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Discovery of Lu AA33810: A highly selective and potent NPY5 antagonist with in vivo efficacy in a model of mood disorder

Mathivanan Packiarajan [a,*], Mohammad R. Marzabadi [a], Mahesh Desai [a], Yalei Lu [a], Stewart A. Noble [a], Wai C. Wong [a], Vrej Jubian [a], Gamini Chandrasena [a], Toni D. Wolinsky [b], Hualing Zhong [b], Mary W. Walker [b], Ove. Wiborg [c], Kim Andersen [d]

[a] Department of Chemical & Pharmacokinetic Sciences, Lundbeck Research USA, 215 College Road, Paramus, NJ 07652, USA
[b] Synaptic Transmission, Disease Biology Unit, Lundbeck Research USA, 215 College Road, Paramus, NJ 07652, USA
[c] Center for Psychiatric Research, Aarhus University Hospital, Aarhus, Denmark
[d] Neuroscience Drug Discovery DK, H. Lundbeck A/S, Ottiliavej 9, Denmark 2500 Copenhagen Valby, Denmark

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Chronic mild stress (CMS)

ABSTRACT

The structure–activity relationship of a series of tricyclic-sulfonamide compounds **11**–**32** culminating in the discovery of *N*-[*trans*-4-(4,5-dihydro-3,6-dithia-1-aza-benzo[e]azulen-2-ylamino)-cyclohexylmethyl]-methanesulfonamide (**15**, **Lu AA33810**) is reported. Compound **15** was identified as a selective and high affinity NPY5 antagonist with good oral bioavailability in mice (42%) and rats (92%). Dose dependent inhibition of feeding was observed after i.c.v. injection of the selective NPY5 agonist ([cPP$^{1-7}$,NPY$^{19-23}$, Ala^{31},Aib^{32},Gln^{34}]-hPP). In addition, ip administration of **Lu AA33810** (10 mg/kg) produced antidepressant-like effects in a rat model of chronic mild stress.

Neuropeptide Y (NPY) is a 36 amino acid neuropeptide belonging to the pancreatic polypeptide family. NPY is widely distributed throughout the central and peripheral nervous systems[1] and has been demonstrated to modulate numerous physiological processes such as: appetite,[2–4] metabolism, and mood.[5] It exerts its biological effects via interaction with a family of specific membrane bound GPCR's. A total of five receptor subtypes have been cloned and pharmacologically characterized (NPY Y1, Y2, Y4, Y5, and Y6). The stimulation of feeding behavior by NPY is thought to occur in part through activation of the hypothalamic NPY5 receptors.[6,7] Therefore, antagonists of the NPY5 receptor were considered to be potentially useful in controlling appetite. The NPY5 receptor is also expressed in limbic regions[8] which raises the possibility that it could play a role in mood disorders as well. Recently two NPY5 antagonists from Merck and Shionogi, MK-0577[9a] and Velneperit,[9b] respectively, have advanced to human clinical trials. Both compounds decreased body weight relative to placebo.

The starting point for the medicinal chemistry effort described herein was the weakly potent NPY receptor antagonist lead benextramine (compound **1**,[10a,b] Fig. 1).

Benextramine **1** displays non-selective micromolar binding to the NPY1 (K_i = 1.8 μM) and NPY5 receptors (K_i = 5 μM). In early lead discovery efforts (data not shown), it was found that clipping the molecule in half, via disulfide disconnection and capping with

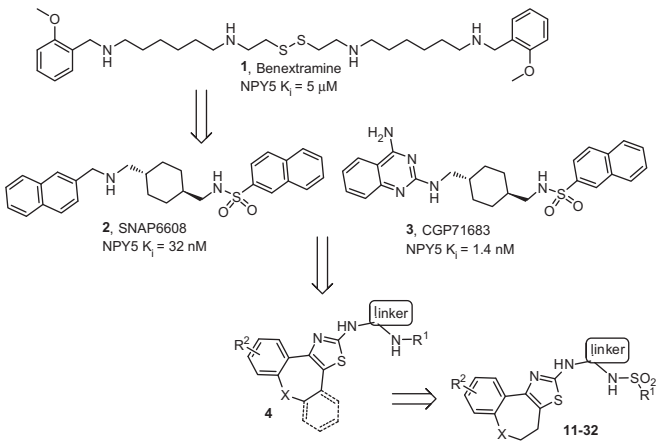

Figure 1. The evolution of tricyclic NPY5 antagonists.

