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Discovery of aryl ureas and aryl amides as potent and selective histamine H_3 receptor antagonists for the treatment of obesity (Part I)

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ABSTRACT

A series of structurally novel aryl ureas was derived from optimization of the HTS lead as selective histamine H_3 receptor (H_3R) antagonists. The SAR was explored and the data obtained set up the starting point and foundation for further optimization. The most potent tool compounds, as exemplified by compounds **2l**, **5b**, **5d**, and **5e**, displayed antagonism potencies in the subnanomolar range in in vitro human-H_3R FLIPR assays and rhesus monkey H_3R binding assays.

Obesity[1] is characterized by accumulation of excess body fat and can be conceptualized as the physical manifestation of chronic energy excess. Thus, the fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. Obesity is rising globally and severe obesity (body mass index BMI of 40 kg/m^2 or 35 kg/m^2 with co-morbidity)[2] is growing at an even faster rate. Worldwide obesity has more than doubled since 1980. Today, one American in three is obese and this epidemic threatens many other countries, too. Obesity is an important risk factor for both cardiovascular disease and diabetes.

Eating behavior is regulated by a complex interplay of central neurotransmitter systems, peripheral endocrine stimuli, the circadian rhythm, and environmental cues, all factors that change the behavioral state and alter homeostatic aspects of appetite and energy expenditure. Brain histamine has long been considered a satiety signal in the central nervous system.[3] Histaminergic neural circuits arise in the tuberomammillary nucleus, and project into the satiety centers of the hypothalamus and participate in regulation of energy homeostasis.[4] The intrasynaptic level of histamine is primarily controlled by feedback signals from pre-synaptic histamine H_3 receptors (H_3R) that inhibit both the conversion of L-histidine to histamine and the release of histamine into the synaptic cleft. Despite conflicting preclinical data, insights are emerging into the potential role of H_3R as a target of anti-obesity therapeutics.[5] This has attracted many pharmaceutical companies to set up programs in discovery of H_3R antagonists for the treatment of obesity. The efforts in the field have resulted in many H_3R antagonists in diverse structural types.[6] When we started our H_3R program, we aimed to identify H_3R antagonists that inhibit food intake in the standard animal models, possess a predictable pharmacokinetic (PK) profile consistent with once-a-day dosing in humans, and demonstrate an acceptable safety profile.

High-throughput screening (HTS) of the in-house collections against a HEK293 cell-line stably expressing human H_3 receptors (h-H_3R) using imetit as an agonist under the standard calcium mobility based assay conditions (FLIPR)[7] in 384 well-format identified among other hits, a set of biaryl urea derivatives exemplified by compound **1**.

This set of the compounds is unique in that the molecules are symmetrical, with phenethylamines connected by a urea core. However, researchers from Johnson and Johnson[8] outlined issues observed with diamine compounds such as **1a** (JNJ-5207852) showing excessive brain residence time. Coincidentally, Zulli et al.[6n] also disclosed a lead compound (**1b**) which displayed an extremely high brain to plasma ratio (B/P = 50). It was hypothesized that the exceptionally long brain residence of the diamines might

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be either associated with their high basicity or due to the di-basic property of the molecule. We hence aimed at replacing one basic side chain with a non-basic functional group to reduce the overall basicity and, at the same time, eliminate one of the two basic centers of the molecule. In further modification of the lead compound, we adopted phenoxy-propylamine, a privileged fragment in the H_3R antagonist research field, to replace the other side chain (Fig. 1, **1** and **2**). Herein we describe the discovery of a novel series of selective H_3R antagonists through series of structural modifications of the lead structure **1** and SAR studies and identification of the new lead series (**2**) as tool compounds for further optimization in our H_3R program.

Analogs **2a**–**2p** were synthesized by coupling of commercially available substituted phenylamines **3** with substituted 4-aminophenyether derivatives **4** using carbonyl di-imidazole (CDI) as coupling agent at elevated temperature in DMF (Scheme 1).

Analogs **2a**–**2p** were tested in h-H_3R FLIPR assays (using the same protocol as for HTS) and their antagonism was confirmed by GTPγS assays.[9,10] The data are collected in Table 1. In general, lipophilic substituents with variations of substitution positions in the left-hand side aromatic ring were tolerated. The length of the side chain was found to be critical (**2a** vs **2b**; **2f** vs **2g**; **2j** vs **2k**; and **2o** vs **2n**) with $n = 3$ being optimal. Introduction of a piperidine as NR^1R^2-group improved potency (**2o** vs **2g**; and **2n** vs **2e**).

