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Development of a selective and potent radioactive ligand for histamine H_3 receptors: A compound potentially useful for receptor occupancy studies

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ABSTRACT

Radioligands are powerful tools for examining the pharmacological profiles of chemical leads and thus facilitate drug discovery. In this study, we identified and characterized 3-([1,1,1-^{3}H]methyl)-2-(4-{[3-(1-pyrrolidinyl)propyl]oxy} phenyl)-4(3*H*)-quinazolinone ([^{3}H]**1**) as a potent and selective radioligand for histamine H_3 receptors. Radioligand [^{3}H]**1** exhibited appreciable specific signal in brain slices prepared from wild-type mice but not from histamine H_3 receptor-deficient mice, demonstrating the specificity and utility of [^{3}H]**1** as a selective histamine H_3 receptor radioligand for ex-vivo receptor occupancy assays.

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The histamine H_3 receptor, one of the G protein-coupled receptors (GPCRs) for histamine, is predominantly expressed in the central nervous system (CNS) and is known to play important roles in various physiological functions. Accumulating evidence has suggested that histamine H_3 receptor antagonists/inverse agonists may be promising agents for the treatment of cognitive dysfunction, epilepsy, hypersomnia, and obesity.[1] Several classes of imidazole- and non-imidazole H_3 receptor antagonists/inverse agonists have been developed to target CNS H_3 receptors.[2–4] Among them, BF2.649,[5,6] ABT-239[7], and GSK189254,[8] which are non-imidazole compounds, have entered clinical trials for treatment of CNS disorders, for indications which include excessive daytime sleepiness, schizophrenia, and cognitive dysfunction (Fig. 1).

In drug discovery, potent and selective radioligands for target molecules are powerful tools and facilitate not only in vitro characterization but also in vivo pharmacodynamic profiling (i.e., determination of relationships between efficacy and levels of target engagement) of test compounds. Several radioligands including positron emission tomography (PET) tracers have been developed and characterized for histamine H_3 receptors.[9–12] However, low brain penetrability and/or high background noise limit the application of these radioligands as reliable and useful radiotracers.[13,14] For example, despite its high performance in in vitro assays, [^{11}C]JNJ-10181457 exhibited no discernable specific binding in rodent brains, suggesting that demonstration of in vivo selectivity (e.g., loss of specific signals in receptor-deficient mice) remains an essential but as yet unmet goal integral for the development of useful and reliable radiotracers.[14]

In histamine H_3 receptor biological and pharmacological studies, [^{3}H] N^α-methylhistamine, a H_3 receptor agonist, has often been used as a standard radiotracer for ligand binding assays of tissues and cells. However, the lack of selectivity of N^α-methylhistamine for histamine H_4 receptors limits the usefulness of [^{3}H] N^α-methylhistamine for whole-tissue studies.[15]

Recently, we have reported the synthesis of a number of quinazolinone analogues that exhibited high potency and selectivity for histamine H_3 receptors.[16,17] In the present study, we report the synthesis and pharmacological characterization of [^{3}H]**1** as a potent and selective inverse agonist radioligand for histamine H_3 receptors (Fig. 2).

We previously reported novel 2-[4-(alkoxy)phenyl]-4(3*H*)-quinazolinone derivatives which exhibited potent human histamine H_3 receptor binding activity.[9] In the course of structure–activity relationship (SAR) studies, a highly potent compound, [^{3}H]**1** was identified as a promising radioligand candidate with low hydrophilicity (Log $D_{7.4}$ = 1.2). With respect to P-glycoprotein (P-gp) transporter susceptibility, the transcellular transport ratios ((B-to-A)/(A-to-B)) of compound **1** for human *MDR1* and mouse *mdrla* proteins were 1.9 and 2.1, respectively, indicating the low susceptibility of compound **1** to P-gp. Consistent with these observations, compound **1** exhibited high brain penetrability 2 h after 10 mg/kg oral administration to SD rats (brain = 2.22 nmol/g, plasma = 1.36 μM, brain-to-plasma ratio = 1.6) (Table 1).