* Corresponding author. Tel.: +1 201 350 0313; fax: +1 201 261 0623.
E-mail address: matp@lundbeck.com (M. Packiarajan).

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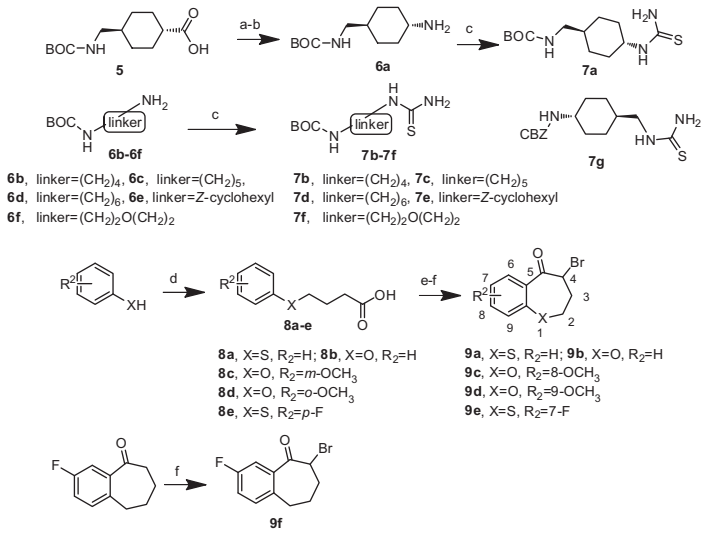
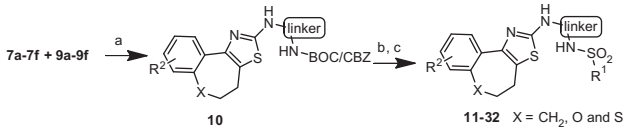


Scheme 1. Reagents and conditions: (a) $(PhO)_2P(O)N_3$, NEt_3, BnOH; (b) H_2/Pd, EtOAc, EtOH, RT, overnight, 94% for two steps; (c) PhC(O)NCS, RT, overnight, 74% then K_2CO_3, MeOH, RT, overnight, 95%; (d) dihydrofuran-2(3H)-one, NaOMe, MeOH, reflux, overnight; (e) PPA, 100 °C, overnight, 52%, and (f) Br_2, HBr in AcOH, AcOH, three steps >80%.

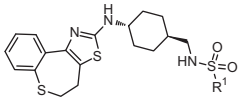
an aryl group, retained micromolar receptor affinity. It was also found that the length and the rigidity of the linker between the amines greatly influenced NPY5 binding. Furthermore, replacement of the amine with a sulfonamide significantly enhanced NPY5 receptor binding. The initial optimization efforts resulted in the discovery of the high affinity NPY5 receptor antagonists **2**, **SNAP6608**[10c] (NPY5 K_i = 32 nM) and **3**, **CGP71683**[11] (NPY5 K_i = 3 nM). Compound **2** was found to be a subtype-selective NPY5 antagonist and did not display cross-reactivity when tested against a panel of 18 GPCR targets. However, several issues were identified with the compound (high in vitro clearance, low solubility and potent CYP3A4 inhibition) likely due to the high lipophilicity (*c* Log *P* = 6.7). Conversely, compound **3** was shown to be selective among the NPY subtypes. However, **3** was not selective against a panel of 24 GPCRs and ion channels (serotonin uptake K_i = 6.2 nM and muscarinic receptor K_i = 2.7 nM) and was thus unusable as an NPY5 antagonist tool compound.[12] We attributed the cross-reactivity issues to the aminoquinazoline group of



Scheme 2. Reagents and conditions: (a) DIEA, EtOH, reflux, overnight, 84%; (b) TFA, 60% if BOC, or H_2/Pd, EtOAc, EtOH, RT, overnight if CBZ and (c) RSO_2Cl, DIEA, CH_2Cl_2, 4 h, RT, 60-85%.

compound and high lipophilicity (*c* Log *P* = 5.91). SAR studies related to chemotype **2** and **3** results tetracyclic compounds **4** with high NPY5 affinity.[13] Optimization of tetracyclic scaffold **4** led to identification of dihydrobenzo[2,3]thiepine analogs (**11**–**32**). Herein we describe our efforts to optimize a tricyclic dihydrobenzo[2,3]thiepine sulfonamide series[13,14] (Fig. 1, Scheme 2) leading to the discovery of the selective NPY5 antagonist **15** suitable for use in behavioral models of mood disorders.