The species variability of the H_3R affinity of this series across human, rhesus monkey, and rat cell lines were then evaluated. Selected compounds (**2m**, **2o** and **2p**) were assessed in in vitro radioligand binding assays[11] by displacement of [^{3}H]*N*-α-methylhistamine in membranes isolated from a CHO cell line stably transfected with the cloned human H_3 receptors (h-H_3R), the rhesus monkey H_3 receptors (rh-H_3R) or the rat H_3 receptors (r-H_3R) (Table 2).

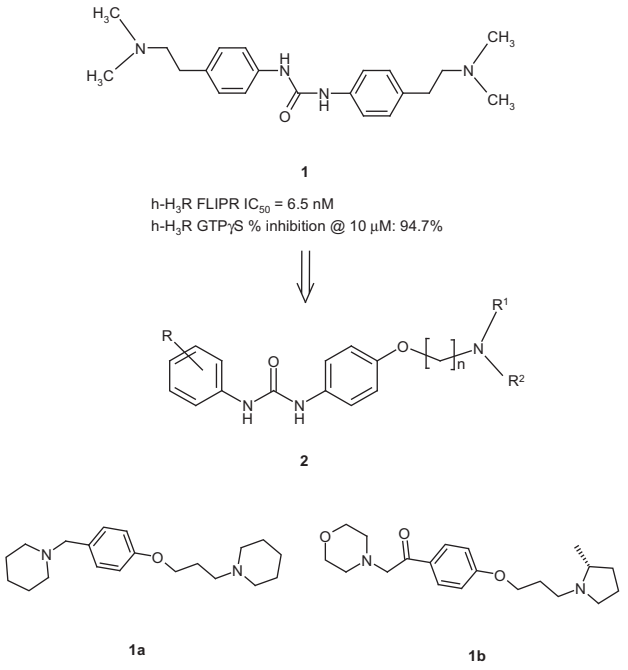


Figure 1. Structures of H_3R antagonists.

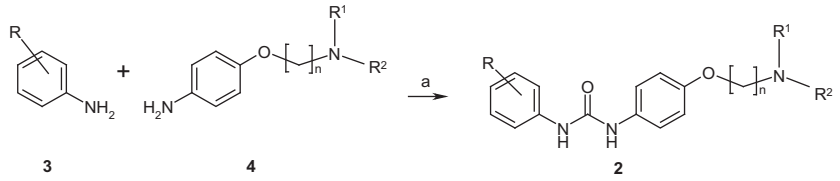
As shown in Table 2, the assessed compounds (**2m**, **2o**, and **2p**) displayed reasonably low species variations even though the numbers of available data points were limited. The project team took advantage of this by using rhesus monkey H_3R binding data as the first tier screening for all the synthesized compounds. The species variability was checked from time to time for selected compounds to ensure that the most interested compounds and each of the new series displayed good human and rat affinity. The choice of rhesus monkey H_3R binding data as the first tier screening was driven by the rationale that the data could be more useful for in vivo pharmacological screening of a few more structurally diverse novel compounds in rhesus monkey acute models in the future when the program advanced to that stage. The selected compounds could be profiled further and the development candidate could be selected.

Due to the less than optimal permeability and metabolic stability of analogs **2a**–**2p** (e.g., **2m** displayed solubility of 0.006 mg/mL, and metabolic liability of 10%, 34%, and 36% in human, mouse, and rat liver microsomes, respectively), we decided to optimize the side chain further. In this regard, the rigid bipyrrolidine side chain was introduced to replace the flexible side chain in **2** to give analogs **5** (Fig. 2).

Analogs **5a**–**5u** were synthesized effectively by condensing commercially available BOC-protected 3-pyrrolidinone (**6**) with 2-methyl-pyrrolidine (**7**) under reductive amination conditions to obtain intermediate **8** (Scheme 2). After de-protection, the desired intermediate **9** ([1,3′]-pyrrolidinyl-pyrrolidine) was realized. Condensation of **9** with *para*-fluoro-nitrobenzenes afforded intermediates **10**. Hydrogenation of **10** yielded anilines **11**, which were then coupled with various amines by using CDI in DMF to obtain arylurea analogs **5a**–**5u**.

