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Discovery of novel orally active ureido NPY Y5 receptor antagonists

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Abstract—We have derived a novel series of neuropeptide Y (NPY) Y5 receptor antagonists from the biphenylurea **3**. Cyclohexylurea **21c,** a member of the series, is a potent NPY Y5 receptor antagonist that exhibits excellent pharmacokinetic parameters in rats and dogs. On chronic oral administration to diet-induced obese rats, **21c** displayed an anti-obesity profile, causing a modest reduction in food intake, a significant decrease in body weight gain, a decrease in adipose mass, and an increase in lean tissue mass.

Neuropeptide Y (NPY) is a 36-amino acid neuropeptide that is widely distributed in the mammalian central and peripheral nervous systems.[1] Over the last two decades, a large body of knowledge related to the NPY system has accumulated. NPY mediates a number of physiological functions through several G protein-coupled receptor subtypes designated Y1, Y2, Y4, Y5, and y6.[2] Pharmacological studies have implicated the hypothalamic Y5 subtype as a mediator of the effects of NPY on food intake and energy homeostasis.[3] Consequently, over the past decade there have been numerous reports of small molecule NPY Y5 receptor antagonists as potential anti-obesity therapies.[4] Until recently, evaluation of these in various rodent studies has yielded inconclusive and often conflicting results as to the role of the Y5 receptor in food intake and body weight maintenance. The recent report that the Y5 antagonist **1** had significant anti-obesity effects in mice under conditions of diet-induced obesity has provided clarity to this issue.[5] Thus, chronic administration of **1** to diet-induced obese (DIO) mice resulted in suppression of body weight gain, while there was no effect on body weight of lean mice or of $Y5^{-/-}$ mice. Amelioration of the obese state by chronic administration of **1** was shown to result from reduced food intake and increased energy expenditure.[6] Moreover, modest but significant anti-obesity effects of the Y5 antagonist, MK-0557 (**2**), have recently been demonstrated in obese human subjects.[7] Herein we describe the design and SAR of a novel, structurally distinct ureido series of Y5 antagonists, and report the profile of the optimized Y5 antagonist **21c** in DIO rats.

Our first generation lead NPY Y5 antagonist biphenylurea **3** is a high affinity antagonist of human and rat Y5 receptors (human Y5 $K_i = 0.4$ nM, rat Y5 $K_b = 0.4$ nM).[8,9] In rats, oral administration of compound **3** (ED_{50} 0.4 mg/kg) potently blocked food intake induced by centrally

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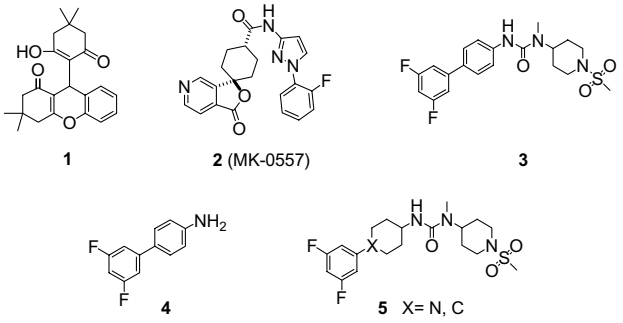
administered D-Trp[34]-NPY, a selective Y5 agonist.[10] However, given the known potent mutagenicity of biphenylamines, the possibility of liberation in vivo of the biphenylamine fragment **4**, a substructure of **3**, posed an unacceptable safety risk. We therefore sought to eliminate this liability by replacing the phenylene linker with a piperidinyl or cyclohexyl ring, represented by structure **5**.