Tritium-labeled compound **1** was synthesized as outlined in Scheme 1. The 2-aminobenzamide **2** was thermally condensed

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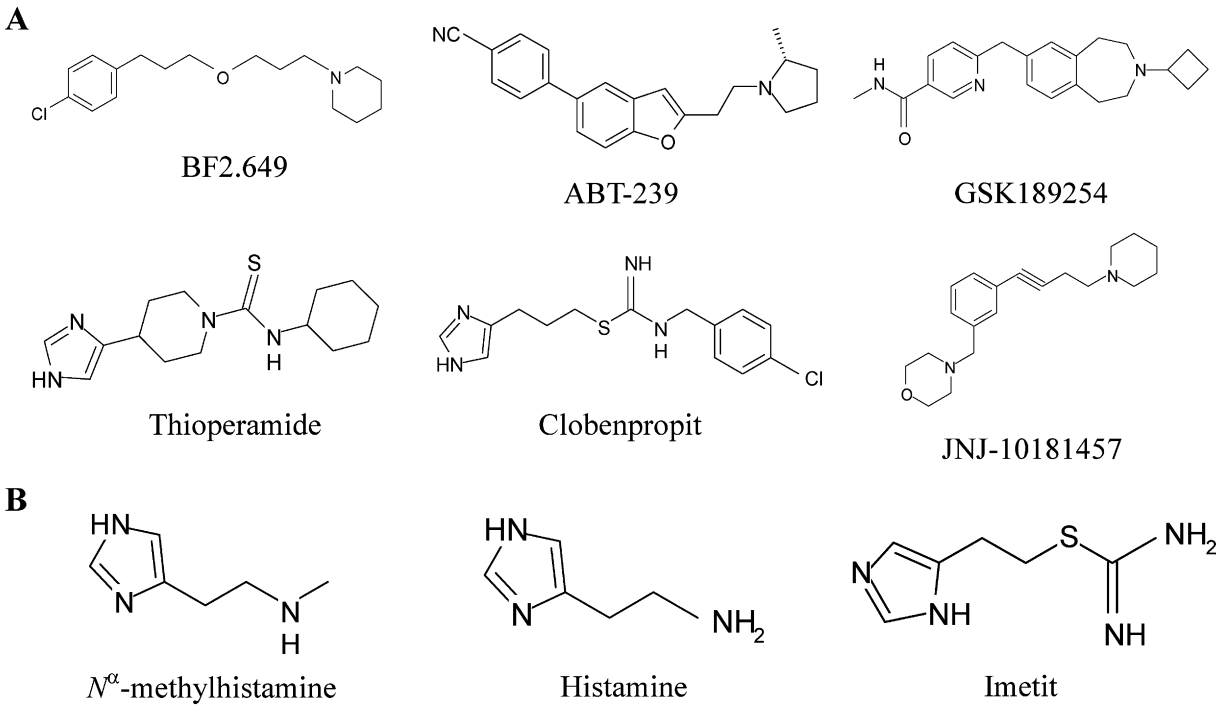


Figure 1. Structures of histamine H_3 receptor (A) antagonists/inverse agonists and (B) agonists.

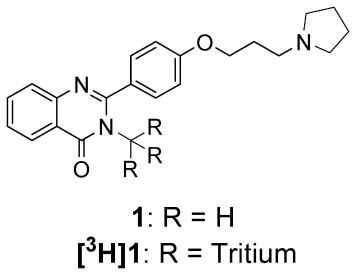


Figure 2. Structure of compound **1**.

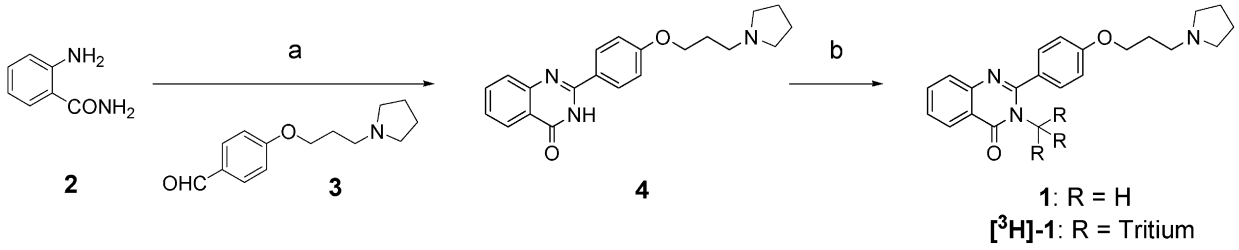
with 4-[3-(pyrrolidin-1-yl)propoxy]benzaldehyde **3** in the presence of a catalytic amount of *p*-toluenesulfonic acid, followed by oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone to obtain **4**. Methylation of **4** then furnished **1**, establishing the route of synthesis of [^{3}H]**1** from the corresponding precursor **4** using a [^{3}H]methyl iodide as a radiolabel donor. Subsequently, radioligand [^{3}H]**1** was purified using radio-HPLC analysis, resulted in the production of a high-specific radioligand with appreciable purity (83.7 Ci/mmol, >95% purity) .