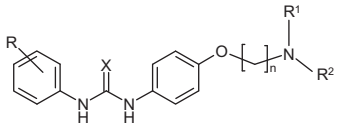
The analogs **5a**–**5u** were tested in human H_3R FLIPR and GTPγS functional assays, and rhesus monkey H_3R radioligand binding assays. The data are collected in Table 3.

As shown in Table 3, introduction of the more rigid bi-pyrrolidine side chain did not cause a significant drop of the potency (**5a** vs **2l**). On the contrary, the potency was substantially enhanced compared with the open chain analogs **2a**–**2p** for most of the compounds. The substituents at the central aryl residue were explored first. Compounds with R′ = H or 2-methyl were usually approximately equipotent (**5a**–**5b** and **5d**–**5e**). These compounds were more potent than compounds with R′ = 3-methyl, except **5g**. When analyzing the influence of substituents at the left-hand side aromatic ring towards rh-H_3R K_i, clear trends were observed: (1) lipophilic substituents were preferred over polar functional groups (**5d** vs **5g** and **5e** vs **5h**); (2) the orientation of the hydrogen-bond forming atoms (oxygen and nitrogen) appeared not critical (K_i of **5j** was comparable with that of **5n**), suggesting that hydrogen bonding interactions might play a less important role in this portion of the molecules; (3) the size of the substituents was important. For example, affinity of **5t** was twofold lower than that of **5a** and **5u** was 3-fold lower in affinity than **5c**. It is worth noting



Scheme 1. Syntheses of analogs **2a**–**2p**. Reagents and conditions: (a) CDI, DMF, 80 °C, 2–14 h.

Table 1
H_3R antagonist activity of compounds **2a–2p**



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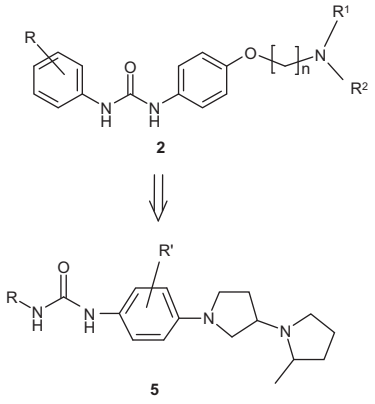
Compd No.	R	*n*	X	R^1R^2N–	h-H_3R FLIPR IC_{50}[a] (nM)	h-H_3R GTPγS I% @ 10 μM[b] (%)
2a	2,3-Di-Me	2	O	Me_2N–	73.1	94
2b	2,3-Di-Me	3	O	Me_2N–	14.7	125
2c	4-Cl	2	O	Me_2N–	16.4	99
2d	2,4-Di-Cl	2	O	Et_2N–	39.1	118
2e	2,4-Di-F	2	O	Me_2N–	148.6	102
2f	2,4-Di-F	2	O	Et_2N–	111.4	117
2g	2,4-Di-F	3	O	Et_2N–	7.9	124
2h	2-EtO, 5-Me	2	O	Et_2N–	9.1	107
2i	2,6-Di-Cl	2	O	Et_2N–	1.0	116
2j	4-Cl	2	O	Piperidinyl	27.5	105
2k	4-Cl	3	O	Piperidinyl	0.7	119
2l	3,5-Di-Cl	3	O	Piperidinyl	0.5	125
2m	4-Me, 3,5-di-Cl	3	O	Piperidinyl	0.2	133
2n	2,4-Di-F	2	O	Piperidinyl	40.5	115
2o	2,4-Di-F	3	O	Piperidinyl	0.5	128
2p	4-iBuO–	2	S	Et_2N–	0.6	129

[a] h-H_3R FLIPR IC_{50} were determined in HTS with a 384-well format, using Imetit agonist.
[b] h-H_3R GTPγS assays were performed in triplicates at 10 μM of the compounds tested; the data were expressed as percent of inhibitions (I%) of basal GTPγS binding.

Table 2
Assessment of species variation of H_3R binding (K_i) across human, rhesus monkey, and rat

Compd No.	h-H_3R FLIPR IC_{50} (nM)	h-H_3R binding K_i[a] (nM)	rh-H_3R binding K_i[a] (nM)	r-H_3R binding K_i[a] (nM)	h-H_3R GTPγS I% @ 10 μM[b] (%)
2m	0.2	112.3	18.2	135.0	133
2o	0.56	52.0	3.4	33	128
2p	0.63	16.8	15.7	39.9	129

[a] K_i values were averages of three or more determinations.
[b] h-H_3R GTPγS assays were performed in triplicates at 10 μM of the compounds tested; the data were expressed as percent of inhibitions (I%) of basal GTPγS binding.