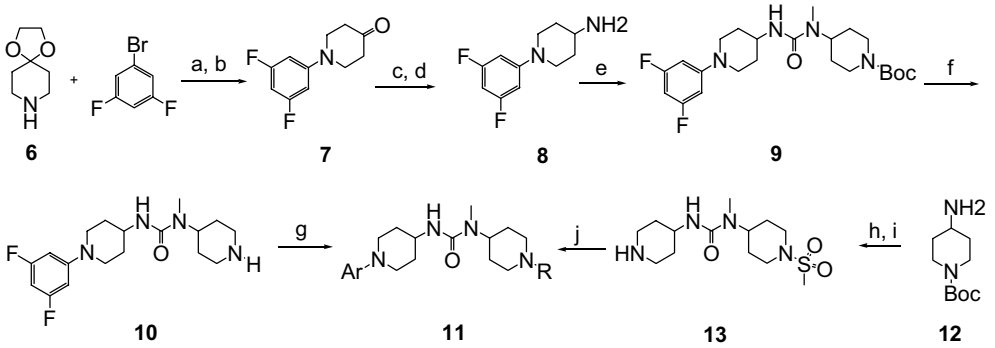
The synthesis of piperidine analogs **11a**–**h** (Scheme 1) commenced from 1,4-dioxa-azaspiro(4,5)decane **6**, which was first *N*-arylated using Buchwald–Hartwig conditions and then subjected to acid hydrolysis of the ketal to give piperidin-4-one **7**. Reductive amination of **7** with benzylamine, followed by reductive cleavage of the benzyl group, gave amine **8**, which was coupled with 1-Boc-4-methylaminopiperidine in the presence of disuccinimidyl carbonate (DSC) in THF to afford the urea **9**. Following removal of the Boc protecting group the resulting amine **10** was derivatized with anhydrides, acyl chlorides or sulfonyl chlorides to provide **11a**–**h** (Table 1). In order to further explore the SAR within this series, we fixed the (4-methylamino)piperidinesulfonamide group and varied the *N*-aryl substituent (compounds **11i**–**z**, Table 1). Thus, Cu(II)-mediated arylation of intermediate **13** with aryl boronic acids afforded targets **11i**–**z** (Scheme 1).[11]

We were encouraged to find that 3,5-difluorophenyl-substituted derivatives **11a**–**h** retained single digit nanomolar Y5 receptor binding affinity (Table 1). While monosubstitution at the 3-position afforded compounds that were generally less potent (**11m**–**s**), these analogs were generally 10–30-fold more potent than the unsubstituted derivative **11i**. The most favorable monosubstitution was 3-bromo, followed by chloro, methyl, cyano, and fluoro. Less well tolerated were 3-methoxy and 3-acetyl, as well as all *para*-substitution. 3,5-Disubstituted analogs afforded the best potency in the series; 3,5-difluorophenyl **11a** and 3,5-dichlorophenyl **11z** were substantially more potent than **11i**. Compounds **11a**–**h** and **11z** were subjected to a rat oral pharmacokinetic screen at a dose of 10 mg/kg and while substantial plasma levels were typically observed, these analogs suffered from poor brain penetration. For example, acetamide **11e** exhibited a plasma $AUC_{0-6\,h}$ of 104 μM.h and a 6 h plasma concentration of 10 μM, however **11e** was not detected in brain extracts from rats 6 h post-dose.

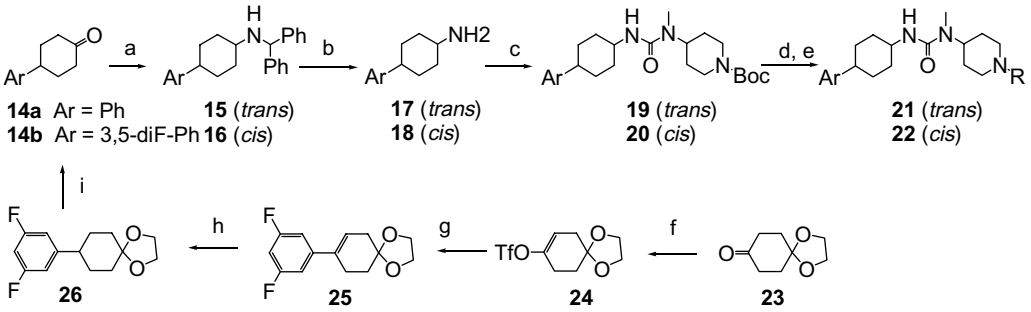
Table 1. Human Y5 binding affinity of compounds **11a**–**z**