In the [^{35}S]GTPγS binding assay, which measures the functional activity of ligands for G-protein coupled receptors,[18] we observed that compound **1** potently decreased basal [^{35}S]GTPγS binding activity in human embryonic kidney (HEK) 293 cell membranes overexpressing recombinant mouse histamine H_3 receptors, with

Table 1
Brain penetration and P-gp transport ratio of compound **1**

Compd	Brian penetration in SD rats[a]			P-gp transporter assay[b] Transcellular transport ratio (B-to-A)/(A-to-B)	
	Plasma (μM)	Brain (nmol/g)	CSF (μM)	*MDR1*	*mdr1a*
1	1.36	2.22	0.134	1.9	2.1

[a] The values represent the means from three animals. The concentrations were determined at 2 h after 10 mg/kg po.
[b] Transcellular transport ratios ((B-to-A)/(A-to-B)) for human and mouse P-glycoproteins were determined in the cellular transporter assay using porcine renal epithelial cells expressing human *MDR1* and murine *mdr1a* proteins.



Scheme 1. Reagents and conditions: (a) 4-[3-(pyrrolidin-1-yl)propoxy]benzaldehyde, *p*-toluenesulfonic acid, toluene, 100 °C, 15 h, then 2,3-dichloro-5,6-dicyanobenzoquinone, rt, 6 h, 67%; (b) NaH, CH_3I or CT_3I, DMF.

Table 2
EC_{50} values and maximum inhibition (% basal control) of H_3 receptor ligands for mouse histamine H_3 receptor in the [^{35}S]-GTPγS binding assay

Compound	Potency EC_{50} (nM)	Efficacy max. inhibition (%)
Compound **1**	9.6	24
Thioperamide	11	27
Clobenpropit	1.7	14

The values represent the means from two independent experiments.

an EC_{50} of 9.6 nM, indicating that compound **1** is a potent inverse agonist for mouse histamine H_3 receptors (Table 2). The maximum efficacy (i.e., inverse agonism) of compound **1** was comparable to that of thioperamide. It has been reported that thioperamide is a potent inverse agonist of rodent histamine H_3 receptors while exhibiting significantly lower activity for human and monkey histamine H_3 receptors,[19] limiting the utility of thioperamide in higher species. In contrast, compound **1** exhibited high intrinsic potencies for rodents and human histamine H_3 receptors in competitive ligand binding assays (human K_i = 4.2 nM, rat K_i = 9.6 nM, mouse K_i = 12 nM). In addition, compound **1** displayed high degrees of selectivity over other histamine receptor subtypes (IC_{50} >10 μM for histamine H_1, H_2, and H_4 receptors) and a panel of 86 GPCRs (data not shown). Furthermore, compound **1** exhibited low lipophilicity as described above (Log $D_{7.4}$ = 1.2), which might be advantageous in terms of decreasing the background noise that is often associated with non-specific binding.

With these promising biochemical and physicochemical properties, the potential of [^{3}H]**1** as a radioligand for histamine H_3 receptors was examined in in vitro membrane binding and ex vivo brain slice binding assays.

Scatchard plot analysis of saturation binding of [^{3}H]**1** against recombinant mouse histamine H_3 receptors revealed that radioligand [^{3}H]**1** bound to a single site with a K_d value of 2.6 ± 0.36 nM, consistent with its high potency in the GTPγS functional assay (Fig. 3). To further examine the receptor binding profile of compound **1**, we conducted a competitive binding assay using [^{3}H]**1** as a radioligand. Binding of compound **1** to mouse histamine H_3 receptors was completely replaced by a series of known histamine H_3 receptor ligands including agonists (N^{α}-methylhistamine, histamine, and imetit) and the antagonists/inverse agonists thioperamide, clobenpropit, and compound **1**. The K_i values of compound **1**, thioperamide, clobenpropit, N^{α}-methylhistamine, histamine, and imetit are 2.4, 4.8, 0.60, 19, 68, and 2.0 nM, respectively. These findings suggest that radioligand [^{3}H]**1** will be useful for studies of histamine H_3 receptor ligands regardless of their functional classes (Fig. 4).