2

5

Figure 2. Structures of H_3R antagonists.

that urea analogs of secondary amines (**5i**–**5n**) were also active, suggesting that the left-hand side NH of the urea moiety was not essential for binding. This piece of the data provided the foundation for our amide bioisosteric replacement.

In the next step, the selectivity of the compounds toward H_1R, H_2R, and MCH_1R[12] was assessed. The compounds in Table 3 were tested for human H_1R and H_2R binding as well as in human MCH_1R FLIPR assays. They all showed weak to no activities toward these targets (percent inhibition <40% @ 10 μM).

In spite of these positive results, this series was not optimal for being an oral drug candidate due to multiple drawbacks (poor solubility, hERG and Cyp inhibition; e.g., **5b** displayed solubility of 0.0105 mg/mL, cyp 3A4 inhibition with IC_{50} of 13 μM, and hERG percent inhibition of 74% at 10 μM). However, the series merited further optimization due to its potency at H_3R, low interspecies variability, and high selectivity. The continued work will be described in a subsequent letter (Part II) dealing with the amide replacement of the urea moiety.

In summary, we have described the SAR around an initial urea series of H_3R ligands. The HTS derived hit compound **1** was modified through a series of medicinal chemistry approaches, such as re-scaffolding to remove the di-basic property of **1**, and side chain optimization including installation of rigidity. These efforts yielded a series of H_3R antagonists with subnanomolar affinity in a rh-H_3R binding assay as exemplified by compounds **2l**, **5b**, **5d**, and **5e**. This series provided a set of tool compounds for further optimization to derive a drug candidate for preclinical and clinical evaluations. Future efforts around this chemotype will be focused on maintaining the excellent potency and selectivity while improving the pharmacokinetic profile.

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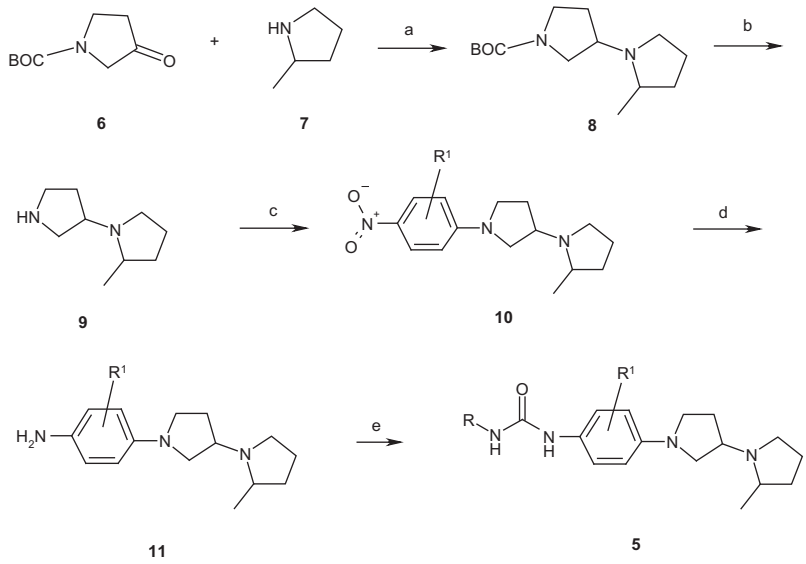
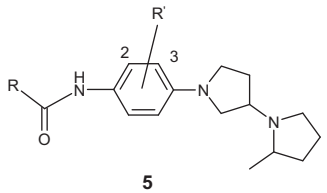
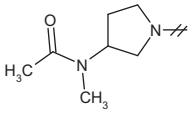
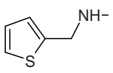


Scheme 2. Syntheses of analogs **5a**–**5u**. Reagents and conditions: (a) $NaBH(OAc)_3$, DCE, rt overnight, 84% yield; (b) 4 M HCl in dioxane, rt, 1 h, 100% yield; (c) (optionally further substituted) *para*-fluoro-nitro-benzene, K_2CO_3, DMSO, 80 °C, 2 h; 80% yield; (d) H_2, 10% Pd–C, MeOH, rt 2 h, 100% yield; (e) amine, CDI, DMF, 80 °C, 2–14 h, 80–84% yield.