Compound	R	Ar	K_i[a] (nM)
11a	CH_3SO_2–	3,5-di-F-Ph	3.2
11b	$EtSO_2$–	3,5-di-F-Ph	2.8
11c	*n*-$PrSO_2$–	3,5-di-F-Ph	3.4
11d	*i*-$PrSO_2$–	3,5-di-F-Ph	3.2
11e	CH_3CO–	3,5-di-F-Ph	7.4
11f	EtCO–	3,5-di-F-Ph	6.3
11g	*n*-PrCO–	3,5-di-F-Ph	5.9
11h	*i*-PrCO–	3,5-di-F-Ph	2.6
11i	CH_3SO_2–	Ph	303
11j	CH_3SO_2–	3-Pyridyl	405
11k	CH_3SO_2–	2-Me-Ph	52
11l	CH_3SO_2–	2-F-Ph	88
11m	CH_3SO_2–	3-F-Ph	35
11n	CH_3SO_2–	3-Cl-Ph	13
11o	CH_3SO_2–	3-Me-Ph	20
11p	CH_3SO_2–	3-Br-Ph	8.8
11q	CH_3SO_2–	3-CN-Ph	27
11r	CH_3SO_2–	3-MeO-Ph	311
11s	CH_3SO_2–	3-Acetyl-Ph	164
11t	CH_3SO_2–	4-F-Ph	799
11u	CH_3SO_2–	4-Cl-Ph	130
11v	CH_3SO_2–	4-Br-Ph	819
11w	CH_3SO_2–	3,4-di-Cl-Ph	48
11x	CH_3SO_2–	3,4-di-F-Ph	312
11y	CH_3SO_2–	3-Cl, 4-F-Ph	160
11z	CH_3SO_2–	3,5-di-Cl-Ph	1.1

[a] Average of two determinations.



Scheme 1. Reagents and conditions: (a) $Pd(OAc)_2$, dppp, $NaOBu^t$, anhydrous toluene, N_2, 90 °C, 16 h, 86%; (b) 5 N HCl, THF, rt, 16 h, 79%; (c) benzylamine, $NaBH(OAc)_3$, DME, 80%; (d) 10% Pd/C, HCO_2H/MeOH, 83%; (e) DSC, Py, THF, 0 °C, then 1-Boc-4-methylaminopiperidine, 80%; (f) 10% TFA/CH_2Cl_2, 86%; (g) RCOCl, Et_3N, 86–96% or RSO_2Cl, Et_3N, 70–84%; (h)DSC, Py, THF, 0 °C, then 1-methanesulfonyl-4-methylaminopiperidine, 58%; (i) 4 N HCl/dioxane in CH_2Cl_2, 88%; (j) $ArB(OH)_2$, Et_3N, $Cu(OAc)_2$, CH_2Cl_2, yields range from 7% to 26%.



Scheme 2. Reagents and conditions commencing with **14a**: (a) $NaBH(OAc)_3$, Ph_2CHNH_2, DME, 82%; (b) 10% Pd/C, HCO_2H/MeOH, 84%; (c) DSC, Py, THF, 0 °C, then 1-Boc-4-methylamino-piperidine in THF, 88%; (d) 4 N HCl in dioxane, rt, 96%; (e) $(RCO)_2O$, Et_3N, 77–84% or RSO_2Cl, Et_3N, CH_2Cl_2, 0 °C, 45–66%; (f) LDA, followed by $PhNTf_2$ in THF, −78 °C, 92%; (g) 3,5-difluorophenylboronic acid, $Pd(PPh_3)_4$, LiCl, Na_2CO_3, DME/H_2O, 55%; (h) 10% Pd/C, H_2; (i) TFA/CH_2Cl_2/H_2O, 64%.

To examine the cyclohexylene linker as a replacement for the phenylene of biphenylurea **3**, 4-phenylcyclohexanone **14a** (Scheme 2) was subjected to reductive amination with benzhydrylamine and sodium triacetoxyborohydride which gave a 1:3 mixture of *trans* and *cis* isomers **15** and **16** (Ar = Ph), which were readily separable. Each of the isomers was transformed into the *trans* and *cis* targets **21a**,**b** and **22a**,**b** (Table 2), respectively. The *trans* isomers were at least 10-fold more potent than the *cis* isomers, presumably because the topology of *trans* diequatorial 1,4-cyclohexyl substituents more closely resembles that of the biphenyl urea **3**.

To identify cyclohexyl analogs with improved Y5 receptor affinity, 3,5-difluorophenyl substitution was incorporated. The requisite 4-(3,5-difluorophenyl)-cyclohexanone **14b** was synthesized (Scheme 2) via Suzuki coupling of 3,5-difluorophenylboronic acid with enol triflate **24** derived from cyclohexanone **23**. Hydrogenation of the styrene **25** to give **26**, followed by ketal hydrolysis, afforded **14b** which were converted to the *trans* 3,5-difluorophenylcyclohexyl targets (**21c**–**h**, Table 2).