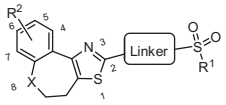
Table 1
The in vitro NPY5 affinity and selectivity profiles of compounds **11**-**16**

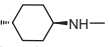
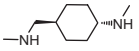
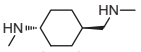
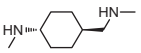
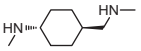


Compound	R^1	hNPY5 K_i (nM)[11,12,14]	hNPY5 IC_{50} (nM)[11,12,14]	hNPY1,2,4 IC_{50} (nM)[11,12,14]
11		4.9 ± 1.1	34.6 ± 2.7	>1000
12		13.9 ± 2.9	43.6 ± 3.7	ND
13		21.5 ± 5.3	10.4 ± 1.3	ND
14		1.9 ± 0.3	3.8 ± 0.5	>1000
15	CH_3	1.5 ± 0.1	1.8 ± 0.2	>1000
16	CH_2CH_3	2.8 ± 0.8	30.8 ± 0.3	>1000

For a description of the pharmacological assays (K_i, IC_{50} and hNPY1,2,4 cross-reactivity assays) see Refs. 11,12,14 The K_i and IC_{50} values are reported as an average ± SEM of n ⩾3 determinations. hNPY5 = human cloned NPY5 receptor; ND = not determined.

Table 2
SAR of tricyclic aminothiazole with variations of R^2, X and R^1 groups



Compound	R^2	X	-Linker-	R^1	hNPY5 K_i (nM)[11,12,14]	hNPY5 IC_{50} (nM)[11,12,14]
17	5-F	C	$-HN(CH_2)_5NH-$	CH_3	5.5 ± 0.6	2.3 ± 0.7
18	5-F	C	$-HN(CH_2)_5NH-$	*i*-Pr	46 ± 19	98.7 ± 13.8
19	5-F	C	$-HN(CH_2)_5NH-$	CH_3	1.4 ± 0.3	0.9 ± 0.2
20	5-F	C	$-HN(CH_2)_5NH-$	CH_2CF_3	7.5 ± 0.7	9.9 ± 1.4
21	5-F	C	$-HN(CH_2)_5NH-$	CH_2CH_3	0.9 ± 0.4	1.3 ± 0.3
22	H	S	$-HN(CH_2)_5NH-$	CH_3	8.8 ± 1.5	5.0 ± 0.8
23	H	S	$-HN(CH_2)_5NH-$	CH_2CH_3	2.5 ± 0.8	1.3 ± 0.3
24	H	S	$-HN(CH_2)_5NH-$	CH_2CH_3	0.8 ± 0.2	0.6 ± 0.1
25	5-F	C		CH_3	27 ± 3	8.8 ± 1.5
26	5-F	C	$-HN(CH_2)_4NH-$	CH_2CF_3	3.4 ± 0.5	1.9 ± 0.4
27	5-F	C	$-HN(CH_2)_2O(CH_2)_2NH-$	*i*-Pr	2.4 ± 0.5	ND
28	6-OCH_3	O		CH_3	6.7 ± 2.1	ND
29	7-OCH_3	O		CH_3	1.1 ± 0.3	ND
30	5-F	S	$-HN(CH_2)_5NH-$	CH_3	1.7 ± 0.4	ND
31	5-F	C		CH_3	1.1 ± 0.3	ND
32	H	O		CH_3	16.1 ± 3.0	3.9 ± 0.9

The K_i and IC_{50} values are reported as average ± SEM of $n \geqslant 3$ determinations. hNPY5 = human cloned NPY5 receptor; ND = not determined.