Table 3
SAR of arylurea analogs **5a**–**5u**



Compd No.	R	R′	h-H_3R FLIPR IC_{50} (nM)	h-H_3R GTPγS I% @ 10 μM[a] (%)	rh-H_3R binding K_i[b] (nM)
2l			0.5	125	3.4
5a	3,5-Di-Cl-phenyl-NH–	H	1.2	103	19.1
5b	3,5-Di-Cl-phenyl-NH–	2-Me	0.9	126	22.2
5c	3,5-Di-Cl-phenyl-NH–	3-Me	3.1	103	43.0
5d	Cyclohexyl-NH–	H	0.1	122	4.1
5e	Cyclohexyl-NH–	2-Me	0.2	110	4.6
5f	Cyclohexyl-NH–	3-Me	0.2	130	11.2
5g	1-Methyl-piperazinyl	H	0.2	132	25.7
5h	1-Methyl-piperazinyl	2-Me	0.8	120	10.5
5i	1-Methyl-piperazinyl	3-Me	1.1	118	12.7
5j	1-Ac-piperazinyl	H	0.5	127	34.1
5k	1-Ac-piperazinyl	3-Me	2.3	115	23.0
5l	1-Phenyl-piperazinyl	H	0.4	122	10.6
5m	Piperidinyl	H	0.3	108	6.0
5n		H	1.1	109	35.5
5o		3-Me	1.1	118	89.7
5p		H	0.1	129	1.9
5q		3-Me	0.2	125	8.4
5r	3,5-Di-Cl-benzyl-NH–	H	0.2	144	7.1
5s	3,5-Di-Cl-benzyl-NH–	3-Me	1.1	130	28.8
5t	4-PhO-Ph-NH–	H	1.4	113	46.8
5u	4-PhO-Ph-NH–	3-Me	0.6	126	115.2

[a] h-H_3R GTPγS assays were performed in triplicates at 10 μM of the compounds tested; the data were expressed as percent of inhibitions (I%) of basal GTPγS binding.
[b] K_i values were averages of three or more determinations.

tributions and to the Sanofi Analytical Department for their support on analytical studies in confirmation of the structures.

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9. The rhesus H_3 GTPγS radioligand binding assay was performed utilizing a modified standard protocol [*Br. J. Pharmacol.*, **2002**, *135*, 383]. Assay mixture contained 50 μl of rhesus H_3 receptor membranes (prepared as described in the literature; 20–30 μg protein/well), diluted in assay buffer (20 mM HEPES–NaOH, pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 3 μM GDT) along with 50 μl of 1 nM [^{35}S] GTPγS (Amersham), 50 μl of WGA SPA beads (0.1 μg) (Amersham) and 50 μl of compound in a 96-well opti-plate (Packard). Total assay volume 200 μl. Assay plates were sealed with TopSeal (Perkin Elmer), incubated (25 °C, 90 min) and centrifuged (1000 rpm, 5 min). Assay plates were read on TopCount scintillation counter (Packard). Non-specific binding was determined by running the assay in the presence of 20 μM GTPγS (Sigma). Inverse agonism was measured by percent inhibition of basal GTPγS binding. This assay was also evaluated using parental cell membranes (Flp-In T-REx 293 Cell Line, Invitrogen) to confirm that the measured GTPγS binding was rhesus H_3-mediated and not due to endogenous receptors.
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11. H_3 radioligand binding assay was performed using H_3 receptor membranes prepared from the Flp-In T-REx 293 Cell Line (Invitrogen) stably transfected with pcDNA5/FRT/TO (Invitrogen) containing the human or rhesus monkey (Macacca Mulatta) or rat 445 amino acid H_3 receptor. [^{3}H]-methylhistamine (Perkin Elmer) and WGA SPA beads (wheat germ agglutinin scintillation proximity assay) beads (Amersham). The assay was performed in 96-well opti-plates (Packard). Each reaction contained 50 μl H_3 membranes (20–30 μg total protein), 50 μl WGA SPA beads (0.1 μg) and 50 μl of 83 Ci/mmol [^{3}H]-methylhistamine (final concentration 2 nM) and 50 μl of tested compound. The exemplified compounds and/or vehicle were diluted with binding buffer from 10 mM DMSO stocks. Assay plates were sealed with TopSeal (Perkin Elmer) and mixed on shaker (25 °C, 1 h). Assay plates were read on TopCount scintillation counter (Packard). Results were analyzed by Hill transformation and K_i values were determined by Cheng–Prusoff equation.
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