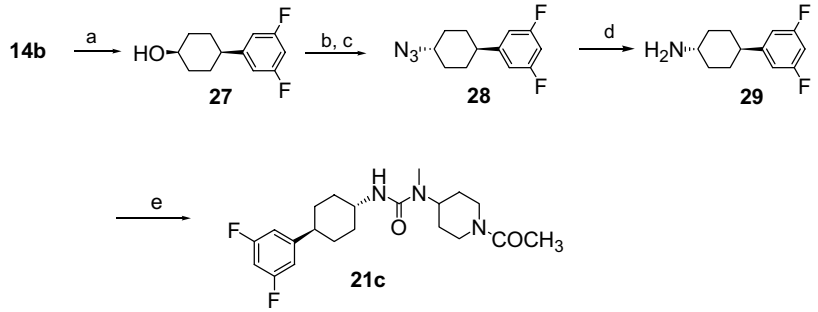
Table 2. Structure–activity relationship of **21a**–**h** and **22a**–**b**

Compound	Ar	R	Y5 K_i[a] (nM)
22a	Ph	CH_3CO–	>1000
22b	Ph	CH_3SO_2–	500
21a	Ph	CH_3CO–	59
21b	Ph	CH_3SO_2–	52
21c	3,5-di-F-Ph	CH_3CO	1.8
21d	3,5-di-F-Ph	EtCO–	1.5
21e	3,5-di-F-Ph	*n*-PrCO	2.0
21f	3,5-di-F-Ph	CH_3SO_2–	1.2
21g	3,5-di-F-Ph	$EtSO_2$–	0.75
21h	3,5-di-F-Ph	*n*-PrCO–	2.4

[a] Average of two determinations.

Trans-cyclohexyl analogs with 3,5-difluorophenyl substitution were uniformly more potent at the Y5 receptor compared to the non-fluorinated analogs (Table 2). Of the analogs prepared, Y5 antagonist **21c** offered an attractive overall profile with respect to Y5 receptor binding affinity, functional activity, and pharmacokinetic properties, and was selected for additional profiling. In order to provide gram quantities of **21c** for further in vivo studies, we developed an efficient stereoselective synthesis (Scheme 3). Reduction of **14b** with L-Selectride at −78 °C produced the *cis*-alcohol **27** in 78% yield. Mesylation of **27** followed by S_N2 displacement with NaN_3 gave *trans* azide **28** in 71% yield. Reduction of **28** with tin(II) chloride quantitatively afforded *trans*-4-(3,5-difluorophenyl)cyclohexylamine **29** which was then coupled with 1-acetyl-4-methylaminopiperidine in the presence of DSC to provide **21c** in 70% yield.

Compound **21c** has high affinity for the Y5 receptor (human Y5 K_i = 1.8 nM), negligible affinity for human Y1, Y2, and Y4 receptors (K_i > 10,000 nM), and is a functional antagonist of the rat Y5 receptor (K_b = 2.7 nM). In counterscreening assays, **21c** did not significantly cross-react with a panel of 60 receptors, enzymes, and



Scheme 3. Reagents and conditions: (a) L-Selectride, THF, −78 °C, 78%; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C–rt; (c) NaN_3, DMSO, 40 °C, 71% for two steps; (d) $SnCl_2$ $2H_2O$, THF/H_2O; (e) DSC, Py, THF, 0 °C, then 1-acetyl-4-methylaminopiperidine in THF, 70% for two steps.

ion channels (<50% inhibition of binding at 10 μM).[12] In rats, **21c** has excellent oral bioavailability (%F = 100), a brain/plasma ratio of 0.3, and a relatively short IV $t_{1/2}$ of 1.4 h. The half-life of **21c** is considerably longer in beagle dogs (IV $t_{1/2}$ = 24 h) and bioavailability remained high in this species (%F = 94).

In vivo, oral administration of **21c** blocked D-Trp[34]NPY-induced feeding with an ED_{50} of 2.3 mg/kg, and did not antagonize food intake induced by centrally administered galanin, consistent with a Y5-specific mechanism for inhibition of feeding. Compound **21c** administered orally to DIO rats for 28 days caused a dose-dependent reduction in food intake that reached statistical significance at the 10 mg/kg/day dose (Fig. 1a).[13] Administration of **21c** also caused a dose-dependent reduction in body weight gain at the 3 and 10 mg/kg doses that was significantly different from the control group (Fig. 1b). Analysis of body composition by DEXA scanning demonstrated a dose-dependent decrease in adipose mass, while lean body mass increased (Fig. 1c). In contrast, control animals gained adiposity and exhibited no change in lean mass over the course of the study. The significant increase in lean mass that accompanied the decrease in body mass at the 10 mg/kg/day dose is presumably a consequence of enhanced fat utilization as energy source, and partitioning of nutrients to lean tissue. This highly interesting observation indicates that measurement of body weight alone does not adequately reflect underlying changes in body composition over the course of treatment.