The synthesis of tricyclic sulfonamide analogs **11**–**32** and their intermediates are outlined in Schemes 1 and 2. The thiourea intermediates **7a**–**7f** and the α-haloketone analogs **9a**–**9f** were synthesized from either commercially available or custom-synthesized starting materials as shown in Scheme 1. Curtius rearrangement[15] of an *N*-protected amino acid **5** followed by hydrogenolysis gave mono-protected diamine **6a**. This was readily transformed to the thiourea **7a** via the reaction with benzoylisothiocyanate and subsequent methanolysis. Reversing the sequence of the deprotection of the Curtius rearrangement product from *N*-protected amino acid **5** using trifluoroacetic acid gave benzyl (trans 4-(aminomethyl)cyclohexyl)carbamate which reacted with benzoylisothiocyanate followed by methanolysis to afford compound **7g**. Similarly, commercially available mono *N*-Boc protected diamines **6b**–**6f** were readily manipulated to give the analogous *N*-Boc protected thiourea intermediates **7b**–**7f.**

The α-haloketone analogs **9a**–**9f** were synthesized in a sequence of three steps in >80% yields. The reaction between a substituted phenol or a substituted thiophenol with dihydrofuran-2(3)-one gave the carboxylic acid intermediates **8a**–**8e**. Acid catalyzed intramolecular cyclization of compounds **8a**–**8e** afforded the fused cyclic ketones which were brominated to produce the corresponding α-bromoketone compounds **9a**–**9e**.[14] Similarly the compound **9f** was prepared by bromination of the commercially available 8-fluoro benzosuberone.

The compounds **11**–**32** were prepared as outlined in Scheme 2. A condensation reaction between α-haloketone compounds **9a**–**9f** and N-BOC- or N-CBZ-protected thiourea linker analogs **7a**–**7f** produced aminothiazole intermediates **10**.[14] Deprotection of the amino group followed by reaction with an appropriate sulfonyl chloride generated compounds **11**–**32** in moderate to good yields.

The SAR of the sulfonamide R^1-group, depicted in Table 1, was investigated keeping the tricyclic template and cyclohexyl linker constant. Simple aliphatic substituents (**15**–**16**) were well tolerated. The naphthyl group (**12**) and polar imidazole (**13**) analogs yielded compounds with reduced NPY5 affinities. However, low nanomolar affinities were realized with substituted phenyl isosteres. The NPY cross-reactivities at NPY1, NPY2, and NPY4 receptors were periodically checked, and in general, excellent NPY subtype selectivity was observed (Table 1). Compounds **11**–**32** were also found to behave as antagonists in a FLIPR based calcium mobilization assay (Tables 1 and 2). The R^1 substituent was limited to small alkyl groups as we examined the rest of the SAR including: different linkers; X substitution; and the R^2 group. This work is summarized in Table 2.

It was shown that optimal affinity could be achieved with compounds having a four to six carbon linker, in combination with a set of small R^1 (CH_3, Et, *i*-Pr, CH_2CF_3) and R^2 (H, F, OCH_3) groups (compounds **17**–**32**). Reversal of the attachment points of the cyclohexyl linker in compound **28** and placement of an *O*-heteroatom in the linker, compound **27**, was also well tolerated. All isosteres where X = S, C or O were essentially equipotent at the NPY5 receptor. The SAR of analogs **11**–**32** is summarized in Figure 2.

In order to triage compounds for the next tier of experiments, several compounds that displayed reasonable NPY5 affinities were screened in human and rat microsomal preparations and in single dose pharmacokinetic experiments to determine brain and plasma levels (10 mg/kg, po). Compound **15** displayed the best overall profile, with moderate rat in vitro clearance and reasonable brain/plasma levels at 4 h (Table 3) and was chosen for detailed analysis.

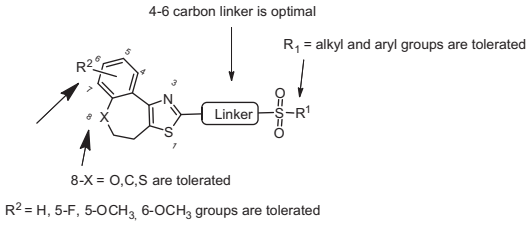


Figure 2. SAR summary.