Finally, we examined the profiles of binding of radioligand [^{3}H]**1** to brain membranes and slices prepared from wild-type and histamine H_3 receptor knockout mice.[20] Consistent with the findings obtained with recombinant mouse histamine H_3 receptors described above, radioligand [^{3}H]**1** displayed high binding affinity to the brain membranes from wild-type mice (K_d = 3.1 nM) while no significant specific binding to brain membranes was observed in the receptor knockout mice (data not shown) .

In the receptor occupancy (RO) assay, the striatum was used as a target area, since histamine H_3 receptors are widely and abundantly expressed in this region.[21–23] Coronal brain sections (20 μm) including striatum were incubated with 3 nM of radioligand [^{3}H]**1** in RO assay buffer (pH 7.4, 150 mM NaH_2PO_4, 2 mM $MgCl_2$, Tris–HCl) for

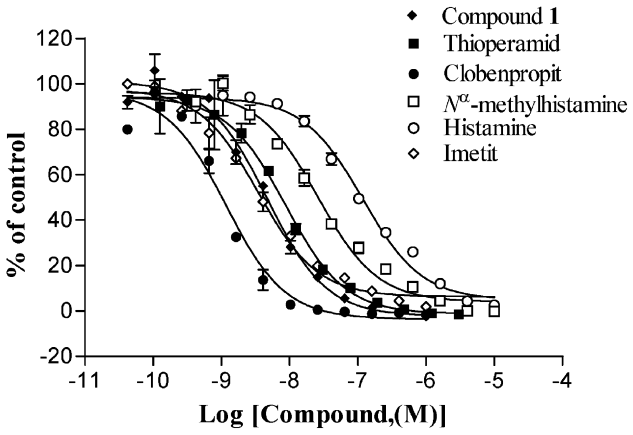


Figure 4. Displacement curves for radioligand [^{3}H]**1** in competition with H_3 ligand in membranes expressing recombinant mouse H_3 receptors. The membranes from HEK293 cells expressing mouse H_3 receptors were incubated with 2 nM [^{3}H]**1** for competition binding assays in the presence of various compound concentrations in buffer (50 mM Tris–HCl, pH 7.4) at 25 °C for 1 h. Following three washes, membrane-bound radioactivity was measured by a liquid scintillation counter. Non-specific binding was determined in the presence of excessive cold (*R*)-α-methylhistamine (10 μM). The result shown is one of two independent experiments.

