (**26**) was quite different. Substitutions at C4″ position, such as methyl (**26a**), isopropyl (**26b**), isobutyl (**26c**) and phenyl (**26f**), all significantly enhanced the NPY Y_1 binding affinity by approximately 4–5-folds with K_i values reaching a range from 50 nM to 60 nM. The substitutions on the 4″-phenyl group on the dihydrothiazine were further investigated. The *ortho* substitution on the C4″-phenyl represented by the *o*-chloro analog **26j** had a ~10-fold decrease in activity with K_i 956 nM compared to its corresponding *p*- and *m*-chloro isomers **26h** (122 nM) and **26i** (107 nM). Eudismic ratio of both thiazolines and dihydrothiazines were found to be quite high (>15-fold) with *R*-enantiomer of C4′-phenyl thiazoline **25f** (K_i 175 nM) and *S*-enantiomer of C4″-phenyl dihydrothiazine **26k** (K_i 30 nM) being the more active enantiomers. Functional antagonism and selectivity against other NPY subtype receptors were further evaluated for compound **26k**. Functionally, **26k** was an NPY Y_1 antagonist, blocking the NPY mediated inhibition of the forskolin-stimulated cAMP accumulation in HEK 293 cells with a K_b of 15.4 nM[19] (Table 3). For comparison, the K_b for **1a** was 58.5 nM. Compound **26k** was highly selective against NPY Y_2, Y_4, and Y_5 receptors. Its K_i values for the Y_2 and Y_4 receptors were >10 μM, and for the Y_5 receptor was 6 μM (Table 3).

The steep SAR observed on the cyclic isothiourea is notable. The aminothiazole (**13**, R_1, R_2 = H) as a replacement of the thiazoline led to complete loss of NPY1 binding affinity, suggesting that a pK_a difference may play a role in activity. We then explored more basic six- and seven-membered cyclic amidines (pK_a ~8)[17] **19a** and **19b** (Scheme 5) as isosteres of cyclic isothioureas (pK_a ~7)[20] thiazoline **25f** and dihydrothiazine **26k.** However, they showed much lower activity with K_i 2.9 μM and 4.6 μM, respectively, despite their structural similarities and their close presentations of the electron density and H-bonding donor/acceptors. Similar scaffolds with further reduced basicity would be of high interest to further probe the SAR.

In conclusion, we have identified a series of novel NPY Y_1 antagonists that bears a cyclic isothiourea group. Multiple efforts in replacement of this group were not successful. Further substitutions on the isothiourea group led to identification of 4*S*-phenyl-dihydro[1,3]thiazine compound (**26k)** as the NPY Y_1 antagonist having the best potency with K_i 30 nM. This antagonist may serve as an important tool for studying the biological functions of NPY Y_1 receptor.

Acknowledgment

Assistance from Mr. Robert Novotny for the LCMS analysis is gratefully acknowledged.

Supplementary data

Supplementary data (competition binding curves: (1) Inhibition of radioligand binding by NPY Y1 receptor antagonists **1a** and **26k** and (2) Inhibition of NPY functional activity by NPY Y1 receptor antagonists **1a** and **26k**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.048.

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13. *Synthesis of* **26k**: A mixture of 2-aminobenzothiazole (**5**, 10 equiv), *p*-nitrophenol carbonate Wang resin (**4**, 1 equiv) and DIEA (6 equiv) in DMF was agitated at 60 °C overnight, followed by treatment of methanol/DBU to cap the unreacted resin as methyl carbonate, to give **6**. This resin bound **6** was treated with aminophenylethanol, PPh_3 and ADDP (5 equiv each) in DCM/THF (1:1) at room temperature overnight to give resin bound **7**, which subsequently reacted with di-2-pyridylthionocarbonate (6 equiv) to give **10** (Scheme 1). This resin bound **10** (300 mg) reacted with (*S*)-3-amino-3-phenyl-1-propanol (5 equiv) in 2 mL DCM at room temperature overnight followed by cleavage with 20% TFA in DCM. The resulting material was treated with neat TFA at 75 °C overnight to give **26k** after purification. ^{1}H NMR ($CDCl_3$, ppm), δ 7.59–7.55 (d, 1H), 7.52–7.48 (d, 1H), 7.42–7.35 (m, 4H), 7.34–7.25 (m, 2H), 7.18–7.11 (m, 4H), 7.09–7.05 (m, 1H), 4.69–4.62 (m, 1H), 3.70–3.60 (m, 2H), 3.12–3.04 (m, 1H), 2.98–2.88 (m, 3H), 2.37–2.26 (m, 1H), 2.05–1.92 (m, 1H). ES-LCMS, *m/z* = 445 $[M+H]^+$.
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18. Human NPY receptor binding assay. Membranes (5–10 μg) from CHO-K1 cells expressing the one of the human NPY receptors were incubated with 0.2 nM [^{125}I] porcine PYY (Y_1, Y_2 and Y_5) or 0.2 nM [^{125}I] human PP (Y_4) and various concentrations of antagonist in a buffer containing 50 mM HEPES, pH 7.2, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1% bovine serum albumen for 90 min at 30 °C. Non-specific binding was defined as binding in the presence of 1 μM human/rat NPY. The reaction mixtures were filtered through Millipore MACF glass fiber filter plates pre-soaked in 0.5% polyethyleneimine. Filters were washed twice with 150 μl of Dulbecco's phosphate-buffered saline (4 °C), and filter-associated radioactivity was measured in a Packard TopCount scintillation counter. The K_i values were calculated using the Cheng–Prussof equation.
19. The functional activity of the compounds was determined using a cAMP assay as described previously.[4b] Briefly, HEK 293 cells expressing the human NPY Y1 receptor were plated at 1.5×10^4 cells/well in 24-well dishes. At confluence, the cells were washed with Hank's balanced salt solution (HBSS) and incubated for 20 min in HBSS supplemented with 4 mM $MgCl_2$, 0.2% bovine serum albumin, 3-isobutyl-1-methyl xanthine (1 mM) and antagonist (1 μM). Forskolin (2.5 μM) and various concentrations of NPY were then added, and the incubation continued for 10 min. Cyclic AMP was extracted from the cell layer using ethanol and measured using a radioimmunoassay (Perkin–Elmer Flash Plate). K_b values were determined using the Schild equation.
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