Table 3
Selected compounds in vitro metabolic clearance and rat plasma and brain exposure data with calculated properties

Compound	hCl_{int}[a] (L/min)	rCl_{int}[a] (mL/min)	[Plasma][b] (ng/mL)	[Brain][c] (ng/g)	MW (g/mole)	*c* Log *P*
14	6.9	120	8	10	491.7	6.0
15	2.5	40	320	460	423.6	4.3
23	ND	ND	14	0	425.6	5.0
24	ND	ND	2	2	411.6	3.9
30	6.8	89	61	140	415.6	4.0

[a] The hCl_{int} and rCl_{int} are human (L/min) and rat (mL/min) intrinsic clearances, respectively, and the clearances were determined according Obach et al.,[16] The rat and human maximum liver blood flow corresponds to 20 mL/min and 1.5 L/min, respectively.
[b] The single dose plasma PK was assessed at 10 mg/kg po in two animals at 4 h with averaged values are shown. Compound limit of quantification (LOQ) is 2 ng/mL.
[c] The brain exposures were determined at the conclusion of the experiment at 4 h and the compound limit of quantification (LOQ) in the brain homogenate was 2 ng/g..

Table 4
In vivo PK properties of **Lu AA33810**[19]

Species	%*F*	CL_p (L/h/kg)	C_{max}	T_{max} (h)	V_{ss} (L/kg)	$T_{1/2}$ (h)
Rat	92	1.1	288	1.9	3.3	6.3
Mouse	42	0.3	126	1.3	3.6	8.2

%*F* = absolute oral bioavailability; CL_p = plasma clearance (L/h/kg); C_{max} = maximum plasma concentration; T_{max} = time to reach maximum plasma concentration (h); V_{ss} = volume of distribution at steady state (L/kg); $T_{1/2}$ = plasma half-life (h).

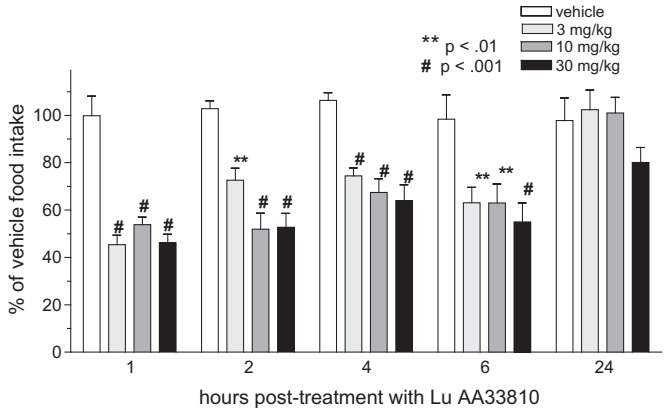


Figure 3. Effects of **Lu AA33810** on 1 h food intake in response to cPP.[20] Results are presented as mean 1-h food intake (g) ± SEM from 16–20 animals per group. Data for each time point were expressed as percent of food intake relative to the vehicle + cPP group and analyzed with one-way analysis of variance. The Newman–Keuls test was used for post-hoc analysis.

Compound **15** (**Lu AA33810**) displayed high selectivity when tested against a panel of 70 GPCR, transporter, channel and enzyme targets, having closest cross-reactivity for $h5HT_{2B}$ (K_i = 245 nM) and $h5HT_{1A}$ (K_i = 478 nM). The hERG IC_{50} of compound **15** was determined to be >10 μM in a patch clamp cellular assay. Plasma protein binding (PPB) data showed compound **15** was highly bound, 99.4% and 98.2% in rat and human, respectively.

Lu AA33810 exhibited moderate inhibition of Cytochrome P450 CYP3A4 (IC_{50} = 4.1 μM) and was inactive against CYP1A2, CYP2C9, CYP2C19 and CYP2D6 (IC_{50} >10 μM). Caco-2[17] permeability studies with compound **15** show good intrinsic permeability ($Papp_{[A-B]}$ = 36.7 cm/s $*$ 10^{-6}) and low potential for Pgp substrate liability ($Papp_{[B-A]}$ = 40.4 cm/s $*$ 10^{-6}, B–A/A–B efflux ratio = 1.1). Compound **15** exhibited acceptable PK properties in rats and mice (Table 4). The optimal rodent pharmacokinetic profile, selectivity and good brain penetration, rendered **Lu AA33810** a suitable tool compound for further in vivo studies. **Lu AA33810** did not inhibit food intake in rats following a 24 h starvation period, consistent with similar reports for other NPY5 antagonists. However, it produced a dose dependent inhibition of centrally induced feeding caused by the intracerebrovascular (i.c.v.) injection of the NPY5 selective agonist ([cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]-hPP) or (cPP) (0.75 nmole in 0.9% saline) with an MED of 3 mg/kg po (Fig. 3). These initial findings prompted a more extensive in vivo screening of **Lu AA33810.** A summary of in vivo experiments that culminated in the finding of efficacy with implications for mood disorders is described elsewhere.[18]