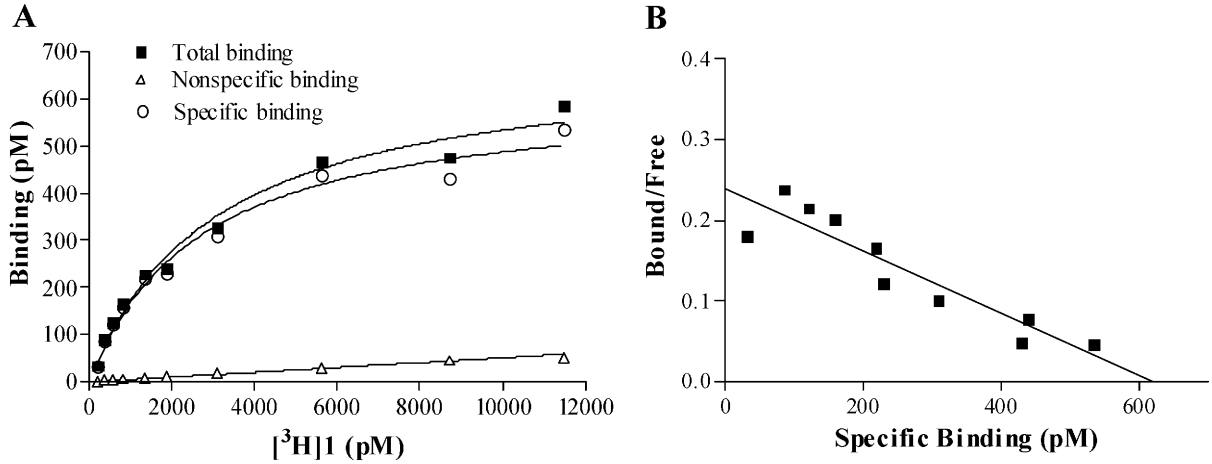


Figure 3. Saturation binding of radioligand [^{3}H]**1** binding in membranes expressing recombinant mouse H_3 receptors. Membranes from HEK293 cells expressing mouse H_3 receptors were incubated with several concentrations of [^{3}H]**1** in the presence of DMSO (total binding) or 1 μM compound **1** in assay buffer (pH 7.4, 50 mM Tris–HCl) at 25 °C for 1 h. The membranes were filtered with GF/C filter and washed with washing buffer (pH 7.4, 50 mM Tris–HCl) three times. The residual radioactivities on the dried filter were measured by microplate scintillation counter. The results shown are from a typical experiment from among three independent experiments. (A) Saturation binding isotherm. (B) Scatchard plot analysis of binding of [^{3}H]**1** resulted in a K_d of 3.9 ± 0.49 nM and a B_{max} of 6.2 ± 0.12 pmol/mg protein.

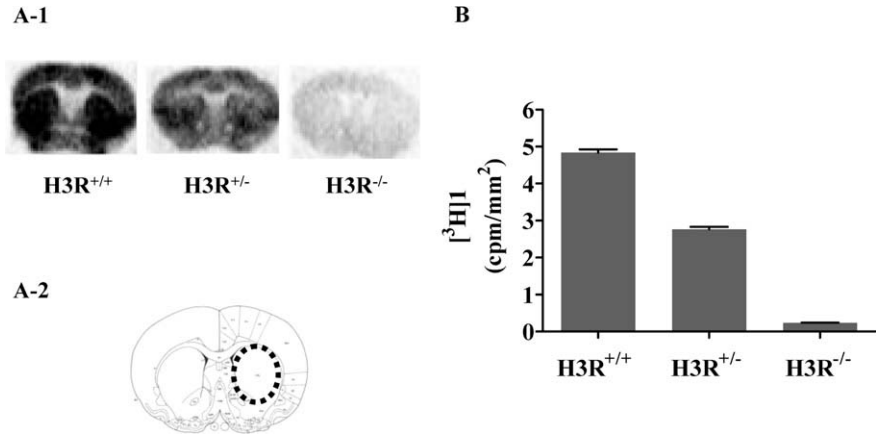


Figure 5. Ex vivo autoradiography of radioligand [^{3}H]**1** binding to the striatum in mouse brains. (A-1) Ex vivo autoradiography with β-imager detection of radioligand [^{3}H]**1** binding to brain sections including striatum. Total binding to wild-type (H3R$^{+/+}$), histamine H3 receptor hetero knockout (H3R$^{+/-}$), and homo knockout (H3R$^{-/-}$) mouse brain sections. (A-2) In this schematic, the striatal region examined in this study is surrounded by a circle. (B) Quantitative analysis of signal potency of [^{3}H]**1** binding to the striatum in the mouse brains. The signal potency of the striatum in the each section was determined in cpm/mm^2 using the β-imager.

30 min at 25 °C. After three washes at 4 °C, residual radioactivities in the striatum were imaged and quantified (in cpm/mm^2) with the β-imager (BioSpace, Paris, France). Radioligand [^{3}H]**1** yielded appreciable signal in wild-type brains but negligible signal in receptor knockout brains, demonstrating the high selectivity of [^{3}H]**1** in vivo (Fig. 5). Moreover, we observed gene-dosage-dependent signal in the wild-type, hetero, and homo knockout brains, suggesting that radioligand [^{3}H]**1** can be used to quantitate levels of expression of histamine H_3 receptors in the brain.

In summary, we developed a highly potent and selective radioligand [^{3}H]**1**, which displayed competitive binding to histamine H_3 receptorxs (vs known histamine H_3 receptor ligands) and a specific signal in brain sections. The high selectivity of and appreciable brain penetration by compound **1** make it promising as a radiotracer for in vitro and in vivo studies. Given the importance of pharmacodynamic findings for compounds in drug discovery, radioligand [^{3}H]**1** will be a useful tool for the development of histamine H_3 receptor agents.

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