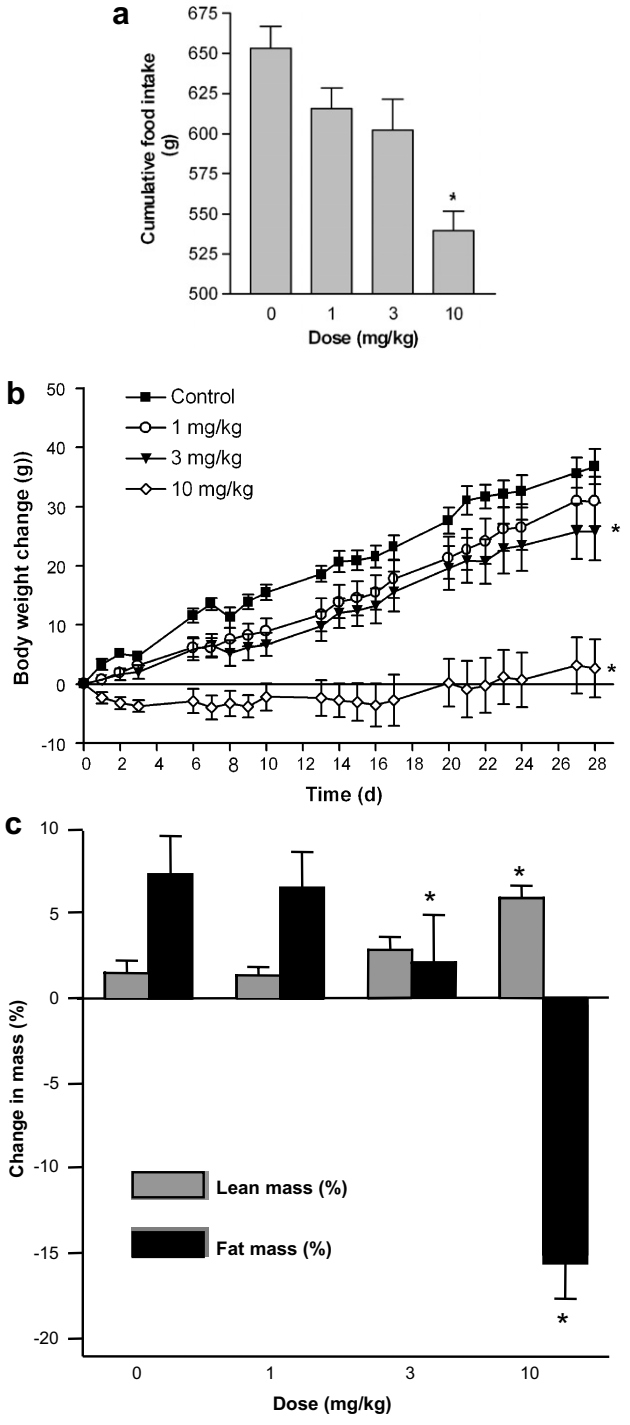


Figure 1. (a) Food intake of DIO rats dosed with **21c** for 28 days. (b) Body weight change of DIO rats dosed with **21c** for 28 days. (c) % Change in fat and lean mass of DIO rats dosed with **21c** for 28 days.

In summary, a series of novel and potent cyclohexylureido NPY Y5 receptor antagonists have been discovered by modifying a biaryl urea lead. The cyclohexylurea **21c** displayed excellent pharmacokinetic properties and produced significant inhibition of food intake and body weight gain in a 28 day DIO rat model at an oral dose of 10 mg/kg/day. This corresponded to a significant decrease in adipose mass and increase in lean mass over the course of the study. The present study contributes to a growing body of evidence demonstrating that significant Y5 mechanism-specific anti-obesity effects can be obtained with chronic administration of Y5 antagonists to obese mammals, and that in DIO rats, through either neuronal or hormonal mechanisms that result from Y5 receptor blockade, these effects are not limited to adipose tissue alone.[5–7]

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