Significant inhibition of cPP-induced feeding by **Lu AA33180** occurred with pretreatment intervals of 1, 2, 4, and 6 h, but not at 24 h. The brain and plasma levels at each dose and time point are shown in Table 5. No detectable brain and plasma levels were observed at the 24 h time point consistent with the behavior. The lowest estimated free brain levels (~0.4 nM, rat PPB = 99.4%) corresponding with efficacy are consistent with the rat Y5 potency (K_i = 1.4 nM).

The effect of **Lu AA33810** in the chronic mild stress (CMS) test of sucrose drinking was examined (Fig. 4). CMS model is considered to be a model of reward deficit or anhedonia and may also capture motivational deficit or apathy. In this model, rats are exposed to chronic stress (or non-stressful conditions) over a 5 week period and sucrose consumption is measured at weekly intervals. Stress-sensitive animals show a reduction in sucrose consumption compared to nonstressed animals. Drug administration is then initiated and chronic stress is continued, with weekly sucrose consumption measurements for a total of four weeks. Chronic stress is associated with a robust decrease in sucrose drinking in approximately 60% of animals, which are then selected for drug testing throughout the remaining portion of the study. Drug-responders are defined as stress-sensitive animals for which drug treatment is associated with an increase sucrose drinking greater >10%.

Table 5
Rat plasma and brain exposure data of **Lu AA33810** from dose dependent food intake study

Dose	3 mg/kg		10 mg/kg		30 mg/kg	
Time (h)	[Plasma] (ng/mL)	[Brain] (ng/g)	[Plasma] (ng/mL)	[Brain] (ng/g)	[Plasma] (ng/mL)	[Brain] (ng/g)
1	54 ± 24	54 ± 24	159 ± 3	179 ± 32	267± 42	288 ± 24
2	87 ± 38	87 ± 38	260 ± 58	244 ± 54	337 ± 178	319 ± 81
4	52 ± 22	52 ± 22	141± 8	172 ± 32	519 ± 185	643±312
6	30 ± 36	30 ± 36	46 ± 38	124 ± 46	181± 305	401±181
24	0	0	0	0	0	0

Plasma (ng/ml)) and brain (ng/g tissue) concentration (mean ± SD) after 3, 10 and 30 mg/kg po dosing in 20% HP-β cyclodextrin suspension.

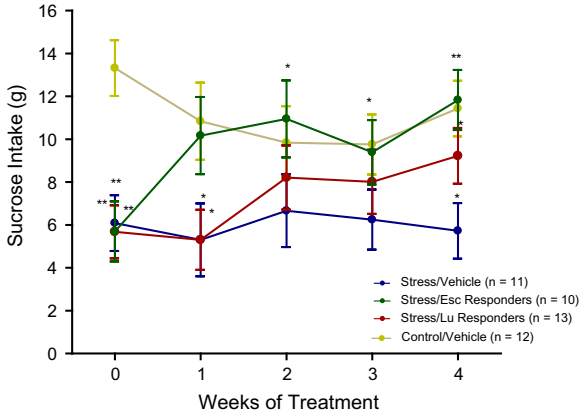


Figure 4. Effect of **Lu AA33810** in the chronic mild stress study in rats.[21] Effects of chronic treatment with vehicle (1 ml/kg), escitalopram (Esc, 5 mg/kg), or **Lu AA33810** (Lu, 10 mg/kg) on the consumption of 1% sucrose solution in rats exposed to chronic mild stress.

Peripheral administration of escitalopram (Esc, 5 mg/kg ip twice a day) significantly increased sucrose consumption in drug-responders after 1 week of dosing (Fig. 4) with a sustained effect though week 4. Similarly, peripheral administration of **Lu AA33810** (Lu, 10 mg/kg ip twice a day) significantly increased sucrose drinking in drug responders with a significant effect after 2 weeks and a sustained effect through week 4 (Fig. 4). These results for **Lu AA33810** are in good agreement with results from an earlier chronic mild stress study performed using a different experimental paradigm[18] and further implicate a potential role for Y5 in modulation of stress sensitivity.

In summary, template hopping and optimization of the known NPY5 leads resulted in the discovery of compound **15**, **Lu AA33180**, (methanesulfonamide,-[[trans-4-[(4,5-dihydro[1] benzothiepino[5,4-d]thiazol-2-yl)amino]cyclohexyl]methyl]-sulfonamide). **Lu AA33180** has a good PK profile in rats and mice with acceptable CNS exposure. **Lu AA33180** showed an oral dose dependent inhibition of a centrally induced cPP feeding effect but no significant inhibition of feeding in a 24 h rat starvation model. In the CMS study, **Lu AA33810** normalized stress-induced deficits in a manner resembling that of established antidepressants. **Lu AA33180** represents a good tool for exploring the role of the NPY5 receptor in disease-relevant preclinical models.

Acknowledgments

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19. The animals ($n = 2$) were dosed at 1 and 2 mg/kg iv and po, respectively, in a crossover manner following a 24 h washout. Both jugular and carotid artery cannulated Sprague–Dawley male rats with averaged body weight of 200–250 g were dosed with compound **15** dissolved in 20% beta cyclodextrin, pH adjusted with methane sulfonic acid to afford a solution. The serial blood samples consist of 12 time points were collected using an automated blood sampling device (Dilab, Lund, Sweden) over a 24 h post dose period. The plasma samples were collected by centrifugation of blood samples and the plasma concentrations were determined using a tandom, Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA) and a TSQ Quantum MS (ThermoFinnigan, San Jose, CA) with Xcalibur software. The PK parameters were determined performing noncompartmental analysis of plasma concentration-time profile using WinNonlin 5.2 Software (Pharsight, Cary, NC)
20. Rats were obtained from Charles River Labs (Kingston, NY) with cannulae implanted into the lateral cerebral ventricle. In order to achieve the number of animals for statistical power, experiments were performed using two different shipments of rats. Animals were dosed with either 20% cyclodextrin vehicle or **Lu AA33810** (3, 10, and 30 mg/kg) at 1, 2, 4, 6, or 24 h prior to i.c.v. administration of cPP (0.6 nmol) and monitoring of food intake over the following 1 h time period. Each of these 20 conditions (4 doses × 5 time points; $n = 2$/condition) was tested weekly on the same day, with 6–7 days intervening between tests. The drug treatment received by a given animal was randomized from week to week, as was the pretreatment time. Each animal was tested 4–5 times. Each week, brain and plasma samples were obtained from a parallel satellite group of animals ($n = 2$/dose). Results are presented as mean 1-h food intake (g) ± SEM from 16–20 animals per group. Data for each time point were expressed as percent of food intake relative to the vehicle + cPP group and analyzed with one-way analysis of variance. The Newman–Keuls test was used for post-hoc analysis.
21. The stress protocol was performed as described previously by Jayatissa et al.[22] Male SD rats, 200–250 g (at start of study) were acclimatized (singly housed) for 1 week before the start of the stress period. Animals were not handled during the acclimatization period (except for cage maintenance). During the drug administration period, drugs were injected intraperitoneally twice a day, using an injection volume of 1.0 ml/kg and a vehicle of 0.25% hydroxypropylmethylcellulose (HPMC). The reference compound, escitalopram (5 mg/kg) was dissolved by vortexing in vehicle. **Lu AA33810** (10 mg/kg) was added to vehicle, sonicated for 30 min until a fine suspension was formed and then injected as a suspension. Drug treatment groups were composed of $n = 25$; drug responders (those showing increase in sucrose consumption >10% after drug administration) were differentiated from nonresponders. Only drug responders were included in the analysis. Values

are the means ± SEM of $n = 10–13$ animals per group. All data were analyzed using a one-way ANOVA followed by post hoc Fischer LSD tests for pairwise comparisons of groups. Significant effect with escitalopram is evident after 1, 2, 3 and 4 weeks of treatment. For **Lu AA33810** there is a significant effect after 4 weeks of treatment, after 2 and 3 weeks there is an obvious tendency to a significant group effect. $^{*}P < 0.05$; $^{**}P < 0.01$.

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